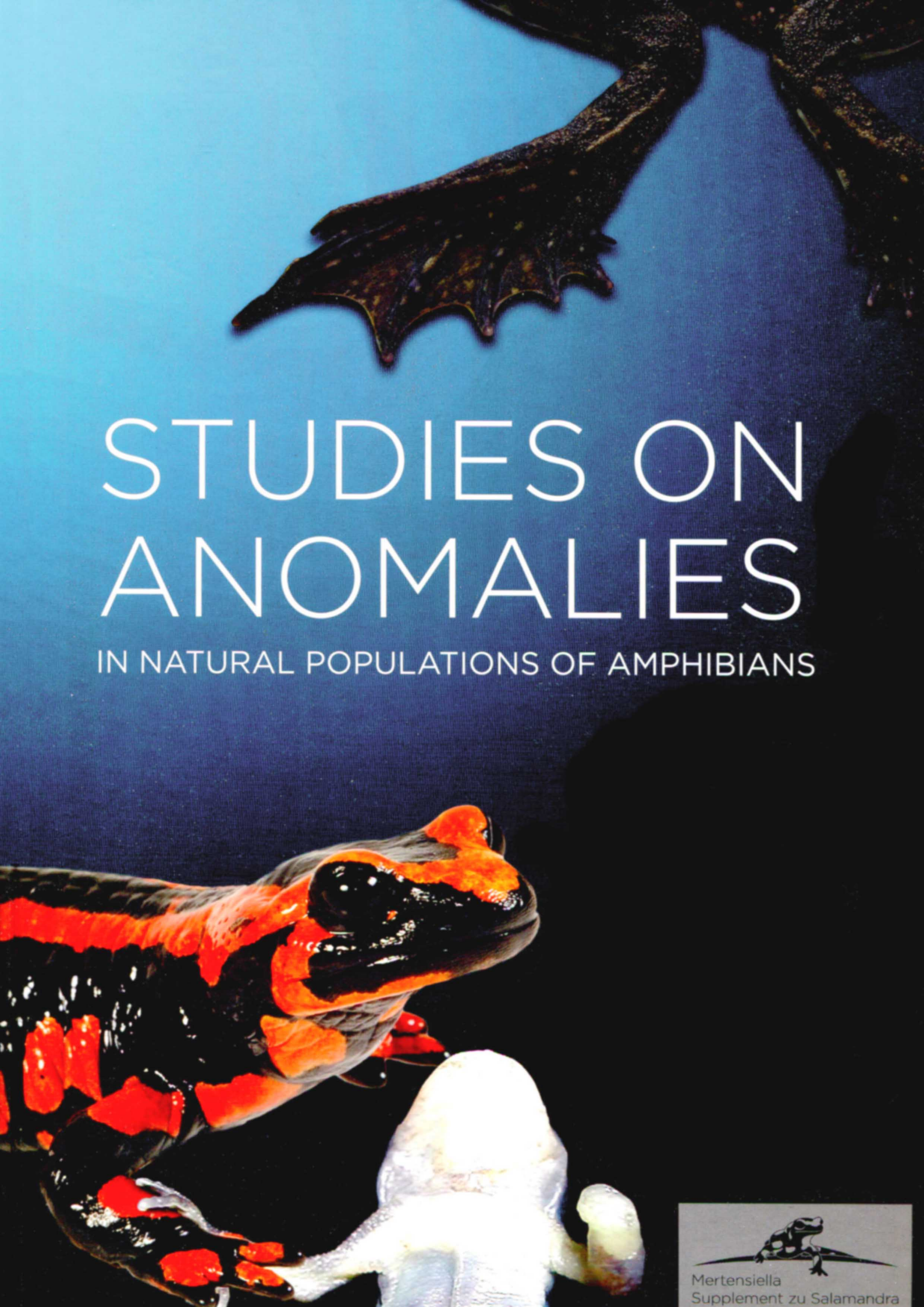


STUDIES ON ANOMALIES

IN NATURAL POPULATIONS OF AMPHIBIANS

Mertensiella
Supplement zu Salamandra

MERTENSIELLA

Studies on Anomalies in Natural Populations of Amphibians

Untersuchungen zu Anomalien in natürlichen Populationen von Amphibien

Herausgegeben von

Klaus Henle & Alain Dubois

im Autrag der
Deutschen Gesellschaft für Herpetologie und Terrarienkunde e.V.

Mertensiella 25
Supplement zu Salamandra
Mannheim, 15. August 2017

A catalogue record for this publication is available from the German National Library (Deutsche Nationalbibliothek) at http://dnb.dnb.de

Studies on Anomalies in Natural Populations of Amphibians
Edited by Klaus Henle & Alain Dubois
Distributor: Chimaira Buchhandelsgesellschaft mbH
Heddernheimer Landstr. 20
60439 Frankfurt am Main, Germany
Telefon: +49 69 497223
Fax: +49 69 497826
E-Mail: frogbook@aol.com
Internet: www.chimaira.de

Layout: Andreas Mendt, Office of the DGHT
Typesetter: Ogarit Uhlmann, Leipzig
using Minion Pro
Cover design: Darina Schmidt, with photos of: Front cover top: *Pelophylax ridibundus* with polydactyly, Ekaterinburg, Russia (photo: Vladimir Vershinin). Front cover bottom left: captive bred erythristic *Salamandra salamandra* (photo: Benny Trapp). Front cover center: adactyly in a juvenile *Bufotes viridis*, Magnitogorsk, Russia (photo: Vladimir Vershinin). Back cover top left: juvenile *Bufotes viridis* with polymely from an anomaly hotspot near Roßwag, Germany (photo: Klaus Henle). Back cover top right: Captive bred *Dendrobates auratus* with polymely (photo: Harald Divossen). Back cover centre: incomplete duplication of the hand in a *Salamandra atra*, Hintersteiner Tal, Germany, July 2009 (photo: Ulrich Schulte).
Printed and bound by: Westermann Druck Zwickau GmbH, Zwickau, Germany

ISBN 978-3-89973-570-3
ISSN 0934-6643

Table of contents

Preface

Anomalies in humans and animals have attracted curiosity for centuries. Long ago scientific writers started to collate their observations of anomalies (primarily of humans and their domesticated animals). Apart from the odd early observation, amphibian anomalies in natural populations only really came on the scene about 150 years ago. Many amphibians develop externally and therefore abnormal development during ontogeny is comparably easy to detect. It is largely because of this that amphibians have played, and still play, such a pivotal role in the emerging science of developmental biology. However, natural history and developmental biology were only merged in the early phases of research on anomalies in natural populations of amphibians and, until recently, the latter largely remained the domain of non-professional naturalists and the spare time of professional herpetologists, without entering mainstream science. Nevertheless, an impressive amount of literature has accumulated on anomalies in natural populations.

More recently, increased environmental awareness, the global decline in amphibians and the increased number of observations of populations exhibiting mass anomalies have created renewed interest in amphibian anomalies as potential indicators for environmental perturbations. Observations of mass anomalies have started to raise questions about why and what it means for the affected individual, humans and the environment. The same applies to the senior editor of this issue of MERTENSIELLA, when he stumbled by accident on a population of green toads (*Bufotes viridis*) in a quarry near Roßwag in southern Germany that exhibited a range of different types of anomalies and a high frequency of affected individuals that later turned out to make it one of the hottest hotspots of anomalies in amphibians ever discovered.

This discovery was the start of a long and heated controversial discussion about the causes of these anomalies. The final official conclusion of the German authorities was that these anomalies were of a natural and not anthropogenic origin, being a combination of inbreeding, hybridization and atavism. While it was clear that this explanation does not have a sound scientific basis, the widely scattered literature on the subject has made it challenging to evaluate the case appropriately. Biologists in North America experienced the same difficulty when they rediscovered mass anomalies in amphibians in the mid–1990s.

At the time when the case near Roßwag was discovered in 1980, specialists on anomalies in natural populations of amphibians were almost non-existent and even today very few scientists or research groups focus on this topic. The junior editor of this volume, Alain Dubois, was called in as a witness for the suggestion that the anomalies observed in Roßwag had a natural cause and that the case was nothing unusual. He was introduced to the study of anomalies in amphibians by Jean Rostand, an amateur naturalist. At that time Jean Rostand was the world's leading expert on anomalies in natural populations of amphibians and the first person to systematically investigate the cause of a certain type of mass anomalies affecting European water frogs (*Pelophylax* species), which he termed anomaly P. At some stages during the debate about the Roßwag case, it was suggested that the mass anomalies observed in Roßwag were also a form of anomaly P in spite of very incompatible patterns of anomalies as was immediately clear to A. Dubois.

The contributions to this volume of MERTENSIELLA reflect the experiences that we made during the debates about the causes of the anomalies of the green toads from the quarry near Roßwag, as well as the difficulties we encountered in reviewing and analysing the relevant literature, and the observations that others in other parts of the world have faced similar challenges. One of the challenges was the absence of a comprehensive terminology with synonyms, as terminology is often inconsistently used and changed over time. We therefore begin with a contribution on this topic. The second contribution provides an updated review of anomaly P.

Although there have been a large number of reviews of specific issues within the research field of amphibian anomalies, there was no comprehensive overview at the time of our discovery in Roßwag. Even today, it is challenging to find an appropriate overview of what has been observed about amphibian anomalies in the wild and what the assumed causes are. We therefore provide a comprehensive review of these issues in the form of a compendium with the aim of helping others to find their way to the relevant literature.

The lack of a comprehensive overview at the time of our discovery also created challenges to develop and test hypotheses about potential causes. The same difficulties reappeared when North American scientists started to study North American anomaly hotspots and the lasting controversy about potential causes stress how difficult it still is to get to the bottom of them. This controversy also highlights the urgent need for a comprehensive reference to the types of anomalies that can be caused by various factors. By presenting the research on the Roßwag case we summarize the results of experimental studies including all factors that have so far been suggested as potential culprits for anomalies in natural populations of amphibians.

Addressing mass anomalies in natural populations creates challenges well beyond science, often involving very sensitive issues in politics and society. Natural scientists are not trained to handle such issues, but rather have to learn these skills by trial and error, usually without guidance. The experiences of Judy Helgen in her book 'Perils in the Pond' demonstrate this for the work on North American hotspots. Our experiences were very similar. To help others that might encounter similar problems, whether with amphibians or other species, we wrote about our failures and success and provide recommendations that could help to avoid the worst pitfalls.

Finally, as appropriate study designs are a prerequisite for improving our knowledge of anomalies in natural populations and their causes, we provide guidelines for such studies.

We would like to use this opportunity to thank all of our friends and colleagues that helped us over the years of work in compiling this volume, either in the field, with the literature or by commenting on various parts of our manuscripts. Above all, we would like to thank Jean-Claude Beetschen, Toulouse, and Alain Collenot, Paris, for the painstaking work of carefully reviewing and commenting on all of our manuscripts. Their life-long research experience on anomalies in amphibians has considerably helped to improve our manuscripts. Vladimir Vershinin facilitated access to the Russian literature. KH cordially thanks Marlies Uhlig, Leipzig, for the tremendous help in finding and acquiring literature on amphibian anomalies, cataloguing it in databases and helping to check the reference lists in our manuscripts. He is also most grateful to Natalia Rumyantseva, Leipzig, for help with translations of Russian publications and for enduring endless hours of his work on the manuscripts.

Klaus Henle
Leipzig, August 2017

Alain Dubois
Paris, August 2017

MERTENSIELLA 25 | 9–48 | 2017

Commented glossary, terminology and synonymies of anomalies in natural populations of amphibians

Klaus Henle[1], Alain Dubois[2] & Vladimir Vershinin[3,4]

[1]UFZ – Helmholtz Centre for Environmental Research,
Department of Conservation Biology, Permoserstr. 15, 04318 Leipzig, Germany; email: klaus.henle@ufz.de

[2]Institut de Systématique, Évolution, Biodiversité ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP 30, 75005, Paris, France; email: adubois@mnhn.fr

[3]Institute of Plant and Animal Ecology, Uralian Division, Russian Academy of Science,
8 Marta St 202, 620144, Ekaterinburg, Russia; email: vol_de_mar@list.ru

[4]Ural Federal University, Ekaterinburg, Mira St 19, 620002, Russia

Abstract. To allow comparison of observations an unequivocal definition of terms is essential. While early scholars of amphibian anomalies already introduced specific terms and provided extensive definitions, some of these terms have changed their meaning over time, some are no longer used and others have been replaced more recently with alternative terms by some but not all authors. Most of the more recently published glossaries are rather narrow in their coverage, addressing primarily a limited set of skeletal anomalies, few provide synonyms and none of them discuss inconsistencies among different terminologies. To facilitate an understanding of the contributions in this volume and to facilitate comparison of publications in general, we provide an extensive glossary and synonymy of the terms used to describe external anomalies of amphibians. This includes colour and pattern anomalies, morphological anomalies, anomalies related to edema or tumours and anomalies of embryos. We discuss the inconsistencies among different terminologies and make recommendations for a future standardized use of terms. Our most important recommendation is to explicitly refer to a particular terminology and to describe the anomaly sufficiently to enable readers to understand what has been observed even if they are accustomed to using another terminology.

Keywords. Amphibia, anomalies, colour anomalies, skeletal anomalies, synonymies, terminology

Zusammenfassung. Eine eindeutige Definition von Begriffen ist für den Vergleich von Beobachtungen essentiell. Zwar haben bereits die ersten Forscher, die sich mit Anomalien in natürlichen Populationen von Amphibien beschäftigt haben, Begriffe zur Differenzierung verschiedener Typen von Anomalien eingeführt und dafür ausführliche Definitionen gegeben, jedoch haben einige Begriffe im Laufe der Zeit ihre Bedeutung geändert, andere fielen in Vergessenheit und wieder andere wurden von manchen, jedoch nicht allen neueren Autoren durch neue Begriffe ersetzt. Die meisten neueren publizierten Glossare sind im Umfang relativ beschränkt und beziehen sich vorwiegend auf eine kleine Auswahl von Skelettanomalien. Die meisten von ihnen gehen weder auf Synonyme noch auf unterschiedliche Definitionen von Begriffen durch verschiedene Autoren ein. Um das Verständnis der folgenden Beiträge in diesem Band und den Vergleich unterschiedlicher Publikationen zu erleichtern, stellen wir hier ein umfangreiches Glossar inklusive Synonyme zusammen. Dies umfasst Farb- und Musteranomalien, morphologische Anomalien, ödem- sowie tumorähnliche Anomalien und Anomalien von Embryonen. Wir diskutieren abweichende Verwendungen von Begriffen durch verschiedene Autoren und machen Vorschläge für eine konsistente Begriffsverwendung. Die wichtigste Empfehlung besteht darin, sich eindeutig auf eine bestimmte Terminologie zu beziehen und Beobachtungen ausführlich genug zu beschreiben, dass Leser die Art der Anomalie eindeutig identifizieren können, auch wenn sie eine andere Terminologie gewöhnt sind.

Schlagwörter. Amphibia, Anomalien, Farbanomalien, Skelettanomalien, Synonyme, Terminologie

1 Introduction

Natural populations of amphibians show a large diversity of deviations from the normal range of variation (Henle et al. 2017a). Various terms have been used for such deviations. For deviations in morphology, for example, the terms malformation, deformity, abnormality or anomaly are used frequently. Johnson et al. (2001) suggested defining malformation as a "permanent structural defect resulting from abnormal development", deformity as an "alteration in an organ or structure that originally formed correctly" and abnormality as "any gross deviation from the normal range in morphological variation". These definitions were adopted by the US Fish & Wildlife Service (USFWS 2007) for the standard operating procedure of abnormal amphibian surveys in the USA.

Malformations and deformities as defined here are often difficult to distinguish without a detailed study of the case. For example, the absence of a digit (ectrodactyly) could either result from perturbations of ontogenesis (e.g. Martin & Signoret 1968, Degitz et al. 2000) or from amputation after a normal ontogenesis (e.g. Dearlove & Dresden 1976, Muneoka et al. 1986). Some authors (e.g. McCallum & Trauth 2003) do not include injuries when reporting on abnormal amphibians. However, unless a wound is still open or bleeding, it is often very difficult to separate injuries from other anomalies, and in the field this is usually impossible (Henle et al. 2017a). Therefore, we will consider both kinds of abnormal phenotypes under the general term "anomaly", a practice that is followed by many authors. We also include all non-morphological deviations from the normal phenotype in this term.

The term "anomaly" is a relative one that has to do with the taxonomic status of specimens. What is an anomaly in a species or in a genus may not be so in another one. Thus, "albinism" is not an anomaly in *Proteus anguinus* or in other cavernicolous amphibians. White eggs are normal in species that deposit their eggs in places that are not exposed to sunlight, such as fast-running water, concealed sites on the ground or in vegetation above water (Duellman & Trueb 1986), but are an anomaly in species in which eggs are exposed to sunlight, such as most European species, the eggs of which are deposited in water (e.g. Rostand 1946, Bender 1997). Likewise, black-eyed individuals are rare mutations in some species (Henle et al. 2017a), whereas they are the normal condition in others (Glaw & Vences 1997). In *Plethodon cinereus*, a red colour morph is frequent in some regions but very rare in others (Lotter & Scott 1977) and thus might only classify as an anomaly in parts of the range.

Even gross morphological anomalies in a given species may be the normal condition in another one: thus, toads of the genus *Brachycephalus* have only two or three fingers and three or four toes, a condition, which would qualify as ectrodactyly in most other anuran genera. In some salamanders, toe morphology is highly variable and it is difficult to set the limit between the range of normal and abnormal variation, e.g. in *Batrachuperus pinchonii* (Chang & Boring 1935), *Ambystoma maculatum* (Worthington 1974) and *Salamandrella keyserlingii* (Borkin 1999, Vershinin 2002). This variation may or may not be described by one of the terms included in the glossary below. Thus, the use of these terms does not necessarily imply an anomaly. However, we recommend using these terms only if such an implication is intended. In any case, one needs to be precise about whether one regards a particular phenotype as normal or abnormal.

It is impossible to provide a list of all possible anomalies in amphibians, as virtually all characters, in eggs, embryos (see overview by Bantle et al. 1991), larvae and adults may be concerned. Some of these anomalies are very spectacular, such as polymely, and have attracted more attention than others (Ouellet 2000).

Early scholars of amphibian anomalies already provided extensive definitions of anomalies (e.g. Geoffroy Saint-Hilaire 1832) and several more recent glossaries for amphibian anomalies also exist (e.g. Dyrkacz, 1981, Tyler 1989, Meteyer 2000, Meteyer et al. 2000, Ouellet 2000, Green & Harshbarger 2001, Johnson et al. 2001, 2010, Vershinin 2002, 2015, Sessions 2003, USFWS 2007, Lannoo 2008, 2009, Nekrasova 2008). Most of them address and are (almost) limited to selected skeletal anomalies. The only relative comprehensive osteopathological glossary for amphibians and reptiles was published recently by Rothschild et al. (2012). Dyrkacz (1981) provided definitions for terms related to albinism and Green & Harshbarger (2001) for terms related to tumours.

Unfortunately, the terminology of amphibian anomalies is not always consistent among the different glossaries and among different scientists (Borkin et al. 2012). Moreover, terminology has changed over time; some terms are no longer used, some have changed their meaning and others have been replaced with new terms by some but not all authors. Only Borkin et al. (2012) and Rothschild et al. (2012) provided some synonymies and only the former authors discussed deviating definitions used by different authors. Therefore, it is not rare that the type of anomaly reported remains unclear even when a specific term was used.

To facilitate an understanding of the remaining publications in this issue, and to help identify the type of anomalies reported in the literature, we provide a commented glossary containing synonyms and explaining the different definitions given to a particular term. We also make recommendations towards future standardization of the use of terms. Our recommendations are guided by the following criteria: The recommended terms should reduce as much as possible any existing inconsistency and confusion. The terminology must be applicable in the field as in environmental monitoring it is not feasible to collect hundreds of individuals for detailed analyses in the lab. As a third criterion, with less weighing, we strived for an internally and etymologically consistent terminology.

We are aware that not everybody may agree with all of our recommendations. In any case, we strongly urge scientists to explicitly state which definition they followed and to describe the anomaly in sufficient details that readers are able to follow what they were referring to. An illustration of the observed anomalies using photographs should be considered, especially if they do not fit any of the given definitions very well. Anomalies may be rather complex, e.g. supernumerary limbs that are incompletely developed. For such cases we recommend only using the term applicable for the primary anomaly, in this instance polymely, and not to additionally call it ectromely. If one believes it to be relevant, one may rather provide a detailed description of the supernumerary limb.

2 Terms, definitions and synonyms

2.1 Anomalies of colouration and pattern

Terminology in this domain either follows external appearance or refer to the cells or pigments that were absent or modified. In most publications the presumed modified or absent chromatophore type was not verified by histological examination. However, inference from external colouration is not always a reliable indicator of the absence of a particular chromatophore type. Therefore, we recommend using a terminology based on the absence of pigments or chromatophore types only if histological examinations were made. Otherwise descriptive terms and statements, such as "black eyes", "blackish frogs", "blue frogs", "erythristic", "flavistic", "golden frogs", or "green frogs", are preferable, ideally in combination with colour photographs (compare Nishioka 1977).

Dyrkacz (1981) provided a short glossary of terms related to albinism. Richard & Nace (1983) classified dark variants in amphibians according to the presence or absence of chromatophore types on different parts of the body.

Colouration and pattern and thus deviations from the normal phenotype are usually species-specific. Here we limit our glossary to types of colour and pattern anomalies that are not species-specific.

Fig. 1a: Albino-like white diurnal colouration of *Chiromantis xerampelina* is attained physiologically and thus not called albinism. Road South of Lower Sabie, Kruger NP, South Africa. Source: Wikipedia Commons. Photo: B. Dupont.

Fig. 1b: Two large albinistic and a normal tadpole of *Bufotes viridis*; albinistic individuals may be difficult to classify: individuals, such as the one on the left, are often called leucistic despite of them still having a few slightly pigmented spots; the middle one is closer to albinistic pinto with a few normal coloured dark spots and normal coloured hindlegs; Roßwag, Germany, September 1980. Photo: K. Rimpp.

Albinism | Partial or complete absence of integumentary pigmentation giving an individual a whitish, yellowish to golden or pinkish to reddish appearance (Figs. 1b, 6, 7, 11, 15, 16, 21 & 23b). Individuals with transparent skin are included, if the whole body is affected and if a golden, yellowish, reddish or whitish tone is present; however, cases in which only parts of the body show abnormal → translucent skin are never included despite partially lacking integumentary pigmentation. Likewise, individuals in which red colour replaces the normal yellow colour and blue frogs are generally not included in the term albinism. Sometimes albinism is used in a more restrictive sense as a synonym of → leucism, but most frequently it is either unspecified or used in the broader sense given here. Since albinism has been used in such a variable way, we recommend using a more specific term whenever possible. Terms for specific types of albinism are based either on external appearance [→ albinistic pinto, → complete albino, → erythrism (partim), → flavism, → leucism] or in relation to the presence/absence of pigments or chromatophores [→ amelanism, → axanthism (partim), → hypomelanism (partim), → hypopigmentation (partim)]. The terms of these two types of classifications cannot be fully matched because the absence of a particular chromatophore type will lead to different external appearance depending on the normal colouration of the species involved. Therefore, we strongly recommend using the terminology based on appearance unless histological examinations were made. Further subcategories of albinism are → partial albinism (only parts of the body affected) and → transient albinism (individuals increasingly acquire pigmentation during development). Some species can change physiologically to an albino-like white colouration but are not called albino (Fig. 1a)

Albinistic pinto | Normal pigmentation only present as small scattered spots (Fig. 1b); a subcategory of → albi-

nism. The term is not often used in the herpetological literature; instead, pinto individuals are often described under the more general term → albinism or, when normal pigmentation covers larger spots, → partial albinism

Albino | Usually a synonym of → true albino but sometimes additionally includes → leucism

Amelanism | Melanin or melanophores absent (a subcategory of → albinism). If absence is not verified histologically, individuals in which melanin or melanophores are reduced but not completely absent may be called amelanistic, but correctly speaking such cases fall into the category → hypomelanism. Sometimes amelanism is used as a synonym of → flavism (e.g. Williams et al. 2013) but this should be avoided because the absence of melanin does not necessarily result in yellowish individuals

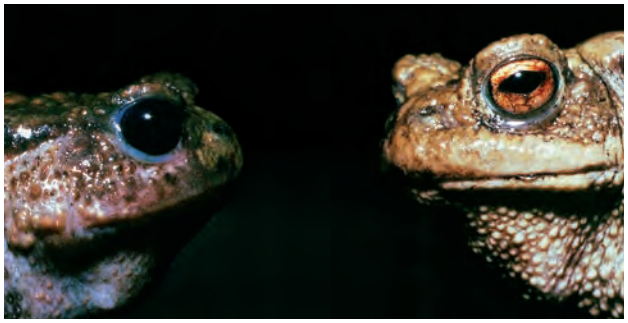


Fig. 2: Adult *Bufo bufo* with a black eye (left), due to a recessive mutation causing absence of iridophores as demonstrated by a crossing experiment, and normal phenotype (right); Carnelle Forest near Paris, France; 1983. Photo: A. Dubois.

Anomaly N | → Black eyed (Fig. 2)

Axanthism | Xanthophores or carotenoid vesicles in xanthophores absent. If the normal phenotype is green such individuals appear blue and are usually called → blue (frogs). In species with another normal colouration, it may be a subcategory of → albinism or may result in a species-specific abnormal colour pattern that is not reminiscent of albinism

Black eyed | Iris black coloured (Fig. 2); in some species, such as various dendrobatids (Fig. 9) and *Brachycephalus ephippium* (Fig. 12), black eye colouration is the normal phenotype (Glaw & Vences 1997). Unilateral black-eyedness is a subcategory of → heterochromia

Blue frogs | Frogs with blue colouration (Fig. 5). The anomaly is due to a reduction or lack of xanthophores or their yellow pigments in species in which green is the normal phenotype. Thus, blue frogs are a special type of → axanthism but usually not included in the term → albinism. Notwithstanding, the term axanthism is seldom applied to blue frogs. In a few frog species, such as *Aplastodiscus arildae* (Fig. 3), blue is part of the normal phenotype and male *Rana arvalis* (Fig. 4a) may turn blue during breeding

Fig. 3: Blue as part of the normal colour pattern is rare in amphibians; in male *Aplastodiscus arildae* it is combined with transparency on hidden surfaces; Teresopolis, Brazil, 29.11.2004. Photo: K. Henle.

Complete albino | A subcategory of → albinism, in which all integumentary pigmentation is lacking (Fig. 6), including in the eyes (eyes that completely lack pigmentation may appear red due to blood vessels or may be vestigial; the body may appear pinkish due to blood vessels). Sometimes, the term is also used for individuals that lack all integumentary pigmentation but whose eyes have the normal colour. We recommend using the term → leucism for such cases and urge to use photos or descriptions of the eye colour to avoid confusion. A few cavernicolous species, notably *Proteus anguinus anguinus* (Fig. 7), are complete albinos in the normal phenotype

Depigmentation | Normal colouration of the body, or parts thereof, lost, usually due to exposure to chemicals or disease

Depigmentation of beaks and/or denticles | Tadpoles lacking pigmentation of the beak and/or denticles of the tooth rows, which are usually dark brown to black due to keratinization. Depigmentation may be due to pollution or infection by *Batrachochytrium dendrobatides,* i.e., an anomaly, but also due to cold temperature, i.e., not an anomaly (reviewed by Henle et al. 2017a)

Erythema | Reddening of the skin due to increased blood flow

Fig. 4a: *Rana arvalis*, blue colour is the normal phenotype for males in some regions during the breeding season; Elbe floodplain, Dessau, 19.4.2011. Photo: A. Künzelmann.

Fig. 4b: *Rana arvalis*, normal phenotype outside the breeding season; Tschekalin, Russia, 20.7.2001. Photo: K. Henle.

Erythrism | Red individuals. There are two types of abnormally red individuals: 1) xanthophores are replaced by erythrophores but the remaining pigmentation is normal (Fig. 8); 2) integumentary pigmentation is absent except for erythrophores. The latter case but not the former is included in the term → albinism; Dyrkacz

Fig. 5a: Blue, an abnormal phenotype of *Pelophylax esculentus*; Braunschweig, Germany. Photo: A. Miralles.

Fig. 5b: *Pelophylax esculentus* normal phenotype; Tschekalin, Oka, Russia, July 2001. Photo: K. Henle.

Fig. 6: Normal phenotype and complete albino of *Xenopus laevis*. Source: Wikipedia Commons. Photo: Nashville Zoo.

Fig. 7: The normal phenotype of *Proteus a. anguinus* is complete albino; Baredine, Croatia, 23.6.2011. Photo: B. Trapp.

Fig. 8a: *Salamandra salamandra*: normal colouration of an adult; Maourine pond, Toulouse, France, 30.10.1975. Source: Wikipedia Commons. Photo: D. Descouens.

Fig. 8b: *Salamandra salamandra*: erythristic adult, captive bred individual; Germany. Photo: B. Trapp.

Fig. 9: *Oophaga pumilio*, a species in which red colour and black eyes are the normal phenotypes; Zoological Garden Wilhelma, Stuttgart. Photo: K. Henle.

(1981) called this category partial albino with erythrophores, but this terminology is rarely used. Some species have a red colour as the normal phenotype (Fig. 9); these are usually not called erythristic, with the red eft of *Notophthalmus viridescens* (Fig. 10) and the red morph of *Plethodon cinereus* being exceptions

Fig. 10: In immature *Notophthalmus viridescens* red is the normal phenotype; North Fork Mountain, USA. Source: Wikipedia Commons. Photo: J. Quinn.

Fig. 11a: *Pelophylax esculentus*; normal phenotype; adult individual; Oka, Tschekalin, Russia, August 2007. Photo: K. Henle.

Fig. 11b: *Pelophylax esculentus*; flavistic individual; Ballertasche, Germany. Photo: M. Vences.

Flavism | A subcategory of → albinism that covers yellowish or golden-to-yellowish individuals (integumentary pigmentation absent except for xanthophores) (Fig. 11 & 23b). Dyrkacz (1981) referred to this category as partial albino with xanthophores, but this terminology is rarely used. Williams et al. (2013) used → amelanism as a synonym of flavism but this should be avoided as amelanism does not necessarily result in yellowish in-

dividuals. Yellow or yellow-to-orange colouration of the body as normal phenotype appears in aposematically-coloured frogs (Fig. 12) and the males of some frog species, e.g. in the genus *Dendropsophus* (Fig. 13), turn completely yellow during the breeding season; usually, they are not called flavistic

Golden morphs | A subcategory of → flavism in which individuals appear golden (Fig. 23b)

Hemorrhage | Reddening due to bleeding (e.g. Miller et al. 2011)

Heterochromia | The two eyes differ in their pigmentation (Fig. 14); sometimes used for unilateral → black-eyed individuals. As many different types of heterochromia exist, we recommend stating explicitly whether one eye was black; otherwise it is not possible to assign them to the black-eyed type of anomaly

Hyperpigmentation | Pigmentation (of body parts) increased in intensity compared to the normal phenotype; usually applied to cases that result from an increase in melanophores or melanin expansion; Richard & Nace (1983) called such individuals dark variants; if individuals are very dark but not completely black, they are called → melanoid

Fig. 12: *Brachycephalus ephippium*, a species in which black eyes and a yellowish-orange colour belong to the normal phenotype; Serra dos Orgãos National Park, Brazil, 1.12.2004. Photo: K. Henle.

Fig. 14: Heterochromia in an adult female *Bufo bufo;* Glubochenskoye pond, Ural, Russia, June 2004. Photo: V. Vershinin.

Fig. 13: *Dendropsophus minutus*, a species in which males turn yellow during the breeding season; near Ilhéus, Bahia state, Brazil, 23.8.2013. Photo: A. Kwet.

Fig. 15: Hypomelanistic adult male *Triturus cristatus*. Those body parts that are normally black show a faint grey pattern; Nordstemmen, Germany. Source: Meyer (2009). Photo: S. Meyer.

Hypomelanism | Melanin or melanophores substantially reduced but present (Fig. 15) or melanophores permanently contracted; if the reduction is substantial, individuals may be called → albinistic

Hypopigmentation | Pigmentation (of body parts) reduced compared to normal individuals; if reduction is substantial, individuals are often called → albinistic

Incomplete albinism | Occasionally used instead of → leucism (e.g. Spadola & Insacco 2010) but this should be avoided since all forms of albinism, except for → complete albinism, are incomplete albinism

Leucism | (Almost) all integumentary pigmentation absent but eyes pigmented (Fig. 1 & 16); in the literature sometimes confused with → flavism

Fig. 16: Leucistic *Epidalea calamita,* offspring from a leucistic male, Germany. Photo: B. Trapp.

Melanism | A recent replacement term for → nigrinism; body or parts thereof completely black (Fig. 17); often used more broadly to include → melanoid individuals and → hyperpigmentation. If only parts of the body are affected, often called partial → melanism. A few species are completely black as normal phenotype, e.g. *Salamandra atra* (Fig. 18), and some, such as *Salamandrella keyserlingii* (Henle et al. 2017a), may change colour physiologically and become completely black

Melanization | Individuals gain darker pigmentation over time from melanin synthesis, melanin expansion or an increase in melanophore numbers. Phenotypically, individuals may either become darker on larger parts of the body or dark spots may increase in size (Vershinin 2002, 2015); may or may not be an abnormal condition

Melanoid | Very dark but not completely black individuals; a few species, e.g. *Salamandrella keyserlingii*, may change colour physiologically and become almost black (Fig. 19b)

Mid-dorsal stripe absent | Abnormal in species, in which the normal pattern generally includes a mid-dorsal stripe

Mid-dorsal stripe abnormal | Mid-dorsal stripe broken, bent or irregular in shape (Fig. 20)

Mid-dorsal stripe present | Abnormal in species in which a mid-dorsal stripe does not belong to the normal range of variation

Nigrinism | Old term for → melanism (e.g. Klunzinger 1903)

Novel colours | Parts of the body show colours that do not belong to the normal range of variation (e.g. yellow patches in *Bufotes viridis*: Henle et al. 2017a)

Partial albinism | The term is used inconsistently; most commonly, it is used for individuals, in which integumentary pigmentation is only absent on parts of the body (Fig. 21) but some authors also applied the term to individuals, in which melanophores are absent or strongly reduced but which have iridophores (then a synonym of → amelanism respectively → hypomelanism); others apply it to → flavistic individuals and Kronshage & Hildmann (1988) used the term instead of → transient albinism. To reduce ambiguity and confusion, we recommend restricting the term to the definition given by us and using other subcategories of → albinism for other types of reduced pigmentation; this also makes it consistent with the use of the term → partial melanism

Partial black | → Partial melanism

Fig. 17: Melanistic *Salamandra salamandra almanzoris*; born in captivity. Photo: U. Seidel.

Fig. 18: Black is the normal phenotype in *Salamandra atra*; adult near Bichlbach, Austria, 8.8.2014. Photo: A. Grimm.

Partial melanism | Individuals in which parts of the body but not the entire body are completely black; the remaining body shows normal colouration; a subcategory of → melanism

Pattern lacking | → Unicolor

Periodic albinism | → Transient albinism

Semi-albino | Rarely used; if used, then either as a synonym of → flavism (e.g. Capanna 1967) or → leucism (e.g. Smallcombe 1949, Tunner 1979); we discourage the use of this term because this inconsistency may create confusion and established widely used terms are available for these two types of colour anomaly

Transient albinism | An anomaly in which the eggs are white but the tadpoles increasingly acquire pigmentation after hatching; in rare cases, pigmentation does not appear before metamorphosis; the light coloured tadpoles in early stages are called depigmented by some authors (Vershinin 2015) but → depigmentation is usually applied to cases in which individuals initially were pigmented and later lost pigmentation. Kronshage &

Fig. 19a: Colour variation in *Salamandrella keyserlingii*: normal phenotype; Nishni Angarsk, Lake Baikal, Russia, 26.7.2014. Photo: K. Henle.

Fig. 19b: Colour variation in *Salamandrella keyserlingii*: almost black subadult taken in a very dark and cold place. This specimen looks like an abnormal melanoid individual but changed to normal colouration in a warmer environment. It also shows oligodactyly on the right hand; Ekaterinburg, Russia, 7.8.2007. Photo: V. Vershinin.

Hildmann (1988) called transient albinism → partial albinism, which is inconsistent with the usual definition of the latter term. Many amphibians that deposit their eggs at concealed sites have white eggs but the larvae or froglets that hatch from them are pigmented; these cases are usually not called transient albinism

Translucent | Pigmentation lacking and skin transparent (Figs. 3, 23b & 47) (Nekrasova 2008). In some species, notably in the family Centrolenidae, parts of the skin are translucent as the normal phenotype (Fig. 22)

Transparent | → Translucent

True albino | → Complete albino

Unicolor | Individuals that lack the dark or light pattern typical for the species, e.g. uniform green backs in the hylid *Acris crepitans* (Gray 1995)

Xanthism | Yellowish individuals in which melanin is strongly reduced or absent (i.e., a synonym of → flavism, e.g. Palis 1997, Williams et al. 2013)

2.2 Morphological anomalies

Several glossaries of skeletal anomalies have been published (e.g. Geoffroy Saint-Hilaire 1832, Tyler 1989, Meteyer 2000, Meteyer et al. 2000, Ouellet 2000,

Fig. 20: Middorsal stripe abnormally bent, subadult *Pelophylax lessonae*; Tschekalin, Russia, 9.9.2002. Photo: K. Henle.

Fig. 21a: Normal phenotype in an adult male *Triturus carnifex*, Stanjel, Slowenia, 24.2.2014. Photo: A. Grimm.

Johnson et al. 2001, 2010, Sesssions 2003, USFWS 2007, Lannoo 2008, 2009, Nekrasova 2008, Rothschild et al. 2012). Most of them also provided definitions of a few non-skeletal morphological anomalies but no comprehensive glossary exists for non-skeletal morphological anomalies in amphibians. Moreover, recent glossaries often ignored the earlier terminology that already existed and most did not provide synonymies, thus creating considerable potential for confusion. Only Borkin et al. (2012) discussed deviating use of some terms by different authors.

2.2.1 Anomalies of limbs

Acheiria | A synonym of → apody that is rarely used in studies of amphibian anomalies

Acheiropodia | A rarely used synonym of → apody in the herpetological literature (Rothschild et al. 2012); in the medical literature (e.g. Ianakiev et al. 2001), it is used as a synonym of → hemimely; to reduce confusion, we recommend using the historically established term, i.e. → apody

Fig. 21b: Partial albinism in *Triturus carnifex*; in this male, the normal colouration is maintained on the ventral parts of the body; thus, it is not a leucistic individual (but one might call it partial leucism); born in captivity. Photo: S. Meyer.

Fig. 22: Translucent skin is the normal phenotype in many centrolenid frogs, here *Hyalinobatrachium fleischmanni*; Guayabo, Costa Rica, 17.5.2003. Photo: A. Kwet.

Fig. 23a: *Scinax fuscovarius*: Normal phenotype of an adult; Intervales National Park, Brazil, 6.11.2004. Photo: K. Henle.

Fig. 23b: *Scinax fuscovarius*: abnormal transparency combined with flavism in a recently metamorphosed individual. Transparency is most obvious on the head and digits and most of the body is of a golden yellow colour. Such individuals are often called flavistic or are described under the generic term albinism. It is best, however, to refer to such individuals as "golden morph" and to provide a colour photo; Pro Mata Reserve, São Francisco do Sul, Brazil, 12.1.2001. Photo: A. Kwet.

Adactyly | Absence of all digits (Fig. 32a; Ouellet 2000, Rothschild et al. 2012); thus a specific form of → ectrodactyly; not defined in most recent glossaries of amphibian malformations; some authors use it as a synonym of → oligodactyly (e.g. Johnson et al. 2010)

Amely | One or several limbs completely lacking (Fig. 24; Tyler 1989, Meteyer 2000, USFWS 2007, Lannoo 2008, 2009, Nekrasova 2008, Johnson et al. 2010, Rothschild et al. 2012); a specific form of → ectromely; some recent glossaries (e.g. Johnson et al. 2001, Sessions 2003) define → ectromely in the same way as we define here amely; they were followed e.g. by Romansic et al. (2011); presumably, this resulted from an inconsistency in the definition of ectromely by Tyler (1989) (see: ectromely); this definition of ectromely creates confusion as it deviates from historically established definitions that are still commonly used; therefore, we strongly recommend to keep the historically established definitions in which amely is treated as a subcategory of ectromely. Absence of limbs is the normal phenotype only in the order Gymnophiona (Fig. 25)

Ankylodactyly | A rarely used synonym of → syndactyly

Ankylosis | Various definitions exist: growing together (Rothschild et al. 2012), stiffness of a joint (*http://en.wikipedia.org/wiki/Ankylosis*), fusion of two bones into one (*http://www.merriam-webster.com/dictionary/ankylosis*); not commonly used in studies of amphibian anomalies, and if so, mainly for the fusion of bones of a limb across a joint, sometimes combined with → taumely; we recommend limiting its use in the literature of anomalies of amphibians to the fusion of bones across a joint

Anomaly E | A dominant mutation in *Rana temporaria* that causes → clinodactyly, → ectrodactyly, → syndactyly, → ectromely and → brachymely; it may further involve → ectropolydactyly (Fig. 33; Rostand 1958, Dubois 1977)

Anomaly No | A dominant mutation in *Rana temporaria* that causes → symphalangy, → brachyphalangy, → clinodactyly and → hypophalangy but affected toes are different from those affected in → Anomaly Ro (Dubois 1977)

Anomaly P | A syndrome of anomalies characterized by → polydactyly in benign forms, either only in the hindlimbs or both in the hind- and forelimbs, whereas in its severe forms (Fig. 26) it includes → brachymely, inguinal → tumours, bony excrescences and even → polymely; it shows a postero-anterior gradient and a good, although not always perfect, bilateral symmetry (Rostand 1971, Dubois 2016)

Anomaly Ro | A dominant mutation in *Rana temporaria* that causes → symphalangy, → brachyphalangy, → clinodactyly and → hypophalangy but affected toes

Fig. 24: Amely, a special form of ectromely, in *Rana arvalis;* Ekaterinburg, Russia, 13.7.2000. Photo: V. Vershinin.

Fig 25: Complete absence of limbs is the natural phenotype in the order Gymnophiona, here a female *Herpele squalostoma* with its litter; Yaoundé, Cameroon, 26.6.2012. Source: Kouete et al. (2012). Photo: M.T. Kouete.

are different from those affected in → Anomaly No (Dubois 1977)

Anteversion | A joint of (long) bones oriented in a forward direction (Ouellet 2000), leading to an abnormal positioning of the foot or hand (Figs. 27 & 28); thus,

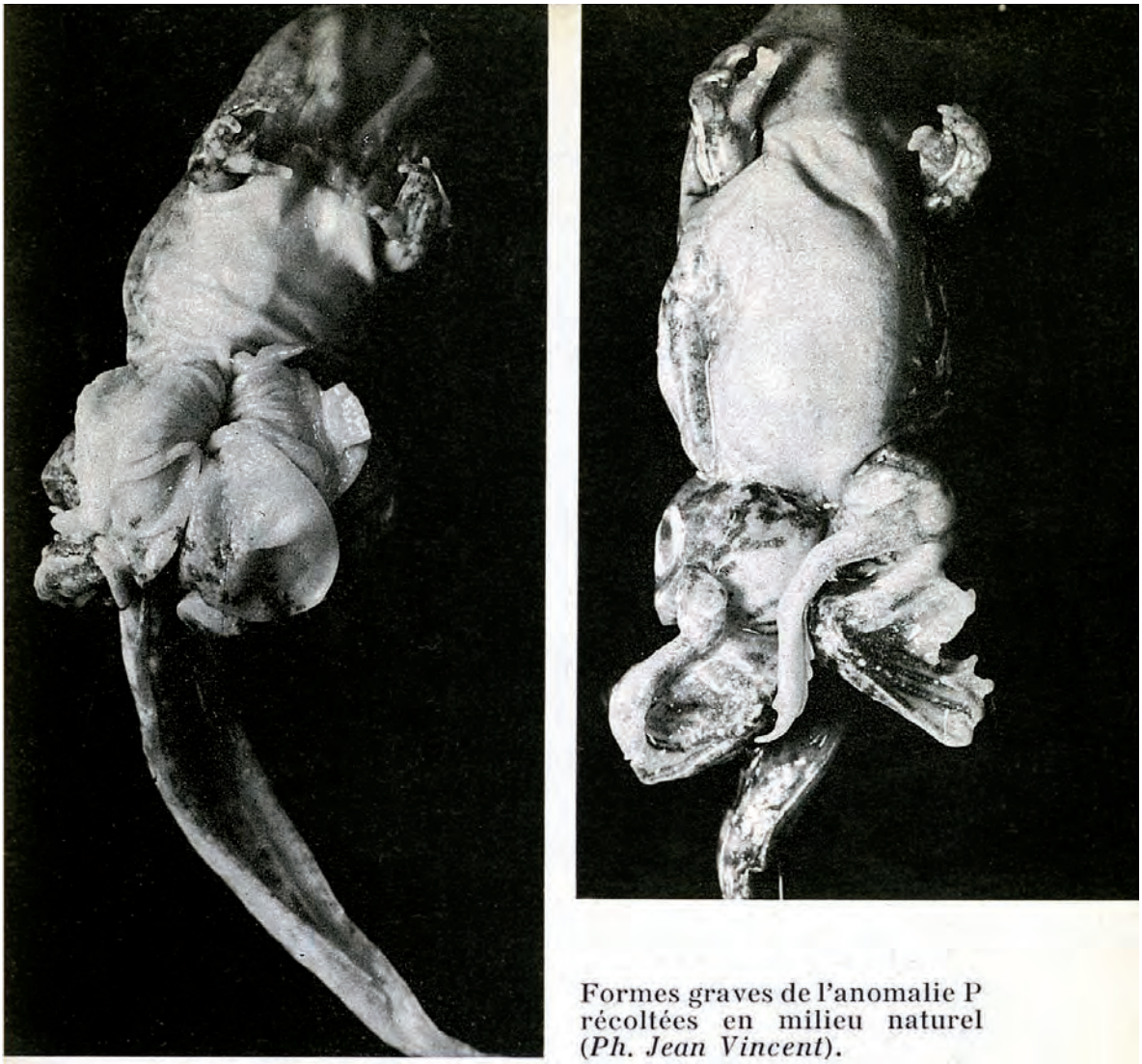


Fig. 26: Severe form of anomaly P in *Pelophylax* synkl. *esculentus,* France. The bottom figure shows particularly well the bilateral symmetry that is very frequently observed in anomaly P. Source: Rostand (1971).

anteversion is a specific form of → rotation (a term not used by Ouellet 2000). Hoppe (2005) applied anteversion more broadly, including → bony triangles, which is a specific form of → taumely; Lannoo (2008) defined anteversion very differently: as cork-screw shaped bones (which according to him usually is combined with taumely); based on the figure given by him, we assume that it is the same as what we call → taumely; because of these divergent and often unclear usages of the term, we discourage its further use, unless accompanied by a detailed description and then preferentially restricted to the definition given by Ouellet (2000)

Aphalangy | Absence of all phalanges of a digit (Figs. 19b & 32a,c); Rothschild et al. (2012) defined it as the absence of some phalanges of fingers, which is incorrect etymologically and for which another term exists: → hypophalangy; aphalangy and → oligodactyly may be used for the same type of anomaly, but the reference differs: the digit in the case of aphalangy and the hand or foot in the case of oligodactyly; both are subcategories of → ectrodactyly

Apody | Foot (hand) partially or completely missing (Fig. 29); a specific form of → ectromely

Arthrogryposis | It literally means curved joint, implying that it is fixed or stuck in the curved position (Staheli et al. 2008); in the herpetological literature, it is occasionally used in the same sense, especially when combined with an underdeveloped musculature (in German → "Streichholzbeinchen") (e.g. Zwart 1985)

Fig. 27: Anteversion combined with brachymely in a *Rana arvalis*; Ekaterinburg, Russia, 29.7.2001. Photo: V. Vershinin.

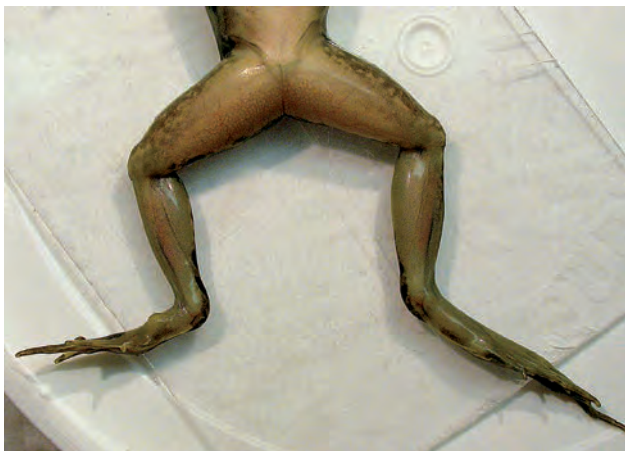


Fig. 28: Anteversion of the right hindleg combined with a shortened foot (brachypody); unidentified species. Photo: USFWS.

and rarely also for → bent bones (Lannoo 2008); most frequently, however, the English term → stiff limbs is used instead of the technical term. We suggest restricting the use of the term to its original meaning

Bent bones | → Curved bones

Bifidy | A rarely used synonym of → schizodactyly (e.g. D'Amen et al. 2006)

Bony bridge | → Bony triangle

Bony extension | → Bony projection

Bony projection | Digit-like microappendices projecting from the region of a (limb) bone (Meteyer 2000); these microappendices can be small rudimentary elements of a supernumerary limb (i.e., → polymely) or rudiments of distal parts of an incomplete limb (→ hypomorphic limb); in the latter case, bony projection is either part of → ectromely (if the microappendix is

Fig. 29: Apody in *Indosylvirana temporalis*; Morningside, Sri Lanka, 2010. Photo: P. Janzen.

a rudiment of the next distal bone, and thus should be classified as ectromely) or a form of → phocomely (if the microappendix originates from the region of a proximal long bone and is a complete or rudimentary digit) and thus should be scored as phocomely; → femoral projection is a subcategory of bony projection in which the microappendices originate from the region of the femur

Bony protuberance | → Bony projection

Bony triangle | → Bent long bone(s) forming a bony triangle or pyramid (Fig. 42; Ouellet 2000, Rohr et al. 2009), also called → bony bridge (e.g. Meteyer 2000, Meteyer et al. 2000, Nekrasova 2008); it is a subcategory of → taumely; Johnson et al. (2001, 2010) regarded it as the main form of → taumely and used the latter term when referring to bony triangles

Brachydactyly | Abnormally short digits; may be due to a reduced number (→ hypophalangy; Fig. 32b,d) or length (→ brachyphalangy) of phalanges or a combination thereof (Fig. 32b). A range of different definitions exists. Meteyer (2000) restricted the definition to the one given here for brachyphalangy but did not include hypophalangy in any of the terms defined by her; however, USFWS (2007), which builds on her definitions, simply defined brachydactyly as short digits, which is equivalent to the definition given here; Nekrasova (2008) defined brachydactyly as we define hypophalangy. Lannoo (2008, 2009) defined brachydactyly as normal number of metatarsals but an abnormal number of phalanges, which would also include → schizodactyly and → polyphalangy (and thus → polydactyly according to our definition); this definition is discouraged as it creates considerable confusion of disparate patterns; besides, it is difficult to diagnose in the field whether the number of metatarsals is normal or not; Vershinin (2015) defined brachydactyly as symmetrically shortened digits. Zaks (2008) used the term → microdactyly as a synonym of brachydactyly; according to Borkin et al. (2012), Zaks also used the term → ectromely for the anomaly we define as brachydactyly, but this may be a lapsus calami; brachydactyly is a specific form of → ectrodactyly; note that several species, especially within Urodela, have a highly variable number of phalanges in their digits; thus brachydactyly is part of the normal range of variability in these species and one needs to know this normal range to decide whether a particular phenotype is abnormal or not; different authors may differ in their opinion for a particular species

Brachymely | Proportionally shortened limb (Figs. 27, 28 & 30), i.e., all long bones of the limbs are present but at least one is abnormally short (Tyler 1989, Ouellet 2000, Kupferberg et al. 2009, Rothschild et al. 2012, Vershinin 2015); in the recent North American literature, and especially in the nation-wide surveys, the

Fig. 30: Brachymely in an adult *Rana dalmatina*; Waldsteinberg, Germany, May 2015. Photo: K. Henle.

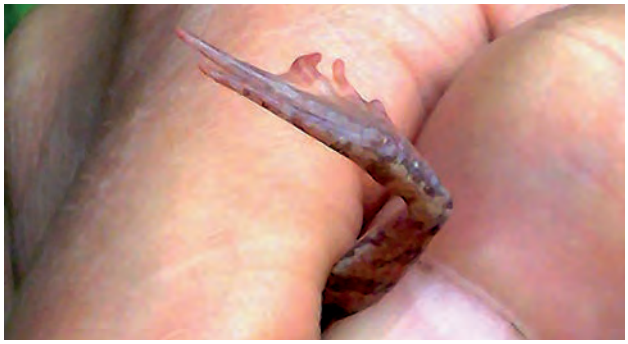


Fig. 31: Clinodactyly and oligodactyly of the right hindleg in a juvenile *Rana temporaria*; Tschekalin, Russia, 16.9.2013. Photo: K. Henle.

term → hemimely is often used for this type of anomaly (e.g. Meteyer et al. 2000, USFWS 2007, Lannoo 2008, Nekrasova 2008, Williams et al. 2008). While this definition is also commonly used in the literature on humans (e.g. Cohn & Bright 1999) it deviates from the historically established definitions that we provide here and that is widely used in the literature on anomalies in amphibians. As this recent change in the definition of hemimely can introduce considerable confusion, we discourage its use and recommend sticking to the original use of the term, which is also etymologically correct ("shortened limb"). Hemimely on the other hand means "half limb" and is thus inappropriate for a shortened limb; Johnson et al. (2001, 2010) defined brachymely as "abnormal shortness of one or more limbs"; while the definition is not explicit enough, comparing it with the definition they provided for ectromely and micromely suggests that they used it in the sense given here for brachymely; Sessions (2003) defined the term → micromely in the same way as we define brachymely; Rothschild et al. (2012) regarded the two terms as synonyms; here we regard micromely as a subcategory of brachymely in which all limb bones are shortened (as well as being proportionally smaller in diameter); some Russian authors apply the term ectrodactyly to the anomaly defined by us a brachymely (fide Borkin et al. 2012); → brachypody is a further subcategory in which the tarsal or carpal bones are shortened

Fig. 32a: Specific forms of ectrodactyly: adactyly (right arm), adult *Bufotes viridis*; Magnitogorsk, Russia, 8.5.1977. Photo: V. Vershinin.

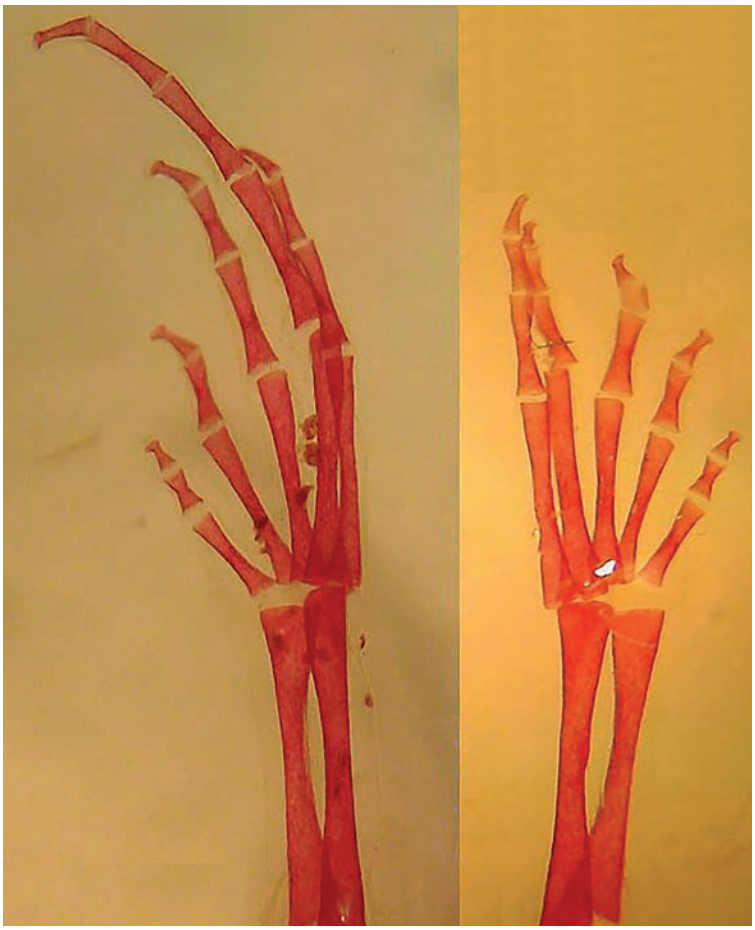


Fig. 32b: Specific forms of ectrodactyly: two subcategories of brachydactyly: hypophalangy (digits 3 and 4) and brachyphalangy (digit 4) of the right foot compared to a normal foot (left); *Rana arvalis*; Ekaterinburg, Russia, 1.7.2000. Photo: V. Vershinin.

Brachyphalangy | A bony element of a digit reduced in length (Fig. 32b), thus, a specific form of → brachydactyly; Eaton-Poole et al. (2003) also included missing proximal or middle phalanges in this category, which is inconsistent with the established use in the literature; we recommend sticking to its established use and to refer to such cases as → hypophalangy or to include them in the more inclusive term → brachydactyly

Brachypody | Hand or foot completely developed but some or all bones of the hand or foot shortened (Fig. 28). Not all authors consider this term and include such cases in the more inclusive term brachymely (Vershinin 2015)

Carpalia abnormal | Bones of the hand fused, absent or additional bones present; often difficult to assess without radiographs

Fig. 32c: Specific forms of ectrodactyly: aphalangy (when referred to a single digit) or oligodactyly (when referred to the foot), juvenile *Rana temporaria*; Samarowo, Russia, 18.8.2012. Photo: K. Henle.

Fig. 32d: Specific forms of ectrodactyly: brachydactyly, left hind limb of a *Rana arvalis*; Elizavet, Russia, 26.8.2010. Photo: V. Vershinin.

Cartilaginous spike | A cartilaginous outgrowth from the end of a limb with missing distal bones (Fig. 35; Sessions 2003); only individuals that show ectromely can have this type of anomaly; thus we do not recommend counting it as an additional anomaly when totalling the number of anomalies or types of anomalies per individual

Clinodactyly | Curvature of digit caused by the presence of an intercalary little asymmetrical bone between two phalanges (Fig. 31). The definitions provided by Tyler (1989) and Rothschild et al. (2012) do not include the intercalary bone, thus also including → curvature of bones in this category; however, the explanation given by Tyler (1989) excludes such cases from clinodactyly. Note that when digits are split (→ schizodactyly) one part of the duplicated digit often bends outwards (Fig. 39b); therefore, and because the figure in Tyler (1989) can be misunderstood as a schizodactylous individual, some authors (e.g. Vershinin 2002; note that since 2015 his group follows the definition given here – Vershinin 2015) used the term clinodactyly for cases of schizodactyly; as this differs from the established use of schizodactyly and can create confusion, we discourage this usage of the term clinodactyly and recommend always referring to such cases as schizodactyly. Note that clinodactyly is difficult to diagnose in preserved specimens unless radiographs are used

Clinomely | Curvature of limb (Fodor & Puky 2002); judging from a poorly reproduced photograph they seem to use the term as a synonym of → taumely. We strongly advice not to replace the established term taumely by clinomely. The term clinomely has rarely been used; an appropriate use could be as a technical term for → curved long bones as defined here

Curved long bones | Bones are curved; → torsion of limbs is sometimes used as a synonym for this type of anomaly but includes other types of anomalies as well. Cases in which the curvature involves a bending back of a bone on itself by > 90° or a misalignment with the rest of the bones are called → taumely; if the abnormal bones have the shape of a triangle or a pyramid, the terms → bony triangle or → bony bridge are often used interchangeably to describe this subcategory of → taumely (Johnson et al. 2001); we suggest using these latter terms to specify these types of bent bones or to describe the type of bending explicitly

Cutaneous fusion | → A band of skin crosses a joint of long limb bones restricting the motion of that limb; → skin webbing (Meteyer 2000, Lannoo 2009) and → skin fold (Vershinin 2015) are commonly used synonyms

Digits swollen | Thickening of the epidermis or the musculature of (parts of) a digit

Diplopody | Complete symmetrical duplication of the hand or foot including all digits (a specific form of → polypody)

Dipygus parasiticus | A specific form of → polymely in which the extra limb originates from the distal end of the pelvic girdle (Grimme 1907); superficially, it may be misidentified as a retained tail; the recent literature rarely differentiates between the different types of polymely

Ectrodactyly | Partial or complete absence of one or more digits (Figs. 19b, 31-33) or digits shortened (Geoffroy Saint-Hillaire 1832, Tyler 1989, Ouellet 2000). Some recent North American glossaries (e.g. Meteyer et al. 2000, Johnson et al. 2001, Sessions 2003, USFWS 2007, Lannoo 2008, 2009) restricted the term to the complete absence of a digit (including the metatarsal bone), making it a synonym of → oligodactyly as defined here. This contradicts with most of the earlier literature and the definition given here that is also the common usage outside of North America. We recommend adhering to the established terminology as given here using → oliogodactyly for a complete absence of at least one digit (e.g. Tyler 1989) and treating → adactyly (Fig. 32a), → oligodactyly (Fig. 32c) and → brachydactyly (Fig. 32b,d) and their subcategories as special cases

of ectrodactyly; Rothschild et al. (2012) recommended the spelling ectodactyly instead of ectrodactyly; we discourage this, as we never found this spelling in the literature on amphibian anomalies nor in the medical literature. Note that some species, e.g. *Proteus anguinus* (Fig. 7), have less than four fingers and five toes – the most common numbers in amphibians – as the normal phenotype

Ectromely | Partial or complete absence of a limb (Figs. 24, 29, 35) (Tyler 1979, Nekrasova 2008), except for cases where proximal bones are missing but the foot or digits are present (which is called → phocomely); ectromely thus includes → amely, → apody and → hemimely. A few recent authors (e.g. Meteyer 2000, USFWS 2007) include phocomely in the term ectromely but most authors do not; a few authors use the term more restrictively or differently: (1) for cases where the femur is present but the distal parts of the limb are missing (Lannoo 2008), which we define as → hemimely; (2) for cases where a limb is completely absent (e.g. Harris et al. 2001, Sessions 2003, Piha et al. 2006, Romansic et al. 2011), which we define as → amely. We recommend using the term amely for this specific subcategory as otherwise most readers will not realize that a specific subcategory is meant; moreover, etymologically amely is the correct term for a completely missing limb; note that while Tyler (1989) also defined ectromely as the complete absence of a limb, he explained that ectromely can also be partial and included → hemimely as a subcategory under ectromely; (3) Rothschild et al. (2012) recommended using the term "ectomely" and regarded "ectromely" as a synonym of → phocomely; to our knowledge ectromely has never been used as a synonym of phocomely and the term ectomely is never used at all. They also included → adactyly as a subcategory of ectromely, which again contradicts historical and current usage and thus should be avoided; (4) Zaks (2008) used the term ectromely for what we call → brachydactyly (fide Borkin et al. 2012)

Ectropolydactyly | One or several digits are absent while another digit is duplicated on the same hand or foot (Fig. 33; Dubois 1977); it may be part of the → anomaly E syndrome. Superficially the hand or foot may look normal in this type of anomaly; word combinations are not often used to define the co-occurrence of two different types of anomalies on the same body part; instead, most authors would call it → ectrodactyly plus → polydactyly

Femoral projection | A specific form of → bony projection in which the digit-like microappendages originate from the tissue of the femur; they may contain ossified elements (Johnson et al. 2001, Kupferberg et al. 2009); only those individuals exhibiting → ectromely, → phocomely, or → polymely can have this type of anomaly; thus we recommend not counting it as an additional anomaly when counting the number of anomalies per individual

Fracture of bone | Broken bone

Front leg(s) remaining covered by the opercular fold | The front legs of anurans usually remain covered by the opercular fold until the final stages of metamorphosis, when they break through the skin; in this rare anomaly one or both arms do not break through the skin (Fig. 34)

Fig. 33: Bilaterally symmetrical ectropolydactyly (anomaly E) in an adult female *Rana temporaria* (see Dubois 1977); the location of the metatarsal shows that toe I is duplicated; toe II is absent. At a glance, the individual might be scored as normal; Nointel near Paris, France, 1968. Photo: A. Dubois.

Gastromely | A supernumerary limb inserted at the venter between the front and hind legs (originating from the anterior part of the pelvis) (Geoffroy Saint-Hilaire 1832); a specific form of → polymely; in the recent literature the term is rarely used

Hemimely | Complete loss of the distal half of a limb (Fig 35; Tyler 1989, Ouellet 2000, Johnson et al. 2001, 2010, Sessions 2003); hemimely is a subcategory of → ectromely. Vershinin (2015) defined it as the absence of the distal parts of a limb, which may also include → apody; Rothschild et al. (2012) defined the term as "defective limbs, especially distal components", which is rather ambiguous and could refer to a range of different types of anomalies. Some recent North American and Russian glossaries (e.g. Meteyer 2000, USFWS 2007, Lannoo 2008, 2009, Nekrasova 2008) defined it as we define → brachymely here; this is inconsistent with all of the earlier herpetological literature and usage outside of North America but follows the definition of some authors working with other vertebrates (e.g. Cohn & Bright 1999). The term is also inconsistently defined in medicine: older influential lexica (e.g. Dornblüth 1927) defined it as we do here; more recently, a wider definition is often used that includes → brachymely, but then usually only in combination with the affected bone. As the definition provided by us here is traditional, long-standing and well-established and still most frequently used in the literature on anomalies in amphibians, we discourage recent deviations from the established use of the term as this only creates considerable confusion. Etymologically this is also preferable as "hemi" and "brachy" literally mean "half" and "short", respectively; thus, applying hemimely to shortened limbs is etymologically incorrect

Hyperdactyly | A specific form of → polydactyly in which there is an entire extra digit present, with or without the duplication of a metacarpal or metatarsal bone (Fig. 37a); the term is rarely used (e.g. Weddeling & Geiger 2011). Nekrasova (2008) restricted the term polydactyly to this type of anomaly and Rothschild et al. (2012) regarded polydactyly and hyperdactyly as synonyms but the traditional use of the term polydactyly also includes incompletely duplicated digits

Hyperphalangy | Extra phalange present in a digit (Fedak & Hall 2004, Rothschild et al. 2012); thus it is a synonym of → polyphalangy; sometimes, it is used more broadly as a synonym of → polydactyly (e.g. Swett 1926); for a consistent terminology we suggest limiting it to cases in which additional phalanges are linearly arranged within a digit without the splitting of a digit, which is covered by the term → schizodactyly, and without a complete extra digit (→ polydactyly)

Hypodactyly | A synonym of → oligodactyly

Fig. 34: Right frontleg remaining covered by the opercular fold in a very large tadpole of *Bufotes viridis*; Roßwag, Germany, September 1980. Photo: K. Henle.

Fig. 35: Hemimely in a metamorph of *Bufotes viridis* with a short cartilaginous spike; September 1980, Roßwag, Germany, MNHN 1984.2326. Photo: H. Steinicke.

Hypomorphic limb | The presence of one or more non-articulating microappendices from the soft tissue of a limb (Sessions 2003); contrary to the opinion of Sessions (2003) this is not diagnostic of amputation (see Henle et al. 2017a). In the field, it may be difficult to separate from → bony projection. Also, ethymologically, the definition is incorrect as "hypomorphic" means that a limb is incompletely formed and will therefore include other types of limb anomalies, such as → ectromely, → phocomely and → brachymely, as well as their subcategories

Hypophalangy | One or several but not all bones of a digit are absent (Fig. 32b,d), i.e. a specific form of → brachydactyly, which in turn is a specific form of → ectrodactyly; it includes → monophalangy as a special case

Interdigital webbing | Webbing between digits in species that normally do not have such webbing

Joint dislocation | → Luxation

Limb hyperextension | Excessive or rigid flexure of a limb joint (Johnson et al. 2001); Lannoo et al. (2003) and Lannoo (2008) used the term for an anomaly where the legs are immobile and straight, with the anomaly being caused by the knee being locked in extension and the hip joint also being immobile; thus, his definition is a subcategory of → stiff limbs

Luxation | Displacement of the joint between two limb bones causing an abnormal articulation of bones; if dislocation is incomplete, it is called → subluxation

Melomely | → Polymely of the front limbs (Fig. 40) (Geoffroy Saint-Hilaire 1832); the term is rarely used in the recent literature

Meromely | Definitions vary; Rothschild et al. (2012) regarded it as a synonym of → adactyly, however, we could not find any publication that used it in that restrictive sense. In the medical literature and for other vertebrates often defined as partial absence of the limb skeleton (Cohn & Bright 1999); this definition includes → ectromely, → phocomely and → ectrodactyly and all subcategories thereof; further definitions exist. Therefore and as it has rarely, if at all, been used in the literature on anomalies in natural populations of amphibians, we recommend using other existing terms; if it is used, it must be explicitly defined

Metacarpal/metatarsal tubercle lacking | An anomaly only found in species that usually have a metacarpal or a metatarsal tubercle, respectively

Metatarsal tubercle abnormally enlarged | Metatarsal tubercle larger than within the normal range of variation

Microdactyly | Zaks (2008) used it as a synonym of → brachydactyly

Micromely | Limbs complete but all elements proportionally smaller than normal (Meteyer 2000, Ouellet 2000, USFWS 2007, Nekrasova 2008, Lannoo 2009). Rothschild et al. (2012) treated it as a synonym of → brachymely, which they defined as we do; Johnson et al. (2001, 2010) and Sessions (2003) defined micromely as "abnormal smallness of a limb", which may or may not include brachymely as defined by us; Sessions (2003) did not include brachymely in his glossary and thus might have regarded both terms as synonyms; in contrast, Johnson et al. (2010) also provided a definition of brachymely: "shorter limb or limbs" and thus it is likely that they did not regard the terms as synonyms. In many publications, it remains unclear as to whether brachymely or micromely as we define these terms is what is actually meant; we recommend using both terms as defined here, thus regarding micromely as a specific form of → brachymely and describing the anomaly in sufficient detail to unequivocally identify the type of anomaly observed

Monodactyly | A special case of → oligodactyly, in which only one digit is present

Monophalangy | A special case of → hypophalangy, in which only one phalange is present

Nanomely | A rarely used synonym of → micromely (Borkin et al. 2012, Rothschild et al. 2012)

Notomely | A special case of → polymely, in which the supernumerary limb originates from the dorsum

Oligodactyly | Less than the normal number of digits but at least one digit present (Figs. 19b, 31, 32c) (Nekrasova 2008, Rothschild et al. 2012, Vershinin 2015). Tyler (1989) is not explicit enough to know whether he regarded oligodactyly as a synonym of ectrodactyly or as a special case of it as we do; oligodactyly and → aphalangy may be used for the same type of anomaly, but the reference differs: the digit in the case of aphalangy and the hand or foot in the case of oligodactyly

Phocomely | An incomplete limb with a proximal bone missing but distal bones present (Fig. 36) (Meteyer 2000, Nekrasova 2008, Rothschild et al. 2012); often the foot (hand) or toes (fingers) are attached directly to the shoulder or the pelvic girdle; Meteyer (2000) regarded this anomaly as a special case of → ectromely but most authors treat the two types of anomalies as separate categories; therefore, we recommend following the established use regarding them as separate categories; Bacon et al. (2006) used phocomely for complete but malformed limbs, which is inconsistent with the usual definition of phocomely

Pleuromelophorus tetrachirus | A specific case of → melomely, with four arms; melomely in turn is a subcategory of → polymely; the term is rarely used in the recent literature

Pleuromelochirus tetrascelus | A specific case of → pygomely, with four hind legs; pygomely in turn is a subcategory of → polymely; the term is rarely used in the recent literature

Polydactyly | Duplication of digit(s) (Figs. 37 & 38) or parts thereof (Figs. 39a,b) (Fischer 1977, Tyler 1989, Johnson et al. 2001, Sessions 2003); this definition includes → schizodactyly (Figs. 39a,b) in which only parts of a digit are duplicated, → synpolydactyly (Fig. 38) in which the proximal parts of the duplicated digit are fused, and → hyperdactyly, in which the whole digit is duplicated (Fig. 37). Johnson et al. (2010) defined polydactyly as "supernumerary digit(s)", which presumably but not definitely includes schizodactyly. Vershinin (2015) followed the definition given here but earlier publications of him and some other Russian authors used the term as a synonym of hyperdactyly (e.g. Nekrasova 2008); some authors (e.g. Meteyer 2000, Meteyer et al. 2000, Lannoo 2008, 2009, Rothschild et al. 2012) and presumably Ouellet (2000) restricted the term polydactyly further to cases in which more than the normal number of metatarsal/metacarpal bones is present; Rothschild et al. (2012) further regarded polydactyly and → hyperdactyly as synonyms but the definition of hyperdactyly generally does not include the presence of an additional metatarsal/metacarpal; they referred to a complete duplication of digits without an extra metatarsal/metacarpal bone as → polyphalangy, which also deviates from the commonly used definition of polyphalangy, which is used for linearly arranged duplicated phalanges; → schizodactyly was not included in the definition of the above authors nor did they provide a term for this type of anomaly. Whether a metacarpal or metatarsal bone is duplicated is very difficult to assess in the field and therefore this distinction has rarely been made for field samples; therefore, and because the definition given by Meteyer (2000) deviates from the more widely used definition, we recommend adhering to the traditional definition given here

Fig. 36: Phocomely – left tarsus attached to the femur – in a juvenile *Bufo bufo*; Sagra, Russia, 2002. Photo: V. Vershinin.

Polymely | Duplication of a complete limb or parts thereof (if more than the digits are duplicated) (Figs. 40 & 41; Geoffroy Saint-Hilaire 1832, Tyler 1989, Johnson et al. 2001, Sessions 2003, Nekrasova 2008, Vershinin 2015); the definition includes → schizomely and → polypody as subcategories. Meteyer (2000) provided a similar definition but excluded polypody; Lannoo (2008, 2009) went one step further and restricted the term to complete extra limbs. We suggest following the more widely used broader definition given here. In the early herpetological literature, but rarely in the recent one, the following terms and their subcategories were used to differentiate between the different types or the position of the supernumerary limb: → gastromely, → melomely, → notomely, → pygomely

Polyphalangy | An extra bone inserted into a digit without splitting the digit (i.e. in a linear arrangement). Vari-

Fig. 37: Polydactyly in *Pelophylax ridibundus*; Oka, Tschekalin, Russia, 12.8.2007. Photo: K. Henle.

Fig. 38: Polydactyly, with proximal fusion of the duplicated fingers, in *Pelophylax esculentus*. Although one could call this anomaly polydactyly plus syndactyly or synpolydactyly, we recommend counting only the primary anomaly, in this case polydactyly; Seelenhofer Ried, Germany (compare Machado et al. 2010). Photo: C. Machado.

Fig. 39a: Schizodactyly, a specific form of polydactyly, in the right hind limb of a *Lissotriton vulgaris*; Ekaterinburg, 2002. Photo: V. Vershinin.

Fig. 39b: Schizodactyly in the foot of an adult *Rana arvalis*. Note: the supernumerary toe bends away from the normal toe; although one could call it polydactyly plus clinodactyly we recommend not counting it as clinodactyly because the bending is a consequence of the primary anomaly schizodactyly; Kalinovsky forest park, Ekaterinburg, Russia, 19.7.2009. Photo: V. Vershinin.

ous other definitions exist: Nekrasova (2008) included → schizodactyly as defined by us in her definition of polyphalangy and Hall (2005) applied the term to any extra phalanges, whether linearly arranged (i.e. polyphalangy in our definition) or with splitting (→ schizodactyly in our definition) or a complete duplication of digits (→ hyperdactyly in our definition); thus he used it as a synonym of → polydactyly. Meteyer (2000) and Meteyer et al. (2000) used the term for another type of → polydactyly, in which there is (are) extra digit(s) present without a duplication of the metatarsal bone(s); except for in the recent North American literature (e.g. McDaniel et al. 2004), this definition is rarely applied in the literature on anomalies of amphibians (and very

difficult to apply in the field). The definition provided by Rothschild et al. (2012) is not very precise but presumably means the same as the definition provided by Meteyer (2000). To reduce ambiguity, to make terminology consistent and in particular because the distinction between the different types of duplicated digits made by Meteyer (2000) is difficult in the field and therefore has rarely been made for field samples, we recommend using the definition given here for → polyphalangy, → schizodactyly and → hyperdactyly if one can identify the nature of the duplication and otherwise using the more inclusive term → polydactyly

Polypody | A limb with one or more extra feet (hands) or parts thereof (Johnson et al. 2001, 2010, Sessions 2003) but more than the duplication of digits (Fig. 41); a specific form of → polymely

Pseudomely | Vizotto et al. (1977) used the term as a synonym of → polymely

Fig. 40: Melomely, a specific case of polymely in a captive offspring of *Dendrobates tinctorius*. Photo: S. Koehler.

Pygomely | A specific form of → polymely, in which the hind limb(s) are duplicated (Fig. 41)

Rotation (of limbs) | Limbs appear twisted and the foot (hand) is not flat with the surface; as defined here, this type of anomaly can either be caused by a distortion of the direction of the bone growth (→ curved bones) or an abnormal articulation of two bones (→ anteversion; Figs. 27 & 28); Meteyer (2000) and Nekrasova (2008) restricted the term to the first case; a rotation of limbs is always combined with → stiff limbs

Schizodactyly | Forked digits (Figs. 39a,b); this is a specific form of → polydactyly; rarely called → bifidy (e.g. D'Amen et al. 2006). The definition given by Nekrasova (2008) for → polyphalangy includes schizodactyly but not our definition. Note that the supplementary phalanges often bent away from the base of the toe (Fig. 39b); therefore and because the figure of → clinodactyly in Tyler (1989) – but not his definition of it – is misleading, some authors (e.g. Vershinin 2002) referred to cases of schizodactyly as clinodactyly. As incomplete duplication of a digit is the primary type of anomaly, we discourage this usage and recommend to call such cases schizodactyly to distinguish them from cases of → clinodactyly that do not involve the partial duplication of a digit. Since 2015 Vershinin (2015) follows the definition given here

Schizomely | Completely duplicated limbs in which the proximal parts of the limbs are fused; thus it is a specific form of → polymely

Skin fold | A synonym of → cutaneous fusion (Vershinin 2015)

Fig. 41: An unusual form of polypody, which is a specific form of polymely, in an adult female *Lissotriton vulgaris;* the two supernumerary feet show different degrees of duplication, the lower one would not be regarded as duplication of the foot but rather as a bony protuberance following the classification of Meteyer (2000); Bayreuth, Germany, spring 2011. Photo: K. Szepanski.

Skin webbing | A synonym of → cutaneous fusion (USFWS 2007, Nekrasova 2008)

Spindly limbs | English term for → Streichholzbeinchen

Stiff limbs | Limbs are immovable; stiffness is linked to other anomalies of bones, such as → polymely (Fig. 39), → rotation of limbs (Figs. 27 & 28) or → taumely (Fig. 41), to → cutaneous fusion or the reduction of musculature; the latter is called → Streichholzbeinchen in the German amphibian husbandry literature

Streichholzbeinchen | The German term for → stiff limbs with degenerative musculature, giving the limbs a spindly appearance. This anomaly is called the fixed limb syndrome by Kovalenko (2000). It is a subcategory of stiff limbs

Subluxation | Incomplete or partial → joint dislocation (Ouellet 2000)

Symmely | Partial or complete fusion of two limbs (Puky & Fodor 2002, Rothschild et al. 2012). We only know this type of anomaly from supernumerary limbs that are partially fused with the normal limb, from *Bombina bombina* in Hungary (Puky & Fodor 2002) and from a single individual of *Pelophylax esculentus* described by Bruch (1864) – he called it → sympody – in which the hindlegs were fused at the level of the femur and not just the feet. In the case of the fusion of supernumerary limbs, the primary anomaly is polymely; therefore, we recommend counting such cases as polymely as opposed to listing them additionally as symmely. Ouellet (2000) and Johnson et al. (2010) provided a different definition for symmely: fusion of a limb or parts of a limb to a body part; we have not found any publication on anomalies in natural populations of amphibians that described such an anomaly; symmely is a specific expression of → synostosis

Symphalangy | Two phalanges of the same digit are fused (Dubois 1977); it is a subcategory of → syndactyly

Sympody | Only the distal parts of two limbs are fused (Bruch 1864); a very rare type of anomaly; the individual described by Bruch (1864), however, would rather classify as → symmely to which sympody belongs as a subcategory; sympody is a specific expression of → synostosis

Syndactyly | The partial or complete fusion of two or more digits due to failure of the soft tissue to break down between digits or due to the fusion of bones of different digits (Tyler 1989, Ouellet 2000, Johnson et al. 2001, 2010, Sessions 2003, USFWS 2007, Lannoo 2008, 2009, Rothschild et al. 2012, Vershinin 2015), the latter being termed → symphalangy; syndactyly is a specific expression of → synostosis

Synostosis | Fusion of bones

Synpolydactyly | Duplication of digits, with digits fused by soft tissue or with proximal phalanges partially fused (Fig. 38; Cohn & Bright 1999); → schizodactyly is similar but the proximal phalanges are single rather than duplicated and fused; synpolydactyly is a subcategory of → polydactyly

Tarsalia abnormal | Bones of the foot are either fused or absent or additional bones are present

Taumely | Gross disturbance of the limb plan, whereby the misalignment of a long bone is more than 90° (Fig. 42; Tyler 1989, Vershinin 2002, 2015). The degree to which the bone bends back may vary and some authors (e.g. Gardiner & Hoppe 1999) included any degree of bending back within the term taumely, whereas others (e.g. Tyler 1989, Meteyer 2000, Meteyer et al. 2000, Johnson et al. 2001) restricted it to the definition given here. We suggest including also cases with a lesser degree of bending if the bauplan of the limb is grossly disturbed. The abnormal bone may have the shape of a pyramid or triangle; such cases are often called → bony triangle or → bony bridge (e.g. Meteyer et al. 2000); however, it is not entirely clear whether Meteyer (2000) and Johnson et al. (2001) regarded bony triangles as a synonym or as a subcategory of taumely; we recommend the latter to separate these anomalies from other types of taumely; we further suggest calling slightly bent bones → curved bones to separate them from grossly misaligned bones

Fig. 42: Particularly grossly malformed left hindlimb that looks like a bony triangle, which is a special case of taumely. The malformation is, however, complex, since the tibia is either missing or completely synostosed with the femur and the foot is reduced in size and the number of digits; in such cases it is best to describe and provide a figure of the observed individual and not simply allocate them to a particular term of anomaly; juvenile *Rana arvalis*; Ekaterinburg, Russia, 5.8.2003. Photo: V. Vershinin.

Toe pad swollen | Epidermis of toe pad thickened

Torsion of limbs | A synonym of → rotation of long limb elements

Triphalangy | A specific form of → oligodactyly, in which three digits are present (Fig. 19b)

Twisted limbs | A synonym of → rotation of limbs

2.2.2 Anomalies of the head, body or tail

Aglossia | Tongue absent (Nekrasova 2008)

Agnathia | Jaw completely missing (Nekrasova 2008)

Anadydimus | Two tails (Wallach 2007)

Anal tube closed | Anal tube distally covered by skin

Anophthalmy | Eye(s) lacking (blindness) (Fig. 43; Tyler 1989, Meteyer 2000, Johnson et al. 2001, Sessions 2003, USFWS 2007, Nekrasova 2008, Lannoo 2009, Vershinin 2015); Lannoo (2008) called this type of anomaly microphthalmy, which was a lapsus calami; unilateral anophthalmy is sometimes called → cyclopy by Russian authors (e.g. Zaks 2008, Fayzulin 2011) but cyclopy usually is defined differently

Asyntaxia caudalis | Duplication of tail due to a failure of the anal fold to close; it is a specific expression of → anadydimus

Axial flexure | Torsion of the vertebral column

Axial incurvation | → Axial flexure

Beak lacking keratinization | The jaw sheaths of the mouths in tadpoles are developed but not keratinized or the keratin was destroyed by pollution or disease; therefore beaks are not pigmented; note that lack of keratinization may also be due to low temperature (Rachowicz 2002) and thus not an abnormal condition

Bent tail | The tail is bent instead of being straight; → kinky tail is a synonym

Bicephaly | Head duplication; a synonym of → dicephaly and → catadydimus

Brachycephaly | Head abnormally short but of normal width; in post-embryonic stages, this type of anomaly is rare (Vershinin 2015, Henle et al. 2017a)

Brachygnathia | Abnormal shortness of the lower jaw (Meteyer 2000, Lannoo 2009); usually either used as a subcategory or a synonym of → mandibular hypoplasia

Brachyuria | Shortened tail (Rothschild et al. 2012); note that the term is used only for inherited shortness of tails and not for lost parts of a tail; this term is rarely used in the literature on amphibian anomalies

Catadydimus | Two headed (Wallach 2007); a synonym of → dicephaly and → bicephaly that is frequently used for snakes but rarely for amphibians

Cataract | Clouding of the eye lens, which leads to a decrease in vision

Cauda bifida | Terminal part of tail duplicated (Fig. 44)

Cleft lip | Lip with a fissure (Fig. 45)

Curvature of tail | → Bent tail

Cyclopia | Eyes completely fused into one single median eye (Adelmann 1936). Note that some Russian authors (e.g. Fayzulin 2011) call unilateral → anophthalmy cyclopy

Dicephaly | Two heads (Fig. 46; Ouellet 2000); → bicephaly and → catadydimus are synonyms

Fig. 43: Anophthalmy in a captive bred *Cruziohyla* cf. *calcarifer*, 18.1.2009. Photo: J. Kaesling.

Fig. 44: Cauda bifida in a tadpole of *Hyla arborea*; Thriptis Mountains, Crete, Greece, 30.8.1999. Source: Henle et al. (2012); Photo: K. Henle.

Duplicitas anterior | Duplication of anterior parts of the body; → Janus twins is a specific form of it; Schmidt (1930) restricted the term to duplication of the head, i.e. used it as a synonym of → dicephaly; Kabisch (1990) included → polymely of the forelimbs (i.e. → melomely) under this term

Duplicitas posterior | Duplication of posterior parts of the body; Kabisch (1990) included → polymely of the hindlimbs (i.e. → pygomely) under this category

Exophthalmy | Eyes protruding abnormally

Eye displacement | Eye displaced laterally, medially, cranially or caudally (Lannoo 2008, 2009, Nekrasova 2008); called abnormal eye position by Vershinin (2015)

Gigantism | Body size much larger than the normal maximum size

Gut miscoiling | Abnormal coiling of the gut; most common is a loose coiling

Hump | → Kyphosis of the spine

Hypognathia | A synonym of → mandibular hypoplasia and → mandibular dysplasia

Fig. 45: Cleft lip, presumably due to injury, in an adult *Bufo bankorensis*; Taroko, Taiwan, 2.12.2012. Photo: K. Henle.

Fig. 46: Dicephaly in a captive born larval *Salamandra salamandra* (Grollich & Grosse 2013). Photo: C. Grollich.

Janus twins | Duplication of dorso-anterior structures in the absence of duplication of posterior structures; i.e. a specific form of → duplicitas anterior

Jaw shape abnormal | Jaw fully developed but upper and lower jaw differ in shape and mouth does not close completely

Jaw sheaths abnormal | A specific form of abnormal → mouthparts in larval amphibians, in which there are unnatural breaks, gaps or other deformities in the jaw sheaths or the jaw sheaths may lack keratinization

Keratinized denticles absent | The labial tooth rows lack denticles or denticles are not keratinized in species that normally have keratinized denticles; a specific form of → tooth rows abnormal

Kinking | Torsion of notochord, vertebral column or tail; → kyphosis, → lordosis and → scoliosis are specific expressions of kinking

Kinky tail | → Bent tails (Tyler et al. 1989); a specific form of → kinking, in which the tail is affected

Kyphosis | Abnormal backward curvature of the spine (tail or body) (Meteyer 2000, Ouellet 2000, Johnson et al. 2010); a specific expression of → kinking; note that Meteyer (2000) does not include → lordosis in her glossary, while USFWS (2007) included it but not kyphosis; they seem to use the two terms as synonyms, as is also indicated by photos on the website *http://www.fws.gov/contaminants/amphibian/PictureThumbs.html*

Labial papillae abnormal | Labial papillae underdeveloped, interrupted or absent

Laceration | Skin wound (Fig. 47)

Lesion | Pathological area of an organ (Rothschild et al. 2012); may be due to injury, disease or → tumour

Lordosis | Abnormal forward curvature of the spine (tail or body) (Ouellet 2000, Johnson et al. 2010, Rothschild et al. 2012, Vershinin 2015); a specific expression of → kinking; note that USFWS (2007) does not include → kyphosis in its glossary, while Meteyer (2000) included it but not lordosis; they seem to use the two terms as synonyms, as is also indicated by photos on the website *http://www.fws.gov/contaminants/amphibian/PictureThumbs.html*

Macrophthalmia | Abnormally large eyes (Fodor & Puky 2002)

Mandibular dysplasia | A synonym of → mandibular hypoplasia (Johnson et al. 2001, Sessions 2003), the latter being more commonly used

Mandibular hypoplasia | Lower jaw underdeveloped or completely missing (Tyler 1989, Johnson et al. 2010) but the term may be used also in a more restrictive way that excludes completely missing jaws. → Brachygnathia and → agnathia are specific types of mandibular hypoplasia as defined here. The following terms are less commonly used synonyms: → mandibular dysplasia, → hypognathia and → micrognathia

Maxillary hypoplasia | Upper jaw underdeveloped or completely missing

Microcephaly | Abnormally small head (Meteyer 2000, USFWS 2007, Nekrasova 2008, Rothschild et al. 2012); either the whole head or parts thereof may be reduced in size or shortened. Lannoo (2008, 2009) defined the term differently: "blunt nose; shortened upper jaw"; while such an anomaly is a specific form of microcephaly, most authors define the term more broadly; for a shortened upper jaw a different technical term exists: → maxillary hypoplasia; moreover, etymologically, "micro" means "small" and "cephaly" means "head" Microcephaly is a rare type of anomaly with the exception of embryos

Micrognathia | A synonym of → mandibular hypoplasia (Nekrasova 2008)

Microphthalmy | Eye(s) abnormally small (Fig. 48) (Meteyer 2000, Ouellet 2000, Nekrasova 2008, Lannoo 2009, Johnson et al. 2010, Vershinin 2015); sometimes also spelled microophthalmy; as a lapsus

Fig. 47: Laceration (skin wound) on the head of a juvenile *Rana arvalis*; in early stages of healing, the skin is transparent; Samarowo, Russia, 17.8.2012. Photo: K. Henle.

calami, Lannoo (2008) called this type of anomaly anophthalmy

Monorhyny | Having a single nostril (Rostand 1958, Ouellet 2000, Rothschild et al. 2012, Vershinin 2015); so far only cases are known that are caused by a fusion of the nostrils, i.e. → synrhyny

Mouthparts abnormal in larvae | Beaks, labial teeth rows or labial papillae reduced in size, number or form, misplaced or abnormal in shape

Mydriasis | Excessive dilation of the eye (Streicher et al. 2010)

Myiasis | Lesions caused by the maggots of parasitic flies that deposit eggs on living individuals; in severe cases, the nasal openings may be destroyed (Fig. 49)

Nanism | Body size much smaller than normal; in natural populations this type of anomaly is very difficult to distinguish from small individuals that are still in the process of growing

Nares closed | Failure of nostrils to open

Nasal opening destroyed | This anomaly is caused by maggots of the parasitic fly *Lucilia bufonivora* that deposits eggs on the nostrils or other body parts (Fig. 49); the larvae migrate to the nostrils and destroy them killing the infected individual; usually *Bufo bufo* is the host but rarely other species are also parasitized (Henle et al. 2017a)

Nose blunt | Nose truncated in species that usually have a rounded snout

Opening underneath opercular fold unusually wide | Resorption of the opercular fold was abnormally large

Ophistocony | Used as a synonym of → lordosis by Flindt & hemmer (1967)

Oral labial papillae swollen | A specific form of the category → mouthparts abnormal, in which the labial papillae are expanded in size

Osteolathyrism | Decreased connective tissue strength (Snawder & Chambers 1993); this anomaly leads to notochord and tail deformities in embryos and to → joint dislocation and → bent long bones in metamorphosing anurans

Otocephaly | Absent or underdeveloped lower jaw (Rothschild et al. 2012); this definition includes → mandibular hypoplasia and → agnathia; both terms were not included in the glossary of Rothschild et al. (2012); whereas we did not find any use of the term otocephaly in the literature on amphibian anomalies, the latter terms are commonly used

Palatine eye | Eye in the mouth (Ouellet 2000)

Panophthalmitis | Infection of the whole eye

Polyophthalmy | More than two eyes (Fodor & Puky 2002)

Sacrum asymmetric | Sacral processes inserted on different vertebrae

Scars | Skin wound, scratches; may not be abnormal in territorial species that fight with each other

Scoliosis | Abnormal lateral curvature of the spine (tail or body) (Meteyer 2000, Ouellet 2000, Lannoo 2008, Johnson et al. 2010, Rothschild et al. 2012, Vershinin 2015); a subcategory of → kinking

Siamese twins | Two almost complete individuals that are fused to each other and share most body parts

Skeletal kinking | → kinking involving the skeleton

Snout pointed | Snout is pointed instead of being round in species that normally have a round snout

Spiraculum number abnormal | More or fewer than the normal number of spiracula (Héron-Royer 1884); most tadpoles have a single spiraculum as the normal phenotype but tadpoles of rhynophrynids, pipids and *Lepidobatrachus* have two spiracula (McDiarmid & Altig 1999)

Spiraculum misplaced | Spiraculum on the wrong side of the body or ventrally in species in which the normal position is lateral; the most common normal phenotype is a single spiraculum on the left side of the body (McDiarmid & Altig 1999)

Synrhyny | Fused nostrils; so far this type of anomaly is the only known form of → monorhyny

Tail bifurcation | → Cauda bifida

Tail duplication | Tail is partially or completely duplicated; includes → Asyntaxia caudalis and → Cauda bifida as subcategories

Tail fin reduced | Parts of the tail fin lacking or reduced in height

Tail retention in metamorphosed anurans | Tail is not completely reabsorbed during metamorphosis; note that in some species tail resorption may be completed only after individuals have left the water (Vershinin 2015, Henle et al. 2017a)

Tail stunted | Tail shorter than normal (Tyler 1989)

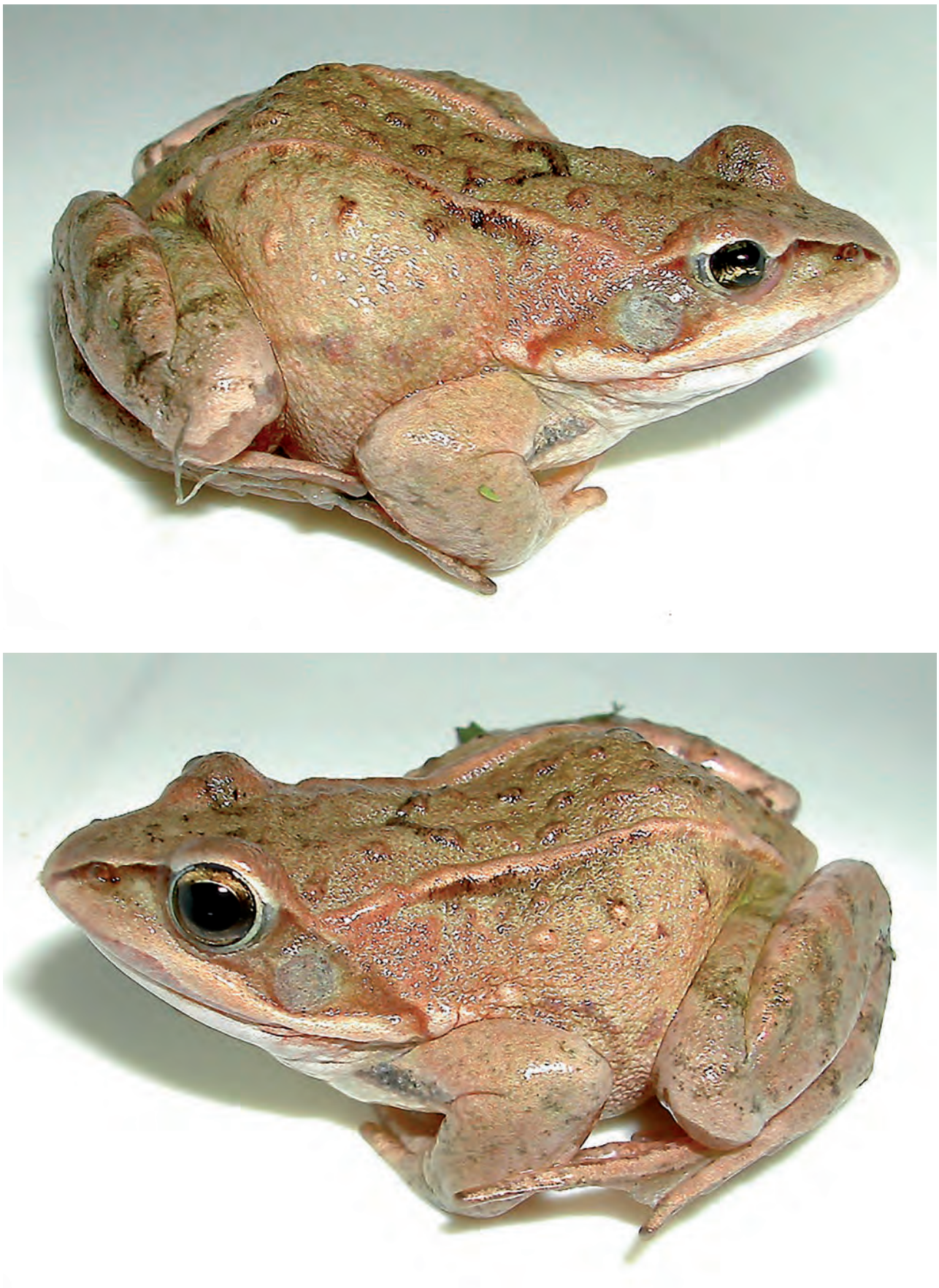


Fig. 48: Microphthalmy (top) in an adult male *Rana arvalis*; for comparison the normal eye of the left side is shown (bottom); Ekaterinburg, Russia, 5.9.2003. Photo: V. Vershinin.

Tooth rows abnormal | Tooth rows in tadpoles misshaped, interrupted, lacking denticles or → keratinization of denticles absent

Torsion of body | → kinking

Torsion of tail | → Bent tails

Twinning | Duplication of body parts; → catadydimus, → dicephaly, → duplicitas anterior, → duplicitas posterior, → Janus twins, → Siamese twins are specific forms of twinning; duplications that involve only the limbs, the tail or parts thereof are usually not called twinning

Tympanum absent | Abnormal in species whose normal phenotype is an externally visible tympanum; note that in some species this is the normal condition

Urostyle bent | Curved bones of the urostyle (Vershinin 2015)

Vertebral column stiff | Vertebral column immovable, usually due to → vertebral fusion

Vertebral fusion | Two or several vertebrae are fused

Vertebral column truncated | Vertebral column considerably reduced in length (Henle et al. 2017b: Fig. 9)

2.3 Edema, tumour and similar types of anomalies

Green & Harshbarger (2001) provided a terminology for anomalies related to tumours and Bantle et al. (1991) and Green (2001) explained terms related to edema. Here we only include terms for externally visible anomalies. Many of these types of edema occur frequently in embryos but are rarely observed in the field at later stages of development.

Ascites | → Edema of the peritoneal cavity (belly) (Fig. 50; Ouellet 2000); → hydrocoelom is a synonym; since amphibians do not have separate thoracic and abdominal cavities, the term is regarded as inappropriate for amphibians by Green (2001); some authors (e.g. Fernandez & Beetschen 1975) used the term more broadly as a synonym of edema

Blister | A vesicle of the skin containing fluid (Bantle et al. 1991); a specific type of → edema

Bloatedness | Swelling of the entire body; usually no differentiation is made as to whether gas or liquid accumulation caused bloatedness (e.g. Lannoo 2009), and it is not always easy to make this distinction in the field; we recommend using either → gas accumulation or → edema when the cause (gas / liquid) of the swelling is known

Fig. 49: Nasal openings destroyed by maggots of *Lucilia bufonivora* in an adult *Bufo bufo*; Bialogard, Poland. Source: Wikipedia Commons. Photo: R. Altenkamp

Fig. 50: Edema in a juvenile *Lissotriton vulgaris;* Ekaterinburg, Russia, 16.9.1980. Photo: V. Vershinin.

Carcinoma | Malignant form of → neoplasia; contains → melanoma as a subcategory

Cephalic edema | → Edema of the head; → hydrocephaly and → hydroencephaly are generally used as synonyms, though the later does not necessarily involve the whole head in tadpoles or later stages

Cyst | Swelling caused by abnormal tissue growth to encapsulate parasites; also used for swellings that contain mushy material; the definition for cysts makes it a subcategory of → tumour but usually the term cyst is regarded as a separate category

Dysplasia | Abnormal development of structures, such as muscles or organs, conventionally used for non-malignant abnormal structures (Rothschild et al. 2012)

Edema | Subcutaneous accumulation of fluid (Fig. 50; Johnson et al. 2001, 2010, Sessions 2003, Vershinin 2015); → hygroma is a rarely used synonym; → hydrops is used as another synonym by some authors but regarded as a subcategory by others; edema is a subcategory of → bloatedness and contains the following subcategories: → ascites, → hydrocephaly, → hydrocoelom and → hydroencephaly

Epithelioma | A synonym of → papilloma (Pfeiffer et al. 1979)

Gas accumulation | A subcategory of → bloatedness, in which the swelling of the body is caused by gas

Granuloma | → Tumour caused by an inflammatory swelling (Green & Harshbager 2001)

Gut miscoiling | Abnormal coiling of the gut; most common is a loose coiling

Hydrocephaly | → Edema of the head (e.g. Hertwig 1911); → cephalic edema is a synonym; because the most common form is an enlargement of the brain ventricles due to an excessive amount of fluid; hydrocephaly and → hydroencephaly are generally used interchangeably (e.g. Pacces-Zaffaroni et al. 1978)

Hydrocoelom | Fluid accumulation in the body cavity (Fig. 50); a synonym of → ascites, if ascites is defined as it is here

Hydroencephaly | → Edema of the brain (Bantle et al. 1991, Vershinin 2015); → hydrocephaly is often used as a synonym, although strictly speaking hydroencephaly is a specific form of hydrocephaly in which the brain is affected

Hydrops | A synonym of → edema; Green (2001) preferred to use this term only for cases in which the subcutis, tissues and coelomic cavity of larval amphibians are jointly affected; Elkan (1976) used it for edema of the subcutaneous lymph sacs in larval and adult amphibians

Hygroma | Used as a synonym of → edema by Lannoo (2008)

Hyperplasia | Abnormally strong development of structures, such as muscles or organs; a subcategory of → tumour

Melanoma | Malignant → neoplasia arising from the melanocytic system of the skin (Rothschild et al. 2012) or other organs; melanomas are characterised by black pigmentation

Neoplasia | Development of abnormal new structures (Green & Harshbarger 2001, Rothschild et al. 2012, Vershinin 2015); a subcategory of → tumours; contains → carcinoma as a subcategory

Nodule | → Swelling or lump; may be filled with lipids, viral particles, spores of fungi or parasitic microorganisms; often an initial stage in the formation of tumours (e.g. Lucké & Schlumberger 1949)

Papilloma | → Neoplasia in which the ectoderm shows an abnormal outgrowth in the form of a horny epithelium or warts (Fig. 51); plural: papillomas or papillomata; → epithelioma is a synonym

Ulceration | Sore or lump; may originate from infection or tumour (e.g. Lucké & Schlumberger 1949)

Tumour | Abnormal mass of tissue resulting from excessive cell division (Fig. 51); a tumour may be inflammatory (→ granulomata), parasitic (encysted immature trematodes), → hyperplastic or → neoplastic (Green & Harshbarger 2001); malignant tumours are called → carcinoma; note that abnormal tissue mass produced to encapsulate parasites is most commonly referred to as a cyst and not as a tumour, though the definition of tumours also includes cysts

2.4 Anomalies of eggs and early embryonic stages

As most of these anomalies are only studied in the laboratory, here we only list a few types of anomalies that can easily be detected in the field with a hand lens. See Bantle et al. (1991) for a more detailed description of various embryonic anomalies

Acephaly | Head structures lacking

Asyntaxia medullaris | A synonym of → spina bifida

Axial duplication | → Spina bifida

Clutch abnormal | Common clutch anomalies are clutches lacking eggs (Fig. 52), eggs without embryos, twin embryos, membrane lacking between embryos (Fig. 52) and abnormally white eggs (see → albinism and → transient albinism in section 2.1) (Vershinin 1991, 2002)

Cyclopia | Eyes completely fused into one single median eye (Adelmann 1936, Signoret 1960); Zaks (2008) and Fayzulin (2011) used the term as a synonym of unilateral → anophthalmy

Microcephaly | Head reduced in size (Nekrasova 2008)

Spina bifida | Duplication of (parts of) the vertebral column, usually combined with duplications of major parts of the body (Hertwig 1892); ontogenetically, the

Fig. 51: Papilloma in *Cynops pyrrhogaster*; Japan. Photo: M. Asashima & V.B. Meyer-Rochow.

correct name should be → asyntaxia medullaris (Barfurth 1900) but this name has not become established (see also → twinning; → Siamese twins)

Supernumerary appendages | Duplication of embryonic structures

Synophthalmy | Eyes are close-set, can adhere more or less to each other but remain distinct, which distinguishes it from → cyclopy (Signoret 1960)

2.5 Other terms

Abnormality | Gross deviation from the normal range in morphological variation (Johnson et al. 2001, USFWS 2007)

Aneuchrony | The speed of development is accelerated or delayed compared to the normal condition (Dubois 1987); → heterochronic and → homochronic aneuchrony are subcategories

Anomaly | Any deviation of the phenotype (morphological and non-morphological) from the range of variation of the phenotype considered to be normal and irrespective of its cause

Deformity | Alteration of an organ or structure that originally formed correctly (Johnson et al. 2001, USFWS 2007)

Heterochronic aneuchrony | Dissociation of the development rate of characters, some being either accelerated or decelerated compared to the normal development rate of other characters (Dubois 1987); → neoteny is a common form of heterochronic aneuchrony

Homochronic aneuchrony | The speed of development deviates from the normal rate for all characters (Dubois 1987)

Fig. 52: Abnormal clutches of *Salamandrella keyserlingii*: left: eggs arranged in strings lacking individual membranes; right: abnormally low number of eggs; Ekaterinburg, Russia, 7.5.2002. Photo: V. Vershinin.

Malformation | A permanent structural defect resulting from abnormal development (Johnson et al. 2001, USFWS 2007)

Neoteny | A specific form of → heterochronic aneuchrony, in which development of the somatic characters is delayed or arrested but not that of gonads, so that the resulting giant larvae are able to reproduce (Fig. 53) (Dubois 1979)

Paedomorphosis | Retention of juvenile traits in adults (Dubois 1979)

3 Acknowledgments

We thank Marlies Uhlig, Leipzig, and the UFZ library team, for their assistance in compiling the literature. Please note that copyrights may apply to all those photos published with this article. Photos retrieved from Wikimedia Commons (*http://commons.wikimedia.org*) were published under the creative commons licence (*https://creativecommons.org/licenses/by-nc-sa/2.0/*). See the cited webpage for conditions of using these photos. Photos of the United States Fish and Wildlife Service are in the public domain and were retrieved from *http://www.fws.gov/contaminants/amphibian/PictureThumbs.html*. We owe thanks to all photographers who made photos available via these platforms or who provided us with their photos directly. VV was supported by Act

Fig. 53: Neoteny in a *Lissotriton vulgaris meridionalis;* Croatia 25.6.2010. Photo: B. Trapp.

211 Government of the Russian Federation, agreement № 02.A03.21.0006. Above all, we would like to thank Jean-Claude Beetschen, Toulouse, and Alain Collenot, Paris, for carefully reviewing and commenting on the glossary, which helped us to clarify synonyms and deviating definitions. KH thanks Natalia Rumyantseva for her help in translating Russian literature and for her patience during many hours of work on the manuscript.

4 References

Adelmann, H.B. (1936): The problem of cyclopia. — Q. Rev. Biol. **11**: 161–182, 284–304.

Bacon, J.P., D.W. Linzey, R.L. Rogers & D.J. Fort (2006): Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part I. Frequencies and distribution of abnormalities. — Appl. Herpetol. **3**: 39–65.

Bantle, J.A., J.N. Dumont, R.A. Finch & G. Linder (1991): Atlas of Abnormalities – A Guide for the Performance of FETAX. — Stillwater (Oklahoma State Publications Department).

Barfurth, D. (1900): Die experimentelle Herstellung der Cauda bifida bei Amphibienlarven. — Arch. Entwickl.-mech. **9**: 1–26.

Bender, B. (1997): Temporärer Albinismus bei der Erdkröte (*Bufo bufo*). — Z. Feldherpetol. **4**: 212–214.

Borkin, L.J. (1999): *Salamandrella keyserlingii* Dybowski, 1870 – Sibirischer Winkelzahnmolch. Pp. 21–55 in Grossenbacher, K. & B. Thiesmeier (eds.): Handbuch der Reptilien und Amphibien Europas. Schwanzlurche I. — Wiesbaden (Aula).

Borkin, L.J., O.S. Bezman-Mosekyo & S.N. Litvinchuk (2012): Evaluation of animal deformity occurence in natural populations (an example of amphibians). — Trudy zoologicheskogo instituta RAN **316**: 324–343.

Bruch, C. (1864): Über Mißbildungen der Chorda dorsalis (Dichordus), nebst Bemerkungen über Doppelbildungen. — Würzburger med. Zeitschr. **5**: 1–35.

Capanna, E. (1967): Osservazioni sul semialbinismo degli Anfibi. — Boll. Zoll. **34**: 100–101.

Chang, T.K. & A.M. Boring (1935): Studies in variation among Chinese Amphibia. I. Salamandridae. — Peking nat. Hist. Bull. **9**: 327-360, pl. I–III.

Cohn, M.J. & P.E. Bright (1999): Molecular control of vertebrate limb development, evolution and congenital malformations. — Cell Tissue Res. **296**: 3–17.

D'Amen, M., D. Salvi, L Vignoli, P. Bombi & M.A. Bologna (2006): Malformation occurrence in two newt species (*Triturus carnifex* and *Lissotriton vulgaris*) from three localities in central Italy: description and possible causes. — Atti Congr. Naz. Soc. Herpetol. Ital., Roma 27.9.–1.10.2006, **6**: 33–40.

Dearlove, G.E. & M.H. Dresden (1976): Regenerative abnormalities in *Notophthalmus viridescens* induced by repeated amputations. — J. exp. Zool. **196**: 251–262.

Degitz, S.J., P.A. Kosian, E.A. Makynen, K.M. Jensen & G.T. Ankley (2000): Stage- and species-specific developmental toxicity of all-trans retinoic acid in four native North American ranids and *Xenopus laevis*. — Toxicol. Sci. **57**: 264–274.

Dornblüth, O. (1927): Klinisches Wörterbuch (13./14. Aufl.). — (*http://www.textlog.de/klinisches.html*).

Dubois, A. (1977): Une mutation dominante déterminant l'apparition de diverses anomalies digitales chez *Rana temporaria* (Amphibiens, Anoures). — Bull. Soc. zool. Fr **102**: 197–213.

Dubois, A. (1979): Néoténie et pédogénèse. A propos d'une anomalie du développement chez *Bombina variegata* (Amphibiens, Anoures). — Bull. Mus. natn. Hist. nat. Paris, 4. sér., sect. A **1**: 537–546.

Dubois, A. (1987): Neoteny and assoicated terms. — Alytes **4**: 122–130.

Dubois, A. (2017): Rostand's anomaly P and similar anomalies in amphibians. — Mertensiella **25**: 49–56.

Duellman, W.E. & L. Trueb (1986): Biology of Amphibia. — New York (McGraw-Hill).

Dyrkacz, S. (1981): Recent instances of albinism in North American amphibians and reptiles. — Soc. Study Amph. Rept. Herpetol. Circ. **11**: 1–32.

Eaton-Poole, L., A.E. Pinkney, D.E. Green, D.R. Sutherland & K.J. Babbitt (2003): Investigation of frog abnormalities on national wildlife refuges in the Northeast U.S.. Pp. 63–78

in Linder, G.L., S. Krest, D. Sparling & E.E. Little (eds.): Multiple Stressor Effects in Relation to Declining Amphibian Populations. — West Conshohocken (ASTM).

Elkan, E. (1976): Pathology in the Amphibia. Pp. 273–312 in Lofts, B. (eds.): Physiology of the Amphibia. Vol. III. — London (Academic Press).

Fayzulin, A.I. (2011): About the morphological anomalies of anurans (Anura, Amphibia) of the Volga basin. — Prazi Ukrainskogo herpetologichnogo tovaristva **3**: 201–207.

Fedak, T.J. & B.K. Hall (2004): Perspectives on hyperphalangy: patterns and processes. — J. Anat. **204(3)**: 151–163.

Fernandez, M. & J.C. Beetschen (1975): Recherches sur le rôle de la température dans la réalisation du phénotype chez des embryons de l'Amphibien *Pleurodeles waltlii* homozygote pour la mutation thermosensible ac (ascite caudale). — J. Embryol. exp. Morphol. **34**: 221–252.

Fischer, J.-L. (1977): Un mime morphologique de la polydactylie faible: la fissuration des phalanges distales chez *Rana temporaria*. — Bull. Soc. Linn. Lyon **46**: 143–146.

Flindt, R. & H. Hemmer (1967): Nachweis natürlicher Bastardierung von *Bufo calamita* und *Bufo viridis*. — Zool. Anz. **178**: 419–429.

Fodor, A. & M. Puky (2002): Herpetological methods: II. Protocol for monitoring amphibian deformities under temperate zone conditions. — Opusc. Zool. Budapest **34**: 35–42.

Gardiner, D.M. & D.M. Hoppe (1999): Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). — J. exp. Zool. **284**: 207–216.

Geoffroy Saint-Hilaire, I. (1832): Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux. Tome 1. — Paris (Imprimerie de Cosson).

Glaw, F. & M. Vences (1997): Anuran eye colouration: definitions, variation, taxonomic implications and possible functions. Pp. 125-138 in Böhme, W., W. Bischoff & T. Ziegler (eds.): Herpetologia Bonnensis. — Bonn (Societas Europaea Herpetologica).

Gray, R.H. (1995): An unusual color pattern variant in cricket frog (*Acris crepitans*) from southern Illinois. — Trans. Illinois State Acad. Sci. **88**: 137–138.

Green, D.E. (2001): Pathology of Amphibia. Pp. 401–485 in Wright, K.M. & B.R. Whitaker (eds.): Amphibian Medicine and Captive Husbandry. — Malabar (Krieger Publ. Comp.).

Green, D.E. & J.C. Harshbarger (2001): Spontaneous neoplasia in Amphibia. Pp. 335–400 in Wright, K.M. & B.R. Whitaker (eds.): Amphibian Medicine and Captive Husbandry. — Malabar (Krieger Publ. Comp.).

Grimme (1907): Eine Missbildung von *Rana temporaria* Ant.. — Abh. Ber. Ver. Naturkde Kassel **51**: 126.

Grollich, C. & W.-R. Grosse (2013): Beobachtungen an einem zweiköpfigen Feuersalamander (*Salamandra salamandra*) in menschlicher Obhut. — Mitt. Sächs. Feldherpetol. Ichthyofaunisten **2013**: 18–19.

Hall, B.K. (2005): Bones and Cartilage: Developmental and Evolutionary Skeletal Biology. — San Diego (Elsevier).

Harris, M.L., C.A. Bishop & T.V. McDaniel (2001): Assessment of rates of deformity in wild frog populations using in situ cages: a case study of leopard frogs (*Rana pipiens*) in Ontario, Canada. — Biomarkers **6(1)**: 52–63.

Henle, K., B. Mester, S. Lengyel & M. Puky (2012): A review of a rare type of anomaly in amphibians, tail duplication and bifurcation, with description of three new cases in European species (*Triturus dobrogicus*, *Triturus carnifex*, and *Hyla arborea*). — J. Herpetol. **46**: 451–455.

Henle, K., A. Dubois & V. Vershinin (2017a): A review of anomalies in natural populations of amphibians and their potential causes. — Mertensiella **25**: 57–164.

Henle, K., A. Dubois, K. Rimpp, & V. Vershinin (2017b): Mass anomalies in green toads (*Bufotes viridis*) at a quarry in Roßwag: inbred hybrids, radioactivity or an unresolved case? — Mertensiella **25**: 185–242.

Héron-Royer, L.F. (1884): Cas tératologiques observés chez quelques têtards de batraciens anoures et de la possibilité de prolonger méthodiquement l'état larvaire chez les batraciens. — Bull. Soc. zool. Fr **1884(9)**: 162–168.

Hertwig, O. (1892): Urmund und Spina bifida. — Arch. mikr. Anat. **39**: 353–499.

Hertwig, O. (1911): Die Radiumkrankheit tierischer Keimzellen. Ein Beitrag zur experimentellen Zeugungs- und Vererbungslehre. — Arch. mikr. Anat. **77**: 1–164.

Hoppe, D.M. (2005): Malformed frogs in Minnesota: history and interspecific differences. Pp. 103-108 in Lannoo, M. (ed.): Amphibian Declines. The Conservation Status of United States Species. — Berkeley (University of California Press).

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie, J.K. Reaser & A.E. Launer (2001): Morphological abnormality patterns in a California amphibian community. — Herpetologica **57**: 336–352.

Johnson, P.T.J., M.K. Reeves, S.K. Krest & A.E. Pinkney (2010): A decade of deformities: advances in our understanding of amphibian malformations and their implications. Pp. 511–536 in Sparling, D.W., G. Linder, C.A. Bishop & S.K. Krest (eds.): Ecotoxicology of Amphibians and Reptiles (2nd ed.). — Boca Raton (CRC Press).

Kabisch, K. (1990): Wörterbuch der Herpetologie. — Jena (Fischer).

Klunzinger, C.B. (1903): Über Melanismus bei Tieren im allgemeinen und bei unseren einheimischen insbesondere. — Jh. Ver. Vaterl. Naturkde Württemberg **59**: 267–297.

Kouete, M.T., M. Wilkinson & D.J. Gower (2012): First reproductive observations for *Herpele* Peters, 1880 (Amphibia: Gymnophiona: Herpelidae): evidence of extended parental care and maternal dermatophagy in *H. squalostoma* (Stuchbury, 1836). — Zoology **2012**: Article ID 269690.

Kovalenko, E.E. (2000): Mass anomalies of extremities in Anura. — Zh. Obshch. Biol. **61**: 412–427.

Kronshage, A. & C. Hildmann (1988): Untersuchungen zum Vorkommen von Amphibien und Reptilien und zur Kleingewässersituation in Schwelm. — Beitr. Heimatkunde Stadt Schwelm Umgebung **38**: 9–36.

Kupferberg, S.J., A. Catenazzi, K. Lunde, A.J. Lind & W.J. Palen (2009): Parasitic copepod (*Lernaea cyprinacea*) outbreaks in foothill yellow-legged frogs (*Rana boylii*) linked to unusually warm summers and amphibian malformations in northern California. — Copeia **2009**: 529–537.

Lannoo, M.J. (2008): Malformed Frogs. The Collapse of Aquatic Ecosystems. — Berkeley (Univ. California Press).

Lannoo, M.J. (2009): Amphibian malformations. Pp. 3089–3111 in Heatwole, H. & J.W. Wilkinson (eds.): Amphibian Biology. Vol. 8: Amphibian Decline: Diseases, Parasites, Maladies, and Pollution. — Baulkham Hills, Australia (Surrey Beatty).

Lannoo, M.J., D.R. Sutherland, P. Jones, D. Rosenberry, R.W. Klaver, D.M. Hoppe, P.T.J. Johnson, K.B. Lunde, C. Facemire & J.M. Kapfer (2003): Multiple causes for the malformed frog phenomenon. Pp. 233–262 in Linder, G., S. Krest, D. Sparling & E. Little (eds.): Multiple Stressor Effects in Relation to Declining Amphibian Populations. — West Conshohocken (ASTM International).

Lotter, F. & N.J. Scott (1977): Correlation between climate and distribution of the color morphs of the salamander *Plethodon cinereus*. — Copeia **1977**: 681–690.

Lucké, B. & H.G. Schlumberger (1949): Neoplasia in cold-blooded vertebrates. — Physiol. Rev. **29**: 91–126.

Machado, C., A. Kwet & A. Schlüter (2010): Polydactyly and polymely in two populations of *Rana temporaria* and *Pelophylax esculentus* (Anura, Ranidae) in southern Germany. — Salamandra **46**: 239–242.

Martin, R. & J. Signoret (1968): Contribution à l'étude de la mutation «short toes» chez l'Axolotl. — Ann. Embr. Morph. **1**: 141–149.

McCallum, M.L. & S.E. Trauth (2003): A fourty-three year museum study of northern cricket frog (*Acris crepitans*) abnormalities in Arkansas: upward trends and distributions. — J. Wildlife Diseases **39(3)**: 522–528.

McDaniel, T.V., M.L. Harris, C.A. Bishop & J. Struger (2004): Development and survivorship of northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) exposed to contaminants in the water and sediments of the St. Lawrence River near Cornwall, Ontario. — Water Qual. Res. J. Canada **39(3)**: 160–174.

McDiarmid, R.W. & R. Altig (1999): Tadpoles. The Biology of Anuran Larvae. — Chicago (The University of Chicago Press).

Meteyer, C.U. (2000): Field Guide to Malformations of Frogs and Toads with Radiographic Interpretations. — Biol. Sci. Report **USGS/BRD/BSR-2000-0005**: 1–16.

Meteyer, C.U., I.K. Loeffler, J.F. Fallon, K.A. Converse, E. Green, J.C. Helgen, S. Kersten, R. Levey, L. Eaton-Poole & J.G. Burkhart (2000): Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. — Teratology **62**: 151–171.

Meyer, S. (2009): Schwarz und Weiß – Farbanomalien in einer Urodelen-Zucht. — amphibia **8(2)**: 27–28.

Miller, D., M. Gray & A. Storfer (2011): Ecopathology of ranaviruses infecting amphibians. — Viruses **3**: 2351–2373.

Muneoka, K., G. Holler-Dinsmore & S.V. Bryant (1986): Intrinsic control of regenerative loss in *Xenopus laevis* limbs. — J. exp. Zool. **240**: 47–54.

Nekrasova, O.D. (2008): Classification of amphibian anomalies. — Prazi Ukrainskogo herpetologitchnogo tovaristva **1**: 55–58.

Nishioka, M. (1977): Color variants induced by radiation and their inheritance in *Rana nigromaculata*. — Sci. Rep. Lab. Amphibian Biol. Hiroshima Univ. **2**: 25–89.

Ouellet, M. (2000): Amphibian deformities: current state of knowledge. Pp. 617-661 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. — Pensacola, Florida (SETAC Press).

Pacces-Zaffaroni, N., E. Arias, G. Capodanno & T. Zavanella (1978): The toxicity of manganese ethylenebisdithiocarbamate to the adult newt, *Triturus cristatus*. — Bull. Environ. Contam. Toxicol. **20**: 261–267.

Palis, J.G. (1997): *Eurycea quadridigitata* (dwarf salamander). Coloration. — Herpetol. Rev. **28**: 82.

Pfeiffer, C.J., T. Nagai, M. Fujimura & T. Tobe (1979): Spontaneous regressive epitheliomas in the Japanese newt, *Cynops pyrrhogaster*. — Cancer Res. **39**: 1904–1910.

Piha, H., M. Pekkonen & J. Merilä (2006): Morphological abnormalities in amphibians in agricultural habitats: a case study of the common frog *Rana temporaria*. — Copeia **2006**: 810–817.

Puky, M. & A. Fodor (2002): Occurrence of amphibian deformities along the Hungarian section of the River Danube, Tisza and Ipoly. — Limnol. Rep. Internatl. Assoc. Danube Res. **34**: 845–852.

Rachowicz, L.J. (2002): Mouthpart pigmentation in *Rana muscosa* tadpoles: seasonal changes without chytridiomycosis. — Herpetol. Rev. **33**: 263–265.

Richards, C.M. & G.W. Nace (1983): Dark pigment variants in anurans: classification, new descriptions, color changes and inheritance. — Copeia **1983**: 979–990.

Rohr, R., T. Raffel & S.K. Sessions (2009): Digenetic trematodes and their relationship to amphibian declines and deformities. Pp. 3067-3088 in Heatwole, H. & J.W. Wilkinson (eds.): Amphibian Biology. Vol. 8. — Baulkham Hills, Australia (Surrey Beatty).

Romansic, J.M., P.T.J. Johnson, C.L. Searle, J.E. Johnson, T.S. Tunstall, B.A. Han, J.R. Rohr & A.R. Blaustein (2011): Individual and combined effects of multiple pathogens on Pacific treefrogs. — Oecologia **166**: 1029–1041.

Rostand, J. (1946): L'albinisme chez les Batraciens Anoures. — Rev. Sci. **84**: 564–565.

Rostand, J. (1958): Les anomalies des Amphibiens Anoures. — Paris (Sedes).

Rostand, J. (1971): Les étangs à monstres. Histoire d'une recherche (1947–1970). — Paris (Stock).

Rothschild, B.M., H.-P. Schultze & R. Pellegrini (2012): Herpetological Osteopathology. — New York (Spinger).

Schmidt, G.A. (1930): Ein Fall einer Doppelmißbildung Duplicitas anterior assimmetricus bei *Rana arvalis* aus der Natur. — Bull. Station biol. Soc. Amis Sci. Nat., Anthropol. Éthnogr. Bolshevo Gouvernement Moscou **4**: 58.

Sessions, S.K. (2003): What is causing deformed amphibians? Pp. 168-186 in Semlitsch, R.D. (ed.): Amphibian Conservation. — Washington & London (Smithsonian Books).

Signoret, J. (1960): Céphalogenèse chez le triton *Pleurodeles waltlii* Michah. après traitement de la gastrula par le chlorure de lithium. — Mém. Soc. Zool. France **32**: 1–117, 7 plates.

Smallcombe, W.A. (1949): Albinism in *Rana temporaria*. — J. Genet. **49**: 286–291.

Snawder, J.E. & J.E. Chambers (1993): Osteolathyrogenic effects of malathion in *Xenopus* embryos. — Toxicol. appl. Pharmacol. **121**: 210–216.

Spadola, F. & G. Insacco (2010): Incomplete albinism in *Discoglossus pictus* (Otth, 1837). — Acta Herpetol. **5(2)**: 245–253.

Staheli, L.T., J.G. Hall, K.M. Jaffe & D.O. Paholke (2008): Arthrogryposis. A Text Atlas. — Cambridge (Cambridge University Press).

Streicher, J.W., C.J. Franklin, E.N. Smith & M.H. Yánez-Muñoz (2010): *Smilisca phaeota* (masked treefrog). Unilateral mydriasis. — Herpetol. Rev. **41**: 208–209.

Swett, F.H. (1926): On the production of double limbs in amphibians. — J. exp. Zool. **44**: 419–473.

Tunner, H.G. (1979): The inheritance of morphology and electrophoretic markers from homotypic crosses of the hybridogenetic *Rana esculenta*. — Mitt. Zool. Mus. Berlin **55(1)**: 89–109.

Tyler, M.J. (1989): Australian Frogs. — South Yarra (Viking O'Neil).

USFWS (2007): US Fish & Wildlife Service Standard Operating Procedures. Abnormal Amphibian Surveys. Abnormality Classification Sop. — *http://www.fws.gov/contaminants/Amphibian/pdfs/AbnormalitySOP_woutpics_Jano8.pdf*.

Vershinin, V.L. (1991): Anomalous amphibian egg deposits in area of urban agglomeration. — Russ. J. Ecol. **22**: 153–157.

Vershinin, V.L. (2002): Ecological specifity and microevolution in amphibian populations in urbanized areas. — Adv. Amphib. Res. Former Soviet Union **7**: 1–161.

Vershinin, V.L. (2015): Osnovy metodologii i metody issledovanija anomalii i patologii amphibii. — Ekaterinburg (Uralskii Federalnyi Universitet).

Vizotto, L.D. & V.A. Taddei (1977): Pseudomelia em *Phyrnohyas hebes* (Cope, 1862) (Anura-Hylidae). — Naturalia **3**: 35–43.

Wallach, V. (2007): Axial bifurcation and duplication in snakes. Part I. A synopsis of authentic and anectodal cases. — Bull. Maryland Herpetol. Soc. **43**: 57–95.

Weddeling, K. & A. Geiger (2011): 3.9 Erdkröte – *Bufo bufo.* Pp. 583–622 in Hachtel, M., M. Schlüpmann, K. Weddeling, B. Thiesmeier, A. Geiger & C. Willigalla (eds.): Handbuch der Amphibien und Reptilien Nordrhein-Westfalens. — Bielefeld (Laurenti Verlag).

Williams, L.A., A.D. Cameron & J.D. Groves (2013): *Aneides aeneus* (green salamander). Color variation. — Herpetol. Rev. **44**: 114–115.

Williams, R.N., D.H. Bos, D. Gopurenko & J.A. DeWoody (2008): Amphibian malformations and inbreeding. — Biol. Lett. **4**: 549–552.

Worthington, R.D. (1974): High incidence of anomalies in a natural population of spotted salamanders, *Ambystoma maculatum*. — Herpetologica **30**: 216–220.

Zaks, M.M. (2008): On the morphological anomalies of green frogs (*Rana ridibunda*, *R. lessonae*) in Penza-city (Russia). — Izv. Penz. gos. Pedagog. Univ. imeni V.G. Belinskogo **10(14)**: 63–65.

Zwart, P. (1985): 14. Amphibien. Pp. 351–362 in Gabrisch, K. & P. Zwart (eds.): Krankheiten der Heimtiere. — Hannover (Schlütersche Verlagsanstalt).

MERTENSIELLA 25 | 49–56 | 2017

Rostand's anomaly P in Palaearctic green frogs (*Pelophylax*) and similar anomalies in amphibians

Alain Dubois

Institut de Systématique, Évolution, Biodiversité
ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE
Muséum national d'Histoire naturelle, Sorbonne Universités
57 rue Cuvier, CP 30, 75005, Paris, France
adubois@mnhn.fr

Abstract. The anomaly P is a polymorphic syndrome that affects many individuals in some populations of Palearctic green frogs (genus or subgenus *Pelophylax*). In its benign forms, it consists in simple polydactyly, either only in hind limbs or both in hind and fore limbs, whereas in its severe forms it includes brachymely, inguinal tumours, bony excrescences and even polymely. Tadpoles showing these extreme forms do not survive to metamorphosis. This anomaly is characterized by several constant features, including a postero-anterior gradient and a good, although not always perfect, bilateral symmetry. The anomaly is widely distributed in Europe and northern Africa. It affects several taxa of *Pelophylax* but is not known to occur in other amphibian groups. Despite extensive work on this anomaly, especially in the period 1949–1984, its cause remains unknown, although several potential factors can be discarded. It is not of genetic origin, not being transmitted to the offspring. It is caused by an unknown exogenous teratogenic factor, possibly a virus transmitted by fish, that acts very early in the development, as amputation of an abnormal hind limb bud in a young tadpole is followed by regeneration of a normal limb. It would appear appropriate to resume scientific work on this syndrome in order to solve the still unsolved questions, as the results might have unexpected implications in developmental biology and in conservation biology.

Keywords. Polydactyly, polymely, tumours; gradient, symmetry, lethality; regeneration; virus.

Zusammenfassung. Anomaly P stellt ein polymorphes Syndrom an Missbildungen dar, das in manchen Populationen paläarktischer Grünfrösche (Gattung oder Untergattung *Pelophylax*) an zahlreichen Individuen auftritt. In seiner milden Form besteht es aus einfacher Polydactyly, die entweder nur an den Hinterbeinen oder sowohl an den Hinter- als auch an den Vorderbeinen auftritt. Bei schwerwiegenden Formen kommen Brachymely, Tumore in der Leistenregion, Knochenwucherungen und selbst Polymely hinzu. Kaulquappen, die schwere Formen des Syndroms aufweisen, überleben die Metamorphose nicht. Anomaly P weißt mehrere konstante Merkmale auf. Hierzu gehören ein postero-anterior Gradient (d.h., die Schwere der Missbildungen nimmt von den Hinter- zu den Vorderbeinen ab) und eine gute, wenn auch nicht immer perfekte bilaterale Symmetrie. Anomaly P hat eine weite Verbreitung in Europa und Nordafrika. Sie befällt mehrere Taxa von *Pelophylax,* aber ist von anderen Amphibiengruppen bisher nicht nachgewiesen. Trotz umfangreicher Untersuchungen über diese Anomalie, die besonders in den Jahren 1949-1984 stattfanden, bleibt die Ursache ungeklärt. Verschiedene potentielle Faktoren können jedoch ausgeschlossen werden. Da Anomaly P nicht auf Nachkommen vererbt wird, kommen genetische Ursachen nicht in Frage. Sie wird vielmehr durch einen unbekannten exogenen, teratogen wirkenden Faktor hervorgerufen, bei dem es sich möglicherweise um ein von Fischen übertragenen Virus handelt. Dieser Faktor entfaltet seine Wirkung sehr früh in der Individualentwicklung: die Amputation missgebildeter Hinterbeinknospen in jungen Kaulquappen führt zur Regeneration normaler Hinterbeine. Die Wiederaufnahme wissenschaftlicher Forschung zu diesem Syndrom scheint angeraten, um die noch immer offenen Fragen klären zu können, denn die Antworten könnten entscheidende Kenntnisse für die Entwicklungsbiologie und den Naturschutz liefern.

Schlüsselwörter. Polydactylie, Polymelie, Tumore; Gradient, Symmetrie, Lethalität; Regeneration; Virus.

Résumé. L'anomalie P est un syndrome polymorphe qui affecte de nombreux individus dans certaines populations de grenouilles vertes Paléarctiques (genre ou sous-genre *Pelophylax*). Ses formes bénignes consistent en une simple polydactylie, qui touche seulement les membres postérieurs ou également les membres antérieurs. Ses formes sévères incluent d'autres anomalies, comme la brachymélie, des tumeurs inguinales, des excroissances osseuses ou même la polymélie. Les têtards qui manifestent ces symptômes extrêmes ne survivent pas à la métamorphose. Cette anomalie se caractérise par plusieurs particularités constantes, notamment l'existence d'un gradient postéro-antérieur et une bonne, quoique souvent imparfaite, symétrie bilatérale. L'anomalie est largement répandue en Europe et en Afrique du Nord. Elle touche plusieurs taxons de *Pelophylax* mais n'est pas connue pour affecter les Amphibiens d'autres groupes. En dépit d'importants travaux sur cette anomalie, particulièrement dans la période 1949–1984, sa cause reste

inconnue, bien que plusieurs facteurs potentiels aient pu être mis hors de cause. Elle n'est pas d'origine génétique, n'étant pas transmise à la descendance. Elle est causée par un facteur tératogène exogène inconnu, peut-être un virus transmis par des poissons, qui agit très tôt dans le développement, puisque l'amputation d'un bourgeon de patte postérieure anormale chez un jeune têtard est suivie de la régénération d'une patte normale. Il serait souhaitable que des travaux soient de nouveau effectués pour élucider les questions que pose encore ce syndrome, car les réponses à ces questions pourraient avoir des implications inattendues en biologie du développement et en biologie de la conservation.

Mots-clés. Polydactylie, polymélie, tumeurs; gradient, symétrie, léthalité; régénération; virus.

1 Introduction

One of the longest studied cases of mass anomalies in amphibians is the syndrome of European green frogs now known as *anomaly P*. Although the first report of this syndrome was by Bonnet & Rey (1937), this isolated observation did not attract attention until the anomaly had been rediscovered and studied by Jean Rostand.

Within the frame of his survey of anomalies in natural populations of French amphibians, Jean Rostand found several examples of polydactylous common toads, *Bufo bufo* (Linnaeus, 1758), with six toes on each foot. In some cases, crossing one of them with a normal toad resulted in normal offspring, whereas crosses between two polydactylous specimens produced polydactylous toadlets. Therefore, this anomaly was interpreted as caused by a recessive mutation (Rostand 1947a,b, 1950a, 1951b). In other cases, a dominant mutation seemed to be involved, as polydactylous toads were obtained from crossing a single abnormal toad with a normal one (Rostand 1949a, 1951b).

2 The anomaly P syndrome

In the hope of finding also mutations responsible for polydactyly in the genus *Rana*, Rostand examined thousands of specimens of this genus and was thus led to the discovery, at Trévignon in Finistère (Brittany), of a population of green frogs (then referred to *Rana esculenta* Linnaeus, 1758) including many adult polydactylous specimens (Rostand 1949b). This polydactyly was very polymorphic and showed a postero-anterior gradient (Rostand 1949b, 1950d, 1951b): in all abnormal specimens, the hind limbs were touched by polydactyly (from 6 to 9 toes), whereas the fore limbs were touched only in some cases, when polydactyly on hind limbs was strong (more than 6 toes). Other morphological peculiarities of this syndrome included a good, although not always perfect, bilateral symmetry (Fig. 1): i.e., the number of additional digits was always roughly the same on both sides, with sometimes one more toe or finger on one side, but never an excess of several digits on one side (e.g., 6-7, 7-7 or 7-8 toes, but never 6-8). The first additional toe was usually longer than the "first" normal toe, which gives the feet of these frogs a very special aspect (Fig. 2D, E), quite different from that of usual polydactyly, e.g. in *B. bufo* (Fig. 2B). This polydactyly turned out to be only the weakest form of a very polymorphic syndrome, which could take much more severe forms in tadpoles, including stronger polydactyly (up to 15 toes), polymely (presence of small supernumerary hind limbs; Fig. 3), brachymely (shortened limbs) and various types of bony excrescences and tumours especially in the inguinal region (Rostand 1952a, 1955a, 1958a). Rostand (1952a) gave this syndrome the name of "*anomaly P*".

The anomaly touched a very high proportion of the larvae in the population, but most of them would die during metamorphosis or shortly afterwards, mostly from internal haemorrhages due to mechanical problems of the malformed specimens. This explains why the rate of anomalies is much higher in tadpoles than in adults (Rostand 1971, Dubois 1979, 1984).

Fig. 1: Severe expression of anomaly P in *Pelophylax esculentus*, with the forelimbs being affected by polydactyly in addition to the hindlegs. Characteristic for anomaly P is also the symmetric expression of the anomaly. Source: Rostand (1958a: Fig. 37).

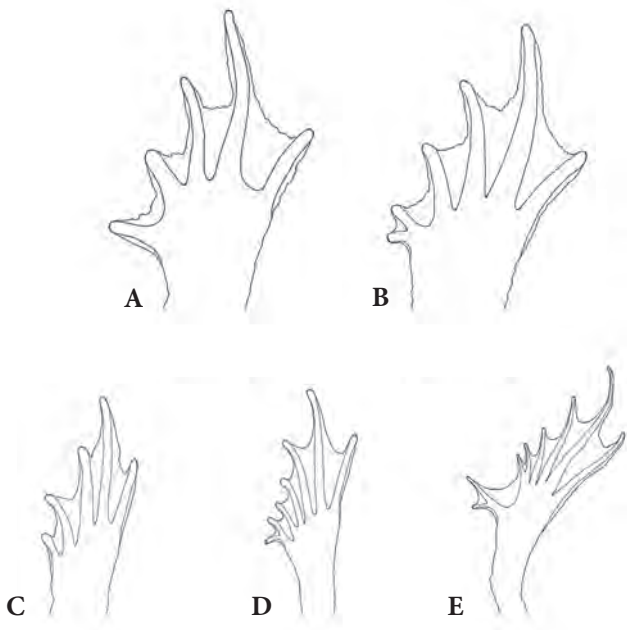


Fig. 2: Polydactyly in hind limbs of *Bufo bufo* and *Pelophylax esculentus*. (A) Normal specimen of *B. bufo* (5 toes) (Rostand 1955a: Fig. 14). (B) Polydactylous specimen of *B. bufo* (6 toes), polydactyly caused by a dominant mutation (Rostand 1955a: Fig. 15). (C) Normal specimen of *P. esculentus* (5 toes) (Rostand 1955a: Fig. 25). (D) Polydactylous specimen of *P. esculentus* (7 toes) (Rostand 1955a: Fig. 27). (E) Polydactylous specimen of *P. esculentus* (8 toes) (Rostand 1955a: Fig. 28). The two latter specimens are from Trévignon (Bretagne, France) and show different severities of polydactyly belonging in the anomaly P syndrome.

Fig. 3: Severe expression of anomaly P in *Pelophylax esculentus*; specimen cleared with potassium and stained with alizarine red; the hindlegs show small supernumerary limbs (polymely), gross malformation of the bones (taumely) and a large number of supernumerary toes (polydactyly). Source: Rostand (1958a: Fig. 38).

At the scale of a whole population, the anomaly shows a strong variability in time, being very frequent some years and very rare or absent in other years, with a possible return after several years of absence. In Trévignon, from 1949 to 1967, the frequency of the anomaly P varied from 0 to 80 % in tadpoles and from 2.6 to 14.5 % in adults; in another population from central France, Champdieu (Loire), from 1950 to 1976 the frequency varied from 0 to 6.3 %, whereas in a third one, Lingé (Indre), from 1961 to 1970 the frequency in tadpoles varied from 14 to 70 % (Dubois 1979, 1984, 2014). The frequency of the most severe forms, lethal at metamorphosis, is also variable from year to year (e.g., from 26.9 to 47.1 % in Trévignon from 1952 to 1958), but is not higher when the frequency of abnormal specimens is higher (Rostand 1959). The frequency of the anomaly is also different, within the same year, at different dates and seems to grow when the temperature in the ponds gets higher (Rostand & Darré 1969, Rostand 1971).

First discovered in a Brittany pond, the anomaly P was found or suspected to occur in many other populations of green frogs of the Palearctic region (for details, see Dubois 1984). The complete syndrome in adults and larvae was observed in several regions of France (see map in Dubois 1984), in the Netherlands and Morocco. Mass polydactyly, or polydactyly which on morphological grounds closely resembles that of the anomaly P, was reported from various other regions of France and from Austria, Belarus, Germany, Switzerland and Turkey (European part). Finally, isolated cases of polydactyly, possibly also belonging to the anomaly P, were reported from France, Germany, Greece, the Netherlands, Poland and Russia.

3 The search for the cause

Contrary to his expectation, when Rostand crossed specimens affected by the anomaly P, he obtained only normal offspring, even if both parents were polydactylous, some of them severely (Rostand 1950d). Despite these negative results, he first remained convinced that a genetic factor, possibly polygenic or transmitted by cytoplasm, was involved (Rostand 1951a,b). However, when the stronger polymorphism of anomalies in tadpoles was discovered, Rostand (1952a) started considering the possibility of its infectious determinism.

In a crucial series of experiments, Rostand (1952b) showed that amputation, in young tadpoles, of the distal part of a hind limb touched by the anomaly P, was followed by regeneration of a normal limb, which demonstrated that the teratogenic factor, whatever it may have been, had stopped being active at the time of regenera-

tion. Such a result, associated with those of the crossings, strongly suggested that the anomaly was caused by an exogenous teratogenic factor having a precocious and temporary action on the limbs of tadpoles. As many such factors could be suspected, Rostand carried out varied experiments in the hope of reproducing artificially, in the laboratory, polydactyly and the other anomalies observed in the anomaly P syndrome. However, he did not succeed in reproducing such anomalies by the action of various chemicals (Rostand 1950b,c), by raising eggs and larvae in brackish water or in water from the ponds where the anomaly was present (Rostand 1952c, 1958a), by the action of various physical aggressions, including thermal shocks, long exposure to high or low temperature or to sun, dehydration, etc. (Rostand 1950d, 1959) or even in provoking overripeness of egg before fertilization (Rostand 1951c).

Through ultraviolet irradiation of just hatched larvae of common brown frogs (*Rana temporaria* Linnaeus, 1758), Rostand (1955b, 1958b) obtained a few cases of polydactyly, polymely and even brachymely, but these anomalies were morphologically very different from those observed in the anomaly P syndrome. In 1957, many abnormal green frogs were found in a canal of Amsterdam where nuclear waste had been thrown, which elicited various papers in popular newspapers incriminating radioactivity as the cause of these anomalies (Rostand 1971). However, the anomalies observed were similar to those of Trévignon and were soon referred to the anomaly P (Hillenius 1959). Artificial radioactivity linked either to civil or military human activities can be dismissed as the possible cause of the anomaly P, as isolated cases of the latter have been reported since the beginning of the 19th century (Virey 1819) and mass occurrence of polydactyly clearly referable to the anomaly P have been known since before the Second World War (Bonnet & Rey 1937). Rostand (1957, 1959) also gave arguments to reject the hypothesis of an action of natural radioactivity. So other causes had to be sought for this syndrome.

All these observations led Rostand to think that the anomaly P was not due to a chemical or physical agent and to favour the hypothesis, suggested to him by Caullery in 1949 (Rostand 1952a), of an infectious agent, which could be a teratogenic virus. Furthermore, as eggs of green frogs collected in the Trévignon ponds gave birth, in the laboratory, only to normal larvae (Rostand 1959), it was possible to suppose that this factor was not active during embryogenesis, but between hatching and the appearance of the hind limb buds.

After various laboratory experiments that did not bring additional support for the virus hypothesis (Rostand 1952c, 1959, 1960, 1971), a series of experiments realized in the field brought new interesting insights. Whereas tadpoles raised from eggs hatching in large cages directly submerged in a pond where the anomaly P was present did not show any anomaly (Rostand et al. 1967), tadpoles raised in such cages but with fishes, i.e., tenches [*Tinca tinca* (Linnaeus, 1758)] and eels [*Anguilla anguilla* (Linnaeus, 1758)], collected in this pond showed severe anomalies belonging to the anomaly P syndrome (Rostand & Darré 1967). The sensitive period for the induction of anomalies in such conditions turned out to be limited to the first days of free larval life (Rostand & Darré 1968). Finally, Rostand & Darré (1969) reported having obtained abnormal specimens in the laboratory by feeding just hatched tadpoles with intestinal contents of fish from the pond: in such conditions some specimens showed even some anomalies more severe than those observed until then in the field. Thus the factor responsible for the anomaly P seems to be present in the digestive tract of fish living in some ponds. This does not mean that this factor exists only there: it could as well be present in some plants or aquatic invertebrates on which the fish feed. This factor has not yet been isolated but the hypothesis that it could be a teratogenic virus agrees with the data published so far.

This factor appears not to be evenly distributed in the habitat, as the frequency of the anomaly varies among different parts of ponds (Rostand et al. 1967, Rostand & Darré 1968, 1969, Rostand 1971). The zones in which the anomaly P appears with high frequencies change from year to year and, after ecological changes that are not understood, it may even disappear completely, sometimes temporarily, sometimes definitively (Rostand 1971).

The variability, and hence the unpredictability, of the appearance, frequency and severity of the anomaly P in a given pond and at a given spot is a serious handicap for the study of its causes. On several occasions, rather heavy experimental protocols, involving the comparison of control tadpoles with tadpoles fed with fish intestinal contents, either intact or submitted to ebullition, freezing, filtration or chromatographic fragmentation, did not give any results, but this was not surprising in view of the fact that, in the same year, the anomaly P proved to have been absent from the ponds (Dubois 1979, 1984). In the world of contemporary research, where obtaining rapidly publishable results is a constraint for obtaining funding for research, this difficulty is a major one for pursuing the study of this question. This probably explains in part why this question remains unresolved today, as after the patient research, for several decades, by Jean Rostand, an individual "amateur" without funding or support of any kind, the study of this anomaly has never been seriously undertaken by any institutional laboratory over the whole of Europe.

As of today, a teratogenic virus appears the best hypothesis to account for the various observations summarized above, but it is still not demonstrated. If it proved true, and if this virus could be isolated, it would provide research with a very powerful teratogenic factor, which could throw some lights on problems of cell proliferation and differentiation, with possible impacts on research on cancers and other pathologies that imply uncontrolled cell multiplication.

4 Ecological significance of anomaly P

Whatever its causes, the anomaly P is an important phenomenon for the populations of green frogs where it occurs, as it affects the survival of frogs (Rostand 1962, Rostand & Darré 1968). Before metamorphosis, the survival of strongly affected larvae does not seem to be affected and artificial or spontaneous prolongation of the larval condition allows keeping these specimens alive for very long periods, up to one year and a half (Rostand 1959). However, even in protected conditions in captivity, specimens exhibiting the severe forms of the anomaly (with brachymely and various supernumerary bony formations) all die within the first weeks after metamorphosis, merely because of internal injuries caused by the bony excrescences in the hind limbs (Rostand 1955c). Thus, for purely mechanical reasons, the anomaly in its severe forms is totally lethal at metamorphosis or just after and only the frogs showing the benign form of the anomaly (simple polydactyly) do survive. Since the frequency of the severe forms often reaches 40 or 50 % of the abnormal specimens, which in turn may represent up to 80 % of the tadpoles of a population, in some cases about 40 % of the tadpoles of a given pond may die at metamorphosis. Such a mortality rate, especially if repeated for several years, might have a significant impact on the dynamics of these natural populations. However, until now no study has been devoted to this question.

5 Species affected

Most of the observations on the anomaly P summarized above are older than the discovery that Palaearctic green frogs consist of several "normal species" and of several *kleptons*, i.e., particular species derived from hybridization between two "normal species" but with a particular meiosis that allows the maintenance of "first generation hybrids" over many generations (Tunner 1974, Dubois 1977, 1991, 2011, Dubois & Günther 1982, Graf & Polls Pelaz 1989). Therefore, it is currently unknown which of these species and kleptons are touched by the anomaly, but the distribution of the latter, briefly surveyed above, is large enough to be sure that it concerns at least several taxa, if not all European green frogs, currently placed in the subgenus *Pelophylax* Fitzinger, 1843 of the genus *Rana* Linnaeus, 1758 (Dubois 1992) or even by some authors (Fei et al. 1990, Frost et al. 2006) in a full genus *Pelophylax*. Currently, 20 species and 3 kleptons are recognized by taxonomists in this group, among which 9 species and 3 kleptons occur in Europe and northern Africa (Dubois & Ohler 1995, Dubois 1998, Ohler 2007). From the distributional data on the anomaly P (see above), at least the species *Pelophylax lessonae* Camerano, 1882, *Pelophylax ridibundus* Pallas, 1771, *Pelophylax perezi* Seoane, 1885 and *Pelophylax saharicus* Boulenger, 1913, and the kleptons *Pelophylax* kl. *esculentus* Linnaeus, 1758 and *Pelophylax* kl. *grafi* Crochet, Dubois, Ohler & Tunner, 1995 appear to be affected by the anomaly. Possibly other taxa might also be involved in some cases.

No report of anomalies clearly belonging to the anomaly P in other groups of frogs traditionally referred to the genus *Rana* but now placed in several subgenera or genera (Dubois 1992, Frost et al. 2006) has been published so far. In particular, this syndrome is unknown in Palaearctic brown frogs (genus or subgenus *Rana* s. str.) and in North American green frogs, now placed in the subgenus *Aquarana* Dubois, 1992 (Dubois 1992) or in the genus *Lithobates* Fitzinger, 1843 (Frost et al. 2006). In any case, the data available now are enough to state that the anomaly P is not species-specific and can touch several taxa in the *Pelophylax* group (Dubois 2014). Almost everything remains to be done to answer the following questions: (1) Are some of the taxa of *Pelophylax* more sensitive than others to the anomaly P and are some of them immune from it? (2) More specifically, in the mixed populations involving two or more distinct taxa of *Pelophylax*, does the anomaly P touch them indiscriminately or not? (3) Does the sensitivity to the anomaly P causative factor depend in any way on the special kind of meiosis that occurs in kleptons?

These questions make sense especially as no hard data exist to document the fact that amphibian species of groups other than the genus or subgenus *Pelophylax* can be touched by the anomaly P, even in the habitats where this anomaly is present in green frogs. In a few species – particularly *Lissotriton helveticus* (Razoumowsky, 1789), *Triturus cristatus* (Laurenti, 1768) and *Rana temporaria* – various limb anomalies have been found in ponds where the anomaly P occurs, or obtained by rearing larvae in contact with fish from such ponds, but it is not at all established whether these anomalies were indeed caused by the anomaly P factor (Dubois 1974, 1979, 1984).

Various other kinds of anomalies have been described so far in natural populations of many amphibian species. They are reviewed in another paper of this volume (Henle et al. 2017). The causes of only a small proportion of them have been scientifically established so far, but none of them seems to result from the anomaly P factor, whatever it is, or from similar causes. In a few cases the causes were shown or supposed to be non-genetic (either through crosses or through regeneration experiments) but remain mysterious (Dubois 1979, 1984) and, in this respect only, remind the anomaly P. Few of them have been studied as thoroughly as the anomaly P and re-examination of these few cases might prove rewarding.

6 Conclusion

Much still remains to be known about the anomaly P: its cause, its geographic distribution, exactly which taxa are affected and why, what is the impact of this syndrome on frog populations, etc. Although this problem attracted the attention, especially of an amateur naturalist, Jean Rostand, mostly in the years 1950-1970, no

studies are apparently under way nowadays, in any laboratory or European country, to elucidate these questions. This is surprising and even shocking, especially in view of the strong interest raised in recent years by amphibian anomalies in conservation biology (Henle et al. 2017). Given the fact that this syndrome involves facts of cellular abnormal multiplication and tissue differentiation and growth, its understanding might throw interesting or important lights on some developmental biology problems. More attention should certainly be paid to this unsolved problem by the international scientific community.

7 Acknowledgements

Jean-Claude Beetschen (Toulouse) and Alain Collenot (Paris) provided valuable suggestions for improving the manuscript.

8 References

Bonnet, A. & M. Rey (1937): Sur quelques cas de polydactylie et de schistodactylie observés en série chez la grenouille. – Bull. Soc. Zool. France **62**: 21–25.

Dubois, A. (1974): Polydactylie massive, associée à la clinodactylie, dans une population de *Rana graeca*. Remarques sur la polydactylie faible et la clinodactylie chez *Bufo bufo* (Amphibiens, Anoures). – Bull. Soc. Zool. Fr. **99**: 505–521, pl. 1–2.

Dubois, A. (1977): Les problèmes de l'espèce chez les Amphibiens Anoures. In: Bocquet, C., J. Génermont & M. Lamotte (eds.): Les problèmes de l'espèce dans le règne animal, **2**. – Mém. Soc. Zool. Fr. **39:** 161–284.

Dubois, A. (1979): Anomalies and mutations in natural populations of the *Rana esculenta* complex (Amphibia, Anura). – Mitt. Zool. Mus. Berlin **55**: 59–87, pl. 1.

Dubois, A. (1984): L'anomalie P des Grenouilles vertes (complexe de *Rana* kl. *esculenta* Linné, 1758) et les anomalies voisines chez les Amphibiens. Pp. 215-221 in Vago, C. & G. Matz (eds.): Comptes rendus du Premier Colloque international de Pathologie des Reptiles et des Amphibiens. – Angers (Presses Univ. Angers).

Dubois, A. (1991): Nomenclature of parthenogenetic, gynogenetic and "hybridogenetic" vertebrate taxons: new proposals. – Alytes **8**: 61–74.

Dubois, A. (1992): Notes sur la classification des Ranidae (Amphibiens, Anoures). – Bull. mens. Soc. linn. Lyon **61(10)**: 305–352.

Dubois, A. (1998): List of European species of amphibians and reptiles: will we soon be reaching "stability"? – Amphibia-Reptilia **19(1)**: 1–28.

Dubois, A. (2011): Species and "strange species" in zoology: do we need a "unified concept of species"? – C. r. Palevol. **10**: 77–94.

Dubois, A. (2014): The anomaly P in Palaearctic green frogs of the genus *Pelophylax* (Ranidae). Pp. 96–104 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. – Ekaterinburg (Uralskii Federalnii Universitet).

Dubois, A. & R. Günther (1982): Klepton and synklepton: two new evolutionary systematics categories in zoology. – Zool. Jb. Syst. **109**: 290–305.

Dubois, A. & A. Ohler (1995): Frogs of the subgenus *Pelophylax* (Amphibia, Anura, genus *Rana*): a catalogue of available and valid scientific names, with comments on name-bearing types, complete synonymies, proposed common names, and maps showing all type localities. – Zool. Polon. "1994", **39(3–4)**: 139–204.

Fei, L, C. Ye & Y. Huang (1990): Key to Chinese Amphibia. – Chongqing (Eds. Sci. Tech.,): [i-iv] + 1–2 + 1–364. [In Chinese].

Frost, D.R., T. Grant, J. Faivovich, R.H. Bazin, A. Haas, C.F.B. Haddad, R.O. de Sá, A. Channing, M. Wilkinson, S.C. Donnellan, C.J. Raxworthy, J.A. Campbell, B.L. Blotto, P. Moler, R.C. Drewes, R.A. Nussbaum, J.D. Lynch, D.M. Green & W.C. Wheeler (2006): The amphibian tree of life. – Bull. Amer. Mus. Nat. Hist. **297**: 1–370.

Graf, J.-D. & M. Polls Pelaz (1989): Evolutionary genetics of the *Rana esculenta* complex. Pp. 289–302 in Dawley, R.M. & J.P. Bogart (eds.): Evolution and Ecology of Unisexual Vertebrates. – Albany (New York State Museum).

Henle, K., A. Dubois & V. Vershinin (2017): A review of anomalies in natural populations of amphibians and their potential causes. – Mertensiella 25.

Hillenius, D. (1959): Ein zweiter Fall der "Anomalie P" (Rostand) bei *Rana esculenta* Linné. – Med. Klinik **54**: 99–100.

Ohler, A. (2007): New synonyms in specific names of frogs (Raninae) from the border regions between China, Laos and Vietnam. – Alytes, **25** (1–2): 55–74.

Rostand, J. (1947a): Gynogenèse et polydactylie chez le crapaud ordinaire. – C. r. Soc. Biol. **141**: 629–630.

Rostand, J. (1947b): De quelques anomalies digitales chez le crapaud ordinaire. – Rev. sci. **85**: 1121–1125.

Rostand, J. (1949a): Hérédité de la polydactylie chez le crapaud ordinaire (*Bufo bufo*). – C. r. Acad. Sci. **228**: 778–779.

Rostand, J. (1949b): Polydactylie naturelle chez la grenouille verte (*Rana esculenta*). – C. r. Acad. Sci. **228**: 1666–1667.

Rostand, J. (1950a): Sur la gynogenèse comme moyen d'exploration du patrimoine héréditaire chez le crapaud ordinaire. – C. r. Acad. Sci. **230**: 2130–2131.

Rostand, J. (1950b): Essais de chimiotératogenèse chez les Batraciens Anoures. – C. r. Soc. Biol., **144**: 915–917.

Rostand, J. (1950c): La chimiotératogenèse chez les Batraciens Anoures. – Rev. sci. **88**: 155–157.

Rostand, J. (1950d): Sur la descendance des grenouilles polydactyles. – C. r. Acad. Sci. **231**: 496–498.

Rostand, J. (1951a): La génétique des Batraciens. – Paris (Hermann).

Rostand, J. (1951b): Sur la polydactylie des Batraciens Anoures. – Bull. Biol. Fr. Belg. **85**: 113–136.

Rostand, J. (1951c): Surmaturité des œufs et anomalies chez *Rana temporaria*. – C. r. Soc. Biol., **145**: 624–625.

Rostand, J. (1952a): Sur la variété d'expression d'une certaine anomalie (P) chez la grenouille verte (*Rana esculenta* L.). – C. r. Acad. Sci. **235**: 583–585.

Rostand, J. (1952b): Régénération de membres normaux chez les larves de *Rana esculenta* présentant l'anomalie P. – C. r. Soc. Biol. **146**: 1530–1531.

Rostand, J. (1952c): Sur une certaine anomalie (P) de la grenouille verte (*Rana esculenta* L.). – Rev. sci. **90**: 353–365.

Rostand, J. (1955a): Les crapauds, les grenouilles, et quelques grands problèmes biologiques. – Paris (Gallimard), pp. 1–217, 48 pl.

Rostand, J. (1955b): Effets tératogènes des rayons ultraviolets sur les larves de grenouilles. – C. r. Soc. Biol. **149**: 905–907.

Rostand, J. (1955c): Nouvelles observations sur l'anomalie P chez la grenouille verte (*Rana esculenta* L.). – C. r. Soc. Biol. **149**: 2075–2077.

Rostand, J. (1957): Grenouilles monstrueuses et radioactivité. – C. r. Acad. Sci. **245**: 1175–1176.

Rostand, J. (1958a): Les anomalies des Amphibiens Anoures. – Paris (Sedes).

Rostand, J. (1958b): Effet tératogène des rayons ultraviolets sur les larves d'Amphibiens. – C. r. Acad. Sci. **247**: 972–974.

Rostand, J. (1959): L'anomalie P chez la grenouille verte (*Rana esculenta* L.). – Bull. Biol. Fr. Belg. **93**: 7–15.

Rostand, J. (1960): Le problème des grenouilles monstrueuses. – Sciences **10**: 25–32.

Rostand, J. (1962): Sur la distribution de l'anomalie *P* chez la grenouille verte (*Rana esculenta* L.). – C. r. Acad. Sci. **255**: 2189–2190.

Rostand, J. (1971): Les étangs à monstres. Histoire d'une recherche (1947–1970). – Paris (Stock).

Rostand, J. & P. Darré (1967): Sur les conditions d'apparition de l'anomalie *P* chez *Rana esculenta*. – C. r. Acad. Sci. **265**: 761–762.

Rostand, J. & P. Darré (1968): Conditions déterminantes de l'anomalie P chez *Rana esculenta*. – C. r. Soc. Biol. **162**: 1682–1683.

Rostand, J. & P. Darré (1969): Action tératogène des déjections de certains poissons sur les larves de *Rana esculenta*. – C. r. Soc. Biol. **163**: 2033–2034.

Rostand, J., M. Jacquot & P, Darré (1967): Nouvelles expériences sur les causes de l'anomalie *P* chez *Rana esculenta*. – C. r. Acad. Sci. **264**: 2395–2397.

Tunner, H.G. (1974): Die klonale Struktur einer Wasserfroschpopulation. – Z. zool. Syst. Evol.-Forsch. **12**: 309–314.

Virey, J.-J. (1819): Observation et description de deux individus sexdigitaires. – J. complém. Dict. Sci. méd. **4**: 327–330.

Jean Rostand (1894, Paris – 1977, Ville-d'Avray), French biologist, author and philosopher. Source: private.

MERTENSIELLA 25 | 57–164 | 2017

A review of anomalies in natural populations of amphibians and their potential causes

Klaus Henle[1], Alain Dubois[2] & Vladimir Vershinin[3,4]

[1]UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstr. 15, 04318 Leipzig, Germany, klaus.henle@ufz.de

[2]Institut de Systématique, Évolution, Biodiversité
ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE
Muséum national d'Histoire naturelle, Sorbonne Universités
57 rue Cuvier, CP 30, 75005, Paris, France
adubois@mnhn.fr

[3]Institute of Plant and Animal Ecology, Uralian Division, Russian Academy of Science, 8 Marta St 202, 620144, Ekaterinburg, Russia; email: vol_de_mar@list.ru

[4]Ural Federal University, Ekaterinburg, Mira St 19, 620002, Russia

Abstract. Amphibian anomalies have been a source of curiosity for centuries and the literature on this topic is enormous. We provide a comprehensive overview of the anomalies that have been observed in natural populations of amphibians and discuss their suspected and demonstrated causes. We review the evidence for different sensitivity of species to acquire anomalies and the individual and population level consequences of anomalies as well as their conservation implications. We then go on to review the evidence for an increase in the prevalence of anomalies in natural populations of amphibians over recent decades. Finally, we provide recommendations for future studies and argue that in spite of knowledge gaps, we have sufficient information to reduce the anthropogenic contribution to the plights of amphibians for the sake of amphibian conservation and ecosystem and human health.

For our review we traced any publication on anomalies in natural populations of amphibians starting from our extensive literature collections. We compiled the published data in a database, treating each species from a particular location as a separate case. Likewise, we entered each population as a separate case, if population specific data were provided for at least one population. In such cases, we ignored additional data provided for lumped populations that included this population.

The earliest documents of amphibian anomalies are provided by the fossil record. Excluding fossils, we compiled data for 3517 natural populations from 98 countries for which anomalies have been reported. These belong to 6, 117 and 386 living species of Gymnophiona, Urodela and Anura, respectively. The oldest definite report of non-fossil anomalies dates back to 1554 and was a toad with malformed limbs. Since the 1860s publications on the topic have appeared almost every year and rapidly increased. Three major increments in the annual number of publications are noticeable, the first occurring after the Second World War, the second in the mid–1970s and the most recent one in 1996 (the year after which malformation hotspots were widely publicized in the USA).

Most published cases involve only one or two individuals (57% of 3034 cases with data). More than 1000 individuals were only affected in 11 cases (9 cases from single populations). Likewise, the vast majority (81% of 3238 cases with data) involved only one or two types of anomalies. Only six cases (four of them being single populations) comprise more than 15 types of anomalies. The highest number (32) was found in a population of the green toad (*Bufotes viridis*) in Roßwag, Germany.

Abnormal individuals have been reported on all continents, with the vast majority of reports from Europe and North America. The same applies to hotspots of anomalies. Hotspots differ strongly in the pattern of anomalies. Besides limb anomalies, tumours, edema, colour anomalies (especially albinism) and malformation of mouthparts in larvae were detected with high prevalences at hotspots. Only one hotspot (a population of *B. viridis* at Roßwag, Germany) showed a high prevalence of various skeletal anomalies, as well as several types of colour anomalies and edema and tumours. The different patterns of anomalies at different hotspots can only be explained by different causative factors or combinations thereof. Opinions on them vary among scientists and their relative importance differs from continent to continent but include chemical pollution, radioactivity, infection by the trematode *Ribeiroia ondatrae* and other parasites, predator attacks, diseases and various combinations of these factors.

We next review the occurrence of different types of externally visible anomalies: skeletal duplications (polymely, polydactyly, supernumerary bones at the jaw, tail bifurcation and duplication, dicephaly and Siamese twins), absence and reduction of skeletal elements (ectromely, phocomely, ectrodactyly, tail partially or completely missing, brachycephaly, microcephaly, pointed snouts, mandibular hypoplasia, absence of nostrils), other skeletal anomalies (clinodactyly, syndactyly, taumely including bony bridges, rotated limbs, truncated stiff vertebral column, torsion of the tail or body), non-skeletal morphological anomalies (duplication of the eyes and the spiraculum, absence of the tympanum, microphthalmy and anophthalmy, tail retention in anurans after metamorphosis, arm not emerging from the branchial cavity, skin webbing, abnormal spiraculum and ventral tube, malformed mouthparts in tadpoles, giant size and nan-

ism, edema and bloatedness, tumours and tumour-like structures) and colour anomalies (albinism, black-eyed and blue individuals, erythrism, flavism, melanism, translucent skin). For each type of anomaly we tallied the number of natural populations and species for which the anomaly had been reported. We discuss suggested and potential causes, focussing on cases, where more than ten individuals and more than 10% of the population had been affected.

Species may show very different degrees of sensitivity to the different environmental factors that cause anomalies, such as pollutants, predators or parasites. However, there is no evidence that urodeles are less sensitive than anurans. The factors determining differential sensitivity are still insufficiently understood. While phylogeny had no effect in the single study that has tested it so far, some studies indicated that more aquatic species are more sensitive (to water-born factors) than less aquatic species, whereas others suggest the opposite, proposing that rapidly developing species are the more sensitive ones.

Only a few experimental studies addressed individual level effects of anomalies. With few exceptions they showed adverse effects, such as failure to metamorphose, reduced mobility, growth, body condition and survival. The occurrence of an effect may depend on the environment, such as food type or the presence of predators.

At the population level, the effects of anomalies are less evident. Even though populations declined and species became extinct at several hotspots, this may not necessarily have been an effect of anomalies. Direct death and anomalies may have been caused by the same factor, and at some hotspots, anomalies and populations persisted for decades. Population effects need further study to understand the relevance of anomalies for amphibian declines.

It is widely believed that the prevalence of anomalies has increased over recent decades. Evidence from comparisons of historical museum collections and recent surveys as well as resurveys of former hotspot sites tend to support this assumption, although declines in the frequency of anomalies have also been observed and the few long-term monitoring studies available show that the frequency of abnormal individuals can fluctuate widely without an apparent trend. As further evidence for an increasing trend, mechanistic links between eutrophication and high rates of anomalies have been demonstrated and various chemical pollutants are also known to cause elevated rates of anomalies at environmentally relevant concentrations. Because environmental pollution increased since the Second World War, there should indeed have been an increase in anomaly prevalences over recent years. In addition, there is evidence that background rates of anomalies are about an order of magnitude lower than currently assumed.

We finish our review with recommendations for future directions in the research of anomalies in natural populations of amphibians. We conclude that in spite of gaps in our knowledge, sufficient information is available for use to reduce human-induced plights of amphibians.

Keywords. Amphibia, anomalies, hotspots, colour anomalies, disease, edema, individual effects, morphological anomalies, parasites, pollution, population effects, predators, radioactivity, skeletal anomalies, trends, tumours.

Zusammenfassung. Anomalien bei Amphibien haben seit Jahrhunderten die Neugier des Menschen geweckt. Entsprechend existiert eine sehr umfangreiche Literatur über dieses Thema. Wir geben einen umfassenden Überblick über im Freiland an Amphibien beobachtete Anomalien und deren vermuteten und nachgewiesenen Ursachen. Wir stellen Kenntnisse zu einer unterschiedlichen Empfindlichkeit von Arten, Anomalien zu entwickeln, sowie zu den Konsequenzen von Anomalien auf der individuellen und Populationsebene zusammen. Abschließend diskutieren wir Hinweise für eine Zunahme an Anomalien in natürlichen Populationen von Amphibien während der letzten Jahrzehnte und enden mit Empfehlungen bezüglich Forschungsfragen, die helfen können, die Entstehung von Anomalien bei Amphibien und deren Bedeutung für den Amphibienschutz und die Umwelt besser zu verstehen. Trotz Wissenslücken reichen unsere Kenntnisse aus, um den vom Menschen verursachten Anteil an hohen Raten von Anomalien bei Amphibien reduzieren zu können, wenn der Wille hierzu vorhanden ist.

Für unsere Übersicht haben wir, ausgehend von unseren umfangreichen privaten Literatursammlungen, jede uns zugängige Publikation ausgewertet, in der Anomalien für natürliche Populationen von Amphibien erwähnt werden. In der erstellten Datenbank betrachten wir jede Art und jede Population als separaten Fall, sofern populationsspezifische Daten publiziert wurden. Wenn gleichzeitig summarische Daten publiziert wurden, die populationsspezifische Daten einschließen, haben wir nur letztere berücksichtigt.

Fossiles Material liefert die frühesten Dokumente von Anomalien bei Amphibien. Ohne deren Berücksichtigung umfasst unsere Datenbank 3517 Populationen aus 98 Ländern. Diese stammen von 6, 117, und 386 Arten der Ordnungen Gymnophiona, Urodela bzw. Anura. Der älteste definitive Bericht stammt aus dem Jahr 1554 und betrifft eine Kröte mit missgebildeten Beinen. Seit dem frühen 18. Jahrhundert erschienen Publikationen, die sich spezifisch mit Anomalien von Amphibien in natürlichen Populationen beschäftigen, seit den 1860iger Jahren jährlich und in rasch wachsender Zahl. Dabei sind drei Stufen in der Anzahl jährlich erschienener Publikationen ersichtlich: nach dem Zweiten Weltkrieg, Mitte der 1970iger Jahre sowie im Jahr 1996, dem Jahr, in dem Missbildungen an Amphibien in den USA eine weite Aufmerksamkeit in der Öffentlichkeit erhielten.

Die meisten Fälle betreffen 1–2 Individuen (57% der 3034 Fälle mit Daten). Mehr als 1000 anomale Individuen wurden in elf Fällen (9 davon sind Einzelpopulationen) beobachtet. Ebenso umfasst die überwiegende Zahl der Fälle (81%, n = 3238) nur 1–2 unterschiedliche Typen von Anomalien. Nur in sechs Fällen (vier davon sind Einzelpopu-

lationen) traten mehr als 15 verschiedene Typen auf. Der extremste Fall waren 32 unterschiedliche Typen in einer Population der Wechselkröte (*Bufotes viridis*) in Roßwag, Deutschland.

Anomalien wurden auf allen Kontinenten festgestellt, am häufigsten in Europa und Nordamerika. Dasselbe gilt für deren massenhaftes Auftreten, sogenannte Hotspots. Hotspots zeigen sehr unterschiedliche Muster an Anomalien. Je nach Hotspot traten entweder Missbildungen der Gliedmaßen, Tumore, Ödeme, Farbanomalien (besonders Albinismus) oder Missbildungen der Mundpartien von Kaulquappen in großer Häufigkeit auf. Nur an einem Hotspot (einer Population von *B. viridis* bei Roßwag, Deutschland) wurden sowohl verschiedene Skelettmissbildungen als auch verschiedene Farbanomalien, Ödeme und Tumore in großer Anzahl festgestellt. Die unterschiedlichen Muster an Anomalien zeigen, dass es unterschiedliche (Kombinationen von) Ursachen für die Hotspots geben muss. Die Meinungen über deren Ursachen werden häufig kontrovers diskutiert, sie umfassen jedoch chemische Stoffe, Radioaktivität, Infektion durch den Saugwurm *Ribeiroia ondatrae*, andere Parasiten, Verletzungen durch Prädatoren, Krankheiten und verschiedene Kombinationen dieser Faktoren.

Wir geben anschließend eine Übersicht über das Auftreten verschiedener Typen von äußerlich sichtbaren Anomalien: Skelettanomalien (Polymelie, Polydactylie, zusätzliche Knochen am Kiefer, Bifurkation und Verdoppelung von Schwänzen, Doppelköpfigkeit und Siamesische Zwillinge), fehlende oder reduzierte Skelettteile (Ectromelie, Phocomelie, Ectrodactylie, reduzierte oder fehlende Schwänze, Brachycephalie, Microcephalie, abnormal zugespitze Schnauzen, reduzierte Kiefer, Fehlen von Nasenöffnungen), weitere Skelettanomalien (Clinodactylie, Syndactylie, Taumelie, Verdrehung von Knochen der Arme oder Beine, gestauchte steife Wirbelsäulen, verkrümmte Körper und Schwänze), andere morphologische Anomalien (Verdoppelung der Augen und des Spiraculums, Fehlen des Tympanums, Microcephalie, Anophthalmie, Beibehalten des Schwanzes nach der Metamorphose bei Froschlurchen, Arme, die unter der Kiemenfalte verbleiben, Hautfalten an Arm- oder Beingelenken, die die Beweglichkeit einschränken, missgebildetes Spiraculum und Afterröhre, missgebildete Mundfelder von Kaulquappen, Riesen- und Zwergwuchs, Ödeme und Aufblähungen, Tumore und tumor-ähnliche Strukturen) sowie Farbanomalien (Albinismus, anomale schwarze Augen, blaue Frösche, Erythrismus, Flavismus, Melanismus, durchscheinende Haut). Wir diskutieren Erklärungen für die Ursachen dieser Anomalien, wobei wir den Schwerpunkt auf Fälle mit mehr als zehn betroffenen Individuen und 10% der Population legen.

Die Empfindlichkeit gegenüber Umweltfaktoren, wie Chemikalien, Parasiten oder Prädatoren, die Anomalien hervorrufen können, kann artspezifisch sehr unterschiedlich sein. Die dafür verantwortlichen Faktoren sind noch ungenügend geklärt. Während Phylogenie in der einzigen Untersuchung, die deren Einfluss geprüft hat, keine Wirkung hatte, zeigten einige Studien eine höhere Empfindlichkeit stärker aquatischer Arten (gegenüber wassergebundenen Faktoren) als weniger aquatischer Arten. In anderen Studien waren dagegen die sich am schnellsten entwickelnden Arten am empfindlichsten. Es gibt keine Hinweise, dass Froschlurche generell empfindlicher sind als Schwanzlurche.

Die Auswirkungen von Anomalien auf Individuen wurde nur selten experimentell untersucht. Mit wenigen Ausnahmen hat sich eine nachteilige Wirkungen gezeigt, z.B. Verhinderung von Metamorphose oder eine reduzierte Beweglichkeit, geringere Körperkondition, langsameres Wachstum oder niedrigere Überlebenswahrscheinlichkeit. Das Auftreten negativer Wirkungen kann von der Umwelt abhängen, zum Beispiel der Art der Nahrung oder dem Vorhandensein von Fressfeinden.

Die Auswirkungen von Anomalien auf Populationsebene sind bisher wenig verstanden. Obwohl an einigen Anomalie-Hotspots die Abundanz der betroffenen Art(en) abgenommen hat und Populationen erloschen sind, bedeutet dies nicht, dass dafür die Anomalien verantwortlich gewesen sein müssen. Vielmehr kann einer oder mehrere Faktoren gleichzeitig hohe Raten von Anomalien und Mortalität verursacht haben. Die Bedeutung von Anomalien für die Populationdynamik bedürfen gezielter Untersuchungen, bevor deren Relevanz für den globalen Rückgang von Amphibien eingeschätzt werden kann.

Oft wird angenommen, dass die Häufigkeit von Anomalien in den letzten Jahrzehnten zugenommen hat. Vergleiche mit historischen Sammlungen sowie erneute Erfassungen an bekannten Hotspots geben dafür Anhaltspunkte, allerdings gab es auch Abnahmen in der Häufigkeit. Außerdem zeigen die wenigen Langzeiterfassungen, dass die Häufigkeit von Jahr zu Jahr sehr stark schwanken kann. Andererseits gibt es mechanistische Zusammenhänge zwischen der Häufigkeit von Anomalien und Eutrophierung und verschiedene Chemikalien können hohe Raten an Anomalien bei Konzentrationen bewirken, die umweltrelevant sind. Da die Eutrophierung und die Verschmutzung von Gewässern seit dem Zweiten Weltkrieg zugenommen hat, müsste als Konsequenz dieser Zusammenhänge auch die Häufigkeit von Anomalien zugenommen haben. Weiterhin scheinen die natürlichen Hintergrundraten an Anomalien etwa eine Größenordnung niedriger zu sein als oft angenommen wird.

Abschließend geben wir Empfehlungen für künftige Forschungen zum Auftreten von Anomalien bei Amphibien im Freiland und deren Ursachen. Trotz Forschungsbedarf besteht jedoch ausreichend Wissen zum Handeln, um den von Menschen verursachten Anteil am Auftreten von Anomalien bei Amphibien im Freiland zu verringern.

Schlüsselwörter. Amphibia, Anomalien, Auswirkungen auf Individuen, Auswirkungen auf Populationen, Farbanomalien, Fressfeinde, Krankheiten, morphologische Anomalien, Ödeme, Parasiten, Radioaktivität, Skelettanomalien, Trend, Tumore, Verschmutzung

1 Introduction

Amphibian anomalies have been a source of human curiosity for centuries (e.g., Wurffbain 1683, Vallisneri 1706, De Superville 1740, Geoffroy Saint-Hilaire 1832, 1836) to the extent that they have even become part of Chinese folklore and art (Fig. 1). Amphibian anomalies also played a pivotal role in the emerging of the science of developmental biology (e.g., Hertwig 1892, Brandt 1924, Woerdeman 1936) and they still have this function today (e.g., Gardiner & Bryant 1996, Kovalenko & Kovalenko 1996, Nye et al. 2003).

Increased environmental awareness, the global decline of amphibians (e.g., Henle & Streit 1990, Stuart et al. 2004) and the recent rediscovery of populations exhibiting mass anomalies (Burkhart et al. 2000, Souder 2002, Lannoo 2008, 2009) have instigated renewed interest in amphibian anomalies as potential indicators for environmental perturbations (e.g., Tyler 1983, Burkhart et al. 2000, Souder 2002, Vershinin 2002). In response to findings of mass anomalies in North America, the North American Reporting Centre for Amphibian Anomalies (NARCAM) was established (Lannoo 2008). Mass anomalies were also reported in other organisms from polluted environments, for example, plants (Hesse-Honegger 2008, Yablokov et al. 2009, Taira et al. 2014), invertebrates (Vuori & Parkko 1996), reptiles (Ernst 1995, Bishop & Gendron 1998), birds and humans (Hoffmann et al. 1988, Savchenko 1995, van der Schalie et al. 1999, Møller et al. 2007, Yablokov et al. 2009), but, so far only humans (e.g., Winter et al. 1988, Savchenko 1995, Feshchenko et al. 2002, Scherb & Weigelt 2003, Lannoo 2008, Yablokov et al. 2009) and domestic animals (Taruffi 1881–1886, Noden & de Lahunta 1985, Yablokov et al. 2009) have received similar attention to amphibians.

Not surprisingly, the literature on amphibian anomalies is enormous (Henle 2014). There is a considerable number of published reviews; the first comprehensive one appeared more than a century ago (Taruffi 1881–1886). Most reviews focus on rather specific topics and, with few exceptions (noticeably Rostand 1971), do not discuss to any extent the potential and limitations to infer causes from observed patterns of anomalies. Many factors have the potential to cause anomalies in amphibians (reviewed e.g., by Rostand 1971, Dubois 1979b, Tyler 1989, Ferraro & Burgin 1993, Ouellet 2000, Henle et al. 2017a), but the relevance for natural populations of many of them remains debated (compare Skelly & Benard 2010 and Sessions & Ballengée 2010). Linking cause to pattern is a central issue in ecology (Wiegand et al. 2003) and crucial for the use of amphibian anomalies as an indicator of the nature of an environmental perturbation that has occurred in a habitat.

Fig. 1: Chinese artwork figuring a toad with a single hindleg (ectromely) based on a Chinese legend. Photo: K. Henle.

The most recent comprehensive review by Ouellet (2000) addressed primarily skeletal anomalies. While he discussed a range of potential causes, no systematic evaluation of the known or suspected causes of mass anomalies or particular types of anomalies was made. Here we provide a comprehensive overview of the anomalies observed in natural populations of amphibians and discuss their suspected and potential causes. We cover mass anomalies on all continents as well as observations on single individuals. We focus (but do not limit) our review on external anomalies that have been frequently reported or observed in incidences of mass anomalies. We further assess the evidence for an increase in the prevalence of anomalies in natural populations of amphibians over recent decades and review the individual and population level consequences of anomalies as well as their conservation implications. Finally, we provide perspectives for future research.

2 Methods

2.1 Data collation

We attempted to find any citations made on anomalies in natural populations of amphibians starting with our own extensive literature collection, the literature on amphibian anomalies compiled by The North American Reporting Centre for Amphibian Malformations, the literature on field herpetology compiled by Schriftenschau für Feldherpetologie and a manual search for any anomaly-related key words in the Zoological Record for 1945–1985 and in Biosis for 1945–1985 and 1996–1999. More recent volumes were searched through the Web of Science using the headings of the results section of this publication as key words (latest access January 2017). In the review that follows we included only those publications that we could check ourselves except for a limited number of papers published more than 100 years ago. Such publications were included, when we recognized verbal citations of relevant parts of these publications, or if at least two later publications cited them consistently with different details provided so that these authors were likely to have examined the original source independently.

Reviewing the occurrence of anomalies poses major challenges. Even gross morphological anomalies in a given species may be the norm in another one; thus, spe-

cies in the genus *Brachycephalus* have only two or three fingers and three or four toes. This condition would qualify as ectrodactyly in most other anuran genera. In some salamanders, toe morphology is highly variable and it is difficult to set the limit between the range of normal and abnormal variation (Henle et al. 2017b). In *Plethodon cinereus*, a red colour morph is frequent in some regions but very rare in others (Lotter & Scott 1977) and thus might only classify as an anomaly in parts of its range. Particularly complex is the situation for *Salamandra salamandra.* It is a highly polymorphic species with some colour morphs, such as highly or completely yellow or highly black, being very common in some subspecies but a rare anomaly in other subspecies. Then again, there may be small areas within a subspecies, at which a colour morph that is a rare anomaly in other parts of the distribution area of the subspecies, is common, such as the highly yellow colour morph in *S. s. terrestris* near Holzminden, Germany (Seidel & Gerhardt 2016). In these cases we only considered data if the individuals were referred to as abnormal by the respective author(s).

Moreover, some authors included injuries (e.g. Reeves et al. 2013) in the figures for anomalies whereas others did not and even excluded ectrodactyly and amely because they regarded them as the result of injuries (e.g. Flindt 1985a). As injuries may develop into deformed bones (Magnusson et al. 1999) and may be difficult to differentiate from developmental anomalies, we also included injuries in our tallies of anomalies. However, we excluded superficial scars that are regularly found in some species that frequently fight in territorial disputes. Contrary to Reeves et al. (2013), we did not include injuries that resulted from capture. Whereas Reeves et al. (2013) scored the presence of parasites as abnormal, we only did this if the presence was accompanied by, explicitly mentioned, morphological (including lesions) or colour anomalies.

There is no generally accepted terminology for amphibian anomalies. Some of the early terms have changed in meaning over time, some are no longer used and others have been replaced by other terms by some but not all authors. Unfortunately, it is often the case that neither a definition of the terms used nor an explicit reference to a glossary is provided. Our definitions follow those suggested by Henle et al. (2017b). As far as possible we translated the terms used by others into these terms.

Data on anomalies are also provided in a highly inconsistent way. Sometimes data apply to the number of limbs that are affected or the number of anomalies observed and not to the number of abnormal individuals. In our database we only included numbers that refer to individuals. Some authors scored the same complex of anomalies, e.g., an incomplete duplication of a limb, as two anomalies. We follow Dubois & Henle (2017) and regard such cases as a single anomaly. Some publications provided sample sizes for each population but anomalies were merged across populations and vice versa. In such cases we merged all data for all populations. If authors provided overlapping data for specific populations and across sites, we only used data for specific populations. To enable comparisons with Ouellet (2000), we pooled data across years and authors for the same population(s), if the data did not overlap. If the degree of overlap remained uncertain, we only used the data from the most recent publication(s). However, for trend analyses, we split, when possible, data that covered more than 5 years of surveys into 5-years-intervals starting with the first survey year. Unless otherwise stated, frequencies are averages across samples weighed by sample size. If a publication contained information on several species, we regarded each species as a separate case.

Names and status of taxa have changed over time. With few exceptions, we follow Frost (2016). When easily possible, we updated names but we did not attempt to trace specimens and locations or morphological descriptions to revise species identities.

Currently, our database on amphibian anomalies covers 3341 publications, 1775 of them reporting anomalies from natural populations, 1046 publications presenting results from experiments and 247 reviewing specific topics within the field of amphibian anomalies.

2.2 Causal inference

Causes for observed anomalies in natural populations are frequently suggested with limited knowledge of the relevant experimental literature. We therefore evaluated suggested causes of anomalies against a comprehensive assessment of experimental studies. Not all experimental studies provided test results that included controls and others lumped together different types of anomalies. Therefore, we accepted a suggested factor as a potential cause for a particular type of anomaly, if any of the following applied: (a) a mechanistic causative pathway between the factor and the anomaly in question was known, (b) at least one study showed a statistically significant difference between treatment and control, (c) there was a significant correlation between the rate of anomalies and the intensity of the causative factor even if no control data were provided, (d) more individuals were affected in the experimental treatment than in the control in more studies than expected at random, or (e) 100 % of individuals were affected and more than 50 individuals were examined, if no data on controls were provided.

Whereas some authors (e.g., Reaser & Johnson 1997) suggested that, albeit limited, inferences can be made from the percentage of individuals affected, others (e.g., Kovalenko 2000) believed that there was no relationship between the frequency of anomalies and environmental quality. Numerous experimental studies have shown that the strength of a teratogenic factor can have a strong influence on the incidence of anomalies. This applies in many but not all cases involving chemicals, irradiation, parasite abundance (e.g., Nishioka et

al. 1981, Power et al. 1989, Zavanella et al. 1989, Johnson et al. 1999), egg retention time and temperature (Briggs 1941, Witschi 1952, Mikamo & Hamaguchi 1973). High incidences of anomalies can also be caused by several other factors, such as disease (e.g., Bradford 1991), predators (e.g., Caldwell 1982, Veith & Viertel 1993), hybridization (e.g., Delarue 1982, Mable & Rye 1992) and inbreeding (e.g., Humphrey 1948, Droin & Fischberg 1984).

Sensitivity also differs among stages. Gene expression – gastrulation is the first stage at which parental genes become active (Davidson 1976) – as well as structural, physiological and biochemical transformations are particularly intensive during the rapid transition of stages (i.e., embryogenesis and metamorphosis) (Duellman & Trueb 1986), explaining why in general, but not invariably, sensitivity is higher at these stages compared to stasis (Tschumi 1954). For example, factors that can cause skeletal deviations are most effective when they act before the aggregation of skeletal mesenchyme at stage 40–42 (Kovalenko 2000) [i.e., approx. stage 30 of Gosner (1960)]. Likewise, osteolathyrogenic compounds are most effective during the stages of limb development and have no effect on earlier or later stages (Riley & Weil 1987). Furthermore, the same chemical may cause polydactyly only early on in limb regeneration but oligodactyly across a longer period of development or regeneration (Gebhardt & Faber 1966b). Effects of exposure to the trematode parasite *Ribeiroia ondatrae* may also be stage dependent, both in the percentage of individuals affected and the dominant types of anomalies (Johnson et al. 2011).

As a consequence, the percentage of affected individuals does not provide information about the cause of the observed anomalies. The only certain inference that can be made from a high incidence of anomalies is that the factor must have been strong and most individuals of the population must have been exposed to it.

3 Occurrence of anomalies in natural populations

The earliest documents of amphibian anomalies are provided by the fossil record. Wang et al. (2016) described 14 salamanders of the species *Chunerpeton tianyiensis* that showed abnormal polydactyly. Polymely, polyphalangy and brachydactyly were present in two, two, and four, respectively, of these individuals. Mlynarski (1961) observed an asymmetric urostyle in fossil *Rana* sp. and Ippen & Heinrich (1977) described abnormally healed broken bones with a callus in three fossil *Rana* sp. Fejérváry (1916) discovered a fossil *Rana mehelyi* with a fractured ilium. The oldest fossil record of skeletal anomalies in amphibians known to us is a capitosauroid, presumably *Eocyclotosaurus wellesi,* from the Middle Triassic of Arizona (Witzmann 2007).

Excluding fossils, our database covers 3517 natural populations from 98 different countries for which anomalies have been reported. These belong to 6, 117 and 386 living species of Gymnophiona, Urodela and Anura, respectively. The oldest definite report of non-fossil anomalies dates back to 1554 and was a toad, possibly *Bufo bufo*, with malformed limbs that Gesner (1554) observed in Zurich, Switzerland. He also discussed bicephalic salamanders supposedly depicted in hieroglyphs that have been referred to by ancient Greek writers. He regarded them, presumably correctly, as misinterpretations. A century later, Aldrovandi (1645) reported on an earlier mentioning of melanistic toads from Italy but he was also sceptical about its reliability. A few decades later, Wurffbain (1683) again mentioned occasional stories involving bicephalic salamanders and provided a first concrete example: a two-headed salamander that was exhibited by Mercerus. He further illustrated an albino *Salamandra s. salamandra* from Italy (Plate II, Fig. 1) that later was described as *Salamandra candida* by Laurenti (1768). Another figure (Plate I, Fig. 2) may be a flavistic individual faintly showing a pattern.

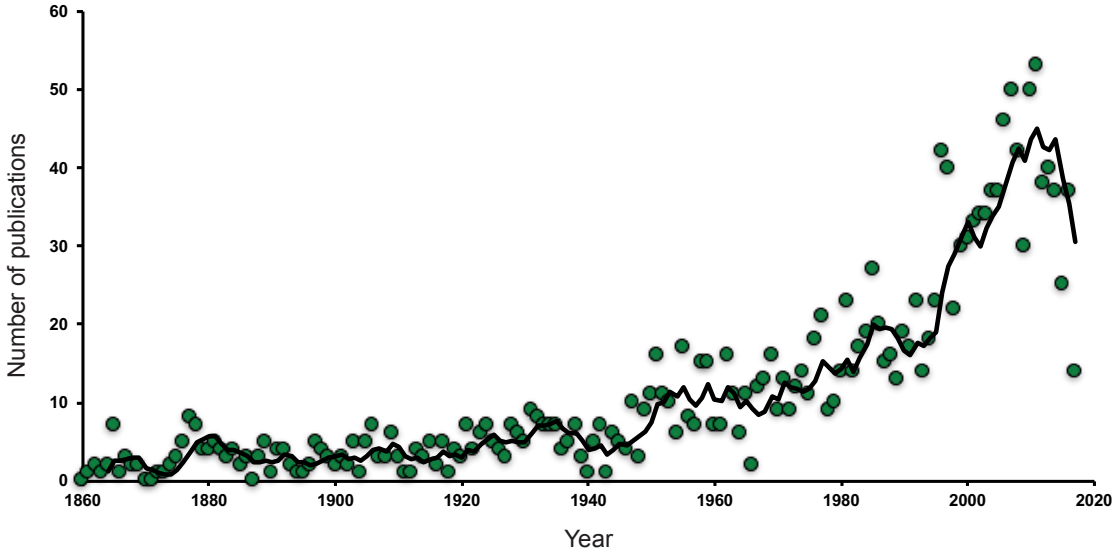


Fig. 2: Temporal pattern of publications reporting or reviewing anomalies in natural populations of amphibians since 1860, with 5-years running average. Source: our database as of 7.6.2017.

Publications that explicitly targeted abnormal amphibians started to appear from the early 18th Century (Vallisneri 1706). In the 18th Century and the first half of the 19th Century ten and 16 publications, respectively, addressed abnormal amphibians in natural populations. Since the 1860s, publications on abnormal amphibians appeared almost annually and rapidly increased in numbers (Fig. 2). Three major increments in the number of annual publications are noticeable: the first occurring after the Second World War, the second in the mid-1970s and the most recent in 1996 (the year after which malformation hotspots had been widely publicized in the USA). Whereas the first increment is also apparent in the graph presented by Johnson & Chase (2004), the second one occurred earlier than indicated in their graph and the last one occurred too late to be assessed by them.

Most published cases involve only one or two individuals (57% of 3034 cases with data; Fig. 3) and background rates are usually around or well below 1% (Tab. 1; see also Henle et al. 2017a). More than 1000 individuals were only reported for 11 populations (9 of them being from single populations), with the largest number for a single population being 2458 adult *Ambystoma tigrinum* with tumours (approx. 0.2% also had edema) living in a sewage pond contaminated by polycyclic hydrocarbons (Rose 1976, 1977, 1981, Rose & Harshbarger 1977). Likewise, the vast majority (81% of 3238 cases with data) involved only one or two types of anomalies (Fig. 4). Only six cases (four of them being from single populations) comprised more than 15 types of anomalies. The highest number of different types of anomalies (32) was exhibited by a population of the green toad (*Bufotes viridis*) in Roßwag, Germany (Henle et al. 2017a).

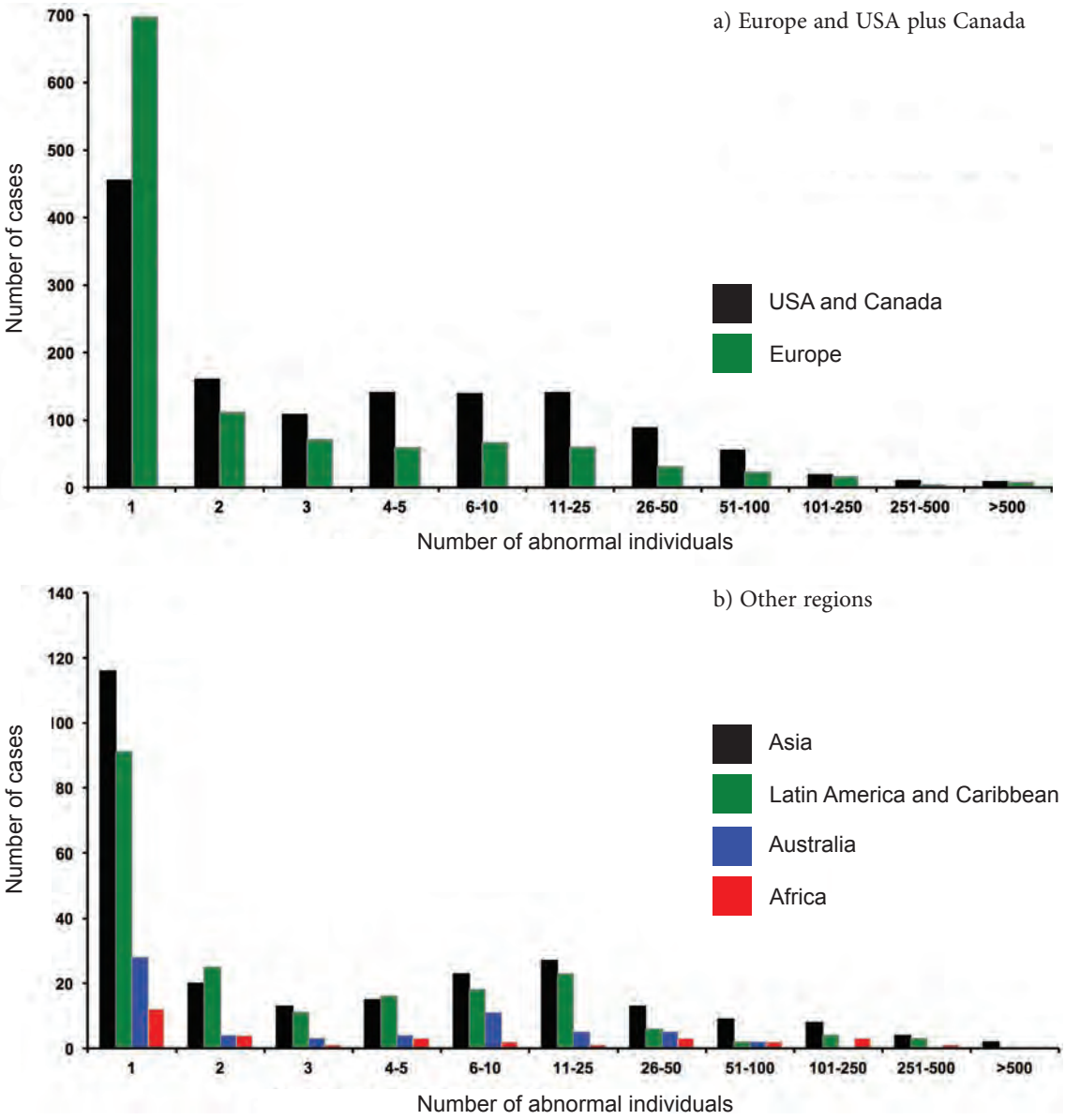


Fig. 3: Frequency distribution of the number of abnormal individuals observed in natural populations; a) Europe and USA plus Canada and b) other regions. Source: our database as of 7.6.2017.

Abnormal individuals have been reported on all continents with the vast majority of cases from Europe and North America (Fig. 3), which is likely to be due to the higher number of naturalists in these regions combined with easier access to locally published observations and the large survey program of amphibian anomalies across U.S. wildlife refuges (Reeves et al. 2013). The frequency distribution of the number of abnormal individuals observed per case (Fig. 3) differs highly significantly among continents (Europe, USA-Canada, remaining parts of America, Asia, Australia+Africa; categories > 500 affected individuals combined) (χ^2_{40} = 216; α = 0). The significance is primarily due to an overrepresentation of cases involving a single individual in Europe and USA-Canada relative to the other groups. Contrary to the opinion of Lannoo (2008) the number of affected individuals does not show a bimodal distribution with most cases either being a single individual or ten or more individuals; rather the distribution follows a steady decline. An exponential decline was also observed in the largest spatial survey that covered 135 wildlife refuges in the USA, in which 48,081 individuals were assessed (Reeves et al. 2013).

On all continents, observations of a single type of anomaly clearly dominate (Fig. 4). The frequency distribution differs highly significantly among continents (Europe, USA-Canada, remaining parts of America, Asia, Australia, Africa; categories > 5 types of anomalies combined) (χ^2_{20} = 227 α = 0). Cases with a single type of anomalies are overrepresented in Europe and cases with more types of anomalies are underrepresented, whereas it is the opposite in North America. In Asia cases with two types of anomalies are underrepresented. For all other categories and continents the frequencies are similar to the global average.

In the following, we first review hotspots of anomalies that involve several types of anomalies or several species, followed by a review of specific types of anomalies.

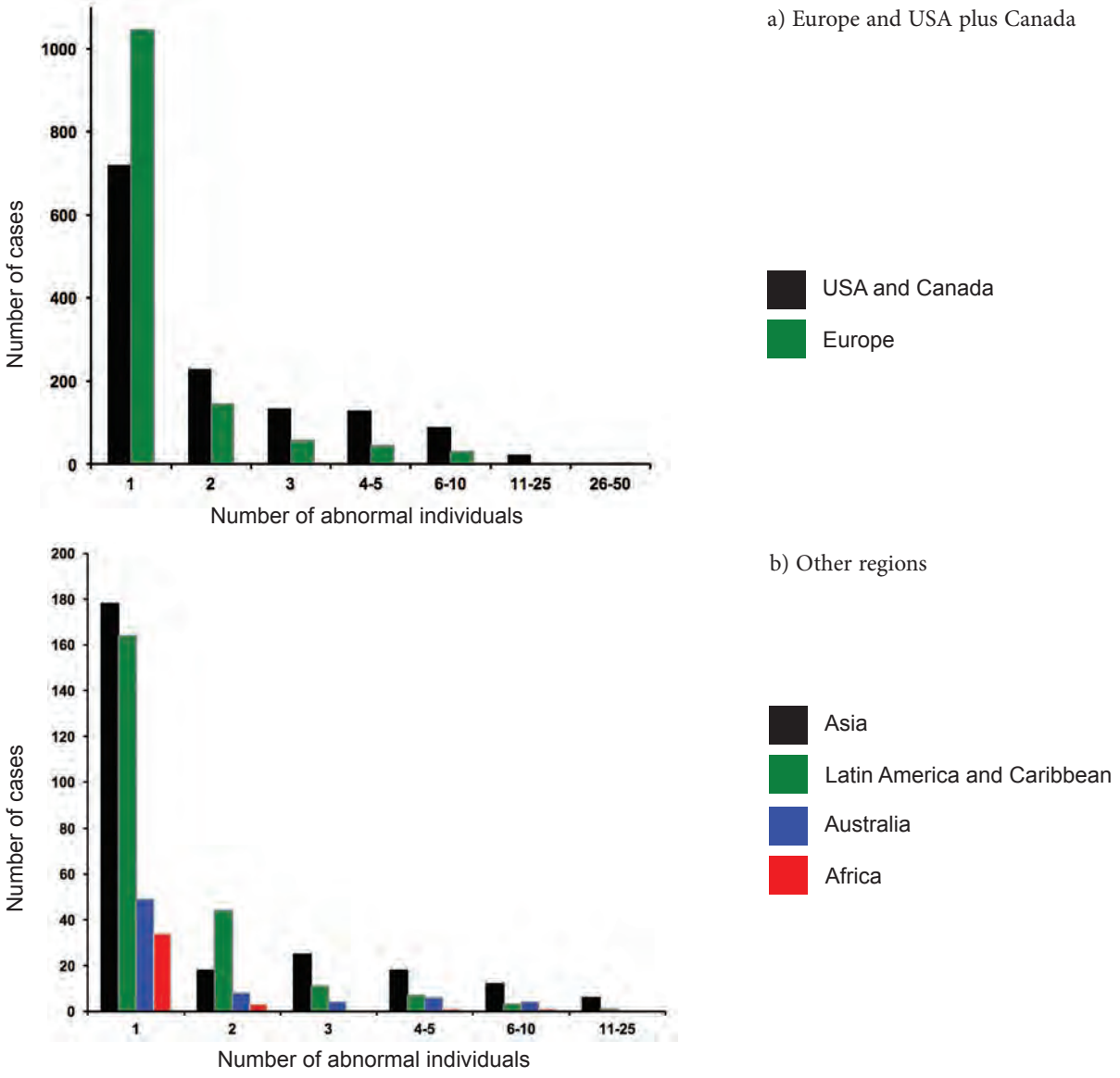


Fig. 4: Frequency distribution of the number of different types of anomalies observed in natural populations of amphibians; a) Europe and USA plus Canada and b) other regions. Source: our database as of 7.6.2017.

Table 1: Background rates of specific types of anomalies in amphibians from presumably "healthy" environments; only studies with samples sizes $N > 5{,}000$ included.

Anomaly	Frequency	*N*	Species	References
Abnormal limbs	2.5%	14,962	*Bufo bufo*	Gittins 1983
Albinism	0.000005%	219,269	*Bufo bufo*	Oerter & Kneitz 1994
Albinism	0.006%	15,657	*Ambystoma opacum*	Mitchell & Church 2002
Albinism	0.006%	16,147	*Necturus maculosus*	Hutt 1945
Albinism	0.008%	25,000	*Necturus maculosus*	Hutt 1945
Albinism	≈ 0.01%	Several 10,000	*Rana temporaria*	Kordges 2002
Albinism	0.01%	9,387	*Ambystoma opacum*	Campbell 2011
Albinism	0.01%	9,000	*Eurycea cirrigera*	Miller & Braswell 2006
Albinism	0.35%	13,852	*Clinotarsus curtipes*	Desai & Pancharatna 2003
Albinism	1%	6,500	*Scaphiopus holbrooki holbrooki*	Johnston & Johnston 2006
Anophthalmy	0.036%	99,992	*Bufo bufo*	Wolf 1994
Anophthalmy	0.07%	7,260	*Lithobates sylvaticus*	Eaton et al. 2004
Anophthalmy	0.15%	5,243	*Lithobates sylvaticus*	Eaton et al. 2004
Blue colouration	0.2–0.3%	1,000–7,000	*Lithobates clamitans*	Berns & Uhler 1966
Clinodactyly	0.23%	44,000	*Bufo bufo*	Rostand 1949a, 1951a,b
Clutch anomaly: eggs without membranes; membranes without eggs	0.08%	11,200	*Rana temporaria*	Vershinin 2002
Ectromely	0.24%	7,260	*Lithobates sylvaticus*	Eaton et al. 2004
Ectromely	0.29%	10,600	*Bufo bufo*	Van Gelder & Strijbosch 1995
Ectromely	0.38%	5,243	*Lithobates sylvaticus*	Eaton et al. 2004
Ectromely	2.4%	9,407	*Bufo bufo*	Wisniewski 1979
Edema	0.01%	7,175	*Pseudacris regilla*	Johnson et al. 2001a
Edema	0.04% (range 0–0.4%)	14,691	*Rana arvalis*	Vershinin 2005
Edema	0.09%	99,992	*Bufo bufo*	Wolf 1994
Flavism	0.007%	14,483	*Bufo bufo*	Thomas et al. 2002
Flavism	<0.02%	50.000	*Pelobates fuscus*	Bisping et al. 2016
Mandibular hypoplasia	0.03%	6,689	*Lithobates pipiens*	Hoppe 2000
Melanoid	<0.02%	50.000	*Pelobates fuscus*	Bisping et al. 2016
Oligodactyly	0.004%	25,000	*Necturus maculosus*	Hutt 1945
Oligodactyly	0.035%	99,992	*Bufo bufo*	Wolf 1994
Oligodactyly	0.34%	7,407	*Bufo bufo*	Rostand 1948
Oligodactyly	0.68%	44,000	*Bufo bufo*	Rostand 1949a, 1951a,b
Oligodactyly	0.82%	5,243	*Lithobates sylvaticus*	Eaton et al. 2004
Oligodactyly	1.32%	13,815	*Cynops pyrrhogaster*	Meyer-Rochow & Asashima 1988
Nasal bones destroyed by maggots of *Lucilia bufonivora*	0%	16211	*Bufo bufo*	Meisterhans & Heusser 1970

Table 1: Continuation.

Anomaly	Frequency	*N*	Species	References
Polydactyly	0%	7,260	*Lithobates sylvaticus*	Eaton et al. 2004
Polydactyly	0.007%	≈30,000	*Ichthyosaura alpestris*	Hachtel 2011
Polydactyly	0.02%	10,000	*Pelophylax lessonae*	Borkin & Pikulik 1986
Polydactyly	0.02%	5,243	*Lithobates sylvaticus*	Eaton et al. 2004
Polydactyly	0.11%	7,407	*Bufo bufo*	Rostand 1948, 1951a
Polydactyly	0.12%	44,000	*Bufo bufo*	Rostand 1949a, 1951a,b
Polydactyly	0.13%	5,350	*Rana temporaria*	Borkin & Pikulik 1986
Polydactyly	0.54%	5,215	*Rana arvalis*	Borkin & Pikulik 1986
Polydactyly	1.04%	13,815	*Cynops pyrrhogaster*	Meyer-Rochow & Asashima 1988
Polymely	0%	5,243	*Lithobates sylvaticus*	Eaton et al. 2004
Polymely	0.000005%	219,269	*Bufo bufo*	Oerter & Kneitz 1994
Polymely	0.002%	45,000	*Pelophylax* synkl. *esculentus*	Duméril 1865
Polymely	0.003%	29,730	*Xenopus laevis*	Hobson 1958
Polymely	0.006%	15,000	*Bufo bufo*	Johnson et al. 2001b
Polymely	0.006%	17,935	*Ambystoma talpoideum*	Semlitsch et al. 1981
Polymely	0%	5,567	*Cynops pyrrhogaster*	Fukui et al. 1996
Polymely	<0.01%	>9,600	*Hoplobatrachus tigerinus*	Mahendra 1936
Polymely	0.01%	10,000	*Lithobates pipiens*	Levey et al. 2003
Polymely	0.03%	7,260	*Lithobates sylvaticus*	Eaton et al. 2004
Polymely	0.025%	48,081	Average across 32 anuran species collected in 135 US national wildlife refuges	Reeves et al. 2013
Polymely	0.05%	13,815	*Cynops pyrrhogaster*	Meyer-Rochow & Asashima 1988
Syndactyly	0%	>50.000	*Bufo bufo*	Rostand 1951d
Syndactyly	0.04%	5,243	*Lithobates sylvaticus*	Eaton et al. 2004
Tail bifurcation	0.003%	≈30,000	*Ichthyosaura alpestris*	Hachtel 2011
Tail bifurcation	0.011%	17,935	*Ambystoma talpoideum*	Semlitsch et al. 1981
Transient albinism	0.5%	9,473	*Pelodytes punctatus*	Rivera et al. 2001
Tumour (skin)	1.8%	13,613	*Cynops pyrrhogaster*	Asashima & Komazaki 1980, Asashima et al. 1982

3.1 Hotspots of anomalies

The rediscovery of malformation hotspots by school children in Minnesota, USA, in 1995 (Helgen 2012) boosted renewed interest in malformation hotspots for amphibians, with most of the literature focussing on North America. However, hotspots are neither limited to North America nor are they only recent. Hotspots have been discovered on all continents.

Reviewing hotspots of anomalies presents several major challenges. First, the frequency of anomalies varies continuously from the single rare individual to

hundreds and even thousands of affected animals. Second, so far there is no common definition of a hotspot. Because of the large number of populations (72; cases that merged data from different populations not considered), in which at least 100 affected individuals were found, it is not possible to discuss each hotspot in detail. Therefore, we focus our review on cases, in which more than 10% of the sampled individuals were affected and additionally either several types of anomalies were observed or where several species exhibited anomalies at the same location. Additional cases in which the anomalies showed a strong correlation with a potential cause or in which the causes were experimentally tested are mentioned as well. This overview is structured on a geographical basis rather than on assumed causes or types of anomalies because causes were not always explored and are contentious in other cases and because too many different patterns of anomalies occur in hotpots.

3.1.1 Europe

One of the earliest report of a hotspot of anomalies comes from Europe. In 1929 Bonnet & Rey (1937) discovered 21 tadpoles of *Pelophylax* synkl. *esculentus* at Villars-les-Dombes, France, that exhibited polydactyly and schizodactyly. Because of the number affected they assumed a genetic cause but did not test this hypothesis. The same type of anomaly was rediscovered by Rostand and became one of the longest studied mass anomaly in amphibians (Rostand 1971). The anomaly now known as anomaly P appears in its benign form as simple polydactyly; its severe forms include schizodactyly, brachymely, inguinal tumours, bony excrescences and even polymely (Dubois 2014, 2017). In the most intensively studied population in Trévignon in France, prevalence varied from 0 to 80% in tadpoles and from 2.6 to 14.5% in adults (Rostand 1971, Dubois 2014, 2017). Anomaly P is characterized by several constant features, including a postero-anterior gradient and a good, although not always perfect, bilateral symmetry (Fig. 5). Mass occurrence of this anomaly is known for at least 13 locations in France (Rostand 1971, Dubois 1984). It affects only species within the *Pelophylax esculentus* complex. Rostand conducted comprehensive investigations on the cause testing experimentally, among others, various chemicals, UV, temperature, overripe eggs and a genetic basis (see Rostand 1971 and Dubois 1984, 2014, 2017 for a summary). It is caused by an unknown exogenous teratogenic factor, possibly a virus, transmitted by fish that acts very early in the development.

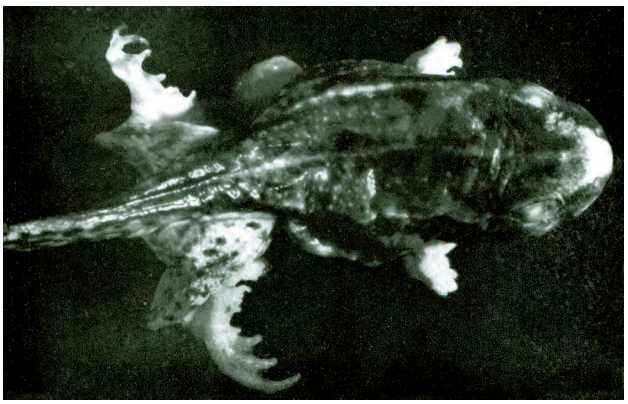


Fig. 5: Anomaly P in a metamorphosing *Pelophylax synkl. esculentus*. Source: Rostand (1971: Fig. 30).

Hillenius (1959) discovered a further hotspot for polydactylous *P. esculentus* in Amsterdam, The Netherlands. He assumed low-level radioactive contamination as the cause but because of a similar pattern of the anomalies Rostand (1971) assigned the case to anomaly P. In Belorussia, Borkin & Pikulik (1986) reported another case of mass polydactyly in the hindlimbs but not the forelimbs, which (based on morphological similarity) is another case of anomaly P. Of 114 juvenile *P. lessonae* (a few were possibly *P. esculentus*) collected at the Alba fishery ponds in the Minsk region, 42 individuals were affected. Furthermore, four out of 47 adults were affected.

Globally the most serious hotspot in terms of different types of anomalies observed was discovered in Europe: a population of *Bufotes viridis* in a quarry near Roßwag, Germany (Henle et al. 2017a). Over a five-year study period (1980–1984), 32 different types were registered, involving among others polymely (Figs. 6a, 10c), ectromely (Fig. 6b), phocomely, a range of further skeletal anomalies, edema, tumours, giant size and

Fig. 6: Examples of anomalies observed in the *Bufotes viridis* population at Roßwag, Germany, in October 1980; a) polymely (voucher: Museum National d'Histoire Naturelle 1984.2325). Photo: H. Steinicke; b) amely, Photo: K. Rimpp.

various colour anomalies including, abnormal yellow patches (Fig. 32), albinism and melanism. At least 245 toadlets and 1,000–2,500 tadpoles were abnormal. An official enquiry did not class ectromely as an anomaly because it was supposedly caused by predators (in spite of its occurrence in the absence of predators) and concluded that hybridization combined with atavism and inbreeding were the culprits for the remaining anomalies (Flindt 1985a). These explanations are incompatible with the range of anomalies observed that can only be explained by a strong mutagenic agent. As high levels of radioactivity were recorded at several places of an earthern deposit and no indication for chemical pollutants were found, radioactive pollution was the most likely cause. See the accompanying papers by Henle et al. (2017a,c) for a detailed documentation and discussion of this case.

In an Italian population of *Triturus carnifex* exposed to low doses of radiation from radon D'amen et al. (2006, 2008) observed eight different types of anomalies (primarily ectrodactyly, polydactyly and webbing between the toes) and a slightly elevated rate of anomalies (5.2%; $n = 1522$). At another site that was surrounded by fields with intensive agriculture and a wide range of pesticide applications, 8.5% ($n = 340$) and 5.6% ($n = 267$) of *T. carnifex* and *Lissotriton vulgaris*, respectively, were abnormal. In contrast, at a third, fully protected, non-polluted site 1.8% ($n = 114$) and 1% ($n = 96$) of *T. carnifex* and *L. vulgaris*, respectively, were found to be abnormal.

Further hotspots were discovered at several contaminated sites. In the Dnepropetrovsk Province in eastern Ukraine, Flax & Borkin (1997, 2004) sampled 1749 *P. ridibundus*, 766 *Bombina bombina* and 484 *Bufotes viridis* between 1991 and 1995. Of these 37.4%, 30.8% and 25.7%, respectively, were abnormal. Tumours, polydactyly, ectromely and ectrodactyly were the most common types of anomalies observed. In addition, 39.2%, 33.5% and 40.8% ($n = 400–500$), respectively, of the tadpoles exhibited anomalies, primarily asymmetric hindlimbs, body curvature and microcephaly. Flax & Borkin (2004) discussed the edge of the range, hybridization, infection and environmental pollution as possible causes. The latter was the most likely cause, with significant correlations found between the frequencies of anomalies and levels of environmental contamination (heavy metals and organic compounds). Likewise, in heavily polluted reservoirs in the vicinity of Kiev up to 42% ($n = 65$) of the sampled *P. ridibundus* exhibited polymely; the average frequency of anomalies (polymely and four other types of anomalies) for the years 2000–2003 and 2006 was 18% ($n = 190$) (Nekrasova et al. 2007). At sites of the Sviyaga and Usa rivers in Russia that were heavily polluted with metals, 61.5% ($n = 343$) and 30.2% ($n = 63$), respectively, of *P. ridibundus* were abnormal (Spirina 2009, Borkin et al. 2012). Of 2400 and 3600 tadpoles of *Rana arvalis* and *R. temporaria*, respectively, that were collected from six polluted sites (heavy metals, petroleum products and/or household waste in Moscow and its surroundings, 32% and 18% showed edema, torsion of the body, gill anomalies, light patches of skin, skin damage and retarded development (Aguillón Gutiérrez & Severtsova 2012).

Other European hotspots were likely to be due to predators and parasites. Malkmus (2008) collected 430 tadpoles of *R. temporaria* in a pond in Germany of which 18% lacked feet (some also lacked the tibia) and 99% lacked parts of the tail. He assumed that sticklebacks (*Gasterosteus aculeatus*) had inflicted these injuries but did not test this assumption experimentally. Veith & Viertel (1993) and Bohl (1997) reported a population of *Bufo bufo* in Rhineland-Palatinate and Bavaria, respectively, in which 15.5% ($n = 1689$) and 42.7% ($n \approx 500$) of the tadpoles and toadlets suffered anomalies (mainly ectromely, but also ectrodactyly). Veith & Viertel (1993) additionally found rare incidences of other skeletal anomalies, such as syndactyly and fusion of legs (skin webbing?). Transplant experiments in the field and laboratory tests demonstrated that these cases were due to predatory attacks from the leech *Erpobdella octoculata* and that the leeches did not inflict these anomalies on other syntopic frogs and newts. Another publication reporting more than 100 affected individuals and a slightly elevated frequency that was attributed to predators is known to us for Europe. Wisniewski (1979) observed 221 (2.4%) adult *B. bufo* with legs partially or completely missing. As metamorphs leaving the pond also exhibited ectromely, he assumed small predators (shrews or carabid beetles) to be the culprits. One frog (*R. temporaria*?) was also affected.

One case that could be attributed to the parasitic fly *Lucilia bufonivora* was recorded by Kordges (2000) from Germany. The fly deposits its eggs on anurans, especially *B. bufo*, and the larvae destroy the nasal bones leading to a wide opening of the narines, invariably leading to the death of the attacked individual (Neumann & Meyer 1994). Although only small numbers of infected individuals are usually observed (Tab. 1), 46% ($n = 260$) of the toads examined by Kordges (2000) at a drift fence in a former mining area in 1999 had destroyed narines.

On European mesic and moist grasslands a large number of frogs may fall victim to mowing machinery (Classen et al. 1996, Oppermann et al. 1997). The authors did not examine whether such injuries resulted in elevated rates of anomalies at breeding sites or whether most injured frogs died. In a population study of *P.* synkl. *esculentus* in an agricultural landscape in Northrhine-Westfalia, Germany, about 100 individuals showed toe injuries and 46 ectromely (*n* approx. 2500) and in total approx. 6.5% exhibited anomalies (Schmidt & Hachtel 2011) – note that the percentages published by the authors are too low for the sample size given. The authors stated that leg injuries had only healed superficially and attributed them to harvesting machinery. Van Gelder & Strijbosch (1995) counted 35 toads (*B. bufo*) ($n = 10,600$) with ectromely in a long-term study and also attributed them to mowing. In a large-scale study in Finland covering 26 farmland breedings sites (includ-

ing grasslands), anomalies in froglets of *R. temporaria* occurred at a low frequency (max. 4%; mainly ectromely and ectrodactyly) (Piha et al. 2006). Adults were not assessed. The effects of mowing and crop harvesting machinery on anomalies (especially ectromely and ectrodactyly) of amphibians at breeding sites still requires more experimental studies to understand whether (and if so, to which extent) agricultural machinery may contribute to the occurrence of mass ectromely.

Bezman-Moseyko et al. (2014) reported another case of mass anomalies affecting 62% (n = 235) and 75% (n = 212) of syntopic *P. ridibundus* and *P. esculentus,* respectively, from Bugornia in the Pridnestrovie District of Russia. At another location the rate of anomalies was also above 60% but sample sizes were much lower. Ectrodactyly and limb deformities were the most common anomalies but in *P. ridibundus* polydactyly was also frequent. In total 5–7 different types of anomalies occurred. The locations did not show any sign of human impact and the cause remains unclear. Lada (1999) reported a further case of mass polydactyly for two Russian populations of *B. bufo*, for which the cause also remains unclear.

3.1.2 Asia

Anomaly hotspots in Asia are known in Russia, India, Malaysia, Kazakhstan and Japan. The second highest incidence on a global scale in terms of different types of anomalies was discovered in Siberia. In 1993, an accident at the Siberian Chemical Combinate in Seversk (Tomsk) contaminated approx. 120 km² with Ruthenium-106, Niobium-95, Cerium-95, Plutonium and Uranium, with the total activity being 500–900 Curie (Saveliev et al. 1996, Kuranova 1998, 2003, Moskvitina et al. 2011). In 1993–1994, these authors observed a range of clutch and embryonal anomalies, including the complete disintegration of embryos of *Rana arvalis*. In surviving tadpoles and froglets, they found a range of internal anomalies, tumours, ectromely and brachymely. While they did not provide detailed information on tadpoles and froglets, they referred to a total of 21 different types of anomalies in embryos and in total to at least 25 different types. Whereas the rate of embryonal anomalies was only 0.9% in control areas, it reached 36.5% in the contaminated area and at least 49% of the embryos died (Moskvitina et al. 2011). Kuranova & Saveliev (1997, 1999) further examined *Salamandrella keyserlingi* from the same contaminated sites. This species also suffered from a range of severe embryonal and clutch anomalies, including the autolysis of bodies. Surviving larvae showed various colour anomalies, absence of a tail, anomalies of axial organs, among many other types of anomalies, most of them not specified. In total, 21 different types of anomalies were observed. Unfortunately, further details about the types and numbers of anomalies in larvae or metamorphs of the two species were only included in disclosed reports. Moskvitina et al. (2011) further reported that the rate of embryonal anomalies in small mammals was also 3.3 times higher in the contaminated area compared to control areas.

Hotspots due to radioactive pollution are known from two further locations in the former Soviet Union. In the East Uralian Radioactive Trace caused by the accident in Mayak, the rate of morphological anomalies in metamorphs of *R. arvalis* was also significantly higher (17%, n = 60) than in control populations (Pyastolova et al. 1996). At locations polluted by nuclear tests at Semipalatinsk, Kazakhstan, 16.3% of *Pelophylax ridibundus* showed ectrodactyly, syndactyly, asymmetrical limbs, melanism and dorsal pattern anomalies (Rakhimzanova & Khromov 1998). Unfortunately, no further details were provided.

In Japan, the region around Hiroshima is a hotspot for colour anomalies; most cases occurred within the immediate vicinity of 6000 km². Between 1963 and 1975 at least 152 (10.7%, n = 1422) albinistic tadpoles of *Dryophytes japonicus* were collected from 12 sites (Daito 1968, Nishioka & Ueda 1977b, 1983). Three different recessive albino genes and two kinds of dominant melanising genes, which affect parts of the albino body, were detected in the sample. At one location, a dominant gene for black-eyedness was linked to the albino gene. At another location a dominant colouring gene linked to the albino gene gives a light greenish colouration to frogs carrying the gene. Nishioka & Ueda (1985c) reported on three adult black-eyed individuals from a further location close to Hiroshima and at another location recessive black-eyedness was discovered in a female by applying gynogenetic techniques. Nishioka & Ueda (1985a) and Nishioka et al. (1987) also collected 428 albinistic tadpoles of *P. nigromaculatus* and two adult albinistic *P. porosus* from an area of < 250 km around Hiroshima. They detected five different recessive genes causing albinisms in these populations. In addition, one *P. porosus* with a transparent blackish back was found (Nishioka & Ueda 1985e) but the anomaly was not inheritable. Furthermore, one adult flavistic and more than 18 albinistic tadpoles of *Rhacophorus schlegelii* were discovered in four populations in the same region (Nishioka & Ueda 1985b). In all populations recessive inheritance was demonstrated. In addition, more than 42 black-eyed individuals were found, with the anomaly being inheritable. These accumulations of colour anomalies across species and the large number of genes controlling them are truly unique on a global scale (see the sections on "albinism, hypomelanism and depigmentation", "black eyes" and "flavism" below). In most species background rates of albinism do not exceed 0.01% (Tab. 1). The authors never discussed the potential cause(s) of this exceptional accumulation of mutations. Only a mutagenic factor operating across species and a large area can explain such an extreme elevation above background rates. Irradiation after the atomic bomb was dropped on Hiroshima could be one explanation.

Chemical pollution also created anomaly hotspots. In 1979, Mizgireuv et al. (1984) collected *Bufo gargarizans, Rana amurensis and R. pirica* (as *R. chensinensis*) of various age classes in three locations on Sakhalin Island in the Far East of Russia that were polluted

by the discharge from a paper mill (one location) and municipal sewage works (two locations). All three species suffered from ectromely and, in the larval stage, also from edema, microcephaly and unilateral anophthalmy. More detailed data were only provided for *R. pirica*. This species was additionally affected by bone tumours, with 2–3 weeks post-metamorphosis being the youngest age at which the tumours became macroscopically visible. At the discharge site of the paper mill, 42% showed abnormal limbs and 11.5% had tumours, whereas the frequencies were 31–39% and 0–5.5% for the sites polluted with municipal wastewater (all $n > 1000$). The level of organic contamination correlated with the frequencies of anomalies but the nature of the contaminants was not assessed. Although the effect of the polluted water was not tested against controls in the laboratory, the correlation suggests a causal relationship. In support of this conclusion it may be noticed that necrosis following disease, chemicals and irradiation are the only factors in experimental tests that induce high numbers and frequencies of both ectromely and tumours (Henle et al. 2017a). Mizgireuv et al. (1984) did not discuss diseases, but it is likely that they would have discovered and mentioned it given their detailed examination of abnormal *R. pirica*.

In the same region, Levinskaya & Barinova (1978) observed that 37.5% (*n* not provided) of immature *R. amurensis* in 1972 suffered from syndactyly, brachydactyly and clinodactyly. They also originated from a site that was polluted with the discharge from a paper mill, but it remains unclear as to whether it is the same paper mill as in the study conducted by Mizgireuv et al. (1984). While experimental tests were not carried out, these hotspots on Sakhalin Island, Russia, call for a systematic testing of the teratogenic effects of paper mill discharges in the field and in the laboratory.

In Ekaterinburg, Vershinin (2002) found significant difference in the frequency of anomalies of *R. arvalis* among different zones of urbanization (Fig. 7). Frequencies were highest in areas of multi-story buildings (juveniles: 4.2%, $n = 3422$; adults: 14.1%, $n = 170$) intermediate in areas of low-story buildings (juveniles: 2.7%, $n = 1004$; adults: 9.4%, $n = 53$) and urban forest parks (juveniles: 1.7%, $n = 5309$; adults: 4.4%, $n = 183$) and low in rural forest control sites (juveniles: 0.78%; $n = 2939$; adults: 1.8%; $n = 57$). In total, eleven different types of anomalies were observed, including abnormal limbs (Figs. 21, 25), digits (Fig. 8a, 12) and mouth (Fig. 17), black-eyes (Fig. 28a) and other eye anomalies, abnormal patterns (Figs. 8b) and edema. *Lissotriton vulgaris* showed the same tendency but sample sizes were much smaller and the difference to other zones was only significant for the low-story buildings zone (Vershinin et al. unpubl.). For *Pelophylax ridibundus*, which occured only in the multi-story buildings zone, the frequency of anomalies was also high (13.4%; $n = 2178$), with the frequency increasing over the last 15 years (Vershinin et al. 2002, unpubl.). Higher concentrations of sulphates, petrol and detergents were associated with these differences as well as a higher biological consumption of oxygen in the urban sites. However, pollution was not the only factor associated with anomalies in *R. arvalis*. Heavy infections by the trematode *Holostephanus volgensis* contributed to skeletal anomalies. although primarily to externally not visible anomalies of vertebrae and the urostyle (Vershinin & Neustroeva 2011).

In India, Gurushankara et al. (2007) compared the prevalence of anomalies in four frog species (*Fejervarya brevipalmata, F. keralensis, F. limnocharis, F. rufescens*) from rice paddy fields, coffee plantations and forests. Of 6303 frogs collected between 2001 and 2003 229 were abnormal (mainly ectrodactyly, ectromely and anophthalmy). The incidence was highest in coffee plantations (4.6%), followed by rice paddies (4%) and water bodies (3.9%). None of the 661 frogs collected in the forests displayed any form of anomaly. Prevalence also differed among species, with the highest rates (8.4–12%) occurring in *F. rufescens*. The authors assumed agricultural contaminants to be the culprits but noted that this hypothesis still needs testing as they did not measure the contamination of the sites surveyed. Likewise, Patel et al. (2008) found elevated rates of anomalies (10.8%; $n = 259$; ectromely, ectrodactyly, tumours) in adult *F. limnocharis* collected in rice paddy fields.

Mass occurrence of tumour-like lesions were reported in Malaysia. Of 471 *Duttaphrynus melanostictus* collected in the region of Kuala Lumpur, 218 were affected externally (Dhaliwal & Griffiths 1963). Attempts to infect toads with fungal mycelium through the epidermis failed but were highly successful after subcutaneous inoculation. The identity of the fungus was not provided and no investigations were made into the reasons for the high rate of infection.

An enigmatic malformation hotspot was discovered by Woitkewitsch (1955, 1959, 1961, 1964, 1965) in Kazakhstan. Between 1947 and 1962 he examined more than 6000 *P. ridibundus* from six water bodies in one particular area of the Ala-Tau Mountains, of which 546 were malformed. The average annual percentage of individuals affected was 12%. Strangely, in all affected individuals the right hindleg was duplicated and only in nine severe cases the left side was also polymelous. Some of the supernumerary legs additionally exhibited bony triangles, other forms of taumely, or polydactyly. Mainly tadpoles exhibited the anomaly, but a few subadults and adults also suffered. The duplication of legs already appeared at limb bud stages. Eighteen individuals (tadpoles; 1-year-old frogs) showed ectromely and at least one giant tadpole was found. Woitkewitsch (1959) provided a very detailed description of the development and morphology of the abnormal limbs. All supernumeray limbs were immobile. In some but not all individuals the supernumerary legs were mirror-image duplications.

A laboratory cross of two polymelous individuals resulted in 472 fertilized eggs of which 285 reached metamorphosis; none of them was abnormal. In addition, the original and the duplicated legs were able to regenerate normally after amputation in the early stages of limb differentiation. Therefore a genetic cause is unlikely. Woit-

Fig. 7: Types of monitoring sites for anomalies of amphibians in the region of Ekaterinburg, Russia; a) multi-story urban area; b) low-story urban area; c) urban forest park; d) rural forest control site. Photos: V. Vershinin.

Fig. 8: Examples of anomalies observed in *Rana arvalis* in Ekaterinburg, Russia; a) oligodactyly in a juvenile found in the multi-story building zone, 3.9.1986; b) abnormal pattern (spotted) in an adult female observed in the multi-story building zone, 4.7.2002. Photos: V. Vershinin.

kewitsch (1959, 1961) discussed traumatism, parasites, radioactivity, UV-irradiation, viruses, chemicals and temperature as potential causes. He considered cold temperatures to be the likely cause because all water bodies with malformed tadpoles were fed by cold mountain springs. However, this is inconsistent with the results of laboratory studies for other species. Low temperatures did not induce polymely in laboratory experiments using *Ambystoma mexicanum* and *Xenopus laevis* (Swett 1926, Detwiler 1938, Kovalenko 2000; see Henle et al. 2017a for a review of temperature effects on skeletal anomalies). Furthermore, the species also occurred in other water bodies in the same region but without any individual showing polymely. No detailed comparison of the temperature or chemistry of the water bodies was provided. Moreover, two other frog species (*Rana asiatica?* and a „green" frog) living in the same water bodies as the malformed *P. ridibundus* did not exhibit anomalies. Skin webbing and bony triangles that usually are associated with trematode infections (e.g., Johnson et al. 2001b) were absent and no parasites were discovered in spite of very detailed anatomical studies of the malformed limbs (Woitkewitsch 1959, 1961).

3.1.3 Australia and Oceania

We only know four hotspots of anomalies in Australia. In Jabiru, Northern Territory, Australia, Tyler (1989) collected 261 abnormal frogs belonging to 14 species, with the frequency of abnormal specimens ranging from 3.8% to 16.1% (n = 26–914). The most common type of anomaly was ectrodactyly, followed by syndactyly, mandibular hypoplasia and clinodactyly. At least four other types of anomalies were detected (but not specified). The largest series was taken from a small body of water from a radioactive source trapped within a retention pond of the uranium mine before its filling; pesticides and herbicides were also detected but at low levels (0.5 ppm), just above detection thresholds. Ten other species collected in Jabiru did not exhibit anomalies but it is not known whether they were collected at the same site and how many of them were sampled. There are no data available on the level of radioactivity nor are there any data available from when the retention pond went into operation.

At the Olympic Dam mine in South Australia, prevalence of limb anomalies (mainly ectrodactyly) in *Neobatrachus centralis* was as high as 9%, with a mean of 1.6% (Read & Tyler 1990, 1994). Levels of radionuclides in frogs and water were low and not related to the incidence of anomalies. However, the data and statistics used are unclear as the location where radionuclide measurements and abnormality assessments were carried out only marginally overlapped. It may be worth mentioning that at another location, Paralana Springs, Flinders Range, South Australia, that naturally exhibits a relatively high level of radon (2,000 pCi/l), 35% (n not given) of the froglets of *Crinia riparia* exhibited abnormal hands.

In contaminated rice paddies in New South Wales 7% of the examined frogs (n = 1209; three species combined) exhibited external anomalies (Spoylarich et al. (2011). *Limnodynastes fletcheri* (7.1%; n = 694) and *L. tasmaniensis* (8.2%; n = 428) had high rates of anomalies, whereas only one out of 87 *Ranoidea raniformis* was abnormal. Ectrodactyly was the most frequently observed anomaly in the former two species, with crooked dorsal stripes also being frequent in *L. tasmaniensis.*

3.1.4 Africa

Only a few anomaly hotspots are known in Africa. Hassine et al. (2011) discovered an anomaly hotspot in Tunisia. At the Lema Dam, 74 of 221 (33%) metamorphs of *Pelophylax saharicus* and 13 of 52 (25%) adult *Discoglossus pictus* were abnormal. We did not count incompletely reabsorbed tails in metamorphs as an anomaly because this is a common feature in *Pelophylax* species in various parts of their distribution. While the authors claimed in the legend to a photo that an adult specimen also had an incompletely reabsorbed tail, the error bar shows that this individual had approximately the average size of a metamorph. Unfortunately, the terminology used by Hassine et al. (2011) is, in part, difficult to convert to historically established terminology. Moreover, the authors sometimes counted the same anomaly as different types of anomalies, e.g., incomplete additional limbs as polymely, ectromely (as hemimely), ectrodactyly and brachydactyly. Therefore, it remains partly unclear as to which types and how many different types of anomalies were involved (approx. 10 and 5 different types for *P. saharicus* and *D. pictus*, respectively). However, skeletal anomalies dominated, with polymely, polydactyly and presumably also ectromely and ectrodactyly having been common. The location was ectremely eutrophic and impacted by pesticides; at a second location, the Oasis of Gabes, where agriculture recently became more intensive, Hassine et al. (2011) found 6 (13%; n = 47) abnormal *D. pictus*. Out of 386 *P. saharicus* and 384 *D. pictus* collected in non-agricultural areas, none exhibited any anomaly. Therefore the authors regarded contamination with herbicides and pesticides as the most likely explanation, with some anomalies presumably also having been caused by predation.

Rostand (1971) mentioned that 25% of a Moroccan *P. saharicus* population was affected by anomaly P (polydactyly). Unfortunately, no further details were presented.

Of 353 and 107 tadpoles of *Kassina* sp. and *Phrynomantis microps* tadpoles from two large temporary ponds in the Ivory Coast 85.8% and 78.8%, respectively, showed tail injuries (Rödel 1998). The rate of tail injuries was significantly correlated with the number of Anisoptera dragonflies and giant water bugs (*Bellostoma*) in the samples taken from different sections of the ponds.

In Comoé National Park, Ivory Coast, 65–80% of *Phrynobatrachus francisci* examined in three populations (n = 45–116) had orange cysts containing *Endotrombicula pillersi* but none of the other 33 sympatric species was affected, with the exception of a single individual of *Sclerophrys maculata* (Spieler & Linsenmair

1999). Spieler & Linsenmair (1999) regarded the mite as a host-specific parasite. More recently, Wohltmann et al. (2007) collected the same parasite from 235 (n = 1183) *P. alleni* in the Tai National Park, Ivory Coast. They also found cysts in four more *Phrynobatrachus* species, *Petropedetes natator* and a single *Ptychadena aequiplicata* but with a lower infection rate. None of the 34 other species examined had cysts.

In the northern Drakensberg of South Africa, Smith et al. (2007) sampled tadpoles of *Strongylopus hymenopus* at 13 sites. Of 256 individuals, 16% had abnormal mouthparts; 39 of these 41 individuals were infected with *Batrachochytrium dendrobatidis*. In a second species sampled at 19 different sites, only 1.7% (n = 294) showed abnormal mouthparts; all were infected with *B. dendrobatidis*.

3.1.5 South and Central America

Seven hotspots of anomalies are known in South and Central America. Three of the cases with more than 100 affected individuals and high frequencies involve tadpoles infected by *Batrachochytrium dendrobatidis*. In Costa Rica, 100% (n = 150) of the tadpoles of *Isthmohyla calypsa* were affected by the absence of dark keratinization of mouthparts and/or the absence of tooth rows (Lips 1998). In Guatemala, Mendelson et al. (2004) observed more than 1000 tadpoles of *Ptychohyla hypomykter* with malformed mouthparts; in moribund individuals tail tips were discoloured. In Argentina, pigmentation of mouthparts was absent in 46.5% (n = 243) of the tadpoles of *Hylodes* cf. *ornatus* (Vieira et al. (2013). Elevated rates of abnormal mouthparts also occurred in four anuran species in the Departamento Yerba Buena, Argentina, albeit with lower numbers of individuals affected. The frequency was highest in the species with a longer larval period (*Boana riojana;* 38.9%, n = 211; *Odontophrynus americanus*: 23.7%, n = 97) compared to the species with shorter larval periods (*Pleurodema borellii*: 19.4%, n = 67; *Rhinella arenarum*: 16.3%, n = 49) (Medina et al. 2013). Elevated prevalence of tadpoles with abnormal mouthparts and/or mass mortality due to *B. dendrobatidis* has been observed at other locations in South and Central America (e.g., Lips 1999) but anomalies usually were not recorded for individual locations and species.

A striking hotspot occurs on the oceanic island Fernando de Noronha, Brazil. In the introduced Cururu toad (*R. jimi*), 17% (n = 179) of the adult toads collected in 2009 and 2010 lacked one or both eyes (Tolledo & Toledo 2015). No other anomalies were reported. In 2008, only 8.2% (n = 159) of the individuals had anophthalmy but 44.6% of the sampled individuals were abnormal (Toldeo & Ribeiro 2009). Besides anophthalmy, ectrodactyly was very common (at least 23.3%). Tadpoles also exhibited a high rate of anomalies (52.5%; n = 413) in 2009 and 2010 (Tolledo et al. 2014). In total 18 different types of anomalies were observed (Toledo & Ribeiro 2009, Tolledo et al. 2014, 2015). The causes are unknown but could be a combination of inbreeding caused by founder effects, absence of competitors and predators, and other unknown factors (Toledo & Ribeiro 2009, Tolledo & Toledo 2014).

In Argentina Agostini et al. (2013) compared the prevalences of anomalies in five species from ponds in two farmland areas. Ponds in the area with intensive and extensive crop production and cattle breeding were contaminated by endosulfan, cypermethrin, and chlorpyrifos. No contamination was detected in the ponds in the reference area with cattle breeding at low density. The prevalences in the populations from cultivated and reference areas were as follows: *R. fernandezae* (37.1 and 10.2%, respectively), *Leptodactylus latrans* adults (28.1 and 9.2%) and juveniles (32.9 and 15.3%), and *H. pulchellus* (11.6 and 2.8%). *Scinax granulatus* populations did not show abnormalities. *Pseudis minuta*, which was only detected in the reference area, exhibited a prevalence of 13.3%. For the former three species prevalences were significantly higher at contaminated ponds than at ponds from the reference area. Ectrodactyly and ectromely were the most common anomalies, with abnormal eyes, pigment defects, microcephaly, and torsion of the vertebral column having also been found at elevated rates. In the laboratory endosulfan can cause ectromely at environmentally relevant concentrations (Rohr et al. 2003).

An enigmatic mass occurrence of polymely was observed by M. Copping in an Ecuadorian *Leptodactylus* species (Rostand 1958b) but unfortunately there is no further information available on the case.

At three locations in Panama, the rate of anomalies in adult *Dendrobates auratus* averaged 13.3% (n = 592) (Gray et al. 2002). At two of these locations an average of 4.3% (n = 230) adult *Physalaemus pustulosus* also exhibited anomalies and at one of them 2.7% of the juveniles of the latter species were abnormal. Ectrodactyly was found to be the main type of anomaly and attributed to attacks from ants and other invertebrates. Although this explanation is plausible, no study has been undertaken to test this hypothesis.

3.1.6 The Caribbean islands and Bermuda

Studies on amphibian anomalies are largely lacking for Carribean islands, but Patel et al. (2012) observed mass occurrence (62%; n = 645) of abnormal mouthparts in tadpoles of the introduced *Rhinella marina* that were associated with infections with *Batrachochytrium dendrobatidis.* The frequency of anomalies increased with development from stage 27 to 41 of Gosner (1960).

Mass anomalies in *R. marina* are also known from Bermuda. Bacon et al. (2006b) examined 13,856 toadlets, subadults and adults at 38 sites across Bermuda. Of these, 21% showed anomalies, with the incidence for some ponds reaching up to 58%. In total 20 different types of anomalies (skeletal, eye-anomalies) were detected, with ectrodactyly, syndactyly, polydactyly, other digital anomalies and ectromely being the most common ones. Run-off from roads and agricultural fields were assumed to be the cause. Surface water and sediments from four hotspots and one control pond showed

high concentrations of metals and petroleum hydrocarbons. In the laboratory these samples induced polydactyly, ectrodactyly, ectromely and abnormal mouths in toadlets of *R. marina* (Fort et al. 2006b, Bacon et al. 2013). The data indicated that metals and hydrocarbons were individually capable of inducing the observed anomalies but that they seemed to act synergistically.

3.1.7 North America

Even though the majority of hotspots in North America were discovered recently (since the 1990s), the earliest hotspot known was also found in North America. At Boulder Creek in California 98.6% (n = 74) of the *Taricha torosa* sampled between 1926 and 1942 had tumours (extreme proliferation of fibrous connective tissue) (Wolterstorff 1935, Twitty 1942, Brattstrom & Warren 1953). The frequency dropped to 10% in 1953. The cause of this anomaly is unknown.

The world's most comprehensive survey of amphibian anomalies was also carried out in the USA. Between 2000 and 2009 the Fish and Wildlife Service conducted a nationwide survey of amphibian anomalies in national wildlife refuges. The core dataset comprises 48,081 anurans, representing 462 sites, 32 species and 41 states. The nation-wide prevalence of morphological anomalies averaged 2.0%, which is consistent with expected background frequencies (Lunde & Johnson 2012, Reeves et al. 2013). From 675 collection events 152 yielded frequencies higher than the 5% that is generally used in the USA as the classification criterion for hotspots. Partially or completely missing limbs or digits clearly dominated; polymely and polydactyly together comprised only 1.7% of all anomalies registered. Up to 20 different types of anomalies were observed [a population of *Lithobates sphenocephalus* at Colin's Ditch, Great Swamp National Wildlife Refuge, in which 11% (n = 583) of the individuals sampled were abnormal]. Clusters of high-abnormality sites were found in the Mississippi River Valley, in California and Alaska (Reeves et al. 2013). In Alaska, 9269 metamorphosing *L. sylvaticus* were examined from 86 breeding sites in five National Wildlife Refuges (Reeves et al. 2008). Prevalence of skeletal and eye anomalies ranged from 1.5% to 7.9% and were as high as 20% at individual breeding sites. Ectromely, black eyes, micromely and brachydactyly were the most common types of anomalies. Polymely and polydactyly were rare.

Several other state-wide surveys were carried out in the USA. In randomly selected wetlands in north-central USA that were sampled between 1998 and 2000, 1.4–2.3% ($n \geq 389$) of the sampled individuals exhibited either skeletal or eye anomalies (Schoff et al. 2003). In Minnesota, out of 25,000 frogs that were sampled at 100 different ponds until the year 2000, 8.2% were malformed and more than 37 sites contained "significant" numbers of deformed frogs (Vandenlangenberg et al. 2003, Helgen 2012). The observed rate is well above the expected baseline of below 2% (Lunde & Johnson 2012, Reeves et al. 2013, Henle et al. 2017a). As in the nationwide survey, ectromely and ectrodactyly were the most common types of anomaly, whereas polymely was rare. Above baseline rates were also recorded at several sites in various other states (Helgen 2012). For example, Lannoo (2008) described 12 hotspot sites from Minnesota and mentioned a hotspot site each for Iowa, Wisconsin, Ohio, California and Indiana. Unfortunately, no quantitative data on observed anomalies or their frequencies were provided. Sessions & Ruth (1990) and Johnson et al. (2001a, 2006, 2013) reported other hotspot sites in California. Hotspots are also known in Vermont (Fort et al. 1999a, Burkhard et al. 2000, Levey et al. 2003) and British Columbia (Roberts & Dickinson 2012).

We found quantitative data for 19 sites, for which prevalence was at least 10% and at least 100 abnormal individuals of a single species were recorded (Tab. 2). Two of the sites harboured populations of larval amphibians that exhibited non-skeletal anomalies. Two hotspots were characterized by cysts caused by mites of the genus *Hannemania* and another one by pustules of unknown ethiology. At the remaining hotspot sites, mainly skeletal anomalies occurred. At most Californian sites, polymely dominated. Parasites were the most frequently assumed or experimentally demonstrated cause of the hotspots. At three sites chemical pollution and at two sites predators were experimentally identified as causes. Cannibalistic attacks presumably contributed to high frequencies of anomalies at two sites and the cause(s) for some or all anomalies remains unknown or controversial for six sites.

Hypothesized causes for skeletal anomalies at other hotspot sites include parasites, predators, chemicals, diseases, UV-irradiation and radioactivity and interactions among these factors, with the first three being the most frequent suspects (Johnson et al. 2004, Ballengée & Sessions 2009, Helgen 2012). Although several authors provided evidence that there are multiple causes and that the causes differ among hotspots (e.g., Meteyer et al. 2000a, Reeves et al. 2010, 2013, Lunde & Johnson 2012), there is still considerable disagreement (e.g., see Lannoo 2008 versus Sessions 2009; Ballengée & Sessions 2009, Ballengée 2010 and Sessions & Ballengée 2010 versus Skelly & Benard 2010). Opinions range from assuming direct effects of chemicals as the main cause at most hotspots (Lannoo 2008, McCoy & Guillette 2009, Mann et al. 2009, Helgen 2012) to the belief that chemicals only play an indirect role through immunosuppression, making tadpoles more sensitive to attacks from trematodes (e.g., Rohr et al. 2009), to an outright exclusion of chemicals and the assumption that all anomalies can be explained entirely by predators and parasites (e.g., Sessions 2009, Ballengée & Sessions 2009, Sessions & Ballengée 2010).

The CWB site in Minnesota, which sparked renewed interest in amphibian anomalies in the USA, is a prime example for the controversy and challenges for establishing the cause(s) of mass anomalies. Three species had high prevalences of anomalies, with cutaneous fusion, polymely, ectromely and anteversion having frequently been observed (Tab. 2). Another four spe-

cies were studied at the site that had much lower sample sizes and prevalences of anomalies. Water samples showed a deficit of micronutrients that caused anomalies in the laboratory (Garber et al. 2004) and lipophilic extracts from water solutions induced skin webbing in a high percentage of the tested individuals (Bridges et al. 2004). Based on the spectrum of anomalies but without studying water samples, Gardiner & Hoppe (1999) suggested that environmental retinoids were the cause of the anomalies. Stopper et al. (2002) disagreed and argued that cysts of the trematode *Ribeiroia ondatrae* induced the malformations as experimental infections of amphibians supposedly resulted in all of the anomalies reported by Gardiner & Hoppe (1999). However, Stopper et al. (2002) did not study the site and contrary to their statement, ectromely and ectrodactyly do not occur beyond background levels in experimental infections with *R. ondatrae,* except for *Pseudacris regilla* (see section 3.1.7.1). While *R. ondatrae* occurred at CWB (Vandenlangenberg et al. 2003), to our knowledge no experimental or correlational study addressed its association with the observed anomalies at the site. Notwithstanding, given that *R. ondatrae* has been shown to be the cause of polymely and cutaneous fusion at other hotspot sites and very few other factors have been shown experimentally to cause elevated rates of polymely (section 3.2.1.1), they presumably contributed to the anomalies observed at CWB. In conclusion, current evidence suggests that *R. ondatrae* and chemical pollution were the main causes for the hotspot. Whether other factors also played a role (see Helgen 2012 for a discussion) remains speculative in the absence of any relevant study at the site.

Studies to unravel the causes were only carried out at a few sites, primarily at the hottest hotspot sites listed in Table 2. Another drawback for inferences is that, as with CWB, most studies that attempted to reveal causes were limited to a single potential factor. Notwithstanding, given the large differences in the pattern of anomalies at North American hotspot sites, different causes must be responsible for the anomalies at different sites. Existing evidence suggests that most hotspots at which either polymely and cutaneous fusion or cysts dominated were caused mainly by parasites (compare Table 2 and the overview table of anomalies that have been produced experimentally published by Henle et al. 2017a), although polymely and cutaneous fusion may also be caused by chemical pollution (see sections 3.2.1.1 and 3.3.2.3). Hotspots at which ectromely and ectrodactyly dominated were likely to have been caused by chemical pollution or predators, including their interactions, and hotspots at which other types of anomalies dominated were likely to have been caused by chemical pollution or diseases. In the following we review the role of these factors for the hotspots in North America.

3.1.7.1 Parasites. Infection by cysts of the trematode *Ribeiroia ondatrae* is a comparably well-studied process that creates limb anomalies in some North American hotspots. Signalling molecules produced by cells set up the three primary limb axes by interacting with regulatory genes (Johnson & Tabin 1997, Martin 1998, Pearse & Tabin 1998, Chen & Johnson 1999). Any factor that disturbs the spatial organization of the cells and thus interferes with their positional values or disrupts the signalling pathways usually causes pattern duplication in a variety of organisms (Bryant et al. 1981, Wallace 1981, Chapron 1986). Cysts of *R. ondatrae* disturb this spatial organization. As a consequence, experimental infection of tadpoles produces polymely, with the frequency increasing with the number of cercariae of *R. ondatrae* in the water to which tadpoles are exposed. Cutaneous fusion and bony triangles also often appear in high frequencies (e.g., Johnson et al. 2001b, 2006, Schotthoefer et al. 2003). Ectromely and ectrodactyly are usually either absent or only occur at low frequency. The only exception is *Pseudacris regilla* (Johnson et al. 1999, Romansic et al. 2011), a species, which seems to be particularly sensitive to *R. ondatrae* infection. In this species, there is a narrow critical window, stages 24–28 of Gosner (1960), during which infection causes high rates of anomalies. Exposure at earlier stages resulted in high mortality and no limb anomalies. When individuals were exposed at stage 24, at which mortality was still high, ectromely occurred. Exposure after stage 26 never caused ectromely or ectrodactyly. In exposures at stages 26–28, bony triangles, skin webbing and polymely dominated (Fig. 9). After stage 28, only a few anomalies were induced (Johnson et al. 2011), explaining why not all infected individuals develop anomalies. Furthermore, effects are greater in small-scale (laboratory) experiments rather than in larger scale experiments using cages or full-scale lake manipulations (Lunde et al. 2012).

Cysts of *R. ondatrae* have been found in the limbs of amphibians at all but two North American hotspots, at which large numbers of individuals with polymely occurred (e.g., Johnson et al. 2001b, 2003, 2006). As *R. ondatrae* readily causes polymely in the laboratory in many frog species, it is plausible to assume that this trematode is (part of) the cause for these hotspots. However, this hypothesis has only been tested in the field once in a British Columbian hotspot (Roberts & Dickinson 2012). At this hotspot 23% of 521 *P. regilla*

Fig. 9: *Pseudacris regilla* with polymely that was induced by *Ribeiroia ondatrae* cysts. Source: Goodman & Johnson (2011a).

Table 2: North American hotspots of anomalies, with more than 100 individuals and 10% of the population of at least one species being affected. Data are sums (*N-abn*) and weighted averages (Freq) across cited references. Stage: ad: adult, juv: juvenile, l: larval, m: metamorphs, p: stages post metamorphosis; Freq: % of the population affected; *N*-abn: number of abnormal individuals; main type: types of anomalies that affected at least 10% of all abnormal individuals (at least 10% of a subsample of at least 50 individuals if data are not available for all samples); Cause: (e): the cause was supported by experimental studies, all other causes are assumptions.

Location	Species (Stage)	Freq	*N-abn*	Years	Main type	Cause	References
Aptos Pond, California	*Ambystoma macrodactylum croceum* (l, juv, ad) *Pseudacris regilla* (m)	14.8% 54.2%	1738 212	1986–1987+1998–2000 1986–1987, 1999	Polydactyly, polymely, ectromely Polymely, ectromely, cutaneous fusion	*Ribeiroia ondatrae,* trauma (conspecifics[3], predators, and their interactions) (e)	Sessions & Ruth 1990, Sesssions et al. 1999, Johnson et al. 2003, 2006
Hidden Pond, California	*Pseudacris regilla* (l, m) *Taricha torosa* (l, ad) *Anaxyrus boreas* (l, m)	12.3% 15.4% 3.3%	1097 148 18	1986, 1987, 2006–2009 1997–1998 1997–1998	Polymely Ectromely, ectrodactyly Ectromely, ectrodactyly	*Ribeiroia ondatrae* (e) Unknown[1] Unknown[1]	Johnson et al. 2001a, Lunde et al. 2012
Sheep Pond, California	*Pseudacris regilla* (m, ad)	43.4%	681	2009–2011	Polymely, cutaneous fusion	*Ribeiroia ondatrae*	Goodman & Johnson 2011a
Frog Pond, California	*Pseudacris regilla* (l, m) *Taricha torosa* (l) *Anaxyrus boreas* (l, m) *Lithobates catesbeianus* (l, m, ad)	25.2% 41.3% 3.1% 3.7%	578 267 51 32	1986, 1987, 2006–2009 1997–1998 1997–1998 1997–1998	Polymely, cutaneous fusion Ectromely, ectrodactyly Ectromely, ectrodactyly Polymely	*Ribeiroia ondatrae* (e) Unknown[1] Unknown[1] Unknown	Johnson et al. 2001a, Lunde et al. 2012
Fortynine Palms Oasis, Joshua Tree National Monument, California	*Pseudacris cadaverina* (p)	98.8%	253	1969–1970	Cysts	Mites: *Hannemania hylae* (histology)	Welbourn & Loomis 1975
Hog Lake, California	*Pseudacris regilla* (p)	26.2%	207	2006–2008	No site specific data available but presumably polymely, bony triangles, polydactyly, skin webbing	*Ribeiroia ondatrae* (e)	Lunde et al. 2012
CWB site, Minnesota	*Lithobates septentrionalis* (m) *Lithobates pipiens* (m) *Lithobates clamitans* (m) *Anaxyrus americanus* (m) 3 further species with sample size < 100	67.5% 12.8% 33.5% 6.7% 2.9%	587 253 82 8 4	1996–1999 1996–1999 1996–1999 1996–1999 1996–1999	Cutaneous fusion, polymely; presumably also torsion of limbs, ectromely Cutaneous fusion, polymely, ectrodactyly, anteversion Cutaneous fusion, polymely, anteversion, ectromely No data provided No data provided	Competing hypotheses[2]: *Ribeiroia ondatrae*, retinoids, micronutrient deficiency, and pollution	Vandenlangenberg et al. 2003, Hoppe 2005
ROI site, Minnesota	*Lithobates pipiens* (m) (5 further species were found but no data on anomalies were provided)	20.3%	283	1996–1999	Ectromely, ectrodactyly	Unknown; *Ribeiroia ondatrae* was not found but estrogenic substances and pesticides	Vandenlangenberg et al. 2003
Duck Pond, Minnesota	*Anaxyrus americanus* (m) *Dryophytes versicolor* (m)	59.5% 3.2%	368 8	2004 2004	Bony triangle, skin webbing, polymely, ectromely –	*Ribeiroia ondatrae* Unknown	Johnson & Hartson 2009
Dor site, Minnesota	*Lithobates pipiens* (m)	18.3%	115	1996–1997	No data provided	Unknown	Canfield et al. 2000, Helgen et al. 2000
Potholes, Oregon	*Ambystoma macrodactylum* (juv)	35%	141	2002, 2004	Ectromely, ectrodactyly	Trauma (conspecifics[3])	Johnson et al. 2006
Lake Aspen, Oregon	*Anaxyrus boreas* (l, m)	12.9%	1734	1998–2008	Ectromely, ectrodactyly, tail injuries	Injury from *Gasterosteus aculeatus* (e)	Bowerman et al. 2010, Johnson & Bowerman 2010
Jette Pond, Montana	*Pseudacris regilla* (l, juv) *Ambystoma macrodactylum* (l, ad)	47.6% 11.5%	161 60	1958–1959, 1999–2001 1998–2004[4]	Polymely, cutaneous fusion Brachymely, skin webbing, polydactyly, polymely	*Ribeiroia ondatrae* *Ribeiroia ondatrae* (but see sction 3.3.2.3)	Hebard & Brunson 1963, Johnson et al. 2003, 2006

Table 2: Continuation.

Location	Species (Stage)	Freq	*N-abn*	Years	Main type	Cause	References
Savannah River National Environmental Research Park (coal ash deposition swamp), Aiken, South Carolina	*Lithobates catesbeianus* (l) *Dryophytes cinereus* (l)	78.5% 0%	1171 0 of 32 tadpoles	1995, 1996 1995, 1996, 1998	Labial tooth rows partially missing, bent tails –	Coal combustion waste (e)	Rowe et al. 1996, Hopkins et al. 2000
Savannah River National Environmental Research Park (coal ash drainage swamp), Aiken, South Carolina	*Lithobates catesbeianus* (l)	34.8%	286	1995, 1996, 1998	Labial tooth rows partially missing, bent tails	Coal combustion waste (e)	Rowe et al. 1996, Hopkins et al. 2000
Rhine Pond, Orange County, North Carolina	*Lithobates palustris* (a, m)	12.6%	162	1961–1963	Cysts, faulty eruption of forelimbs	Mites: *Hannemania dunni* (histology) and unknown	Murphy 1965
Tunica, Mississippi	*Lithobates catesbeianus* (p)	?	350	1958	Polymely	Pesticides assumed	Volpe & Rosenbaum 2000
UPM02 site (Fish Farm Mounds), Upper Mississippi River National Wildlife and Fish Refuge, Iowa	*Lithobates pipiens* (l, m) *Lithobates clamitans* (l, m)	66.5% 4.9%	119 2	2002, 2003, 2006 2002	Small translucent pustules –	Unknown –	Reeves et al. 2013
Isobel Lake, British Columbia, Canada	*Pseudacris regilla* (m) *Rana luteiventris* (m)	23.0% 30.7%	120 86	2011	Polymely, cutaneous fusion Cutaneous fusion, rotation of limbs, ectromely	*Ribeiroia ondatrae* (e) Unknown (*R. ondatrae* assumed[5])	Roberts & Dickinson 2012

[1] The pattern is inconsistent with results from experimental infections with *Ribeiroia ondatrae* (see section 3.1.7.1).

[2] For competing hypotheses see Gardiner & Hoppe (1999), Stopper et al. (2002), Lannoo (2008), Helgen (2012) and section 3.1.7.

[3] Johnson et al. (2006) assumed cannibalism as the cause because when two individuals were housed together in 1.5 l containers without food high frequencies of limb injuries occurred. However, these conditions are very artificial and need to be corroborated under more natural conditions.

[4] No anomalies were observed in 1958–1961 (Hebard & Brunson 1963).

[5] *Ribeiroia ondatrae* usually does not produce elevated rates of ectromely and other causes likely were more relevant for the hotspot (see section 3.1.7.1).

and 30.7% of 280 *Rana luteiventris* metamorphs but only a few *Ambystoma macrodactylum* and *Anaxyrus boreas* were affected. *Pseudacris regilla* and *R. luteiventris* primarily exhibited polymely and cutaneous fusion, respectively. When tadpoles were reared in closed cages that prevented the entry of *R. ondatrae*, no anomalies occurred. Roberts & Dickinson (2012) concluded that *R. ondatrae* caused the anomaly hotspot. A caveat remains as the authors did not provide any information as to whether the cages also excluded access to sediment and thus to pollutants that may have accumulated in the sediment, which could confound results (Helgen 2012). Moreover, predators likely were also excluded and, like chemicals, could be responsible for the elevated rates of ectromely in *Rana luteiventris* (compare section 3.2.2.1). Furthermore, no explanation has been provided why the other two species were only mildly affected in spite of them being highly sensitive to *R. ondatrae* infection in the laboratory (Johnson et al. 2001b, 2006).

In California and the northwestern states of the USA, a significant relationship between the number of *R. ondatrae* infections and the frequency of malformed *P. regilla* was discovered (Johnson et al. 2002, 2013, Lunde et al. 2012). However, such relationships do not exist for frogs in Alaska, Vermont and Michigan (e.g., Gillilland & Muzzall 2002, Levey et al. 2003, Skelly et al. 2007, Reeves et al. 2008, 2010); for Minnesota it still remains unclear whether such a relationship exists

(Helgen 2012). Further, at one of the hotspots with numerous polymelous individuals of *Lithobates catesbeianus* (Anonymous 1954), *R. ondatrae* was not detected in preserved polymelous frogs, nor in the resurvey, but anomalies were also absent in the resurvey (Johnson et al. 2003). The other exception (Volpe & Rosenbaum 2000) was discovered at a time when *R. ondatrae* was not yet known as a cause of limb anomalies. The anomaliy appeared only in one year. While the abnormality rate may vary from year to year (see section 6), to be absent in all years and very frequent in another year is at odds with the dynamics of *R. ondatrae*. Volpe & Rosenbaum (2000) assumed pesticides that were heavily used in the surrounding environment to be the cause.

Despite these exceptions and the fact that factors other than *R. ondatrae* have caused mass occurrences of polymely elsewhere (see 3.2.1.1), it is likely that *R. ondatrae* was one of the main causes in most North American hotspots in which polymely dominated, especially if bony triangles and/or cutaneous webbing were also prominent. At several of these hotspots other anomalies that are absent or very rare in experimental infections were also frequent, notably ectromely and ectrodactyly (e.g., Johnson et al. 2001b, 2006, Johnson & Hartson 2009). Thus, it is rather unlikely that trematodes were the only cause and trematodes cannot explain hotspots where ectromely and ectrodactyly were dominant but polymely was rare. This seems to be meanwhile consensus even among those scientists that otherwise have rather opposing opinions about the causes of amphibian anomalies.

Ribeiroia ondatrae is not the only parasite that may create anomaly hotspots. The database of Reeves et al. (2013) contains two populations of *L. clamitans*, in which 21 out of 53 and 52 out of 137, respectively, metamorphosing individuals had cysts. The trematode *Clinostomum* was the cause in the former population and unidentified metacercariae in the second population. High prevalences of individuals with cysts may also be due to chigger mites of the genus *Hannemania*. For example, prevalences well above 50% have been observed in *Desmognathus fuscus, Eurycea bislineata, Plethodon ouachitae, Anaxyrus punctatus, Dryophytes arenicolor, Pseud-acris cadaverina, L. berlandieri,* and *L. palustris* (Pope & Pope 1951, Murphy 1965, Duszynski & Jones 1973, Shoemaker & Clark 1975, Welbourn & Loomis 1975, Jung et al. 2001, Anthony et al. 2004, Westfall et al. 2008). Often reddening of the skin around the cysts is also observed (e.g. Pope & Pope 1951) and ectrodactyly may occur if digits are heavily infested (e.g. Winter et al. 1986 for *Desmognathus brimleyorum*).

Copepods are yet another group of parasites that are likely to have contributed to elevated rates of anomalies. Significantly more *Rana boylii* individuals with copepod parasites (*Lernaea cyprinacea*) were malformed at South Fork Eel River in California than those individuals without copepod parasites in 2006, but no difference occurred in 2008 (Kupferberg et al. 2009).

3.1.7.2 Chemical pollution. As an alternative explanation to infection by *R. ondatrae,* retinoids and the insecticide methoprene have been suspected as culprits for anomaly hotspots because retinoids play a major role in limb differentiation, can induce polymely in laboratory experiments and biologically active retinoids were detected by Gardiner et al. (2003) in water samples from sites where large numbers of malformed frogs were found. Other chemicals contributing to pollution have also been suggested as causes for anomaly hotspots.

Retinoids are metabolic derivatives of vitamin A. They are part of the thyroid hormone dependent system that activates the genes involved in limb differentiation (Kliewer et al. 1992, Maden & Corcoran 1996, Lee et al. 2004). Feeding retinoids to tadpoles can lead to ectromely and bony triangles (Degitz et al. 2000, Alsop et al. 2004). In regenerating limbs, the effects depend on the dose and duration of the application (Maden 1983b). At high and low doses polymely is not observed. High doses suppress regeneration completely and at low doses ectrodactyly occurs (Saxena & Niazi 1977, Maden 1983b, Thoms & Stocum 1984, Scadding & Maden 1986b).

Most authors observed polymely only in regenerating limbs but not in developing limbs (Scadding & Maden 1986a,b, Degitz et al. 2000) and in a review Niazi (1996) concluded that retinoids suppress differentiation in developing limbs. However, Das & Mohanty-Hejmadi (2000) obtained significantly elevated rates of polymely in the developing hindlimbs of *Duttaphrynus melanostictus* tadpoles raised in vitamin A solutions after the amputation of their tails. Moreover, Bruschelli & Rosi (1971) and Gardiner et al. (2003) observed polymely in developing limbs without surgical manipulation. No statistical comparisons were made with controls in both studies but the frequency was very high in the latter. Gardiner et al. (2003) emphasized that polymely only occurred when stage 52 tadpoles (*Xenopus laevis*, stages after Nieuwkoop & Faber 1994) were treated, which may explain the discrepancy with Degitz et al. (2000), who only treated tadpoles at stage 48 and 51. From studies on mice and rats it is also well known that the effects of retinoids are highly stage-specific (Lee et al. 2004).

On the other hand, embryos (of *Xenopus laevis*) are far more sensitive to retinoids than tadpoles and show 100% mortality at doses, which are ineffective in tadpoles (Degitz et al. 2003a). Degitz et al. (2003a) therefore concluded that retinoids and methoprene cannot play a significant role in malformation hotspots. However, this does not exclude a role of chemicals in the development of mass occurrences of polymely, since relevant chemicals may only contaminate water at times when tadpoles have reached later developmental stages. Moreover, recent research showed that various agrochemicals, including the widely used glyphosate, polychlorinated pollutants and persistent pharmaceuticals, influence retinoic acid activity at environmentally relevant doses or bind to retinoic acid receptors (Alsop et al. 2004, Lee et al. 2004, Mann et al. 2009, Paganelli

et al. 2010). Retinoid metabolism can be influenced by agricultural contamination (Bérubé et al. 2005, Boily et al. 2009) and one study showed that compounds in pulp mill effluent (probably originating from the wood used) bound to retinoid acid receptors (Alsop et al. 2004). Moreover, Wu et al. (2012) discovered that cyanobacteria blooms release teratogenic retinoic acids. Thus, one cannot dismiss *a priori* the direct effect of pollutants at hotspots with a duplication of limbs or digits. Notably, outside North America two cases are known in which a high number of polymelous frogs were found in polluted habitats where *Ribeiroia ondatrae* does not occur and no other potential cause has been found (see section 3.2.1.1). Notwithstanding, as yet there is no hard evidence that chemicals directly contributed to mass polymely at North American hotspots. However, field experiments with agrochemicals that influence retinoid activity and cyanobacteria are still in dire need. In any case, there is strong evidence of an indirect, meanwhile widely accepted, effect through eutrophication favouring snails that are intermediate hosts of *R. ondatrae* (see section 3.1.7.5).

In contrast to hotspots that are dominated by polymely, a direct chemical effect is likely to explain, at least in part, North American hotspots in Vermont, Minnesota, California, Alaska and Canada, where ectromely and ectrodactyly are frequent and where polymely is comparably rare or absent. Some authors reject such conclusions, putting all such cases down to predation attempts (e.g., Ballengée & Sessions 2009, Sessions 2009, Sessions & Ballengée 2010). Contrary to the statement made by Sessions & Ballengée (2010) there is strong evidence of a direct effect from chemicals for several sites.

In Minnesota and Vermont, water and sediment from hotspot sites caused high rates of anomalies in embryos but water from control ponds did not (Burkhart et al. 1998, Fort et al. 1999a,b). Garber et al. (2004) challenged these results because in their laboratory study an addition of sodium and potassium removed the teratogeneity of the water samples from three hotspot sites. However, later tests with laboratory prepared water of similar ionic strength as in the tested hotspots did not induce any anomalies (Burkhart et al. 2000). Thus, low ionic concentrations as such cannot explain the observed anomalies. Moreover, water from hotspot sites running through activated charcoal filters no more induced anomalies (Burkhart personal communication to Helgen 2012). Furthermore, fat-soluble extracts from water samples from malformation hotspots caused anomalies in tadpoles, including bony bridges, whereas those from a control pond did not (Bridges et al. 2004). Given these results and the fact that many biocides are capable of inducing ectromely and ectrodactyly at environmentally relevant concentrations (Tab. 3), it is very likely that chemicals were a main direct cause at the assessed hotspots. Note that *R. ondatrae* infection cannot explain most hotspots where ectromely or ectrodactyly dominate as so far only one species, *Pseudacris regilla*, has developed high rates of these anomalies in laboratory experiments, whereas other species affected in ectromely hotspots did not (see 3.2.2.1 for more details).

In Vermont, Taylor et al. (2005) examined 5264 metamorphs of six frog species collected in 42 wetlands for malformations. The rate of non-traumatic limb malformations varied by location from 0–10.2%. Ectromely and other limb anomalies were the most frequent types of anomalies, with only two individuals showing polymely. The proximity to agricultural land was associated with an increased risk of limb malformations, with an odds ratio of 2.26.

In California, Sparling et al. (2015) raised tadpoles of *Pseudacris regilla* in cages in three ponds each in the Yosemite, Sequoia and Lassen National Parks in 2001 and 2002. Volatile pesticides from the very agricultural Central Valley have been deposited in these ecosystems. Between 6.2% and 25.5% of the metamorphs exhibited brachymely (called hemimely by Sparling et al. 2015). Tissue concentrations of DDE, trans-nonachlor, cis-nonachlor, a-chlordane and polybrominated diphenyl ethers were significantly correlated with the percentage of malformed metamorphs. In 2002, when tissue loads were lower on average, no correlation was significant. Genotoxic effects were detected in tadpoles from all three parks.

For 21 sites in the Kenai Wildlife Refuge, Alaska, organic and inorganic pollutants in addition to predators (Odonata) best explained the probability of *L. sylvaticus* acquiring skeletal anomalies (Reeves et al. 2010). While water plus sediment from six of the ponds did not induce skeletal anomalies in the laboratory, pollutants at the site with the highest rate of anomalies exceeded toxic thresholds and Odonata were not abundant. This indicates that pollution was a direct cause of the anomalies.

In Quebec in Canada out of 853 metamorphosing anurans (*Anaxyrus americanus, Lithobates catesbeianus, L. clamitans, L. pipiens*) from 14 farmland habitats 106 (12%, range 0–69%) had severe ectromely or ectrodactyly compared to only two (0.7%, range 0–7.7%) out of 271 individuals from 12 control sites (Ouellet et al. 1997). Malformed frogs had chromosomal anomalies and flow cytometry revealed genomic disruption in adult and metamorphosing individuals from all farmland sites (Bonin et al. 1997, Lowcock et al. 1997). Water samples taken from these sites showed correspondingly high genotoxicity values – observations that cannot be explained by parasites or predators.

Sewage also created abnormality hotspots. Labial tooth rows were partially missing in 96.2% of 1048 *L. catesbeianus* tadpoles from a coal ash deposition pond and 85.1% (n = 208) in an associated drainage swamp but only 2.9% (n = 589) were affected in control ponds (Rowe et al. 1996). When eggs were transplanted 97–100% of the tadpoles that developed in the contaminated ponds suffered from the same anomalies (Rowe et al. 1998). In a sewage pond of the Reese Airforce Base adult *Ambystoma tigrinum* suffered from tumours, with the overall rate of anomalies being 6.6% (n = 37,143; range among years 0.04–50%) (Rose 1976, 1981). None

Table 3: Overview of chemicals that caused ectromely in experimental treatments.

Chemical	Species	References
a) Whole body application at environmentally relevant concentrations		
Carbaryl	*Ambystoma barbouri*	Rohr et al. 2003
Copper	*Xenopus laevis*	Fort & Stover 1996, 1997
Endosulfan	*Ambystoma barbouri*	Rohr et al. 2003
Malaoxon (organophosphate)	*Xenopus laevis*	Snawder & Chambers 1989
Maneb 80 (carbamate)	*Triturus carnifex*	Zavanella et al. 1984
Maneb (carbamate)	*Xenopus laevis*	Fort et al. 1999b
Methylmecury	*Lithobates sphenocephalus*	Unrine et al. 2004
Octylphenol	*Ambystoma barbouri*	Rohr et al. 2003
s-methoprene (field application as Altosid)	*Lithobates pipiens*	Sparling 2000
Retinoids (only if exposed after hatching)	*Ambystoma mexicanum, Xenopus laevis, Lithobates sylvaticus*	Scadding & Maden 1986a,b, Degitz et al. 2000
Seawater (8 ppm)	*Rhinella marina*	Rios-López 2008
b) Whole body application after limb or tail amputation		
Retinoids, vitamin A	*Ambystoma mexicanum, Xenopus laevis, Duttaphrynus melanostictus, Rana temporaria*	Jangir & Niazi 1978, Maden 1983a,b, Scadding 1983, Niazi & Alam 1984, Niazi & Ratnasamy 1984, Scadding & Maden 1986a,b, Das & Mohanty-Hejmadi 2000
c) Teratogeneity index low (i.e., mortality high in treatments causing anomalies) or conditions required that are unlikely in natural habitats		
Methoprene	*Lithobates pipiens*	Ankley et al. 1998
Nitrogen mustard	*Ambystoma maculatum, Taricha torosa*	Gillette & Bodenstein 1946, Bodenstein 1947
Thyroxine (after amputation)	*Notophthalmus viridescens*	Hay 1956
d) Test conditions environmentally not relevant or relevance not assessed		
Acetylhydrazide	*Xenopus laevis*	Fort & Stover 1997
Aflatoxine	*Rana temporaria*	Gabor et al. 1973
Atropine	*Notophthalmus viridescens*	Singer et al. 1960
Citral	*Eleutherodactylus coqui*	Lee & Elinson 2008
Colchicine (treatment of regenerating limbs)	*Xenopus laevis, Rana temporaria*	Bretscher 1949, Crézé 1950, Rostand 1950c, Tschumi 1954
Cyclophosphamide	*Xenopus laevis*	Dumont et al. 1983
Estradiol	*Pelobates cultripes*	Collenot 1965
Thalidomide and analogues EM_{12} and EM_{87}	*Notophthalmus viridescens, Xenopus laevis*	Bazzoli et al. 1977, Dumpert & Zietz 1984

of the 19,802 individuals examined from the non-sewage ponds suffered from tumours. Edema occurred at a frequency of 0.2% in the non-sewage ponds and at a similar frequency in the Reese Airforce Base sewage pond. The sewage sludge was contaminated by numerous polycyclic aromatic hydrocarbons (PAH), especially perylene. Physiological studies indicated that PAH derivatives induced the tumours.

In wetlands constructed with wastewater treatment in Georgia 20.9% (n = 833) of the sampled *L. catesbeianus* tadpoles showed anomalies, whereas in four control ponds only 1.6% (n = 190) were affected (Ruiz et al. 2010). Edema, scoliosis and nodules consisting of calcium phosphate were the most frequently observed anomalies. For the later two types of anomalies the frequency dropped substantially with the distance of the sampling site from the discharge point. These data strongly indicate that the anomalies were caused by chemicals that retained in the treated wastewater.

In floodplain ponds of a river contaminated with petroleum in the south central US, froglets from three out of four species (*L. sphenocephalus, L. blairi* and *L. clamitans*) had elevated rates of anomalies (> 11%; $n \geq 83$) (with face, eye, mouth and hindlimbs affected) compared to reference sites (≤ 0.7%; $n \geq 192$) (Fort & McLaughlin 2003). The spectrum of anomalies was similar to that obtained in in-situ caged tadpoles that excluded predators and in laboratory settings with water from the contaminated sites.

Anomaly hotspots in North America that are due to chemical pollution are not limited to amphibians but have also been reported for birds (Hoffmann et al. 1988) and reptiles (Ernst 1995, Bishop & Gendron 1998). There are also a number of publications that link increased rates of anomalies in humans in North America to chemical pollution, including those regions where amphibian anomaly hotspots also occur. However, such links have been even more rigorously rejected for humans than for amphibians (discussed by Lannoo 2008).

3.1.7.3 Predators. Predators were suggested as alternative explanation to chemicals for hotspots where ectromely and ectrodactyly were prevalent (Ballengée & Sessions 2009, Sessions 2009, Sessions & Ballengée 2010). Ballengée & Sessions (2009) derived their hypothesis from their own laboratory study with larval dragonflies of the genus *Sympetrum* and the European toad *Bufo bufo.* They cited several papers to support their claim that partial predation is common and the cause of anomalies at North American hotspots with ectromely. We have examined all the papers cited by them but none of them is relevant as none reported on staged predation experiments with amphibians; the large body of literature on that subject was ignored. In most staged predation experiments, including odonates and beetles, ectromely did not occur, and if it occurred, damage to the tails of tadpoles was also common (Henle et al. 2017a), which, unfortunately, is rarely assessed in the field. Moreover, ectromely was extremely rare (< 0.03%; n = 36,151) in eight anuran species examined from 37 ponds in Michigan in which larval *Sympetrum* achieved high densities (> 1 individual / m²) (Skelly & Benard 2010). This inconsistency between these field results and their laboratory study was rejected by Sessions & Ballengée (2010) as irrelevant for their conclusion that predator attacks are responsible for most cases of ectromely in natural populations of amphibians. However, inconsistencies between laboratory studies and field studies question the potential to extrapolate from laboratory conditions to field conditions.

Only three studies seem to have experimentally assessed the predator hypothesis for North American anomaly hotspots. One was negative (see the study above by Fort & McLaughlin 2003) and two were positive. At two hotspot sites in Oregon, Lake Aspen and two ponds on Broken Top volcano, the annual frequency of anomalies (mainly ectromely and ectrodactyly) in metamorphosed *Anaxyrus americanus* and *Lithobates cascadae* was as high as 35% (weighted mean 13%) n = 13,443) and 5–21% (n = ?), respectively (Bowerman et al. 2010). Tail damage was common in tadpoles at both sites. At Lake Aspen, annual rates of anomalies were related to stickleback (*Gasterosteus aculeatus*) abundance and at sites without sticklebacks less than 1% of the toadlets showed limb anomalies. Raising tadpoles in cages that excluded predators stopped the anomalies in tadpoles and toadlets but also excluded access to mud that could have contained teratogenic chemicals. Notwithstanding, laboratory experiments demonstrated that attacks from *G. aculeatus* were sufficient to explain the observed anomalies. Laboratory experiments also showed that nymphs of the dragonfly *Somatochlora albicincta* can inflict the same types of anomalies in *L. cascadae* as observed in the field while pond water and mud without predators resulted in normal froglets. As predators that are capable of inflicting ectromely also damage tails (Henle et al. 2017a), it is recommended that other studies testing the hypothesis of predators as a cause for hotspots dominated by ectromely also assess tail damage in tadpoles, which unfortunately is almost never done.

Although not severe hotspots, the prevalence of skeletal and eye anomalies at 38 sites of five Alaskan Wildlife Refuges ranged from 1.5–7.9% and was as high as 20% at one breeding site close to a former mine (Reeves et al. 2008). Ectromely, black eyes, micromely and brachydactyly were the most common types of anomalies. Polymely and polydactyly were rare and the trematode *Ribeiroia ondatrae* was absent. Proximity to roads significantly increased the risk of skeletal but not of eye anomalies. In a later study, Reeves et al. (2010) tested the association of anomalies with organic and inorganic contaminants, parasite infection, abundance of predatory invertebrates, UV-B and temperature for 21 wetlands in Kenai Wildlife Refuge, south-central Alaska. Logistic regression and model comparison using the Akaike information criterion (AIC) identified dragonflies and both organic and inorganic contaminants to be the best predictors for the probability of frogs acquiring skeletal

anomalies (individual frogs used as repeated measure). In predator exclusion experiments no tadpole had limb anomalies but 6.3% of the wild tadpoles at the same sites in the same years showed anomalies, which was significantly different. The cages also removed access of the tadpoles to mud so that two factors are confounded. Staged predation experiments were not carried out but limb amputation resulted in limb anomalies, which is an observation that has frequently been made before and depends on the stage, species and the degree of damage to the apical pit in the developing limb (see section 3.2.2.1). However, raising tadpoles in pond water with mud did not result in any limb anomalies, strongly supporting the hypothesis that predators were indeed responsible for the anomalies, except for the site with the highest rate of skeletal anomalies. At that site, several metals and organic contaminants exceeded toxic thresholds and odonate predators were not abundant, suggesting that pollution was a direct cause of the anomalies.

For eye anomalies, surprisingly, predatory beetles were the best predictors. Reeves et al. (2010) speculated that interaction with predators may induce tadpoles to take refuge in suboptimal habitats where they have to feed on a poor diet. However, while nutrition may change skin colouration and may cause limb anomalies, no effect on eye colouration has been discovered so far (reviewed by Henle et al. 2017a) and black-eyedness, which was the main eye anomaly observed by Reeves et al. (2010), is usually due to a recessive mutation (see section 3.3.5.2). The correlation may be spurious as predator exclusion did not change the frequency of eye anomalies and eye anomalies occurred when tadpoles were raised in the laboratory in pond water with access to mud. Also, temperature tested alone was found to be negatively associated with eye anomalies as well, although less strongly than predatory beetles. Interestingly, Vershinin (2004) noted in a long-term study that in years where no frosts occurred during the spawning period, no black-eyed *Rana arvalis* were discovered. Thus, a genetic basis with temperature acting epigenetically, may have been responsible for the black-eye anomalies.

The only other larger-scale correlational study using AIC model selection we know of found strong correlational evidence for predators (trouts – *Oncorhynchus mykiss*) and large conspecifics as cause for elevated rates of tail loss and ectromely (and other injuries?) in *Dicamptodon temebrosus* at 32 sites along the Fox Creek, Northern California (Munshaw et al. 2014). The average rate of injuries was 17% (n = 354) and increased with the size of individuals. Munshaw et al. (2014) found strong support for an effect of density of young-of-the-year *O. mykiss* and of the largest size class of the salamanders on the frequency of injuries. They explained the intraspecific effects with agonistic behaviour and not with attempted predation.

There are other North American studies that have implicated predators as the causes for ectromely and/or ectrodactyly (e.g., Martof 1956, Harris et al. 2001, Gridi-Papp & Gridi-Papp 2005, Kupferberg et al. 2009). Martof (1956) and Gridi-Papp & Gridi-Papp (2005) did not test their hypothesis. The percentage of abnormal individuals and/or sample sizes were low in the other two studies.

3.1.7.4 Disease and other causes. Diseases also caused anomaly hotspots in North America. At seven sites in Maine, Utah and North Dakota, hundreds and even thousands of dead larvae of *Ambystoma maculatum* and *A. tigrinum* were found. Sick individuals suffered from reddish skin and edema or swollen venter and legs (Worthylake & Hovingh 1989, Docherty et al. 2003). An iridovirus was isolated from sick larvae at six sites. At the 7th site (in Utah) no attempts to isolate viruses were made and anomalies were attributed to *Acinetobacter* bacteria (Worthylake & Hovingh 1989) but were most likely to be only secondary infections (Docherty et al. 2003).

In a population of *Rana muscosa* 41% (n = 387) of the tadpoles had depigmented and abnormal mouthparts and the anomalies were associated with *Batrachochytrium dendrobatidis* infection (Fellers et al. 2001). By comparison, in a study across 78 Californian ponds, the prevalence of the absence of dark keratinization of mouthparts in tadpoles of *Anaxyrus boreas, A. canorus, Pseudacris regilla* and *Lithobates catesbeianus* was not related to *B. dendrobatidis* infection (Padgett-Flohr & Goble 2007).

Finally, a hotspot of albinism is known. Childs (1953) discovered 298 (21%) albino tadpoles of *Spea hammondii,* which must have developed from 3–5 clutches.

3.1.7.5 Interactions among factors. It has been suggested that interactions among factors contribute to the appearance of hotspots of anomalies (e.g., Kiesecker 2002, Sessions 2009) but only two studies seem to have addressed this hypothesis in situ. Reeves et al. (2010) found that a significant negative interaction between odonate predators and metals best explained the probability of a frog having limb anomalies in *Lithobates sylvaticus* for 21 wetlands in Kenai Wildlife Refuge, Alaska. They came up with three possible explanations for this subadditive interaction. The one that was most compatible with the observed increased mortality rate of tadpoles raised in the laboratory with pond water and sediment was that in wetlands with high metal concentrations there was a higher mortality rate and thus fewer injured tadpoles survived to metamorphosis.

In field and laboratory experiments, Kiesecker (2002) discovered synergisms between anomalies caused by trematodes and water pollution. Larvae of *L. sylvaticus* were raised in enclosures in ponds that varied in exposure to agricultural run-off. In each pond, half of the enclosures prevented the cercariae of *Ribeiroia* from entering. The frequency of limb anomalies was higher in ponds exposed to contaminants than in those that had no detectable contaminants. No anomalies occurred in enclosures to which cercariae had no access and only 4% of the individuals in ponds without contamination but

exposed to cercariae were abnormal. Besides polymely, ectromely also accounted for a considerable number of the anomalies. This is surprising as in laboratory experiments polymely but not ectromely occurred when this frog species was exposed to *R. ondatrae* (Stopper et al. 2002). Either chemicals from agricultural run-off need not only have increased the susceptibility of the tadpoles to *R. ondatrae* but also changed the pattern of anomalies inflicted by them or the presence of the parasite made the tadpoles more sensitive to the effect of chemical pollution. In the laboratory, exposure to atrazine, malathion and esfenvalerate at low concentrations increased the rate of cercarial encystment and had a dramatic effect on immune response (Kiesecker 2002). However, currently no mechanism is known that could have caused a change in the types of anomalies produced by trematodes. This discrepancy requires further study. An increased sensitivity of tadpoles to agrochemicals in the presence of parasites might be related to the significant retardation of regeneration that was observed for larvae of *Ambystoma macrodactylum* with limb amputation exposed to *R. ondatrae* cercariae by Johnson et al. (2006). Furthermore, larvae with limb amputation developed 3–5 times more anomalies than those without amputation. Whether or not such a synergism also occurs when conspecifics or predators inflict injuries remains an open question.

Atrazine also increased the susceptibility of *L. pipiens* to infection by various trematode species in the field and a combination of atrazine concentration and phosphate accounted for 74% of the variation in trematode abundance across 18 wetlands in Minnesota, USA (Rohr et al. 2008). Unfortunately, the study did not quantify these associations for *R. ondatrae*, the only trematode known to cause mass anomalies in North America. Also, in a laboratory study, the infection rate of *L. sylvaticus* with the trematode *Echinostoma trivolvis* did not increase with atrazine treatment because both the host and the parasite were sensitive to atrazine (Koprivnikar et al. 2007). Notwithstanding, a relationship between eutrophication and anomalies induced by *R. ondatrae* is likely. Many of the hotspots described by Lannoo (2008) were strongly affected by eutrophication and nitrogen-based eutrophication has increased 20-fold in the USA over the past 50 years (Rouse et al. 1999). With increasing eutrophication shifts in snail communities occur and larger species, such as those that are intermediate hosts of *R. ondatrae,* increased in abundance (Johnson & Lunde 2005, Johnson et al. 2007). Furthermore, eutrophication enhances survival of infected snails, thereby increasing the period over which they release parasites. These changes can lead to higher infection rates in amphibians and consequently higher frequencies of anomalies.

Interactions among stressors, however, may also reduce the frequency of malformations, at least in mesocosms (Michel & Burke 2011). The presence of predators reduced the frequency of torsion of the tail in *L. sylvaticus* tadpoles, especially under low density.

3.2 Skeletal anomalies

3.2.1 Supernumerary elements

3.2.1.1. Polymely. Following Henle et al. (2017b), we include schizomely and polypody in the term polymely. Polymely is one of the most widely studied amphibian anomalies and a range of reviews on natural occurrences and/or potential causes is available (Taruffi 1880, 1881–1886, Ercolani 1881, Przibram, 1921, Guyénot & Schotté 1926, Guyénot et al. 1948, Butler & Schotté 1949, Rostand 1951b, Bennett 1957, Brunst 1961, Woitkewitsch 1961, Rose 1964, Thornton 1968, Scadding 1981, Tank & Holder 1981, Wallace 1981, Michael & Hassona 1982, Borkin & Pikulik 1986, Chapron 1986, Ouellet 2000). We found reports of 352 natural populations from 93 extant species. Two fossil *Chunerpeton tianyiensis* also showed abnormal polymely (Wang et al. 2016). Reported background rates are well below 0.1% (Tab. 1). Mass occurrence of polymely (at least 100 abnormal individuals, at least 10% of the abnormal individuals with polymely) was observed in 14 populations of seven species (*Ambystoma macrodactylum croceum, Anaxyrus americanus*, *Pseudacris regilla, Lithobates catesbeianus, L. clamitans, L. pipiens and L. septentrionalis*) in North America (Tab. 2). With six cases, *P. regilla* seems to be a particularly sensitive species. Outside North America, only six populations with high numbers or frequencies of polymely are known: a population of *Pelophylax* synkl. *esculentus* in Trevignon, France (Rostand 1952a,c, 1955b, 1958b, 1971, Dubois 1979b, 2014, 2017), a population of *P. ridibundus* near Almaty, Kazakhstan (Woitkewitsch 1955–1965), a Tunisian population of *P. saharicus* (Hassine et al. 2011), an Ecuadorian population of *Leptodactylus* sp. (Rostand 1958b) and a *Bufotes viridis* population in Roßwag, Germany (Henle et al. 2017a). In addition, 27 of 65 juvenile *P. ridibundus* sampled in two lakes in Kiev in 2001 were polymelous, but only a few individuals were affected in other years (Nekrasova et al. 2007). All cases, except *P. ridibundus* from Kazakhstan and *Leptodactylus* sp. from Ecuador, came from malformation hotspots, where various anomalies were observed.

The *B. viridis* case differs from all other cases of mass polymely because polymely is confined to the front legs as opposed to only or predominantly the hindlegs being affected in all other cases. The *P. ridibundus* population from Kazakhstan is unusual in that polymely always occurred on the right hindlimb with the left hindlimb being abnormal in only a few particularly severely malformed individuals (Woitkewitsch 1961). While supernumerary limbs in North American hotspots frequently show mirror symmetry (Rohr et al. 2009), this is usually not the case outside of North America (Figs. 6a, 10a,b). In fact, the supernumerary limb may develop incompletely and even be only a short stump as in some *B. viridis* from the Roßwag population mentioned above (Fig. 10c).

All North American cases, except for two populations of *L. catesbeianus*, with mass occurrences of polymely

Fig. 10: Different types of non-symmetrical duplications of limbs or parts thereof; a) asymmetric duplication of a hind limb in *Rana arvalis*, Chelyabinsk, Russia, 19.8.1981. Photo: V. Vershinin; b) incomplete duplication of the hand in a *Salamandra atra*, Hintersteiner Tal, Germany, July 2009. Photo: U. Schulte; c) rudimentary supernumerary limb in a juvenile *B. viridis,* Roßwag, Germany, October 1980. Photo: K. Henle.

were associated with high rates of infection with the trematode (*R. ondatrae*) and experimental infections invariably caused elevated rates of polymely (Johnson et al. 2001b). While only one field experiment identified *R. ondatrae* as the cause for a mass occurrence of polymely, there is strong evidence that it is the main factor for these mass occurrences in North America (see section 3.1.7.1 for further discussion and section 3.1.7.2 for an alternative explanation).

The cause for the hotspot in Kazakhstan remains unclear but is non-hereditary (see detailed discussion in section 3.1.2). The cause for the *Leptodactylus* sp. case from Ecuador (Rostand 1958b) also remains enigmatic. The French case is the most severe expression of anomaly P, which is characterized by severe polydactyly. Anomaly P is transmitted by fish; the causal factor remains to be identified but is most likely to be a virus (Dubois 1979b, 2014, 2017).

Hassine et al. (2011) and Nekrasova et al. (2007) assumed that pollution caused the anomalies, including polymely, in the Tunisian and Ukrainian populations, respectively, studied by them. Retinoids, vitamin A and colchicine are the only chemicals – save for surgically implanted crystals – for which the potential to induce polymely in regenerating limbs of amphibians has been repeatedly demonstrated (Tschumi 1954, Bruschelli & Rosi 1971, Maden 1982, 1983a,b, Syuzyumova 1985, Niazi & Ratnasamy 1984, Scadding & Maden 1986a,b, Bryant & Gardiner 1992, Gardiner & Bryant 1996). Colchicine treated amputated limbs produced polymely only at doses that were lethal when applied to the whole animal (Tschumi 1954). For a discussion of retinoids, agrochemicals and other pollutants that interact with the retinoid signalling pathway as a potential cause of polymely in natural populations of amphibians see section 3.1.7.2.

The cause for the *B. viridis* case in Germany was controversial. Flindt (1985a) linked it to hybridization and overripeness of eggs but only a strong mutagenic factor, most likely radioactivity, is consistent with the observations made for that case (Henle et al. 2017a). Contrary to the opinion of Flindt (1985a) polymely is an exception in hybrids. Only few, statistically insignificant cases of polymely have been observed in two independent crosses of *P. lessonae* × *P. esculentus* but not in 45 other crosses (Guex et al. 2001). In numerous crosses performed within *P.* synkl. *esculentus* by Berger (1971), only a single individual showed polymely.

Contrary to the belief of Flindt (1985a), polymely is not a characteristic developmental anomaly caused by overripe eggs. While Witschi (1920, 1922, 1925, 1952) reported polymely in *Rana temporaria* that were raised from overripe eggs (i.e., forced retention of the eggs in the uterus), he obtained only seven individuals in all his experiments (Witschi 1952). Five of these individuals originated from eggs from the same female and were raised at 28°C but none of 548 offspring of the same female raised at temperatures of 10–20°C were affected (Witschi 1920, 1925). Others (e.g., Bataillon & Tchou Su 1932, Briggs 1941, Rostand 1951c) did not succeed in inducing polymely in anurans, including *R. temporaria,* by the forced retention of eggs. Possibly, temperature exerted an epigenetic effect on a recessive mutation borne by the parental female.

Isolated cases of polymely are usually explained by hyperregeneration. Decades of research has shown that any mechanical perturbation that causes a physical rearrangement of cells in developing or regenerating limbs, such as the implantation of foreign tissue (Nassanov 1930, Bodemer 1959; reviewed by Ruben 1960), crystals of chemicals (Balls & Ruben 1964), or inert objects like resin beads (Sessions & Ruth 1990), cysts of

the trematode *Ribeiroia ondatrae* (Sessions & Ruth 1990, Johnson et al. 1999, Schotthoefer et al. 2003) and surgical rotation, ligature of legs, or cuts at an angle (e.g., Tornier 1901, Nassanov 1930, Scadding 1981, Tank & Holder 1981, Michael & Hassona 1982, Nye et al. 2003) can cause polymely. Injuries to the bones of the shoulder girdle or pelvis that cause a sliding partial separation of the injured parts may also lead to a duplication of the shoulder girdle or pelvis and associated limbs (Tornier 1901). However, simple amputation does not usually lead to a rearrangement of cells and thus does not usually induce hyperregeneration (e.g., Lecamp 1935, Holder et al. 1979, Stopper et al. 2002). Therefore, it is not surprising that polymely has never been reported in staged predation experiments. We only know three documented cases in which polymely developed after the partial loss of legs caused by conspecific attacks (Duméril 1867, Hellmich 1929a,b, Wolterstorff 1941).

Ming (2001) suggested that the ectroparasitic copepod *Lernaea cyprinacea* may also cause polymely by attaching deep in the dermis of the host. While such an attachment may cause a mechanical rearrangement of cells, it remains to be tested experimentally whether this parasite can actually induce polymely.

Two tadpoles of *Lithobates sylvaticus* that were exposed to a strong magnetic field (17,700 Gauss) at the egg stage developed supernumerary legs during the climax stage of metamorphosis (Levengood 1969) but the mechanism for this effect – and whether there is indeed a causal link – remains unclear.

Rostand (1951b) reviewed the genetic basis of polymely. The genetics of polymely has rarely been studied probably because such individuals are usually severely handicapped and only exceptionally survive to sexual maturity. Witschi & Chang (1954) demonstrated inheritance in a toad (species not provided) and Rostand (1958b) in a *Bufo bufo*. Polymely appeared in the first and second generation of irradiated eggs and sperm of *Pelophylax nigromaculatus* (Kawamura & Nishioka 1978).

3.2.1.2. Polydactyly and polyphalangy. Following Henle et al. (2017b), we include schizodactyly (split fingers) (Fig. 11) and polyphalangy (serial duplication of a phalange within a digit) in the term polydactyly. Cases in which the additional digit severely bends outwards (Fig. 12) were called clinodactyly rather than schizodactyly by some authors (e.g., Vershinin 2002). Here we include such cases under polydactyly, if we were confident that the anomaly in question was a split or additional digit, and restrict clinodactyly to the abnormal bending of a finger without any duplication.

Meteyer (2000) introduced a new terminology for polyphalangy and called all cases of polydactyly, in which the metatarsal or metacarpal is not duplicated, polyphalangy. However, absence or presence of these bones usually cannot be assessed in the field (see for example Fig. 13) and her definition deviates from the es-

Fig. 11: Schizodactyly, a specific form of polydactyly, in *Lissotriton vulgaris*, Ekaterinburg, Russia, 2002. Photo: V. Vershinin.

Fig. 12: Polydactyly, combined with brachydactyly (and lack of musculature), in a male *Rana arvalis*, Kalinovsky Forest Park, Ekaterinburg, Russia, 26.9.2009. This type of polydactyly is called clinodactyly by some authors because the additional finger is bent outwards. Photo: V. Vershinin.

Fig. 13: Symmetrical polydactyly in an adult *Pelophylax ridibundus*, Southern Ural, Russia, 2012. Photo: V. Vershinin.

tablished terminology (Henle et al. 2017b). Thus, one needs to be aware that recent publications mentioning polyphalangy may not actually be polyphalangy in our definition.

In urodeles abnormal polydactyly and polyphalangy may be difficult to distinguish from the normal pattern of variability in skeletal elements of the foot and hand. For example, in *Salamandrella keyserlingii,* in some but not all regions there is a high variation in digital bones, including polyphalangy and supernumerary digits (Fig. 34b), and only 30% or fewer of the animals may have the normal set of digits. Therefore, some authors (e.g., Borchvardt & Ivaschintsova 1993, Borkin 1999, Zmeeva 2014) regarded deviations from the normal number of digits as natural variation. However, others referred to such deviations as abnormal (e.g., Vershinin 2002, Kuzmin & Maslova 2003). Therefore, we included those cases classed as abnormal by authors in our database but not those cases classed as normal.

Excluding those publications where it remains unclear which definition of polyphalangy was used, polyphalangy has only been reported for 20 cases from 5 extant urodele and 7 extant anuran species, all except for one involving only a few specimens. In addition, Wang et al. (2016) reported it for the fossil *Chunerpeton tianyiensis*. Zamaletdinov (2014) reported polyphalangy for ten populations belonging to five species (*Bufo bufo, Bufotes viridis, Pelophylax lessonae, P. ridibundus, Rana arvalis*) in Tatarstan, Russia. In most locations, only a few individuals were affected but at Dogaya Polyana 47 out of 64 *P. ridibundus* exhibited it. Mikitinez (2014) observed polphalangy in Ukrainian *Pelobates fuscus, Pelophylax esculentus* and *P. ridibundus* and Svivin (2014) in Russian *P. lessonae* and *P. ridibundus*. Diego-Rasilla et al. (2007) described it for a male and an albinistic female *Triturus marmoratus* in Spain, D'Amen et al. (2006) for a *T. carnifex* in Italy, Hanken (1983) for one *Plethodon cinereus* ($n = 100$) in Canada and Chang & Boring (1935) for at least three Chinese *Batrachuperus pinchonii* (as *T. sinensis*) and one *Hypselotriton orientalis*. These cases are not included in the figures of polydactyly given below unless the duplication of digits was reported as well.

Several reviews of polydactyly in natural populations are available (Rostand 1951a, 1958b, 1971, Van Valen 1974, Dubois 1979, Borkin & Pikulik 1986, Ouellet 2000) but none on its potential causes. Our review resulted in 281 cases from 87 extant species. Wang et al. (2016) also observed abnormal polydactyly in 14 individuals of the fossil salamander *Chunerpeton tianyiensis*. Reported background rates ranged up to 1% (Henle et al. 2017a). The earliest illustration of a polydactylous frog is in Seba (1735: Tab. XIII, Fig. 3). However, it is unclear whether it is truly a polydactylous frog or the liberty of the artist because both front limbs have five fingers and symmetrical front limb polydactyly is very rare. On the other hand, all other amphibians illustrated show only four fingers. Virey (1819) was the first who explicitly mentioned polydactylous frogs.

Most cases of mass occurrences of polydactyly were reported either in green frogs of the genus *Pelophylax*, for North American malformation hotspots, or for polluted sites in Asia and Africa. Mass occurrence of polydactyly was first discovered in *P.* synkl. *esculentus* (Bonnet & Rey 1937) in France and termed anomaly P by Rostand (1952a). Anomaly P is characterized by, albeit not always perfect, symmetrical polydactyly that primarily affects the hindlimbs (Fig. 5). Anomaly P has been detected at 13 locations in France, with the frequency varying from 0 to 80% in tadpoles and from 2.6% to 14.5% in adults in different years (Bonnet & Rey 1937, Dubois 1968, Rostand 1952a-c, 1957, 1962, Rostand & Darré 1967, 1968, reviewed by Rostand 1958b, 1971 and by Dubois 1979, 1984, 2014, 2017). Mass occurrences that probably were related to anomaly P were further observed in one population of *P.* kl. *esculentus* in The Netherlands, with 45% of the sample being affected (Rostand 1957, Hillenius 1959), in populations of *P. esculentus* (7.9% affected; $n = 69$) and *P. lessonae* (27% affected; $n = 161$) at the Alba Fisheries in Byelorussia (Borkin & Pikulik 1986) and in *P. saharicus* (25% affected) in Morocco (Rostand 1971). At the hotspot site in Bugornia, Pridnestrovie district, Russia (section 3.1.1), 5% of the *P. ridibundus* individuals exhibited polydactyly. However, more than 40% showed ectrodactyly and most probably there is no relationship to anomaly P. In two populations of *Bufo bufo* from near Tambov, Russia, 24.9% ($n = 229$) of the adult toads showed symmetrical polydactyly of the hindlimb (Lada (1999). The symmetry of the anomaly is reminiscent of anomaly P although so far anomaly P is known only for green frogs of the genus *Pelophylax*.

Rostand and co-workers conducted a range of experiments to discover the cause(s) of anomaly P. It is not inherited and the causative factor is still unknown but likely to be a virus transmitted by fish (Rostand 1971; see Dubois 2014, 2017 for recent reviews). Rostand failed to evoke polydactyly in *P. esculentus* and/or *R. temporaria* by applying various physical stresses, such as thermic shocks, long exposure to high or low temperature, dehydration, long-term insolation (Rostand 1950a, 1959), or by overripeness of eggs (Rostand 1951c). Rearing eggs and tadpoles of *P. esculentus* in brackish water did not produce polydactyly (Rostand 1952b, 1958b). Rostand (1950b) also obtained negative results with various chemicals and this is also the case for all chemicals in our database except when combined with amputation (see below). We also failed to find a single published case of experimental hybridization that resulted in polydactyly. After irradiation of embryos, polydactyly occured in low numbers in *B. bufo* (Rostand 1955a, 1958a, Oerter 1985).

In North America, elevated rates of polydactyly have been observed in six populations of five species (Tab. 4). All of these cases were from hotspots where other types of anomalies were common as well. Apart from the population studied by Williams et al. (2008), for which there is no indication of a cause, these cases were associated with and presumably due, at least in part, to

Table 4: Elevated rates of polydactyly in North American populations of amphibians (≥ 5% of the population abnormal, ≥ 2.5% of the population with polydactyly, ≥ 10 polydactylous individuals). *N*: sample size; only cases with *N* ≥ 50 included; % polydactyly refers to the sample size. Cause: (e): the cause was supported by experimental studies; all other causes are assumptions.

Species	*N* (% abnormal)	% polydactyly	Location	Cause	References
Ambystoma macrodactylum	11,732 (14.8%)	≥4.4%	Aptos Pond, California	*Ribeiroia ondatrae* (e)	Sessions & Ruth 1990, Johnson et al. 2003, 2006
Ambystoma macrodactylum	520 (11.5%)	≥2.7%	Jette Pond, Montana	*Ribeiroia ondatrae* (e)	Johnson et al. 2003, 2006
Ambystoma tigrinum	1946 (8%)	4.5%	Wetland in Tippecanoe County, Indiana	Unknown	Williams 2007, Williams et al. 2008
Anaxyrus americanus	618 (59.5%)	3.4%	Duck Pond, Minnesota	*Ribeiroia ondatrae*	Johnson & Hartson 2009
Lithobates septentrionalis	869 (67.5%)	3.2%	CWB, Minnesota	Competing hypotheses[1]: *Ribeiroia ondatrae*, retinoids, micronutrient deficiency, and pollution	Vandenlangenberg et al. 2003, Hoppe 2005

[1] For competing hypotheses see Gardiner & Hoppe (1999), Stopper et al. (2002), Lannoo (2008), Helgen (2012) and section 3.1.7.

infections by the trematode *Ribeiroia ondatrae* (see section 3.1.7 for a discussion).

Chemicals have been suggested as an alternative explanation for mass anomalies observed in North American amphibians, not specifically for polydactyly, but rather for polymely (see section 3.1.7.2). As for polymely, retinoic acid (Scadding & Maden 1986b, Crawford & Vincenti 1998), colchicine (Tschumi 1954) and aminopterin (Gebhardt & Faber 1966a,b) treatments applied to amputated limbs caused polydactyly – aminopterin only, when amputation occurred proximally and only when applied at an early stage of limb regeneration. For thalidomide and its analogue EM_{12} inconsistent results have been published. Whereas Bazzoli et al. (1977) obtained increased rates of polydactyly, others did not report such an effect (Gebhardt & Faber 1966a, Dumpert & Zietz 1984). The difference may be due to stage-specific effects. Unfortunately, no North American study experimentally tested the hypothesis that mass occurrences of polydactyly may have been caused by chemicals.

While field tests on the possible effect of chemicals on the occurrence of polydactyly are lacking, a few studies showed an association of polydactyly with pollution of breeding sites, both in the Old and the New World. Bacon et al. (2006b) found at least 122 (approx. 1%) polydactylous *Rhinella marina* in Bermuda. Breeding ponds with elevated rates of anomalies were contaminated with petroleum hydrocarbons and metals at teratogenic concentrations (Fort et al. 2006a,b). Microcosm experiments with sediment and water from polluted sites also induced high rates of polydactyly (Bacon et al. 2013) and trematodes could be excluded as a cause (Bacon et al. 2006b).

In Tunisia, 19 of 221 *P. saharicus* metamorphs exhibited polydactyly (and various other skeletal anomalies) at the Lebna Dam, which is situated in intensively used agricultural area and contaminated by agrochemicals, whereas anomalies were absent in non-agricultural areas (Hassine et al. 2011). At polluted sites in Russia 18 (0.3%) *Rana pirica,* 230 (4.4%) *P. ridibundus and* 74 (2.5%) *Bombina bombina* exhibited polydactyly (Borkin & Pikulik 1986, Flax & Borkin 2004). In the latter two species, the frequency of limb anomalies (ectromely and ectrodactyly in addition to polydactyly) increased with the biological oxygen demand of the breeding sites. Whether chemicals played a direct or indirect role for these cases remains to be studied. The facts that retinoic acid can induce polymely and various agrochemicals (Mann et al. 2009, Paganelli et al. 2010), polychlorinated toxicants and estrogen may increase internally cycling levels of retinoic acids and that in one study compounds in pulp mill effluents (probably originating from the wood used) bound to retinoic acid receptors (Alsop et al. 2004), support the assumption of a direct effect.

Vershinin (2010) collected 10 (n = 294) *Bufotes viridis* with schizodactyly (he referred to it as clinodactyly) in presumably polluted sites in the vicinity of industrial premises in Magnitogorsk, South Ural (Russia); on the other hand, *Bufo bufo* from a relatively unpolluted site, Kuzino village (Sverdlovsk District, Russia), had similar

high rates (22 of 183 individuals). Slightly elevated rates of polydactyly (0.7–1.4%, n_{total} = 13,815) were further observed in *Cynops pyrrhogaster* from Honshu, Japan, in the years 1981–1985 (Meyer-Rochow & Asashima 1988). The rate dropped to less than 0.1% in 1995 (Fukui et al. 1996). The decline was explained by improved water quality – but no data on water quality were provided.

Elevated rates of polydactyly were further reported for a population of *Triturus pygmaeus* (2.3%, *n* = 557) in Portugal (Caetano 1999), *B. bufo* (5%, *n* = 317) in Russia (Borkin & Pikulik 1986) and *R. arvalis* (2.6%, *n* = 464) in Byelorussia (Borkin & Pikulik 1986). The cause for these cases is unknown.

Isolated cases of polydactyly are most frequently explained by hyperregeneration. As for polymely (see section 3.2.1.1), polydactyly can be induced by mechanical perturbation that causes a physical rearrangement of cells in the developing or regenerating digits, such as the surgical implantation of inert objects such as resin beads (Sessions & Ruth 1990), surgical rotation (Lecamp 1935) and cell death caused by irradiation (Rieck 1954). In contrast to polymely, polydactyly (schizodactyly) can occur in high numbers after amputation without further manipulation as observed after toe-clipping in *Ambystoma talpoideum* (Semlitsch et al. 1981). Experimental infections with the trematode *Ribeiroia ondatrae* also caused significantly elevated rates of polydactyly in *Pseudacris regilla* and *Lithobates pipiens* (Johnson et al. 1999, Schotthoefer et al. 2003) but experiments with the trematode genus *Alaria* were negative (Johnson et al. 1999). In experiments with Sri Lankan monostome-type trematodes, three *Polypedates crucifer* (*n* = 218) developed polydactyly (Rajakaruna et al. 2008) but this was not significantly different from controls (χ^2= 1.084; $\alpha > 0.2$).

Dubois (1974) observed polydactyly, linked to clinodactyly, in a population of *Rana graeca* for which he hypothesized a genetic cause, based on crossing experiments in *B. bufo*. He reported recessive genetic transmission of polydactyly linked to clinodactyly in *B. bufo*. Genetic transmission of polydactyly is well-documented in *B. bufo*. Rostand (1949a) detected 53 polydactylous adult male *B. bufo* among the 44,000 that were examined. The breeding experiments conducted by him revealed that at least some of them were of genetic origin, being caused either by a dominant or recessive allele (Rostand 1947, 1949b, 1950d, 1951a, 1958b). Inheritance of polydactyly was further demonstrated in *Xenopus laevis* (Uehlinger 1969, Droin & Fischberg 1980). Hereditary polydactyly is also well known in mice and humans (Cohn & Bright 1999, Gurrieri et al. 2002).

3.2.1.3. Supernumerary bone at lower jaw. Christaller (1983) described a single individual of *Bufotes viridis* collected at Roßwag in Germany with a supernumerary bone at the lower jaw and regarded it as an extension of the lower mouth. Flindt (1985*a*) suggested that this anomaly was an additional limb and not a jaw malformation. This may be the case but the printed photograph is inconclusive. In the same population, a toadlet had a very short bony protuberance present at the angle of the left yaw and an additional protuberance on the right side of the head (Henle et al. 2017a). A supernumerary limb originating from the jaw was illustrated for a *Pelophylax nigromaculatus* by Iwasawa & Takasu (1985) and for an experimentally produced adult hybrid *Ceratophrys cranwelli* × *C. ornata* by Miranda (1989).

3.2.1.4. Tail bifurcation and duplication. Tail duplications have rarely been reported from natural populations (reviewed by Henle et al. 2012). They listed 11 publications that mentioned a total of 22 affected larval amphibians belonging to 15 species. Twenty publications described the incidence for 36 individual postlarval urodeles from 13 species. In experiments, the duplication of tails was induced by destroying parts of the chorda dorsalis, by injecting tar into the tail or by irradiating the lumbar region.

We found eight additional papers on this topic, adding seven more species. One *Ichthyosaura alpestris* from approx. 30,000 individuals examined in an area near Bonn, Germany (Hachtel 2011), and one adult female *Taricha torosa* among 22 adults examined from Boulder Creek, California (Wolterstorff 1935), had a split tail. Smirnov (2014) reported one male *Lissotriton montandoni* with tail bifurcation. Reeves et al. (2013) listed one (*n* = 52) *Pseudacris triseriata,* one (*n* = 121) *Lithobates pipiens,* one (*n* = 53) *L. sphenocephalus* and seven tadpoles of *L. sylvaticus* originating from six populations (10,345 individuals and > 100 populations exmined) in US national wildlife refuges. Levey et al. (2003) also reported one *L. pipiens* with a forked tail. Finally, Medina et al. (2013) listed one tadpole (*n* = 211) of *Boana riochana* from Argentina. Two papers added observations for species, for which tail bifurcation or duplication was already reported. Fukui et al. (1996) printed a photo of tail bifurcation in a *Cynops pyrrhogaster*. Up to four individuals may have been affected. Hiller (1986) observed two lateral tail appendages in a *Pelobates fuscus* tadpole.

3.2.1.5. Dicephaly and Siamese twins. Externally visible duplications of body parts other than limbs seem to be very rare in amphibians and were observed mainly in captivity. While there are various ancient but unreliable stories (discussed by Gesner 1554), Wurffbain (1683) was presumably the first to report a reliable observation: a two-headed salamander that was exhibited by Mercerus. Funk (1827) provided the first more detailed description of a dicephalic embryo of *Salamandra salamandra*. Jourdain (1877) briefly mentioned an individual with two heads that were almost completely fused but exhibited two faces and a Siamese twin that was fused at the sternum and umbilical scar. Furthermore, Braun (1875), Susebach (1941) and Wolterstorff & Freytag (1941) reported further dicephalic larvae and/or Siamese twins of the same species. While most if not all of these observations derived from individuals born

in captivity, the following observations presumably apply to individuals caught in the wild: a twin embryo (Lébédinsky (1921) and a dicephalic tadpole of *Rana temporaria* (Loyez 1897) and a *Bufo bufo* tadpole with a partially duplicated head (Canella 1935).

The following observations were definitely from natural populations. Schwind (1942) reported a total of 5 and 29 eggs with twin embryos of *Ambystoma maculatum* and *Lithobates sylvaticus*, respectively (plus further ones that were manipulated before the twins were detected). Lynn (1944) found one egg with twin embryo of *Eleutherodactylus alticola* among 20 eggs examined, Lindberg (1995) three eggs (n = 7846) with twin embryos of *Ambystoma tigrinum* and Hamed et al. (2015) one egg (n = 14,379) with twin embryos of *Hemidactylium scutatum*. Dragoiu & Busnitza (1927) detected a dicephalic embryo in a clutch of *Bombina bombina*. Donaire (2016) found a Siamese a twin larvae of *S. algira* and Rivera et al. (1993) collected a larval *S. salamandra* with two heads near Barcelona, Spain, which metamorphosed successfully in captivity. Marvin & Hutchison (1997) found a *Plethodon kentucki* with two heads, Pereira & Rocha (2004) found one such larval *Chioglossa lusitanica* and Schmidt (1930) found one such tadpole of *R. arvalis*. Fernández-Álvarez et al. (2011) collected a dicephalic embryo of *Lissotriton boscai*, which soon died. Bei et al. (2011) discovered a dicephalic larval *Ichthyophis bannanicus*.

Tornier (1901) suggested that the duplication of heads and Siamese twins result if the vertebrae and the adjacent skin are ruptured in embryos He further suggested that only supernumerary vertebrae will develop if the adjacent skin does not rupture as well. He did not provide any experimental evidence for his hypothesis. Experimental splitting of the head in young tadpoles did not result in head duplications. Rather, the injury healed normally (Vulpian 1862).

3.2.2 Absence and reduction of skeletal elements

3.2.2.1 Ectromely. The term ectromely is not used consistently in the literature. Following Henle et al. (2017b), we define ectromely as the partial or complete absence of a limb from distal to proximal. The term thus includes apody, hemimely and amely but neither phocomely nor brachymely.

So far, ectromely in natural populations has only been reviewed by Ouellet (2000). Our database contains 666 cases from 129 species. Reported background rates reach up to 2.4% (Tab. 1). Most mass incidences of ectromely have been observed in North America but cases are also known from Europe and Asia. From North America at least 15 cases belonging to 11 species are known, in which at least 5% of the population were abnormal and at least 2.5% of the population and ten individuals were affected by ectromely (Tab. 5). For regions outside of North America there are ten cases from six species. There are other cases with high numbers of ectromelous individuals but either insufficient data were provided to include them in Table 5 or they were summed across several populations. Suggested causes were parasitism, trauma due to attacks from predators or conspecifics and chemicals for the North American cases and trauma, chemicals and radioactivity for the remaining cases.

The cause(s) of the North American cases are controversial. Most frequently, infection from the trematode *Ribeiroia ondatrae* has been assumed as the cause (Tab. 5). However, in experiments, such infections did not result in elevated frequencies of ectromely and ectromely was even absent in some experiments: *Ambystoma macrodactylum* (Johnson et al. 2006), *Anaxurus americanus* (Johnson & Hartson 2009), *A. boreas* (Johnson et al. 2001b), *Dryophytes versicolor* and *D. cinereus* (Johnson & Hartson 2009, Johnson et al. 2012), *Lithobates pipiens* and *L. sylvaticus* (Stopper et al. 2002, Schotthoefer et al. 2003). The only known exception is *Pseudacris regilla* (Johnson et al. 1999, Romansic et al. 2011). Thus, *R. ondatrae* is unlikely to be a cause of elevated rates of ectromely, except perhaps for the one case involving *P. regilla*. Notwithstanding, *R. ondatrae* may have contributed to other anomalies observed in the same populations – see section 3.1.7.1 for further discussion).

Parasites other than *R. ondatrae* have been suggested once for a North American case of elevated rates of ectromely (not listed in Table 5). Kupferberg et al. (2009) reported a significantly higher percentage of abnormal *Rana boylii* (ectromely and other anomalies combined) in 2006 that were carrying parasitic copepods *Lernaea cyprinacea* attached to their limbs compared to those individuals that were free of the parasite. However, this was not the case in 2008 and the cause was not tested experimentally.

Trauma as an alternative explanation was only tested experimentally for the two German populations of *Bufo bufo* listed in Table 5. These exeriments demonstrated respectively made it very likely that the leech *Erpobdella octoculata* was the culprit (Bohl 1987, Veith & Viertel 1993). For the German population of *Rana temporaria* in Table 5, observations in aquaria supported the hypothesis that attacks from sticklebacks (*Gasterosteus aculeatus*) were the cause (Malkmus 2008). In addition, for two North American cases with elevated rates of ectromely, *A. boreas* in Lake Aspen and *Lithobates cascadae* at Broken Top, that are not listed in Table 5 because insufficient data were published, in-situ and laboratory experiments identified trauma inflicted by *G. aculeatus* and dragonfly larvae (*Somatochlora albicincta*) as the cause (Bowerman et al. 2010). While the exclusion experiments in the field also removed the contact with mud and thus results cannot unequivocally be attributed to the exclusion of predators, laboratory experiments, in which *L. cascadae* tadpoles had access to mud, did not produce elevated rates of ectromely or ectrodactyly whereas nymphs of *S. albicincta* did.

Sessions (2009) and Ballangée & Sessions (2009) attributed all cases of ectromely in natural populations to such failed predation attacks. However, one must be

Table 5: Elevated rates of ectromely in natural populations of amphibians (≥ 5% of the population abnormal, ≥ 2.5% of the population with ectromely, ≥ 10 ectromelous individuals). *N*: sample size; only cases with *N* ≥ 50 included; % ectromely refers to the sample size. Cause: (e): the cause was supported by experimental studies, (c): correlational evidence; all other causes are assumptions.

Species	*N* (% abnormal)	% ectromely	Location	Cause	References
North American cases					
Cryptobranchus bishopi	96 (39.6%)	≈ 6%	Eleven Point River, Arkansas	Trauma (fishing and conspecifics)	Hiler et al. 2005
Ambystoma macrodactylum croceum *Pseudacris regilla*	11,732 (14.8%) 391 (54.2%)	≥2.8% 11.3%	Aptos Pond, California	*Ribeiroia ondatrae*[1] *Ribeiroia ondatrae* (e)	Sessions & Ruth 1990, Johnson et al. 2003, 2006
Ambystoma macrodactylum	183 (15.3%)	≥10.4%	Toolman, Montana	Trauma (conspecifics), *Ribeiroia ondatrae*[1]	Johnson et al. 2006
Ambystoma macrodactylum	403 (35%)	≥18.1%	Potholes, Oregon	Trauma (conspecifics)	Johnson et al. 2006
Taricha torosa	647 (41.3%)	≥5.7%	Frog Pond, California	Unknown	Johnson et al. 2001a
Taricha torosa	961 (15.4%)	≥7.5%	Hidden Pond, California	Unknown	Johnson et al. 2001a
Anaxyrus americanus	618 (59.5%)	≥5.8%	Duck Pond, Minnesota	Unknown (*Ribeiroia ondatrae* assumed[1])	Johnson & Hartson 2009
Dryophytes cinereus	100 (17%)	12%	Dahomey National Wildlife Refuge (DHM03), Mississippi	Unknown	Reeves et al. 2013
Dryophytes chrysoscelis or *D. versicolor*	50 (24%)	24%	Oil pit, D'Arbonne National Wildlife Refuge (DRB03), Louisiana	Contamination by petrochemicals	Reeves et al. 2013
Lithobates clamitans *Lithobates septentrionalis*	245 (33.5%) 869 (67.5%)	6.1% 4.3%	CWB site, Minnesota	Competing hypotheses[2]: *Ribeiroia ondatrae*, retinoids, micronutrient deficiency and pollution	Vandenlangenberg et al. 2003, Hoppe 2005
Lithobates pipiens	1394 (20.3%)	8.8%	ROI site, Minnesota	Unknown; *Ribeiroia ondatrae* was not found but estrogenic substances and pesticides	Helgen et al. 2000, Garber et al. 2001, Vandenlangenberg et al. 2003
Lithobates pipiens	2493 (7.4%)	≈ 5%	NEY pond, Minnesota	Competing hypotheses: *Ribeiroia ondatrae*[1], chemical pollution	Canfield et al. 2000, Vandenlangenberg et al. 2003, Lannoo 2008
Rana luteiventris	280 (30.7%)	3.2%	Isobel Lake, British Columbia, Canada	Unknown (*Ribeiroia ondatrae* assumed[1])	Roberts & Dickinson 2012

Table 5: Continuation.

Species	*N* (% abnormal)	% ectromely	Location	Cause	References
Other regions					
Salamandra salamandra terrestris	456 (13.4%)	9%	Hummingenbach, Harburg, Germany	Trauma [conspecifics, dragonfly larvae (*Cordulegaster boltoni*)]	Giesenberg 1991
Salamandra salamandra terrestris	768 (11.8%)	11.6%	Künningbach, Niederbergisches Land, Germany	Trauma (conspecifics, *Neomys fodiens*)	Thiesmeier-Hornberg 1988, Thiesmeier 1990, 2004
Bufo bufo	≈ 500 (42.7%)	Most[3]	Fish pond, Aufseß, Germany	Trauma [leeches (*Erpobdella octoculata*)] (e)	Bohl 1997
Bufo bufo	2480 (14.8%)	≈ 10%[4]	Fish pond, Remagen-Oedingen, Germany	Trauma [leeches (*Erpobdella octoculata*)] (e)	Veith & Viertel 1993
Bufotes viridis	675 (7.4%)[5]	3.7%[5]	Roßwag, Germany	Radioactivity (e)	Henle et al. 2017a
Hyla arborea	87 (18.4%)	17.4%	Schmielteich near Wurzen, Germany	Trauma or chemical pollution	Grosse & Bauch 1988
Rana temporaria	430 (99.1%)	18%	Spring fed pond, Lehngrund, Spessart, Germany	Trauma (*Gasterosteus aculeatus*)	Malkmus 2008
Rana pirica	1095 (≥ 42%)	42%	Point A, South Sakhalin Island, Russia	Sewage effluent of paper and pulp mill (c)	Mizgireuv et al. 1984
Rana pirica	1614 (≥ 31%)	31%	Point B, South Sakhalin Island, Russia	Pollution from municipal sewage (c)	Mizgireuv et al. 1984
Rana pirica	3651 (≥ 39%)	39%	Point C, South Sakhalin Island, Russia	Pollution from municipal sewage (c)	Mizgireuv et al. 1984

[1] Experimental infections with *Ribeiroia ondatrae* do not result in elevated frequencies of ectromely, except for *Pseudacris regilla* (see section 3.1.7.1).
[2] For competing hypotheses see Gardiner & Hoppe (1999), Stopper et al. (2002), Lannoo (2008), Helgen (2012) and section 3.1.7.
[3] No figure provided but most individuals from a separate experiment with caged individuals showed ectromely.
[4] Calculated based on a subsample for which numbers of abnormal individuals were provided for different types of anomalies.
[5] Data for 1983, similar in 1980 but no exact counts available, lower in 1982 and 1984

careful when extrapolating from staged predation experiments under laboratory conditions to the field because whether predators amputate (parts of) legs or not depends on the predator and prey species and details of the experimental set-up (Henle et al. 2017a). Therefore, the hypothesis needs testing under field condition to demonstrate that it is the cause of an observed mass occurrence of ectromely (see section 3.1.5.3 for further discussion).

Meteyer et al. (2000a), Ouellet (2000) and Lannoo (2008) suggested that ectromely caused by predators can be differentiated from malformation during

limb development by disruption of the pigment pattern or the appearance of a regenerative spike but no study has tested this hypothesis explicitly. However, in *Pelobates fuscus* and *L. sylvaticus* the wound healed without the development of a spike and rapidly returned to a normal colour pattern after amputation (Kammerer 1905, Reeves et al. 2010). Similar observations were made in amputation studies by Lannoo (unpublished, fide Levey et al. 2003). On the contrary, a spike was observed in several hemimelous individuals of *Bufotes viridis* from the hotspot at Roßwag in Germany that developed in a predator-free pond. The spikes must therefore have originated from abnormal development, most likely due to radioactive contamination (Henle et al. 2017a). Likewise, legs may atrophy and the pigment pattern may be disrupted in diseased individuals, as reported by Wisniewski (1984) for *Cynops ensicauda popei* and *Paramesotriton chinensis* that were infected by *Saprolegnia*. Further, correlational evidence exists that infection from the pox virus may lead to ectromely with a disrupted colour pattern due to necrosis (Cunningham et al. 1993). Thus, ectromely can only be attributed with certainty to trauma if the wound is fresh.

Besides conspecifics and predators, trauma may be caused by machinery. Storer (1925) attributed a case to lawn mowing, in which approximately 50% of the sampled *A. boreas* lacked (parts of) limbs. In Poland Classen et al. (1996) found that large numbers of anurans were injured or killed during grassland mowing with the impact depending on the type of mowing machines used; however, they did not provide figures about the number of ectromelous anurans surviving. Injuries from agricultural machinery was also the likely cause for a case of mass occurrence of ectromely in waterfrogs (*Pelophylax* synkl. *esculentus*) living in an agricultural landscape dominated by crop fields in Germany, as in many of the individuals their wounds had only partially healed (Schmidt & Hachtel 2011).

Amputated limbs may regenerate in amphibians, with urodeles retaining this capacity more widely and for later stages in ontogeny than anurans (Sánchez Alvarado & Tsonis 2006)). In anurans it is a complex phenomenon (Bryant et al. 1987, Gardiner & Bryant 1996) that depends, among other factors, on the level of amputation, severity and the larval stage (Muneoka et al. 1986, Girvan et al. 2002, Ballengée & Sessions 2009). In early larval stages ectromely requires the removal of the apical ectodermal ridge of the amputated limb or cell death in the apical 200 mm of the mesenchyme (reviewed by Nye et al. 2003). Only structures distal to the amputation plane are formed. Late stage tadpoles have reduced ability and adults lose the ability of limb regeneration in most anuran species (Thornton 1968, Scadding 1981, Girvan et al. 2002) but some species are still able to produce cones or rods (reviewed by Kurabuchi & Inoue 1982).

Pollution by chemicals has also been suggested as a cause for the mass occurence of ectromely but is debated for North American hotspots. Whereas tadpoles raised in water samples from the CWB site in Minnesota developed elevated rates of anomalies, those raised in unpolluted controls did not (Volpe & Rosenbaum 2000, Bridges et al. 2004). However, ectromely was not among the types of anomalies reported at an elevated rate. Notwithstanding, there is evidence of chemical contamination from agricultural run-off as cause for elevated rates of ectromely in some regions of Minnesota, Alaska and Canada (Ouellet et al. 1997, Burkhart et al. 1998, 2000, Fort et al. 1999a, Bridges et al. 2004, Lannoo 2008, Reeves & Trust 2008). For example, Fort et al. (1999b) demonstrated for a Minnesota case that only some fractions of the water and sediment samples were able to induce anomalies and these contained a mixture of herbicides and pesticides, of which maneb is capable of causing ectromely (Zavanella et al. 1984, Fort et al. 1999b).

In addition to maneb, various other chemicals are capable of inducing ectromely at environmentally relevant doses, including some widely used pesticides, such as the mosquito-control agent s-methoprene (commercially known as Altosid) and malaoxon (a metabolite of the insecticide malathion) (Tab. 3). The effects of retinoids differ among species, are limited to a narrow window of development (Degitz et al. 2000) and might not even have an effect with continuous flow exposure (Degitz et al. 2003a for *Xenopus laevis*). Only those limb elements are suppressed that start differentiation when retinoids are administered (Niazi 1996). Thus, rather specific conditions would be required for them to contribute to mass occurences of ectromely in natural populations of amphibians.

Outside of North America four cases of mass occurrence of ectromely were associated with polluted sites (Tab. 5). The globally most severe cases were reported by Mizgireuv et al. (1984) for three points on southern Sakhalin Island, Russia. They discovered almost 2000 *R. pirica* (as *R. chensinensis*) (31–42% of the samples) with ectromely in ponds contaminated with municipal and paper and pulp mill sewage. The frequency of ectromely correlated with the oxygen demand of water samples from the breeding sites. For two respectively one Ukrainian district(s) mass occurrence of ectromely of *P. ridibundus* (535 individuals; 10% of the samples; several populations combined) and *Bombina bombina* (280 individuals; 10% of the samples; several populations combined) was reported by Flax & Borkin (1997, 2004). The frequency was also strongly correlated with oxygen demand and was significantly higher in the districts heavily polluted by heavy metals and organics compared to the area with lower contamination (all $\chi^2 > 4.5$, $\alpha < 0.05$). In Ekaterinburg, Russia, ectromely was significantly more common in *R. arvalis* from contaminated ponds compared to control sites (Vershinin 2002) ($\chi^2 = 4.02$, $\alpha < 0.05$), although the percentage of individuals affected was not high. In Argentina, 9% ($n = 124$) of the *Rhinella fernandezae* and 17% ($n = 785$) of the juvenile *Leptodactylus latrans* collected at ponds in cultivated areas that were contaminated by endosulfan,

cypermethrin, and chlorpyrifos, were ectrodactylous (Agostini et al. 2013). In the laboratory, endosulfan has been shown to cause ectromely at environmentally relevant concentrations (Rohr et al. 2003).

One case of elevated levels of ectromely, a population of *Bufotes viridis* in a quarry at Roßwag, Germany (Tab. 5), was associated and most likely due to radioactive pollution (Henle et al. 2017a). Ectromely frequently appears in anurans that develop from irradiated sperm, eggs and/or embryos (*Bufo bufo, Rana temporaria*: Rostand 1955a, 1958a, Oerter 1985; *P. nigromaculatus*: Kawamura & Nishioka 1978; *R. japonica*: Nishioka 1978). In *P. nigromaculatus* and *R. japonica* ectromely still occurred in the second and third generation, respectively. Inheritance of ectromely has further been shown in laboratory strains of *Ambystoma mexicanum* (Humphrey 1973), *Pleurodeles waltl* (Lauthier 1971, Dournon 1983) and *X. laevis* (Droin & Fischberg 1980). In the case of *P. waltl,* temperature acted epigenetically with the highest penetration when larvae were raised under high temperature before the development of limb buds (Dournon 1983). Heritable ectromely is also known in humans (Cohn & Bright 1999).

Ankley et al. (1998) observed high rates of ectromely in *Lithobates pipiens* tadpoles that were exposed to UV-B throughout hind-limb development. Pahkala et al. (2001) also found ectromely when embryos of *R. temporaria* were exposed to increased UV-B irradiation but the frequency was not different to that occurring under natural UV-B irradiation and Smith et al. (2000) did not report this effect in *L. blairi*.

Other factors exist that may cause ectromely but so far have not been suggested for mass occurrences of ectromely in natural populations. Ectromely appeared in metamorphs of experimental hybrids within the *Pelophylax esculentus* complex (Berger 1971, Berger & Uzzell 1977, Günther 1982, 1990, 1996b), in crosses of *Incilius bocourti* males with females of *Anaxyrus speciosus* and *A. cognatus*, in a cross between a male *A. nelsoni* with a female *A. punctatus* (9 of 11 metamophs) (Blair 1972) and among *Triturus* sensu lato species (Hamburger 1935), with only the latter case probably being significant (data however are insufficient for conclusions). None of the 54 hybridization experiments involving European bufonid species in our database reported any offspring with ectromely.

Rearing conditions may also cause ectromely (Meijer 1962, Dournon 1983). Christmann (1995) reported it for tadpoles that were fed on food tablets. A diet consisting only of enchytraea had the same effect in *Ambystoma mexicanum* and *Pleurodeles waltl* but not in *Triturus* (sensu lato) species (Reinhardt 1939, Vogt 1939). Witschi (1952) mentioned atrophied legs in *R. temporaria* developing from overripe eggs but does not provide any details.

3.2.2.2. Phocomely. Following Henle et al. (2017b) we define phocomely as the absence of the proximal long bones of limbs whereby the distal part of the limb is directly attached to the shoulder, pelvic girdle, elbow, or knee. So far there have not been any reviews of phocomely and it is rare in natural populations of amphibians. Our database contains 15 cases from at least ten species. The only mass incidence for a natural population was discovered by Kurtyak (2010), who found 31 (n = 89) individuals of *Pelophylax esculentus* with phocomely in the Transcarpathian lowlands of the Ukraine. He assumed hybridization as the cause but did not test his hypothesis. Except for one individual in each of the crosses of *Triturus cristatus* × *Lissotriton helveticus* and *T. cristatus* × *L. vulgaris* (Hamburger 1935), phocomely was never recorded from artificial hybridization experiments, including among *Pelophylax* species.

At least five phocomelous individuals were observed in a population of *Bufotes viridis* with the cause very likely being radioactive contamination (Henle et al. 2017a). Phocomelous individuals have been repeatedly observed after experimental irradiation of tadpoles, eggs or sperm (Rostand 1958*a*, Kawamura & Nishioka 1978, Nishioka 1978). The anomaly was still present in the second generation of *P. nigromaculatus* and the third generation of *Rana japonica* (Kawamura & Nishioka 1978, Nishioka 1978). A recessive gene *ph* causes phocomely in *Ambystoma mexicanum* (Humphrey 1975, Malacinski 1978).

In natural populations, phocomely has further been reported for four *Lithobates sphenocephalus* and one *L. sylvaticus* from three and one sites, respectively (Reeves et al. 2013), and two and one *L. pipiens* at a site in Minnesota (Meteyer 2000, Meteyer et al. 2000a,b) and Wisconsin (Lannoo (2008), respectively. Phocomely also occurred in *L. pipiens* in Douglas County, Minnesota, but the number of individuals remains unclear (Helgen 2012). It was further observed in one *A. tigrinum* (Williams et al. 2008), a larval *T. marmoratus* from Spain (Diego-Rasilla et al. 2007a), two albino *Bufo bufo* tadpoles in the Czech Republic (Wenig (1913), a metamorph of *Epidalea calamita* from Spain (Garin et al. 2009) and a *Hyla meridionalis* from the Canary Islands (Luis & Baez 1987). In addition, Peltzer et al. (2011) observed three phocomelous individuals in Argentina but did not provide the identity of the species.

The most extreme forms of anomaly P may also involve phocomely (Surlève-Bazeille et al. 1969b) but no concrete data for natural populations were given. Picariello et al. (2006) published a figure of *R. dalmatina,* which could possibly be phocomely but it is not certain. Bacon et al. (2006a) reported ≥ 26 (n = 13,856) metamorphs of *Rhinella marina* with "phocomely" from across Bermuda but they were not phocomelous in our definition because their definition of phocomely as "complete but malformed limbs" deviates from the general use of the term phocomely.

A few cases of mass incidences occurred in captivity. These were *P. ridibundus* that were fed on fish food as tadpoles but the causative mechanism remains unknown (Schchupak, pers. comm.). Another mass incidence occurred in the offspring of neoteneous *L. vulgar-*

is, with a few phocomelous individuals also occurring in the offspring of normal parents (Hartwig & Rotmann 1940), All offspring that developed from a single F_3-generation clutch of *Discoglossus pictus* were phocomelous, whereas all individuals from the remaining clutches were normal (Glaw 1987). Glaw (1987) assumed nutritional deficiencies of the parents to be the cause. Vogt (1939) showed that feeding on a pure diet of enchytraea caused phocomely in *Pleurodeles waltl* but not in *Triturus* (sensu lato) spp.

Thymus oil (Wink & Wurmbach 1967) and colchicine (Tschumi 1954) are known to cause phocomely in *Xenopus laevis*. Retinoids and vitamin A also caused phocomely when given for the short sensitive period when the thigh and shank start to differentiate but foot differentiation has not yet started (Liotti & Bruschelli 1969, Niazi 1996). Fort & McLaughlin (2003) reported that a diesel fraction of petroleum hydrocarbons induced phocomely in *Lithobates sphenocephalus* but no details were provided.

3.2.2.3. Ectrodactyly. The term ectrodactyly is not used consistently in the literature (Henle et al. 2017b). We follow the definition of these authors and include any complete (oligodactyly; Fig. 14) or partial absence (brachydactyly; Fig. 15) of one or more digits in the term ectrodactyly. Some salamander species show a high variability in foot and hand morphology, e.g., *Salamandrella keyserlingii* (Fig. 16) and *S. tridactyla* (Basarukin & Borkin 1984, Borkin 1999, Vershinin 2002, Zmeeva 2014), *Ambystoma maculatum* (Worthington 1974), *Batrachuperus pinchonii* (Chang & Boring 1935), *Lissotriton vulgaris* (Roberts & Verrel 1984), *Taricha granulosa* (Shubin et al. 1995), *Triturus carnifex* (Paces-Zaffaroni et al. 1992, 1996) and *T. marmoratus* (Caetano 1991). Brachydactyly is common in these species and even more than 50% of the individuals may show deviations from the normal number of digits, for example in *S. tridactyla* on Sakhalin Island, Russia (Basarukin 1984). In *S. keyserlingii* the percentage of individuals whose digit morphology deviates from the normal pattern may be small in some regions but high in others (Borkin 1999). As it is difficult to decide which cases are normal or abnormal, we only included the loss of phalanges or complete digits in urodeles if the individuals observed were regarded as abnormal in the assessed publication.

Fig. 14: Oligodactyly in a juvenile *Pelophylax ridibundus*, Kosolapowo, Oka, Russia, 30.8.2011. Photo: K. Henle.

Fig. 15: Brachydactyly in *Rana arvalis*, Tschekalin, Russia, 29.8.2011. Photo: K. Henle.

Fig. 16: Subadult *Salamandrella keyserlingii,* a species with a high variability of the number of phalanges: oligodactyly (right front limb), brachydactyly (left front limb) and shortened tail; depending on location and author the digital variability may be regarded as part of the normal variation or as abnormal. Note that four toes are the normal phenotype; Ekaterinburg, Russia, 29.7.2009. Photo: V. Vershinin.

So far, only Ouellet (2000) has reviewed the occurrence of ectrodactyly in natural populations. Our database contains 697 cases from 145 extant species. Wang et al. (2016) also observed abnormal brachydactyly in the fossil salamander *Chunerpeton tianyiensis.* Reported background rates reach up to 0.68% (Tab. 1). Elevated rates of ectrodactyly were recorded across several regions of North America (Bonin et al. 1997, Ouellet et al. 1997, Burkhart et al. 2000, Hoppe 2000, Sower et al. 2000, Johnson et al. 2003, Guderyahn 2006, Reeves et al. 2008, 2010, Bowerman et al. 2010) and at least eight cases belonging to six or seven species are known, in which at least 5% of the population were abnormal and at least 2.5% of the population and ten individuals were affected by ectrodactyly (Tab. 6). For regions outside North America the figures are 12 cases from ten species. Trauma inflicted by conspecifics and small invertebrates or parasites were suggested as causes for the North American cases and trauma inflicted by small invertebrates, chemical pollution, radioactivity, and unknown for the remaining cases but these hypotheses were not tested for any of these cases.

For one case not listed in Table 6, elevated rates of ectrodactyly in adult *Cryptobranchus alleganiensis*

Table 6: Elevated rates of ectrodactyly in amphibian populations (≥ 5% of the population abnormal, ≥ 2.5% of the population with ectrodactyly, ≥ 10 ectrodactylous individuals). *N:* sample size; only cases with *N* ≥ 50 included; % ectrodactyly refers to the sample size.

Species	*N* (% abnormal)	% ectrodactyly	Location	Cause	Reference
North American cases					
Ambystoma macrodactylum	403 (35%)	≥8.9%	Potholes, Oregon	Trauma (conspecifics)	Johnson et al. 2006
Ambystoma tigrinum	1926 (8%)	2.7%	Tippecanoe County, Indiana	Unknown	Williams et al. 2008
Taricha torosa	961 (15.4%)	≥3.9%	Hidden Pond, California	Unknown	Johnson et al. 2001a
Taricha torosa	647 (41.3%)	≥11.4%	Frog Pond, California	Unknown	Johnson et al. 2001a
Anaxyrus americanus	618 (59.5%)	≥3.2%	Duck Pond, Minnesota	*Ribeiroia ondatrae*[1]	Johnson & Hartson 2009
Anaxyrus species	170 (14.7%)	10.0%	Bald Knob National Wildlife Refuge (BLD-09), Arkansas, USA	Unknown	Reeves et al. 2013
Pseudacris streckeri	437 (11.4%)	≈ 11.4%	Barton Creek Habitat Preserve, Texas	Trauma (terrestrial invertebrates)	Gridi-Papp & Gridi-Papp 2005
Lithobates pipiens	1394 (20.3%)	7.7%	ROI site, Minnesota	Unknown; *Ribeiroia ondatrae* was not found but estrogenic substances and pesticides	Helgen et al. 2000, Garber et al. 2001, Vandenlangenberg et al. 2003
Other regions					
Bufo bufo	183 (30.1%)	25.7%	Kuzno village, Svertlowsk District, Russia	Unknown	Vershinin 2010
Bufotes viridis	294 (7.8%)	4.1%	Severnyi Plyazh beach, Magnitogorsk, Russia	Unknown	Vershinin 2010
Rhinella jimi	159 (44.6%)	>23.3%	Fernando de Noronha	Unknown	Toledo & Ribeiro 2009
Cyclorana australis *Cyclorana longipes* *Litoria rubella* *Platyplectrum ornatum*	914 (7%) 522 (7.5%) 479 (8.1%) 467 (13.5%)	5.9% 4.8% 4% 5.6%	Jabiru, Australia	Radioactivity, pesticides	Tyler 1989
Dendrobates auratus	231 (14.7%)	13.4%	Isla Tobago, Panama	Trauma (ants, other small invertebrates)	Gray et al. 2002
Dendrobates auratus	274 (10.4%)	9.9%	Cerro Ancon, Panama	Trauma (ants, other small invertebrates)	Gray et al. 2002
Dendrobates auratus	87 (19.5%)	14.9%	Cerro Bruja, Panama	Trauma (ants, other small invertebrates)	Gray et al. 2002
Pelophylax esculentus *Pelophylax ridibundus*	212 (62.3%) 235 (75.9%)	40.6% 65.5%	Bugornia, Russia	Unknown	Borkin et al. 2012, Bezman-Moseyko et al. 2014, pers. comm.

[1] Inconsistent with results from experimental infections with *Ribeiroia ondatrae* (see text).

(Miller & Miller 2005), the hypothesis of trauma as the cause is supported by observations in captivity. Captive *C. alleganiensis* do bite off the limbs and digits of conspecifics during the breeding time. Similar observations were made for larval *A. talpoideum* in laboratory experiments (Semlitsch & Reichling 1989). Cannibalism caused significantly elevated rates of lost digits (or feet – not specified), which increased with density.

Agricultural machinery has been suggested as another source of trauma. Schmidt & Hachtel (2011) attributed a case of mass ectrodactyly in waterfrogs (*Pelophylax* synkl. *esculentus*) in Germany to it. No tests were made to support the hypothesis.

For one case listed in Table 6, *Anaxyrus americanus*, infection with cysts of the trematode *Ribeiroia ondatrae* has been suggested as cause. However, ectrodactyly was absent or occurred at low, statistically insignificant numbers in most amphibian species that were experimentally infected with *R. ondatrae* (Johnson et al. 1999, 2001b, Stopper et al. 2002, Schotthoefer et al. 2003, Johnson & Hartson 2009). The only exception was *Pseudacris regilla* in the study of Romansic et al. (2011) in which ectrodactyly was slightly (5%) elevated. However, in another study of the same species, it was absent in most treatments and rare in the treatment with the highest number of cercariae (Johnson et al. 1999). Experiments with the trematode *Alaria mustelae* did not cause any limb anomaly (Johnson et al. 1999). In contrast, experimental infections of limb bud stage tadpoles (Gosner stage 27 and 28) of the Sri Lankan rhacophorid frog *Polypedates crucifer* with monostome-type cercariae (*Acanthostomum burminis* according to Jayawardena et al. 2013) caused significantly elevated levels of ectrodactyly (Rajakaruna et al. 2008). Limb anomalies did not occur when pre-limb bud stage tadpoles (Gosner stage 25 and 26) were exposed (Jayawardena et al. 2010). Clearly, more systematic studies across larval development with different amphibian and trematode species are needed before the potential of trematodes to induce high levels of ectrodactyly in natural populations can be understood.

Another parasite, chigger mites of the genus *Hannemania*, can cause ectrodactyly, if infestation of feet is heavy. Winter et al. (1986) and Brown et al. (2006) observed this effect in wild *Desmognathus brimleyorum* and *Plethodon metcalfi*, respectively.

For the four cases listed in Table 6 from retention ponds at the Jabiru Uranium mine in Australia, pesticides and/or radioactivity have been suggested as the cause. Ectrodactyly was observed in ten additional species from the same area (Tyler 1989). The extent to which the biocides or radioactivity were responsible could not be established. Ectrodactyly developed at low frequency in *Bufo bufo* and *Rana temporaria* that were irradiated as hatchlings (Rostand 1955a, 1958a) – for chemicals, see the next paragraph. At another uranium mine, the Olympic Dam in South Australia, Read & Tyler (1990, 1994) collected 19 *Neobatrachus pictus* (as *N. centralis*) with ectrodactyly at sites with very low radionuclide concentrations. The malformation rate did not differ significantly from areas outside the mine but the radioactivity level at one control site (and its frequency of ectrodactyly) was higher than for the mine sites (Read & Tyler 1990). Radioactivity was most likely to be the cause of several individuals of *Bufotes viridis* exhibiting ectrodactyly in a quarry at Roßwag in Germany (Henle et al. 2017a).

While not suggested for any other case listed in Table 6, evidence exists for chemical pollution as the cause for elevated rates of ectrodactyly from studies in Russia and Tunesia. Ectrodactyly in *Pelophylax ridibundus* and *Bombina bombina* was significantly more common (all $\chi^2 > 56$, $\alpha < 0.001$) in two and one Ukrainian area(s), respectively, strongly polluted by heavy metals and organics compared to an area with lower contamination (Flax & Borkin 1997, 2004). In Sakhalin (Russia), 37.5% of immature *R. amurensis* showed ectrodactyly (together with syndactyly and clinodactyly; sample size and number affected by ectrodactyly not given) in a habitat polluted by a paper and pulp mill but none in other populations (Levinskaya & Barinova 1978). In Ekaterinburg, Russia, ectrodactyly was significantly more common in *R. arvalis* from contaminated ponds than in control sites ($\chi^2 = 8.3$ $\alpha < 0.01$) (Vershinin 2002). In Indian rice paddies that were heavily contaminated by pesticides 6% (n = 259) of the collected *Feyervarja limnocharis* were ectrodactylous (Patel et al. 2008). In Argentina, 12% (n = 124) of the *Rhinella fernandezae* and 11% (n = 785) of the juvenile *Leptodactylus latrans* collected at ponds in cultivated areas that were contaminated by endosulfan, cypermethrin, and chlorpyrifos, were ectrodactylous (Agostini et al. 2013). The frequency of all anomalies was significantly higher than for individuals collected at control ponds without detectable pollution. Finally, ectrodactyly was one of the most common anomalies (exact figures not clear because of partial double counting) in *P. saharicus* metamorphs collected at the Lemna Dam in Tunisia, which is contaminated by agricultural run-off, whereas indivduals from non-agricultural areas did not exhibit any anomaly (Hassine et al. 2011).

Several chemicals are known to cause ectrodactyly in developing limbs: the fungicide maneb at environmentally relevant concentrations (Fort 1999b), the antifouling agent tributyltin oxide but only at concentrations that cause high mortality (Scadding 1990), retinoids (Scadding & Maden 1986a,b), citral (an inhibitor of retinoic acid synthesis) when treatment started at late neurulation (Lee & Elinson 2008), thymus oil and other seed oils (Wink & Wurmbach 1967), estradiol (Takahashi 1957, 1958, Collenot 1965) and trypoflavin (in *R. temporaria* but not in *P. esculentus*: Rostand 1950c).

Besides the factors discussed above, several others have experimentally been shown to cause ectrodactyly. A significantly elevated frequency of ectrodactyly has been observed in *R. temporaria* tadpoles treated with the mucus from the skin of fish from ponds in which anomaly P occurred (Surlève-Bazeille et al. 1969a).

The causative factor is still not clear but is assumed to be a virus (Dubois 2014, 2017). Correlational evidence exists that infection by poxvirus, *Aeromonas hydrophila* and *Batrachochytrium dendrobatidis* may cause oligodactyly due to necrosis in various species (Hine et al. 1975, Frye 1985, Cunningham et al. 1993, Brodman & Briggler 2008). Approximately 4% of 377 metamorphosed individuals of crosses within *P.* synkl. *esculentus* showed ectrodactyly (Berger 1971) and Hamburger (1935) obtained two such individuals in crosses between *Triturus* species.

Ankley et al. (1998, 2000, 2002) observed high rates of ectrodactyly in *Lithobates pipiens* tadpoles exposed to UV-B (full sunlight) throughout hind-limb development but exposure conditions were not natural. Under more realistic exposure conditions, Pahkala et al. (2001) obtained significantly elevated rates in *R. temporaria* metamorphs that were exposed to enhanced UV-B as embryos.

Inheritance of ectrodactyly has been demonstrated in *Ambystoma mexicanum* (Humphrey 1966, 1967b, Martin & Signoret 1968, Malacinski 1978), *Pleurodeles waltl* (Lauthier 1971, Dournon 1983), *Xenopus laevis* (Droin & Fischberg 1980) and *R. temporaria* (Dubois 1977). In the case of *P. waltl* temperature acted epigenetically with the highest penetration when larvae were raised under high temperature before the development of the limb buds (Dournon 1983). Heritable ectrodactyly is also known in humans (Cohn & Bright 1999).

3.2.2.4. Tail completely or partially missing. Tail autotomy occurs naturally in various plethodontid salamanders (Wake & Dresner 1967). Such cases are not included in this review. Our database contains 140 cases of partially (Fig. 16) or completely missing tails from 56 species.

Tail loss has rarely been reported for juvenile, subadult or adult urodeles. Our database contains 18 cases, involving ten species. In most cases only a few individuals were found and the cause remained unclear. The largest number reported was seven out of eight abnormal (n = 681) *Plethodon* individuals [as *P. glutinosus* (Highton 1956), but fide Hayslett et al. (1998) *P. grobmani*], although presumably more individuals were affected in a population of *Dicamptodon tenebrosus* (Munshaw et al. 2014). The cause in the former species remains unknown but was conspecifics and steelhead trouts (*Oncorhynchus mykiss*) for the latter species. Reported causes for partially or completely missing tails in adult urodeles were injuries from cars (for *Salamandra salamandra*: Feldmann & Klewen 1981, Klewen 1988), a bird attack (*S. atra*: Klewen 1986) and injuries from a spate (for *Triturus cristatus*: Böhm 2013).

In tadpoles frequencies are often high. For example, Bleakney (1958) reported that in a sample of hundreds of tadpoles of *Lithobates sylvaticus* all lacked parts of the tail. He assumed cannibalism under high density to have been the cause of this mass occurrence. In the same species and in *L. catesbeianus* Blair & Wassersug (2000) found that in samples of 98–100 tadpoles from six different ponds 37–87% of the tadpoles had shortened tails. They assumed predators to be the cause. In a population of *L. sylvaticus* sampled at the Great Bay National Wildlife Refuge 11.1% (n = 90) of the tadpoles lacked parts of their tails (Reeves et al. 2013).

Rates of tadpoles with shortened tails may differ among habitat types. For example, in *Acris crepitans* 38–51% of the tadpoles had damaged tails, with the frequency being significantly lower in lake habitats compared to creek and pond habitats (Caldwell 1982).

Most cases of mass occurrences of shortened or missing tails outside of North America were also attributed to either cannibalism or predation attempts. Bruch (1864) collected more than 100 tadpoles of *Bufotes viridis* with partially missing tails. He attributed the damage to gnawing by other tadpoles. In a sample of 430 tadpoles of *Rana temporaria*, 99% lacked parts of their tails; sticklebacks (*Gasterosteus aculeatus*) were assumed to be the cause (Malkmus 2008). Kordges et al. (2013) discovered tail damage in 93% of 83 *Pelobates syriacus* tadpoles examined and regarded predation attempts by fish and larvae of the dytiscid beetle *Cybister tripunctatus lateralis* to have been the cause. For one case, a population of *T. cristatus*, in which 9.7% (n = 179) of the tadpoles had partially or completely missing tails, cannibalism and attempted predation were suggested as the causes (Kupfer & von Bülow 2011).

None of the studies cited above tested their hypotheses experimentally or statistically but correlational evidence for predators as culprits of high rates of tail loss was provided by Rödel (1998) for two African species. Of 353 and 107 tadpoles of *Kassina* sp. and *Phrynomantis microps*, respectively, from two large temporary ponds in the Ivory Coast 85.8% and 78.8%, respectively, showed tail injuries. The rate of tail injuries correlated significantly with the number of Anisoptera larvae and giant water bugs (*Bellostoma*) in the samples taken from different parts of the ponds.

Only one study, Bowerman et al. (2010), experimentally tested the hypothesis of predator attacks being the cause of elevated rates of partially lost tails in tadpoles. At Lake Aspen, central Oregon, *Anaxyrus boreas* tadpoles typically lacked small pieces of their tails and at Broken Top in central Oregon less than 20% of the *L. cascadae* tadpoles had incomplete tails. The exclusion of predators effectively eliminated tail injuries in *Anaxyrus boreas* and laboratory experiments showed that sticklebacks (*Gasterosteus aculeatus*) inflict tail injuries at significantly elevated rates.

In another laboratory study, the mosquito fish (*Gambusia holbrooki*) injured tails of *L. capito* at a mean rate of 50% but rarely in *L. sphenocephalus* (Gregoire & Gunzburger 2008). Laboratory tests further showed that nymphs of various Odonata species frequently cause tail injuries or loss in tadpoles of all tested species (reviewed by Henle et al. 2017a). Salamanders are also known to inflict tail injuries in tadpoles (Parichy & Kaplan 1992) and staged interspecific interactions of

Ambystoma talpoideum and *A. mavortium nebulosum* also resulted in high rates of tail injuries or partial tail loss (Semlitsch & Reichling 1989, Harvey 2003). Likewise, fighting among courting male urodeles may lead to tail loss (*Eurycea cirrigera*: Halliday & Tejedo 1995; *Ommatotriton ophryticus*: Raxworthy 1989).

Radioactive contamination following a nuclear accident caused tail agenesia in *Salamandrella keyserlingii* embryos and larvae (Kuranova & Saveliev 1997, 1999). Tail loss after irradiation has been demonstrated experimentally for *A. mexicanum* and *Salamandra salamandra* (Luther 1939).

Besides the causes suggested for natural populations, feeding paraquat to tadpoles of *L. berlandieri* resulted in high rates of individuals with shortened tails (Bauer Dial & Dial 1995). Beetschen & Jaylet (1965) discovered a recessive mutant for short tails in *Pleurodeles waltl.*

3.2.2.5. Brachycephaly, microcephaly and pointed snouts. Microcephaly and other reductions of the head are extremely common anomalies in amphibian embryos resulting from experimental hybridization (Delarue 1974, 1982), chemical treatment (e.g., Brachet et al. 1964, Pérez-Coll et al. 1986) and irradiation (e.g., Kawamura & Nishioka 1978, Nishioka 1978) and may have a genetic basis (e.g. Beetschen & Jaylet 1965). However, they are rarely observed in natural populations. Several toadlets of *Bufotes viridis* from a quarry in Roßwag, Germany, exhibited both pointed snouts and brachycephaly, presumably due to radioactive contamination (Henle et al. 2017a). In Argentina, Agostini et al. (2013) observed microcephaly in 10 (n = 329) *Rhinella fernandezae* and in 6 (n = 2116) *Leptodactylus latrans.* Brachycephaly without pointed snouts was also discovered in ten out of 781 juvenile *Rana arvalis* from Ekaterinburg (Vershinin & Neustroeva 2011, Vershinin unpubl.), in one adult female *Rhinella jimi* out of 159 individuals sampled (Toledo & Ribeiro 2009) and in a recently metamorphosed *Bufo bufo* from Sagra, near Ekaterinburg (Vershinin, unpubl.), as well as in three metamorphs of *Lithobates pipiens* (Meteyer 2000, Reeves et al. 2013).

For tadpoles, most observations of microcephaly were made at heavily polluted sites in Sakhalin, Russia [*B. gargarizans, Rana amurensis and R. pirica* (as *R. chensinensis*): Mizgireuv et al. 1984] and the Ukraine [18 (4.5%) *Bombina bombina,* 9 (1.8%) *Bufotes viridis* and 34 (6.8%) *Pelophylax ridibundus*: Flax & Borkin 2004]. Additionally, Reeves et al. (2008, 2013) found four microcephalic individuals of *Lithobates sylvaticus* when sampling 38 breedings sites in the Kenai Wildlife Refuge, Alaska. Reeves et al. (2013) further listed one metamorphosing individual of *R. aurora*, Levey et al. (2003) one tadpole of *L. pipiens*, and Bregulla (1987) and Veith (1988) each reported a *Salamandra salamandra* larva with microcephaly.

In the laboratory, microcephaly is occasionally retained in early tadpole stages in individuals developing from overripe eggs (Briggs 1941). Pisanets (1992) reported that 100% of the metamorphs from crosses between 11 male *B. danatensis* and 8 female *Bufotes viridis* (from the Ukraine) were brachycephalic but none were found in other crosses with the two species from other geographic origins. Brandt (1933) observed a young larval *Triturus marmoratus* × *T. karelini* hybrid with microcephaly and the only surviving adult hybrid *Ceratophrys cranwelli* × *C. ornata* had a shortened snout (Miranda 1989). Kawamura & Nishioka (1978) observed microcephaly in tadpoles developing from irradiated eggs or sperm. Heritable brachycephaly was discovered by Lipsett (1941) in one strain of *Ambystoma mexicanum*. Tetraploid or pentaploid larval *Notophthalmus viridescens* and *Cynops pyrrhogaster* usually show pointed snouts (Fankhauser 1945) similar to the *B. viridis* individuals with pointed snout in the Roßwag population.

3.2.2.6 Mandibular hypoplasia. No review exists for this type of anomaly. Mandibular hypoplasia (Fig. 17) has been reported for 70 natural populations of amphibians. Twenty-six species were affected. Background rates are very low: 0.03% (n = 6,689) in *Lithobates pipiens* (Hoppe 2000) and 0.1% (n = 12,674) in juvenile *R. arvalis* in Ekaterinburg and its vicinity in Russia (Vershinin 2002). Only three cases involved more than ten individuals: 33 out of 13,856 *Rhinella marina* from 38 sites across Bermuda, of which many were polluted with petroleum hydrocarbons and metals (Bacon et al. 2006b), 15 (n = 479) *Litoria rubella* (plus one *L. caerulea*) that developed in ponds contaminated with pesticides and radioactivity from the Jabiru Uranium Mine in Australia (Tyler 1989) and more than ten out of 295 toad-

Fig. 17: Mandibular hypoplasia, juvenile *Rana arvalis*, Ekaterinburg, Russia, 3.7.1997. Photo: V. Vershinin.

lets of *B. viridis* sampled in a quarry in Roßwag, Germany, in 1980, in which radioactive contamination was found (Henle et al. 2017a). *Litoria rubella* shared mass ectrodactyly and several other skeletal anomalies with the Roßwag population but not polymely; non-skeletal anomalies were not listed in the study by Tyler (1989).

In laboratory experiments, a few chemicals and radioactivity turned out to be potent causes of mandibular hypoplasia: 2-methyl-4-chlorophenoxy acetic acid in *Triturus carnifex* (Zavanella et al. 1989), corticosterone in *Kassina senegalensis* (Hayes et al. 1997, Hayes 2000), jodothyrin (Romeis 1918), thymus oil, various seed oils that inhibit the growth of the thyroid gland and the sulfonamids aristamid and nadisan (they inhibit sugar and energy metabolism) in *Xenopus laevis* (Wurmbach et al. 1964) and possibly diquat in *Rana temporaria* (Gelnarova 1987a,b). DDT induced hyperactivity in tadpoles of *R. temporaria,* which resulted in the lower jaw colliding with the inner surface of the upper jaw and eroding the jaw (Osborn et al. 1981). DDT treatment also caused mandibular hypoplasia in *Spea hammondi, Pseudacris regilla* and *Lithobates catesbeianus* (Hayes 1997).

Many *R. japonica* originating from irradiated sperm or eggs developed mandibular hypoplasia and other anomalies of the jawbones (Nishioka 1978). An inherited mandibular hypoplasia was found in *Pleurodeles waltl* (Jaylet 1971). Deformed lower jaws, which may include mandibular hypoplasia, were also observed in haploid larvae of urodeles (Fankhauser 1945).

3.2.2.7. Monorhyny and obscure or lacking nostrils or nasals. Apart from parasitism by the fly *Lucilia bufonivora* (Figs. 18&19) only very few cases are known in which nostrils or nasals were obscure or lacking. Kolobaev (2000) reported the absence of nostrils in *Rana dybowskii;* it remains unclear whether one or two individuals were affected. Rostand (1955b) raised 567 tadpoles of *Bufo bufo* collected in the wild to metamorphosis; 27 of them exhibited monorhyny. He published photographs of two individuals (Rostand 1958b). The cause remains unclear but might be genetic. One tadpole of *Rhinella jimi* from the island of Fernando de Noronha lacked one nostril and another one (n = 413) had a shortened nostril (Tolledo et al. 2014). In an experimental cross between *Pelophylax lessonae* and *P. esculentus* 4.1% (n = 491) individuals exhibited fused nostrils (Guex et al. 2001).

All other cases in our database apply to the destruction of nasal openings and bones by maggots of *L. bufonivora.* It is a wellknown phenomenon in *B. bufo* (Fig. 19) but the percentage of individuals affected usually is low (Meisterhans & Heusser 1970). Our database contains reports from at least 111 populations of *B. bufo* from Belgium (Preudhomme de Borre 1876, Lestage 1926), the Czech Republic (summaried by Zavadil et al. 1997), Denmark (Mortensen 1892, Kryger 1921), France (Moniez 1876, Brumpt 1934, Rostand 1947), Germany (e.g., Kordges 2000, Sowig & Laufer 2007), Poland (Sandner 1955), Russia (Kuranova 1998, Kuzmin 2013), Sweden (Löwegren 1939), Switzerland (Meisterhans & Heusser 1970), the Netherlands (Strijbosch 1980) and the UK (Richards 1926). In most cases only a few individuals with parasites were observed but the reported parasitization rate was up to 50% (Brumpt 1934). It usually increased with the size of the toads and juveniles were never infested (Strijbosch 1980). Infested toads always die but recovery was usually observed if all maggots were removed (Neumann & Meyer 1994).

Destruction of nasal bones by maggots of *L. bufonivora* occurs rarely in other species (Zumpt 1965, Neumann & Meyer 1994). Two exceptions are 20–25% and 4.5–9.5% of the *R. arvalis* sampled being infested in the Wolga-Kama region of Russia in 1972 (Garanin & Shaldybin 1976) and in Siberia in different years (Kuranova 1998), respectively. Rare cases have been observed in the following species: *Salamandra salamandra* (1 case; Keilin 1915), *Alytes obstetricans* (Meisterhans & Heusser 1970, Kordges 2003), *Bombina variegata* (Veith

Fig. 18: *Lucilia bufonivora* depositing eggs on an adult *Bufo bufo,* Botanical Garden, Munich. Photo: S. Swoboda.

Fig. 19: Adult *Bufo bufo* with the nasal openings destroyed by maggots of the fly *Lucilia bufonivora*; the fly on the head is not the culprit. Photo: R. Stawikowski.

1996), *Pelobates cultripes* (Taton 1877), *Pelobates fuscus* (Silantev 1898, Sigov 1936), *Bufo verrucosissimus* (Kidov 2010), *Bufotes viridis* (Mertens 1921, Garanin & Shaldybin 1976), *Epidalea calamita* (5 cases; reviewed by Zavadil 1996), *Pelophylax* synkl. *esculentus* (Sandner 1955, Albrecht et al. 1996, Schmidt & Hachtel 2011), *R. amurensis* (Kuranova 1998, Kuzmin 2013) and *R. temporaria* (Brumpt 1934, Sandner 1955, Koskela et al. 1974, Riedel 1988). Goverse (2009) further reported one case in *Hyla arborea,* despite eggs usually easily dropping off of *H. arborea* (Meisterhans & Heusser 1970).

In earlier publications the species *Lucilia splendida* and *L. silvarum* were also implicated as relevant parasites (e.g., Hesse 1906, Stadler 1930) but these were likely to be mis-identified (Zumpt 1965, Neumann & Meyer 1994). Eberle (1937) further reported one case in which *Muscina pabulorum* but no *L. bufonivora* hatched from the deceased toad. However, it cannot be excluded with certainty that this was a secondary infestation, as the deceased toad was not immediately placed securely under a mesh.

3.2.3 Other externally visible skeletal anomalies

3.2.3.1 Clinodactyly. Clinodactyly is an abnormal bending of a toe caused by the presence of an intercalary little asymmetrical bone between two phalanges. Note that when toes are split (schizodactyly, a specific form of polydactyly) the supplementary phalanges often bend away from the other phalanges (see Fig. 12). Therefore, and as an illustration (but not the definition) given by Tyler (1989) can easily be confused with schizodactyly, some authors used the term clinodactyly for such cases (e.g., Vershinin 1991, 2002). Where obvious from illustrations or photographs, we excluded such cases from the review of clinodactyly.

Clinodactyly has not yet been reviewed for amphibians. Our database contains 70 cases from natural populations of 38 species, all but two involving only a few individuals. The exceptions are 100 (n = 44,000) adult male *Bufo bufo* from France, for which the cause is unknown (Rostand 1949a). In addition, high frequencies of clinodactyly (37.5% but including syndactyly and ectrodactyly; the sample size and the number affected by clinodactyly were not given) were reported for *Rana amurensis* breeding in sites polluted by the discharge of a paper and pulp mill in Sakhalin (Russia) (Levinskaya & Barinova 1978). It is possible that the anomalies observed might have been schizodactyly rather than clinodactyly. Tyler (1989) reported low rates of clinodactyly for seven species collected in retention ponds of the uranium mine at Jabiru, Australia, that were additionally polluted with herbicides and pesticides.

Laboratory experiments demonstrated that several chemicals induce clinodactyly at elevated rates (often together with other limb anomalies): the antifouling agent tributyltin oxide (Scadding 1990), thiosemicarbacide, which is a potent osteolathyritic chemical (Riley & Weil 1986, 1987), thymus oil (Wink & Wurmbach 1967) and estradiol (Takahashi 1958, Collenot 1965). Percutaneous treatment of amputated legs of *Triturus carnifex* with the fungicid maneb also caused clinodactyly (Arias & Zavanella 1979, Zavanella et al. 1984). Clinodactyly further developed in hatchery raised *Pelophylax perezi* tadpoles fed on artificial diets but not when they were fed on fresh lettuce (Martínez et al. 1992). It was also observed in a few *B. bufo* tadpoles developing from irradiated embryos (Rostand 1958a, Oerter 1985).

Dubois (1977) discovered a heritable form of clinodactyly in *R. temporaria* that occurred together with other anomalies of the digits. Clinodactyly is also part of another syndrome of limb anomalies in the same species, the so-called anomaly E, which has a dominant inheritance (Rostand 1956a,b, 1958b). Temperature may act epigenetically to induce clinodactly, as Muto (1969a,b, 1970) observed (in some experiments, but not in others) that clinodactyly developed in *B. formosus* reared at 30°C, but not in controls reared at 20°C.

3.2.3.2 Syndactyly. So far, no review exists for syndactyly. In our database we have reports for 137 natural populations from 64 species. The number of affected individuals was low except for three, possibly four cases. Eleven *Lithobates sylvaticus* with syndactyly (0.2%; n = 5716) were found in a survey of 38 sites in the Kenai Wildlife Refuge in Alaska (Reeves et al. 2008, 2013). Out of 467 *Limnodynastes ornatus* collected in a water body derived from a radioactive source that was additionally polluted by herbicides and pesticides 32 (6.9%) exhibited syndactyly; several other species were also affected but at a lower rate (Tyler 1989). At least 191 out of 13,856 individuals of *Rhinella marina* sampled across Bermuda showed syndactyly, with the pollution of breeding ponds resulting from the run-off from roads and agricultural fields, and the spraying of pesticides presumably being the cause (Bacon et al. 2006b, Fort et al. 2006a). In a *Rana amurensis* population living in the wastewater from a paper and pulp mill in Sakhalin, Russia, 37.5% of immature frogs exhibited syndactyly, clinodactyly or ectrodactyly (the sample size and the number affected by syndactyly were not specified) (Levinskaya & Barinova 1978).

Various chemical treatments have caused syndactyly in laboratory experiments. All *Pelodytes punctatus* treated with testosterone developed syndactyly (Collenot 1965). Likewise, chloraethylamin treatment of limb buds and feeding retinoic acid to tadpoles caused a very high rate of syndactyly in *Xenopus laevis* (Tschumi 1954, Alsop et al. 2004). Limb amputation together with vitamin A treatment caused syndactyly in 5–7.5% (n = 40) of the treated larval *Duttaphrynus melanostictus* (Das & Mohanty-Hejmadi 2000). However, seemingly inconsistent results were reported for several other chemicals. Trypaflavine caused syndactyly in *R. temporaria* but not in *Pelophylax esculentus* (Rostand 1950b,c). For colchicine, syndactyly was noted in *X. laevis* by Tschumi

(1954) but not by Bretscher (1949), Crézé (1950) and Rostand (1950b,c); estradiol was effective in *R. pirica* but not in *Bufo formosus* (Takahashi 1957, 1958). According to Tschumi (1954), syndactyly is a mild expression of a syndrome that is caused by a reduction in cell numbers of the blasteme, which causes brachydactyly, ectrodactyly, or ectromely with an increased reduction of the number of living cells. Thus, these seemingly inconsistent results may be due to different degrees of damage of the blasteme, perhaps combined with a varying degree of sensitivity of species.

A high frequency of syndactyly has further been observed in *R. temporaria* tadpoles treated with the skin mucus of fish from ponds in which anomaly P occurred (Surlève-Bazeille et al. 1969a) but not in *P.* synkl. *esculentus* (Surlève-Bazeille et al. 1969a, Rostand 1971). Low to high rates were also found in *B. formosus* reared at 30°C, but none in controls reared at 20°C (Muto 1969a,b, 1970). It was further observed in several species after limb or toe amputation, especially under higher temperatures (e.g., Bonnet 1779, Schmalhausen 1925, Tschumi 1954, Dearlove & Dresden 1976, Semlitsch et al. 1981).

Syndactyly rarely occurs in anurans infected with the trematode *Ribeiroia ondatrae* (Johnson et al. 1999, 2001b, Schotthoefer et al. 2003). Hamburger (1935) obtained one syndactylous individual in experimental crosses of *Triturus cristatus* × *Lissotriton helveticus*. Syndactyly occurred rarely in *B. bufo* tadpoles developing from irradiated embryos (Rostand 1958a, Oerter 1985).

Dubois (1977) demonstrated the dominant inheritance of a syndrome of digital anomalies that included syndactyly in one *R. temporaria* individual. Syndactyly is also part of another dominant syndrome of limb anomalies in the same species, the so-called anomaly E (Rostand 1956a,b, 1958b). In humans and mice, several mutations of single regulatory genes are also known to cause syndromes of limb anomalies involving syndactyly (Cohn & Bright 1999, Gurrieri et al. 2002).

3.2.3.3 Symmely and related anomalies. Apart from the fusion of supernumerary limbs, we know this type of anomaly only from *Bombina bombina* (n = ?) in Hungary (Puky & Fodor 2002) and from a single individual of *Pelophylax esculentus* (Bruch 1864). In an individual of *Lithobates pipiens* the tibiofibula, the femur and the tail were fused together (Reeves et al. 2013).

3.2.3.4 Bony bridges, bony triangles and other forms of taumely. Taumely is a gross disturbance of the bauplan of the limb (Fig. 20), with a long bone being out of alignment (Henle et al. 2017b). The degree of misalignment may vary and some authors (e.g., Gardiner & Hoppe 1999) included any degree of bending back in the term taumely, whereas others restrict it to a misalignment of at least 90° (e.g., Tyler 1989, Meteyer et al. 2000a, Henle et al. 2017b). We additionally include cases in which the mis-alignment is smaller but the bauplan of the limb is grossly disturbed. Bony bridges,

Fig. 20: Taumely, *Rana arvalis*, Berdanish Lake, Ural, Russia, 6.9.1993. Photo: V. Vershinin.

and its synonym bony triangles, are a specific form of taumely in which the bent long bones have a sharp midshaft angle and form a triangle or pyramid. In spite of being called "bony", these anomalies are variably either ossified or chondrified (Gardiner & Hoppe 1999).

Our database contains 55 cases from natural populations of 27 species. Most of the cases from North America relate to bony triangles. High numbers and frequency of individuals with bony triangles have been reported for a population of *Anaxyrus americanus* (33%, n = 618) from Duck Pond, Minnesota (Johnson & Hartson 2009) and a population of *Lithobates clamitans* (≤ 9.8%, n = 245) and *L. septentrionalis* (≤ 5%, n = 869) from the CWB site, Minnesota (Vandenlangenberg et al. 2003, Hoppe 2005), respectively. Individuals of *A. americanus* were parasitized by the trematode *Ribeiroia ondatrae* and *R. ondatrae* also occurred at the CWB site. Johnson & Hatrson (2009) assumed *R. ondatrae* as the cause for bony triangles in the population studied by them. In contrast, Vandenlangenberg et al. (2003) and Hoppe (2005) regarded pollution as the cause for the cases at the CWB site. Likewise, Gardiner & Hoppe (1999) and Gardiner et al. (2003) assumed that retinoids were the cause of mass occurrences of bony bridges in natural populations, whereas Stopper et al. (2002) dismissed this explanation and assumed that infection with *R. ondatrae* caused elevated rates of bony triangles in natural populations.

Bony triangles are often regarded as being characteristic of *R. ondatrae* infection since they appeared frequently in experimental infections of anurans (*A. americanus, A. boreas, Pseudacris regilla*) with this trematode (Johnson et al. 2001b, 2006, 2010, Johnson & Hartson 2009). However, in other experimentally infected species they were only observed rarely or were even ab-

sent (e.g., in *Ambystoma macrodactylum croceum, Dryophytes versicolor* and *L. pipiens* Schotthoefer et al. 2003, Johnson et al. 2006, Johnson & Hartson 2009).

In a laboratory setting, bony triangles were found when rearing *L. pipiens* in a lipophilic water sample from a malformation hotspot and treating them with carbaryl in combination with atrazine (Bridges et al. 2004). Feeding retinoic acid to tadpoles of *Xenopus laevis* caused high rates of bony triangles (Degitz et al. 2000, Alsop et al. 2004). It should be noted that the effects of chemicals can be highly stage specific. For example, the synthetic retinoic acid receptor-specific activator TTNPB caused primarily bony triangles when *X. laevis* was treated at stage 52 but other anomalies or no anomalies appeared at other stages (Gardiner et al. 2003). In conclusion, both *R. ondatrae* and chemical pollution may have directly contributed to North American malformation hotspot at which bony triangles occurred with high frequencies.

In regions outside of North America, it is usually unclear as to what type of taumely had been observed. Rostand (1949a) reported about the rare occurrence of a hump-shaped tarsus in *Bufo bufo* and Brandt (1933) published a drawing of a *Pelophylax esculentus* that indicates a bony bridge. Landois (1882) described bony triangles in two ranid frogs from Germany. Woitkewitsch (1959) reported at least 47 individuals with bony bridges; however, most or even all affected limbs were supernumerary legs or legs with duplications of the foot. The causes for the cases outside North America remain unknown but in single individuals may be due to traumatism, as experimental rotation of the limb buds can cause high rates of bony triangles (Hecker & Sessions 2001, Stopper et al. 2002).

3.2.3.5 Rotation and curvature of bones; torsion and stiffness of limbs. Various terms have been used for these types of anomalies (see Henle et al. 2017b). Rotation of limbs is an anomaly in which limbs are twisted and the foot (hand) is not in a plain with the surface but the limb is not grossly malformed. Rotation may be caused by an abnormal joint or by a distortion of the direction of bone growth. Following Henle et al. (2017b) we regard torsion of the limbs as a synonym and anteversion as a specific form of rotation but note that some authors include bony bridges or all forms of taumely under the term anteversion (e.g., Hoppe 2005). We exclude studies from this section in which it is unclear as to whether authors used the term anteversion in such a broad meaning. In addition, the term curved long bones has been used as a synonym in cases where bones are curved but not more than 90° out of alignment (Sanchiz & Pérez 1974).

Rotation of limb bones is always combined with stiffness of limbs. However, limbs may be stiff without rotation, e.g., in the case of polymely, cutaneous fusion, or when the musculature is reduced or disfunct; the latter is often referred to as "Streichholzbeinchen" (Fig. 21) in the German literature. Here, we only consider forms of stiffness that are not linked to cutaneous fusion, polymely, or taumely (the latter being discussed in the previous section).

No review exists for rotation or for stiffness of the limbs. We found reports of rotation for 50 populations. These cases involve one urodele (*Notophthalmus viridescens*: Collins 1932) and 22 anuran species. In addition, Sanchiz & Pérez (1974) described three abnormally curved urostyles among 497 that were examined from a population of *Discoglossus pictus*. Further, Fodor & Puky (2002) and Puky (2006) mentioned clinomely for *Bombina bombina* and *Pelophylax* synkl. *esculentus* from two different respectively one location(s) at the Danube River. It is unclear as to whether these anomalies are the same as what we refer to as rotation and we do not know of others that have used the term clinomely for abnormal limbs.

Most of the studies that reported rotation for natural populations mentioned only few individuals but in a population of *Rana luteiventris* from British Columbia, Canada, 4.3% (n = 280) of the metamorphs had stiff limbs (Roberts & Dickinson 2012). All abnormal individuals carried cysts of *Ribeiroia ondatrae* in their limbs. Individuals reared in closed cages did not develop any anomaly. Therefore, Roberts & Dickinson (2012) concluded that *R. ondatrae* was the cause for the anomalies. In experiments with the trematode, rotation of limbs occurred as a rare anomaly in *Anaxyrus boreas* and *Ambystoma macrodactylum* (Johnson et al. 2001b, 2006). In spite of this rarity in experiments, *R. ondatrae* infection is a parsimonious explanation. However, a caveat re-

Fig. 21: Stiff front legs with disfunct musculature (called Streichholzbeinchen in the German literature) in a juvenile *Rana arvalis*, Rezhevskoy road, non-urban natural habitat near Ekaterinburg, Russia, 23.7.2003. Photo: V. Vershinin.

mains as no information was provided as to whether access to mud and predators was also excluded. Chemical pollution and/or predation and once a virus (anomaly P) have been assumed to be the cause(s) for rotation in other studies. However, no attempts have been made to test these assumptions in any of the cases. Whereas we do not know any staged predation experiment resulting in elevated rates of rotation, it was reported once (two tadpoles of *Hymenochirus boettgeri*) after experimental limb amputation (Girvan et al. 2002). In contrast, various chemicals are known to cause rotation and/or stiffness of limbs (see below).

In five of the cases of rotation, stiffness has also been mentioned explicitly and in two of them also atrophied musculature (Canfield et al. 2000, Helgen et al. 2000, Vandenlangenberg et al. 2003, Reeves et al. 2013, Henle et al. 2017a). We suggest that it is likely that in most cases of rotation stiffness was simply not mentioned. In one of the five cases, a *Bufotes viridis* population in a quarry at Roßwag, Germany, in more than 25 out of 295 toadlets sampled in 1980 the limbs were rotated and stiff; the anomalies were most likely induced by radioactive pollution (Henle et al. 2017a). In a population of *Lithobates sphenocephalus* from Goose Pond, Patuxent Research Reserve, one of 33 (sample size 595) abnormal individuals had a twisted bone and another one a stiff limb. In populations of *L. pipiens* at the NEY and the ROI hotspot site in Minnesota, respectively, approximately 35 (n = 2493) and 4 (n = 1394) individuals exhibited rotation of long bones and approximately 38 and 29–30 individuals showed muscle atrophy (Helgen et al. 2000, Vandenlangenberg et al. 2003).

In addition to the cases in which stiff limbs and torsion of legs co-occurred, we found 27 cases of stiff limbs covering 13 anuran species, in which rotation was not mentioned. All of these cases involved only a few individuals or stiff limbs occurred in low frequency.

In contrast to the scarce observations in natural populations, torsions and stiffness of limbs have been observed repeatedly in captive raised offspring of many anurans and urodelans, especially in Dendrobatidae (e.g., Klatt 1927, Krefft 1938, Ensinck 1978, Heselhaus 1983, Conaghan 1987, Cummins 1987, 1989, Glaw 1987, Krintler 1988, Birkhahn 1991, Meyer 1996, Zimmermann & Aeschbach 2005). Nutritional deficiencies, especially in Ca^{2+}, inbreeding and too high temperatures were frequently assumed to be the causes but were rarely addressed experimentally. Marshall et al. (1980) and Leibovitz et al. (1982) showed that diets low in calcium or vitamin C, respectively, caused twisted limbs in cultured *L. catesbeianus* larvae and that an enrichment of the diet with these nutrients significantly reduced the rate of deformities. Similarly, hatchery reared *P. perezi* tadpoles developed elevated rates of stiff legs and torsions of the vertebral column and tail when fed on artificial diets but no anomalies appeared when they were fed on fresh lettuce (Martínez et al. 1992). Likewise, stiffness and rotation of the legs developed after hatching in all *Lissotriton vulgaris* fed on a diet of mussle meat as larvae but not in controls (Klatt 1927, Krefft 1938). Both anomalies also occurred in *A. mexicanum* and *Pleurodeles waltl* fed on a pure enchytraea diet; however *Triturus* (sensu lato) spp. were insensitive to such a diet (Reinhardt 1939, Vogt 1939). Stiff legs also appeared in more than 50% of metamorphosing *Gastrotheca marsupiata* from two clutches that were raised at 26°C but none in 11 clutches of the same parents that were reared at lower temperatures (Ensinck 1978). However, the clutches were deposited at different times so that confounding factors cannot be ruled out completely.

Stiffness of legs was very frequent in hybrids of *T. cristatus* × *L. helveticus* and *T. cristatus* × *L. vulgaris* (Hamburger 1935). Stiffness and torsion of legs also occurred often in experimental hybrids within the *Pelophylax esculentus* complex (Berger 1971, Günther 1990, Berger & Berger 1992). Flindt & Hemmer (1967) and Hemmer (1973) reported stiffness of limbs (frequency not given) in one, but not in a second, experimental cross of *Epidalea calamita* × *Bufotes viridis*. As other authors never observed it in any experimental hybridization among European bufonid species, a causal relationship with hybridization is questionable.

Chemical treatment can also induce stiffness and torsion of legs. Stiffness is induced frequently when tadpoles are reared in hydrogen-enriched tapwater (Sladden 1932), water contaminated by petroleum (Danilova 1992), hydrocarbons (Fort et al. 2006a) and sugar cane solutions (Tornier 1908, Sladden 1932, Piiper 1933). It also appeared in a few individuals that were exposed as tadpoles to DDT, DDE (Cooke 1971, 1973), or endosulfan (Gelnarova 1987a,b). Various PCBs that act as endorcrine disruptors (Qin et al. 2005) as well as thymus oil, various other oils that inhibit the growth of the thyroid gland, the sulfonamids nadisan and aristamid that interfere with sugar and energy metabolisms (Wurmbach et al. 1964, Wink & Wurmbach 1967) and estradiol (Takahashi 1958) can cause stiffness and torsion of legs.

These anomalies also appeared in tadpoles of *Rana temporaria* reared in pH 4 under low to medium density (4–8 tadpoles/l) but not under high density (16 tadpoles/l) (Cummins 1987). However, the effect of pH could not be isolated from that of the diet. Indeed, no anomalies appeared when the diet was changed. While low pH itself probably does not cause torsion of legs, it enhances the effect of vitamin C deficiency in the diet on the appearance of twisted legs in *Lithobates catesbeianus* (Leibovitz et al. 1982).

Bent knees appeared in metamorphs as a carry-over effect when embryos of *R. temporaria* were exposed to enhanced UV-B, with doses still within natural variation (Pahkala et al. 2001). Irradiation of eggs, sperm, or embryos caused torsion of legs in a few to many metamorphs of *Xenopus laevis* (Schinz & Fritz-Niggli 1954), *B. bufo* (Oerter 1985), *P. nigromaculatus* (Kawamura & Nishioka 1978) and *R. temporaria* (Rostand 1955a, 1958a, Roth 1988). For *P. nigromaculatus* stiff legs without rotation were also reported and both types

of anomalies still occurred in the second generation (Kawamura & Nishioka 1978).

Inheritance of stiff legs was further shown by Krotoski et al. (1985), Kovalenko (2000) and Kovalenko & Kovalenko (2000) in inbred *X. laevis*. In some laboratory stocks of *Pleurodeles waltl,* anteversion of the knee joint seems to be inherited, with temperature acting epigenetically (Dournon 1983). In humans, bent bones may also be due to mutations and may appear as a syndrome together with brachymely, brachydactyly and knee joint anomalies (Cohn & Bright 1999).

3.2.3.6 Stiff and truncated vertebral column and torsion of the vertebral column. We only know four populations in which truncated stiff vertebral columns were observed: more than ten toadlets of *Bufotes viridis* in a quarry near Roßwag, Germany (Henle et al. 2017a), with radioactive contamination most likely being the cause. Jarvis (2011) and Raichoudhury & Das (1931) found one adult male *Triturus cristatus*, and one adult *Duttaphrynus melanostictus*, respectively, in which the lower vertebral column was fused and stiff; in the latter case, the anomaly was not visible externally. Schmelcher & Hellmich (1951) reported one larval *Salamandra salmandra* with a truncated body that was born by a recently captured female.

While fusion of vertebrae has been reported for various species, generally no information is given as to whether fusion resulted in a truncated stiff vertebral column (e.g., Benham 1894, Grewal & Dasgupta 1967, Worthington 1974, Prigioni & Langone 1985, Gollmann 1991, Vershinin & Neustroeva 2011). In some species (e.g., *B. bufo, Pelophylax esculentus*) fusion of some vertebrae is regarded as normal variation (e.g., Adolphi 1893, 1895).

The oldest record of torsion of the vertebral column is a capitosauroid, presumably *Eocyclotosaurus wellesi* from the Middle Triassic of Arizona (Witzmann 2007). For extant amphibians, only one study reported elevated rates of torsion of the vertebral column. Agostini et al. (2013) observed it in 73 (n = 2030) juvenile *Leptodactylus latrans* and 13 (n = 427) *Boana pulchella*. It has also been observed in two *Eurycea junaluska* (Ryan 1998), one *Plethodon idahoensis* (Peterson et al. 1999), two *P. glutionosus,* two *P. kentucki* (Marvin 1995, Marvin & Hutchison 1997), one *Pseudotriton ruber* (Haines-Eitzen 2016), in *Notophthalmus viridescens* (Collins 1932), one adult female *Lissotriton vulgaris* (D'amen et al. 2006), one metamorph of *Pseudacris regilla (*Reeves et al. 2013), one *Pelobates fuscus* (Nöllert 1990), *Bufo bufo* (Weddeling & Geiger 2011), one *Trachycephalus typhonius* (as *Pseudohyas hebes*: Vizotto et al. 1977), one female each of *Sphaerotheca breviceps* and of *Fejervarya kirtisinghei* (De Silva 2009), in at least five *Lithobates pipiens* (Levey et al. 2003, Lannoo 2008, Reeves et al. 2013), one *L. sylvaticus* (Reeves et al. 2013), one *L. septentrionalis* (Schoff et al. 2003) and in an unstated number of *Pelophylax ridibundus* (Spirina 2009). Lannoo (2008) assumed pollution to be the cause for the case observed by him but this assumption was not tested. Other authors did not discuss potential causes.

Irradiation of the eggs of *Pleurodeles waltl* and the sperm of *Xenopus laevis* resulted in offspring with a truncated vertebral column (Labrousse 1967, Hart & Armstrong 1984). Malformed backbones were further reported by Nishioka (1978) in the offspring produced by irradiating eggs or sperm of *Rana japonica*.

Using gynogenesis, Krotoski et al. (1985) discovered a case of inherited truncated vertebral column in *X. laevis* and Kovalenko (1994) observed vertebral fusion, together with asymmetry of the vertebral column, in an inbred line of the same species. Dasgupta & Grewal (1968) revealed a single dominant gene responsible for the fusion of vertebrae in five populations of *Euphlyctis cyanophlyctis* around Dehli.

The cause(s) of torsion of the vertebral column has been neglected so far in amphibians. In humans, it may be due to trauma, cancer, neuromuscular disease, inflammation of the vertebral column, oxygen deficiency and in rare cases mutations (McMaster 2001, Asher & Burton 2006).

3.2.3.7 Torsion of the tail or body in tadpoles and torsion of the tail in adult urodeles. In natural populations torsion of the tail and/or body has been noticed in 57 populations of 22 anuran and two urodele species but probably often remains undetected or unreported. Only two cases are known for urodele larvae: one *Salamandra salamandra longirostris* with a shortened bent tail from Spain (Escoriza & Garcia-Cardenete 2005) and one *S. salamandra* with a bent tail from Germany (Henle unpubl.). The latter lost the torsion during metamorphosis. Only Collins (1932) reported bent tails in adult newts: one male *Notophthalmus viridescens* with marked lateral undulatory twists in the tail.

Elevated rates of bent tails or bodies in tadpoles were reported from several sites. In a population of *Lithobates pipiens* from Maryland, 28.4% (n = 74) were affected (Hardy (1964). Bruch (1864) found 39 tadpoles of *Pelobates fuscus* with bent tails among more than 1000 individuals examined. In both cases the cause remains unknown. In a population of *Bufotes viridis* from a quarry in Roßwag, Germany, in which elevated levels of radioactivity were measured, 35–55% of the tadpoles had bent tails (total tadpole population size 2000–5000) (Henle et al. 2017a). In tadpoles of *L. catesbeianus* from wetlands constructed with treated wastewater in Georgia 54 out of 833 individuals were affected but none out of 190 individuals from reference sites (Ruiz et al. 2010). Also, the frequency dropped substantially with the distance from the discharge point. At the Savannah River National Research Park, 17.7% and 36.8% of the *L. catesbeianus* tadpoles sampled in two water bodies polluted by coal combustion waste were affected by torsion of tails whereas only 0% and 4.4% were affected at two control sites (all $n \geq 350$) (Hopkins et al. 2000). In water bodies of the Nikopol District, Ukraine, that were polluted by chemical and metallurgical discharge from factories,

5.8% (n = 400), 2.6% (n = 500) and 7.2% (n = 500) of the *Bombina bombina*, *Bufotes viridis* and *Pelophylax ridibundus* tadpoles, respectively, had torsions of the body (Flax & Borkin 2004). In Sakhalin, Russia, the percentage of recently hatched tadpoles of *Bufo gargarizans, Rana amurensis* and *R. pirica* with torsions of the body or tail increased with a decrease of water temperature and pH (Flax 1986). In the city of Moscow, 10% of the tadpoles of *R. temporaria* were affected by torsion of the body, whereas this was only the case in 4% in a pond at the Biological Station of the Lomonosov University 50 km west of Moscow (Aguillón Gutiérrez & Severtsova 2012). The cause for the difference is unknown.

Torsion of the body and/or tail is a very common anomaly in experimental studies of amphibian embryos, but much less is known for tadpoles. In embryos it develops, for example, after mechanically induced defects and constrictions (reviewed by Shen 1938), under malnutrition (Krefft 1938), after high intensity ultrasound treatment (Bonhomme & Pourhadi 1957, Bonhomme et al. 1960, Pourhadi et al. 1968), from overripe eggs (Briggs 1941) and in experimental hybridization, including all European bufonid species (Hertwig et al. 1959, Flindt & Hemmer 1967, Flindt et al. 1968, Schipp et al. 1968, Delarue 1974, 1982), within the *P. esculentus* complex (Berger 1967, Berger & Uzzell 1977, Tunner 1980) and in crosses of *Pseudophryne corroboree* from different geographic origins (Osborne & Norman 1991). Hybrid tadpoles of tetraploid *Bufotes viridis* × *B. pewzowi* may also show bent tails and curvature of the body (according to photographs in Stöck 1998). Tadpoles that developed from a clutch of a *R. arvalis* female that mated with a *R. temporaria* male in nature showed bent tails and all died before hindlimbs emerged (Luttenberger 1976).

A wide range of chemicals also causes torsions of the tail or body in embryos and some of them also in tadpoles (partially reviewed by Power et al. 1989). Among these are DDT and other organochlorides (e.g., Cooke 1971, Marchal-Ségault & Ramade 1981), carbamate insecticides (e.g., Rzehak et al. 1977, Cooke 1981, Raj et al. 1988, Bridges 2000), nitrate and nitrite (Hecnar 1995, Marco et al. 1999), some metals (Plowman et al. 1994), excess Vitamin A (e.g., Syuzyumova 1985) and low or high pH (Ferraro & Burgin 1993). These experimental studies corroborate the conclusion of pollution being the cause for the elevated rates of torsion of the tail or body in the field studies of polluted sites mentioned above.

When reared at elevated temperature (18°C), a high proportion of the larvae of *Euproctus asper* developed bent tails (Clergue-Gazeau 1971). Likewise, some larvae of *Lissotriton helveticus* hatching from eggs that were kept at a high temperature (22°C) showed a curvature of the tail, whereas those hatching from eggs maintained at 18°C or 14°C did not (Galloy & Denoël 2010). Unfortunately, the difference was not tested statistically. Notwithstanding, these observations and those of Flax (1986) mentioned above suggest that both too high and too low temperatures may result in increased frequencies of bent tails or bodies.

Exposure to radium bromide, γ- and X-rays very frequently result in torsions of the bodies or tails in embryos and tadpoles (e.g., Hertwig, O. 1911, Hertwig, G. 1913, Stachowitz 1914, Labrousse 1967, Nishioka 1977, 1978, Kawamura & Nishioka 1978, Oerter 1985, Trux 1985). The same applies for high intensity light (Ruehmekorf 1958) and unnaturally high doses of UV-B (e.g., Worrest & Kimeldorf 1975, Kashiwagi 1980, Grant & Licht 1995). Hays et al. (1996) found elevated rates of torsion of the tails in *Pseudacris regilla* when treated with moderate UV-A+UV-B, but not when treated only with UV-A. Furthermore, the treatment was only effective if tadpoles were kept in dim laboratory light but not under moderate light; the dim light may have inhibited photo-induced repair mechanisms of UV damage.

Besides the factors suggested for natural populations, amputation of tails at an angle may cause regeneration that leads to bent tails in various anurans and urodeles (Barfurth 1891).

Bent tails in tadpoles and bent bodies of metamorphosed individuals also occurred after experimental infection at the pre-limb bud stage (Gosner stages 25 and 26) tadpoles of the Sri Lankan *Polypedates crucifer* with *Acanthostomum burminis* cercariae (Jayawardena et al. 2010, Jayawardena & Rajakaruna 2013) but not in controls (difference not tested statistically). Only other types of anomalies were observed when limb bud stage tadpoles (Gosner stages 27 and 28) were exposed to the cercariae (Rajakaruna et al. 2008).

A genetic basis of torsion of the tail was discovered in a laboratory strain of *Pleurodeles waltl,* with temperature acting epigenetically (Fernandez & Beetschen 1975), in tadpoles of *Lithobates pipiens* (Browder 1972), in *Xenopus borealis* (Droin 1985) and in *X. laevis* (Elsdale et al. 1958, Droin & Chavane 1976, Hart & Armstrong 1984). *Rana japonica* and *Pelophylax nigromaculatus* originating from irradiated eggs or sperm maintained torsion of bodies and tails and other anomalies up to the third generation; in the fourth generation the frequency was similar to controls (Nishioka 1977, 1978, Kawamura & Nishioka 1978). Torsion of tails may have a genetic basis in other species as well (Freytag 1955, Sládecek 1964).

3.3 Non-skeletal morphological anomalies

3.3.1 Duplications and absence of structures

3.3.1.1 Duplication of eyes or spiraculum. Héron-Royer (1884) discovered an embryo of *Alytes obstetricans* with four eyes. He also observed a *Pelobates fuscus* tadpole with two spiracula instead of one.

3.3.1.2 Tympanum absent. While several amphibian species and genera lack an externally visible tympa-

num (Passmore & Carruthers 1995), i.e., absence is the normal condition, we found only five publications that mention an abnormal absence of the tympanum. Martof (1956) detected one *Lithobates clamitans* that lacked a tympanum and four more individuals in which the tympanum was ruptured. He assumed attacks by rock bass (*Amblopites rupestris*) to be the cause. Grosse (2015) published a photo of an adult *Rana temporaria* that lacked an externally visible tympanum. Reeves et al. (2013) listed one metamorph of *L. pipiens* and of *L. sphenocephalus* with the tympanum lacking. Bleakney (1963) discovered an *Anaxyrus americanus*, whose tympanum, and parotoid glands, had been destroyed by maggots of *Lucilia silvarum*. Finally, Peltzer et al. (2011) reported the absence of the tympanum in a sample of 12 Argentinian anuran species, without providing numbers or the species affected; they illustrated the anomaly for one *Leptodactylus mystacinus*.

3.3.1.3 Macrophthalmy, microphthalmy, anophthalmy and cyclopy. Macrophthalmy, microphthalmy (Fig. 22), anophthalmy (Fig. 23) and cyclopy in natural populations of amphibians have not yet been reviewed. Our database contains eight individuals with macrophthalmy: an adult *Bufo bufo* from Russia (Korzikov & Alekseev 2014) and one metamorphosing individual of *Acris* species, *Lithobates blairi, L. palustris*, *L. pipiens*, *L. sphenocephalus,* as well as two individuals of *L. sylvaticus* from the USA (Reeves et al. 2013).

Our data base further contains 53 cases of microphthalmy involving 20 species. Most of them are from surveys across many sites, involving only a few individuals. Bacon et al. (2006b) observed 18 and 21 *Rhinella marina* (n = 13,856) with microphthalmy and anophthalmy, respectively, in a survey of 38 sites in Bermuda and assumed pollution (metals and pesticides) to be the cause. To test this hypothesis, Fort et al. (2006b) exposed embryos to sediments from contaminated sites and raised them until metamorphosis had been completed in laboratory microcosms. This treatment resulted in elevated rates of eye anomalies (12.1%; n = 272) whereas no control individual showed any anomaly.

For individual populations, Haensch (1985), Harris et al. (2001) and Burton et al. (2008) each reported a single *Salamandra salamandra, L. pipiens* and *L. catesbeianus*, respectively. Maruschak & Muravynets (2013, unpubl.) further reported it for adult *Pelobates fuscus* and *Pelophylax ridibundus*. Burton et al. (2008) suggested ranavirus FV3 to be the cause, as they observed FV3 particles in the granuloma. Experimental studies assessing the potential of virus to cause microphthalmy or anophthalmy are still lacking.

Anophthalmy is known from 159 cases, involving 55 species. Only eight of these species are urodeles. The rarity of anophthalmy in natural populations of urodeles can be explained by their high capability of regeneration. In *Cynops pyrrhogaster,* extirpation of the eye lens 18 times within 16 years was followed each time by regeneration (Eguchi et al. 2011).

All but three publications mentioned less than ten affected individuals. A striking hotspot occurs on the oceanic island Fernando de Noronha, Brazil. In the introduced Cururu toad (*Rhinella jimi*) 17% (n = 179) of the adult toads collected in 2009 and 2010 lacked one or both eyes (Tolledo & Toledo 2015). In 2008, only 8% (n = 159) of the toads showed anophthalmy (Toledo & Ribeiro 2009). The cause is unknown but could be the result of inbreeding caused by founder effects (Tolledo & Toledo 2015). Gurushankara et al. (2007) collected 12 (n = 1670) *Fejervarya keralensis* with anophthalmy in contaminated rice paddies in India, whereas not one of the 239 individuals collected in forests was abnormal. Three further species (*F. brevipalmata, F. limnocharis, F. rufescens*) were also affected by anophthalmy or microphthalmy but at slightly lower frequencies.

Reeves et al. (2008) found 12 (n = 5716) *L. sylvaticus* with anophthalmy in a survey of 38 sites in Kenai Wildlife Refuge, Alaska. They also assumed that pollution, together with predators, were the cause of the eye anomalies observed. While many chemicals cause eye anomalies in embryos, much less is known of their effects on tadpoles. Tadpoles developing from clutches of *Dryophytes chrysoscelis* that invaded mesocosms contaminated with atrazine and chlorpyrifos developed anophthalmy (Britson & Threlkeld 1998). Feeding *Xenopus laevis* tadpoles with retinoic acid resulted in high rates of anophthalmy and microphthalmy (up to 45.5%), with stage 46 tadpoles being more sensitive than later stages (Alsop et al. 2004).

Fig. 22: Microphthalmy in an adult male *Rana arvalis*, Verhnaya Serga village, Russia, 21.4.2012. Photo: V. Vershinin.

Fig. 23: Unilateral anophthalmy in *Bombina bombina*, Morava-Dyje floodplains, Austria, 6.8.2012. Photo: U. Nüsken.

Klewen (1986) mentioned a *S. atra* that lost its eyes and received tail injuries due to the attack of a bird. Attacks from predators and injuries were also suggested for several other cases involving single individuals (e.g., Martof 1956, Gollmann & Gollmann 2012). Anophthalmy occurred occasionally in *B. bufo* tadpoles that were experimentally exposed to dragonfly nymphs (Ballengée & Sessions 2009). Taton (1877) and Grosse & Simon (2015) each reported a case in which the eyes of a *B. bufo* had been destroyed by maggots of the parasitic fly *Lucilia bufonivora*. In captivity, one *D. versicolor* lost its eye due to infestation with maggots of *L. illustris* (Anderson & Bennett 1963). Mitchell et al. (2004) suggested a genetic origin for anophthalmy in a single *Plethodon cinereus* that they found but they did not test their hypothesis. However, Jaylet (1971) and Krotoski et al. (1985) demonstrated the heritability of microphthalmy in *Pleurodeles waltl* and *X. laevis*, respectively.

Besides the factors suggested for observations in natural populations, hybridization may also result in microphthalmy or anophthalmy. Natural hybridization of a *R. ictericus* male with a *R. crucifer* female resulted in four individuals with anophthalmy and three with microphthalmy out of eight individuals that had successfully metamorphosed (Haddad et al. 1990). Anophthalmy may also be common in hybrids of *D. versicolor* × *D. cinereus* (Mable & Rye 1992) and in offspring of *Pelophylax lessonae* × *P. esculentus* (Berger & Uzzel 1977).

Whereas cyclopy is a rather common anomaly in embryos (Tornier 1908, Shen 1938), for postembryonic stages from natural populations, it was reported only for one albinistic larval *S. salamandra* (Cabela 1987).

3.3.2 Other non-skeletal morphological anomalies

3.3.2.1 Retention of tail in anurans after metamorphosis. Only a few observations have been made for natural populations. Olivier (1893) reported it for the first time in an adult *Bufo bufo* (see also photograph in Dubois 1979a). This remains the only publication for an adult anuran but De Silva (2009) mentioned an extension of the spine beyond the rump in an unidentified adult(?) *Philautus* species from Sri Lanka that may also be an incomplete resorption of the tail. Note that the figures of two adult toads with tails published by Aldrovandi (1645) are based on fake specimens. In addition, Hassine et al. (2011) labelled a photograph of a *Pelophylax saharicus* with incomplete tail retention as adult but this age class is inconsistent with the size bar and recently metamorphosed *Pelophylax* spp. may retain their tail for some time (Fig. 24).

All other observations relate to juveniles. Lannoo (2008) reported two recently metamorphosed *Lithobates catesbeianus* as well as *L. pipiens* and one recently metamorphosed *Acris blanchardi* with incompletely resorpted tails. He assumed, but did not test, that chicken waste fertilizer and other chemicals were the cause and suggested that unresorbed tails are common. Likewise, Helgen et al. (2000) collected one recently metamorphosed *L. pipiens* with a very long tail and McCallum & Trauth (2003) reported 37 *A. crepitans* with retained tails. García-Muñoz et al. (2010) collected a metamorphosed *Bufotes boulengeri* with an almost complete tail. Eikamp (1980) discovered a 28 mm long metamorphosed juvenile *Pelophylax esculentus,* which retained a long tail with tail fins still present and Hassine et al. (2011) four such *P. saharicus*. These observations may not be abnormal. In various frog species [e.g., *Bufo bufo, Osteocephalus elkejungingerae, P. ridibundus* (Fig. 24), *Rana temporaria*] metamorphosing individuals may leave the water with long tails that will be resorbed later (Henle & Vershinin, unpubl.). On the other hand, treating larval stages of *Xenopus laevis* with substances that interfere with the thyroid axis (rape-seed oil, wheat-germ oil, sesame-seed oil, cottonseed oil, thymus oil, labrafil, methimazole, or pentachlorophenol) does result in incomplete or delayed tail resorption (Pohland 1962, Wurmbach et al. 1964, Wink & Wurmbach 1967, Fort & Stover 1997).

To enable a more certain conclusion that retained tails in metamorphs are abnormal and not within the normal variation of a species, we recommend comparing and reporting the distribution of tail length relative to snout-vent length for sufficiently large samples across several sites.

3.3.2.2 Arms not emerging from the branchial cavity. We found reports of 48 individuals from natural populations in which one arm remained hidden within the branchial cavity: 13 *Rhinella marina* (n = 13,856; Bacon et al. 2006b), ten tadpoles of *Bufotes viridis* in the final stages of metamorphosis in a quarry in Roßwag, Germany (Henle et al. 2017a), three individuals in Argentina (one *Leptodactylus latrans*, identity not provided for

Fig. 24: Juvenile *Pelophylax ridibundus*, with tail still very long; additionally, the opecular chamber is still open, Ekaterinburg, Russia, 17.8.2005. Photo: V. Vershinin.

the other two individuals) (Peltzer et al. 2001), three *Lithobates pipiens* (Meteyer 2000, Levey et al. 2003, Lannoo 2008) and one individual each of *Bombina bombina* (Fayzulin 2011), *B. variegata* (Dubois 1979a), *Ranoidea aurea* (Richardson & Barwick 1957), *Pseudacris regilla* and *Lithobates sylvaticus* (Reeves et al. 2013). Annandale (1905) reported a tadpole of *Clinotarsus alticola* in which both forearms remained hidden beneath the skin; the individual also lacked both hindlimbs. Murphy (1965) collected 12 *L. palustris* (n = 990) in which one or both forelegs only partly emerged. In addition, we have a photograph of a juvenile *Rana arvalis* (Fig. 25) from Russia in which one foreleg did not erupt.

The cause of the *B. viridis* case was most likely to be radioactive pollution (Henle et al. 2017a). Murphy (1965) considered a genetic cause; no cause has been suggested for the remaining cases.

Failure of forelimbs to emerge from the branchial cavity occurred in several offspring of *Incilius valliceps* whose parents descented from the cross of an irradiated male and a control female (Blair 1960).

Several factors not considered for natural populations had been associated with the failure of a limb to emerge from the branchial cavity in experiments and in husbandry. Günther (1990, 1996b) occasionally observed it in experimental crosses within the *Pelophylax esculentus* complex. In tadpoles of a F_3 clutch of *Discoglossus pictus auritus* that developed thin and stiff front legs, these also occasionally remained covered by the opercular fold (Glaw 1987). Likewise, in captive *Gastrotheca riobambae,* the front legs often had difficulties to break through the skin and the skin had to be opened surgically (Hammermeister 1991). In some *Pleurodema cinereum* that were fed with food tablets the front leg remained hidden within the branchial cavity (Christmann 1995). After termination of the feeding of food tablets, the remaining tadpoles developed normally.

Fig. 25: One arm remaining covered underneath the opercular fold of skin in a juvenile *Rana arvalis*, Ekaterinburg, 8.7.1987. Photo: V. Vershinin.

Disruption of the thyroid axis often leads to one arm remaining within the branchial cavity. When tadpoles are fed with mammalian thyroids (Romeis 1914/15, 1918, Kahn 1916) or thymus oil extracts (Wink & Wurmbach 1967), one arm often fails to break through the skin that covers the branchial cavity, presumably due to a thickening of the skin (Romeis 1918). Many tadpoles of *Dryophytes versicolor* treated with perchlorate from the early larval stages onwards and iodine added after 70 days died during metamorphosis, often with only one arm having emerged (Sparling et al. 2003). Perchlorate strongly competes with iodine for storage in the thyroid and thus interferes with thyroid hormone production. When larval *Hoplobatrachus tigerinus* were treated with the pesticide methyl parathion, front legs also failed to break through the branchial cavity (Kennedy & Sampath 2001) but no data were presented; thus, the frequency and whether the effect was significant or not cannot be assessed.

In one individual of *L. sylvaticus* exposed to a strong magnetic field at the egg stage (17,700 Gauss), the arm did not emerge from the branchial cavity (Levengood 1969).

3.3.2.3 Skin webbing. Although skin webbing (synonym: cutaneous fusion), together with bony triangles, has been regarded as diagnostic for abnormal development induced by infection with the trematode *Ribeiroia ondatrae* (Johnson & Hatrson 2009), neither a review of its occurrence in natural populations nor in experimental teratogenesis exists. We collated 41 cases in our database, all but one from anurans. Except for seven, all originated from North America, involving the species *Ambystoma macrodactylum croceum* (Johnson et al. 2006), *Anaxyrus americanus* (Johnson & Hartson 2009), *A. boreas* (Johnson et al. 2001b), *Lithobates blairi, L. palustris, L. sphenocephalus, L. sylvaticus* (Reeves et al. 2013), *L. clamitans, L. pipiens* (Vandenlangenberg et al. 2003, Hoppe 2005, Reeves et al. 2013), *Rana luteiventris* (Johnson et al. 2002, Richards & Dickinson 2012), *L. septentrionalis* (Gardiner & Hoppe 1999, Vandenlangenberg et al. 2003, Hoppe 2005), *L. sylvaticus* (Reeves et al. 2008), *Pseudacris regilla* (Sessions & Ruth 1990, Johnson et al. 2003, Roberts & Dickinson 2012), *R. aurora* (Johnson et al. 2002) and *R. pretiosa* (Bowerman & Johnson 2003).

The seven cases outside North America are a ranid frog from Germany (Landois 1882), in which skin webbing extended along the entire upper and lower shank, up to twelve *Bufo bufo* – it is unclear how many of them had skin webbing and how many syndactyly – from Germany (Veith & Viertel 1993), one adult each of *B. bufo* and *Hyla arborea* from the Ukraine (Maruschak & Muravynets 2013), one *Hyla meridionalis* from France (Delcourt 1963), three individuals of *Rhinella marina* from Bermuda (Bacon et al. 2006b) and a juve-

nile *R. jimi* from a population introduced to the island of Fernando de Noronha (Toledo & Ribeiro 2009). In the German *B. bufo* the anomaly was likely due to limb damage from leeches (*Erpobtella octoculata*).

While the cause(s) of the North American cases has been controversial (Sessions & Ruth 1990, Gardiner & Hoppe 1999, Stopper et al. 2002, Bridges et al. 2004, Lannoo 2008), many of these cases were associated with *Ribeiroia ondatrae.* Skin webbing is a frequent anomaly in experimental infections with *R. ondatrae* (e.g., Johnson et al. 1999, 2001b), albeit not always. For example, experimental infection of *Ambystoma macrodactylum* did not produce a single individual with cutaneous fusion but the anomaly was common in a field site (Jette Pond), in which the parasite was present. In spite of this inconsistency all anomalies were explained by infection with *R. ondatrae* (Johnson et al. 2003, 2006). It should be noted that, while chemicals have been rejected categorically as an explanation for mass anomalies (e.g., Stopper et al. 2002, Sessions 2009), a lipophilic extract of a water sample from the CWB hotspot site, where *L. pipiens, L. septentrionalis* and *L. clamitans* exhibited skin webbing (Vandenlangenberg et al. 2003, Hoppe 2005), induced the same anomaly in the laboratory (Bridges et al. 2004). Notably, two commonly used agricultural chemicals, carbaryl in combination with atrazine, significantly increased the frequency of skin webbing in *L. pipiens* (up to 80% of the treated individuals) (Bridges et al. 2004).

In some locations in which skin webbing was detected, e.g., in the Kenai Wildlife Refuge in Alaska, *R. ondatrae* could not be found and pollution is a more likely explanation (Reeves et al. 2008). Moreover, *R. ondatrae* is absent from Bermuda (Bacon et al. 2006b) and Europe. The Bermuda cases have been explained by pollution (Bacon et al. 2006b) and the German cases by damage inflicted by leeches (Veith & Viertel 1993). Thus, skin webbing can be caused by factors other than infection by *R. ondatrae* and both *R. ondatrae* and pollution were likely to have contributed to the reported cases.

3.3.2.4 Misplaced or abnormal spiraculum, ventral tube, or operculum. We only know of three publications that mention this anomaly for natural populations. In a tadpole of *Pelobates fuscus* the spiraculum was situated on the wrong side of the body (Héron-Royer 1884). In one out of 47 *Leptobrachium pullum* the spiraculum was dextral instead of sinistral (Sunderasan & Rowley 2012). Additionally, the ventral tube was sinistral instead of dextral and various internal organs also showed reversed positions. One albinistic tadpole of *Bufotes viridis* collected in a quarry near Roßwag, Germany (an anomaly hotspot most likely due to radioactive contamination), had a mid-ventrally instead of laterally placed spiraculum (Henle et al. 2017a).

Only in two experimental studies a misplaced or abnormal spiraculum was mentioned. Berger (1967) detected two tadpoles with the spiraculum on the left instead of the right side and one on the belly among approximately 3000 individuals obtained in experimental crosses among the species *Pelophylax esculentus, P. lessonae* and *P. ridibundus*. In addition, Oerter (1985) noted one individual with a malformed operculum in a *B. bufo* that had been irradiated as an embryo.

3.3.2.5 Mouthparts abnormal in tadpoles. We do not know of any review of this type of anomaly. Our database contains 94 cases covering at least 74 anuran species. In 37 out of 72 cases with a sample size of at least 50, more than 10% and up to 100% of the individuals were affected and Mendelson et al. (2004) reported that 60–70% of several thousand tadpoles of *Ptychohyla hypomykter* showed abnormal mouthparts.

Of the cases with abnormal mouthparts 59% were attributed to infection by the chytrid fungus *Batrachochytrium dendrobatidis.* Infected individuals typically showed absence of dark keratinization of mouthparts, had missing, misshaped or interrupted labial tooth rows, swollen oral papillae or malformed jaw sheaths (e.g., Lips 1998, 1999, Fellers et al. 2001, Lips et al. 2004, Rachowicz & Vredenburg 2004, Knapp & Morgan 2006, Felger et al. 2007, Patel et al. 2012, Vieira et al. 2013). Therefore, malformation and absence of dark keratinization of mouthparts were used as characteristics to diagnose this disease in early studies. However, this is not possible. Infected tadpoles may appear normal and depigmentation can have other causes. For example, 27–94% of the tadpoles of four Californian anurans had malformed mouthparts without an infection with *B. dendrobatidis* (Padgett-Flohr & Goble 2007). Similarly, Drake et al. (2007) counted mouthpart anomalies relating to labial papillae, tooth rows and keratinized jaw sheaths in 13 populations of North American anurans belonging to the genera *Anaxyrus, Hyla* and *Lithobates*. Malformed mouthparts were common in all populations (13.5–98%) but *B. dendrobatidis* was only found in three of them. Some anomalies were minor and would probably be regarded as normal natural variation by others, such as Nichols (1937) in his study of *A. fowleri, Pseudacris triseriata* and *L. pipiens.* Rachowicz (2002) showed experimentally for *Rana muscosa* that pigmentation of mouthparts changes seasonally, with an absence of pigmentation under cold temperatures and reversibility of depigmentation when tadpoles were transferred to higher temperatures.

In a population of *Rhinella jimi* introduced to the oceanic island Fernando de Noronha 52.5% (n = 413) of the tadpoles collected from 2009 to 2010 had abnormal mouthparts, including missing tooth rows and lack of dark keratinization of mouthparts (Tolledo et al. 2014). Likewise, 45% out of 113 tadpoles of *Peltophryne fustiger* collected on Cuba had abnormal mouthparts including lack of dark keratinization (Alfonso Bosch et al. 2017). The cause for these high rates of anomalies remains unknown.

Abnormal mouthparts are often observed in captive raised tadpoles, e.g., in *A. americanus* (Tubbs et al.

1993), *Sclerophrys gutturalis* (Wager 1986) and *R. arvalis* (Grillitsch & Grillitsch 1989, Trubetskaya 2006). They appear frequently when tadpoles are raised at high but rarely when they are raised at low temperatures (Bresler 1954), which is opposite to what Rachowicz (2002) observed for the presence of dark keratinization of mouthparts.

Abnormal mouthparts were twice as common in the hybrid zone of *Bombina bombina* and *B. variegata* compared to populations from both species (Szymura & Barton 1986), but statistical tests were not presented.

Two cases of abnormal mouthparts were due to chemical pollution. In *L. catesbeianus* living in a pond contaminated by coal combustion waste, mouthparts lacked dark keratinization and labial tooth rows in 96% of the tadpoles (n = 1048). Rowe et al. (1998) showed experimentally that the pollution was responsible. In a *Bufotes viridis* population from a quarry in Roßwag, Germany, more than 250 (n = 2000–5000) tadpoles showed malformed mouthparts that were most likely due to radioactive contamination (Henle et al. 2017a).

Chemicals and irradiation also frequently induce abnormal mouthparts in experimental exposure studies, e.g., aminocarb in *L. clamitans* (Lyons et al. 1976 fide Power et al. 1989), DDT and DDE in *Bufo bufo* and *R. temporaria* (Cooke 1971, 1972, 1973), Zn in *Xenopus laevis* (Dawson et al. 1988), jodothyrin and thyroid extracts in *R. temporaria* (Romeis 1918). Oerter (1985) and Trux (1985) observed missing and interrupted labial tooth rows, partial absence of denticles and the keratinized beaks and lack of labial papillae in tadpoles developing from irradiated embryos of *B. bufo*. Moreover, *R. japonica* and *Pelophylax nigromaculatus* raised from irradiated eggs or sperm transmitted malformation of the labial teeth to the second generation (Nishioka 1977, 1978, Kawamura & Nishioka 1978). In one F_3 cross of *X. laevis,* Droin & Beauchemin (1975) detected a recessive genetic degeneration of the lower jaw in tadpoles. Rostand (1947) and Volpe & Dasgupta (1962) obtained gynogenetic tadpoles with missing or poorly developed denticles in *B. bufo* and *L. pipiens*, respectively.

3.3.3 Abnormal size

3.3.3.1 Giant larvae and adults. It is often difficult to decide whether large size is still within the expected normal range of size or abnormal. We include cases in which the author(s) referred to their observation(s) as gigantism or talked about abnormal size. We further include cases in which the reported size was at least 25% larger than the maximum size reported in other studies for the species.

Giant adults have been reported for one *Speleomantis flavus* (Lanza et al. 1986), two *Ichthyosaura alpestris* (Arribas 2008), two *Pleurodeles waltl* (Wolterstorff 1925b), one female *Anaxyrus terrestris* (Means & Richter 2007) and one female *Rana luteiventris* (Hayes & Cassidy 2013).

Various urodeles exhibit neoteny, in which larvae grow to adult size and reproduce without metamorphosis (e.g., Henle 1983). We exclude such cases from this review. Anuran larvae, in contrast, generally do not reach sexual maturity as larvae, although there are two reports of giant tadpoles of *Pelophylax ridibundus* and *P. esculentus*, in which one individual of the former species had eggs in the oviducts and three individuals developed testes; of the latter species one individual had well developed paired gonads (Lusis & Tsaune 1984, Milto 2009). Likewise, giant *Xenopus laevis* tadpoles may develop fully differentiated gonads but do not reproduce (Rot-Nikcevic & Wassersug 2004).

Gigantism in tadpoles was reviewed for green frogs (genus *Pelophylax*) by Borkin et al. (1981, 1982) and for European anurans by Günther (1990). However, there have been no reviews of other anurans or urodeles. Giant larvae were found in 78 natural populations of 23 anuran species and 9 urodele species. They have been reported most frequently in tadpoles of the *P. esculentus* complex (Borkin et al. 1981, 1982, Günther 1990, 1996b, Atakhanova & Bigaliev 1995, Fagotti et al. 2005). Mass incidences with at least ten giant larvae in samples of at least 50 individuals and an abnormaility rate of at least 2.5% were observed three times: 95.8% (n = 166) *Lissotriton helveticus* from Belgium (Giltay 1932), 33.2% (n = 177) *L. vulgaris* in Germany (Kordges et al. 2008), and at least 5–12.5% (n = 2000–5000) *Bufotes viridis* in Germany (Henle et al. 2017a). The latter case was most likely due to radioactive pollution (Henle et al. 2017a). Whereas 31 other types of anomalies occurred in this population, only a few other anomalies (if at all) were observed in all other cases in which giant tadpoles or larvae were discovered.

One other case, 30 (n = ?) giant tadpoles of *P. ridibundus*, originated from fishponds in Kazakhstan. The fishponds were probably contaminated by an emergency chemical discharge from a copper combinate (Atakhanova & Bigaliev 1995), which likely was the cause for the giant larvae.

Borkin et al. (1981, 1982) discussed the hypotheses suggested to explain giant tadpoles and concluded that only a disruption of the hypothalamus-pituitary-thyroid axis evoked by genetic factors and the continued presence of larval growth hormones could explain their occasional occurrence. However, it is also conceivable that external factors or disease could cause a disruption to the hypothalamus-pituitary-thyroid axis.

Laboratory studies corroborate that any disruption of the endocrine thyroid axis, which regulates metamorphosis (Kaltenbach 1996, Hayes 2000), can cause giant larvae. Thyroid ectomy (Allen 1917), mutations and other factors that block or modulate the effects of thyroid hormones or the enhancing effects of corticosterone on thyroid hormones as well as a lack of thyroid receptors will lead to the failure of metamorphic changes (Kobayashi & Gorbman 1962, Galton 1992, Hayes 2000) and continued growth (e.g., Pohland 1962, Wink & wurmbach 1967). Thus, hypophyseal hypertrophy (Hahn 1912, Dodd

& Dodd 1976), low light intensity, which inhibits the development of the thyroid gland (Toivonen 1952), and various chemicals can cause giant larvae. For example, perchlorate inhibits iodide uptake by the thyroid gland at environmentally relevant concentrations (Goleman et al. 2002) and may lead to giant tadpoles (Coleman et al. 1968) and estradiol blocks the conversion of thyroxine into the more potent triiodothyronine (Nishimura et al. 1997). Feeding tadpoles on defenuron (Paulov 1977), thymus oil (Pohland 1962, Wink & Wurmbach 1967), labrafil and oils prepared from plants (rape seed, cotton seed, sesame seed, wheat germ) (Pohland 1962, Wink & Wurmbach 1967) also results in giant individuals. Administration of thyroid hormone to giant tadpoles can induce metamorphosis (Guex et al. 2001).

Occasionally, giant albinistic tadpoles were observed in experimental crosses within the *P. esculentus* complex (Tunner 1980, Guex et al. 2001), in *Epidalea calamita* × *Bufo bufo* and *Bufotes viridis* × *Bufo bufo* (Hertwig & Weiss 1955, Hertwig et al. 1959, Weiss 1960).

Ponse (1941a,b) obtained giant tadpoles of *B. bufo* from eggs that were taken from Bidder's organ in castrated females but the mechanism for this result is unclear.

A genetic origin for giant growth has frequently been assumed (e.g., Ponse 1941a,b, Freytag 1956, Borkin et al. 1981, 1982, Tarkhnishvili & Serbinova 1998) – a plausible explanation given the tight genetic control of the thyroid axis and its pivotal role in metamorphosis (Hayes 2000). While congenital absence or reduction of the thyroid has been observed several times (Toivonen 1952, Saxén 1957, Rot-Nikcevic & Wassersug 2003), so far a genetic origin for gigantism in larval amphibians has only been demonstrated in a female *Xenopus laevis* obtained from nuclear grafting (Uehlinger 1965).

3.3.3.2 Nanism. It is difficult to judge whether small individuals found in natural populations can be classified as abnormal dwarf specimens or whether they are still growing (e.g., tadpoles derived from late clutches or individuals that underwent an early metamorphosis) and will eventually reach a normal size. Reports of small individuals from natural populations generally do not allow a clear differentiation among these alternative explanations, except for in a few cases.

Ippisch (1928) collected an unstated number of dwarf tadpoles of *Bombina variegata* that did not grow when transferred to an aquarium with ample food. Lenk (1995) reported on an abnormally small leucistic tadpole of *Pelobates fuscus*. Meteyer (2000) illustrated two emaciated dwarf postmetamorphic *Lithobates pipiens*. Metamorphosed *Rana arvalis* in the Uralian radioactive trace of the Chelabynsk oblast, Russia, were significantly smaller than individuals from non-contaminated control sites (Pyastolova & Vershinin 1999, Vershinin & Seredyuk 2000). In a population of *Bufotes viridis* from a quarry in Roßwag, Germany, more than 100 dwarf tadpoles were observed in 1980. All dwarf individuals also showed various anomalies of the mouthparts, which probably reduced their ability to ingest food. The combination of small size and abnormal mouthparts puts forward a case for a developmental anomaly in this case. The ultimate cause was presumably radioactivity (Henle et al. 2017a).

Experimental studies have shown that many factors can cause growth retardation or nanism in amphibian larvae or metamorphs. For example, dwarf metamorphs result if the dorsal chorda is injured or the tail amputated in embryos of *R. temporaria* (Aron 1929). Dwarf tadpoles occasionally occur in experimental crosses between *Epidalea calamita* × *Bufo bufo, E. calamita* × *Bufotes viridis* and *Bufotes viridis* × *Bufo bufo* (Hertwig & Weiss 1955, Hertwig et al. 1959, Delarue 1982), in crosses within the *Pelophylax esculentus* complex (Berger 1967) and in crosses of *Pseudophryne corroboree* from different geographic origins (which most likely represent different species) (Osborne & Norman 1991).

Almost any chemical in high enough concentration will reduce, retard, or stop the growth in amphibian larvae and for many chemicals, effects on growth start well below lethal concentrations (e.g., Marian et al. 1983, Power et al. 1989, Materna et al. 1995). Irradiation of embryos also frequently inhibits growth (e.g., Brunst & Figge 1951, Gallien 1969b, Oerter 1985). Heritable recessive dwarfism has been discovered by Lipsett (1941) in an inbred line of *Ambystoma mexicanum,* by Kaylor (1940) in *Cynops pyrrhogaster* and by Droin (1988) in *Xenopus laevis.*

3.3.4 Edema and bloatedness, tumours and nodules, cysts and swellings

3.3.4.1 Edema and bloatedness. The literature on amphibian anomalies often does not differentiate between edema (Fig. 26), i.e., intercellular accumulation of fluids and inflation of the body from gas. Bloatedness is often used for both types of anomalies, sometimes only for the latter one. Thus, some of the cases included here under edema may actually be cases of the accumulation of gas in the body cavity rather than edema.

Fig. 26: Edema in a metamorph of *Pelophylax ridibundus,* Ekaterinburg, Russia, 16.9.1980; Photo: V. Vershinin.

We found 158 cases of bloatedness (edema or gas accumulation), covering 51 species. Reported background rates of edema are very low (0.01–0.4%; Tab. 1) and we only know of four cases in which at least 5% of the population were abnormal and at least 2.5% of the population and ten individuals were affected by edema. In three out of ten years, Wittouck (1980) detected a total of 50 edemateous *Pelophylax esculentus* with an average frequency of 17% in those three years. He suggested a genetic cause. In a population of *Bufotes viridis* in a quarry near Roßwag, Germany, more than 250 tadpoles (n = 2000–5000) and more than 30 (n = 1630) toadlets exhibited severe edema (Flindt 1985a, Henle et al. 2017a), with radioactivity being the most likely cause (Henle et al. 2017a). In wetlands constructed with treated wastewater in Georgia, 39 (n = 833) tadpoles of *Lithobates catesbeianus* exhibited edema, whereas none out of 190 individuals from reference sites was affected (Ruiz et al. 2010). Slightly elevated rates of edema occurred in tadpoles of *Bombina bombina* (1.5%, n = 400), *Bufotes viridis* (0.8%, n = 500) and *P. ridibundus* (2.2%, n = 500) collected in polluted habitats in the Ukraine (Flax & Borkin 2004). Finally, O'Connor et al. (2016) observed at least 100 tadpoles of *Lithobates sylvaticus* with swollen – presumably edemateous – hindlimbs floating on the surface. Ten of the sampled tadpoles were infected with *Ranavirus*.

In cases where only a few individuals were affected by edema, a range of causes have been suggested, most often disease (e.g., Densmore & Green 2007), pollution (e.g., Mizgireuv et al. 1984), or hybridization (e.g., Hemmer 1973, Gollmann et al. 1984). Occasionally, edema was also observed in toads (*Bufo bufo*) parasitised by the maggots of *Lucilia bufonivora* (Stadler 1930, Neumann & Meyer 1994) and in *Pseudacris regilla* parasitized by the trematode *Ribeiroia ondatrae* (Johnson et al. 2001a). In all of these cases no attempts have been made to test the cause experimentally. A causal relationship is unlikely in the case of *R. ondatrae* infection, since it generally does not appear in experimental infections.

Six cases were definitely gas accumulations. Guderyahn (2006) discovered ten tadpoles/recently metamorphosed *Lithobates sylvaticus* with gas accumulation when surveying four national wildlife refuges in Alaska (n = 164). Reeves et al. (2013) additionally listed two tadpoles of *Rana aurora* from one site and three tadpoles of *L. sphenocephalus* from two sites that exhibited gas accumulations. In rare cases, the pulmonary nematode *Rhabdias tokyoensis,* a parasite of *Cynops pyrrhogaster,* penetrates the lungs of the infected individuals allowing the peritoneal cavity to fill with air while inhaling; individuals affected are greatly distended and float at the surface (Pfeiffer-Asashima 1997). Mutschmann (2010) found a greatly distended adult *B. bufo* that died after three days. He assumed injuries from predator attacks as cause.

Laboratory experiments have shown that many factors can cause edema: e.g., bacterial and viral infections, pronephric defects, degenerative and toxic kidney and liver diseases, heart and lymph heart insufficiencies (e.g., Granoff et al. 1969, Wolf et al. 1969, Newcomer et al. 1982, Olson et al. 1992, Green 2001, Ny et al. 2005, Lee & Elinson 2008), unbalanced nutrition and deprivation of food (Reinhardt 1939, Grimm 1953, Reichenbach-Klinke 1961, Jara 1963), which in some cases is reversible (Mangold 1955), forced overripeness of eggs (Witschi & Chang 1954), chilling of embryos in a refrigerator (Coghill 1936), temperature shock (Fischberg 1948), rearing at high temperature (Muto 1969a; but see Muto 1969b) and treatment with high intensity of light (Ruehmekorf 1958) or ultrasound (Pourhadi et al. 1968). It also becomes frequently manifest in experimental hybridizations (e.g., Berger 1967, Berger & Uzzell 1977 Kuramoto 1983), including experimental crosses of European species of toads (e.g., Pflüger & Smith 1883, Born 1886, Montalenti 1933, Hertwig & Weiss 1955, Hertwig et al. 1959, Kawamura et al. 1980, Delarue 1974, 1982). Edema also occurred in tadpoles of *Polypedates crucifer* when they were exposed at the pre-limb bud stage (Gosner stage 25 and 26) to *Acanthostomum* cercariae (Jayawardena et al. 2010, Jayawardena & Rajakaruna 2013) but not when limb bud stage tadpoles (Gosner stage 27 and 28) were exposed (Rajakaruna et al. 2008).

A very wide range of chemicals can cause edema in embryos or tadpoles, often at very high rates. These include various organochlorine, organophosphate, carbamate (reviewed by Power et al. 1989) and pyrethroid insecticides (e.g., Fort et al. 1999b), various herbicides and fungicides (e.g., Harris et al. 1998; partly reviewed by Power et al. 1989), atrazine (Morgan et al. 1996), hydrazine and derivates (e.g., Greenhouse 1976, 1977), vitamin A and retinoids (e.g., Syuzyumova 1985, DeYoung et al. 1991), various metals (e.g., Pérez-Coll et al. 1985), low pH (e.g., Haidacher & Fachbach 1991), nitrates and nitrite (e.g., Hecnar 1995, Marco et al. 1999), as well as PCBs (Birge et al. 1978).

Exposure to X-rays, γ-rays, or neutron irradiation of any developmental stage of amphibians induces severe edema often at high frequency (e.g., Stachowitz 1914, Rugh 1950, Labrousse 1967, Nishioka 1977, Kawamura & Nishioka 1978, Oerter 1985, Trux 1985; partly reviewed by Ahmad 1976). High intensitiy UV-B irradiation also causes severe edema (e.g., Kashiwagi 1980, Nishioka et al. 1981, Grant & Licht 1995) but whether or not natural levels can induce elevated rates is still unclear. Hays et al. (1996) attributed high rates of edema in metamorphs of *Lithobates cascadae* and *Pseudacris regilla* and in tadpoles of the latter species to treatment with moderate levels of UV-B. However, the main difference in the appearance or frequency was related to light levels in the laboratory, with dim lights having more severe effects than moderate lights. This finding may be related to the need for light as the most important repair mechanism of UV-B damage: the photoreactivation with the help of the enzyme photolyase (Sinha & Häder 2002). In the study by Pahkala et al. (2002) UV-B was only effective at low pH, which can

cause edema on its own (e.g., Haidacher & Fachbach 1991) and, unfortunately, their figures combine edema with kinky tails. In any case, most authors (e.g., Calfee et al. 2006) did not report edema under natural UV-B levels (but see e.g., Blaustein et al. 1997 for *Ambystoma macrodactylum*).

Several studies demonstrated – usually recessive – heritability of edema in *A. mexicanum* (e.g., Humphrey 1964, 1972, Smith & Armstrong 1990; reviewed by Malacinski 1978), *Pleurodeles waltl* (Gallien & Collenot 1964, Signoret et al. 1966) and in *Xenopus borealis, X. laevis, X. muelleri* and *X. tropicalis* (e.g., Uehlinger & Reynaud 1965, Droin & Colombelli 1982, Hart & Armstrong 1984, Krotoski et al. 1985).

In conclusion, edema is a very general expression of a developmental disorder and, in the field, does not pinpoint to a specific cause.

3.3.4.2 Tumours and nodules. A tumour is an abnormal mass of tissue resulting from excessive cell division. Tumours can affect all organs. We restrict this review to externally visible tumours. Nodules are swellings that contain lipids or particles of microorganisms (Henle et al. 2017b). They often are initial stages in the formation of tumours (Lucké & Schlumberger 1949). Some authors (e.g. Ruiz et al. 2010) call swellings caused by atypical deposition of chrystals nodules and some (e.g., Green et al. 2002) call swellings caused by parasites nodules whereas we treat them under the heading "cysts and other forms of swellings" (chapter 3.3.4.3). Where possible, depending on the information provided by the authors, we adjusted terminology to the one used here.

The occurrence of tumours in amphibians has been repeatedly reviewed (Schlumberger & Lucké 1948, Willis & Collins 1948, Lucké & Schlumberger 1949, Balls 1962a, Balls & Ruben 1964, Mizell 1969, Balls & Clothier 1974, Balls et al. 1978, Asashima et al. 1987, Green 2001). These reviews did not differentiate between internal and externally visible tumours. Balls & Clothier (1974) cited more than 100 reports involving 53 species. Our review covers 95 cases of externally visible tumours from 35 species and 21 cases of nodules from 11 species.

Most cases of mass occurrence or elevated rates of tumours are from polluted sites (Tab. 7). Between 1970 and 1980 Rose (1976, 1991) and Rose & Harshbarger (1977) collected more than 2458 (annual mean 6.6%, range 0.04–50%; n = 37,143) *Ambystoma tigrinum* in a sewage pond at the Reese Airforce Base, Texas, that were affected by tumours. At 19 non-sewage ponds, by contrast, no tumours were detected (n = 19,802). The pond was contaminated by polycyclic aromatic hydrocarbons, especially perylene, and physiological studies indicated that metabolic derivatives of polycyclic aromatic hydrocarbons were the most likely inductor for the tumours. Flax & Borkin (1997, 2004) found 20 (n = 1980) *Bombina bombina* and 117 (n = 3505) *Pelophylax ridibundus* with tumours at sites in eastern Ukraine that were polluted by sewage and Mizgireuv et al. (1984) counted 126 (n = 1095) and 202 (n = 3651) *Rana pirica* with tumours at sites in Sakhalin, Russia, that were polluted with the wastewater of a paper and pulp mill and municipal sewage, respectively. Patel et al. (2008) reported 11 *Fejervarya limnocharis* with tumours from contaminated agricultural sites. In a polluted site in Horicon National Wildlife Refuge, USA, 16 of 125 tadpoles and recently metamorphosed *Lithobates pipiens* had small translucent pustules (Reeves et al. 2013); in four other National Wildlife Refuges 22–83% ($n \geq 86$) of the tadpoles had pustules or nodules. In a quarry near Roßwag, Germany, 10–25 (n = 295) toadlets of *Bufotes viridis* with tumours were encountered in 1980, with radioactivity being the most likely cause (Henle et al. 2017a).

Besides pollution, microorganisms, fungi, and virus have been found or suggested as cause for elevated rates of tumours. In Malaysia, Dhaliwal & Griffiths

Table 7: Elevated rates of tumours in amphibian populations (≥ 5% of the population abnormal, ≥ 2.5% of the population and ≥10 individuals with tumours. *N:* sample size; only cases with $N \geq 50$ included; % tumours refers to the sample size. Cause: (e): the cause was supported by experimental studies; all other causes are assumptions.

Species	*N* (% abnormal)	% tumours	Location	Cause	References
Ambystoma tigrinum	37,143 (6.6%)	(mean 6.6%, range 0.04-50%)	Reese Airforce Base, Texas, USA	Polycyclic aromatic hydrocarbons (e)	Rose 1976, Rose & Harshbarger 1977
Duttaphrynus melanostictus	471 (46.3%)	46.3%	Kuala Lumpur, Malaysia	Fungus (e)	Dhaliwal & Griffiths 1963
Pleurodema cinereum and/or *Pleurodema marmoratum*	663 (9.7%)	19.5%	La Paz, Bolivia	Unknown (bacilli assumed)	Machicao & La Paca 1954
Lithobates pipiens	74 (28.4%)	28.4%	Long Beach, Maryland, USA	Unknown	Hardy 1964

(1963) discovered 218 *Duttaphrynus melanostictus* with tumour-like lesions. An unidentified fungus caused the lesions, which were transmissible. In Bolivia Machicao & La Paca (1954) found tumours in 19.5% (n = 663) of the *Pleurodema cinereum* and/or *Pleurodema marmoratum* collected in the vicinity of La Paz. Bacilli were associated with the lesions.

Pfeiffer et al. (1979), Asashima & Komazaki (1980) and Asashima et al. (1982) collected 286 (n = 12,167) *Cynops pyrrhogaster* with skin papilloma in Japan, with the frequency varying geographically between 0% and 6.3%. Tsonis (1984) reported another 22 individuals with the same type of tumour. A virus was the cause for these cases, with temperature influencing its expression (Asashima et al. 1987). Since then Meyer-Rochow & Asashima (1988) and Fukui et al. (1996) collected another 170 (1.2%) and 67 (1.6%) affected individuals, respectively, from regions that overlapped with the sampling areas of these earlier publications. However, whether the same type of virus was involved or not is not mentioned.

Experimental studies showed that in many different organisms, including amphibians (Asashima et al. 1987), several forms of tumours have a viral ethiology (Gross 1961). Bacteria of the genus *Mycobacterium* can also lead to visceral tumour-like nodules (Inoue & Singer 1970, Hardwick & Philpott 2015). However, they do not have a neoplastic nature (Green 2001). In rare cases, the fungus *Mucor amphibiorum* induced tumour-like skin nodules and ulceration in *Rhinella marina* (Speare et al. 1997) and in one free-ranging *Ranoidea caerulea* (Berger et al. 1997), and in Africa, the fungus *Rhinosporidium rwandae* caused similar pathologies in *Hyperolius viridiflavus* and *H. lateralis* (Scheid et al. 2015). See Hardwick & Philpott (2015) for an overview of the developmental pathways that are involved in the proliferation of tumours.

Embryos from overripe eggs show abnormal ectodermal growth, some of them resembling papilloma (Briggs 1941). At later developmental stages, no tumours were observed.

Early reviews (e.g., Balls & Ruben 1964) stated that many attempts to induce tumours chemically failed because of rapid removal of tumourous tissue and the ability of amphibians to regenerate damaged tissue. While amphibians are relatively resistant to spontaneous and transplanted tumours (Hardwick & Philpott 2015), a range of chemicals is known to induce tumours in amphibians, such as tar, benzopyrene, dibenzanthracene, methylcholanthrene, fluorenacene, fluoraphene, diethylnitrosamine, potassium perchlorate and various aldehyde blocking agents, such as semicarbazides, hydrazines, nitriles and urea (Koch et al. 1939, Levy 1958, Pflugfelder 1959, Neukomm & Luder-Huguenin 1960, Arffmann & Collatz Christensen 1961, Balls 1962b, Seilern-Aspang & Kratochwil 1962, Ingram 1971, Khudoley et al. 1979). Therefore, newts are used as a standard test system for carcinogenic substances in Russia (Pliss & Khudoley 1979).

Irradiation can also induce tumours. Tumours developed in most adults of *Triturus carnifex* after intensive (1.3×10^5 J/m^2) UV-B irradiation (Zavanella & Losa 1981). Solid tumour-like swellings appeared on the back of some metamorphosed *Pelophylax nigromaculatus* developing from irradiated eggs or sperm (Kawamura & Nishioka 1978). The anomaly was still present in the second generation. Oerter (1985) observed tumour-like proliferation of the epidermis in early developmental stages of *Bufo bufo* that were irradiated as embryos.

3.3.4.3 Cysts and other forms of swellings. Cysts are swellings to encapsulate parasites. Some authors (e.g., Green et al. 2002) call swellings caused by parasites nodules. We include these anomalies under the term cysts, except for when the parasites are microorganisms. Our review covers 180 cases of cysts from 87 species (21 Urodela, 66 Anura) and 50 cases of other forms of swellings in 22 different species (2 Urodela, 20 Anura).

Reeves et al. (2013) listed two populations of *Lithobates clamitans*, in which 21 (n = 53) individuals in the final stages of metamorphosis had cysts. Trematodes (*Clinostomum*) were the cause. Trematodes presumably were also the cause in a population of *L. palustris,* a population of *L. pipiens* and two populations of *L. sphenocephalus*, in which 16 (n = 166), 54 (n = 67), 16 (n = 88) and 31 (n = 124), respectively, individuals showed small cysts. In two populations of *L. pipiens* and *L. clamitans* 46 (n = 52) and 52 (n = 137) individuals, respectively, had cysts but potential causes were not mentioned.

Infection by *Amphibiocystidium ranae* induced cysts in 12 out of 200 Swiss *Rana temporaria* and several *Triturus cristatus* sampled (Guyénot & Naville 1922), in 23 out of 43 Italian *Pelophylax bergeri* and eight out of 50 *P. hispanicus* (Pascolini et al. 2003). Several other cases involving fewer individuals exhibiting cysts were also attributed to infection with *Amphibiocystidium* spp. (e.g., Carini 1940) or to trematode parasites (e.g., Green et al. 2002).

Trombiculid mites of several genera usually, but not always, bury within the skin of their amphibian hosts, inducing the development of externally visible cysts. Some authors mentioned in addition a red or orange colour of the cysts (e.g., Pope & Pope 1951, Regester 2001, Anthony et al. 2004, Westfall et al. 2008). Heavy infection of the digits can result in ectromely (reported for *Desmognathus brimleyorum* by Winter et al. 1998). Species of the genus *Hannemania* parasitize anurans and urodeles in North, Central and South America (e.g., Sambon 1928, Duszynski & Jones 1973, McAllister et al. 1995, Jung et al. 2001, Regester 2001, Bradley et al. 2002, Hatano et al. 2007, Westfall et al. 2008). We found 80 published cases, involving 11 urodelan and 39 anuran host species, in which externally visible cysts caused by species of *Hannemania* were explicity mentioned.

Prevalences well above 50% have been repeatedly observed, e.g., in *D. fuscus, Eurycea bislineata, Plethodon ouachitae, Anaxyrus punctatus, Dryophytes arenicolor, Pseudacris cadaverina, Hylodes phyllodes*, *Lithobates*

berlandieri and *L. pipiens* (Pope & Pope 1951, Loomis 1956, Duszynski & Jones 1973, Shoemaker & Clark 1975, Welbourn & Loomis 1975, Jung et al. 2001, Anthony et al. 2004, Hatano et al. 2007, Westfall et al. 2008). Prevalences may vary substantially among years (Welbourn & Loomis 1975) and among host species (Jung et al. 2001, Regester 2001, Anthony et al. 2004, Hatano et al. 2007, Westfall et al. 2008). Primarily, terrestrial species are infested (Hatano et al. 2007). Aquatic and arboreal species usually do not harbour encysted mites (Wohltmann et al. 2007) but Quinzio & Goldberg (2015) also observed cysts in the aquatic *Telmatobius atacamensis*. Prevalences also differ among habitats. For example, significantly more *Leptodactylus chaquensis* were infected in rice and soybean fields than in native forests (Attademo et al. 2012).

Some but not all species of the genus *Vercammenia* also induce cysts (Domrow et al. 1983). For example, *V. hasseltii* induced tiny cysts in three terrestrial Malaysian frog species (Audy & Nadchatram 1958). *Vercammenia zweifelorum* caused externally visible orange cysts in the Australian microhylid *Cophixalus neglectus* but did not protrude above the skin level in *Taudactylus acutirostris* (Domrow et al. 1983). Likewise, larvae of *V. gloriosa* did not protrude beyond the lesion they created in *Taudactylus* spp. (Domrow et al. 1983).

In Africa and Madagascar *Endotrombicula* spp. induce orange cysts in the skin of terrestrial anurans, especially in species of the genus *Phrynobatrachus* (5 species, for which externally visible cysts were explicitly mentioned: Spieler & Linsenmair 1999, Wohltmann et al. 2007). Spieler & Linsenmair (1999) further mentioned a single infected individual of *Sclerophrys maculata*. On Madagascar, tiny cysts caused by *E. madagascariensis* were found in *Gephyromantis luteus* (Sambon 1928).

Larvae of the dipteran *Batrachomyia krausi* caused skin swellings in five populations of four frog species from Papua New Guinea (Kraus 2007). In one population of *Papurana supragrisea* 13 out of 16 examined individuals were parasitized.

Most other forms of swellings reported from wild populations were caused by parasites or fungal or viral infections. Many adult *Notophthalmus viridescens* with plaquelike swellings of the caudal half of the body, the rump and the proximal half of the body caused by a fungal disease (*Ichthyophonus*-like) were found in Virginia and Vermont (Herman 1984, Green 2001). In *L. clamitans* from Vermont an *Ichthyophonus* infection was associated with the swelling of the dorsal pelvic musculature (Green et al. 2002). A similar swelling was found in five recent metamorphs of *L. catesbeianus* from Massachusettes (Goodchild 1953). Whereas Goodchild (1953) regarded *Amphibiocystidium ranae* to be the cause, Green (2001) argued that *Ichthyophonus* also was the cause in this case.

The ciliate *Tetrahymena pyriformis* caused a swelling of the head and chest in larvae of *Ambystoma maculatum* that hatched in the laboratory from eggs collected in ponds with the ciliate present but not from eggs collected in a pond without the ciliate (Ling & Werner 1988). Swelling was followed by rapid death, usually within 24 h. A *Ranavirus* infection caused ulcers and swellings of the head, limbs and ventral surface in 61% (n = 570) of farmed *Andrias davidianus* (Geng et al. 2011).

Of 833 tadpoles of *L. catesbeianus* collected in wetlands constructed with treated wastewater in Georgia, USA, 110 had swellings that were completely filled with calcium phosphate (Ruiz et al. 2010).

Five publications mention abnormally swollen (but not clearly edemateous) digits. A juvenile *Bufotes viridis* from a quarry near Roßwag, Germany, in which radioactive pollution was discovered, had an abnormally swollen finger and another one showed an abnormally swollen shank (Henle et al. 2017a). Two *Anaxyrus canorus* in a population presumably suffering from immuno-depression due to an unknown factor had swollen toes (Green & Kagarise Sherman 2001). One recently metamorphosed *Rana muscosa* infected with *Batrachochytrium dendrobatidis* exhibited a thickened epidermis of the toe pad (Fellers et al. 2001) but none of the numerous other studies on chytrid infections of anurans mentioned the same kind of anomaly. One *R. amurensis* had a (several?) thickened phalange(s) (Gurvich 2014). In an Argentinian collection examined by Medina et al. (2013), four, five, and two individuals of *Leptodactylus chaquensis, Pleurodema borellii* and *Rhinella arenarum*, respectively, had swollen parts of digits.

In humans broadened thumbs and big toes are well-known clinical manifestations of the Rubinstein-Taybi-syndrome, which is caused by a mutation of the gene encoding the CRB-binding protein – a co-activator of cyclic AMP-regulated gene expression (Gurrieri et al. 2002). Whether swellings of digits in amphibians can also have a genetic cause is unknown.

3.3.5 Colour anomalies

The colours and patterns of amphibians are produced by the relative densities and arrangements of three basic types of pigment cells (melanophores, iridophores and xanthophores) and the types, presence, abundance and distribution of pigments within them (Frost-Mason et al. 1984). Any mutation or exogenous factor, which affects the distribution or abundance of any type of chromatophores, the distribution of the pigments within the chromatophores, or the synthesis chain of any kind of pigment, either totally or partially, will result in a "pigment pattern variant" or "colour variant". As most patterns are species specific so are colour anomalies and thus require a sufficient knowledge of the species in question to decide whether a particular phenotype is still within the "expected" normal variation or should be scored as an anomaly. Therefore, we limit our review to types of colour anomalies that occur across different species.

Most colour anomalies are due to genetic factors. However, depigmentation and paling may be due to temperature, disease, or chemicals. Likewise, darkening may be due to chemicals, UV-irradiation, or diseases.

Reddening in the form of haemorrhage may be caused by disease or injury and cysts produced by parasites may also have red or orange colour (e.g. Domrow et al. 1983, Westfall et al. 2008).

3.3.5.1 Albinism, hypomelanism and depigmentation. The terminology for whitish amphibians is inconsistent in the literature and the term albinism is either used in a narrow or a broader sense (Dyrkacz 1981, Henle et al. 2017b). Unfortunately, it is often impossible to know whether a particular publication used the term in the narrow or the broader sense. Consequently, in this section, we use the term in the broader sense but exclude cases that clearly belong to flavism or erythrism (see below). Thus, we include in this section any individual that completely or partially lacks pigmentation resulting in a white (complete albinos when eyes are red; leucism when eyes have normal colour), whitish, light pinkish (due to blood vessels) or paler than normal appearance. We further include individuals in which melanophores or melanin are absent (amelanistic) or strongly reduced (hypomelanistic), i.e., individuals, in which the body parts that are normally black appear abnormally faded to whitish but in which other types of pigments are present (Fig. 33b). We further review factors that can cause discolouration or depigmentation if the affected individuals show a whitish or pale colouration.

For some species, e.g., *Proteus anguinus*, albinism is the normal phenotype. Likewise, amphibian species that deposit their eggs in concealed sites tend to have white eggs but larvae or froglets that emerge from them are pigmented (Duellman & Trueb 1986, Passmore & Carruthers 1995). Such cases are not covered in the following review and the latter cases are usually not called transient albinism.

Albinism has received great attention and several reviews exist, most of them with a regionally limited coverage and/or restricted to specific taxa (Noble 1931, Rostand 1946, 1955b, Brame 1962, Gilboa & Dowling 1974, Hensley 1959, Dubois 1979b, Dyrkacz 1981, Frost et al. 1984a, 1986a,b, Klemz & Kühnel 1986, Klewen 1988, Günther 1996a, Rivera et al. 1993, Schlüpmann et al. 1995, Thiesmeier & Schulte 2010, Seidel & Gerhardt 2016). Browder (1975), Bagnara et al. (1978), Malacinski (1978), Dubois (1979b), Nishioka & Ueda (1985a,b), Sumida & Nishioka (2000), and Seidel & Gerhardt (2016) reviewed the heritability of albinism in selected taxa of amphibians. To our knowledge neither transient albinism nor discolouration has been reviewed.

Albinism is one of the most commonly reported anomalies in Amphibia. Our database contains 595 cases from 150 species (86 Anura, 64 Urodela) with partial or complete albinism. These include 15 cases that were termed depigmentation or discolouration, implying that pigmentation initially was normal but was lost later on. However, only in three cases a cause was suggested: skin discolouration in *Lithobates pipiens* presumably due to *Aeromonas hydrophila* infection (Hine et al. 1975), pale skin patches in *L. sylvaticus* due to infection with a ranavirus (Krynak & Dennis 2014) and discoloured tail tips in *Ptychohyla hypomykter* due to infection with *Batrachochytrium dendrobatidis* (Mendelson et al. 2004).

The earliest report of albinism dates back to Wurffbain (1683) who illustrated an albino *Salamandra salamandra.* Background rates of albinism are up to 1% but usually much lower (Tab. 1). The most exceptional case occurred in Japan where albinism was found in several species in an area of up to 250 km around Hiroshima, with most of them in the immediate vicinity of 6000 km². In this region, two albinistic adult *Pelophylax porosus* and 428 albinistic tadpoles of *P. nigromaculatus* were discovered (Nishioka & Ueda 1985a, Nishioka et al. 1987). Five different recessive genes caused albinism in these populations. In addition, at least 152 albinistic tadpoles (10.7%, $n = 1422$) of *Dryophytes japonicus* were collected at 12 sites from 1963 to 1975 (Daito 1968, Nishioka & Ueda 1977b, 1983). Nishioka & Ueda (1977b, 1983) identified three different recessive albino genes and two kinds of dominant melanising genes, which affected parts of the albino body. Moreover, one flavistic adult and more than 18 albinistic tadpoles of *Rhacophorus schlegelii* were found (Nishioka & Ueda 1985b). In all four populations, from which these individuals originated, recessive inheritance was demonstrated.

Another exceptional case is the finding of 100–250 ($n = 2000–5000$) albinistic tadpoles of *Bufotes viridis* in a quarry near Roßwag, southern Germany, which was most likely caused by irradiation from radioactive pollution (Henle et al. 2017a). It is interesting to note that a considerably elevated frequency of partial albinism (13–15%) that was caused by an increased rate of germline mutations was also observed in barn swallows (*Hirundo rustica*) from the contaminated area of Chernobyl (Ellegren et al. 1997).

Large numbers of albinistic tadpoles were also observed by Childs (1953), Johnston & Johnston (2006) and Hensley (1959): 298 (21%), 65 (1%) and 65 *Spea hammondi, Scaphiopus holbrooki* and *Gastrophryne carolinensis*, respectively. Martof (1962) collected 43 albinistic *Desmognathus marmoratus* between 1954 and 1960, with the frequency increasing from 0.7% to 8.9%. In 1963, the percentage had increased to 15% (Martof & Walton 1965). In the years 1997, 1998, 2000 and 2001 Nijs & Keller (2002) detected between 9 and 109 albinistic *Rana temporaria* tadpoles. While it is not rare that larvae that hatch from white eggs acquire pigmentation during larval development (see below), their observation is the only one known to us in which all eggs from clutches deposited were black and some larvae became gradually pale after some weeks. Dandová et al. (1995) and Kotlík & Zavadil (1997) observed 71 albinistic *P. lessonae* tadpoles. They assumed that chemical pollution had induced mutations that resulted in albinism.

While a genetic origin is likely for all the cases compiled, except the 15 cases termed discolouration and perhaps for the case reported by Nijs & Keller (2002), heritability has been demonstrated only for the Japanese

species studied by Nishioka and colleagues. For *P. nigromaculatus* heritable albinism has also been detected by Tokunaga (1949). In addition to these Japanese species, heritable albinism – always recessive – has been demonstrated in individuals collected in the wild from at least 11 further species: *Ichthyosaura alpestris* (Parent & Thorn 1983), *Triturus carnifex* (Capanna 1967, Capanna & Foresti 1974), *Salamandra salamandra* (Concaro 2004, Seidel et al. 2012), *Alytes obstetricans* (Héron-Royer 1886), *Bombina orientalis* (Ellinger 1980), *B. variegata* (Genthner & Hölzinger 2007), *Bufotes viridis* (Boschwitz 1963), *Pseudacris maculata* (Corn 1986), *Phrynobatrachus* sp. (Oeser 1952), *Lithobates pipiens* (Gibbs et al. 1971, Browder 1972) and *R. temporaria* (Smallcombe 1949). Only a single study failed to demonstrate a genetic origin (Nishioka & Ueda 1985e for partial albinism in *Pelophylax porosus*).

Albinistic individuals sometimes appeared in low numbers in experimental hybrids, probably due to gynogenesis (which results in haploid individuals), e.g., in crosses of *Epidalea calamita* × *Bufo bufo* (Hertwig et al. 1959), *E. calamita* × *Bufotes viridis* (Siboulet (1971), *B. viridis* × *B. bufo* (Born 1883, 1886, Hertwig & Weiss 1955, Hertwig et al. 1959), within the *P. esculentus* complex (Tunner 1980, Pabijan et al. 2004) and in *Geocrinia laevis* × *G. victorina* (Littlejohn et al. 1971, Gollmann 1991). In crosses within the *P. esculentus* complex transient albinism has also occasionally been discovered (Ogielska-Nowak 1985, Pabijan et al. 2004).

Mutations leading to albinism have been induced by treating adults with ethyl methanesulfonate and ethyl nitrosurea (Hart & Armstrong 1984) and by irradiation of eggs and sperm (Nishioka 1977, Nishioka & Ueda 1977a, 1985a, Kawamura & Nishioka 1978). In *P. nigromaculatus* these mutations were still retained in the 10th generation (Nishioka & Ohtani 1986). Genes controlling the expression of albinism may operate at different levels (Bagnara et al. 1978, Nishioka & Ueda 1985a). Some are involved in the production of tyrosinase, while others affect the melanosomal matrix.

While most attempts to establish heritability of albinism in individuals collected in the wild were successful, other factors may also cause whitish individuals. Experimental infection with Sri Lankan monostome-type trematode cercariae caused partial or complete lack of pigmentation in metamorphs of *Polypedates cruciger* (Rajakaruna et al. 2008) (significantly different to controls: $\chi^2 = 12.4$; $\alpha < 0.001$) but the mechanism for this effect and whether it is truly depigmentation remains obscure. Colour anomalies were not reported in experiments with the North American trematodes *Ribeiroia ondatrae* and *Alaria* sp. (Johnson et al. 1999, 2001b, Stopper et al. 2002, Schotthoefer et al. 2003). Disease (psittacosis, bacterial infection, frog virus 3 and *Batrachochytrium dendrobatidis*) can also cause (partial) depigmentation (Granoff et al. 1969, Newcomer et al. 1982, Nichols et al. 2001, Wright & Whitaker 2001).

Tornier (1907) observed that a diet consisting mainly of algae (with a minimum amount of meat required to allow growth) caused albinism in *Pelobates fuscus*. Though his observations have been questioned (see Freytag 1952a), bleaching due to nutritional deficiencies has been shown repeatedly for *Lissotriton vulgaris* (Klatt 1927, Krefft 1938) and an appropriate diet reverses it (Klatt 1927). Presumably, nutrition interfered with melanin synthesis in these cases.

There is a range of chemicals that are able to cause depigmentation or bleach amphibian skin: ammonium nitrate (Hecnar 1995), the insecticides aldrin (Rane & Mathur 1978), endosulfan (Brunelli et al. 2009), fenitrothion, benzene hexachloride, carbofuran (Pawar & Katdare 1983) and karbatox 75 (Rzehak et al. 1977), the herbicides maneb, nabam (Pawar et al. 1983) and their metabolite ethylene-thiourea (Ghate 1986) and coal gasification effluents (Dumont & Schultz 1980). Malathion strongly reduced pigmentation in *Microhyla ornata* and *Xenopus laevis* (Pawar et al. 1983), but no such effect was reported in *Rhinella arenarum* (Rosenbaum et al. 1988). Depigmentation is transient for sodium diethyldithiocarbamate treatment (Bancroft & Prahlad 1973, Ghate & Mulherkar 1980). S-methoprene might also cause amelanism but it was not significantly more frequent than in controls (Sparling 2000). Depigmentation also commonly develops when embryos receive high doses of irradiation: e.g., *Pleurodeles waltl* (Labrousse 1967), *I. alpestris* (Mangold & Peters 1956, Sanides 1956), *X. laevis* (Hart & Armstrong 1984) and *B. bufo* (Oerter 1985, Trux 1985).

Transient albinism is an anomaly in which some or all eggs are abnormally white and pigmentation is acquired during development (Henle et al. 2017b). Note that many amphibian species that deposit their eggs in concealed sites have white eggs as the normal phenotype and the larvae get increasingly pigmented. Such cases are usually not called transient albinism and not covered in our review. Our database contains 32 cases of transient albinism from 18 species. Most cases are white clutches (Fig. 27b) from which tadpoles hatched that increasingly acquired pigmentation soon after hatching. Most of these observations are based on single clutches; the maximum concrete number reported are three clutches in a population of *Bufotes viridis* (Christaller 1983, Flindt 1985b).

Completely white larvae of *S. salamandra* frequently develop the yellow colour pattern after metamorphosis (e.g., Opatrny 1979, Pastors & Greven 2016). There are only four other cases in which pigmentation was acquired at later developmental stages. In a *Pelodytes punctatus* population in Spain, 48 individuals were completely white as tadpoles, with red eyes, but acquired pigmentation after metamorphosis (Rivera et al. 2001). One adult female *T. cristatus* collected in the wild had a pigmentless chin and reduced pigmentation on the belly. It turned whitish within a few months in captivity; two years later it regained increasingly normal pigmentation (Freytag 1947). A cause was not provided but nutritional deficiency is known to be able to cause reversible albinism (Klatt 1927). From laboratory stocks

two further exceptions are known. A female *Ambystoma mexicanum* from a cross between a white and a black parent was initially white but became pigmented within 1.5 years (Häcker 1906). In a laboratory strain of *Dryophytes japonicus* a dominant colouring gene was only activated after metamorphosis (Nishioka & Ueda 1977b).

A genetic basis of transient albinism has also been demonstrated in laboratory strains of *A. mexicanum* (Humphrey 1975) and *X. laevis* (Hoperskaya 1975, 1981, MacMillan 1979, Droin & Fischberg 1984) and in *Pelophylax nigromaculatus* that were derived from irradiated eggs or sperm (Nishioka 1977, Nishioka & Ueda 1977a, Kawamura & Nishioka 1978). Heritability for albinism has further been demonstrated for *A. mexicanum* (e.g., Humphrey 1967a,b, Gallien 1969a, Malacinski & Brothers 1974; reviewed by Frost et al. 1984, 1986a,b), *A. tigrinum* (Humphrey 1967a), *Pleurodeles waltl* (Lacroix & Capuron 1970) and *X. laevis* (Krotoski et al. 1985, Droin 1992). Albinism (silvery tadpoles) in tadpoles of *L. pipiens* with congenital absence of the pituitary and/or the infundibulum and normal colouration in sibs that had both also indicates a genetic origin (Underhill 1967). These effects can be explained by the central role of melanotropic substances and tyrosine in melanin synthesis (Allen 1916, Smith 1916, Frost-Mason et al. 1984).

3.3.5.2 Black eyes. In black-eyed amphibians the eyes may either be completely or partially black. However, phenotypically all share a lack of the glittering iris (Nishioka 1977). A black iris is the normal phenotype in some species of anurans, e.g., in some species of the families Pelobatidae, Microhylidae, Bufonidae, Leptodactylidae, Hylidae, Dendrobatidae, Brachycephalidae and Ranidae (Glaw & Vences 1997).

Nishioka (1977), Dubois (1979b) and Richard & Nace (1983) reviewed the black-eyed anomaly focussing on ranid frogs. Our database contains data for 123 natural populations belonging to 29 species in which black-eyed individuals were observed; all but a single individual of *Lissotriton helveticus* (Dubois et al. 1973) were anurans. In most cases the number of individuals and frequencies were very low and only six publications reported more than 10 individuals from natural populations. Nishioka & Ueda (1985b) detected more than 42 black-eyed tadpoles in one population of *Rhacophorus schlegelii*, with the anomaly being heritable. Among 13,856 toadlets, juvenile and adult *Rhinella marina* examined in Bermuda Bacon et al. (2006b) discovered 19 with a dark brown or missing (i.e. black) iris. Within four years Rostand & Darré (1970) found approximately 60 *Pelophylax esculentus* with black eyes in the West of France.

Within 23 years Vershinin (2004) discovered 194 black-eyed *Rana arvalis* (Fig. 28a) among 15,803 individuals examined from four different environmental zones in Ekaterinburg and its vicinity. The frequency was higher in juveniles than in adults and in urban populations compared to populations from forests. He assumed that inbreeding, together with a higher mutation rate in polluted urban habitats, was responsible for the elevated frequency. In years in which no black-eyed individuals were discovered, no frosts occurred during the spawning period. This indicates that temperature possibly acted epigenetically on the expression of a mutation.

Reeves et al. (2008) found 154 black-eyed individuals among 9268 *Lithobates sylvaticus* examined in a four-years survey of 86 Alaskan breeding sites. They also regarded pollution to be the likely cause. In a later study (Reeves et al. 2010), they surprisingly found that the abundance of predatory beetles was the best predictor for the probability that *L. sylvaticus* exhibited eye anomalies, mainly black eyes, at a site. However, the anomaly was also negatively associated with temperature, which, as in *R. arvalis* in Ekaterinburg, was most likely to be the proximate factor responsible, acting either through differential survival or epigenetically (see section 3.1.7.3 for further discussion).

Most cases of black-eyedness are likely to be due to recessive mutations but in one breeding stock of *Dryophytes japonicus* the mutation was dominant. Inbreeding in a captive group of *Osteocephalus elkejungin-*

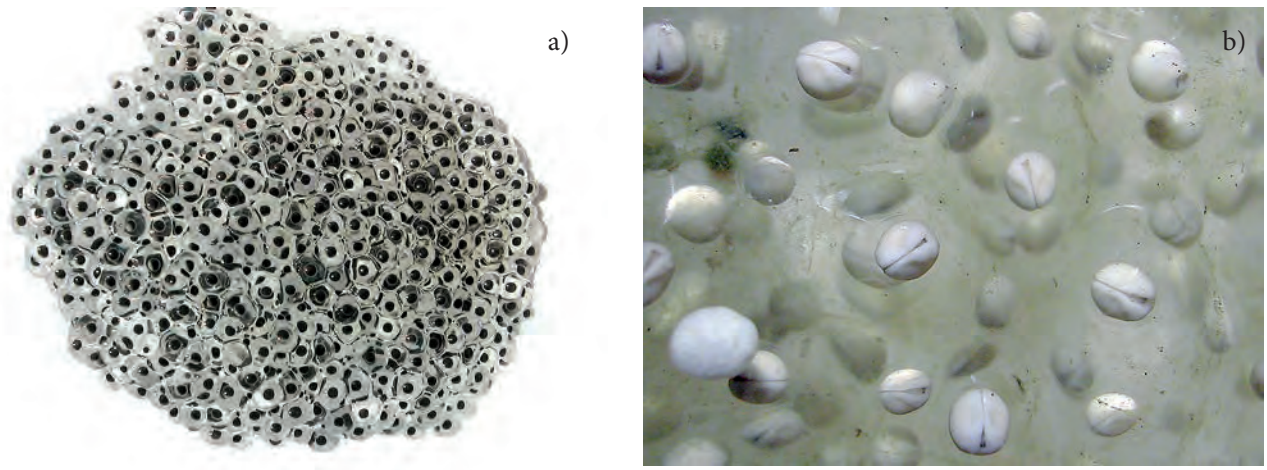


Fig. 27: a) Normal and b) abnormal white clutch (transient albinism) of *Rana arvalis*; the tadpoles that hatched from the white eggs acquired pigmentation during the larval stages; Ekaterinburg, Russia, 2005. Photos: V. Vershinin.

Fig. 28: a) Black-eyed adult female *Rana arvalis,* Polevskoi, Russia, June 2004. Photo: V. Vershinin; b) normal eye colour in an adult female *R. arvalis*, Tschekalin, Russia, 5.8.2008. Photo: K. Henle.

gerae revealed a recessive mutation for black-eyedness (Henle 1992). Nishioka (1977) obtained three recessive black-eyed mutations from 64 irradiated gametes of *P. nigromaculatus;* all mutations occurred at the same locus. Genetic studies in other species, however, showed that mutations leading to black-eyedness usually are non-homologous, which explains the differences in the morphological expression of the anomaly among species (*D. japonicus*: Nishioka & Ueda 1977b, 1985c; *L. clamitans, L. pipiens, L. sylvaticus*: Richards et al. 1969, Richards & Nace 1983; *Rhacophorus schlegelii*: Nishioka & Ueda 1985b).

Only in three cases involving *P. porosus* and *P. nigromaculatus* heritability could not be demonstrated (Nishioka & Ueda 1985d,e). In addition, Günther (1996c) wrote, without presenting details, that occasionally *P. ridibundus* acquires black-eyedness after long periods of captivity. Also, UV-irradiation produced a greatly increased number of melanophores in the cornea of *L. catesbeianus* and *L. pipiens* but only when tadpoles were kept in dark after irradiation (Zimskind & Schisgall 1956).

Fig. 29: Male *Ichthyosaura alpestris*, one of the few urodele species in which the normal phenotype is blue (only in males), Naturtheater Renningen, Germany, May 2005. Photo: K. Henle.

3.3.5.3 Blue, bluish and bluish-grey colour. Blue is a structural colour in most animals and occurs only in a few amphibian species as normal phenotype, such as male *Ichthyosaura alpestris* (Fig. 29). In addition, male *Rana arvalis* may turn blue during the breeding season. Such cases are not reviewed here.

Berns & Uhler (1966), Dubois (1979b) and Murphy (1980) reviewed the occurrence of blue individuals in the genera *Pelophylax* and *Lithobates.* Most cases involved only few specimens. Rivera et al. (1993) and García-Paris et al. (2004) reviewed the occurrence of blue frogs in Spain; most cases were observed in *Hyla meridionalis.*

Our database contains 92 cases covering either completely or mottled green / blue individuals from 20 species. Except for seven individuals, all belong to the genera *Hyla* (3 species) (Fig. 30) *Dryophytes* (1 species), *Lithobates* (5 species) or *Pelophylax* (6 species). The ex-

Fig. 30: *Hyla arborea;* a) abnormal colouration (blue), Saxony, 1980s. Photo: L. Berger; b) normal phenotype of an adult male, Waldsteinberg, Germany, April 2006. Photo: K. Henle.

ceptions are a single bluish-grey (axanthic) toad (*Anaxyrus fowleri*) (Bechtel 1995), a mottled green / blue *Acris crepitans* (Niccoli 2013), three juvenile *Pseudarcis regilla* (Altig & Brodie 1968), a *P. cultripes* with a bluish eye (Gonzáles-Fernández & Valladolid 2004), two bluish-grey *Pelobates fuscus* tadpoles (Sacher 1985), and an adult female *Ichthyosaura alpestris* with a greyish blue venter (Dandová & Zavadil 1993).

Most cases involve only a few individuals and the prevalence of blue frogs is usually low although it can reach higher rates in some populations. Berns & Uhler (1966) and Uhler (1971) obtained 69 blue *L. clamitans* among 2 million individuals from suppliers in Wisconsin and Minnesota; they further obtained 15 individuals from Barre, Massachusetts. Rivera et al. (1993, 2001) and Arribas et al. (1996) found 51 (2%) and 28 (4%) blue *H. meridionalis* in different populations near Barcelona, Spain. Furthermore, Dubois (1979b) observed 27 (4.5%) and 23 (4.0%) blue *P.* synkl. *esculentus* in two French populations and Nekrasova (2014) ten (16.7%) blue individuals in a mixed population of *P. lessonae/P. esculentus/P. ridibundus* in the Ukraine.

Bagnara et al. (1978) reviewed the genetic and physiological basis of blue colouration in frogs. Blue mutants either lack partially or totally the xanthophore cell type (Nishioka & Ueda 1985d) or show a diminuation or complete absence of pigments in xanthophores (Bagnara et al. 1978). Presumably, most blue frogs carry recessive mutations for this type of anomaly. For example, up to 40% of the offspring of a female *P. nigromaculatus* whose eggs were irradiated or fertilized with irradiated sperm were blue (Nishioka 1977, Nishioka & Ohtani 1986). Blue frogs were homozygous for a recessive gene coding for the absence of carotenoid vesicles in the xanthophores and possessed a dominant allele for the expansion of all three kinds of chromatophores. However, one case is known in which blue colouration was not heritable: two juvenile female *Dryophytes japonicus* that lacked xanthophores (Nishioka & Ueda 1985d). Although the cause remains unclear, a somatic mutation could explain it.

3.3.5.4 Erythrism. In many amphibian species red colouration belongs to the normal phenotype. Whether a red colour morph is regarded as normal or abnormal may depend on the origin of the individual. For example, in *Plethodon cinereus* an erythristic morph exists, which is rather common in some regions (Lotter & Scott 1977), yet very rare in others (Thurow 1961). Here we include only species or individuals in which red colouration was regarded as abnormal by the author(s). It is caused either by yellow pigment being replaced by red or orange pigment (e.g., in *Salamandra salamandra*), by a reddish brown colour replacing normal brown colouration, e.g., in *Rana temporaria* (Fig. 31; Rösel von Rosenhof 1758, Deichsel 2013) and *Discoglossus* spp. (Vences et al. 1996), or by widely expanded red spots, e.g., in *Bufotes viridis* (Schreiber 1912, Bons & Geniez 1996, Lanza & Canestrelli 2002).

Our database contains 58 cases involving 11 species – excluding an individual of *S. salamandra* that was orange in a reprint published by Schmidtler (2016), as the individual is yellow in the original publication by Meyer (1748–1756). Except for a population of *S. salamandra* near Barcelona, Spain, where 14 (5%; $n = 286$) individuals were erythristic (Rivera et al. 2001) and a population of *R. dybowskii* in the Far East of Russia, in which 14 (5.6%; $n = 250$) adults had an abnormal reddish colouration (Kolobaev 2000), all cases involved only few individuals. Apart from the species already mentioned, erythrism has been discovered in *Ichthyosaura alpestris* (Hachtel 2011), *S. corsica* (Michelot 1980), *R. arvalis* (Schreiber 1912, Henle unpubl.) and *Bufo bufo* (Luef 2009).

Tornier (1907) stated that *Pelobates fuscus* tadpoles fed on a diet rich in meat turned an orange colour. While his observations need confirmation, it is known that food rich in carotinoids intensifies orange colours in newts (*Triturus* s.l.) (Wolterstorff 1924 fide Freytag 1952) and red colour in other taxa (e.g., Chatzifotis et al. 2011).

Wolf (1994) observed that *B. bufo* migrating across a field that was treated with N-fertilizer developed reddish bellies. Reddish skin patches due to hemorrhage is a commonly observed pathology in diseased individu-

Fig. 31: Juvenile *Rana temporaria;* a) with normal colouration, Samarowo, Russia, September 2012; b) erythristic individual, Samarowo, Russia, 16.9.2013. Photos: K. Henle.

als, especially after *Aeromonas hygrophila* infection (see Henle et al. 2017a for a short overview). These types of reddening can easily be differentiated from an erythristic colour pattern.

Heritability of erythrism has been demonstrated in populations of *Salamandra salamandra* (Schweizerbarth 1909, Rivera et al. 2001, Seidel & Gerhardt 2016). No other causes have been suggested for erythrism in natural populations.

3.3.5.5 Flavism and abnormal yellow patches. Yellow pigmentation that is not part of the normal colouration is called flavism. Very few species are entirely yellow but in many species yellow colouration belongs to the normal phenotype, e.g. *Salamandra salamandra* (Fig. 33a). In addition, males of several species, notably in the genus *Scinax,* become completely yellow during the breeding season. Such cases are usually not called flavism and are not reviewed here.

Parent & Thorn (1983) reviewed the occurrence of flavism in amphibians. They listed ten species for which it had been reported. Our database contains 134 cases involving 26 urodelan and 20 anuran species. The earliest report of flavism is by Wurffbain (1683) for a *Triturus cristatus* and presumably also a *Salamandra salamandra*. Natural background rates are below 0.1% (Tab. 1) and only two cases cover more than ten specimens: 26 (sample size not provided) *Lithobates catesbeianus* (Peters 1962) and 12 *Lissotriton helveticus* and/or *L. vulgaris* (Smith 1950).

We know of only one case of transient flavism. A yellowish-beige paedomorphic larva of *L. vulgaris* was reddish-brown when recaptured two years later (Thiesmeier 2014). In addition a yellow adult female *T. cristatus* that was almost completely yellow in spring developed increasingly large black patches till October (Reichenbach 1865).

Flavism usually affects the whole body. However, in an adult *Pseudopaludicola mystacalis* only the anterior dorsal surface was yellow. Similarly, in a *Lithobates clamitans* flavism was restricted to those body parts that are normally green (Pinder 2010) and Guttmann (1972) published a photo of an individual of the same species with a yellow saddle-back. Rimpp (2007b) and Sewell (2007) each also reported a partially flavistic *T. cristatus*.

The affected bodyparts may be further reduced to small yellow, orange-yellow, or golden-yellow patches while the rest of the body has the normal colour: for example, more than ten metamorphs and tadpoles of *Bufotes viridis* from Roßwag, Germany, with yellow or ocre patches on different parts of the body (Fig. 32) (Henle et al. 2017a), two male *Chioglossa lusitanica* with an irregular yellow dorsal patch (Sequeira et al. 1999), an adult *Salamandrina perspicillata* with yellow spots on the back (Lanza & Canestrelli 2002), golden-yellow dorsal spots on a melanistic *Hyla arborea* (Bitz & Schader 1996), an adult female *L. clamitans melanota* (Vorps 1976) and an adult male *Rana temporaria* with sulphur-coloured patches on the back (Goethe 1972). Yellow spots on the back also occur occasionally in *Bombina variegata* (Pracht & Rogner 1987, Herrmann 1990, Gollmann & Gollmann 2012), a species that has a bright yellow venter. In one of the *Bufotes viridis* individuals from Roßwag the anomaly was heritable (presumably dominant) and the mutation was most likely caused by irradiation (Henle et al. 2017a).

In *Salamandra salamandra* yellow is part of the normal pattern (Fig. 33a) and in some subspecies a substantial expansion to almost completely yellow belongs to the normal range of patterns; in the subspecies *S. s. terrestris* a substantial extension of the yellow pattern has been observed often in a small area near Holzminden, Germany (Seidel et al. 2012) but we know only one publication that mentioned a single individual for another locality, Ludwigsburg, in southern Germany (Klewen et al. 2016).

Recessive mutations coding for flavism have been discovered in *Ambystoma mexicanum* (Bagnara & Obika 1964), *Ichthyosaura alpestris* (Parent & Thorn 1983), *S. salamandra* (Nath 1940, Seidel et al. 2012) and *T. carnifex* (Capanna & Foresti 1974). Wolterstorff (1925a) obtained an albinistic larva from a cross between two flavistic *S. salamandra.*

3.3.5.6 Melanism and darkening. A few species, such as *Salamandra atra* (but not the subspecies *S. a. aurorae*) and *S. lanzai,* are completely black in the normal phenotype; these species are not reviewed here. Some species, e.g., *Salamandrella keyserlingii* (Fig. 34), may physiologically change to complete black under dark and cold conditions. If returned to light and warm condition, their colouration reverses to the normal pattern. Such physiological changes are not considered in this review.

Browder (1975), Bagnara et al. (1978), Malacinski (1978), Richards & Nace (1983), Frost et al. (1984, 1986a), Klewen (1988) and Seidel & Gerhardt (2016) reviewed abnormal melanism and its genetic origin in *Ambystoma mexicanum, S. salamandra*, ranid frogs and other amphibians. Richard & Nace (1983) additionally suggested a classification for different types of melanism. Schlüpmann et al. (1995) reviewed melanism in amphibians from Northrhine-Westphalia, Germany.

Fig. 32: Abnormal ocre patch in a juvenile *Bufotes viridis*, Roßwag, Germany, September 1980. Photo: K. Henle.

Fig. 33: *Salamandra salamandra;* a) normal pattern of the subspecies *S. s. salamandra*, individual born in captivity, parents from Austria; b) albinistic individual, collected as larva in Wuppertal, Germany; c) melanistic individual born in captivity. Photos: B. Trapp.

Our database contains data from 111 natural populations of 24 urodelan and 25 anuran species, in which individual(s) were completely or partially black or abnormally dark. The earliest mentioning of melanistic individuals (*Bufo* species from Italy) was by the poet Guntheus (Aldrovandi 1645). However, Aldrovandi (1645) slightly doubted this claim as no other author had ever mentioned toads that were completely black. We were unable to track the original source. Completely black individuals are rare exceptions (e.g., Rivera et al. 2001, Manenti 2006) and only five cases comprise more than ten partially or completely melanistic individuals for a single population: a population of *Pelodytes punctatus* from France, in which thousands of tadpoles were almost black on the back (Boulenger 1891), a high altitude population of *Bufotes viridis* in the Alps, in which most tadpoles were black (Andrä & Deuringer-Andrä 2011), and two German populations of *Bufo bufo,* in which 27 out of 386 and 13 out of 26 individuals, respectively, had large black spots on the back or belly (Mattes 2013). In another population of *B. bufo*, only 4 out of 1121 individuals were affected. Mattes (2013) assumed that a fungal disease was the cause. Kliemt (2017) observed black spots on 15 (n = several thousands) juvenile *B. bufo* that were caused by a fungus of the genus *Cladosporium*. In addition, Richards et al. (1969) and Richards & Nace

Fig. 34: *Salamandrella keyserlingii;* a) normal phenotype, Nishni Angarsk, Lake Baikal, Russia, 26.7.2014. Photo: K. Henle; b) physiological colour change to black in an individual kept under dark and cold conditions, Ekaterinburg, Russia, May 2003; this individual also shows polydactyly (left front leg), which sometimes is regarded as part of natural variation in this species. Photo: V. Vershinin.

(1983) received 32 melanistic *Lithobates pipiens* from various dealers (sample size and locations not given).

A genetic origin is assumed for most cases but has rarely been tested, except in laboratory stocks of commonly maintained species. In melanistic laboratory stocks of *A. mexicanum,* at least 16 different recessive genes cause dark pigmentation (Malacinski 1978). Melanoid mutants are characterized by an overproduction of eumelanin, usually through the differentiation of an excessive number of melanophores. Melanoid mutants exhibit a great diminuition in xanthophore and iridophore number and it is assumed that this is caused by a genetic defect involving the xanthine dehydrogenase (Bagnara et al. 1978). Inheritance of melanism has also been shown in laboratory stocks of *L. pipiens* (Gibbs et al. 1971, Richards & Nace 1983). Moreover, gynogenesis revealed mutations that caused darkening of tadpoles in *Xenopus laevis* (Krotoski et al. 1985) and melanism in *L. clamitans* (Richards & Nace 1983). Heritability of melanism has also been demonstrated in captive stocks of *S. salamandra* (Freytag & Susebach 1942, 1949, Freytag 1955, Seidel & Gerhardt 2016; Fig. 33c).

Apart from mutations, a few other factors may be considered as causing an abnormal black colouration. Tornier (1907) observed that feeding *Pelobates fuscus* tadpoles on a diet consisting exclusively of meat and ant eggs resulted in a very dark, almost black colouration. According to Freytag (1952) the dark colouration may have been due to reversible physiological changes. However, rare cases of darkening to almost completely black have been observed in captivity also for *Speleomantes flavus, Salamandra a. aurorae* (Steinfartz 1998), *Triturus carnifex* and *T. cristatus* (Werner 1930, Mertens 1941, Mutz 1999). Reichenbach-Klinke (1956) reported increased melanisation of necrotic tissue in *Pleurodeles waltl* and Cummer et al. (2005) and Bachhausen (2016) observed the same in an individual of *Plethodon neomexicanus* infected with *Batrachochytrium dendrobatidis* and *S. salamandra* infected with *B. salamandrivorans*, respectively. An infection may also have been the cause in a *B. bufo* in which the head showed pathologically altered black skin (Glaw et al. 2014).

Two (frequency < 1%) darkened individuals appeared from experimental crosses between *Pelophylax esculentus* and *P. lessonae* (Tunner 1980). Their uniqueness questions the relationship with hybridization.

Several chemicals may cause darkening of tadpoles by affecting melanophore number, distribution, size or contraction or melanin synthesis (Pogonowska 1914, Pohland 1962, Pandey & Tomar 1985). Tadpoles of *Euphlyctis hexadactylus* develop abnormal black spots when treated with the pesticide carbamate (Raj et al. 1988). Furthermore, dichlorvos, an organophosphorous insecticide, may cause the release of the hypophyseal melanocyte-stimulating hormone, which results in darkening at low doses but lightening at high doses (Tomar & Pandey 1988). Lindane causes darkening by dysfunction of the intermediate lobe of the hypophysis that controls pigmentation (Marchal-Ségault & Ramade 1981).

Unnaturally high doses of UV-B irradiation will lead to enhanced pigmentation and darkening of tadpoles (Worrest & Kimeldorf 1976, Grant & Licht 1995). At ambient levels of UV-B, darkening has been observed in *Hyla arborea* tadpoles but not in *T. cristatus, Bufo bufo, Epidalea calamita* or *Rana temporaria* (Langhelle et al. 1999). Tadpoles that develop from irradiated eggs, sperm or embryos often show high rates of melanism (e.g.: *Bombina orientalis*: Ueda 1980; *Glandirana rugosa*: Kashiwagi 1980; *P. nigromaculatus*: Nishioka 1977, Nishioka & Ueda 1977a).

3.3.5.7 Translucent skin. While a translucent skin on parts of the body is the natural condition for some species, notably in the family Centrolenidae, translucent patches of skin may also be an abnormal condition. An abnormal absence of the epidermis or lack of pigmentation giving the affected skin patch a grey to blackish translucent appearance has rarely been observed in amphibians collected from the wild. Our database contains 26 cases from 18 species, all but four involving a single individual. Absence of the epidermis due to injury was the likely cause in three out of 24 *Rana arvalis* examined by Henle (unpubl.; see Fig. 47 in Henle et al. 2017b) in Samarowo, Russia, that had a translucent patch of skin on the belly, head and back, respectively. Dubois (1968) described a translucent ventral skin for four *Pelophylax esculentus* affected by anomaly P and Rostand & Darré (1970) mentioned several tadpoles from another population that were also affected by anomaly P. The fourth case is several *Bufotes viridis* metamorphs in the Roßwag population in Germany that showed translucent blackish patches of skin; the anomalies were presumably due to irradiation (Henle et al. 2017a).

A translucent blackish female *Pelophylax porosus* collected in the wild ($n = 29$) lost its pattern over the course of two years (Nishioka & Ueda 1985e). Among its 36 gynogenetic and F_2 offspring, one acquired a blackish colouration one year after metamorphosis and the anomaly was not heritable. The involvement of phagocytes in the formation of these colour anomalies was indicated by their contact with xanthophores and melanophores and pieces of melanophores in their cytoplasm. No causes for the remaining cases of singular individuals observed were suggested.

Out of 218 Sri Lankan *Polypedates crucifer* experimentally infected with monostome-type cercariae (species not identified) nine exhibited translucent skin but none of the 78 controls did (Rajakaruna et al. 2008) ($\chi^2 = 3.32$; $0.1 > \alpha > 0.05$). Such anomalies were not reported in experiments with the North American trematodes *Ribeiroia ondatrae* and *Alaria* sp. (Johnson et al. 1999, 2001b, Stopper et al. 2002, Schotthoefer et al. 2003).

In experimental crosses within the *Pelophylax esculentus* complex, tadpoles and metamorphs showed patches of translucent skin (Berger 1971, 1976, Günther 1990). Similarly, the posterior body part was translucent in 30 surviving tadpoles from a clutch obtained from natural hybridization between a *Rhinella icterica* male and a *R. crucifer* female.

Translucent patches of skin further appeared in *Bufo bufo* tadpoles raised from irradiated embryos (Oerter 1985, Trux 1985) and in *P. nigromaculatus* raised from irradiated eggs or sperm (Nishioka 1977, Nishioka & Ueda 1977a, Nishioka & Ueda 1985a, Nishioka & Ohtani 1986). In the latter species, it was always combined with the black-eye anomaly.

In laboratory stocks of *Lithobates pipiens* translucent patches of skin occur occasionally and the anomaly has an incomplete dominant inheritance (Browder 1968). Recessive inheritance of translucent grey body colour has also been discovered in a *B. bufo* (Rostand 1951e) and in *Rana temporaria* raised from clutches of females affected by anomaly E (Rostand 1956a, 1958b).

4 Species differences in sensitivity

Several suggestions of differences in the sensitivity of species and between anurans and urodeles to develop anomalies have been made. For example, occasionally it has been suggested that the phenomenon of mass occurrence of anomalies is limited to the Temperate Zone of the Northern Hemisphere and absent from tropical areas. However, the few studies with large sample sizes available for tropical areas show that this is not the case. Mass occurrences have been observed in Malaysia, India, northern Australia, the Ivory Coast, Costa Rica, Guatemala, Panama and Ecuador. These involved skeletal and other morphological anomalies and were associated with disease, predators, parasites as well as chemical and radioactive pollution (see section 3.1 for further details).

Ankley et al. (2004) and Lannoo (2008) suggested that anurans are more prone to acquire limb anomalies than urodeles. Lannoo (2008) derived his conclusion from the higher percentage of North American anuran species for which limb anomalies have been reported compared to urodele species. In contrast, Litvinchuk (2014) stated that urodeles are more sensitive. He derived his ideas from a limited set of data on the percentage of individuals observed with anomalies. Globally, anomalies are known for a higher fraction of the total number of species of Urodela than for Anura or Gymnophiona (Fig. 35; ($\chi^2_2 = 126$; $\alpha = 0$). Borkin (2014) noticed a similar pattern in Russian amphibians but asked whether this may not be a sampling effect. Indeed, such inferences depend on the strong assumption that both anuran and urodele species were exposed to the same teratogenic factors and that the same percentage of species and the same (average) number of populations have been assessed with sufficiently large sample sizes. These assumptions are certainly violated. For example, our global comparison rather reflects the fact that a larger percentage of urodele species are found in Europe and North America than is the case for anuran species and Gymnophiona (Duellman 1999), i.e. for regions that have a much longer and broader natural history tradi-

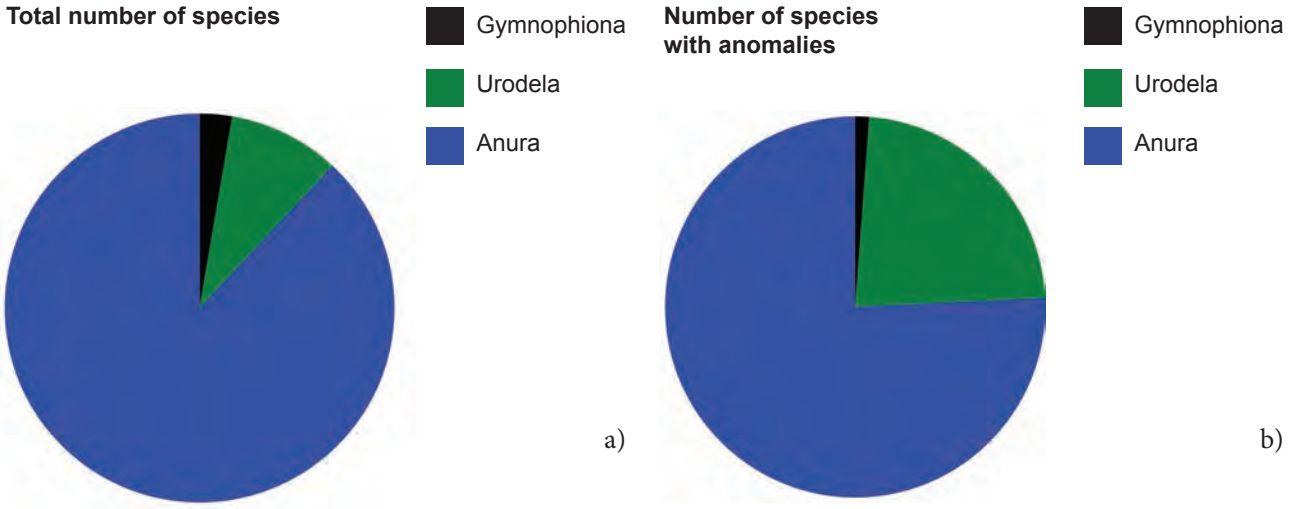


Fig. 35: Comparison between extant amphibian orders for a) global number of species (Frost 2017) and b) the number of species for which anomalies have been reported. Source: our database as of 7.6.2017.

tion than other regions of the world. To obtain reliable inferences surveys are required that assess all urodele and anuran species for anomalies across a larger set of sites.

One of the authors of this paper (Vershinin 2002) conducted such a study that allows comparisons of the sensitivity between anurans and urodeles. Since 1977 all amphibians found in three zones of urbanization within the city of Ekaterinburg, Russia, and rural areas 23 km away from the city were scored for anomalies. In the most urbanized zone the frequency of abnormal *Lissotriton vulgaris* was intermediate between *Rana arvalis* (the most affected species) and *R. temporaria*. It was higher compared to adult but lower than for juvenile *Pelophylax ridibundus*. In the second most urbanized zone, *L. vulgaris* was also less affected than *R. arvalis* but similar to *R. temporaria*. In the forest park zone *Salamandrella keyserlingii* was the most severely affected species, followed by *R. arvalis, L. vulgaris* and *R. temporaria*. In the rural area *S. keyserlingii* was most frequently affected followed by *Rana arvalis*. Thus, sensitivity apparently was consistent when comparing the same species declining from *S. keyserlingii* to *R. arvalis* to *L. vulgaris*, with *R. temporaria* being the least sensitive species. In the Danube floodplains at Gemenc, Hungary, *L. vulgaris* scored intermediately between six anuran species in terms of the number of types of anomalies (Puky & Fodor 2002). These data indicate that neither anurans nor urodeles as a group were more prone to have anomalies but that there may be a consistent ranking of species.

At two sites in California, Johnson et al. (2001) surveyed all amphibians. The newt *Taricha torosa* exhibited the highest rate of anomalies, followed by *Pseudacris regilla, Anaxyrus boreas* and *Lithobates catesbeianus*. Anomalies were probably caused by the trematode *Ribeiroia ondatrae*. At Duck Pond, Minnesota, the prevalence of anomalies was high for *A. americanus* (60% abnormal; n = 618) and *L. pipiens* (~50%) but low for *Dryophytes versicolor* (3%; n = 251) (Johnson & Hartson 2009). The results from another study across 345 Californian wetland sites were only partially consistent with these results. Whereas *P. regilla* showed on average the highest frequency of anomalies, it was followed by *Rana draytonii* and *L. catesbeianus*, but *A. boreas* had only a low (2.6%) average frequency of anomalies (Johnson et al. 2013).

The sensitivity ranking obtained in laboratory experiments that compared the effects of the trematode *Ribeiroia ondatrae* on anomalies in 13 amphibian species agreed only weakly with the ranking from field sites. The two urodele species tested (*Ambystoma macrodactylum, T. torosa*) were intermediate in sensitivity between the most sensitive species (*Anaxyrus* spp., *Pseudacris* spp., *L. cascadae*, *Rana pretiosa*) and *D. cinereus, D. versicolor*, *L. catesbeianus* and *Xenopus laevis*, which were immune (Johnson et al. 2012). Moreover, mesocosm studies showed that the infection of *P. regilla* with *Ribeiroia ondatrae* depended on amphibian species composition (Johnson et al. 2013). Whereas the addition of *L. catesbeianus* tadpoles significantly reduced infection this was not the case when adding *L. cascadae* tadpoles.

Across 462 wetland sites in US wildlife refuges, anuran species were not differentially sensitive to obtain anomalies (Reeves et al. 2013). This is to be expected as the factor(s) that caused anomalies are likely to differ considerably across the USA.

In the laboratory study of Johnson et al. (2012) discussed above, species that develop quickly and metamorphose at a small size were most prone to develop anomalies but phylogeny had no effect on sensitivity. In contrast, the rapidly developing Asian *Duttaphrynus melanostictus* was less prone to develop anomalies when experimentally exposed to cercariae of the trematode *Acanthostomum burminis* compared to *Polypedates cruciger*, which has a long developmental time (Jayawardena et al. 2013). The types of anomalies were also less severe. Other studies, in contrast, suggested that aquatic species are more sensitive than semi-aquatic and terrestrial species (Borkin 2014). At the CWB hotspot site in

Minnesota, for example, the two most aquatic species with the longest larval period exceeded by far the remaining species in terms of the frequency of anomalies (Hoppe 2005). These contradictory observations among studies and locations can be explained best by different causal factors occurring in the field and species-specific differences in the susceptibility to different teratogenic factors. The different sensitivity ranking of species at CWB compared to sites at which *R. ondatrae* likely was the main causative factor of anomalies (see previous paragraph) supports the conclusion of Helgen (2012) that parasites at most played a minor role at the CWB site.

A higher sensitivity of those species that have longer larval periods compared to those with shorter larval periods to anomalies caused by pollution is corroborated by amphibians at breeding sites contaminated by sewage in the Ukraine (Flax & Borkin 1997, 2004). The most aquatic species, *Pelophylax ridibundus* showed the highest rate of anomalies, *Bombina bombina* was intermediate and the most terrestrial species, *Bufotes viridis,* was least affected. Likewise, 96% of the tadpoles of *L. catesbeianus* partially lacked labial tooth rows in a coal ash deposit pond, whereas *D. cinereus* was not affected (Rowe et al. 1996). The former species has a much longer larval period than the latter (Lannoo 2005). At a site in Argentina, tadpoles of the two species with the longest larval period, *Boana riojana* and *Odontophrnus americanus*, had higher rates of anomalies than the two species with a shorter larval period, *Pleurodema borellii* and *Rhinella arenarum* (Medina et al. 2013).

Another suggestion is a difference between urodeles and anurans in their proneness to show different types of anomalies. For example, Asashima et al. (1987) assumed that anurans are more prone to develop tumours than urodeles. They based their proposition on the higher regeneration capacity of urodeles compared to anurans. However, there is a lack of comparative studies in the laboratory or the field in which anurans and urodeles were exposed to the same carcinogenic factors. A corollary of this hypothesis is that tumours should be found less frequently relative to other types of anomalies and in a smaller percentage of sampled populations of urodeles compared to anurans. Similarly, because of the high regeneration capacity of urodeles for the eye (Eguchi et al. 2011), anophthalmy should be observed less frequently relative to other types of anomalies in urodeles than in anurans. Extending the argument about the regeneration capacity, one should find the same relationships for ectromely and ectrodactyly, which are often assumed to be primarily caused by trauma (but see section 3.2.2.1).

Excluding populations for which the types of anomalies found was not specified, there is a striking highly significant difference (χ^2-Tests) between urodeles and anurans in the percentage of populations for which these four types of anomalies were reported (Tab. 8). As predicted anophthalmy, ectromely and ectrodactyly were reported for a much smaller fraction of the surveyed urodele populations compared to anuran populations. Contrary to prediction, the opposite was the case for tumours.

The reporting patterns, assessed as the relative numbers of populations for which particular types of anomalies were reported, also differed strongly between urodeles and anurans (Figs. 36–38). These differences are not rigorous tests of a different sensitivity of urodeles and anurans to develop a particular type of anomaly, as other factors than a different sensitivity (e.g. sampling in different regions and habitats that differ in teratogenic agents, types of anomalies targeted in one but not the other taxonomic group) might have contributed to these differences. Notwithstanding, they are indicative of the types of anomalies that are promising to target in a well-designed study that compares the sensitivity of urodeles with that of anurans.

In terms of limb anomalies ectromely has been reported much less frequently relative to other types of anomalies in urodeles compared to anurans and the opposite is the case for polydactyly (Fig. 36). For polydactyly this parallels the much higher natural variability in the phalangeal formula of urodeles compared to anurans (Alberch & Gale 1985). The relative frequency of other limb anomalies is similar in urodeles and anurans, with a slightly higher rate for syndactyly in uordeles.

In terms of non-skeletal morphological anomalies, anophthalmy has been reported less frequently and tumours more frequently relative to other types of anomalies in urodeles compared to anurans (Fig. 37). Giant larvae were also reported relatively more frequently and microphthalmy, skin webbing and bloatedness less frequently in urodeles compared to anurans. Oral anomalies in larval amphibians have only been studied for anurans.

Albinism is by far the most frequently reported type of colour anomaly for natural populations of Urodela and Anura and the only one reported for Gymnophio-

Table 8: Comparion between Anura and Urodela of the number of populations with and without a particular type of anomaly having been reported. Bold: amphibian order with the higher percentage of populations for which the respective type of anomaly was reported.

Type of Anomaly	Urodela		Anura		Significance
	with	without	with	without	
Anopthalmy	13	806	**145**	**2531**	$\alpha < 0.0001$
Tumours	**38**	**781**	57	2617	$\alpha < 0.001$
Ectromely	53	766	**609**	**2066**	$\alpha = 0$
Ectrodactyly	103	716	**589**	**2086**	$\alpha = 0$

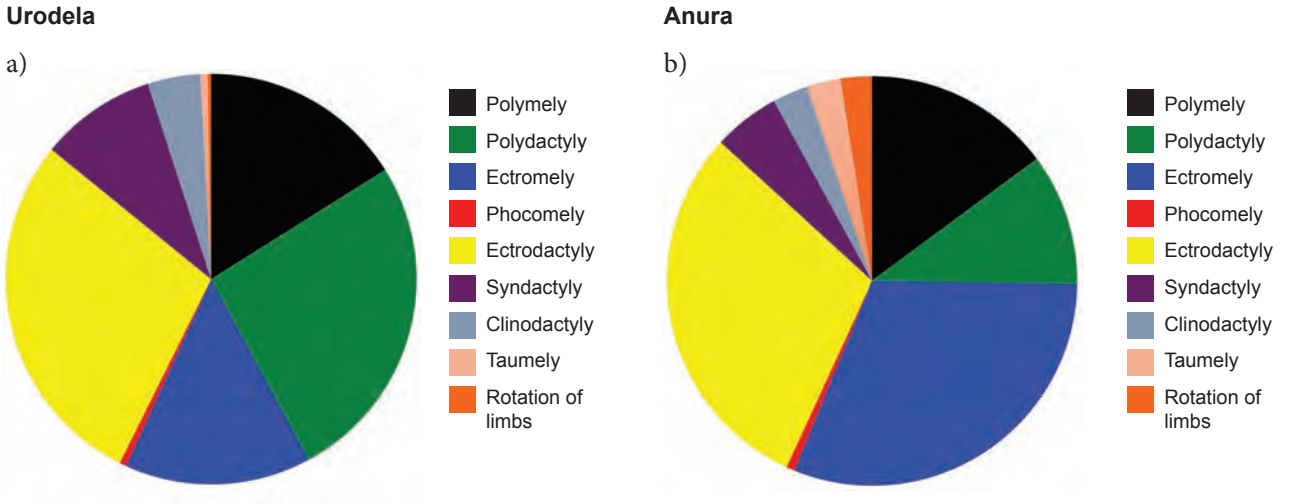


Fig. 36: Comparison of the reporting frequency of different types of skeletal anomalies for a) urodeles (n = 361) and b) anurans (n = 1981). Source: our database as of 7.6.2017.

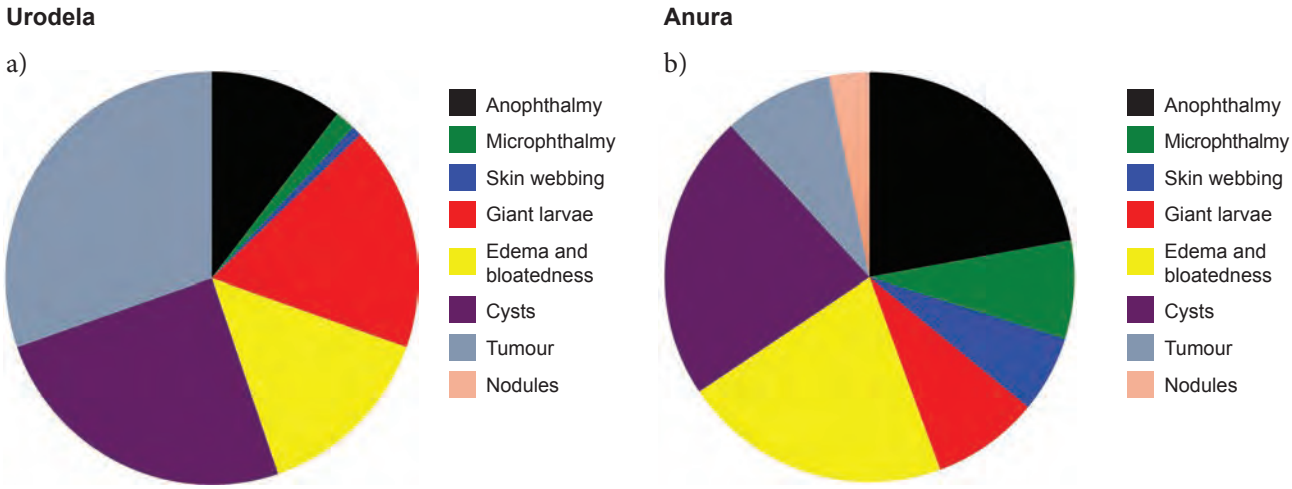


Fig. 37: Comparison of the reporting frequency of different types of non-skeletal morphological anomalies for a) urodeles (n = 125) and b) anurans (n = 660). Source: our database as of 7.6.2017.

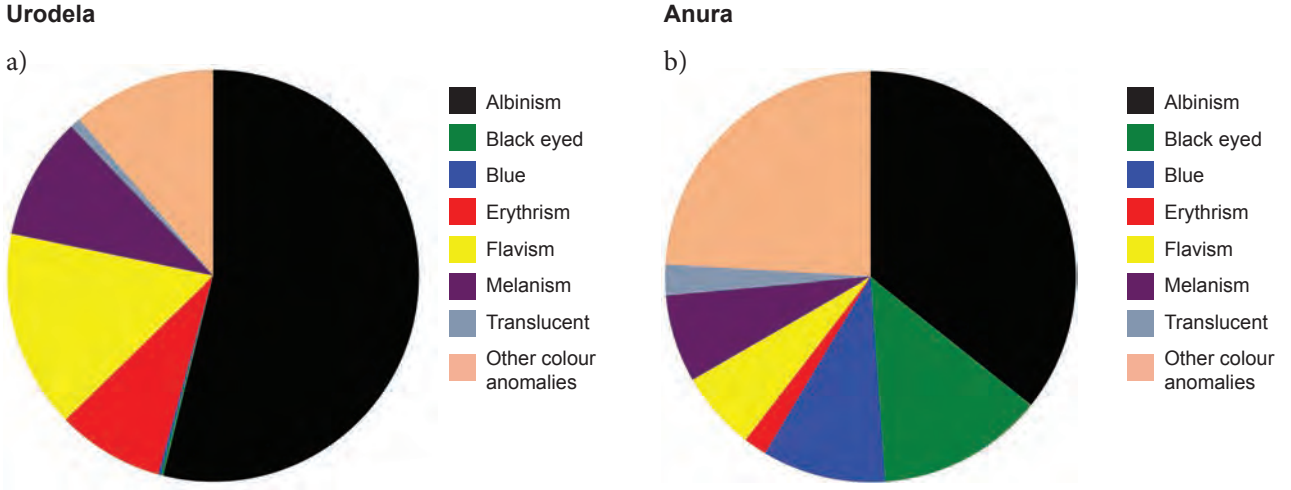


Fig. 38: Comparison of the reporting frequency of different types of colour anomalies for a) urodeles (n = 456) and b) anurans (n = 954). Note: albinism does not include flavism and erythrism. Source: our database as of 7.6.2017.

na. For urodeles it dominates much more strongly than for anurans (Fig. 38). Likewise, relative to other types of colour anomalies, erythrism, flavism, and to a lesser extend melanism have been reported more frequently in urodeles compared to anurans, whereas the opposite is the case for abnormal black-eyedness and blue colour.

Some anomalies are rather species-specific, with syntopic species not being affected at all. Such species-specific reactions seem to take place primarily if parasites or diseases are involved. For example, anomaly P only affects species within the genus *Pelophylax* (Dubois 2014, 2017). Similarly, *P. ridibundus* was the only species suffering from mass polymely at the Kazakh locations studied by Woitkewitsch (1959, 1965), whereas two other syntopic ranid species did not exhibit any anomaly. Maggots of the fly *Lucilia bufonivora* destroy the nasal bones of *Bufo bufo* but other species are rarely parasitized. Similarly, at six sites in Maine, USA, *Ambystoma maculatum* and *A. tigrinum* were infected by an iridovirus and suffered from edema, while non-ambystomatid urodeles and anurans were not affected (Docherty et al. 2003). The reasons for these highly species-specific reactions are still unknown.

As for predators, the leech *Erpobtella octoculata* causes ectromely in *Bufo bufo* but not in syntopic *Rana temporaria* (Bohl 1997); it can also damage limbs in *Ichthyosaura alpestris* (Hachtel 2011). Laboratory experiments demonstrated further that predator effects on tail injuries and/or ectromely of larval amphibians is often rather specific to the combination and size of the species involved and the larval stage (reviewed by Henle et al. 2017a).

In summary, species may show very different sensitivities to particular environmental factors that cause anomalies, such as pollutants and biological agents, with the latter sometimes being rather species-specific. However, there is no evidence that urodeles are less sensitive than anurans or vice-versa and phylogeny had no effect in the only study that has tested it so far. While several studies have indicated that more aquatic species are more sensitive (to water born factors) compared to less aquatic species, others have suggested the opposite with rapidly developing species being the more sensitive ones. These seemingly contradictory observations may be explained by different causal factors in the field. Analyses that simultaneously assess different potential causes and differentiate different types of anomalies are in dire need to better understand the differential sensitivity of species to teratogenic factors in the field.

5 Individual and population level effects

Concerns have been voiced that increased incidences of anomalies contribute to the global decline of amphibians (Lannoo 2008, Helgen 2012) but only a few studies have attempted to assess the impacts of anomalies at the individual or population level. As expected, most of these show adverse effects on the individual level, which may or may not translate to population level effects.

5.1 Individual level effects

Helgen et al. (2000) found a significantly smaller snout-vent-length (SVL) of *Lithobates pipiens* metamorphs at malformation hotspot sites in Minnesota in 1996 compared to reference sites. However, this does not necessarily mean that anomalies were responsible for the smaller mean SVL. It could have simply been that a noxious factor caused both anomalies and the smaller SVL. In 1997, they compared the SVL of abnormal and normal individuals within seven sites. At three sites the former were significantly smaller and at two other sites the mass was significantly lower. At a sixth site, the Ney pond, the difference was significant for mass on one but not on another sampling date.

Between 1997 and 1999, Canfield et al. (2000) also compared SVL and mass of *L. pipiens* metamorphs at seven sites in Minnesota. Three of these sites were the same as those used in the study by Helgen et al. (2000). In this study, abnormal metamorphs were smaller than normal ones at four sites but larger at three sites. The difference was significant for two of the latter sites (CWB, NEY). An ANOVA showed that most of the variance was explained by the sites and not by the status of being normal or abnormal. Abnormal metamorphs had a lower mass at six sites and a higher one at one site (CWB). The differences were only significant for one site (ROI). Canfield et al. (2000) explained the difference at the ROI site with mass loss from missing limbs and the handicap in capturing prey for affected individuals. An ANOVA showed that most of the variability was explained by site effects, followed by the interaction of sites and the logratio of mass of abnormal / mass of normal metamorphs. While there is an overall tendency of abnormal froglets to have a lower mass compared to normal froglets, the assessments of SVL do not provide a consistent picture. More studies are needed that also include the main types of anomalies observed and the sampling dates, and also on tadpoles before metamorphosis took place, before we can better understand the effects that anomalies have on the size and mass of metamorphs.

In a larval guild of *Ambystoma opacum, A. tigrinum* and *A. maculatum,* in which invertebrate predators were rare, severely injured individuals of *A. opacum* (partially missing gills, digits, and/or limbs) had smaller snout-vent-lengths than individuals that were not injured and individuals only suffering from tail injuries (Mott & Steffen 2014). They also showed an increased use of benthic microhabitats, a higher risk of intraspecific aggression and reduced agonistic displays. However, foraging success was not reduced. Similarly, *Dicamptodon tenebrosus* that lacked limbs or tails or parts thereof had a significantly lower mass compared to individuals that had not been injured, but only in the largest size class (Munshaw et al. 2014). In recently metamorphosed individuals from a population of *Lissotriton vulgaris* in Romania SVL was very similar between animals that had not been injured and animals with ectromely, ectrodactyly or injured tails (Nemes 2005).

Early stage larvae of *Ambystoma jeffersonianum* with torsion of the body caused by boron treatment had substantially reduced swimming speed compared to normal larvae (Laposata & Dunson 1998).

Blind Cururu toads (*Rhinella jimi*) on the oceanic island Fernando de Noronha, Brazil, where the species is invasive, have significantly lower body condition than individuals with two functional eyes (Tolledo & Toledo 2015). Half blind toads were intermediate. Moreover, the number of oocytes and reproductive effort were lowest in blind individuals, intermediate in half blind individuals and best in normal toads, with the difference being significant.

The few studies available that were either conducted in the laboratory or using enclosures indicate that anomalies often but not always reduce growth and development in tadpoles and may reduce survival. For example, tadpoles of *Rhinella jimi* with oral anomalies foraged less efficiently than tadpoles without oral anomalies (Tolledo et al. 2014). *Rana arvalis* tadpoles with numerous anomalies of the mouthparts were sluggish, had reduced growth and reached Terent'ev (1950) stage 29 6–12 days later than normal tadpoles (Trubetskaya 2006). Most of them died during metamorphosis and surviving postmetamorphic juveniles differed from their siblings in morphophysiological parameters. Tadpoles of *L. sylvaticus* with bent tails had lower survival than normal individuals when exposed to a beetle (*Dytiscus*) predator while the presence of predators reduced the percentage of tadpoles that developed bent tails (Michel & Burke 2011).

Effects may depend on the environment. Tadpoles of *L. catesbeianus* that suffered from deformed labial teeth rows and papillae were less able to graze on periphyton compared to normal tadpoles, when tested in the laboratory (Rowe et al. 1996). They had lower (negative) growth rates compared to those with normal teeth rows when periphyton was the only food source. However, when particulate food was also available, tadpoles grew well regardless of their deformities. When exposed together to juvenile snapping turtles (*Chelydra serpentina*) in outdoor tanks (Raimondo et al. 1998), survival was lower in individuals with abnormal mouth parts compared to normal individuals. In the absence of predators, however, survival was similar.

Pelobates cultripes tadpoles with tail injuries had a marginally lower survival rate than uninjured tadpoles, with the difference increasing with the number of times that the tail had been injured (Nunes et al. 2010). The effects did not dependent on food availability and development and growth were not affected. In *Dryophytes chrysoscelis*, swimming performance (sprint speed and distance swum) and the ability of tadpoles with damaged or incomplete tails to escape the attacks of dragonfly larvae (*Tramea lacerata*) was significantly reduced (Semlitsch 1990, Figiel & Semlitsch 1991). In *D. versicolor,* maximum speed and minimum escape time were also impaired but only if at least 30% of the tail was removed (Van buskirk & McCollum 2000). As an exception to the increased sensitivity of abnormal tadpoles to predation, there was no statistical difference between the prevalence of deformities and the median time to predation of *L. cascadae* tadpoles by the newt *Taricha torosa* under laboratory conditions (Romansic et al. 2009).

Whereas the survival of albino tadpoles of *Spea hammondii* to metamorphosis did not differ from normal tadpoles when predators were excluded, it was only one third of that of normal tadpoles in a natural pond to which predators had access (Childs 1953). While several albino tadpoles metamorphosed successfully, albeit later than normal ones, all albino froglets died soon after metamorphosis. Albinism also prolonged the time required for development and metamorphosis in *Phrynohyas mesophaea* (Sazima 1974); such prolongation usually increases the mortality of amphibians in natural populations (e.g., Semlitsch et al. 1988).

Anomalies can also reduce survival rates in the absence of predators. For example, in a *Bufotes viridis* population in Roßwag, Germany, none of the more than 100 albinistic tadpoles metamorphosed despite the absence of predators (Henle et al. 2017a). Notwithstanding, albinism is not always lethal and in captivity albinistic individuals have been raised through many generations (e.g., *Ambystoma mexicanum*: Malacinski & Brothers 1974; *Salamandra salamandra*: Concaro 2004). Whereas albinistic tadpoles may complete metamorphosis, giant tadpoles do not metamorphose nor do they reproduce.

Ambystoma larvae infected by an iridovirus and suffering from edema were lethargic and slow moving. They swam in circles with obvious buoyancy problems (Docherty et al. 2003). Larvae of *A. maculatum* with swellings due to infection from the ciliate *Tetrahymena pyriformis* died within 24 hours (Ling & Werner 1988). Likewise, edemateous tadpoles in a population of *B. viridis* had obvious buoyancy problems and soon died (Flindt 1985a, Henle et al. 2017a). Moreover, incidences of anomalies in tadpoles often decrease with season (e.g., Flindt 1985a, Burkhart et al. 2000), indicating decreased survival. Likewise, a decrease in the spectrum of anomalies during development, indicating the elimination of (sub-)lethal types of anomalies, was observed in several cases (e.g., Flindt 1985a, Kuranova 1997, Pyastolova & Vershinin 1999, Vershinin 2004).

Limb anomalies caused by *Ribeiroia ondatrae* did not affect the development rate of *Pseudacris regilla* tadpoles; however, the infection itself retarded development irrespective of the malformation status (Romansic et al. 2011).

Frogs with supernumerary or missing limbs often fall on their back when attempting to jump (Woitkewitsch 1959, Helgen 2012). *Pseudacris regilla* that were malformed due to *R. ondatrae* infection (mainly polymely or skin-webbing) allowed simulated predators to approach more closely than did normal frogs (Goodman & Johnson 2011b). They had reduced jumping distances (41% reduction), slower swimming speed (37% reduction), decreased endurance (66% reduction), low-

er body condition and poorer foraging success, which resulted in a 22% lower biweekly survival rate relative to infected frogs without malformations (Goodman & Johnson 2011a). By contrast, in enclosures without predators, survival was similar to normal frogs. Moreover, malformed individuals selected warmer microhabitats than normal frogs, which resulted in higher body temperatures of the malformed frogs (Goodman & Johnson 2011b). They were more likely to use open ground microhabitats relative to vertical refugia and selected less-angled perches closer to the ground compared to normal frogs. This altered habitat selection is likely to expose them more to avian predators, which should facilitate the transmission of the parasite to its definitive host and thus can be regarded as an extended phenotype of the parasite that facilitates transmission to the definitive host. Selection of warmer sites may also increase the risk of desiccation and overheating but this risk has not yet been assessed in the field.

Lower survival rates of abnormal individuals are reflected in higher percentages of abnormal juveniles compared to adults. Johnson et al. (1999), Goodman & Johnson (2011a) and Lunde et al. (2012) found less than 5% abnormal adult *P. regilla* at ponds in California even after years in which up to almost 60% of the metamorphosing frogs were abnormal. Black-eyed *Rana arvalis* were significantly more frequent among juveniles than adults in Ekaterinburg, Russia (Vershinin 2006). In three Australian frog species in Jabiru the frequency of abnormal individuals was 2–8% lower in adults than in juveniles (Tyler 1989). In a population of *Bufo bufo* from Rhineland-Palatinate, Germany, only 1% of the adults but 15.5% of tadpoles and toadlets suffered from ectromely or oligodactyly (Veith & Viertel 1993). Likewise, in a Californian population of *A. macrodactylum* infected by *Ribeiroia ondatrae* only 4.6% of the adults but 38.5% of the juveniles showed limb anomalies (Sessions & Ruth 1990). In populations of *Pelophylax* synkl. *esculentus* affected by anomaly P (Dubois 1979b) and the *P. ridibundus* populations studied by Woitkewitsch (1959, 1965), the frequency of anomalies was much higher in tadpoles than adults; Woitkewitsch (1959, 1965) even had difficulties finding any affected adults. These differences indicate that most abnormal individuals died before reaching sexual maturity. However, when anomalies are due to abnormal regeneration or injuries, then they sometimes accumulate with age (Vershinin 2002, Gridi-Papp & Gridi-Papp 2005).

5.2 Population level effects

Because anomalies are usually detrimental at the individual level, one might expect that anomalies contribute to the global decline of amphibians (Dubois 1979b, Lannoo 2008, Helgen 2012). For example, a population of *Anaxyrus americanus* failed to breed after years of high rates of malformations (Johnson & Hartson 2009) and in some wetlands 90–100% of the larval *Pseudacris regilla* were abnormal leading to an almost outright recruitment failure (Lunde et al. 2012). At Veteran's Park, Idaho, and Muskee Lake, California, *P. regilla* and *Ambystoma tigrinum*, showed high rates of anomalies (albeit sample size was low for the latter species) in 1988 and 1946–1951, respectively, and were extirpated in 1999. Similarly, at Morgan Pond in Texas *Lithobates catesbeianus* had an abnormality rate of approx. 5% in 1961 and was extinct in 2001 (Johnson et al. 2003). A neotenic population of *A. tigrinum* from the Reese Air Force Base, Texas, USA, in which up to 45% of the individuals developed tumours between 1970 and 1980 rapidly declined from approx. 18.000 mature individuals in 1976 to around 3.000 in 1980 (Rose 1981). However, similar declines also occurred in populations from non-polluted sites and might have been due to low rainfall rather than the anomalies.

Mass mortality and declines, however, are not necessarily due to anomalies. For example, at the CWB malformation hotspot in Minnesota, *L. sylvaticus* and *L. pipiens* became extinct and *Anaxyrus americanus* seriously declined (Hoppe 2005, Lannoo 2008). Concomittantly, increasing numbers of dead and dying tadpoles and frogs of several species were found in the lake (Hoppe 2002). It should be noted that the frequency of abnormal individuals was the same in dead and living samples, indicating that mortality was irrespective of the abnormality status. Moreover, dead fish were observed as well. This indicates that anomalies may not have been the direct cause of decline. Rather, one (or multiple) factor(s) probably simultaneously caused anomalies and directly killed the frogs and fish.

Mass occurrence of anomalies linked to major mortality events caused by infection with *Ranavirus*, an iridiovirus, *Areomonas hydrophila*, or *Batrachochytrium dendrobatidis* have been discovered in various amphibian species in different regions of the world (e.g., Cunningham et al. 1993, 1996, Lips 1999, Docherty et al. 2003, Lips et al. 2004, Berger et al. 2009, Teacher et al. 2010). Infections with *Ranavirus* and *B. dendrobatidis* have been implicated in the long-term decline of populations and even the extinction of species (Berger et al. 2009). However, in all of these cases, the observed anomalies, such as edema or hemorrhage, are rather an epiphenomenon than the cause of the mortality. For example, *B. dendrobatidis* infection causes mortality by disrupting cutaneous functions in sensitive species (Voyles et al. 2009).

In spite of observed declines and extinctions, evidence exists that some anomalies may be maintained in a given population over rather long periods of time, at least for 27 years (Rostand 1971, Dubois 1984, Vershinin 2002) and even longer (Dubois, unpublished data). Anomaly P, for example, was retained in several populations of *Pelophylax* synkl. *esculentus* in France for more than a decade (27 years at Champdieu) at variable, often high rates (up to 80% in tadpoles) (Rostand 1971, Dubois 1979b, 1984, 2014, 2017). At Seascape, California, and Jette Pond, Montana, for example, *P. regilla* showed high rates of anomalies 1986–1987 and

1958–1959 and were still extant one and four decades later, respectively, again exhibiting high rates of anomalies (Johnson et al. 2003). The reason why populations remained extant over years despite high frequencies of anomalies may be explained by a combination of several factors. First, there may be an overestimation of the percentage of abnormal metamorphs as these may be easier to observe and may disperse less from breeding locations than normal individuals. The extent to which dispersal differs in abnormal individuals from normal ones has not been studied and only Goodman & Johnson (2011a) seemed to have addressed catchability: there was no difference between malformed and normal *P. regilla*. Secondly, mortality may be compensatory, which is common in larval and recently metamorphosed amphibians (e.g., van Buskirk & Smith 1991, Patrick et al. 2008). Therefore, mortality in post-metamorphic stages of pond breeding amphibians is more likely to contribute to declines rather than the mortality of eggs, larvae and recently metamorphosed individuals (Biek et al. 2000, Salice 2012). While it is clear that many types of anomalies incur costs to the abnormal individual, the population level effects are less clear and urgently need to be addressed to understand the role of anomalies for the conservation of amphibians.

6 Temporal patterns in the occurrence of anomalies and their reporting

In the North American literature it is generally assumed that the occurrence of anomalies has increased considerably since the 1990s, (e.g., Blaustein & Johnson 2003, Sessions 2003, Hoppe 2005, Johnson & Chase 2004, Johnson & Lunde 2005, Lannoo 2008; Anderson & Hoppe 2010, Helgen 2012). Several pieces of evidence were advocated to support these conclusions, such as lower rates of anomalies in historical museum collections and in early surveys at locations that have been resurveyed more recently. Also, average rates of anomalies above the baseline rates in several recent large-scale surveys and the appearance of hotspots have been regarded as evidence of an increase in the frequency of anomalies over recent decades. Finally, mechanistic arguments that link an increase in anomalies to the eutrophication of ponds and lakes have been put forward.

To evaluate whether an increase in the occurrence and frequency of anomalies has occurred over time, and if so, when and how strongly, is challenging because of the almost complete absence of standardized monitoring programs with an appropriate site selection design. Moreover, greater public awareness following the rediscovery of anomaly hotspots in North America (Souder 2002) and concomitantly substantially increased survey efforts likely contributed to the considerable increase in publications reporting anomalies from natural populations since the 1990s (see Fig. 2). In addition, populations without anomalies are rarely reported and in spite of guidelines for data collecting (Meteyer 2000, Lunde & Johnson 2012) anomalies are still examined, scored and reported in inconsistent ways.

In the following we discuss what can be inferred despite these challenges. We first summarize the results from comparisons of recent data with historical museum collections. We then evaluate trends from resurveys and long-term monitoring of sites and analyse whether there is evidence for an increase in the prevalence of anomalies in recent large-scale surveys and from the record of publications. Finally, we outline mechanistic arguments that link an increase in anomalies to environmental change over recent decades.

6.1 Historical collections

The few comparisons of more recent and historical museum collections provide some support for the notion of increased frequencies of abnormal amphibians over recent decades. In recent (1993–1999) collections of tadpoles of *Rana muscosa* from the Sierra Nevada, California, 18% of the individuals showed oral anomalies but none of those collected between 1955–1976; the difference was highly significant (Fellers et al. 2001). The anomalies were caused by infection with *Batrachochytrium dendrobatidis*.

Of the museum specimens of *Acris crepitans* housed at the University of Arkansas, 3.3% were scored as abnormal (mainly ectrodactyly and syndactyly and retained tails) for the period 1957–1979 (McCallum & Trauth 2003). In the 1980s, the rate was 5.5%, increasing to 6.9% in the 1990s and to 8.5% by 2000. This increase was statistically highly significant. There are two caveats for inferences from these data that must be mentioned. Firstly, frequencies of abnormal frogs were not uniformly distributed across Arkansas; they were rather high at locations in the Ozark highlands and no data were given whether the percentage of examined individuals that originated from these highlands were the same in all compared periods. Secondly, one third of the abnormalities were retained tails and presumably occurred in recently metamorphosed individuals – tail retention in adults has been reported only extremely rarely and retarded tail resorption is a normal feature in some anuran species (see section 3.3.2.1). Unfortunately, McCallum & Tauth (2003) did not provide the age of these individuals nor whether age distribution was constant across the periods under comparison.

Juvenile and adult amphibians of 19 species collected at six localities in Tucumán Province, Argentina, between 1940 and 2010 also did not show uniform distributions of the prevalence of anomalies across decades (Medina et al. 2013). The highest prevalence was observed in the decade 1960–1969. Similar caveats as for the study by McCallum & Trauth 2003) apply. Prevalences differed (marginally significant) among sites and it was not assessed whether all sites were equality represented in all decades. In addition, digital amputations were considered as toe-clipping and not as anomalies but criteria how amputations by toe-clipping and natural loss of digits could be

differentiated in museum specimens were not provided – and is unlikely to be possible (see section 3.2.2.1).

6.2 Resurveys

Resurveys and monitoring of sites showed mixed results. Resurveys conducted within a few years most frequently showed limited differences in the percentage of individuals affected between the survey and the resurvey (e.g., Canfield et al. 2000, Levey et al. 2003, Vandenlangenberg et al. 2003, Johnson et al. 2001, 2003). In a nation-wide survey of US wildlife refuges the differences among surveys was most frequently below 10% but reached more than 30% at one site, with the variability being higher at sites within hotspot clusters compared to outside of hotspot clusters (Reeves et al. 2013). This is not too surprising as hotspot clusters include more hotspots that by definition have higher prevalences of anomalies and thus can vary more than those areas outside hotspot clusters.

At a few sites, the percentage of abnormal frogs had declined considerably in the resurveys (Canfield et al. 2000, Lunde et al. 2012) or even completely disappeared. For example, at Granite Falls, Minnesota, many malformed frogs were observed in 1993 but none were discovered among hundreds examined in a resurvey the following year (Helgen 2012). At Hog Lake in California the percentage of abnormal *Pseudacris regilla* was approximately 50% in the 2006–2007 and 2007–2008 breeding seasons and then dropped to approx. 5% in the 2008–2009 and 2009–2010 breeding seasons (Lunde et al. 2012). At Muskee Lake, Colorado, a high prevalence of polydactyly suddenly appeared in *Ambystoma tigrinum* in 1945 and disappeared again after 1953 (Bishop 1947, Bishop & Hamilton 1947).

Surveys that were made more than a decade apart showed a similar mixed picture. At Macinaw River, Illinois, for example, no abnormal *Acris crepitans* was found in 1968–1971 ($n = 345$) but in a resurvey in 1998 three out of 140 individuals had a missing digit (Gray 2002). The increase is statistically significant (Fisher's exact test: $P = 0.02$), albeit small. Field surveys at nine sites in Minnesota sampled between 1975 and 1992 revealed three abnormal *Lithobates pipiens* out of the 1772 examined. A resurvey in 1996–1997 resulted in 2.3% of 2548 frogs being abnormal, which is a small but significant increase (Hoppe 2000). Unfortunately, no data were provided as to whether the earlier surveys comprised the same percentage of juveniles as the later resurveys.

Of 276 juvenile *L. pipiens* specimens collected from 1958 to 1963 by Merrell and stored at the Bell Museum of Natural History, University of Minnesota, from five sites, at which the species was still found in 1997, one was abnormal (0.4%). In 1997, 15 out of 611 individuals were malformed; the increase is significant (Hoppe 2000). A limitation of the study is that Hoppe (2000) did not include individuals of the historic collection with missing body parts (limbs?) in the number of abnormal individuals because he regarded them to be due to predators. However, trauma as cause can be identified with certainty only if wounds are still fresh, which is not the case in old collections (see 3.2.2.1). Notwithstanding, further surveys during 1998–1999 yielded frogs at four more sites at which museum specimens were available for comparison. Across the nine sites, the frequency of abnormal individuals was higher than the historic frequency when individulas with amputations were included in the number of abnormal specimens (2.4 % versus 0.7%) (Hoppe 2005, Anderson & Hoppe 2010). At one of these sites, in Douglas County, none of the museum specimens collected between 1958 and 1963 was abnormal ($n = 40$), only a single one ($n = 85$) was abnormal in a resurvey from 1996 to 1999 (Hoppe 2005) but 14 ($n = 147$) were abnormal in 2011 (Helgen 2012). Moreover, the malformation risk in the 1990s was about six times that of the historical period (calculated without counting individuals with amputations as abnormal) (Anderson & Hoppe 2010)

At Frog Pond, California, 15% and 25% of the *P. regilla* were abnormal in 1997 and 1998, respectively (Johnson et al. 2003). A decade later (2006–2009), the frequency was slightly higher (25–35%). At Jette Pond, Montana, the anomaly rate increased in three species (*Ambystoma macrodactylum* from 0% to 11%, *Anaxyrus boreas* from 0% to 6%, *P. regilla* from 20% to 46%) between 1958 and 2002 (Johnson et al. 2003). A very strong increase of the abnormality rate also occurred in *Cryptobranchus bishopi* in the Spring River, Arkansas: from 12.5% ($n = 45$) in museum specimens collected between 1970 and 1975 to 90% ($n = 10$) in field samples in 2003–2004 (Hiler et al. 2005). The difference is highly significant ($\chi^2 = 25.4$, $\alpha < 0.0001$). Tumours, ectromely, ectrodactyly and necrotic limbs were the most common anomalies.

Four declines in abnormality rates were detected in resurveys. At Ripley Pond, Ohio, 8% of *L. catesbeianus* suffered from polymely, polydactyly, ectromely, or ectrodactly in 1954 (Anonymous 1954) but only a single abnormal individual (with schizodactyly) was found in a resurvey in 2001 (Johnson et al. 2003). At Seascape, California, the abnormality rate in *P. regilla* and *Ambystoma macrodactylum croceum* dropped substantially (from 72% to 13% and from 28% to 0.9%, respectively, between 1986 and 2000 (Johnson et al. 2003). Slightly elevated rates of polydactyly (0.7–1.4%) were observed in *Cynops pyrrhogaster* from Honshu, Japan, in the years 1981–1985 (Meyer-Rochow & Asashima 1988), which fell to less than 0.1% in 1995 (Fukui et al. 1996). The fall was explained by improved water quality – but no explicit data on water quality were provided.

In summary, resurveys tend to indicate a slight dominance of increases in abnormality rates. However, from eight increases only four were strong and four declines occurred as well, two of which were very severe. Given the very high year-to-year fluctuations that have been reported for several populations, little can be inferred about historic trends from these resurveys until more resurveys become available to enable assessments about whether increases occurred significantly more often than declines.

6.3 Long-term monitoring of sites

Very few long-term monitoring studies of the same site(s) are available. Most of them did not show a clear trend of the prevalence of anomalies through time. For example, at Hidden Pond in California the percentage of abnormal *Pseudacris regilla* was approximately 10% in 1997 and 1998 (Johnson et al. 2001a), increased to 58% in 2006 and then fell to 28–36% in the years 2007–2009 (Lunde et al. 2012). Between 1997 and 2001 the perecentage of abnormal *Lithobates pipiens* metamorphs fluctuated between 1% and 20% at one site (Otter Creek) in the Lake Chamberlain Region of Vermont, showed little fluctuations at three sites, and declined from 35% to 1% at one Site (Wards Marsh) (Levey et al. 2003). Between 1949 and 1967, the frequency of *Pelophylax* synkl. *esculentus* suffering from anomaly P at Trévignon in France varied from 0% to 80% in tadpoles and from 2.6% to 14.5% in adults (Dubois 1979b, 2014, 2017). In another population from central France, Champdieu (Loire), the frequency varied from 0% to 6.3% between 1950 and 1976, whereas in a third one, Lingé (Indre), from 1961 to 1970 the frequency in tadpoles fluctuated between 14% and 70% (Dubois 1979b, 1984, 2014, 2017). At the same time in Champdieu the frequency of black-eyed individuals ranged from 0% to 0.7%; black-eyedness is not related to anomaly P. The percentage of black-eyed individuals of *Rana arvalis* in Ekaterinburg, Russia, fluctuated between 0% and 10% in 1980–1998, with the fluctuations being highest in the multistory urban zone and lowest in rural forest areas (Vershinin 2002, 2004, 2005). It is noteworthy that even for the same type of anomaly and the same cause the interannual variability in the frequency of anomalies may differ considerably among sites and may be huge.

Besides the one site in Vermont mentioned above, only five studies witnessed a clear trend in the percentage of abnormal individuals for a specific location. Martof (1962) collected 43 albinistic *Desmognathus marmoratus* between 1954 and 1960, with their frequency increasing from 0.7% to 8.9%. In 1963, the percentage had increased to 15% (Martof & Walton 1965). Rose (1976, 1981) monitored an *Ambystoma tigrinum* population in a sewage pond at the Reese Airforce Base, Texas, that suffered from tumours. Polycyclic aromatic hydrocarbons were identified as the cause of the anomalies. In 1970, only one larva out of 2430 was abnormal. The anomaly rate rose continuously to approx. 50% in 1975 and then fell again to approx. 20% in 1980, with the fall happening simultaneously with a strong decline in abundance.

In two Russian populations of *Bufo bufo* the incidence of polydactyly dropped from 29.8% in 1990 to 0% in 1997 (Lada 1999). From 1926 to 1934 all 46 individuals of *Taricha porosa* that were sampled at Boulder Creek, California, exhibited a pathological warty skin (Brattstrom & Warren 1953) that Twitty (1942) identified as an extreme proliferation of fibrous connective tissue, i.e., tumour. In 1942, this was the case for 27 out of 28 individuals sampled. Prevalence had dropped to 26% (n = 19) in 1948 and to 10% (n = 148) in 1953 (Brattstrom & Warren 1953). The anomaly was not transmitted among individuals maintained in captivity nor influenced by the maintenance temperature and the cause of the anomaly remains obscure.

6.4 Prevalence of anomalies in large-scale surveys and background rates

Following the re-discovery of anomaly hotspots in Minnesota, a range of large-scale surveys on abnormal frogs was carried out in various states of the USA and nationwide in wildlife refuges (Helgen 2012, Reeves et al. 2013). The nation-wide frequency of anomalies across 135 national wildlife refuges was 2% (Reeves et al. 2013). In the surveys outside national wildlife refuges in various states, anomaly rates well above this rate were repeatedly found (reviewed by Helgen 2012). Unfortunately, some of these surveys also targeted known anomaly hotspots and thus may not have provided data for unbiased comparisons (Reeves et al. 2013). Also, a considerable number of surveys resulted in anomaly prevalences around the presumed baseline level (Lunde & Johnson 2012), which would weaken the conclusion that contemporary rates of anomalies are elevated. On the other hand, Table 1 indicates that true baseline rates might even have been an order of magnitude lower than the 2% calculated in the nation-wide survey in wildlife refuges (see Tab. 1 and Henle et al. 2017a), which would strengthen the conclusion about an elevated frequency of anomalies over recent decades. It is worth noting, however, that, although not targeted, the survey of Reeves et al. (2013) included some hotspots. One third of the sites did not reveal any anomaly and at about 50% of the sites the frequency of anomalies was below 2%. This further strengthens the results from Table 1 on true baseline rates of well below 1%. In the absence of comparative data from the past uncertainty remains about true baseline rates.

Based on an analysis of the compilation of data from publications of skeletal anomalies by Ouellet (2000), Lannoo (2008) argued that the frequency of anomalies increased in recent decades. He coded all records in a binary fashion, with "fewer than ten individuals" as one category and "ten or more individuals" as the second category. A plot of these data against publication date indicated that the category "more than ten" started to appear around 1950 and became increasingly more prevalent. He concluded that these reports of "ten or more" constitute the malformed frog problem and that this problem started to appear from about 1950.

There are several caveats with this conclusion. Firstly, the number of publications has strongly increased (Fig. 2) creating a strong overlap of dots on his graph for recent decades. Therefore, it is not really clear as to whether the percentage of surveys that resulted in "ten or more" has indeed increased over recent decades. Lannoo (2008) did not test for a temporal trend in this percentage. Secondly, several cases of the category "ten or more" that were recorded for the first half of the last

century were missing in the compilation of Ouellet (2000).

We redraw the graph based on our more comprehensive database that includes all types of externally visible anomalies (Fig. 39). If sampling covered more than one year, we used the median survey year (the earlier year in cases of equal numbers of sampling years). To reduce noise created by long time spans, we divided the data, if possible, into 5-years-intervals, starting with the first year that the site was sampled. Figure 39 clearly shows that there is a considerable number of "ten or more" cases that occurred in the first half of the 20th Century.

Lannoo's (2008) conclusion further requires the assumption that sample sizes did not increase over time as this would bias trend analyses based on the number of abnormal individuals observed. Elsewhere we will show that the median value of the percentage of abnormal individuals observed per case increased with the year of observation (Henle et al. submitted).

6.5. Mechanistic explanations linking an increased frequency of anomalies to environmental changes

Mechanistic links of anomalies to environmental factors that have changed over recent decades provide the best evidence that the frequency of anomalies has indeed increased over recent decades. One comparably well understood link is to eutrophication. Many of the North American hotspots occur in highly eutrophic water bodies (Lannoo 2005) and eutrophication has substantially increased in North America (Rouse et al. 1999) and in many other parts of the world (Ellenberg 1991). This increase already started many decades ago, well before the 1990s, and thus is in line with Lannoo's (2008) suggestion that the the malformed frog problem already started to appear around the 1950s (or even slightly earlier – see Figure 39; and not only in the 1990ies as assumed by many authors, see introduction to section 6) and that the collapse of wetlands caused by eutrophication and other pollutants was a key driver of the malformed frog problem.

The eutrophication of ponds fosters planorbid snails that are intermediate hosts to the trematode *Ribeiroia ondatrae* (Johnson & Chase 2004). Eutrophication further enhances the survival of infected snails, thereby increasing the period over which they release cercariae of the parasite. A higher abundance and an enhanced survival of infected snails will increase the number of cercariae in a pond that can infect amphibian hosts. These changes should lead to higher infection rates in amphibians and as a consequence higher frequencies of anomalies. While a range of observational and experimental studies support these mechanisms (Johnson & Chase 2004, Johnson & Lunde 2005, Johnson et al. 2007), it can only explain an increase in anomalies that are typical for an infection with *R. ondatrae*, such as polymely, polydactyly, bony triangles and skin webbing. Several of the hotspots in the northwestern states of the USA and California are characterised by these types of anomalies (Johnson et al. 2002) but in many other re-

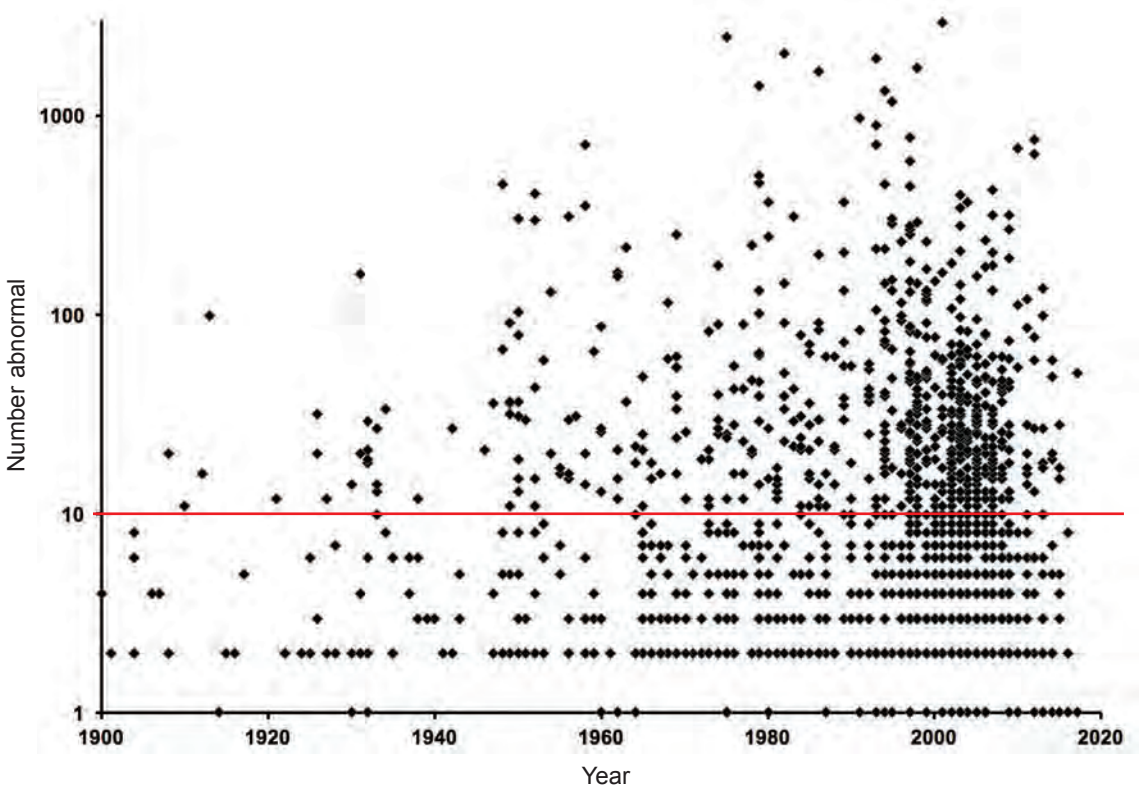


Fig. 39: Plot of the number of anomalous individuals reported per case against year of observation. Note the logarithmic scale. The red line separates the two categories used by Lannoo (2008) for inferring an increase in the frequency of abnormal individuals since about 1950.

gions of the USA and in all other parts of the world the spectrum of anomalies does not fit the pattern typical for this parasite (see section 3.1).

Lannoo (2008) suggested that eutrophication and the resulting collapse of wetland ecosystems is also a direct cause for increased frequencies of anomalies, independent of the presence of *R. ondatrae*. While fertilizers are not known to cause most of the anomalies observed in hotspots, eutrophication is an indicator of intensive agricultural practices. Intensive agriculture in turn is characterised by an intensive use of pesticides (Henle et al. 2008, Lannoo 2008) that can contribute to an increase in the prevalence of anomalies through at least two mechanisms. Firstly, they may enhance infection rates of tadpoles by *R. ondatrae* (Kiesecker 2002). Secondly, various pesticides are teratogenic for larval amphibians at environmentally relevant concentrations. Among other anomalies, they can induce ectromely and ectrodactyly (see e.g. Table 3 and section 3.1.7.2), which are dominant at many North American hotspot sites.

Recently, a further mechanism that may link eutrophication to increased frequencies of anomalies was discovered. Eutrophication may cause blooms of cyanobacteria that produce teratogenic retinoic acids (Wu et al. 2012). Together, these mechanisms should have contributed to an increase in the prevalence of anomalies in landscapes dominated by intensive agriculture that relates to the intensification of agriculture. While historical comparisons are not available to document such a temporal correlation, comparisons of sites close to intensively used agricultural fields with control sites away from intensive agriculture tend to support such a causal relationship; for examples from Europe, Asia, Africa, North and South America see section 3.1. Moreover, several of the hotspot clusters identified in the USA are located in areas with intensive agriculture, particularly in the Midwest (Lannoo 2008, Helgen 2012, Reeves et al. 2013). However, quantitative assessments that relate abnormality prevalences to land cover types are rare and the few existing ones used only crude proxies for the intensity of agriculture (e.g., Ouellet et al. 1997, Piha et al. 2006), with measures of eutrophication or the use of pesticides missing in large-scale assessments. Because agricultural land use is spatially and temporally heterogeneous, showing strong non-linearity across scales (Tzanopoulos et al. 2013), results may also differ, depending on the scale of assessment. This heterogeneity in time and space may further explain why historical comparisons and resurveys show both increases and decreases in the percentage of abnormal individuals.

If pollution associated with more intensive land use has contributed significantly to an increased prevalence of anomalies at a large scale (be it directly or indirectly), then prevalences of anomalies should be higher outside compared to inside national parks, wildlife reserves and forest reserves, which generally have a lower land use intensity. Elsewhere we show that this is the case for North America (Henle et al. submitted).

Some hotpots of anomalies in Europe, Asia, Australia, Bermuda and North America were linked to industrial pollution (see section 3.1). A wide range of anomalies can be caused in amphibians by industrial chemicals (Henle et al. 2017a) and the causal relationships have been documented experimentally for several hotspot sites. Like eutrophication, the pollution of water bodies from industrial sources has also increased substantially worldwide after the Second World War (e.g., Widener 1970, Koch & Vahrenholt 1983, Dubois 2012) and this should also have contributed to an increase in the prevalence of amphibian anomalies since then. On the other hand, progress in water protection in some countries should have also reversed the trend of the prevalence of anomalies. Indeed, Fukui et al. (1996) related a decline in the rates of polydactyly in *Cynops pyrrhogaster* in Honshu, Japan, from 0.7% to 1.4% in the years 1981–1985 to less than 0.1% in 1995 to improved water quality. Unfortunately, he did not provide data on water quality and data that relate changes in the frequency of anomalies to changes in water quality are generally lacking. In any case, progress in water protection is limited in many regions of the world. Even in Europe with its very stringent legal requirements, water pollution still jeopardizes the health of freshwater ecosystems on a continental scale (Malaj et al. 2014) – but note that no large-scale data are available for small, especially ephemeral water bodies that are preferred breeding sites for many amphibians.

7 Conclusion and perspective

Since the first reliable report of an observed malformed amphibian in 1554 (Gesner 1554) a considerable number of publications and observations have accrued. Much of this literature is widely dispersed and addresses anectodal observations of 1–2 individuals and types of anomalies. Anomalies have been reported from all continents and from more than 400 species. Large numbers and percentages of affected individuals (so-called hotspots) are also known from all continents. The largest number of reports originates from Europe and North America, likely due to a much higher number of naturalists being active in these countries and greater chances of finding their publications.

A considerable number of causes have been suggested for observed anomalies but attempts to unravel them have only been undertaken in a minority of cases. This also applies to hotspots and much of the inferences rely on correlations and extrapolations – which may be indicative but have limitation – and less on experiments conducted for specific hotspots. Nevertheless, it is clear that a range of different factors is involved. This also applies to hotspots in North America (Reeves et al. 2013) in spite of the still existing controversy. Demonstrated causative factors are parasites, chemical pollution, predators, disease, injuries from mowing and radioactive pollution, with the relative importance differing among regions. All but the last were demonstrated for at least one hotspot site in North America as well. These factors may not only

co-occur at a particular hotspot site but may interact with each other, even though co-occurrences and interactions are still insufficiently understood.

It is widely assumed that prevalences of anomalies have increased over recent decades. Evidence from museum collections and resurveys tend to support this assumption, although declines were observed over recent decades as well and the few long-term monitoring studies available show that the frequency of abnormal individuals can fluctuate widely without an apparent trend. Despite these fluctuations, across all published cases, there is a significant increase over time in the percentage of individuals that are abnormal and this is especially apparent in the most severe cases (Henle et al. submitted). However, in the absence of large-scale long-term monitoring programs these patterns are only indicative but cannot prove a change over time. Initiation of well-designed long-term monitoring studies and resurveys of sites where anomalies were assessed in the past are urgently needed.

Notwithstanding, the revelation of mechanistic relationships does support an increase in the prevalence of anomalies over the last 50 years. Mechanistic links of eutrophication with high rates of anomalies have been demonstrated and various chemical pollutants are also known to cause elevated rates of anomalies. Because pollution increased in many parts of the world since the Second World War and still jeopardizes the health of freshwater ecosystems on a continental scale (USEPA 2009, Malaj et al. 2014), it is likely that the prevalence of anomalies has indeed had an increasing tendency over recent decades. Further, there is evidence that background rates of anomalies are about an order of magnitude lower than currently assumed. However, in the absence of repeated standardized large-scale surveys based on sampling design theory, in which potential causative factors and also absences of anomalies are reported, it is difficult to establish baseline rates and to quantify the role different factors play in increased rates of anomalies.

Little is also known about the population level consequences of elevated rates of anomalies. Even at the individual level, few studies quantitatively addressed the effects of particular types of anomalies. Although most types of anomalies hamper performances of affected individuals, this often depend on other environmental factors, such as presence or absence of predators and food availability. Also, decreased performance, including reduced survival, does not necessarily translate into population level effects because of compensatory mortality. Moreover, anomalies may not be the direct cause of an observed increase in mortality and population decline; rather the same factor may cause the anomalies and directly kill normal and abnormal individuals. Therefore, it is currently not possible to know whether elevated rates of anomalies contribute to the decline of amphibians and studies that unravel population level effects are urgently needed. Likewise, studies on other organisms in regions where several hotspots of amphibian anomalies occur could be enlightening to better understand the relevance of amphibian anomalies for ecosystem and human health.

In conclusion, while studies on single individuals or types of anomalies are still valuable, especially for countries and species for which only limited observations have been published, one should always examine all individuals, report sample sizes and the number of abnormal individuals, and describe the anomalies using a clearly defined terminology. In population studies, anomalies should be routinely assessed in such a way that they can be used in rigorous meta-analyses and it should be reported if none were detected.

In addition, long-term monitoring studies across larger regions based on sampling design theory that include the monitoring of potential causative factors need to be established to better understand trends. Such studies should not only assess populations in wildlife areas (as e.g. Reeves et al. 2010, 2013) but should include other land use types. Evaluating the correlation between anomaly prevalences and land cover type and quantitative indicators of land use intensity, such as indicators of agricultural intensity (Ouellet et al. 1997, Taylor et al. 2005), urbanization (Vershinin 2002) or industrialization (Mizgireuv et al. 1984), across large scales would further allow major progress in identifying likely causative factors of elevated rates of anomalies. Hypotheses derived from such studies could then be tested experimentally for targeted sites or landscapes. Experimental studies to unravel potential causes should not be limited to a single factor; rather they should address all of those factors that are consistent with the pattern of anomalies observed in the targeted natural population and thus possible candidates as culprits. In this respect the work of the amateur biologist Jean Rostand to elucidate the cause(s) of anomaly P (summarized in Rostand 1971 and Dubois 2017) still remains a highly recommendable example.

Finally, studies are needed that unravel the population level consequences and the consequences for amphibian declines and ecosystem and human health of elevated rates of anomalies. Whether or not, and if so to which extent, they contribute to amphibian declines, it is an ethical obligation to minimize eutrophication and pollution of wetlands to reduce the anthropogenically driven plight of amphibians. This, at the same time, will benefit many other organisms, ecosystem services to humans, reduce human health risks, and improve the quality of human life.

8 Acknowledgments

Please note that copyrights may apply to all those photos published with this article. Photos retrieved from Wikimedia Commons (http://commons.wikimedia.org) were published under the creative commons licence (https://creativecommons.org/licenses/by-nc-sa/2.0/). See the cited webpage for conditions of using these photos. Photos of the United States Fish and Wildlife Service are in the public domain and were retrieved from

http://www.fws.gov/contaminants/amphibian/PictureThumbs.html.

We owe thanks to all photographers who made photos available via these platforms or who provided us personally their photos. VV was supported by Act 211 Government of the Russian Federation, agreement № 02.A03.21.0006. We thank Marlies Uhlig, Leipzig, and the UFZ library team, for their assistance in compiling the literature. Spartak Litvinchuk, St. Peterburg, and Sergius L. Kuzmin, Moscow, helped with access to Russian literature. Annegret Grimm-Seyfarth carried out part of the statistical analyses. Above all, we would like to thank Jean-Claude Beetschen, Toulouse, and Alain Collenot, Paris, for carefully reviewing and commenting on the manuscript. KH thanks Natascha Rumyantseva for her help in translating Russian literature and for her patience during many hours of work on the manuscript.

9 References

Adolphi, H. (1893): Über Variation der Spinalnerven und der Wirbelsäule anurer Amphibien, I (*Bufo variabilis* Pall.). — Morphol. Jb. **19**: 313–375.

Adolphi, H. (1895): Über Variation der Spinalnerven und der Wirbelsäule anurer Amphibien, II, (*Pelobates fuscus* Wagl. und *Rana esculenta* L.). — Morphol. Jb. **22**: 449–490.

Agostini, M.G., F. Kacoliris, P. Demetrio, G.S. Natale, C. Bonetto & A.E. Ronco (2013): Abnormalities in amphibian populations inhabiting agroecosystems in northeastern Buenos Aires Province, Argentina. — Dis. Aquat. Org. **104**: 163–171.

Aguillón Gutiérrez, D.R. & E.A. Severtsova (2012): Malformations in larvae of common and moor frogs in Moscow and surrounding areas. — Proc. Congr. Herpetol. Soc. A.M. Nikolsky, Minsk, Belarus **5**: 370–373.

Ahmad, M. (1976): The effects of ionizing radiations on prometamorphic anuran tadpoles. — J. Sci. (Univ. Karachi) **4**: 26–37.

Alberch, P. & E.A. Gale (1985): A developmental analysis of an evolutionary trend: digital reduction in amphibians. — Evolution **39**: 8–23.

Albrecht, C., T. Esser & J. Weglau (1996): Auftreten der parasitären Fliege *Lucilia bufonivora* (Insecta: Diptera) an Amphibien (Amphibia) im Bereich des rekultivierten Tagebaus Frechen, NRW. — Acta Biol. Benrodis **8**: 161–163.

Aldrovandi, U. (1645): De quadrupedibus digitatis viviparis libri tres et de quadrupedibus digitatis oviparis libri duo. — Bologna (Nicolai Tebaldini).

Allen, B.M. (1916): Experimental ablation of the hypophysis and thyroid glands. — Science **44**: 755–757.

Allen, B.M. (1917): The results of thyroid removal in the larvae of *Rana pipiens*. — J. exp. Zool. **26**: 499–519.

Alonso Bosch, R., L.Y. García Padrón, M. Almaguer & M. Valle (2017): First reports of tadpole mouth anomalies in a Cuban toad (Anura: Bufonidae: *Peltophryne*). — Herpetol. Rev. **48**: 58–62.

Alsop, D.H., S.B. Brown & G.J. van der Kraak (2004): Dietary retinoic acid induces hindlimb and eye deformities in *Xenopus laevis*. — Environ. Sci. Technol. **38**: 6290–6299.

Altig, R. & E. Brodie (1968): Albinistic and cyanistic frogs from Oregon. — Wasmann J. Biol. **26**: 241–242.

Anderson, J.E. & D.M. Hoppe (2010): Malformed frogs: Bayesian and random-effect model analyses . — IJDATS **2(2)**: 103–121.

Anderson, R.C. & G.F. Bennett (1963): Ophthalmic myiasis in amphibians in Algonquin Park, Ontario, Canada. — Can. J. Zool. **41**: 1169–1170.

Andrä, E. & M. Deuringer-Andrä (2011): Höchstgelegenes Laichhabitat der Wechselkröte (*Bufo viridis*) in Mitteleuropa nördlich des Alpenhauptkammes im Grenzbereich zwischen Bayern und Tirol. Ergebnisse einer 10-jährigen Langzeitbeobachtung. — Z. Feldherpetol. **18**: 19–68.

Ankley, G.T., J.E. Tietge, D.L. DeFoe, K.M. Jensen, G.W. Holcombe, E.J. Durhan & S.A. Diamond (1998): Effects of ultraviolet light and methoprene on survival and development of *Rana pipiens*. — Environ. Toxicol. Chem. **17**: 2530–2542.

Ankley, G.T., J.E. Tietge, G.W. Holcombe, D.L. DeFoe, S.A. Diamond, K.M. Jensen & S.J. Degitz (2000): Effects of laboratory ultraviolet radiation and natural sunlight on survival and development of *Rana pipiens*. — Can. J. Zool. **78**: 1092–1100.

Ankley, G.T., S.A. Diamond, J.E. Tietge, G.W. Holcombe, K.M. Jensen, D.L. DeFoe & R. Peterson (2002): Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hindlimb malformations in the northern leopard frog (*Rana pipiens*). — Environ. Sci. Technol. **36**: 2853–2858.

Ankley, G.T., S.J. Degitz, S.A. Diamond & J.E. Tietge (2004): Assessment of environmental stressors potentially responsible for malformations in North American anuran amphibians. — Ecotoxicology and Environmental Safety **58**: 7–16.

Annandale, N. (1905): On abnormal ranid larvae from north-eastern India. — Proc. zool. Soc. London **1905**: 58–61, pl. VI.

Anonymous (1954): Many-legged frogs. — Sci. News Let. **66**: 327.

Anthony, C.D., J.R. Mendelson & R.R. Simons (1994): Differential parasitism by sex on plethodontid salamanders and histological evidence for structural damage to the nasiolabial groove. — Am. Midl. Nat. **132**: 302–307.

Arffmann, E. & B. Collatz Christensen (1961): Studies on the newt test for carcinogenicity. — Acta Pathol. Microbiol. Scand. **52**: 330–342.

Arias, E. & T. Zavanella (1979): Teratogenic effects of manganese ethylenebisdithiocarbamate (MANEB) on forelimb regeneration in the adult newt, *Triturus cristatus carnifex*. — Bull. Environm. Contam Toxicol. **22**: 297–304.

Aron, M. (1929): Recherches chez les batraciens. La croissance embryonnaire. Rôle du système nerveux central. — Arch. Biol. **39**: 609–675.

Arribas, O. (2008): Neotenia y longitud excepcional en *Mesotriton alpestris* de Fuentes Carrionas (Palencia). — Bol. Asoc. Herpetol. Esp. **19**: 23–24.

Arribas, O., J. Rivera & F. Marti (1996): Nuevos datos sobre la presencia de individuos azules de *Hyla meridionalis* en el noreste Ibérico. — Bol. Asoc. Herpetol. Esp. **7**: 25–28.

Asashima, M. & S. Komazaki (1980): Spontaneous progressive skin papilloma in newt (*Cynops pyrrhogaster*). — Proc. Japanese Acad. **56**: 638–642.

Asashima, M., S. Komazaki, C. Satou & T. Oinuma (1982): Seasonal and geographical changes of spontaneous skin papillomas in the Japanese newt *Cynops pyrrhogaster*. — Cancer Res. **42**: 3741–3746.

Asashima, M., T. Oinuma & V.B. Meyer-Rochow (1987): Tumors in Amphibia. — Zool. Sci. **4**: 411–425.

Asher, M.A. & D.C. Burton (2006): Adolescent idiopathic scoliosis: natural history and long term treatment effects. — Scoliosis **1**: 1–10.

Atakhanova, K.Y. & A.B. Bigaliev (1995): Current status of amphibian populations in Central Kazakhstan. Pp. 141–143 in Kuzmin, S.L., C.K. Dodd & M.M. Pikulik (eds.): Amphibian Populations in the Commenwealth of Independent States: Current Status and Declines. — Moscow (Pensoft).

Attademo, A.M., P.M. Peltzer, R.C. Lajmanovich, C. Junges, A. Basso & M. Cabagna-Zenklusen (2012): Trombiculid mites (*Hannemannia* sp.) in *Leptodactylus chaquensis* (Amphibia: Anura) inhabiting selected soybean and rice agroecosystems of Argentina. — J. Zoo Wildl. Med. **43**: 579–584.

Audy, J.R. & M. Nadchatram (1958): *Vercammenia*, a new genus of chiggers hypodermal in Amphibia (Acarina, Trombiculidae). — Stud. Inst. Med. Res. Malaya **28**: 95–102.

Bachhausen, R. (2016): *Batrachochytrium salamandrivorans*. Die neue Gefahr für Schwanzlurche. — Terraria/elaphe **2016(3)**: 28–37.

Bacon, J.P., J.A. Gray & L. Kitson (2006a): Status and conservation of the reptiles and amphibians of the Bermuda islands. — Appl. Herpetol. **3**: 323–344.

Bacon, J.P., D.W. Linzey, R.L. Rogers & D.J. Fort (2006b): Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part I. Frequencies and distribution of abnormalities. — Appl. Herpetol. **3**: 39–65.

Bacon, J.P., C.E. Fort, B. Todhunter, M. Mathis & D.J. Fort (2013): Effects of multiple chemical, physical, and biological stressors on the incidence and types of abnormalities observed in Bermuda's cane toads (*Rhinella marina*). — J. exp. Zool. (Mol. Dev. Evol.) **9999B**: 1–20.

Bagnara, J.T. & M. Obika (1964): Differences in integumental pteridines between black and white axolotls. — Anat. Rec.. **148**: 358.

Bagnara, J.T., S.K. Frost & J. Matsumoto (1978): On the development of pigment patterns in amphibians. — Am. Zool. **18**: 301–312.

Ballengée, B. (2010): Malamp. The Occurrence of Deformities in Amphibians. — London & West Yorkshire (The Arts Catalyst & Yorkshire Sculpture Park).

Ballengée, B. & S.K. Sessions (2009): Explanation for missing limbs in deformed amphibians. — J. exp. Zool. **312B**: 770–779.

Balls, M. (1962a): Spontaneous neoplasms in Amphibia: a review and descriptions of six new cases. — Cancer Res. **22**: 1142–1154.

Balls, M. (1962b): Methylcholantrene-induced lymphosarcoma in *Xenopus laevis*. — Nature **196**: 1327–1328.

Balls, M. & R.H. Clothier (1974): Spontaneous tumours in Amphibia. — Oncology **29**: 501–518.

Balls, M. & L.N. Ruben (1964): A review of the chemical induction of neoplasms in Amphibia. — Experientia **20**: 241–247.

Balls, M., R.H. Clothier & L. Ruben (1978): Neoplasia of Amphibia. Pp. 48–62 in Gershwin, M.E. & E.L. Cooper (eds.): Animal Models of Comparative Developmental Aspects of Immunity and Diseases. — New York (Pergamon Press).

Bancroft, R. & K.V. Prahlad (1973): Effects of ethylenebis (dithiocarbamic acid) disodium salt (nabam) and ethylenebis (dithiocarbamato) manganese (maneb) on *Xenopus laevis* development. — Teratology **7**: 143–150.

Barfurth, D. (1891): Versuche zur functionellen Anpassung. — Arch. mikr. Anat. **37**: 392–405.

Basarukin, A.M. (1984): On some morphological anomalies in amphibians of Sakhalin and Kurile Islands. — Yuzhno-Sakhalinsk (PhD Thesis, Univ. Yuzhno-Sakhalinsk).

Basarukin, A.M. & L.J. Borkin (1984): Distribution, ecology and morphological variability of the Siberian salamander, *Hynobius keyserlingii*, of the Sakhalin Island. — Proc. Zool. Inst. Leningrad **124**: 12–54.

Bataillon, E. & M. Tchou Su (1932): Étude comparative des processus cinétique initiaux chez l'œuf d'*Hyla* fecondé aux divers stades d'immaturité et de surmaturité. — C.r. Acad. Sci. Paris **195**: 353–355.

Bauer Dial, C.A. & N.A. Dial (1995): Lethal effects of the consumption of field levels of paraquat-contaminated plants on frog tadpoles. — Bull. Environ. Contam. Toxicol. **55**: 870–877.

Bazzoli, A.S., J. Manson, W.J. Scott & J.G. Wilson (1977): The effects of thalidomide and two analogues on the regenerating forelimb of the newt. — J. Embryol. exp. Morph. **41**: 125–135.

Bechtel, H.B. (1995): Reptile and Amphibian Variants: Colors, Patterns, and Scales. — Malabar (Krieger).

Beetschen, J.C. & A. Jaylet (1965): Sur un facteur récessif semi-létal déterminant l'apparition d'ascite caudale (ac), chez le Triton *Pleurodeles waltlii*. — C.r. Acad. Sci. Paris **261**: 5675–5678.

Bei, Y., S. Meng, J. Li, J. Feng, J. Zhou & G. Li (2011): A dicephalic caecilian larva, *Ichthyophis bannanicus* (Amphibia: Gymnophiona: Ichthyophiidae), from southeast Guangxi, China. — Asian Herpetol. Res. **2**: 230–233.

Benham, W.B. (1894): Notes on a particularly abnormal vertebral column of the bull-frog; and on certain other variations in the anuran column. — Proc. zool. Soc. London **33**: 477–481.

Bennett, G.A. (1957): The bones. Pp. 1189–1250 in Anderson, W.A.D. (ed.): Pathology. — London (Henry Kimpton).

Berger, L. (1967): Embrional and larval development of F_1 generation of green frogs different combinations. — Acta Zool. Cracoviensia **7**: 123–160.

Berger, L. (1971): Viability, sex and morphology of F_2 generation within forms of *Rana esculenta* complex. — Zool. Polon. **21**: 345–393.

Berger, L. (1976): Hybrids of F_2 generations of European water frogs (*Rana esculenta* complex). — Ann. Zool. **33**: 201–214.

Berger, L. & W.A. Berger (1992): Progeny of water frog populations in central Poland. — Amphibia-Reptilia **13**: 135–146.

Berger, L. & T. Uzzell (1977): Viability and growth of progeny from different egg size classes of *Rana esculenta* L. (Amphibia, Salientia). — Zool. Polon. **26**: 292–317.

Berger, L., R. Speare & J. Humphrey (1997): Mucormycosis in a free-ranging green tree frog from Australia. — J. Wildl. Dis. **33**: 903–907.

Berger, L., J.E. Longcore, R. Speare, A. Hyatt & L.F. Skerratt (2009): Fungal diseases of amphibians. Pp. 2986–3052 in Heatwole, H. & J.W. Wilkinson (eds.): Amphibian Biology. Vol. 8. — Chipping Norton, Australia (Surrey Beatty).

Berns, M.W. & L.D. Uhler (1966): Blue frogs of the genus *Rana*. — Herpetologica **22**: 181–183.

Bérubé, V.E., M.H. Boily, C. DeBlois, N. Dassylova & P.A. Spear (2005): Plasma retinoid profile in bullfrogs, *Rana catesbeiana*, in relation to agricultural intensity of sub-watersheds in the Yamaska River drainage basin, Quebec, Canada. — Aquat. Toxicol. **71**: 109–120.

Bezman-Moseyko, O.S., L.J. Borkin, J.M. Rosanov & S.N. Litvinchuk (2014): Mass hindlimb deformities of green frogs (*Pelophylax esculentus* complex) in Pridnestrovie: causes and bioindication. Pp. 13–18 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Biek, R., W.C. Funk, B.A. Maxell & L.S. Mills (2000): What is missing in amphibian decline research: insights from ecological sensitivity analysis. — Conserv. Biol. **16**: 728–734.

Birge, , W.J., J.A. Black & A.G. Westerman (1978): Effects of polychlorinated biphenyl compounds and proposed PCB-replacement products on embryo-larval stages of fish and amphibians. — Lexington (Univ. Kentucky).

Birkhahn, H. (1991): Neue Erkenntnisse über die Aminosäureversorgung bei Dendrobatiden. — herpetofauna, Weinstadt **13 (74)**: 23–28.

Bishop, C.A. & A.D. Gendron (1998): Reptiles and amphibians: shy and sensitive vertebrates of the Great Lakes basin and St. Lawrence River. — Environ. Monitor. Assess. **53**: 225–244.

Bishop, D.W. (1947): Polydactyly in the tiger salamander. — J. Hered. **38**: 290–293.

Bishop, D.W. & R. Hamilton (1947): Polydactyly and limb duplication occurring naturally in the tiger salamander, *Ambystoma tigrinum*. — Science **106**: 641–642.

Bisping, M., C. Göcking, F. Kraskes, N. Menke & B. Thiesmeier (2016): Farbanomalien bei Larven der Knoblauchkröte. — Feldherpetol. Mag. **6**: 14–19.

Bitz, A. & H. Schader (1996): Laubfrosch – *Hyla arborea arborea* (Linné, 1758). Pp. 231–248 in Bitz, A., K. Fischer, L. Simon, R. Thiele & M. Veith (eds.): Die Amphibien und Reptilien in Rheinland-Pfalz. — Landau (GNOR).

Blair, J. & R.J. Wassersug (2000): Variation in the pattern of predator-induced damage to tadpole tails. — Copeia **2000**: 390–401.

Blair, W.F. (1960): Radiation-induced genetic damage in the Mexican toad (*Bufo valliceps*). — Texas J. Sci. **12**: 216–227.

Blair, W.F. (1972): Evidence from hybridization. Pp. 196–232 in Blair, W.F. (ed.): Evolution in the Genus *Bufo*. — Austin (Univ. Texas Press).

Blaustein, A.R. & P.T. Johnson (2003): Explaining frog deformities. — Scientific American **288**: 60–65.

Blaustein, A.R., J.M. Kiesecker, D.P. Chivers & R.G. Anthony (1997): Ambient UV-B radiation causes deformities in amphibian embryos. — Proc. Natl. Acad. Sci. **94**: 13735–13737.

Bleakney, J.S. (1963): First North American record of *Bufolucilia silvarum* (Meigen) (Diptera: Calliphoridae) parasitizing *Bufo terrestris americanus* Holbrook. — Can. Entomol. **95**: 107.

Bleakney, S. (1958): Cannibalism in *Rana sylvatica* tadpoles a well known phenomenon. — Herpetologica **14**: 34.

Bodemer, C.W. (1959): Observations on the mechanism of induction of supernumerary limbs in adult *Triturus viridescens*. — J. exp. Zool. **140**: 79–99.

Bodenstein, D. (1947): The effects of nitrogen mustard on embryonic amphibian development. — J. exp. Zool. **104**: 311–341.

Bohl, E. (1997): Mißbildungen der Extremitäten von Amphibienlarven in Aufseß (Oberfranken). Versuche zur Ursachenermittlung. — Münchener Beitr. Abwasser-, Fischerei- Flussbiol. **50**: 160–189.

Böhm, S. (2013): Amphibienbeobachtungen im Gartenteich. — elaphe/Terraria **2013(5)**: 80–82.

Boliy, M.H., J. Thibodeau & M. Bisson (2009): Retinoid metabolism (LRAT, REH) in the liver and plasma retinoids of bullfrog, *Rana catesbeiana*, in relation to agricultural contamination. — Aquat. Toxicol. **91**: 118–125.

Bonhomme, C. & R. Pourhadi (1957): Sur quelques larves monstrueuses obtenues par applications d' ultrasons sur divers stades de l'œuf de *Triton*. — C.r. Acad. Sci. Paris **245**: 224–226.

Bonhomme, C., R. Pourhadi & J.P. Turchini (1960): Tératogenèse expérimentale par applications d'ultrasons sur des œufs de *Triton*. — Z. Biol. **3**: 357–366.

Bonin, J., M. Ouellet, J. Rodrigue, J.-L. DesGranges, F. Gagné, T.F. Sharbel & L.A. Lowcock (1997): Measuring the health of frogs in agricultural habitats subjected to pesticides. Pp. 246–257 in Green, D.M. (ed.): Amphibians in Decline: Canadian Studies of a Global Problem. — St. Louis (Soc. Study Amphibians Reptiles).

Bonnet, A. & M. Rey (1937): Sur quelques cas de polydactylie et de schistodactylie observés en série chez la grenouille. — Bull. Soc. Zool. Fr. **62**: 21–25.

Bonnet, C. (1779): Expériences sur la reproduction des membres de la Salamandre aquatique. Second mémoir. Pp. 1–18 in Rozier, M.V. & M.J.A. Mongez (eds.): Observations et Mémoires sur la Physique, sur l'histoire Naturelle et sur les arts et Métiers. — Paris (Cuchet).

Bons, J. & P. Geniez (1996): Amphibiens et Reptiles du Maroc (Sahara occidental compris). Atlas biogéographique. — Barcelona (Asoc. Herpetol. Española).

Borchvardt, V.G. & E.B. Ivaschintsova (1993): Some regularities in the variation of the paw skeleton of the Siberian salamander *Salamandrella keyserlingii* (Amphibia, Hynobiidae). — Zool. Zh. Moskva **72(6)**: 66–73.

Borkin, L.J. (1999): *Salamandrella keyserlingii* Dybowski, 1870 – Sibirischer Winkelzahnmolch. Pp. 21–55 in Grossenbacher, K. & B. Thiesmeier (eds.): Handbuch der Reptilien und Amphibien Europas. Schwanzlurche I. — Wiesbaden (Aula).

Borkin, L.J. (2014): Morphological abnormalities in natural populations of amphibians: what do we study and how do we measure? Pp. 25–36 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Borkin, L.J. & M.M. Pikulik (1986): The occurrence of polymely and polydactyly in natural populations of anurans of the USSR. — Amphibia-Reptilia **7**: 205–216.

Borkin, L.J., L. Berger & R. Günther (1981): On giant tadpoles of the green frogs of the *Rana esculenta* complex. — Trudy zool. inst. Leningrad **101**: 29–47.

Borkin, L.J., L. Berger & R. Günther (1982): Giant tadpoles of water frogs within *Rana esculenta* complex. — Zool. Polon. **29**: 103–127.

Borkin, L.J., O.S. Bezman-Mosekyo & S.N. Litvinchuk (2012): Evaluation of animal deformity occurence in natural populations (an example of amphibians). — Trudy zool. Inst. RAN **316**: 324–343.

Born, G. (1883): Beiträge zur Bastardierung zwischen den einheimischen Anurenarten. — Pflüger's Arch. **32**: 453–518.

Born, G. (1886): Weitere Beiträge zur Bastardierung zwischen den einheimischen Anurenarten. — Arch. mikr. Anat. **27**: 192–271.

Boschwitz, D. (1963): Histological investigation of inherited incomplete albinism. — Zool. Anz. **170**: 19–23.

Boulenger, G.A. (1891): A synopsis of the tadpoles of the European batrachians. — Proc. R. Soc. London **1891**: 593–627.

Bowerman, J. & P.T.J. Johnson (2003): Timing of trematode-related malformations in Oregon spotted frogs and Pacific treefrogs. — Northwestern Nat. **84**: 142–145.

Bowerman, J., P.T.J. Johnson & T. Bowerman (2010): Sublethal predators and their injured prey: linking aquatic predators and severe limb abnormalities in amphibians. — Ecology **91**: 242–251.

Brachet, J., H. Denis & F. de Vitry (1964): The effects of actinomycin D and puromycin on morphogenesis in amphibian eggs and *Acetabularia mediterranea*. — Develop. Biol. **9**: 398–434.

Bradford, D.F. (1991): Mass mortality and extinction in a high-elevation population of *Rana mucosa*. — J. Herpetol. **25**: 174–177.

Bradley, G.A., P.C. Rosen, M.J. Sredl, T.R. Jones & J.E. Longcore (2002): Chytridiomycosis in native Arizona frogs. — J. Wildl. Dis. **38**: 206–212.

Brame, A.H. (1962): A survey of albinism in salamanders. — Abh. Ber. Naturk. Vorgeschichte Magdeburg **11**: 65–81.

Brandt, W. (1924): Extremitätentransplantationen an *Triton taeniatus*. Ein experimenteller Beitrag zum Determinationsproblem. — Roux Arch. Entwickl.-mech. **103**: 517–554.

Brandt, W. (1933): Weitere Mitteilungen über Abnormitäten bei Amphibien. — Bl. Aquar. Terrarienkde **44**: 395–401.

Brattstrom, B.H. & J.W. Warren (1953): On the validity of *Taricha torosa klauberi* Wolterstorff. — Herpetologica **9**: 180–182.

Braun, M. (1875): Notiz über Zwillingsbildungen bei Wirbelthieren. — Verh. Physik.-med. Ges. Würzburg N.F. **10**: 68–73.

Bregulla, D. (1987): Fund einer anencephalen und teilalbinotischen Feuersalamander-Larve *Salamandra salamandra* (Linnaeus, 1758) (Caudata: Salamandridae). — Salamandra **23**: 65–67.

Bresler, J. (1954): The development of labial teeth of salientian larvae in relation to temperature. — Copeia **1954**: 207–211.

Bretscher, A. (1949): Die Hinterbeinentwicklung von *Xenopus laevis*, Daud. und ihre Beeinflussung durch Colchicin. — Rev. suisse Zool. **56(2)**: 33–96.

Bridges, C.M. (2000): Long-term effects of pesticide exposure at various life stages of the southern leopard frog (*Rana sphenocephala*). — Arch. Environ. Contam. Toxicol. **39**: 91–96.

Bridges, C.M., E. Little, D. Gardiner, J. Petty & J. Huckins (2004): Assessing the toxicity and teratogenicity of pond water in north-central Minnesota to amphibians. — Environ. Sci. Pollut. Res. **11**: 233–239.

Briggs, R.W. (1941): The development of abnormal growths in *Rana pipiens* embryos following delayed fertilization. — Anat. Rec. **81**: 121–135.

Britson, C.A. & S.T. Threlkeld (1998): Abundance, metamorphosis, developmental, and behavioral abnormalities in *Hyla chrysoscelis* tadpoles following exposure to three agrichemicals and methyl mercury in outdoor mesocosms. — Bull. Environ. Contam. Toxicol. **61**: 154–161.

Brodman, R. & J.T. Briggler (2008): *Batrachochytrium dendrobatidis* in *Ambystoma jeffersonianum* larvae in southern Indiana. — Herpetol. Rev. **39**: 320–321.

Browder, L.W. (1968): Pigmentation in *Rana pipiens*. I. Inheritance of the speckle mutation. — J. Hered. **59**: 162–166.

Browder, L.W. (1972): Genetic and embryological studies of albinism in *Rana pipiens*. — J. exp. Zool. **180**: 149–156.

Browder, L.W. (1975): Frogs of the genus *Rana*. Pp. 19–33 in King, R.C. (ed.): Handbook of Genetics. — New York (Plenum Press).

Brown, J.D., M.K. Keel, M.J. Yabsley, T. Thigpen & J.C. Maerz (2006): Clinical challenge. — J. Zoo Wildl. Med. **37**: 571–573.

Bruch, C. (1864): Über Mißbildungen der Chorda dorsalis (Dichordus), nebst Bemerkungen über Doppelbildungen. — Würzburger med. Z. **5**: 1–35.

Brumpt, E. (1934): Spécificité parasitaire et déterminisme de la ponte de la mouche *Lucilia bufonivora*. — C.r. Acad. Sci. Paris **198**: 124–126.

Brunelli, E., I. Bernabò, C. Berg, K. Lundstedt-Enkel, A. Bonaccoi & S. Tripepi (2009): Environmentally relevant concentrations of endosulfan impair development, metamorphosis and behaviour in *Bufo bufo* tadpoles. — Aquat. Toxicol. **91**: 135–142.

Brunst, V.V. (1961): Some problems of regeneration. — Q. Rev. Biol. **36**: 178–206.

Brunst, V.V. & F.H.J. Figge (1951): The development of secondary tails in young axolotl after local irradiation. — J. Morphol. **89**: 111–133.

Bruschelli, G.M. & G. Rosi (1971): Polimelia indotta dalla vitamina A in embrioni di *Bufo vulgaris*. — Riv. Biol. **64**: 271–283.

Bryant, S.V. & D.M. Gardiner (1992): Retinoic acid, local cell-cell interactions, and pattern formation in vertebrate limbs. — Develop. Biol. **152**: 1–25.

Bryant, S.V., V. French & P.J. Bryant (1981): Distal regeneration and symmetry. — Science **212**: 993–1002.

Bryant, S.V., D.M. Gardiner & K. Muneoka (1987): Limb development and regeneration. — Am. Zool. **27**: 675–696.

Burkhart, J.G., J.C. Helgen, D.J. Fort, K. Gallagher, D. Bowers, T.L. Propst, M. Gernes, J. Magner, M.D. Shelby & G. Lucier (1998): Induction of mortality and malformation in *Xenopus laevis* embryos by water sources associated with field frog deformities. — Environ. Health Perspectives **106**: 841–848.

Burkhart, J.G., G. Ankley, H. Carpenter, D. Fort, D. Gardiner, H. Gardner, R. Hale, J.C. Helgen, P. Jepson, D. Johnson, M. Lannoo, D. Lee, J. Lary, R. Levey, J. Magner, C. Meteyer, M.D. Shelby & G. Lucier (2000): Strategies for assessing the implications of frog malformations for environmental and human health. — Environ. Health Perspectives **108**: 83–90.

Burton, E.C., D.L. Miller, E.L. Styer & M.J. Gray (2008): Amphibian ocular malformation associated with frog virus 3. — Vet. J. **177**: 442–444.

Butler, E.G. & O.E. Schotté (1949): Effects of delayed denervation on regenerative activity in limbs of Urodela larvae. — J. exp. Zool. **112**: 361–391.

Cabela, A. (1987): Fund einer teilalbinotischen und cyclopischen Feuersalamanderlarve. — Österr. Ges. Herpetol. Nachr. **10/11**: 19–27.

Caetano, M.H. (1991): Anomalies et régénération des membres chez *Triturus marmoratus* (Latreille, 1800). — Bull. Soc. Herpétol. France **57**: 53–58.

Caldwell, J.P. (1982): Disruptive selection: a tail color polymorphism in *Acris* tadpoles in response to differential predation. — Can. J. Zool. **60**: 2818–2827.

Calfee, R.D., C.M. Bridges & E.E. Little (2006): Sensitivity of two salamander (*Ambystoma*) species to ultraviolet radiation. — J. Herpetol. **40**: 35–42.

Campbell, J.R. (2011): *Ambystoma opacum* (marbled salamander). Albinism. — Herpetol. Rev. **42**: 80–81.

Canella, M.F. (1935): Osservazioni teratologiche in Anfibi anuri. — Boll. Zool. **6**: 195–201.

Canfield, J.T., S.M. Kersten & P. Vanselow (2000): 1997–1999 Field Season Report Minnesota Malformed Frog Investigation. — Minnesota (Minnesota Pollution Control Agency).

Capanna, E. (1967): Osservazioni sul semialbinismo degli Anfibi. — Boll. Zool. **34**: 100–101.

Capanna, E. & V. Foresti (1974): Developmental analysis of a semi-albino mutant of *Triturus cristatus*. — Riv. Biol. **67**: 37–45.

Carini, A. (1940): Sobre um parasito semelhante ao "*Rhinosporidium*," encontrado em quistos da pele de uma "*Hyla*". — Arq. Inst. Biol. **11**: 93–98.

Chang, T.K. & A.M. Boring (1935): Studies in variation among Chinese Amphibia. I. Salamandridae. — Peking nat. Hist. Bull. **9**: 327–360, pl. I–III.

Chapron, C. (1986): La régénération. Pp. 303–319 in Grassé, P.P. & M. Delsol (eds.): Traité de Zoologie. Anatomie, Systématique, Biologie. Tomé 14: Les Batraciens. — Paris (Masson).

Chatzifotis, S., I. vaz Juan, P. Kyriazi, P. Divanach & M. Pavlidis (2011): Dietary carotenoids and skin melanin content influence the coloration of farmed red porgy (*Pagrus pagrus*). — Aquaculture Nutrition **17**: e90–e100.

Chen, H. & J.L. Johnson (1999): Dorsoventral patterning of the vertebrate limb: a process governed by multiple events. — Cell Tissue Res. **296**: 67–73.

Childs, H.E. (1953): Selection by predation on albino and normal spadefoot toads. — Evolution **7**: 228–233.

Christaller, J. (1983): Vorkommen, Phänologie und Ökologie der Amphibien des Enzkreises. — Jh. Ges. Naturkde. Württ. **138**: 153–182.

Christmann, S.P. (1995): *Pleurodema cinerium* [sic!], eine schaumnest-produzierende Kröte aus Bolivien. — elaphe **3(4)**: 2–7.

Classen, A., A. Hirler & R. Oppermann (1996): Auswirkungen unterschiedlicher Mähgeräte auf die Wiesenfauna in Nordost-Polen. — Naturschutz und Landschaftsplanung **28**: 139–144.

Clergue-Gazeau, M. (1971): L'Euprocte pyrénéen. Conséquences de la vie cavernicole sur son développement et sa reproduction. — Ann. Spéléol. **26**: 825–960.

Coghill, G.E. (1936): Effects of chilling on structure and behavior of embryos of *Amblystoma punctatum* Cope. — Proc. Soc. exp. Biol. Med. **35**: 71–74.

Cohn, M.J. & P.E. Bright (1999): Molecular control of vertebrate limb development, evolution and congenital malformations. — Cell Tissue Res. **296**: 3–17.

Coleman, R., P.J. Evennett & J.M. Dodd (1968): Ultrastructural observations on some membranous cytolasmic inclusion bodies in follicular cells of experimentally-induced goitres in tadpoles and toads of *Xenopus laevis* Daudin. — Z. Zellforsch. **84**: 497–505.

Collenot, A. (1965): Recherches comparatives sur l'inversion sexuelle par les hormones stéroides chez les amphibiens. — Mém. Soc. Zool. Fr. **33**: 1–141.

Collins, H.H. (1932): Morphological anomalies in the amphibian, *Triturus viridescens*. — Pennsylvania Acad. Sci. **6**: 165–167.

Conaghan, P. (1987): Freaky froglets. — BBC Wildlife **5**: 573.

Concaro, J.C. (2004): Zucht einer Albinoform von *Salamandra salamandra terrestris* (Lacépède, 1788) über 25 Jahre. — elaphe **12(3)**: 28–34.

Cooke, A.S. (1971): The effects of pp'-DDT on tadpoles of the common frog (*Rana temporaria*). — Environ. Pollut. **1**: 57–71.

Cooke, A.S. (1972): The effects of DDT, Dieldrin and 2,4-D on amphibian spawn and tadpoles. — Environ. Pollut. **3**: 51–68.

Cooke, A.S. (1973): Response of *Rana temporaria* tadpoles to chronic doses of pp'-DDT. — Copeia **1973**: 647–652.

Cooke, A.S. (1981): Tadpoles as indicators of harmful levels of pollution in the field. — Environ. Pollut. Ser. A **25**: 123–133.

Corn, P.S. (1986): Genetic and developmental studies of albino chorus frog. — J. Hered. **77**: 164–168.

Crawford, K. & D.M. Vincenti (1998): Retinoic acid and thyroid hormone may function through similar and competitive pathways in regenerating axolotls. — J. exp. Zool. **282**: 724–738.

Crézé, J. (1950): Action de la colchicine sur les têtards de *Xenopus laevis*. — C.r. Soc. Biol. **144**: 1317–1318.

Cummer, M.R., D.E. Green & E.M. O'Neill (2005): Aquatic chytrid pathogen detected in terrestrial plethodontid salamander. — Herpetol. Rev. **36**: 248–249.

Cummins, C.P. (1987): Factors influencing the occurrence of limb deformities in common frog tadpoles raised at low pH. — Ann. Soc. R. Zool. Belg. (Suppl. 1) **117(1)**: 353–364.

Cummins, C.P. (1989): Interaction between the effects of pH and density on growth and development in *Rana temporaria* L. tadpoles. — Func. Ecol. **3**: 45–52.

Cunningham, A.A., T.E.S. Langton, P.M. Bennett, S.E.N. Drury, R.E. Gough & J.K. Kirkwood (1993): Unusual mortality associated with poxvirus-like particles in frogs (*Rana temporaria*). — Vet. Rec. **133**: 141–142.

Cunningham, A.A., T.E.S. Langton, P.M. Bennett, J.F. Lewin, S.E.N. Drury, R.E. Gough & S.K. MacGregor (1996): Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). — Phil. Trans. R. Soc. Lond. B **351**: 1539–1557.

D'Amen, M., D. Salvi, L Vignoli, P. Bombi & M.A. Bologna (2006): Malformation occurrence in two newt species (*Triturus carnifex* and *Lissotriton vulgaris*) from three localities in central Italy: description and possible causes. — Atti Congr. Naz. Soc. Herpetol. Ital., Roma **6**: 33–40.

D'Amen, M., D. Salvi, A. Antoccia, P. Bombi, V. Bordoni, S. Chiesa, G. Gibertini, M. Marino, A. Ruzza, M. Scalici, C. Tanzarella, L. Vignoli & M.A. Bologna (2008): Studio degli effetti delle emissioni naturali del gas radon su *Triturus carnifex* (Amphibia, Salamandridae): un approccio multidisciplinare. Pp. 180–183 in Corti, C. (ed.): Herpetologia Sardiniae. — Latina (Belvedere).

Daito, Y. (1968): On albinos in the tree frog, *Hyla arborea japonica* Guenther. — Zool. Mag. (Dobutsugaku Zasshi) **77**: 92–96.

Dandová, R. & V. Zavadil (1993): Atypické zbarveni brisni strany colka horského *Triturus alpestris* (Laur., 1768) z Jeseniku. — Terrarista **4(2/3)**: 43–44.

Dandová, R., P. Kotlik & V. Zavadil (1995): Ein Fall von Albinismus beim Kleinen Wasserfrosch. — Salamandra **31**: 57–60.

Danilova, M.N. (1992): Eksperimentalnoe Issledovanie Vliyaniya Benzina na Rannie Stadii Ontogeneza Amfibii. — Ekaterinburg (PhD Diss. Abstr., Inst. Plant Animal Ecol., USSR Acad. Sci.).

Das, P. & P. Mohanty-Hejmadi (2000): Vitamin A mediated limb deformities in the commom Indian toad, *Bufo melanostictus* (Schneider). — Ind. J. exp. Biol. **38**: 258–264.

Dasgupta, S. & M.S. Grewal (1968): The selective advantage of temperature tolerance among the progeny of frogs with vertebral fusions. — Evolution **22**: 87–92.

Davidson, E.H. (1976): Chapter 2: The onset of genome control in embryogenesis. Pp. 27–55 in Davidson, E.H. (ed.): Gene Activity in Early Development (2nd ed.)— New York (Acad. Press).

Dawson, D.A., E.F. Stebler, S.L. Burks & J.A. Bantle (1988): Evaluation of the developmental toxicity of metal-contaminated sediments using short-term fathead minnow and frog embryo-larval assays. — Environ. Toxicol. Chem. **7**: 27–34.

De Silva, A. (2009): The incidence and pattern of malformations, abnormalities, injuries, and parasitic infection of amphibians in Sri Lanka. — Gampola (Unpublished Final Report Amphibian Specialists Group, Seed Grant).

De Superville, D. (1740): Some reflections on generation, and on monsters, with a description of some particular monsters. — Philos. Trans. R. Soc. London **41**: 294–307.

Dearlove, G.E. & M.H. Dresden (1976): Regenerative abnormalities in *Notophthalmus viridescens* induced by repeated amputations. — J. exp. Zool. **196**: 251–262.

Degitz, S.J., P.A. Kosian, E.A. Makynen, K.M. Jensen & G.T. Ankley (2000): Stage- and species-specific developmental toxicity of all-trans retinoic acid in four native North American ranids and *Xenopus laevis*. — Toxicol. Sci. **57**: 264–274.

Degitz, S.J., G.W. Holcombe, P.A. Kosian, J.E. Tietge, E.J. Durhan & G.T. Ankley (2003): Comparing the effects of stage and duration of retinoic acid exposure on amphibian limb development: chronic exposure results in mortality, not limb malformations. — Toxicol. Sci. **74**: 139–146.

Deichsel, G. (2013): Forensische Herpetologie. Ein Teichkrimi. — elaphe/Terraria **1**: 92–94.

Delarue, M. (1974): Étude de l'hybridation par la greffe nucléaire et par croisement chez les bufonidés (Amphibiens, Anoures). — Ann. Embryol. Morphol. **7**: 425–443.

Delarue, M. (1982): Embryogenèse et relations nucléocytoplasmiques chez les hybrides expérimentaux entre *Bufo bufo* L., *Bufo viridis* Laur. et *Bufo calamita* Laur. — Amphibia-Reptilia **3**: 161–178.

Delcourt, A. (1963): Observations sur la Rainette, *Hyla arborea meridionalis* Boettger, dans les Bouchse-du-Rhône. — Bull. Soc. Linn. Provence **23**: 18–26.

Densmore, C.L. & D.E. Green (2007): Diseases of amphibians. — ILAR J. **48**: 235–254.

Desai, R.N. & K. Pancharatna (2003): *Rana curtipes* (NCN). Coloration. — Herpetol. Rev. **34**: 53–54.

Detwiler, S.R. (1938): An experimental study of limb development in relation to temperature. — J. exp. Zool. **79**: 355–360.

DeYoung, D.J., J.A. Bantle & D.J. Fort (1991): Assessment of the developmental toxicity of ascorbic acid, sodium selenate, coumarin, serotonin, and 13-cis retinoic acid using FETAX. — Drug Chem. Toxicol. **14(1–2)**: 127–141.

Dhaliwal, S.S. & D.A. Griffiths (1963): Fungal disease in Malayan toads: an acute lethal inflammatory reaction. — Nature **197**: 467–469.

Diego-Rasilla, F.J., R.M. Luengo & L. Rodríguez-García (2007): *Triturus marmoratus* (marbled newt). Limb abnormalities. — Herpetol. Rev. **38**: 68.

Docherty, D.E., C.U. Meteyer, J. Wang, J. Mao, S.T. Case & V.G. Chinchar (2003): Diagnostic and molecular evaluation of three iridovirus-associated salamander mortality events. — J. Wildl. Dis. **39**: 556–566.

Dodd, M.H. & J.M. Dodd (1976): The biology of metamorphosis. Pp. 467–599 in Loft, B. (ed.): Physiology of the Amphibia. — New York (Acad. Press).

Domrow, R., R.B. Loomis & L.N. Lester (1983): The genus *Vercammenia* Audy and Nadchatram in Australian frogs (Acari: Trombiculidae). — J. Aust. ent. Soc. **22**: 205–209.

Donaire, D. (2016): Sobre un caso de salamandra magrebí siamesa. — Butll. Soc. Catalana Herpetol. **23**: 79–81.

Dournon, C. (1983): Développement somatique et inversion sexuelle sous l'action de la température chez les amphibiens. — Proc. Int. Colloq. Pathol. Reptiles Amphibians, Angers, France **1**: 227–231.

Dragoiu, J. & T. Busnitza (1927): Sur un tétard de *Bombinator igneus* bicéphale. — C.r. Soc. Biol. Fr. **97**: 1015–1017.

Drake, D.L., R.Altig, J.B. Grace & S.C. Walls (2007): Occurrence of oral deformities in larval anurans. — Copeia **2007**: 449–458.

Droin, A. (1985): Wrinkled oedema' (w. oe), une nouvelle mutation recessive chez *Xenopus borealis*. — Rev. suisse Zool. **92(4)**: 863–869.

Droin, A. (1988): Three new analogous mutations in *Xenopus laevis*. — Acta Embryol. Morphol. Exp. **9(2)**: 115–127.

Droin, A. (1992): Genetic and experimental studies on a new pigment mutant in *Xenopus laevis*. — J. exp. Zool. **264**: 196–205.

Droin, A. & M.L. Beauchemin (1975): Immobile' (im), a recessive lethal mutation of *Xenopus laevis* tadpoles. — J. Embryol. exp. Morph. **34**: 435–449.

Droin, A. & M.C. Chavane (1976): A recessive semi-lethal mutation, "distended lungs" (dl) affecting the tadpoles of *Xenopus laevis*. — Acta Embryol. Exp. **3**: 273–289.

Droin, A. & B. Colombelli (1982): "Bloated-1" and "bloated-2", two recessive mutations in *Xenopus borealis* and *Xenopus muelleri*. — Acta Embryol. Morphol. Exp. **3(2)**: 117–126.

Droin, A. & M. Fischberg (1980): Abnormal limbs (abl), a recessive mutation affecting the tadpole of *Xenopus l. laevis*. — Experientia **36**: 1286–1288.

Droin, A. & M. Fischberg (1984): Two recessive mutations with maternal effect upon color and cleavage of *Xenopus laevis laevis* eggs. — Wilhelm-Roux's Arch. Dev. Biol. **193(2)**: 86–89.

Dubois, A. (1968): Sur deux anomalies de la grenouille verte (*Rana esculenta*). — Bull. Soc. Linn. Lyon **37**: 316–320.

Dubois, A. (1974): Polydactylie massive associée à la clinodactylie, dans une population de *Rana graeca*. Remarques sur la polydactylie faible et la clinodactylie chez *Bufo bufo* (Amphibiens, Anoures). — Bull. Soc. Zool. Fr. **99**: 505–521.

Dubois, A. (1977): Une mutation dominante déterminant l'apparition de diverses anomalies digitales chez *Rana temporaria* (Amphibiens, Anoures). — Bull. Soc. Zool. Fr. **102**: 197–213.

Dubois, A. (1979a): Néoténie et pédogénèse. A propos d'une anomalie du développement chez *Bombina variegata* (Amphibiens, Anoures). — Bull. Mus. natn. Hist. nat. Paris, 4. sér., sect. A **1**: 537–546.

Dubois, A. (1979b): Anomalies and mutations in natural populations of the *Rana* "*esculenta*" complex (Amphibia, Anura). — Mitt. Zool. Mus. Berlin **55**: 59–87.

Dubois, A. (1984): L'anomalie P des grenouilles vertes (complexe de *Rana* kl. *esculenta* Linné, 1758) et les anomalies voisines chez les amphibiens. — C.r. Coll. int. Pathol. Reptiles Amphibiens **1**: 215–221.

Dubois, A. (2012): Jean Rostand. Un biologiste contre le nucléaire. — Paris (Berg International).

Dubois, A. (2014): The anomaly P in Palaearctic green frogs of the genus *Pelophylax* (Ranidae). Pp. 96–104 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Dubois, A. (2017): Rostand's anomaly P in Palaearctic green frogs (*Pelophylax*) and similar anomalies in amphibians. — Mertensiella **25**: 49–56.

Dubois, A. & K. Henle (2017): Methodological recommendations for studying the causes of anomalies in natural populations of amphibians. — Mertensiella **25**: 243–260.

Dubois, A., J.-L. Fischer & D. Payen (1973): Un triton palmé (*Triturus helveticus*) aux yeux noirs. — C.r. Soc. Biol. **167**: 1733–1735.

Duellman, W.E. (1999): Patterns of Distribution of Amphibians. A Global Perspective. — Baltimore (John Hopkins Univ. Press).

Duellman, W.E. & L. Trueb (1986): Biology of Amphibia. — New York (McGraw-Hill).

Duméril, A. (1865): Observations sur la monstruosité dite polymélie ou augmentation du nombre des membres chez des batraciens anoures. — Mém. Nouv. Arch. Mus. Hist. Nat. **1**: 309–319.

Duméril, A. (1867): Description de diverses monstruosités observées à la Ménagerie des Reptiles du Muséum d'Histoire Naturelle sur les batraciens urodèle à branchies extérieures dits axolotls. — Nouv. Arch. Mus. Hist. Nat. **3**: 119–130.

Dumont, J.N. & T.W. Schultz (1980): Effects of coal-gasification sour water on *Xenopus laevis* embryos. — J. Environ. Sci. Health **15A**: 127–138.

Dumont, J.N., T.W. Schultz & R.G. Epler (1983): The response of the FETAX model to mammalian teratogens. — Teratology **27**: 39A–40A.

Dumpert, K. & E. Zietz (1984): Platanna (*Xenopus laevis*) as a test organism for determining the embryotoxic effects of environmental chemicals. — Ecotoxicol. Environ. Safety **8**: 55–74.

Duszynski, D.W. & K.L. Jones (1973): The occurrence of intradermal mites, *Hannemannia* spp. (Acarina: Trombiculidae), in

anurans in New Mexico with a histological description of the tissue capsule. — Int. J. Parasitol. **3**: 531–538.

Dyrkacz, S. (1981): Recent instances of albinism in North American amphibians and reptiles. — SSAR Herpetol. Circ. **11**: 1–32.

Eaton, B.R., S. Eaves, C. Stevens, A. Puchniak & C.A. Paszkowski (2004): Deformity levels in wild populations of the wood frog (*Rana sylvatica*) in three ecoregions of Western Canada. — J. Herpetol. **38**: 283–287.

Eberle, G. (1937): Befall und Tötung von Erdkröten (*Bufo vulgaris*) durch Fliegenbrut. — Natur und Volk **67**: 250–254.

Eguchi, G., Y. Eguchi, K. Nakamura, M.C. Yadav, J.L. Millian & P.A. Tsonis (2011): Regenerative capacity in newts is not altered by repeated regeneration and aging. — Nature Communications **2**: 384.

Eikamp, H. (1980): Neotenie-Merkmale beim Wasserfrosch *Rana esculenta*. — Aquarium **138**: 658.

Ellegren, H., G. Lindgren, C.R. Primmer & A.P. Møller (1997): Fitness loss and germline mutations in barn swallows breeding in Chernobyl. — Nature **389**: 593–596.

Ellenberg, H. (1991): Ökologische Veränderungen in Biozönosen durch Stickstoffeintrag. Pp. 75–90 in Henle, K. & G. Kaule (eds.): Arten- und Biotopschutzforschung für Deutschland. — Jülich (Forschungszentrum).

Ellinger, M.S. (1980): Genetic and experimental studies on a pigment mutation, Pale (Pa), in the frog, *Bombina orientalis*. — J. Embryol. exp. Morph. **56**: 125–137.

Elsdale, T.R., M. Fischberg & S. Smith (1958): A mutation that reduces nucleolar number in *Xenopus laevis*. — Exp. Cell Res. **14**: 642–643.

Ensinck, F.H. (1978): Zur Zucht des Beutelfrosches *Gastrotheca marsupiata*. — Das Aquarium **111**: 401–405.

Ercolani, G.B. (1881): Della polidactylia, della melomelia e della polimelia nei rettili e di alcuni forme di monstruosità con queste corrispondenti, osservate negli organi della locomozione nei pesci. — Mem. Accad. Sci. Ist. Bologna **(4)3**: 810–824.

Ernst, C.H. (1995): Freshwater and terrestrial turtles of the United States: status and prognosis. — Bull. Chicago Herpetol. Soc. **30**: 225–233.

Escoriza, E. & L. Garcia-Cardenete (2005): Polimelia en *Alytes dickhilleni* y *Salamandra salamandra longirostris*. Dos casos de ejemplares con seis extremidades. — Bol. Asoc. Herpetol. Esp. **16(1–2)**: 39–41.

Fagotti, A., L. Morosi, I. Di Rosa, R. Clarioni, F. Simoncelli, R. Pascolini, R. Pellegrino, G.-D. Guex & H. Hotz (2005): Bioaccumulation of organochlorine pesticides in frogs of the *Rana esculenta* complex in central Italy. — Amphibia-Reptilia **26**: 93–104.

Fankhauser, G. (1945): The effects of changes in chromosome number on amphibian development. — Q. Rev. Biol. **20**: 20–78.

Fayzulin, A.I. (2011): About the morphological anomalies of anurans (Anura, Amphibia) of the Volga basin. — Prazi Ukrain. Gerpetol. Tovaristva **3**: 201–207.

Fejérváry, G.J. von (1916): Beiträge zur Kenntnis von *Rana mehelyi* Bolkay. — Mitt. Jb. Kgl.-ungar. Geol. Reichsanstalt **23**: 133–155.

Feldmann, R. & R. Klewen (1981): Feuersalamander – *Salamandra salamandra terrestris* (Lacépède 1788). — Abh. Landesmus. Naturkde Münster Westfalen **43**: 30–44.

Felger, J., J. Enssle, D. Mendez & R. Speare (2007): Chytridiomycosis in El Salvador. — Salamandra **43**: 122–127.

Fellers, G.M., D.E. Green & J.E. Longcore (2001): Oral chytridiomycosis in the mountain yellow-legged frog (*Rana muscosa*). — Copeia **2001**: 945–953.

Fernandez, M. & J.C. Beetschen (1975): Recherches sur le rôle de la température dans la réalisation du phénotype chez des embryons de l'Amphibien *Pleurodeles waltlii* homozygote pour la mutation thermosensible ac (ascite caudale). — J. Embryol. exp. Morph. **34**: 221–252.

Fernández-Álvarez, F.Á., E. Recuero, Í. Martínez-Solano & D. Buckley (2011): First record of bicephaly in *Lissotriton boscai* (Amphibia, Caudata, Salamandridae). — North-Western J. Zool. **7**: 161–163.

Ferraro, T.J. & S. Burgin (1993): Review of environmental factors influencing the decline of Australian frogs. Pp. 205–218 in Lunney, D. & D. Ayers (eds.): Herpetology in Australia: A Diverse Discipline. — Chipping Norton, Australia (Surrey Beatty).

Feshchenko, S.P., H.C. Schroder, W.E.G. Muller & G.I. Lazjuk (2002): Congenital malformations among newborns and developmental abnormalities among human embryos in Belarus after Chernobyl accident. — Cell. Mol. Biol. **48**: 423–426.

Figiel, C.R. & R.D. Semlitsch (1991): Effects of nonlethal injury and habitat complexity on predation in tadpole populations. — Can. J. Zool. **69**: 830–834.

Fischberg, M. (1948): Experimentelle Auslösung von Heteroploidie durch Kältebehandlung der Eier von *Triturus alpestris* aus verschiedenen Populationen. — Genetica **24**: 213–329.

Flax, N.L. (1986): Influence of acidity and water temperature on the survival of Sakhalin anurans. — Proc. Zool. Inst. USSR Akad. Sci. **157**: 152–166.

Flax, N.L. & L.J. Borkin (1997): High incidence of abnormalities in anurans in contaminated industrial areas (eastern Ukraine). Pp. 119–123 in Böhme, W., W. Bischoff & T. Ziegler (eds.): Herpetologia Bonnensis. — Bonn (Societas Europaea Herpetologica).

Flax, N.L. & L.J. Borkin (2004): Morphological abnormalities and heavy metal concentrations in anurans of contaminated areas, eastern Ukraine. — Appl. Herpetol. **1**: 229–264.

Flindt, R. (1985a): Untersuchungen zum Auftreten von mißgebildeten Wechselkröten (*Bufo viridis*) in einem Steinbruch in Vaihingen-Roßwag. — Jh. Ges. Naturkde. Württ. **140**: 213–233.

Flindt, R. (1985b): Latenter Albinismus und Mißbildungen bei Kaulquappen von Wechselkröten *Bufo viridis* Laurenti, 1768. — Salamandra **21**: 298–303.

Flindt, R. & H. Hemmer (1967): Nachweis natürlicher Bastardierung von *Bufo calamita* und *Bufo viridis*. — Zool. Anz. **178(5–6)**: 419–429.

Flindt, R., H. Hemmer & R. Schipp (1968): Zur Morphogenese von Mißbildungen bei Bastardlarven *Bufo calamita* ♀ × *Bufo viridis* ♂: Störungen in der Ausbildung des Axialskeletts. — Zool. Jb. Anat. **85**: 51–71.

Fodor, A. & M. Puky (2002): Herpetological methods: II. Protocol for monitoring amphibian deformities under temperate zone conditions. — Opusc. Zool. Budapest **34**: 35–42.

Fort, D.J. & D.W. McLaughlin (2003): Establishing cause-effect relationships for chemical stressors in amphibians: providing adequate data for the ERA. Pp. 219–232 in Linder, G.L., S. Krest, D. Sparling & E.E. Little (eds.): Multiple Stressor Effects in Relation to Declining Amphibian Populations. — West Conshohocken (Am. Soc. Testing Material).

Fort, D.J. & E.L. Stover (1996): Effect of low-level copper and pentachlorophenol exposure on various early life stages of *Xenopus laevis*. Pp. 188–203 in Bengtson, D.A. & D.S. Henshel (eds.): Environmental Toxicology and Risk Assessment: Biomakers and Risk Assessment. Vol. 5. — West Conshohocken (Am. Soc. Testing Material).

Fort, D.J. & E.L. Stover (1997): Development of short-term, whole-embryo assays to evaluate detrimental effects on am-

phibian limb development and metamorphosis using *Xenopus laevis*. Pp. 376–390 in Dwyer, F.J., T.R. Doane & M.L. Hinman (eds.): Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment (Vol. 6). — West Conshohocken, PA (Am. Soc. Testing Material).

Fort, D.J., T.L. Propst, E.L. Stover, J.C. Helgen, R.B. Levey, K. Gallagher & J.G. Burkhart (1999a): Effects of pond water, sediment, and sediment extracts from Minnesota and Vermont, USA, on early development and metamorphosis of *Xenopus*. — Environ. Toxicol. Chem. **18**: 2305–2315.

Fort, D.J., R.L. Rogers, H.F. Copley, L.A. Bruning, E.L. Stover, J.C. Helgen & J.G. Burkhart (1999b): Progress toward identifying causes of maldevelopment induced in *Xenopus* by pond water and sediment extracts from Minnesota, USA. — Environ. Toxicol. Chem. **18**: 2316–2324.

Fort, D.J., R.L. Rogers & J.P. Bacon (2006a): Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part II. Progress towards characterization of chemical stressors. — Appl. Herpetol. **3**: 143–172.

Fort, D.J., R.L. Rogers, B.O. Buzzard, G.D. Anderson & J.P. Bacon (2006b): Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part III. Microcosm-based exposure pathway assessment. — Appl. Herpetol. **3**: 257–277.

Freytag, G.E. (1947): Bemerkungen über abnorme Farbkleidveränderungen bei Tritonen. — Mitt. Mus. Naturkde. Vorgeschichte Magdeburg **1947**: 13–19.

Freytag, G.E. (1952): Über das Farbkleid der Salamandriden in seiner Abhängigkeit von äußeren und inneren Faktoren. — Veröff. Zool. Staatssammlung München **2**: 80–123.

Freytag, G.E. (1955): Feuersalamander und Alpensalamander. — Wittenberg (Neue Brehm Bücherei (142)).

Freytag, G.E. (1956): Weitere Naturfunde albinotischer Amphibien. — Zool. Garten **21**: 383–385.

Freytag, G.E. & E. Susebach (1942): Beitrag zur Kenntnis des Farbkleides des Feuersalamanders (*Salamandra salamandra* L.). — Zool. Anz. **138(5–6)**: 127–138.

Freytag, G.E. & E. Susebach (1949): Zweiter Beitrag zur Kenntnis des Farbkleides des Feuersalamanders (*Salamandra salamandra* L.). Die Weiterzucht nigrinotischer Feuersalamander. — Mitt. Mus. Magdeburg **1(2)**: 95–120.

Frost, D. (2017): Amphibian Species of the World: an Online Reference. Version 6.0. — New York (Am. Mus. Natl. Hist.).

Frost, S.K., F. Briggs & G.M. Malacinski (1984): A color atlas of pigment genes in the Mexican axolotl (*Ambystoma mexicanum*). — Differentiation **26**: 182–188.

Frost, S.K., L.G. Epp & S.J. Robinson (1986a): The pigmentary system of developing axolotls. III. An analysis of the albino phenotype. — J. Embryol. exp. Morph. **92**: 255–268.

Frost, S.K., L.G. Epp & S.J. Robinson (1986b): The pigmentary system of developing axolotls. IV. An analysis of the axanthic phenotype. — J. Embryol. exp. Morph. **95**: 117–130.

Frost-Mason, S., R. Morrison & K. Mason (1994): Pigmentation. Pp. 64–97 in Heatwole, H. & G.T. Barthalmus (eds.): Amphibian Biology Vol. 1: The Integument. — Chipping Norton, Australia (Surrey Beatty).

Frye, F.L. (1985): An unusual epizootic of anuran aeromoniasis. — JAVMA **187**: 1223–1224.

Fukui, A., M. Asashima & V.B. Meyer-Rochow (1996): 1995 update of morphological abnormalities in wild populations of the Japanese newt *Cynops pyrrhogaster* Boie (Urodela, Salamandridae). — Acta Hydrobiol. **38**: 19–24.

Funk (1827): De Salamandrae terrestris vita, evolutione, e formatione. — Berlin (Diss. Univ. Berlin).

Gabor, M., F. Puscariu & C. Deac (1973): Action tératogène des aflatoxine sur les tétards. — Arch. roum. Path. exp. Microbiol. **32**: 269–275.

Gallien, L. (1969a): Anomalies chromosomiques provoquées et mutations chez les amphibiens. — Ann. Biol. **8**: 319–331.

Gallien, L. (1969b): Spontaneous and experimental mutations in the newt *Pleurodeles waltlii* Michah. Pp. 35–42 in Mizell, M. (ed.): Biology of Amphibian Tumors. — New York (Springer).

Gallien, L. & A. Collenot (1964): On a lethal recessive mutant, the syndrome of which is associated with mitotic disturbances, in the newt, *Pleurodeles waltlii*. — C.r. Hebd. Seances Acad. Sci. Paris **259**: 4847–4849.

Galloy, V. & M. Denoël (2010): Detrimental effect of temperature increase on the fitness of an amphibian (*Lissotriton helveticus*). — Acta Oecol. **36**: 179–183.

Galton, V.A. (1992): Thyroid hormone receptors and iodothyronine deiodinases in the developing Mexican axolotl, *Ambystoma mexicanum*. — Gen. Comp. Endocrinol. **85**: 62–70.

Garanin, V.I. & S.L. Shaldybin (1976): On parasitism of the larvae of *Lucilia bufonivora* Moniez, 1876 on Anura. — Parazitologiya **10**: 286–288.

Garber, E.A.E., J.L. Erb, J.G. Downward, E.M. Priuska, J.L. Wittliff, W. Feng, J. Magner & G.L. Larsen (2001): Biosensor, ELISA, and frog embryo teratogenesis assay: *Xenopus* (FETAX) analysis of water associated with frog malformations in Minnesota. — Proc. SPIE **4206**: 147–158.

Garber, E.A.E., J.E. Erb, J. Magner & G. Larsen (2004): Low levels of sodium and potassium in the water from wetlands in Minnesota that contained malformed frogs affect the rate of *Xenopus* development. — Environ. Monitor. Assess. **90**: 45–64.

García-Muñoz, E., F. Jorge, C. Rato & M.A. Carretero (2010): Four types of malformations in a population of *Bufo boulengeri* (Amphibia, Anura, Bufonidae) from the Jbilet Mountains (Marrakech, Morocco). — Herpetol. Notes **3**: 267–270.

García-París, M., A. Montori & P. Herrero (2004): Fauna Iberica. Vol. 24 Amphibia. Lissamphibia. — Madrid (Mus. Nac. Cienc. Nat. Consejo Sup. Investigaciones Científicas).

Gardiner, D.M. & S.V. Bryant (1996): Molecular mechanisms in the control of limb regeneration: the role of homeobox genes. — Int. J. Dev. Biol. **40**: 797–805.

Gardiner, D.M. & D.M. Hoppe (1999): Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). — J. exp. Zool. **284**: 207–216.

Gardiner, D.M., A. Ndayibagira, F. Grün & B. Blumberg (2003): Deformed frogs and environmental retinoids. — Pure Appl. Chem. **75**: 2263–2273.

Garin, I., O. San Sebastían, A. Bandrés & X. Rubio (2009): Malformaciones en ejemplares de sapo corredor (*Epidalea calamita*) de la costa vasca. — Munibe **57**: 163–167.

Gebhardt, D.O.E. & J. Faber (1966a): The influence of aminopterin and other teratogenic substances on the regenerating fore limb of *Ambystoma mexicanum*. — Acta Physiol. Pharmacol. Neerlandica **13**: 480.

Gebhardt, D.O.E. & J. Faber (1966b): The influence of aminopterin on limb regeneration in *Ambystoma mexicanum*. — J. Embryol. exp. Morph. **16**: 143–158.

Gelnarova, J. (1987a): Larvy obojzivelnici (Amphibian larvae). Pp. 196–205 in Svobodova, Z., J. Gelnarová, J. Justýn, V. Krupauer, J. Máchová, L. Simanov, V. Valentová, B. Vykusová & E. Wohlgemuth (eds.): Toxikologie Vodnich Zivocichu (Toxicology of Water Animals). — Prague (Ministry Agriculture).

Gelnarova, J. (1987b): Effects of pesticides on the development of frogs. Pp. 18–21 in Ruzicka, V. (ed.): Research Activity Re-

port 1984–1986. — Prague (Dept Biodiagnostics, Inst. Landscape Ecol., Czech. Acad. Sci.).

Geng, Y., K.Y. Wang, Z.Y. Zhou, C.W. Li, J. Wang, M. He, Z.Q. Yin & W.M. Lai (2011): First report of a *Ranavirus* associated with morbidity and mortality in farmed Chinese giant salamanders (*Andrias davidianus*). — J. Comp. Pathol. **145**: 95–102.

Genthner, H. & J. Hölzinger (2007): Gelbbauchunke *Bombina variegata* (Linnaeus, 1758). Pp. 271–292 in Laufer, H., K. Fritz & P. Sowig (eds.): Die Amphibien und Reptilien Baden-Württembergs. — Stuttgart (Ulmer).

Geoffroy Saint-Hilaire, I. (1832): Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux. Tome 1. — Paris (Cosson).

Geoffroy Saint-Hilaire, I. (1836): Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux. Tome 3. — Paris (Cosson).

Gesner, C. (1554): Historiae animalium liber II. de quadrupedipus ovipari. — Zürich (C. Froshover).

Ghate, H.V. (1986): Teratogenic effects of ethylenethiourea in frog embryo. — Geobios **13**: 82–85.

Ghate, H.V. & L. Mulherkar (1980): Effects of sodium diethylthiocabamate on developing embryos of the frog *Microhyla ornata*. — Ind. J. exp. Biol. **18**: 1040–1042.

Gibbs, E.L., G.W. Nace & M.B. Emmons (1971): The live frog is almost dead. — BioScience **21**: 1027–1034.

Giesenberg, A. (1991): Untersuchungen zur Entwicklung der Larven von *Salamandra salamandra terrestris* Lacépède, 1788 aus dem Landkreis Harburg (Niedersachsen). — Salamandra **27**: 97–107.

Gilboa, I. & H.G. Dowling (1974): A bibliography of albinism in amphibians and reptiles. — HISS Publ. Herpetol. **6**: 1–11.

Gillette, R. & D. Bodenstein (1946): Specific development inhibitions produced in amphibian embryos by a nitrogen mustard compound. — J. exp. Zool. **103**: 1–32.

Gillilland, M.G. & P.M. Muzzall (2002): Amphibians, trematodes, and deformities: an overview from Southern Michigan. — Comp. Parasitol. **69(1)**: 81–85.

Giltay, L. (1932): Note sur quelques larves néoténiques de *Molge palmata* Schneid., trouvées en campine anversoise. — Bull. Mus. Roy. Hist. Nat. Belg. **8(24)**: 1–9.

Girvan, J.E., W.M. Olson & B.K. Hall (2002): Hind-limb regeneration in the dwarf African clawed frog, *Hymenochirus boettgeri* (Anura: Pipidae). — J. Herpetol. **36**: 537–543.

Gittins, S.P. (1983): Population dynamics of the common toad (*Bufo bufo*) at a lake in Mid-Wales. — J. Anim. Ecol. **52**: 981–988.

Glaw, F. (1987): Probleme mit Streichholzbeinen bei der Nachzucht von *Discoglossus pictus*. — herpetofauna, Weinstadt, **9(51)**: 30–33.

Glaw, F. & M. Vences (1997): Anuran eye colouration: definitions, variation, taxonomic implications and possible functions. Pp. 125–138 in Böhme, W., W. Bischoff & T. Ziegler (eds.): Herpetologia Bonnensis. — Bonn (Societas Europaea Herpetologica).

Glaw, F., J. Morinière, K. Glaw & D. Doczkal (2014): Myiasis bei der Erdkröte (*Bufo bufo*) verursacht durch die Schmeißfliege *Lucilia ampullacea*. — Z. Feldherpetol. **21**: 83–95.

Goethe, F. (1972): Über Lurche und Kriechtiere im Teutoburger Wald und im Lipperland. — Lippische Mitt. Geschichte Landeskde **41**: 311–330.

Goleman, W.L., L.J. Urquidi, T.A. Anderson, E.E. Smith, R.J. Kendall & J.A. Carr (2002): Environmentally relevant concentrations of ammonium perchlorate inhibit development and metamorphosis in *Xenopus laevis*. — Environ. Toxicol. Chem. **21**: 424–430.

Gollmann, B. & G. Gollmann (2012): Die Gelbbauchunke (2. Aufl.). — Z. Feldherpetol. Beih. **4**: 1–135.

Gollmann, G. (1991): Osteological variation in *Geocrinia laevis*, *Geocrinia victoriana*, and their hybrid populations (Amphibia, Anura, Myobatrachinae). — Z. zool. Syst. Evolutionsforsch. **29**: 289–303.

Gollmann, G., W. Hödl & A. Ohler (1984): A tadpole from a *Bombina* hybrid population – a hopeless monster. — Amphibia-Reptilia **5**: 411–413.

González-Fernández, J.E. & M. Valladolid (2004): Un caso de polimelia natural en *Pelobates cultripes*, Cuvier, 1829. — Bol. Asoc. Herpetol. Esp. **15(1):** 26–29.

Goodchild, C.G. (1953): A subcutaneous cyst-parasite of bullfrogs: *Histocystidium ranae*, N.G., N. Sp. — J. Parasitol. **39**: 395–405.

Goodman, B.A. & P.T.J. Johnson (2011a): Disease and the extended phenotype: parasites control host performance and survival through induced changes in body plan. — PLOS One **6(5)**: e20193. doi:10.1371/journal.pone.0020193.

Goodman, B.A. & P.T.J. Johnson (2011b): Ecomorphology and disease: cryptic effects of parasitism on host habitat use, thermoregulation, and predator avoidance. — Ecology **92**: 542–548.

Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. — Herpetologica **16**: 183–190.

Goverse, E. (2009): *Hyla arborea* (tree frog). Blowfly parasitism. — Herpetol. Rev. **40**: 71.

Granoff, A., M. Gravell & R.W. Darlington (1969): Studies on the viral etiology of the renal adenocarcinoma of *Rana pipiens* (Lucké Tumor). Pp. 279–295 in Mizell, M. (eds): Biology of Amphibian Tumors. — Berlin (Springer).

Grant, K.P. & L.E. Licht (1995): Effects of ultraviolet radiation on life-history stages of anurans from Ontario, Canada. — Can. J. Zool. **73**: 2292–2301.

Gray, H.M., M. Ouellet, D.M. Green & A.S. Rand (2002): Traumatic injuries in two Neotropical frogs, *Dendrobates auratus* and *Physalaemus pustulosus*. — J. Herpetol. **36**: 117–121.

Gray, R.H. (2002): Amphibian malformations and population declines may not be linked. — Proc. Int. Symp. Pathol. Reptiles Amphibians **6**: 49–53.

Green, D.E. (2001): Pathology of Amphibia. Pp. 401–485 in Wright, K.M. & B.R. Whitaker (eds.): Amphibian Medicine and Captive Husbandry. — Malabar (Krieger).

Green, D.E. & C. Kagarise Sherman (2001): Diagnostic histological findings in Yosemite toads (*Bufo canorus*) from a die-off in the 1970s. — J. Herpetol. **35**: 92–103.

Green, D.E., K.A. Converse & A.K. Schrader (2002): Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. — Ann. N.Y. Acad. Sci. **969**: 323–339.

Greenhouse, G. (1976): Evaluation of the teratogenic effects of hydrazine, methylhydrazine and dimethylhydrazine on embryos of *Xenopus laevis*, the South African clawed toad. — Teratology **13**: 167–178.

Greenhouse, G. (1977): Toxicity of N-phenyl-a-naphthylamine and hydrazine to *Xenopus laevis* embryos and larvae. — Bull. Environ. Contam. Toxicol. **18**: 503–511.

Gregoire, D.R. & M.S. Gunzburger (2008): Effects of predatory fish on survival and behavior of larval gopher frogs (*Rana capito*) and southern leopard frogs (*Rana sphenocephala*). — J. Herpetol. **41**: 97–103.

Grewal, M.S. & S. Dasgupta (1967): Skeletal polymorphism and genetic drift in a Delhi frog, *Rana cyanophlictis*. — Genet. Res. Camb. **9**: 299–307.

Gridi-Papp, M. & C.O. Gridi-Papp (2005): Abnormal digits in Strecker's chorus frogs (*Pseudacris streckeri,* Hylidae) from Central Texas. — Southwestern Naturalist **50**: 490–494.

Grillitsch, B. & H. Grillitsch (1989): Teratological and ontogenetic alterations to external oral structures in some anuran larvae (Amphibia: Anura: Bufonidae, Ranidae). — Fortschritte Zool. **35**: 276–282.

Grimm, H. (1953): Ödemkrankheit bei Krallenfröschen. — Zool. Garten **20**: 98–101.

Gross, L. (1961): Oncogenic Viruses. — New York (Pergamon Press).

Grosse, W.-R. (2015): Grasfrosch – *Rana temporaria* (Linnaeus, 1758). — Ber. Landesamt Umweltschutz Sachsen-Anhalt **4**: 357–370.

Grosse, W.-R. & S. Bauch (1988): Fehlentwicklung beim Laubfrosch *Hyla a. arborea* L. — Feldherpetologie **1988**: 25–28.

Grosse, W.-R. & B. Simon (2015): Feinde und Krankheiten heimischer Lurche und Kriechtiere. — Ber. Landesamt Umweltschutz Sachsen-Anhalt **4**: 597–602.

Guderyahn, L. (2006): Nationwide assessment of morphological abnormalities observed in amphibians collected from United States national wildlife refuges. — U.S. Fish & Wildl. Serv. **Report CBFO-C06-01**.

Guex, G.-D., H. Hotz, T. Uzzell, R.D. Semlitsch, P. Beerli & R. Pascolini (2001): Developmental disturbances in *Rana esculenta* tadpoles and metamorphs. — Mitt. Mus. Naturkde. Berlin Zool. Reihe **77**: 79–86.

Günther, R. (1982): Ergebnisse experimenteller Kreuzungen zwischen Wasserfröschen (Anura, Ranidae) aus verschiedenen Ländern Europas und Mittelasiens. — Vertebrata Hungarica **21**: 157–167.

Günther, R. (1990): Die Wasserfrösche Europas. — Wittenberg Lutherstadt (Ziemsen).

Günther, R. (1996a): Die Amphibien und Reptilien Deutschlands. — Jena (Fischer).

Günther, R. (1996b): 6.20. Teichfrosch – *Rana* kl. *esculenta* Linnaeus, 1758. Pp. 455–475 in Günther, R. (ed.): Die Amphibien und Reptilien Deutschlands. — Jena (Fischer).

Günther, R. (1996c): 6.22. Seefrosch – *Rana ridibunda* Pallas, 1771. Pp. 490–507 in Günther, R. (ed.): Die Amphibien und Reptilien Deutschlands. — Jena (Fischer).

Gurrieri, F., K.W. Kjaer, E. Sangiorgi & G. Neri (2002): Limb anomalies: developmental and evolutionary aspects. — Am. J. Med. Genetics **115**: 231–244.

Gurushankara, H.P., S.V. Krishnamurthy & V. Vasudev (2007): Morphological abnormalities in natural populations of common frogs inhabiting agroecosystems of central Western Ghats. — Appl. Herpetol. **4**: 39–45.

Gurvich, A.N. (2014): Variability of the skeleton and anomalies of juvenile Siberian frogs (*Rana amurensis*). Pp. 75–80 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Guttmann, S.I. (1972): Colour photo of a yellow "saddle back" *Rana clamitans*. — BioScience **22(4)**: Cover.

Guyénot, E. & A. Naville (1922): Un nouveau protiste, du genre *Dermocystidium*, parasite de la Grenouille. *Dermocystidium ranae* nov. spec. — Rev. suisse Zool. **29(3)**: 133–145.

Guyénot, E. & O.E. Schotté (1926): Démonstration de l'existence des territoires spécifiques de régénération par la méthode de la déviation des troncs nerveux. — C.r. Soc. Biol. **94**: 1050–1052.

Guyénot, E., J. Dinichert & M. Galland (1948): L'exploration du territoire de la patte antérieure du triton. — Rev. suisse Zool. Suppl. **2**: 1–120.

Hachtel, M. (2011): 3.2 Bergmolch – *Mesotriton alpestris*. Pp. 337–374 in Hachtel, M., M. Schlüpmann, K. Weddeling, B. Thiesmeier, A. Geiger & C. Willigalla (eds.): Handbuch der Amphibien und Reptilien Nordrhein-Westfalens. — Bielefeld (Laurenti).

Häcker, V. (1906): Über Mendelsche Vererbung bei Axolotln. — Zool. Anz. **31**: 99–102.

Haddad, C.F.B., A.J. Cardoso & L.M. Castanho (1990): Hibridaçao natural entre *Bufo ictericus* e *Bufo crucifer* (Amphibia: Anura). — Rev. Brasil. Biol. **50(3)**: 739–744.

Haensch, G. (1985): Beobachtungen an einer albinotischen Larve des Feueralamanders. — elaphe **85(3)**: 45–47.

Hahn, A. (1912): Einige Beobachtungen an Riesenlarven von *Rana esculenta*. — Arch. mikr. Anat. **80**: 1–38.

Haidacher, S. & G. Fachbach (1991): Experimentelle Säuretoleranzanalysen von Laich und Larven heimischer Amphibien. — Salamandra **29**: 108–118.

Haines-Eitzen, E. (2016): *Pseudotriton ruber* (northern red salamander). — Herpetol. Rev. **47**: 276–277.

Halliday, T. & M. Tejedo (1995): Intrasexual selection and alternative mating behaviour. Pp. 419–468 in Heatwole, H. & B.K. Sullivan (eds.): Amphibian Biology. Vol. 2: Social Behaviour. — Chipping Norton, Australia (Surrey Beatty).

Hamburger, V. (1935): Malformations of hind limbs in species hybrids of *Triton taeniatus* (and *palmatus*) ♀ × *Triton cristatus* ♂. — J. exp. Zool. **70**: 43–54.

Hamed, M.K., M.J. Gray & T.F. Lauglin (2015): *Hemidactylium scutatum* (four-toed salamander). Polyembryony. — Herpetol. Rev. **46**: 227.

Hammermeister, J. (1991): Zur Haltung und Vermehrung von *Gastrotheca riobambae* (Fowler 1913). Pp. 31–34 in Herrmann, H.-J. (ed.): Amphibienforschung und Vivarium. — Schleusingen (Naturhistorisches Museum).

Hanken, J. (1983): High incidence of limb skeletal variants in a peripheral population of the red-backed salamander, *Plethodon cinereus* (Amphibia: Plethodontidae), from Nova Scotia. — Can. J. Zool. **61**: 1925–1931.

Hardwick, L.J.A. & A. Philpott (2015): An oncologist's friend: how *Xenopus* contributes to cancer research. — Develop. Biol. **408**: 180–187.

Hardy, J.D. (1964): The spontaneous occurrence of scoliosis in tadpoles of the leopard frog, *Rana pipiens*. — Chesapeake Sci. **5(1/2)**: 101–102.

Harris, M.L., C.A. Bishop & T.V. McDaniel (2001): Assessment of rates of deformity in wild frog populations using in situ cages: a case study of leopard frogs (*Rana pipiens*) in Ontario, Canada. — Biomarkers **6(1)**: 52–63.

Harris, M.L., C.A. Bishop, J. Struger, B. Ripley & J.P. Bogart (1998): The functional integrity of northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*) populations in orchard wetlands. II. Effects of pesticides and eutrophic conditions on early life stage development. — Environ. Toxicol. Chem. **17**: 1351–1363.

Hart, D.R. & J.B. Armstrong (1984): Assessment of mutagenic damage by monofunctional alkylating agents and gamma radiation in haploid and diploid frogs, *Xenopus laevis*. — Environ. Mutagenesis **6**: 719–735.

Hartwig, H. & E. Rotmann (1940): Experimentelle Untersuchungen an einem Massenauftreten von neotenen *Triton taeniatus*. — Roux Arch. Entwickl.-mech. **140**: 195–251.

Harvey, E. (2003): Evaluation of fluorescent marking techniques using cannibalistic salamander larvae. — Herpetol. Rev. **34**: 119–121.

Hassine, J.B., V. de Buffrénil & S. Nouria (2011): First record of morphological abnormalities in natural populations of two amphibian species in Tunesia. — J. Herpetol. **45**: 465–471.

Hatano, F.H., D. Gettinger, M. Van Sluys & C.F.D. Rocha (2007): Parasitism of *Hylodes phyllodes* (Anura: Cycloramphidae) by *Hannemania* sp. (Acari: Trombiculidae) in an area of Atlantic forest, Ilha Grande, southeastern Brazil. — Lincoln (Univ. Nebraska). http://digitalcommons.unl.edu/parasitologyfacpubs/687

Hay, E.D. (1956): Effects of thyroxine on limb regeneration in the newt, *Triturus viridescens*. — Bull. John Hopkins Hospital **99**: 262–285.

Hayes, M.P. & K.M. Cassidy (2013): *Rana luteiventris* (Columbia spotted frog). Maximum size. — Herpetol. Rev. **44**: 128.

Hayes, T.B. (1997): Hormonal mechanisms as developmental constraints on evolution: examples from the Anura. — Am. Zool. **37**: 482–490.

Hayes, T.B. (2000): Endocrine disruption in amphibians. Pp. 573–593 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. — Pensacola (SETAC Press).

Hayes, T.B., T.-H. Wu & T.N. Gill (1997): DDT-like effects as a result of corticosterone treatment in an anuran amphibian: is DDT a corticoid mimic or a stressor? — Environ. Toxicol. Chem. **16**: 1948–1953.

Hays, J.B., A.R. Blaustein, J.M. Kiesecker, P.D. Hoffmann, I. Pandelova, D. Coyle & T. Richardson (1996): Developmental responses of amphibians to solar and artificial UVB sources: a comparative study. — Photochem. Photobiol. **64**: 449–456.

Hayslett, M.S., G.L. Wilson & J.C. Mitchell (1998): *Plethodon hubrichti* (Peaks of Otter salamander). Albinism. — Herpetol. Rev. **29**: 229–230.

Hebard, W.B. & R.B. Brunson (1963): Hind limb anomalies of a Western Montana population of Pacific tree frog, *Hyla regilla* Baird and Girard. — Copeia **1963**: 570–572.

Hecker, L. & S.K. Sessions (2001): Developmental analysis of limb deformities in amphibians. — BIOS **72(1)**: 9–13.

Hecnar, S.J. (1995): Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from Southern Ontario. — Environ. Toxicol. Chem. **14**: 2131–2137.

Helgen, J.C. (2012): Peril in the Ponds. Deformed Frogs, Politics, and a Biologist's Quest. — Amherst (Univ. Massachussetts Press).

Helgen, J.C., M.C. Gernes, S.M. Kersten, J.W. Chirhart, J.T. Canfield, D. Bowers, J. Haferman, R.G. McKinnell & D.M. Hoppe (2000): Field investigations of malformed frogs in Minnesota 1993–97. — J. Iowa Acad. Sci. **107(3)**: 96–112.

Hellmich, W. (1929a): Mehrfachbildungen von Extremitäten bei Amphibien (Naturfunde). — Roux Arch. Entwickl.-mech. **115**: 409–414.

Hellmich, W. (1929b): Überzählige Gliedmaßen bei Amphibien (Naturfunde). — Bl. Aquar. Terrarienkde **40**: 302–304.

Hemmer, H. (1973): Die Bastardierung von Kreuzkröte (*Bufo calamita*) und Wechselkröte (*Bufo viridis*). — Salamandra **9**: 118–136.

Henle, K. (1983): Eine neue neotenische Population des Bergmolches *Triturus alpestris* (Laurenti, 1768) (Caudata: Salamandridae). — Salamandra **19**: 151–157.

Henle, K. (1992): Zur Amphibienfauna Perus nebst Beschreibung eines neuen *Eleutherodactylus* (Leptodactylidae). — Bonn. zool. Beitr. **43**: 79–129.

Henle, K. (2014): Anomalies in natural populations of amphibians: overview, patterns and causes, methodological recommendation. Pp. 183–190 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Henle, K. & B. Streit (1990): Kritische Betrachtungen zum Artenrückgang bei Amphibien und Reptilien und zu dessen Ursachen. — Natur und Landschaft **65**: 347–361.

Henle, K., D. Alard, J. Clitherow, P. Cobb, L. Firbank, T. Kull, D. McCracken, R.F.A. Moritz, J. Niemelä, M. Rebane, D. Wascher, A. Watt & J. Young (2008): Identifying and managing the conflicts between agriculture and biodiversity conservation in Europe – a review. – Agric. Ecosyst. Environm. **124**: 60–71.

Henle, K., B. Mester, S. Lengyel & M. Puky (2012): A review of a rare type of anomaly in amphibians, tail duplication and bifurcation, with description of three new cases in European species (*Triturus dobrogicus, Triturus carnifex*, and *Hyla arborea*). — J. Herpetol. **46**: 451–455.

Henle, K., A. Dubois, K. Rimpp & V. Vershinin (2017a): Mass anomalies in green toads (*Bufotes viridis*) at a quarry in Roßwag, Germany: inbred hybrids, radioactivity, or an unresolved case? — Mertensiella **25**: 185–242.

Henle, K., A. Dubois & V. Vershinin (2017b): Commented glossary, terminology, and synonymies of anomalies in natural populations of amphibians. — Mertensiella **25**: 9–48.

Henle, K., K. Rimpp, J. Margraf & A. Dubois (2017c): The Roßwag affair: history and controversy of the discovery of mass anomalies in the greeen toad (*Bufotes viridis*) in a German quarry. — Mertensiella **25**: 165–184.

Hensley, M. (1959): Albinism in North American amphibians and reptiles. — Publ. Mus. Michigan State Univ. **1**: 133–159.

Herman, R.L. (1984): *Ichthyophonus*-like infection in newts (*Notophthalmus viridescens* Rafinesque). — J. Wildl. Dis. **20**: 55–56.

Héron-Royer, L.F. (1884): Cas tératologiques observés chez quelques têtards de batraciens anoures et de la possibilité de prolonger méthodiquement l'état larvaire chez les batraciens. — Bull. Soc. Zool. Fr. **1884(9)**: 162–168.

Héron-Royer, L.F. (1886): Sur la reproduction de l'albinisme par voie héréditaire chez l'Alyte accoucheur et sur l'accouplement de ce batracien. — Bull. Soc. Zool. Fr. **1886(11)**: 671–679.

Herrmann, H.-J. (1990): Partiell abnorme Dorsalfärbung bei *Bombina variegata variegata*. — Veröff. Naturhist. Museum Schleusingen **5**: 85.

Hertwig, G. (1913): Parthenogenesis bei Wirbeltieren, hervorgerufen durch artfremden radiumbestrahlten Samen. — Arch. mikr. Anat. **81**: 87–127.

Hertwig, G. & I. Weiss (1955): Die Auswirkung der Vereinigung von Genomen, die ungleichen Kernklassen angehören, auf die Entwicklungsfähigkeit reziproker Krötenbastarde. — Gegenbaurs Morphol. Jb. **95**: 1–34.

Hertwig, G., I. Weiss & C. Ziemann (1959): Unterschiedliche Ergebnisse reziproker Kreuzungen der drei europäischen Krötenarten unter besonderer Berücksichtigung ihrer Kern- und Zellgrößen. — Biol. Zentralbl. **78**: 675–702.

Hertwig, O. (1892): Urmund und Spina bifida. — Arch. mikr. Anat. **39**: 353–499.

Hertwig, O. (1911): Die Radiumkrankheit tierischer Keimzellen. Ein Beitrag zur experimentellen Zeugungs- und Vererbungslehre. — Arch. mikr. Anat. **77**: 1–164.

Heselhaus, R. (1983): Zum Problem der "Streichholzvorderbeine" bei Dendrobatidennachzuchten. — herpetofauna, Weinstadt, **5(26)**: 22–24.

Hesse, E. (1906): *Lucilia* in *Bufo vugaris* Laur. schmarotzend. — Biol. Zentralbl. **26**: 633–640.

Hesse-Honegger, C. & P. Wallimann (2008): Malformation of true bug (Heteroptera): a phenotype field study on the possible influence of artificial low-level radioactivity. — Chemistry & Biodiversity **5**: 499–539.

Highton, R. (1956): The life history of the slimy salamander, *Plethodon glutinosus*, in Florida. — Copeia **1956**: 75–92.

Hiler, W.R., B.A. Wheeler & S.E. Trauth (2005): Abnormalities in the Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) in Arkansas: a comparison between two rivers with a historical perspective. — J. Arkansas Acad. Sci. **59**: 88–94.

Hillenius, D. (1959): Ein zweiter Fall der "Anomalie P" (Rostand) bei *Rana esculenta* Linné. — Medizin. Klinik **54**: 99–100.

Hiller, A. (1986): Über die Entwicklung einer Larve der Knoblauchkröte (*Pelobates fuscus*) mit Mehrfachbildung an der Schwanzwirbelsäule. — Salamandra **22**: 93–94.

Hine, R.L., B.L. Les, B.F. Hellmich & R.C. Vogt (1975): Preliminary report on leopard frog (*Rana pipiens*) populations in Wisconsin. — Dept. Natural Resources Res. Report **81**: 1–31.

Hobson, B.M. (1958): Polymely in *Xenopus laevis*. — Nature **181**: 862.

Hoffmann, D.J., H.M. Ohlendorf & T.W. Aldrich (1988): Se teratogenesis in natural populations of aquatic birds in central California. — Arch. Environ. Contam. Toxicol. **17**: 519–525.

Holder, N., S.V. Bryant & P.W. Tank (1979): Interactions between irradiated and unirradiated tissues during supernumerary limb formation in the newt. — J. exp. Zool. **208**: 303–310.

Hoperskaya, O.A. (1975): The development of animals homozygous for a mutation causing periodic albinism (aP) in *Xenopus laevis*. — J. Embryol. exp. Morph. **34**: 253–264.

Hoperskaya, O.A. (1981): Induction – The main principle of melanogenesis in early development. — Differentiation **20**: 104–116.

Hopkins, W.A., J. Congdon & J.K. Ray (2000): Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal-burning power plant. — Environ. Toxicol. Chem. **19**: 862–868.

Hoppe, D.M. (2000): History of Minnesota frog abnormalities: do recent findings represent a new phenomenon? — J. Iowa Acad. Sci. **107(3)**: 86–89.

Hoppe, D.M. (2002): Mortality and population declines associated with a Minnesota malformed frog site. — Proc. Int. Symp. Pathol. Reptiles Amphibians **6**: 77–85.

Hoppe, D.M. (2005): Malformed frogs in Minnesota: history and interspecific differences. Pp. 103–108 in Lannoo, M. (ed.): Amphibian Declines. The Conservation Status of United States Species. — Berkeley (Univ. California Press).

Humphrey, R.R. (1948): A lethal fluid imbalance in the Mexican axolotl, inherited as a simple Mendelian recessive. — J. Hered. **39**: 255–261.

Humphrey, R.R. (1964): Genetic and experimental studies on a lethal factor (r) in the axolotl which induces abnormalities in the renal system and other organs. — J. exp. Zool. **155**: 139–150.

Humphrey, R.R. (1966): A recessive factor (o, for ova deficient) determining a complex of abnormalities in the Mexican axolotl (*Ambystoma mexicanum*). — Develop. Biol. **13**: 57–76.

Humphrey, R.R. (1967a): Albino axolotls from an albino tiger salamander through hybridization. — J. Hered. **58**: 95–101.

Humphrey, R.R. (1967b): Genetic and experimental studies on a lethal trait ("short toes") in the Mexican axolotl (*Ambystoma mexicanum*). — J. exp. Zool. **164**: 281–295.

Humphrey, R.R. (1972): Genetic and experimental studies on a mutant gene (c) determining absence of heart action in embryos of the Mexican axolotl (*Ambystoma mexicanum*). — Develop. Biol. **27**: 365–375.

Humphrey, R.R. (1973): Experimental studies on a new lethal trait in Mexican axolotls of the Wista Institute white strain. — J. exp. Zool. **183**: 201–208.

Humphrey, R.R. (1975): The axolotl, *Ambystoma mexicanum*. Pp. 3–17 in King, R.C. (ed.): Handbook of Genetics. — New York (Plenum Press).

Hutt, F.B. (1945): Complete albinism in the mud puppy, *Necturus*. — J. Hered. **36**: 145–147.

Ingram, A.J. (1971): The reactions to carcinogens in the axolotl (*Ambystoma mexicanum*) in relation to the regeneration field control hypothesis. — J. Embryol. exp. Morph. **26**: 425–441.

Inoue, S. & M. Singer (1970): Experiments on a spontaneously originated visceral tumor in the newt, *Triturus pyrrhogaster*. — Ann. N.Y. Acad. Sci. **174**: 729–764.

Ippen, R. & W.-D. Heinrich (1977): Pathologische Veränderungen an fossilen Extremitätenknochen von Anuren aus dem fossilen Tierbautensystem von Pisede bei Malchin. — Wiss. Ze. Humboldt Univ. Berlin, Math.-Nat. R. **26**: 301–305.

Ippisch, G. (1928): Beobachtungen über Albinismus usw. bei *Bombinator pachypus* Laur. — Bl. Aquar. Terrarienkde **39**: 380–383.

Iwasawa, H. & T. Takasu (1985): Study of thumb pad regions developed by the administration of testosterone in a young female *Rana nigromaculata* with a supernumerary forelimb. — Jap. J. Herpetol. **11**: 5–10.

Jangir, O.P. & I.A. Niazi (1978): Stage dependent effects of vitamin A excess on limbs during ontogenesis & regeneration in tadpoles of the toad *Bufo melanostictus* (Schneider). — Ind. J. exp. Biol. **16**: 438–445.

Jara, Z. (1963): O niektórych konsekwencjach przystosowania ryb do zycia w srodowisku wodnym. — Przeglad Zool. Wroclaw **7**: 207–218.

Jarvis, L.E. (2011): *Triturus cristatus* (great crested newt): malformation. — Herpetol. Bull. **117**: 40–41.

Jayawardena, U.A. & R. Rajakaruna (2013): Understanding possible ethiologies of amphibian crisis in Sri Lanka. — Froglog **21**: 47–49.

Jayawardena, U.A., R.S. Rajakaruna, A.N. Navaratne & P.H. Amerasinghe (2010): Monostome cercariae induced malformations in amphibians: effect of infection at the pre-limb-bud stage tadpoles of *Polypedates cruciger* Blyth. — J. Natl. Sci. Foundation Sri Lanka **38**: 241–248.

Jayawardena, U.A., V.V. Tkach, A.N. Navaratne, P.H. Amerasinghe & R.S: Rajakaruna (2013): Malformations and mortality in the Asian common toad induced by exposure to pleurolophocercous cercariae (Trematoda: Cryptogonimidae). — Parasitol. Internatl. **62**: 246–252.

Jaylet, A. (1971): Change in the karyotype by a homozygous pericentric inversion in the newt *Pleurodeles waltlii* Michahelles. — Chromosoma **35**: 288–99.

Johnson, P.T.J. & T. Bowerman (2010): Do predators cause frog deformities? The need for an eco-epidemiological approach. — J. exp. Zool. (Mol. Dev. Evol.) **314B**: 5151–518.

Johnson, P.T.J. & J.M. Chase (2004): Parasites in the food web: linking amphibian malformations and aquatic eutrophication. — Ecol. Letters **7**: 521–526.

Johnson, P.T.J. & R.B. Hartson (2009): All hosts are not equal: explaining differential patterns of malformations in an amphibian community. — J. Anim. Ecol. **78**: 191–201.

Johnson, P.T.J. & K.B. Lunde (2005): Parasite infection and limb malformations: a growing problem in amphibian conserva-

tion. Pp. 124–138 in Lannoo, M. (ed.): Amphibian Declines. The Conservation Status of United States Species. — Berkeley (Univ. California Press).

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie & A.E. Launer (1999): The effect of trematode infection on amphibian limb development and survivorship. — Science **284**: 802–804.

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie, J.K. Reaser & A.E. Launer (2001a): Morphological abnormality patterns in a California amphibian community. — Herpetologica **57**: 336–352.

Johnson, P.T.J., K.B. Lunde, R.W. Haight, J. Bowerman & A.R. Blaustein (2001b): *Ribeiroia ondatrae* (Trematoda: Digenea) infection induces severe limb malformations in western toads (*Bufo boreas*). — Can. J. Zool. **79**: 370–379.

Johnson, P.T.J., K.B. Lunde, E.M. Thurman, E.G. Ritchie, S.N. Wray, D.R. Sutherland, J.M. Kapfer, T.J. Frest, J. Bowerman & A.R. Blaustein (2002): Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. — Ecol. Monogr. **72**: 151–168.

Johnson, P.T.J., K.B. Lunde, D.A. Zelmer & J.K. Werner (2003): Limb deformities as an emerging parasitic disease in amphibians: evidence from museum specimens and resurvey data. — Conserv. Biol. **17**: 1724–1737.

Johnson, P.T.J., D.R. Sutherland, J.M. Kinsella & K.B. Lunde (2004): Review of the trematode genus *Ribeiroia* (Psilostomidae): ecology, life history and pathogenesis with special emphasis on the amphibian malformation problem. — Adv. Parasitol. **57**: 192–253.

Johnson, P.T.J., E.R. Preu, D.R. Sutherland, J.M. Romansic, B. Han & A.R. Blaustein (2006): Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. — Ecology **87**: 2227–2235.

Johnson, P.T.J., E. Kellermanns & J. Bowerman (2011): Critical windows of disease risk: amphibian pathology driven by developmental changes in host resistence and tolerance. — Funct. Ecol. **25**: 726–734.

Johnson, P.T.J., J.R. Rohr, J.T. Hoverman, E. Kellermanns, J. Bowerman & K.B. Lunde (2012): Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. — Ecol. Letters **15**: 235–242.

Johnson, P.T.J., D.L. Preston, J.T. Hoverman & K.L.D. Richgels (2013): Biodiversity decreases disease through predictable changes in host community competence. — Nature **494**: 5.

Johnson, R.L. & C.J. Tabin (1997): Molecular models for vertebrate limb development. — Cell **90**: 979–990.

Johnston, G.R. & J.C. Johnston (2006): *Scaphiopus holbrookii holbrookii* (eastern spadefoot toad). Albinism. — Herpetol. Rev. **37**: 211–212.

Jourdain, M. (1877): Monstre ectromèle rencontré dans un individu de *Rana temporaria*. — Bull. Soc. Sci. Nancy **2(3)**: 10.

Jung, R.E., S. Claeson, J.E. Wallace & W.C. Welbourn (2001): *Eleutherodactylus guttilatus* (spotted chirping frog), *Bufo punctatus* (red-spotted toad), *Hyla arenicolor* (canyon tree frog), and *Rana berlandieri* (Rio Grande leopard frog). Mite infestation. — Herpetol. Rev. **32**: 33–34.

Kahn, R.H. (1916): Zur Frage der Wirkung der Schilddrüse und Thymus auf Froschlarven. — Pflüger's Arch. gesamte Physiol. **163**: 384–404.

Kaltenbach, J.C. (1996): Endocrinology of amphibian metamorphosis. Pp. 403–431 in Gilbert, L.I., J.R. Tata & B.G. Atkinson (eds.): Metamorphosis. — New York (Acad. Press).

Kammerer, P. (1905): Über die Abhängigkeit des Regenerationsvermögens der Amphibienlarven von Alter, Entwicklungsstadium und spezifischer Größe. — Arch. Entwicklungsmech. **14**: 148–180.

Kashiwagi, K. (1980): Mature haploids and their reproductive capacity in *Rana rugosa*. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **4**: 217–237.

Kawamura, T. & M. Nishioka (1978): Abnormalities in the descendants of *Rana nigromaculata* produced from irradiated eggs or sperm. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **3**: 1–187.

Kawamura, T., M. Nishioka & H. Ueda (1980): Inter- and intraspecific hybrids among Japanese, European and American toads. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **4**: 1–125.

Kaylor, C.T. (1940): Studies on experimental haploidy in salamander larvae. — Biol. Bull. **79**: 397–408.

Keilin, D. (1915): Recherches sur les larves de diptères cyclophaphes. Cycle évolutif de "*Pollenia rudis* Fabr.", parasite d'"*Allolobophora chlorotica* Sav." – Biologie comparée des larves de diptères. — Bull. Sci. Fr. Belg. **49**: 15–198.

Kennedy, I.J.J. & K. Sampath (2001): Short-term and long-term survival studies in *Rana tigrina* tadpoles with reference to methyl parathion toxicity. — J. exp. Biol. **22(4)**: 267–271.

Khudoley, V.V., I.V. Anikin, O.A. Sirenko & G.B. Pliss (1979): The possibility of using fish and Amphibia to reveal the carcinogenic effect of nitrosomorpholine and its chemical precursors. — Voprosy Onkologii (Leningrad) **25(6)**: 70–75.

Kidov, A.A. (2010): Parasitism of larval *Lucilia bufonivora* on *Bufo verrucosissimus*. — Estestvennye i Tekhnicheskie Nauki **3**: 99–101.

Kiesecker, J.M. (2002): Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? — Proc. Natl. Acad. Sci. **99**: 9900–9904.

Klatt, B. (1927): Fütterungsversuche an Tritonen. II. (Die Bedeutung der "Ausgangsgröße"). — Roux Arch. Entwickl.-mech. **109**: 176–187.

Klemz, C. & K.-D. Kühnel (1986): Ein Beitrag zum Albinismus beim Moorfrosch *Rana arvalis* Nilsson, 1842 (Salientia: Ranidae). — Salamandra **22**: 190–195.

Klewen, R. (1986): Untersuchungen zur Verbreitung, Öko-Ethologie und innerartlichen Gliederung von *Salamandra atra* Laurenti 1768. — Köln (Unpubl. PhD Thesis., Math.-Nat. Faculty, Univ. Köln).

Klewen, R. (1988): Die Landsalamander Europas, Teil I. — Wittenberg Lutherstadt (Ziemsen).

Kliemt, M. (2017): Verschiedene Auffälligkeiten bei juvenilen Erdkröten und einem Moorfrosch. — Feldherpetol. Mag. **7**: 27–29.

Kliewer, S.A., K. Umesono, D.J. Mangelsdorf & R.M. Evans (1992): Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamine D3 signalling. — Nature **355**: 446–449.

Knapp, R.A. & J.A.T. Morgan (2006): Tadpole mouthpart depigmentation as an accurate indicator of chytridiomycosis, an emerging disease of amphibians. — Copeia **2006**: 188–197.

Kobayashi, H. & A. Gorbman (1962): Thyroid function in *Amphiuma*. — Gen. Comp. Endocrinol. **2**: 279–282.

Koch, C., B. Schreiber & G. Schreiber (1939): Tumeurs produites par le goudron et le benzopyrène chez les amphibiens urodèles. — Bull. Assoc. Franç. étude cancer **28**: 852–859.

Koch, E.R. & F. Vahrenholt (1983): Die Lage der Nation. — Hamburg (GEO).

Kolobaev, N.N. (2000): Dybovsky's frog (*Rana dybowskii*) in the Upper Zeya Basin, Amurland. — Adv. Amph. Res. Former Soviet Union **5**: 47–78.

Koprivnikar, J., M.R. Forbes & R.L. Baker (2007): Contaminant effects on host-parasite interactions: atrazine, frogs, and trematodes. — Environ. Toxicol. Chem. **26**: 2166–2170.

Kordges, T. (2000): Starker Befall der Erdkröte (*Bufo bufo*) durch die Krötengoldfliege (*Lucilia bufonivora* Moniez, 1876). — Z. Feldherpetol. **7**: 211–218.

Kordges, T. (2002): Notizen zur Phänologie und Larvalökologie von Amphibien in Hochgebirgslagen der Alpen. — Z. Feldherpetol. **9**: 119–123.

Kordges, T. (2003): Zur Biologie der Geburtshelferkröte (*Alytes obstetricans*) in Kalksteinbrüchen des Niederbergischen Landes (Nordrhein-Westfalen). — Z. Feldherpetol. **10**: 105–128.

Kordges, T., B. Thiesmeier, C. Hornberg, U. Möller & H. Greven (2008): Kropfbildung und Pädomorphose in einer Teichmolch-Population in Nordrhein-Westfalen. — Z. Feldherpetol. **15**: 29–41.

Kordges, T., B. Thiesmeier, M. Franzen & P. de Wijer (2013): Die Syrische Schaufelkröte (*Pelobates syriacus*) in der Südwest-Türkei mit Beschreibung neuer Fundorte und Angaben zur Larvalbiologie. — Z. Feldherpetol. **20**: 79–89.

Korzikov, V.A. & S.K. Alekseev (2014): Study on morphological anomalies in anuran amphibians of the territory of the Kaluga region. Pp. 123–127 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Koskela, P., J. Itämies & S. Pasanen (1974): *Lucilia bufonivora* Moniez (Dipt., Calliphoridae), a lethal parasite in *Rana temporaria* L. (Anura). — Ann. Zool. Fennici **11**: 105–106.

Kotlík, P. & V. Zavadil (1997): Long-term presence of *Rana lessonae* albinos in Western Bohemia (with some notes on the albinism in hybridogenetic frogs). Pp. 115–116 in Rocek, Z. & S. Hart (eds.): Herpetology '97. Abstracts of the Third World Congress of Herpetology 2–10 August 1997, Prague, Czech Republic. — Prague (Charles Univ.).

Kovalenko, E.E. (1994): The compound sacrum in individual variability of common platanna (*Xenopus laevis*). — Russ. J. Herpetol. **1**: 172–178.

Kovalenko, E.E. (2000): Mass anomalies of extremities in Anura. — Z. Gen. Biol. **61**: 412–427.

Kovalenko, E.E. & Y.I. Kovalenko (1996): Certain pelvic and sacral anomalies in Anura. — Russ. J. Herpetol. **3**: 172–177.

Kovalenko, E.E. & Y.I. Kovalenko (2000): Anomalii zadnich konetschnostei u laboratornoi schporschevoi ljaguschki *Xenopus laevis* (Anura, Pipidae). 1. Fenomen massobysch anomalii [Hind limb anomalies in laboratory clawed frogs *Xenopus laevis* (Anura, Pipidae). 1. The phenomenon of mass anomalies]. — Vestnik Sankt-Peterburgskogo Univ., Ser. 3 (Biol.) **1(3)**: 3–22.

Kraus, F. (2007): Fly parasitism in Papuan frogs, with a discussion of ecological factors influencing evolution of life-history differences. — J. Nat. Hist. **41**: 1863–1874.

Krefft, G. (1938): Fütterungsversuche an Tritonen. V. (Zur Frage der Vitamine). — Roux Arch. Entwickl.-mech. **137**: 566–590.

Krintler, K. (1988): Beobachtungen zum Problem der "Streichholzbeine" bei Dendrobatiden und Hyliden. — herpetofauna, Weinstadt, **10(52)**: 30–31.

Krotoski, D.M., D.C. Reinschmidt & R. Tompkins (1985): Developmental mutants isolated from wild-caught *Xenopus laevis* by gynogenesis and inbreeding. — J. exp. Zool. **233**: 443–449.

Kryger, J.P. (1921): *Lucilia sylvarum* Meig. som Snylter paa *Bufo vulgaris*. — Videnskabelige meddeleser Dansk Naturhist. Forening i Kjøbenhavn **72**: 99–113.

Krynak, T.J. & P.M. Dennis (2014): Detection of *Ranavirus* in northeastern Ohio's wetlands. — Herpetol. Rev. **45**: 601–602.

Kupfer, A. & B. von Bülow (2011): 3.3 Kammmolch – *Triturus cristatus*. Pp. 375–406 in Hachtel, M., M. Schlüpmann, K. Weddeling, B. Thiesmeier, A. Geiger & C. Willigalla (eds.): Handbuch der Amphibien und Reptilien Nordrhein-Westfalens. — Bielefeld (Laurenti).

Kupferberg, S.J., A. Catenazzi, K. Lunde, A.J. Lind & W.J. Palen (2009): Parasitic copepod (*Lernaea cyprinacea*) outbreaks in foothill yellow-legged frogs (*Rana boylii*) linked to unusually warm summers and amphibian malformations in northern California. — Copeia **2009**: 529–537.

Kurabuchi, S. & S. Inoue (1982): Limb regenerative capacity of four species of Japanese frogs of the families Hylidae and Ranidae. — J. Morphol. **173**: 129–135.

Kuramoto, M. (1983): Studies on the speciation of pond frogs in East Asia. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **6**: 253–267.

Kuranova, V.N. (1998): Fauna i ekologia zemnovodnych i presmykayushichsia yugo-vostoka zapadnoi sibiri. — Tomsk (PhD., Univ. Tomsk).

Kuranova, V.N. (2003): Antropiceskoe vosdeistie na zemnovodnysh i presmykayushishtshsja. Proble-my ish oshrany na yugo-vostoke Zapadnoi Sibiri (Anthropogenic impacts on amphibians and reptiles. Problems of conservation in southeastern West-Siberia). Pp. 39–51 in Erdakov, L.N. & O.N. Tshernyshtshova (eds.): Amphibii i Reptilii v Zapadnoi Sibiri (Amphibians & Reptiles of Western Siberia). — Novosibirsk (Novosibirskii Gosudarstennyi Pedagogitsheskii Univ.).

Kuranova, V.N. & S.V. Saveliev (1997): The effect of radiation and chemicals on amphibian ontogeny. — Adv. Amph. Res. Former Soviet Union **2**: 167–168.

Kuranova, V.N. & S.V. Saveliev (1999): Disturbances of ontogeny in natural populations of amphibians on industrially polluted territories. — Programme and book of abstracts, Ord. Gen. Meet SEH. **10**: 94.

Kurtyak, F.F. (2004): Amfibii ravninnogo zakarpat'ya: sostoyaniye fauny i analiz problemnykh grupp. — Kiev (PhD, Inst. Zool., Natl. Acad. Sci.).

Kuzmin, S.L. (2013): The Amphibians of the Former Soviet Union. 2nd revised edition. — Sofia (Pensoft).

Kuzmin, S.L. & I.V. Maslova (2003): The Amphibians of the Russian Far East. — Adv. Amph. Res. Former Soviet Union **8**: 1–459.

Kwet, A., P. Werner & U. Schulte (2016): Salamander lebe hoch. Lurch des Jahres 2016. Terraria/elaphe **3/2016**: 14–21.

Labrousse, M. (1967): Analyse des effets des rayonnements embryonaires de l'Amphibien Urodèle *Pleurodeles waltlii* Michah. — Bull. Soc. Zool. Fr. **91**: 491–588.

Lacroix, J.-C. & A. Capuron (1970): Sur un facteur récessif, modifiant le phénotype pigmentaire de la larve, chez l'Amphibien Urodèle *Pleurodeles waltlii* Michahelles. — C.r. Acad. Sci. Paris **270**: 2122–2123.

Lada, G.A. (1999): Polydactyly in anurans in the Tambov region (Russia). — Russ. J. Herpetol. **6**: 104–106.

Landois, H. (1882): Monströse Beine des Landfrosches *Rana platyrrhina* Steenstr. — Verh. Naturhist. Ver. Preuss. Rheinlande Westfalens **39**: 236–244.

Langhelle, A., M.J. Lindell & P. Nyström (1999): Effects of ultraviolet radiation on amphibian embryonic and larval development. — J. Herpetol. **33**: 449–456.

Lannoo, M.J. (2005): Amphibian Declines. The Conservation Status of United States Species. — Berkeley (Univ. California Press).

Lannoo, M.J. (2008): Malformed Frogs. The Collapse of Aquatic Ecosystems. — Berkeley (Univ. California Press).

Lannoo, M.J. (2009): Amphibian malformations. Pp. 3089–3111 in Heatwole, H. & J.W. Wilkinson (eds.): Amphibian Bio-

logy. Vol. 8: Amphibian Decline: Diseases, Parasites, Maladies, and Pollution. — Chipping Norton, Australia (Surrey Beatty).

Lanza, B. & D. Canestrelli (2002): Atypische Färbung bei *Salamandra terdigitata* (Lacépède, 1788) und *Bufo viridis viridis* Laurenti, 1768. — Salamandra **38**: 105–108.

Lanza, B., G. Nascetti & L. Bullini (1986): A new species of *Hydromantes* from eastern Sardinia and its generic relationships with the other Sardinian plethodontids (Amphibia: Urodela). — Boll. Mus. Reg. Sci. Nat. Torino **4(1)**: 261–289.

Laposata, M.M. & W.A. Dunson (1998): Effects of boron and nitrate on hatching success of amphibian eggs. — Arch. Environ. Contam. Toxicol. **35**: 615–619.

Laurenti, J.N. (1768): Austriaci viennensis specimen medicum, exhibens synopsin reptilium emendatam cum experimentis circa venena et antidota reptilium austriacorum. — Wien (Joan. Thom. De Trattnern).

Lauthier, M. (1971): Étude descriptive d'anomalies spontanées des membres postérieurs chez *Pleurodeles waltlii* Michah. — Ann. Embryol. Morphol. **4**: 65–78.

Lébédinsky, N.-G. (1921): Sur un tétard de *Rana temporia* L. bicéphale. — C.r. Soc. Biol. Fr. **85**: 791–792.

Lecamp, M. (1935): Les formations multiples dans la greffe et la régénération des membres chez le crapaud accoucheur (*Alytes obstetricans*, Laur.). — Bull. biol. Fr. Belg. Suppl. **19**: 1–149.

Lee, G.S., D.M. Kochhar & M.D. Collins (2004): Retinoid-induced limb malformations. — Current Pharmaceutical Design **10**: 2657–2699.

Lee, S.Y. & R.P. Elinson (2008): Abnormalities of forelimb and pronephros in a direct developing frog suggest a retinoic acid deficiency. — Appl. Herpetol. **5**: 33–46.

Leibovitz, H.E., D.D. Culley & J.P. Geaghan (1982): Effects of vitamin C and sodium benzoate on survival, growth and skeletal deformities of intensively cultured bullfrog larvae *Rana catesbeiana* reared at two pH levels. — J. World Maricul. Soc. **13**: 322–328.

Lenk, P. (1995): Zum Fund einer leukistischen Larve der Knoblauchkröte (*Pelobates fuscus*) aus Alzenau, Unterfranken. — Z. Feldherpetol. **2**: 221–223.

Lestage, J.A. (1926): La batrachomyase. — Les Naturalistes belges **7**: 157–159, 162–163.

Levengood, W.C. (1969): A new teratogenic agent applied to amphibian embryos. — J. Embryol. exp. Morph. **21**: 23-31.

Levey, R., N., Shambaugh, D. Fort & J. Andrews (2003): Investigations into the Causes of Amphibian Malformations in the Lake Chamberlain Basin of New England. — Waterbury (Vermont Dept. Environ. Conserv.).

Levinskaya, I.K. & S.S. Barinova (1978): On the ecology of the brown frogs of the south of Sakhalin. Pp. 21–23 in Bromlej, G.F. (ed.): Amfibii Dalnego Vostoka i Sibiri. — Vladivostok (Acad. Nauk.).

Levy, B.M. (1958): New method for the rapid determination of lathyrogenic agents. — Science **129**: 720.

Lindberg, A.J. (1995): *Ambystoma tigrinum tigrinum* (eastern tiger salamander). Reproduction and twinning. — Herpetol. Rev. **26**: 142.

Ling, R.L. & J.K. Werner (1988): Mortality in *Ambystoma maculatum* larvae du to *Tetrahymena* infection. — Herpetol. Rev. **19**: 26.

Liotti, F.S. & G. Bruschelli (1969): Ricerche sperimentali sulle malformazioni embrionali da vitamina A negli anfibi. — Riv. Biol. **42**: 99–133.

Lips, K.R. (1998): Decline of a tropical montane amphibian fauna. — Conserv. Biol. **12**: 106–117.

Lips, K.R. (1999): Mass mortality and population declines of anurans at an upland site in western Panama. — Conserv. Biol. **13**: 117–125.

Lips, K.R., J.R. Mendelson, A. Muñoz-Alonso, L. Canseco-Márquez & D.G. Mulcahy (2004): Amphibian population declines in montane southern Mexico: resurveys of historical localities. — Biol. Conserv. **119**: 555–564.

Lipsett, J.C. (1941): Disproportionate dwarfism in *Amblystoma*. — J. exp. Zool. **86**: 441–459.

Littlejohn, M.J., G.F. Watson & J.J. Loftus-Hills (1971): Contact hybridization in the *Crinia laevis* complex (Anura: Leptodactylidae). — Aust. J. Zool. **19**: 85–100.

Litvinchuk, S.N. (2014): Anomalies in two species of newts from north-western Russia: a comparative analysis. Pp. 127–135 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Loomis, R.B. (1956): The chigger mites of Kansas (Acarina, Trombiculidae). — Univ. Kansas Sci. Bull. **32(2)**: 1195–1443.

Lotter, F. & N.J. Scott (1977): Correlation between climate and distribution of the color morphs of the salamander *Plethodon cinereus*. — Copeia **1977**: 681–690.

Lowcock, L.A., T.F. Sharbel, J. Bonin, M. Ouellet, J. Rodrigue & J.-L. DesGranges (1997): Flow cytometric assay for in vivo genotoxic effects of pesticides in green frogs (*Rana clamitans*). — Aquat. Toxicol. **38**: 241–255.

Löwegren, Y. (1939): Nagra fall av batrachomyasis hos paddor i Sverige förorsakade av *Lucilia bufonivora* Mon. — Opuscula Entomol. **4**: 10–15.

Loyez, M. (1897): Sur un têtard de *Rana temporaria* bicéphale. — Bull. Soc. Zool. Fr. **22**: 146–148.

Lucké, B. & H.G. Schlumberger (1949): Neoplasia in cold-blooded vertebrates. — Physiol. Rev. **29**: 91–126.

Luef, H.-J. (2009): Beobachtung einer amelanistischen Erdkröte (*Bufo bufo*) in Niederösterreich. — Draco **38**: 40–43.

Luis, R. & M. Baez (1987): Anomalías morfológicas en los anfibios de las Islas Canarias (Amphibia, Anura). — Vieraea **17**: 295–296.

Lunde, K.B. & P.T.J. Johnson (2012): A practical guide for the study of malformated amphibians and their causes. — J. Herpetol. **46**: 429–441.

Lunde, K.B., V.H. Resh & P.T.J. Johnson (2012): Using an ecosystem-level manipulation to understand host-parasite interactions and how they vary with study venue. — Ecosphere **3(10)**: 84 (http://dx.doi.org/10.1890/ES12-00001.1).

Lusis, Y.Y. & I.A. Tsaune (1984): Giant tadpoles of green frogs in Latvia. — Zool. Muz. Rak. P. Stuckas Latvij Valsts Univ. **1984**: 117–128.

Luther, W. (1939): Die Strahlenwirkung auf Amphibienhaut vor und nach der Metamorphose. — Naturwissenschaften **43**: 713–720.

Luttenberger, F. (1976): Zur Ökologie der Braunfrösche, *Rana t. temporaria* und *Rana arvalis wolterstorffi*. — Das Aquarium **82**: 173–179.

Lynn, W.G. (1944): Duplicitas anterior in the toad, *Eleutherodactylus alticola*. — Anat. Rec. **89**: 345–355.

Lyons, D.B., C.H. Buckner, B.B. McLeod & K.M.S. Sundaram (1976): The Effects of Fenitrothion, Matacil, and Orthene on Frog Larvae. — Ottawa (Chem. Control Inst., Environ. Canada (Report CC-X-129)).

Mable, B.K. & L.A. Rye (1992): Developmental abnormalities in triploid hybrids between tetraploid and diploid tree frogs (genus *Hyla*). — Can. J. Zool. **70**: 2072–2076.

Machicao, N. & E. La Placa (1954): Lepra-like granulomas in frogs. — Laboratory Investigation **3**: 219–227.

MacMillan, G.J. (1979): An analysis of pigment cell development in the periodic albino mutant of *Xenopus*. — J. Embryol. exp. Morph. **52**: 165–170.

Maden, M. (1982): Vitamin A and pattern formation in the regenerating limb. — Nature **295**: 672–675.

Maden, M. (1983a): The effect of vitamin A on limb regeneration in *Rana temporaria*. — Develop. Biol. **98**: 409–416.

Maden, M. (1983b): The effect of vitamin A on the regenerating axolotl limb. — J. Embryol. exp. Morph. **77**: 273–295.

Maden, M. & J. Corcoran (1996): Role of thyroid hormone and retinoid receptors in the homeotic transformation of tails into limbs in frogs. — Develop. Genetics **19**: 85–93.

Magnusson, W.E., A.P. Lima, J.-M. Hero, & M.C. de Araujo (1999): The rise and fall of a population of *Hyla boans*: reproduction in a Neotropical gladiator frog. — J. Herpetol. **33**: 647–656.

Mahendra, B.C. (1936): A case of polymely in the Indian bullfrog, *Rana tigrina* Daud.. — Proc. Indian Acad. Sci (B) **4**: 483–493.

Malacinski, G.M. (1978): The Mexican axolotl, *Ambystoma mexicanum*: its biology and developmental genetics, and its autonomous cell-lethal genes. — Am. Zool. **18**: 195–206.

Malacinski, G.M. & A.J. Brothers (1974): Mutant genes in the Mexican axolotl. — Science **184**: 1142–1147.

Malaj, E., P.C. von der Ohe, M. Grote, R. Kühne, C.P. Mondy, P. Usseglio-Polatera, W. Brack & R.B. Schäfer (2014): Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. — PNAS **111**: 9549–9554.

Malkmus, R. (2008): Der Stichling (*Gasterosteus aculeatus*) als Prädator von Larven des Grasfrosches (*Rana temporaria*). — Nachr. Naturwiss. Mus. Aschaffenburg **109**: 35–42.

Manenti, R. (2006): Un cas de mélanisme chez *Euproctus montanus* (Savi, 1838). — Bull. Soc. Herpétol. France **117**: 65–66.

Mangold, O. (1955): Die Entwicklung kleinster und größter Keime von *Triton alpestris* bei Fütterung und Hunger. Wachstum, Hinterextremitäten, Hungererscheinungen. — Roux Arch. Entwickl.-mech. **147**: 373–404.

Mangold, O. & T. Peters (1956): Über die Wirkung gleicher Röntgendosen auf verschiedene Stadien der Frühentwicklung von *Triton alpestris*. — Beitr. pathol. Anat. allgem. Pathol. **116**: 478–516.

Mann, R.M., R.V. Hyne, C.B. Choung & S.P. Wilson (2009): Amphibians and agricultural chemicals: review of the risks in a complex environment. — Environ. Pollut. **157**: 2903–2927.

Marchal-Ségault, D. & F. Ramade (1981): The effects of lindane, an insecticide, on hatching and postembryonic development of *Xenopus laevis* (Daudin) anuran amphibian. — Environ. Res. **24**: 250–258.

Marco, A., C. Quilchano & A.R. Blaustein (1999): Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. — Environ. Toxicol. Chem. **18**: 2836–2839.

Marian, M.P., V. Arul & T.J. Pandian (1983): Acute and chronic effects of carbaryl on survival, growth, and metamorphosis in the bullfrog (*Rana tigrina*). — Arch. Environ. Contam. Toxicol. **12**: 271–275.

Marshall, G.A., R.L. Amborski & D.D. Culley (1980): Calcium and pH requirements in the culture of bullfrog (*Rana catesbeiana*) larvae. — Proc. World Maricul. Soc. **11**: 445–453.

Martin, G.R. (1998): The roles of FGFs in early development of vertebrate limbs. — Genes Develop. **12**: 571–586.

Martin, R. & J. Signoret (1968): Contribution à l'étude de la mutation «short toes» chez l'Axolotl. — Ann. Embryol. Morphol. **1**: 141–149.

Martínez, I., R. Álvarez, I. Herráez & P. Herráez (1992): Skeletal malformations in hatchery reared *Rana perezi* tadpoles. — Anat. Rec. **233**: 314–320.

Martof, B.S. (1956): Factors influencing size and composition of populations of *Rana clamitans*. — Am. Midl. Nat. **56**: 224–245.

Martof, B.S. (1962): Some aspects of the life history and ecology of the salamander *Leurognathus*. — Am. Midl. Nat. **67**: 1–35.

Martof, B.S. & K.G. Walton (1965): A study of the unusual pigmentation in the salamander *Leurognathus marmoratus*. — Physiol. Zool. **38**: 349–352.

Maruschak, A. & A. Muravynets (2013): Morphological abnormalities in natural populations of anurans (Amphibia, Anura) in Ukraine. — Prazi Ukrain. Gerpetol. Tovaristva **4**: 87–94.

Marvin, G.A. (1995): *Plethodon glutinosus* (slimy salamander). Morphology. — Herpetol. Rev. **26**: 30.

Marvin, G.A. & V.H. Hutchison (1997): *Plethodon kentucki* (Cumberland Plateau woodland salamander). Morphology. — Herpetol. Rev. **28**: 199.

Materna, E.J., C.F. Rabeni & T.W. LaPoint (1995): Effects of the synthetic pyrethroid insecticide, esfenvalerate, on larval leopard frogs (*Rana* spp.). — Environ. Toxicol. Chem. **14**: 613–622.

Mattes, D. (2013): Schwärzepilz bei Erdkröten am Niederrhein entdeckt. — Z. Feldherpetol. **20**: 249.

McAllister, C.T., C.R. Bursey, S.J. Upton, S.E. Trauth & D.B. Conn (1995): Parasites of *Desmognathus brimleyorum* (Caudata: Plethodontidae) from Ouachita Mountains of Arkansas and Oklahoma. — J. Helminthol. Soc. Wash. **62**: 150–156.

McCallum, M.L. & S.E. Trauth (2003): A fourty-three year museum study of northern cricket frog (*Acris crepitans*) abnormalities in Arkansas: upward trends and distributions. — J. Wildl. Dis. **39**: 522–528.

McCoy, K.A. & L.J. Guillette (2009): Endocrine disrupting chemicals. Pp. 3208-3238 in Heatwole, H. & J.W. Wilkinson (eds.): Amphibian Biology. Vol. 8: Amphibian Decline: Diseases, Parasites, Maladies, and Pollution. — Chipping Norton, Australia (Surrey Beatty).

McMaster, M. (2001): Congenital scoliosis. Pp. 161–177 in Weinstein, S.L. (ed.): The Pediatric Spine. Principles and Practice. — Phildelphia (Lippincott Williams & Wilkins).

Means, D.B. & S.C. Richter (2007): Genetic verification of possible gigantism in a southern toad, *Bufo terrestris*. — Herpetol. Rev. **38**: 297–298.

Medina, R.G., M.L. Ponssa, C. Guerra & E. Aráoz (2013): Amphibian abnormalities: historical records of a museum collection in Tucumán Province, Argentina. — Herpetol. J. **23**: 193–202.

Meijer, R.M. (1962): De kweek van *Phyllomedusa hypochondrialis*. — Lacerta **9**: 67–72.

Meisterhans, K. & H. Heusser (1970): *Lucilia*-Befall an vier Anuren-Arten (Dipt. Tachinidae). — Mitt. Schweiz. Entomol. Ges. **43**: 41–44.

Mendelson, J.R., E.D. Brodie, J.H. Malone, M.E. Acevedo, M.A. Baker, N.J. Smatresk & J.A. Campbell (2004): Factors associated with the catastrophic decline of a cloudforest frog fauna in Guatemala. — Rev. Biol. tropcial **52**: 991–1000.

Mertens, R. (1921): Zoologische Streifzüge in Rumänien. — Bl. Aquar. Terrarienkde **32**: 247–252.

Mertens, R. (1941): Ist "*Molge macrosoma* Boulenger" eine gute Spezies? — Zool. Anz. **137(7–8)**: 128–131.

Meteyer, C.U. (2000): Field Guide to Malformations of Frogs and Toads with Radiographic Interpretations. — Biol. Sci. Report **USGS/BRD/BSR-2000-0005**: 1–16.

Meteyer, C.U., I.K. Loeffler, J.F. Fallon, K.A. Converse, E. Green, J.C. Helgen, S. Kersten, R. Levey, L. Eaton-Poole & J.G. Burkhart (2000a): Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. — Teratology **62**: 151–171.

Meteyer, C.U., R.A. Cole, K.A. Converse, D.E. Docherty, M. Wolcott, J.C. Helgen, R. Levey, L. Eaton-Poole & J.G. Burkhart (2000b): Defining anuran malformations in the context of a developmental problem. — J. Iowa Acad. Sci. **107(3)**: 72–78.

Meyer, E. (1996): Eine oliv-gelbe Variante von *Dendrobates granuliferus* aus dem zentral-pazifischen Tiefland Costa Ricas: erste Beobachtungen zur Fortpflanzungsbiologie. — herpetofauna, Weinstadt **18(100)**: 21–26.

Meyer, J.D. (1748–1756): Angenehmer und nützlicher Zeit-Vertreib mit Betrachtung curioser Vorstellungen allerhand kriechender, fliegender und schwimmender, auf dem Land und im Wasser sich befindender und nährender Thiere..... — Nürnberg (Johann Joseph Fleischmann).

Meyer-Rochow, V.B. & M. Asashima (1988): Naturally occurring morphological abnormalities in wild populations of the Japanese newt *Cynops pyrrhogaster* (Salamandridae; Urodela; Amphibia). — Zool. Anz. **221**: 70–80.

Michael, M.I. & A.A. Hassona (1982): Intercalary and supernumery regeneration in limbs of stages of the Egyptian toad, *Bufo regularis* Reuss. — J. exp. Zool. **220**: 207–218.

Michel, M.J. & S. Burke (2011): Consequences of an amphibian malformity for development and fitness in complex environments. — Freshwater Biol. **56**: 1417–1425.

Michelot, M. (1980): Quelques notes sur la faune herpétologique de la Corse. — Bull. Soc. Herpétol. France **15**: 27–44.

Mikamo, K. & Hamaguchi, H. (1973): Chromosomal disorders caused by preovulatory overripeness of oocytes. Pp. 72–97 in Blandau, R.J. (ed.): Aging Gametes International Symposium. — Basel (Karger).

Mikitinez, G.I. (2014): Geographic occurences of morhological anomalies in populations of anurans in the steppe zone in Ukraine. Pp. 136–144 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Miller, B.T. & J.L. Miller (2005): Prevalence of physical abnormalities in eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) populations of middle Tennessee. — Southeastern Naturalist **4**: 513–520.

Miller, J.E. & A.L. Braswell (2006): *Eurycea cirrigera* (southern two-lined salamander). Coloration. — Herpetol. Rev. **37**: 198.

Milto, K.D. (2009): A giant tadpole record of *Rana esculenta* in northwestern Russia. — Russ. J. Herpetol. **16**: 143–145.

Ming, L.T. (2001): Parasitic copepods responsible for limb abnormalities? — Froglog **46**: 2–3.

Miranda, M. (1989): Supervivencia de hibridos de *Ceratophrys ornata* × *Ceratophrys cranwelli* (Anura: Ceratophryinae). — Bol. Asoc. Herpetol. Argentina **5**: 7–8.

Mitchell, J.C. & D.R. Church (2002): Leucistic marbled salamanders (*Ambystoma opacum*) in Virginia. — Banisteria **20**: 67–69.

Mitchell, J.C., E. Lanham, M. Kangas, H. Germaine & W. Brown (2004): *Plethodon cinereus* (red-backed salamander). Anophthalmia. — Herpetol. Rev. **35**: 366.

Mizell, M. (1969): Biology of amphibian tumors. — Recent Results Cancer Res. Special Suppl. **17**: 1–484.

Mizgireuv, I.V., N.L. Flax, L.J. Borkin & V.V. Khudoley (1984): Dysplastic lesions and abnormalities in amphibians associated with environmental conditions. — Neoplasma **31**: 175–181.

Mlynarski, M. (1961): Plazy (Amphibia) z pliocenu Polski. — Acta Palaeontol. Polon. **3**: 261–282.

Møller, A.P., T.A. Mousseau, F. de Lope & N. Saino (2007): Elevated frequency of abnormalities in barn swallows from Chernobyl. — Biol. Lett. **3**: 414–417.

Moniez, R. (1876): Un diptère parasite du crapaud (*Lucilia bufonivora* n. sp.). — Bull. Sci. Hist. Litt. Depart Nord **8(2)**: 25–27.

Montalenti, G. (1933): L'ontogenesi degli ibridi fra *B. vulg.* e *B. viridis*. — Physiol. Zool. **6**: 329–395.

Morgan, M.K., P.R. Scheuermann, C.S. Bishop & R.A. Pyles (1996): Teratogenic potential of atrazine and 2,4-d using FETAX. — J. Toxicol. Environ. Health **48**: 151–168.

Mortensen, R.C. (1892): *Lucilia sylvarum* Meig. als Schmarotzer an *Bufo vulgaris*. — Zool. Anz. **15**: 193–195.

Moskvitina, N.S., V.N. Kuranova & S.V. Saveliev (2011): Abnormalities of embryonal development of vertebrates under the condition of technogenic environmental pollution. — Contemporary Probl. Ecol. **4**: 366–372.

Mott, C.L. & M.A. Steffen (2014): Associations between nonlethal injury, body size, and foraging ecology in an amphibian intraguild predator. — Ethology **120**: 42–52.

Muneoka, K., G. Holler-Dinsmore & S.V. Bryant (1986): Intrinsic control of regenerative loss in *Xenopus laevis* limbs. — J. exp. Zool. **240**: 47–54.

Munshaw, R.G., W.I. Atlas, W.J. Palen, D.M. Courcelles & Z.L. Monteith (2014): Correlates and consequences of injury in a large, predatory stream salamander (*Dicamptodon tenebrosus*). — Amphibia-Reptilia **35**: 107–116.

Murphy, J.C. (1980): Green, blue, and yellow frogs. — Bull. Chicago Herpetol. Soc. **15**: 103–106.

Murphy, T.D. (1965): High incidence of two parasitic infestations and two morphological abnormalities in a populaton of the frog, *Rana palustris* Le Conte. — Am. Midl. Nat. **74**: 233–239.

Muto, Y. (1969a): Hindlimb development and malformations of toes in the larvae reared at high temperature in the toad, *Bufo vulgaris formosus*. — Cong. Anom. **9**: 1–12.

Muto, Y. (1969b): Anomalies in the hindlimb skeletons of the toad larvae reared at a high temperature. — Cong. Anom. **9**: 61–73.

Muto, Y. (1970): Digital malformations in the forelimbs of the toad larvae reared at a high temperature. — Cong. Anom. **10**: 135–147.

Mutschmann, F. (2010): Erkankungen der Amphibien. 2. Aufl.. — Stuttgart (Enke).

Mutz, T. (1999): Sardische Höhlensalamander. — elaphe **7(2)**: 72–73.

Nassanov, N.V. (1930): Die Regeneration der Axolotlextremitäten nach Ligaturanlegung. — Roux Arch. Entwickl.-mech. **115**: 637–657.

Nath, K. (1940): Von Albinos des Feuersalamanders und von Embryonen der Perleidechse. — Wschr. Aquar. Terrarienkunde **37**: 7–8.

Nekrasova, O.D. (2014): Some aspectes of the manifestation of anomalies in the colouration of amphibians. Pp. 144–149 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Nekrasova, O.D., S.V. Mezhzherin, S.Y. Morozov-Leonov & Y.N. Sytnik (2007): Cases of mass polymelia in the lake frog (*Rana ridibunda* Pall., 1771) from Kiev. — Sci. Bull. Uzhgorod Univ. (Ser. Biol.), **21**: 92–95.

Nemes, S. (2005): Comparative morphometric analysis of injured and uninjured newly metamorphosed smooth newts (*Triturus vulgaris*). — North-Western J. Zool. **1**: 61–63.

Neukomm, S. & M. Luder-Huguenin (1960): Investigations on the neoplasic power of chemical compounds in newt. — Oncologia **13**: 294–303.

Neumann, V. & F. Meyer (1994): *Lucilia bufonivora* Moniez, 1876 – ein euryxener Amphibienparasit (Insecta: Diptera: Calliphoridae). — Mitt. Zool. Mus. Berlin **70**: 331–341.

Newcomer, C.E., M.R. Anver, J.L. Simmons, B.W. Wilcke & G.W. Nace (1982): Spontaneous and experimental infections of *Xenopus laevis* with *Chlamydia psittaci*. — Lab. Anim. Sci. **32**: 680–686.

Niazi, I.A. (1996): Background to work on retinoids and amphibian limb regeneration: studies on anuran tadpoles – a retrospect. — J. Biosci. **21**: 273–297.

Niazi, I.A. & S. Alam (1984): Regeneration of whole limbs from shank stumps in toad tadpoles treated with vitamin A. — Roux's Arch. Dev. Biol. **193**: 111–116.

Niazi, I.A. & C.S. Ratnasamy (1984): Regeneration of whole limbs in toad tadpoles treated with retinol palmitate after the wound-healing stage. — J. exp. Zool. **230**: 501–505.

Niccoli, J.R. (2013): *Acris crepitans* (northern cricket frog). Axanthism. — Herpetol. Rev. **44**: 117.

Nichols, D.K., E.W. Lamirande, A.P. Pessier & J.E. Longcore (2001): Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. — J. Wildl. Dis. **37**: 1–11.

Nichols, R.J. (1937): Taxonomic studies on the mouth parts of larval anurans. — Illinois Biol. Monogr. **15(4)**: 1–73.

Nieuwkoop, P.D. & F. Faber (1994): Normal table of *Xenopus laevis*. — New York (Garland).

Nijs, J. & H. Keller (2002): *Rana temporaria* (European common frog). Albino larvae. — Herpetol. Rev. **33**: 131.

Nishimura, N., Y. Fukazawa, H. Uchiyama & T. Iguchi (1997): Effects of estrogenic hormones on early development of *Xenopus laevis*. — J. exp. Zool. **278**: 221–233.

Nishioka, M. (1977): Color variants induced by radiation and their inheritance in *Rana nigromaculata*. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **2**: 25–89.

Nishioka, M. (1978): Abnormalities in the descendants of *Rana japonica* produced from irradiated eggs or sperm. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **3**: 189–384.

Nishioka, M & H. Ohtani (1986): Detection of chromosomes bearing the loci for blue and olive mutations in *Rana nigromaculata*. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **8**: 1–27.

Nishioka, M. & H. Ueda (1977a): An electro-microscopic study on six kinds of color variants induced by radiation in *Rana nigromaculata*. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **2**: 91–102.

Nishioka, M. & H. Ueda (1977b): Genetic and morphologic studies on ten albino stocks in *Hyla arborea japonica*. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **2**: 103–164.

Nishioka, M. & H. Ueda (1983): Studies on polyploidy in Japanese treefrogs. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **6**: 207–252.

Nishioka, M. & H. Ueda (1985a): Genetics and morphology of 13 albino stocks in the *Rana nigromaculata* group. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **7**: 1–121.

Nishioka, M. & H. Ueda (1985b): Electron-microscopic observation on the dermal chromatophores of normal frogs and three kinds of color variants in *Rhacophorus schlegelii*. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **7**: 123–155.

Nishioka, M. & H. Ueda (1985c): Two kinds of black-eyed variants in *Hyla arborea japonica*. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **7**: 157–179.

Nishioka, M. & H. Ueda (1985d): Blue variants in *Hyla arborea japonica*. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **7**: 181–197.

Nishioka, M. & H. Ueda (1985e): Non-inheritable color variants in *Rana brevipoda porosa*. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **7**: 199–217.

Nishioka, M., H. Okumoto & Y. Kondo (1981): Effects of ultraviolet rays on the sperm of *Rana japonica*. I. Production of gynogenetic haploids. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **5**: 47–67.

Nishioka, M, H. Ohtani & M. Sumida (1987): Chromosomes and the sites of five albino gene loci in the *Rana nigromaculata* group. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **9**: 1–52.

Noble, G.K. (1931): The Biology of the Amphibia. — New York (McGraw-Hill).

Noden, D.M. & A. de Lahunta (1985): The Embryology of Domestic Animals: Developmental Mechanisms and Malformations. — Baltimore, Maryland (Williams & Wilkins).

Nöllert, A. (1990): Die Knoblauchkröte. — Wittenberg-Lutherstadt (Ziemsen).

Nunes, A.L., M.J. Cruz, M. Tejedo, A. Laurila & R. Rebelo (2010): Nonlethal injury caused by an invasive alien predator and its consequences for an anuran tadpole. — Basic Appl. Ecol. **11**: 645–654.

Ny, A., M. Koch, M. Schneider, E. Neven, R.T. Tong, S. Maity, C. Fischer, S. Plaisance, D. Lambrechts, C. Héligon, S. Terclavers, M. Ciesiolka, R. Kälin, W. Yan Man, I. Senn, S. Wyns, F. Lupu, A. Brändli, K. Vleminckx, D. Collen, M. Dewerchin, E.M. Conway, L. Moons, R.K. Jain & P. Carmeliet (2005): A genetic *Xenopus laevis* tadpole model to study lymphangiogenesis. — Nature Medicine **11**: 998–1004.

Nye, H.L.D., J.A. Cameron, E.A.G. Chernoff & D.L. Stocum (2003): Regeneration of the urodele limb: a review. — Develop. Dynamics **226**: 280–294.

O'Connor, K.M., T.A.G. Rittenhouse & J.L. Brunner (2016): *Ranavirus* is common in wood frog (*Lithobates sylvaticus*) tadpoles throughout Connecticut, USA. — Herpetol. Rev. **47**: 494–397.

Oerter, K. (1985): Morphologische und histopathologische Untersuchungen zur Entwicklung von *Bufo bufo* nach Bestrahlung mit Co-60 im Schwanzknospenstadium. — Bonn (Unveröff. Diplomarb., Univ. Bonn).

Oerter, K. & G. Kneitz (1994): Zur Wirksamkeit von Ersatzlaichgewässern für Amphibien beim Bundesfernstraßenbau. — Forschung Straßenbau Straßenverkehrstechnik **675**: 3–211.

Oeser, G. (1952): Albinotische Amphibien. — DATZ **5**: 306–308.

Ogielska-Nowak, M. (1985): Stages of normal and spontaneous abnormal development of the natural hybrid, *Rana esculenta* L. (Amphibia, Anura). — Zool. Polon. **32(1-2)**: 37–62.

Olivier, E. (1893): Un crapaud phénomène. — Rev. Sci. Bourbon. Centr. Fr. **6**: 105, pl. II.

Olson, M.E., S. Gard, M. Brown, R. Hampton & D.W. Morck (1992): *Flavobacterium indologenes* infection in leopard frogs. — J. Am. Vet. Med. Assoc. **201**: 1766–1770.

Opatrny, E. (1979): Fund eines melaninlosen Feuersalamanders *Salamandra salamandra* (L.). — Acta Univ. Palackianae Olomucensis Fac. Rerum Natur. **63**: 239–242.

Oppermann, R., Y. Liczner & A. Classen (1997): Auswirkungen von Landmaschinen auf Amphibien und Handlungsempfehlungen für Naturschutz und Landwirtschaft. — Singen (Inst. Landschaftsökol. Naturschutz).

Osborn, D., A.S. Cooke & S. Freestone (1981): Histology of a teratogenic effect of DDT on *Rana temporaria* tadpoles. — Environ. Pollut. Ser. A **25**: 305–319.

Osborne, W.S. & J.A. Norman (1991): Conservation genetics of corroboree frogs, *Pseudophryne corroboree* Moore (Anura: Myobatrachidae): population subdivision and genetic divergence. — Aust. J. Zool. **39**: 285–297.

Ouellet, M. (2000): Amphibian deformities: current state of knowledge. Pp. 617–661 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. — Pensacola (SETAC Press).

Ouellet, M., J. Bonin, J. Rodrigue, J.-L. DesGranges & S. Lair (1997): Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. — J. Wildl. Dis. **33**: 95–104.

Pabijan, M., E. Czarniewska & L. Berger (2004): Amelanistic phenotypes in Western Palearctic water frogs from Poland. — Herpetozoa **17**: 127–134.

Pacces-Zaffaroni, N., E. Arias & T. Zavanella (1992): Natural variation in the limb skeletal pattern of the crested newt, *Triturus carnifex* (Amphibia: Salamandridae). — J. Morphol. **213**: 265–273.

Pacces-Zaffaroni, N., E. Arias, S. Lombardi & T. Zavanella (1996): Natural variation in the appendicular skeleton of *Triturus carnifex* (Amphibia: Salamandridae). — J. Morphol. **230**: 167–175.

Padgett-Flohr, G.E. & M.E. Goble (2007): Evaluation of tadpole mouthpart depigmentation as a diagnostic test for infection by *Batrachochytrium dendrobatidis* for four California anurans. — J. Wildl. Dis. **43**: 690–699.

Paganelli, A., V. Gnazzo, H. Acosta, S.L. López & A.E. Carrasco (2010): Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. — Chem. Res. Toxicol. **23**: 1586–1595.

Pahkala, M., A. Laurila & J. Merilä (2001): Carry-over effects of ultraviolet-B radiation on larval fitness in *Rana temporaria*. — Proc. R. Soc. London, Ser. B **268**: 1699–1706.

Pahkala, M., K. Räsänen, A. Laurila, U. Johanson, L.O. Björn & J. Merila (2002): Lethal and sublethal effects of UV-B/pH synergism on common frog embryos. — Conserv. Biol. **16**: 1063–1073.

Pandey, A.K. & V. Tomar (1985): Melanophores in *Bufo melanostictus* (Schneider) tadpoles following exposure to the insecticide dimethoate. — Bull. Environ. Contam. Toxicol. **35**: 796–801.

Parent, G.H. & R. Thorn (1983): Un cas de flavisme chez le Triton alpestre (*Triturus alpestris* Laur.) au Grand-Duché de Luxembourg. — Rev. fr. Aquariol. **10**: 21–24.

Parichy, D.M. & R.H. Kaplan (1992): Developmental consequences of tail injury on larvae of the oriental fire-bellied toad, *Bombina orientalis*. — Copeia **1992**: 129–137.

Pascolini, R., P. Daszak, A.A. Cunningham, S. Tei, D. Vagnetti, S. Bucci, A. Fagotti & I. Di Rosa (2003): Parasitism by *Dermocystidium ranae* in a population of *Rana esculenta* complex in Central Italy and description of *Amphibiocystidium* n. gen. — Dis. Aquat. Org. **56**: 65–74.

Passmore, N.I. & V.C. Carruthers (1995): South African Frogs. A Complete Guide. Revised Edition. — Johannesburg (Southern Book).

Pastors, J. & H. Greven (2016): Die schwierige Klassifizierung von Farbabweichungen bei einheimischen Amphibien. — Feldherpetol. Magazin **5**: 8–15.

Patel, A.M., P.A. Kulkarni, K.G. Girish, H.P. Gurushankara & S.V. Krishnamurthy (2008): *Fejervarya limnocharis* (Indian cricket frog). Morphology. — Herpetol. Rev. **39**: 77.

Patel, Y.A., J.N. Cavin & M.K. Moore (2012): Morphological anomalies as indicators of chytrid infection in *Bufo marinus* from Trinidad, West Indies. — BIOS **83**: 75–80.

Patrick, D.A., E.B. Harper, L. Malcolm, L. Hunter & A.J.K. Calhoun (2008): Terrestrial habitat selection and strong density-dependent mortality in recently metamorphosed amphibians. — Ecology **89**: 2563–2574.

Paulov, S. (1977): The effects of N-phenyl-N'-methylurea (Defenuron) on the development of Amphibia (*Rana temporaria* L.). — Biologia (Bratislava) **32**: 607–611.

Pawar, K.R. & M. Katdare (1983): Effect of the insecticide sumithion (fenitrothion) on embryonic development in a frog. — Experientia **39**: 297–298.

Pawar, K.R., H.V. Ghate & M. Katdare (1983): Effect of malathion on embryonic development of the frog *Microhyla ornata* (Duméril & Bibron). — Bull. Environ. Contam. Toxicol. **31**: 170–176.

Pearse, R.V. & C.J. Tabin (1998): The molecular ZPA. — J. exp. Zool. **282**: 677–690.

Peltzer, P.M., M.L. Ponssa & R.C. Lajmanovich (2001): Caso de malformacion en *Leptodactylus mystacinus* (Anura, Leptodactylidae). — Natura Neotropicalis **32**: 165–168.

Peltzer, P.M., R.C. Lajmanovich, L.C. Sanchez, A.M. Attademo, C.M. Junges, C.L. Bionda, A.L. Martino & A. Bassó (2011): Morphological abnormalities in amphibian populations from the Mid-Eastern region of Argentina. — Herpetol. Conserv. Biol. **6**: 432–442.

Pereira, R. & S. Rocha (2004): *Chioglossa lusitanica* (golden-striped salamander): Dicephalic larva. — Herpetol. Bull. **87**: 29–30.

Pérez-Coll, C.S., J. Herkovits & A. Salibian (1985): Efectos del cadmio sobre el desarrollo de un anfibio. — Arch. Biol. Med. Exp. **18**: 33–40.

Pérez-Coll, C.S., J. Herkovits & A. Salibian (1986): Teratogenic effects of cadmium on *Bufo arenarum* during gastrulation. — Experientia **42**: 1174–1176.

Peters, B. (1962): Albino bullfrog (*Rana catesbeiana*) population found in Leandro Creek, Alameda County, California. — Turtox News **40(1)**: 7.

Peterson, S.M., A.G. Wilson & E.M. Wilson (1999): *Plethodon idahoensis* (coeur d'Alene salamander). Skeletal abnomality. — Herpetol. Rev. **30**: 222.

Pfeiffer, C.J. & M. Asashima (1997): Ultrastructure of *Rhabdias tokyoensis*, a pulmonary nematode of the Japanese newt (*Cynops pyrrhogaster*). — Amphibia-Reptilia **18**: 283–294.

Pfeiffer, C.J., T. Nagai, M. Fujimura & T. Tobe (1979): Spontaneous regressive epitheliomas in the Japanese newt, *Cynops pyrrhogaster*. — Cancer Res. **39**: 1904–1910.

Pflüger, E. & W.J. Smith (1883): Untersuchungen über Bastardierung der anuren Batrachier und die Principien der Zeugung. I. Teil: Experimente über Bastardierung der anuren Batrachier. — Arch. ges. Physiol. Mensch Thiere **32**: 519–541.

Pflugfelder, O. (1959): Atypische Gewebsdifferenzierungen bei *Xenopus laevis* Daudin nach experimenteller Verhinderung der Metamorphose. — Roux Arch. Entwickl.-mech. **151**: 229–241.

Picariello, O., F.M. Guarino & F. Barbieri (2006): *Rana dalmatina* Bonaparte, 1838. Pp. 352–357 in Sindaco, R., G. Doria, E. Razzetti & F. Bernini (eds.): Atlante degli Anfibi e dei Rettili d'Italia. — Firenze (Polistampa).

Piha, H., M. Pekkonen & J. Merilä (2006): Morphological abnormalities in amphibians in agricultural habitats: a case study of the common frog *Rana temporaria*. — Copeia **2006**: 810–817.

Piiper, E. (1933): The development of *Rana temporaria* under the influence of cane sugar solution. — Proc. R. Soc. London, ser. B **112**: 359–365.

Pinder, R. (2010): *Rana clamitans* (green frog). Coloration. — Herpetol. Rev. **41**: 66.

Pisanets, E.M. (1992): Evidence of hybridization between di- and tetraploid toads (*Bufo viridis*-complex) from Eurasia (Anura: Bufonidae). Pp. 357–360 in Korsós, Z. & I. Kiss (eds.): Proc. 6th Ord. Gen. Meet. SEH. — Budapest (Hungarian Natl. Mus.).

Pliss, G.B. & V.V. Khudoley (1979): Oncogenesis and cancerogenious factors in primitive vertebrates and invertebrate animals. Pp. 167–185 in Sokolov, V.E. (ed.): Ekologicheskoe Prognozirovanie. — Moscow (Akad. Nauk.).

Plowman, M.C., S. Grbac-Ivankovic, J. Martin, S.M. Hopfer & F.W. Sunderman (1994): Malformations persist after metamorphosis of *Xenopus laevis* tadpoles exposed to Ni^{2+}, Co^{2+}, or Cd^{2+} in FETAX assays. — Teratogenesis, Carcinogenesis, Mutagenesis **14**: 135–144.

Pogonowska, I. (1914): Über den Einfluß chemischer Faktoren auf die Farbveränderung des Feuersalamanders. I. Mitteilung: Einfluß von Kochsalzlösung. — Arch. Entwicklungsmech. **39**: 352–361.

Pohland, H.-L. (1962): Die Wirkung von Thymuswirkstoffen auf Wachstum und Metamorphose sowie deren Einfluss auf endokrine Drüsen bei *Xenopus laevis* Daudin. — Roux Arch. Entwickl.-mech. **153**: 621–663.

Ponse, K. (1941a): Sur l'ectromélie bithoracique et diverses anomalies présentées par les descendants issus d'une ponte bidérienne d'un crapaud femelle (Note préliminaire). — Rev. suisse Zool. **48**: 545–551.

Ponse, K. (1941b): Une mutation dominante chez le Crapaud: l'ectromélie bithoracique. — C.r. Soc. Phys. Hist. nat. Genève **1949**: 174–176.

Pope, C.H. & S.H. Pope (1951): A study of the salamander *Plethodon ouachitae* and the description of an allied form. — Bull. Chicago Acad. Sci. **9(8)**: 129–152.

Pourhadi, R., C. Bonhomme & J.P. Turchini (1968): Action tératogène des ultrasons sur des oeufs de Batraciens. — Arch. Anat. Microscop. **57**: 255–274.

Power, T., K.L. Clark, A. Harfenist & D.B. Peakall (1989): A review and evaluation of the amphibian toxicological literature. — Techn. Rep., Can. Wildl. Serv. **61**: 1–91.

Pracht, A. & M. Rogner (1987): Farbanomalien bei der Gelbbauchunke *Bombina variegata* (Linnaeus, 1758) (Anura: Discoglossidae). — Salamandra **23**: 68–69.

Preudhomme de Borre, A. (1876): Sur un crapaud dévoré vivant par des larves des Muscides. — C.r. Soc. Entomol. Belg. **19 (7. oct. 1876)**: LXIV.

Prigioni, C.M. & J.A. Langone (1985): Anomalias anatomicas registradas en anfibios de la coleccion herpetologica del Museo Nacional de Historia Natural. — Actas Jorn. Zool. Uruguay **1985**: 73–75.

Przibram, H. (1921): Die Bruch-Dreifachbildung im Tierreich. — Arch. Entwicklungsmech. Organismen **48**: 205–444.

Puky, M. (2006): Amphibian deformity frequency and monitoring methodology in Hungary. — Froglog **74**: 3–4.

Puky, M. & A. Fodor (2002): Occurrence of amphibian deformities along the Hungarian section of the River Danube, Tisza and Ipoly. — Limnol. Rep. Int. Assoc. Danube Res. **34**: 845–852.

Pyastolova, O.A. & V.L. Vershinin (1999): Some cytological features of *Rana arvalis* Nilss. frogs on the territory of the Eastern Ural radioactive trace. — Russ. J. Ecol. **30**: 30–35.

Pyastolova, O.A., V.L. Vershinin, E.A. Trubezkaja & E.Z. Gatiyatullina (1996): Utilization of amphibians in bioindication research on territories of the Eastern Urals radioactive trace. — Russ. J. Ecol. **27**: 361–365.

Qin, Z.F., J.M. Zhou, L. Cong & X.-B. Xu (2005): Potential ecotoxic effects of polychlorinated biphenyls on *Xenopus laevis*. — Environ. Toxicol. Chem. **24**: 2573–2578.

Quinzio, S. & J. Goldberg (2015): Intradermal infections by chigger mites (*Hannemannia* spp.) in the Andean frog *Telmatobius atacamensis* (Anura, Telmatobiidae). — Salamandra **51**: 263–268.

Rachowicz, L.J. (2002): Mouthpart pigmentation in *Rana muscosa* tadpoles: seasonal changes without chytridiomycosis. — Herpetol. Rev. **33**: 263–265.

Rachowicz, L.J. & V.T. Vredenburg (2004): Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. — Dis. Aquat. Org. **61**: 75–83.

Raichoudhury, D.P. & B.K. Das (1931): Observations on the malformations in the common Bengal toad, *Bufo melanostictus*. — Anat. Anz. **71**: 120–131.

Raimondo, S.M., C.L. Rowe & J.D. Congdon (1998): Exposure to coal ash impacts swimming performance and predator avoidance in larval bullfrogs (*Rana catesbeiana*). — J. Herpetol. **32**: 289–292.

Raj, T.P., A. Jebanesan, M. Selvanayagam & G.J. Manohar (1988): Effect of organophosphorus (Nuvan) and carbamate (Baygon) compounds on *Rana hexadactyla* (Lesson) with a note on body protein and liver glycogen. — Geobios **15**: 25–32.

Rajakaruna, R.S., P.M.J.R. Piyatissa, U.A. Jayawardena, A.N. Navaratne & P.H. Amerasinghe (2008): Trematode infection induced malformations in the common hourglass treefrogs. — J. Zool. **275**: 89–95.

Rakhimzhanova, A.K. & V.A. Khromov (1998): Morphological study of the green frog (*Rana ridibunda* Pallas, 1771) from eastern Khazakstan province. — Asian Herpetol. Meet. Almaty Abstracts **3**: 31.

Rane, P.D. & D.S. Mathur (1978): Toxicity of aldrin to *Rana cyanophlyctis* (Schn.). — Science and Culture **44**: 128–130.

Raxworthy, C.J. (1989): Courtship, fighting and sexual dimorphism of the banded newt, *Triturus vittatus ophryticus*. — Ethology **81**: 148–170.

Read, J.L. & M.J. Tyler (1990): The nature and incidence of postaxial skeletal abnormalities in the frog *Neobatrachus centralis* Parker at Olympic Dam, South Australia. — Trans. R. Soc. S. Aust. **114**: 213–217.

Read, J.L. & M.J. Tyler (1994): Natural levels of abnormalities in the trilling frog (*Neobatrachus centralis*) at the Olympic Dam mine. — Bull. Environ. Contam. Toxicol. **53**: 25–31.

Reaser, J.K. & P.T. Johnson (1997): Amphibian abnormalities: a review. — Froglog **24**: 2–4.

Reeves, M.K. & K.A. Trust (2008): Contaminants as contributing factors to wood frog abnormalities on the Kenai National Wildlife Refuge, Alaska. Final Report. — U.S. Fish and Wildl. Serv. Techn. Rep. **AFWFO TR#2008-01**: 1–56.

Reeves, M.K., C.L. Dolph, H. Zimmer, R.S. Tjeerdema & K.A. Trust (2008): Road proximity increases risk of skeletal abnormalities in wood frogs from national wildlife refuges in Alaska. — Environ. Health Perspectives **116**: 1009–1014.

Reeves, M.K., P. Jensen, C.L. Dolph, M. Holyoak & K.A. Trust (2010): Multiple stressors and the cause of amphibian abnormalities. — Ecol. Monogr. **80**: 423–440.

Reeves, M.K., K.A. Medley, A.E. Pinkney, M. Holyoak, P.T.J. Johnson & M.J. Lannoo (2013): Localized hotspots drive continental geography of abnormal amphibians on U.S. wild-

life refuges. — PLOS One **8(11)**: e77467. doi:10.1371/journal.pone.0077467.

Regester, K.J. (2001): Intradermal mite, *Hannemania* sp. (Acarina: Trombiculidae), infestations differ in populations of syntopic plethodontids in central Tennessee. — Herpetol. Nat. Hist. **8**: 69–73.

Reichenbach, L. (1865): Ein zweifelhafter Triton. — Nova Acta Leopolina Carolinensis **21**: XXX1, pl. 1–3.

Reichenbach-Klinke, H.-H. (1956): Knochendegeneration beim Rippenmolch (*Pleurodeles waltli* Michahelles) im Zusammenhang mit intramuskulärer Melanophorenanreicherung. — Biol. Zentralbl. **75**: 407–416.

Reichenbach-Klinke, H.-H. (1961): Krankheiten der Amphibien. — Stuttgart (Fischer).

Reinhardt, F. (1939): Beeinflussung der Hinterbeinentwicklung des Rippenmolches durch einseitige Fütterung. — Anat. Anz. **89**: 45–51.

Richards, C.M. & G.W. Nace (1983): Dark pigment variants in anurans: classification, new descriptions, color changes and inheritance. — Copeia **1983**: 979–990.

Richards, C.M., D.T. Tartof & G.W. Nace (1969): A melanoid variant in *Rana pipiens*. — Copeia **1969**: 850–852.

Richards, O.W. (1926): Notes on the British species of *Lucilia* (Diptera). — Trans. Entomol. Soc. Lond. **74**: 255–260.

Richardson, L.R. & R.E. Barwick (1957): Faulty eruption of the fore-limb in *Hyla aurea*. — Trans. R. Soc. New Zealand **84**: 941–942.

Rieck, A.F. (1954): The effects of ultraviolet, and of photorecovery, on the developing forelimb of *Amblystoma*. — J. Morphol. **94**: 367–400.

Riedel, W. (1988): Die Wirbeltiere des Bannwaldes Eisenbachhain im Schönbuch. — Veröff. Naturschutz Landschaftspfl. Bad.-Württ. **63**: 151–162.

Riley, E.E. & M.R. Weil (1986): The effects of thiosemicarbazide on development in the wood frog, *Rana sylvatica*. I. Concentration effects. — Ecotoxicol. Environ. Safety **12**: 154–160.

Riley, E.E. & M.R. Weil (1987): The effects of thiosemicarbazide on development in the wood frog, *Rana sylvatica*. II. Critical exposure length and age sensitivity. — Ecotoxicol. Environ. Safety **13**: 202–207.

Rimpp, K. (2007): Nördlicher Kammmolch *Triturus cristatus* (Laurenti, 1768). Pp. 207–222 in Laufer, H., K. Fritz & P. Sowig (eds.): Die Amphibien und Reptilien Baden-Württembergs. — Stuttgart (Ulmer).

Rios-López, N. (2008): Effects of increased salinity on tadpoles of two anurans from a Caribbean coastal wetland in relation to their natural abundance. — Amphibia-Reptilia **29**: 7–18.

Rivera, J., A. Vilagrosa & O. Arribas (1993): El albinismo y otros fenomenos aberrantes en los anfibios ibericos. — Acuamar **53**: 16–19.

Rivera, X., O. Arribas & F. Marti (2001): Anomalías pigmentarias en anfibios y reptiles. — Quercus **180**: 18–22.

Roberts, C.D. & T.E. Dickinson (2012): *Ribeiroia ondatrae* causes limb abnormalities in a Canadian amphibian community. — Can. J. Zool. **90**: 808–814.

Roberts, J.M. & P.A. Verrell (1984): Physical abnormalities of the limbs of smooth newts (*Triturus vulgaris*). — Brit. J. Herpetol. **6**: 416–418.

Rödel. M.-O. (1998): Kaulquappengesellschaften ephemerer Savannengewässer in Westafrika. — Frankfurt/Main (Chimaira).

Rohr, J.R., A.A. Elskus, B.S. Shepherd, P.H. Crowley, T.M. McCarthy, J.H. Niedzwiecki, T. Sager, A. Sih & B.D. Palmer (2003): Lethal and sublethal effects of atrazine, carbaryl, endosulfan, and octylphenol on the streamside salamander (*Ambystoma barbouri*). — Environ. Toxicol. Chem. **22**: 2385–2392.

Rohr, J.R., A.M. Schotthoefer, T.R. Raffel, H.J. Carrick, N. Halstead, J.T. Hoverman, C.M. Johnson, L.B. Johnson, C. Lieske, M.D. Piwoni, P.K. Schoff & V.R. Beasley (2008): Agrochemicals increase trematode infections in a declining amphibian species. — Nature **455**: 1235–1240.

Rohr, J.R., T. Raffel & S.K. Sessions (2009): Digenetic trematodes and their relationship to amphibian declines and deformities. Pp. 3067–3088 in Heatwole, H. & J.W. Wilkinson (eds.): Amphibian Biology. Vol. 8. — Chipping Norton, Australia (Surrey Beatty).

Romansic, J.M., A.A. Waggener, B.A. Bancroft & A.R. Blaustein (2009): Influence of ultraviolet-B radiation on growth, prevalence of deformities, and susceptibility to predation in Cascades frog (*Rana cascadae*) larvae. — Hydrobiologia **624**: 219–233.

Romansic, J.M., P.T.J. Johnson, C.L. Searle, J.E. Johnson, T.S. Tunstall, B.A. Han, J.R. Rohr & A.R. Blaustein (2011): Individual and combined effects of multiple pathogens on Pacific treefrogs. — Oecologia **166**: 1029–1041.

Romeis, B. (1914/15): Experimentelle Untersuchungen über die Wirkung innersekretorischer Organe. II. Der Einfluß von Thyroidea- und Thymusfütterung auf Wachstum, die Entwicklung und die Regeneration von Anurenlarven. — Arch. Entwicklungsmech. **40/41**: 58–119.

Romeis, B. (1918): Die Beeinflussung von Wachstum und Entwicklung durch Fett-, Lipoid- und Eiweißstoffe sowie eiweißfreie Extrakte der Schilddrüse und der Thymus. — Z. exp. Med. **6**: 101–288.

Rose, F.L. (1976): Tumorous growths of the tiger salamander, *Ambystoma tigrinum*, associated with treated sewage effluent. — Prog. exp. Tumor Res. **20**: 251–262.

Rose, F.L. (1977): Tissue lesions of tiger salamanders (*Ambystoma tigrinum*): relationship to sewage effluents. — Ann. N.Y. Acad. Sci. **298**: 270–279.

Rose, F.L. (1981): The tiger salamander (*Ambystoma tigrinum*): A decade of sewage associated neoplasia. Pp. 91–100 in Dawe, C.J., J.C. Harshbarger & S. Kondo (eds.): Phyletic Approaches to Cancer. — Tokio (Japan Sci. Soc. Press).

Rose, F.L. & J.C. Harshbarger (1977): Neoplastic and possibly related skin lesions in neotenic tiger salamanders from a sewage lagoon. — Science **196**: 315–317.

Rose, S.M. (1964): Regeneration. Pp. 545–622 in Moore, J.A. (ed.): Physiology of the Amphibia. Vol. I. — London (Acad. Press).

Rösel von Rosenhof, A.J. (1758): Historia naturalis Ranarum Nostratium. Die natürliche Historie der Frösche hiesigen Landes. — Nürnberg (Johann Jakob Fleischmann).

Rosenbaum, E.A., A. Caballero de Castro, L. Gauna & A.M. Pechen de D'Angelo (1988): Early biochemical changes produced by malathion on toad embryos. — Arch. Environ. Contam. Toxicol. **17**: 831–835.

Rostand, J. (1946): L'albinisme chez les Batraciens Anoures. — Rev. Sci. **84**: 564–565.

Rostand, J. (1947): La vie des crapauds. — Paris (Stock).

Rostand, J. (1948): Ectrodactylie naturelle chez le Crapaud ordinaire (*Bufo bufo*). — C.r. Soc. Biol. **226**: 1398–1399.

Rostand, J. (1949a): Sur diverses anomalies relevées dans une population de crapauds (*Bufo bufo*). — C.r. Soc. Biol. **143**: 758–760.

Rostand, J. (1949b): Hérédité de la polydactylie chez le crapaud ordinaire (*Bufo bufo*). — C.r. Acad. Sci. Paris **228**: 778–779.

Rostand, J. (1950a): Sur la descendance des grenouilles polydactyles. — C.r. Acad. Sci. Paris **231**: 496–498.

Rostand, J. (1950b): Essais de chimiotératogenèse chez les Batraciens Anoures. — C.r. Soc. Biol. **144**: 915–917.

Rostand, J. (1950c): La chimiotératogenèse chez les Batraciens Anoures. — Rev. Sci. **88**: 155–157.

Rostand, J. (1950d): Sur la gynogenèse comme moyen d'exploration du patrimoine héréditaire chez le crapaud ordinaire (*Bufo bufo*). — C.r. Acad. Sci. Paris **230**: 2130–2131.

Rostand, J. (1951a): Sur la polydactylie des Batraciens Anoures. — Bull. biol. Fr. Belg. **85**: 113–136.

Rostand, J. (1951b): Génétique des Batraciens Anoures: anomalies héréditaires et phénocopies. — Bull. Soc. Hist. nat. Toulouse **86**: 230–236.

Rostand, J. (1951c): Surmaturité des œufs et anomalies chez *Rana temporaria*. — C.r. Soc. Biol. **145**: 624–625.

Rostand, J. (1951d): La génétique de Batraciens. — Paris (Hermann).

Rostand, J. (1951e): Albinisme partiel d'orgine génétique chez le Crapaud ordinaire (*Bufo bufo*). — C.r. Acad. Sci. Paris **233**: 761–762.

Rostand, J. (1952a): Sur la variété d'expression d'une certaine anomalie (P) chez la grenouille verte (*Rana esculenta* L.). — C.r. Acad. Sci. Paris **235**: 583–585.

Rostand, J. (1952b): Sur une certaine anomalie (P) de la grenouille verte (*Rana esculenta* L.). — Rev. Sci. **90**: 353–365.

Rostand, J. (1952c): Polydactylie, polypodie et polymélie chez les têtards de *Rana esculenta* L. — C.r. Acad. Sci. Paris **235**: 322–324.

Rostand, J. (1955a): Effets tératogènes des rayons ultraviolets sur les larves de grenouilles. — C.r. Soc. Biol. **149**: 905–907.

Rostand, J. (1955b): Les Crapauds, les Grenouilles, et quelques grands problèmes biologiques. — Paris (Gallimard).

Rostand, J. (1956a): Polymorphisme de l'anomalie E chez *Rana temporaria* L. — C.r. Acad. Sci. Paris **243**: 973–974.

Rostand, J. (1956b): Sur une nouvelle anomalie (E) de *Rana temporaria* L: ectrodactylie, torsion de la queue et pupille claire. — C.r. Acad. Sci. Paris **242**: 3000–3002.

Rostand, J. (1957): Grenouille monstrueuses et radioactivité. — C.r. Acad. Sci. Paris **245**: 1175–1176.

Rostand, J. (1958a): Effet tératogène des rayons ultraviolets sur les larves d'Amphibiens. — C.r. Acad. Sci. Paris **247**: 972–974.

Rostand, J. (1958b): Les anomalies des Amphibiens Anoures. — Paris (Sedes).

Rostand, J. (1959): L'anomalie P chez la grenouille verte (*Rana esculenta* L.). — Bull. biol. Fr. Belg. **93**: 7–15.

Rostand, J. (1962): Sur la distribution de l'anomalie P chez la grenouille verte (*Rana esculenta* L.). — C.r. Acad. Sci. Paris **255**: 2189–2190.

Rostand, J. (1971): Les étangs à monstres. Histoire d'une recherche (1947–1970). — Paris (Stock).

Rostand, J. & P. Darré (1967): Sur les conditions d'apparition de l'anomalie P chez *Rana esculenta*. — C.r. Acad. Sci. Paris **265**: 761–762.

Rostand, J. & P. Darré (1968): Conditions déterminantes de l'anomalie P chez *Rana esculenta*. — C.r. Soc. Biol. **162**: 1682–1683.

Rostand, J. & P. Darré (1970): Une mutation de *Rana esculenta*: la grenouille aux yeux noirs. — C.r. Acad. Sci. Paris **217**: 1414–1415.

Rot-Nikcevic, I. & R.J. Wassersug (2003): Tissue sensitivity to thyroid hormone in athyroid *Xenopus laevis* larvae. — Develop. Growth Differ. **45**: 321–325.

Rot-Nikcevic, I. & R.J. Wassersug (2004): Consequences of gigantism in *Xenopus laevis* tadpoles. — ICVM Abstracts **7**: 324.

Roth, M. (1988): The 2-stage neuroskeletal pathomechanism of developmental deformities of the limb skeleton – a contribution to the discussion on the McCredie-McBride hypothesis. — Anat. Anz. **167**: 271–279.

Rouse, J.D., C.A. Bishop & J. Struger (1999): Nitrogen pollution: an assessment of its threat to amphibian survival. — Environ. Health Perspectives **107**: 799–803.

Rowe, C.L., O.M. Kinney, A.P. Fiori & J.D. Congdon (1996): Oral deformities in tadpoles (*Rana catesbeiana*) associated with coal ash deposition: effects on grazing ability and growth. — Freshwater Biol. **36**: 723–730.

Rowe, C.L., O.M. Kinney & J.D. Congdon (1998): Oral deformities in tadpoles of the bullfrog (*Rana catesbeiana*) caused by conditions in a polluted habitat. — Copeia **1998**: 244–246.

Ruben, L.N. (1960): An immunobiological model of implant-induced urodele supernumerary limb formation. — Am. Nat. **94**: 427–434.

Ruehmekorf, E. (1958): Beiträge zur Ökologie mitteleuropäischer Salientia. — Z. Morphol. Ökol. Tiere **47**: 1–53.

Rugh, R. (1950): Inhibition of growth and the production of oedema by X-irradiation. — J. exp. Zool. **114**: 137–157.

Ruiz, A.M., J.C. Maerz, A.K. Davis, M.K. Keel, A.R. Ferreira, M.J. Conroy, L.A. Morris & A.T. Fisk (2010): Patterns of development and abnormalities among tadpoles in a constructed wetland receiving treated wastewater. — Environ. Sci. Technol. **44**: 4862–4868.

Ryan, T.J. (1998): *Eurycea junaluska* (Junaluska salamander). Morphology. — Herpetol. Rev. **29**: 163.

Rzehak, K., A. Maryanska-Nadachowska & M. Jordan (1977): The effect of karbatox 75, a carbaryl insecticide, upon the development of tadpoles of *Rana temporaria* and *Xenopus laevis*. — Folia Biol. Krakow **25**: 391–399.

Sacher, P. (1985): Bemerkenswerte Farbkleidanomalie bei der Knoblauchkröte. — Feldherpetologie **1985**: 29–31.

Salice, C.J. (2012): Multiple stressors and amphibians: contributions of adverse health effects and altered hydroperiod to population decline and extinction. — J. Herpetol. **46**: 675–681.

Sambon, L.W. (1928): The parasitic acarians of animals and the part they play in the causation of the eruptive fevers and other diseases of man. — Ann. Trop. Med. Parasitol. **22**: 67–132.

Sánchez Alvarado, A. & P.A. Tsonis (2006): Bridging the regeneration gap: genetic insights from diverse animal models. — Nature Reviews Genetics **7**: 873–884.

Sanchiz, F.B. & P.J. Pérez (1974): Frecuencia de anomalías óseas en la poblacíon de *Discoglossus pictus* (Anura, Discoglossidae) de Campos (Asturias). — Bol. Estacion Central Ecol. **3(6)**: 69–77.

Sandner, H. (1955): *Lucilia bufonivora* Moniez 1876 (Diptera) w Polsce. — Acta Parasitol. Polon. **2(16)**: 319–329, 2 Tables.

Sanides, F. (1956): Die letale und teratogene Wirkung von Röntgenstrahlen auf ungefurchte Keime und Gastrulen von *Triton alpestris* in verschiedenartigem Milieu. — Biol. Zentralbl. **75**: 149–177.

Savchenko, V.K. (1995): The Ecology of the Chernobyl Catastrophe. — Paris (UNESCO).

Saveliev, S.V., N.S. Moskvitina, B.D. Kuranov & V.N. Kuranova (1996): Narushenie embriogeneza v prirodnysh populjatshijash pozvovotshnysh kak indikator sostojanija sredy obitanija. — Tomsk (Akad. Nauk.).

Saxén, L. (1957): Schilddrüsenaplasie als Ursache von partieller Neotenie. — Acta Endocrinol. **24**: 271–281.

Saxena, S. & I.A. Niazi (1977): Effect of vitamin A excess on hind limb regeneration in tadpoles of the toad, *Bufo andersonii* (Boulenger). — Ind. J. exp. Biol. **15**: 435–439.

Sazima, I. (1974): An albino hylid frog, *Phrynohyas mesophaea* (Hensel). — J. Herpetol. **8**: 264–265.

Scadding, S.R. (1981): Limb regeneration in adult Amphibia. — Can. J. Zool. **59**: 34–46.

Scadding, S.R. (1983): Effect of retinoic acid on limb regeneration in *Xenopus laevis*. — Can. J. Zool. **61**: 2698–2702.

Scadding, S.R. (1990): Effects of tributyltin oxide on the skeletal structures of developing and regenerating limbs of the axolotl larvae, *Ambystoma mexicanum*. — Bull. Environ. Contam. Toxicol. **45**: 574–581.

Scadding, S.R. & M. Maden (1986a): Comparison of the effects of vitamin A on limb development and regeneration in *Xenopus laevis* tadpoles. — J. Embryol. exp. Morph. **91**: 35–53.

Scadding, S.R. & M. Maden (1986b): Comparison of the effects of vitamin A on limb development and regeneration in the axolotl, *Ambystoma mexicanum*. — J. Embryol. exp. Morph. **91**: 19–34.

Scheid, P., C. Balczun, J.M. Dehling, A. Ammon & U. Sinsch (2015): Rhinosporidiosis in African reed frogs *Hyperolius* spp. caused b a new species of *Rhinosporidium*. — Dis. Aquat. Org. **115**: 111–120.

Scherb, H. & E. Weigelt (2003): Congenital malformation and stillbirth in Germany and Europe before and after the Chernobyl nuclear power accident. — Environ. Sci. Pollut. Res. Special Issue **1**: 117–125.

Schinz, H.R. & H. Fritz-Niggli (1954): Mißbildungen adulter Krallenfrösche (*Xenopus laevis* Daud.) nach Röntgen-Bestrahlung im Embryonalstadium. — Strahlentherapie **94**: 147–151.

Schipp, R., H. Hemmer & R. Flindt (1968): Vergleichende licht- und elektronenmikroskopische Untersuchungen an Chordomen von Krötenbastardlarven. — Beitr. pathol. Anat. **138**: 109–133.

Schlumberger, H.G. & B. Lucké (1948): Tumours of fishes, amphibians and reptiles. — Cancer Res. **8**: 657–754.

Schlüpmann, M., A. Kronshage & A. Geiger (1995): Bibliographie zur Herpetofauna Nordrhein-Westfalens. — Z. Feldherpetol. **2**: 97–220.

Schmalhausen, J. (1925): Über die Beeinflussung der Morphogenese der Extremitäten vom Axolotl durch verschiedene Faktoren. — W. Roux Arch. Entwicklungsmech **105**: 483–500.

Schmelcher, D. & W. Hellmich (1951): Über eine merkwürdige Larve eines Feuersalamanders. — DATZ **4**: 300–302.

Schmidt, G.A. (1930): Ein Fall einer Doppelmißbildung Duplicitas anterior assimmetricus bei *Rana arvalis* aus der Natur. — Bull. Station biol. Soc. Amis Sci. Nat., Anthropol. Éthnogr. Bolshevo Gouvernement Moscou (Zapiski Biol. Stancii Obyesyecestva Ljubitelej Estestvozanija, Antr. Biol. Stancija, Moskva) **4**: 58.

Schmidt, P. & M. Hachtel (2011): 3.16 Wasserfrösche – *Pelophylax esculentus*-Komplex. Pp. 841–896 in Hachtel, M., M. Schlüpmann, K. Weddeling, B. Thiesmeier, A. Geiger & C. Willigalla (eds.): Handbuch der Amphibien und Reptilien Nordrhein-Westfalens. — Bielefeld (Laurenti).

Schmidtler, J.F. (2016): Zur frühen Erforschungsgeschichte der Gattung *Salamandra*. Pp. 14–41 in Seidel, U. & P- Gerhardt (eds.): Die Gattung *Salamandra*. — Frankfurt/M. (Chimaira).

Schoff, P.K., C.M. Johnson, A.M. Schotthoefer, J.E. Murphy, C. Lieske, R.A. Cole, L.B. Johnson & V.R. Beasley (2003): Prevalence of skeletal and eye malformations in frogs from north-central United States: estimations based on collections from randomly selected sites. — J. Wildl. Dis. **39**: 510–521.

Schotthoefer, A.M., A.V. Koehler, C.U. Meteyer & R.A. Cole (2003): Influence of *Ribeiroia ondatrae* (Trematoda: Digenea) infection on limb development and survival of northern leopard frogs (*Rana pipiens*): effects of host stage and parasite-exposure level. — Can. J. Zool. **81**: 1144–1153.

Schreiber, E. (1912): Herpetologia europaea. — Jena (Fischer).

Schweizerbarth, von E.M. (1909): Der rotfleckige Feuersalamander. — Bl. Aquar. Terrarienkde **20**: 382–385.

Schwind, J.L. (1942): Spontaneous twinning in the Amphibia. — Am. J. Anat. **71**: 117–151.

Seba, A. (1735): Locupletissimi rerum naturalium thesauri accurata descriptio, et iconibus artificiosissims expressio. Tomus II. — Amsterdam (Janssonio-Waesbergius, J. Wetstenium & Gul Smith).

Seidel, U., E. Hartmann & A. Hein (2012): Farb- und Zeichnungsanomalien beim Feuersalamander (*Salamandra salamandra*). — amphibia **11(2)**: 4–18.

Seidel, U. & P. Gerhardt (2016): Die Gattung *Salamandra*. — Frankfurt (Chimaira).

Seilern-Aspang, F. & K. Kratochwil (1962): Induction and differentiation of an epithelial tumour in the newt (*Triturus cristatus*). — J. Embryol. exp. Morph. **10**: 337–356.

Semlitsch, R.D. (1990): Effects of body size, sibship, and tail injury on the susceptibility of tadpoles to dragonfly predation. — Can. J. Zool. **68**: 1027–1030.

Semlitsch, R.D. & S.B. Reichling (1989): Density-dependent injury in larval salamanders. — Oecologia **81**: 100–103.

Semlitsch, R.D., G.B. Moran & C.A. Shoemaker (1981): *Ambystoma talpoideum* (mole salamander). Morphology. — Herpetol. Rev. **12**: 69.

Semlitsch, R.D., D.E. Scott & H.K. Pechmann (1988): Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. — Ecology **69**: 184–192.

Sequeira, F., H. Gonçalves, C. Meneses & M. Mouta-Faria (1999): Morphological abnormalities in a population of *Chioglossa lusitanica*. — Bol. Asoc. Herpetol. Esp. **10**: 35–36.

Sessions, S.K. (2003): What is causing deformed amphibians? Pp. 168–186 in Semlitsch, R.D. (ed.): Amphibian Conservation. — Washington & London (Smithsonian).

Sessions, S.K. (2009): Book review: Malformed Frogs, the Collaps of Aquatic Ecosystems by Michael Lannoo 2008. — Herpetol. Rev. **40**: 121–124.

Sessions, S.K. & B. Ballengée (2010): Explanations for deformed frogs: plenty of research left to do (a reply to Skelly and Benard). — J. exp. Zool. (Mol. Dev. Evol.) **314B**: 341–346.

Sessions, S.K. & S.B. Ruth (1990): Explanation for naturally occuring supernumerary limbs in amphibians. — J. exp. Zool. **245**: 38–47.

Sessions, S.K., R.A. Franssen & V.L. Horner (1999): Morphological clues from multilegged frogs: are retinoids to blame? — Science **284**: 800–801.

Sewell, D. (2007): The masked newt: variation of body coloration in a great crested newt, *Triturus cristatus*. — Herpetol. Bull. **102**: 32–33.

Shen, G. (1938): Experimente zur Analyse der Regulationsfähigkeit der frühen Gastrula von *Triton*, zugleich ein Beitrag zum Problem der Cyclopie. — Roux Arch. Entwickl.-mech. **137**: 271–316.

Shoemaker, J.P. & J.G. Clark (1975): The occurrrence of *Hannemania dunni* Sambon, 1928, (Acari: Trombiculidae) on *Eurycea bislineata* (Green) from West Virginia. — Proc. West Virginia Acad. Sci. **47**: 5–6.

Shubin, N., D.B. Wake & A.J. Crawford (1995): Morphological variation in the limbs of *Taricha granulosa* (Caudata: Sala-

mandridae): evolutionary and phylogenetic implications. — Evolution **49**: 874–884.

Siboulet, R. (1971): L'élevage des embryons et larves des Amphibiens anoures. — Aquarama **5(14)**: 38–39.

Signoret, J., A. Collenot & L. Gallien (1966): Description d'un nouveau mutant récessif léthal (u) et de son syndrome chez le triton *Pleurodeles waltlii*. — C.r. Acad. Sci. Paris **262**: 699–701.

Sigov, V.A. (1936): To the problem of the role of anurans of local carp ponds. — Trudy Voronezhskogo NII Prudovogo i Rybnogo Khozyaistva **2**: 1–100.

Silantev, A.A. (1898): Zoologicheskie issledovanija i nabludenija 1894–1898 godov (Zoological studies and observations in the years 1894–1898). Department of Forestry, St. Petersburg.

Singer, M., M.H. Davis & M.R. Scheuing (1960): The influence of atropine and other neuropharmacological substances on regeneration of the forelimb in the adult urodele, *Triturus*. — J. exp. Zool. **143**: 33–45.

Sinha, R.P. & D.-P. Häder (2002): UV-induced DNA damage and repair: a review. — Photochem. Photobiol. Sci. **1**: 225–236.

Skelly, D.K. & M.F. Benard (2010): Mystery unsolved: missing limbs in deformed amphibians. — J. exp. Zool. (Mol. Dev. Evol.) **314B**: 179–181.

Skelly, D.K., S.R. Bolden, L.K. Freidenberg, N.A. Freidenfels & R. Levey (2007): *Ribeiroia* infection is not responsible for Vermont amphibian deformities. — EcoHealth **4**: 156–163.

Sladden, D.E. (1932): Experimental distortion of development in amphibian tadpoles. Part II. — Proc. R. Soc. London, Ser. B **112**: 1–12.

Sládecek, F. (1964): The development of "white eggs" mutants of *Rana temporaria* L. in normal conditions and in parabiotic and chimaeric combinations with pigmented embryos. — Folia Biol. Prague **10**: 23–29.

Smallcombe, W.A. (1949): Albinism in *Rana temporaria*. — J. Genet. **49**: 286–291.

Smirnov, N.A. (2014): About the morphological abnormalities in newts of the genus *Lissotriton* (Salamandridae, Caudata) in western Ukraine. Pp. 165–171 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Smith, G.R., M.A. Waters & J.E. Rettig (2000): Consequences of embryonic UV-B exposure for embryos and tadpoles of the plains leopard frog. — Conserv. Biol. **14**: 1903–1907.

Smith, K.G., C. Weldon, W. Conradie & L.H. du Preez (2007): Relationships among size, development, and *Batrachochytrium dendrobatidis* infection in African tadpoles. — Dis. Aquat. Org. **74**: 159–164.

Smith, M. (1950): Neoteny in British newts. — Brit. J. Herpetol. **1**: 91–92.

Smith, P.E. (1916): Experimental ablation of the hypophysis and thyroid glands. — Science **44**: 280–282.

Smith, S.C. & J.B. Armstrong (1990): Heart induction in wild-type and cardiac mutant axolotls (*Ambystoma mexicanum*). — J. exp. Zool. **254**: 48–54.

Snawder, J.E. & J.E. Chambers (1989): Toxic and developmental effects of organophosphorus insecticides in embryos of the South African clawed frog. — J. Environ. Sci. Health **B24(3)**: 205–218.

Souder, W. (2002): A Plague of Frogs. Unraveling an Environmental Mystery. — Minneapolis (Univ. Minnesota Press).

Sower, S.A., K.L. Reed & K.J. Babbitt (2000): Limb malformations and abnormal sex hormone concentrations in frogs. — Environ. Health Perspectives **108**: 1085–1090.

Sowig, P. & H. Laufer (2007): Erdkröte *Bufo bufo* (Linnaeus, 1758). Pp. 311–3334 in Laufer, H., K. Fritz & P. Sowig (eds.): Die Amphibien und Reptilien Baden-Württembergs. — Stuttgart (Ulmer).

Sparling, D.W. (2000): Effects of altosid and abate-4E on deformities and survival in southern leopard frogs under semi-natural conditions. — J. Iowa Acad. Sci. **107(3)**: 90–91.

Sparling, D.W., G. Harvey & V. Nzengung (2003): Interaction between perchlorate and iodine in the metamorphosis of *Hyla versicolor*. Pp. 143–158 in Linder, G.L., S. Krest, D. Sparling & E.E. Little (eds.): Multiple Stressor Effects in Relation to Declining Amphibian Populations. — West Conshohocken (Am. Soc. Testing Material).

Sparling, D.W., J. Bickham, D. Cowman, G.M. Fellers, T. Lacher, C.W. Matson & L. McConnell (2015): In situ effects of pesticides on amphibians in the Sierra Nevada. — Ecotoxicol. **24**: 262–278.

Speare, R., L. Berger, P. O'Shea, P.W. Ladds & A.D. Thomas (1997): Pathology of mucormycosis of cane toads in Australia. — J. Wildl. Dis. **33**: 105–111.

Spieler, M. & K.E. Linsenmair (1999): The larval mite *Endotrombicula pillersi* (Acarina: Trombiculidae) as a species-specific parasite of a West African savannah frog (*Phrynobatrachus francisci*). — Am. Midl. Nat. **142**: 152–161.

Spirina, E.V. (2009): Morfologitsheskie anomalii *Rana ridibunda* Pall. kak indikatori katshestva okryshajushtshej sredy. — Isvestija Orenburgkogo Gosudarstennogo Agrarnogo Univ. (Biol. Nauki) **1(21)**: 228–230.

Spoylarich, N., R.V. Hyne, S.P. Wilson, C.G. Palmer & M. Byrne (2011): Morphological abnormalities in frogs from a rice-growing region in NSW, Australia, with investigations into pesticide exposure. — Environ. Monit. Assess. **173**: 397–407.

Stachowitz, W. (1914): Veränderungen in der Entwicklung von Amphibienembryonen, die auf dem Stadium der Medullarplatte mit Radium bestrahlt wurden. — Arch. mikr. Anat., Abt. 1 **85**: 521–554.

Stadler, H. (1930): Über den Befall einer Kröte (*Bufo vulgaris* Laur.) durch die Larven von *Lucilia sylvarum* Meig.; Krankheitsgeschichte und Sektionsbefund. — Z. Parasitenkunde **2**: 360–367.

Steinfartz, S. (1998): Über eine interessante Farbkleidveränderung bei *Salamandra atra aurorae*. — Salamandra **34**: 69–72.

Stöck, M. (1998): Tetraploid toads (*Bufo viridis* complex) from north-western China and preliminary taxonomic conclusions for *Bufo nouettei* Mocquard, 1910. — Z. Feldherpetol. **5**: 139–166.

Stopper, G.F., L. Hecker, R.A. Franssen & S.K. Sessions (2002): How trematodes cause limb deformities in amphibians. — J. exp. Zool. (Mol. Dev. Evol.) **294**: 252–263.

Storer, T.I. (1925): A Synopsis of the Amphibia of California. — Berkeley (Univ. California Press).

Strijbosch, H. (1980): Mortality in a population of *Bufo bufo* resulting from the fly *Lucilia bufonivora*. — Oecologia **45**: 285–286.

Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischmann & R.W. Waller (2004): Status and trends of amphibian declines and extinctions. — Science **306**: 1783–1786.

Sumida, M. & M. Nishioka (2000): Sex-linked genes and linkage maps in amphibians. — Comp. Biochem. Physiol. **B126**: 257–270.

Sunderasan, L. & J.J.L. Rowley (2012): *Leptobrachium pullum* (Vietnam spadefoot toad). Situs inversus. — Herpetol. Rev. **43**: 464.

Surlève-Bazeille, J.-E., R. Cambar & R. Mauget (1969a): Essai de transmission de l'anomalie P à diverses espèces d'Am-

phibiens. Premiers resultats obtenus sur *Rana temporaria*. — Actes Soc. Linn. Bordeaux, Ser. A **106(2)**: 1–7.

Surlève-Bazeille, J.-E., R. Cambar & A. Calas (1969b): Premières observations histologiques sur les membres des larves de Grenouille verte (*Rana esculenta* L.) atteints de l'anomalie P. — C.r. Acad. Sci. Paris, Sér. D **269**: 2240–2242.

Susebach, E. (1941): Eine zweiköpfige Feuersalamanderlarve. — Wschr. Aquar. Terrarienkunde **38**: 366.

Svinin, A.O. (2014): The occurrence of morphological anomalies in green frog population systems (*Pelophylax* Fitzinger, 1843) from the north-eastern part of its distributuon area. Pp. 156–161 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Swett, F.H. (1926): On the production of double limbs in amphibians. — J. exp. Zool. **44**: 419–473.

Syuzyumova, L.M. (1985): Effects of ecological factors on growth and development of frog limbs upon inhibition of thyroid function by vitamin A. — Ekaterinburg (Akad. Nauk. SSSR).

Szymura, J.M. & N.H. Barton (1986): Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. — Evolution **40**: 1141–1159.

Taira, W., C. Nohara, A. Hiyama & J.M. Otaki (2014): Fukushima's biological impacts: the case of the pale grass blue butterfly. — J. Hered. **105**: 710–722.

Takahashi, H. (1957): The malformed development of toad larvae treated with estradiol. — Annot. zool. jap. **30**: 199–203.

Takahashi, H. (1958): Time relation between the administration of estradiol and the occurrence of abnormalities in limbs and gonads of frog larvae. — J. Fac. Sci. Hokkaido Univ., Ser VI, Zool. **14**: 101–110.

Tank, P.W. & N. Holder (1981): Pattern regulation in the regenerating limbs of urodele amphibians. — Q. Rev. Biol. **56**: 113–142.

Tarkhnishvili, D.N. & I.A. Serbinova (1998): Giant larvae of the Caucasian salamander (*Mertensiella caucasica*). — Adv. Amph. Res. Former Soviet Union **3**: 187–191.

Taruffi, C. (1880): Nota storica sulla polimelia delle rane. — Atti Soc. Ital. Sci. Nat. **23**: 112–122.

Taruffi, C. (1881–1886): Storia della Teratologia. Parte Seconda. Note ed Osservazioni. Fascicolo I–IV. — Bologna (Regia Tipografia).

Taton, E. (1877): (No title). — Bull. Soc. Entomol. France **23. Mai 1877**: 119–120.

Taylor, B., D. Skelly, L.K. Demarchis, M.D. Slade, D. Galusha & P.M. Rabinowitz (2005): Proximity to pollution sources and risk of amphibian limb malformation. — Environ. Health Perspectives **113**: 1497–1501.

Teacher, A.G.F., A.A. Cunningham & T.W.J. Garner (2010): Assessing the long-term impact of *Ranavirus* infection in wild common frog populations. — Anim. Conserv. **13**: 514–522.

Terent'ev, P.V. (1950): Lyagushka. Moscow (Akad. Nauk. SSSR).

Thiesmeier, B. (1990): Freilandbeobachtungen über Abortivgebilde und larvale Extremitätenschäden beim Feuersalamander (*Salamandra salamandra terrestris*). — herpetofauna, Weinstadt, **12(68)**: 6–10.

Thiesmeier, B. (2004): Der Feuersalamander. — Z. Feldherpetol. Suppl. **4**: 1–192.

Thiesmeier, B. (2014): Wiederfunde eines pädomorphen Teichmolchs. — Feldherpetol. Magazin **2**: 27–28.

Thiesmeier, B. & U. Schulte (2010): Der Bergmolch. — Beih. Z. Feldherpetol. **13**: 1–159.

Thiesmeier-Hornberg, B. (1988): Zur Ökologie und Populationsdynamik des Feuersalamanders (*Salamandra salamandra terrestris* Lacépède, 1788) im Niederbergischen Land unter besonderer Berücksichtigung der Larvalphase. — Essen (Dissertation Universität-Gesamthochschule).

Thomas, M., R. Rottscheidt & M. Hachtel (2002): Ein Fall von Flavismus bei der Erdkröte *Bufo bufo* (Linnaeus, 1758) nahe Bonn. — Salamandra **38**: 119–123.

Thoms, S.D. & D.L. Stocum (1984): Retinoic acid induced pattern duplication in regenerating urodele limbs. — Develop. Biol. **103**: 319–328.

Thornton, C.S. (1968): Amphibian limb regeneration. — Adv. Morphogen. **7**: 205–249.

Thurow, G.R. (1961): A salamander color variant associated with glacial boundaries. — Evolution **15**: 281–287.

Toivonen, S. (1952): Ein Fall von partieller Neotenie bei *Xenopus laevis* Daudin und experimentelle Untersuchungen zu seiner kausalen Erklärung. — Archiv. Soc. Zool. Bot. Fennicae Vanamo **6**: 107–123.

Tokunaga, C. (1949): Albino frogs produced by artificial parthenogenesis. — J. Hered. **40**: 279–281.

Toledo, L.F. & R.S. Ribeiro (2009): The archipelago of Fernando de Noronha: an intriguing malformed toad hotspot in South America. — EcoHealth **6**: 351–357.

Tolledo, J. & L.F. Toledo (2015): Blind toads in paradise: the cascading effects of vision loss on a tropical archipelago. — J. Zool. **296**: 167–176.

Tolledo, J., E.T. Silva, C.H.L. Nunes-de-Almeida & L.F. Toledo (2014): Anomalous tadpoles in a Brazilian oceanic archipelago: implications of oral anomalies on foraging behaviour, food intake and metamorphosis. — Herpetol. J. **24**: 237–243.

Tomar, V. & A.K. Pandey (1988): Sublethal toxicity of an insecticide to the epidermal melanophores of *Rana* tadpoles. — Bull. Environ. Contam. Toxicol. **41**: 582–588.

Tornier, G. (1901): Neues über das natürliche Entstehen und experimentelle Erzeugen überzähliger und Zwillingsbildungen. — Zool. Anz. **24**: 488–504.

Tornier, G. (1907): Nachweis über das Entstehen von Albinismus, Melanismus und Neotenie bei Fröschen. — Zool. Anz. **32**: 284–288.

Tornier, G. (1908): Über experimentelles Hervorrufen und Naturentstehen von Mopsköpfen, Cyclopen und anderen vorgebürtlichen Kopfverbildungen bei Wirbeltieren. — Sitz.-ber. Ges. Nat.-forsch. Freunde, Berlin **24**: 298–315.

Trubetskaya, E.A. (2006): Anomalies of mouthparts in *Rana arvalis* Nilss. tadpoles and ecological conditions providing for their emergence. — Russ. J. Ecol. **37**: 193–199.

Trux, A. (1985): Histopathologische Untersuchung zur Entwicklung des visuellen Systems von *Bufo bufo* (Amphibia) nach Bestrahlung mit Co-60. — Bonn (Unveröff. Diplomarb., Univ. Bonn).

Tschumi, P. (1954): Konkurrenzbedingte Rückbildungen der Hinterextremität von *Xenopus* nach Behandlung mit einem Chloraethylamin. — Rev. suisse Zool. **61(6)**: 177–270.

Tsonis, P.A. (1984): Limb regeneration in newts with spontaneous skin cancer. — Can. J. Zool. **62**: 2681–2685.

Tubbs, L.O.E, R. Stevens, M. Wells & R. Altig (1993): Ontogeny of the oral apparatus of the tadpole of *Bufo americanus*. — Amphibia-Reptilia **14**: 333–340.

Tunner, H.G. (1980): Kreuzungsexperimente mit Wasserfröschen aus österreichischen und polnischen Mischpopulationen (*Rana lessonae* + *Rana esculenta*). Eine Analyse biochemischer und morphologischer Merkmale. — Z. zool. Syst. Evolutionsforsch. **18**: 257–297.

Twitty, V.C. (1942): The species of Californian *Triturus*. — Copeia **1942**: 65–76.

Tyler, M.J. (1983): Natural pollution monitors. — Aust. Nat. Hist. **21**: 31–33.

Tyler, M.J. (1989): Australian Frogs. — South Yarra (Viking O'Neil).

Tzanopoulos, J., R. Mouttet, A. Letourneau, I.N. Vogiatzakis, S.G. Potts, K. Henle, R. Mathevet & P. Marty (2013): Scale sensitivity of drivers of environmental change across Europe. — Global Environmental Change **23**: 167–178.

Ueda, H. (1980): The sex of triploids and gynogenetic diploids. — Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **4**: 185–199.

Uehlinger, V. (1965): Une forme de goitre héréditaire chez le Batracien *Xenopus laevis* D. — Experientia **21**: 271.

Uehlinger, V. (1969): Une mutation récessive (pd) déterminant la polydactylie chez *Xenopus laevis* D. (Batraciens anoures). — J. Embryol. exp. Morph. **21**: 207–218.

Uehlinger, V. & J. Reynaud (1965): Une anomalie héréditaire "kt" (kinky tailtip) chez *Xenopus laevis* D. — Rev. suisse Zool. **72**: 680–685.

Uhler, L.D. (1971): Blue frogs: familiar figures in strange apparel. — The Conservationist **1971(8–9)**: 4.

Underhill, D.K. (1967): Spontaneous silvery color and pituitary abnormality in tadpoles from a laboratory cross of *Rana pipiens*. — Copeia **1967**: 673–674.

Unrine, J.M., C.H. Jagoe, W.A. Hopkins & H.A. Brant (2004): Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenocephala*) larvae. — Environ. Toxicol. Chem. **23**: 2964–2970.

USEPA (2009): A national assessment of impaired waters within or near US Fish and Wildlife Service properties – Phase 2 final report. – Washington (US Environmental Protection Agency).

Vallisneri, A. (1706): Galleria di Minerva overo notizie universali di quanto e stato scritto da letterati di Europa. Vol. 5. — Venice (Galleria di Minerva).

Van Buskirk, J. & S.A. McCollum (2000): Influence of tail shape on tadpole swimming performance. — J. Exp. Biol. **203**: 2149–2158.

Van Buskirk, J. & D.C. Smith (1991): Density-dependent population regulation in a salamander. — Ecology **72**: 1747–1756.

Van der Schalie, W.H., H.S. Gardner, J.A. Bantle, C.T. deRosa, R.A. Finch, J.S. Reif, R.H. Reuter, L.C. Backer, J. Burger, L.C. Folmar & W.S. Stokes (1999): Animals as sentinels of human health hazards of environmental chemicals. — Environ. Health Perspectives **107**: 309–315.

Van Gelder, J.J. & H. Strijbosch (1995): Adult common toads with mutilated legs. — Alytes **13**: 105–108.

Van Valen, L. (1974): A natural model for the origin of some higher taxa. — J. Herpetol. **8**: 109–121.

Vandenlangenberg, S.M., J.T. Canfield & J.A. Magner (2003): A regional survey of malformed frogs in Minnesota (USA) (Minnesota malformed frogs). — Environ. Monitor. Assess. **82**: 45–61.

Veith, M. (1988): Teilalbinotische Larven des Feuersalamanders (*Salamandra salamandra terrestris* Lacépède, 1788) aus dem Hunsrück (BRD, Rheinland-Pfalz, Rheinisches Schiefergebirge) (Amphibia: Caudata: Salamandridae). — Fauna Flora Rheinland-Pfalz **5(1)**: 92–99.

Veith, M. (1996): Gelbbauchunke – *Bombina variegata* (Linnaeus, 1758). Pp. 151–164 in Bitz, A., K. Fischer, L. Simon, R. Thiele & M. Veith (eds.): Die Amphibien und Reptilien in Rheinland-Pfalz. — Landau (GNOR).

Veith, M. & B. Viertel (1993): Veränderungen an den Extremitäten von Larven und Jungtieren der Erdkröte (*Bufo bufo)*: Analyse möglicher Ursachen. — Salamandra **29**: 184–199.

Vences, M., F. Glaw & K. Hirschberger (1996): Auf der Suche nach roten Scheibenzüngler. — DATZ **49**: 44–47.

Vershinin, V.L. (1991): Anomalous amphibian egg deposits in area of urban agglomeration. — Russ. J. Ecol. **22**: 153–157.

Vershinin, V.L. (2002): Ecological specifity and microevolution in amphibian populations in urbanized areas. — Adv. Amph. Res. Former Soviet Union **7**: 1–161.

Vershinin, V.L. (2004): Frequency of iris depigmentation in urban populations of *Rana arvalis* frogs. — Russ. J. Ecol. **35**: 58–62.

Vershinin, V.L. (2005): Morphological deviations in population *Rana arvalis* Nilss. on urbanized territories: spectrum, topography, frequency. Pp. 311–313 in Ananjeva, N. & O. Tsinenko (eds.): Herpetologia Petropolitana. Proc. 12th Ord. Gen. Meeting Soc. Eur. Herpetol., August 12–16, 2003, St. Petersburg. — St. Petersburg (Societas Europaea Herpetologica).

Vershinin, V.L. (2006): Significance of recessive and dominant mutations in adaptive processes of the genus *Rana* in the modern biosphere. Pp. 197–200 in Vences, M., J. Köhler, T. Ziegler & W. Böhme (eds.): Herpetologica Bonnensis II. — Bonn (Societas Europaea Herpetologica).

Vershinin, V.L. (2010): Topography of morphological abnormalities and mate selection in the toads *Bufo bufo* L., 1758 and *B. viridis* Laur., 1768. — Russ. J. Ecol. **41**: 340–344.

Vershinin, V.L. & N.S. Neustroeva (2011): The role of trematode infestation in the specifics of skeleton morphogenesis of *Rana arvalis* Nilsson, 1842. — Dokl. Biol. Sci. **440**: 290–292.

Vershinin, V.L. & S.D. Seredyuk (2000): Trophic specifity of the *Rana arvalis* population from the Eastern Ural radioactive trace in relation to the state of the soil mesofauna. — Russ. J. Ecol. **31**: 330–335.

Vieira, C.A., L.F. Toledo, J.E. Longcore & J.R. Longcore (2013): Body length of *Hylodes* cf. *ornatus* and *Lithobates catesbeianus* tadpoles, depigmentation of mouthparts, and presence of *Batrachochytrium dendrobatidis* are related. — Braz. J. Biol. **73**: 195–199.

Virey, J.-J. (1819): Observation et description de deux individus sexdigitaires. — J. Complém. Dict. Sci. Méd. **4**: 327–330.

Vizotto, L.D. & V.A. Taddei (1977): Pseudomelia em *Phyrnohyas hebes* (Cope, 1862) (Anura-Hylidae). — Naturalia **3**: 35–43.

Vogt, W. (1939): Über alimentäre Unterdrückung der Hinterbeine beim Rippenmolch (*Pleurodeles w.*). — Verh. Ges. Anat. **47**: 137–144.

Volpe, E.P. & S. Dasgupta (1962): Gynogentic diploids of mutant leopard frogs. — J. exp. Zool. **151**: 287–301.

Volpe, E.P. & P.A. Rosenbaum (2000): Understanding Evolution (6nd ed.). — New York (McGraw-Hill).

Vorps, H.M. (1976): A „specle phenotype in *Rana clamitans*. — Can. Field Nat. **90**: 57–58.

Voyles, J., S. Young, L. Berger, C, Campbell, W.F. Voyles, A. Dinudom, D. Cook, R. Webb, R.A. Alford, L.F. Skerratt & R. Speare (2009): Pathogenesis of chytriodiomycosis, a cause of catastrophic amphibian declines. — Science **326**: 582–585.

Vulpian, A. (1862): Über künstlich erzeugte Doppelbildungen bei Froschlarven. — Gazette méd. **108**: 188.

Vuori, K. & M. Parkko (1996): Assessing pollution of the river Kymijoki via hydropsychid caddis flies: population age structure, microdistribution and gill abnormalities in the *Cheumatopsyche lepida* and *Hydropsyche pellucidula* larvae. — Arch. Hydrobiol. **136**: 171–190.

Wager, V.A. (1986): Frogs of South Africa, their Fascinating Life Stories. — Craighall (Delta Books).

Wake, D.B. & I.G. Dresner (1967): Functional morphology and evolution of tail autotomy in salamanders. — J. Morphol. **122**: 265–306.

Wallace, H. (1981): Vertebrate Limb Regeneration. — New York (Wiley).

Wang, Y., L. Dong & S.E. Evans (2016): Polydactyly and other limb abnormalities in the Jurassic salamander *Chunerpeton* from China. — Palaeobio Palaeoenv **96**: 49–59.

Weddeling, K. & A. Geiger (2011): 3.9 Erdkröte – *Bufo bufo*. Pp. 583–622 in Hachtel, M., M. Schlüpmann, K. Weddeling, B. Thiesmeier, A. Geiger & C. Willigalla (eds.): Handbuch der Amphibien und Reptilien Nordrhein-Westfalens. — Bielefeld (Laurenti).

Weiss, I. (1960): Umwandlung von Disnothoi der Bastardkombination *Bufo calamita* × *Bufo viridis* in Eunothoi durch experimentelle Verdoppelung des mütterlichen Genoms. — Roux Arch. Entwickl.-mech. **152**: 552–564.

Welbourn, W.C. & R.B. Loomis (1975): *Hannemannia* (Acarina: Trombiculidae and their anuran hosts at Fortynine Palms Oasis, Joshua Tree National Monument, California. — Bull. S. Calif. Acad. Sci. **74**: 15–19.

Wenig, J. (1913): Der Albinismus bei den Anuren, nebst Bemerkungen über den Bau des Amphibien-Integuments. — Anat. Anz. **43**: 113–135.

Werner, F. (1930): Die Wirkung ausschließlicher Fütterung mit Säugetierfleisch auf Molche. — Bl. Aquar. Terrarienkde **41(2)**: 30.

Westfall, M.C., K.K. Cecala, S.J. Price, & M.E. Dorcas (2008): Patterns of trombiculid mite (*Hannemania dunni*) parasitism among plethodontid salamanders in the western Piedmont of North Carolina. — J. Parasitol. **94**: 631–634.

Widener, D. (1970): Timetable for Disaster. – Los Angeles (Nash Publ.).

Wiegand, T., F. Jeltsch, I. Hanski & V. Grimm (2003): Using pattern-oriented modeling for revealing hidden information: a key for reconciling ecological theory and application. – Oikos **100**: 209–222.

Williams, R.N., D.H. Bos, D. Gopurenko & J.A. DeWoody (2008): Amphibian malformations and inbreeding. — Biol. Lett. **4**: 549–552.

Willis, R.A. & W.H. Collins (1948): Pathology of Tumours. — London (Butterworth).

Wink, U. & H. Wurmbach (1967): Die Wirkung der Schilddrüsenhemmung durch Thymusöl bei Xenopuskaulquappen auf die Zellteilung, die Bildung von Knorpel und Muskeln sowie die Entstehung von Extremitätenmißbildungen. — Zool. Jb. Physiol. **73**: 343–374.

Winter, D.A., W.M. Zawada & A.A. Johnson (1986): Comparison of the symbiotic fauna of the family Plethodontidae in the Ouachita Mountains of western Arkansas. — Proc. Arkansas Acad. Sci. **40**: 82–85.

Winter, R.M., S.A.S. Knowles, F.R. Bieber & M. Baraitser (1988): The Malformed Fetus and Stillbirth. — New York (Wiley).

Wisniewski, P.J. (1979): Notes on leg-loss in the common toad. — ASRA J. **1**: 43–47.

Wisniewski, P.J. (1984): Limb degeneration in two species of Asian newts. — Brit. Herpetol. Soc. Bull. **10**: 54.

Witschi, E. (1920): Über merogenetische Entwickung äquipotentieller Teile. — Festschr. Zschokke, Universität Basel **36**: 3–15.

Witschi, E. (1922): Überreife der Eier als kausaler Faktor bei der Entstehung von Mehrfachbildungen und Teratomen. — Verh. Naturf. Ges. Basel **34**: 33–40.

Witschi, E. (1925): Die Überreife des Eies als teratogenetischer Faktor. — Verh. Dtsch. Zool. Ges. **30**: 91–94.

Witschi, E. (1952): Overripeness of the eggs as a cause of twinning and teratogenesis: a review. — Cancer Res. **12**: 763–786.

Witschi, E. & C.Y. Chang (1954): Hereditary polymelia combined with edema and sex reversal in the toad. — Anat. Rec. **118**: 370.

Wittouck, P. (1980): L'œdème sous-cutané, œdema (œ), une maladie de la postmétamorphose, observée chez des têtards de *Rana esculenta*. — Bull. Soc. Zool. Fr. **105**: 545–550.

Witzmann, F. (2007): A hemivertebra in a temnospondyl amphibian: the oldest record of scoliosis. — J. Vertebrate Paleontol. **27**: 1043–1046.

Woerdeman, M.W. (1936): Embryonic "induction" by chemical substances. — Proc. K. Akad. Weten. Amsterdam Sect. Sci. **39**: 306–314.

Wohltmann, A., L. du Preez, M.-O. Rödel, J. Köhler & M. Vences (2007): Endoparasitic mites of the genus *Endotrombicula* Ewing, 1931 (Acari: Prostigmata: Parasitengona: Trombiculidae) from African and Madagascan anurans, with description of a new species. — Folia Parasitol. **54**: 225–235.

Woitkewitsch, A.A. (1955): Magnification of limb in frogs. — Priroda (Moscow) **12**: 100–102.

Woitkewitsch, A.A. (1959): Natürliche Mehrfachbildungen an Froschextremitäten. — Jena (Fischer).

Woitkewitsch, A.A. (1961): Le dévelopment des extremités surnuméraires chez les amphibiens. — Bull. biol. Fr. Belg. **7**: 569–600.

Woitkewitsch, A.A. (1964): Stoikost duplikatsii pri sverchkomplektnom obrazovanii zadnich konetsnostei u ljagushki v prirodne. Pp. 15–16 in Dubrovskaya, I.P. (ed.): Materialy herpetologitsheskoi Konferenzii 12–14 Okt. 1964. — Leningrad (Univ. Leningrad).

Woitkewitsch, A.A. (1965): Mass formation of additional hind extremities in the lake frog. — Zh. obschtschej biol. **26**: 56–62.

Wolf, K.-R. (1994): Untersuchungen zur Biologie der Erdkröte *Bufo bufo* L. unter besonderer Berücksichtigung des Einflusses von Migrationshindernissen auf das Wanderverhalten und die Entwicklung von vier Erdkrötenpopulationen im Stadtgebiet von Osnabrück. — New York (Mellen Univ. Press).

Wolf, K.W., G.L. Bullock, C.E. Dunbar & M.C. Quimby (1969): Tadpole edema virus: pathogenesis and growth studies and additional sites of virus infected bullfrog tadpoles. Pp. 327–336 in Mizell, M. (ed.): Biology of Amphibian Tumors. — Berlin (Springer).

Wolterstorff, W. (1925a): Albinismus bei *Salamandra maculosa*. — Bl. Aquar. Terrarienkde **36**: 77–79.

Wolterstorff, W. (1925b): Katalog der Amphibien-Sammlung im Museum für Natur- und Heimatkunde zu Magdeburg. Erster Teil: Apoda, Caudata. — Abh. Ber. Mus. Natur- Heimatkunde Naturwiss. Ver. Magedeburg **4**: 231–310.

Wolterstorff, W. (1935): Über eine eigentümliche Form des kalifornischen Wassermolches, *Taricha torosa* (Rathke). — Bl. Aquar. Terrarienkde **46**: 178–184.

Wolterstorff, W. (1941): Abnorme Regeneration einer Hintergliedmaße bei einer Larve von *Alytes obstetricans* (Geburtshelferkröte). — Bl. Aquar. Terrarienkde **52**: 326–327.

Wolterstorff, W. & G.E. Freytag (1941): Bemerkungen zu Susebachs brieflicher Mitteilung: Eine zweiköpfige Feuersalamanderlarve. — Wschr. Aquar. Terrarienkunde **38**: 367–68.

Worrest, R.C. & D.J. Kimeldorf (1975): Photoreactivation of potentially lethal, UV-induced damage to boreal toad (*Bufo boreas boreas*) tadpoles. — Life Sciences **17**: 1545–1550.

Worrest, R.C. & D.J. Kimeldorf (1976): Distortions in amphibian development induced by ultraviolet-B enhancement

(290–315 nm) of a simulated solar spectrum. — Photochem. Photobiol. **24**: 377–382.

Worthington, R.D. (1974): High incidence of anomalies in a natural population of spotted salamanders, *Ambystoma maculatum*. — Herpetologica **30**: 216–220.

Worthylake, K.M. & P. Hovingh (1989): Mass mortality of salamanders (*Ambystoma tigrinum*) by bacteria (*Acinetobacter*) in an oligotrophic seepage mountain lake. — Great Basin Naturalist **49**: 364–372.

Wright, K.M. & B.R. Whitaker (2001): Amphibian Medicine and Captive Husbandry. — Malabar (Krieger).

Wu, X., J. Jiang, Y. Wan, J.P. Giesy & J. Hu (2012): Cyanobacteria blooms produce teratogenic retinoic acids. – PNAS **109**: 9477–9482.

Wurffbain, J.P. (1683): Salamandrologia h.e. Descriptio Historico-Philologico-Philosophico-Medica Salamandrae quae vulgo in igne vivere creditur. — Nürnberg (Georg Scheurer).

Wurmbach, H., A. Biwer, L. Schneider, H-L. Pohland & U. Borchert (1964): Zur antithyreoidalen und Mißbildungen erzeugenden Wirkung pflanzlicher und tierischer Öle bei Kaulquappen. — Forschungsber. Landes Nordrhein-Westfalen **1371**: 1–71.

Yablokov, A.V., V.B. Nesterenko & A.V. Nesterenko (2009): Chernobyl: consequences of the catastrophe for people and the environment. — Ann. N.Y. Acad. Sci. **1181**.

Zamaletdinov, R.I. (2014): Materials on the occurrence of morphological abnormalities in natural populations of anurans in the Tatarstan Republic. Pp. 105–111 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Zavadil, V. (1996): Ropucha kratkonoha, *Bufo calamita* Laurenti 1768. — Ochrana Prirody **51**: 135–138.

Zavadil, V., P. Kolman & J. Marík (1997): Frogs myiasis in the Czech Republic with regard to its occurrence in the Cheb district and comments on the bionomics of *Lucilia bufonivora* (Diptera, Calliphoridae). — Folia Fac. Sci. Nat. Univ. Masarykianae Brunensis, Biol. **95**: 201–210.

Zavanella, T. & M. Losa (1981): Skin damage in adult amphibians after chronic exposure to ultraviolet radiation. — Photobiol. **34**: 487–492.

Zavanella, T., N. Pacces Zaffaroni & E. Arias (1984): Abnormal limb regeneration in adult newts exposed to the fungicide MANEB 80. A histological study. — J. Toxicol. Environ. Health **13**: 735–745.

Zavanella, T., E. Arias & N. Pacces-Zaffaroni (1989): Evaluation of the carcinogenic risk of 2-methyl-4-chlorophenoxy acetic acid (MCPA) in the adult newt. — Herpetopathologica **1**: 51–56.

Zimmermann, P. & B. Aeschbach (2005): Freilandbeobachtungen, Terrarienhaltung und Nachzucht der Bribri-Variante von *Dendrobates pumilio* Schmidt, 1857. — elaphe **13(3)**: 34–39.

Zimskind, P.D. & R.M. Schisgall (1956): Photorecovery from ultraviolet-induced pigmentation changes in anuran larvae. — J. Cell. Comp. Physiol. **45**: 167–175.

Zmeeva, D.V. (2014): Variants of the anatomical structure in Siberian salamander populations (*Salamandrella keyserlingii* Dybowsky, 1870, Amphibia, Hynobiidae). Pp. 112–118 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. — Ekaterinburg (Uralskii Federalnii Univ.).

Zumpt, F. (1965): Myiasis in man and animals in the Old World. — London (Butterworth).

MERTENSIELLA 25 | 165–184 | 2017

The Roßwag affair: history and controversy of the discovery of mass anomalies in the green toad (*Bufotes viridis*) in a German quarry

Klaus Henle[1], Kurt Rimpp[2], Josef Margraf[3†] & Alain Dubois[4]

[1]UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstr. 15, 04318 Leipzig, Germany, klaus.henle@ufz.de

[2]Brunnenfeldstr. 10/3, 71272 Renningen, Germany

[3]Tian Zi Biodiversity Research & Development Centre, Mekong Hill Garden, International Hill, 666100 Jinghong, Xishuangbanna, Yunnan, China; [†]deceased

[4]Institut de Systématique, Évolution, Biodiversité
ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE
Muséum national d'Histoire naturelle, Sorbonne Universités
57 rue Cuvier, CP 30, 75005, Paris, France
adubois@mnhn.fr

Abstract. Increased rates of anomalies in natural animal populations raise concerns about hazards to wildlife and human health. The discovery of mass anomalies may spark intensive political, social and scientific controversies. How we deal with increased rates of anomalies is an indicator of our maturity in dealing with environmentally and politically sensitive issues. Here we report on controversies that developed after the discovery of an extreme case of mass anomalies in a population of green toads (*Bufotes viridis*) in a quarry in Roßwag, southern Germany, in 1980. Screening for potential environmental causes indicated increased levels of radioactivity inside a fill of earthen material in the quarry. After follow-up measurements, governmental organizations argued that artificial radioactivity as a potential cause could be excluded with certainty. In the ensuing controversy, responsible administrations jumped from one explanation to another once the previous one was no longer tenable. They refused to seriously consider any non-natural causes, ignored concerns voiced by scientists and misinformed the public. We summarize the history of the discovery, attempts to unravel the causes and ensuing chain-reactions of controversies about potential causes. All potential causes except mutagenic factors can be excluded, and in 2006 a key protagonist confessed that official measurements were unsuitable for detection of radioactivity if buried in the earth deposit. We summarize open questions and suggest lessons learnt.

Keywords. Anura: *Bufotes viridis*, chronology, environmental politics, hazard management, mass anomalies, radioactivity, science & truth, wildlife hazard

Zusammenfassung. Ein besonders extremer Fall massenhaften Auftretens von Anomalien wurde 1980 bei Wechselkröten in einem Steinbruch bei Roßwag, Baden-Württemberg, Deutschland, entdeckt. Bei der Suche nach möglichen Ursachen haben die Entdecker der Missbildungen erhöhte Werte an Radioaktivität an einer Erddeponie gemessen, deren Fuß in das Laichgewässer ragte. An Erdspalten erreichten die Werte das 100-fache der Umgebung. Die alarmierten Behörden unternahmen ad-hoc-Messungen, aufgrund derer sie die von den Entdeckern gemessenen Werte als Artefakte zurückwiesen und das Vorhandensein einer künstlichen radioaktiven Quelle mit Sicherheit ausschlossen. Bevor chemische Analysen der Wasserproben vorlagen, erlaubten die Behörden dem Steinbruch-Unternehmen, noch am Tage der Anfrage, das Wasser abzupumpen. Auch das Sediment wurde komplett beseitigt.

In der sich anschließenden Kontroverse sprangen die verantwortlichen Behördenvertreter von einer zur nächsten natürlichen Ursache als Erklärung für die Missbildungen, sobald die vorhergehende nicht länger haltbar war. Ein als Kronzeuge für die von ihnen favorisierte Erklärung zitierter Wissenschaftler wurde später als verantwortungslos bezeichnet, nachdem er die Unhaltbarkeit der behördlichen Erklärungen aufzeigte und darlegte, dass die Anomalien keine natürlichen Ursachen haben, sondern nur durch Radioaktivität oder Chemikalien erklärt werden können. Die Behörden ignorierten jegliche nicht-natürliche Erklärung und sämtliche kritisch fragende Wissenschaftler wurden in der Presse mit Unwahrheiten gezielt in ein schlechtes Licht gerückt. So wurden beispielsweise missgebildete Kröten weggeworfen, statt diese zu untersuchen; das Ausbleiben von Untersuchungsergebnissen wurde der Arbeitsüberlastung eines unabhängigen Wissenschaftlichen Institutes angelastet, obwohl dieses nicht mit Untersuchungen beauftragt wurde. Erst durch öffentlichen Druck führten sie eine Anhörung im Landwirtschaftsausschuss des Landtags von Baden-Württemberg durch. Als Ergebnis der Anhörung wurde eine drei-jährige wissenschaftliche Untersuchung in Auftrag gegeben, die allerdings nur den Zweck hatte, Hybridisierung mit einer weiteren Krötenart als Ursache nachzuweisen. Sämtliche Ergebnisse der Untersuchung widersprachen dieser Hypothese. Dennoch wurde sie, zusammen mit Inzucht, als abschließende offizielle Erklärung verkündet.

Inzwischen können alle möglichen Ursachen außer einem starken mutagenen Faktor (Radioaktivität oder mutagene Chemikalien) mit Sicherheit ausgeschlossen werden. Auch ist klar, dass die Behördenmessungen nicht geeignet waren, etwaiges in der Erddeponie vergrabenes radioaktives Material nachzuweisen.

In der vorliegenden Veröffentlichung geben wir eine ausführliche Übersicht über den Verlauf der Affaire, wobei wir uns auf die politischen und öffentlichen Kettenreaktionen konzentrieren, die durch die Kontroversen über mögliche Ursachen ausgelöst wurden. Wir zeigen auf, was aus der Affaire für ähnliche Fälle gelernt werden kann, und fragen unter anderem, inwieweit zuständige Behörden und Politiker inzwischen besser mit potentiellen Umweltgefahren umzugehen wissen, oder ob noch immer eine Bereitschaft besteht, die Öffentlichkeit falsch zu informieren. Unklar ist immer noch, wie und von wem mutagene Stoffe in den Steinbruch verbracht wurden, ob es ein Einzelfall war und inwieweit sichergestellt werden kann, dass heute und in Zukunft von der Erddeponie keine Risiken mehr ausgehen können.

Schlagwörter. Anura: *Bufotes viridis*, Wechselkröte; Chronologie, Gefahrenmanagement, massenhafte Missbildungen, Radioaktivität, Umweltpolitik, Wissenschaft & Wahrheit

1 Introduction

Scientists and the public have been interested in anomalies of animals for centuries (e.g., Geoffroy Saint-Hillaire 1832–1836, Taruffi 1881–1886, Rostand 1971). Increased environmental awareness has created a recent resurge of interest in anomalies as an indicator of environmental and human health (e.g., Tyler 1983, 1989, Gilbertson et al. 1991, Vuori & Parkko 1996, Burkhart et al. 2000, Ouellet 2000, Feshchenko et al. 2002, Souder 2002, Vershinin 2002, Lannoo 2008). Therefore, it is not surprising that the discovery of increased incidences of malformed animals raises concern about the environment and possible hazards of causative agents for wildlife and human health (Ferraro & Burgin 1993, Ellegren et al. 1997, Burkhart et al. 2000, Blaustein & Johnson 2003, Møller et al. 2007, Lannoo 2008, Helgen 2012). Despite an impressive literature on anomalies in amphibians and other animals, it still remains a major challenge to identify factor(s) responsible, whether they are natural or anthropogenic (e.g., Rostand 1971, Burkhart et al. 2000, Ouellet 2000, Blaustein & Johnson 2003, Henle et al. 2017a) and what needs to be done to solve the problem (Lannoo 2008). Not all increased incidences of anomalies are necessarily due to anthropogenic pollution (e.g., Veith & Viertel 1993, Sessions et al. 1999), but many environmental pollutants, including radioactivity, are known to cause anomalies in amphibians and other organisms (Semenov & Ivanova 1995, Ouellet 2000, Feshchenko et al. 2002, Møller et al. 2007, Hesse-Honegger & Wallimann 2008, Henle et al. 2017a).

Discoveries of increased rates of anomalies are a highly sensitive issue that frequently sparks intense controversy and heated debate over causal factors (e.g., Bär 1981, Henle 1981, Kneissler 1981, Allen 1996, Schmidt 1997, Møller et al. 2007, Hessse-Honegger 2008). Amphibians have been repeatedly involved in such controversies (e.g., Rostand 1971, Henle 1981, Kneissler 1981, Allen 1996, Souder 2002, Lannoo 2008, Helgen 2012) and environmental history has many records of premature dismissal and even denial of hazards of anthropogenic pollutants to wildlife and human welfare (e.g., Carson 1962, Widener 1970, Conrad 1977, Lenssen & Flavin 1996, UCS 2004, Møller et al. 2007, 2008). So far, science and society has largely failed to deal with such challenges (Lannoo 2008). Histories of past cases can enlighten us concerning both pitfalls and proactive methods to help us meet these challenges.

Despite valuable insights that can be gained from histories of controversies about mass anomalies in amphibians and scientific attempts to identify causes, they have been rarely compiled and published (but see e.g., Rostand 1971, Dubois 1979, Burkhart et al. 2000, Souder 2002, Lannoo 2008, Helgen 2012). Furthermore, with the exception of Ouellet (2000), existing reviews on amphibian anomalies cover only a small number of known anomalies or causes.

Among the observations of anomalies in natural animal populations that have received broad attention in the mass media and in the political arena was the discovery of malformed amphibians in Amsterdam (Rostand 1971), Mississipi (Volpe & Rosenbaum 2000), Minnesota (Souder 2002, Lannoo 2008, Helgen 2012) and of green toads (*Bufotes viridis*) in a quarry in Roßwag, southern Germany, in 1980 (Fig. 1). While the discov-

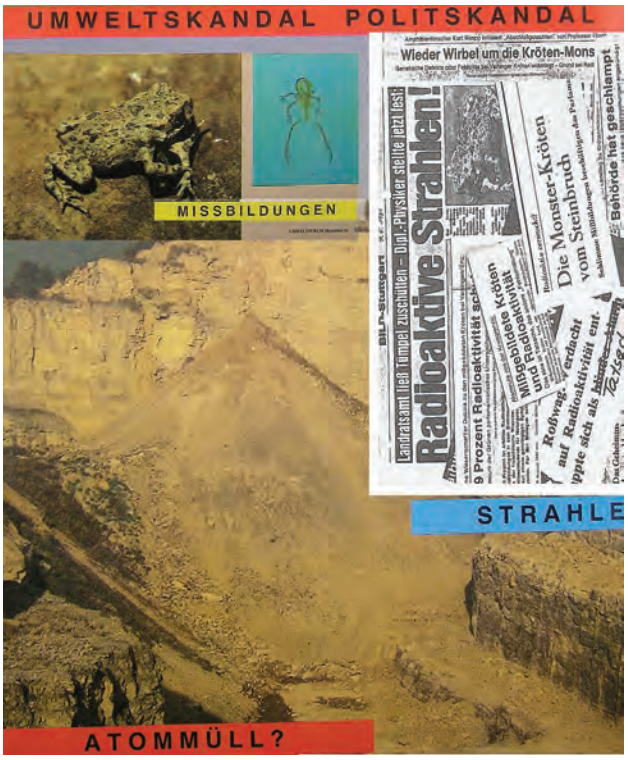


Fig. 1: Mass media coverage of the controversy about the malformed toads of Roßwag, showing the quarry in the background after the destruction of the breeding pond.

Fig. 2: White tadpole of the green toad (*Bufotes viridis*); Roßwag quarry, September 1980. Photo: K. Henle.

eries and controversies about cases in North America have been summarized in three books (Souder 2002, Lannoo 2008, Helgen 2012), only parts of the history of the German case have been published (Beck-Oberdorf 1981a, Henle 1981, 1982, Henle & Schmitt 1981, Kneissler 1981, Rimpp 1981a, Dubois 1984). Most information is widely scattered, difficult to access or not yet made public.

The discovery in Roßwag fuelled an intense controversy regarding potential cause(s), with explanations ranging from a normal natural phenomenon to chemical pollution respectively illegally dumped radioactive material. Strong evidence now exists that only a highly mutagenic factor could have caused the anomalies (Henle et al. 2017a). Therefore, the possibility of continuous existence of hazards to wildlife or humans cannot be excluded. Furthermore, the knowledge regarding amphibian anomalies has advanced considerably in the meantime (e.g., Tyler 1989, Ouellet 2000, Ankley et al. 2004, Lannoo 2008, Henle et al. 2017a,b). To comprehend the controversy, a chronology of key issues is essential. Here we report on the history of the discovery, the scientific attempts to unravel the causes and the ensuing chain-reactions in the political and public arena. We summarize open questions and suggest lessons learnt.

2 History of the affair

30.8.1980. – On their way home from a project to map the distribution of amphibians and their habitats, Kurt Rimpp (an amateur herpetologist) and Klaus Henle (then an undergraduate student of biology) visited a quarry owned by KG Zimmermann in Roßwag, near Stuttgart, Germany. In an abandoned part of the quarry, they encountered a pond of ca. 100 x 25 m with a maximum depth of 1 m (Fig. 4a). A large deposit of earthen material bordered one side of the pond. They detected a large number of tadpoles of *B. viridis*, including white individuals (Fig. 2) and many giant ones, which appeared similar to tadpoles of *B. viridis* but by far exceeded the maximum known size.

6.9.1980. – K. Rimpp and K. Henle returned to the quarry to survey the tadpole population in more detail. They estimated a tadpole population of a few thousand individuals. In the surrounding area, they counted 65 toadlets, all belonging to *B. viridis*. More than 1,000 tadpoles and approximately 50% of the toadlets exhibited major colour and/or morphological anomalies (Fig. 3). No other amphibians were found.

8.9.1980. – K. Henle and K. Rimpp again surveyed amphibians in the quarry yielding only *B. viridis*. They collected water samples and measured radioactivity with a detector (type Minicont, trade mark of Herfurth) borrowed from Hermann Schreiber (Institut für Physik und Meteorologie, Universität Stuttgart-Hohenheim – Institute of Physics and Meteorology). They expected this would exclude from further consideration one known cause of anomalies. They made radioactivity measurements in a parking area east of the quarry (con-

Fig. 3: Recently metamorphosed *Bufotes viridis* with supernumerary legs, Roßwag quarry, September 1980. Photo: K. Henle.

trol side), at the entrance of the quarry, along the pond edge and across the base of the earthen deposit. All measurements were made ca. 1 m above ground. These measurements resulted in slightly elevated activity at the earthen deposit compared to the other sites. Because of the twofold increase at the earthen deposits, K. Henle decided to select large cracks at the base of the deposit for additional sampling, since they provided potential hiding places for toads. When approaching these cracks with the instrument, radioactivity increased exponentially and attained a maximum directly above the openings, reaching more than 100 times background level for the largest crack. Measurements close to the surface of the earthen deposit at sites where no cracks were present did not result in elevated levels (see Henle et al. 2017a for details).

K. Henle and K. Rimpp made considerable effort to test for potential artefacts or malfunctioning of the instrument. They hold the instrument upside down and vertical, shook it heavily, placed it firmly upon stones collected from within the quarry and rapidly approached the ground while making measurements. All tests were repeated several times but did not elicit deviations from background measurements. Some tests were later repeated together with Schreiber in his institute with the same negative results.

The same day, K. Rimpp informed the Ministerium für Ernährung, Landwirtschaft, Umwelt und Forsten Baden-Württemberg (MELUF – Ministry of Food, Agriculture, Environment and Forestry) about these measurements via Umwelttelefon – a phone number for the public to report environmental problems.

10.9.1980. – At 9:00 h, K. Rimpp phoned the Katastrophenschutzamt Ludwigsburg (Office for Disaster Control, District Ludwigsburg) and requested that a specialist repeat the radioactivity measurements. At 9:45 h, K. Rimpp was ordered to the quarry within an hour. When he arrived, about 40 vehicles were present: police, fire brigade, ABC-Schutz (emergency task force for nuclear, biological and chemical disasters) of the Districts Enzkreis and Ludwigsburg, physicists of the Institut für Immissions-, Arbeits- und Strahlenschutz, Landesanstalt für Umweltschutz (LfU) (Institute for Pollution Control, Workplace Safety and Protection against Radiation of the State Institute for Environmental Protection in Baden-Württemberg) and many journalists. LfU measured radioactivity and collected one sample each of pond water, of quarry rocks and of earthen material. The entire field protocol consisted of six values for radioactivity without local differentiation. Neither information on the locations surveyed within the quarry and control points nor numbers of survey points and measurements per survey point, height above ground and distance to the opening of cracks were provided. LfU informed media representatives that existence of unnatural levels and sources of radioactivity in the quarry could be excluded with certainty.

Werner Burckhart (Deputy District Administrator of the Landratsamt Enzkreis) threatened K. Rimpp with fines because he had collected malformed toads without a permit (Rimpp 1981a).

Later, all officials had already left, K. Henle returned to the quarry to reconfirm or reject his earlier measurements. H. Schreiber, who was still highly supportive, lent him another more sophisticated instrument, a Xenon-based detector. The cracks could not be approached as closely as with the Herfurth Minicont instrument because the Xenon detector was broader and bulkier and was more expensive. Therefore, a larger safety margin for not touching the ground was taken to comply with instructions by H. Schreiber. Consequently, a much smaller part of the instrument could be exposed to the crack openings. Measurements reconfirmed a raised level of radioactivity at the earthen deposit compared to the edge of the pond. Measurements 10 cm above the crack that showed the highest level on 8.9.1980 revealed 3–6 times the level of background radioactivity, in accordance with measurements made two days earlier (Henle et al. 2017a).

The same day, Wolfram Morgenstern (Director of the Institut für Immissions-, Arbeits- und Strahlenschutz, LfU; he was a fellow student with H. Schreiber) phoned H. Schreiber and complained because he loaned a radioactivity detector to a student.

11.-12.9.1980. – Newspapers reported on the malformed toads and radioactivity measurements. They explained away K. Henle's results with arguments provided by LfU as "frequency interferences" or "operational errors" (Anonymous 1980a-g, Einzel 1980, Theegarten 1980).

11.9.1980. – TV Südfunk Stuttgart interviewed K. Ruge (a biologist from LfU), K. Rimpp and K. Henle in the quarry, which was broadcast the same day in "Die Abendschau". K. Ruge collected malformed toads for analysis of residues of chlorinated hydrocarbons, as some of these are teratogenic.

12.9.1980. – K. Henle tried once again to borrow a radioactivity detector from Schreiber, but this time H. Schreiber refused because of the media attention and the phone call from Morgenstern. After an extended discussion, K. Henle persuaded H. Schreiber to meet privately on the weekend at the quarry to make joint measurements.

13.9.1980. – K. Henle, H. Schreiber, Schreiber's wife and Martin Weiss (a fellow biology student of K. Henle, University Stuttgart-Hohenheim) met at the quarry. H. Schreiber made a few initial measurements in the quarry. They indicated levels of radioactivity at the earthen deposit approximately twice that measured at the shore of the pond. An employee of the quarry operation Zimmermann KG interrupted the team at that early stage and threatened it with financial compensation claims. H. Schreiber left the quarry without finishing measurements (Anonymous 1981a,g,h, Kneissler 1981). Therefore, neither the initial control points nor cracks were assessed, nor are field protocols available. H. Schreiber informed MELUF at some stage, presumably via LfU, that he made measurements

Fig. 4a

Fig. 4c

Fig. 4b

Fig. 4: Roßwag quarry and breeding pond of *Bufotes viridis*:
(a) before destruction (8.9.1980);
(b) after destruction (28.9.1980);
(c) on 28.5.2006.
The black lines in (a) and (b) encircle the area where the initial measurements of elevated radioactivity had been made; notice in (b) the lack of water and the recent deposits of material at this area. The white arrow in (c) indicates the approximate location of the back edge of the former pond; for better orientation, the black arrow in (a) indicates the platform from which the earthen material shown in (a) and (b) was dumped. Photos: K. Henle.

together with Henle in the quarry. Either he did not mention that these measurements were interrupted and not done directly at the opening of cracks or this information was suppressed in a letter of Minister Gerhard Weiser (MELUF) to the president of the State Parliament dated 10.6.1981. [Note that in a TV interview in 2006, H. Schreiber clearly stated that his measurements could not have detected radioactive material hidden within the earthen deposit; see below.]

15.9.1980 (Monday). – Steinbruch Zimmermann KG asked for a permit to pump out the pond water and fill the pond with stones and earth. Regarding on-going quarry operation, there was no need for such an action (see Appendix I). W. Burckhart provided permission immediately despite results of the water analyses not yet being available (Henle 1982). This speed of action was termed "not unusually rapid" by Minister G. Weiser in his letter to the president of the State Parliament dated 10 June 1981 as a response to a parliamentary inquiry of the Green Party. Additionally, Minister G. Weiser pretended that the chemical water analyses had been almost completed when the request was granted but the first results were not available until 9.10.1980 (Schnorbach 1981, Henle et al. 2017a).

18.9.1980. – K. Henle called Friedrich Veigel (general manager of Steinbruch Zimmermann KG) to ask for permission to visit the quarry and collect toads. F. Veigel refused and explained that a visit would be senseless since the habitat was destroyed.

K. Rimpp wrote to R. Nöthiger (Zoologisches Institut, Universität Zürich, Switzerland – Zoological Institute), about the malformed toads (Knapp 1980) to get his opinion about potential causes.

20.9.1980. – K. Henle and K. Rimpp visited the quarry and noticed that those places where they had measured high levels of radioactivity (Fig. 4a-b) were covered with 3-5 m of earth. Also, the pond water had been completely removed, as was the pond sediment (Anonymous 1980h, Henle 1981, 1982, Kneissler 1981, Rimpp 1981a).

24.9.1980. – K. Henle and Josef Margraf (PhD student of biology, University Stuttgart-Hohenheim) visited the quarry to survey for additional toads. They could not get access but observed that depositing of earth and

alterations of the empty pond were continuing under floodlight well into the night (after 22:00 h).

28.9.1980. – Alerted by media reports, the Institut für Energie- und Umweltforschung, Heidelberg (IFEU – Institute for Energy and Environmental Research) (see Appendix I) decided to start a series of radioactivity measurements in the quarry. The measurements were terminated on 7.12.1980 only, because of drastic alterations of the earth deposit and the original pond and because access to the quarry became increasingly difficult. The measurements indicated elevated radioactivity (Franke et al. 1981, see also Knapp 1980) but they were disputed by LfU (see below).

9.10.1980. – Chemisches Untersuchungsamt der Stadt Pforzheim (Chemical Laboratory of the City of Pforzheim) presented results of the water analysis to the Landratsamt Enzkreis (LRE – Administrative District Enzkreis). No indications of pesticides were found (Anonymous 1981e) – gas chromatography was used. Toxicity or mutagenicity tests (e.g. Birge et al. 1983) were not carried out.

18.10.1980. – Based on information launched by LRE, newspapers reported that citizens with sound knowledge and interest in the affair believe that the malformations were due to detonations during quarry operations (Anonymous 1980i,j).

22.10.1980-19.1.1981. – Correspondence was exchanged between Friedrich Vogel (Institut für Anthropologie und Humangenetik, Universität Heidelberg – Institute of Anthropology and Human Genetics) and W. Burckhart. F. Vogel expressed concerns and asked that the area around the pond be left unaltered. W. Burckhart ignored these concerns. He explained his reluctance to take them seriously due to high costs of further investigations and failure of official measurements (water and radioactivity) to detect anything unusual. A newspaper article launched by W. Burckhart dated 31.1.1981 (Schmid 1981) reads: "Prof. Vogel schweigt seit Oktober beharrlich auf die Frage, was die Behörden… [unternehmen sollten]" – Prof. Vogel stubbornly remains silent since October when questioned by the administration… [about what actions they should take].

28.10.1980. – Schauz (1980) reported in a regional newspaper that the malformed toads collected by LfU were sent to Tierhygienisches Institut, Universität Freiburg (TIF – Institute of Veterinary Hygiene). He noted that results could not be expected within the next three months because of an extremely heavy workload at TIF. In reality, the malformed toads collected by Klaus(?) Ruge disappeared at LfU (Kneissler 1981, Puhlmann 1981a). Only by coincidence the head of TIF learned that he was supposed to be examining the toads (Schnorbach 1982). On 21.1.1981, K. Ruge had to ask K. Rimpp to send him additional malformed toads, which were examined in a short time by TIF. B. Funsch, a colleague of K. Ruge at LfU, complained in a parliamentary hearing on 6.11.1981 that he never received malformed toads for examination of radioactive residuals.

18.11.1980-7.1.1981. – Correspondence was exchanged between R. Nöthiger and LRE (W. Burckhart). R. Nöthiger excluded the possibility that mutations were the cause of the malformations, suggested chemical examinations and indicated that Rostand's (1971) scientific work on "anomaly P" of green frogs (*Pelophylax esculentus* complex) should also be considered. On 7.1.1981, he announced that he no longer wanted to be involved in this affair because of the unscientific treatment it received in the public. From this time onward, LRE pretended that it is proven that the anomalies in the Roßwag population were the same as anomaly P. LRE launched a newspaper article (Schmid 1981) stating that the malformations were a usual, natural phenomenon and that Nöthiger had demonstrated that a virus was the cause. Rostand (1971) had favoured viral infections as the cause of anomaly P, but its cause still remains obscure (Dubois 2017). Schmidt's newspaper article further stated that R. Nöthiger regarded the hypothesis that chemicals may be the cause of the anomalies as highly speculative and unqualified. In reality (Nöthiger in litt., Rimpp 1981b) he criticized a report (Klein 1981a), which insisted without scientific support that chemicals were the cause of the anomalies, and asked for experimental proof of this hypothesis.

7.12.1980. – IFEU stopped its radioactivity measurements because considerable alterations to the site prevented continuation (Anonymous 1981p). The measurement series contained four temporal data points. It was consistent with the hypothesis of a level of radioactivity in the deposit of earth that was elevated relative to a control area outside the quarry and declining with time (Henle et al. 2017a). The report acknowledged that inferences are only tentative because of limited sample size, technical reasons and particularly because of alterations to the site. These results were communicated to the press (Anonymous 1980h,k, Schnedler 1980, Puhlmann 1981b) and created an intense dispute with LfU about inferences that can be drawn from the measurements.

18.12.1980. – Hinrich Rahmann (Director of the Institut für Zoologie, Universität Stuttgart-Hohenheim – Institute of Zoology) expressed in writing his concern about the malformations to W. Burckhart. Again, these concerns were ignored (Rahmann, pers. comm.).

2.1.1981. – Radio Südwestfunk Channel I broadcast an interview with Eckhard Krüger (leader of the IFEU team) about the measurements of IFEU and the alterations in the quarry.

January 1981. – Klein (1981a) published excellent photos of malformed toads but, without providing data, insisted that sprayings in vineyards around the quarry had been the cause.

The science magazine *Bild der Wissenschaft* reported on the IFEU measurements and criticized the alterations in the quarry (Kovacsics 1981), despite of Ewe (Institut für Zoologie, Universität Stuttgart-Hohenheim) advising them not to publish the article because the malformations could well have had natural causes (e.g., injuries to eggs by bird pecking).

31.1.1981. – Referring to information received from W. Burckhart, Schmid (1981) wrote about the scientific debate, regarded it as useless and took the virus hypothesis suggested by R. Nöthiger as proven. Most facts, for which documentations exist, were distorted. This article, and those of Klein (1981a) and Kovacsics (1981) and the interview of E. Krüger, caused a dispute between K. Henle and H. Rahmann (at whose institute Henle was preparing his MSc thesis). H. Rahmann felt that the observations should have been published under his supervision in a scientific paper before any public or political steps were undertaken.

February 1981. –Several newspapers again reported on the controversy concerning causes for the malformations and the IFEU measurements (Anonymous 1981b-d).

20.2.1981. – Wolf-Dieter Hasenclever (Deputy Leader of the Green Party) made an inquiry in the Landtag Baden-Württemberg (State Parliament of Baden-Württemberg) whether or not the government can definitely exclude radioactivity as cause of the malformations and what steps the government intends to undertake (Hasenclever 1981). Marieluise Beck-Oberdorf (chairwomen of the Green Party in Baden-Württemberg) sent a similar letter to LRE (Anonymous 1981e). In his answer, W. Burckhart complained that IFEU had not yet contacted him and that he was unable to find their phone number. [This phone number could easily be found by consulting the Heidelberg phone book]. He stated that he was willing to discuss the results, but not in public (Anonymous 1981f,u, Bär 1981, Ignatz der Zornige 1981).

11.3.1981. – Rimpp (1981b) wrote an open letter to W. Burckhart, asking him why he incorrectly cited R. Nöthiger's letter as having proved a natural cause for the Roßwag anomalies although the letter only briefly mentioned Rostand's (1971) research as worth considering. He drew attention to the contradiction that W. Burckhart gave immediate permission to destroy the habitat of *B. viridis*, a protected species, while threatening Rimpp with prosecution because he collected malformed toads. W. Burckhart provided permission to collect a few malformed toads for further studies by K. Rimpp only after intervention by journalists that were present during official measurements in the quarry (Rimpp 1981a). Rimpp (1981b) summarized additional false information given to the public by W. Burckhart.

26.3.1981. – Minister G. Weiser's answer (Landtag von Baden-Württemberg 1981) to W.-D. Hasenclever's inquiry (Hasenclever 1981) stated that the Roßwag malformations were not a singular case and the government can definitely exclude radioactivity as the cause of the malformations. He justified his statement with the measurements made by LfU, information from "various sources" that anomalies in the green toad have been observed elsewhere and Rostand's (1971) research [on anomaly P in green frogs]. He also stated that no further investigations were intended (Puhlmann 1981b-c). According to an internal protocol of a meeting of the Landwirtschaftsausschuss (Parliamentary Committee for Food, Agriculture and Environment) at the State Parliament of Baden-Württemberg, the "various sources" consisted of an amateur geologist (Mr. Regelmann) and supposed hearsay by local people (see below). K. Rimpp and K. Henle met Regelmann on the day of their discovery of the toads. They showed him malformed specimens and he told them that he had seen such toads before in other quarries that were in the process of being filled. When questioned further, he could remember one case for certain. J. Margraf and K. Henle decided to visit this specifically named quarry in the municipality of Ilsfeld on 14.5.1981 (see Anonymous 1981r). [This quarry is close to a nuclear power station.] A worker confirmed that during the phase of filling the quarry malformed toads were present but he could not provide details. J. Margraf and K. Henle tried to contact Regelmann but learned that he had died.

30.3.1981. – The city council of Vaihingen rejected a motion by its SPD (Social Democrats) fraction to take necessary steps for excavations in the quarry as a precautionary step in the interest of the quarry workers and inhabitants of Roßwag (Anonymous 1981z).

April 1981. – Wolfgang Böhme (ZFMK: Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn – Zoological Research Institute and Museum Alexander Koenig) and Henle discovered a publication of the Laboratory for Amphibian Biology in Hiroshima on abnormalities in frogs caused by experimental irradiation (Kawamura & Nishioka 1978, Nishioka 1978). K. Henle presented a talk on the Roßwag toads at University Stuttgart-Hohenheim. He discussed his radioactivity measurements and those made by IFEU with H. Schreiber. H. Schreiber agreed that the approach of IFEU to measure radioactivity within the quarry relative to a control outside the quarry are correct in principle but he questioned the possibility of any reliable inference because of the few data points available and insufficient precision. He regarded the mathematics of the analyses by IFEU as insufficient and admitted that the protocol of LfU was inadequate. He confirmed that when he tested the Herfurth Minicont instrument (which he had loaned to K. Henle) it was functioning correctly and that he had no satisfactory explanation for K. Henle's measurements. Therefore, newspapers regarded it as surprising that H. Schreiber later pretended at the parliamentary inquiry that K. Henle's measurements were definitely wrong (Anonymous 1981n).

4.5.1981. – IFEU presented a detailed report to the press on their radioactivity measurements and commented on those of LfU. The report and information that "results from Hiroshima have become available" – the laboratory studies on the effects of radioactivity on amphibians by Kawamura & Nishioka (1978) and Nishioka (1978) – created renewed interest in the affair in the mass media (Anonymous 1981h-l, Ignatz der Zornige 1981, Pooth 1981, Puhlmann 1981c-d).

7.5.1981. – Referring to information obtained by W. Burckhart, a newspaper article (Anonymous 1981i)

informed the public that LRE wanted to resolve the controversy by dialogue. Two days later in an interview W. Burckhart stated that he had invited IFEU to a symposium (Anonymous 1981ac). However, this was not true (Steinhilber-Schwab, pers. comm.); to the opposite, he subsequently refused to participate in any symposium (see below).

9.5.1981. – Puhlmann (1981d) reported that Gewerbeaufsichtsamt (Trade Regulations Office) Karlsruhe informed him that they had become active in efforts to resolve the radioactivity controversy, but we have no documentation of such activity.

18.5.1981. – Motivated by IFEU's presentation of their radioactivity measurements, Hasenclever et al. (1981) made a motion to the State Parliament of Baden-Württemberg to organize a symposium on the Roßwag affair (Ignatz der Zornige 1981).

July 1981. – Minister G. Weiser, W. Burckhart and LfU in unison refused to organize a symposium. Therefore, the Green Party of the Landtag Baden-Württemberg invited all authorities involved in the affair, the IFEU team, as well as H. Schreiber, K. Henle, K. Rimpp and Alain Dubois to a symposium in Pforzheim (Anonymous 19810,u, Klein 1981b, Kneissler 1981, Schnedler 1981a-b, Buchholz 1982, Henle 1982). A. Dubois was invited because he was a pupil of Jean Rostand and the leading expert on anomaly P.

In the Federal German Parliament, the politician Paul (?) Laufs (CDU/CSU – Christian Democratic Union and Christian Social Union) requested that the Federal Government informs him whether the Government knew that "IFEU alarmed the public with grossly wrong information about the malformed toads". In an attempt to cut funding for IFEU, he further asked how the Federal Government rates the scientific quality of IFEU as a basis for decisions on further grant support (Deutscher Bundestag 1981). Appendix I provides further information on the involvement of the Federal Government and Parliament.

May-August 1981. – Monika Kovacsics prepared a TV program on the affair for Wissenschaftsmagazin, which was broadcast by Südwestfunk Baden-Baden, Channel III, in August 1981. She documented that she had difficulties getting an interview with LfU representatives because in their opinion the whole story was entirely a political affair and not to be dealt with by a scientific administration. In contrast, Minister G. Weiser of MELUF (the supervising Ministry for LfU – see Appendix I) refused an interview arguing the story was entirely a scientific issue.

16.9.1981. – The Green Party of the Landtag Baden-Württemberg (State Parliament of Baden Württemberg) organized a symposium in Pforzheim (Beck-Oberdorf 1981a) with the following invited participants: M. Beck-Oberdorf (Green Party, Baden-Württemberg), Heinz Bopp (representative of the city of Vaihingen), A. Dubois, W.-D. Hasenclever (Green Party, Baden-Württemberg), K. Henle, E. Krüger (IFEU), K. Rimpp, Winfried Scheuermann (Deputy Mayor, Municipality Illingen) and Barbara Steinhilber-Schwab (IFEU).

Several journalists attended the symposium, but none of the invited officials nor H. Schreiber or the quarry owner. Minister G. Weiser justified this arguing that the symposium was a private meeting. According to Bächle (1981), G. Weiser issued a directive forbidding administrations to participate. Note that the Ministry gave the public the incorrect impression that LfU was a scientific organisation independent of the Ministry (Schmutz 1981) (see Appendix I for their dependency). IFEU presented a more detailed analysis of its radioactivity measurements (Franke et al. 1981) and commented on the critique made by LfU (Ignatz der Zornige 1981). Dubois rejected any similarity with anomaly P [the official advocated cause of the Roßwag anomalies]. A final result of the symposium stated that the malformations were not natural and had probably been caused by artificial radioactivity. This conclusion was widely reported in the regional radio (e.g., Südwestfunk Baden-Baden, Südfunk Stuttgart) and the press (e.g., Anonymous 1981q-u, Bächle 1981, Bogen 1981, Kneissler 1981, Puhlmann 1981e, Schmidt et al. 1981).

18.9.1981. – Landwirtschaftsausschuss of the Landtag Baden-Württemberg internally discussed the Roßwag case and results of the symposium. Minister G. Weiser called A. Dubois an irresponsible scientist and seriously questioned his qualifications because he left a 1% chance that chemicals, instead of radioactivity, were the cause of the anomalies and could not say that radioactivity was the cause with 100% certainty (unpubl. protocol of the meeting; Landtag von Baden-Württemberg 1981). At the end of the meeting, Landwirtschaftsausschuss decided to organize a public hearing (Anonymous 1981v).

6.11.1981. – This public hearing was held with the following participants: W. Böhme (ZFMK), A. Dubois, B. Funsch (LfU), K. Henle, Konrad Klemmer (Senckenbergmuseum), E. Krüger (IFEU), W. Morgenstern (LfU), K. Rimpp, Walter Sachsse (Institut für Humangenetik, Universität Mainz – Institute of Human Genetics), Klaus Sander (Zoologisches Institute, Universität Freiburg – Zoological Institute), H. Schreiber, Helmut Schönnamsgruber (LfU) and B. Steinhilber-Schwab (IFEU). In addition a paper by J. Hacke (Hahn-Meitner-Institut für Kernforschung Berlin GmbH – Hahn-Meitner Institute of Nuclear Research) was distributed, as he could not appear.

Though the hearing was public, interested visitors had difficulties getting admission (Schuldes 1981). All scientists were asked to provide their interpretations of the case except K. Henle, who was not allowed to make a statement. Minister G. Weiser was the only person allowed to interrupt at any time. He cross-examined the participants that did not share the official opinion and gave the floor to "his experts" to reject any uncomfortable statement made by other participants. All other members of Landwirtschaftsausschuss were allowed to ask questions only when it was agreed to open a new round of questions. Invited experts were not allowed to

talk unless questioned (Schuldes 1981). All biologists except K. Sander agreed that the malformations were exceptional and not natural. Consequently, he was allowed the longest time to speak. He insisted that malformations were a common phenomenon caused by hybridization of two species of toads and backcrossing. He stated that the quarry manager, F. Veigel, identified with certainty spawn of the common toad [*Bufo bufo* (Linnaeus, 1758)] in the quarry. Apparently, K. Sander did not know that morphologically the egg strings of *B. bufo* and *Bufotes viridis* are almost identical and practically impossible to discriminate (Mertens 1964, Arnold & Burton 1978, Nöllert & Nöllert 1992). Regarding radioactivity, LfU and H. Schreiber insisted that the IFEU approach was invalid and that IFEU interpreted their data incorrectly. They also insisted that LfU measurements were sophisticated and correct despite absence of a field protocol. [Note, in an LfU paper commenting on IFEU measurements and dated 27.5.1981, LFU more appropriately called their own measurements "ad-hoc radiation assessment", which, in W. Morgenstern's opinion, does not require a systematic sampling scheme]. Schreiber pretended that until shortly before the hearing he had never received the IFEU data (which is not true, see Anonymous 1981a,g,p).

Minister G. Weiser had to admit carelessness by LfU regarding loss of sampled toads. The official conclusion of the public hearing was: artificial radioactivity in the quarry does not exist; the malformations are definitely natural; hybridization very likely is the cause; however, a concrete biological explanation cannot yet be given; thus, a research project will be initiated (Anonymous 1981w,x, Puhlmann 1981a, Schnorbach 1981). MELUF estimated the costs of the research at several hundred thousand Deutsch Marks, i.e., ca. US $ 250,000 (Anonymous 1981y).

1982. – The German Federal Ministry of the Interior (under Minister Gerhard Baum) initiated a research project on the influence of low-level radioactivity on toads at the University of Bonn, presumably because of the controversy about the Roßwag toads and other controversies about the effects of radioactivity (e.g., Bär 1981). G. Baum was no longer in office when the project finished. [Results of the project have not been published to our knowledge, but three unpublished MSc theses are available showing a broad range of types of anomalies and a high frequency of malformed individuals (Schneider 1984, Oerter 1985, Trux 1985, see Henle et al. 2017a for a summary of results)].

1982–1984. – MELUF contracted research on amphibians living in the area surrounding the quarry, malformations of *B. viridis,* and a search for evidence of hybridization as their cause to Rainer Flindt (Pädagogische Hochschule Ludwigsburg, Baden-Württemberg – College of Tertiary Education). Other hypotheses were not addressed.

January 1984. – Flindt (1984) reported his results to the State Parliament. He stated that the malformations were neither unique to the quarry nor new, but had formerly existed there in 1908 and 1932. He briefly discussed different potential causes, did not suggest unequivocally a single cause and preferred different explanations for each type of anomaly. In conclusion, he regarded past hybridization followed by inbreeding as the best explanation (see also Anonymous 1984a-d, Cichowicz 1984a-c, Landesregierung von Baden-Württemberg 1984, Schnedler 1984). He used serum allozymes and morphological characters to test for hybridization. Previously, he and others regarded these methods as unequivocally capable of detecting hybrids (Flindt & Hemmer 1967, 1973, Kabisch & Engelmann 1975). Since the results were negative, he had to base his arguments entirely on an anecdote (Anonymous 19810, 1984d,e) and on his observation (Flindt 1985a,b) that the upper labial tooth rows of tadpoles appeared more like those of the natterjack (*Epidalea calamita* Laurenti, 1768) than those of *B. viridis*. However, this characteristic is similar in all German toad species and problematic even for species identification (Nöllert & Nöllert 1992, Stresemann 1995, see Henle et al. 2017a for further discussion). Flindt (1984 1985a) mentioned Fritz Burkhardt as the source for the dates and the proof of hybridization. In Anonymous (19810) using local dialect, F. Burkhardt disclosed his source as an elderly lady that came to visit him to tell him that all the nervousness about the malformed toads are utter nonsense and he should remember that malformed toads were around when they were young. Her words read "I do not really know when, but I would say round about 1908". J. Margraf and K. Henle contacted F. Burkhardt on 17.11.1984. He told them he had never seen a malformed toad, that according to his knowledge no other witnesses exist except an elderly lady, Pauline Vögele, born 1894. He claimed that she was a highly reliable witness and mentally still extremely agile in 1981 when the visit supposedly happened. He refused to disclose her address and explained that the lady meanwhile was suffering from advanced Alzheimer's disease. Therefore, a contact would make no sense.

8.2.1984. – The State Government of Baden-Württemberg stated its position vis-à-vis R. Flindt's report. Though it admitted that the cause of the anomalies remained unresolved, it regarded the anomalies as not unusual, stated that they had already been occurring in the quarry and at other locations for decades, rejected any causative environmental factor and assumed that characteristics of the genome of the toads combined with inbreeding and hybridizations were the explanations. Though it admitted that additional analyses would be helpful, it indicated no further action would be taken.

Referring to the position of the State Government, some newspapers reported that the anomalies were caused by hybridization or inbreeding (e.g. Anonymous 1984a-c, 1985). Others criticized this conclusion and the State Government's position of not attempting

to resolve the cause (e.g. Anonymous 1984d,e, Cichowicz 1984a-c, Schnedler 1984).

1980–1984. – K. Rimpp attempted to raise malformed juveniles; most were unable to capture food and died soon after metamorphosis. A single individual with a large yellow spot on the flank survived. K. Rimpp managed to cross it with an individual originating from Malmsheim. Several metamorphosed offspring showed the same anomaly. K. Henle and R. Flindt attempted to raise tadpoles and clutches collected in the field; most developed anomalies after being transferred to aquaria and died, suggesting a genetic basis for the anomalies (Henle et al. 2017a).

1984–2002. – Lack of financial resources prevented a continuation of attempts to clarify the cause of the anomalies. Thus, the affair ended. Because of succinct and sometimes direct pressure experienced throughout the affair (Beck-Oberdorf 1981a, Deutscher Bundestag 1981), B. Steinhilber-Schwab left IFEU and K. Henle decided to leave Germany to continue his scientific career abroad. J. Margraf, meanwhile owner of a publishing book house, had a manuscript of the Roßwag toads, which he announced as a special issue in a forthcoming book "Ökologie aktuell". He experienced his phone being tapped and his private visitors being followed by police. J. Margraf moved his publishing house away from Baden-Württemberg soon after these incidents.

16.9.2002, Sheremetyevo airport, Moscow. – Russian customs police arrested K. Henle suspecting him of smuggling radioactive material when they detected increased levels of radioactivity while screening his luggage. To the surprise of Russian authorities and K. Henle, a detailed investigation identified his camera as the culprit. One part of the camera was contaminated beyond legal limits (3 kBq ^{232}Thorium). The contaminated lens was used in the Roßwag quarry but no other parts of the camera had been exposed. The camera was not carried while radioactivity measurements were made in Roßwag.

31.3.2003. – In an answer to a query by K. Henle, the producer of the lens, Canon, answered that "a radioactive contamination of the lens in the production process can be definitely excluded". Though K. Henle was not convinced by this statement, the incident at Moscow airport sparked renewed interest in revisiting the Roßwag case. This reassessment allows a definitive exclusion of any natural cause and is consistent only with the hypothesis of a mutagenic factor, with radioactivity being the most parsimonious explanation (Henle et al. 2017a).

28.5.2006. – A visit to the quarry showed that the location of the previous pond was covered by at least 50 m of fill and therefore difficult to recognize (Fig. 4c).

29.5.2006. – In an interview made by Chris Hammer (sbs Television Australia), Schreiber (meanwhile retired) still regarded radioactivity as an unlikely cause because its presence could not be demonstrated with absolute certainty and because other factors, such as chemicals, also induce mutations. However, when questioned by C. Hammer if his measurements (and those of LfU) would have been able to measure a radioactive source buried under 1–2 m of fill, he clearly stated "no, definitely no chance at all" (Hammer 2006). He further stated that already a cover of 20 cm of fill would have made it impossible to detect such a source unless measured directly at an opening reaching sufficiently deep into the deposit.

3 Discussion

While several cases of mass anomalies in amphibians are known, histories of the discovery and ensuing investigations apparently have been compiled only for anomaly P (Rostand 1971) and for recent mass anomalies in North American amphibians (Souder 2002, Lannoo 2008, Helgen 2012). Rostand (1971) focussed on the research undertaken by him and his collaborators in search for the cause(s) of anomaly P. Environmental issues have been touched rather briefly: the attention of the mass media to the observation of mass incidences of polydactyly in a Dutch population of *Pelophylax* synkl. *esculentus* that was found in a water body contaminated by radioactivity. The cause for these anomalies was presumed to be the same as for anomaly P (Rostand 1971) but a detailed analysis seems never to have been carried out. It remains unknown whether this discovery received continued public attention or political action.

The three books about recent cases of mass anomalies in North American amphibians focused on the scientific controversies and competitions that surfaced among involved scientists and scientific organisations but Helgen (2012) also discussed political and institutional support and resistance. While it is clear that these discoveries created widespread attention and, partly, controversies among scientists, by the mass media, in the political arena and among institutions, unfortunately implications for environmental policies and interactions of scientists and institutions with politics were covered only marginally, except as pertinent to the investigations of scientists and scientific organisations. Drawing on these case histories and general environmental literature, we first discuss lessons that may be learned from the Roßwag affair. We conclude with summarizing open questions and recommendations for further actions in the Roßwag case.

3.1 Lessons learnt

There were four decisive elements in the affair that may have prevented it from taking a different course. Clearly, the discovery got out of control when pressure was exerted on the administration to become active and check initial radioactivity measurements. K. Henle and K. Rimpp could not have imagined the public and political chain-reactions that their discovery would cause. K. Rimpp expected that authorities may show some inertia but would act appropriately once they understood that

there was really a need for action. Misunderstandings and distortions in information-transfer may have contributed to the scientifically inadequate ad-hoc measurements at the quarry by administrative bodies. With the permit for quarry modifications, the chance of a careful field assessment was gone soon after the discovery. This contrasts strongly to the North American cases, in which a range of scientific investigations was initiated as a response to the initial discovery (Souder 2002, Lannoo 2008), albeit there was quite strong institutional resistance for some time by the Minnesota Pollution Control Agency to support the malformed frog research. However, federal institutions, such as the Environmental Protection Agency (EPA) and the (NIEHS) partially made up for this deficit. Whether a more sympathetic attitude of federal institutions and local politicians in North America (Souder 2002, Helgen 2012) facilitated this difference or whether it was due to radioactivity being implicated early on in the Roßwag case remains an open question. Radioactivity features among the politically most sensitive environmental issues, with obvious repercussions far beyond abnormal amphibians and extending to human health and major economic and state interests (Feshchenko et al. 2002, Scherb & Weigelt 2003, Savchencko 1995, Körblein & Hoffmann 2006, Møller et al. 2008, Dubois 2012, Radkau & Hahn 2013). In the North American cases, in contrast, initially there were only vague ideas what might be the cause(s) and local citizen were concerned about their health (Souder 2002, Lannoo 2008, Helgen 2012).

Second, the discovery might have taken a different course were it not for extreme pressure put upon H. Schreiber by W. Morgenstern and the quarry manager, which prevented an adequate repetition of the initial radioactivity measurements. In North America, in contrast, owners of properties, where malformed amphibians were found, mainly seemed to have supported investigations into the causes of the anomalies (Souder 2002, Helgen 2012), and at the time of the discovery of anomaly P, neither mainstream science nor environmental scientists were interested in mass anomalies in natural populations of amphibians (Rostand 1971). However, given the extreme distortions and selectivity of scientific information shown by authorities involved in the Roßwag affair, they might not have acted differently had such measurements confirmed the initial results. Institutions often have a tendency to develop their own "truth" and to ignore or distort information that is incompatible with it (Gadamer 1989, Cornwell 2003, Radkau & Hahn 2013). The histories of chemical pollution (e.g., widespread DDT usage, Seveso and Bhophal), the mission of the Atomic Bomb Casualty Commission, Chernobyl and Fukushima (Carson 1962, Widener 1970, Savchenko 1995, Kriener 2011, Dubois 2012, The Independent Investigation Commission on the Fukushima Nuclear Accident 2014), the culture of maintaining secret any problems with human or environmental risks created by the use of nuclear energy in many countries (Dubois 2012) or attempts to protect habitat of endangered species (Brown & Mesrobian 2005) show that this is rather the rule than the exception when environmental protection and conservation clash with economic, personal or institutional interests. For example, recently it was uncovered that in 1983 the German government under Helmut Kohl has manipulated a key study that initially casted doubt on the safety of the proposed German final deposal site for radioactive waste in Gorleben towards it being suitable (Wendel 2009). Disturbingly, in 2008 it became clear that the German storage facility for low to intermediate level nuclear waste, the mine called ASSE II, which up to then was always pretended to be safe, was in danger of collapse, that radioactive waste had been mishandled, and that it was unknown what material, including plutonium, had been stored. And the clean-up of the waste is still extremely challenging (Anonymous 2008, BfS 2010).

Third, ignorance of the precautionary principle, political unwillingness to take the issue seriously and an inappropriate crisis management prevented an easy and early resolution of the radioactivity controversy. Without disturbance to quarry operations, the drilling of holes into the earthen deposit could have demonstrated once and for all the absence or presence of any artificial source of elevated radioactivity. For the cost of the research project that the Ministry initiated later, the whole earthen deposit could have been removed. This was expressly requested by A. Dubois, when he, because he was a foreign expert, finally obtained the right to speak again before the end of the public hearing of 6.11.1981 in the State Parliament of Baden-Württemberg. It remains an open question why the authorities and the quarry owner let this excellent chance to prove that they were correct pass by.

Finally, when forced by public pressure to commission a research project, the Ministry still had the chance to initiate careful assessments of all reasonable hypotheses suggested as explanations for the anomalies. Unfortunately, the Ministry was not interested in such assessments. Rather, it asked only to find evidence for hybridization, and when all evidence of the commissioned project rejected their favoured hypothesis, they opted to ignore the facts.

3.1.1 Handling sensitive discoveries

Since early actions after the discovery decisively influenced the affair, what could have been done differently? The most difficult question one has to answer in such cases is: Is there any imminent hazard to humans or the environment? If risks appear high, there is no option but to immediately inform relevant authorities and the public with the hope that they will act appropriately. The Roßwag affair showed it is essential that the person, who discovers a potential hazard, makes contact with relevant authorities, so that the risk of misunderstandings is minimized. If possible, photographic or video documentation of any initial measurements should be made.

If immediate hazards to humans are low, it is better to do more exhaustive surveys in as short a period of time as possible and document evidence for and against different causes. Given that high levels of radioactivity decreased with distance from the surface of the deposit to double the background level, this might have been justified in the Roßwag case. Believing in the existence of an adequate crisis management by relevant authorities, Rimpp felt the burden of possibly acting too late as too high.

Without institutional backing, it is difficult to make detailed assessments of environmental perturbations potentially responsible for mass incidences of amphibian anomalies. Unfortunately, different causes may result in similar symptoms and guidelines that could help screening for potential causes were lacking. The extensive literature review by Henle et al. (2017a,b) may help to separate more likely from less likely causes. If an ecotoxicological laboratory is accessible, teratogenicity and mutagenicity assays of water samples and suspect material should be conducted (Birge et al. 1983, Burkhart et al. 2000). It is essential to have the support of a reliable institution that does not back down from industrial or governmental pressure (compare the North American case documented by Helgen 2012). Environmental organizations can help establish such contacts but one must be aware that they will not be acceptable to administrations that failed to do the job they should do. All measurements should be repeated independently by different teams and carefully documented, including photographic records. Evidence for and against all potential causes should be carefully collected, even for causes considered unlikely. Whenever feasible, experiments for hypothesis testing should be conducted (Lunde & Johnson 2012, Dubois & Henle 2017).

3.1.2 Involvement of the mass media

For anomaly P, the mass media did not play a significant role (Rostand 1971) and published histories of the North American cases only marginally cover their involvement (Souder 2002, Lannoo 2008) except of the huge time burden placed on the key scientists investigating malformed frogs in Minnesota (Helgen 2012). While the account of Helgen (2012) implicates, without explicitly discussing it, that the mass media were highly influential in the North American investigations, they were clearly a driving force in the case of the abnormal toads in Roßwag. Without strong pressure from the media, neither the workshop organized by the Green Party, nor the Parliamentary hearing, nor later investigations would have occurred. This public pressure is the major advantage of involving mass media. Indeed, it is essential to draw authorities into action when administrators, governments or industry dispute and try to downgrade the severity of observations and question the reliability of measurements, as in the Roßwag case. The history of environmental protection is replete of many such examples (e.g., Widener 1970, Koch & Vahrenholt 1983, UCS 2004, Hesse-Honegger & Wallimann 2008, Borchert 2009, Dubois 2012, Radkau & Hahn 2013, this study).

On the other hand, early involvement of the mass media can have disadvantages. Few journalists will have sufficient training to fully understand the science behind the contended issue. Uncritical presentation of contradictory or distorted information will leave the public confused. This partly happened early in the Roßwag affair when local and regional media reported in contradictory ways about the discoveries. A similar situation seems to have been present at some stage in North America (see Helgen 2012). This and the inexperience of the discoverers of the malformed toads in dealing with journalists effectively worked against a broad and thorough assessment and treatment of the arguments for and against the suggested hypotheses.

When involving mass media, one needs to understand that they follow their own rules. Mass media need to sell their news; thus crime, sex and sensations are essential ingredients that carry a story to the front pages of newspapers, radio or TV (Reaser & Johnson 1997, Souder 1997, Jacklyn 2001). Thus, it is no surprise that the Roßwag affair resulted in an explosion of media reports. Likewise, interest faded rapidly with time. Only coincidental discovery of a study of malformations in amphibians caused by radioactivity undertaken at Hiroshima University and leakage of "new information from Hiroshima" to the media created a sufficient resurgence of interest to prevent the story from dying.

3.1.3 The role of science and scientists

Discoveries like those made in Roßwag necessarily pose the question of whether and to which extend scientists should engage in policy processes (Lackey 2007). This question typically hinges on the issue of advocacy. Some argue that scientists have an ethical obligation to act as advocates (e.g., Shrader-Frechette 1996, Blockstein 2002), whereas others are adamant that advocacy oversteps the bounds of appropriate scientific activity (e.g., McCoy 1996, Lackey 2007). In discussions of credibility, values and policy advocacy, the fundamental concern is with beliefs about what constitutes science, beliefs that vary widely within and beyond the scientific community. Scholars in the field of social studies of science have critiqued the modern scientific enterprise, including its tenets of objectivity, neutrality and logical positivism. They argue instead that science is a historically and culturally situated activity that extends a particular vision of reality through networks of power (Latour 1987, Hacking 1999). As the boundaries between science and policy are not fixed, it is essential that scientists disclose conflicts of interest (Orr 2004) and make clear the role they play at the outset (Niemelä et al. 2005).

Some scientists were dissatisfied with the whole affair and with the early involvement of administrations and the public. They argued that the discovery should have been investigated and published first in a scientific journal before going public. While this is the best option if risks to human or environmental health can be excluded with certainty, this position is inappropriate in situations like the Roßwag case. When major haz-

ards cannot be excluded with confidence, it takes too long to complete a scientific investigation and wait for its publication before action is taken. Also, the availability of a scientific publication may make no difference. Besides Roßwag, there are many cases in which politicians and administrators intentionally ignored published scientific facts and developed their own "truth" (Widener 1970, Gadamer 1989, Orr 2004, UCS 2004).

Scientists are not without blame. Out of frustration from being misrepresented and to avoid further negative experience, scientists may withdraw from debates when they get uncomfortable. Scientists should make such cases public, because this is the only language those who have no scruples about misconduct understand. It may be excusable that scientists succumb to intensive pressure, and the Roßwag affair gives at least a little insight to the sorts of pressures, which occur in such cases, however, the carelessness and low scientific standards exhibited by some scientists contracted by the responsible authorities in the Roßwag affair are not excusable. Unfortunately, willing scientists are not the exception when major political interests are at stake, not only discrediting their own profession, but also betraying the public and putting it at risk (McDaniel 2004, Orr 2004, UCS 2004).

Science has developed mechanisms to protect against fraud by data manipulation, and the lack of scientific recognition among colleagues helps protect science against poor professional standards. However, the same usually is not the case for environmental testimony by scientists. Mechanisms urgently need to be developed, such as personal liability, for the use of poor science that puts the stakes higher. One such mechanism could be the mandatory consideration of all debated hypotheses until they can be excluded beyond any reasonable doubt. Scientists may tend to weight arguments in favour of their hypothesis more strongly than arguments against it and vice versa for alternative hypotheses (see e.g. Lannoo 2008 versus Sessions 2009). The way a question is posed determines at least partially the answer obtained (e.g., Russell 1979, Gould 1980, Latour 1999, Dubois 2006). Therefore, scientists should explicitly state in a verifiable document what types of results might be achieved and which ones will support respectively reject the tested hypothesis before any test is undertaken.

3.1.4 Management of the affair by responsible authorities

Governmental environmental organisations have the responsibility to protect the environment and humans from hazards. This requires an appropriate crisis management when potentially serious hazards are discovered and necessitates to ask what the facts are and to reassess these facts until any current or future hazard to wildlife and humans can be excluded beyond reasonable doubt (Burkhart et al. 2000, Souder 2002, Helgen 2012). A careful repetition of critical measurements instead of superficial approaches, speculations and denials is a fundamental principle of adequate crisis management and should be made mandatory for such situations.

The history of the Roßwag affair is full of inadequate crisis management. First of all, an adequate crisis management would have included the repetition of the original radioactivity measurements of K. Henle in his presence and the presentation of a measurement protocol that fulfils high scientific standards. Second, the controversy about radioactivity measurements addressed wrong issues instead of focusing on the critical ones. The discussion was raised to a level of technical detail that was hard to follow by non-specialists. In non-technical terms, IFEU regarded the malformations as sufficiently important to warrant assessment of any potential cause until it could be excluded beyond reasonable doubt. In contrast, LfU asked whether IFEU measurements, stand alone, could definitely prove a contamination of the quarry with radioactivity. LfU's answer to the question it asked is correct but the question one asks determines the answers one will get (Russell 1979, Gould 1980, Latour 1999) – and LfU obviously asked the wrong question.

IFEU measurements also have limitations that do not allow an unequivocal proof of the presence of artificial radioactive material (Franke et al. 1981, Henle et al. 2017a). For example, the addition of material to the earthen deposit could have caused the decline in the level of measured radioactivity. IFEU acknowledged limitations of their study. Notwithstanding, the results are consistent with the hypothesis of radioactive contamination. In the same way LfU measurements cannot prove the absence of radioactive material in the earthen deposit (confirmed by Schreiber in a sbs Television interview). Measurements close to openings of cracks would have been vital to reject, or confirm, the initial measurements of K. Henle. Measurements of LfU are only comparable to his background measurements, and these are consistent (Henle et al. 2017a). It is a fundamental principle of science and good crisis management to test disputed critical measurements by repeating them exactly and not to reject them on the basis of ad-hoc assessments and speculations of what could have gone wrong in other scientists' measurements.

Unfortunately, the Roßwag affair is not an exception. There have been numerous other controversies concerning radioactivity in the environment, usually with a categorical denial of any health risks by some stakeholders (see e.g. Kaatsch et al. 2007, Hesse-Honegger & Wallimann 2008, Møller et al. 2008, Rosenkranz 2010, Friebe 2011, Dubois 2012, Radkau & Hahn 2013, The Independent Investigation Commission on the Fukushima Nuclear Accident 2014). Some of these controversies involved some of the same public organizations as in the Roßwag case (e.g., Anonymous 1981aa, Beck-Oberdorf 1981b, Burchard in litt., 1980). Interim storage of radioactive waste at the Asse location was called save beyond any doubt by the

responsible organizations and the government for years, but it turned out to be a huge mess requiring very expensive removal (Bunse 2009, Rosenkranz 2010). Manipulations of scientific facts related to the deposition of radioactive waste by the German government have been discovered also quite recently (Wendel 2009). Furthermore, environmental controversies are not unique to Germany (e.g., Widener 1970, Bär 1981, Savchenko 1995, McDaniel 2004, Orr 2004, UCS 2004, Hesse-Honegger & Wallimann 2008, Rosenkranz 2010, Dubois 2012). In fact, statements that environmental disasters are too unlikely to be possible are frequently made and a cloud of secret enshrines all subsequent accidents, resulting in tremendously different figures even for fatalities in humans (Lepage 2011, Dubois 2012).

Up to 1982 the nuclear energy industry of Germany and other European countries dumped more than 100,000 tons of radioactive waste in the Sea (SWR 2011) using cheapest containers not designed to contain the waste even for tens of years (Baxter et al. 1995). The documented official motivation was to reduce costs of the interim deposition of radioactive waste as much as possible (SWR 2011). Dumping at sea was at its highest level in 1980 (OSPAR-RSC 2010). Not surprisingly, rust-eaten and cracked containers and elevated levels of radioactivity, including plutonium, were later discovered at marine dumping sites (OSPAR-RSC 2010, SWR 2011). Part of the dumping took place even in near offshore regions, such as in the English Channel. Notwithstanding, the current German government does not see any need to monitor radioactivity at marine dumping sites (SWR 2011). It may be just a temporal coincidence with the Roßwag affair but in 1980 plans existed in Germany to export radioactive waste to developing countries. Clearly, there remains much to be improved in how our administrative and political institutions handle environmental issues with potential risks to wildlife and humans (Dubois 2012, Radkau & Hahn 2013).

3.2 Open questions

A number of other questions remain for the Roßwag affair: Did those that rejected any anthropogenic cause and resisted further investigations ever consider hazards to workers of the quarry (Anonymous 1981z,ab)? How strong are our scientific and administrative institutions subject to political pressure? Was it only incapability in crisis management or did somebody know something he/she wanted to cover up (Anonymous 1981m, Beck-Oberdorf 1981a,b)?

Since the discovery of malformed toads, considerable time has passed and many key players are no longer in their former positions. After the impact of Chernobyl (Savchenko 1995) environmental administration has seen a considerable reorganisation in Germany, and in other countries, and environmental sensitivity has increased. Have the responsible authorities learnt from the Roßwag case and adopted a more precautionary position? Willingness of senior officials at the Ministry of the Environment of Baden-Württemberg to give an interview to a TV journalist on very short notice is a good sign that attitudes have changed (though LfU declined again) but cover-up of problems related to civil use of radioactive material, including inadequate or illegal deposition, is still attempted in Germany and many other countries (Lenssen & Flavin 1996, Bunse 2009).

What really happened in Roßwag? It is clear that Roßwag is the most extreme case of malformations ever reported for a natural population of amphibians and that anomalies must have been caused by a powerful mutagenic factor (Henle et al. 2017a). All natural factors suggested require biologically impossible assumptions leaving only radioactivity or mutagenic chemicals as an explanation. We realize that this conclusion is difficult to accept for many individuals and institutions but illegal deposition of radioactive waste is also known from other countries (e.g., Lenssen & Flavin 1996). Can we be sure beyond reasonable doubt that there are no longer risks in Roßwag now and in the future for wildlife, ecosystems and human health? Even the 100 m of fill may not prevent leakage into the ground water, if the material buried is still active. Has the same happened elsewhere, e.g. in the quarry near Ilsfeld, as suggested by two independent witnesses and can we be sure that it will not happen again (Anonymous 1981p)? We urge to undertake any endeavour to try to answer these questions and to guarantee that no serious hazards remain.

4 Acknowledgments

We thank all those persons and institutions that supported us in unravelling the Roßwag affair. K. Henle thanks Natalia Rumyantseva (Tula) for support at Moscow airport (and beyond). Doris Wolst (Leipzig), Lauren Brown (Urbana), Birgit Felinks (†), Ulrich Joger (Braunschweig), Scott Jones (Lichfield), Hans-Konrad Nettmann (Bremen), Jean-Claude Beetschen (Toulouse), Alain Collenot (Paris) and three anonymous reviewers provided valuable suggestions for improving the manuscript. Marlies Uhlig (Leipzig) was invaluable in helping to compile relevant literature.

5 References

Allen, S. (1996): Amphibious abnormalities: scientists probing cause of frogs' missing parts. – Boston Globe **9.12.1996**.

Ankley, G.T., S.J. Degitz, S.A. Diamond & J.E. Tietge (2004): Assessment of environmental stressors potentially responsible for malformations in North American anuran amphibians. – Ecotoxicol. Environ. Safety **58**: 7–16.

Anonymous (1980a): Jungkröten mit sechs Beinen stellen Umweltschützer vor Rätsel. – Ludwigsburger Kreiszeitung 11.9.1980.

Anonymous (1980b): Roßwag: Verdacht auf Radioaktivität entpuppte sich als blinder Alarm. – Vaihinger Kreiszeitung **11.9.1980**.

Anonymous (1980c): Rätselhafte Krötenkrüppel. – Stuttgarter Zeitung **11.9.1980**: 25.

Anonymous (1980d): Radioaktivität im Steinbruch? – Bietigheimer Zeitung **11.9.1980**.

Anonymous (1980e): Als im Steinbruch der Geigerzähler ausschlug. – Mühlacker Tagblatt **12.9.1980**.

Anonymous (1980f): Alarmrufe von der Kreisgrenze: mißgebildete Kröten und Radioaktivität. – Pforzheimer Zeitung **12.9.1980 (212)**: 24.

Anonymous (1980g): Horror à la Hollywood. Monsterkröten aus dem Steinbruch. – Taz, Berlin **11.9.1980**.

Anonymous (1980h): Roßwag: Verdacht auf Radioaktivität entpuppte sich als ~~blinder Alarm~~ Tatsache! – Löwenzahn, Vaihingen/Enz **4/1980**: 1, 18–19, 24.

Anonymous (1980i): Krötenprüfung dauert an. Mißbildungen möglich durch Sprengerschütterungen. – Ludwigsburger Kreiszeitung **18.10.1980**.

Anonymous (1980j): Krötenmißbildungen nicht außergewöhnlich. – Pforzheimer Zeitung **18.10.1980 (243)**.

Anonymous (1980k): Krötenmonster durch Chemiereste möglich. – Ludwigsburger Kreiszeitung **20.12.1980**.

Anonymous (1981a): Roßwager Kröten Drama. – Löwenzahn, Vaihingen/Enz **6/1981**: 15.

Anonymous (1981b): Mißgebildete Kröten im Steinbruch: Wissenschaftler haben jetzt das Wort. – Vaihinger Kreiszeitung **6.2.1981**.

Anonymous (1981c): Auch nach fünf Monaten noch Rätsel um Krötenmonster. – Ludwigsburger Kreiszeitung **7.2.1981**.

Anonymous (1981d): Abnormale Kröten aus Roßwager Tümpel sorgen für Aufregung. – Mühlacker Tagblatt **9.2.1981**.

Anonymous (1981e): Mißgebildete Kröten Dauerbrenner? – Pforzheimer Zeitung **20.2.1981**.

Anonymous (1981f): In Sachen Kröten. – Löwenzahn, Vaihingen/Enz **5/1981**: 5.

Anonymous (1981g): Krötenmißbildungsfall: Gegendarstellung und Erwiderung. – Pforzheimer Zeitung **15.6.1981**.

Anonymous (1981h): Krötenmißbildungen auch weiterhin im Meinungsstreit der Kontrahenten. – Pforzheimer Zeitung **6.5.1981**.

Anonymous (1981i): Vom Pforzheimer Landratsamt angestrebt: Streitgespräch um die Krötenmonster von Roßwag soll endlich Klarheit bringen. – Ludwigsburger Kreiszeitung **7.5.1981**: 8.

Anonymous (1981j): Landesanstalt für Umweltschutz: keine künstliche Radioaktivität. – Vaihinger Kreiszeitung **8.5.1981**.

Anonymous (1981k): Zu keinem Zeitpunkt Umgang mit chemischen oder radioaktiven Abfällen (Stellungnahme der Kalk- und Schotterwerke Gebr. Zimmermann, Roßwag). – Vaihinger Kreiszeitung **9.5.1981**.

Anonymous (1981l): Strahlenmessungen krottenfalsch? – Schwäbische Zeitung, Rottweil **12.5.1981 (108)**.

Anonymous (1981m): Krötenmißbildungsfall hochgejubelt oder vertuscht? – Pforzheimer Zeitung **29.5.1981**.

Anonymous (1981n): Superstrahlung ein Kuckucksei? – Pforzheimer Zeitung **6.6.1981**.

Anonymous (1981o): Froschperspektiven. – Vaihinger Kreiszeitung **2.12.1981**.

Anonymous (1981p): Le crapaud nucléaire. – Ca m'intéresse, Paris **Juin 1981 (4/1981)**: 41.

Anonymous (1981q): Wolf-Dieter Hasenclever, MdL: "Dies wird ein Fall der Unklarheiten bleiben". – Pforzheimer Kurier **17.9.1981 (214)**: 21.

Anonymous (1981r): Vergebliche Suche nach Strahlungsquelle. – Vaihinger Kreiszeitung **17.9.1981**.

Anonymous (1981s): Wuchs Kröten durch Strahlen ein fünftes Bein? – Rhein-Neckar-Zeitung, Mannheim **19.9.1981**.

Anonymous (1981t): Kröten-Mißbildungen "zu 99 Prozent durch Radioaktivität verursacht". – Südwest-Presse, Villingen-Schwenningen **18.9.1981**.

Anonymous (1981u): Verschleiert die LfU Umweltverbrechen? – S'Gärtle **Herbst/Winter 1, Nr. 2**: 22–23.

Anonymous (1981v): Mißgebildete Kröten. Anhörung im Landtag. – Stuttgarter Nachrichten **19.9.1981 (216)**.

Anonymous (1981w): Das Geheimnis der Strahlenden Kröten. – Schwäbische Zeitung, Trossingen **9.11.1981**.

Anonymous (1981x): Krötendiskussion im Landtag. – Leonberger Kreiszeitung **10.11.1981**.

Anonymous (1981y): Krötenmißbildungen. 1982 verstärkte Forschungen. – Stuttgarter Nachrichten **27.11.1981**.

Anonymous (1981z): "Strahlen-Grabung" abgelehnt. Vaihinger Gemeinderat will erst ein Ergebnis der Fachleute sehen. – Vaihinger Kreiszeitung **30.3.1981**.

Anonymous (1981aa): Weil eben nicht sein konnte, was nicht sein durfte: „Vaihinger Krüppel-Kröten durch Radioaktivität". – Nachbar GKN **Nr. 1/IV**: 3.

Anonymous (1981ab): Auch an Kröten zu erkennen. – Pforzheimer Zeitung **14.3.1981 (61)**: 17.

Anonymous (1981ac): Die Publizität scheint diesen Leuten wichtiger. – Pforzheimer Kurier **9.5.1981 (106)**: 38.

Anonymous (1984a): Mißbildungen bei Kröten gibt es schon seit langem. – Kreiszeitung Böblingen **23.2.1984**.

Anonymous (1984b): Missbildungen bei Kröten gibt es schon seit langem. – Südwest-Presse, Villingen-Schwenningen **23.2.1984**.

Anonymous (1984c): Die Krötenmonster waren schon immer da. – Stuttgarter Zeitung **21.2.1984**.

Anonymous (1984d): Grüne bezweifeln Krötengutachten. – Stuttgarter Zeitung **21.3.1984**.

Anonymous (1984e): Krötenmonster in Vaihingen. Grüne: Ursache nicht geklärt. – Stuttgarter Nachrichten **6.3.1984**.

Anonymous (1985): Verkrüppelte Kröten durch Inzucht. – Frankfurter Rundschau **12.1.1985**.

Anonymous (2008): Radioactive Leakage. Berlin Takes Steps to Address Nuclear Waste Scandal. Spiegel online *http://www.spiegel.de/international/germany/0,1518,576362,00.html* (accessed 1.7.2011).

Arnold, E.N. & J.A. Burton (1978): A Field Guide to the Reptiles and Amphibians of Britain and Europe. – London (Collins).

Bächle, G. (1981): Im Fall der Krötenmonster von Roßwag: Grüne werfen Landesbehörden "Schlamperei und Missachtung" vor. – Ludwigsburger Kreiszeitung **18.9.1981**.

Bär, W. (1981): Schöne neue Tierwelt. – Tell, Zürich **19.6.1981 (41)**: 16-19.

Baxter, M.S., S. Ballestra, J. Gastaud, T.F. Hamilton, I. Harms, L. Huynh-Ngoc, L. Liong Wee-Kwong, L. Osvath, P. Parsi, H. Petterssen, P.P. Povinec & A. Sanchez (1995): Marine radioactivity studies in the vicinity of sites with potential radionuclide releases, Proc. Int. Symp. Environm. Impact Radioactive Releases. –Vienna (IAEA).

Beck-Oberdorf, M. (1981a): Politische Bedeutung des Krötenfalls. – Vaihinger Löwenzahn **7**: 16–18.

Beck-Oberdorf, M. (1981b): Schlamperei oder Vertuschung. – Umweltforum **6/1981**: 16–17.

Birge, W.J., J.A. Black, A.G. Westerman & B.A. Ramey (1983): Fish and amphibian embryos – a model system for evaluating teratogeneicity. – Fund. Appl. Toxicol. **3**: 237–242.

Blaustein, A.R. & P.T.J. Johnson (2003): The complexity of deformed amphibians. – Front. Ecol. Environ. **1**: 87–94.

Blockstein, D.E. (2002): How to lose your political virginity while keeping your scientific credibility. – BioScience **52**: 91–96.

Bogen (1981): "Grünes" Symposium über Illinger Umweltskandal. Licht ins Dunkel der Krötenmonsterei. – Pforzheimer Kreiszeitung **17.9.1981**.

Borchert, T. (2009): Vattenfall patzt auch in Schweden. – Leipziger Volkszeitung, Leipzig **115 (157)**: 2.

Brown, L.E. & A. Mesrobian (2005): Houston toads and Texas politics. Pp. 150–167 in Lannoo, M.L. (ed.): Amphibian Declines. The Conservation Status of United States Species. – Berkeley (Univ. Calif. Press).

Buchholz, M. (1982): Kröten, die keiner sehen soll. – Konkret, Hamburg **3/1983**: 100–102.

BfS (2010): Endlager Asse II. Aktueller Stand der Arbeiten zur Stabilisierung und sicheren Schließung, September 2010. – Salzgitter (Bundesamt für Strahlenschutz): urn:nbn:de:0221-201007142816.

Bunse, W. (2009): Vertrauen ist erschüttert. Radioaktiv belastete Lauge im Atommülllager Asse/Umweltminister Gabriel legt Jahresbericht vor. – Leipziger Volkszeitung, Leipzig **115 (163)**: 3.

Burkhart, J.G., G. Ankley, H. Carpenter, D. Fort, D. Gardiner, H. Gardner, R. Hale, J.C. Helgen, P. Jepson, D. Johnson, M. Lannoo, D. Lee, J. Lary, R. Levey, J. Magner, C. Meteyer, M.D. Shelby & G. Lucier (2000): Strategies for assessing the implications of frog malformations for environmental and human health. – Environ. Health Perspectives **108**: 83–90.

Carson, R.L. (1962): Der stumme Frühling. – München (Biederstein).

Cichowicz, A. (1984a): Dicke Kröte. – Vaihinger Kreiszeitung **3.3.1984**: 13.

Cichowicz, A. (1984b): Natürliche Ursachen für Krötenmonster? – Vaihinger Kreiszeitung **3.3.1984**: 13.

Cichowicz, A. (1984c): Waren die Monster schon immer da? – Vaihinger Kreiszeitung **3.3.1984**: 13.

Conrad, B. (1977): Die Giftbelastung der Vogelwelt Deutschlands. – Greven (Kilda).

Cornwell, J. (2003): Hitler's Scientists. – New York (Viking).

Deutscher Bundestag (1981): Schriftliche Fragen mit den in der Woche vom 13. Juli 1981 eingegangenen Antworten der Bundesregierung. – Deutscher Bundestag, Drucksache **9/672**: 60.

Dubois, A. (1979): Anomalies and mutations in natural populations of the *Rana esculenta* complex (Amphibia, Anura). – Mitt. Zool. Mus. Berlin **55**: 59-87, pl. 1.

Dubois, A. (1984): L'anomalie P des Grenouilles vertes (complexe de *Rana* kl. *esculenta* Linné, 1758) et les anomalies voisines chez les Amphibiens. Pp. 215–221 in Vago, C. & G. Matz (eds.): Comptes rendus du Premier Colloque international de Pathologie des Reptiles et des Amphibiens. – Angers (Presses Univ. Angers).

Dubois, A. (2006): Species introductions and reintroductions, faunistic and genetic pollution: some provocative thoughts. – Alytes **24**: 147–164.

Dubois, A. (2012): Jean Rostand, un biologiste contre le nucléaire. –Paris (Berg International).

Dubois, A. (2017): Rostand's anomaly P and similar anomalies in amphibians. – Mertensiella **25**: 49–56.

Dubois, A. & K. Henle (2017): Methodological recommendations for the study of the causes of anomalies in natural populations of amphibians. – Mertensiella **25**: 243–260.

Einzel, T. (1980): Kröten und Quappen mit Beinen am Kopf. – Pforzheimer Zeitung **12.9.1980 (212)**.

Ellegren, H., G. Lindgren, C.R. Primmer & A.P. Møller (1997): Fitness loss and germline mutations in barn swallows breeding in Chernobyl. Nature **389**: 593–596.

Ferraro, T.J. & S. Burgin (1993): Amphibian decline: a case study in western Sydney. Pp. 197–204 in Lunney, D. & D. Ayers (eds.): Herpetology in Australia: A Diverse Discipline. – Sydney (Surrey Beatty).

Feshchenko, S.P., H.C. Schroder, W.E.G. Muller & G.I. Lazjuk (2002): Congenital malformations among newborns and developmental abnormalities among human embryos in Belarus after Chernobyl accident. – Cell. Mol. Biol. **48**: 423–426.

Flindt, R. (1984): Gutachten über das Vorkommen von Amphibien im Raum Illingen/Roßwag und das Auftreten missgebildeter Wechselkröten (*Bufo viridis*) im Steinbruch Zimmermann in Vaihingen-Roßwag. – Unpubl. Report to the Ministry of Food, Agriculture, Environment, and Forestry, Baden-Württemberg, Stuttgart: 1–29.

Flindt, R. (1985a): Untersuchungen zum Auftreten von mißgebildeten Wechselkröten (*Bufo viridis*) in einem Steinbruch in Vaihingen-Roßwag. – Jh. Ges. Naturk. Württ. **140**: 213–233.

Flindt, R. (1985b): Latenter Albinismus und Mißbildungen bei Kaulquappen von Wechselkröten *Bufo viridis* Laurenti, 1768 (Salientia: Bufonidae). – Salamandra **21**: 298–303.

Flindt, R. & H. Hemmer (1967): Nachweis natürlicher Bastardierung von *Bufo calamita* und *B. viridis*. – Zool. Anz. **178**: 419–429.

Flindt, R. & H. Hemmer (1973): Die Bedeutung des Serumeiweißbildes zur Diagnose von *Bufo calamita* Laur., *Bufo viridis* Laur. und deren Bastarden (Amphibia, Anura, Bufonidae). – Experientia **29**: 361–364.

Franke, B., K. Henle, M. Kötter, E. Krüger & B. Steinhilber-Schwab (1981): Radioaktivitätsmessungen im Steinbruch Zimmermann – Detaillierte Auswertungen mit Berücksichtigung der Kritik der Landesanstalt für Umwelt. – Heidelberg (Unpubl. Ber. IFEU).

Friebe, R. (2011): Tschernobyl 25 Jahre danach. Blühende Landschaften, strahlende Wiesel. FAZ.NET **25.3.2011**: *http://www.faz.net/-o1q3en* (accessed 7.4.2011)

Gadamer, H.G. (1989): Truth and Method (2nd ed.). – New York (Crossroad).

Geoffroy Saint-Hillaire, I. (1832–1836): Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux, Vol. 1-3. – Paris (Baillière).

Gilberston, M., T.J. Kubiak, J.P. Ludwig & G. Fox (1991): Great Lakes embryo mortality, edema and deformities syndrome (GLEMEDS) in colonial fish-eating birds: Similarities to chicken-edema disease. – J. Toxicol. Environ. Health **33**: 455–520.

Gould, S.J. (1980): The Panda's Thumb. – Harmondsworth (Penguin Books).

Günther, R. (1996): Die Amphibien und Reptilien Deutschlands. – Jena (Fischer).

Hacking, I. (1999): The Social Construction of what? – Cambridge (Harvard Univ. Press).

Hammer, C. (2006): Toad in the Hole – Germany. *http://www.youtube.com/watch?v=z2uf2x6yruM&feature=youtu.be* (accessed 6.7.2011).

Hasenclever, W.-D. (1981): Kleine Anfrage des Abg. Hasenclever, Grüne: Missbildungen von Wechselkröten (*Bufo viridis*) im Steinbruch KG Zimmermann, Gemarkungen Illingen und Roßwag. – Landtag von Baden-Württemberg Drucksache **8/1034**: 1–3.

Hasenclever, W.-D., H. Heimann, G. Kretschmann, E. Mordo & Erichsen (1981): Antrag der Abgeordneten Hasenclever u.a., Missbildung von Wechselkröten (*Bufo viridis*) im Steinbruch Zimmermann, KG, Gemarkungen Illingen und Roßwag. – Landtag von Baden-Württemberg Drucksache **8/1386**: 1–3.

Helgen, J. (2012): Peril in the Ponds. Deformd Frogs, Politics, and a Biologist's Quest. – Amherst (University of Massachussetts Press).

Henle, K. (1981): A unique case of malformations in a natural population of the green toad (*Bufo viridis*) and its meaning for environmental politics. – Brit. Herp. Soc. Bull. **4**: 48–49.

Henle, K. (1982): Ein außergewöhnlicher Fall von Mißbildungen bei der Wechselkröte (*Bufo viridis*). – Unicornis, Schwäbisch Gmünd **2(1)**: 40–42.

Henle, K. & W. Schmitt (1981): Pressespiegel. Mißgebildete Wechselkröten im Steinbruch Zimmermann bei Vaihingen/Enz. Parlamentarische und Außerparlamentarische Initiativen der Grünen. – Stuttgart (Private Publication of "Die Grünen, Landtag Stuttgart").

Henle, K., A. Dubois, K. Rimpp & V. Vershinin (2017a): Mass anomalies in green toads (*Bufo viridis*) at a quarry in Roßwag: inbred hybrids, radioactivity or an unresolved case? – Mertensiella **25**: 185–242.

Henle, K., A. Dubois & V. Vershinin (2017b): A review of anomalies in natural populations of amphibians and their potential causes. – Mertensiella **25**: 57–164.

Hesse-Honegger, C. & P. Wallimann (2008): Malformation of true bug (Heteroptera): a phenotype field study on the possible influence of artificial low-level radioactivity. – Chemistry & Biodiversity **5**: 499–539.

Ignatz der Zornige (1981): Krötenmonster im Landtag. – Taz, Berlin **26.5.1981**: 10.

Jacklyn, P.M. (2001): Using the mass media in networks. Pp. 589–693 in Saunders, D.A., J.L. Cragg & E.M. Mattiske (eds.): Nature Conservation 4: The Role of Networks. – Sydney (Surrey Beatty).

Kaatsch, P., C. Spix, S. Schmiedel, R. Schulze-Rath, A. Mergenthaler & M. Blettner (2007): Epidemiologische Studie zu Kinderkrebs in der Umgebung von Kernkraftwerken: (KiKK-Studie); Zusammenfassung / summary; Teil1: Fall-Kontroll-Studie ohne Befragung; Teil 2: Fall-Kontroll-Studie mit Befragung. – Salzgitter (Bundesamt für Strahlenschutz). *http://nbn-resolving.de/urn:nbn:de:0221-20100317939* (accessed 4.1.2012).

Kabisch, K. & E. Engelmann (1975): Vergleichende elektrophoretische Untersuchung der α-Naphthylacetat-Esterase im Serum mitteleuropäischer Discoglossiden und Bufoniden (Amphibia, Anura). – Biol. Zentralbl. **94**: 211–214.

Kawamura, T. & M. Nishioka (1978): Abnormalities in the descendants of *Rana nigromaculata* produced from irradiated eggs or sperm. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **3**: 1–187.

Klein, J.F. (1981a): Krötenmonster im Steinbruchsee. – Kosmos, Stuttgart **1/1981:** 24–25.

Klein, J.F. (1981b): Gen verkehrt. – Sonntag Aktuell, Stuttgart **6.9.1981**.

Knapp, U. (1980): Schöne neue Kröten. – Schaffhausener Nachrichten **13.11.1980**.

Kneissler, N. (1981): Die Krötenmonster von Roßwag. – Umweltforum, München **6**: 14–19.

Koch, E.R. & F. Vahrenholt (1983): Die Lage der Nation. – Hamburg (GEO).

Körblein, A. & W. Hoffmann (2006): Background radiation and cancer mortality in Bavaria: an ecological analysis. – Arch. Environ. Occup. Health **61**: 109–114.

Kovacsics, M. (1981): Kröten-Mißbildungen: Spuren verwischt? – Bild der Wissenschaft, Stuttgart **2/1981** (Akzent **9**): 3.

Kriener, M. (2011): Ein GAU pro Jahr schadet nicht. Wie gefährlich ist radioaktive Strahlung wirklich? Darüber gehen die Meinungen seit Röntgens Entdeckung heftig auseinander. – Die Zeit **14.4.2011 (16)**: 20.

Lackey, R.T. (2007): Science, scientists, and policy advocacy. – Conserv. Biol. **21**: 12–17.

Landesregierung von Baden-Württemberg (1984): Stellungnahme zu Beschlüssen des Landtages. 1. Mißbildungen von Wechselkröten (*Bufo viridis*) im Steinbruch Zimmermann KG, Gemarkungen Illingen und Roßwag. – Landtag von Baden-Württemberg, Stuttgart, Drucksache **8/4596**: 1–3.

Landtag von Baden-Württemberg (1981): Kleine Anfrage des Abg. Hasenclever GRÜNE und Antwort des Ministeriums für Ernährung, Landwirtschaft, Umwelt und Forsten: Missbildungen von Wechselkröten (*bufo viridis*) [sic] im Steinbruch KG Zimmermann, Gemarkungen Illingen und Roßwag. – Landtag von Baden-Württemberg, Drucksache **8/1034**: 1–3.

Lannoo, M. (2008): Malformed Frogs. The Collapse of Aquatic Ecosystems. – Berkeley (Univ. Calif. Press).

Latour, B. (1987): Science in Action: How to Follow Scientists and Engineers through Society. – Cambridge (Harvard Univ. Press).

Latour, B. (1999): Pandora's Hope: Essays on the Reality of Science Studies. – Cambridge (Harvard Univ. Press).

Lenssen, N. & C. Flavin (1996): Meltdown. – World Watch **May/June 1996**: 23–31.

Lepage, C. (2011): La Vérité sur le nucléaire: le choix interdit. – Paris (Albin Michel).

Lunde, K.B. & P.T.J. Johnson (2012): A pracical guide for the study of malformated amphibians and their causes. – J. Herpetol. **46**: 429–441.

McCoy, E.D. (1996): Advocacy as as part of conservation biology. – Conserv. Biol. **10**: 919–920.

McDaniel, C.N. (2004): The human cost of ideology as science. – Conserv. Biol. **18**: 869–871.

Mertens, R. (1964): Welches Tier ist das? Kriechtiere und Lurche. – Stuttgart (Kosmos).

Møller, A.P., T.A. Mousseau, F. de Lope & N. Saino (2007): Elevated frequency of abnormalities in barn swallows from Chernobyl. – Biol. Lett. **3**: 414–417.

Møller, A.P., T.A. Mousseau, F. de Lope & N. Saino (2008): Anecdotes and empirical research in Chernobyl. – Biol. Lett. **4**: 65–66.

Niemelä, J., J. Young, D. Alard, M. Askasibar, K. Henle, R. Johnson, M. Kurttila, T.B. Larsson, S. Matouch, P. Nowicki, R. Paiva, L. Portoghesi, R. Smulders, A. Stevenson, U. Tartes & A. Watt (2005): Identifying, managing and monitoring conflicts between forest biodiversity conservation and other human interests in Europe. – Forest Policy Econ. **7**: 877–890.

Nishioka, M. (1978): Abnormalities in the descendants of *Rana japonica* produced from irradiated eggs or sperm. – Sci. Rep. Lab. Amph. Biol. Univ. Hiroshima **3**: 189–384.

Nöllert, A. & C. Nöllert (1992): Die Amphibien Europas – Bestimmung-Gefährdung-Schutz. – Stuttgart (Franckh-Kosmos).

Oerter, K. (1985): Morphologische und histopathologische Untersuchungen zur Entwicklung von *Bufo bufo* nach Bestrahlung mit Co-60 im Schwanzknospenstadium. – Bonn (Unpubl. Master Thesis, Univ. Bonn).

Orr, D.W. (2004): The corruption (and redemption) of science. – Conserv. Biol. **18**: 862–865.

OSPAR-RSC (Radioactive Substance Committe of OSPAR) (2010): Position paper on the implications of deep sea disposal of radioctive waste. – London (Oslo and Paris Conventions for the Protection of the Marine Environment of the North-East Atlantic: Dokument **RSC 10/4/3-E**).

Ouellet, M. (2000): Amphibian deformities: current state of knowledge. Pp. 617-661 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. – Pensacola, Florida (SETAC).

Pooth, L. (1981): Radioaktive Strahlen! Steinbruch bei Vaihingen doch verseucht! – BILD, Stuttgart **7.5.1981**: 4.

Puhlmann, P. (1981a): Anhörung im Landtag zu Krötenmonstern – Minister Weiser: Behörde hat geschlampt. – Stuttgarter Nachrichten **7.11.1981**.

Puhlmann, P. (1981b): Doch Hinweis auf Radioaktivität. – Stuttgarter Nachrichten **16.4.1981**.

Puhlmann, P. (1981c): Tausendmal mehr Radioaktivität. – Stuttgarter Nachrichten **7.5.1981**.

Puhlmann, P. (1981d): Streit um Radioaktivität geht weiter. – Stuttgarter Nachrichten **12.5.1981**.

Puhlmann, P. (1981e): Der französische Wissenschaftler Dubois zu den mißgebildeten Kröten bei Vaihingen/Enz: "zu 99 % Radioaktivität schuld". – Stuttgarter Nachrichten **18.9.1981 (215)**: 15.

Radkau, J.& L. Hahn (2013): Aufstieg und Fall der deutschen Atomwirtschaft. – München (oekom).

Reaser, J.K. & P.T. Johnson (1997): Amphibian abnormalities: a review. – Froglog **24**: 2–4.

Rimpp, K. (1981a): Bericht über einen Fund mißgebildeter Wechselkröten (*Bufo viridis*). – Herpetofauna, Weinstadt **3(11)**: 25–28.

Rimpp, K. (1981b): Zur Sachlage Krötenmißbildungen im Steinbruch Zimmermann. – Vaihinger Kreiszeitung **11.3.1981**.

Rosenkranz, G. (2010): Mythen der Atomkraft. Wie uns die Energielobby hinters Licht führt. – München (oekom).

Rostand, J. (1971): Les étangs à monstres. Histoire d'une recherche (1947–1970). – Paris (Stock).

Russell, B. (1979): Die Philosophie des logischen Atomismus. Aufsätze zur Logik und Erkenntnistheorie 1908–1918. – München (dtv 4327).

Savchenko, V.K. (1995): The Ecology of the Chernobyl Catastrophe. – Paris (UNESCO).

Schauz, R. (1980): Spritzmittel ist die Ursache. – Stuttgarter Nachrichten **28.10.1980**.

Scherb, H. & E. Weigelt (2003): Congenital malformation and stillbirth in Germany and Europe before and after the Chernobyl nuclear power accident. – Environ. Sci. Pollut. Res. **10** (Special Issue **1**): 117–125.

Schmid, W. (1981): Gelehrtenstreit um das Geheimnis der Krötenmonster. – Stuttgarter Zeitung **31.1.1981**.

Schmidt, C.W. (1997): Amphibian deformities continue to puzzle researchers. – Environ. Sci. Technol. **31**: 324–326.

Schmidt, E.A., U. Schmidt & V. Schucht (1981): Symposium über Krötenmißbildung ergab: Mit 99 %iger Sicherheit radioaktive Strahlung. – Löwenzahn, Vaihingen/Enz **7/1981**: 14–18.

Schmutz, S. (1981): Vorwürfe nicht mit Zahlen belegt. Missgebildete Kröten ziehen politische Kreise/Experten reagieren gelassen. – Pforzheimer Kurier **7.5.1981**.

Schnedler, L. (1980): Radioaktive Strahlung doch schuld? – Stuttgarter Nachrichten **20.12.1980**.

Schnedler, L. (1981a): Die Grünen und die Kröten. Einladung zum Symposium. – Leonberger Kreiszeitung **16.7.1981**.

Schnedler, L. (1981b): Weiser: Falsche Messungen. – Stuttgarter Nachrichten **22.7.1981**.

Schnedler, L. (1984): Wieder Wirbel um die Krötenmonster. – Stuttgarter Nachrichten **24.2.1984**.

Schneider, M. (1984): Strahlenbedingte Veränderungen des Nervensystems bei Amphibien. – Bonn (Unpubl. Master Thesis, Univ. Bonn).

Schnorbach, N. (1981): Die Monsterkröten vom Steinbruch. – Die Zeit **26.11.1981**: 22.

Schnorbach, N. (1982): Stuttgarts Krötenkrüppel vom Steinbruch. – Das Tier, Frankfurt/M. **März 1982 (3/1982)**: 19.

Schuldes, H. (1981): Wo kommen denn mißgebildete Kröten her? Die läßt der liebe Gott vom Himmel fallen. – Virus, Fachschaft Biologie, Universität Stuttgart-Hohenheim **1**: 10–12.

Semenov, D.V. & S.A. Ivanova (1995): Embryonic abnormalities in lizards *Lacerta vivipara* (Sauria, Lacertidae) inhabiting a radioactive contaminated territory. – Russ. J. Herpetol. 2(2): 166–169.

Sessions, S.K. (2009): Malformed frogs, the collapse of aquatic ecosystems (book review). – Herpetol. Rev. **40**: 121–124.

Sessions, S.K., R.A. Franssen & V.L. Horner (1999): Morphological clues from multilegged frogs: are retinoids to blame? – Science **284**: 800–801.

Shrader-Frechette, K. (1996): Throwing out the bathwater of positivism, keeping the baby of objectivity: relativism and advocacy in conservation biology. – Conserv. Biol. **10**: 912–914.

Souder, W. (1997): New reports of deformed frogs trigger US ecological alarms. – Washington Post **29.1.1997**.

Souder, W. (2002): A Plague of Frogs. Unraveling an Environmental Mystery. — Minneapolis (Univ. Minnesota Press).

Stresemann, E. (1995): Exkursionsfauna von Deutschland. Bd. **3**. Wirbeltiere. – Jena & Stuttgart (Gustav Fischer).

SWR (Südwestfunk Radio) (2011): Strahlende Altlast. *http://www.swr.de/report/strahlende-altlast/-/id=233454/vv=printall/nid=233454/did=8815982/1x70gty/index.html* accessed 4.1.2012).

Taruffi, C. (1881–1886): Storia della teratologia. Parte seconda. Fascicolo I-IV. – Bologna (Regia Tipografia).

The Independent Investigation Commission on the Fukushima Nuclear Accident (2014): The Fukushima Daiichi Nuclear Power Station Disaster. Investigating the Myth and Reality. – New York (Routledge).

Theegarten, F. (1980): Kröten mit fünf Beinen. – Stuttgarter Nachrichten **12.9.1980**.

Trux, A. (1985): Histopathologische Untersuchung zur Entwicklung des visuellen Systems von *Bufo bufo* (Amphibia) nach Bestrahlung mit Co-60. – Bonn (Unpubl. Master Thesis, Univ. Bonn).

Tyler, M.J. (1983): Natural pollution monitors. – Austr. Nat. Hist. **21**: 31–33.

Tyler, M.J. (1989): Australian Frogs. – South Yarra (Viking O'Neil).

UCS (2004): Scientific Integrity in Policymaking: An Investigation into the Bush Administration's Misuse of Science. – Cambridge, Massachusetts (Union of Concerned Scientists). [available via usc@ucsusa.org].

Veith, M. & B. Viertel (1993): Veränderungen an den Extremitäten von Larven und Jungtieren der Erdkröte (*Bufo bufo*). Analyse möglicher Ursachen. – Salamandra **29**: 184–199.

Vershinin, V.L. (2002): Ecological specificity and microevolution in amphibian populations in urbanized areas. – Adv. Amph. Res. Former Soviet Union *7*: 1–161.

Volpe, E.P. & P.A. Rosenbaum (2000): Understanding Evolution. – New York (McGraw-Hill).

Vuori, K. & M. Parkko (1996): Assessing pollution of the river Kymijoki via hydropsychid caddis flies: population age structure, microdistribution and gill abnormalities in the *Cheumatopsyche lepida* and *Hydropsyche pellucidula* larvae. – Arch. Hydrobiol. **136**: 171–190.

Wendel, M. (2009): Unüberbrückbare Gräben. Gorleben-Streit dauert an/Geschöntes Gutachten bei Auswahl als Endlager? – Leipziger Volkszeitung **115 (211)**: 2.

Widener, D. (1970): Timetable for Disaster. – Los Angeles (Nash Publ.).

6 Appendix I
Background information relevant for understanding the development of the conflict

6.1 German quarries: operations and characteristics

In the early 1980s, the German Mining Act (Bergbaugesetz) required owners to recultivate quarries after cessation of mining. This usually consisted of filling abandoned parts of the quarry with earthen material from the opening up of new parts of the quarry and from commercial or public construction sites. Thus, deposition of material was a normal activity that did not attract any attention except of naturalists disliking this practice because abandoned quarries provide habitat for many rare or endangered species. Accumulating water was pumped-off from active parts of quarries but never in abandoned parts of quarries to save costs.

The pond in the Roßwag quarry was fed by rain, surface run-off and lateral seepage. As usual in quarries, it had no vegetation and was shallow. Such water bodies are highly suitable breeding habitats for the green toad (*Bufotes viridis*) and the natterjack toad (*Epidalea calamita*) but are rarely used by the common toad (*Bufo bufo*) (Günther 1996). The former two species were strictly protected by law but abandoned quarries were not listed as protected habitats.

At the time of discovery of the malformed toads, quarries were still favoured places to illegally dump waste, such as old electrical equipment, housing material, tyres and construction material. With increased environmental awareness and (partly) free dumping of material at official refuse tips, this illegal custom declined in the 1980s. We found no signs of such illegal waste deposits in the Roßwag quarry.

6.2 Administrative structures and political responsibilities

Germany is a federal state with complicated administrative structures and political responsibilities. Most environmental issues fall within the responsibility of the states, but the federal government sets the legal framework for the states. Before Chernobyl, environmental ministries and agencies did not exist and environmental issues fell within the portfolio of other ministries. In Baden-Württemberg, this was the Ministry of Agriculture, Food, Environment and Forestry. Most ministries supervise agencies that act as their advisory scientific and technical bodies. Among others, they are responsible for the implementation of state policies and legislation. In Baden-Württemberg, the Landesamt für Umweltschutz (LfU – State Institute for Environmental Protection) has this responsibility.

Below the state level, there are several political and administrative levels. Responsible for issuing mining operation permits for the Roßwag affair was the Landkreis administration (Administrative District). The border between two districts (Enzkreis and Ludwigsburg) runs through the Roßwag quarry. The City of Pforzheim was an independent administrative unit in the Enzkreis district. Each district is headed by an elected political representative and has its own administrative unit for Protection Against Natural Disasters (Katastrophenschutzamt) that is responsible for protecting the public in the case of nuclear, biological or chemical disasters. However, these units relied on the Landesamt für Umweltschutz for equipment and coordinated emergency plans.

6.3 Institut für Energie- und Umweltforschung (IFEU)

The IFEU (Institute for Energy and Environmental Research) is an independent centre of excellence for environmental research. It was founded in 1978 – two years before the discovery of the malformed toads in Roßwag. It is financed entirely by research contracts and does not receive core funding from governmental sources. In 1980, it depended heavily on funds from state sources.

6.4 Involvement of the Federal Government

We have few sources documenting the role of the Federal Government in the affair. However, one document of the German Parliament (Deutscher Bundestag 1981) shows that politicians of the CDU/CSU (conservative parties) in the Federal Parliament attempted to cut off IFEU from federal funding. The Minister for Research, Andreas von Bülow, SPD, did not follow this request and stated that the Federal Government trusts the relevant authorities in Baden-Württemberg. Rather, the controversy of the case, together with other controversies about radioactivity (e.g., Bär 1981), seems to have been the motivation for commissioning a study on the impact of low levels of radioactivity on amphibians. The study was initiated by G. Baum, FDP, Minister of the Interior, the ministry that was responsible for nuclear safety at the federal level. He was no longer in office when the project terminated because the government had changed.

A collation of newspaper stories indicating the media attention received and the controversies of the discovery of a hotspot of abnormal toads in a quarry near Roßwag, Germany. Photo: K. Henle.

MERTENSIELLA 25 | 185–242 | 2017

Mass anomalies in green toads *(Bufotes viridis)* at a quarry in Roßwag, Germany: inbred hybrids, radioactivity or an unresolved case?

Klaus Henle[1], Alain Dubois[2], Kurt Rimpp[3] & Vladimir Vershinin[4,5]

[1]UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstr. 15, 04318 Leipzig, Germany, klaus.henle@ufz.de

[2]Institut de Systématique, Évolution, Biodiversité, ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE, Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, 75005 Paris, France, adubois@mnhn.fr

[3]Brunnenfeldstr. 10/3, 71272 Renningen, Germany, rimpp@t-online.de

[4]Institute of Plant and Animal Ecology, Uralian Division, Russian Academy of Science, 8 Marta Str. 202, 620144, Ekaterinburg, Russia, wow@ipae.uran.ru

[5]Ural Federal University, Ekaterinburg, Mira Str. 19, 620002, Russia

Abstract. Amphibian anomalies have attracted human curiosity for centuries and the literature on this issue is very extensive. The vast majority of publications on natural populations refer to less than ten affected individuals. Recent observations of mass anomalies in amphibians and increased environmental awareness reignited the interest in amphibian anomalies as potential indicators of environmental perturbations and triggered concerns about environmental conditions and human health. A particularly severe case of mass anomalies in a natural amphibian population was discovered in a quarry close to the village of Roßwag in southern Germany in 1980. Until now, only very brief preliminary data have been published on this case and the cause(s) of the anomalies have remained controversial. Here we provide a detailed account of the anomalies observed and on the studies undertaken to assess the potential cause(s). Based on an extensive literature review on experimentally induced anomalies in amphibians, we evaluate all known causes of amphibian anomalies as potential explanations for the observations in Roßwag.

At least 245 recently metamorphosed individuals and 1,000–2,500 tadpoles of the green toad (*Bufotes viridis*) exhibited anomalies, thus scoring fourth highest among the 2782 cases from natural populations compiled by us for which concrete data were available. Thirty-two different types of anomalies were observed, which exceeded the next extreme case known (25 types of anomalies in a population of *Rana arvalis* that was exposed to a nuclear accident; 2990 cases with data available). Therefore, the anomalies observed clearly do not represent a normal natural occurrence. The green toad was the only species spawning in the pond and, with the exception of three white clutches of the same species in a neighbouring quarry, no anomalies were found in any other population of amphibians in the vicinity of the quarry.

Based on an evaluation of results from 1025 publications of experimental studies, we show that all potential non-genetic factors taken together could at most explain a fraction of the observed types of anomalies, except for a cocktail of chemicals. Only one individual with a squashed head can be attributed with certainty to a non-genetic cause: traumatism. Chemical analyses of water samples from the breeding pond revealed a copper concentration at a level at which it might cause bent tails and edema in tadpoles. The water samples did not provide any other indication of chemical pollution and no pesticides were detected in abnormal toads. Notwithstanding, pesticides aerially sprayed on neighbouring vineyards might have contaminated the breeding pond well before water samples were taken.

The appearance of various anomalies in tadpoles and recently metamorphosed individuals raised from eggs transferred to the laboratory indicates that several types of anomalies were of genetic origins. The successful breeding of one abnormal individual verified this for one colour anomaly. Inbreeding, hybridization, as well as radioactivity and other mutagenic factors can explain genetically-based anomalies. Hybridization followed by inbreeding was put down as one explanation resulting from an official inquiry. However, genetic and morphological analyses as well as amphibian surveys conducted in the area of Roßwag clearly contradict hybridization as a potential cause. The great interindividual variability and high number of different types of anomalies are not compatible with inbreeding as an explanation. No evidence of mutagenic chemicals was found, though it cannot be excluded with absolute certainty that such substances had been present but were already degraded at the time of chemical sampling. In contrast, high levels of radioactivity were measured at the opening of cracks at a deposit of earth bordering the breeding pond. Alerted officials rejected these measurements as artefacts but did not take any measurements at the opening of cracks. Here it is shown that all radioactivity measurements are consistent with one another and with the hypothesis of radioactive material being burrowed in the deposit of earth and accessible to toads via cracks. This hypothesis is the only explanation that is consistent with all observations and measurements taken and with the results from a huge body of literature on experimental studies. There is no evidence for any other potential cause for the anomalies and biological knowledge clearly contradicts all of them with the exception of mutagenic substances.

Keywords. Anura: Bufonidae: *Bufotes viridis*, Germany, chemicals, genetic causes, hybridization, inbreeding, mass anomalies, radioactivity, teratogenetic causes.

Zusammenfassung. Anomalien bei Amphibien haben seit Jahrhunderten Aufmerksamkeit erzeugt. Entsprechend existiert eine umfangreiche Literatur über dieses Thema. Allerdings beziehen sich die meisten Veröffentlichungen zu freilebenden Populationen auf Beobachtungen von weniger als zehn betroffenen Individuen. In jüngster Zeit wurde jedoch wiederholt ein massenhaftes Auftreten von Missbildungen bei Amphibien beobachtet. Dies sowie ein erhöhtes Umweltbewusstsein haben das Interesse am Auftreten von Anomalien als Indikator für Umweltbelastungen erneut geweckt und zu Sorgen über den Umweltzustand und Gesundheitsrisiken für den Mensch geführt. Ein besonders extremer Fall von Anomalien wurde im Jahre 1980 in einem Steinbruch bei Roßwag, Baden-Württemberg, Deutschland, entdeckt. Bisher wurden nur vorläufige Beobachtungen über den Fall publiziert und die Ursache(n) blieben kontrovers. In diesem Beitrag geben wir erstmals eine ausführliche Übersicht über die beobachteten Anomalien sowie die Untersuchen, die zur Aufklärung der Ursachen unternommen wurden. Basierend auf einer Auswertung von 1025 Publikationen über Experimente diskutieren wir alle Faktoren, die als Erklärung vorgeschlagen wurden oder experimentell Anomalien verursachen können.

In den Jahren 1980–1984 wiesen mindestens 245 metamorphosierte Jungtiere und 1000–2500 Kaulquappen der Wechselkröte (*Bufotes viridis*) Anomalien auf. Abgesehen von drei weißen Laichschnüren der Wechselkröte in einem benachbarten Steinbruch konnten in keiner Amphibienpopulation der weiteren Umgebung von Roßwag Anomalien gefunden werden. Bezüglich der Anzahl missgebildeter Individuen stellt die Roßwag-Population den viert-extremsten, je im Freiland beobachteten Fall dar (n=2782 Fälle mit expliziten Daten). Insgesamt wurden an Kaulquappen und Kröten 32 verschiedene Typen von Anomalien beobachtet, was alle anderen 2990 Fälle übertrifft, für die wir Daten haben (maximal 25 verschiedene Typen innerhalb einer Population von *Rana arvalis*, die radioaktiver Strahlung ausgesetzt war). Diese Zahlen belegen, dass die Anomalien kein normales, häufig auftretendes Phänomen darstellen.

Fast alle betroffenen Individuen verstarben spätestens wenige Monate nach der Metamorphose. Nur ein Individuum mit einer Farbanomalie (großer gelber Fleck an der Flanke) konnte zur Fortpflanzung gebracht werden. Ein Viertel der Nachkommen wiesen dieselbe Anomalie auf, d.h., die Anomalie war vermutlich dominant vererbt. Das Auftreten unterschiedlicher Typen von Anomalien bei der Entwicklung von Eiern und Kaulquappen, die ins Labor transferiert wurden, spricht dafür, dass auch weitere Anomalien eine genetische Ursache hatten.

Die Auswertung der Literatur über experimentell erzeugte Anomalien zeigt, dass alle bekannten, nicht-genetischen Ursachen von Anomalien bei Amphibien zusammengenommen höchstens einen kleinen Anteil der beobachteten Anomalien erklären könnten, abgesehen von einem Cocktail an Chemikalien. Lediglich ein Individuum mit Quetschungen des Schädels kann mit Sicherheit auf eine nicht-genetische Ursache zurückgeführt werden. Chemische Analysen von Wasserproben aus den Laichgewässern haben eine Kupferkonzentration ergeben, bei der gekrümmte Schwänze und Ödeme auftreten können. In den umliegenden Weinbergen wurden handelsübliche Pestizide mit Helikoptern versprüht, jedoch ließen sich Rückstände von Pestiziden weder in den Wasserproben noch in gesammelten Jungkröten nachweisen.

Für eine genetische Erklärung der Anomalien kommen Hybridisierung, Inzucht und mutagene Faktoren in Frage. Hybridisierung gefolgt von Inzucht wurde von der zuständigen Landesregierung als Erklärung angenommen. Inzucht kann ausgeschlossen werden, da bei Inzucht alle Individuen dieselbe Art von Anomalien aufweisen (sowohl theoretisch als auch empirisch umfassend belegt), was im krassen Widerspruch zum beobachteten breiten, von Individuum zu Individuum stark unterschiedlichen Spektrum an Anomalien in Roßwag steht. Alle genetischen und morphologischen Analysen widersprechen der Hypothese einer Hybridisierung mit einer weiteren heimischen Art. Außerdem war die Wechselkröte die einzige im Steinbruch laichende Amphibienart, das Steinbruchgewässer ist als Laichgewässer für die Erdkröte (*Bufo bufo*) ungeeignet und die nächsten Populationen der Erdkröte und der Kreuzkröte (*Epidalea calamita*) sind durch Barrieren und eine Entfernung von Roßwag getrennt, die ein kurzzeitiges Zuwandern einer höheren Anzahl an Individuen dieser Arten ausschließen.

Hinweise auf mutagene Chemikalien liegen nicht vor; es kann jedoch nicht mit absoluter Sicherheit ausgeschlossen werden, dass solche Substanzen zur Fortpflanzungszeit vorhanden, aber zum Zeitpunkt der Wasseranalysen bereits abgebaut waren. Dagegen wurden an einer Erddeponie, die in das Laichgewässer ragte, erhöhte Werte an Radioaktivität gemessen, die das zweifache von Kontrollwerten an anderen Stellen des Steinbruchs betrugen; an der Öffnung von Spalten stiegen sie bis zum 100-fachen der Kontrollwerte an. Die alarmierten Behörden fanden ebenfalls eine Verdopplung der Werte an der Erddeponie, nahmen jedoch keine Messungen unmittelbar an der Öffnung von Spalten vor; stattdessen wiesen sie die hier gemessenen hohen Werte als Artefakte zurück. Hier zeigen wir, dass die verschiedenen Messergebnisse zu Radioaktivität nicht im Widerspruch zueinander stehen, sondern sich decken, soweit es die an vergleichbaren Stellen durchgeführten Messungen betrifft. Sie stimmen auch mit der Hypothese überein, dass in der Erddeponie radioaktive Stoffe verborgen waren, zu denen die Wechselkröten über Spalten Zugang hatten. Diese Erklärung ist die einzige, die mit allen Beobachtungen und Messungen sowie der sehr umfangreichen Literatur zu experimentell erzeugten Anomalien bei Amphibien in Einklang gebracht werden kann.

Schlüsselwörter. Anura: Bufonidae: *Bufotes viridis*, Deutschland, Chemikalien, genetische Ursachen, Hybridisierung, Inzucht, massenhafte Anomalien, Radioaktivität, teratogene Ursachen

1 Introduction

Amphibian anomalies have attracted human curiosity for centuries (e.g., Vallisneri 1706, Geoffroy Saint-Hilaire 1832, 1836). Amphibian anomalies also played a pivotal role in the emerging science of developmental biology, aiming to understand morphogenesis (e.g., Hertwig 1892, Sladden 1930, Woerdeman 1936) and they still assume this role today (e.g., Kovalenko & Kovalenko 1996, Nye et al. 2003).

Increased environmental awareness, the global decline in amphibians (e.g., Henle & Streit 1990, Blaustein & Wake 1990, Mendelson et al. 2004) and recent observations of populations exhibiting mass anomalies (Burkhart et al. 2000, Meteyer et al. 2000, Souder 2002, Lannoo 2008) have prompted renewed interest in amphibian anomalies as potential indicators for environmental perturbations (e.g., Tyler 1983, 1989, Read & Tyler 1990, 1994, Ouellet et al. 1997, Pyastolova & Vershinin 1999, Burkhart et al. 2000, Ouellet 2000, Souder 2002, Vershinin 2002). One species, the African clawed frog (*Xenopus laevis)* is even used as a standard test system for the teratogenicity of chemicals and natural water bodies (Bantle et al. 1989) and, in Russia, newts (*Triturus* spp.) are used as a standard test system for potentially carcinogenic substances (Pliss & Khudoley 1979). A micronucleus test has been used on several amphibian species for *in vivo* monitoring of genotoxic aquatic pollution (Gauthier 1996).

In response to findings of mass anomalies in North America, the North American Reporting Centre for Amphibian Anomalies (NARCAM) was established (Lannoo 2008). Mass anomalies and/or high genetic loads have also been reported in other organisms from polluted environments, for example, plants (Yablokov et al. 2009), invertebrates (Vuori & Parkko 1996, Saura et al. 1998, Hesse-Honegger & Wallimann 2008, Taira et al. 2014), reptiles (Ernst 1995, Bishop & Gendron 1998), birds (van der Schalie et al. 1999, Møller et al. 2007, Yablokov et al. 2009), mammals (Yablokov et al. 2009) and humans (Gilberston et al. 1991, Savchenko 1995, Guizard et al. 2001, Yablokov et al. 2009). However, these organisms, with the exception of humans (e.g., Winter et al. 1988, Savchenko 1995, Feshchenko et al. 2002, Scherb & Weigelt 2003, Lannoo 2008, Yablokov et al. 2009) and domestic animals (Taruffi 1881–1886, Noden & de Lahunta 1985, Yablokov et al. 2009), have still not received the same level of attention as amphibians.

Not surprisingly, the literature on amphibian anomalies is enormous (Henle 2014). Many factors have been shown to cause developmental anomalies in amphibians (reviewed e.g. by Hertwig, O. 1894, Hertwig, G. 1918, Rostand 1971, Dubois 1979, Tyler 1989, Ferraro & Burgin 1993, Ouellet 2000, Blaustein & Johnson 2003), but the relevance for natural populations of many of them remains obscure. Likewise, a considerable number of reviews of amphibian anomalies have been published – the first ones more than a century ago (Geoffroy Saint-Hilaire 1832, 1836, Taruffi 1881–1886). Most reviews focussed on narrow topics and, with few exceptions (noticeably Rostand 1971), did not discuss to any extent the potential and limits to infer causes from the observed patterns of anomalies. Linking cause to pattern is a central issue in ecology (Wiegand et al. 2003) and crucial for the use of amphibian anomalies as an indicator of the nature of an environmental perturbation that has occurred in the habitat.

The large majority of publications refer to malformations in less than 10 individuals per species at a particular site (Ouellet 2000, Henle 2014; this publication). However, this is not the phenomenon of concern. It is only when frequencies and types of abnormalities grossly exceed the baseline level (currently usually assumed to be below 1–5%: e.g., Hoppe 2000, Ouellet 2000, Johnson et al. 2010) that there is any reason to suspect the presence of an unusual factor (Burkhart et al. 2000, Henle et al. 2017a). Whatever the actual causes of mass anomalies, the biggest challenges will be to determine whether they are natural or anthropogenic and to assess their relevance for environmental hazards and human health (Tyler 1989, Burkhart et al. 2000, Souder 2002).

A particularly striking case of mass anomalies in a natural amphibian population was discovered by Rimpp and Henle in a green toad (*Bufotes viridis*) population in a quarry in southern Germany in 1980 (Henle 1981, 1982, Rimpp 1981, Dubois 1984). This discovery received intensive public attention in the mass media (e.g., Kneissler 1981, Kovacsics 1981, Henle et al. 2017b) and resulted in a highly controversial debate about its potential causes with major environmental and political implications (Henle 1981, Rimpp 1981, Flindt 1985a, Henle et al. 2017b). Suggested explanations ranged from a completely normal observation to inbred hybrids and victims of radioactive contamination but only preliminary observations of the anomalies and very limited assessments of potential causes have been published (Henle 1981, 1982, Rimpp 1981, Flindt 1985a).

Elsewhere in this volume, we present the first detailed history of the discovery and its treatment in the scientific, public and political arena and discuss its political and environmental implications (Henle et al. 2017b). Here we describe the affected population and provide a comprehensive description of the observed anomalies. We continue with the investigations into potential causes. We then compare the Roßwag case to other cases of anomalies in natural populations of amphibians and review experimental studies taking into account factors that caused the types of anomalies observed in Roßwag. After evaluating all the causes suggested for the Roßwag population as well as all other causes put forward for other cases of mass anomalies in amphibians, we conclude with a parsimonious explanation that is consistent with all of the observations made.

2 Habitat of the abnormal toad population – The Roßwag quarry

The abnormal toads were found at the end of August 1980 by Rimpp and Henle in a quarry owned by the company Steinbruch Zimmermann KG near a small village called Roßwag approximately 25 km NW of Stuttgart in the State of Baden-Württemberg, southern Germany. A road and the river Enz border the quarry to the South, while the rest of the quarry is bordered by agricultural land (mainly vineyards, but also an extensively-used orchard, fields and a small copse) (Fig. 1). In an abandoned part of the quarry, there was a large water body approximately 100 m × 25 m in size, with an average depth of approximately 0.5 m, a maximum depth of approximately 1 m and shallow shores. One side of the pond had been filled with earth as part of restoration activities (Fig. 2). The pond was free of vegetation and had a soft silty bottom. The shore was sparsely covered with herbs (*Atriplex* sp.).

Within three weeks of discovering the abnormal toads – a great controversy about radioactivity as a potential cause was already raging at that time (Henle et al. 2017b) – the quarry management pumped out all of the water, destroying the pond, but in 1981 some water collected again in the same place forming a small puddle of less than 200 m² and a maximum depth of less than 0.3 m. In addition, there were also two shallow (< 0.3 m depth) depressions of approx. 35–100 m² at the border of the quarry in 1980 and 1981. They were completely free of vegetation and had a loamy respectively silty bottom. In 1982 and 1983, these water bodies were still present and another depression within the quarry and a concrete pool at the entrance of the quarry contained water (Flindt 1985a). In 1984, none of the water bodies contained sufficient water to allow a successful completion of larval development of green toads (Flindt 1985a).

The original pond area was continually filled with soil material and by 2005 this fill had reached a height of approximately 100 m, making it impossible for water to collect at this location. On 15.5.2005, the only existing water body was a very small depression in a more recent part of the quarry. On 28.5.2005, there were two small water bodies with an estimated size of 10 m × 5 m and two road ditches with an estimated size of 10 m × 3 m and 5 m × 3 m, respectively. Furthermore, the concrete pool at the entrance of the quarry contained water.

3 Amphibian surveys in the Roßwag quarry

Before the pond was destroyed in September 1980, one detailed survey of tadpoles and four surveys of the terrestrial habitat in the vicinity of the pond were carried out by Henle, Rimpp and Weiss. On 30.8.1980 and 6.9.1980, tadpoles were visually counted on five plots (each 3 m in length) extending from the shore 2 m into the pond. The total population size was estimated by multiplying the average of the counts with the number of plots, which visually contained similar densities. Toadlets were surveyed within the abandoned part of the quarry by systematically inspecting accessible potential hiding places, such as cracks in the earthen deposit and objects providing cover. In addition, Henle surveyed the area surrounding the existing water bodies (up to a distance of 1 km) on 7.5.1981 and 30.5.1981. The surveys of 1980 were carried out during the day, whereas those of 1981 were carried out during the evening and early night.

Amphibians in the quarry were also surveyed by Christaller and Körbele from May to August 1981 (Christaller 1983) and by Flindt from 1982 to 1984 (Flindt 1985a). No further information is available for these surveys.

Only the green toad (*B. viridis*) was detected in the Roßwag quarry (Rimpp 1981, Henle 1982, Christaller 1983, Flindt 1985a). According to quarry workers the population had existed since 1976 and had presumably been founded by individuals originating from a quarry 1–1.5 km away (Christaller 1983). In May 1981, Christaller (1983) observed 30–50 calling males alone in the remnant of the destroyed pond and conservatively assumed a population size of approximately 200 adults. [Note that Christaller (1983) inconsistently called the Roßwag quarry 'quarry A' in one part of his publication but normally referred to it as 'quarry B']. Over the same period, Henle (unpubl.) counted a minimum of 50 males and 12 females during two surveys. Flindt (1985a) observed 46 adult males and eight adult females in 1982 and 23 adult males and six females in 1983. Given the limited survey days and the fact that most *B. viridis* females only stay one day at the breeding site (Nehring 1988), the population size must have been considerably larger than the minimum counts. The minimum numbers observed show that the population was large to very large, falling within the upper 10% of known population sizes for Baden-Württemberg (Laufer & Pieh 2007).

Data on the number of clutch strings are not available for any year. Tadpole numbers on 6.9.1980 were conservatively estimated as a minimum of 2,000–5,000 individuals in the main pond of the quarry, with the real size probably having been an order of magnitude higher. No tadpoles were observed in the two shallow depressions at the border of the quarry in 1980, but in May 1981 tadpoles were found living in one of them, roughly estimated at 1,000–5,000 individuals (Henle unpubl.).

Minimum numbers of toadlets are available for 1980–1983: > 300 in 1980 (Henle & Rimpp unpublished), 191 in 1981 (Christaller 1983), 361 in 1982 and 675 (591 from water depressions within the quarry plus 84 from the depression at the border of the quarry) in 1983 (Flindt 1985a). In 1984, all water bodies dried out before larval development was completed. In 2005, the population still existed, with a minimum of 150 tadpoles observed in the concrete pool. The water was very turbid; therefore, the total number of tadpoles must have been much higher.

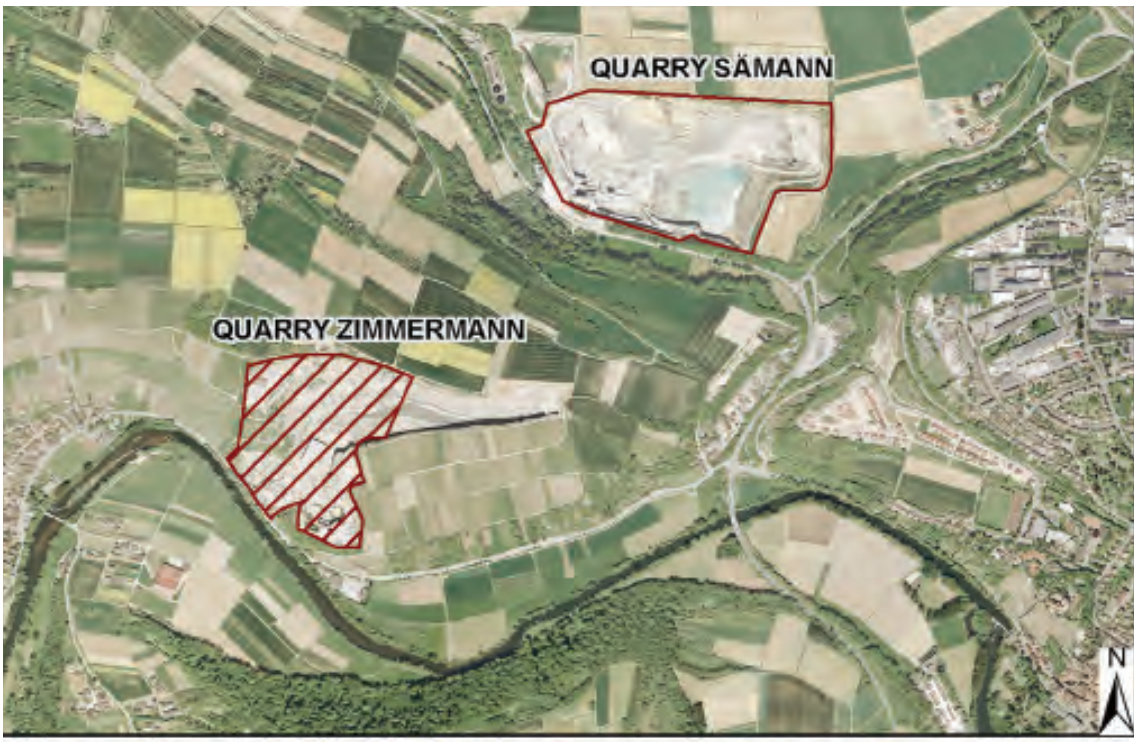


Fig. 1: Landscape structure in the surroundings of the Roßwag quarry and Roßwag's location within Germany.

Fig. 2: The breeding pond of *Bufotes viridis* in the Roßwag quarry photographed on 8.9.1980 before its destruction. Photo: K. Henle.

4 Assessment of anomalies

4.1 Methods

Our assessment of anomalies is based on our own field notes of sampled individuals, photographs and 12 malformed voucher specimens deposited at the National Museum of Natural History in Paris (MNHN 1984.2316–2326, MNHN 1984.2331). We also include data presented by Christaller (1983) and Flindt (1985a,b).

Tadpoles briefly described below were staged according to the table of Gosner (1960). We measured snout-vent length (SVL) and total length (TL) of preserved tadpoles using a ruler and of live tadpoles from photographs placed on a mm grid.

Table 1: Summary of anomalies observed in recently metamorphosed individuals of *Bufotes viridis* from Roßwag. *N*: Number; +++: > 25 individuals, ++: > 10 individuals, +: ≤ 10 individuals registered; +(D): anomaly not reported by Flindt (1985a) but detected by Alain Dubois in individuals collected by Flindt (collection year unknown); *: based on individuals raised in the laboratory, **: figures in the body of the table may not add to these sums because of multiple anomalies in some individuals and because anomalies were not specified for all individuals, ***: includes all individuals inside and at the border of the quarry; †: average of three samples; ††: figures in parentheses count different types of ectromely and ectrodactyly separately. Sources: 1980 data – Henle 1981, 1982, Rimpp 1981, Henle, Vershinin & Dubois unpubl.; 1981 data – Christaller 1983, Henle unpubl.; 1982–1984 data – Flindt 1985a.

	1980	1981 Christaller	1981 Henle*	1982	1983	1984*
N sampled	295	191	21	361	675***	87
N malformed**	173	4	4	6	50***	8
Frequency	53±9%†	2%	19%	1.7%	7.4%	9.2%
Polymely	+++	≥1		1	18	
Polydactyly (including schizodactyly)	+				1	
Ectromely: amely	++	1(?)		?	1	2
Ectromely: hemimely	++		1	2	3	
Ectromely: apody	+			1	21	
Ectrodactyly: oligodactyly	+		1	1	3	
Ectrodactyly: brachydactyly	+					
Phocomely	+				1	2
Syndactyly			?	+(D)	+(D)	
Clinodactyly				+(D)	+(D)	
Stiff legs	+++		1		1	
Rotation of legs	+++		?		1	
Vertebral column truncated and stiff	++					
Brachycephaly	+					
Pointed snout	+					
Mandibular hypoplasia	++					
Supernumerary bone at the angle of the jaw	1	1?				
Upper and lower jaw differ in shape and do not close	+					
Head injury (squashed)				1		
Swollen finger	+					
Atrophied finger musculature	+					
Partial melanism	+					
Novel colours	++					
Abnormal pattern	++					
Edema	+++		1			5
Tumours	++					
N types of anomalies††	20 (23)	∑1981: 5 or 6 (6 or 7)		4–6 (5–8)	6–8 (9–11)	3

The terminology for describing amphibian anomalies has not yet been standardized in the literature (Henle et al. 2017c). Whereas some terms are used consistently, such as polymely and anophthalmy, others are applied to different types of anomalies by different authors. Similarly, the same type of anomaly may be given different names. In this publication, we follow the terminology outlined in the companion paper by Henle et al. (2017c).

We group anomalies that occurred on the same body part of an individual (e.g., ectrodactyly or polydactyly on supernumerary legs) as a single anomaly (in this case polymely) and also merge some categories of anomalies that are tallied separately by some but not all authors. Thus, we combine apody, hemimely and amely in the category ectromely and brachydactyly, brachyphalangy and oligodactyly in the category ectrodactyly.

To quantify anomalies, Henle, Rimpp and Weiss counted all observed metamorphosed and adult individuals during their surveys in 1980 and 1981 and noted whether they had any easily visible external anomaly. They listed all types of anomalies observed but counted only the most obvious anomalies. Other anomalies were placed in classes of abundance only. To avoid double counting, individuals were kept in containers and released at the end of sampling. Henle and Rimpp also took four samples of > 80 tadpoles and one sample of 50 tadpoles from different parts of the pond. Sampled individuals were classified with the naked eye as normal or abnormal (e.g., whitish, giant, strong edema, strongly bent bodies).

During two surveys conducted in June and August 1981, Christaller (1983) counted the number of toadlets with very obvious external anomalies. In 1982 and 1983, Flindt (1985a) examined all adult and recently metamorphosed individuals that he found in the quarry for externally visible malformations. Furthermore, he sampled tadpoles and examined them for anomalies.

4.2 Frequency of anomalies

From a minimum of 149 adult males and 26 females examined from 1980 to 1983, only a single male collected by Christaller (1983) in 1981 showed an anomaly: an approximately 7–8 mm long supernumerary bone at the lower angle of the jaw. According to Flindt (1985a), it is a duplication of the upper arm. The accompanying photo does not allow a clear distinction between these two interpretations.

At least 245 toadlets exhibited anomalies. Most of them were found in 1980, when the percentage of abnormal individuals was approximately 50% (Tab. 1; see also Henle 1981, 1982, Rimpp 1981). The frequency of anomalies was 19% in 1981 (in individuals raised in the laboratory) and below 10% in the years 1982–1984 (Tab. 1). The figures for 1982–1984 are minimum numbers since one of us (AD) detected anomalies in individuals conserved by Flindt that had been overlooked by him.

The frequency of abnormal tadpoles in the sample of 6.9.1980 ranged from 30% to 55% (Henle 1982) or 35–55% (Henle 1981), if the smallest sample with 50

Table 2: Summary of anomalies observed in tadpoles. *N*: Number; n.a.: not assessed; +++: > 250 individuals; ++: > 100 individuals; +: > 10 individuals; *: based on individuals raised in the laboratory; **: figures in the body of the table may not add to these sums because of multiple anomalies in some individuals and because anomalies were not specified for all individuals; †: average of five samples taken in September. Sources: 1980 data – Henle 1981, 1982, Rimpp 1981, Henle, Vershinin & Dubois unpubl.; 1981 data – Henle unpubl.; 1982–1984 data – Flindt (1985a).

	1980	**1981**	**1982**			**1984***
			Sample 1	Sample 2	Sample 3	
N sampled	2,000–5,000	80	153	59	286	103
N malformed**	1,000–2,500	27	25	1	1	12
Frequency	35–55%†	33.8%	16.3%	1.7%	0.35%	11.7%
Ectrodactyly	+					
Arm remaining within branchial cavity	10					
Mouthparts malformed	+++	n.a.	++			
Curvature of tail	+++	6	5			
Spiraculum mid-ventrally	1					
Giant tadpoles	+++					
Nanism	++					
Partial albinism	++		2			
Novel colours	+					
Edema	+++	27	20	1	1	12
Tumours	1					
Other anomalies: reduced tail fin	1					
Other anomalies: asymmetric bodies	++					
N types of anomalies	13	2	4	1	1	1

individuals is excluded. Given the estimated number of tadpoles, at least 1,000 and up to 2,500 individuals were abnormal (Tab. 2). On two later occasions before the destruction of the pond, approximately 300 and 100, respectively, abnormal tadpoles were captured. In the 1982 field surveys, the percentage of abnormal tadpoles was 16.3% in the first sample falling within two and five weeks to 1.7% and 0.35%, respectively, due to mortality of abnormal individuals (Flindt 1985a). In 2005, no conspicuous anomalies could be detected but tadpoles could not be captured for detailed inspection.

4.3 Types of anomalies observed in the Roßwag population

In total, 23 different types of anomalies were observed in toads (26, if different types of ectromely and of ectrodactyly are counted separately), 13 in tadpoles and 32 (35) in total. In 1980, 20 (23) different types of anomalies were found in recently metamorphosed toads (Tab. 1) and 13 in tadpoles (Tab. 2). These numbers dropped considerably in subsequent years.

4.3.1 Recently metamorphosed and adult individuals

A) Skeletal anomalies of limbs

A1) Polymely (Figs. 3a–c & 4): From 1980 to 1983 at least 50 recently metamorphosed individuals with supernumerary limbs were found. In 1980, approximately 20% of the abnormal individuals showed polymely. Combining data from 1982 and 1983, 1.8% of all toadlets exhibited polymely. Duplications differed considerably among individuals. Flindt (1985a) found five different types of 5-limbed toadlets alone and two types of 6-limbed toadlets. Additionally, an individual with seven limbs was found (Rimpp 1981). Most common in 1980 were complete but stiff supernumerary limbs originating from the shoulder girdle, which may or may not be split at the elbow or the hand (e.g., Fig. 3a; MNHN 1984.2325), movable supernumerary limbs originating from the shoulder girdle but with both limbs covered to the elbow by the same skin sheet (e.g., Fig. 3b; MNHN 1984.2322) and small additional stumps originating from the shoulder region (Fig. 3c).

Two cleared and stained individuals (Fig. 4 and another one shown in Kneissler 1981) and X-rays of MNHN 1984.2325 demonstrated that at least some supernumerary limbs were associated with a duplication of elements of the shoulder girdle. The shoulder girdle of the individual pictured in Figure 4 was grossly malformed and the duplicated bones of the lower arm on the right side were partly fused. Supernumerary limbs were usually smaller in size and thinner than the normal ones (Flindt 1985a), but in a few cases, they were similar in size (e.g., figure in Klein 1981). The incidence of limb duplication was similar for the left side (registered for 10 specimens) and right side (9 specimens) (Flindt 1985a). It is remarkable that all cases of polymely except for one found in 1983 by Flindt (1985a) affected the front legs.

A2) Polydactyly (including schizodactyly) (Flindt 1985a: Fig. 6H): Polydactyly only occurred on supernumerary limbs (not counted as separate anomaly), except for two individuals unilaterally exhibiting six fingers and seven toes, respectively (Flindt 1985a). The cleared-and-stained individual figured by Kneissler (1981: 15) shows schizodactyly of the terminal phalangeal bone of finger three on the supernumerary right arm.

A3) Ectromely (Fig. 5): Individuals without legs (amely; Fig. 5a; see also Kneissler 1981: figure on page 18), lacking a limb from the elbow or knee (hemimely; Fig. 5b; MNHN 1984.2323, MNHN 1984.2326) or lacking a foot or hand (apody) occurred. Over the period 1980–1984 at least 50 toadlets were observed with incomplete

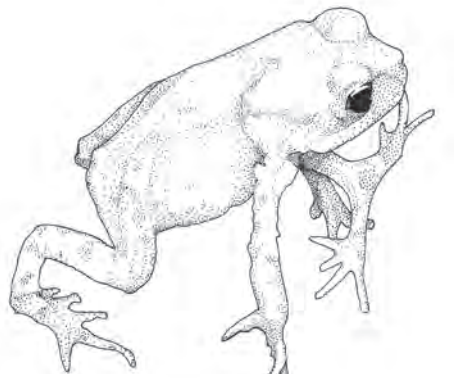


Fig. 3a

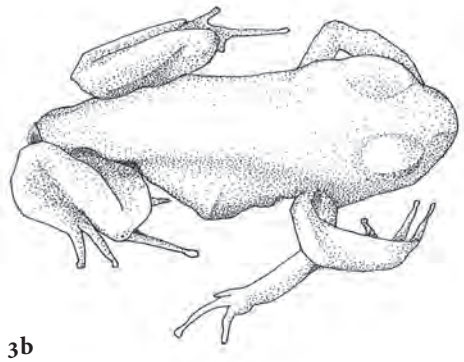


Fig. 3b

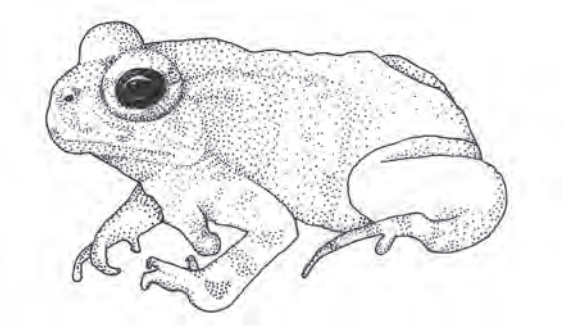


Fig. 3c

Fig. 3: Three common types of polymely observed in recently metamorphosed individuals in September 1980; a) complete stiff supernumerary arm originating from the shoulder girdle and split at the elbow; b) supernumerary arm originating from the shoulder girdle but covered to the elbow by the same skin sheet as the normal leg; and c) bony stump. Drawings from photographs (individuals not preserved) by R. Lange.

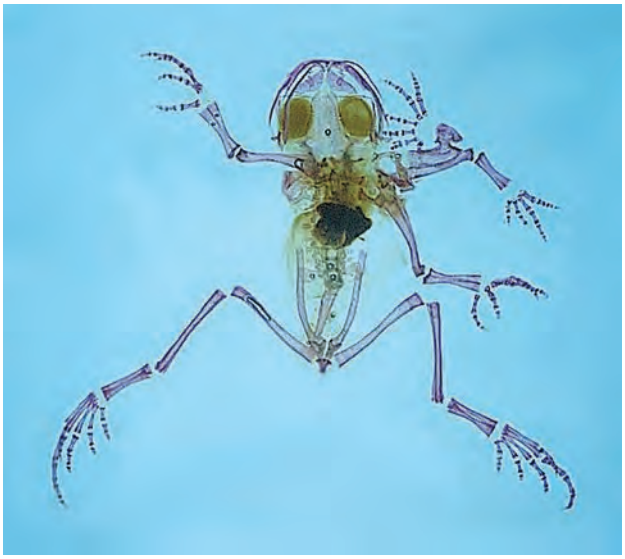


Fig. 4: Cleared and stained specimen of a six-legged frog showing duplications of elements of the right shoulder girdle; specimen collected in September 1980.

Fig. 5a

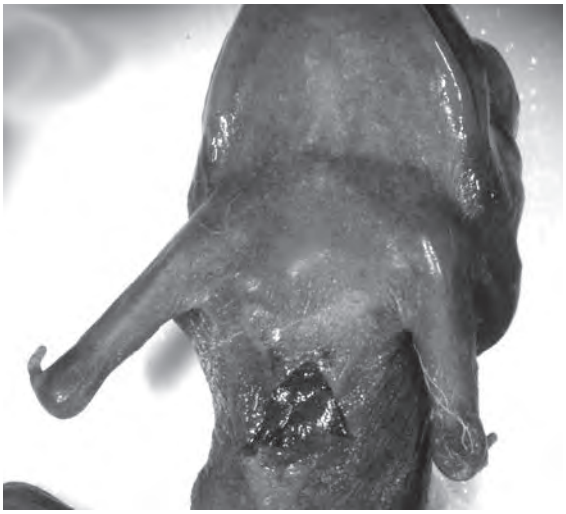


Fig. 5b | Fig. 5: Different types of ectromely observed in September 1980; a) amely (individual not preserved). Photo: K. Henle; b) hemimely (MNHN 1984.2323). Photo: H. Steincke.

hind or front legs. In 1982 and 1983, the frequency was 2.7%, but Flindt (1985a) assumed that such individuals were underrepresented in his samples (Tab. 1) since they had difficulties leaving the water and easily drowned. This is probably also the reason why apody occurred more frequently than hemimely. Both types of anomaly occurred mainly in front legs in 1980, but mainly in hind legs in the following years. In 1983, Flindt (1985a) found eight individuals without a right hind foot and ten individuals without a left hind foot; two individuals lacked both feet. In addition, three individuals were lacking a lower shank. Because Flindt (1985a) assumed that these malformations were due to cannibalism and predation by dragonfly larvae (see discussion), he did not count them as anomalies.

A4) Ectrodactyly (oligodactyly and brachydactyly) (Fig. 6): We did not take note of any individuals with a digit completely lacking (oligodactyly) in our field notes except for the case of incomplete supernumerary legs, but an examination of the voucher specimens revealed one case in an individual with a normal number of legs (MNHN 1984.2325: Fig. 6a). Flindt (1985a) reported such individuals in 1982 and 1983. Individuals with digits reduced in length (brachydactyly) were also not listed in our field notes or by Flindt (1985a), although an examination of voucher specimens revealed two individuals with brachydactyly (MNHN 1984.2319: Fig. 6b; MNHN 1984.2322). In addition, digits on supernumerary limbs were often shortened.

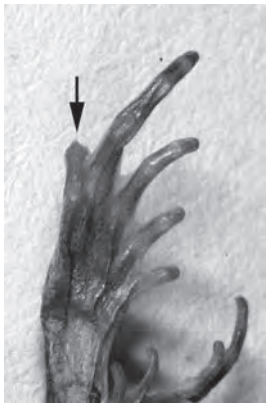


Fig. 6a

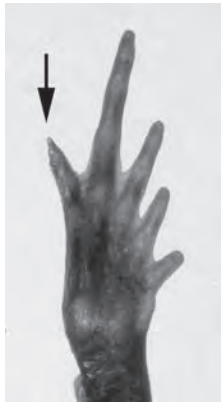


Fig. 6b

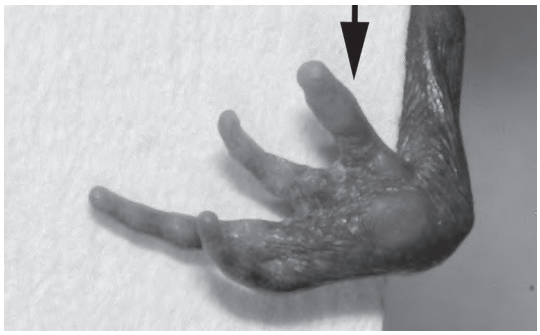


Fig. 6c | Fig. 6: Anomalies of toes and fingers observed in September 1980; a) oligodactyly (MNHN 1984.2325); b) brachydactyly (MNHN 1984.2319); c) swollen first finger (MNHN 1984.21). Photos: H. Steinicke.

A5) Phocomely (Fig. 7): In 1980, we found a few toadlets with one or several fingers or the hand attached directly to the shoulder. Flindt (1985a) observed phocomely in individuals raised in the laboratory from eggs that he had collected in 1984. He assumed that such individuals were not represented in his 1982 and 1983 samples (Tab. 1) because they experienced difficulties leaving the water and easily drowned.

A6) Syndactyly: Dubois (unpubl.) detected cases of syndactyly in recently metamorphosed toads collected by Flindt. This anomaly was overlooked by Flindt (1985a). Henle & Rimpp did not assess this type of anomaly in the field.

A7) Clinodactyly: Dubois (unpubl.) detected cases of clinodactyly in recently metamorphosed toads collected by Flindt. This anomaly was overlooked by Flindt (1985a). Henle & Rimpp did not assess this type of anomaly in the field.

A8) Stiffness and rotation of limbs (see Henle et al. 2017c for terminology) (Fig. 8): Stiffness and rotation of limbs were one of the most frequently observed appendicular anomalies in 1980. Only hind limbs showed rotations, invariably combined with stiffness and vice-versa. Stiffness also occurred in front legs but only when they were supernumerary. Except for one case reported by Flindt (1985a), the stiffness of legs was due to a reduction or an absence of musculature. Rotations were either caused by twisted long bones or an anomalous insertion at the joints.

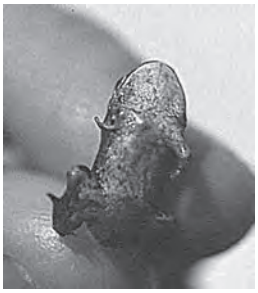


Fig. 7: Phocomely (right arm) and hemimely (left arm) (individual not preserved); September 1980. Photo: K. Rimpp.

Fig. 8: Combination of stiffness and rotation – a specific form of torsion – of both hind legs (individual not preserved); September 1980. Photo: K. Henle.

B) Skeletal anomalies of the body and head

B1) Stiff and shortened vertebral column (Fig. 9): Several individuals exhibited a shortened and stiff vertebral column in 1980, but the number was not quantified. These individuals had a very stout appearance. In most of them, this anomaly was combined with a shortened head.

B2) Brachycephaly (Fig. 9): A shortened head was observed in several individuals in 1980, but their number was not quantified. In most, but not all cases, brachycephaly was combined with a stiff and shortened vertebral column.

B3) Pointed snout (Fig. 10): In 1980, at least ten individuals with an acuminate instead of a broad trapezoidal snout were observed.

B4) Mandibular hypoplasia (Fig. 11): The lower jaw was more or less severely reduced in several individuals and in extreme cases it was completely lacking (agnathia).

B5) Other skeletal anomalies: Christaller (1983) collected one male in 1981 that showed an approximately 7–8 mm long supernumerary bone at the lower right angle of the jaw (figured in Flindt 1985a). According to Flindt (1985a), this bone is a supernumerary limb. The accompanying photograph indicates that this may be the case but does not allow an unequivocal discrimination between both interpretations. In any case, in MNHN 1984.2325, there is a very short bony protuberance present at the angle of the left jaw, an additional protuberance on the right side of the head and the shoulder blades are strongly expanded. In MNHN 1984.2324, the upper jaw is almost quadrangular, but the lower jaw round and thus not completely closing.

Fig. 9: Individual with a stiff and shortened vertebral column, brachycephaly and very few small dark green spots on a grey back (individual not preserved); September 1980. Photo: K. Henle.

Fig. 10: Individual with a strongly pointed snout and a large yellow spot on the back; September 1980; drawing from photograph (individual not preserved) by R. Lange.

C) Colour anomalies

C1) Partial melanism (Fig. 12): In some individuals, the head and body was very dark grey to black dorsally with the exception of small red dots on the warts. In one individual, this colouration extended to the dorsal surface of the hind legs, with arms and feet showing normal colouration and pattern. All partially melanistic individuals exhibited other anomalies as well. Some individuals had blackish but slightly transparent patches on the upper surface of the legs, in the groin or on the back. These individuals were not counted as partial melanistic, but were instead allocated to the category "abnormal pattern".

C2) Appearance of novel colours (Figs. 10 & 13): In 1980, many individuals showed patches of ochre to an orangey yellow colour. The normal colour and pattern of *B. viridis* does not include such colours. The patches were either present on the flanks or on the dorsum and of a variable size. The crossing of one such individual with a normal individual originating from the vicinity of Renningen in Baden-Württemberg, Germany, resulted in 25% ($n = 20$) of the offspring raised beyond metamorphosis exhibiting the same orange yellow patch as their parent. Thus, the anomaly must have had a genetic basis.

C3) Abnormal patterns: Some individuals had transparent blackish patches on the upper surface of their legs, in the groin or on the back. With few exceptions, they were also affected by skeletal anomalies.

D) Edema and tumours

D1) Edema (Figs. 12–14; see also Kneissler 1981: Photo on p.18): In 1980, this was the most common anomaly observed in recently metamorphosed individuals. In mild cases, it was mainly the upper shank that was distended (Figs. 12 & 13). In more severe cases, edema also affected the lower shank and in extreme cases, the whole legs looked like water-filled balloons (Fig. 14).

D2) Tumours (Fig. 15; see also Klein 1981: Photo on p. 24; Kneissler 1981: Photo on p. 18): Tumours were frequent in 1980. They occurred mainly on the head and in a few cases also on the back.

E) Other anomalies

E1) Flindt (1985a) observed one individual with a squashed partly regenerated head in 1982. In the voucher specimens collected in 1981, two additional anomalies were observed: thumb of right hand broadly swollen (MNHN 1984.2321) (Fig. 6c); a phalange of one toe with degenerated musculature (MNHN 1984.2322).

Fig. 11: Mandibular hypoplasia (individual not preserved); September 1980. Photo: K. Rimpp.

Fig. 12: Individual with black dorsal surfaces and edema on the hind legs (individual not preserved); September 1980. Photo: K. Henle.

Fig. 13: Individual with a very large yellow patch on the right flank extending to the back and onto the base of the shank; mild edema on the upper hind leg (individual not preserved); September 1980. Photo: K. Henle.

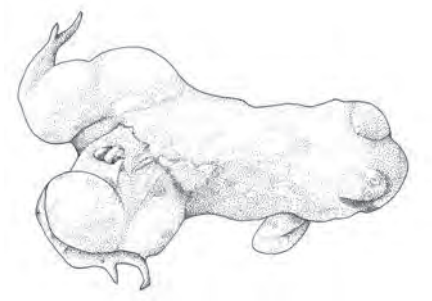


Fig. 14: Severe edema of the hind legs observed in September 1980. Drawing from photograph (individual not preserved) by R. Lange.

Fig. 15: Head tumour observed in September 1980. Drawing from photograph (individual not preserved) by R. Lange.

4.3.2 Tadpoles

A) Morphological anomalies

A1) Ectrodactyly: This anomaly was observed in the field in 1980 in one slightly oversized tadpole (total length approx. 50 mm).

A2) Forelimb enclosed in the opercular fold of skin (Fig. 17): In 1980, more than 10 tadpoles with this type of anomaly were observed in the field. One tadpole transferred to an outdoor tank developed this anomaly, but died before completing metamorphosis.

A3) Mouthparts malformed (Fig. 16): In 1980, many tadpoles of all sizes and colouration showed various irregularities of mouthparts, mainly labial tooth rows interrupted in places where they are normally complete (Rimpp 1981). In a few cases, only a few scattered keratinized denticles were present. In addition, labial papillae were reduced or completely absent in many specimens (Fig. 16a). Furthermore, in a few specimens, one or both beaks lacked keratinization. Flindt (1985a) reported that tadpoles with bent tails and/or (?) edema had unusually broad gaps in the second upper labial tooth row (Fig. 16b).

A4) Curvature of tail (Rimpp 1981: Photo 1): Curvature of tail was observed frequently in 1980. It occurred in giant tadpoles, in whitish tadpoles and in tadpoles that had normal body size and colouration. Some of these individuals had two bents in the tail or strongly asymmetric bodies. Flindt (1985a) also reported individuals with bent tails (Tab. 2).

A5) Mid-ventral spiraculum: One whitish tadpole examined in 1980 had its spiraculum mid-ventrally positioned instead of laterally on the left side as in the majority of Anura (McDiarmid & Altig 1999).

A6) Giant tadpoles (Fig. 17, see also Klein 1981: Photo on page 25; Rimpp 1981: Photo 3): *B. viridis* tadpoles normally reach a maximum total length of 52 mm (Günther & Podloucky 1996). In 1980, many tadpoles had a total length of approximately 40–50 mm. Only those individuals were counted as giant tadpoles that reached a total length of 60 mm and over. More than 100 such individuals were observed in 1980. The largest measured tadpole reached a total length of approximately 85 mm (Henle 1981). None of the giant tadpoles developed forelegs and all ten individuals transferred to outdoor water tanks died without attempting to metamorphose. In 1983, tadpoles reached a total length of only 38 mm (Flindt 1985a).

A7) Stunted growth (Klein 1981: Photo on page 25; Rimpp 1981: Fig. 2): Many tadpoles showed stunted growth with a body length of approximately 5 mm and a total length below 15 mm (Rimpp 1981), a size that tadpoles usually attain soon after hatching. These individuals were blackish – the typical colouration of small tadpoles of that size – and most of them had abnormal mouthparts (various forms of interruptions or lack of one or several labial tooth rows, lack of papillae and lack of keratinized beaks).

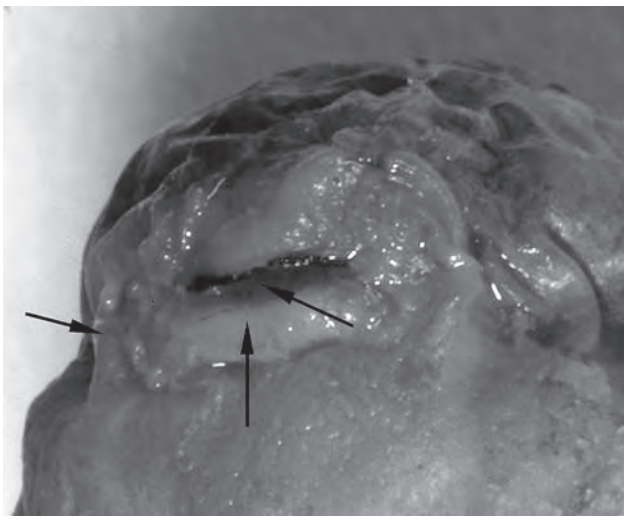


Fig. 16a

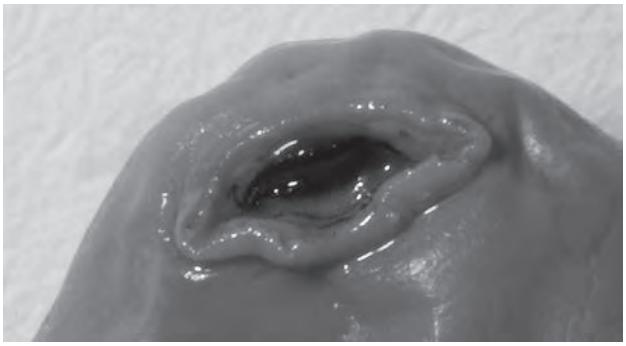


Fig. 16b

Fig. 16: Abnormal mouths of tadpoles collected in September 1980; a) grossly malformed (see arrows), with lower beak lacking keratinization, labial teeth rows with few scattered denticles only and labial papillae reduced and partly absent (MNHN 1984.2317); b) minor deviation: abnormally broad gap of the second upper labial tooth row and reduced keratinization of the teeth (MNHN 1984.2316). Photos: H. Steinicke.

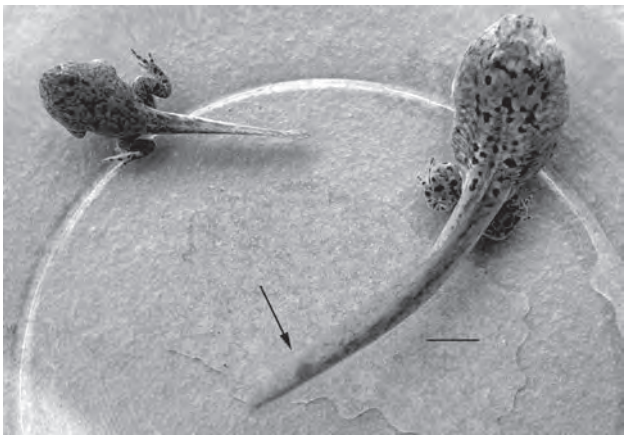


Fig. 17: Giant (> 80 mm total length) and large (approx. 50 mm total length) tadpoles (individuals not preserved), September 1980; the giant tadpole has a small yellow spot on the tail; a skin fold covers the left arm of the large tadpole. Black bar: 1 mm. Photo: K. Henle.

B) Colour anomalies

B1) Whitish tadpoles (Fig. 18): More than 100 whitish tadpoles were observed in 1980 (Henle 1981, 1982, Rimpp 1981) but only two conspicuously light-coloured tadpoles in 1982 (Flindt 1985a). In most individuals, the background colour was dirty white, but in some individuals it had a more or less pronounced brownish tinge (Rimpp 1981: photo 1). In whitish tadpoles, eyes were normally coloured and fine light grey dots and reticulation were present (Fig. 18). In some individuals, parts of the body were translucent. All individuals examined in 1980 were slightly oversized (total length 45–55 mm). Several whitish individuals were affected by slight to strong torsions of the tail and asymmetric bodies. In one individual, the spiraculum was positioned mid-ventrally.

B2) Appearance of novel colours (Fig. 17): In 1980, several tadpoles including giant ones, showed small to large patches of ochre to an orangey-yellow colour. The normal pattern of *B. viridis* tadpoles does not include such colouration. The colouration appeared on the body, the tail fin and the musculature of the tail.

Fig. 18: Large whitish tadpole (approx. 50 mm total length) (individual not preserved); September 1980. Photo: K. Henle.

C) Edema and tumours

C1) Edema (Flindt 1985a: Fig. 4): Edema was very common in 1980 and 1981. Its expression was highly variable. In most individuals, only a small part of the body was affected and individuals were still able to swim under water but some individuals had a balloon-like appearance and floated on the surface of the water. Flindt (1985a) observed edematous tadpoles in the field in 1982 and in tadpoles reared in the laboratory from a clutch in 1984.

C2) Tumours (Fig. 19): A photograph of a giant tadpole taken in September 1980 shows a tumour-like knot in the distal part of the caudal musculature. This anomaly was only noticed while the photograph for the preparation of this publication were being closely examined.

Fig. 19: Tumour-like knot in the tail tip of a giant tadpole (individual not preserved); September 1980. Photo: K. Rimpp.

D) Other anomalies

D1) Other anomalies: In 1980, some tadpoles exhibited strongly asymmetric bodies. Whereas extreme cases were clearly abnormal, it is difficult to establish at which degree of asymmetry it should be classed as an anomaly. Therefore, we did not include asymmetric individuals in the tally of the number of abnormal individuals.

4.4 Individual variability in anomalies

Many individuals were affected by several types of anomalies (up to 4). The average number of different types of anomalies per abnormal individual in the specimens preserved at MNHN is 1.8 (n = 12).

In 1980, few abnormal individuals resembled each other even if they showed the same type of anomaly. This is well exemplified by the following descriptions of voucher specimens:

MNHN 1984.2318 (toadlet): Dorsum completely black without any pattern; this is no longer visible in the preserved individual (examined June 2008).

MNHN 1984.2319 (toadlet): 4th finger of left hand and 5th toe of right foot without terminal phalange (brachydactyly) (Fig. 6b); strong edema with expanded skin on the upper shanks of both hind legs still obvious after preservation (examined June 2008).

MNHN 1984.2320 (toadlet): Edema at the hind leg shanks; in the preservative only visible as wrinkled skin (examined June 2008).

MNHN 1984.2321 (toadlet): 1st finger (thumb) of right hand broadly swollen (Fig. 6c).

MNHN 1984.2322 (toadlet): Supernumerary right arm, both arms covered to the elbow by a single skin sheet, supernumerary arm twisted at the elbow, both hands with four fingers but on one hand a very short thumb (brachydactyly); a phalange of one toe with degenerated musculature.

MNHN 1984.2323 (toadlet): Hemimely on both arms, with a finger-like protuberance at the elbow (Fig. 5b); the individual represents one of the few cases of symmetrical anomaly.

MNHN 1984.2324 (toadlet): Snout slightly malformed (upper jaw almost quadrangular, lower jaw rounded and not completely closing).

MNHN 1984.2325 (toadlet): Supernumerary left and right arms inserted at the shoulder; shoulder blades strongly expanded and probably duplicated; one arm on both sides normal; both supernumerary arms stiff; left supernumerary arm formed by a single long bone with a strongly broadened asymmetrical hand attached to it; hand with seven fingers, five of them brachydactylous; right supernumerary arm split at the elbow (probably two humeri present but fused); both elbows forming a 90° angle with the upper arm; one hand almost normal, the other one strongly reduced but with four fingers, only one of them normal; the remaining fingers partly or completely lacking terminal phalanges (brachydactyly); musculature of the penultimate phalange of one finger absent; outer toe of the left foot (almost) completely

absent (oligodactyly) (Fig. 6a); a bony protuberance present at the angle of the left jaw; an additional protuberance on the right side of the head.

MNHN 1984.2326 (toadlet): Left arm missing from elbow onwards (hemimely), with a tiny finger-like protuberance.

MNHN 1984.2316 (tadpole, Gosner stage 35): TL approx. 45 mm; SVL 18 mm; whitish (light brown in the preservative); eyes completely covered by skin and slightly reduced; the right posterior part of the body with strong edema (only clearly visible in radiographs); mouthparts slightly abnormal: only a few scattered keratinized denticles present on the upper lip, in two rows, the second broadly interrupted medially (gap covering approximately 50% of the row: Fig. 16b).

MNHN 1984.2317 (tadpole, Gosner stage 38): TL approx. 52 mm; SVL 20 mm; upper shanks slightly swollen (edema); mouthparts grossly abnormal (Fig. 16a): only upper beak keratinized (with serrate denticles), lower beak completely lacking keratinization; no tooth row on the upper lip; only a few keratinized denticles scattered across two tooth rows on the lower beak, the first with a narrow median gap, the second complete and almost as broad as the keratinized upper beak; only a few labial papillae showing at the lateral margin of the beak.

MNHN 1984.2331 (tadpole, Gosner stage 25): TL approx. 18 mm; SVL approx. 6 mm, retarded growth; upper and lower beak well keratinized (normal); no tooth row visible but preservation condition is poor and it is not certain that tooth rows were really absent (abnormal).

5 Investigations into potential causes

In short, the investigations undertaken can be summarized as follows. Tadpoles and clutches were transferred to the laboratory to study the further development of abnormal individuals and to find out whether anomalies also developed under laboratory conditions. Surveys on potential predators that could inflict injuries were undertaken in the Roßwag quarry. In the vicinity of the quarry amphibian surveys were carried out to assess whether anomalies occurred in other populations and to search for species that could have possibly hybridized with *B. viridis* from the Roßwag population. Additionally, *B. viridis* sampled at the Roßwag quarry were morphologically and genetically screened for markers characteristic of other toad species. Water samples were taken for chemical analyses and collected toads were analysed for residuals of pesticides. Finally, various radioactivity measurements were taken. Most measurements were severely constrained due to the great controversy about potential causes and the major alterations to the breeding habitat in the Roßwag quarry that followed soon after the discovery of the toads (see Henle et al. 2017b, for relevant background information).

5.1 Amphibian surveys in the wider vicinity of the Roßwag quarry

Amphibian surveys were conducted in the vicinity of the Roßwag quarry to assess whether anomalies were present in other populations and whether other toad species that might hybridize with *B. viridis* were present in the vicinity of Roßwag. Surveys were carried out in the area between the village of Roßwag, the country road between Roßwag and Vaihingen and the major road B10, i.e. approximately 1–1.5 km around the quarry, by Rimpp and Henle in September 1980 and by Henle in May 1981. Flindt (1985a) surveyed an area that extended approximately 8 km to the north and 1.5 km to the south of the quarry in 1982 and 1983. In addition, since 1978, amphibian surveys had been carried out in the administrative district to which Roßwag belongs and in surrounding areas (Christaller 1983).

Within a ca. 6 km radius around the Roßwag quarry, breeding populations of the following amphibians were found: fire salamander (*Salamandra salamandra*), Alpine newt (*Ichthyosaura alpestris*), crested newt (*Triturus cristatus*), midwife toad (*Alytes obstetricans*), yellow-bellied toad (*Bombina variegata*), common toad (*Bufo bufo*), green toad (*B. viridis*), water frogs (*Pelophylax* synkl. *esculentus*), agile frog (*Rana dalmatina*) and grass frog (*R. temporaria*) (Christaller 1983, Flindt 1985a). The only other population of *B. viridis* was found in a quarry (Steinbruch Sämann) approx. 1–1.5 km NNW of the Roßwag quarry. In the early 1970's, the population supposedly comprised several hundred individuals but in 1979, only 30 calling males could be heard (Christaller 1983). In 1982, approximately 25 adults were still present (Flindt 1985a). In 2005, a visual inspection of the quarry from the distance revealed that it still contained a large pond of approx. 75 m × 75 m, two medium-sized ponds and a very small pond, all of which were suitable for *B. viridis* to breed. No amphibian surveys were conducted during this brief visit. This was the only other population in which anomalies were observed: three clutches of white eggs and edema in 13% (*n* not given) of the free-living tadpoles of the same species. Two of these clutches were found in 1982 but were destroyed when the water was deliberately pumped out of the pond (Christaller 1983). For the third clutch and the edematous tadpoles the year of observation was not provided (Flindt 1985b).

5.2 Laboratory studies of anomalies

Eggs, tadpoles and recently metamorphosed individuals were transferred to the laboratory to study the development of anomalies and to assess whether they would develop irrespective of the quarry environment.

On 6.9.1980, Henle transferred eight small blackish tadpoles that otherwise appeared to be normal, ten large (approx. 50 mm TL) whitish, ten giant and three average-sized normal tadpoles to an outdoor elliptical water tank 2 m × 1 m in diameter. Commercial aquarium sand

was used as a substrate and the tank was filled with tap water to a depth of 0.4 m. Half of the water was changed every week. None of the abnormal tadpoles survived, but two of the normal tadpoles metamorphosed without developing any anomaly. The third individual died during metamorphosis. Its right forearm did not emerge from the opercular chamber. All small black tadpoles developed strong edema and died within a few days. The whitish and the giant tadpoles did not develop any signs of front legs and in some whitish individuals hind legs were also completely absent. All whitish and giant tadpoles died before the onset of metamorphosis.

On 7.5.1981, Henle collected part of an egg string that was approximately 20 cm long from a pair spawning at the small water-filled depression on the border of the quarry and transferred it to an outdoor tank. Eggs hatched within 14 days but hatching embryos soon died. Several of them showed torsions of the body, anencephaly, microcephaly, gross distortions and edema.

On 30.5.1981, Henle collected 80 tadpoles with a normal appearance and raised them under the same conditions as in 1980. Of these, 34% developed edema (Tab. 2) and died. In all affected tadpoles, edema rapidly increased in severity until, in the final stage, tadpoles had an inflated balloon-like appearance (as in Fig. 4 of Flindt 1985a). These individuals were unable to dive and soon died. In six of these tadpoles, the tail was bent. Of the 21 individuals that metamorphosed successfully, one showed hemimely of one arm, one had stiff hind legs, one showed ectrodactyly and one had mild edema of the hind legs (Tab. 1).

In 1982, Flindt (1985a) transferred 45 tadpoles to the laboratory, of which 20 showed edema. Of the latter, the last two died during metamorphosis whereas all but one of the 25 tadpoles that appeared normal metamorphosed successfully. In the field, the mortality of edematous tadpoles also appeared to be very high as reflected by a seasonal decline in their frequency (Tab. 2).

In 1984, Flindt (1985a) transferred part of a recently deposited egg string containing approximately 120 eggs to the laboratory, of which 103 individuals hatched. Of these, 11.7% showed severe edema (Tab. 2) and 87 survived to metamorphosis, of which eight developed anomalies (Tab. 1).

Flindt (1985b) also transferred approximately 120 eggs to the laboratory from a white egg string found in the Sämann quarry. Approximately 20% of the embryos developed gross distortions of the body and died. Survivors started to develop pigmentation two days after hatching and although they appeared normal one week after hatching, edema developed in approximately 40% of them. Some tadpoles showed abnormal screw-like swimming behaviour. Ten individuals metamorphosed successfully and were reported not to show any obvious anomaly.

Flindt (1985a) and Rimpp (unpubl.) attempted to raise metamorphosed toads exhibiting polymely, ectromely, lacking the lower jaw or showing large yellow patches on the flanks. Most individuals were severely handicapped, had difficulties or were unable to catch prey and showed uncoordinated movements. As a consequence, most individuals died soon after metamorphosis (Flindt 1985a, Henle 1982) and only a single individual with a large orange yellow patch on the flank could be raised to maturity (Rimpp unpubl.). This individual was crossed with a specimen collected in the vicinity of Renningen, Baden-Württemberg, Germany. Of the 20 offspring 25% showed the same anomaly as their parent.

In summary, the laboratory studies show that several types of anomalies also developed under laboratory conditions, indicating that they might have been of genetic origin. By crossing, a genetic origin could be confirmed for the yellow patch of one individual.

5.3 Hybridization

Flindt (1985a) analysed the serum albumin variation of nine adult *B. viridis* that he had collected in the Roßwag quarry in 1982–1983 to test the hypothesis that the anomalies were caused by hybridization. Additionally, he assessed species-specific patterns and morphological characteristics of – presumably 60 – adults.

The serum albumin bands of all nine individuals were diagnosed as *B. viridis* (Flindt 1985a). Likewise, pattern, colouration and the index "body length/hind leg length" of all individuals were typical for this species. In the survey years no other species of toads was found in the quarry (Henle 1981, 1982, Rimpp 1981, Christaller 1983, Flindt 1985a) and in 1980 none of the malformed individuals showed patterns typical for *Bufo bufo* or *Epidalea calamita* (Rimpp 1981). Only one adult examined by Flindt (1985a) and MNHN 1984.2321, a recently metamorphosed individual with a swollen finger, showed a divided instead of a single subarticular tubercle, as is the case in *E. calamita*. However, this characteristic occurs in about 5% of individuals in *B. viridis* populations (Flindt & Hemmer 1969).

The closest breeding population of *B. bufo* was located at approximately 1–1.5 km SSE of the quarry in cut-off oxbows of the River Enz (Flindt 1985a), with two country roads and the river Enz between the populations (Fig. 20). The next closest population was located NNW of Illingen at a distance of approximately 2.5–3 km from the Roßwag quarry (Christaller 1983), with a village and a major road separating the population from Roßwag. Before 1980, the occasional calling of the natterjack toad (*E. calamita*) was reported at the Roßweiher near Maulbronn at a distance of approximately 10 km from the Roßwag population and isolated from it by two major roads and several country roads (Christaller 1983). The nearest extant population known was very small and located close to Heimsheim (Betzenbuckel), approximately 15 km SSW of Roßwag. The nearest existing large *E. calamita* population known was located approx. 18 km away near Bretten.

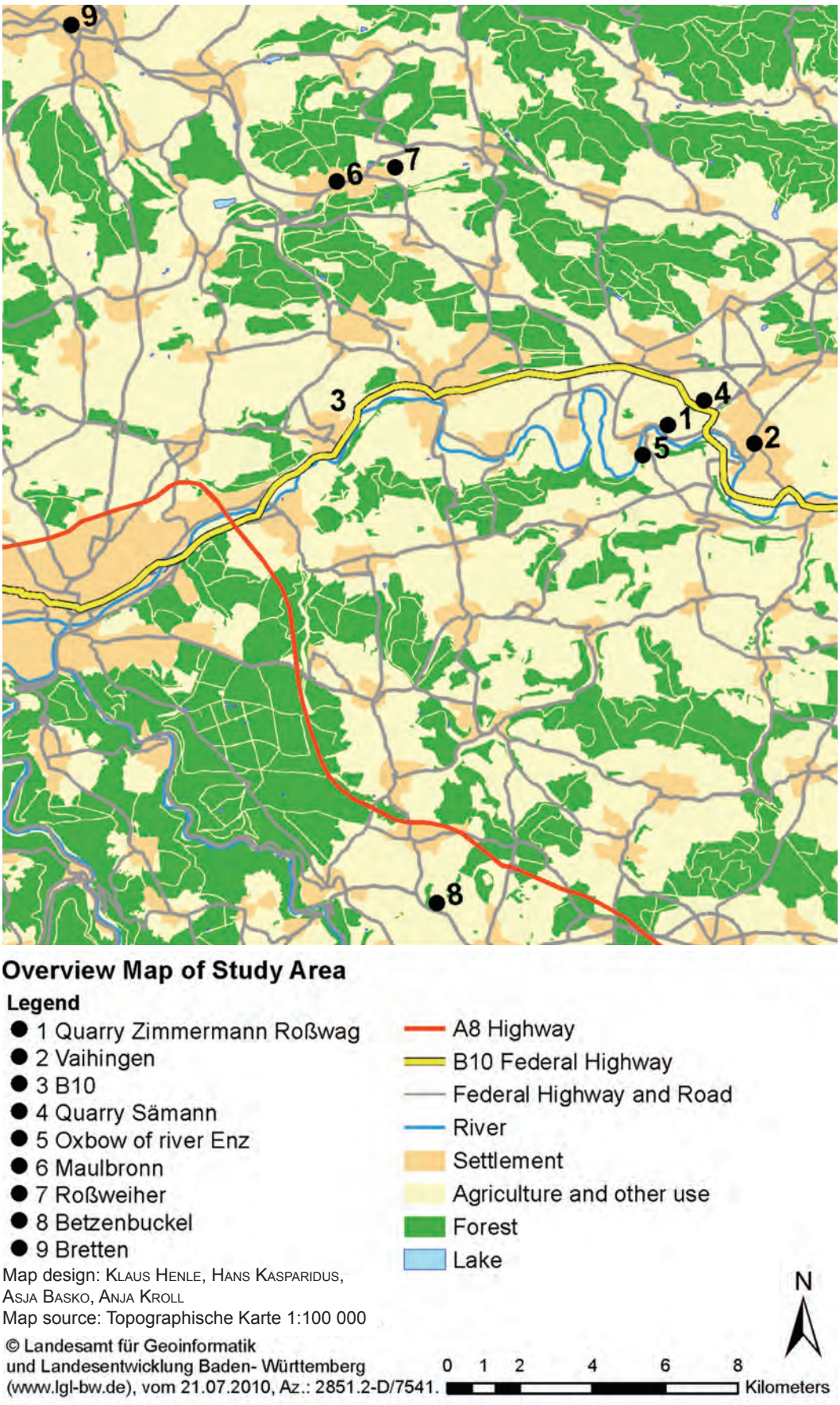


Fig. 20: Locations of landscape barriers and breeding populations of toads in the Roßwag region.

5.4 Potential predators

In 1980, Henle & Rimpp surveyed the shoreline of the pond visually and with dip-nets for dragonfly larvae, dytiscid beetles, leeches and other potential predators. The clear water of the pond enabled good visibility to the bottom of the pond even at its deepest part. The pond was not connected with any other water bodies and free of fish. Neither dragonfly larvae nor dytiscid beetles, leeches or any other potential predator species could be observed in any of the surveys.

In 1983, Flindt (1985a) observed many dragonfly larvae in a pond on the border of the quarry but did not report any predators for the remaining four water bodies. He implied that the dragonfly larvae had bitten off the hind legs of tadpoles. In addition, he explicitly stated that in the same pond he had observed cannibalistic attacks directed at the tails and hind legs of other tadpoles.

5.5 Chemical analyses

Rimpp collected water samples from the main pond on 8.9.1980 and sent them to the chemical laboratory of a private company. Three additional water samples were taken on 10.9.1980 by the Police Department of the City of Pforzheim and analysed by the Chemical Laboratory of the City of Pforzheim as well as by the Landesamt für Umweltschutz, Karlsruhe (LfU; State Institute for Environmental Protection, Baden-Württemberg). These samples were screened for metals, nitrate, nitrite, ammonium, phosphate and calcium. Gas chromatography was carried out by the Chemical Laboratory of Pforzheim to assess the presence of pesticides. Teratogenicity or mutagenic tests (see e.g. Birge et al. 1983, Lowcock et al. 1997) were not carried out by any of the laboratories. Abnormal toads collected in 1980 by Rimpp were also analysed for residues of chlorinated hydrocarbons by the Institute of Animal Hygiene at the University of Freiburg.

In the vineyards surrounding the quarry, the following pesticides were sprayed using helicopters: pomuran (i.e., captan + mancozeb), rovral [i.e., iprodion = 3-(3,5-dichlorophenyl)-N-(1-methylaethyl)-2,4-dioxo-1-imidazolidincarboxamid], copper sulphate and sulphur (Buck pers. comm.). In the early 1980s these pesticides were commonly applied to vineyards.

The analysis of the water samples collected by Rimpp did not provide any suspicious results but no concrete data are available. Gas chromatography by the Chemical Laboratory of the City of Pforzheim did not detect any traces of pesticides (neither the methods nor the minimum detection levels were given). Copper was below the detection level and no excessive concentrations of any other metals were found although sulphate concentration was very high (Tables 3 & 4). No residues of chlorinated hydrocarbons could be detected in the abnormal toads analysed by the Institute of Animal Hygiene at the University of Freiburg (Kneissler 1981, Henle 1982, Schnorbach 1982).

Table 3: Results of the chemical analyses of water samples from the Roßwag pond collected by the Police Department Pforzheim at 15:00 on 10.9.1980. Bdl: below detection level (detection level not given). Source: unpublished protocols of the Chemical Laboratory of the City of Pforzheim, dated 7.10.1980.

Chemicals	**Concentration (in mg/l)**		
	Sample 1	Sample 2	Sample 3
$KMnO_4$ consumption	8.7	6.0	6.7
Chloride	43.4	41.5	43.4
Nitrate	19.70	23.66	23.82
Nitrite	0.12	bdl	bdl
Ammonium	0.32	bdl	bdl
Phosphate	0.22	0.05	0.11
Iron	23.0	72.5	87.0
Manganese	0.8	1.35	1.6
CaO	250.5	245.0	240.0
MgO	135.5	123.7	135.9
Copper	bdl	0.1	0.1
Cadmium	bdl	bdl	bdl
Chromium	bdl	bdl	bdl
Nickel	bdl	bdl	bdl
Zinc	0.2	0.6	0.5
Plumbum	0.05	0.15	0.1
Mercury	bdl	bdl	bdl
Silver	bdl	bdl	bdl
Tin	bdl	0.4	0.85

Table 4: Results of the chemical analyses of water samples from the Roßwag pond that were received by the Institute of Water and Waste Management, State Agency for Environmental Protection, Karlsruhe (LfU) on 11.9.1980. Bdl: below detection level (detection level not given). Source: unpublished protocols, dated 9.10.1980.

Chemicals	**Concentration (in mg/l)**
pH	7.5
Ammonium	< 0.1
Calcium	90
Fe (total)	approx. 0.1
K	10
Mg	80
Na	25
Cyanide (total)	bdl
Chloride	43
Sulphate	approx. 400

5.6 Radioactivity studies

5.6.1 Assessment methods

The first assessment was made by Henle, in the presence of Rimpp, on 8.9.1980 using a Geiger counter sensitive to α and β radiation (type Minicont, trade mark of Herfurth). Measurements took place at the shore of the pond and across the basis of the earthen deposit within the quarry as well as at control sites outside of the quarry. All measurements were taken approximately 1 m above the ground. Large cracks at the base of the earthen deposit were selected as additional sampling points since they provided potential hiding places for toads. At these locations, measurements were taken at various distances above the opening of the cracks (Tab. 5a). Before and after the measurements, batteries were checked and showed sufficient charge. Because unexpected high levels of radioactivity were indicated, the instrument used was tested for potential artefacts and malfunctioning after terminating the assessments by taking measurements while holding the instrument vertical, upside down, at different angles, by shaking it heavily, by placing it upon the ground and upon stones collected from the earthen deposit and by rapidly approaching the surface of the earthen deposit at sites without cracks. Each test was repeated at least twice.

Upon a request made by Rimpp to repeat the measurements (Henle et al. 2017b), the Institute for Radiation Assessment at LfU measured the level of radioactivity in the quarry around mid-day of 10.9.1980. Rimpp but not Henle was informed and asked to attend immediately. The field protocol of LfU does not contain any information about the concrete locations or any other details regarding the measurements taken (Tab. 5c). A stone from the quarry wall, one water sample from the pond and one sample of rubble, presumably from the earthen deposit, were collected and spectrally analysed in the laboratory. No sediment samples were taken, which is unfortunate since some radionuclides are readily filtered out of the solution (Olympic Dam Operations 1990) and the tadpoles of *B. viridis* mainly inhabit the bottom of the pond.

On the evening of 10.9.1980, Henle once again measured the level of radioactivity at the shore of the pond, across the base of the earthen deposit and at three distances above the opening of cracks in the earthen deposit using a Xenon Geiger counter sensitive to α and γ radiation (Tab. 5b). The same instrument was used for additional measurements by Schreiber, Professor of Physics at the University of Stuttgart-Hohenheim, in the presence of Henle on 13.9.1980. They were stopped by the intervention of the quarry manager so that only very preliminary measurements at the shore of the pond and above the earthen deposit could be made and no field notes were taken.

The Institute for Energy and Environmental Research (Institut für Energie- und Umweltfragen – IFEU) in Heidelberg compared the level of radioactivity at three sites of the earthen deposit with five control points within and outside the quarry on 28.9., 9.11. and 7.12.1980. All measurements were taken just above the surface of the earthen deposit. It is important to note that, after the measurements taken by Henle, the earthen deposit had been covered by 3–10 m of fill. Large cracks with openings to the surface were no longer present. Due to a continual covering of the earthen deposit, measurements were abandoned thereafter (Franke et al. 1981).

5.6.2 Assessment results

Results of the field measurements are summarized in Table 5. All measurements that compared the level of radioactivity between control sites outside the quarry and/or the vicinity of the pond with the earthen deposit in September 1980 showed approximately 2–3 times higher levels at the earthen deposit, a difference that is within the expected normal range of variation (LfU in lit., Landtag von Baden-Württemberg 1981, Landesregierung von Baden-Württemberg 1984). In November and December 1980, the relative intensity at the earthen deposit compared to the control sites was lower (Tab. 5d).

Measurements close to the openings of cracks were made only by Henle in September 1980. These measurements showed an exponential increase of radiation intensity when approaching the opening of the cracks. They were 1–2 orders of magnitude higher directly at the opening of the cracks compared to 1 m above them (Tab. 5a,b). Tests carried out before and immediately after the measurements showed that the batteries were sufficiently charged. All tests for artefacts made for the Herfurth Minicont instrument were unable to elicit any elevated values. When returned to the laboratory none of the instruments showed any damage or malfunctioning.

The laboratory results of the three samples taken by LfU did not reveal any traces of artificial radionuclides (internal protocol by Funsch LfU, Landtag von Baden-Württemberg 1981, Landesregierung von Baden-Württemberg 1984) but showed considerably higher levels of natural radionuclides in the sample of the earthen deposit compared to stones from the quarry (Tab. 6).

Table 5: Summary of field measurements of radioactivity in the Roßwag quarry

a) Henle, 8.9.1980: Herfurth Mincont (α and β sensitive). Note, the tests for the correct functioning of the instrument are not included.

Location	**Measurements**	**Intensity: min – max (Impulses per second)**
Car park	once at 17:00 h	5–7
Entrance to the quarry	10 times, starting 17:15 h	5–7
Along the pond	repeatedly while moving along the shore; during the period 17:30-18:30	5–7
Across the basis of the earthen deposit	continuously while moving across the deposit; 30 min	12–15
Close above 2 cracks at the left side of the earthen deposit	3 times, ≥ 2 min each	100
Close above 3 cracks at the right side of the earthen deposit	2 times, ≥ 2 min each	500–1000
At the surface of the lowest of the 3 cracks	once, 2 min	1000–5000
5 cm above the lowest crack	2 times	100
25 cm above the lowest crack	2 times	10–15
At the surface 1 m away from the lowest crack	once	10–15
Opposite side of the pond	5 times	5–7
1 m above the earthen deposit	2 times	10–15
20 min later at the surface of the largest crack	2 times	1500-2000; values decreased exponentially with distance from crack opening
Car park	once	5–7

b) Henle, 10.9.1980: Xenon Geiger counter (α and γ sensitive)

Location	**Measurements**	**Intensity: min – max (Impulses per second)**
At the pond	5 times, 2 min each	300–500
At the basis of the earthen deposit (3 locations)	2 times, 2 min each	700–1000
30 cm above the same crack as on 8.9.1980	2 times, 2 min each	1000
20 cm above the same crack as on 8.9.1980	2 times, 2 min each	1200
10 cm above the same crack as on 8.9.1980	2 times, 2 min each	1500–1800
Opposite side of the pond	2 times, 2 min each	300–500

c) LfU, 13.9.1980; S: scintillation counter; C: contamination counter. Source: unpublished protocol of the LfU, dated 11.9.1980

Location	**Measurements**	**Intensity: min – max (Impulses per second)**
Within the quarry	Not recorded	S: 70–150; C: 15–25
Location unspecified: "Normal level (plain)"	Not recorded	S: 50; C: 10

d) IFEU, various instruments and dates. Relative sensitivity: Earthen deposit / control site; Source: Franke et al. (1981)

	Intensity (Impulses per second)		
Date	**Control sites**	**Earthen deposit**	**Relative intensity**
28.9.1980	0.25±0.02	0.47±0.021	1.88±0.19
9.11.1980	0.49±0.02	0.72±0.02	1.47±0.10
7.12.1980	0.44±0.02	0.61±0.03	1.39±0.11

Table 6: Summary protocol of the laboratory measurements made for radioactivity of samples taken in the Roßwag quarry by the LfU. Source: unpublished protocol of the LfU, dated 11.9.1980.

Sample	Elements	Activity
Stone of the quarry wall	Ra-226	≈ 0.65 pCi/g
	AC-228	≈ 0.12 pCi/g
	K-40	≈ 2 pCi/g
Earthen deposit	Ra-226	≈ 1.5 pCi/g
	AC-228	≈ 1.8 pCi/g
	K-40	≈ 38 pCi/g
Pond water	Ra-226	< 0.01 pCi/ml
	Ac-228	< 0.01 pCi/ml
Pond water (residues)	total α	≈ 0.03 pCi/ml
	Rest β	≈ 0.06 pCi/ml
	K-40	≈ 0.008 pCi/ml

5.6.3 Experimental studies on the effects of radioactivity on amphibians

The Federal Ministry of the Interior commissioned a study on the effects of radioactivity on amphibian development (Henle et al. 2017b) carried out by the Zoological Institute of the University of Bonn. The common toad (*B. bufo*) was used as a test species. Three unpublished Masters theses resulting from this study are known to us. Schneider (1984) analysed the effects on the nervous system, Trux (1985) studied the histopathology of the developing visual system and Oerter (1985) assessed general developmental anomalies in tadpoles. Since the latter that is particularly relevant as a comparative basis for the field observations in Roßwag is unpublished and in German, we provide a brief summary of the study.

Embryos at tailbud stage III [after Cambar & Gipouloux (1956); equivalent to Gosner (1960) stage 19] (5.5–8 days old) received 320, 640 or 800 rad Co-60 γ irradiation (distance of irradiation source: 50 cm). The number of embryos used ranged from 200 to 1400 depending on the treatment. At each stage usually 10 individuals were conserved for analyses of anomalies. The study terminated with the completion of metamorphosis but the results were only presented up to stage IV_{13} (Gosner stage 40).

Growth retardation (total body length) of 2.6, 4 and 5.7 mm was observed for the 320, 640 and 800 rad treatments, respectively. Mortality increased strongly in the 640 rad treatment group. Histological examinations of individuals in stage III_8–IV_1 (Gosner stages 23–26) showed that all structures were disorganised or destructed. The brains showed incomplete cellular arrangements as well as disorganisation and necrosis of cell nuclei due to chromatin coagulation. The pronephros was degenerated and the epidermis exhibited necrosis, a condensation of pigment granula and abnormally distributed cells. The histological anomalies were less pronounced in later stages. A summary of the external anomalies recorded is provided in Table 7. They included among others brachymely, ectromely, clinodactyly, syndactyly, polydactyly, polymely, torsion of legs, malformed mouthparts and depigmentation.

Table 7: Anomalies of common toad (*Bufo bufo*) embryos and tadpoles after Co-60 γ-irradiation of stage III5; stages after Cambar & Gipouloux (1956) and, in parenthesis, after Gosner (1960); *n*: sample size; Source: Oerter (1985: Tab. 5–7) and text; note minor differences exist between her text and her tables.

Stage	Anomaly	Exposure (rad)	Frequency (*n*)
III_8	–	320	0% (10)
(23)	Gills deformed and underdeveloped; proliferation of epidermis	640 800	11% (9) 30% (10)
IV_1	–	320	0% (10)
(26)	Mouth closed, denticles missing on the denticle bearing labial ridges	640 800	50% (10) 100% (10)
	Hind leg buds retarded or missing	640 800	50% (10) 100% (10)
	Edema	640 800	50% (10) 50% (10)
IV_{12} (39)	Labial papillae abnormal, rows of denticles abnormal, broken or missing, mouth closed, horny beak partially missing	320 640 800	10% (10) 78% (9) 67% (15)
	Massive edema	640 800	89% (9) 33% (15)
	Depigmentation and transparency	640 800	78% (9) 33% (15)
	Brachymely, ectromely, clinodactyly, syndactyly, polydactyly, torsion of legs	320 640 800	10% (10) 67% (9) 20% (15)
	Dorsal torsions, hyperplasm of skin		not given
IV_{13}	Labial papillae abnormal, rows of denticles abnormal, broken or missing, mouth closed, horny beak partially missing	640	84% (25)
(40)	Edema	640	100% (25)
	Depigmentation and transparency	640	36% (25)
	Torsion of legs; polydactyly, polymely (tarsus), femur absent and lower leg attached to body (phocomely)	640	12% (25)

6 Discussion

The observations in the Roßwag quarry can be summarized as follows. A large number, high frequency and broad range of anomalies were found in *B. viridis,* all declining from 1980 to 1984. Anomalies could not be detected in any other amphibian population in the region except for three white clutches and edema in some tadpoles of the same species in a neighbouring quarry. Breeding showed that at least one type of the observed anomalies (yellow patches) was inherited. The appearance of various anomalies in tadpoles transferred to the laboratory or hatched from transferred clutches indicates that other anomalies are also of a genetic origin, although a teratogenic factor present at the time of egg deposition but not showing any effects until later on in development cannot be excluded with certainty.

With respect to potential causes for amphibian anomalies, all tests for hybridization failed and no other toad species was present in the same quarry. Potential predators were only found in one small water body and only in one year. Pesticides were sprayed by helicopter on vineyards bordering the quarry but no unusual chemical contamination of pond water or animals could be observed. High levels of radioactivity were found although disputed (see below).

Numerous hypotheses about the causes of the anomalies have been put forward, including "normal occurrence of a natural phenomenon" (Tab. 8). Some of these hypotheses would not normally appear in a scientific paper but because of the highly controversial debate about potential causes (Henle et al. 2017b) we scrutinise all of them.

The official report commissioned by the Ministry of Food, Agriculture, Environment and Forestry of the State of Baden-Württemberg excluded most of the potential causes from consideration, suggested different explanations for each type of anomaly and concluded that the anomalies are a normal natural phenomenon that have existed in the Roßwag quarry since at least 1908 (Landesregierung von Baden-Württemberg 1984). It acknowledged that the commissioned study could not provide a sound final explanation of the factors causing the anomalies but regarded past hybridization combined with atavism and inbreeding as the likely cause. It compared the anomalies observed to a small number of other cases in natural populations and experimental studies (Flindt 1984, 1985a, Landesregierung von Baden-Württemberg 1984). No review of the relevant literature was attempted. Such an approach is common in the literature on natural occurrences of anomalies in amphibians but it is not sufficient (see Souder 2002 for a parallel in recent mass anomalies in North America).

Whereas some authors (e.g., Reaser & Johnson 1997) suggest that, albeit limited, inferences can be made from the percentage of individuals affected, others disagree (e.g., Kovalenko 2000a,b). Numerous experimental studies have shown that the frequency often, but not always, depends on the strength of the causative factor (e.g., Nishioka et al. 1981, Power et al. 1989, Johnson et al. 1999) and differs among stages (e.g., Tschumi 1954, Kovalenko 2000a,b) and species (e.g., Johnson et al. 2001a, Vershinin 2002, Dubois 2014, 2017). The only conclusion from high incidences of anomalies that can be made is that something unusual must have happened and that the causative factor was strong.

Inferences are made even more difficult by the fact that all types of anomalies can have different causes (see

Table 8: Hypotheses suggested as explanations for the anomalies observed in *Bufotes viridis* from Roßwag.

Hypothesis	Suggested by (Reference)
Normal regular occurrence	Landesregierung von Baden-Württemberg 1984
Traumatism: falling stones	Flindt 1985a
Traumatism: failed predator attacks	Flindt 1985a
Traumatism: picking of eggs	Ewe, Institute of Zoology, University of Hohenheim (see Kovacsics 1981)
Parasites	Not suggested previously
Same cause as that of anomaly P	Landratsamt Enzkreis (see Schmid 1981, Henle et al. 2017b), Landtag von Baden-Württemberg 1981, Flindt 1985a
Virus	Landratsamt Enzkreis (see Schmid 1981), Flindt 1985a
Sound pressure from detonations	Landratsamt Enzkreis (see Anonymous 1980a,b)
Temperature extremes or shock	Not suggested previously
Electromagnetic fields	Once suggested in a discussion but never considered seriously
Overripeness of eggs	Flindt 1984, 1985a
Chemicals	Anonymous 1981a, Klein 1981, Flindt 1985a
Hybridization	Flindt 1984, 1985a, Landesregierung von Baden-Württemberg 1984
Inbreeding and genetic drift (recessive gene)	Flindt 1984, 1985a, Landesregierung von Baden-Württemberg 1984
Atavism	Flindt 1984, 1985a
UV-B (increased ambient levels)	Not suggested previously
Radioactivity	Henle 1981, 1982, Kneissler 1981 Rimpp 1981

Tab. 9) and by the lack of comprehensive reviews covering many types of anomalies and attempts to extract a pattern from a comparison of experimental studies in spite of at least 236 reviews on amphibian anomalies. We therefore conducted a thorough review of the pertinent literature to provide a sound basis for evaluating potential causes in the Roßwag case. We tried to trace any citation on amphibian anomalies starting with our own extensive literature collection, the literature on amphibian anomalies compiled by The North American Reporting Centre for Amphibian Malformations, the literature on field herpetology compiled by Schriftenschau für Feldherpetologie and key word searches in the Zoological Record for 1945–1985 and in Biosis for 1945–1985 and 1996–1999. More recent volumes and additional references were searched through the internet. Only publications that we could read were retained. This resulted in an evaluation of 3117 publications involving 3183 cases with natural populations; 1025 publications addressed experimental studies.

We regarded each species as a separate case if a publication contained information on several species. The same applies for several populations of a species, if sample sizes and the anomalies were counted separately for each population. Otherwise, we lumped data for all populations. If authors provided data for concrete populations and across sites, we only used data for concrete populations. For comparison with Ouellet (2000), we summed data across years and authors for the same population, if there was no data overlap. Otherwise, we only used the data from the most recent publication.

In many experimental studies no statistical comparisons with controls were made or different anomalies were lumped for tests. Therefore, we accepted a potential causative relationship, if (a) a mechanistic causative pathway between the factor and the anomaly in question is known, (b) at least one study showed a statistical significant difference between treatment and control, (c) there was a significant correlation between the rate of anomalies and the intensity of the causative factor even if no control data were provided, (d) more individuals were affected in the experiment treatment than in the control in more cases than expected at random or (e) 100% (n>50) of individuals were affected if no data of the controls were available.

6.1 Evaluation of hypotheses suggested for the Roßwag population

6.1.1 Normal natural occurrence

Observations of single or a few malformed individuals are nothing new or exceptional and the Roßwag population of *B. viridis* shares several anomalies with other populations. The State Government of Baden-Württemberg regarded the anomalies observed in the Roßwag population of *B. viridis* as a normal natural occurrence (Landtag von Baden-Württemberg 1981, Landesregierung von Baden-Württemberg 1984). However, the natural background rate of anomalies in "healthy" environments is usually around or well below 1% (Tab. 10), with previous authors assuming a baseline value of 5% or lower (reviewed by Johnson et al. 2010, Lunde & Johnson 2012, Reeves et al. 2013, Borkin et al. 2012 and Borkin 2014). Notably, von Humboldt (1797) pointed out that anomalies of frogs are exceedingly rare in spite of thousands of frogs having been dissected. Only ten other cases out of 2782 cases with concrete data available exceed 1000 abnormal individuals (Fig. 21). Of these, two comprise pooled data for several locations. Similar numbers within a population like Roßwag were found in *Ambystoma tigrinum* (tumours, edema) from a sewage pond (Rose 1976, 1977, Rose & Harshbarger 1977), in *Ambystoma macrodactylum* (ectrodactyly, syndactyly, polydactyly, ectromely, polymely and a further unspecified anomaly) in a pond with high prevalence of the trematode *Ribeiroia ondatrae* (Sessions & Ruth 1990, Johnson et al. 2003, 2006), in an *Anaxyrus boreas* population (ectrodactyly, ectromely, micromely, polymely, tail injuries) with the introduced fish *Gasterosteus aculeatus* being the cause (Bowerman et al. 2010) and in *Rana pirica* (ectromely, edema, microcephaly, anophthalmy, tumours) from a sewage pond (Mizgireuv et al. 1984). Considerably more individuals were affected only in a population of *A. tigrinum* (swollen legs and venter, hemorrhage) presumably dying from bacterial infection [Worthylake & Hovingh (1989); but see Docherty et al. (2003) who suggest that an iridovirus may have been the cause].

With 32 different types of anomalies detected, the Roßwag population of *B. viridis* far exceeds any other case (n=2990). The vast majority (81%) involved less than three types of anomalies; only for five other cases at least 15 different types of anomalies were reported for a single species (Fig. 22). One of them pooled data from 38 sites from across Bermuda and mentioned 20 different types in *Rhinella marina* (Linzey et al. 2003, Bacon et al. 2006a,b, 2013, Fort et al. 2006a,b). In terms of a single affected population, 15 types of anomalies were observed in the *Lithobates pipiens* population at Ney Pond, USA, which sparked off renewed interest in mass anomalies in North America (Helgen et al. 1998, 2000, Canfield et al. 2000, Garber et al. 2001, 2004, Souder 2002, Lannoo et al. 2003, Vandenlangenberg et al. 2003, Lannoo 2008). Parasites and chemical pollution were suggested as alternative explanations (e.g., Helgen et al. 1998, Lannoo 2008). For a *L. sphenocephalus* population at Colin's roadsite ditch, Great Swamp National Wildlife Refuge, USA, 20 types of anomalies were reported (Reeves et al. 2013). Of 583 metamorphosed individuals and tadpoles sampled 2003–2009, 11% were abnormal, with most types of anomalies being observed in one or very few individuals only. No potential cause was provided. Only one case approaches the observations in Roßwag. After the nuclear accident at the Siberian Chemical Combinat at Seversk (Tomsk, Russia), at least 25 different types of anomalies were observed in clutches, embryos, tadpoles and juveniles of *Rana arvalis* collected in the radioactive trace zone (Kuranova & Saveliev

Table 9: Overview of the factors that caused anomalies in tadpoles or recently metamorphosed juveniles in experiments. ++: significant relationship experimentally shown (see text for criteria) or 100% of individuals affected and $n > 50$ if no data of controls are available; (+): anomaly observed in treatments but not significantly more frequent than in controls, confounding factors not excluded or no statistical tests available; +?: association suggested but contradicted by other studies; –?: anomaly observed in an experimental treatment but a causative association with the treatment is unlikely; –: never observed in ≥ 10 experiments or never more frequent than in controls, with ≥ 10 experiments and ≥ 2 species; 1: see text for discussion; 2: epigenetic effect; 3: only reddish skin or haematonoms; 4: correlational evidence; 5: elevated rate in hybrid zone; 6: fused vertebrae; 7: only in specific host-parasite combinations; negative in most combinations; 8: apart from rare exceptions, only reddish skin or haematonoms. Source: References in text and Henle et al. (2017a).

	Non-genetic									**Genetic**			
Type of anomaly	Traumatism	Parasites	Mucous or excrements of fish	Virus	Other diseases	Temperature	Electrosmog and magnetic field	Overripeness of eggs	Chemicals	Hybridization	Inbreeding	Increased natural level of UV-B	Radioactivity & other mutagenic factors
Polymely	++[1]	++	(+)[1]		–	+?[1,2]	(+)	+?	++[1]	–?		–	++
Polydactyly	++	++	++			–		1	++[1]	–	++	–	++
Ectromely	++	++[7]	–	(+)[4]	++	++[2]	++	(+)	++	(+)[1]	++	++	++
Ectrodactyly	++[1]	++[7]	++	(+)[4]		++	+?	–	++	(+)	++	++	++
Phocomely	–		(+)[1]	–	–	–		–	++[1]	–?	–?	–	++
Clinodactyly	–	(+)	(+)[1]			(+)			++	–			(+)
Syndactyly	++	(+)	++			(+)			++	–?			(+)
Stiffness and rotation of limbs	(+)[1]	(+)	(+)[1]			++[1]			++	++	++	+?	++
Arms remaining within branchial cavity	–	–	–	–	–	–	(+)	–	++	(+)	(+)	–	(+)
Stiff and truncated vertebral column	–	–	–					–		–	++[6]	–	++
Brachycephaly & microcephaly	–		–					(+)		++	++	–	++
Mandibular hypoplasia	–		–		++	–		–	++	–	++	–	++
Mouthparts malformed in larvae	–		–		++				++	(+)[5]			++
Swollen fingers or body (solid)		–	–		(+)[1]	–		–		–	–	–	
Torsion of tail or body		(+)						(+)	++	(+)	++	++	++
Spiraculum misplaced	–	–	–	–	–	–		–	–	(+)		–	–
Giant larvae	–	–	–	–	–	–		–	++	(+)	++	–	–
Stunted growth				(+)					++	(+)	++		++
Melanism and darkening	–	(+)[4]	–	–	(+)	–		–	++	–?	++	++	++
Albinism and depigmentation	–	–	–	++	++	–		–	++	(+)	++	–	++
Other divergent patterns or colouration	–	++[8]	–	(+)[3,4]	++[3]	–		–	++		++	–	++
Edema		(+)	(+)[1]	++	++	+?	++	(+)	++	++	++	+?[1]	++
Tumours			(+)[1]	++	++				++	–			++

1997, unpubl.; Kuranova 1998, 2003). In small mammals, the rate of anomalies was 3.3. times higher than in control areas (Moskvitina et al. 2011). Concomitantly, 21 different types of anomalies were detected in *Salamandrella keyserlingii* embryos and larvae (Saveliev et al. 1996, Kuranova 1998, 2003). Thus, the Roßwag population clearly outruns all other natural populations in terms of the spectrum of anomalies.

Table 10: Background rates of anomalies in amphibians; only studies with samples sizes $N > 5{,}000$ included. We exclude cases that address only one type of anomalies; see Henle et al. (2017a) for a review of such cases.

Types of anomalies	Frequency	*N*	Species	Reference
–	0%	19,802	*Ambystoma tigrinum*	Rose 1981
Albinism, ectrodactyly	0.01%	25,000	*Necturus maculosus*	Hutt 1945
Oligodactyly & syndactyly	0.01%	10,000	*Rana temporaria*	Koskela 1974
Polymely of hind legs, shizodactyly, syndactyly, duplication of parts of the tail	0.02%	17,935	*Ambystoma talpoideum*	Semlitsch et al. 1981
Ectromely, deformed limbs	0.03%	36,151	8 anuran species in Michigan	Skelly & Benard 2010
Ectromely, deformed limbs	0.07%	22,482	*Lithobates sylvaticus*	Skelly & Benard 2010
Ectromely, oligodactyly, mandibular hypoplasia, polymely, digits malformed	0.4%	9,987	*Acris crepitans*	Gray 2000a,b, 2002
Unspecified	< 0.5%	> 5,300	*Rana pretiosa*	Bowerman & Johnson 2003
Ectromely, ectrodactyly, syndactyly, polydactyly, polymely, anophthalmy, microphthalmy	0.7% (range 0.2–1.7%)	21,050	*Lithobates sylvaticus*	Eaton et al. 2004
Clinodactyly, polydactyly	0.13%	5,350	*Rana temporaria*	Borkin & Pikulik 1986
Polydactyly, polymely, ectrodactyly, clinodactyly, brachydactyly, syndactyly, hump-shaped tarsus (bony triangle?), iris lacking, anophthalmy	1.02%	44,000	*Bufo bufo*	Rostand 1949, 1951a,b
Ectromely, ectrodactyly, broken bones, nasal bones destroyed, blindness, edema (adults and juveniles)	0.97%	99,992	*Bufo bufo*	Wolf 1994

Moreover, many of the anomalies observed in Roßwag were rarely reported from natural populations: phocomely, swollen fingers, atrophied musculature on digits, supernumerary bone at angle of lower jaw, spiraculum misplaced, stiff limbs, rotation of limbs, arms covered underneath a fold of skin, stiff and truncated vertebral column, mandibular hypoplasia and yellow patches as a novel colouration (Henle et al. 2017a). Notably, the last six types were frequent in Roßwag. In conclusion, the Roßwag case is the most extreme one ever reported far beyond any normal natural occurrence.

6.1.2 Non-genetic factors

6.1.2.1 Traumatism. ***Evidence put forward for this hypothesis.*** Flindt (1985a) found an individual with head injuries and, without supporting evidence, explained all cases of ectromely and ectrodactyly in juveniles as injuries inflicted by stones. He attributed ectromely and ectrodactyly in tadpoles to cannibalism and failed predation attacks by dragonfly larvae, because he observed dragonfly larvae in one of the water-filled depressions in 1983.

Anomalies known to be caused by traumatism. Anomalies caused by traumatism are summarized in Table 9. While ectromely is common following experimental amputations (e.g., Girvan et al. 2002), Loeffler et al. (2001) and Lannoo (2008) considered it as unlikely for a predator to amputate the limb bud of a tadpole without causing fatal injury to the tadpole itself. Loeffler et al. (2001) assumed that non-lethal limb bud injuries are more likely to be due to intraspecific interactions and abrasions against plants or the substrate. In line with their idea, the only case known to us in which staged predation caused ectromely without other injuries is an adult *Notophthalmus viridescens* (n=14), whose front leg was amputated by a *Chelydra serpentina* (Hurlbert 1970). However, we do not know of any experimental study that demonstrated abrasion as the cause of limb injuries.

Ectromely occurred in a few staged predation experiments but results were often inconsistent with one another or only occurred under specific conditions. The leech *Erpobdella octoculata* can cause high incidences of ectromely in *Bufo bufo* tadpoles, and additionally tail injuries, but not in *Rana temporaria* tadpoles (Viertel & Veith 1992, Bohl 1997). It may also cause limb anomalies in the newt *Ichthyosaura alpestris* (Kneitz et al. unpublished report, fide Hachtel 2011). Whereas sticklebacks (*Gasterosteus aculeatus*) did not inflict ectromely in *B. bufo* tadpoles (Ballengée & Sessions 2009), experiments using *Anaxyrus boreas* resulted in ectromely (or ectrodactyly; it is not stated which one of these) but also high rates of tail damage (Bowerman et al. 2010). Likewise, mosquito fish (*Gambusia holbrooki*) injured tadpoles of *Lithobates capito* at very high rates (mainly the tail) but rarely those of *L. sphenocephalus* (Gregoire & Gunzburger 2008). Experimental exposures of *B. bufo* and *R. arvalis* tadpoles to adult newts (*Lissotriton helveticus, L. vulgaris*), diving beetles (*Dytiscus marginalis* and *D. lapponicus*), water scorpions (*Nepa cinerea*) and various other insects did not result in ectromely (Henrikson 1990, Manteifel & Reshetnikov 2002, Ballengée & Sessions 2009). For dytiscid beetles this

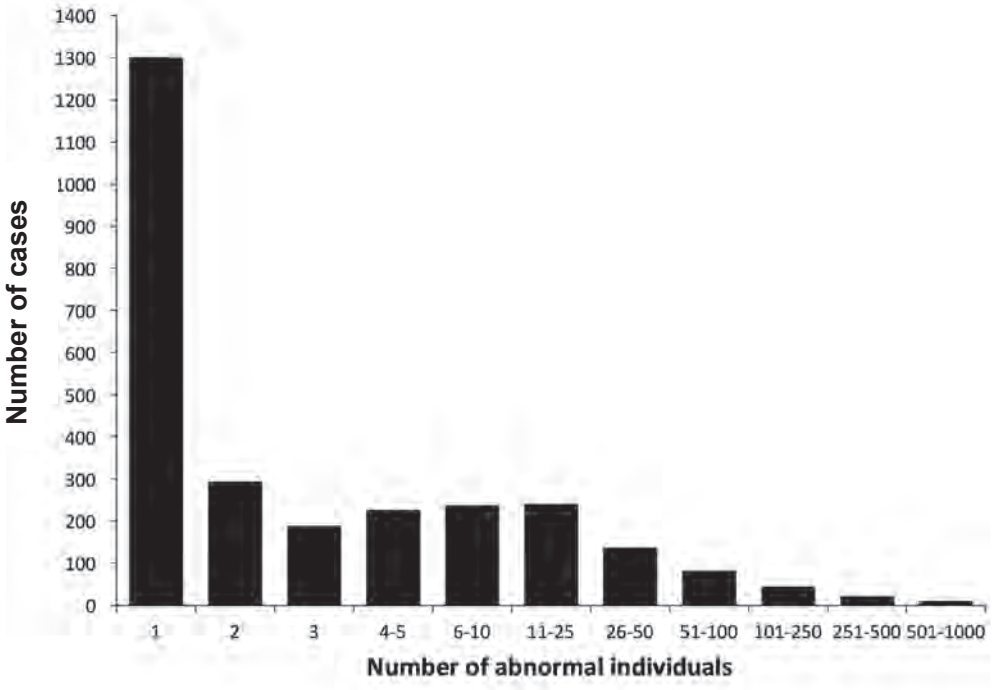


Fig. 21: Frequency distribution of the number of abnormal individuals reported from natural populations.

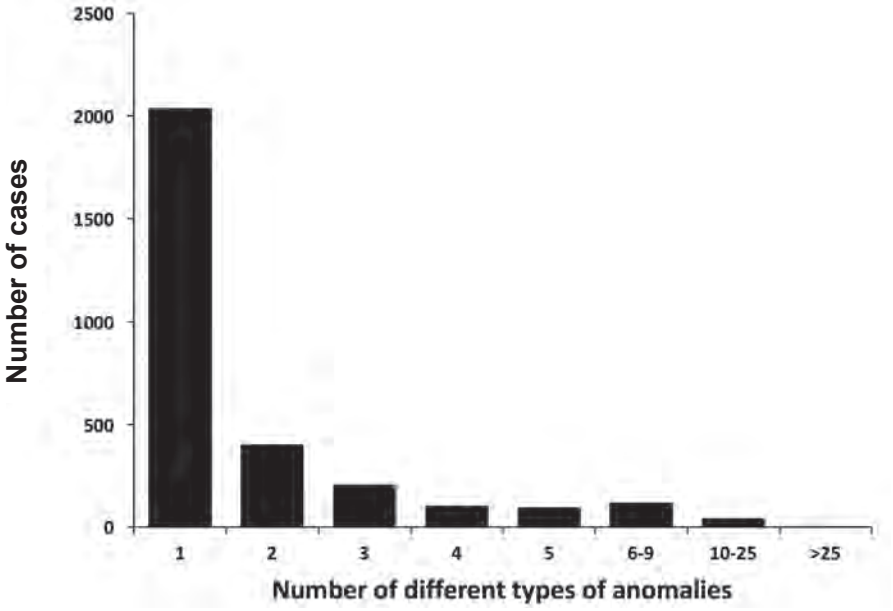


Fig. 22: Frequency distribution of the number of different types of anomalies reported from natural populations.

may be explained by them grapping tadpoles by the body. In contrast, crayfish (*Cambarus diogenes* and/or *Orconectes propinquus*) may capture tadpoles either by the body or by the hind legs (Formanowicz & Brodie 1982) but these authors did not mention whether crayfish attacks resulted in ectromely in larval amphibians. Attacks by crayfish may cause ectromely in adult frogs – although it remains unclear as to whether the observed attacked individual managed to escape predation (Niemiller & Miller 2005).

With two exceptions (Ballengée & Sessions 2009, Bowerman et al. 2010), ectromely or ectrodactyly was never reported from staged predation experiments with odonate nymphs, whereas tail injuries occurred frequently (e.g., Caldwell 1982, Morin 1985, Petranka 1989, Henrikson 1990, Richards & Bull 1990, Wilbur & Semlitsch 1990, Chovanec 1992, Parichy & Kaplan 1992, Thiesmeier 1992, Manteifel & Reshetnikov 2002, Casanovas & Úbeda 2006, Malkmus 2009). The exceptions were nymphs of *Aeshna mixta, Libellula depressa* and especially *Sympetrum* (probably either *S. striolatum* or *S. sanguineum*) that amputated parts of the limbs of Gosner (1960) stage 32–37 tadpoles of *B. bufo* without killing them (Ballengée & Sessions 2009)

and *Somatochlora albicincta* that caused missing and abnormal limbs in *Rana cascadae* (Bowerman et al. 2010). However, in all experiments with *B. bufo* tadpoles, tail injuries and other injuries were very common as well (no data is available for *R. cascadae*) – which unfortunately is not assessed in most studies on hotspots of amphibian anomalies. Moreover, in staged experiments, other species of the same dragonfly genera only inflicted tail injuries without amputating or mutilating limbs: *Sympetrum nigrifemur* in *Pelophylax perezi* tadpoles (Malkmus 2009), *A. cyanea* and *Aeshna* spp. in *B. bufo, Bombina bombina, Hyla arborea, R. dalmatina* and *R. temporaria* tadpoles (Hendrikson 1990, Chovanec 1992, Manteifel & Reshetnikov 2002, Van Buskirk et al. 2003). Along these lines, ectromely was extremely rare in Michigan and Connecticut across many ponds that contained high densities (> 1 individual / m^2) of larval *Sympetrum* (*S. internum, S. obstrusum, S. rubicundulum, S. semicinctum, S. vicinum*) (Skelly & Benard 2010).

Likewise, conspecific interactions result rarely in ectromely or ectrodactyly but injuries are common (e.g., *Ambystoma laterale*: Van Buskirk & Smith 1991; *Eurycea cirrigera*: Halliday & Tejedo 1995; *Salamandra salamandra*: Thiesmeier 1992; *Ommatotriton ophryticus*: Kosswig 1951, Raxworthy 1989; *Dendropsophus minutus*: Peixoto & dos Reis Gomes 1997; *Nannophrys ceylonensis*: Wickramasinghe et al. 2005). Exceptions are the carnivorous larval *Ambystoma talpoideum* and *A. macrodactylum,* which inflict significantly elevated rates of ectromely and ectrodactyly in staged interspecific interactions (Semlitsch & Reichling 1989, Johnson et al. 2006). In the former, high rates of tail losses also occurred – in the latter (Johnson et al. 2006) it was not assessed. Also, in captivity *Cryptobranchus bishopi* may frequently bite-off the legs of conspecifics during the breeding season (Ettling et al. 2013) and the same is the case in larval *Triturus pygmaeus* (Malkmus 2007); in the latter case, the limbs regenerated within 1.5 months. The only other case of apody caused by conspecifics known to us is a juvenile *Hynobius tsuensis* (Wallays 1998).

In conclusion, ectromely caused by predation attempts is more of an exception than the rule in staged predation experiments; i.e. it only occurs for specific combinations of predators, amphibian species and larval stages exposed and potentially other details of the interactions. For example, the opposing results reported for *Sympetrum* and *Aeshna* dragonfly nymphs and for sticklebacks (*Gasterosteus aculeatus*) (see above) suggest that congeneric predators and even the same predator species may have a different potential to amputate or mutilate limbs or that differences in details of the staging experiments may have caused opposing results. In any case, dominance of tail injuries and rarity or absence of ectromely and ectrodactyly in staged experiments with dragonfly larvae and with conspecifics are incompatible with the conclusion of the official inquiry (Landesregierung von Baden-Württemberg 1984, Flindt 1985a) that ectromely and ectrodactyly in the Roßwag population were caused by dragonfly larvae and conspecific attacks. Moreover, ectromely was also observed in the absence of dragonfly larvae in the quarry pond in 1980 (this study) and in the laboratory by Flindt (1985a).

Hyperregeneration following traumatism is often assumed as cause for polymely. While numerous studies (e.g., Tornier 1896, 1905, Lissitzky 1910, Puppé 1925, Brandt 1940, Kleinebeckel 1975, Michael & El Mekkawy 1977, Michael & Hassona 1982, Tsonis & Eguchi 1985, Stopper et al. 2002) demonstrated that experimental injuries, amputation or transplantation of amphibian legs or toes can result in hyperregeneration, i.e., the duplication of the affected part, duplications occur only if injuries cause physical rearrangement of cells or death of some but not all cells in the apical epidermal cap of the blastema (Stopper et al. 2002, Nye et al. 2003). Simple amputation usually does not lead to a rearrangement of cells and thus usually does not induce hyperregeneration (Lecamp 1935, Stopper et al. 2002). To our knowledge, staged predation experiments never resulted in polymely and we know of only two cases in which the partial loss of legs from attacks by conspecifics induced polymely: an *Alytes obstetricans* tadpole (Hellmich 1929, Wolterstorff 1941) and one *Ambystoma mexicanum* (Duméril 1867).

In numerous experiments involving amputations of limbs or parts thereof the torsion of legs was only mentioned once (2 tadpoles in *Hymenochirus boettgeri*) (Girvan et al. 2002). In contrast, traumatism often results in injuries of fins (e.g., Kosswig 1951; dragonfly studies discussed above), eyes (Kluge 1981, Ballengée & Sessions 2009), scars on the body or head in fighting males (e.g., Martins et al. 1998, Kwet & Di-Bernardo 1999) and on the head and body of tadpoles attacked by dragonfly nymphs (Ballengée & Sessions 2009), as well as schizodactyly and syndactyly (Semlitsch et al. 1981). These anomalies were, however, rare or absent in Roßwag.

Evaluation. It is difficult to imagine how stones could cause the amputation of fingers and legs without inflicting any other injuries to metamorphosed individuals. Toad tadpoles avoid injured conspecifics (demonstrated for *B. bufo*: Kulzer 1954, Pfeiffer 1966, Kiselva & Manteifel 1982) and cannibalism has never been observed in tadpoles of European toad species. Likewise, there is no evidence that dragonfly larvae may have caused ectromely or ectrodactyly in the tadpoles. Only few species of dragonflies are known to amputate limbs of anuran tadpoles without killing them. Significantly, tail injuries that are frequently inflicted when predators attack tadpoles did not occur in the Roßwag population of *B. viridis*. Moreover, no potential predators were detected in the pond in the Roßwag quarry in 1980 or in 1981 and in 1983 dragonfly larvae were only observed in one of five ponds (Flindt 1985a). Finally, ectromely also occurred in laboratory raised individuals in the absence of predators. In conclusion, traumatism is only a likely explanation for the one individual with head injuries or rare cases of partially missing legs.

6.1.2.2 Parasitism. ***Evidence put forward for this hypothesis.*** This cause was never suggested for the Roßwag population but widely discussed for recent incidences of mass anomalies in North American amphibians and therefore evaluated here as well.

Anomalies known to be caused by parasitism are summarized in Table 9. Anomalies strongly differ among the parasites involved and species differ strongly in their sensitivity. The largest range of anomalies is caused by the trematode *Ribeiroia ondatrae*. In most experimental infestations, polymely, polydactyly, taumely (bony triangles), femoral projections and cutaneous fusion were significantly more frequent than in controls (Johnson et al. 1999, 2001b, Stopper et al. 2002, Schotthoefer et al. 2003, Johnson & Hartson 2009). Ectromely, ectrodactyly, brachymely, rotation of limbs, clinodactyly, syndactyly, reduced or misshaped ilia and edema occasionally appeared in experiments. However, the rates of these anomalies were usually similar to controls; so the causal relationship is not certain. Two studies conducted on *Pseudacris regilla* are an exception (Johnson et al. 1999, Romansic et al. 2011), in which ectromely was frequent, presumably statistically significantly higher than in controls but the method of counting anomalies was not explained sufficiently for a rigorous formal test. In contrast, experimental infestations of limb bud stages (Gosner stages 27 and 28) of Sri Lankan *Polypedates crucifer* tadpoles with monostome-type cercariae (*Acanthostomum* species according to Jayawardena & Rajakaruna 2013) resulted in significantly elevated rates of ectromely, ectrodactyly, micromely, deformations of the vertebral column, translucent skin and partially or completely lacking pigmentation (Rajakaruna et al. 2008). While a few individuals also showed skin webbing and polydactyly, the difference to controls was not significant. The results were stage-specific. If pre-limb bud stage tadpoles (Gosner stages 25 and 26) tadpoles were exposed, edema, bent tails and ulcers of the skin occurred whereas metamorphosed individuals showed bent bodies and skin ulcers (Jayawardena et al. 2010a). Experiments with the genus *Alaria* failed to produce anomalies (Johnson et al. 1999).

The parasitic fly *Lucilia bufonivora* deposits its eggs in the nasal opening of anurans (mainly in *Bufo bufo*; other species are rarely attacked) and the larvae destroy the nasal openings, leading to the death of the parasitized toad (reviewed by Neumann & Meyer 1994 and Henle et al. 2017a). The North American species *L. illustris* is able to destroy the eyes (Anderson & Bennett 1963) and trombiculid mites of the genus *Hannemannia* may encyst in the skin of amphibians in North-, Central and South America (reviewed by Henle et al. 2017a). The only deviating colour patterns known to be caused by parasites are haematonoms, e.g. by the small ostracod parasite *Argulus foliaceus* that clinged to the digits in a breeding population of *Pelobates fuscus* (Schäperclaus 1954).

Evaluation. Although the trematode *Ribeiroia ondatrae* can cause several of the limb anomalies observed in Roßwag, the pattern differs considerably, with three typical anomalies caused by the trematode – taumely, femoral projections and cutaneous fusions – being absent and another typical one, polydactyly, being rare in Roßwag. Furthermore, stiff and truncated vertebral columns, phocomely, colour anomalies (except for haematonoms), tumours and giant larvae are not induced by *Ribeiroia ondatrae* or any other parasite. Moreover, *R. ondatrae* does not occur in Europe. All types of anomalies known to be caused by parasitism could together explain at most a small fraction of the types of anomalies observed in Roßwag (Tab. 9). Moreover, symptoms of parasitic infections are similar among individuals in contrast to the expression of anomalies in the Roßwag population of *B. viridis*. Finally, the development of anomalies in tadpoles from eggs transferred to the laboratory in the absence of parasites is not consistent with parasitic infections as an explanation of the anomalies.

6.1.2.3 Skin mucus and fish excrements (anomaly P factor). ***Evidence put forward for this hypothesis.*** Suggestions to consider anomaly P by Nöthiger (in lit.) was taken as a proof by the responsible administration that the anomalies of the Roßwag toads are caused by the same factor (see Henle et al. 2017b for details). Flindt (1985a) was vaguer but regarded the anomalies as sufficiently similar to justify such an explanation. He further justified this link by his assumption that the anomalies observed in Roßwag were due to hybridization and the fact that anomaly P affects species within the *Pelophylax esculentus* synklepton, which includes taxa of hybridogenetic origin. He faild to provide an explanation for the role of skin mucus and fish excrements in this context.

Anomalies known to be caused by the anomaly P factor. The anomaly P syndrome is characterised by mass polydactyly of the hind limbs often combined with massive edema (reviewed by Rostand 1971, Dubois 2014, 2017). Other morphological peculiarities include a good, although not always perfect, bilateral symmetry (i.e., the number of additional digits was always roughly the same on both sides, with sometimes one more toe or finger on one side, but never an excess of several digits on one side) and a first toe longer than the second and following. The most severely affected tadpoles may additionally show polymely, brachymely and in the most extreme cases phocomely, various types of bony excrescences and tumours, especially in the inguinal region, but these anomalies never occurred without severe polydactyly (Rostand 1955, 1958, 1971, Surlève-Bazeille et al. 1969b). No quantitative data are available for these anomalies. Exposure of *Pelophylax esculentus* and *Rana temporaria* tadpoles to skin mucus or feeding them with fish excrements from ponds in which anomaly P occurred caused significantly elevated rates of polydactyly (Rostand & Darré 1968, 1969) and ectrodactyly plus syndactyly, respectively (Surlève-Bazeille et al. 1969a).

Evaluation. The only similarity between anomaly P and the anomalies in Roßwag are the very high percentage of animals affected. Polydactyly was rare in the Roßwag population but is invariably present in anomaly P. Most of the anomalies observed in Roßwag have never been detected in populations affected by anomaly P. Moreover, anomaly P is known only in green frogs of the genus *Pelophylax* and only in large ponds inhabited by tenches (*Tinca tinca*) and eels (*Anguilla anguilla*) (Dubois 2014, 2017). Fish therefore seem to be vectors of the causal factor of anomaly P (whatever it is) but it is not known whether this factor can also be active in the absence of fish. In any case, fish were absent from the Roßwag quarry.

6.1.2.4 Viral and other diseases. ***Evidence put forward for this hypothesis.*** Schmid (1981) and Flindt (1985a) suggested a viral cause for the Roßwag anomalies based on the argument that the anomalies observed in Roßwag were supposedly similar to those observed in anomaly P and that a virus is probably the cause of anomaly P.

Anomalies known to be caused by viral and other diseases are summarized in Table 9. Many pathogens cause diseases, with hemorrhage, skin lesions and edema being commonly observed non-specific clinical manifestations (Densmore & Green 2007, Hemingway et al. 2009). For example, viral (herpes virus, iridovirus and tadpole edema virus), bacterial (e.g., *Aeromonas hydrophila, Flavobacterium indologenes, Chlamydophila* and other gram-negative aeromonads, pseudomonads and enterobacteria) and probably fungal (*Basidiobolus ranarum)* infections may lead to edema (Emerson & Norris 1905, Wolf et al. 1968, Newcomer et al. 1982, Olson et al. 1992, Taylor et al. 1999, Crawshaw 2000, Densmore & Green 2007, Gray et al. 2009), skin lesions and the loss of eyes (Polder 1973, Rose 1976, Bennati et al. 1994, Taylor et al. 1999, Densmore & Green 2007). A reddish colour due to hemorrhage is a symptom called "red leg" disease that may be caused by *A. hydrophila* (Emerson & Norris 1905, Hunsaker & Potter 1960, Nyman 1986, Bradford 1991, Carey 1993), other gram-negative aeromonads, pseudomonads and enterobacteria (Caselitz 1966, Densmore & Green 2007) and *Ranavirus* (Gray et al. 2009) and is also found in individuals affected by *Batrachochytrium dendrobatidis* (e.g., Brodman & Briggler 2008, Venesky & Brem 2008). Often, but not always, bloatedness or edema are noted as well (e.g., Emerson & Norris 1905, Densmore & Green 2007, Gray et al. 2009). Note, however, that bloatedness and hemorrhage may also be caused by supersaturation of the water with gases and, according to Colt et al. (1984), may be the primary etiology in many observed cases, as supersaturation commonly occurs in lakes in the spring (Harvey 1967).

Partial or complete depigmentation has been observed in infected individuals of *Ambystoma mexicanum* (Brunst 1968), *Pipa carvalhoi* (Leja 1981), *Xenopus laevis* (Newcomer et al. 1982), *Agalychnis callidryas* (Wright & Whitaker 2001) and tadpoles of *Ptychohyla hypomykter* (Mendelson et al. 2004). Symptoms of infection with *B. dendrobatidis* are malformations of the mouthparts in tadpoles, especially depigmentation of keratinized parts (e.g., Fellers et al. 2001, Lips et al. 2004, Rachowicz & Vredenburg 2004), discolouration (usually brown) and in some species reddening of the skin, usually of the ventral body and toes and feet (reviewed by Carey et al. 2003) and sometimes grossly visible lesions (reviewed by Carey et al. 2003) or black flakes of unshed skin (Cummer et al. 2005). However, depigmented mouthparts are not diagnostic for *B. dendrobatidis* (Rachowicz 2002); likewise, infected tadpoles may appear normal (Blaustein et al. 2005, Padgett-Flohr & Goble 2007). An abnormal deposition of melanin may be an inflammatory reaction to skin infection with helminths (Mutschmann & Manzke 2006).

Wisniewski (1984) noticed atrophy of the face, jaw and legs in the urodeles *Cynops ensicauda popei* and *Paramesotriton chinensis* after infection by *Saprolegnia.* Correlational evidence also exists that poxvirus, *B. dendrobatidis* and bacterial infection with *Aeromonas* may cause ectromely and oligodactyly due to necrosis, ulceration, as well as a reddening of skin (Frye 1985, Cunningham et al. 1993, Brodman & Briggler 2008).

Tumours are further well-known pathologies of viral infections in amphibians (reviewed by Balls & Ruben 1964, Mizell 1969, Balls et al. 1978, Asashima et al. 1987) and other organisms (Gross 1961). Experimental infections with fungi of the genus *Mycobacterium* and other unidentified fungi may also cause tumours in amphibians (Dhaliwal & Griffiths 1963, Inoue & Singer 1970). Green (2001) reported a swelling on the caudal half of the body and the proximal half of the tail in *Notophthalmus viridescens* that was associated with an *Ichthyophonus*-like fungal infection. Infection with *Saprolegnia* may also result in ulcerated skin and erythema (Densmore & Green 2007).

Evaluation. All known diseases cause a narrow range of anomalies. Most types of anomalies observed in Roßwag have never been seen in any infected amphibian (compare Tables 1 and 9). Furthermore, no evidence for any disease is available for the Roßwag population and infected animals always show the same symptoms whereas the Roßwag individuals differed strikingly in their anomalies. Thus disease can be excluded as a potential cause.

6.1.2.5 Temperature extremes and shocks. ***Evidence put forward for this hypothesis.*** This was never suggested as a cause for the Roßwag population but for a few other natural occurrences of mass anomalies, although never proven for any of them (Woitkewitsch 1961, Worthington 1974, Harkey & Semlitsch 1988).

Anomalies known to be caused by temperature extremes and temperature shocks are summarized in

Table 9. Most studies have been conducted on embryos (reviewed by Hertwig 1894, 1898, Lillie & Knowlton 1897, Hoadley 1938). Under permanent high temperature, a typical haploid syndrome manifests itself and almost all individuals die in early embryonic stages. Temperature shock led to triploids in hybrid eggs of *Bufotes viridis* × *Bufo bufo* and *B. viridis* × *Epidalea calamita,* most of which died as embryos although a few developed normlly or showed edema and giant sizes (Hertwig & Weiss 1955). Temperature also acts synergistically with overripeness in early embryonic anomalies (Briggs 1941, Witschi 1952, Rugh 1965, Mikamo 1968, Mikamo & Hamaguchi 1973).

Temperature effects on larvae have been less well studied. At a constantly low temperature (6–9°C), larval *Ambystoma mexicanum* reabsorbed digits, resulting in brachydactyly or oligodactyly (Blount 1950). Low temperature also resulted in a slightly albeit significantly elevated (1.8%) rate of abnormal limbs in *Pseudacris ornata* (Harkey & Semlitsch 1988). Under an elevated temperature (18°C), a high proportion of *Euproctus asper* larvae developed bent tails (Clergue-Gazeau 1971). A constantly high temperature caused a significantly elevated frequency of ectrodactyly in the developing limbs of *B. b. formosus.* Clinodactyly and edema was occasionally observed, but at low frequency and only in a few experiments (Muto 1969a,b, 1970, 1971). Likewise, in *Pseudacris ornata* the frequency of limb anomalies was significantly increased (1.3%) (Harkey & Semlitsch 1988). Under a constantly high temperature, regenerating limbs of *A. mexicanum* showed a fusion of tarsal bones and occasionally syndactyly (Schmalhausen 1925). In *Pleurodeles waltl,* temperature acted epigenetically on an inherited anomaly causing ectromely, ectrodactyly, twisted feet and knee anteroversion (Dournon 1983, Dournon et al. 1998). At high temperature (30°C) and when the treatment started early in development, the frequency, severity and variability of anomalies were greater than at control temperatures (20°C) (Dournon 1983, Dournon et al. 1998). Epigenetic effects of temperature are also known for some embryonic anomalies of the same species (Conter & Jaylet 1974, Fernandez & Beetschen 1975).

Witschi (1920, 1925) reported that *Rana temporaria* raised at a constantly high temperature (27–28°C) from overripe eggs developed polymely. An epigenetic effect is likely because most polymelous individuals were derived from a single female and never appeared in batches raised at lower temperatures (19–20°C).

Ensinck (1978) suggested that constantly high temperatures may cause stiffness of legs in *Gastrotheca marsupiata* but confounding effects cannot be entirely excluded.

Evaluation. There is no possibility that the Roßwag population received artificial temperature shocks. The large volume of the pond where the largest number of abnormal individuals was found excludes the possibility of exposure to exceptionally high temperatures during development. It is not known whether the temperature of the small water bodies in which *B. viridis* spawned after 1981 was exceptionally high. However, the years when anomalies were observed were not exceptional and anomalies also appeared in individuals transferred to the laboratory. Although it is highly unlikely, it cannot be excluded with certainty that high temperature contributed to the few individuals with ectrodactyly, syndactyly or clinodactyly found by Flindt (1985a). In any case the vast majority of anomalies detected in Roßwag cannot be explained by temperature extremes (see Tab. 9).

6.1.2.6 Electro smog and strong magnetic fields. *Evidence put forward for this hypothesis.* This cause was once raised in a non-public discussion of the Roßwag toads. It was also suggested by a layperson for the mass anomalies observed in North America (Souder 2002) and there is a great deal of controversy over the potential health hazards of electromagnetic radiation for humans (Coleman & Beral 1988, Ross 1988). Anomalies in the strength of the magnetic field have never been suggested as a cause for amphibian anomalies.

Anomalies known to be caused by electromagnetic fields and abnormal magnetic fields are summarized in Table 9. The effect of the electromagnetic field on amphibian development has, to our knowledge, never been studied. We only know of two studies on the regeneration of limbs in newts. Landesman & Douglas (1990) observed a significantly ($\chi^2 = 6.47$; $\alpha < 0.01$) elevated rate, compared to controls, of abnormal limb regeneration in *Notophthalmus viridescens* when amputated individuals were exposed for 30 days to a pulsed electromagnetic field. Anomalies reported are fused carpal bones, a lower or larger number of carpal bones than normal variation, oligodactyly, ectromely and malformed long bones. In contrast, Smith & Pilla (1981) did not obtain elevated rates of anomalies using similar waveforms (for 21 days) but regeneration was completely inhibited when they used other waveforms.

The effects of abnormally strong magnetic fields in amphibian devleopment was studied by Levengood (1969). When de-jellied eggs of *Ambystoma maculatum* or *Lithobates sylvaticus* were exposed to strong magnetic fields (6300–17700 Gauss), hatching larvae showed edema, scoliosis, microcephaly and stunted growth at rates higher than controls. Tadpoles of *L. sylvaticus* that reached climax stages of metamorphosis showed various (non-specified) leg anomalies, edema, two cases of polmely, one case of one arm not emergig from the branchial cavity, one case of unilateral anophthalmy and one case of neoteny. The percentage of animals affected was significantly higher for both levels of the strength of the magnetic field.

Evaluation. The lack of experimental studies makes evaluation difficult. The few studies only show a small range of the types of anomalies observed in Roßwag. In any case, no potential sources of pulsed electromagnetic fields or for a severely elevated strength of the magnetic field existed in the quarry. Thus, it is an unlikely cause

for anomalies of *B. viridis* in Roßwag although it merrits studies on its teratogenic and mutagenic potential in amphibian larvae.

6.1.2.7 Sound pressure and shock waves. ***Evidence put forward for this hypothesis.*** Launched by the district president, newspapers reported that citizens with sound knowledge and interest in the Roßwag case believe that the anomalies of the Roßwag toads were due to detonation shock waves from quarry operations (Anonymous 1980a,b).

Anomalies known to be caused by sound pressure and shock waves are summarized in Table 9**.** Only a few studies are available on the teratogenic effects of sound pressure in amphibians. Bombardement with ultrasound (0.7 WA/cm², 1000 kHz, 5–15 min) caused duplication of body, bicephaly, microcephaly, acephaly, anophthalmy, torsion of the body, torsion of the tail and edema in embryos of *Ichthyosaura alpestris* and *Lissotriton helveticus* (Bonhomme & Pourhardi 1957, Bonhomme et al. 1960, Pourhardi et al. 1968). The majority of individuals died at the neurula stage at the latest and no anomalies developed when post-gastrula stages were treated. We do not know of any study that experimentally exposed late larval stages to ultrasound or to explosion shock waves.

Evaluation. If detonations were responsible for the anomalies, we would expect anomalies to occur in all active quarries since detonations are part of regular operations of active quarries. More than 100 quarries have been surveyed in Baden-Württemberg by herpetologists without reporting elevated rates of anomalies (Laufer & Pieh 2007). Furthermore, the eggs transferred to the laboratory were not exposed to any sound pressure waves but developed anomalies and no mechanism is known by which sound pressure waves can induce mutations (though this deserves study). Moreover, sound pressure is ineffective at late embryonic stages and lethal at earlier stages. Therefore, detonations cannot have contributed to the anomalies observed in Roßwag.

6.1.2.8 Overripeness of eggs. ***Evidence put forward for this hypothesis.*** Flindt (1985a) argued that the malformations of the front legs are astonishingly similar to the symptoms that develop when raising overripe eggs. Although he raised the open question as to why females would have produced overripe eggs only in the Roßwag population, he concluded that this could have been due to warm water in the quarry (which is unlikely: see section on temperature extremes above).

Anomalies known to be caused by overripeness of eggs are summarized in Table 9. Overripeness of eggs leads to a range of anomalies in cleaving eggs and embryos resulting in the death of most individuals at early embryonic stages. Anomalies include irregular cleavage, incomplete blastopore closure with a persistent yolk-plug, exogastrula, incomplete gastrula, abnormal neurula, tumour-like growth, spina bifida, microcephaly, acephaly, microphthalmy and anophthalmy. The frequency of anomalies increases with the time of retention and temperature (Briggs 1941, Witschi 1952, Rugh 1965, Mikamo 1968, Mikamo & Hamaguchi 1973). O_2-deficiency and CO_2 excess (Witschi 1952) and degeneration of egg cytoplasm as well as the disturbance of chromosome distribution during meiosis (Zimmerman & Rugh 1941, Witschi 1971) have all been suggested as mechanisms leading to the observed anomalies. Witschi & Chang (1954) speculated that mutations could also be caused by overripeness of eggs but did not provide an explanation for this hypothesized effect.

The majority of the few individuals surviving beyond embryonic stages are normal (Briggs 1941, Rostand 1951c). Anomalies reported for very early tadpole stages are bent bodies and microcephaly in *Lithobates pipiens* (Briggs 1941). Witschi (1920, 1922, 1925, 1952) obtained *R. temporaria* with polymely and individuals with polydactyly of duplicated hands that developed from overripe eggs. Witschi (1952) counted the total as seven polymelous individuals and two polydactylous individuals. He also made reference to ectromely but did not provide any figures. Polymely only appeared in batches raised at high temperatures (27–28°C) (Witschi 1925) but not in batches raised at 10–20°C and all the affected individuals were the offspring of a single female. Data on controls (non-overripe eggs) were not provided. Thus, skeletal anomalies in individuals developing from overripe eggs are rare, contrary to the statement by Flindt (1985a). Later experiments (e.g., Rostand 1951c) were unable to induce any skeletal anomalies by overripeness of eggs in *R. temporaria*. Therefore, it is very likely that in the study of Witschi (1925) temperature exerted an epigenetic effect on a recessive mutation borne by the parental female.

Evaluation. All results contradict the hypothesis of overripeness as a cause of anomalies in the Roßwag population: the low number of surviving embryos, the absence of anomalies, such as giant tadpoles or colour anomalies, the rarity of skeletal anomalies, the inability to reproduce early reports of polymely as a consequence of overripeness and the lack of an explanation as to why females of the Roßwag population and only them, should retain eggs so long that overripeness comes into play.

6.1.2.9 Chemicals. ***Evidence put forward for this hypothesis.*** Anonymous (1981a) and Klein (1981) suggested that chemicals sprayed on adjacent vineyards or illegally dumped in the quarry caused the anomalies but did not provide any supporting evidence. The chemical teratogenic literature was apparently unknown to Flindt (1985a) and he therefore did not further discuss chemicals as a potential cause of the anomalies observed in Roßwag.

Anomalies known to be caused by chemicals. There is a considerable body of literature on the teratogenicity of a wide range of chemicals for developing amphibians. In addition, partial reviews of anomalies caused by a range

of different types of chemicals are available: acidification (Pierce 1985, 1993, Ferraro & Burgin 1993), alkalinity, salinity and metals (Ferraro & Burgin 1993), LiCl (Hall 1942, Pasteels 1945, Bustuoabad et al. 1977, Kao & Elinson 1998), an anaerobic environment (Büchner 1948, 1955, Rübsaamen 1955, Mangold & Peters 1956), endocrine disruptors (Hayes 2000) and various pesticides (Power et al. 1989, Cowman & Mazanti 2000, Johnson et al. 2010).

Chemicals can cause a range of different types of anomalies (Tables 9 & 11). It was only for three types of the anomalies considered that we could not find any report – a stiff and truncated vertebrate column, microcephaly in larval or metamorphosed individuals and a misplaced spiraculum; these are rare anomalies. In addition, only retinoids and vitamin A applied under specific conditions can induce polymely or polydactyly unless the limbs are simultaneously manipulated surgically (amputation or insertion of crystals – see Henle et al. 2017a for discussion). Furthermore, apart from darkening, depigmentation, hemorrhage and the use of dyes, few colour anomalies are known to be induced by chemicals (see Henle et al. 2017a).

Evaluation. *Salts, metals and pH.* The teratogenicity of metals depends on the concentration and differs among organisms and metals (Ferraro & Burgin 1993, Power et al. 1989, Peskova 2000). Of the chemicals analysed in the water samples from Roßwag (Tables 3 & 4), only iron was measured at a concentration that could potentially cause anomalies in embryos or tadpoles of anurans (Miller & Landesmann 1978, Dawson et al. 1988, Haidacher & Fachbach 1991, Nechitailo & Peskova 1999, Peskova 2000). When eggs of *Ommatotriton vittatus* were exposed to 0.5 mg/l $FeSO_4$ – this is below the value measured for iron by the Chemical Laboratory of the City of Pforzheim (Tab. 3), but above the value measured by the Landesanstalt für Umweltschutz (Tab. 4) – they developed a curvature of the axial skeleton (Nechitailo & Peskova 1999, Peskova 2000). Copper sulphate was sprayed by helicopters in vineyards adjacent to the Roßwag quarry (Buck, pers. comm.). Known teratogenic effects of copper in tadpoles are curvature of the axial skeleton in *O. vittatus* (at 0.5 mg/l $CuSO_4$: Nechitailo & Peskova 1999, Peskova 2000) and ectromely in *Xenops laevis* (significant effects at ≥ 0.25 mg/l copper: Fort & Stover 1997). Both values are above those measured for copper in the water samples from Roßwag (Tables 3 & 4) but below that measured for sulphate (Tab. 4). In any case, *B. viridis* is exceptionally tolerant to high salt concentration (Zavadil & Prikryl 2003). In conclusion, it is unlikely that pH, salts or metals contributed to the observed anomalies.

Nutritional deficiencies. An absence of essential micronutrients has been invoked as a contributor to the mass anomalies of frogs in Minnesota, USA (Fort et al. 1999, Souder 2002). Nutritional deficiencies (vitamin C, calcium, possibly vitamin D and other essential nutrients) may cause rachitis, growth retardation, torsion of the tail and body, changes in head proportions and bleaching in urodelans (Klatt 1927, Krefft 1938), stiff legs, torsion of the legs (Klatt 1927, Krefft 1938, Reinhardt 1939, Vogt 1939, Bruce & Parkes 1950, Marshall et al. 1980, Leibovitz et al. 1982, Pollack & Liebig 1989, Martínez et al. 1992), hypertrophy of the lower jaw and anomalies of the urostyle, pelvic girdle and the spine (Bruce & Parkes 1950) and phocomely and ectromely in *Ambystoma mexicanum* and *Pleurodeles waltl* but not in *Triturus* s.l. spp. (Reinhardt 1939, Vogt 1939). In *Ambystoma* sp., dietary deficits (probably arginine) caused anemia and short and curled gills (Patch 1936). Edema is also often regarded as a symptom of nutritional deficiencies (Grimm 1953, Reichenbach-Klinke 1961, Jara 1963) but few experimental analyses have shown this (e.g., Reinhardt 1939). In anuran husbandry, it has further been suggested that ectromelous offspring (observed in dendrobatids, discoglossids and hylids) are due to deficiencies in vitamins and minerals of the parents or the offspring (e.g., Schmidt 1985, Glaw 1987, Krintler 1988) but this hypothesis has not yet been tested experimentally. Poor nutrition led to the fusion of tarsal bones and occasionally ectrodactyly in regenerating limbs of *A. mexicanum* (Schmalhausen 1925). The results of the analysis of the water samples taken from the Roßwag quarry (Tables 3 & 4) are inconsistent with mineral deficiencies and the large size of many tadpoles and most toadlets further contradicts nutritional deficiencies as a potential cause. Moreover, the patterns of anomalies resulting from nutritional deficiencies differ in most respects from the pattern observed in Roßwag.

Fertilizer. Although the water samples do not provide any evidence for fertilizer contamination (Tables 3 & 4), fertilizer was probably used in fields close to the Roßwag quarry. Wolf (1994) observed inflamed reddish bellies in adult *Bufo bufo* that migrated across fields that had been treated the year before with *N*-fertilizers. Nitrate and nitrite caused edema and bent tails in tadpoles of some but not all species at lethal concentrations (Marco et al. 1999). Hecnar (1995) detected eye and head anomalies, complete depigmentation and digestive tract deformities in tadpoles treated with ammonium nitrate. These types of anomalies clearly differ from those observed in Roßwag.

Pesticides. The water samples and tissue analyses did not reveal any indication for pesticide contamination; however, pomuran (captan + mancozeb) and rovral (= iprodion; [3-(3,5-dichlorophenyl)-2,4-dioxoimidazolidinyl]-N-(methylethyl)carboxamide) were sprayed by helicopter to vineyards bordering the quarry and thus were probably drifted into the water bodies of the Roßwag quarry as well. Mancozeb can cause edema, abnormal notochord, bent tail and gill displacement in embryos (Ghate 1985, Harris et al. 1998) but we do not know any experiment involving tadpoles. We found no studies testing the teratogenicity of the other substances for amphibians. Captan is usually assumed to be weakly mutagenic in vitro but requires

bioactivation and is not mutagenic in vivo (Osaba et al. 2002, Rahden-Staron 2002). For mancozeb, assessments of biomarkers for genotoxicity in pesticide workers have provided positive and negative results (reviewed by Bull et al. 2006). For iprodion, we found no information regarding mutagenicity. Captan and mancozeb are classified as carcinogens and iprodion is classified as a probable carcinogen (Pastor et al. 2003).

Anomalies reported for most other pesticides are mainly of a general teratogenic type, such as exogastrula, abnormal neurula, anophthalmy, microcephaly, bent bodies or tails and edema in embryos and bent bodies and tails, growth retardation, mouth anomalies and edema in tadpoles (e.g., Cooke 1981, Power et al. 1989). In addition, many pesticides can induce pale tadpoles (Henle et al. 2017a). Only few pesticides are known to cause darkening: diamethoate and benzene hexachloride (Pandey & Tomar 1985), dichlorvos (Tomar & Pandey 1988) and lindane (Marchal-Ségault & Ramade 1981). No other pigmentary changes resulting from exposure to pesticides are known. Defenuron is the only pesticide known to us that results in giant tadpoles (Paulov 1977). Several pesticides cause various skeletal anomalies (Tab. 11) and, according to Levy (1958), semicarbazide may induce tumours but this needs confirmation. In any case, at environmentally relevant concentrations, agrochemicals (no differentiation of data among glyphosate, dimethoate, chlorpyrifos and propanil) may cause skin ulcers in tadpoles (Jayawardena et al. 2010b).

In summary, the fungicides sprayed on neighbouring vineyards may potentially have contributed to the tumours observed in the Roßwag toads. However, pesticides are not known to cause any of several conspicuous anomalies, notably polymely, stiff and truncated vertebral columns, yellow spots and abnormal patterns. Also, if pesticides contributed significantly to the anomalies, we would expect higher rates and more severe anomalies in the years 1981–1984 compared to 1980 because the water bodies were much smaller, which in turn should lead to higher and thus more teratogenic, concentrations of pesticides in the breeding sites. This expectation is opposite to the observed frequency and severity of the anomalies. Moreover, the pesticides sprayed on neighbouring vineyards were in common use and one would expect many other populations with anomalies unless their application was unusually careless on vineyards in Roßwag. Finally, treatments with specific pesticides result in similar anomalies in all affected individuals and not in a hige range of individually differing anomalies, as observed in Roßwag.

Industrial waste. Crankcase oil and crude oil can cause edema and a deformed axial skeleton in amphibian larvae (Pyastolova & Danilova 1986, Mahaney 1994), coal combustion waste result in bent tails and missing labial teeth rows (Rowe et al. 1998, Hopkins et al. 2000), PCBs can lead to lordosis, scoliosis and edema in embryos of toads (Birge et al. 1978) and stiff legs in metamorphosed individuals (Qin et al. 2005), whereas tar and benzpyrene can cause high rates of tumours (Koch et al. 1939). These are but a small fraction of the different types of anomalies observed in Roßwag and there were no signs of dumping industrial waste in the quarry.

Endocrine disruptors and endocrine substances (hormones). A range of chemicals, including various pesticides and/or their metabolits, such as DDT (Osborn et al. 1981), maneb 80 (Fort et al. 1999) and methoprene (Ankley et al. 1998, La Clair et al. 1998), as well as medical drugs (e.g. thalidomide: Bazzoli et al. 1977, dumpert & Zietz 1984) may interfere with the endocrine pathways of developing organisms and cause various anomalies in amphibians. Anomalies caused by pesticides are reviewed above. For a review of the effects of endocrine disruptors on amphibian development and their environmental significance see the review of Hayes (2000). The insecticide methoprene, which can mimic natural retinoid acids (Harmon et al. 1995) and chemicals inferring with the thyroid axis have received particular attention and are briefly reviewed below.

Endocrine substances – retinoids. In North America concerns have been raised that mass occurrence of leg malformations may be attributable to the disruption

Table 11: Skeletal anomalies caused by pesticides.

Substance	Species	Effects	Author(s)
Acetyl hydrazine	*Xenopus laevis*	Ectromely and torsion of legs	Fort & Stover 1997
DDT, DDE	*Rana temporaria, Bufo bufo*	Stiff legs, abnormal snouts, mandibular hypoplasia (together with loss of keratinized beaks, torsion of body and tail and transient colour change in tadpoles)	Cooke 1971, 1972, 1973, Osborn et al. 1981
Diquat	*Rana temporaria*	Mandibular hypoplasia	Gelnarová 1987a,b
Endosulfan	*Rana temporaria*	Stiff legs	Gelnarová 1987a,b
Malaoxon	*Xenopus laevis*	Ectromely, bent vertebral column, abnormal acetabular joint	Snawder & Chambers 1989
Maneb	*Xenopus laevis*	Ectromely, torsion of legs	Fort et al. 1999
Methoprene	*Lithobates pipiens*	Ectrodactyly, ectromely and severe axial distortions (at concentrations causing 100% mortality in the laboratory)	Ankley et al. 1998
S-methoprene	*Lithobates sphenocephalus*	Ectromely (in addition anophthalmy, amelanism at non-significant rates) under field conditions	Sparling 2000
Thiosemicarbazide	*Xenopus laevis, Lithobates sylvaticus*	Clinodactyly, abnormal limb articulations (dislocation of joints), bent long bones	Newman & Dumont 1983, Riley & Weil 1986

of retinoid signalling pathways (Gardiner & Hoppe 1999). Retinoids are metabolic derivatives of vitamin A that act as signalling molecules and regulate many processes critical to early embryonic development (Sucov & Evans 1995), in the initiation of pattern formation of limbs and in limb regeneration (Lee et al. 2004). Alterations in retinoid levels, whether excess or deficiency, resulted in developmental anomalies in nearly all of the vertebrate species studied (Degitz et al. 2000, Lee et al. 2004). The teratogenic effects depend on the concentration, the duration of treatment and the developmental stage at treatment. Effects also differ among normal and regenerating limbs (Maden 1983, Niazi 1996) and according to whether tadpoles are raised in retinoid acid solutions or fed retinoid acid.

In regenerating limbs, low doses of retinoids and synthetic activators of retinoid acid receptors cause ectrodactyly, intermediate doses induce polymely and high doses suppress regeneration (Saxena & Niazi 1977, Maden 1983, Thoms & Stocum 1984, Scadding & Maden 1986b). In developing limbs, it suppresses differentiation causing shortened skeletal elements, oligodactyly or ectromely as well as triangular bony excrescences (Scadding & Maden 1986a, Degitz et al. 2000, Gardiner et al. 2003). In contrast to other studies, Bruschelli & Rosi (1971) and Gardiner et al. (2003) also obtained polymely in developing limbs. In rodents polydactyly has also been observed following a reduction in the dose of Hox gene products, which are involved in the signalling pathways of retinoids (Lee et al. 2004). The disparity regarding polymely may be explained by the high stage specificity of the teratogenic effects (Niazi 1996). In the experiment of Gardiner et al. (2003), polymely only occurred when stage 51 (after Nieuwkoop & Faber 1994) tadpoles of *X. laevis* were treated. Limb elements are only sensitive at the beginning of their differentiation and this sensitivity seems to progress sequentially and proximodistally; therefore phocomely can only be produced if retinoid treatment is restricted to the short period of the initial differentiation of shanks or thighs (Niazi 1996).

Increased circulating retinoid acid also caused significantly elevated rates of bony triangles and ectrodactyly but it additionally significantly increased syndactyly, anophthalmy and microphthalmy in *Xenopus laevis* (Alsop et al. 2004). It also caused polyphalangy and duplicated digits (polydactyly), albeit at non-significant levels. Effects further depended on the stages that were exposed, with stages 46 (after Nieuwkoop & Faber 1994) having been more sensitive than later stages for eye anomalies; limbs tended to be more affected when stage 49 or 52 were fed retinoid acid compared to stage 46. Various agrochemicals (Mann et al. 2009, Paganelli et al. 2010), polychlorinated toxicants, estrogen, pharmaceutica and inflammation may increase internal cycling levels of retionoid acids and one study showed that compounds in pulp mill effluents that probably originated from the wood used bound to retinoid acid receptors (Alsop et al. 2004, Lee et al. 2004).

Embryo lethality occurs at much lower concentration than those necessary to cause reductions and deletions of the hind limb (Degitz et al. 2000). This suggests that if retinoid mimics in the environment are causing hindlimb malformations, this would only occur under scenarios of pulsed-chemical exposure or when water bodies became contaminated at later larval stages of development. In any case, neither potential sources of retinoids or retinoid mimics are known from the Roßwag quarry and, importantly, the spectrum of anomalies differ, with bony triangles being absent in Roßwag and neither colour anomalies, nor edema or giant tadpoles being induced by retinoids. Thus, retinoids are unlikely to have been a contributor to the anomalies in the Roßwag *B. viridis* population.

Endocrine disruptors – thyroid axis. In the laboratory, antithyroidal substances are among the most potent teratogenic chemicals. Feeding antithyroidal substances (thymus oil, various plant seed oils or extracts of the thyroidea) to tadpoles of *X. laevis* resulted in 8–13 anomalies (phocomely, oligodactyly, clinodactyly, reduction or absence of bones of the pelvic girdle, torsion of the legs, stiff legs, front legs covered by a skin fold, mandibular hypoplasia, edema, giant tadpoles, tail resorption incomplete, exophthalmy, degeneration of keratinized mouthparts and papillae, dark pigmentation) (Romeis 1918, Pohland 1962, Wurmbach et al. 1964, Wink & Wurmbach 1967). Thymus oil suppresses cell division but it is not yet clear whether it acts directly on the cell or indirectly via the thyroid gland. Thiourea causes similar changes to the basophilic cells of the thyroid as thyroidectomy (Gasche 1946) and estradiol (Nishimura et al. 1997), which blocks the conversion of thyroxine into the more potent triiodothyronine (Maclatchy et al. 1986). Estradiol and the pesticide defenuron (Paulov 1977) cause giant tadpoles with estradioal sometimes also causing goitre. Raising tadpoles in solutions of estradiol benzoate may cause high frequencies of ectrodactyly and clinodactyly and testosterone proprionate may cause syndactyly and edema (Collenot 1965). Thus, contamination by antithyroidal substances or substances that have steroidal effects could have contributed to the anomalies observed in Roßwag. However, no potential source for any contamination with such substances is known for the Roßwag quarry. In any case, they cannot explain all conspicuous and frequent anomalies, especially polymely, ectromely, yellow spots, albinism and tumours. Moreover, these substances do not accelerate growth in some tadpoles but retard it in others and individuals exposed to similar concentrations show similar symptoms.

Mutagenic chemicals. To our knowledge, all studies undertaken to test the mutagenicity of chemicals were made on the cellular level (e.g., Siboulet et al. 1984, Fernandez et al. 1993) except for studies by Armstrong and colleagues (e.g., Armstrong & Gillespie 1980, Hart & Armstrong 1984), who immersed adult males of *A. mexicanum* and *X. laevis* in ethyl methanesulfonate or ethyl nitrosurea and examined anomalies in the offspring. They focused on

chromosomal anomalies and morphological anomalies of embryos. Without providing details they listed edema, torsion of body, microcephaly, microphthalmy, vertebral truncation and reduced pigmentation in hatchlings (Hart & Armstrong 1984). Additionally, Armstrong & Gillespie (1980) showed an individual with ectrodactyly and amely. Since no details for tadpoles or metamorphosed individuals are available, a comparison with the Roßwag population of *B. viridis* is difficult. However, anomalies differed strongly among individuals, which is consistent with the observations in Roßwag and included vertebral truncation, which is an anomaly that has been infrequently reported for amphibians (see Henle et al. 2017a) but had appeared in several individuals in Roßwag.

Summary evaluation. Apart from the chemicals sprayed on the neighbouring vineyards, which may have contributed to edema, ectromely, bent tails and tumours, no other evidence for potential chemical contamination in the Roßwag quarry is available. However, it is conceivable that the earthen deposit in the quarry could have contained undetected contaminants because of degradation at the time samples were taken and because the chemical analyses undertaken were of limited scope.

Different types of chemicals taken together can induce most of the anomalies observed in Roßwag. Notwithstanding, undetected chemical contamination in the quarry could not explain the occurrence of anomalies in individuals developing in rain puddles at the border or outside the quarry. Mutagenic substances are an exception since they may have affected adults that spawned in the rain puddles in later years, but the pesticides sprayed on the neighbouring vineyards are not regarded as potent mutagens (see above). Moreover, mutagenic chemicals excluded, individuals treated with a particular chemical substance show the same types of anomalies, not different ones, which is in contradiction to the observations made in Roßwag. Furthermore, most chemical substances, for which we have data, are able to induce only a small number of different types of anomalies. Embryonic anomalies and mutagenic chemicals excluded, only the feeding of tadpoles on antithyroidal substances (thymus oil, various plant seed oils or extracts of the thyroidea) resulted in more than ten types of anomalies (a maximum of 13; see above). The highest number for any pesticide we found was seven types when newts (*Triturus carnifex*) with amputated legs were exposed to maneb 80, which most likely also interferes with the thyroid axis (Arias & Zavanella 1979, Zavanella et al. 1984). Thus, only a cocktail of pesticides and other contaminants, with individual tadpoles exposed to different chemicals within the cocktail or mutagenic substances could explain a considerable part of the anomalies observed in Roßwag. Still, non-mutagenic chemicals cannot explain yellow spots and abnormal colour patterns, nor the fact that anomalies developed in eggs transferred to the laboratory and that yellow spots were heritable. Finally, it is difficult to imagine how individuals living in the same, partly small water body should become exposed to different chemicals within a chemical cocktail. In conclusion, while mutagenic chemicals would be consistent with the observations of anomalies made, we have no indication that such substances could have contaminated the Roßwag quarry as well as the rain pools on its border.

6.1.3 Factors revealing or inducing genetic-based anomalies

6.1.3.1 Traumatic parthenogenesis (pricking of eggs). Parthenogenesis means that the egg develops solely from genetic material from the ovum, without any participation of DNA from a spermatozoon. Parthenogenetic offspring may be haploid, homozygous diploid or polyploid. Parthenogenesis can be achieved in various ways: it is "spontaneous" in nature in many groups of organisms and it can be artificially induced by several kinds of intervention, such as the chemical treatment of the sperm (e.g., inactivation of the nucleus by acriflavine, toluidine blue, methylene blue or thionine) or by physical action (e.g., cold and hot temperature shocks, sub-lethally high temperatures, high hydrostatic pressure, irradiation of the sperm, pricking the egg with a fine glass needle or cross fertilization with distantly related species of the same or a different genus) (e.g., Hertwig 1898, Bataillon 1910, Fischberg 1948, Moore 1955, Miyada 1960, 1977, Weiss 1960, Gallien et al. 1965, Pogany 1976, Gillespie & Armstrong 1979).

Evidence put forward for this hypothesis. This explanation was made by Ewe (Univ. Stuttgart-Hohenheim) in an attempt to suppress a planned publication on the Roßwag toads that nevertheless appeared in a popular science magazine (Kovacsics 1981). No supporting evidence was provided.

Anomalies known to appear in traumatic parthenogenetic individuals. Usually, the pricking of eggs mainly results in haploids (e.g., Parmenter 1933), where anomalies generally manifest at the beginning of the gastrula stage. This is the earliest stage at which paternal genes are activated (Davidson 1976). Most individuals die at this stage (e.g., Hertwig 1923, Volpe & dasgupta 1962). Surviving embryos often develop edema, microcephaly, defective eyes, defects of the circulatory and digestive system, as well as abnormal otocyst and brain formation. Individuals that reach free-swimming larval stages and metamorphose are rare exceptions (Baltzer 1922, Parmenter 1933, Miyada 1960, 1977, Nishioka & Kondo 1978). If individuals survive the embryonic stage, they will usually develop normally (Böök 1941, 1943, Fischberg 1948). One of the reasons for the lethality of haploidy may be that any lethal recessive allele, which in a diploid embryo is "silenced" by a dominant "wild" allele, can be expressed in the haploid and entails death.

Pricking at the 2-cell stage stops further development of the pricked cell and incomplete or partially duplicated embryos develop from the other cell (Endres & Walter 1895).

Evaluation. Traumatic parthenogenesis through the pricking of the egg is very difficult to achieve (e.g., Bataillon 1910, Parmenter 1933). One needs to use a very fine needle, polluted by a few drops of blood, as some material must be pushed into the egg (Maiorca et al. 1975). Hundreds of eggs must be individually pricked before one develops; this is a very painstaking procedure. The idea that birds could prick toads' eggs (but not eat or even kill them!) and induce parthenogenesis is *completely absurd*, both from a biological point of view regarding bird behaviour and from the point of view of traumatic parthenogenesis, as a bird's beak is much too large to have the action of a very fine needle.

6.1.3.2 Atavism. ***Evidence put forward for this hypothesis.*** Being aware of the shaky grounds for hybridization as an explanation of the anomalies in the absence of any evidence for it and the presence of many contradictory results, Flindt (1985a) argued that hybridization in the past combined with atavism caused the anomalies. As evidence, he provided hearsay from an elderly lady (Anonymous 1981b), who soon afterwards supposedly suffered from advanced Alzheimer's disease (Henle et al. 2017b). He cautiously introduced the hearsay but when he came to his final conclusions, it was solid as a rock. He additionally took the adult individual with a supernumerary bone at the angle of the jaw found by Christaller (1983) in 1981 as proof that the anomalies must have existed before 1980 in the quarry. In combination with inbreeding and hybridization, this reasoning was taken as justification to regard the anomalies observed in Roßwag as a normal occurrence by the official inquiry (Landesregierung von Baden-Württemberg 1984).

Anomalies known to be caused by atavism. Only epigenetic processes may lead to the expression of genes that are silenced under normal conditions, which then may lead to abnormal development. For example, high temperatures during larval development may induce ectromely and ectrodactyly in a specific line of *Pleurodeles waltl* that carries a mutation sensitive to temperature (Lauthier 1971, Dournon et al. 1998).

Evaluation. Atavism is the sudden reappearance of ancient characters. It is often used in a metaphysical frame with no reference to any mechanism that may cause this reappearance. Placed in a modern genetic context, one might explain "atavism" under the concept of epigenetics as genes that have been silenced in previous generations and then have been reactivated. Although methylation of the DNA that may silence genes is usually completely erased shortly after fertilization, examples of transgenerational persistence of methylation of silenced genes are meanwhile known for a range of organisms (Gilbert & Epel 2008, Vargas 2009). However, no mechanism is known that would silence many genes coding for a range of different morphological characters and colouration simultaneously for decades and then suddenly erase this silencing in a single year. Beyond that, it would not provide an explanation for the origin of the anomalies, it would only shift the timeframe.

Lethal mutations do not accumulate with time (Falconer 1989, Hedrick 1994, Kirkpatrick & Jarne 2000). On the contrary, in their presence even mildly deleterious mutations are rapidly purged (Gilligan et al. 1997). The malformed adult discussed by Flindt (1985a) may or may not have been born before 1980, the year of the discovery of the mass anomalies. *Bufotes viridis* grows rapidly and may reach adult size within a year (Günther & Podloucky 1996). Of two marked individuals recaptured by Flindt & Hemmer (1970b), one had reached 52 mm in the second year, making it feasible that the adult in question had metamorphosed in 1980, especially since the quarry operated intensively under flood light in 1980 (Henle et al. 2017b); flood light attracts insects and toads, including *B. viridis,* gather under the light to feed on these insects (Baumgart 2003).

6.1.3.3 Hybridization. ***Evidence put forward for this hypothesis.*** Sander suggested this hypothesis in a parliamentary hearing (Henle et al. 2017b). He based his hypothesis on hearsay: the supposedly definitive observation of *B. bufo* spawn in the quarry by the manager of the quarry. Independently, Flindt (1985a,b) explained three phenomena observed in Roßwag by hybridization between *Bufotes viridis* and an unspecified species (*B. bufo* or *Epidalea calamita*): (1) various anomalies (edema, curvature of the tail, malformed mouthparts, etc.) of young tadpoles and the anomalies of the tadpoles that had hatched from the white clutch collected in the neighbouring quarry, because he regarded them as characteristic for interspecific hybridizations of amphibians; (2) the giant tadpoles, which he attributed to heterosis (hybrid vigor); (3) polymely of metamorphosed individuals, which he related in a diffuse way to anomaly P of green frogs (*Pelophylax* synkl. *esculentus*) and the hybrid character of *P. esculentus*. The official position of the State Government of Baden-Württemberg explained all anomalies with hybridization in combination with inbreeding and atavism (Landesregierung von Baden-Württemberg 1984).

Anomalies reported from experimental hybridizations are summarized in Table 9. Anomalies depend on the species involved. Artificial hybridizations between European toad species and other genera fail to develop (Pflüger & Smith 1883, Born 1886, Tchou-su 1931). The results of hybridization experiments among European toad species strongly depend on the species involved, the paternal and maternal species, the geographic origin of the specimens and the individuals involved in the cross (different results may be obtained with different parents of the same species). All crosses between *B. bufo* males and *B. viridis* females died as embryos (Born 1883, 1886, Hertwig 1918, 1930, 1953, Montalenti 1933, Perri 1946, Hertwig & Weiss 1955, Vlcek 1995, Hertwig et al. 1959, Delarue 1982, Zavadil & Roth 1997), with the exception of one of two crosses performed by Blair (1972), in which

6.1% metamorphosed. No further details were provided by Blair (1972). Death at early embryonic stages can be explained by the expression of parental genes, which starts at the gastrula stage (Davidson 1976). Reciprocal crosses also either died as embryos or, if they were viable and reached advanced tadpole stages, at most a few individuals showed anomalies (Born 1883, 1886, Pflüger & Smith 1883, Hertwig 1918, 1953, Lang 1926, Montalenti 1933, 1939, Perri 1946, Hertwig & Weiss 1955, Meusel 1955, Hertwig et al. 1959, Weiss & Ziemann 1959, Flindt & Hemmer 1970a, Delarue 1974, 1982, Kawamura et al. 1980, Zavadil & Roth 1997, Bressi et al. 2000, Zavadil et al. 2003, Duda 2008). The only anomalies repeatedly obtained at elevated rates in experiments are edema, the torsion of tails and microcephaly in embryos and young tadpoles, which do not complete their development. Occasionally, a few albinistic, dwarf or giant tadpoles and missing eyes in metamorphosed individuals were reported but never any skeletal anomalies.

Similarly, crosses between *E. calamita* males and *B. viridis* females either died at early tadpole stages at the latest (Born 1886, Meusel 1955, Blair 1972, Delarue 1982) or metamorphosed normally, with at most a small percentage showing anomalies (maximum frequency reported: 5.9%; $n = 341$) (Hertwig et al. 1959, Flindt & Hemmer 1967b, 1970a,b, Siboulet 1971, Grosse 1976, 1977). The observed anomalies were edema, microcephaly, the torsion of tails, stiff legs, large and dwarf tadpoles and one albinistic individual. Reciprocal crosses almost invariantly died at early embryonic stages (Hadorn 1955, Meusel 1955, Hertwig et al. 1959, Weiss 1960, Flindt & Hemmer 1967b, Flindt et al. 1968, Schipp et al. 1968, Siboulet 1971, Delarue 1982), with a single exception in which nine individuals survived the hatching stage and developed normally (Weiss 1960).

Although gonads appear normal in some juveniles (Perri 1949), the rare hybrids of Europen toads that reached sexual maturity and were tested were sterile (Hertwig & Weiss 1955, Weiss & Ziemann 1959).

Evaluation. Morphologically, the strings of *B. bufo* and *B. viridis* are almost identical and it is practically impossible to differentiate between them, even for specialists (Arnold & Burton 1978, Nöllert & Nöllert 1992, Günther 1996), which seriously questions the only supposed field evidence put forward for a hybrid origin of the Roßwag anomalies. Whereas it is known that hybridization can lead to different types of anomalies (Tab. 9), the anomalies attributed to hybridization by Flindt (1985a) are not at all typical for hybridization. Rather, they are common anomalies that can have a very wide range of causes (Tab. 9). Moreover, only approximately 50% of the different types of anomalies observed in Roßwag have ever been reported from hybridization experiments even if anomalies are included that have been observed only rarely or in a single experiment (Tab. 9; based on 78 different combinations of hybridizing species).

In spite of Flindt's (1985a) insistence on the hybrid hypothesis, it is impossible as the cause of the anomalies observed in Roßwag. First of all, only *B. viridis* was found in the quarry between 1980 and 1983. A large number of individuals of a second species is an essential prerequisite because of the low viability of hybrids (see above) – and even then the frequency of abnormal individuals would be much lower than observed. It is very unlikely that large numbers of *E. calamita* males or *B. bufo* females (only their crosses with *B. virdis* can survive beyond embryonic stages – see above) were overlooked in all surveys given the high visibility at the breeding sites in Roßwag. Moreover, the habitat is typical for *B. viridis* but only marginally suitable for *E. calamita*. *B. bufo* does not occur in similar habitats in the region (own unpubl. observations). In general, it rarely breeds in quarries in Baden-Württemberg and tends to spawn much earlier (Sowig & Laufer 2007).

No mixed populations are known in the region of Roßwag (Christaller 1983, Flindt 1985a). The next population of *B. bufo* lived 1–1.5 km SSE of the quarry (Flindt 1985a), with two country roads and the river Enz separating it from the quarry. The next closest population occurred NNW of Illingen at a distance of approximately 2.5–3 km (Christaller 1983). A village and a major road separate it from Roßwag. Switching breeding sites that are separated by more than 1 km is rare for *B. bufo* even in the absence of barriers (Reading et al. 1991). The closest existing large population of *E. calamita* was separated by a distance of approximately 18 km. This distance is far beyond the known dispersal capacity of the species, ranging to a maximum of 3–5 km, with the movement of most males being restricted to a radius of less than 600 m around the breeding site (Sinsch 2009) – and only males can produce viable offspring when hybridizing with *B. viridis* (see above)! Blab (1986) suggested that under very high density, the maximum distance for founding new populations is 8-10 km although there is no supporting documentation for this dispersal distance. In any case, the closest extant population known in the region was located well beyond that distance (Christaller 1983) and even the closest extinct population was more than twice the documented dispersal distance away from Roßwag. Moreover, it is highly inconsistent to assume that many individuals of other toad species immigrated purely for mating from great distances and to state at the same time that the population was inbreeding and consequently closed to immigration from a much closer conspecific population in the neighbouring quarry.

Importantly, Flindt (1985a) collected eggs from a pair that was genetically and morphologically *B. viridis*. Tadpoles and toadlets raised from the clutch developed anomalies. Moreover, all of Flindt's (1985a) morphological and genetic analyses contradicted hybridization as a cause. Previously, all authors, including Flindt himself, regarded serum albumin

electrophoresis and the selected combination of morphological characters studied by him as reliable for the discrimination of European toad species and their hybrids (Flindt & Hemmer 1973, Kabisch & Engelmann 1975, Siboulet et al. 1975). However, for the official report, Flindt (1985a) rejected the reliability of genetic diagnostics arguing that hybridization may have taken place in the distant past and that all diagnostic characters were lost while genetic incompatibility was maintained – a position that is biologically impossible. The chance of fixation is highest for neutral or mildly deleterious mutations (Lande 1995). It is theoretically impossible that neutral genetic markers would be lost but a large number of (semi-)lethal genes are maintained in the population (Falconer 1989, Kirkpatrick & Jarne 2000) and the studies of Kawamura & Nishioka (1978) and Nishioka (1978) have confirmed this experimentally for amphibians. Moreover, adult *B. viridis* × *B. bufo* hybrids are sterile (Hertwig & Weiss 1955, Weiss & Ziemann 1959).

Finally, it is hard to explain why hybridization should have a decreasing effect from 1980 to 1984, why the Roßwag population should show a much wider range of anomalies than experimental hybridizations and why such anomalies were never reported for populations, in which *B. viridis* lives sympatrically with other toad species and especially from those for which natural hybridizations have been demonstrated (e.g., Flindt & Hemmer 1967a,b, Glaw & Vences 1989, Andrä 1994). In summary, to consider hybridization as an explanation, one must first make several highly unlikely assumptions followed by several biologically impossible ones and even then only a few of the anomalies observed in the Roßwag toads could be explained.

6.1.3.4 Inbreeding and low genetic variability. *Evidence put forward for the cause.* Flindt (1985a) introduced inbreeding as an alternative explanation to hybridization. The official position of the state government (Landesregierung von Baden-Württemberg 1984) combined inbreeding, hybridization and atavism in their explanation of the anomalies. No supporting evidence for inbreeding was given.

Anomalies known to be caused by the factor (Tab. 9). We found only two studies on the effects of genetic variability on anomalies in amphibians. Weyrauch & Grubb (2006) reported a significant influence of genetic diversity (as measured by arbitrary RAPD primers) on rates of bent and wavy tails in tadpoles of *Lithobates sylvaticus*. In contrast, Williams et al. (2008) did not find any significant difference in several measures of inbreeding between abnormal and normal individuals of *Ambystoma tigrinum*.

With the exception of stiff legs, inbreeding has never been a major issue in amphibian husbandry and thus to cause anomalies infrequently even in small founder populations typical for captive breeding. Heselhaus (1983) observed stiff legs in all offspring of all crosses of one pair of *Epipedobates tricolor* (as *Dendrobates*) and in all offspring of a *Colosthetus* sp., but only when sibs were bred. Schmidt (1985) also attributed ectromely and ectrodactyly that appeared in captive offspring of dendrobatid frogs to inbreeding but he obtained the same anomalies in offspring of individuals caught in the wild, making the explanation unlikely. Inbred *Osteocephalus elkejungingerae* exhibited black eyes (Henle 1992). Oeser (1952) obtained albinistic *Phrynobatrachus* sp. from inbreeding and maintained them for several generations.

Stocks of *Pleurodeles waltl, L. pipiens* and especially *A. mexicanum* and *X. laevis* have been maintained in the laboratory for many generations (Moore 1955, Gallien 1969a,b, Armstrong 1985, Gurdon & Woodland 1975). They have been subjected to inbreeding and various other genetic techniques to discover recessive genes. These studies revealed a genetic basis for the following anomalies: polydactyly in *X. laevis* (Uehlinger 1969), ectrodactyly in *A. mexicanum* (Humphrey 1967b) and *X. laevis* (Droin & Fischberg 1980), phocomely in *A. mexicanum* (Humphrey 1975), stiff legs (Krotoski et al. 1985, Kovalenko 2000a, Kovalenko & Kovalenko 2000), fused vertebrae (Kovalenko 1994), truncated vertebral column (Krotoski et al. 1985), lack of otoliths (Droin 1967) and degeneration of the lower jaw in tadpoles (Droin & Beauchement 1975) of *X. laevis*, as well as abnormal eyes of *A. mexicanum* and *X. laevis* (Armstrong 1985, Krotoski et al. 1985). Edema, the torsion of tails and microcephaly in embryos, also frequently appeared in breeding experiments with *A. mexicanum, P. waltl, X. laevis* and *L. pipiens* as well as in other *Xenopus* species (e.g., Elsdale et al. 1958, Gallien & Collenot 1964, Beetschen & Jaylet 1965, Uehlinger & Reynaud 1965, Uehlinger 1966, Humphrey 1967b, Droin et al. 1970, Droin 1978, Droin & Colombelli 1982, Korotoski et al. 1985). Albinistic and melanistic lines were obtained in *A. mexicanum* (e.g., Bagnara & Obika 1964, Humphrey 1967a, Borack 1972, Frost et al. 1984) and in *L. pipiens* (Gibbs et al. 1971, Browder 1972), albinistic ones in *P. waltl* (Lacroix & Capuron 1970), transient albinism in *A. mexicanum* (Humphrey 1975) and *X. laevis* (e.g., Hoperskaya 1975, 1981, Mikhailov & Korneev 1980, Droin & Fischberg 1984) and transient and permanent absence of yellow pigmentation in *Pleurodeles waltl* (Collenot & Collenot 1985, Collenot et al. 1989). Furthermore, maintenance of the brown embryonic colouration to the tadpole stage in *X. laevis* (Uehlinger & Droin 1969) and bright yellow patches of xanthophores on the head of *A. mexicanum* (Tompkins 1970) appeared in breeding stocks. A female descendent from an irradiated male *Pleurodeles waltl* carried a recessive mutation in which melanophores were morphologically normal but lacked the ability of expansion and contraction for matching the brightness of the background (Jaylet et al. 1980).

Apart from embryonic anomalies, it is rare that more than two types of anomalies that form a syndrome occurred in single inbred lines, despite the fact that some of these lines have been maintained in the laboratory for decades (Gurdon & Woodland 1975). In some mutants of *A. mexicanum,* albinism is combined with ectromely, a lack of ribs and edema (Humphrey 1967b, 1973, Martin & Signoret 1968, Malacinski 1978), in others edema is combined with abnormal kidneys and gills, microphthalmy and feet that do not differentiate (Humphrey & Chung 1978) or with abnormal gills and the distribution of melanin (Humphrey 1972). Jaylet (1971) obtained a line of *P. waltl* with abnormal vertebrae, poorly formed eyes and an open mouth. In another line, ectromely, ectrodactly, twisted feet and a reversed knee joint (anteversion) are inherited, with high temperature acting epigenetically, with the severity and variability of the anomalies increasing the sooner the heat treatment began (Lauthier 1971, Dournon et al. 1998). The lethal recessive mutation "ulcer" causes ulcers, reduced gills, weak ventral edema and ectromely (Signoret et al. 1966, Gounon & Collenot 1975). A single recessive gene causes brachymely, syndactyly, polydactyly and brachydactyly in *X. laevis* (Droin & Fischberg 1980) and another one brachydactyly, brachymely, brachycephaly, nanism, kyphosis and bulging eyes in *A. mexicanum* (Lipsett 1941). In the latter case, most individuals die at early larval stages. In all of the cited cases, all individuals show the same type of anomalies (or syndrome of anomalies). Three unlinked mutant genes that cause lethal albinism respectively microcephaly, edema and bent tails have been observed within one family line of *X. laevis* (Droin 1992).

Evaluation. What is fundamental to genetic theory (Falconer 1989) and has been demonstrated in numerous experiments (see above) is that inbreeding reduces variability. If anomalies appear they are always of the same type(s). This is incompatible with the extreme variablility in the Roßwag population of *B. viridis*, with few abnormal individuals resembling each other. Moreover, it is inconsistent to assume that many individuals of other toad species immigrated solely for the purpose of mating from great distances (see previous section) while claiming at the same time that the population was inbreeding and consequently closed to immigration from a much closer conspecific population in the neighbouring quarry. The minimum size of the Roßwag population falls within the upper size classes known for the species for Baden-Württemberg and Germany (Günther & Podloucky 1996, Laufer & Pieh 2007). If inbreeding had caused anomalies in one of the largest populations, then most German populations would now show serious anomalies. To explain the extraordinary variation of anomalies in the Roßwag population, one would need to assume that at least 32 inbred lines existed in the quarry without mixing. This would mean that all individuals selected only closely related individuals for breeding. With males being able to mate multiple times, the probability that 32 females selected a related male from the same inbred line out of 32 co-existing ones is

$$\left(\frac{1}{32}\right)^{32} = 7 \times 10^{-49} \; !$$

Thus, to retain the inbreeding hypothesis, one would have to assume that the Roßwag toads (and only these toads) were able to recognize their closest kins and sought incest instead of avoiding it.

6.1.3.5 UV-B (increased natural levels). ***Evidence put forward for the cause.*** Although this was never suggested for the Roßwag toads, increased awareness of ozone depletion and increased levels of UV-B have triggered concerns that amphibian anomalies could be caused by increased levels of UV-B (Grant & Licht 1995, Blaustein et al. 1997).

Anomalies known to be caused by UV-B are summarized in Table 9**.** UV-B can damage the DNA, especially by producing pyrimidine dimers. Enzymes, such as photolyase, can repair damaged DNA but require UV-A or visible light for this (Licht & Grant 1997). The earliest studies examined the effects of UV-B at environmentally irrelevant high doses. Under such conditions, UV-B deactivates the nucleus in newly fertilized eggs, leading to embryos with twisting, abnormal neurula and a curvature of the medullary plate. UV-B treatment before first cleavage may further interfere with egg reorganization leading to more or less severe reductions of the head and tail proportion of embryos (Malacinski et al. 1977, Scharf & Gerhart 1983). When tadpoles are treated, mainly a variety of skin abnormalities, a curvature of the spine (lordosis) and tail kinks are observed and melanin pigmentation on the dorsum and the cornea may increase (Worrest & Kimeldorf 1975, Grant & Licht 1995). Fite et al. (1998) induced various abnormalities in retinal pigment cells of adult *Lithobates pipiens* by exposure to high intensity fluorescent light that resembled the histopathological conditions detected in adult *Rana cascadae* collected from high altitudes in the wild. When UV-B was enhanced 4.8–23% over ambient levels at noon, *Pseudacris regilla* and *R. aurora* developed significantly more skin sores and opaque lenses than controls (Novales Flamarique et al. 2000). Most adult *Triturus carnifex* developed tumours after intensive UV-B irradiation (Zavanella & Losa 1981). In a variety of organisms, including humans, tumours may also develop at environmentally relevant doses (van der Leun & Gruijl 1993) but for amphibians such data are lacking.

The relevance of UV-B for anomalies in natural populations of amphibians has been controversial. Licht & Grant (1997) did not find any anomalies in embryos of *L. clamitans* and *L. sylvaticus* in treatments with environmentally relevant doses. They reviewed earlier studies on the effects of UV-B enhancement or shielding on amphibians and concluded that under natural conditions UV-B is unlikely to cause anomalies. In contrast, Blaustein et al. (1997) argued that natural levels of UV caused the torsion of tails, blisters and edema

in *Ambystoma macrodactylum*. Starnes et al. (2000) did not obtain significantly increased rates of anomalies in *L. sylvaticus* and *A. maculatum* but in *Dryophytes chrysoscelis* and *P. triseriata* (under current taxonomy presumably *P. feriarum*) (blisters, malformed tails, torsion of the spine and edema). Pahkala et al. (2001) observed significantly elevated rates of anomalies (ectromely, ectrodactyly, bent knees and the torsion of tail) in metamorphosed *R. temporaria* that were treated as embryos with enhanced UV-B still in the range of the natural variability. In contrast, Smith et al. (2000) did not obtain such a delayed effect in *L. blairi*.

These studies suggest species differences in sensitivity to UV-B and that the conditions under which the experiments were conducted also strongly influenced the results. However, in most studies the dose actually received by the treated animals was not measured and the conditions used were often unnatural. For example, all *Hyla arborea* tadpoles darkened when they were exposed to ambient levels of UV-B in the laboratory, whereas *T. cristatus, Bufo bufo, E. calamita* and *R. temporaria* did not show such an effect (Langhelle et al. 1999). Edema and the torsion of tails in *R. cascadae* and *P. regilla* treated with moderate levels of UV-A plus UV-B occurred at lower rates or were absent when only UV-A was applied or when tadpoles were kept under moderate compared to dim laboratory light conditions (Hays et al. 1996). Similarly, the filtration of UV-B plus UV-A was most effective in reducing the incidence of ectromely, ectrodactyly and anophthalmy in *L. pipiens* tadpoles (Ankley et al. 2002).

Moreover, the results of studies on the same species may be inconsistent. For example, Merilä et al. (2000) obtained asymmetric bodies and the coiling of tails in embryos of *R. temporaria*, whereas neither Langhelle et al. (1999) nor Pahkala et al. (2000) found significant UV-B effects at ambient UV-B levels. In a later study, UV-B was effective but only at low pH, causing edema and the torsion of tails (Pahkala et al. 2002). In contrast to their previous study, there were significant population effects. It should be noted that low pH can alone induce edema and the torsion of tails (see section 6.1.2.9). Genetic variability of individuals may further interact with the effects of UV (Weyrauch & Grubb 2006: edema, wavy tails and bent tails in *L. sylvaticus*) and may thus partially explain different results within the same species. Moreover, some early studies used cellulose acetate as a filter that was in contact with water. However, cellulose acetate is highly toxic to newly hatched tadpoles (Berrill & Lean 1998) and thus casts doubt on previous experiments that used these filters.

Evaluation. Increased levels of UV radiation have been suggested as a potential cause for anomalies mainly for higher altitudes because of higher UV-B intensity but Roßwag is located at a low altitude and no similar anomalies are known from populations surveyed in the vicinity of Roßwag, especially not from the neighbouring quarry with similar UV-B conditions. Artificial sources of increased UV-B radiation did not exist in the Roßwag quarry. Although UV-B can cause a number of the anomalies observed in the Roßwag *B. viridis* population under laboratory conditions (Tab. 9), most of the anomalies recorded in Roßwag were never detected in experiments with ambient levels of UV radiation and UV-B is unlikely to be a major cause of limb anomalies in nature (see also Johnson et al. 2010). Thus, ambient level of UV-B radiation can be excluded as a cause of the anomalies.

6.1.3.6 Radioactivity (and other mutagenic factors). ***Evidence put forward for the cause.*** Field measurements of radioactivity (see section "radioactivity assessment results") have been put forward as evidence for this cause (Henle 1981, 1982, Henle et al. 2017b), though this evidence has been challenged (Landesregierung von Baden-Württemberg 1984). The similarity in the spectrum of anomalies induced by experimental irradiation of amphibian germ cells (Kawamura & Nishioka 1978, Nishioka 1978) to the one observed in Roßwag and demonstrated heritability of at least one type of anomaly were again regarded as supportive evidence (Puhlmann 1981a,b, Henle 1982). No evidence for any mutagenic factor other than radioactivity has been put forward.

Anomalies known to be caused by irradiation. In amphibian embryology the use of irradiation (X-rays, γ-rays, neutron rays, radioactive substances, high intensity UV-B) to study differentiation processes has a long history. Most of the earlier studies used lethal doses and only assessed individual development for a short period of time (e.g., Hertwig, O. 1911, Hertwig, G. 1913, Stachowitz 1914, Rugh 1950; reviewed among others by Rugh 1939, Brunst 1950, 1965, Peters 1960, Labrousse 1967, Ahmad 1976, Kawamura & Nishioka 1978). More recently, some researchers used lower doses and studied the effects on the descendants from irradiated eggs or sperm throughout most of the larval development (Blair 1960, Oerter 1985, Trux 1985) and even for one or several generation(s) (Nishioka 1977, 1978, Nishioka & Ueda 1985, Kawamura & Nishioka 1978, Kashiwagi 1980, Hart & Armstrong 1984, Nishioka & Ohtani 1986). Mutagenic chemicals have been used much less frequently (on the level of full organisms). Jones & Jackson (1974), Hemsworth & Wardhaugh (1978), McKinnell et al. (1979), Armstrong & Fletcher (1983) and Hart & Armstrong (1984) tested their effects but did not analyse offspring beyond hatching.

All studies showed a wide range of anomalies in individuals descending from treated sperm or eggs (see e.g. Tab. 7). In *Pelophylax nigromaculatus* alone, Kawamura & Nishioka (1978) and Nishioka (1978) reported 18 different types of anomalies, 13 of which appeared frequently in the Roßwag population. Oerter (1985) listed 12 types of anomalies in *B. bufo* tadpoles that were irradiated at an early stage (Tab. 7); Blair (1960) also mentioned 12 different types of anomalies in offspring of irradiated *Incilius valliceps* males and crosses of the

surviving offspring among each other and wild caught individuals. Among these were several individuals with the forelegs remaining covered by the opercular fold, white individuals that failed to metamorphose and with amely. In addition, all types of anomalies found in the Roßwag population have been reported from irradiation experiments except for giant tadpoles (Tab. 9) and rare anomalies that affected only a few individuals and that have never been specifically considered in experimental studies.

As expected for mutagenic factors, the types of anomalies differed among individuals and among experiments, but some unspecific anomalies, such as abnormal cleavage, exogastrula, abnormal neurula, edema, tumours and bent bodies or tails, are commonly observed in early developmental stages (e.g., Schinz & Fritz-Niggli 1953, Jones & Jackson 1974, Oerter 1985) and edema, malformed mouthparts, bent tails and nanism in tadpoles (e.g., Oerter 1985). Recently metamorphosed individuals usually showed a range of leg and colour anomalies (e.g., Schinz & Fritz-Niggli 1954, Nishioka 1977, Kawamura & Nishioka 1978, Oerter 1985, Nishioka & Ohtani 1986).

After irradiation the incidence of anomalies decreases from generation to generation. In the fourth generation offspring of treated *P. nigromaculatus* and *Rana japonica,* the incidence had dropped to the level of the controls (Kawamura & Nishioka 1978, Nishioka 1978) but recessive colour mutations were retained for 10 years (Nishioka & Ohtani 1986, Nishioka et al. 1987).

Evaluation. The evidence from 1025 publications reporting anomalies in experimental studies, including 74 using irradiation (other than UV), shows that mutagenic factors are the only potential cause that can explain the range of anomalies observed in the Roßwag population except for a cocktail of teratogenic chemicals (see section 6.1.2.9) (Tab. 9). Chemicals and radioactivity are the only potent mutagenic factors that could have caused the range and frequency of anomalies under natural conditions. For a discussion of mutagenic chemicals as a potential cause, see section 6.1.2.9). Giant size of tadpoles is the only type of anomaly that was frequently observed in Roßwag but so far has not yet been reported in irradiation experiments. However, increased growth following radioactive contamination was observed, for example, in some plant species after the Chernobyl accident (Savchenko 1995). Furthermore, giant size in larval amphibians is caused by a disruption of the hypothalamus-pituitary-thyroid axis that may be evoked by genetic factors (reviewed by Borkin et al. 1982) and irradiation may interfere with the thyroidal adrenocorticoid hormonal balance (Ahmad 1976). Moreover, Uehlinger (1965) demonstrated that some recessive mutations lead to giant-sized tadpoles. Thus, a giant size is not incompatible with an explanation of the anomalies in Roßwag by radioactive contamination.

The laboratory studies undertaken clearly showed a genetic basis for at least one type of anomaly, orange colour patches, and made a genetic basis very likely for others, which again is in concordance with radioactivity as a cause. Genetic theory (Falconer 1989, Hedrick 1994) and experimental studies (Kawamura & Nishioka 1978, Nishioka 1978) demonstrate a rapid decrease in the range and severity of developmental anomalies with time after exposure to a mutagenic factor as observed in Roßwag from 1980 to 1984. Similar declines of anomalies were observed in barn swallow populations (*Hirundo rustica*) in areas contaminated by the Chernobyl accident (Møller et al. 2007). Thus, this decrease is also consistent with the assumption that toads were exposed to radioactive contamination within the quarry during the breeding period of 1980 and that radioactivity has either decayed and/or the source was covered by fill after 1980 (as happened).

In terms of the number of different types of anomalies, the laboratory studies of Nishioka (1977, 1978) and Kawamura & Nishioka (1978) on the effects of irradiation on the descendents of *R. japonica* and *P. nigromaculatus* (18 different types of anomalies, 13 of which were frequently observed in the Roßwag population) and the field study of Saveliev et al. (1996), Kuranova & Saveliev (1997) and Kuranova (2003) on *R. arvalis* and *Salamandrella keyserlingii* exposed to irradiation from a nuclear accident (at least 25 and 21 types of anomalies, respectively), come closest to the observations in Roßwag. It is worth noting that in barn swallows (*H. rustica*) from the Chernobyl and Fukushima area, in the butterfly *Zizeeria maha*, in the common lizard (*Zootoca vivipara*) from the East Uralian radioactive trace and in true bugs (Heteroptera) from the Chernobyl area and downwind from La Hague, a much higher frequency and wider range of different types of anomalies have been observed than it is known for these species from anywhere else (Semenov & Ivanova 1995, Ellegren et al. 1997, Møller et al. 2007, Hesse-Honegger & Wallimann 2008, Taira et al. 2014, Mousseau & Møller 2014). In areas contaminated with high levels of radioactivity caused by the accident in the Mayak plant, Ural, the frequency of morphological anomalies was also significantly elevated compared to control populations (Pyastolova et al. 1996). In addition, the irradiation of *B. bufo* embryos in the laboratory by Oerter (1985) resulted in more different types of anomalies – many shared with the Roßwag population – than any other experimental study known to us that did not use mutagenic factors. Finally, a wide range of anomalies and huge individual differences like those in Roßwag are expected, if eggs or sperm of the toads were exposed to radioactivity because of the random nature of mutations. These differences are still expected in the second and third year after irradiation because most of the offspring will still be from parents whose germ cells obtained mutations and not from surviving abnormal offspring.

The official enquiry regarded all non-official radioactivity measurements as unreliable and as artefacts (Landesregierung von Baden-Württemberg

1984). However, all official and non-official measurements that can be compared are consistent with one another. All of these showed that the level of radioactivity across the earthen deposit was elevated by a factor of 2–3 compared to the surrounding area. Locations, where measurements resulting in very high values of radioactivity were taken by KH, were not assessed in the official survey. Therefore, the official measurements cannot provide any evidence to confirm or reject them. To reject them, first an explanation for similar artefacts by two technically very different instruments and in repeated measurements with the same instrument is required. Secondly, an explanation is needed as to why these instruments never produced artefacts whenever measurements were made that could be compared with the official measurements. Thirdly, one needs to explain why both instruments produced artefacts only at the same locations (large cracks opening to the interior of the fill) but never when tested at other places and by pressing the instruments against material from the fill, nor in other operational tests for the functioning of one of the instruments. Such explanations have never been given and are exceedingly difficult to construct. Finally, the official enquiry insisted that the results of the official measurements excluded the presence of radioactive contamination in the quarry with absolute certainty (Landesregierung von Baden-Württemberg 1984). In an interview given to sbs Television, Australia, in 2006, Prof. Schreiber, a key physicist involved in the controvery (Henle et al. 2017b), denied such a possibility for the official measurements and his own ones, if the source was covered by fill. Already a cover of more than 20 cm of deposit material makes it impossible to detect any α or β radiation with measurements made 1 m above the surface. In this case, only measurements made close to the opening of cracks that only partially shielded the source or that allowed leakage of radioactive gases could have detected a strongly elevated activity. A necessary corollary is that measurements would need to exponentially decline with the distance from the opening. These predictions are met qualitatively by the non-official measurements, which the official enquiry rejected as artefacts (see Table 5a for measurement results).

6.2 Conclusion

The mass anomalies observed for *B. viridis* in the Roßwag quarry score among the four most severe ones in terms of the number of individuals affected and it is by far the most extreme case regarding the different types of anomalies involved. A normal natural phenomenon can clearly be ruled out.

Most types of anomalies can have different causes (Tab. 9). As a consequence, it is usually impossible to infer the cause from a single type of anomaly as attempted by Flindt (1985a) for some anomalies of the Roßwag population. However, not all factors are able to induce all types of anomalies. For example, with the exception of bleaching, darkening and haemorrhage, colour anomalies caused by non-genetic factors are extremely rare.

Most factors cause few anomalies and usually all affected individuals exhibit the same types of anomalies. More than ten different types of anomalies in larvae and/or juveniles/adults have only been reported in four experiments involving radioactivity or endocrine substances. Most notable is the study on the effects of irradiation of eggs or sperm in *P. nigromaculatus*, in which 18 different types of anomalies were reported in tadpoles, many similar to those observed in Roßwag (Nishioka 1977, Nishioka & Ueda 1977, Kawamura & Nishioka 1978, Nishioka & Ohtani 1986). In a parallel study on *R. japonica,* ten different types were mentioned but several types of anomalies were combined in a single category without providing details (Nishioka 1978). Oerter (1985) listed 13 different types of anomalies in *B. bufo* tadpoles that were irradiated as embryos, many of which were shared by the Roßwag population of *B. viridis*. Feeding *X. laevis* with thymus oil and various antithyreoidal plant seed oils induced 11–13 different types of anomalies, among them phocomely, oligodactyly, torsion of legs, stiff legs, front legs covered by a fold of skin, mandibular hypoplasia, giant larvae and edema but neither colour anomalies nor polymely (Pohland 1962, Wurmbach et al. 1964, Wink & Wurmbach 1967).

Summing across more than 1000 publications on experimentally applied teratogenic factors, more than ten different types of anomalies are known only for three of them: chemicals, irradiation and mutations revealed by breeding experiments (Tab. 9). If experiments are included, in which an anomaly was only occasionally observed and no statistical tests were made, then hybridization also surpasses ten different types of anomalies. Even in combination, all the remaining potential causes could explain at most approximately 50 % of the different types of anomalies observed in Roßwag (Tab. 9). Except for irradiation (Nishioka 1977, Nishioka & Ueda 1977, Kashiwagi 1980, Nishioka & Ohtani 1986), we know of no experimental study, which induced different types of colour anomalies in the same population. Thus, their co-occurrence at elevated rates within a natural population, such as in Roßwag, is an extremely strong indication for a genetic cause. Because the majority of colour anomalies are recessive and impose a reduced, often low viability (e.g., Nishioka 1977, Nishioka & Ohtani 1986; own observations of albinistic tadpoles in Roßwag), the simultaneous co-occurrence of many individuals carrying different types of colour anomalies can only be explained by the recent presence of a strongly mutagenic factor.

Inheritance could be demonstrated for the orange colour spot by the appearance of the same anomaly in the offspring of an affected individual and is supported for other anomalies by their appearance in tadpoles and eggs transferred to the laboratory. The individual with a squashed head aside, there is no convincing evidence

that any non-genetic factor contributed to the anomalies. While the non-mutagenic effects of chemicals could theoretically explain the spectrum of anomalies observed, this would require the assumption that many different substances contaminated the pond and that the tadpoles were exposed to different subsets of these substances, which is highly improbable given the homogeneous nature of the pond. Although there is a possibility that pesticides sprayed in the neighbouring vineyards might have contributed to the appearance of tumours, bent tails, edema and ectromely, the evidence for this hypothesis is weak and evidence against it is strong – and the anomalies can be explained without such an assumption.

Hybridization, inbreeding, mutagenic chemicals, UV-B and radioactivity can result in the appearance of genetically-based anomalies. Genetic and morphological studies attempting to demonstrate hybridization were all negative: anomalies developed from a clutch of a pair that was genetically *B. viridis* and no population of other toad species existed in the near vicinity of the affected population. Supporting hybridization as an explanation requires several biologically extremely unlikely and impossible assumptions (see above for details).

Similarly, inbreeding can also be excluded with certainty on theoretical and empirical grounds. The Roßwag population belongs to the largest populations in southern Germany and it is inconsistent to assume that the population was closed to conspecific immigrants from a nearby population but hybridized intensively with other toads from far more distant populations. Most importantly, however, inbreeding would lead to the same types of anomalies in all affected individuals, not to the huge variability observed. Explaining the anomalies by inbreeding thus requires nothing less than assuming that a fundamental biological law did not apply to the Roßwag toads.

In the Roßwag quarry, no artificial source of UV-B existed and ambient levels of UV-B do not induce a similar spectrum of anomalies as observed in the Roßwag population. Moreover, it should have caused the same mass anomalies in *B. viridis* breeding in the structurally very similar neighbouring quarry. In conclusion, only chemical mutagenes or radioactivity are compatible with the biological observations made in Roßwag and the huge body of experimental literature on amphibian anomalies.

Biologically, it is impossible to differentiate between different mutagenic factors based on externally observed anomalies. However, none of the chemical analyses did provide any kind of indication of chemical pollution of the breeding site. While the chemicals sprayed in the neighbouring vineyards by helicopters presumably contaminated the quarry pond, they are widely used in viniculture and are not known to be potent mutagenic substances (see section 6.1.2.9). Moreover, the declining range of anomalies observed from 1980 to 1984 would require a considerably reduced application of chemicals or manual spraying in the later years as opposed to helicopter spraying in the neighbouring vineyards, especially since the water bodies were much smaller in the years 1981-1984 compared to 1980. Although we cannot exclude with absolute certainty that some mutagenic chemicals contaminated the fill in 1980, there is no indication that any such substance was present in the quarry in sufficient quantities to affect a considerable part of the population.

Radioactivity is another mutagenic factor and measurements were made that indicated the presence of very high unnatural levels, although these measurements were disputed. Casting aside for a moment the disputed measurements and the notion that it is difficult to grasp that humans could have illegally deposited radioactive material in the quarry – though theft and illegal deposition of plutonium in the environment was discovered in 2000 in Baden-Württemberg at a location less than 100 km distant from Roßwag (Struktur- und Genehmigungsdirektion Süd Rheinland-Pfalz 2002) – what would be the predicted observations and measurements, what would contradict and what would support such an explanation?

Firstly, there were large cracks in the fill, which were ideal hiding places for adult toads, especially as few other hiding places were available in the immediate vicinity of the pond and thus it is very likely that a considerable number of toads found a retreat in these hiding places. They would be exposed to elevated radioactivity and contract mutations in their germ cells. As known from the experiments of Kawamura & Nishioka (1978), Nishioka (1978), Oerter (1985) and other studies summarized in Table 9, this would lead to a very wide range and high incidence of anomalies both in tadpoles and in recently metamorphosed individuals, many of which would be lethal. Notably, most of the frequent anomalies in the Roßwag population were also frequently observed in the experimental studies on the decendants of irradiated eggs and sperm of amphibians. The only exception, giant tadpoles, is caused by a disturbance of the thyroid axis, which is tightly regulated genetically and thus can also be caused by mutations as observed in other organisms (Savchenko 1995). Secondly, because of the genetic basis, anomalies would also occur when eggs or tadpoles were transferred to the laboratory as well as in their offspring – all predictions that are met by the observations for the Roßwag population. Finally, since the source was inaccessible to toads after 1980 because of several meters of additional fill, theory (Falconer 1989, Hedrick, 1994) predicts and the experiments of Kawamura & Nishioka (1978) and Nishioka (1978) demonstrated empirically, that the spectrum and frequency of the anomalies would rapidly decline with time as was the case in the Roßwag population (Tables 1 & 2). Moreover, toads spawning in neighbouring water bodies in the following years because of the destruction of the original breeding ponds would bring their anomalies to these water bodies, each differing in the spectrum of anomalies as observed by Flindt (1985a,b).

The single adult individual with an anomaly found in 1981 does not contradict these concluding remarks. Firstly, it is much more parsimonious to explain this single individual with an injury (or another cause) and the rest with a single cause than to use many different causes as explanation and still not being able to explain the majority of the anomalies. Moreover, *B. viridis* grows rapidly and is able to reach adult size in the year after metamorphosis (Flindt & Hemmer 1970b, Günther & Podloucky 1996). In 1980, flood-light was frequently used in the quarry, which attracts insects and *B. viridis* that hunt for insects under the light (Baumgart 2003). Thus, unusually good growth conditions existed and the individual may well have metamorphosed in 1980.

What are the predictions about radioactivity measurements? Already a cover of more than 20 cm with deposit material would make it impossible to detect any α or β radiation with measurements taken 50 cm or 1 m above the deposit of earth. Neither the measurements of IFEU, nor those of LfU or Schreiber would have detected it (confirmed by Schreiber in an interview to sbs television Australia). The same applies to those measurements of Henle that were made 1 m above the ground. All these measurements were consistent with those that were not disputed. Measurements made close to the opening of cracks that only partially shielded the source or allowed radioactive gases to leak would have provided different results: they would have been considerably elevated (depending on the radiation intensity and shielding), exponentially declining with the distance from the opening. Again, these predictions are met qualitatively by the contested measurements (Tab. 5a). There are no contradictions concerning the measurements taken, only in their interpretation.

It is not the cause of the anomalies [highly mutagenic material] that remains a mystery, the open question is rather: exactly which material was deposited, where did it come from, who deposited it in the quarry and why? Since any mutagenic material poses hazards not only to wildlife but also to humans, if they are exposed to it (Savchenko 1995, Lannoo 2008, Mousseau & Møller 2014, Taira et al. 2014), the most important questions that remain are: How long will the material remain active? Is there still a long-term hazard to toads, other wildlife or humans? And was it a single case or has it happened elsewhere as well (Henle et al. 2017b)?

7 Acknowledgements

We owe thanks to many persons and institutions that have supported us with the assessment of the anomalies observed in Roßwag and their potential causes, above all W. Böhme, M. Beck-Oberdorf, B. Franke, B. Hammer, C. Hammer, W.-D. Hasenclever, N. Kneissler, M. Kötter, M. Kovacsics, E. Krüger, J. Margraf, W. Schmitt, H. Schreiber, B. Steinhilber-Schwab and M. Weiss. Without the help of M. Uhlig the literature compilation would have been an impossible task. KH thanks N. Rumyantseva for her patience during many hours of work on the manuscript. M. Liess, J.-C. Beetschen and A. Collenot provided valuable suggestions for improving the manuscript.

Funding: The authors funded most of this study privately but received institutional support for reviewing the relevant literature. Alain Dubois acknowledges financial support by the Green Party of Baden-Württemberg and by the Government of Baden-Württemberg to attend a symposium and a parliamentary hearing organized by these institutions.

Competing interests: All authors are engaged in the conservation of amphibians. The father of the senior author (deceased) was employed at the mid-level management of a company with major involvement in nuclear energy technology in Germany. The second author wrote a book, largely based on the publications of Jean Rostand, pointing to the dangers of nuclear radiations for the health and the genetic integrity of human populations (Dubois 2012). The authors have declared that no other competing interests exist.

8 References

Ahmad, M. (1976): The effects of ionizing radiations on prometamorphic anuran tadpoles. – J. Sci. (Univ. Karachi) **4**: 26–37.

Alsop, D.H., S.B. Brown & G.J. Van Der Kraak (2004): Dietary retinoic acid induces hindlimb and eye deformities in *Xenopus laevis*. – Environ. Sci. Technol. **38**: 6290–6299.

Anderson, R.C. & G.F. Bennett (1963): Ophthalmic myiasis in amphibians in Algonquin Park, Ontario, Canada. – Can. J. Zool. **41**: 1169–1170.

Andrä, E. (1994): Ein überregionales bedeutsames gemeinsames Vorkommen von Wechselkröte (*Bufo viridis*) und Kreuzkröte (*Bufo calamita*) im Landkreis Fürstenfeldbruck, Oberbayern. – Mitt. Landesverband Amph.-Rept.-Schutz Bayern **14**: 27–34.

Ankley, G.T., J.E. Tietge, D.L. DeFoe, K.M. Jensen, G.W. Holcombe, E.J. Durhan & S.A. Diamond (1998): Effects of ultraviolet light and methoprene on survival and development of *Rana pipiens*. – Environ. Toxicol. Chem. **17**: 2530–2542.

Ankley, G.T., S.A. Diamond, J.E. Tietge, G.W. Holcombe, K.M. Jensen, D.L. DeFoe & R. Peterson (2002): Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hindlimb malformations in the northern leopard frog (*Rana pipiens*). – Environ. Sci. Technol. **36**: 2853–2858.

Anonymous (1980a): Krötenprüfung dauert an. Mißbildungen möglich durch Sprengerschütterungen. – Ludwigsburger Kreiszeitung **18.10.1980**.

Anonymous (1980b): Krötenmißbildungen nicht außergewöhnlich. – Pforzheimer Zeitung **18.10.1980 (243)**.

Anonymous (1981a): Mißgebildete Kröten Dauerbrenner? – Pforzheimer Zeitung **20.2.1981**.

Anonymous (1981b): Froschperspektiven. – Vaihinger Kreiszeitung **2.12.1981**.

Arias, E. & T. Zavanella (1979): Teratogenic effects of manganese ethylenebisdithiocarbamate (maneb) on forelimb

regeneration in the adult newt, *Triturus cristatus carnifex*. – Bull. Environ. Contam. Toxicol. **22**: 297–304.

Armstrong, J.B. (1985): The axolotl mutants. – Develop. Genet. **6**: 1–25.

Armstrong, J.B. & W.S. Fletcher (1983): Assessment of mutagenic damage following ethyl methanesulfonate mutagenesis in the axolotl (*Ambystoma mexicanum*). – J. exp. Zool. **226**: 333–340.

Armstrong, J.B. & L.L. Gillespie (1980): Dominant lethal induction by ethyl methanesulfonate in the male axolotl (*Ambystoma mexicanum*). – J. exp. Zool. **212**: 415–421.

Arnold, E.N. & J.A. Burton (1978): A Field Guide to the Reptiles and Amphibians of Britain and Europe. – London (Collins).

Asashima, M., T. Oinuma & V.B. Meyer-Rochow (1987): Tumors in Amphibia. – Zool. Sci. **4**: 411–425.

Bacon, J.P., J.A. Gray & L. Kitson (2006a): Status and conservation of the reptiles and amphibians of the Bermuda islands. – Appl. Herpetol. **3**: 323–344.

Bacon, J.P., D.W. Linzey, R.L. Rogers & D.J. Fort (2006b): Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part I. Frequencies and distribution of abnormalities. – Appl. Herpetol. **3**: 39–65.

Bagnara, J.T. & M. Obika (1964): Differences in integumental pteridines between black and white axolotls. – Anat. Rec. **148**: 358.

Ballengée, B. & S.K. Sessions (2009): Explanation for missing limbs in deformed amphibians. – J. exp. Zool. **312B**: 770–779.

Balls, M. & L.N. Ruben (1964): A review of the chemical induction of neoplasms in Amphibia. – Experientia **20(5)**: 241–247.

Balls, M., R. Clothier & L. Ruben (1978): Neoplasia of Amphibia. – Pp. 48–62 in Gershwin, M.E. & E.L. Cooper (eds.): Animal Models of Comparative Developmental Aspects of Immunity and Diseases. – New York (Pergamon Press).

Baltzer, F. (1922): Über die Herstellung und Aufzucht eines haploiden *Triton taeniatus*. – Verh. Schweiz. Natforsch. Ges. Bern **103**: 248–249.

Bantle, J.A., D.J. Fort & B.L. James (1989): Identification of developmental toxicants using the Frog Embryo Teratogenesis Assay-Xenopus (FETAX). – Hydrobiologia **188/189**: 577–585.

Bataillon, E. (1910): Le problème de la fécondation circonscrit par l'imprégnation sans amphimixie et la parthénogenèse traumatique. – Arch. Zool. Expér. Gén. **5(5)**: 101.

Baumgart, G. (2003): Die Wechselkröte (*Bufo viridis*) in Frankreich: Allgemeine Angaben unter besonderer Berücksichtigung der Entwicklung im Elsass. – Mertensiella **14**: 109–122.

Bazzoli, A.S., J. Manson, W.J. Scott & J.G. Wilson (1977): The effects of thalidomide and two analogues on the regenerating forelimb of the newt. – J. Embryol. exp. Morphol. **41**: 125–135.

Beetschen, J.C. & A. Jaylet (1965): Sur un facteur récessif semi-létal déterminant l'apparition d'ascite caudale (ac), chez le Triton *Pleurodeles waltlii*. – C.r. Acad. Sc. Paris **261**: 5675–5678.

Bennati, R., M. Bonetti, A. Lavazza & D. Gelmetti (1994): Skin lesions associated with herpesvirus-like particles in frogs (*Rana dalmatina*). – Vet. Rec. **135**: 625–626.

Berrill, M. & D.R.S. Lean (1998): Problems in conducting in-situ experiments to evaluate the influence of UV-B radiation on amphibian eggs and larvae. – Environ. Toxicol. Risk Assess. **7**: 21–30.

Birge, W.J., J.A. Black & A.G. Westerman (1978): Effects of Polychlorinated Biphenyl Compounds and Proposed PCB-replacement Products on Embryo-larval Stages of Fish and Amphibians. – Lexington (Univ. Kentucky).

Birge, W.J., J.A. Black, A.G. Westerman & B.A. Ramey (1983): Fish and amphibian embryos – a model system for evaluating teratogeneicity. – Fund. Appl. Toxicol. **3**: 237–242.

Bishop, C.A. & A.D. Gendron (1998): Reptiles and amphibians: shy and sensitive vertebrates of the Great Lakes Basin and St. Lawrence River. – Environ. Monit. Assess. **53**: 225–244.

Blab, J. (1986): Biologie, Ökologie und Schutz von Amphibien. – Greven (Kilda).

Blair, W.F. (1960): Radiation-induced genetic damage in the Mexican toad (*Bufo valliceps*). – Texas J. Sci. **12**: 216–227.

Blair, W.F. (1972): Evidence from hybridization. – Pp. 196–232 in Blair, W.F. (ed.): Evolution in the Genus *Bufo*. – Austin (Univ. Texas Press).

Blaustein, A.R. & P.T.J. Johnson (2003): The complexity of deformed amphibians. – Front. Ecol. Environ. **1(2)**: 87–94.

Blaustein, A.R. & D.B. Wake (1990): Declining amphibian populations: a global phenomenon? – TREE **5**: 203–204.

Blaustein, A.R., J.M. Kiesecker, D.P. Chivers & R.G. Anthony (1997): Ambient UV-B radiation causes deformities in amphibian embryos. – Proc. Natl. Acad. Sci. **94**: 13735–13737.

Blaustein, A.R., J.M. Romansic, E.A. Scheessele, B.A. Han, A.P. Pessier & J.E. Longcore (2005): Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. – Conserv. Biol. **19**: 1460–1468.

Blount, I.H. (1950): The effects of prolonged refrigeration upon the limbs of urodeles. – J. exp. Zool. **113**: 683–715.

Bohl, E. (1997): Mißbildungen der Extremitäten von Amphibienlarven in Aufseß (Oberfranken). Versuche zur Ursachenermittlung. – Münchener Beitr. Abwasser-, Fischerei- und Flussbiol. **50**: 160–189.

Bonhomme, C. & R. Pourhadi (1957): Sur quelques larves monstrueuses obtenues par applications d'ultrasons sur divers stades de l'œuf de *Triton*. – C.r. Acad. Sc. Paris **245**: 224–226.

Bonhomme, C., R. Pourhadi & J.P. Turchini (1960): Tératogenèse expérimentale par applications d'ultrasons sur des œufs de *Triton*. – Z. Biol. **3**: 357–366.

Böök, A. (1941): Induction of haploidy in a cold treatment experiment with egg-cells of the salamander *Triton taeniatus*. – Förk. Fysiogr. Sällsk. Lund **11(12)**.

Böök, A. (1943): Low temperature and cleavage in *Triton taeniatus*. – Hereditas **29**: 195–197.

Borack, L.I. (1972): Gene action on proliferation and migration in the developing neural crest of black and white axolotls, *Ambystoma mexicanum* Shaw. – J. exp. Zool. **179**: 289–298.

Borkin, L. (2014): Morphological abnormalities in natural populations of amphibians: what do we study and how do we measure? – Pp. 25–36 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. – Ekaterinburg (Uralskii Federalnii Universitet).

Borkin, L.J. & M.M. Pikulik (1986): The occurence of polymely and polydactyly in natural populations of anurans of the USSR. – Amphibia-Reptilia **7**: 205–216.

Borkin, L.J., L. Berger & R. Günther (1982): Giant tadpoles of water frogs within *Rana esculenta* complex. – Zool. Poloniae **29**: 103–127.

Borkin, L.J., O.S. Bezman-Mosekyo & S.N. Litvinchuk (2012): Evaluation of animal deformity occurence in natural populations (an example of amphibians). – Trudy zoologicheskogo instituta RAN **316**: 324–343.

Born, G. (1883): Beiträge zur Bastardierung zwischen den einheimischen Anurenarten. – Pflügers Arch. **32**: 453–518.

Born, G. (1886): Weitere Beiträge zur Bastardierung zwischen den einheimischen Anurenarten. – Arch. Mikr. Anat. **27**: 192–271.

Bowerman, J. & P.T.J. Johnson (2003): Timing of trematode-related malformations in Oregon spotted frogs and Pacific treefrogs. – Northwestern Nat. **84**: 142–145.

Bowerman, J., P.T.J. Johnson & T. Bowerman (2010): Sublethal predators and their injured prey: linking aquatic predators and severe limb abnormalities in amphibians. – Ecology **91**: 242–251.

Bradford, D.F. (1991): Mass mortality and extinction in a high-elevation population of *Rana mucosa*. – J. Herpetol. **25**: 174–177.

Brandt, W. (1940): Experimental production of functioning reduplications – a triple and a functioning quintuple hindlimb in the frog. – J. exp. Biol. **17**: 396–401.

Bressi, N., S. Battistella & G.A. Amirante (2000): Note sull'ibridazione spontanea tra *Bufo bufo spinosus* Daudin, 1803 e *Bufo viridis viridis* Laurenti, 1768 (Amphibia, Bufonidae) e sul ritrovamento in natura di esemplari di origine ibrida. – Pp. 377–384 in Giacoma, C. (ed.): Atti del I Congresso Nazionale della Societas Herpetologica Italica (Torino, 1996). – Torino (Mus. reg. Sci. Nat. Torino).

Briggs, R.W. (1941): The development of abnormal growths in *Rana pipien*s embryos following delayed fertilization. – Anat. Rec. **81**: 121–135.

Brodman, R. & J.T. Briggler (2008): *Batrachochytrium dendrobatidis* in *Ambystoma jeffersonianum* larvae in southern Indiana. – Herpetol. Rev. **39**: 320–321.

Browder, L.W. (1972): Genetic and embryological studies of albinism in *Rana pipiens*. – J. exp. Zool. **180**: 149–156.

Bruce, H.M. & A.S. Parkes (1950): Rickets and osteoporosis in *Xenopus laevis*. – J. Endocrinol. **7**: 64–81.

Brunst, V.V. (1950): Influence of local x-ray treatment on the development of extremities of the young axolotl (*Siredon mexicanum*). – J. exp. Zool. **114**: 1–50.

Brunst, V.V. (1965): Effects of ionizing radiation on the development of amphibians. – Q. Rev. Biol. **40**: 1–67.

Brunst, V.V. (1968): Spontaneous transformation of adult dark axolotls (*Siredon mexicanum*) into partial albinos. – Anat. Rec. **160**: 322–323.

Bruschelli, G.M. & G. Rosi (1971): Polimelia indotta dalla vitamina A in embrioni di *Bufo vulgaris*. – Riv. Biol. **64**: 271–283.

Büchner, F. (1948): Experimentelle Entwicklungsstörungen durch allgemeinen Sauerstoffverbrauch. – Klin. Wochenschr. **26**: 38–42.

Büchner, F. (1955): Von den Ursachen der Mißbildungen und Mißbildungskrankheiten. – Münchener Med. Wochenschr. **97**: 1673–1677.

Bull, S., K. Fletcher, A.R. Boobis & J.M. Battershill (2006): Evidence for genotoxicity of pesticides in pesticide applicators: a review. – Mutagenesis **21**: 93–103.

Burkhart, J.G., G. Ankley, H. Carpenter, D. Fort, D. Gardiner, H. Gardner, R. Hale, J.C. Helgen, P. Jepson, D. Johnson, M. Lannoo, D. Lee, J. Lary, R. Levey, J. Magner, C. Meteyer, M.D. Shleby & G. Lucier (2000): Strategies for assessing the implications of frog malformations for environmental and human health. – Environ. Health Perspectives **108**: 83–90.

Bustuoabad, O.D., J. Herkovits & A. Pisanó (1977): Different sensitivity to lithium ion during the segmentation of *Bufo arenarum* eggs. – Acta Embryol. Exper. **3**: 271–282.

Caldwell, J.P. (1982): Disruptive selection: a tail color polymorphism in *Acris* tadpoles in response to differential predation. – Can. J. Zool. **60**: 2818–2827.

Cambar, R. & J.-D. Gipouloux (1956): Table chronologique du développement embryonnaire et larvaire du crapaud commun: *Bufo bufo* (L.). – Bull. Biol. Fr. Belg. **90**: 1–11.

Canfield, J.T., S.M. Kersten & P. Vanselow (2000): 1997–1999 Field Season Report Minnesota Malformed Frog Investigation. – Minnesota (Minnesota Pollution Control Agency).

Carey, C. (1993): Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. – Conserv. Biol. **7**: 355–362.

Carey, C., D.F. Bradford, J.L. Brunner, J.P. Collins, E.W. Davidson, J.E. Longcore, M. Ouellet, A.P. Pessier & D.M. Schock (2003): Biotic factors in amphibian population declines. – Pp. 153–208 in Linder, G., S.K. Krest & D.W. Sparling (eds.): Amphibian Decline: An Integrated Analysis of Multiple Stressor Effects. – Boca Raton (SETAC).

Casanovas, P. & C.A. Úbeda (2006): A*lsodes gargola* (Rana del catedral). Predation. – Herpetol. Rev. **37**: 439–440.

Caselitz, F.-H. (1966): *Pseudomonas-Aeromonas* und ihre humanmedizinische Bedeutung. – Jena (Gustav Fischer).

Chovanec, A. (1992): The influence of tadpole swimming behaviour on predation by dragonfly nymphs. – Amphibia-Reptilia **13**: 341–349.

Christaller, J. (1983): Vorkommen, Phänologie und Ökologie der Amphibien des Enzkreises. – Jh. Ges. Naturk. Württ. **138**: 153–182.

Clergue-Gazeau, M. (1971): L'Euprocte pyrénéen. Conséquences de la vie cavernicole sur son développement et sa reproduction. – Ann. Spéléol. **26**: 825–960.

Coleman, M. & V. Beral (1988): A review of epidemiological studies of the health effects of living near or working with electricity generation and transmission equipment. – Int. J. Epidemiol. **17**: 1–13.

Collenot, A. (1965): Recherches comparatives sur l'inversion sexuelle par les hormones stéroides chez les amphibiens. – Mém. Soc. Zool. France **33**: 1–141.

Collenot, A. & G. Collenot (1985): Une nouvelle mutation récessive du Triton *Pleurodeles waltlii*: apxé, caractérisée par l'absence de pigmentation xanthophorique à l'éclosion. – Arch. Anat. Microscop. Morph. Exp. **74**: 275.

Collenot, A., C. Dournon & M. Lauthier (1989): Genetic evidence for linkage with the Z and W sex chromosomes of two distinct couples of alleles controlling larval and postmetamorphic skin pigmentation in salamander. – Biol. Cell **67**: 1–7.

Colt, J., K. Orwicz & D. Brooks (1984): Gas bubble disease in the African clawed frog, *Xenopus laevis*. – J. Herpetol. **18**: 131–137.

Conter, A. & A. Jaylet (1974): A triple translocation in the newt *Pleurodeles waltlii*. – Chromosoma **46**: 37–58.

Cooke, A.S. (1971): The effects of pp'-DDT on tadpoles of the common frog (*Rana temporaria*). – Environ. Pollut. **1**: 57–71.

Cooke, A.S. (1972): The effects of DDT, dieldrin and 2,4-D on amphibian spawn and tadpoles. – Environ. Pollut. **3**: 51–68.

Cooke, A.S. (1973): Response of *Rana temporaria* tadpoles to chronic doses of pp'-DDT. – Copeia **1973**: 647–652.

Cooke, A.S. (1981): Tadpoles as indicators of harmful levels of pollution in the field. – Environ. Pollut. **25**: 123–133.

Cowman, D.F. & L.E. Mazanti (2000): Ecotoxicology of "new generation" pesticides to amphibians. – Pp. 233–268 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. – Pensacola, Florida (SETAC Press).

Crawshaw, G.J. (2000): Diseases and pathology of amphibians and reptiles. – Pp. 199–231 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. – Pensacola, Florida (SETAC Press).

Cummer, M.R., D.E. Green & E.M. O'Neill (2005): Aquatic chytrid pathogen detected in terrestrial plethodontid salamander. – Herpetol. Rev. **36**: 248–249.

Cunningham, A.A., T.E.S. Langton, P.M. Bennett, S.E.N. Drury, R.E. Gough & J.K. Kirkwood (1993): Unusual mortality associated with poxvirus-like particles in frogs (*Rana temporaria*). – Vet. Rec. **133**: 141–142.

Davidson, E.H. (1976): Chapter 2: The onset of genome control in embryogenesis. – Pp. 27–55 in Davidson, E.H. (ed.): Gene Activity in Early Development (2nd ed.). – New York (Acad. Press).

Dawson, D.A., E.F. Stebler, S.L. Burks & J.A. Bantle (1988): Evaluation of the developmental toxicity of metal-contaminated sediments using short-term fathead minnow and frog embryo-larval assays. – Environ. Toxicol. Chem. **7**: 27–34.

Degitz, S.J., P.A. Kosian, E.A. Makynen, K.M. Jensen & G.T. Ankley (2000): Stage- and species-specific developmental toxicity of all-trans retinoic acid in four native North American ranids and *Xenopus laevis*. – Toxicol. Sci. **57**: 264–274.

Delarue, M. (1974): Étude de l'hybridation par la greffe nucléaire et par croisement chez les bufonidés (Amphibiens, Anoures). – Ann. Embr. Morph. **7**: 425–443.

Delarue, M. (1982): Embryogenèse et relations nucléocytoplasmiques chez les hybrides expérimentaux entre *Bufo bufo* L., *Bufo viridis* Laur. et *Bufo calamita* Laur. – Amphibia-Reptilia **3**: 161–178.

Densmore, C.L. & D.E. Green (2007): Diseases of amphibians. – ILAR Journal **48(3)**: 235–254.

Dhaliwal, S.S. & D.A. Griffiths (1963): Fungal disease in Malayan toads: an acute lethal inflammatory reaction. – Nature **197**: 467–469.

Docherty, D.E., C.U. Meteyer, J. Wang, J. Mao, S.T. Case & V.G. Chinchar (2003): Diagnostic and molecular evaluation of three iridovirus-associated salamander mortality events. – J. Wildl. Diseases **39**: 556–566.

Dournon, C. (1983): Développement somatique et inversion sexuelle sous l'action de la température chez les amphibiens. – Proc. Int. Coll. Path. Rept. Amph. 29.9.–2.10.1982, Angers, France **1**: 227–231.

Dournon, C., A. Bautz, H. Membre, M. Lauthier & A. Collenot (1998): Expression of hindlimb abnormalities under rearing temperature effects during the larval development of the salamander *Pleurodeles waltl* (urodele amphibian). – Develop. Growth Differ. **40**: 555–565.

Droin, A. (1967): Une mutation létale récessive "otl" (otoliths less) chez *Xenopus laevis* Daudin. – Rev. Suisse Zool. **74**: 628–636.

Droin, A. (1978): Two recessive lethal mutations, hooked tail tip and narrow head, affecting development of the young tadpoles of *Xenopus borealis*. – Rev. Suisse Zool. **85**: 809–816.

Droin, A. (1992): Genetic and experimental studies on a new pigment mutant in *Xenopus laevis*. – J. exp. Zool. **264**: 196–205.

Droin, A. & M.L. Beauchemin (1975): ‚Immobile' (im), a recessive lethal mutation of *Xenopus laevis* tadpoles. – J. Embryol. exp. Morphol. **34(2)**: 435–449.

Droin, A. & B. Colombelli (1982): "Bloated-1" and "bloated-2", two recessive mutations in *Xenopus borealis* and *Xenopus muelleri*. – Acta Embryol. Morphol. Exp. **3(2)**: 117–126.

Droin, A. & M. Fischberg (1980): Abnormal limbs (abl), a recessive mutation affecting the tadpole of *Xenopus l. laevis*. – Experientia **36**: 1286–1288.

Droin, A. & M. Fischberg (1984): Two recessive mutations with maternal effect upon color and cleavage of *Xenopus laevis laevis* eggs. – Wilhelm-Roux's Arch. Develop. Biol. **193(2)**: 86–89.

Droin, A., V. Uehlinger & J. Reynaud (1970): A recessive lethal mutation, "bt" (bent tail), in *Xenopus laevis* Daudin. – Rev. Suisse Zool. **77**: 596–603.

Dubois, A. (1979): Anomalies and mutations in natural populations of the *Rana* "*esculenta*" complex (Amphibia, Anura). – Mitt. Zool. Mus. Berlin **55**: 59–87.

Dubois, A. (1984): L'anomalie P des grenouilles vertes (complexe de *Rana* kl. *esculenta* Linné 1758) et les anomalies voisines chez les amphibiens. – C.r. Coll. Int. Pathol. Rept. Amph. **1**: 215–221.

Dubois, A. (2012): Jean Rostand: un biologiste contre le nucléaire. Paris (Berg International).

Dubois, A. (2014): The anomaly P in Palaearctic green frogs of the genus *Pelophylax* (Ranidae). – Pp. 96–104 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. – Ekaterinburg (Uralskii Federalnii Universitet).

Dubois, A. (2017): Rostand's anomaly P in Palearctic green frogs (*Pelophylax*) and similar anomalies in amphibians. – Mertensiella **25**: 49–56.

Duda, M. (2008): First record of a natural male hybrid of *Bufo* (*Pseudepidalea*) *viridis* Laurenti, 1768 and *Bufo* (*Bufo*) *bufo* Linnaeus 1758 from Austria. – Herpetozoa **20**: 184–186.

Duméril, A. (1867): Description de diverses monstruosités observées à la Ménagerie des Reptiles du Muséum d'Histoire Naturelle sur les batraciens urodèle à branchies extérieures dits axolotls. – Mem. Nouv. Arch. Mus. Hist. Nat. **3**: 119–130.

Dumpert, K. & E. Zietz (1984): Platanna (*Xenopus laevis*) as a test organism for determining the embryotoxic effects of environmental chemicals. – Ecotoxicol. Environ. Safety **8**: 55–74.

Eaton, B.R., S. Eaves, C. Stevens, A. Puchniak & C.A. Paszkowski (2004): Deformity levels in wild populations of the wood frog (*Rana sylvatica*) in three ecoregions of Western Canada. – J. Herpetol. **38**: 283–287.

Ellegren, H., G. Lindgren, C.R. Primmer & A.P. Møller (1997): Fitness loss and germline mutations in barn swallows breeding in Chernobyl. – Nature **389**: 593–596.

Elsdale, T.R., M. Fischberg & S. Smith (1958): A mutation that reduces nucleolar number in *Xenopus laevis*. – Exp. Cell Res. **14**: 642–643.

Emerson, H. & C. Norris (1905): "Red-leg" – an infectious disease of frogs. – J. exp. Med. **7**: 32–58.

Endres, H. & H.E. Walter (1895): Anstichversuche an Eiern von *Rana fusca*; Erster Theil. – Arch. Entwickl-mech. **2**: 38–51.

Ensinck, F.H. (1978): Zur Zucht des Beutelfrosches *Gastrotheca marsupiata*. – Das Aquarium **111**: 401–405.

Ernst, C.H. (1995): Freshwater and terrestrial turtles of the United States: status and prognosis. – Bull. Chicago Herpetol. Soc. **30**: 225–233.

Ettling, J.A., M.D. Wanner, C.D. Schuette, S.L. Armstrong & A.S. Pedigo (2013): Captive reproduction and husbandry of adult Ozark hellbenders, *Crytobranchus alleganiensis bishopi*. – Herpetol. Rev. **44:** 605–610.

Falconer, D.S. (1989): Introduction to Quantitative Genetics (3rd ed.). – London (Longman).

Fellers, G.M., D.E. Green & J.E. Longcore (2001): Oral chytridiomycosis in the mountain yellow-legged frog (*Rana muscosa*). – Copeia **2001**: 945–953.

Fernandez, M. & J.C. Beetschen (1975): The role of temperature in phenotypic expression of amphibian embryos

Pleurodeles waltlii homozygous for the heat-sensitive mutation ac (caudal ascites). – J. Embryol. exp. Morphol. **34**: 221–252.

Fernandez, M., J. L'Haridon, L. Gauthier & C. Zoll-Moreux (1993): Amphibian micronucleus test(s): a simple and reliable method for evaluating in vivo genotoxic effects of freshwater pollutants and radiation. Initial assessment. – Mutation Res. **292**: 83–99.

Ferraro, T.J. & S. Burgin (1993): Review of environmental factors influencing the decline of Australian frogs. – Pp. 205–218 in Lunney, D. & D. Ayers (eds.): Herpetology in Australia: A Diverse Discipline. – Sydney (Surrey Beatty).

Feshchenko, S.P., H.C. Schröder, W.E.G. Müller & G.I. Lazjuk (2002): Congenital malformations among newborns and developmental abnormalities among human embryos in Belarus after Chernobyl accident. – Cell. Mol. Biol. **48**: 423–426.

Fischberg, M. (1948): Experimentelle Auslösung von Heteroploidie durch Kältebehandlung der Eier von *Triturus alpestris* aus verschiedenen Populationen. – Genetica **24**: 213–329.

Fite, K.V., A. Blaustein, L. Bengston & H.E. Hewitt (1998): Evidence of retinal light damage in *Rana cascadae*: a declining amphibian species. – Copeia **1998**: 906–914.

Flindt, R. (1984): Gutachten über das Vorkommen von Amphibien im Raum Illingen/Roßwag und das Auftreten missgebildeter Wechselkröten (*Bufo viridis*) im Steinbruch Zimmermann in Vaihingen-Roßwag. Unpublished Report. – Stuttgart (Ministry of Food, Agriculture, Environment and Forestry, Baden-Württemberg).

Flindt, R. (1985a): Untersuchungen zum Auftreten von mißgebildeten Wechselkröten (*Bufo viridis*) in einem Steinbruch in Vaihingen-Roßwag. – Jh. Ges. Naturk. Württ. **140**: 213–233.

Flindt, R. (1985b): Latenter Albinismus und Mißbildungen bei Kaulquappen von Wechselkröten *Bufo viridis* Laurenti, 1768. – Salamandra **21**: 298–303.

Flindt, R. & H. Hemmer (1967a): Variation und wahrscheinliche Hybridisation in einer *Bufo viridis/Bufo calamita*-Population. – Zool. Beitr. (NF) **13**: 149–160.

Flindt, R. & H. Hemmer (1967b): Nachweis natürlicher Bastardierung von *Bufo calamita* und *Bufo viridis*. – Zool. Anz. **178**: 419–429.

Flindt, R. & H. Hemmer (1969): Vergleichende Untersuchung zum Aufbau zweier unterschiedlich zusammengesetzter *Bufo viridis/Bufo calamita*-Populationen. – Zool. Beitr. **15**: 219–235.

Flindt, R. & H. Hemmer (1970a): Kreuzungsversuche mit *Bufo calamita, Bufo viridis viridis* und *Bufo viridis arabicus* (Amphibia, Bufonidae). – Salamandra **6**: 94–98.

Flindt, R. & H. Hemmer (1970b): Vergleichende Untersuchungen über das Larval- und Postmetamorphose-Wachstum von *Bufo calamita* Laur., *Bufo viridis* Laur. und deren Bastarden. – Z. wiss. Zool. **181**: 317–330.

Flindt, R. & H. Hemmer (1973): Die Bedeutung des Serumeiweißbildes zur Diagnose von *Bufo calamita* Laur., *Bufo viridis* Laur. und deren Bastarden (Amphibia, Anura, Bufonidae). – Experientia **29**: 361–364.

Flindt, R., H. Hemmer & R. Schipp (1968): Zur Morphogenese von Mißbildungen bei Bastardlarven *Bufo calamita* ♀ x *Bufo viridis* ♂: Störungen in der Ausbildung des Axialskeletts. – Zool. Jb. Anat. **85**: 51–71.

Formanowicz, D.R. & E.D. Brodie (1982): Relative palatabilities of members of a larval amphibian community. – Copeia **1982**: 91–97.

Fort, D.J. & E.L. Stover (1997): Development of short-term, whole-embryo assays to evaluate detrimental effects on amphibian limb development and metamorphosis using *Xenopus laevis*. – Pp. 376–390 in Dwyer, F.J., T.R. Doane & M.L. Hinman (eds.): Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment (Vol. 6). – West Conshohocken, PA (Amer. Soc. Testing Materials).

Fort, D.J., R.L. Rogers, H.F. Copley, L.A. Bruning, E.L. Stover, J.C. Helgen & J.G. Burkhart (1999): Progress toward identifying causes of maldevelopment induced in *Xenopus* by pond water and sediment extracts from Minnesota, USA. – Environ. Toxicol. Chem. **18**: 2316–2324.

Fort, D.J., R.L. Rogers & J.P. Bacon (2006a): Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part II. Progress towards characterization of chemical stressors. – Appl. Herpetol. **3**: 143–172.

Fort, D.J., R.L. Rogers, B.O. Buzzard, G.D. Anderson & J.P. Bacon (2006b): Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part III. Microcosm-based exposure pathway assessment. – Appl. Herpetol. **3**: 257–277.

Franke, B., K. Henle, M. Kötter, E. Krüger & B. Steinhilber-Schwab (1981): Radioaktivitätsmessungen im Steinbruch Zimmermann. Detaillierte Auswertungen mit Berücksichtigung der Kritik der Landesanstalt für Umwelt. Unveröff. Bericht. – Heidelberg (Institut für Energie- und Umweltfragen).

Frost, S.K., F. Briggs & G.M. Malacinski (1984): A color atlas of pigment genes in the Mexican axolotl (*Ambystoma mexicanum*). – Differentiation **26**: 182–188.

Frye, F.L. (1985): An unusual epizootic of anuran aeromoniasis. – JAVMA **187**: 1223–1224.

Gallien, L. (1969a): Anomalies chromosomiques provoquées et mutations chez les amphibiens. – Ann. Biol. **8**: 319–331.

Gallien, L. (1969b): Spontaneous and experimental mutations in the newt *Pleurodeles waltlii* Michah. – Pp. 35–42 in Mizell, M. (ed.): Biology of Amphibian Tumors. – New York (Springer).

Gallien, L. & A. Collenot (1964): On a lethal recessive mutant, the syndrome of which is associated with mitotic disturbances, in the newt, *Pleurodeles waltlii*. – C.r. Hebd. Seances Acad. Sc. Paris **259**: 4847–4849.

Gallien, L., M. Labrousse & J.C. Lacroix (1965): Modifications expérimentales du caryotype chez un Amphibien Urodèle (*Pleurodeles waltlii* Michah.) par irradiation de l'œuf et la greffe nucléaire. – Rev. Suisse Zool. **72**: 59–85.

Garber, E.A.E., J.L. Erb, J.G. Downward, E.M. Priuska, J.L. Wittliff, W. Feng, J. Magner & G.L. Larsen (2001): Biosensor, ELISA and frog embryo teratogenesis assay: *Xenopus* (FETAX) analysis of water associated with frog malformations in Minnesota. – Proceed. SPIE **4206**: 147–158.

Garber, E.A.E., J.E. Erb, J. Magner & G. Larsen (2004): Low levels of sodium and potassium in the water from wetlands in Minnesota that contained malformed frogs affect the rate of *Xenopus* development. – Environ. Monitor. Assess. **90**: 45–64.

Gardiner, D.M. & D.M. Hoppe (1999): Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). – J. exp. Zool. **284**: 207–216.

Gardiner, D.M., A. Ndayibagira, F. Grün & B. Blumberg (2003): Deformed frogs and environmental retinoids. – Pure Appl. Chem. **75**: 2263–2273.

Gasche, P. (1946): Zur Frage des Angriffspunktes des Thiouracil. Versuche an Xenopuslarven. 1. Mitteilung. – Experientia **2(1)**: 24–26.

Gauthier, L. (1996): The amphibian micronucleus test, a model for in vivo monitoring of genotoxic aquatic pollution. – Alytes **14**: 53–84.

Gelnarová, J. (1987a): Larvy obojzivelnici (Amphibian larvae). – Pp. 196–205 in Svobodova, Z., J. Gelnarová, J. Justýn,

V. Krupauer, J. Máchová, L. Simanov, V. Valentová, B. Vykusová & E. Wohlgemuth (eds.): Toxikologie Vodnich Zivocichu (Toxicology of Water Animals). – Prague (Ministry of Agriculture).

Gelnarová, J. (1987b): Effects of pesticides on the development of frogs. – Pp. 18–21 in Ruzicka, V. (ed.): Research Activity Report 1984–1986. – Prague (Dept. Biodiagnostics, Inst. Landscape Ecol., Czech. Acad. Sci.).

Geoffroy Saint-Hilaire, I. (1832): Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux. Volume 1. – Paris (Baillière).

Geoffroy Saint-Hilaire, I. (1836): Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux. Volume 3. – Paris (Baillière).

Ghate, H.V. (1985): Dithiocarbamate induced teratogenesis in frog embryos. – Riv. Biol. **78**: 288–291.

Gibbs, E.L., G.W. Nace & M.B. Emmons (1971): The live frog is almost dead. – BioScience **21**: 1027–1034.

Gilberston, M., T.J. Kubiak, J.P. Ludwig & G. Fox (1991): Great Lakes embryo mortality, edema and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarities to chicken-edema disease. – J. Toxicol. Environ. Health **33**: 455–520.

Gilbert, S.F. & D. Epel (2008): Ecological Developmental Biology. – Sunderland, MA (Sinauer Associates).

Gillespie, L.L. & J.B. Armstrong (1979): Induction of triploid and gynogenetic diploid axolotls (*Ambystoma mexicanum*) by hydrostatic pressure. – J. exp. Zool. **210**: 117–122.

Gilligan, D.M., L.M. Woodworth, M.E. Montgomery, D.A. Briscoe & R. Frankham (1997): Is mutation accumulation a threat to the survival of endangered species? – Conserv. Biol. **11**: 1235–1241.

Girvan, J.E., W.M. Olson & B.K. Hall (2002): Hind-limb regeneration in the dwarf African clawed frog, *Hymenochirus boettgeri* (Anura: Pipidae). – J. Herpetol. **36**: 537–543.

Glaw, F. (1987): Probleme mit Streichholzbeinen bei der Nachzucht von *Discoglossus pictus*. – herpetofauna, Weinstadt **9(51)**: 30–33.

Glaw, F. & M. Vences (1989): Zur Verbreitung von Wechselkröte (*Bufo viridis* Laurenti, 1768) und Kreuzkröte (*Bufo calamita* Laurenti, 1768) im nördlichen Rheinland. – Jb. Feldherpetol. **3**: 61–75.

Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. – Herpetologica **16**: 183–190.

Gounon, P. & A. Collenot (1975): Analyse expérimentale de l'expression de la mutation léthale récessive ("ulcère") chez l'embryon de *Pleurodeles waltlii* (Amphibien Urodèle). – Experientia **31**: 1461–1464.

Grant, K.P. & L.E. Licht (1995): Effects of ultraviolet radiation on life-history stages of anurans from Ontario, Canada. – Can. J. Zool. **73**: 2292–2301.

Gray, M.J., D.L. Miller & J.T. Hoveman (2009): Ecology and pathology of amphibian ranaviruses. – Diseases Aquat. Organisms **87**: 243–266.

Gray, R.H. (2000a): Morphological abnormalities in Illinois cricket frogs, *Acris crepitans*, 1968–71. – J. Iowa Acad. Sci. **107(3)**: 92–95.

Gray, R.H. (2000b): Historical occurrence of malformations in the cricket frog, *Acris crepitans*, in Illinois. – Trans. Illinois State Acad. Sci. **93**: 279–284.

Gray, R.H. (2002): Amphibian malformations and population declines may not be linked. – Proc. Intern. Symp. Pathol. Reptiles Amphibian **6**: 49–53.

Green, D.E. (2001): Pathology of Amphibia. – Pp. 401–485 in Wright, K.M. & B.R. Whitaker (eds.): Amphibian Medicine and Captive Husbandry. – Malabar (Krieger Publ. Comp.).

Gregoire, D.R. & M.S. Gunzburger (2008): Effects of predatory fish on survival and behavior of larval gopher frogs (*Rana capito*) and southern leopard frogs (*Rana sphenocephala*). – J. Herpetol. **41**: 97–103.

Grimm, H. (1953): Ödemkrankheit bei Krallenfröschen. – Der zool. Garten **20**: 98–101.

Gross, L. (1961): Oncogenic Viruses. – New York (Pergamon Press).

Grosse, W.-R. (1976): Krötenbastarde (Amphibia, Anura, Bufonidae). III. Beitrag zur Herpetofauna des Leipziger Auenwaldes. – Faun. Abh. Staatl. Mus. Tierkde Dresden **6**: 155–161.

Grosse, W.-R. (1977): Krötenbastarde. – Aquar. Terr. **24**: 153.

Günther, R. (1996): Die Amphibien und Reptilien Deutschlands. – Jena (Fischer).

Günther, R. & R. Podloucky (1996): 6.14. Wechselkröte – *Bufo viridis* Laurenti, 1768). – Pp. 322–343 in Günther, R. (ed.): Die Amphibien und Reptilien Deutschlands. – Jena (Fischer).

Guizard, A.V., O. Boutou, D. Pottier, X. Troussard, D. Pheby, G. Launoy, R. Slama, A. Spira & ARKM (2001). The incidence of childhood leukaemia around the La Hague nuclear waste reprocessing plant (France): a survey for the years 1978–1998. – J. Epidemiol. Community Health **55**: 469–474.

Gurdon, J.B. & H.R. Woodland (1975): *Xenopus*. – Pp. 35–50 in King, R.C. (ed.): Handbook of Genetics. – New York (Plenum Press).

Hachtel, M. (2011): 3.2 Bergmolch – *Mesotriton alpestris*. – Pp. 337–374 in Hachtel, M., M. Schlüpmann, K. Weddeling, B. Thiesmeier, A. Geiger & C. Willigalla (eds.): Handbuch der Amphibien und Reptilien Nordrhein-Westfalens. – Bielefeld (Laurenti Verlag).

Hadorn, E. (1955): Letalfaktoren und ihre Bedeutung für Erbpathologie und Genphysiologie der Entwicklung. – Stuttgart (Thieme).

Haidacher, S. & G. Fachbach (1991): Experimentelle Säuretoleranzanalysen von Laich und Larven heimischer Amphibien. – Salamandra **29**: 108–118.

Hall, M. (1942): The mode of action of lithium salts in amphibian development. – J. exp. Zool. **89**: 1–30.

Halliday, T. & M. Tejedo (1995): Intrasexual selection and alternative mating behaviour. – Pp. 419–468 in Heatwole, H. & B.K. Sullivan (eds.): Amphibian Biology. Vol. 2: Social Behaviour. – Sydney (Surry Beatty).

Harkey, G.A. & R.D. Semlitsch (1988): Effects of temperature on growth, development and color polymorphisms in the ornate chorus frog *Pseudacris ornata*. – Copeia **1988**: 1001–1007.

Harmon, J.E., M.F. Boehm, R.A. Heyman & D.J. Mangelsdorf (1995): Activation of mammalian retinoid X receptors by the insect growth regulator methoprene. – Proc. Natl. Acad. Sci. **92**: 6157–6160.

Harris, M.L., C.A. Bishop, J. Struger, B. Ripley & J.P. Bogart (1998): The functional integrity of northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*) populations in orchard wetlands. II. Effects of pesticides and eutrophic conditions on early life stage development. – Environ. Toxicol. Chem. **17**: 1351–1363.

Hart, D.R. & J.B. Armstrong (1984): Assessment of mutagenic damage by monofunctional alkylating agents and gamma radiation in haploid and diploid frogs, *Xenopus laevis*. – Environ. Mutagenesis **6**: 719–735.

Harvey, H.H. (1967): Supersaturation of lake water with a precaution to hatchery usage. – Trans. Am. Fish Soc. **96**: 194–201.

Hayes, T.B. (2000): Endocrine disruption in amphibians. – Pp. 573–593 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. – Pensacola, Florida (SETAC Press).

Hays, J.B., A.R. Blaustein, J.M. Kiesecker, P.D. Hoffmann, I. Pandelova, D. Coyle & T. Richardson (1996): Developmental responses of amphibians to solar and artificial UVB sources: a comparative study. – Photochem. Photobiol. **64(3)**: 449–456.

Hecnar, S.J. (1995): Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from Southern Ontario. – Environ. Toxicol. Chem. **14**: 2131–2137.

Hedrick, P.W. (1994): Purging inbreeding depression and the probability of extinction: full-sib mating. – Heredity **73**: 363–372.

Helgen, J.C., R.G. McKinnell & M.C. Gernes (1998): Investigation of malformed northern leopard frogs in Minnesota. – Pp. 288–297 in Lannoo, M.J. (ed.): Status & Conservation of Midwestern Amphibians. – Iowa (Univ. Iowa Press).

Helgen, J.C., M.C. Gernes, S.M. Kersten, J.W. Chirhart, J.T. Canfield, D. Bowers, J. Haferman, R.G. McKinnell & D.M. Hoppe (2000): Field investigations of malformed frogs in Minnesota 1993–97. – J. Iowa Acad. Sci. **107(3)**: 96–112.

Hellmich, W. (1929): Mehrfachbildungen von Extremitäten bei Amphibien (Naturfunde). – Roux Arch. Entwickl.-mech. **115**: 409–414.

Hemingway, V., J. Brunner, R. Speare & L. Berger (2009): Viral and bacterial diseases of amphibians. – Pp. 2963–2985 in Heatwole, H. & J.W. Wilkinson (eds.): Amphibian Biology. Vol. 8: Amphibian Decline: Diseases, Parasites, Maladies and Pollution. – Chipping Norton (Surrey Beatty).

Hemsworth, B.N. & A.A. Wardhaugh (1978): Embryopathies due to spermatozoal impairment in *Xenopus laevi*s. – Mutation Res. **51**: 45–53.

Henle, K. (1981): A unique case of malformations in a natural population of the green toad (*Bufo viridis*) and its meaning for environmental politics. – Brit. Herp. Soc. Bull. **4**: 48–49.

Henle, K. (1982): Ein außergewöhnlicher Fall von Mißbildungen bei der Wechselkröte (*Bufo viridis*). – Unicornis (Schwäbisch Gmünd) **82(1)**: 40–42.

Henle, K. (1992): Zur Amphibienfauna Perus nebst Beschreibung eines neuen *Eleutherodactylus* (Leptodactylidae). – Bonn. Zool. Beitr. **43**: 79–129.

Henle, K. (2014): Anomalies in natural populations of amphibians: overview, patterns and causes, methodological recommendations. – Pp. 183–190 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. – Ekaterinburg (Uralskii Federalnii Universitet).

Henle, K. & B. Streit (1990): Kritische Betrachtungen zum Artenrückgang bei Amphibien und Reptilien und zu dessen Ursachen. – Natur und Landschaft **65**: 347–361.

Henle, K., A. Dubois & V. Vershinin (2017a): A review of anomalies in natural populations of amphibians and their potential causes. – Mertensiella **25**: 57–164.

Henle, K., K. Rimpp, J. Margraf & A. Dubois (2017b): The "Roßwag affair": history and controversy of the discovery of an exceptional case of mass anomalies in the toad *Bufotes viridis*. – Mertensiella **25**: 165–184.

Henle, K., A. Dubois & V. Vershinin (2017c): Commented glossary, terminology and synonymies of anomalies in natural populations of amphibians. – Mertensiella **25**: 9–48.

Henrikson, B.-I. (1990): Predation on amphibian eggs and tadpoles by common predators in acidified lakes. – Holarctic Ecol. **13**: 201–206.

Hertwig, G. (1913): Parthenogenesis bei Wirbeltieren, hervorgerufen durch artfremden radiumbestrahlten Samen. – Arch. Mikr. Anat. **81**: 87–127.

Hertwig, G. (1918): Kreuzungsversuche an Amphibien. – Arch. Mikr. Anat. **91**: 203–271.

Hertwig, G. (1930): Kern- und Zellgrößenunterschied der Eltern als Ursache des verschiedenen Ausfalls reziproker Krötenbastarde. – Z. anat. Entwickl.-gesch. **92**: 718–739.

Hertwig, G. (1953): Das Auftreten eines Chordoms bei einer Krötenbastardlarve und die möglichen Ursachen seiner Entstehung. – Zbl. allgem. Pathol. Pathol. Anat. **91**: 56–64.

Hertwig, G. & I. Weiss (1955): Die Auswirkung der Vereinigung von Genomen, die ungleichen Kernklassen angehören, auf die Entwicklungsfähigkeit reziproker Krötenbastarde. – Gegenbaurs Morphol. Jb. **95**: 1–34.

Hertwig, G., I. Weiss & C. Ziemann (1959): Unterschiedliche Ergebnisse reziproker Kreuzungen der drei europäischen Krötenarten unter besonderer Berücksichtigung ihrer Kern- und Zellgrößen. – Biol. Zentralbl. **78**: 675–702.

Hertwig, O. (1892): Urmund und Spina bifida. – Arch. Mikr. Anat. **39**: 353–499.

Hertwig, O. (1894): Über den Einfluß äußerer Bedingungen auf die Entwicklung des Froscheies. – Sitz.-ber. kgl. preuss. Akad. Wiss. Berlin **17**: 311–317.

Hertwig, O. (1898): Über den Einfluß der Temperatur auf die Entwicklung von *Rana fusca* und *Rana esculenta.* – Arch. Mikr. Anat. Entwickl.-gesch. **51**: 319–381.

Hertwig, O. (1911): Die Radiumkrankheit tierischer Keimzellen. Ein Beitrag zur experimentellen Zeugungs- und Vererbungslehre. – Arch. Mikr. Anat. **77**: 1–164.

Hertwig, P. (1923): Bastardierungsversuche mit entkernten Amphibieneiern. – Roux Arch. Entwickl.-mech. **100**: 41–60.

Heselhaus, R. (1983): Zum Problem der "Streichholzvorderbeine" bei Dendrobatidennachzuchten. – herpetofauna, Weinstadt **5(26)**: 22–24.

Hesse-Honegger, C. & P. Wallimann (2008): Malformation of true bug (Heteroptera): a phenotype field study on the possible influence of artificial low-level radioactivity. – Chemistry & Biodiversity **5**: 499–539.

Hoadley, L. (1938): The effects of supramaximum temperatures on the development of *Rana pipiens*. – Growth **2**: 25–48.

Hoperskaya, O.A. (1975): The development of animals homozygous for a mutation causing periodic albinism (aP) in *Xenopus laevis*. – J. Embryol. exp. Morphol. **34**: 253–264.

Hoperskaya, O.A. (1981): Induction – The main principle of melanogenesis in early development. – Differentiation **20**: 104–116.

Hopkins, W.A., J. Congdon & J.K. Ray (2000): Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal-burning power plant. – Environ. Toxicol. Chem. **19**: 862–868.

Hoppe, D.M. (2000): History of Minnesota frog abnormalities: do recent findings represent a new phenomenon? – J. Iowa Acad. Sci. **107(3)**: 86–89.

Humphrey, R.R. (1967a): Albino axolotls from an albino tiger salamander through hybridization. – J. Hered. **58**: 95–101.

Humphrey, R.R. (1967b): Genetic and experimental studies on a lethal trait ("short toes") in the Mexican axolotl (*Ambystoma mexicanum*). – J. exp. Zool. **164**: 281–295.

Humphrey, R.R. (1972): Genetic and experimental studies on a mutant gene I determining absence of heart action in embryos of the Mexican axolotl (*Ambystoma mexicanum*). – Develop. Biol. **27**: 365–375.

Humphrey, R.R. (1973): Experimental studies on a new lethal trait in Mexican axolotls of the Wista Institute white strain. – J. exp. Zool. **183**: 201–208.

Humphrey, R.R. (1975): The axolotl, *Ambystoma mexicanum*. – Pp. 3–17 in King, R.C. (ed.): Handbook of Genetics. – New York (Plenum Press).

Humphrey, R.R. & H.-M. Chung (1978): Experimental studies on two mutant genes, r and x, in the Mexican axolotl (A*mbystoma mexicanum*). – Develop. Biol. **62**: 34–43.

Hunsaker, D. & F.E. Potter (1960): "Red leg" in a natural population of amphibians. – Herpetologica **16**: 285–286.

Hurlbert, S.H. (1970): Predator responses to the vermilion-spotted newt (*Notophthalmus viridescens*). – J. Herpetol. **4**: 47–55.

Hutt, F.B. (1945): Complete albinism in the mud puppy, *Necturus*. – J. Hered. **36**: 145–147.

Inoue, S. & M. Singer (1970): Experiments on a spontaneously originated visceral tumor in the newt, *Triturus pyrrhogaster*. – Ann. NY Acad. Sci. **174**: 729–764.

Jara, Z. (1963): O niektórych konsekwencjach przystosowania ryb do zycia w srodowisku wodnym. – Przeglad Zool. Wroclaw **7**: 207–218.

Jayawardena, U.A. & R.S. Rajakaruna (2013): Understanding possible ethiologies of amphibian crisis in Sri Lanka. – Froglog **21(4)**: 47–49.

Jayawardena, U.A., R.S. Rajakaruna, A.N. Navaratne & P.H. Amerasinghe (2010a): Monostome cercariae induced malformations in amphibians: effect of infection at the pre-limb-bud stage tadpoles of *Polypedates cruciger* Blyth. – J. Natn. Sci. Foundation Sri Lanka **38(4)**: 241–248.

Jayawardena, U.A., R.S. Rajakaruna, A.N. Navaratne & P.H. Amerasinghe (2010b): Toxicity of agrochemicals to common hourglass tree frog (*Polypedates cruciger*) in acute and chronic exposure. – Int. J. Agric. Biol. **12**: 641–648.

Jaylet, A. (1971): Change in the karyotype by a homozygous pericentric inversion in the newt *Pleurodeles waltlii* Michahelles. – Chromosoma **35**: 288–99.

Jaylet, A., V. Ferrier & B. Andrieux (1980): Sur un facteur récessif apparu après mutagenèse aux rayons X affectant l'adaptation pigmentaire chez le Triton *Pleurodeles waltlii*. – C.r. Acad. Sc. Paris **291**: 673–675.

Johnson, P.T.J. & R.B. Hartson (2009): All hosts are not equal: explaining differential patterns of malformations in an amphibian community. – J. Anim. Ecol. **78**: 191–201.

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie & A.E. Launer (1999): The effect of trematode infection on amphibian limb development and survivorship. – Science **284**: 802–804.

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie, J.K. Reaser & A.E. Launer (2001a): Morphological abnormality patterns in a California amphibian community. – Herpetologica **57**: 336–352.

Johnson, P.T.J., K.B. Lunde, R.W. Haight, J. Bowerman & A.R. Blaustein (2001b): *Ribeiroia ondatrae* (Trematoda: Digenea) infection induces severe limb malformations in western toads (*Bufo boreas*). – Can. J. Zool. **79**: 370–379.

Johnson, P.T.J., K.B. Lunde, D.A. Zelmer & J.K. Werner (2003): Limb deformities as an emerging parasitic disease in amphibians: evidence from museum specimens and resurvey data. – Conserv. Biol. **17**: 1724–1737.

Johnson, P.T.J., E.R. Preu, D.R. Sutherland, J.M. Romansic, B. Han & A.R. Blaustein (2006): Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. – Ecology **87**: 2227–2235.

Johnson, P.T.J., M.K. Reeves, S.K. Krest & A.E. Pinkney (2010): A decade of deformities: advances in our understanding of amphibian malformations and their implications. – Pp. 511–536 in Sparling, D.W., G. Linder, C.A. Bishop & S.K. Krest (eds.): Ecotoxicology of Amphibians and Reptiles (2nd ed.). – Boca Raton (CRC Press).

Jones, P. & H. Jackson (1974): Actions of antifertility chemicals on *Xenopus laevis* spermatozoa in vitro. – J. Reprod. Fert. **38**: 347–357.

Kabisch, K. & W.-E. Engelmann (1975): Vergleichende elektrophoretische Untersuchung der α-Naphthylacetat-Esterase im Serum mitteleuropäischer Discoglossiden und Bufoniden (Amphibia, Anura). – Biol. Zentralbl. **94**: 211–214.

Kao, K.R. & R.P. Elinson (1998): The legacy of lithium effects on development. – Biol. Cell **90**: 585–590.

Kashiwagi, K. (1980): Mature haploids and their reproductive capacity in *Rana rugosa*. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **4**: 217–237.

Kawamura, T. & M. Nishioka (1978): Abnormalities in the descendants of *Rana nigromaculata* produced from irradiated eggs or sperm. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **3**: 1–187.

Kawamura, T., M. Nishioka & H. Ueda (1980): Inter- and intraspecific hybrids among Japanese, European and American toads. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **4**: 1–125.

Kirkpatrick, M. & P. Jarne (2000): The effects of a bottleneck on inbreeding depression and the genetic load. – Am. Nat. **155**: 157–167.

Kiselva, E.I. & Y.B. Manteifel (1982): Behavioural reactions of *Bufo bufo* and *Rana temporaria* tadpoles to chemical stimuli (in Russian). – Zool. Zhurnal **61**: 1669–1681.

Klatt, B. (1927): Fütterungsversuche an Tritonen. II. (Die Bedeutung der "Ausgangsgröße"). – Roux Arch. Entwickl.-mech. **109**: 176–187.

Klein, J.F. (1981): Krötenmonster im Steinbruchsee. – Kosmos, Stuttgart **1981(1)**: 24–25.

Kleinebeckel, D. (1975): Die Entstehung überzähliger Extremitäten nach Transplantation von Beinanlagen bei *Xenopus laevis*. – Roux Arch. Entwickl.-mech. **178**: 321–331.

Kluge, A.G. (1981): The life history, social organization and parental bahavior of *Hyla rosenbergi* Boulenger, a nest building gladiator frog. – Misc. Publ. Mus. Zool. Univ. Michigan **160**: 1–170.

Kneissler, N. (1981): Die Krötenmonster von Roßwag. – Umweltforum, München **6**: 14–19.

Koch, C., B. Schreiber & G. Schreiber (1939): Tumeurs produites par le goudron et le benzopyrène chez les amphibiens urodèles. – Bull. Assoc. Franç. Étude Cancer **28**: 852–859.

Koskela, P. (1974): Combination of partial adactylism and syndactylism in *Rana temporaria* L. – Aquilo Ser. Zool. **15**: 37–38.

Kosswig, C. (1951): Herpetologisches aus der Türkei. – Mitt. Mus. Naturkde Vorgeschichte **3(3)**: 13–23.

Kovacsics, M. (1981): Kröten-Mißbildungen: Spuren verwischt? – Bild der Wissenschaft, Stuttgart **1981(2)** (Akzent **9**): 3.

Kovalenko, E.E. (1994): The compound sacrum in individual variability of common platanna (*Xenopus laevis*). – Russ. J. Herpetol. **1**: 172–178.

Kovalenko, E.E. (2000a): Mass anomalies of extremities in Anura. – Z. Gen. Biol. **61(4)**: 412–427.

Kovalenko, E.E. (2000b): Variability of postcranial skeleton of tailless amphibians (Amphibia, Anura). – PhD Diss. Abstracts, St. Petersburg State University (in Russian).

Kovalenko, E.E. & Y.I. Kovalenko (1996): Certain pelvic and sacral anomalies in Anura. – Russ. J. Herpetol. **3**: 172–177.

Kovalenko, E.E. & Y.I. Kovalenko (2000): Anomalii sadnich konetschnostei u laboratornoi schporschevoi ljaguschki *Xenopus laevis* (Anura, Pipidae). 1. Fenomen massobysch anomalii [Hind limb anomalies in laboratory clawed frogs *Xenopus laevis* (Anura, Pipidae). 1. The phenomenon of mass anomalies]. – Vest. St-Peterburg. Univ., Ser. 3 (Biol.) **1(3)**: 3–22.

Krefft, G. (1938): Fütterungsversuche an Tritonen. V. (Zur Frage der Vitamine). – Roux Arch. Entwickl.-mech. **137**: 566–590.

Krintler, K. (1988): Beobachtungen zum Problem der "Streichholzbeine" bei Dendrobatiden und Hyliden. – herpetofauna, Weinstadt **10(52)**: 30–31.

Krotoski, D.M., D.C. Reinschmidt & R. Tompkins (1985): Developmental mutants isolated from wild-caught *Xenopus laevis* by gynogenesis and inbreeding. – J. exp. Zool. **233**: 443–449.

Kulzer, E. (1954): Untersuchungen über die Schreckreaktion der Erdkrötenkaulquappen (*Bufo bufo* L.). – Z. vergl. Physiol. **36**: 445–463.

Kuranova, V.N. (1998): Fauna i ekologia zemnovodnych i presmykayushichsia yugo-vostoka zapadnoi sibiri. – Tomsk (PhD, University of Tomsk).

Kuranova, V.N. (2003): Antropiceskoe vosdeistie na zemnovodnysh i presmykayushishtshsja. Problemy ish oshrany na yugo-vostoke Zapadnoi Sibiri (Anthropogenic impacts on amphibians and reptiles. Problems of conservation in southeastern West-Siberia). – Pp. 39–51 in Erdakov, L.N. & O.N. Tshernyshtshova (eds.): Amphibii i Reptilii v Zapadnoi Sibiri (Amphibians & Reptiles of Western Siberia). – Novosibirsk (Novosibirskii Gosudarstennyi Pedagog. Univ.).

Kuranova, V.N. & S.V. Saveliev (1997): The effect of radiation and chemicals on amphibian ontogeny. – Adv. Amph. Res. Former Soviet Union **2**: 167–168.

Kwet, A. & M. Di-Bernardo (1999): Pró-Mata. Anfíbios – Amphibien – Amphibians. – Porto Alegre (EDIPUCRS).

La Clair, J.J., J.A. Bantle & J. Dumont (1998): Photoproducts and metabolites of a common insect growth regulator produce developmental deformities in *Xenopus*. – Environ. Sci. Technol. **32**: 1453–1461.

Labrousse, M. (1967): Analyse des effets des rayonnement embryonaire de l'Amphibien Urodèle *Pleurodeles waltlii* Michah. – Bull. Soc. Zool. France **91**: 491–588.

Lacroix, J.-C. & A. Capuron (1970): Sur un facteur récessif, modifiant le phénotype pigmentaire de la larve, chez l'Amphibien Urodèle *Pleurodeles waltlii* Michahelles. – C.r. Acad. Sc. Paris **270**: 2122–2123.

Lande, R. (1995): Mutation and conservation. – Conserv. Biol. **9**: 782–791.

Landesman, R.H. & W.S. Douglas (1990): Abnormal limb regeneration in adult newts exposed to a pulsed electromagnetic field. – Teratology **42**: 137–145.

Landesregierung von Baden-Württemberg (1984): Stellungnahme zu Beschlüssen des Landtages. 1. Mißbildungen von Wechselkröten (*Bufo viridis*) im Steinbruch Zimmermann KG, Gemarkungen Illingen und Roßwag. – Landtag Baden-Württemberg, Stuttgart, Drucksache **8/4596**: 1–3.

Landtag von Baden-Württemberg (1981): Kleine Anfrage des Abg. Hasenclever GRÜNE und Antwort des Ministeriums für Ernährung, Landwirtschaft, Umwelt und Forsten Missbildungen von Wechselkröten (*bufo viridis*) [sic] im Steinbruch KG Zimmermann, Gemarkungen Illingen und Roßwag. – Landtag von Baden-Württemberg, Drucksache **8/1034**: 1–3.

Lang, K. (1926): Groddjurens och kräldjurens utbredning inom Bara härad (Malmöhus län). – Fauna och Flora **21**: 145–150.

Langhelle, A., M.J. Lindell & P. Nyström (1999): Effects of ultraviolet radiation on amphibian embryonic and larval development. – J. Herpetol. **33**: 449–456.

Lannoo, M. (2008): Malformed Frogs. The Collapse of Aquatic Ecosystems. – Berkeley (Univ. Calif. Press).

Lannoo, M.J., D.R. Sutherland, P. Jones, D. Rosenberry, R.W. Klaver, D.M. Hoppe, P.T.J. Johnson, K.B. Lunde, C. Facemire & J.M. Kapfer (2003): Multiple causes for the malformed frog phenomenon. – Pp. 233–262 in Linder, G., S. Krest, D. Sparling & E. Little (eds.): Multiple Stressor Effects in Relation to Declining Amphibian Populations. – West Conshohocken (ASTM International).

Laufer, H. & A. Pieh (2007): Wechselkröte *Bufo viridis* Laurenti, 1768. – Pp. 357–375 in Laufer, H., K. Fritz & P. Sowig (eds.): Die Amphibien und Reptilien Baden-Württembergs. – Stuttgart (Ulmer).

Lauthier, M. (1971): Étude descriptive d'anomalies spontanées des membres postérieurs chez *Pleurodeles waltlii* Michah. – Ann. Embr. Morph. **4**: 65–78.

Lecamp, M. (1935): Les formations multiples dans la greffe et la régénération des membres chez le crapaud accoucheur (*Alytes obstetricans*, Laur.). – Bull. Biol. France Belg. Suppl. **19**: 1–149.

Lee, G.S., D.M. Kochhar & M.D. Collins (2004): Retinoid-induced limb malformations. – Current Pharmaceutical Design **10**: 2657–2699.

Leibovitz, H.E., D.D. Culley & J.P. Geaghan (1982): Effects of vitamin C and sodium benzoate on survival, growth and skeletal deformities of intensively cultured bullfrog larvae *Rana catesbeiana* reared at two pH levels. – J. World Maricul. Soc. **13**: 322–328.

Leja, M. (1981): Abnormes Anschwellen eines Weibchens der Wabenkröte. – Aquarium **15(150)**: 662–663.

Levengood, W.C. (1969): A new teratogenic agent applied to amphibian embryos. – J. Embryol. exp. Morph. **21(1)**: 23–31.

Levy, B.M. (1958): New method for the rapid determination of lathyrogenic agents. – Science **129**: 720.

Licht, L.E. & K.P. Grant (1997): The effects of ultraviolet radiation on the biology of amphibians. – Amer. Zool. **37**: 137–145.

Lillie, F.R. & F.P. Knowlton (1897): On the effects of temperature on the development of animals. – Zool. Bull. **1**: 179–193.

Linzey, D.W., J. Burroughs, L. Hudson, M. Marini, J. Robertson, J.P. Bacon, M. Nagarkatti & P.S. Nagarkatti (2003): Role of environmental pollutants on immune functions, parasitic infections and limb malformations in marine toads and whistling frogs from Bermuda. – Int. J. Environ. Health Res. **13**: 125–148.

Lips, K.R., J.R. Mendelson, A. Muñoz-Alonso, L. Canseco-Márquez & D.G. Mulcahy (2004): Amphibian population declines in montane southern Mexico: resurveys of historical localities. – Biol. Conserv. **119**: 555–564.

Lipsett, J.C. (1941): Disproportionate dwarfism in *Amblystoma*. – J. exp. Zool. **86**: 441–459.

Lissitzky, E. (1910): Durch experimentelle Eingriffe hervorgerufene überzählige Extremitäten bei Amphibien. – Arch. Mikr. Anat. **75**: 587–633.

Loeffler, I.K., D.L. Stocum, J.F. Fallon & C.U. Meteyer (2001): Leaping lopsided: a review of the current hypotheses regarding etiologies of limb malformations in frogs. – Anat. Rec. **265**: 228–245.

Lowcock, L.A., T.F. Sharbel, J. Bonin, M. Ouellet, J. Rodrigue & J.-L. DesGranges (1997): Flow cytometric assay for in vivo genotoxic effects of pesticides in green frogs (*Rana clamitans*). – Aquat. Toxicol. **38**: 241–255.

Lunde, K.B. & P.T.J. Johnson (2012): A pracical guide for the study of malformated amphibians and their causes. – J. Herpetol. **46**: 429–441.

Maclatchy, D.L., D.G. Cyr & J. Eales (1986): Estradiol-17β depresses T4 to T3 conversion in rainbow trout. – Amer. Zool. **26**: 24a.

Maden, M. (1983): The effect of vitamin A on the regenerating axolotl limb. – J. Embryol. exp. Morphol. **77**: 273–295.

Mahaney, P.A. (1994): Effects of freshwater petroleum contamination on amphibian hatching and metamorphosis. – Environ. Toxicol. Chem. **13**: 259–265.

Maiorca, A., R. Di Pasquale & G. Reverberi (1975): Androgenetici e ginogenetici in *Discoglossus pictus*. – Atti Accad. Naz. Lincei Rend. Ser. 8, **59**: 809–813.

Malacinski, G.M. (1978): The Mexican axolotl, *Ambystoma mexicanum*: its biology and developmental genetics and its autonomous cell-lethal genes. – Amer. Zool. **18**: 195–206.

Malacinski, G.M., A.J. Brothers & H.-M. Chung (1977): Destruction of components of the neural induction system of the amphibian egg with ultraviolet irradiation. – Develop. Biol. **56**: 24–39.

Malkmus, R. (2007): Einige Bemerkungen zur Larve des Südlichen Marmormolches. – amphibia **6(2)**: 9–12.

Malkmus, R. (2009): Bergbachkolke als Reproduktionsgewässer von *Pelophylax perezi* auf Madeira. – Z. Feldherpetol. **16**: 240–244.

Mangold, O. & T. Peters (1956): Über die Wirkung gleicher Röntgendosen auf verschiedene Stadien der Frühentwicklung von *Triton alpestris*. – Beitr. Pathol. Anat. Allgem. Pathol. **116**: 478–516.

Mann, R.M., R.V. Hyne, C.B. Choung & S.P. Wilson (2009): Amphibians and agricultural chemicals: review of the risks in a complex environment. – Environ. Pollut. **157**: 2903–2927.

Manteifel, Y.B. & A.N. Reshetnikov (2002): Avoidance of noxious tadpole prey by fish and invertebrate predators: adaptivity of a chemical defence may depend on predator feeding habitats. – Arch. Hydrobiol. **153**: 657–668.

Marchal-Ségault, D. & F. Ramade (1981): The effects of lindane, an insecticide, on hatching and postembryonic development of *Xenopus laevis* (Daudin) anuran amphibian. – Environ. Res. **24**: 250–258.

Marco, A., C. Quilchano & A.R. Blaustein (1999): Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. – Environ. Toxicol. Chem. **18**: 2836–2839.

Marshall, G.A., R.L. Amborski & D.D. Culley (1980): Calcium and pH requirements in the culture of bullfrog (*Rana catesbeiana*) larvae. – Proc. World Maricul. Soc. **11**: 445–453.

Martin, R. & J. Signoret (1968): Contribution à l'étude de la mutation «short toes» chez l'Axolotl. – Ann. Embr. Morph. **1**: 141–149.

Martínez, I., R. Álvarez, I. Herráez & P. Herráez (1992): Skeletal malformations in hatchery reared *Rana perezi* tadpoles. – Anat. Rec. **233**: 314–320.

Martins, M., J.P. Pombal & C.F.B. Haddad (1998): Escalated aggressive behaviour and facultative parental care in the nest building gladiator frog, *Hyla faber*. – Amphibia-Reptilia **19**: 65–73.

McDiarmid, R.W. & R. Altig (1999): Tadpoles. The Biology of Anuran Larvae. – Chicago (Univ. Chicago Press).

McKinnell, R.G., D.J. Picciano & J.W. Schaad (1979): Dominant lethality in frog embryos after paternal treatment with triethylenemelamine: cytogenetics, morphology and swimming capability. – Environ. Mutagenesis **1**: 221–231.

Mendelson, J.R., E.D. Brodie, J.H. Malone, M.E. Acevedo, M.A. Baker, N.J. Smatresk & J.A. Campbell (2004): Factors associated with the catastrophic decline of a cloudforest frog fauna in Guatemala. – Rev. Biol. Tropcial **52**: 991–1000.

Merilä, J., A. Laurila & M. Pahkala (2000): Effects of ambient UV-B radiation on early development of the common frog (*Rana temporaria*) embryos in the Subarctic. – Ann. Zool. Fennici **37**: 51–58.

Meteyer, C.U., I.K. Loeffler, J.F. Fallon, K.A. Converse, E. Green, J.C. Helgen, S. Kersten, R. Levey, L. Eaton-Poole & J.G. Burkhart (2000): Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota and Vermont suggest multiple etiologies. – Teratology **62**: 151–171.

Meusel, W. (1955): Krötenbastarde. – Aquarien, Terrarien **1955(2)**: 62.

Michael, M.I. & D.A. El Mekkawy (1977): Progress of malformations during limb regeneration in stages of the Egyptian toad, *Bufo regularis* Reuss, after transection at various proximo-distal levels. – Mem. Soc. Zool. France **41**: 203–208.

Michael, M.I. & A.A. Hassona (1982): Intercalary and supernumery regeneration in limbs of stages of the Egyptian toad, *Bufo regularis* Reuss. – J. exp. Zool. **220**: 207–218.

Mikamo, K. (1968): Mechanism of non-disjunction of meiotic chromosomes and degeneration of maturation spindles in eggs affected by intrafollicular overripeness. – Experientia **24**: 75–78.

Mikamo, K. & Hamaguchi, H. (1973): Chromosomal disorders caused by preovulatory overripeness of oocytes. – Pp. 72–97 in Blandau, R.J. (ed.): Aging Gametes International Symposium. – Basel (Karger).

Mikhailov, A.T. & A.Y. Korneev (1980): The lens proteins in adult and embryos of the periodic albino mutant of *Xenopus laevis*. – Roux Arch. Entwickl.-mech. **189**: 155–163.

Miller, J.C. & R. Landesman (1978): Reduction of heavy metal toxicity to *Xenopus* embryos by magnesium ions. – Bull. Environ. Contam. Toxicol. **20**: 93–95.

Miyada, S. (1960): Studies on haploid frogs. – J. Sci. Hiroshima Univ., ser. B, div. 1, **19**: 1–56.

Miyada, S. (1977): A three-year-old haploid frog. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **2**: 213–227.

Mizell, M. (1969): Biology of amphibian tumors. – Recent Results Cancer Res. Spec. Suppl. **17**: 1–484.

Mizgireuv, I.V., N.L. Flax, L.J. Borkin & V.V. Khudoley (1984): Dysplastic lesions and abnormalities in amphibians associated with environmental conditions. — Neoplasma **31**: 175–181.

Møller, A.P., T.A. Mousseau, F. de Lope & N. Saino (2007): Elevated frequency of abnormalities in barn swallows from Chernobyl. – Biol. Lett. **3**: 414–417.

Montalenti, G. (1933): L'ontogenesi degli ibridi fra *B. vulg.* e *B. viridis*. – Physiol. Zool. **6**: 329–395.

Montalenti, G. (1939): Il fenotipo degli ibridi di prima generazione fra *Bufo vulgaris* Laur. (♀) e *Bufo viridis* Laur. (♂). – Arch. Zool. Torino **26**: 1–39.

Moore, J.A. (1955): Abnormal combinations of nuclear and cytoplasmatic systems in frogs and toads. – Adv. Genetics **7**: 139–182.

Morin, P.J. (1985): Predation intensity, prey survival and injury frequency in an amphibian predator-prey interaction. – Copeia **1985**: 638–644.

Moskvitina, N.S., V.N. Kuranova & S.V. Saveliev (2011): Abnormalities of embryonal development of vertebrates under the condition of technogenic environmental pollution. – Contemporary Probl. Ecol. **4(4)**: 366–372.

Mousseau, T.A. & A.P. Møller (2014). Genetic and ecological studies of animals at Chernobyl and Fukushima. J. Heredity **105**: 704–709.

Muto, Y. (1969a): Hindlimb development and malformations of toes in the larvae reared at high temperature in the toad, *Bufo vulgaris formosus*. – Cong. Anom. **9**: 1–12.

Muto, Y. (1969b): Anomalies in the hindlimb skeletons of the toad larvae reared at a high temperature. – Cong. Anom. **9**: 61–73.

Muto, Y. (1970): Digital malformations in the forelimbs of the toad larvae reared at a high temperature. – Cong. Anom. **10**: 135–147.

Muto, Y. (1971): Skeletal anomalies in the feet of toad larvae reared in aerated water at a high temperature. – Cong. Anom. **11**: 159–169.

Mutschmann, F. & U. Manzke (2006): Tierärztlicher Befund von zwei abnorm gefärbten Laubfröschen *Hyla arborea*. – RANA **7**: 41–43.

Nechitailo, T.Y. & T.Y. Peskova (1999): Development of *Triturus vittatus* in solutions of heavy metal salts of low concentrations. – Interrepublican scientific conference Aktualnye voprosyekologii i okhrany prirody ekosistem yuzhnykh regionov Rossii, Krasnodar: 82–84.

Nehring, H. (1988): Beitrag zur Biologie der Wechselkröte (*Bufo viridis*) während der Laichzeit. – Feldherpetologie **1988**: 11–21.

Neumann, V. & F. Meyer (1994): *Lucilia bufonivora* Moniez, 1876 – ein euryxener Amphibienparasit (Insecta: Diptera: Calliphoridae). – Mitt. Zool. Mus. Berlin **70**: 331–341.

Newcomer, C.E., M.R. Anver, J.L. Simmons, B.W. Wilcke & G.W. Nace (1982): Spontaneous and experimental infections of *Xenopus laevis* with *Chlamydia psittaci*. – Lab. Anim. Sci. **32**: 680–686.

Newman, S.M. & J.N. Dumont (1983): Thiosemicarbazide-induced osteolathyrism in metamorphosing *Xenopus laevis*. – J. exp. Zool. **225**: 411–421.

Niazi, I.A. (1996): Background to work on retinoids and amphibian limb regeneration: studies on anuran tadpoles – a retrospect. – J. Biosci. **21**: 273–297.

Niemiller, M.L. & B.T. Miller (2005): *Rana clamitans melanota* (northern green frog). Predation. – Herpetol. Rev. **36**: 440.

Nieuwkoop, P.D. & F. Faber (1994): Normal Table of *Xenopus laevis*. – New York (Garland).

Nishimura, N., Y. Fukazawa, H. Uchiyama & T. Iguchi (1997): Effects of estrogenic hormones on early development of *Xenopus laevis*. – J. exp. Zool. **278**: 221–233.

Nishioka, M. (1977): Color variants induced by radiation and their inheritance in *Rana nigromaculata*. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **2**: 25–89.

Nishioka, M. (1978): Abnormalities in the descendants of *Rana japonica* produced from irradiated eggs or sperm. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **3**: 189–384.

Nishioka, M. & Y. Kondo (1978): Production of gynogenetic haploids by treatment of spermatozoa with toluidine blue in *Rana japonica*. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **3**: 385–398.

Nishioka, M & H. Ohtani (1986): Detection of chromosomes bearing the loci for blue and olive mutations in *Rana nigromaculata*. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **8**: 1–27.

Nishioka, M. & H. Ueda (1977): An electro-microscopic study on six kinds of color variants induced by radiation in *Rana nigromaculata*. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **2**: 91–102.

Nishioka, M. & H. Ueda (1985): Genetics and morphology of 13 albino stocks in the *Rana nigromaculata* group. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **7**: 1–121.

Nishioka, M., H. Okumoto & Y. Kondo (1981): Effects of ultraviolet rays on the sperm of *Rana japonica*. I. Production of gynogenetic haploids. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **5**: 47–67.

Nishioka, M, H. Ohtani & M. Sumida (1987): Chromosomes and the sites of five albino gene loci in the *Rana nigromaculata* group. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **9**: 1–52.

Noden, D.M. & A. de Lahunta (1985): The Embryology of Domestic Animals: Developmental Mechanisms and Malformations. – Baltimore, Maryland (Williams & Wilkins).

Nöllert, A. & C. Nöllert (1992): Die Amphibien Europas – Bestimmung-Gefährdung-Schutz. – Stuttgart (Franckh-Kosmos).

Novales Flamarique, I., K. Ovaska & T.M. Davis (2000): UV-B induced damage to the skin and ocular system of amphibians. – Biol. Bull. **199**: 187–188.

Nye, H.L.D., J.A. Cameron, E.A.G. Chernoff & D.L. Stocum (2003): Regeneration of the urodele limb: a review. – Develop. Dynamics **226**: 280–294.

Nyman, S. (1986): Mass mortality in larval *Rana sylvatica* attributable to the bacterium, *Aeromonas hydrophila*. – J. Herpetol. **20**: 196–201.

Oerter, K. (1985): Morphologische und histopathologische Untersuchungen zur Entwicklung von *Bufo bufo* nach Bestrahlung mit Co-60 im Schwanzknospenstadium. – Univ. Bonn (Unveröff. Diplomarb.).

Oeser, G. (1952): Albinotische Amphibien. – DATZ **1952(5)**: 306–308.

Olson, M.E., S. Gard, M. Brown, R. Hampton & D.W. Morck (1992): *Flavobacterium indologenes* infection in leopard frogs. – J. Amer. Vet. Med. Assoc. **201**: 1766–1770.

Olympic Dam Operations (1990): Environmental Radiation Annual Report June 1989 – May 1990. – South Australia (Olympic Dam).

Osaba, L., M.J. Rey, A. Aguirre, A. Alonso & U. Graf (2002): Evaluation of genotoxicity of captan, maneb and zineb in the wing spot test of *Drosophila melanogaster*: role of nitrosation. – Mutation Res. **518**: 95–106.

Osborn, D., A.S. Cooke & S. Freestone (1981): Histology of a teratogenic effect of DDT on *Rana temporaria* tadpoles. – Environ. Pollut. **25**: 305–319.

Ouellet, M. (2000): Amphibian deformities: current state of knowledge. – Pp. 617–661 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. – Pensacola, Florida (SETAC Press).

Ouellet, M., J. Bonin, J. Rodrigue, J.-L. DesGranges & S. Lair (1997): Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. – J. Wildl. Dis. **33**: 95–104.

Padgett-Flohr, G.E. & M.E. Goble (2007): Evaluation of tadpole mouthpart depigmentation as a diagnostic test

for infection by *Batrachochytrium dendrobatidis* for four California anurans. – J. Wildl. Dis. **43**: 690–699.

Paganelli, A., V. Gnazzo, H. Acosta, S.L. López & A.E. Carrasco (2010): Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. – Chem. Res. Toxicol. **23**: 1586–1595.

Pahkala, M., A. Laurila & J. Merilä (2000): Ambient ultraviolet-B radiation reduces hatchling size in the common frog *Rana temporaria*. – Ecography **23**: 531–538.

Pahkala, M., A. Laurila & J. Merilä (2001): Carry-over effects of ultraviolet-B radiation on larval fitness in *Rana temporaria*. – Proc. R. Soc. London B **268**: 1699–1706.

Pahkala, M., K. Räsänen, A. Laurila, U. Johanson, L.O. Björn & J. Merila (2002): Lethal and sublethal effects of UV-B/pH synergism on common frog embryos. – Conserv. Biol. **16**: 1063–1073.

Pandey, A.K. & V. Tomar (1985): Melanophores in *Bufo melanostictus* (Schneider) tadpoles following exposure to the insecticide dimethoate. – Bull. Environ. Contam. Toxicol. **35**: 796–801.

Parichy, D.M. & R.H. Kaplan (1992): Developmental consequences of tail injury on larvae of the oriental fire-bellied toad, *Bombina orientalis*. – Copeia **1992**: 129–137.

Parmenter, C.L. (1933): Haploid, diploid, triploid and tetraploid chromosome numbers and their origin in parthenogenetically developed larvae and frogs of *Rana pipiens* and *R. palustris*. – J. exp. Zool. **66**: 409–453.

Pasteels, J. (1945): Recherches sur l'action du LiCl sur les œufs des amphibiens. – Arch. Biol. **56**: 105–183.

Pastor, S., A. Creus, T. Parrón, A. Cebulska-Wasilewska, C. Siffel, S. Piperakis & R. Marcos (2003): Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers. – Mutagenesis **18**: 249–258.

Patch, E.M. (1936): Dietary production and prevention of anemia in larval *Amblystoma*. – Science **83**: 560–561.

Paulov, S. (1977): The effects of N-phenyl-N'-methylurea (Defenuron) on the development of Amphibia (*Rana temporaria* L). – Biologia (Bratislava) **32**: 607–611.

Peixoto, O.L. & M. dos Reis Gomes (1997): *Hyla minuta* (NCN). Tadpole behavior. – Herpetol. Rev. **28**: 146–147.

Perri, T. (1946): Sull'ibridazione tra "*Bufo viridis*" e "*Bufo vulgaris*". Esperienze di trapianto tra ibridi e normali. Nota I. – Rend. Sc. Fis. Mat. Nat. Accad. Lincei, ser. 8, **1**: 995–1000.

Perri, T. (1949): Sull'ibridazione tra *Bufo viridis* e *Bufo vulgaris*. Nota II. – Rend. Sc. Fis. Mat. Nat. Accad. Lincei **6**: 255–259.

Peskova, T.Y. (2000): Influence of some technogenic pollutants on amphibian spawn. – Adv. Amph. Res. Former Soviet Union **5**: 275–282.

Peters, T. (1960): Über die Wirkung von Röntgenstrahlen auf befruchtete Eizellen von *Triturus alpestris* unter besonderer Berücksichtigung der Wirkung kleiner Strahlendosen und geringer Schäden. – Strahlentherapie **112**: 525–542.

Petranka, J.W. (1989): Density dependent growth and survival of larval *Ambystoma*: evidence from whole-pond manipulations. – Ecology **70**: 1752–1767.

Pfeiffer, W. (1966): Die Verbreitung der Schreckreaktion bei Kaulquappen und die Herkunft des Schreckstoffes. – Z. vergl. Physiol. **52**: 79–98.

Pflüger, E. & W.J. Smith (1883): Untersuchungen über Bastardierung der anuren Batrachier und die Principien der Zeugung. I. Teil: Experimente über Bastardierung der anuren Batrachier. – Arch. ges. Physiol. Mensch Thiere **32**: 519–541.

Pierce, B.A. (1985): Acid tolerance in amphibians. – BioScience **35**: 239–243.

Pierce, B.A. (1993): The effect of acid precipitation on amphibians. – Ecotoxicology **2**: 65–77.

Pliss, G.B. & V.V. Khudoley (1979): Oncogenesis and cancerogenious factors in primitive vertebrates and invertebrate animals. – Pp. 167–185 in Sokolov, V.E. (ed.): Ekologicheskoe Prognozirovanie. – Moscow (Akad. Nauk.).

Pogany, G.C. (1976): Analysis of a Hertwig effect induced by the irradiation of *Rana pipiens* sperm with ultraviolet. – Develop. Growth Differ. **18**: 339–348.

Pohland, H.-L. (1962): Die Wirkung von Thymuswirkstoffen auf Wachstum und Metamorphose sowie deren Einfluss auf endokrine Drüsen bei *Xenopus laevis* Daudin. – Roux Arch. Entwickl.-mech. **153**: 621–663.

Polder, W.N (1973): Pflege und Fortpflanzung von *Dendrobates azureus* und anderer Dendrobatiden. – DATZ **26**: 424–428.

Pollack, E.D. & V. Liebig (1989): An induced developmental disorder of limbs and motor neurons in *Xenopus*. – Amer. Zool. **29**: 163A.

Pourhadi, R., C. Bonhomme & J.P. Turchini (1968): Action tératogène des ultrasons sur des oeufs de Batraciens. – Arch. Anat. Microscop. **57**: 255–274.

Power, T., K.L. Clark, A. Harfenist & D.B. Peakall (1989): A review and evaluation of the amphibian toxicological literature. – Tech. Rep. Can. Wildl. Serv. **61**: 1–91.

Puhlmann, P. (1981a): Tausendmal mehr Radioaktivität. – Stuttgarter Nachrichten **7.5.1981**.

Puhlmann, P. (1981b): Streit um Radioaktivität geht weiter. – Stuttgarter Nachrichten **12.5.1981**.

Puppé, A. (1925): Experimentell erzeugte Polydaktylie und Bruchdreifachbildung bei *Rana fusca*. – Arch. mikroskop. Anat. Entwickl-mech. **104**: 125–137.

Pyastolova, O.A. & M.N. Danilova (1986): Growth and development of *Rana arvalis* in condition imitating oil contamination. – Sov. J. Ecol. **17**: 209–214.

Pyastolova, O.A. & V.L. Vershinin (1999): Some cytological features of *Rana arvalis* Nilss. frogs on the territory of the Eastern Ural radioactive trace. – Russ. J. Ecol. **30**: 30–35.

Pyastolova, O.A., V.L. Vershinin, E.A. Trubezkaja & E.Z. Gatiyatullina (1996): Utilization of amphibians in bioindication research on territories of the Eastern Urals radioactive trace. – Russ. J. Ecol. **27**: 361–365.

Qin, Z.F., J.M. Zhou, L. Cong & X.-B. Xu (2005): Potential ecotoxic effects of polychlorinated biphenyls on *Xenopus laevis*. – Environ. Toxicol. Chem. **24**: 2573–2578.

Rachowicz, L.J. (2002): Mouthpart pigmentation in *Rana muscosa* tadpoles: seasonal changes without chytridiomycosis. – Herpetol. Rev. **33**: 263–265.

Rachowicz, L.J. & V.T. Vredenburg (2004): Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. – Dis. Aquat. Org. **61**: 75–83.

Rahden-Staron, I. (2002): The inhibitory effect of the fungicides captan and captafol on eukaryotic topoisomerases in vitro and lack of recombinagenic activity in the wing spot test of *Drosophila melanogaster*. – Mutation Res. **518**: 205–213.

Rajakaruna, R.S., P.M.J.R. Piyatissa, U.A. Jayawardena, A.N. Navaratne & P.H. Amerasinghe (2008): Trematode infection induced malformations in the common hourglass treefrogs. – J. Zool. **275**: 89–95.

Raxworthy, C.J. (1989): Courtship, fighting and sexual dimorphism of the banded newt, *Triturus vittatus ophryticus*. – Ethology **81**: 148–170.

Read, J.L. & M.J. Tyler (1990): The nature and incidence of post-axial skeletal abnormalities in the frog *Neobatrachus centralis* Parker at Olympic Dam, South Australia. – Trans. R. Soc. S. Aust. **114**: 213–217.

Read, J.L. & M.J. Tyler (1994): Natural levels of abnormalities in the trilling frog (*Neobatrachus centralis*) at the Olympic Dam mine. – Bull. Environ. Contam. Toxicol. **53**: 25–31.

Reading, C.J., L. Loman & T. Madsen (1991): Breeding pond fidelity in the common toad, *Bufo bufo*. – J. Zool. **225**: 201–211.

Reaser, J.K. & P.T. Johnson (1997): Amphibian abnormalities: a review. – Froglog **24**: 2–4.

Reeves, M.K., K.A. Medley, A.E. Pinkney, M. Holyoak, P.T.J. Johnson & M.J. Lannoo (2013): Localized hotspots drive continental geography of abnormal amphibians on U.S. wildlife refuges. – PLOS One **8(11)**: e77467. doi:10.1371/journal.pone.0077467.

Reichenbach-Klinke, H.-H. (1961): Krankheiten der Amphibien. – Stuttgart (Fischer).

Reinhardt, F. (1939): Beeinflussung der Hinterbeinentwicklung des Rippenmolches durch einseitige Fütterung. – Anat. Anz. **89**: 45–51.

Richards, S.J. & C.M. Bull (1990): Size-limited predation on tadpoles of three Australian frogs. –Copeia **1990**: 1041–1046.

Riley, E.E. & M.R. Weil (1986): The effects of thiosemicarbazide on development in the wood frog, *Rana sylvatica.* I. Concentration effects. – Ecotoxicol. Environ. Safety **12**: 154–160.

Rimpp, K. (1981): Bericht über einen Fund mißgebildeter Wechselkröten (*Bufo viridis*). – herpetofauna, Weinstadt **3(11)**: 25–28.

Romansic, J.M., P.T.J. Johnson, C.L. Searle, J.E. Johnson, T.S. Tunstall, B.A. Han, J.R. Rohr & A.R. Blaustein (2011): Individual and combined effects of multiple pathogens on Pacific treefrogs. – Oecologia **166**: 1029–1041.

Romeis, B. (1918): Die Beeinflussung von Wachstum und Entwicklung durch Fett-, Lipoid- und Eiweißstoffe sowie eiweißfreie Extrakte der Schilddrüse und der Thymus. – Z. exp. Med. **6**: 101–288.

Rose, F.L. (1976): Tumorous growths of the tiger salamander, *Ambystoma tigrinum*, associated with treated sewage effluent. – Prog. exp. Tumor Res. **20**: 251–262.

Rose, F.L. (1977): Tissue lesions of tiger salamanders (*Ambystoma tigrinum*): relationship to sewage effluents. – Ann. New York Acad. Sci. **298**: 270–279.

Rose, F.L. (1981): The tiger salamander (*Ambystoma tigrinum*): a decade of sewage associated neoplasia. – Pp. 91–100 in Dawe, C.J., J.C. Harshbarger & S. Kondo (eds.): Phyletic Approaches to Cancer. – Tokio (Jap. Sci. Soc. Press).

Rose, F.L. & J.C. Harshbarger (1977): Neoplastic and possibly related skin lesions in neotenic tiger salamanders from a sewage lagoon. – Science **196**: 315–317.

Ross, R. (1988): Do power line-generated electromagnetic fields have any association with certain disorders? – J. Amer. Med. Assoc. **259**: 1131–1133.

Rostand, J. (1949): Sur diverses anomalies relevées dans une population de crapauds (*Bufo bufo*). – C.r. Soc. Biol. **143**: 758–760.

Rostand, J. (1951a): Sur la polydactylie des Batraciens Anoures. – Bull. biol. France Belg. **85**: 113–136.

Rostand, J. (1951b): Génétique des Batraciens Anoures: anomalies héréditaires et phénocopies. – Bull. Soc. Hist. nat. Toulouse **86**: 230–236.

Rostand, J. (1951c): Surmaturité des œufs et anomalies chez *Rana temporaria*. – C.r. Soc. Biol. **145**: 624–625.

Rostand, J. (1955): Les Crapauds, les Grenouilles, et quelques grands problèmes biologiques. – Paris (Gallimard).

Rostand, J. (1958): Les anomalies des Amphibiens Anoures. – Paris (Sedes).

Rostand, J. (1971): Les étangs à monstres. Histoire d'une recherche (1947–1970). – Paris (Stock).

Rostand, J. & P. Darré (1968): Conditions déterminants de l'anomalie P chez *Rana esculenta*. – C.r. Soc. Biol. **162**: 1682–1683.

Rostand, J. & P. Darré (1969): Action tératogène des déjections de certains poissons sur les larves de *Rana esculenta*. – C.r. Soc. Biol. **163**: 2033–2034.

Rowe, C.L., O.M. Kinney & J.D. Congdon (1998): Oral deformities in tadpoles of the bullfrog (*Rana catesbeiana*) caused by conditions in a polluted habitat. – Copeia **1998**: 244–246.

Rübsaamen, H. (1955): Mißbildungen durch Sauerstoffmangel im Experiment und in der menschlichen Pathologie. – Naturwissenschaften **42**: 319–325.

Rugh, R. (1939): Developmental effects resulting from exposure to x-rays. I. Effects on the embryo of irradiation of frog sperm. – Proc. Amer. Philos. Soc. **81**: 447–471.

Rugh, R. (1950): Inhibition of growth and the production of oedema by X-irradiation. – J. exp. Zool. **114**: 137–157.

Rugh, R. (1965): Experimental Embryology. – Minneapolis (Burgess Publ. Comp.).

Saura, A., B. Johansson, E. Eriksson & M. Kohonen-Corish (1998): Genetic load in marginal populations of *Drosophila subobscura*. – Hereditas **129**: 283–286.

Savchenko, V.K. (1995): The Ecology of the Chernobyl Catastrophe. – Paris (UNESCO).

Saveliev, S.V., N.S. Moskvitina, B.D. Kuranov & V.N. Kuranova (1996): Narushenie embriogeneza v prirodnysh populjatshijash pozvovotshnysh kak indikator sostojanija sredy obitanija. – (Akad. Nauk.).

Saxena, S. & I.A. Niazi (1977): Effect of vitamin A excess on hind limb regeneration in tadpoles of the toad, *Bufo andersonii* (Boulenger). – Ind. J. exp. Biol. **15**: 435–439.

Scadding, S.R. & M. Maden (1986a): Comparison of the effects of vitamin A on limb development and regeneration in *Xenopus laevis* tadpoles. – J. Embryol. exp. Morphol. **91**: 35–53.

Scadding, S.R. & M. Maden (1986b): Comparison of the effects of vitamin A on limb development and regeneration in the axolotl, *Ambystoma mexicanum*. – J. Embryol. exp. Morphol. **91**: 19–34.

Schäperclaus, W. (1954): Fischkrankheiten. 3. Aufl. – Berlin (Akademie Verlag).

Scharf, S.R. & J.C. Gerhart (1983): Axis determination in eggs of *Xenopus laevis*: a critical period before first cleavage, identified by the common effects of cold, pressure and ultraviolet irradiation. – Develop. Biol. **99**: 75–87.

Scherb, H. & E. Weigelt (2003): Congenital malformation and stillbirth in Germany and Europe before and after the Chernobyl nuclear power accident. – Environ. Sci. Pollut. Res. **10**: 117–125.

Schinz, H.R. & H. Fritz-Niggli (1953): Die Wirkung der Röntgenstrahlen und des cancerogenen Stoffes "Styryl" auf die Embryonalentwicklung. – Z. Radiol. Onkol. **90**: 345–352.

Schinz, H.R. & H. Fritz-Niggli (1954): Mißbildungen adulter Krallenfrösche (*Xenopus laevis* Daud.) nach Röntgen-Bestrahlung im Embryonalstadium. – Strahlentherapie **94**: 147–151.

Schipp, R., H. Hemmer & R. Flindt (1968): Vergleichende licht- und elektronenmikroskopische Untersuchungen an Chordomen von Krötenbastardlarven. – Beitr. Pathol. Anat. **138**: 109–133.

Schmalhausen, J. (1925): Über die Beeinflussung der Morphogenese der Extremitäten vom Axolotl durch verschiedene Faktoren. – Roux Arch. Entwickl.-mech. **105**: 483–500.

Schmid, W. (1981): Gelehrtenstreit um das Geheimnis der Krötenmonster. – Stuttgarter Zeitung **31.1.1981**.

Schmidt, M. (1985): Mißbildungen bei Dendrobatiden als Folge von Inzucht und falscher Ernährung. – Aquarium **19**: 33–35.

Schneider, M. (1984): Strahlenbedingte Veränderungen des Nervensystems bei Amphibien. – Univ. Bonn (Unveröff. Diplomarb.).

Schnorbach, N. (1982): Stuttgarts Krötenkrüppel vom Steinbruch. – Das Tier, Frankfurt/M. **1982/3**: 19.

Schotthoefer, A.M., A.V. Koehler, C.U. Meteyer & R.A. Cole (2003): Influence of *Ribeiroia ondatrae* (Trematoda: Digenea) infection on limb development and survival of northern leopard frogs (*Rana pipiens*): effects of host stage and parasite-exposure level. – Can. J. Zool. **81**: 1144–1153.

Semenov, D.V. & S.A. Ivanova (1995): Embryonic abnormalities in lizards *Lacerta vivipara* (Sauria, Lacertidae) inhabiting a radioactive contaminated territory. – Russ. J. Herpetol. **2**: 166–169.

Semlitsch, R.D. & S.B. Reichling (1989): Density-dependent injury in larval salamanders. – Oecologia **81**: 100–103.

Semlitsch, R.D., G.B. Moran & C.A. Shoemaker (1981): *Ambystoma talpoideum* (mole salamander). Morphology. – Herpetol. Rev. **12**: 69.

Sessions, S.K. & S.B. Ruth (1990): Explanation for naturally occuring supernumerary limbs in amphibians. – J. exp. Zool. **245**: 38–47.

Siboulet, R. (1971): L'élevage des embryons et larves des Amphibiens anoures. – Aquarama **5(14)**: 38–39.

Siboulet, R., F. Loirat & C. Grenot (1975): Variation du phenotype proteique chez des hybrides interspecifiques du genre *Bufo*. – Comp. Biochem. Physiol. **50 B**: 89–95.

Siboulet, R., S. Grinfeld, P. Deparis & A. Jaylet (1984): Micronuclei in red blood cells of the newt *Pleurodeles waltl* Michah.: induction with X-rays and chemicals. – Mutation Res. **125**: 275–281.

Signoret, J., A. Collenot & L. Gallien (1966): Description d'un nouveau mutant récessif léthal (u) et de son syndrome chez triton *Pleurodeles waltlii*. – C.r. Hebd. Seances Acad. Sci. Paris **262**: 699–701.

Sinsch, U. (2009): *Bufo calamita* Laurenti, 1768 – Kreuzkröte. – Pp. 337–411 in Grossenbacher, K. (ed.): Handbuch der Reptilien und Amphibien Europas. Bd. V/II: Froschlurche (Anura) II: (Hylidae, Bufonidae). – Wiebelsheim (Aula).

Skelly, D.K. & M.F. Benard (2010): Mystery unsolved: missing limbs in deformed amphibians. – J. exp. Zool. **314:** 179–181.

Sladden, D.E. (1930): Experimental distortion of development in amphibian tadpoles. – Proc. R. Soc. London, Ser. B **106**: 318–325.

Smith, G.R., M.A. Waters & J.E. Rettig (2000): Consequences of embryonic UV-B exposure for embryos and tadpoles of the plains leopard frog. – Conserv. Biol. **14**: 1903–1907.

Smith, S.D. & A.A. Pilla (1981): Modulation of newt limb regeneration by electromagnetically induced low level pulsating current. – Pp. 137–152 in Becker, R.O. (ed.): Mechanisms of Growth Control: Papers. – Springfield (C.C. Thomas).

Snawder, J.E. & J.E. Chambers (1989): Toxic and developmental effects of organophosphorus insecticides in embryos of the South African clawed frog. – J. Environ. Sci. Health **B24**: 205–218.

Souder, W. (2002): A Plague of Frogs. Unraveling an Environmental Mystery. – Minneapolis (Univ. Minnesota Press).

Sowig, P. & H. Laufer (2007): Erdkröte *Bufo bufo* (Linnaeus, 1758). – Pp. 311–334 in Laufer, H., K. Fritz & P. Sowig (eds.): Die Amphibien und Reptilien Baden-Württembergs. – Stuttgart (Ulmer).

Sparling, D.W. (2000): Effects of altosid and abate-4E on deformities and survival in southern leopard frogs under seminatural conditions. – J. Iowa Acad. Sci. **107(3)**: 90–91.

Stachowitz, W. (1914): Veränderungen in der Entwicklung von Amphibienembryonen, die auf dem Stadium der Medullarplatte mit Radium bestrahlt wurden. – Arch. Mikr. Anat., Abt. 1 **85**: 521–554.

Starnes, S.M., C.A. Kennedy & J.W. Petranka (2000): Sensitivity of embryos of southern Appalachian amphibians to ambient solar UV-B radiation. – Conserv. Biol. **14**: 277–282.

Stopper, G.F., L. Hecker, R.A. Franssen & S.K. Sessions (2002): How trematodes cause limb deformities in amphibians. – J. exp. Zool. (Mol. Dev. Evol.) **294**: 252–263.

Struktur- und Genehmigungsdirektion Süd, Rheinland-Pfalz (2002): Plutonium-Affäre 2001/2002 – Kontamination in der Südpfalz. – Neustadt a.d. Weinstraße (Struktur- und Genehmigungsdirektion Süd, Rheinland-Pfalz).

Sucov, H.M. & R.M. Evans (1995): Retinoic acid and retinoic acid receptors in development. – Mol. Neurobiol. **10**: 169–184.

Surlève-Bazeille, J.-E., R. Cambar & R. Mauget (1969a): Essai de transmission de l'anomalie P à diverses espèces d'Amphibiens. Premiers resultats obtenus sur *Rana temporaria*. – Actes Soc. Linn. Bordeaux, ser. A **106(2)**: 1–7.

Surlève-Bazeille, J.-E., R. Cambar & A. Calas (1969b): Premières observations histologiques sur les membres des larves de Grenouille verte (*Rana esculenta* L.) atteints de l'anomalie P. – C.r. Acad. Sc. Paris, Sér. D **269**: 2240–2242.

Taira, W., C. Nohara, A. Hiyama & J.M. Otaki (2014): Fukushima's biological impacts: the case of the pale grass blue butterfly. – J. Heredity **105**: 710–722.

Taruffi, C. (1881–1886): Storia della Teratologia. Parte Seconda. Note ed Osservazioni. Fascicolo I–IV. – Bologna (Regia Tipografia).

Taylor, S.K., E.S. Williams, E.T. Thorne, K.W. Mills, D.I. Withers & A.C. Piers (1999): Causes of mortality of the Wyoming toad. – J. Wildl. Dis. **35(1)**: 49–57.

Tchou-Su (1931): Étude cytologique sur l'hybridation chez les anoures. – Arch. anat. Microsc. **27(1)**: 1–104.

Thiesmeier, B. (1992): Ökologie des Feuersalamanders. – Essen (Westarp Wissenschaften).

Thoms, S.D. & D.L. Stocum (1984): Retinoic acid induced pattern duplication in regenerating urodele limbs. – Develop. Biol. **103**: 319–328.

Tomar, V. & A.K. Pandey (1988): Sublethal toxicity of an insecticide to the epidermal melanophores of *Rana* tadpoles. – Bull. Environ. Contam. Toxicol. **41**: 582–588.

Tompkins, R. (1970): Biochemical effects of the gene g on the development of the axolotl, *Ambystoma mexicanum*. – Develop. Biol. **22**: 59–83.

Tornier, G. (1896): Über Hyperdactylie, Regeneration und Vererbung mit Experimenten. – Arch. Entwickl.-mech. **3**: 469–476.

Tornier, G. (1905): An Knoblauchkröten experimentell entstandene überzählige Hintergliedmaßen. – Roux Arch. Mikr. Anat. Entwickl.-mech. **20**: 76–124.

Trux, A. (1985): Histopathologische Untersuchung zur Entwicklung des visuellen Systems von *Bufo bufo* (Amphibia) nach Bestrahlung mit Co-60. – Univ. Bonn (Unveröff. Diplomarb.).

Tschumi, P. (1954): Konkurrenzbedingte Rückbildungen der Hinterextremität von *Xenopus* nach Behandlung mit einem Chloraethylamin. – Rev. Suisse Zool. **61(6)**: 177–270.

Tsonis, P.A. & G. Eguchi (1985): The regeneration of newt limbs deformed in nature. – Experientia **41**: 918–919.

Tyler, M.J. (1983): Natural pollution monitors. – Aust. Nat. Hist. **21**: 31–33.

Tyler, M.J. (1989): Australian Frogs. – South Yarra (Viking O'Neil).

Uehlinger, V. (1965): Une form de goitre héréditaire chez le Batracien *Xenopus laevis* D. – Experientia **21**: 271.

Uehlinger, V. (1966): Description chez *Xenopus laevis* D. d'une mutation dominante „Screwy" (S), létale à l'état homozygote. – Rev. Suisse Zool. **73**: 527–534.

Uehlinger, V. (1969): Une mutation récessive (pd) déterminant la polydactylie chez *Xenopus laevis* D. (Batraciens anoures). – J. Embryol. exp. Morphol. **21**: 207–218.

Uehlinger, V. & A. Droin (1969): Origin and heredity of the mutation "rusty" (ry) of the batrachian anuran *Xenopus laevis* (Daudin). – Arch. Julius Klaus Stiftung Vererbungsforsch., Sozialanthropol. Rassenhygiene Suppl. **44(3–4)**: 48–54.

Uehlinger, V. & J. Reynaud (1965): Une anomalie héréditaire "kt" (kinky tailtip) chez *Xenopus laevis* D. – Rev. Suisse Zool. **72**: 680–685.

Vallisneri, A. (1706): Galleria di Minerva overo notizie universali di quanto e stato scritto da letterati di Europa. Vol. 5. – Venice (Girolamo Albrizzi).

van Buskirk, J. & D.C. Smith (1991): Density-dependent population regulation in a salamander. – Ecology **72**: 1747–1756.

Van Buskirk, J., P. Anderwald, S. Lüpold, L. Reinhardt & H. Schuler (2003): The lure effect, tadpole tail shape and the target of dragonfly strikes. – J. Herpetol. **37**: 420–424.

van der Leun, J.C. & F.R. de Gruijl (1993): Influence of ozone depletion on human and animal health. – Pp. 95–123 in Tevini, M. (ed.): UV-B Radiation and Ozone Depletion: Effects on Humans, Animals, Plants, Microorganisms and Materials. – Boca Raton (Lewis Publ.).

van der Schalie, W.H., H.S. Gardner, J.A. Bantle, C.T. de-Rosa, R.A. Finch, J.S. Reif, R.H. Reuter, L.C. Backer, J. Burger, L.C. Folmar & W.S. Stokes (1999): Animals as sentinels of human health hazards of environmental chemicals. – Environ. Health Perspectives **107**: 309–315.

Vandenlangenberg, S.M., J.T. Canfield & J.A. Magner (2003): A regional survey of malformed frogs in Minnesota (USA) (Minnesota malformed frogs). – Environ. Monitor. Assess. **82**: 45–61.

Vargas, A.O. (2009): Did Paul Kammerer discover epigenetic inheritance? A modern look at the controversial midwife toad experiments. – J. exp. Zool. **312B**: 1–12.

Venesky, M.D. & F.M. Brem (2008): Occurrence of *Batrachochytrium dendrobatidis* in Southwestern Tennessee, USA. – Herpetol. Rev. **39**: 319.

Vershinin, V.L. (2002): Ecological specifity and microevolution in amphibian populations in urbanized areas. – Adv. Amph. Res. Former Soviet Union **7**: 1–161.

Viertel, B. & M. Veith (1992): Predation by leeches and regeneration, a factor in larval development of *Bufo bufo* (L.). – Proc. Ord. Gen. Meet. Soc. Europ. Herpetol., Budapest **6**: 479–484.

Vlcek, P. (1995): Mezidruhovy amplexus ropuch. – Ziva, Praha **43(2)**: 83.

Vogt, W. (1939): Über alimentäre Unterdrückung der Hinterbeine beim Rippenmolch (*Pleurodeles w.*). – Verh. Ges. Anat. **47**: 137–144.

Volpe, E.P. & S. Dasgupta (1962): Gynogenetic diploids of mutant leopard frogs. – J. exp. Zool. **151**: 287–301.

Von Humboldt, A. (1797): Versuche über die gereizten Muskel- und Nerven-Faser nebst Vermuthungen über den chemischen Process des Lebens in der Thier- und Pflanzenwelt. Vol. I. – Berlin (Rottmann) & Posen (Decker).

Vuori, K. & M. Parkko (1996): Assessing pollution of the river Kymijoki via hydropsychid caddis flies: population age structure, microdistribution and gill abnormalities in the *Cheumatopsyche lepida* and *Hydropsyche pellucidula* larvae. – Arch. Hydrobiol. **136**: 171–190.

Wallays, H. (1998): Haltung von japanischen *Hynobius*-Arten. – elaphe **6(3)**: 78–79.

Weiss, I. (1960): Umwandlung von Disnothoi der Bastardkombination *Bufo calamita* x *Bufo viridis* in Eunothoi durch experimentelle Verdoppelung des mütterlichen Genoms. – Roux Arch. Entwickl.-mech. **152**: 552–564.

Weiss, I. & C. Ziemann (1959): Erwachsene Bastarde einheimischer Kröten. – Wiss. Z. Univ. Halle, math.-naturwiss. Kl. **8**: 796–798.

Weyrauch, S.L. & T.C. Grubb (2006): Effects of the interaction between genetic diversity and UV-B radiation on wood frog fitness. – Conserv. Biol. **20**: 802–810.

Wickramasinghe, D.D., K.L. Oseen, S.W. Kotagama & R.J. Wassersug (2005): Raising semi-terrestrial tadpoles of *Nannophrys ceylonensis* (Anura, Ranidae) in the laboratory. – Herpetol. Rev. **36**: 23–26.

Wiegand, T., F. Jeltsch, I. Hanski & V. Grimm (2003): Using pattern-oriented modeling for revealing hidden information: a key for reconciling ecological theory and application. – Oikos **100**: 209–222.

Wilbur, H.M. & R.D. Semlitsch (1990): Ecological consequences of tail injury in *Rana* tadpoles. – Copeia **1990**: 18–24

Williams, R.N., D.H. Bos, D. Gopurenko & J.A. DeWoody (2008): Amphibian malformations and inbreeding. – Biol. Lett. **4**: 549–552.

Wink, U. & H. Wurmbach (1967): Die Wirkung der Schilddrüsenhemmung durch Thymusöl bei Xenopus-kaulquappen auf die Zellteilung, die Bildung von Knorpel und Muskeln sowie die Entstehung von Extremitätenmißbildungen. – Zool. Jb. Physiol. **73**: 343–374.

Winter, R.M., S.A.S. Knowles, F.R. Bieber & M. Baraitser (1988): The Malformed Fetus and Stillbirth. – New York (John Wiley).

Wisniewski, P.J. (1984): Limb degeneration in two species of Asian newts. – Brit. Herp. Soc. Bull. **10**: 54.

Witschi, E. (1920): Über merogenetische Entwicklung äquipotentieller Teile. – Festschr. Zschokke, Universität Basel **36**: 3–15.

Witschi, E. (1922): Überreife der Eier als kausaler Faktor bei der Entstehung von Mehrfachbildungen und Teratomen. – Verh. Naturf. Ges. Basel **34**: 33–40.

Witschi, E. (1925): Die Überreife des Eies als teratogenetischer Faktor. – Verh. Dtsch. Zool. Ges. **30**: 91–94.

Witschi, E. (1952): Overripeness of the eggs as a cause of twinning and teratogenesis: a review. – Cancer Res. **12**: 763–786.

Witschi, E. (1971): Developmental causes of malformation. – Experientia **27**: 1245–1247.

Witschi, E. & C.Y. Chang (1954): Hereditary polymelia combined with edema and sex reversal in the toad. – Anat. Rec. **118**: 370.

Woerdeman, M.W. (1936): Embryonic "induction" by chemical substances. – Proc. K. Akad. Weten. Amsterdam Sect. Sci. **39**: 306–314.

Woitkewitsch, A.A. (1961): Le dévelopment des extrémitées surnuméraires chez les amphibiens. – Bull. Biol. France Belgique **7**: 569–600.

Wolf, K., G.L. Bullock, C.E. Dunbar & M.C. Quimby (1968): Tadpole edema virus: a viscerotropic pathogen for anuran amphibians. – J. infect. Dis. **118**: 253–262.

Wolf, K.-R. (1994): Untersuchungen zur Biologie der Erdkröte *Bufo bufo* L. unter besonderer Berücksichtigung des Einflusses von Migrationshindernissen auf das Wanderverhalten und die Entwicklung von vier Erdkrötenpopulationen im Stadtgebiet von Osnabrück. – New York (Mellen Univ. Press).

Wolterstorff, W. (1941): Abnorme Regeneration einer Hintergliedmaße bei einer Larve von *Alytes obstetricans* (Geburtshelferkröte). – Bl. Aquar.-Terrarienkde **1941**: 326–327.

Worrest, R.C. & D.J. Kimeldorf (1975): Photoreactivation of potentially lethal, UV-induced damage to boreal toad (*Bufo boreas boreas*) tadpoles. – Life Sciences **17**: 1545–1550.

Worthington, R.D. (1974): High incidence of anomalies in a natural population of spotted salamanders, *Ambystoma maculatum*. – Herpetologica **30**: 216–220.

Worthylake, K.M. & P. Hovingh (1989): Mass mortality of salamanders (*Ambystoma tigrinum*) by bacteria (*Acinetobacter*) in an oligotrophic seepage mountain lake. – Great Basin Nat. **49**: 364–372.

Wright, K.M. & B.R. Whitaker (2001): Amphibian Medicine and Captive Husbandry. – Malabor (Krieger Publ. Comp.).

Wurmbach, H., A. Biwer, L. Schneider, H-L. Pohland & U. Borchert (1964): Zur antithyreoidalen und Mißbildungen erzeugenden Wirkung pflanzlicher und tierischer Öle bei Kaulquappen. – Forsch.-ber. Nordrhein-Westfalen **1371**: 1–71.

Yablokov, A.V., V.B. Nesterenko & A.V. Nesterenko (2009): Chernobyl. Consequences of the catastrophe for people and the environment. – Ann. New York Acad. Sci. **1181.**

Zavadil, V. & I. Prikryl (2003): Die Wechselkröte (*Bufo viridis* Laurenti, 1768), eine Lurchart mit dem höchsten Anpassungsgrad an Laichgewässer mit hohen Salinitätswerten in Extrembiotopen von Braunkohletagebaugebieten in Westböhmen bei Sokolov – vorläufige Ergebnisse. – Mertensiella **14**: 241–252.

Zavadil, V. & P. Roth (1997): Natural hybridization between *Bufo viridis* and *Bufo bufo* in the Doupovské hory Hills (northwest Bohemia, Czech Republic) with general comments on hybridization of European green and common toads. – Pp. 401–404 in Böhme, W., W. Bischoff & T. Ziegler (eds.): Herpetologia Bonnensis. – Bonn (SEH).

Zavadil, V., P. Vlcek & P. Roth (2003): Beobachtungen zur Entwicklung hybrider Exemplare von *Bufo viridis* x *Bufo bufo* im Labor. – Mertensiella **14**: 263–269.

Zavanella, T. & M. Losa (1981): Skin damage in adult amphibians after chronic exposure to ultraviolet radiation. – Photobiol. **34**: 487–492.

Zavanella, T., N. Pacces Zaffaroni & E. Arias (1984): Abnormal limb regeneration in adult newts exposed to the fungicide maneb 80. A histological study. – J. Toxicol. Environ. Health **13**: 735–745.

MERTENSIELLA 25 | 243–260 | 2017

Methodological recommendations for studying the causes of anomalies in natural populations of amphibians

Alain Dubois[1] & Klaus Henle[2]

[1]Institut de Systématique, Évolution, Biodiversité
ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE
Muséum national d'Histoire naturelle, Sorbonne Universités
57 rue Cuvier, CP 30, 75005, Paris, France
adubois@mnhn.fr

[2]UFZ – Helmholtz Centre for Environmental Research,
Department of Conservation Biology, Permoserstr. 15, 04318 Leipzig, Germany,
klaus.henle@ufz.de

Abstract. The study of anomalies in natural populations of amphibians is a research field of its own, requiring a rigorous, careful and complex methodology. In order to draw reliable conclusions about the distribution, frequency, cause and conditions of appearance of a given anomaly in a population, data must be gathered at all stages of the study, both in the field and in the laboratory, and on both abnormal and normal specimens of all amphibian species occurring on a particular study site. The field survey protocol should involve a careful examination of all specimens and all ages of all amphibian species collected in the studied locations, and not only of those showing gross anomalies. Only then, will one obtain reliable data on the frequency of anomalies, on their distribution among sexes and age classes, on their variation in time and space and on their morpho-anatomy, including data on bilaterality and symmetry, degree of severity and association with syndromes. Specimens showing morphological, pigmentary or other anomalies should be brought back to the laboratory for long-term study on their evolutionary potential, regeneration and hybridization experiments, experimental gynogenesis and other surveys that will enable reliable conclusions to be drawn about their causes. If elevated frequencies of abnormal individuals are found, sites should be investigated for the presence and association of potential causes of the observed anomalies with patterns observed in the field. Field and laboratory experiments should be designed to test the effect(s) of hypothesized causes. Moreover, we stress the importance of differentiating between mutagenic factors (inducing hereditary mutations) and mere teratogenic factors (causing anomalies that are not hereditary). This is a point that is often overlooked and yet it is so important because the subsequent fate of the anomaly in a population can be very different in both cases.

Keywords. Amphibia, anomaly, experimental teratogenesis, field protocols, inheritance experiments, methodology, pattern analysis, regeneration experiments.

Zusammenfassung. Forschung zu Anomalien in Freiland-Populationen von Amphibien stellt ein eigenes Forschungsfeld dar, für das eine rigorose, sorgfältig geplante und komplexe Methodik erforderlich ist. Um verlässliche Schlussfolgerungen über die Verteilung, Häufigkeit, Ursachen und Bedingungen treffen zu können, unter denen bestimmte Anomalien in einer Population auftreten, werden Daten benötigt, die alle Phasen der Untersuchung im Gelände und Labor und sowohl abnormale als auch normale Individuen aller Amphibien-Arten einschließen, die im Untersuchungsgebiet vorkommen. Feldprotokolle sollten eine sorgfältige Dokumentation aller untersuchten Individuen aller Altersklassen aller Arten umfassen und nicht etwa nur stark missgebildete Individuen berücksichtigen. Nur dann erhält man verlässliche Daten über die Häufigkeit von Anomalien, deren Verteilung auf die Geschlechter und Altersklassen, deren Variation in Raum und Zeit sowie deren morphologische und anatomische Ausprägung, inklusive ihrer Bilateralsymmetrie, Gradienten in der Schwere der Ausprägung und der Kombination unterschiedlicher Anomalien in Syndromen. Individuen mit morphologischen oder Färbungsanomalien sollten für langfristige Untersuchungen ins Labor gebracht werden, um das Evolutionspotential zu erforschen sowie um Regenerations-, Kreuzungs- und gynogenetische Experimente und andere Untersuchungen durchzuführen, die verlässliche Rückschlüsse auf die Ursachen der Anomalien ermöglichen. Wir betonen die Wichtigkeit, mutagene Faktoren (die Mutationen hervorrufen, die an die Nachkommen vererbt werden) von teratogenen Faktoren (die Anomalien verursachen, die nicht an die Nachkommen vererbt werden) zu unterscheiden, was häufig ignoriert wird, denn das Schicksal von Anomalien in einer Population kann für die beiden Fälle sehr unterschiedlich sein.

Schlagwörter. Amphibia, Anomalien, experimentelle Teratogenese, Feldprotokolle, Methodik, Musteranalyse, Regenerationsexperimente, Vererbungsexperimente.

Résumé. L'étude des anomalies dans les populations naturelles d'amphibiens est un domaine de recherche particulier, qui exige l'emploi d'une méthodologie rigoureuse, soigneuse et complexe. De manière à pouvoir tirer des conclusions fiables sur la répartition, la fréquence, la cause et les conditions d'apparition d'une anomalie donnée dans une population, des données doivent être recueillies à tous les stages de l'étude, aussi bien sur le terrain qu'au laboratoire, et sur les spécimens anormaux et normaux de toutes les espèces d'amphibiens présentes sur un site d'étude. Le protocole d'étude de terrain doit consister en un examen soigneux de tous les spécimens de tous âges de toutes les espèces d'amphibiens récoltées dans le peuplement étudié, et non pas seulement de ceux qui manifestent des anomalies spectaculaires. Ces observations permettront d'obtenir des données sur la fréquence des anomalies, sur leur distribution au sein des classes de sexe et d'âge, sur leur variation dans le temps et l'espace et sur leur morpho-anatomie, comportant des données sur leur bilatéralité et leur symétrie, sur leurs gradients de gravité et leur association en syndromes. Les spécimens présentant des anomalies morphologiques, pigmentaires ou autres devront être rapportés au laboratoire pour l'étude à long terme de leur évolution potentielle, pour des expériences de régénération, des croisements, des expériences de gynogenèse artificielle et d'autres études qui permettront d'établir leurs causes. La différence entre les facteurs mutagènes (qui induisent des mutations qui seront héritées par les descendants) et les facteurs simplement tératogènes (responsables d'anomalies qui ne seront pas transmises aux enfants), qui est souvent négligée, est ici particulièrement soulignée, car le devenir ultérieur de l'anomalie dans la population peut être fort différent dans les deux cas.

Mots-clés. Amphibia, analyse de variation, anomalies, expérience de régénération, expérience génétique, expérience tératogène, méthodologie, protocole d'étude de terrain.

1 Introduction

Increased environmental awareness has created a resurgence of interest in amphibian anomalies as an indicator of environmental conditions, with potential implications for human health (e.g., Tyler 1989, Burkhart et al. 2000, Ouellet 2000, Vershinin 2002). For an effective use of amphibian anomalies as an indicator of environmental health and potential human hazards, it is of prime importance to distinguish between the various causes of anomalies, and above all between genetic and non-genetic anomalies. This distinction is particularly useful when attempting to understand the possible fate of a given anomaly in a given population and therefore its potential impact on the survival rate and health of that population. A particular situation, however, is that of teratogenic-mutagenic factors, such as radiation, that may have both a direct phenotypic effect on exposed individuals as well as on their gametes, While genetic anomalies are passed on to offspring or part of it (sometimes through complex mechanisms) non-genetic anomalies are not hereditary. This distinction is particularly useful when attempting to understand the possible fate of a given anomaly in a given population, and therefore its potential impact on the survival rate and health of that population.

The distinction between genetic and non-genetic anomalies cannot be made through the phenotype by itself and there is a good reason for this: the possibilities of morphogenesis are constrained by the ontogenetic steps covered at each stage of development. Thus, after the blastula stage, there are three possibilities: (1) the embryo stops developing incurring death; (2) it goes through the gastrula stage, which is the first stage at which parental genes become active (Davidson 1976); (3) it results in an exogastrula that is bound to die. The "errors" leading to an exogastrula can be caused by various factors, such as chemicals (Stanisstreet 1974, Brennan 1991), irradiation (Hertwig 1911, Kawamura & Nishioka 1978), hybridization (Hertwig 1918, Delarue 1982), mutations (Armstrong & Gillespie 1980) or overripeness of eggs (Briggs 1941, Zimmerman & Rugh 1941). On its own, an exogastrula tells us nothing about these causes. The same applies (with some minor differences) at all stages of morphogenesis. Thus, when limb morphogenesis is at the stage of differentiation of digits, whereas a normal development would lead to four fingers and five toes, anomalies can occur with more (polydactyly) or fewer digits (oligodactyly), with fusion of digits (syndactyly), abnormal shape (clinodactyly), etc. These phenotypes may be caused by various factors, some of which are genetic whereas others are not (Rostand 1955, 1958, 1971, Dubois 1979, Henle et al. 2017a,b).

Genetic determinism has been demonstrated so far in various pigmentary anomalies (e.g., "albinism" or "black eyes": Héron-Royer 1887, Rostand 1951a, 1955, 1958, Humphrey 1967a, Humphrey & Bagnara 1967, Lacroix & Capuron 1970, Nishioka 1977, Kawamura & Nishioka 1978) and in several anomalies of the limbs (e.g., some cases of polydactyly or ectrodactyly: Rostand 1951a, 1955, 1958, Witschi & Chang 1954, Humphrey 1967b, Uehlinger 1969, Dubois 1974, 1977, Kawamura & Nishioka 1978) and the mouth (Droin & Beauchemin 1975, Kawamura & Nishioka 1978), as well as for torsion of tails (Browder 1972, Droin 1985) and edema (Uehlinger & Reynaud 1965, Hart & Armstrong 1984). It is also suspected in various other cases (e.g., Rostand 1951a, 1955, 1958, 1971, Dubois 1979, Henle et al. 2017a,b). However, the "same" anomalies, or at least anomalies that appear to be similar, may also be caused by non-genetic factors. With respect to colouration, so-called "albinism" may be caused by deficient hypophyseal or thyroidian physiology, the melanophores being permanently contracted (although melanin is present) (Hayes 2000), "black-eyed frogs" or "blue frogs" may result not only from mutations (Humphrey

& Bagnara 1967, Lacroix & Capuron 1970, Rostand & Darré 1970, Nishioka 1977, Nishioka & Ohtani 1986) but also apparently from other causes, as in some specimens this colouration in not stable (Dubois & Vachard 1971a, Dubois unpublished data). In terms of skeletal anomalies and edema, non-genetic cases can be caused by trematodes (Johnson et al. 2001), leeches (Veith & Viertel 1993), amputation (Wallace 1981, Muneoka et al. 1986), pollution from chemicals (Scadding & Maden 1986, Vershinin 2002), UV irradiation (Ankley et al. 2002) or still unknown teratogenic factors, such as that of anomaly P (Rostand 1971, Dubois 2014, 2017). Hormones in the water are known to cause developmental arrest and various cases of aneuchrony or so-called heterochrony (Rot-Nikcevic & Wassersug 2004). For more detailed reviews of known causes of amphibian anomalies see Rostand (1955, 1958, 1971), Dubois (1979), Ouellet (2000), Johnson et al. (2010) and Henle et al. (2017a,b).

Criteria are needed that distinguish genetic from non-genetic factors. Whereas direct criteria (resulting from controlled crosses) are preferable, in many cases they cannot be used, meaning that indirect criteria must be called upon. A review of potentially suitable criteria is presented below.

In order to distinguish these cases, several methodologies can be used that involve collecting basic data from the populations being studied and from abnormal specimens Thus, in order to be able to formulate hypotheses on the causes of observed anomalies, it is useful to have collected certain information when surveying natural populations of amphibians. As the presence of anomalies in a natural population may go undetected during a variable proportion of the total study of this population, the best approach would certainly be to systematically collect all this information when a new study of an amphibian population is undertaken: in some cases this information will later prove to be "useless", but even if only a fraction of this information is needed, it will be available from an early stage of studying this population. Moreover, because anomalies are not routinely assessed in herpetological fieldwork, it is difficult to establish baseline data for natural background rates of anomalies, and if they are assessed, the scoring is not standardized (Lunde & Jonhson 2012). Therefore, currently published data are available only for populations where anomalies have already been detected (reviewed by Henle et al. 2017a), but only rarely does data exist on the number of individuals examined from populations not showing any anomalies. This means that any attempt to establish a background rate of anomalies is biased upwards.

Our recommendations expand a recent publication by one of us (Dubois 2014). They complement the practical guide by Lunde & Johnson (2012) for the design of field surveys of malformed amphibians and the study of their causes and the recommendations for counting and summarizing anomalies by Borkin (2014).

2 Field survey protocol

The following demonstrates how important information can be obtained or derived from the mere *observation* of phenotypic anomalies in natural populations of amphibians. One important prerequisite however to be able to use this information is that: all specimens reported as *examined* must have been thoroughly observed and their phenotype reliably scored as "normal" or "abnormal" in some way. For this, a precise methodology for the examination of specimens must be followed noting *all* unusual characteristics of *all* specimens handled. As in some cases hundreds of specimens have to be examined in a single day or night, this examination process should not only be rapid, but also very thorough and follow a precise and repeatable protocol. Although many different protocols may be considered, for the sake of illustration we describe the methodology that one of us (AD) has followed in the field since 1962 for the examination of thousands of amphibians in hundreds of populations across Europe, Asia and North Africa (most of the data remains unpublished). An examination of live specimens was conducted in the field, immediately after or, if this was not possible, as soon as possible after the collection of specimens. This avoids harming specimens while they are interacting and limits the stress period, which is imperative when specimens are collected in great numbers during the breeding season.

As a matter of fact, an excellent period for examining large numbers of specimens is during breeding, as it provides immediate access to hundreds, thousands or even more specimens at once, enabling reliable quantitative data to be collected on the frequency of anomalies in natural populations and their variability, etc. However, if this period is chosen one must take care that the examination does not disrupt breeding activity unduly. Similarly, the time of metamorphosis may also be an excellent choice because many individuals are usually available at that time and most skeletal anomalies are already apparent (Lunde & Johnson 2012). For such data to be reliably obtained, it is indispensable that a given specimen be examined, described and counted only once, which requires methodological precaution.

One first possibility is to collect all specimens at once and store them before examination, e.g. in buckets, large basins or jute bags, then to examine them and release them in the same area where they were collected (a large pond, lake or river may be subdivided into distinct zones in order to release the specimens in the vicinity of where they were found). In a species like the common toad (*Bufo bufo*), this is the only way of obtaining reliable quantitative data, as the density of specimens may be very high, thus not allowing them to be released among the others without the risk of confusion. For *B. bufo*, this can be done without disturbing breeding behaviour: both males and females can remain stored for several hours in bags or basins without showing any signs of notable stress (even when hundreds of specimens are kept

together), and they will breed normally after release. In some other species, however, breeding specimens should not be kept for long, as this may induce evacuation of the eggs by the females and/or a loss of motivation to breed in the specimens of both sexes, which may then leave the breeding spot without having laid any eggs. In such species, special care should be taken to avoid any disruptive consequences of the study on breeding. Also, if disease transmission is of concern, precautionary measures, such as wearing gloves and sterilizing equipment, should be taken (Lunde & Johnson 2012).

Another possibility is to examine the specimens directly in the field at the point of capture and to release them immediately (except for those showing some anomalies that call for further study). This is possible for species with lower densities and where breeding specimens are more or less regularly spread along the banks of the water body. However, after examination, specimens do not always remain at the release spot: they may swim rather far, for example, to another unexplored part of the pond. To avoid confusion, it is necessary to mark them after examination, either for a short period (e.g., with colour spots) or more durably (e.g., by toe-clipping, PIT tags or taking photos of patterns that distinguish individuals) – for reviews of marking methods see Donnelly et al. (1994), Henle et al. (1997) and Ferner (2007).

Specimens can be examined standing in or beside the water body, but this is more reliable and complete if carried out under more comfortable conditions, e.g., sitting on a (pliable) chair, and even better using a (pliable) table.

For reliable data, it is crucial that the information on specimens is recorded during the examination phase, and not afterwards. Several methods can be used to record the observations. When a single observer is in the field, this person must both examine the specimens and take down the information, which is not easy as handling wet specimens is not really compatible with writing on paper or typing on a computer keyboard. One can sometimes examine the specimens with one hand and write with the other, but this is not always possible, as sometimes both hands are needed for detailed observation. It is better to use a pencil than a pen, as ink may blot on damp paper and notes may be lost. A good but expensive solution is the use of special writing equipment developed for underwater use. When alone in the field, it is easier to dictate the observations on a voice-recording device, but attention is required to check if the tape has finished or if batteries have run out. It also has another drawback: that of almost doubling the work time, as tapes later have to be copied onto files. The best method is definitely to work as a team, with one observer and one "secretary", taking down the observations as they are made, either on paper or on a portable computer. In the daytime, for most amphibian species, a simple visual survey, without binoculars or a magnifying lens, is sufficient for making all useful observations on specimens in hand, but at night, or with very small specimens, a headlamp is necessary, unless work can be conducted in a building with light.

Several pieces of information must be gathered when conducting such fieldwork. First, the precise place, date and hours of collection of specimens, and their examination, must be noted. Then all amphibian species must be reliably identified, and if there is any uncertainty (i.e. with larvae, young, or even adults of closely-related species), it should be recorded as such. A convenient system is to designate a three-letter code to each species (e.g., BUF for *Bufo bufo*, TEM for *Rana temporaria*, PEL for *Pelophylax* unidentified at species level). Specimens must be referred to a precise sex and phenotypic stage category, which may be recorded using standard abbreviations, e.g., one of the following (Dubois 1974, 1976): egg (ω), developing egg (with embryo) (ε), larva (lar.), or tadpole (tad.), the latter having three possible subcategories (0 L tad., tadpole without limbs; 2 L tad., tadpole with two hindlimbs; 4 L tad., tadpole with four limbs), newly metamorphosed specimen or imago (im.), juvenile (juv.), subadult (sub.) and adult (ad.), the latter having two subcategories, male (♂) and female (♀).

Each specimen must be individually held and carefully examined. It is best to follow a standard course for the examination of all specimens, e.g., first both eyes, then the rest of the head, then the dorsal and lateral parts of the body, followed by the venter, then both forelimbs and finally both hindlimbs. Paired structures (eyes, limbs) must be examined on both sides of the body, as anomalies may be present on one side only. Most specimens will not show any unusual signs and must simply be scored as examined, in their respective category: e.g., BUF, 46 ♂, 5 ♀, 1 juv. Then, among these examined specimens, a small proportion may be briefly described, also in an abbreviated way (see Fig. 1), as showing some phenotypic anomalies or injuries. For the surveys of anomalies in the USA, the USFWS has developed a field form that is available online (http://www.fws.gov/contaminants/amphibian/SOPs.html) and may be adapted for other regions. The advantage of this field form is that many types of anomalies can be ticked-off but for each individual a separate sheet is necessary. Also note that the terminology used in this field form differs in some important aspects from the terminology used by other authors, including from North America [see Henle et al. (2017c) for translations among terminologies]. Care should be taken to record each specimen only once, i.e., for adopting a standard procedure for those specimens for which descriptive information is recorded: either to add them in the field to the total number of examined specimens, or to record them independently.

The abbreviated descriptions (quickly noted in the field) that appear in Figure 1 should be "translated" when transferred to a database, as shown in Table 1. A useful abbreviation for writing the number of digits is by using a digital formula in which the toes are given first, followed by the fingers, and in each case starting with the left side of the body. Thus a normal specimen of *B. bufo* would have the digital formula 5–5/4–4; a polydactylous with 7 toes on the left foot and 6 on the right foot and five fingers on the right hand would have the formula 7–6/4–5;

and an ectrodactylous with only 4 fingers on each foot would have the formula 4–4/4–4. Another useful sign for field notes is an arrow to indicate clino-dactyly, either medial (directed towards the inside of the foot: ←) or lateral (directed towards the outside of the foot: →). Limbs can be given a three-letter abbreviation, such as RHL (right hindlimb) or PAD (patte antérieure droite) in French, and digits are numbered starting from I for the most medial one. A drawing of a frog or salamander, as in the field sheet of the USFWS, may facilitate reporting the observed anomalies but requires more space.

When summarizing the data on anomalies in tables, it is important to be precise about how the figures are tallied. Several possibilities exist and it is rarely stated in the literature, which approach was followed, which makes comparisons among studies difficult or impossible. We therefore recommend including the following methodology as a standard that enables comparisons with other studies even if one uses additionally other approaches for tallying anomalies. As a standard, we recommend counting each type of anomaly only once per individual as it is usually impossible to know from field data whether they are linked or not to a single incidence. If one aims to provide information, for example of the number of limbs partially missing or in how many individuals this was the case for the forelimbs or hindlimbs, one may provide this information as additional information. Otherwise, it is impossible to know how many individuals were affected by ectromely and to compare this with other studies. Likewise, if a portion of a limb is abnormal (e.g. phocomely or an incomplete supernumerary limb), we discourage additionally tallying missing or multiple digits on these limbs as ectrodactyly or polydactyly as they probably have the same origin. Finally, one should tally sample size and the number of abnormal individuals, enabling an easy comparison with other such studies on the frequency of abnormal individuals or the frequency of a particular type of anomaly using c^2-tests, without uncertainties created by the potential interdependence of anomalies within an individual. Together with the information on the number of individuals affected by each type of anomaly obtained by the recommended standard approach, one can estimate the mean number of types of anomalies per individual and from the additional information the mean number of anomalies per individual.

Other additional information that could be of interest is size (snout-vent length or SVL) or mass. This can be useful for etho-ecological purposes, e.g., for studying correlations between injuries and size classes.

3 Patterns of anomalies as clues to potential causes

3.1 Frequency

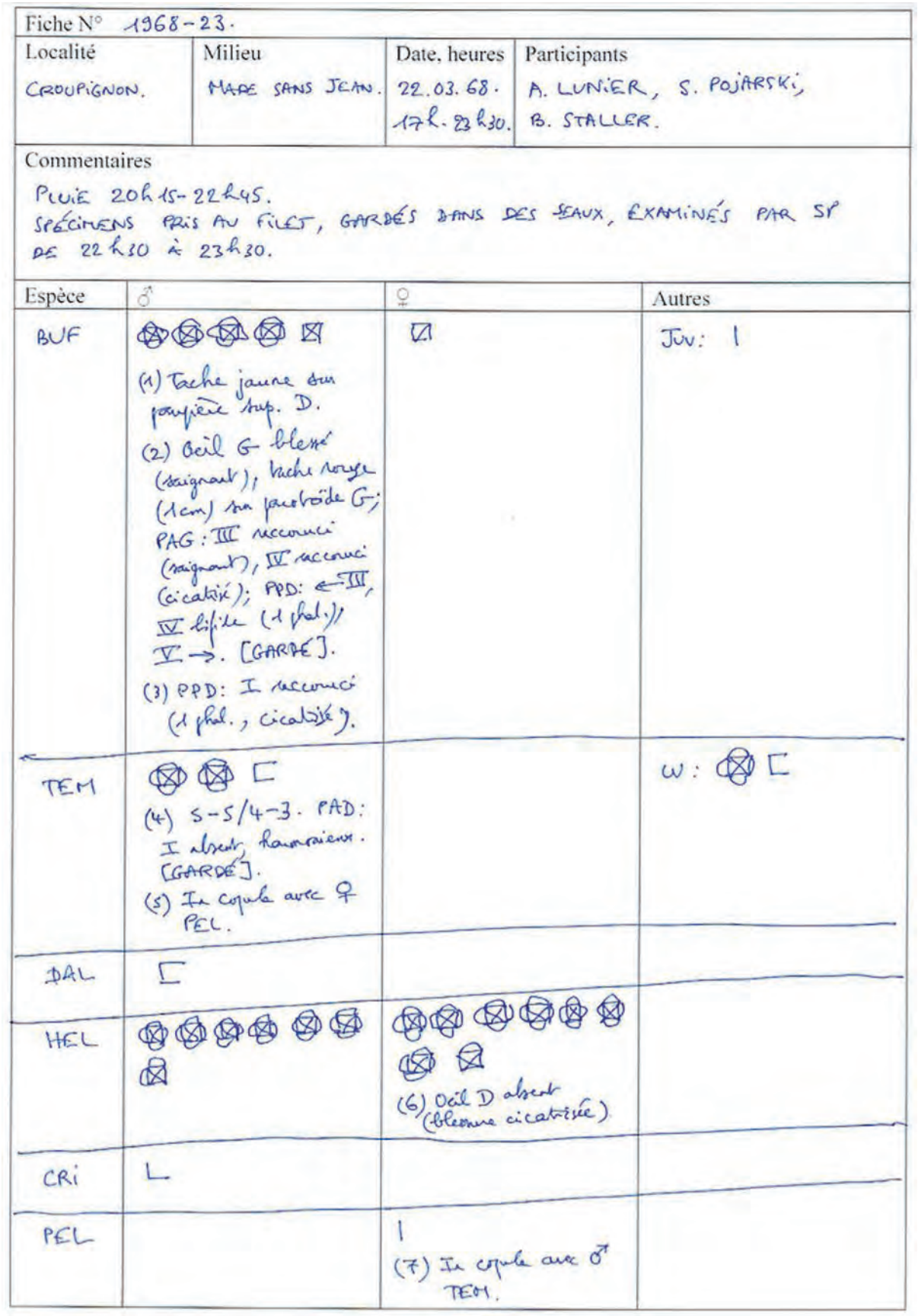
Although the frequency of anomalies is primarily determined by the strength of their cause (Nishioka et al. 1981, Power et al. 1989, Johnson et al. 2001, Henle et al. 2017b), it may also provide a first clue for understanding the cause. Isolated accidents can touch a single individual or a few animals. A teratogenic factor may have direct effects on several individuals. Some factors, such as some chemicals, may have rather specific effects on some parts of the phenotype only (e.g., limbs) or on some processes (e.g., metamorphosis) (Collenot 1965, Rot-Nikcevic & Wassersug 2004, Henle et al. 2017a). If this kind of factor is effective across the breeding site, all affected individuals may tend to show the same kind of anomalies (e.g., Rowe et al. 1998). Genetic-caused anomalies can start from mutation in a single individual and then spread among its offspring, which can either show similar phenotypic expressions of the anomaly (e.g., Humphrey 1967a, Benjamin 1970) or some variability in this respect (e.g., Dubois 1977). If a mutagenic factor is present in the environment, affected individuals will show a large range of different types of anomalies (Kuranova 2003, Henle et al. 2017a).

Any work on the anomalies in natural populations of amphibians requires numerical data on their frequency. This means that a population survey on anomalies must include precise *counts* of specimens examined from the very onset of the study, even before the discovery of anomalies. Therefore, from the first day of the survey, some basic information must be noted in the field, including the number of specimens *examined*, their sex, stage (from egg to adult) and all anomalies observed. Reliable frequency data requires at least some dozens of specimens to have been observed, but preferably several hundreds or, if background rates need to be established, thousands; see Lunde & Johnson (2012) for the sample size needed to obtain a required power. According to our personal experience, in a breeding population of *B. bufo*, an efficient working team of several experienced observers can easily examine several thousand specimens per day and during the first hours of night.

Three main categories can be identified in terms of the frequency of anomalies (or injuries) in natural populations of amphibians: isolated cases, a few per hundred or thousand and mass anomalies.

3.1.1 Isolated cases of anomalies or injuries

In any survey of a natural amphibian population, isolated cases of anomalies or injuries are bound to be observed. In such cases, each anomaly is unique of its kind or only superficially resembles another anomaly observed in another specimen of the same population. Such anomalies are usually of non-genetic origin, having been caused by random perturbations of morphogenesis or by accidents following the latter. These often result from escaped predation attempts (e.g. Ballengée & Sessions 2009; reviewed by Henle et al. 2017a), from interactions between congeners (reviewed by Henle et al. 2017a) or from injuries caused by external parasites, such as leeches (Veith & Viertel 1993) or by commensals, such as bivalve molluscs that sometimes clasp the extremities of amphibian digits (Dubois 1979, Kupferberg et al. 2009). If isolated, such injuries do not properly qualify as "anomalies", but they must be scored



Fiche N° 1968-23.

Localité	Milieu	Date, heures	Participants
CROUPIGNON.	MARE SANS JEAN.	22.03.68. 17h.-23h30.	A. LUNIER, S. POJARSKI, B. STALLER.

Commentaires

PLUIE 20h15-22h45.
SPÉCIMENS PRIS AU FILET, GARDÉS DANS DES SEAUX, EXAMINÉS PAR SP DE 22h30 à 23h30.

Espèce	♂	♀	Autres
BUF	⊠ (1) Tache jaune sur paupière sup. D. (2) Oeil G bleui (saignant), tache rouge (1cm) sur parotide G; PAG: III raccourci (saignant), IV raccourci (cicatrisé); PPD: ← III, IV bifide (1 phal.)// V →. [GARDÉ]. (3) PPD: I raccourci (1 phal., cicatrisé).	☑	Juv: 1
TEM	⊏ (4) S-S/4-3. PAD: I absent, flammaieux. [GARDÉ]. (5) En copule avec ♀ PEL.		W: ⊏
DAL	⊏		
HEL		(6) Oeil D absent (blessure cicatrisée)	
CRI	L		
PEL		1 (7) En copule avec ♂ TEM.	

Fig. 1: An example of a field sheet for scoring a pond in France, with hand-written data as entered in the field (in blue). See text for explanation of abbreviations.

Table 1: The field sheet of Figure 1 after a detailed transcription of the data in explicit form, for entering into the database.

Sheet number: 1968-23			
Locality: Croupignon	**Habitat:** Mare sans Jean	**Date & time:** 22 March 1968 17 h – 23 h 30	**Personnel:** Augustin Lunier, Saturnin Pojarski, Barbara Staller
Comments: Raining from 20 h 15 to 22 h 45 Specimens caught by net, kept in buckets, examined by SP from 22 h 30 to 23 h 30			
Species	♂	♀	**Other**
Bufo bufo	46 [1] Yellow spot on right eyelid [2] Left eye recently wounded (still bleeding), red spot of 1 cm in diameter on skin of left parotoid; left forelimb: finger III injured and shortened (still bleeding) and finger IV injured shortened (ancient injury, now scarred); right hindlimb: toe III with medial clinodactyly (directed towards inside of foot), toe IV bifid (last phalanx duplicated) and toe V with lateral clinodactyly (directed towards outside of foot). **Specimen kept alive:** 1968-23-2 [3] Right hindlimb: toe I shortened (1 phalanx, scarred)	5	1 juvenile
Rana temporaria	23 [4] Right forelimb with ectrodactyly: finger I missing, harmonious. **Specimen kept alive:** 1968-23-4 [5] Found in copula with ♀ [7] of *Pelophylax*	0	13 clutches
Rana dalmatina	3	0	0
Lissotriton helveticus	68	77 [6] Right eye missing (healed injury)	0
Triturus cristatus	2	0	0
Pelophylax sp. (*esculentus* ?)	0	1 [7] Found in copula with ♂ [5] of *Rana temporaria*	0

during the survey, as only at the end of the survey will it be known whether they are isolated or whether a given pattern of occurrence or an unusual frequency calls for specific interpretation because sometimes it is not easy to determine whether an anomaly is due to an injury, a disease or a developmental deviation.

3.1.2 Anomalies observed in a few individuals per hundred

When large numbers of specimens (at least several hundreds) are examined in a population, it is not uncommon for several individuals to show similar abnormal phenotypes, such as unusual colouration or anomalies of limbs or digits. In such cases, the abnormal phenotype is often very similar in all affected specimens. They cannot be considered as individual random accidents, and they call for a specific explanation. The latter may be of two kinds, as discussed above, involving either a genetic basis or a non-genetic factor acting specifically in a certain domain of ontogenesis. Mere observation of the phenotype is not usually sufficient to identify this factor (Henle et al. 2017a,b), but calls for additional studies, which may focus on those factors that are known to have the potential to cause the phenotype in question as compiled by Henle et al. (2017a).

3.1.3 Mass anomalies

We talk about mass anomalies when several per cent of the individuals in a population or even several tens per cent are affected. Often 5% is used as a threshold beyond which incidences are regarded as of concern (Henle et al. 2017a). There are two major, very distinct situations in this respect. The first one is a mass occurrence of a single anomaly or syndrome, as in the anomaly P, all abnormal phenotypes being clearly *related*, e.g., showing various levels (on a severity scale) of polydactyly or ectrodactyly. In all known cases of such mass syndromes in amphibians, the cause is not genetic, but the syndrome is due to a powerful external *teratogenic* factor. In such cases the anomalies can not be passed on to offspring. Theoretically, it is possible that a high percentage of specimens show the same anomaly caused by a single mutation, e.g., if all affected individuals were derived from the same clutch and the tadpole population only originated from a few breeding individuals. For example, more than 100 white tadpoles of *Dryophytes japonicus* were collected from five populations spread over an area of 6000 km² surrounding Hiroshima that were due to at least three recessive albino genes (Nishioka & Ueda 1977). However, such cases can usually be discerned from the data on the total number of individuals collected in the field and known reproductive information on the species of concern.

The second situation is that of a population showing a wide range of anomalies that include unrelated characters (colouration, limbs, tumours, etc.) either in different individuals or even in the same ones. Most potential factors that cause anomalies produce only a rather limited set of, often related, types of anomalies and therefore could explain such cases only if a range of factors co-occur (see review by Henle et al. 2017a). However, such cases can be observed whenever a powerful *mutagenic* factor has acted randomly on the DNA of different individuals, thus producing a variety of unrelated anomalies. As many chemicals are known to be mutagenic, genetic causes for anomalies should not be dismissed from the outset, as in some North American studies (e.g., Meteyer et al. 2000, Loeffler et al. 2001; but see Lowcock et al. 1997). In such cases the anomalies can be passed on to offspring, if the abnormal specimens survive until breeding, and thus breeding experiments should be conducted in such cases.

3.2 Distribution of anomalies among sexes and age classes

So far, no sex-linked anomaly has been reported in amphibians from natural populations, but is known from a laboratory stock of *Pleurodeles waltl* (Collenot et al. 1989). Two mutant recessive alleles affecting the distribution of melanophores and the number of xanthophores in early larval respectively metamorphosed individuals were linked to the W sex chromosome. Any significant difference in the frequency of an anomaly in males and females should therefore be interpreted as suggesting a genetic origin. Exceptions are sexually dimorphic characters, which are only affected in one sex, also by non-genetic teratogenic factors. The only example of this kind known to us is the treatment of male *Hyperolius argus* tadpoles with estrogen at the climax of metamorphosis. The males that develop from such tadpoles adopt a female colouration (Hayes 1997, Hayes & Menendez 1999).

Clues can also be obtained from different frequencies of anomalies in different age classes. Without precise data on age (which requires the use of rather difficult techniques such as skeletochronology: Caetano et al. 1985), data on stage classes, as outlined above (larvae, recently metamorphosed individuals, juveniles, adults), can be useful. Thus, if an anomaly is present, or even abundant, in a given stage class, but completely absent in others, this strongly suggests limited action in time of a non-genetic factor. However, such data should be interpreted with care, as some anomalies, even of genetic origin, can change during the life of an individual: for example, some pigmentary anomalies may show strong changes over years (e.g., Dubois 1968, Dubois & Vachard 1971a, Nishioka & Ueda 1977), and the same is true for some limb anomalies, such as clinodactyly, which develops after some years during the growth of polydactylous individuals (e.g., Dubois 1974). Furthermore, if the anomaly is only present in the younger age classes but absent in older age classes, this may result from complete lethality of the affected individuals or from remodelling during metamorphosis. For example, the torsion of tail in larval *Salamandra salamandra* may disappear during metamorphosis (Henle et al. 2017b).

3.3 Variation of anomalies in time and space

Some anomalies of non-genetic origin, such as anomaly P, show a variable occurrence in time and space in the same population (Rostand 1971, Dubois 1979, 1984, 2014, 2017, Henle et al. 2017b). There are "good years" and "bad years", "good places" and "bad places". Obtaining information on these aspects requires detailed information on the origin of anomalous specimens in various parts of the water body and over different years.

Some mass anomalies may have a significant impact on the survival of individuals, while in other cases they may threaten populations (Dubois 1979; reviewed by Henle et al. 2017b). Nevertheless, evidence exists that some anomalies may be latent in a given population over rather long periods of time, at least for 27 years (Dubois 1984, Vershinin 2002) or longer (Dubois, unpublished data). This can apply to anomalies resulting both from non-genetic and genetic causes. Even syndromes caused by mutations that appear deleterious in the individuals carrying them can remain present in a population for a long time. For example, the *No* syndrome of *Rana temporaria*, associated with various anomalies of limbs, first observed in 1968 (Dubois & Vachard 1969) and shown later to be caused by a dominant mutation (Dubois 1977), was found again in the same population in 1992 (Dubois unpublished data), so that the long-term persistence of an anomaly in a population alone is not informative about the cause of the latter.

3.4 Morpho-anatomy of anomalies

As discussed above, the same anomalous phenotype might result from a number of different reasons: e.g., polydactyly may result from hyperregeneration (Brunst 1961, Rose 1964), trematodes (Johnson et al. 2001) or mutations (Rostand 1951a,b, 1955, 1958, 1971, Uehlinger 1969, Dubois 1974, 1979, Droin & Fischberg 1980). In spite of this, however, a careful examination of the morpho-anatomy of anomalies can provide some useful insights (Henle et al. 2017a). Several distinct pieces of information must be distinguished.

3.4.1 Particularities of anomalies

Some syndromes can be recognised by their "trademark" or "family likeness". For example, in most cases of anomaly P, the first (medial) additional toe is longer than the next one and often almost perpendicular to the latter (Rostand, 1955, 1958, 1971). For weak polydactyly, of apparently complex genetic origin, described in *B. bufo* by Dubois (1974), the abnormal phenotype (distal bifidity of the first toe, the last phalanx of which is duplicated) is always of a similar nature. Despite the diversity of colour anomalies, pigmentary anomalies resulting from the same genetic cause are usually more similar in nature than they are to anomalies of different origins (e.g., among the various "unpigmented" or "black-eyed" frogs: Rostand 1958, Nishioka 1977, Nishioka & Ueda 1977, 1985b).

3.4.2 Bilaterality and symmetry

Important information is provided by anomalies that are bilateral and, above all, symmetrical. Injuries caused by accidents are very rarely bilateral, and in the rare cases where they happen to be so (e.g., Van Gelder & Strijbosch 1995), they are not symmetrical. If the anomalies tend to be bilateral and symmetrical, it indicates either a genetic factor or a non-genetic factor to which both sides of the animal have been similarly exposed, such as elevated UV in laboratory studies (Ankley et al. 2002). Many but not all ontogenetic anomalies, whether genetic or not (such as anomaly P), are bilateral and tend to be symmetrical). The symmetry is rarely "perfect", but it testifies for the existence of a similar impact from the factor responsible for the anomaly (whatever it may be) on both sides of the body. Thus, in anomaly P, the number of additional toes and fingers varies from one frog to another, but there is never a difference of more than one digit between both sides of the specimen's body (Dubois 1979). Likewise, the genetic syndrome *No* in *R. temporaria* always occurs bilaterally (Dubois 1977).

A few exceptional cases of mass anomalies are known that are asymmetrical on each individual and can be found only, or mostly, on one side of the body. Polymely occurring almost exclusively on the right hind limbs of *Pelophylax ridibundus* (sample size > 6000 individuals) was described in detail by Woitkevitch (1959, 1961, 1965) and is a unique case. Such a bias for left or right unilaterality or prevalent laterality calls for a specific explanation. It cannot be due to repeated accidents but must be caused by a specific ontogenetic teratogenetic factor, which has not yet been identified, but which might prove useful for evolutionary developmental studies of bilateralisation.

3.4.3 Gradients of severity

A strong indication of an ontogenetic anomaly is provided by the existence of gradients of severity or seriousness in the expression of anomalies. The most frequently observed kind is that of a *postero-anterior* gradient, e.g., in anomaly P (Rostand 1971, Dubois 1979, 2014, 2017). In such cases, in the less severely affected specimens only the hindlimbs exhibit the anomaly. The forelimbs are only affected if the hindlimbs are already affected and often only if the anomaly reaches a certain level of severity in the hindlimbs. This can be explained by the chronology of development, as in anuran tadpoles the hindlimbs develop much earlier than the forelimbs.

Other gradients that are sometimes observed (e.g., Woitkevitch 1959, 1961, 1965, Rostand 1971, Dubois 1977, Dournon et al. 1998) are *medio-lateral* (most digital anomalies start from the medial side of the limb), *latero-lateral* (from one side of the body to the other), *proximo-distal* (from base to extremity) and *disto-proximal* (from extremity to base). The latter two could be cases of brachydactyly, brachyphalangy, symphalangy or hyperphalangy. For example, Tschumi (1954) observed that the loss of digits and limb elements progress disto-proximally in severity and that the sequence of finger

loss is usually I > II and V > III and IV under the influence of factors causing a reduction in the number of viable cells in the blasteme of the developing limb bud.

3.4.4 Syndromes of anomalies

A syndrome is a family of related anomalies, involving the same organs or tissues but showing variability, particularly in the degree of severity. Identifying a syndrome does not reveal its cause, some being genetic and some non-genetic, but it does facilitate comparisons among cases.

Syndromes that are known to have a genetic origin include various degrees of severity observed in a polydactyly syndrome due to a recessive mutation in *Xenopus laevis* (Uehlinger 1969), syndromes associated with weak polydactyly, hyperphalangy and clinodactyly due to recessive mutations in *B. bufo* and *Rana graeca* (Dubois 1974) and dominant mutations in *R. temporaria* that result in ectrodactyly, clinodactyly and syndactyly (Dubois & Vachard 1971b) and additionally hypophalangy, symphalangy and brachyphalangy (Dubois 1977).

The best known syndromes of non-genetic origin are the anomaly P of European green frogs (genus *Pelophylax*), which is presumably caused by a virus, and the anomalies in several North American amphibian species (especially in *Pseudacris regilla* and in the genera *Lithobates, Anaxyrus* and *Ambystoma*) caused by the trematode *Ribeiroia ondatrae*. Anomaly P involves polydactyly, brachymely, polymely and tumours, i.e., anomalies affecting the limbs and girdles, but no other part of the body (Rostand 1971, Dubois 1979, 2014, 2017). *Ribeiroia ondatrae* parasitizes 71 North American amphibian species (Lannoo 2008). Infected individuals primarily exhibit polymely, polydactyly and skin webbing (Johnson et al. 2003), but may also show (usually to a lesser extent) taumely, femoral projections and limb hyperextensions as well as (in *P. regilla* only) ectrodactyly and ectromely (Johnson et al. 2001, 2003).

3.4.5 Association of various anomalies that do not constitute a syndrome

Some anomalies may be associated without being part of a syndrome. Such associations may exist both at the level of the individual and at that of the population.

3.4.5.1 Individuals. A single individual may show several anomalies that are found separated in other individuals of the same population. Such associations may be purely random or may result from common causes. An extraordinary case of random association between two independent anomalies is that of a specimen of green frog (*Pelophylax*) that was found in a natural population where both anomaly P (caused by an unknown non-genetic factor) and "black eyed" specimens (anomaly caused by a recessive mutation) were known to be present (Dubois 1968): in the sample studied (1,552 frogs), 21 (i.e., 1.35 %) showed anomaly P and four (i.e., 0.25 %) had black eyes, but one specimen had both anomalies. If both anomalies are independent, the statistical chances of finding a specimen with both anomalies is 0.003 %, so that, from a statistical point of view, about 28,675 specimens (instead of 1,552) should have been examined to find a "double abnormal" one! In this case, however, there is no known evidence that either of the two anomalies facilitates the appearance of the other one, so that the "random" hypothesis is the best one, which is also supported by the fact that no such "double abnormal" specimen was ever found again later in the same population (Dubois 1979).

Some other associations of anomalies are found in the same individual because they have the same proximal cause. For example, hypophysectomy or thyroidian deficiencies both result in anomalies of development (aneuchrony) and in pigmentary anomalies (depigmentation) (Bodenstein 1932, Pohland 1962). Finally, others result from having the same ultimate cause, as in the case of individuals exposed to strong aggressions, e.g., radiation: this can then be referred to the next category.

3.4.5.2 Populations. Some mass occurrences of anomalies in some populations show a wide polymorphism: the anomalies are very diverse, as they may concern various systems and organs (e.g., Kuranova 2003, Henle et al. 2017a). Such anomalies are caused by teratogenetic or mutagenetic external aggressions, which indiscriminately touch different characters.

A very high rate of injuries and amputations (ectromely, ectrodactyly) in a population may be a testimony of a very high pressure of predation (Veith & Viertel 1993, Bohl 1997, Ballengée & Sessions 2009) or parasitism (Kupferberg et al. 2009) or may even result from mowing machinery (Storer 1925), but it has also been related to chemical pollution (e.g., Ouellet et al. 1997; reviewed by Henle et al. 2017b). The impact of predation may be very variable within and among species according to the population and developmental stage. For example, the leech *Erpobdella octoculata* inflicts injuries to the limb buds that result in ectromely – only in *B. bufo* but not in *Rana temporaria* (Bohl 1977). The rate of injuries could be used as a criterion for roughly measuring the ecological pressure of predation on amphibian populations (Dubois 1979), as has been done in lizards where the rate of injuries has been used as a measure of predation (Henle 1990, Wilson 1992, Niewiarowksi et al. 1997; but see Jaksić & Busack 1984).

In contrast to ectromely and ectrodactyly, there does not appear to be any data on a single proven case of hyperregeneration due to injuries by invertebrates (e.g., none of the experimental studies with dragonflies or leeches caused polymely; they all resulted only in ectromely and tail injuries; reviewed by Henle et al. 2017b). Thus, hyperregeneration due to predators seems to be very rare under normal conditions (i.e., affecting only some odd individuals). This could be explained by the fact that legs only have this potential during a limited phase of development and the injury must be such

that the positional values of the cells in the limb stumps change (Nye et al. 2003), which can be carried out in experimental manipulations (e.g., Sessions & Ruth 1990) but would not normally occur when attacked by predators. The only proven polymely due to other organisms that is known to regularly occur is that caused by the trematode *Ribeiroia ondatrae* (Johnson et al. 2003). In this case, it is not hyperregeneration, but rather that the limb does not develop normally from the limb buds due to mechanical or chemical – still unresolved – inference with signalling pathways.

Finally, mass anomalies may be due to the action of a strong and non-specific teratogenic or mutagenic factor on eggs, larvae or adults. This factor may be chemical (industrial waste products, pesticides, hormones; e.g., Mizgireuv et al. 1984, Ouellet et al. 1997, Rowe et al. 1998), physical (radiation; e.g., Kuranova 2003, Tyler 1989) or biological (parasites, pathogens; e.g., Johnson et al. 2003). In such cases, it is necessary to look for these possible ultimate causes both in the amphibians and in the habitat. Mutagenic factors are strongly indicated if more than one different abnormal colour type or abnormal colouration, abnormal morphology and tumours occur together within the same population (Henle et al. 2017a).

3.5 Similar anomalies concerning several sympatric species

Another important clue is the presence of similar anomalies in different species living in the same habitat. Such cases indicate that a non-species-specific teratogenic factor may have had an effect on the eggs, tadpoles or adults of several syntopic species during the same period in ontogeny. Examples include the occurrence of ectromely and ectrodactyly in several *Lithobates* species and *Anaxyrus americanus* in ponds contaminated with agricultural run-off in Canada (Ouellet et al. 1997), tumours, ectromely, ectrodactyly and polydactyly in *Bombina bombina, Bufotes viridis* and *Pelophylax ridibundus* breeding in ponds polluted by industrial waste discharge in Russia (Flax & Borkin 1997) and various limb anomalies in *Lissotriton vulgaris* and *Rana arvalis* living in impacted ponds in the city of Yekaterinburg, Russia (Vershinin 2002).

Theoretically, similar selection pressures may also favour similar phenotypes in several species. For example, Vershinin (2002 and references cited therein) showed that striped and un-striped individuals of *Rana arvalis* have different physiologies placing striped individuals in a selective advantage in polluted habitats where they clearly dominate, whereas un-striped morphs dominate unpolluted habitats. A similar case in the North American ranid *Lithobates pipiens* is well-documented. It concerns two dominant mutations affecting the pigmentary pattern, initially described as different species (*Rana burnsi* and *R. kandiyohi*), and the persistence of which in natural populations seems to be due to an equilibrium between different selection pressures, related not only to the colouration but also to other characters, including physiological ones (Merrell 1965, 1972, 1973, Dubois 1980). In 1972, Dubois (unpublished) found unpigmented tadpoles in both *Duttaphrynus stomaticus* and *Hoplobatrachus tigerinus* at the same locality in central Nepal (Pokhara), but this isolated observation may well be due to a pure random phenomenon.

4 Study of environmental factors

In order to identify potential external factors that may be responsible for some anomalies, it is useful to collect information on the parameters of the environment known or suspected to influence the development of anomalies. These may be physical (temperature, irradiation, oxygen, pH, etc.), chemical (pesticides, endocrine substances; particularly in water but also in the soil) or biological (hormones, bacteria, viruses, parasites, predators). Unfortunately, these parameters are potentially numerous and often time-consuming or costly to study. Therefore, the survey of such environmental factors should be based on carefully-developed hypotheses concerning the genuine role of such factors. Furthermore, it should always be remembered that *a correlation is not a cause*, so that the presence, e.g., of a high rate of pesticides or predators does not demonstrate *on its own* that this factor is indeed responsible for the anomalies. Such a factor may have an effect on some other characteristics of the habitat, which are then the direct agents causing the anomalies. For example, there is a link between pesticide exposure and trematode infection facilitating the development of anomalies (Kiesecker 2002). The toxicity and teratogeneity of fluoranthene is enhanced by UV photoinduction (Hatch & Burton 1998) and both enhancement (e.g., Dawson & Bantle 1987, Andrén et al. 1988) and protective effects of chemicals (e.g., Luo et al. 1993) on the teratogenetic effects of other chemicals are known. However, correlations warrant further investigations of the potential role of those environmental factors associated with the observed anomalies, providing the basis of hypotheses, which then need to be tested experimentally. See Eberhardt & Thomas (1972) for designing field studies and Lunde & Johnson (2012) for designing studies to assess the role of trematodes in amphibian anomalies. As far as mutagenic factors are concerned however, the situation is somewhat different. If they are present above a certain threshold they will invariably lead to anomalies, although the details of the latter are usually unpredictable.

5 Direct study of the causes

A survey of anomalies in amphibian populations cannot be complete without direct research of the proximate causes of these anomalies. Even if external factors have been shown to have played a role as ultimate causes, this is not sufficient to understand the final way of acting, and above all to distinguish between mutagenic and non-mutagenic factors. Mutagenic factors will have modified the genome of the amphibians and can thus

be passed on to offspring, whereas anomalies caused by non-mutagenic factors will not be inherited by the progeny. Therefore the fate of the anomaly in the population, and the impact on survival of the latter, will be very different in the two cases. In the laboratory, the following methods can be applied to obtain more precise information than the obviously limited data that can be obtained in the field.

5.1 Detailed anatomy

Detailed studies of the anatomy of abnormal specimens are necessary to go further than the mere description in terms of "polydactyly", "ectrodactyly", etc. Radiographs or even a simple clearing of tissues, e.g., by short exposure to hydrogen peroxide (in tadpoles and imagos), can reveal those bones that are modified, absent or supernumerary (e.g., Bergendal 1889, Dubois 1974, 1977, Stopper et al. 2002). Histology, sometimes after dyeing, can show the direct modifications of tissues (e.g., tumours: Burns & White 1971, Pfeiffer et al. 1979) or the presence of exogenous elements in the tissues (parasites, fungi, etc.: e.g., Pfeiffer & Asashima 1997, Stopper et al. 2002, Lunde & Johnson 2012). To ascertain the nature of colour anomalies, the pigmentary cells and their pigments must be examined (e.g., Bagnara 1966, Nishioka & Ueda 1977, 1985a).

5.2 Long-term observation of phenotype

Once collected, abnormal specimens should not be released or killed and fixed in the field, but preferably be brought back to the laboratory for breeding. First of all, the possibility of change in the phenotype over time should be explored. Several pigmentary anomalies are labile. The colouration of black-eyed, blackish and whitish or blue frogs, etc., may change over months or years (Dubois & Vachard 1971a, Frost et al. 1984, Nishioka & Ueda 1985b, Günther 1996). This may be due to an ontogenetic evolution of the anomaly and the genetic expression of a mutation being variable according to the tissue, age or other factors. The anomaly may also be caused by factors that are still active (e.g., a virus infection that progressively destroys some pigment cells). Some anomalies of limbs also tend to change over time: thus, clinodactyly may only develop after several months or years, as the digits grow (as shown in *Bufo bufo* and *Rana graeca*; Dubois 1974). Stability of an anomaly over time, or its modification, is interesting information that can easily be obtained, simply by keeping the animals alive for a long time and observing them regularly.

5.3 Regeneration experiments

As mentioned above, regeneration experiments may provide important information: if an abnormal structure regenerates normally, this suggests that the anomaly was not genetically determined but caused by an external factor (e.g. Woitkevich 1965, Rostand 1955, 1958, 1971). Such experiments should be repeated with several specimens and controls should be used, as even in normal specimens regeneration can be abnormal, and the reverse may be true.

5.4 Breeding experiments

The most convincing way of establishing the genetic cause of an anomaly is through breeding experiments (e.g., Rostand 1951a,b, 1955, 1958, 1971, Dubois & Vachard 1971b, Dubois 1974, 1977, Kawamura & Nishioka 1978, Nishioka & Ueda 1985a,b, Nishioka & Ohtani 1986). Ideally, such breeding experiments should be made between both abnormal and normal specimens and among abnormal ones, and control breeding using normal specimens should be made under the same conditions. The best procedure is to use the artificial fertilization of eggs obtained after hormonal stimulation of the female with sperm obtained from squeezing one of the testes of the male. This ensures a better fertilization rate than natural fertilization and allows the clutch to be divided and to use several fathers, including controls, for the same mother and several mothers for the same father.

As many mutations are recessive, it is often necessary to cross the offspring among themselves or in backcrosses with the parents to observe the re-appearance of an anomaly that had disappeared in the first generation offspring, so that it will take two generations (i.e., several years) to establish whether or not a given anomaly was caused by a recessive mutation.

5.5 Experimental gynogenesis

Experimental gynogenesis was successfully used by Rostand (1950, 1951a,b, 1955, 1958, 1971) to explore the genetic patrimony of several amphibian species. This is a very powerful technique to test the genetic origin of many anomalies observed in the field. As viable specimens obtained by gynogenesis result from re-incorporation of the last polar body into the egg before the start of the development, they are usually homozygous for all alleles. If a female frog is polydactylous for being homozygous for a recessive allele of polydactyly, and if it reproduces by gynogenesis, all its offspring will be polydactylous and this will have already demonstrated the recessive genetic nature of the anomaly in the first generation – whereas it would have taken two generations to obtain the same result from breeding. This technique was "rediscovered" later by Trottier & Armstrong (1976), who failed to quote Rostand (Dubois et al. 1978), and it has been used by a few other authors since then, but unfortunately not by recent authors, in spite of a recent renewed interest in the anomalies of amphibians. Gynogenesis often produces non-viable specimens, because of the presence of lethal recessive genes that act early in ontogenetic development (e.g., Nishioka & Kondo 1978, Gillespie & Armstrong 1979), but

the viable specimens often exhibit anomalies (albinism, polydactyly, etc.) that were not expressed in the mother as they result from recessive mutations that were in the heterozygous condition in this specimen.

Until the genetic nature of an anomaly has been tested (by regeneration, crossings or gynogenesis), a genetic proximate cause of an anomaly cannot be ruled out. Even so, some mutations are expressed differently in different environmental conditions, called epigenetics e.g., (Lauthier 1971, Conter & Jaylet 1974, Fernandez & Beetschen 1975) or because of other genetic factors that interfere with them. Non-Mendelian heredity (e.g., through cytoplasmic genes) or intervention with regulatory genes (particularly in all aneuchronic anomalies) cannot be ruled out. Therefore, prudence is always necessary in this domain.

5.6 Experimental teratology

If unusual external factors (e.g., pesticides, radiation, heavy metals) have been identified in the environment, they may be hypothesized as being responsible for some of the anomalies observed. It would then theoretically be possible to reproduce exactly the same anomalies using the same factors, the same concentration, the same developing stages, etc. This research domain still has to be developed (Burkhart et al. 2000, Fort et al. 2006). Such tests should be made with the species suffering from anomalies, as species may differ in their susceptibility to teratogenic factors (Lunde & Johnson 2012). Even then, experimental studies can produce a rather wide range of unpredictable abnormal phenotypes. In the case of anomaly P, for example, the characteristic syndrome of the anomaly was produced artificially in a few experiments in cages in the field and in the laboratory (Rostand 1971), but attempts to repeat these experiences have failed (Dubois 1979), and this question should be studied again, until the teratogenic factor responsible for this anomaly is isolated. More constant and partly predictable but still variable results within and across species have been obtained in experiments with some chemicals and with trematodes (e.g., Sessions & Ruth et al. 1990, Johnson et al. 2001, Bridges et al. 2004; compare Lunde & Johnson 2012 and Henle et al. 2017b).

5.7 Long-term studies of populations

While some anomalies may have a significant impact on the survival of the affected individuals (Goodman & Johnson 2011, Lunde & Johnson 2012) and may in some cases threaten the populations (Dubois 1979, Rose 1981, Lannoo 2008), no study has directly looked at whether, and if so to which extent, anomalies pose a risk to the viability of amphibian populations. In some cases, such as red-leg disease or malformed mouthparts in individuals affected by *Batrachochytrium dendrobatidis,* the anomalies may only be an epiphenomenon of infection with the disease directly killing the animals (Bradford 1991, Green 2001, Voyles et al. 2009). In other cases, they may further reduce survival beyond the direct mortality induced by the causative agent. Such studies concerning the role of anomalies on the dynamics of amphibian populations are imperative.

6 Conclusion

The study of anomalies in natural populations of amphibians is a research field of its own, requiring a careful and complex methodology. In order to draw reliable conclusions about the distribution, frequency, cause and conditions of appearance of a given anomaly in a population, data must be collected at all stages of the study, both in the field and in the laboratory, and both on abnormal and normal specimens of all amphibian species occurring on a study site. With some of these pieces of information still lacking, some recent studies of amphibian anomalies still leave many questions unanswered. The adoption of a rigorous methodology should improve the efficiency of such studies in the future.

7 Acknowledgements

Jean-Claude Beetschen (Toulouse), Alain Collenot (Paris) and Mari Reeves (Anchorage) provided valuable suggestions for improving the manuscript. Marlies Uhlig (Leipzig) assisted in compiling and checking the reference list.

8 References

Andrén, C., L. Henrikson, M. Olsson & G. Nilson (1988): Effects of pH and aluminium on embryonic and early larval stages of Swedish brown frogs *Rana arvalis, R. temporaria* and *R. dalmatina.* – Holarctic Ecol. **11**: 127–135.

Ankley, G.T., S.A. Diamond, J.E. Tietge, G.W. Holcombe, K.M. Jensen, D.L. DeFoe & R. Peterson (2002): Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hindlimb malformations in the northern leopard frog (*Rana pipiens*). – Environ. Sci. Technol. **36**: 2853–2858.

Armstrong, J.B. & L.L. Gillespie (1980): Dominant lethal induction by ethyl methanesulfonate in the male axolotl (*Ambystoma mexicanum*). – J. exp. Zool. **212**: 415–421.

Bagnara, J.T. (1966): Cytology and cytophysiology of non-melanophore pigment cells. – Int. Rev. Cytol. **20**: 173–205.

Ballengée, B. & S.K. Sessions (2009): Explanation for missing limbs in deformed amphibians. – J. exp. Zool. **312B**: 770–779.

Benjamin, C.P. (1970): The biochemical effects of the d, m, and a genes on pigment cell differentiation in the axolotl. – Develop. Biol. **23**: 62–85.

Bergendal, D. (1889): Über eine dritte vordere Extremität eines braunen Frosches. – Bihang till K. Svenska Vet.-Akad. Handlingar **14(IV)8**: 1–35.

Bodenstein, D. (1932): Ein *Triton alpestris* Albino. – Zool. Anz. **98**: 322–326.

Bohl, E. (1997): Mißbildungen der Extremitäten von Amphibienlarven in Aufseß (Oberfranken). Versuche zur Ursachenermittlung. – Münchner Beitr. Abwasser-, Fischerei- und Flussbiol. **50**: 160–189.

Borkin, L. (2014): Morphological abnormalities in natural populations of amphibians: what do we study and how do we mea-

sure? Pp. 25-36 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. – Ekaterinburg (Uralskii Federalnii Universitet).

Bradford, D.F. (1991): Mass mortality and extinction in a high-elevation population of *Rana mucosa*. – J. Herpetol. **25**: 174–177.

Brennan, S.M. (1991): Morphogenetic and molecular correlates of teratogenesis in the amphibian embryo. – Teratology **43**: 341–353.

Bridges, C., E. Little, D. Gardiner, J. Petty & J. Huckins (2004): Assessing the toxicity and teratogenicity of pond water in north-central Minnesota to amphibians. – Environ. Sci. Pollut. Res. **11(4)**: 233–239.

Briggs, R.W. (1941): The development of abnormal growths in *Rana pipiens* embryos following delayed fertilization. – Anat. Rec. **81**: 121–135.

Browder, L.W. (1972): Genetic and embryological studies of albinism in *Rana pipiens*. – J. exp. Zool. **180**: 149–156.

Brunst, V.V. (1961): Some problems of regeneration. – Quart. Rev. Biol. **36**: 178–206.

Burkhart, J.G., G. Ankley, H. Carpenter, D. Fort, D. Gardiner, H. Gardner, R. Hale, J.C. Helgen, P. Jepson, D. Johnson, M. Lannoo, D. Lee, J. Lary, R. Levey, J. Magner, C. Meteyer, M.D. Shelby & G. Lucier (2000): Strategies for assessing the implications of frog malformations for environmental and human health. – Environ. Health Perspectives **108**: 83–90.

Burns, E.R. & H.J. White (1971): A spontaneous mesenchymal cell neoplasm in the adult newt, *Diemictylus viridescens*. – Cancer Res. **31**: 826–829.

Caetano, M.H., J. Castanet & H. Francillon (1985): Détermination de l'âge de *Triturus marmoratus marmoratus* (Latreille, 1800) du Parc National de Peneda Gerês (Portugal) par squelettochronologie. – Amphibia-Reptilia **6**: 117–132.

Collenot, A. (1965): Recherches comparatives sur l'inversion sexuelle par les hormones stéroides chez les amphibiens. – Mém. Soc. Zool. France **33**: 1–141.

Collenot, A., C. Dournon & M. Lauthier (1989): Genetic evidence for linkage with the Z and W sex chromosomes of two distinct couples of alleles controlling larval and postmetamorphic skin pigmentation in salamander. – Biol. Cell **67**: 1–7.

Conter, A. & A. Jaylet (1974): A triple translocation in the newt *Pleurodeles waltlii.* – Chromosoma **46**: 37–58.

Davidson, E.H. (1976): Chapter 2: The onset of genome control in embryogenesis. Pp. 27-55 in Davidson, E.H. (ed.): Gene Activity in Early Development (2nd ed.). – New York (Academic Press).

Dawson, D.A. & J.A. Bantle (1987): Coadministration of methylxanthines and inhibitor compounds potentiates teratogenicity in *Xenopus* embryos. – Teratology **35**: 221–227.

Delarue, M. (1982): Embryogenèse et relations nucléocytoplasmiques chez les hybrides expérimentaux entre *Bufo bufo* L., *Bufo viridis* Laur. et *Bufo calamita* Laur. – Amphibia-Reptilia **3**: 161–178.

Donnelly, M.A., C. Guyer, J.E. Juterbock & R.A. Alford (1994): Techniques for marking amphibians. Pp. 277–284 in Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.-A.C. Hayek & M.S. Foster (eds.): Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians. – Washington & London (Smithsonian Inst. Press).

Dournon, C., A. Bautz, H. Membre, M. Lauthier & A. Collenot (1998): Expression of hindlimb abnormalities under rearing temperature effects during the larval development of the salamander *Pleurodeles waltl* (urodele amphibian). – Develop. Growth Differ. **40**: 555–565.

Droin, A. (1985): Wrinkled oedema' (w. oe), une nouvelle mutation recessive chez *Xenopus borealis*. – Rev. Suisse Zool. **92**: 863–869.

Droin, A. & M.L. Beauchemin (1975): Immobile' (im), a recessive lethal mutation of *Xenopus laevis* tadpoles. – J. Embryol. exp. Morphol. **34**: 435–449.

Droin, A. & M. Fischberg (1980): Abnormal limbs (abl), a recessive mutation affecting the tadpole of *Xenopus l. laevis*. – Experientia **36**: 1286–1288.

Dubois, A. (1968): Sur deux anomalies de la Grenouille verte (*Rana esculenta*). – Bull. Soc. linn. Lyon **37:** 316–320.

Dubois, A. (1974): Polydactylie massive, associée à la clinodactylie, dans une population de *Rana graeca*. Remarques sur la polydactylie faible et la clinodactylie chez *Bufo bufo* (Amphibiens, Anoures). – Bull. Soc. zool. Fr. **99:** 505–521, pl. I–II.

Dubois, A. (1976): Les Grenouilles du sous-genre *Paa* du Népal (famille Ranidae, genre *Rana*). – Cahiers népalais. Documents, CNRS, Paris **6**: i-vi + 1–275.

Dubois, A. (1977): Une mutation dominante déterminant l'apparition de diverses anomalies digitales chez *Rana temporaria* (Amphibiens, Anoures). – Bull. Soc. zool. France **102:** 197-213.

Dubois, A. (1979): Anomalies and mutations in natural populations of the *Rana esculenta* complex (Amphibia, Anura). – Mit. zool. Mus. Berlin **55:** 59–87, pl. I.

Dubois, A. (1980): Populations, polymorphisme et adaptation: quelques exemples chez les Amphibiens Anoures. Pp. 141–158 in Barbault, R., P. Blandin & J.-A. Meyer (eds.): Recherches d'écologie théorique. Les stratégies adaptatives. – Paris (Maloine).

Dubois, A. (1984): L'anomalie P des Grenouilles vertes (complexe de *Rana* kl. *esculenta* Linné, 1758) et les anomalies voisines chez les Amphibiens. Pp. 215–221 in Vago, C. & G. Matz (eds.): Comptes rendus du Premier Colloque international de Pathologie des Reptiles et des Amphibiens. – Angers (Presses Univ. Angers).

Dubois, A. (2014): Anomalies in natural populations of amphibians: a general survey and methodological recommendations for study. Pp. 87–96 in Vershinin, V.L., A. Djubuya, K. Henle & M. Puky (eds.): Anomalii i Patologii Amfibii i Reptilii. – Ekaterinburg (Uralskii Federalnii Universitet).

Dubois, A. (2017): Rostand's anomaly P and similar anomalies in amphibians. – Mertensiella **25**: 49–56.

Dubois, A. & D. Vachard (1969): Sur trois anomalies digitales de la grenouille rousse (*Rana temporaria*). – C. r. Soc. Biol. **163**: 2255–2257.

Dubois, A. & D. Vachard (1971a): Sur une anomalie pigmentaire de la Grenouille verte (*Rana esculenta*) et de quelques autres Amphibiens Anoures et Urodèles. – Bull. Soc. linn. Lyon **40:** 40–52.

Dubois, A. & D. Vachard (1971b): Sur la descendance d'une Grenouille rousse (*Rana temporaria*) ectrodactyle. – C. r. Soc. Biol. **165**: 26–29.

Dubois, A., J.-L. Fischer, J.-J. Morère & D. Vachard (1978): Hommage à Jean Rostand. 1. Le biologiste. – Bull. Soc. Herpétol. France **5**: 5–7.

Eberhardt, L.L. & J.M. Thomas (1991): Designing environmental field studies. – Ecol. Monogr. **6**: 53–73.

Fernandez, M. & J.C. Beetschen (1975): The role of temperature in phenotypic expression of amphibian embryos *Pleurodeles waltlii* homozygous for the heat-sensitive mutation ac (caudal ascites). – J. Embryol. exp. Morphol. **34**: 221–252.

Ferner, J.W. (2007): A review of marking and individual recognition techniques for amphibians and reptiles. – Herpetol. Circ. **35**: 1–72.

Flax, N.L. & L.J. Borkin (1997): High incidence of abnormalities in anurans in contaminated industrial areas (eastern Ukraine). Pp. 119–123 in Böhme, W., W. Bischoff & T. Ziegler (eds.):

Herpetologia Bonnensis Proc. 8th Ord. Gen. Meet. SEH., 23–27 August 1995, Bonn, Germany. – Bonn (Societas Europaea Herpetologica).

Fort, D.J., R.L. Rogers & J.P. Bacon (2006): Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part II. Progress towards characterization of chemical stressors. – Appl. Herpetol. **3**: 143–172.

Frost, S.K., F. Briggs & G. Malacinski (1984): A colour atlas of pigment mutants in the axolotl. – Differentiation **26**: 182-188.

Gillespie, L.L. & J.B. Armstrong (1979): Induction of triploid and gynogenetic diploid axolotls (*Ambystoma mexicanum*) by hydrostatic pressure. – J. exp. Zool. **210**: 117–122.

Goodman, B.A. & P.T.J. Johnson (2011): Disease and the extended phenotype: parasites control host performance and survival through induced changes in body plan. – PLOS ONE **6(5)**: e20193. doi:10.1371/journal.pone.0020193.

Green, D.E. (2001): Pathology of Amphibia. Pp 401-485 in Wright, K.M. & B.R. Whitaker (eds.): Amphibian Medicine and Captive Husbandry. – Malabar (Krieger Publ. Comp.).

Günther, R. (1996): Chapter 6.22. Seefrosch – *Rana ridibunda* Pallas, 1771. Pp. 490–507 in Günther, R. (ed.): Die Amphibien und Reptilien Deutschlands. – Jena (Fischer).

Hart, D.R. & J.B. Armstrong (1984): Assessment of mutagenic damage by monofunctional alkylating agents and gamma radiation in haploid and diploid frogs, *Xenopus laevis*. – Environ. Mutagen. **6**: 719–735.

Hatch, A.C. & G.A. Burton (1998): Effects of photoinduced toxicity of fluoranthene on amphibian embryos and larvae. – Environ. Toxicol. Chem. **17(9)**: 1777–1785.

Hayes, T.B. (1997): Hormonal mechanisms as developmental constraints on evolution: examples from the Anura. – Am. Zool. **37**: 482–490.

Hayes, T.B. (2000): Endocrine disruption in amphibians. Pp. 573–593 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. – Pensacola (SETAC).

Hayes, T.B. & K.P. Menendez (1999): The effects of sex steroids on primary and secondary sex differentiation in the sexually dichromatic reedfrog (*Hyperolius argus:* Hyperoliidae) from the Arabuko Sokoke Forest of Kenya. – Gen. Comp. Endocrinol. **115**: 188–199.

Henle, K. (1990): Population ecology and life history of the arboreal gecko *Gehyra variegata* in arid Australia. – Herpetol. Monogr. **4**: 30–60.

Henle, K., J. Kuhn, R. Podloucky, K. Schmidt-Loske & C. Bender (1997): Individualerkennung und Markierung mitteleuropäischer Amphibien und Reptilien: Übersicht und Bewertung der Methoden; Empfehlungen aus Natur- und Tierschutzsicht. – Mertensiella **7**: 133–184.

Henle, K., A. Dubois, K. Rimpp & V. Vershinin (2017a): Mass anomalies in green toads (*Bufo viridis*) at Roßwag: inbred hybrids, radioactivity or an unresolved case? – Mertensiella **25**: 185–242.

Henle, K., A. Dubois & V. Vershinin (2017b): A review of anomalies in natural populations of amphibians and their potential causes. – Mertensiella **25**: 57–164.

Henle, K, A. Dubois & V. Vershinin (2017c): Commented glossary, terminology and synonymies of anomalies in natural populations of amphibians. – Mertensiella **25**: 9–48.

Héron-Royer, L.-F. (1887): Sur la reproduction de l'albinisme par voie héréditaire chez l'alyte accoucheur et sur l'accouplement de ce batracien. – Bull. Soc. Zool. France **"1886" 11**: 671–679.

Hertwig, G. (1911): Radiumbestrahlung unbefruchteter Froscheier und ihre Entwicklung nach Befruchtung mit normalem Samen. – Arch. mikr. Anat. **77**: 165–209.

Hertwig, G. (1918): Kreuzungsversuche an Amphibien. – Arch. mikr. Anat. **91**: 203–271.

Humphrey, R.R. (1967a): Albino axolotls from an albino tiger salamander through hybridization. – J. Heredity **58**: 95–101.

Humphrey, R.R. (1967b): Genetic and experimental studies on a lethal trait ("short toes") in the Mexican axolotl (*Ambystoma mexicanum*). – J. exp. Zool. **164**: 281–295.

Humphrey, R.R. & J.T. Bagnara (1967): A color variant in the Mexican axolotl. – J. Heredity **58**: 251–256.

Jaksić, F.M. & S.D. Busack (1984): Apparent inadequacy of tail-loss figures as estimates of predation upon lizards. – Amphibia-Reptilia **5**: 177–179.

Johnson, P.T.J., K.B. Lunde, R.W. Haight, J. Bowerman & A.R. Blaustein (2001): *Ribeiroia ondatrae* (Trematoda: Digenea) infection induces severe limb malformations in western toads (*Bufo boreas*). – Can. J. Zool. **79**: 370–379.

Johnson, P.T.J., K.B. Lunde, D.A. Zelmer & J.K. Werner (2003): Limb deformities as an emerging parasitic disease in amphibians: evidence from museum specimens and resurvey data. – Conserv. Biol. **17**: 1724–1737.

Johnson, P.T.J., M.K. Reeves, S.K. Krest & A.E. Pinkney (2010): A decade of deformities: Advances in our understanding of amphibian malformations and their implications. Pp. 511-536 in Sparling, D.W., G. Linder, C.A. Bishop & S.K. Krest (eds.): Ecotoxicology of Amphibians and Reptiles (2nd ed.). – Boca Raton (CRC Press).

Kawamura, T. & M. Nishioka (1978): Abnormalities in the descendants of *Rana nigromaculata* produced from irradiated eggs or sperm. – Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **3**: 1–187.

Kiesecker, J.M. (2002): Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? – Proc. Natl. Acad. Sci. **99**: 9900–9904.

Kupferberg, S.J., A. Catenazzi, K. Lunde, A.J. Lind & W.J. Palen (2009): Parasitic copepod (*Lernaea cyprinacea*) outbreaks in foothill yellow-legged frogs (*Rana boylii*) linked to unusually warm summers and amphibian malformations in northern California. – Copeia **2009**: 529–537.

Kuranova, V.N. (2003): Antropiceskoe vosdeistie na zemnovodnysh i presmykayushishtshsja. Problemy ish oshrany na yugovostoke Zapadnoi Sibiri (Anthropogenic impacts on amphibians and reptiles. Problems of conservation in southeastern West-Siberia). Pp. 39–51 in Erdakov, L.N. & O.N. Tshernyshtshova (eds.): Amphibii i Reptilii v Zapadnoi Sibiri (Amphibians & Reptiles of Western Siberia). – Novosibirsk (Novosibirskii Gosudarstennyi Pedagogitsheskii Universitet).

Lacroix, J.-C. & A. Capuron (1970): Sur un facteur récessif, modifiant le phénotype pigmentaire de la larve, chez l'Amphibien Urodèle *Pleurodeles waltli* Michahelles. – C. r. Acad. Sci. **270**: 2122–2123.

Lannoo, M.J. (2008): Malformed Frogs. The Collapse of Aquatic Ecosystems. – Berkeley (University California).

Lauthier, M. (1971): Étude descriptive d'anomalies spontanées des membres postérieurs chez *Pleurodeles waltlii* Michah. – Ann. Embr. Morph. **4**: 65–78.

Loeffler, I.K., D.L. Stocum, J.F. Fallon & C.U. Meteyer (2001): Leaping lopsided: a review of the current hypotheses regarding etiologies of limb malformations in frogs. – Anat. Rec. **265**: 228–245.

Lowcock, L.A., T.F. Sharbel, J. Bonin, M. Ouellet, J. Rodrigue & J.-L. DesGranges (1997): Flow cytometric assay for in vivo genotoxic effects of pesticides in green frogs (*Rana clamitans*). – Aquat. Toxicol. **38**: 241–255.

Lunde, K.B. & P.T.J. Johnson (2012): A pracical guide for the study of malformed amphibians and their causes. – J. Herpetol. **46**: 429–441.

Luo, S.-Q., M.C. Plowman, S.M. Hopfer & F.W. Sunderman (1993): Mg^{2+}-deprivation enhances and Mg^{2+}-supplementation diminishes the embryotoxic and teratogenic effects of Ni^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+} for frog embryos in the FETAX assay. – Ann. Clin. Lab. Sci. **23**(**2**): 121–129.

Merrell, D.J. (1965): The distribution of the dominant burnsi gene in the leopard frog, *Rana pipiens*. – Evolution **19**: 69–85.

Merrell, D.J. (1972): Laboratory studies bearing on pigment pattern polymorphism in wild populations of *Rana pipiens*. – Genetics **70**: 141–171.

Merrell, D.J. (1973): Ecological genetics of anurans as exemplified by *Rana pipiens*. Pp. 329-335 in Vial, J.L. (ed.): Evolutionary Biology of the Anurans. – Columbia (Univ. Missouri Press).

Meteyer, C.U., I.K. Loeffler, J.F. Fallon, K.A. Converse, E. Green, J.C. Helgen, S. Kersten, R. Levey, L. Eaton-Poole & J.G. Burkhart (2000): Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. — Teratology **62**: 151–171.

Mizgireuv, I.V., N.L. Flax, L.J. Borkin & V.V. Khudoley (1984): Dysplastic lesions and abnormalities in amphibians associated with environmental conditions. – Neoplasma **31**: 175–181.

Muneoka, K., G. Holler-Dinsmore & S.V. Bryant (1986): Intrinsic control of regenerative loss in *Xenopus laevis* limbs. – J. exp. Zool. **240**: 47–54.

Niewiarowski, P.H., J.D. Congdon, A.E. Dunham, L.J. Vitt & D.W. Tinkle (1997): Tales of lizard tails: Effects of tail autotomy on subsequent survival and growth of free-ranging hatchling *Uta stansburiana*. – Can. J. Zool. **75**: 542–548.

Nishioka, M. (1977): Color variants induced by radiation and their inheritance in *Rana nigromaculata*. – Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **2**: 25–89.

Nishioka, M. & Y. Kondo (1978): Production of gynogenetic haploids by treatment of spermatozoa with toluidine blue in *Rana japonica*. – Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **3**: 385–398.

Nishioka, M. & H. Ohtani (1986): Detection of chromosomes bearing the loci for blue and olive mutations in *Rana nigromaculata*. – Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **8**: 1–27.

Nishioka, M. & H. Ueda (1977): Genetic and morphologic studies on ten albino stocks in *Hyla arborea japonica*. – Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **2**: 103–164.

Nishioka, M. & H. Ueda (1985a): Genetics and morphology of 13 albino stocks in the *Rana nigromaculata* group. – Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **7**: 1–121.

Nishioka, M. & H. Ueda (1985b): Two kinds of black-eyed variants in *Hyla arborea japonica*. – Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ. **7**: 157–179.

Nishioka, M., H. Okumoto & Y. Kondo (1981): Effects of ultraviolet rays on the sperm of *Rana japonica*. I. Production of gynogenetic haploids. – Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. **5**: 47–67.

Nye, H.L.D., J.A. Cameron, E.A.G. Chernoff & D.L. Stocum (2003): Regeneration of the urodele limb: a review. – Develop. Dynamics **226**: 280–294.

Ouellet, M. (2000): Amphibian deformities: current state of knowledge. Pp. 617–661 in Sparling, D.W., G. Linder & C.A. Bishop (eds.): Ecotoxicology of Amphibians and Reptiles. – Pensacola (SETAC).

Ouellet, M., J. Bonin, J. Rodrigue, J.-L. DesGranges & S. Lair (1997): Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. – J. Wildl. Diseases **33**(1): 95–104.

Pfeiffer, C.J. &. M. Asashima (1997): Ultrastructure of *Rhabdias tokyoensis*, a pulmonary nematode of the Japanese newt (*Cynops pyrrhogaster*). – Amphibia-Reptilia **18**: 283–294.

Pfeiffer, C.J., T. Nagai, M. Fujimura & T. Tobe (1979): Spontaneous regressive epitheliomas in the Japanese newt, *Cynops pyrrhogaster*. – Cancer Res. **39**: 1904–1910.

Pohland, H.-L. (1962): Die Wirkung von Thymuswirkstoffen auf Wachstum und Metamorphose sowie deren Einfluss auf endokrine Drüsen bei *Xenopus laevis* Daudin. – Roux' Arch. Entwickl.-mech. **153**: 621–663.

Power, T., K.L. Clark, A. Harfenist & D.B. Peakall (1989): A review and evaluation of the amphibian toxicological literature. – Tech. Rep. Can. Wildl. Serv. **61**: 1–91.

Rose, S.M. (1964): Regeneration. Pp. 545–622 in Moore, J.A. (ed.): Physiology of the Amphibia. Vol. I. – London (Acad. Press).

Rose, S.M. (1981): The tiger salamander (*Ambystoma tigrinum*): a decade of sewage associated neoplasia. Pp. 91–100 in Dawe, C.J., J.C. Harshbarger & S. Kondo (eds.): Phyletic Approaches to Cancer. – Tokyo (Japan Sci. Soc. Press).

Rostand, J. (1950): Sur la gynogenèse comme moyen d'exploration du patrimoine héréditaire chez le Crapaud ordinaire (*Bufo bufo*). – C. r. Acad. Sci. **230**: 2130–2131.

Rostand, J. (1951a): La génétique des Batraciens. – Paris (Hermann).

Rostand, J. (1951b): Génétique des batraciens anoures: anomalies héréditaires et phénocopies. – Bull Soc. Hist. nat. Toulouse **86**: 230–236.

Rostand, J. (1955): Les Crapauds, les Grenouilles, et quelques grands problèmes biologiques. – Paris (Gallimard).

Rostand, J. (1958): Les anomalies des Amphibiens Anoures. – Paris (SEDES).

Rostand, J. (1971): Les étangs à monstres. Histoire d'une recherche (1947–1970). – Paris (Stock).

Rostand, J. & P. Darré (1970): Une mutation de *Rana esculenta*: la Grenouille aux yeux noirs. – C. r. Acad. Sci. **271**: 1414–1415.

Rot-Nikcevic, I. & R.J. Wassersug (2004): Arrested development in *Xenopus laevis* tadpoles: how size constrains metamorphosis. – J. exp. Biol. **207**: 2133–2145.

Rowe, C.L., O.M. Kinney & J.D. Congdon (1998): Oral deformities in tadpoles of the bullfrog (*Rana catesbeiana*) caused by conditions in a polluted habitat. – Copeia **1998**: 244–246.

Scadding, S.R. & M. Maden (1986): Comparison of the effects of vitamin A on limb development and regeneration in *Xenopus laevis* tadpoles. – J. Embryol. exp. Morph. **91**: 35–53.

Sessions, S.K. & S.B. Ruth (1990): Explanation for naturally occurring supernumerary limbs in amphibians. – J. exp. Zool. **245**: 38–47.

Stanisstreet, M. (1974): Comparison of the effects of lithium, b-phenylethylamine, and tyrosine on *Xenopus* embryos. – Experientia **30**: 1081–1082.

Stopper, G.F., L. Hecker, R.A. Franssen & S.K. Sessions (2002): How trematodes cause limb deformities in amphibians. – J. exp. Zool. (Mol. Dev. Evol.) **294**: 252–263.

Storer, T.I. (1925): A Synopsis of the Amphibia of California. – Berkeley (University of California Press).

Trottier, T.M. & J.B. Armstrong (1976): Diploid gynogenesis in the Mexican axolotl. – Genetics **83**: 783–792.

Tschumi, P. (1954): Konkurrenzbedingte Rückbildungen der Hinterextremität von *Xenopus* nach Behandlung mit einem Chloraethylamin. – Rev. Suisse Zool. **61(6)**: 177–270.

Tyler, M.J. (1989): Australian Frogs. – South Yarra (Viking O'Neil).

Uehlinger, V. (1969): Une mutation récessive (pd) déterminant la polydactylie chez X*enopus laevis* D. (Batraciens anoures). – J. Embryol. exp. Morphol. **21**: 207–218.

Uehlinger, V. & J. Reynaud (1965) : Une anomalie héréditaire "kt" (kinky tailtip) chez *Xenopus laevis* D. – Rev. Suisse Zool. **72**: 680–685.

Van Gelder, J.J. & H. Strijbosch (1995): Adult common toads (*Bufo bufo*) with mutilated legs. – Alytes **13(3)**: 105–108.

Veith, M. & B. Viertel (1993): Veränderungen an den Extremitäten von Larven und Jungtieren der Erdkröte (*Bufo bufo*): Analyse möglicher Ursachen. – Salamandra **29**: 184–199.

Vershinin, V.L. (2002): Ecological specifity and microevolution in amphibian populations in urbanized areas. – Adv. Amph. Res. Former Soviet Union **7**: 1–161.

Voyles, J., S. Young, L. Berger, C. Campbell, W.F. Voyles, A. Dinudom, D. Cook, R. Webb, R.A. Alford, L.F. Skerratt & R. Speare (2009): Pathogenesis of chytriodiomycosis, a cause of catastrophic amphibian declines. – Science **326**: 582–585.

Woitkevitch, A.A. (1959): Natürliche Mehrfachbildungen an Froschextremitäten. – Jena (Fischer).

Woitkevitch, A.A. (1961): Le développement des extrémités surnuméraires chez les amphibiens. – Bull. Biol. France Belgique **7(4)**: 749–762.

Woitkevitch, A.A. (1965): Mass formation of additional hind extremities in the lake frog. – Zhurnali obschtschej biologii **26**: 56–62.

Wallace, H. (1981): Vertebrate Limb Regeneration. – New York (J. Wiley).

Wilson, B.S. (1992): Tail injuries increase the risk of mortality in free-living lizards (*Uta stansburiana*). – Oecologia **92**: 145–152.

Witschi, E. & C.Y. Chang (1954) Hereditary polymelia combined with edema and sex reversal in the toad. – Anat. Rec. **118**: 370.

Zimmermann, L. & R. Rugh (1941): Effect of age on the development of the egg of the leopard frog, *Rana pipiens*. – J. Morph. **68**: 329–345.

Gastrotheca riobambae, an F_2 individual with an abnormal mouth and right side of the head. Photo: D. Schmidt.

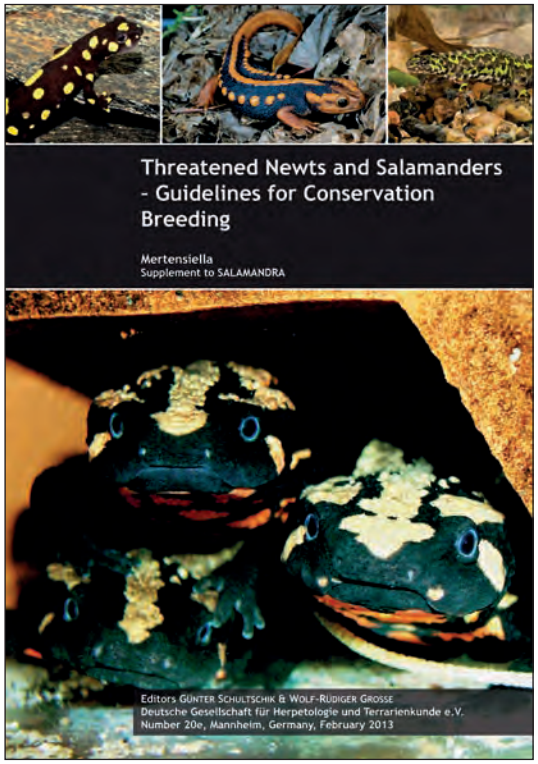
MERTENSIELLA – Supplement to SALAMANDRA

The DGHT (Deutsche Gesellschaft für Herpetologie und Terrarienkunde – German Society for Herpetology and Herpetoculture) was founded in 1964 as a successor of the German herping group "Salamander" going back to the year 1918.

As implied by its name, DGHT's philosophy is to bring together professional scientists and amateurs, thus promoting their dialog at an international scale. This gives the society a unique strength currently uniting about 6,000 members. The society's mission is to contribute to both research on amphibians and reptiles and captive keeping and breeding. DGHT is strongly engaged in herpeto-logical conservation and is accredited by the German Federal Nature Conservation Act.

Among the print media of DGHT are "SALAMANDRA – German Journal of Herpetology", a quarterly open access journal (published online and as printed annual volume) with scientific contributions in English from all fields of herpetology, listed under Current Contents and under the Science Citation Index (Impact Factor 2016: 1,250), and "MERTENSIELLA – Supplement to SALAMANDRA", a sporadically published supplement (in German or English) with special issues on particular topics like the symposium volumes on *Podarcis muralis* (no. 21) or *Bufo bufo* (no. 24).

The next edition of MERTENSIELLA (no. 26) is "Threatened Newts and Salamanders – Guidelines for Conservation Breeding Part II".

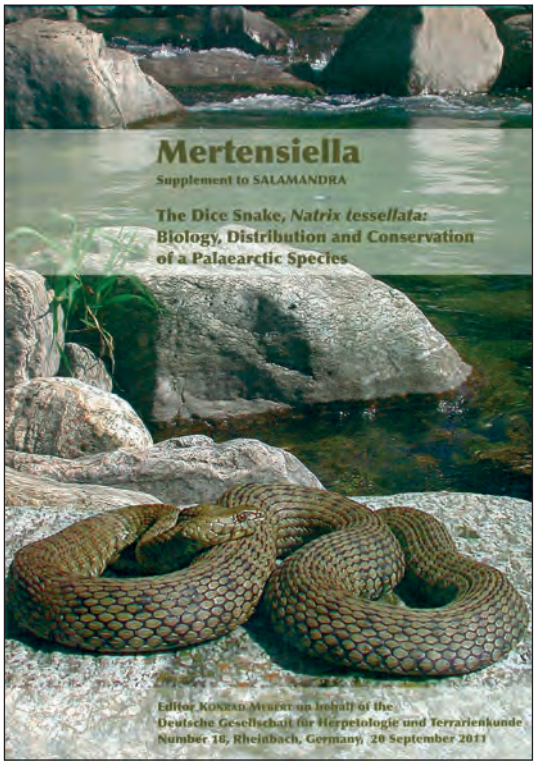


SCHULTSCHIK, G., GROSSE, W.-R. ed.: **Threatened Newts and Salamanders of the World – Guidelines for Conservation Breeding**; Mannheim 2013, hardcover, 224 pages, 298 colour photographs. The book covers breeding advices for the following species: *Ambystoma mexicanum, Paradactylodon persicus, Paradactylodon gorganensis, Calotriton asper, Cynops ensicauda, Cynops orphicus, Euproctus montanus, Euproctus platycephalus, Laotriton laoensis, Liua shihi, Mertensiella caucasica, Neurergus crocatus, Neurergus derjugini, Neurergus kaiseri, Neurergus strauchii, Triturus dobrogicus, Triturus pygmaeus, Tylototriton asperrimus, Tylototriton hainanensis, Tylototriton kweichowensis, Tylototriton shanjing, Tylototriton taliangensis, Tylototriton wenxianensi.*

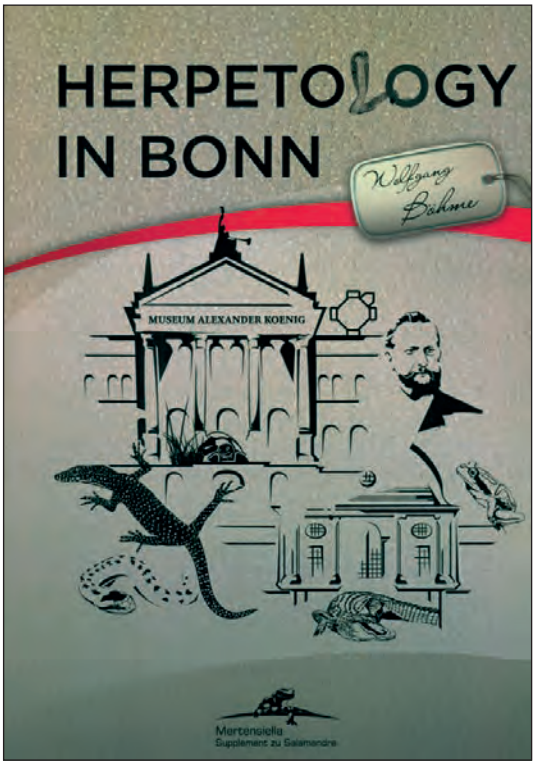
MERTENSIELLA Vol. 20 E	**59,90 €**
DGHT members' price	**39,90 €**

ISBN 978-3-89973-567-3

These and other Mertensiella volumes can be ordered from www.chimaira.de



VOL. 18: MEBERT, K. ed.: **The Dice Snake, *Natrix tessellata* – Biology, Distribution and Conservation of a Palaearctic Species**; Rheinbach 2011, 456 pages, numerous illustrations.
ISBN 978-3-9812565-4-3 **98,00 €**
DGHT members' price **75,00 €**



VOL. 21: BÖHME, W.: **Herpetology in Bonn**; Mannheim 2014, softcover, 256 pages
ISBN978-3-945043-02-8 **29,80 €**

VOL. 26: GROSSE, W.-R. ed.: **Threatened Newts and Salamanders of the World – Guidelines for Conservation Breeding 2**

in preparation!

VOL. 23: KWET, A., NIEKISCH, M.. ed.: **Amphibien und Reptilien der Neotropis. Entdeckungen deutschsprachiger Forscher in Mittel- und Südamerika;** Rangsdorf/ Mannheim 2016, 400 pages, 544 colour illustrations.
ISBN 978-3-941365-53-7 **68,00 €**
DGHT members' price **48,00 €**

VOL. 19: GROSSE, W.-R., et al.: **Verbreitung, Biologie und Schutz des Teichmolches, *Lissotriton vulgaris* (Linnaeus, 1758)**;
Mannheim 2013, 184 pages, 212 colour photos.
ISBN 978-3-89973-565-9 **59,90 €**
DGHT members' price **39,90 €**

VOL. 20 D: SCHULTSCHIK, G., GROSSE, W.-R. eds.: **Gefährdete Molch- und Salamanderarten der Welt – Richtlinien für Erhaltungszuchten**;
Mannheim 2013, 224 pages, 298 colour photos.
ISBN 978-3-89973-566-6 **59,90 €**
DGHT members' price **39,90 €**

VOL. 22: LAUFER, H., SCHULTE, U. ed. **Verbreitung, Biologie und Schutz der Mauereidechse *Podarcis muralis* (Laurenti, 1768)**;
Mannheim 2015, 218 pages, 126 colour photos,
ISBN 978-3-89973-568-0 **59,80 €**
DGHT members' price **39,80 €**

VOL. 24: MALETZKY, A. et al. ed.: **Verbreitung, Biologie und Schutz der Erdkröte *Bufo bufo* (Linnaeus, 1758) mit besonderer Berücksichtigung des Amphibienschutzes an Straßen**;
Mannheim 2016, 232 pages, 359 colour photos.
ISBN 978-3-89973-569-7 **59,80 €**
DGHT members' price **39,80 €**