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MASS OCCURRENCE OF TADPOLE DEFORMITIES IN TOAD SPECIES OF THE GENUS *Duttaphrynus* (BUFONIDAE) IN THE HIMALAYA (UTTARAKHAND, INDIA)

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In April and May 2019, we examined seven tadpole samples of two bufonid species *Duttaphrynus stomaticus* (one site) and *Duttaphrynus himalayanus* (six sites) taken in the Yamuna River and its tributaries, and the Bhagirathi River at altitudes from 687 m up to 2448 m above sea level; the western part of the Garhwal Himalaya, Uttarakhand, India. Seven categories of larval deformities were recorded: scoliosis, kyphosis, shortened tail, rounded tip of the tail, bifurcated tip of the tail, opercular chamber defect, and edema. The occurrence of malformed tadpoles calculated from a sample size ranged from 3.3 to 13.6%. Water mineralization levels were fluctuated in the limits 37 – 330 mg/dm^3, exceeding 100 mg/dm^3 in three cases. There was no significant correlation between share of abnormal animals or abnormalities frequency and mineralization as well as with altitude. Mass occurrence of malformed tadpoles was revealed in two places: the Aglad Nadi (= Algar River, 10%, site 1, 687 m) and the town Purola (9.9 – 11.8%, sites 2 and 3, 1339 – 1349 m). Both places were associated with human activity: cattle grazing and residential agrolandscape, respectively. Enlarged rate of anomalies on the town territory may be explained by a synergy between complex of drivers (mineralization, fertilizers and pesticides) that can lead to amplification of general impact on larval morphogenesis. Thus, we reported the first record of mass larval abnormalities in amphibians for the Garhwal in particular and for the Himalaya in general. Formerly published data evidenced that another malformation hotspot of amphibians was situated at the Western Ghats, peninsular India.

Keywords: larval deformities; *Duttaphrynus stomaticus*; *Duttaphrynus himalayanus*; Bufonidae; the Garhwal Himalaya; Uttarakhand; India.

INTRODUCTION

Amphibian malformations are reported since the mid 16th century (Henle et al., 2017a: 135), and enormous literature has accumulated since then. Originally such cases were regarded as "oddities of nature," however, later, they became the object of studies in terms of morphology, teratology and evolutionary theory. For a long time, as a rule, only isolated cases of deformed amphibians were known, however, since the 1960s and especially 1990s, the number of mass abnormalities has noticeably increased (Hoppe, 2000; Oullet, 2000; Johnson et al., 2003; Lannoo, 2008; Henle et al., 2017a).

Morphological abnormalities have been documented across a broad array of amphibian families and genera that occur in different habitats and microhabitats worldwide. They have been found in both common, widespread and rare, endangered species (e.g., Soto-Rojas et al., 2017). Deformed amphibians have been found both in contaminated areas and even in natural reserves in remote wilderness areas (Reeves et al., 2008).

Therefore, it is not surprising that reports of amphibian anomalies published in many countries are of increasing concern to both scientists and the public, both in terms of environmental health and of species protection. This led to a much greater attention of researchers to the problem of abnormalities in amphibians for two reasons. Firstly, this was due to elucidation of possible factors of population decline observed throughout the world (Blau-

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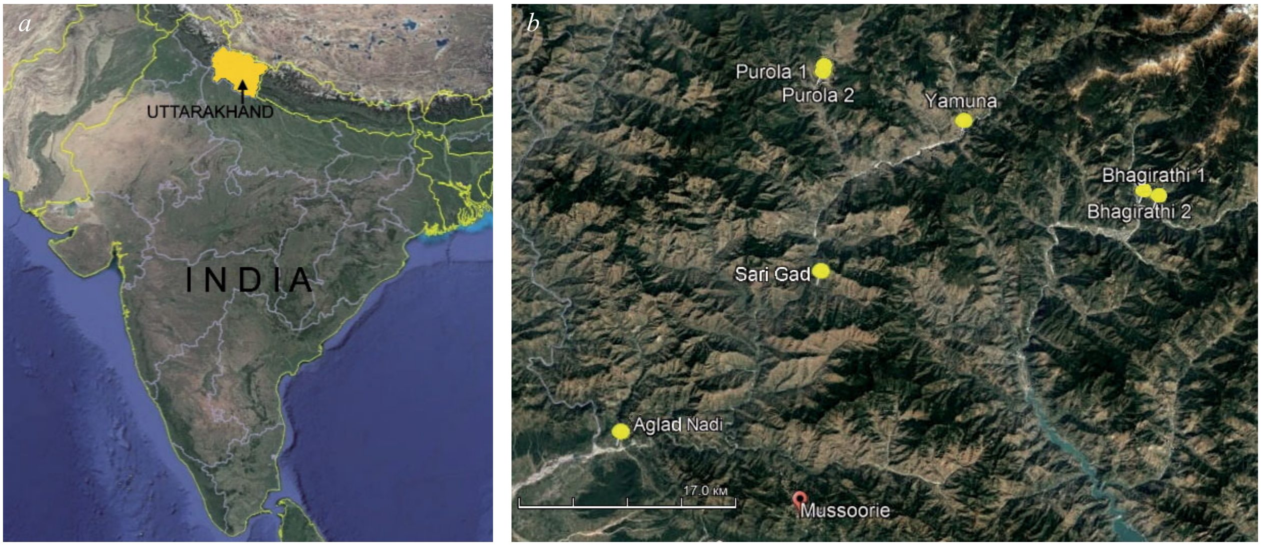

Fig. 1. Localities of larval samples of two toad species of the genus *Duttaphrynus* in the Garhwal Himalaya (Uttarakhand, India). Left, the state of Uttarakhand on the map of India; right, the localities with samples (yellow dots).

stein and Wake, 1990; Pechman et al., 1991; Wake, 1991; Pounds, 2001; Schoff et al., 2003; Beebee and Griffiths, 2005; Wake and Vredenburg, 2008; Hof et al., 2011, Whittaker et al., 2013; Grant et al., 2016), and secondly, to use occurrence of abnormal amphibians for bioindication of environmental quality (Tyler, 1989, 1997; Burkhart et al., 2000; Oullet, 2000; Vershinin, 2002; Flyaks and Borkin, 2004; Simon et al., 2011).

Interestingly, T. Gardner (2001: 27) concluded that many amphibian species declines, extinctions or extirpations have occurred at higher altitude sites (>500 m a.s.l.). According to a model (Daszak et al., 2011: 740 – 741), there is the relation between high-altitude and population decline, in stream-breeding amphibians in particular.

The geographical distribution of mass abnormalities is very uneven. The vast majority of cases in amphibians were recorded in temperate latitudes of the northern hemisphere (Oullet, 2000; Lannoo, 2008; Borkin, 2014; Henle et al., 2017a).

In India, the first record of two deformed larvae of *Rana alticola* (now *Clinotarsus alticola*, Ranidae) from Assam was published by Nelson Annandale (1905). Papers on Indian malformed frogs and toads are still quite scarce and mainly contain information about isolated cases based on single or a few animals of various species (Kurulkar and Deshpande, 1931; Mahendra, 1936; Nair and Kumar, 2005, 2007; Hippargi et al., 2010; Modak et al., 2013; Pardeshi, 2017; Santhoshkumar et al., 2017; Ashaharraza and Mahapatra, 2020). Nevertheless, some of these papers were published under alarmist headlines and were even reflected in major Indian mass media (e.g., Pacha, 2017).

However, mass incidences of deformed frogs belonging to four species of the family Dicroglossidae were registered in some papers (Gurushankara et al., 2007a; Patel et al., 2008; Hegde and Krishnamurthy, 2014). The Western Ghats can be identified as a malformation hotspot in India.

During our field trip (Spring 2019) to the Western Himalaya (Garhwal, Uttarakhand, India), we faced anomalous tadpoles in toads of the genus *Duttaphrynus*, Bufonidae (Borkin et al., 2021, 2022). The purpose of this article is to describe revealed anomalies and their occurrence at different altitudes and under different anthropogenic pressures on local montane ecosystems.

MATERIAL, LOCALITIES, AND METHODS

In April – May 2019, we travelled across the so-called Garhwal Himalaya (Western Himalaya), located in the state of Uttarakhand (formerly known as Uttaranchal), India (Borkin et al., 2021). Our trip in the western part of the Garhwal division of this state largely coincided with the ancient pilgrimage to sacred Hindu places and temples in Yamunotri and Gangotri (Fig. 1). All local rivers belong to the Ganges basin.

Numerous tadpoles were registered in many places of our travel along the Yamuna River with its tributaries (Aglad Nadi, Sari Gad, Tons, and other streams) and the Bhagirathi River. Often in the same water body we observed both long egg threads and tadpoles at different stages of development (before the appearance of hind

Fig. 2. Site 1: Aglad Nadi (= Aglar River).

limbs). However, at higher altitudes, where it was colder, tadpoles in their mass were at earlier stages of development.

Study sites. Large samples were made in seven localities at lower altitudes from 687 to 1349 m above sea level in the Lesser Himalaya (Fig. 1, Table 1).

Site 1: "Aglad Nadi," April 28, 2010 (Fig. 2). The place was located in the lower reaches of the Aglad Nadi (= Aglar River, e.g. Dalai et al., 2004, Fig. 1, 2) nearby to the bridge on the road from Mussoorie to Barkot between Kempti and Nainbagh, the western part of Tehri Garhwal District. This stream is a left (eastern) tributary of the Yamuna River.

Several families of shepherds rested in large tents and in the shade of a shrub near the shore. Goats, few cows and horses crowded nearby, and groups of buffalos bathed in the river. In addition to cattle watching, we did not notice other human activities. Thin egg strings of various length and tadpoles at various stages of development (Fig. 3) were observed in open sunny and shallow temporary water bodies located in the riverbed between the shore and the main stream, and partially littered with plastic trash.

Fig. 3. Egg strings and tadpoles of *Duttaphrynus stomaticus* in a shallow water (site 1: Aglad Nadi).

Site 2: "Sari Gad," April 28, 2010 (Fig. 4). The site was situated near the small settlement Sari Gad (= Sarigad, Saraigad) staying near the road. The Sari Gad (= Sa-

TABLE 1. Some Characteristics of Sampling Sites

Locality	Altitude, m	Latitude, N	Longitude, E	Mineralization, mg/dm^3	pH
1. Aglad Nadi	687	30°30.780′	77°59.805′	330	8.8
2. Sari Gad	973	30°40.460′	78°04.728′	70	8.8
3. Purola-1	1349	30°52.579′	78°04.939′	64	7.9
4. Purola-2	1339	30°52.463′	78°04.852′	108	9.8
5. Yamuna	1232	30°49.580′	78°14.857′	130	8.9
6. Bhagirathi-1	1203	30°45.163′	78°28.373′	37	8.9
7. Bhagirathi-2	1175	30°45.555′	78°27.320′	65	8.5

Fig. 4. Site 2: Sari Gad River.

Fig. 6. Site 4: Purola-2.

Fig. 5. Site 3: Purola-1.

Fig. 7. Site 5: Yamuna (Nirvana camp), a pebbly river bank.

rai Gad) River is a small left (eastern) tributary of the Yamuna River. Tadpoles concentrated in shallow water with a slower current.

Site 3: Purola-1, April 29 – 30, 2010 (Fig. 5). This site was situated in the town Purola, Uttarkashi District. Tadpoles have been found in only one river which the residents called "Kamal Nadi" (= Purola River). The places of concentration of toad tadpoles were represented by natural shallow waters located between stones along the banks of the riverbed above the gas station (Indian Oil Petrol pump Srishti Service) near the border of wheat fields. In general, the site Purola-1 can be regarded as a residential area combined with a well-formed agrolandscape.

Site 4: Purola-2, April 30, 2010 (Fig. 6). This site was also situated in the Purola town, but below the gas station along the river bordered with the agriculture fields from both sides. Shallow water bodies of the Purola River were represented by small depressions among stones.

Site 5: "Yamuna" (the camp Nirvana), May 3, 2010 (Fig. 7). The tourist camp Nirvana was located on the wide picturesque left bank of the mainstream Yamuna River in some distance from the settlement Gangnani (before the town Barkot), Uttarkashi District. Toad tadpoles inhabited small shallow water reservoirs inconspicuously lying on sandy riverbank among large stones and rounded pebbles of various sizes. This camp was isolated from other settlements, and apart from the tourist service (our group), no other human activity was observed.

Site 6: Bhagirathi-1, May 5, 2019 (Fig. 8). The site was a shallow water part of the small inflow of Bhagira-

Fig. 8. Site 6: Bhagirathi-1.

thi River. The sample was taken 150 m from the river mouth under the bridge near the Barahat Range village.

Site 7: Bhagirathi-2, May 11, 2019 (Fig. 9). The site was located at a distance of about 1500 m from the site Bhagirathi-1, on the right bank downstream of the Bhagirathi River, before the Great Ganga Hotel. Tadpoles were sampled in shallow water bodies connected with the main stream of the river and surrounded by large stones and pebbles.

Water parameters. In water bodies with tadpoles, we determined the pH values and level of mineralization (Table 1) by an electronic pH meter (Pocket Pen Type pH Meter PH-009 (I) ATC ROHS) and conductivity/TDS meter DIST 1, "Hanna Instruments."

Material. The larvae were caught using a small net or by hands. For comparison of various samples, we tried to collect swimming tadpoles at similar stages of development: about the stage 28 according to Gosner and Black (1958) and Gosner (1960) or the stage 40 according to Dabagyan and Sleptsova (1975). In accordance with the recommendations (Fodor and Puky, 2002: 36; Borkin et al., 2012: 337; Borkin, 2014: 31), about 100 tadpoles were taken at each site.

Methods. All tadpoles were carefully examined for external abnormalities with general-purpose measuring loop (LOMO GOST 8309-57) with a magnification of 10 times and with an accuracy of 0.1 mm. Anomalous specimens were photographed immediately with a Nikon Coolpix 5000 camera. Any deviations were analyzed in accordance with current terminology (Henle et al., 2017a, 2017b) and our methodological approaches (Borkin et al., 2012, 2014; Vershinin, 2015).

Fig. 9. Site 7: Bhagirathi-2.

The calculations were performed in the Statistica v. 6.0 software package for Windows (license No. AXXR003A622407FAN8).

RESULTS

In total, 717 tadpoles of two toad species from 7 localities were examined (Table 2). Larvae from the lowest locality No. 1 (110 tadpoles) at the Aglad Nadi (altitude

TABLE 2. The Frequencies of Abnormal Tadpoles and Abnormalities in Seven Garhwal Samples

Parameter	Aglad Nadi	Sari Gad	Purola-1	Purola-2	Yamuna	Bhagirathi-1	Bhagirathi-2
Abnormalities, %	10.91	3.64	13.64	10.89	4.55	3.49	3.33
Abnormal tadpoles, %	10	3.64	11.8	9.9	4.55	3.49	3.33
Sample size	110	110	110	101	110	86	90

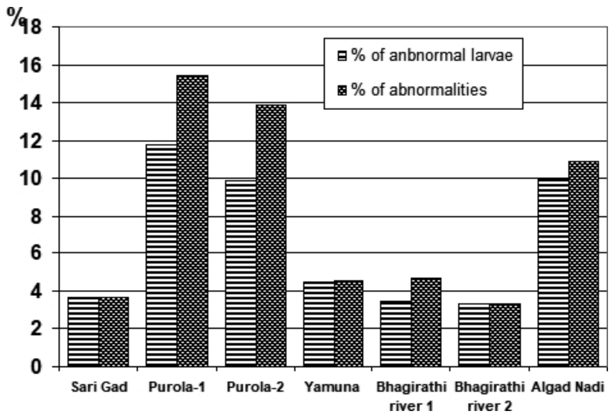

Fig. 10. The share of abnormal tadpoles and the total frequency of abnormalities in *D. stomaticus* (Aglad Nadi) and *Duttaphrynus himalayanus* (other six samples).

687 m a.s.l.) were assigned to *D. stomaticus*, whereas larvae of *D. himalayanus* were identified in other six higher localities (Nos. 2 – 7) at altitudes between 973 m and 1349 m a.s.l., where totally collected 607 tadpoles.

Frequencies of anomalous tadpoles varied from 3.33 to 11.8% across the Garhwal samples (Table 2). We counted 10.0% in *D. stomaticus*, and 3.33 – 11.8% in *D. himalayanus* (average 6.26% for this species).

Higher occurrence of malformed larvae was mentioned in three sites associated with tributaries of Yamuna River, namely in the Aglad Nadi sample of *D. stomaticus*, and in two Purola samples of *D. himalayanus* (Fig. 10). Lower frequencies were revealed in water bodies located on the banks of larger rivers Yamuna and Bhagirathi themselves. However, a sample from the Sari

Fig. 11. Types of abnormalities revealed in toad tadpoles in the Garhwal samples: A, normal; B, round tip of the tail; C, shortened tail; D, opercular chamber defect; E, edema; F, kyphosis; G, bifurcated tail; H, scoliosis.

Gad River, a tributary of the Yamuna, also had a small number of anomalous tadpoles.

Totally, seven types of larval abnormalities were revealed: scoliosis, kyphosis, shortened tail, tail with rounded tip, tail with split tip (two kinds), opercular chamber defect and edema of the abdominal cavity (Fig. 11; Table 3).

All seven types were registered in tadpoles of *D. himalayanus*, while the Aglad Nadi sample of *D. stomaticus* demonstrated only two abnormalities, namely: scoliosis and shortened tail (Fig. 12).

Scoliosis was the most common abnormality in all sites except the Purola-2, where the round tip tail predominated (Fig. 12). The rarest anomaly was bifurcated tail found only once in the Purola-2. Total frequencies of different variants were: scoliosis — 5.16%, rounded tip tail — 1.4%, shortened tail — 0.98%, edema — 0.27%, and kyphosis, opercular chamber defect, and bifurcated tail — each equally 0.14%.

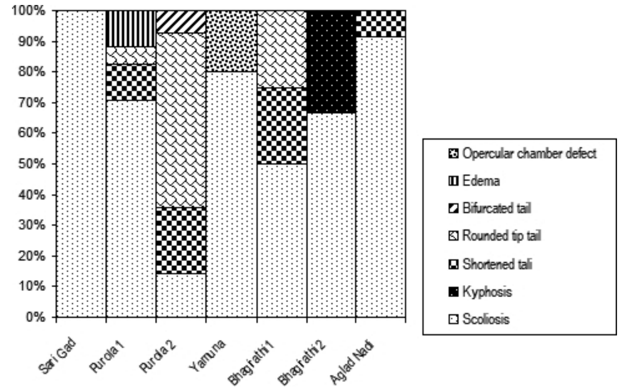

Fig. 12. Total spectra of larval abnormalities in *Duttaphrynus stomaticus* (Aglad Nadi) and *D. himalayanus* (other six samples).

In the Garhwal samples, tadpoles with more than one abnormality were also encountered. The frequency of tadpoles with combined abnormalities calculated from total number of malformed tadpoles in a sample varied from 3 to 13 (Table 4).

TABLE 3. Frequencies, %, of Malformed Tadpoles* and Frequencies, %, of Various Types of Larval Abnormalities** in the Garhwal Samples

Parameter	Aglad Nadi (n = 110)	Sari Gad (n = 110)	Purola-1 (n = 110)	Purola-2 (n = 101)	Yamuna (n = 110)	Bhagirathi-1 (n = 86)	Bhagirathi-2 (n = 90)
Malformed tadpoles, P_{at}	10.0	3.64	13.64	10.89	4.55	3.49	3.33
Defect opercular chamber	—	—	—	—	0.90	—	—
Edema	—	—	1.80	—	—	—	—
Bifurcated tail	—	—	—	0.99	—	—	—
Tail with rounded tip	—	—	0.90	4.95	—	1.16	—
Shortened tail	0.90	—	1.81	0.99	—	1.16	—
Scoliosis	10.0	3.64	10.90	1.98	3.60	2.32	2.22
Kyphosis	—	—	—	—	—	—	1.11

* *The frequency of abnormal tadpoles* (P_{at}) was calculated as the number of abnormal tadpoles (N_{at}) to total number of tadpoles (N_t) in a sample, i.e., $P_{at} = N_{at}/N_t$.

** The frequency of anomaly (so called *partial frequency of anomaly*, A_p, %) indicates the proportion of tadpoles with a given anomaly. It was calculated as the number of tadpoles with a given anomaly to all (normal + abnormal) tadpoles in a sample.

TABLE 4. Frequencies, %, of Tadpoles with Combined Abnormalities* and Frequencies, %, of Various Combinations of Larval Abnormalities** in the Garhwal Samples

Parameter	Aglad Nadi (n = 110)	Sari Gad (n = 110)	Purola-1 (n = 110)	Purola-2 (n = 101)	Yamuna (n = 110)	Bhagirathi-1 (n = 86)	Bhagirathi-2 (n = 90)
Tadpoles with combined abnormalities	0.90	0.90	2.72	0.99	0.90	0.90	0.90
Number of abnormalities per tadpole	0.110	0.036	0.154	0.139	0.045	0.047	0.033
Scolios + shortened tail	0.90	—	—	—	—	—	—
Shortened tail + rounded tip	—	—	0.90	—	—	1.16	—
Shortened tail + edema	—	—	0.90	—	—	—	—
Rounded tail tip + split tip	—	—	—	0.99	—	—	—
Scolios + shortened tail + rounded tip	—	—	0.90	—	—	—	—

* *The frequency of tadpoles with combined abnormalities* was calculated as the number of tadpoles with all combinations to all (normal + abnormal) tadpoles in the sample.

** *The frequency of a combined abnormality* was calculated as the number of tadpoles with a given combination to all (normal + abnormal) tadpoles in the sample.

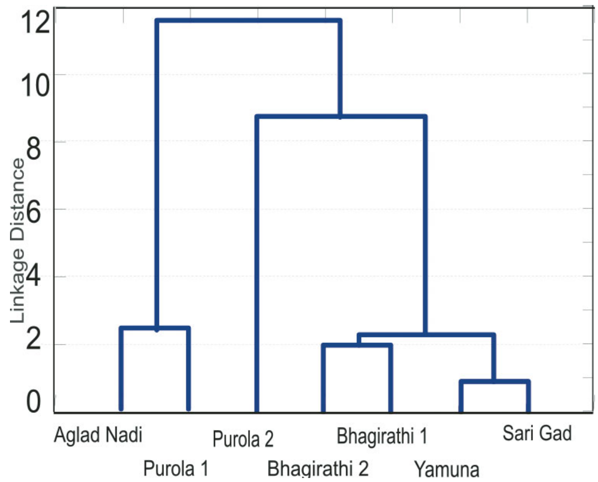

Fig. 13. Distances between studied samples by the spectra of abnormalities.

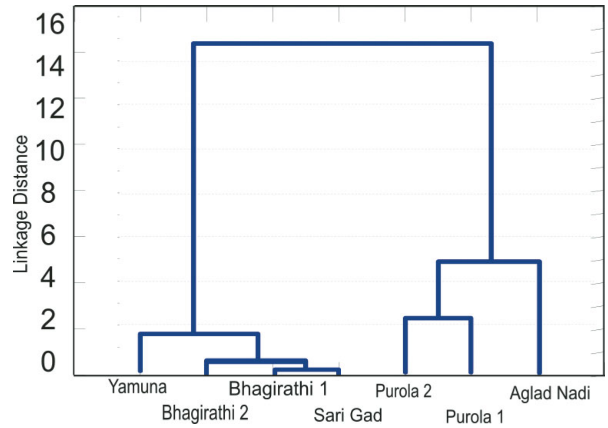

Fig. 14. Distances between seven samples by frequencies of abnormalities and abnormal tadpoles.

The maximum number of malformations recorded for an individual larva was 3. The share of tadpoles with several deformities averaged 6.8%, varying from 3.3 (Bhagirathi-2) to 11.8% (Purola-1). The average number of abnormalities per specimen among abnormal larvae (summary for 7 samples) was equal to 1.2. The abnormalities number per larva for total sample size varied from 0.033 in Bhagirathi-2 to 0.154 in Purola-1.

The following four combinations with two abnormalities were mentioned: scolios + shortened tail, shortened tail + rounded tip, shortened tail + abdominal edema, and rounded tail tip + split tip. In both samples from the Purola River were mentioned the combination of shortened tail with rounded tip (totally four specimens).

Three abnormalities (scolios + shortened tail + rounded tail tip) were expressed in only one tadpole (Purola-1).

Significant differences in the frequency of abnormal tadpoles were founded only for the sample Purola-1 (a residential area combined with a well-developed agro-landscape) which was significantly different from samples taken in relatively low transformed territories: Sari Gad, Yamuna, Bhagirathi-1 and Bhagirathi-2, respectively ($\chi^2 = 5.16$, $p = 0.0231$; $\chi^2 = 3.87$, $p = 0.0490$; $\chi^2 = 4.47$, $p = 0.0346$; $\chi^2 = 4.84$, $p = 0.0278$). However, the differences between the samples Purola-1 and Purola-2 proved to be not significant ($\chi^2 = 0.2$, $p = 0.6553$), the same situation with the samples Purola-1 and Aglad Nadi ($\chi^2 = 0.05$, $p = 0.8318$).

According to total frequency of abnormalities (Fig. 10), the sample Purola-1 differed significantly from samples originated from relatively clean territories: the Sari Gad ($\chi^2 = 8.9$, $p = 0.0029$), Yamuna ($\chi^2 = 7.27$, $p = 0.007$), Bhagirathi-1 ($\chi^2 = 5.89$, $p = 0.0152$) and Bhagirathi-2 ($\chi^2 = 8.08$, $p = 0.0045$). The sample Purola-2 also differed by frequency of abnormalities from Sari Gad ($\chi^2 = 7.05$, $p = 0.0079$), Bhagirathi-1 ($\chi^2 = 4.53$, $p = 0.0333$) and Bhagirathi-2 ($\chi^2 = 6.51$, $p = 0.0108$). The sample Sari Gad was significantly different from Aglad Nadi ($\chi^2 = 4.31$, $p = 0.0378$).

A dendrogram based on spectra of abnormalities (Fig. 13) showed clear division of samples into two clusters with high and low diversity. A similar pattern was observed in the dendrogram using frequencies of abnormalities and abnormal tadpoles (Fig. 14). In both cases, three samples (Purola-1, Purola-2, Aglad Nadi) fell into one cluster, and four samples (Bhagirathi-1, Bhagirathi-2, Sari Gad, Yamuna) into another.

DISCUSSION

Taxonomic distribution in India. Based on own data and scarce published literature, P. Santhoshkumar et al. (2017) listed 12 anuran species of India with records of various abnormalities in the field. These species belonged to six families, namely (alphabetically): Dicroglossidae (*Euphlyctis hexadactylus*, *Fejervarya* sp., *Fejervarya brevipalmata*, *Fejervarya keralensis*, *Fejervarya limnocharis*, *Fejervarya rufescens*, *Hoplobatrachus tigerinus*), Hylidae (*Hyla annectans*), Microhylidae (*Uperodon triangularis*), Ranidae (*Amolops gerbillus*), Ranixalidae (*Indirana beddomii*), and Rhacophoridae (*Polypedates* sp.).

However, taking into account several more publications and our data, one more family Bufonidae with two genera *Duttaphrynus* and *Xanthophryne* as well as genera *Minervarya* and *Sphaerotheca* (Dicroglossidae), and *Clinotarsus* (Ranidae) should be added. Thus, the total

TABLE 5. Distribution of Malformed Anurans in terms of Taxonomy and Geography in India

Current name (original name)	State, Region	Abnormalities	Reference
		I. Bufonidae	
1. *Duttaphrynus himalayanus*	Uttarakhand, Garhwal Himalaya	Various (tadpoles)	Present paper**
2. *Duttaphrynus melanostictus*	a) Maharashtra	a) microphthalmy, brachydactyly	a) Hippargi et al., 2010*
	b) Odisha (= Orissa), Eastern Ghats	b) anophthalmy	b) Ashaharraza, Mahapatra, 2020*
3. *Duttaphrynus scaber*	a) Maharashtra, Western Ghats	a) limb and eye deformities	a) Aravind, Gururaja, 2011*
	b) Maharashtra	b) brachydactyly	b) Modak et al., 2013*
4. *Duttaphrynus stomaticus*	Uttarakhand, Garhwal Himalaya	Various (tadpoles)	Present paper**
5. *Xanthophryne tigerina*	Maharashtra, Western Ghats	Anophthalmy	Pardeshi, 2017*
		II. Dicroglossidae	
6. *Euphlyctis cyanophlyctis* (*"Rana cyanophlyctis*")	Maharashtra	Vocal sacs in a female frog	Isaac, 1969*
7. *Euphlyctis hexadactylus*	a) Meghalaya	a) limbs	a) Mathew, Sen, 2006*
	b) central Western Ghats		b) Gurushankara et al., 2007a**
8. *Fejervarya limnocharis* ("*Limnonectes limnocharis*")	a) Meghalaya	a) limbs, anophthalmy	a) Mathew, Sen, 2006*
	b) central Western Ghats	b) various (limbs, anophthalmy, bulged abdomen, tumors)	b) Gurushankara et al., 2007a**
	c) Karnataka, Western Ghats	c) various (eyes, limbs, skin)	c) Patel et al., 2008**
	d) central Western Ghats		d) Hegde, Krishnamurthy, 2014**
9. *Fejervarya* sp.	Meghalaya	Limbs	Mathew, Sen, 2006**
10. *Hoplobatrachus tigerinus* ("*Rana tigrina*")	a) no data	a) polymely	a) Mahendra, 1936*
	b) Maharashtra	b) absence of fore-limb	b) Kurulkar, Deshpande, 1931*
11. *Minervarya brevipalmata* ("*Limnonectes brevipalmata*")	Central Western Ghats	Various (limbs, eyes, bulged abdomen, tumors)	Gurushankara et al., 2007a**
12. *Minervarya keralensis* ("*Limnonectus keralensis*")	Central Western Ghats	Various (limbs, eyes, bulged abdomen, tumors)	Gurushankara et al., 2007a**
13. *Minervarya rufescens* ("*Tomopterna rufescens*," *Sphaerotheca rufescens*)	a) central Western Ghats	a) various (limbs, anophthalmy, tumors)	a) Gurushankara et al., 2007a**
	b) Karnataka	b) anophthalmy	b) Nair, Kumar, 2007*
14. *Sphaerotheca maskeyi* ("*Sphaerotheca pashchima*")	Gujarat	Unilateral cataract	Patel et al., 2021*
		III. Hylidae	
15. *Hyla annectans* ("*Hyla annectens*")	Nagaland	Missing right foot	Mathew, Sen, 2006*
		IV. Microhylidae	
16. *Uperodon triangularis*	Tamil Nadu, Western Ghats	Missing digits, leg, foot	Santhoshkumar et al., 2017**
		V. Ranidae	
17. *Amolops gerbillus*	Meghalaya	Missing digits	Mathew, Sen, 2006*
18. *Clinotarsus alticola* (*Rana alticola*)	Assam	Skeleton	Annandale, 1905*
		VI. Ranixalidae	
19. *Indirana beddomii* (*Indirana* sp.)	Karnataka	Anophthalmy	Nair, Kumar, 2005*
		VII. Rhacophoridae	
20. *Polypedates* sp.	Nagaland	Limbs	Mathew, Sen, 2006*

* Single or a few malformed specimen(s); ** 5 and more specimens.

number of Indian species with deformity cases increased to 20. These species belong to 13 genera and 7 families (Table 5).

Most publications reported on malformed frogs (after metamorphosis), and only a few works concerned tadpoles (Annandale, 1905; Ashaharraza and Mahapatra, 2020). Our data provide the first registration of larval deformities in toad species of the genus *Duttaphrynus*.

Geographic distribution in India. All abnormal frogs of above mentioned species were recorded south of the Himalaya, in peninsular India, mostly in the Western Ghats region which is a malformation hotspot or in north-eastern India (Table 5). Therefore, as far as we know, our data are the first record of the occurrence of abnormalities in amphibians of the Himalaya.

Categories and composition of abnormalities. When describing deviations of larvae, the curvature of the notochord in various manifestations (scoliosis, lordosis, kyphosis, hard tail, curved tail end, size reduction), reduction in fin folds, edemas of the base of the tail and of the abdominal cavity are most often mentioned (Flindt, 1985; Krishnamurthy and Smith, 2010; Wijesinghe et al., 2011; Henle et al., 2017c: 191; David and Kartheek, 2015).

As mentioned above, we found seven types of abnormalities affected the body and the tail in larval samples of two Garhwal toad species. Some of them were associated with the axial skeleton (Fig. 11). These types of deformities were previously noted in the literature.

According to various authors (Meteyer, 2000: 3; Oullet, 2000: 647; Henle et al., 2017b: 39), *scoliosis* is an abnormal lateral curvature (either left or right) of the normally straight spine (tail or body). *Kyphosis* is abnormally convex (hunchback) thoracic spine (Meteyer, 2000: 3). Sometimes, scoliosis and kyphosis (Fig. 11F and H) are treated to belong to the same phenomenon (Cooke, 1981: 125; Vershinin, 2015: 49) or as a subcategory or specific expression of kinking (Henle et al., 2017b: 38, 39).

From hatching up to stage 26 (hind limb paddles) the most common deformity is simple curvature in the horizontal or vertical plane which can be expressed in various forms (Cooke, 1981: 125, Fig. 1). However, torsion of the body and/or tail was more often observed in experimental studies of amphibian embryos, but much less is known for tadpoles. Changes in the occurrence of kinked tails during larval development of *Rana* temporaria have been described (Cooke, 1981: 125). However, if the tadpoles succeed in metamorphosing into frogs, the adults appear normal (Tyler, 1989: 168). Various chemical pollutants including DDT caused bent tails (Cooke, 1973; Henle et al., 2017a: 104, 105).

In western Garhwal, tadpoles with scoliosis involving the presence of a deep bent at the base of the tail were registered in six sites (Table 3, no record in the sample Sari Gad). The proportion of tadpoles with such tails ranged from 1.98 to 10.90% (in general 5.16%). In contrast, kyphosis was found in only one sample with a frequency of 1.11% (single tadpole in the sample Bhagirathi-2).

Shortened tail (Fig. 11C), "abbreviation of tail" (Tyler, 1989: 168) or "tail stunted" (Henle et al., 2017b: 39) means that tail shorter than normal and may be only one-half of the normal length (Tyler, 1989: 169). However, malformed Gahrwal tadpoles did not show tail shortening to such an extent, and their shortened tails did not exceed 1/4 of the normal length. We recorded such a deformity in four out of seven samples with a frequency of 0.90 – 1.81% (Table 3: Aglad Nadi, Purola-1, Purola-2 and Bhagirathi-1).

Some experiments with Noth American ranid tadpoles suggested that tail loss by anuran larvae incurs little cost and therefore may be an important mechanism to reduce the effect of predation (Wilbur and Semlitsch, 1990). However, other experiments with tail damage in the tadpoles of the East Asian toad *Bufo gargarizans* showed that the effect of 50% tail loss on the swimming performance was significant (Ding et al., 2014). It has been suggested that shorter tailed tadpoles can escape less easily from predators than longer tailed ones. Also, tadpole tails are important energy and calcium reservoirs for metamorphosis eventually resulting in smaller toads (Baier et al., 2016: 12). Laboratory experiments with tadpoles of Neotropical treefrog, *Dendropsophus elegans* showed that tail injury delayed the metamorphose process, which could influence the survival of the individual (Martins et al., 2022).

In addition, we observed two types of deviations in the shape of tadpole tail tip in *D. himalayanus*. Usually the tail is 1.6 – 1.7 times longer than the body and slightly tapering towards the end. However, we registered some tadpoles with rounded or *split tail tips* (Fig. 11B, G).

Round-tailed tadpoles were noted in three out of seven samples with a frequency of 0.90 – 4.95% (Table 3: Purola-1, Purola-2 and Bhagirathi-1).

The category split tail tip requires some explanation. Surveying the literature on axial bifurcation in snakes, Hobart Smith and Gonzalo Pérez-Higareda (1987: 140) proposed the category *urodichotomy* when the tail or part thereof duplicated. In our opinion, three categories of urodichotomy in amphibians (urodeles and larval anurans) could be considered, depending on the extent of tail fission. If the larva has almost two tails, then we can talk about *tail duplication* (duplicated tail). If the tail is only partially split (for example, by a third or half), then we are talking about *tail bifurcation* (bifurcated or forked tail). Finally, if splitting affects only the very tip of the tail, then such cases are referred to *cauda bifida* (tip-forked tail).

Klaus Henle et al. (2012) reviewed the natural occurrence of urodichotomy in 27 species of amphibians from Europe, Asia, North America, South America, and Australia. 14 species of newts and salamanders with that rare malformation expressed at larval and postlarval stages belonged to three families: Ambystomatidae (3 species), Plethodontidae (2), and Salamandridae (9). Various cases of urodichotomy were also recorded in tadpoles of 13 species of frogs and toads from six families: Bufonidae (1), Hylidae (7), Microhylidae (1), Pelobatidae (1), Pelodryadidae (1), and Ranidae (2). All records of "tail duplication and bifurcation" were based on single or a few individuals per species, except 13 adult *Cynops pyrrhogaster* from Japan.

Additional information about seven more species has been published by Henle et al. (2017a: 88). Our data increase this list by two more species and one bufonid genus.

A photo with a tadpole of *Hyla arborea* from Greece, Crete Island (Fig. 4, Henle et al., 2012: 454) demonstrated a "bifurcated tail tip" with a forked axial skeleton at the tip of the tail. This case was later assigned to the category cauda bifida (see Henle et al., 2017b: 36), i.e., "terminal part of tail duplicated." A forked tail seems to be quite rare phenomenon. For instance, among 424 tadpoles (128 with abnormalities) examined in Yerba Buena, Tucumán Province, Argentina, only one specimen of *Hypsiboas riojanus* (currently *Boana riojana*, Hylidae) exhibited a forked tail (Medina et al., 2013). We failed to identify any case with a clear cauda bifida in 717 tadpoles from the seven studied samples.

However, we found another kind of deformation of tail tip shape. A tadpole of *D. himalayanus* from the sample Purola-2 had a normal axial skeleton but an evident notch was expressed on the terminal part of transparent fin (Fig. 11G). This case was only 0.99% of the sample. As far as we know, such a condition has not previously been noted in the literature.

Fabian Baier et al. (2016: 6, Fig. 1A – C) reported two deviant variations in the tail structure in tadpoles of the European toad *Bufo bufo* treated with glyphosate-based herbicides. These were tadpoles with lacerated and curved tail tips; only curved tail tips were considered deformed and included in the analyses. Curiously, such tips were crooked in one direction.

A *defect of the opercular chamber* was expressed in a tadpole from the Yamuna sample (Fig. 11D; Table 3: 0.90%). This type of abnormality was previously noted in an invasive water frog species *Pelophylax ridibundus* under the effect of urbanization in the City of Yekaterinburg, Middle Ural (Vershinin et al., 2018), as well as in experimental studies on the changes in hormonal levels (Kollros, 1961), on the influence of herbicides (glycophosphates) and wastewater on amphibian morphogenesis (Lajmanovich et al., 2003; Ruiz et al., 2010).

Abdominal edemas (Fig. 11) were mentioned in two tadpoles in the sample Purola-1 only (Table 3: 1.80%). Such deviations have previously been observed in *Pelophylax ridibundus* population of urbanized areas (Vershinin, 1989). The malformation was associated with the effect of wastewater (Ruiz et al., 2010), and may also have hereditary causes (Gollmann et al., 1984).

In contrast to single anomalies, the occurrence of combined anomalies (two or three in the same tadpole) in the Garhwal samples was low. We found them only in both samples from the town of Purola. We suppose that urbanization with various pollutants and other complex drivers that acting synergistically may amplified impact and lead to more unpredictable results (Jung and Zafoe, 1993; Dodd and Smith, 2003).

In amphibian larvae, 15 types of abnormalities were previously listed (Vershinin, 2015: 49). In the Garwal samples, we were not able to find eight of them. They affect the head (dicephalism, anencephaly, microphthalmy, anophthalmy, oral disc deformities), limbs, skin pigmentation, skin neoplasms (melanomas and others), hernias, gigantism, and neoteny.

In the last decades, deformation of larval oral structures has attracted special attention. Some authors (Rowe et al., 1998; Lips, 1999; Fellers et al., 2001; Knapp and Morgan, 2006; Drake et al., 2007; Vieira et al., 2013) demonstrated that tadpoles infected with chytrid fungus *Batrachochytrium dendrobatidis* had abnormal oral disc structures and depigmentation of normally black keratinized jaw sheaths (beaks) and toothrows. Importantly, chytridiomycosis is considered as an emerging panzootic fungal disease, which can lead to a catastrophic population declines and extinctions in amphibians globally (Daszak et al., 1999; Skerratt et al., 2007; Fisher et al., 2009; Blaustein et al., 2012; Van Rooij et al., 2015).

However, loss of pigmentation in tadpole mouthparts might not always be associated with chytridiomycosis and their pigmentation might vary seasonally (Rachowicz, 2002). Moreover, some authors (Padgett-Flohr and Goble, 2007; Bosch et al., 2017; Navarro-Lozano et al., 2018) concluded that the occurrence of larval mouthpart abnormalities is neither an accurate nor a reliable diagnostic test for *Batrachochytrium dendrobatidis* infection because tadpoles with normal mouthparts were positive for this fungus and tadpoles with defective mouthparts were not infected.

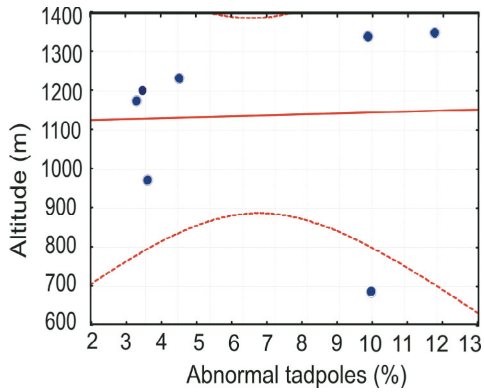

Fig. 15. Relation between the frequency of malformed tadpoles and the altitude.

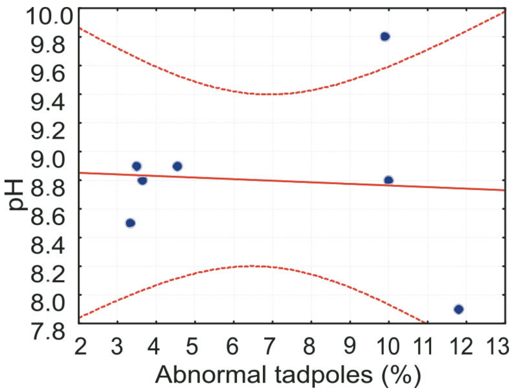

Fig. 16. Relation between the frequency of malformed tadpoles and pH values.

Some partial disturbances in toothrows were observed in a few tadpoles from studied Gahrwal bufonid samples. However, we did not registered any depigmentation of their keratinized jaw sheaths (beaks) and toothrows, as well as swollen and reddened labial papillae. Importantly, we did not test for *Batrachochytrium dendrobatidis* infections by special analysis. This fungal pathogen was not found in Indian amphibians (Fisher et al., 2009: 297, map). Screening various species collected in the Western Ghats for the pathogenic fungus evidenced no *B. dendrobatidis* infection in all (142) frogs, except one individual from the southernmost locality in Kerala (Nair et al., 2011).

Species differences. We found higher number of abnormalities and their combinations in *D. himalayanus* compared to *D. stomaticus*. However, these differences are most likely due to markedly larger number of tadpoles studied (607 versus 10) and larger number of samples (6 versus 1) in the former species.

Environmental parameters. We did not found significant correlations between frequencies of deformed tadpoles and some environmental parameters (Figs. 15 – 17).

The elevation. The Yamuna, the largest tributary of the Ganges, and its tributaries form so called Yamuna River System. The river itself originates near the Yamunotri glacier in the Higher Himalaya and drains the southern slopes of the mountains in its upper reaches. The river Bhagirathi is a large stream of the Ganges fluvial system in the Garhwal Himalaya. It originates from the Gangotri glacier, flows through the deep gorges and traverses around 225 km across the Himalaya before its confluence with the Alaknanda River at Devprayag to form the Ganges itself. The Yamuna and Bhagirathi rivers have similar the discharge and drainage area.

Our travel covered the altitudes from the Indian plain up to the Higher Himalaya namely to the Yamunotri temple (3290 m a.s.l.) below the Yamunotri glacier and to Gomukh (4023 m) near the Gangotri glacier. However, tadpoles were sampled at much lower heights, from 687 to 1349 m a.s.l. in the Lesser Himalaya (Fig. 1, Table 1). Tadpoles were also encountered in higher places, for example, on the Tons River (1629 m), the main tributary of the Yamuna. However, they were at earlier stages of development and therefore were not comparable with our main samples.

Within our vertical range of studies, we did not find any significant dependence of the frequency of abnormal tadpoles on altitudes ($R = 3.8\%$, $p = 0.49$; Fig. 15).

pH. The river waters are mildly alkaline in nature, covering a pH range of 7.7 to 9.2 in the Yamuna River and its tributaries (Dalai et al., 2002) and of 7.1 – 8.4 in the Bhagirathi River (Pandey et al., 1999). In our study sites, pH values varied from 7.9 to 9.8; both limits were noted in the town Purola, the Yamuna basin (Table 1). We did not find significant correlation between of pH values and frequencies of anomalous tadpoles (Fig. 16). The coefficient of correlation of the frequency of malformed tadpoles with pH values was equal to –7% ($p = 0.69$).

Mineralization. Chemical analysis of waters of the Yamuna River with its tributaries and of that of the Bhagirathi River showed that the major cations decreased in the order: Ca > Mg > Na > K > Fe or Ca > Na > Mg > K > Fe, and the molar abundance of anions decreased as ($HCO_3 + CO_3$) > SO_4 > Cl > NO_3 (or SO_4 > HCO_3 > Cl > NO_3). Carbonate and silicate weathering is the dominant source of these components. Anthropogenic contribution to the Yamuna major ion budget is unlikely to be of importance (Pandey et al., 1999; Dalai et al., 2002a; Krishnaswami and Singh, 2005).

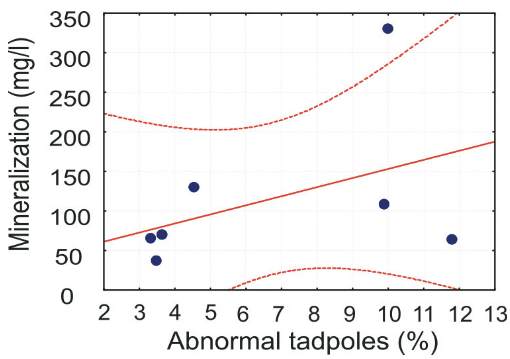

Fig. 17. Relation between the frequency of malformed tadpoles and water mineralization.

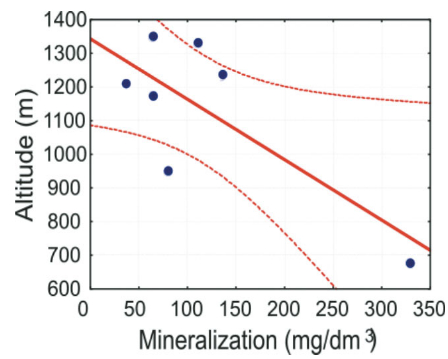

Fig. 18. Correlation of water mineralization with altitude.

One of the conspicuosus characteristics of the Yamuna and the Bhagirathi, like other Himalayan rivers, is their high dissolved concentration of uranium, in orders of magnitude higher than in other large river worldwide (Sarin et al., 1992). The Bhagirathi River supplies water with the highly radiogenic strontium (Tripathy and Singh, 2010). Other average dissolved trace elements, like rhenium, osmium, barium, are significantly higher than the reported global average river water concentration (Dalai et al., 2002b).

The relationship between the occurrence of abnormal larvae and the water mineralization values measured by us turned out to be contradictory. At the places (Sari Gad, Bhagirathi-1, Bhagirathi-2) where lower water mineralization was in the limits 37 – 70 mg/dm^3, the frequency of deformed tadpoles varied within the interval 3.3 – 3.6%, whereas at the sites with higher water mineralization equal to 130 – 330 mg/dm^3 (Aglad Nadi, Yamuna) the frequency of larvae with deviations varied from 4.55% up to 10.0%. The only exception was two samples from the Purola River (Purola-1 and Purola-2), where relatively low or moderate water mineralization (64 – 108 mg/dm^3) and high pH variability (7.9 – 9.8) combined with the highest frequencies of abnormal tadpoles (Fig. 10).

The coefficient of correlation of the frequency of malformed tadpoles with the level of water mineralization was equal to 42.8% ($p = 0.08$, Fig. 17). Nevertheless, regression analysis did not reveal a significant dependence ($R = 42.8\%$, $F = 1.1$, $p = 0.08$).

Curiously, although the level of mineralization (Fig. 18) was negatively correlated with altitude ($R = -0.76$; $F = 7.0$; $p = 0.019$), no significant correlation between the frequency of malformed tadpoles or abnormalities, on the one hand, and altitude, on the other hand, has not been identified.

Thus, the frequency of malformed tadpoles and abnormalities did not show significant correlations either with altitudes, or with salinity of water bodies, or with pH.

Background and mass level of deformed animals. Theoretically, malformed animal(s) may occur in any natural population. Based on her experiments, Elena Kovalenko (2000: 39) suggested to name the *background* abnormalities when they manifest in a small part of the group during normal development (abnormalities against the background of the norm). Indeed, it is important to assess the level of background occurrence of abnormal individuals within a population (“natural levels of abnormalities”) in order to separate it from the *mass* occurrence caused by one or another factors, including environmental contamination by human activity.

Previously, the following values were proposed as a conditional threshold: 1% (Borkin and Pikulik, 1986; Hoppe, 2000: 89; Henle et al., 2017a: 133) or 2% (e.g., Puky and Fodor, 2002; D’Amen et al., 2006; Laurentino et al., 2016: 12). When the frequency was less than these values, such cases with deformed animals were related to the background, and higher to mass occurrence. However, since the 1990s, there was a tendency toward a clear increase in the occurrence of background anomalies, more than 2% (Hoppe, 2000; Johnson et al., 2003; McCallum and Trauth, 2003; Lannoo, 2008; Henle et al., 2017a). Large-scale field surveys across the United States and Canada have identified similar mean abnormality levels (2%) ranging from 0.3 to 4.3% (Lunde and Johnson, 2012).

Moreover, even in nature reserves the situation may be different. So, in the United States in 1997, deformed anurans were registered in 29 of 38 protected areas. A frequency of 1% was detected in 10 reserves, from 1 to 2% in 11 and from 2 to 5% also in 11, and from 5.5 to 9.9% in 4 protected areas (Converse et al., 2000). At five Alaskan refuges, the overall prevalence of abnormal frogs was 6.2% (from 1.5 to 7.9%), with the highest abnormality prevalence at any single breeding site equal to 20% at a Kenai site in 2005 (Reeves et al., 2008). The incidence of abnormal newts at the individual breeding sites ranged from 0.69 to 4.17% in the Serra da Estrela Natural Park, Portugal (Laurentino et al., 2016).

Therefore, it was proposed to increase the conditional baseline that separates background anomalies from mass ones in wild amphibian populations to 5% (e.g., Johnson et al., 2010; Borkin et al., 2012). However, recent studies of frogs in some areas have documented rates as high as 6 – 22% (McCallum and Trauth 2003; Taylor et al., 2005; Rogers et al., 2006; Medina et al., 2013), and the 5% baseline was estimated as a conservative threshold (Lunde and Johnson, 2012: 432).

On the other hand, many data indicates that true baseline rates might even have been an order of magnitude lower than the 2% calculated in the US nation-wide survey in wildlife refuges, and the significant increase in background rate in recent decades has been called into question (Henle et al., 2017a: 133 – 134).

It was recommended to pay special attention to cases where the frequency of abnormal amphibians in the population reaches 10%, since this indicates the presence of environmental factors causing anomalies (Fodor and Puky, 2002). The highest values of mass anomalies were found in the mink frog (*Rana septentrionalis*, now the genus *Lithobates*) in Minnesota, USA, up to 80% in yearlings (Gardiner and Hoppe, 1999), in green frogs of the *Rana esculenta* complex (now the genus *Pelophylax*) in France, up to 80% in tadpoles (Dubois, 1979) and in the red-bellied toad, *Bombina bombina* in Hungary, up to 71% (Puky and Fodor, 2002). Some reports often documented populations in which greater than 10% and even up to 95% of amphibians suffered from severe limb malformations (Lunde and Johnson, 2012).

In our opinion, when assessing the occurrence of anomalous amphibians, it is especially important to provide data for each studied population, and not just for the species as a whole or its geographical segments. It should also be noted that the frequency of abnormal individuals is higher in tadpoles and postmetamorphosed froglets (yearlings) compared with older age cohorts (e.g., Dubois, 1979; Medina et al., 2013; our data) but not always (Flyaks and Borkin, 2004: 238).

Thus, regardless of whether we will accept the baseline of 1, 2, or 5%, then data published by Gurushankara et al. (2007a), Patel et al. (2008), Hegde and Krishnamurthy (2014), and, possibly, Santhoshkumar et al. (2017) as well as our report should be attributed to the mass occurrence of abnormal amphibians in India. Therefore, we can outline two Indian hotspots with malformed amphibians: the Western Ghats and the Garhwal Himalaya (at least some tributaries of the Yamuna River).

Possible causes and human impact. A complicated life cycle that includes necrobiotic metamorphosis, lack of larval envelopes, and ectothermia makes anuran morphogenesis dependent on a large number of factors, whose synergism (Baier et al., 2016) determines both the definitive morphological appearance and the morphology of larval stages; the change of the latter may substantially affect the success of new generation.

Due to the global decline of amphibians, researchers are giving more of their attention to the most vulnerable stages of the life cycle, including larval development and the probabilities of normal realization of ontogenesis in natural populations at various parts of species' range (Bradshaw and Holzapfel, 2001). Two main categories of causes of the appearance of abnormalities could be distinguished: these are natural causes and man-made causes. Among our samples, the highest frequencies of abnormal tadpoles were revealed in two localities associated with tributaries of the Yamuna River (Figs. 1 and 10): Aglad Nadi (10.9%) and the town Purola (with two sites Purola-1 and Purola-2, 13.6 and 10.9%, respectively). The Aglad Nadi River is used for grazing (watering) cattle, while the Purola River for agriculture.

Four other samples were taken in rivers near a tourist camp, a hotel and/or small settlements, where, according to our observations, there was no significant human impact, with the exception of plastic garbage randomly scattered along the banks. In these places, there may be a small drain of household waste from nearby houses. However, a strong river flow is unlikely to allow this waste to accumulate much. The incidence of abnormal tadpoles in these places was lower than in the Aglad Nadi and Purola samles. Therefore, we can conclude that the level and nature of anthropogenic load affect the frequency of abnormal tadpoles.

A clear increase in the incidence of abnormal amphibians (tadpoles, postmetamorphs, and adults) in places used for agriculture was noted in many places globally (e.g., Oullet et al., 1997; Oullet, 2000; Taylor et al., 2005; Piha et al., 2006; Huang et al., 2010; Peltzer et al., 2011; Spolyarich et al., 2011), including India (Gurushankara et al., 2007a; Patel et al., 2008; Hegde and Krishnamurthy, 2014).

Abnormal development that gives rise to malformations could be caused by various genetic or environmental factors (teratogens). Environmental factors include radiation, hyperthermia, low oxygen, high carbon dioxide, poor nutrition, numerous chemical pollutants from agriculture, industry and other human activities (pesticides, herbicides, fertilizers, heavy metals, and a lot of other contaminants), retinoids (vitamin A and its derivatives), parasites, predators, and so on. The same teratogen presented at different developmental ages could initiate different malformations, whereas different teratogens presented at the same developmental age could result in similar errors in development and cause the same malformation (Burkhart et al., 2000; Oullet, 2000; Lannoo, 2008; Henle et al., 2017a).

As is well known, amphibian larvae living in water are very sensitive to various chemical pollutants used in agriculture (Harfenist et al., 1989; Tyler, 1989; Boone and James, 2005; Mann et al., 2009). Large amount of publications devoted to the influence of agricultural chemicals on the survival, development, and morphogenesis of tadpoles. Along with growing of mortality and hyperactivity that associated with toxic effects of pesticides on developing tadpoles, amphibians exposed to high concentrations of pesticides exhibit a number of characteristic abnormalities (e.g., Hazelwood, 1970; Cooke, 1973; Tyler, 1989).

In addition to field observations, it has been experimentally shown in many works that the treatment of aquatic larvae of various amphibian species, including bufonid genus *Duttaphrynus*, with chemicals used in agriculture leads to the appearance of various abnormalities of the body and tail (Table 5). Bufonid tadpoles are suggested to be less sensitive to agrochemicals, at least to glyphosate-based herbicides, compared to larval Pipidae (*Xenopus laevis*), Hylidae, and Ranidae (Hammond et al., 2012: 599; Wagner et al., 2013: 1692).

We do not know if Puroda residents used pesticides and, if so, which ones and how much. Nevertheless, the larval abnormalities obtained in numerous experiments with various agrochemicals (Table 6) surprisingly coincide with those that we found in tadpoles collected in the agrolandscape of Purola. These are deformations of the axial skeleton (notochord): scoliosis, kyphosis, lordosis, malformed tails, as well as edemas. The agricultural field situation may differ from experimental conditions. As a rule, there is a mixture of pollutants, which increasing the frequency of morphological abnormalities in tadpoles (Krishnamurthy and Smith, 2011; Rathanayaka and Rajakaruna, 2018).

The most diverse spectrum of abnormalities, along with their high frequency, was precisely mentioned under effect of residential complex interaction with agrolandscapes (Purola, Fig. 12). The synergy of agricultural pes-

TABLE 6. Some Agrochemicals and Tadpole Deformities

Amphibian current name (original name)	Chemical	Tadpole deformities	Reference
		Alytidae	
Discoglossus galganoi	Ammonium nitrate (fertilizer)	Edema, bent tail, lordosis	Ortiz et al., 2004
		Bufonidae	
Anaxyrus americanus (“*Bufo americanus*”)	Malathion (organophosphate insecticide) and nitrate	Diamond-shaped body, stiff tail	Krishnamurthy and Smith, 2011
Bufo bufo	Glyphosate-based herbicides	Lacerated tail, curved tail tip	Baier et al., 2016
Bufo spinosus (“*Bufo bufo*”)	Ammonium nitrate	Edema, bent tail, lordosis	Ortiz et al., 2004
Duttaphrynus melanostictus (“*Bufo melanostictus*”)	Diazinon (organophosphate pesticide)	Bent tail, curved tail	Sumanadasa et al., 2008
	Carbofuran (carbamate insecticide)	Swelling of head and body, enclosing vesicle	Jayatillake et al., 2011
	Chlorpyrifos (organophosphate pesticide)	Swollen head, coiling of the tip, tail curvature, swelling at the base of the tail	Wijesinghe et al., 2011
	Chlorpyrifos	Gill (reduction in blood vessels, edema), liver, tail muscle fiber atrophy	Bandara et al., 2012
	Chlopyrifos, dimethoate (organophosphorous insecticides), glyphosate, propanil (herbicides)	Kyphosis, scoliosis (tail curvature), edema, skin ulcer	Jayawardena et al., 2011, 2017
	Carbosulfan (carbamate pesticide)	Swelling, tail bending	Samarakoon and Pathiratne, 2017
	Malathion	Scoliosis, kyphosis, lordosis, fin blistering	David, Kartheek, 2015
	a) Profenophos (organophosphate insecticide)	a) Scoliosis, kyphosis	Rathanayaka, Rajakaruna, 2018
	b) Abamectin (mixture of avermectins, insecticide)	b) Edema	
	c) Profenophos + Abamectin	c) Scoliosis, edema	
Epidalea calamita (“*Bufo calamita*”)	Ammonium nitrate	Edema, bent tail, lordosis	Ortiz et al., 2004

TABLE 6 (continued)

Amphibian current name (original name)	Chemical	Tadpole deformities	Reference
		Dicroglossidae	
Fejervarya limnocharis (“*Limnonectus limnocharis*”)	Malathion	Decrease in body and tail length	Gurushankara et al., 2007b
	Nitrate	Swollen body, swollen head, bent tail, depigmentation, intestinal hemorrhage	Krishnamurthy et al., 2008
		Hylidae	
Scinax nasicus	GLYFOS (glyphosate herbicide)	Craniofacial and mouth deformities, eye abnormalities, bent curved tail$ deformations of the hyobranchial apparatus	Lajmanovich et al., 2003
		Leptodactylidae	
Physalaemus albonotatus	Amina Zamba (2,4-dichlorophenoxyacetic acid (2,4-D)-based commercial herbicide Amina)	Decreased pigmentation, oral disc malformations, abdominal edema, intestinal abnormalities (gut uncoiling, diverted gut), altered body shape	Curi et al., 2019
		Nyctibatrachidae	
Nyctibatrachus major	Nitrate	Protruded mouth, swollen body, swollen head, paralysis	Krishnamurthy et al., 2006, 2008
		Pipidae	
Xenopus laevis	Atrazine (herbicide)	Heart malformations, visceral hemorrhaging, intestinal miscoiling, shortened body axis, curved (upward) tail	Lenkowski et al., 2008
	Roundup Classic (glyphosate-based herbicide)	Shortening of the anterior-posterior axis, craniofacial malformations, reduction or loss of the eye, cyclopia	Paganelli et al., 2010
	Roundup Power 2.0 (glyphosate-based herbicide)	Small, narrowed and flattened head with rounded brow, prominent oral sucker, microphthalmia, narrow eyes, forebrain regionalization, uncorrected gut coiling, cardiac edema, facial and abdominal edemas	Bonfanti et al., 2018
		Ranidae	
Lithobates clamitans (“*Rana clamitans*”)	a) Diazinon, basudin (organophosphate insecticide)	a) Abdominal and head edemas, blistering, ventral and lateral flexure of the tail, stunting of the tail, underdevelopment of the gills	Harris et al., 1998
	b) Dithane (ethylenebisdithiocarbamate fungicide)	b) Dorsal and lateral flexure of the tail, abdominal edema, gill displacement	
	c) Thiodan (organochlorine insecticide)	c) Skeletal deformities (dorsal and lateral flexure)	
Lithobates pipiens (“*Rana pipiens*”)	Glyphosate technical, polyethoxylated tallowamine surfactant (POEA), Roundup Original, Roundup Transorb (glyphosate-based herbicides)	Tail damage (necrosis of the tail tip, flexure of the tail tip, fin damage, abnormal growths on the tail tip, blistering on the tail fin)	Howe et al., 2004
Rana pretiosa	Nitrate, nitrite	Edema, bent tail, paralysis	Marco et al., 1999
		Rhacophoridae	
Polypedates cruciger	Chlorpyrifos, dimethoate, glyphosate, propanil	kyphosis, scoliosis, edema, skin ulcer	Jayawardena et al., 2010, 2016

ticides, herbicides, mineral fertilizers and household pollution influence seems to lead to the amplification of ontogenetic deviations increasing their frequency and variability.

Apart from agrochemicals, other factors can also cause abnormalities in tadpoles. Exposure of tadpoles of the common hourglass tree frog, *Polypedates cruciger* (Rhacophoridae) to an infection of “monostome-type” cercariae of trematode, collected from the freshwater snail *Thiara scabra*, under laboratory conditions induced malformations at high frequencies (50 – 90%) with their highest number (76%) in the tail region. Axial deformities, like scoliosis, extension of the spine beyond the rump and kyphosis, as well as edemas and skin malformations included lack of pigment, translucent skin, abnormal patterns of skin pigmentation and skin ulcers were observed in tadpoles; various limb deformities in the metamorphs were also reported (Rajakaruna et al., 2008; Jayawardena et al., 2010).

Later, molecular analysis showed that these cercariae belong to the trematode *Acanthostomum burmini*, family Cryptogonimidae (Jayawardena et al., 2013: 248, 250). Also, laboratory exposure of cercariae of this digenetic trematode species *Acanthostomum burmini*s caused high levels of scoliosis, kyphosis, and edema in tadpoles and metamorphs of the Asian common toad, *Duttaphrynus melanostictus* (Jayawardena et al., 2013). Importantly, the effect of cercariae on malformation incidence increases in combination with the pesticide treatment (Jayawardena et al., 2016, 2017). Polydactyly and heavy forms of the anomaly P syndrome in toad tadpoles of the genera *Bufo* and *Bufotes*, as a result of exposure to trematode *Strigea robusta cercariae*, were observed in the laboratory experiments (Svinin et al., 2022).

Larval malformation could also be caused by extreme (cold or high) water temperatures and nutritional deficiencies (scoliosis). Effects of radioactive and some trace metals may be influenced by dissolved organic carbon, hardness, pH, and temperature of water. Osteolathyrogenic agents can induce the notochord and tail deformities. Larval lordosis, tail malformations, and edemas were experimentally obtained after enhanced level of ultraviolet radiation (Ouellet, 2000; Blaustein et al., 2003; Blaustein and Johnson, 2003). The latter should be given special attention in the mountains. The synergism or interaction of various factors may increase the frequency of malformed tadpoles.

In conclusion, we would like to note that the Himalaya are usually considered relatively "pristine," unpolluted and sparsely populated region in contrast to the peninsular India. However, even here, we found the mass occurrence of abnormal tadpoles of toads, which was associated with human activity (grazing and residential agrolandscape). Previously, the increased incidence of abnormal amphibians was revealed by Indian researchers in agricultural areas of the Western Ghats. Thus, the Western Ghats and the Garwal Himalaya can be considered as two *malformation hotspots*. Therefore, we recommend to assess environmental quality of other regions of India, especially with strong industrial and agricultural human impacts, using tadpoles and metamorphs of common amphibian species as a possible bioindicator of the environment health.

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