
**BIOLOGY, MORPHOLOGY,
AND SYSTEMATICS OF HYDROBIONTS**

Ossification Variability in the Cranial Skeleton of *Lissotriton vulgaris* (L.) (Caudata) from Urbanization Areas

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Abstract—This paper considers the influence of environmental conditions on the specificity of the process of ossification of the skull of yearlings of common newt *Lissotriton vulgaris* (Linnaeus, 1758), depending on the degree of urbanization. It is found that the degree of sagittal suture closure in the cranial skeleton of yearlings depends on the level of mineralization of surface waters in which the common newt develops. The accumulating nature of urban ecosystems promotes the intensification of the mineral metabolism of juveniles due to the abundance of calciphilous animals in newt habitats and their dominance in the diet of newt yearlings. This contributing factor accelerates the formation of cranial ossifications in animals from the residential part of urban agglomeration.

Keywords: common newt, cranial skeleton, urbanization, mineralization

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INTRODUCTION

It is known that the emergence and ossification of cranial bones in tailed amphibians depend on their taxonomic features [19, 21, 28] and intertissue induction interactions [5, 8], as well as on the mechanism of their endocrine regulation [20, 24, 25]. At the same time, the ossification of different cranial elements is differently determined not only by the level of thyroid hormones [9, 26, 27], but also by the specific ecological pattern of the habitat, which influences the new generation that is integrated into this area in the course of its ontogenesis [22].

Common newt *Lissotriton vulgaris* (L.) is a widespread species in Eurasia; it inhabits both natural and anthropogenically changed landscapes and easily adapts to water bodies in urbanized areas [11, 13, 16, 17].

Along with the sensitivity of *L. vulgaris* to environmental pollution and geochemical environmental parameters [10], this species has a certain resistance to anthropogenic transformations of communities. It is known [4] that the common newt is dependent not as much on the composition of plant communities as on the height and density of herbage, which provide favorable microclimatic conditions. As a rule, urbanization in Ural cities is combined with a significant industrial pollution [1] and the populations of common newt that have been covered by the sinurbization process in this area [14] acquire a number of both adaptive and negative features reflecting the state of their habitat [3].

The purpose of this research was to study the features of the formation of the cranium of *L. vulgaris* yearlings in an urbanization gradient.

MATERIALS AND METHODS

The material was collected in 2013–2014 in common newt habitats in urban agglomeration areas in the city of Yekaterinburg. Animals were caught manually soon after the end of their metamorphosis and achievement of the 56th stage [6]. The total number of the studied animals was 72 specimens of *L. vulgaris* yearlings, which included four independent representative and numerically comparable samples (Table 1). The degree of urbanization was assessed by the typification of urban landscapes [29, 30]. The typification was based on the degree of land development by humans (number of stories in the residential area, residential density, land use pattern, and degree of pollution).

Four zones with amphibian habitat areas were distinguished within the urban agglomeration: zone I (the central part of the city (amphibians were not found), including multistory buildings, massive asphalt coverings, water bodies with severe industrial pollution, small rivers, and streams drained by pipes); zone II (areas with multistory buildings, including territories being developed, waste grounds, sites with open soils, and small ponds with high levels of pollution); zone III (low-rise building areas that are occupied by private houses with gardens and kitchen gardens, as well

Table 1. Proportion of yearlings (% of the number of individuals) with different states of the sagittal suture in the cranial skeleton

Suture state	Zone II (n = 18)	Zone III (n = 15)	Zone IV (n = 17)	C (n = 22)
Fully closed	61.11	80.0	41.18	45.45
Partially closed	33.33	20.0	35.29	45.45
Open	5.56	0	23.53	9.09
Body length, mm	$\frac{18.38 \pm 0.26}{15.4-19.7}$	$\frac{18.01 \pm 0.23}{16.3-19.4}$	$\frac{18.67 \pm 0.25}{17.0-20.7}$	$\frac{19.83 \pm 0.23}{18.2-22.0}$

Zone II, multistory buildings; zone III, low-rise buildings; zone IV, forest-park zone; C, control zone (suburban population), and n, number of individuals. Mean value and its error is above the line; min–max is below the line.

as by waste grounds and parks); and zone IV (a forest-park area with habitats that are mainly exposed to the recreation load). A sample from the suburban population 50 km from Yekaterinburg was used as a control (C). The presence of the urbanization gradient corresponding to our typification [30] is annually confirmed by hydrochemical analyses that are carried out at the Laboratory of Physicochemical Analyses, Ural State Mining University. Water samples were collected in water bodies where the common newt developed during the period of its reproduction and during the completion of its metamorphosis. The temperature of spawning ponds was measured using a TP-2 mercury thermometer with a scale division of 0.5°C during the first month after eggs laying.

The animals were caught, dissected, and fixed in 70% alcohol. Their body length was measured using a digital Kraftool caliper (Germany) with a scale division of 0.01 mm. The content of the gastrointestinal tract of yearlings was studied during the laboratory treatment using an MBS-10 binocular. Skeleton structures were studied using a standard protocol of carti-

lage and bone binary staining with alcian blue and alizarin red, respectively [31].

The results were processed using regression analysis and analysis of variance.

RESULTS AND DISCUSSION

The study of the features of formation of the cranium of yearlings established that this phase of the life cycle of the common newt (stage 56 according to [6]) is characterized by three variants of sagittal suture occlusion on the border of frontal and parietal bones: the suture is fully closed, the suture is partially closed and there is a small hole between bones, and the suture is open (Fig. 1).

The highest occurrence of the variant with a fully closed suture was recorded for animals from multistory (zone II) and low-rise building (zone III) zones: 61.1 and 80%, respectively (Table 1). Only the sample from zone III did not have individuals with an open suture and the lowest occurrence of animals with a

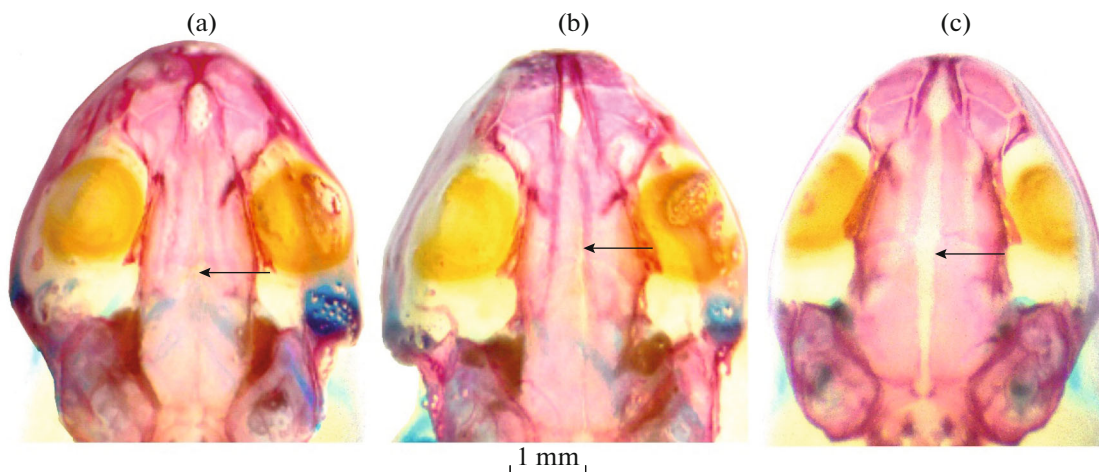


Fig. 1. Variants of occlusion of the sagittal suture in *L. vulgaris* yearlings: (a) the suture is fully closed, (b) the suture is partially closed, and (c) the suture is open. The arrows indicate the sagittal suture (photo by A.G. Trofimov).

Table 2. Coefficient of correlation (r) between some parameters and the proportion of individuals with a fully closed sagittal suture

Parameter	Zone II	Zone III	Zone IV	C	r
Mineralization, mg/L	562.3 ± 31.6 ($n = 8$)	793.5 ± 44.7 ($n = 4$)	81.9 ± 44.7 ($n = 4$)	149.3 ± 63.2 ($n = 2$)	0.985 ($p = 0.015$)
pH	8.19 ± 0.16 ($n = 8$)	7.95 ± 0.22 ($n = 4$)	6.75 ± 0.22 ($n = 4$)	7.16 ± 0.3 ($n = 2$)	0.82 ($p = 0.2$)
Mean-monthly temperature in May, °C	18.9 ± 1.0 ($n = 8$)	21 ± 1.5 ($n = 4$)	13.8 ± 1.5 ($n = 4$)	12 ± 2 ($n = 2$)	0.93 ($p = 0.07$)
Occurrence of mollusks in the GIT, % of the number of intestines	33.8	31.2	3.2	3.8	0.87 ($p = 0.06$)

See the notations of types of urban landscapes in Table 1. GIT, gastrointestinal tract; n , number of measurements.

closed suture was characteristic of forest-park populations and the suburban population (41.2 and 45.5%, respectively).

One feature of the diet of yearlings during the initial period of their terrestrial life in the residential area (multi- and low-story buildings) is the dominance of calciphilous invertebrates such as mollusks; their proportion is 18.0% in the food spectrum of the animals and 19.0% versus 12.5 and 2.0% in forest and forest-park habitats, respectively [2].

An analysis of the relationship of the mineral composition of water and features of the content of the gastrointestinal tract of newt yearlings with the degree of sagittal suture occlusion in the urbanization gradient

(Table 2, Fig. 2) revealed a direct positive correlation ($r = 0.985$, $p = 0.015$) between the occurrence of animals with fully occluded suture and water environment mineralization. The positive relationship of the degree of skull suture ossification with the occurrence of mollusks in the newt gastrointestinal tract, as well as with the water temperature and pH, was recorded as a trend (Table 2). Despite significant ($F(3, 68) = 11.046$, $p = 0.00001$) differences in the body length of yearlings from different zones (Table 1), there was no significant relationship between the absolute body length of yearlings and ossification.

It is known that the most important factor that influences the ossification of the skeleton of fish and amphibian larvae is the level of content of mineral substances in water. It is established that the accumulation of calcium in juvenile fish is more intensive in water with its high concentration [12], while water acidification leads to morphofunctional adaptive changes in the permeability of the gill epithelium, as well as to a decrease in ionic transport [7]. In addition, the direct absorption of calcium (through gills and skin) exceeds its consumption with food both by fish [18] and amphibians [15]. Thus, the larvae of bullfrog *Lithobates catesbeianus* (Shaw, 1802) consume only 5% of calcium entering with food, while the rest of it enters through their gills (70%) and skin (25%). In metamorphosed amphibians, calcium enters only through their skin and gastrointestinal tract [32]. Relatively high temperatures also contribute to an increase in the degree of skeleton ossification in amphibian yearlings [23]. As a result, it can be assumed that there is a synergetic interaction between the abovementioned environmental factors, which significantly determines the entry of calcium into the body, as well as the rate of sagittal suture occlusion in animals from natural *Lissotriton vulgaris* populations.

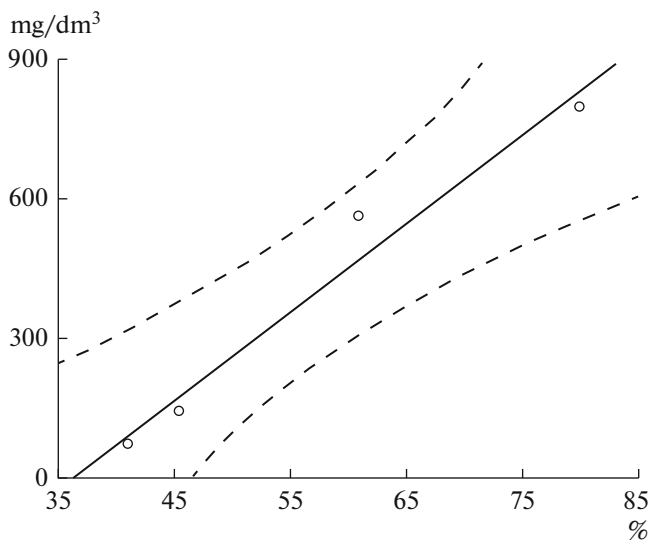


Fig. 2. Relationship between the proportion of individuals with a closed suture, % (x axis), and mineralization of spawning water bodies.

CONCLUSIONS

It was established that the state of the sagittal suture of the skull cap in *L. vulgaris* yearlings can be represented by three well-differentiated variants by the time of metamorphosis completion. An acceleration of *L. vulgaris* yearlings is observed under the conditions of the residential part of the city (multistory and low-rise buildings), which is expressed in the early occlusion of the sagittal suture. Among the possible causes of differences being observed, the main factor is the direct influence of increased mineralization of surface waters and the secondary role is played by factors such as an increase in the proportion of calciphilous organisms (shell gastropods) in the diet of *L. vulgaris* yearlings and an increase in the temperature and pH in the water bodies of the residential part of the city.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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