Conference Paper

Skeletal Anomalies of Juvenile Smooth Newts - *Lissotriton vulgaris*

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Abstract

An article contains first data on internal skeletal anomalies of smooth newt juveniles. The material was collected on the territory of urban agglomeration of Yekaterinburg. We found 16 variants of anomalies. An increase of general frequency of deviations along urbanization gradient were mentioned. The differences and specificity of the populations spectra are related with the profiling of the juveniles definitive morphology by environmental specifics.

Keywords: smooth newt, skeleton, anomaly

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1. Introduction

Amphibians are good indicators of ecological changes because of their two-phase lifecycle, complicated development and moderate ability of migration. As long as their skin is semipermeable, various water-soluble substances, including pollutants, can get easily into their organism. Consequently, the characteristic of species whose embryogenesis and larval development occurs outside the mother's body has high dependence of ontogegesis on the conditions of habitats.

Newts have high sensitivity to changes in the environment and a considerable level of tolerance to adverse conditions, which makes them an important indicator for the condition of the environment. In this article, the influence of environmental geochemistry on the development of caudate amphibians is considered. The aim of the article is to explore the skeletal anomalies of juvenile smooth newts (*Lissotriton vulgaris* Linnaeus, 1758) following the gradient of urbanisation.

Tasks:

1. Learn the alizarin-alcian method of staining skeletons.

2. Explore the skeletal morphology of yearlings from populations of areas with different urbanization levels.
3. Compose spectrums of deviant forms for the explored samples.
4. Make a comparative analysis of identified spectrums.

2. Methods

The material was collected in the Yekaterinburg conurbation and in the countryside in 2011-2015. The animals were caught manually soon after the completion of metamorphosis. The total amount of samples was 72 juvenile *L. vulgaris*: 18 individuals from zone II, 15 from zone III, 17 from zone IV and 22 from the control pool. During the exploration, the overall skeleton structure of the smooth newt was learnt. A classification of skeletal anomalies was compiled based on the available methodical elaborations and data received during the survey of collected material. Hydrochemical indicators were identified in the physical and chemical analysis laboratory of Ural State Mining University. The analysis of the content of the gastrointestinal tract was conducted by Dmitry Berzin. The skeletons were studied via the alizarin-alcian method of staining skeletons [1].

3. Results

The basic variants of skeletal anomalies are represented in table 1. The stages of median seam overgrowth were studied. After the analysis of the interconnection between water chemical cosist and the content of the gastrointestinal tract of juvenile newts with stages of median seam overgrowth was made in urbanization gradient (fig. 1), a direct positive correlation between the occurrence of animals with completely overgrown seams and water mineralization was noted (R=0,99, p=0,0003). A similar tend was noted for the occurrence of shellfish in newts' gastrointestinal tracts and several other environmental parameters (temperature and pH). In this way, one can assume the synergies of the effects of the above factors on seam overgrowth speed.

Spectra composed for each population have specificity and similarity (fig. 2). So the high occurrence of rib anomalies is noted, particularly bifurcation, for all the samples. Equally, in the urbanized area the frequency of this variant changes by 50 to 82%: in the countryside population, it changes from 35 to 45.45%. It should be noted that in the countryside population, the biggest number of unique anomalies were found (anomaly

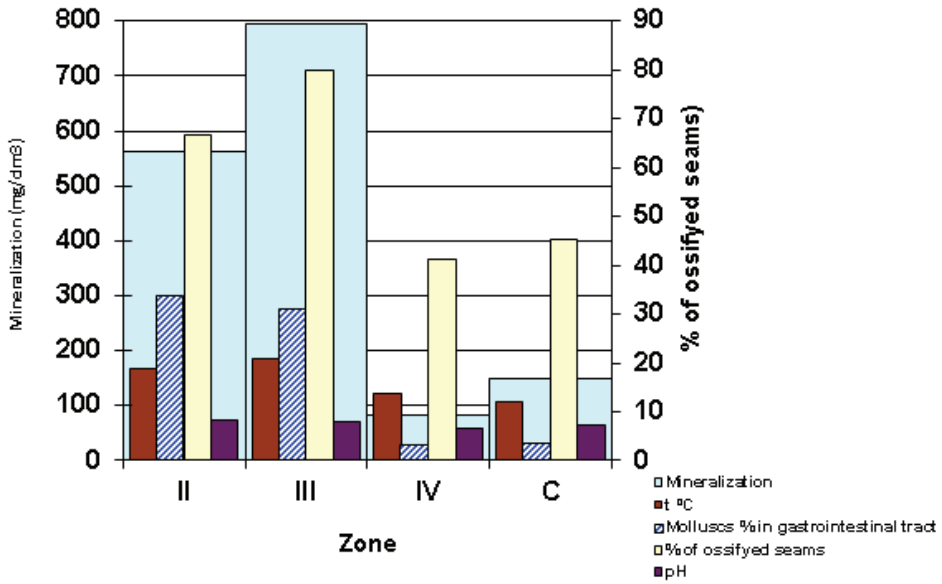

Figure 1: The changing biotic and abiotic characteristics of explored populations and habitats along an urbanization gradient.

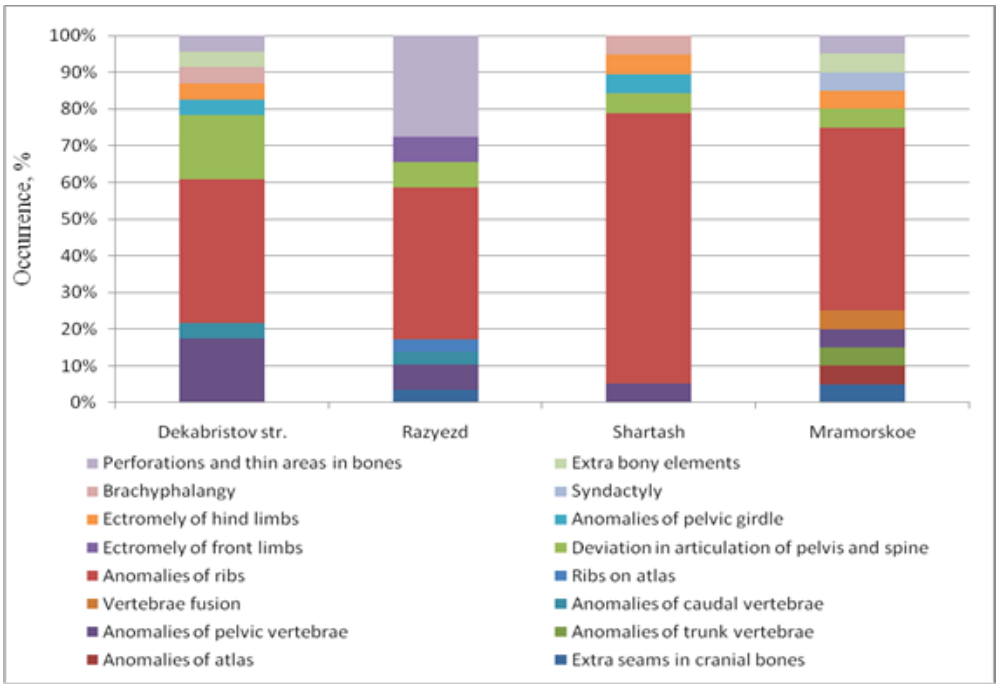

Figure 2: Spectra of skeletal anomalies of juvenile smooth newts along an urbanization gradient.

of atlas, anomalies of trunk vertebrae, fusion of vertebrae and aschistodactylia). The number of axial anomaly variants in the countryside is much higher, while in the urbanized areas there are more peripheral sceleton deviations. Maybe this is the result of regeneration after injuries from more predators. Extra seams in cranial bones are found in zone III and the control area. Perforated bones with a honeycomb structure are most noted in zone III (Shartash) with maximal mineralization. The occurrence of

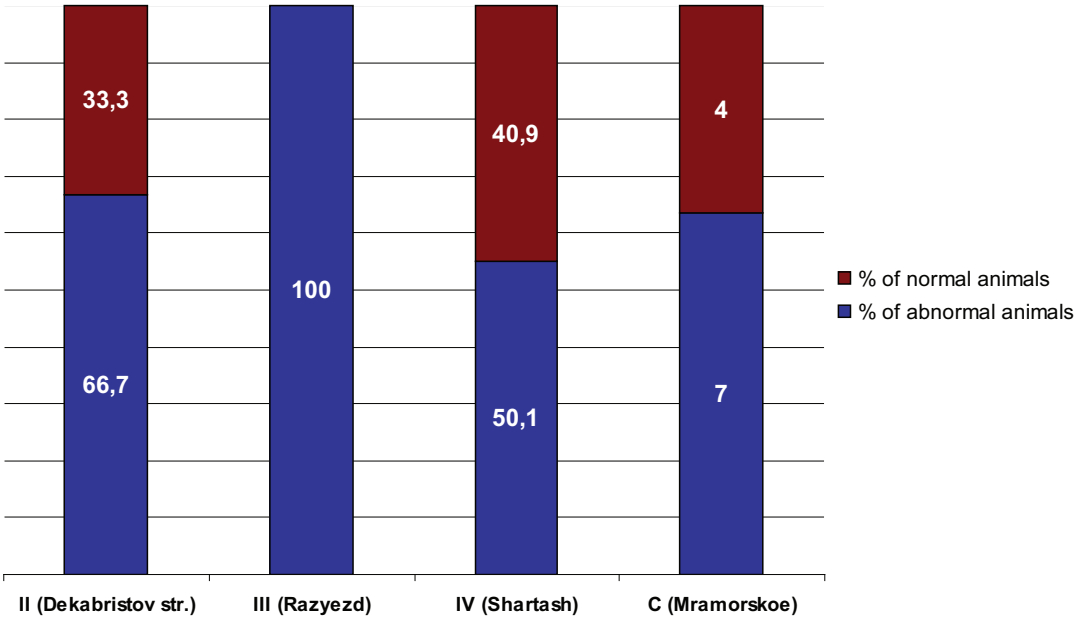

Figure 3: Distances between compared spectra (Euclidean distances).

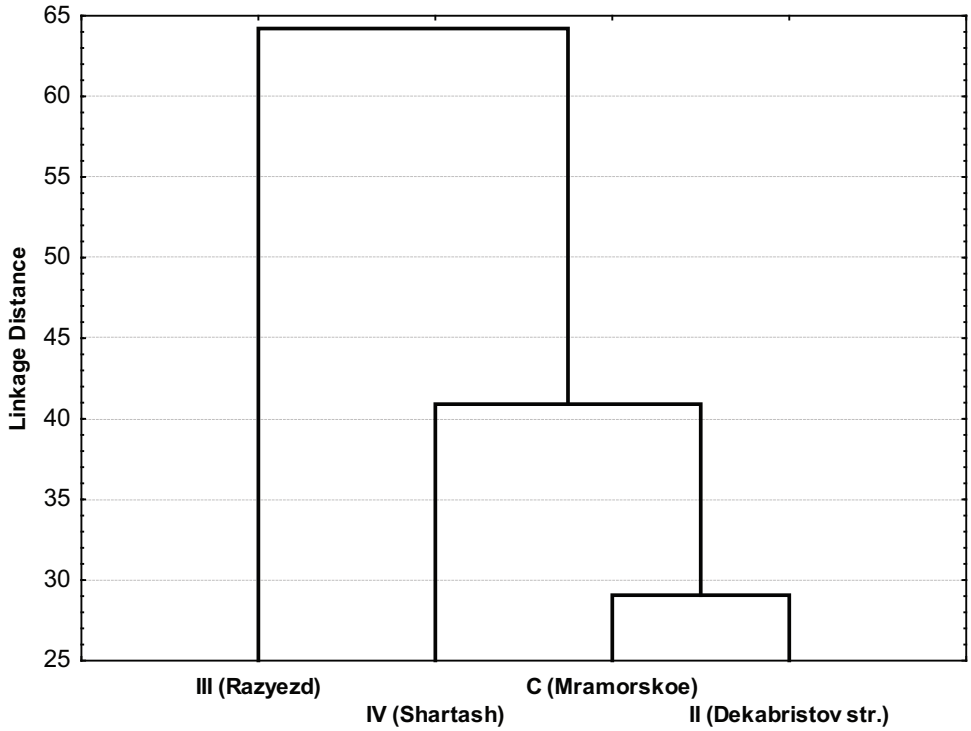

Figure 4: Percentage ratio of abnormal and normal animals.

this deviation decreases to zero in the habitat with the decrease of mineralization (in Shartash 7, where mineralization is below 100 mg/l). Maybe this formation of the bone structure is caused by high mineral substance content in the habitat. The frequency of anomalies of the pelvic vertebrae and the violation of the articulation of the pelvis

Table 1: Types of anomalies and their occurrence.

Type of anomaly / Habitat	II (Dekabristov str.)	III (Razyezd)	IV (Shartash)	C (Mramorskoe)
Extra seams in cranial bones	0	6.67	0	4.55
Anomalies of atlas	0	0	0	4.55
Anomalies of trunk vertebrae	0	0	0	4.55
Anomalies of pelvic vertebrae	22.22	13.33	5.88	4.55
Anomalies of caudal vertebrae	5.56	6.67	0	0
Vertebrae fusion	0	0	0	4.55
Ribs on atlas	0	6.67	0	0
Anomalies of ribs	50	80	82.35	45.45
Deviation in articulation of pelvis and spine	22.22	13.33	5.88	4.55
Ectromely of front limbs	0	13.33	0	0
Anomalies of pelvic girdle	5.56	0	5.88	0
Ectromely of hind limbs	5.56	0	5.88	4.55
Syndactyly	0	0	0	4.55
Brachyphalangy	5.56	0	5.88	0
Extra bony elements	5.56	0	0	4.55
Perforations and thin areas in bones	5.56	53.33	0	4.55

Table 2: Degrees of spectra overlap according to the Morishita index.

	II (Dekabristov str.)	III (Razyezd)	IV (Shartash)	C (Mramorskoe)
II (Dekabristov str.)	\\\\\\\\\\\\\\\\\\			
III (Razyezd)	72.52	\\\\\\\\\\\\\\\\		
IV (Shartash)	84.25	79.98	\\\\\\\\\\\\\\\\	
C (Mramorskoe)	85.79	66.16	82.87	\\\\\\\\\\\\\\\\

and spine increases as urbanization does, from 4.5 to 22,2% in zone II. Perhaps several deviant forms are able to survive in urbanized areas, which have a lower number of predators. Anomalies of the caudal vertebrae were found in zones II and III. It is possible that the appearance of forms like these is connected with an increase of myco infections. The boosting of fungal lesions is caused by the alkaline condition of the habitats in these zones. Ribs on the atlas were noted in one individual in the population of zone III. This phenomenon might be connected with genetic changes, not habitat. Among anomalies of the peripheral skeleton, the most common one is ectromelia, which reached its highest frequency in the sample from the low-rise building zone (Razyezd). Ectromelia might appear as a result of injury. Anomalies of the pelvic girdle (in zones II and IV) were only noted in urbanized areas, where brahifalangiya was noted. Extra bony elements were found in the populations of zones II and III.

The cluster analysis of identified spectrums (fig. 3) shows that the sample from Razyed is much more distanced from all the others. This is confirmed by the fact that zone III has the smallest overlap with the other populations (table 2). One of the reasons for this is the increased mineralization of the surface water. The sample from Shartash made the 2^{nd} level cluster. The low distancing of Mramorskoye and Decabristov was noted, as well as the high overlap of these zones according to the Morishita index. Perhaps this is connected with the similar anamolies of abnormal animals in these populations.

According to the diagram (fig. 4), the highest percentage of abnormal animals was noted in zone II and the least one in the control area. Differences in calcium content in the studied zones should be noted. The highest concentration of this element is observed in zones II and III. It can be assumed that the most common anomaly of abnormal animals, the overgrowth of the median seam on the skull, is connected with the level of mineralization of the surface water and other factors considered in Fig. 1.

4. Conclusion

1. 16 variants of anomalies were found in the skeletons of smooth newts: 9 in the axial skeleton, 6 in the peripheral skeleton and one in different parts.

2. The acceleration of the intergrowth of interparietal sutures was mentioned in juvenile habitats of the residential part of the city (zones II and III). Possible reasons for these phenomenon are the high mineralization of the surface water and the increase in the percentage of calciphilic organisms (Mollusca) in the food of juvenile *L. vulgaris*.

3. The analysis of changes in the deviant form spectra under effect of urbanization shows an increase of the general frequency of deviations along the gradients of anthropogenic habitat transformation, anomaly diversity, reduction in the variants of axial skeleton anomalies and the increase of peripheral ones.

4. 5 unique skeletal deviations not found in the forest population were noted in urbanized areas, while 3 not found in urbanized areas were found in the forest population.

5. The mentioned differences and specificity of the explored populations are related to the "environmental sieve" effect, or the profiling of the definitive morphology of a new generation through a complex of conditions in a certain habitat.

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