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GENERAL BIOLOGY

The Role of Trematode Infestation in the Specifics of Skeleton Morphogenesis of *Rana arvalis* Nilsson, 1842

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Morphological variations in amphibian populations have been described repeatedly. Various authors assumed that morphological deviations are induced by external environmental conditions [1], virus infections [2], abnormal regeneration [3], mutations [4], radioactive background [5], and other environmental pollutions [6]. The cases of mass abnormalities are always of special interest. In junior Nearctic amphibians, the presence of cysts in the areas of active morphogenesis is known to induce polymelia [7], which facilitates the consumption of the trematode-infested specimens by the definitive host, a bird or reptile. Russian publications lack any information on the dependence of skeletal morphogenesis on cercarioses in natural amphibian populations.

This study has been carried out on young-of-the-year *R. arvalis* from the populations inhabiting the urban area of the city of Yekaterinburg. Within the city limits, we distinguished four zones with amphibian habitats: multistory buildings (II), low-rise buildings (III), the forest–park zone (IV), and a plot located 23 km away from Yekaterinburg serving as the control zone (C). The validity of this classification has been confirmed by the results of long-term hydrochemical analysis. The collected material was clarified using Dawson's method [8] before examining under a binocular microscope to detect skeletal deviations and parasite (trematode) cysts, which became clearly distinguishable after treatment (Fig. 1). The blood of 988 junior frogs was studied using the standard hematological techniques. A blood smear was prepared for each animal, and, after Romanowsky–Giemsa staining, the percentages of different types of leukocytes was determined using the unified method for morphological analysis of blood elements [9]. Mass polymelia (multiple limbs) in Palearctic [10] and Nearctic [11] amphibians and the causes of this phenomenon are widely discussed in foreign literature. Trematodes have been unambiguously demonstrated to cause polymelia due to encystations of cercaria in amphibian larvae [7]. Various groups of trematodes with different life cycles are related to amphibians, which serve as intermediate or definitive hosts [12]. Frogs, newts, and water birds (ducks, gulls, and others) may be definitive hosts of trematodes. Monogenetic trematodes (Polystoma) are frog parasites; they have a double cycle: "normal" adults live in the urinary bladder, whereas "summer"

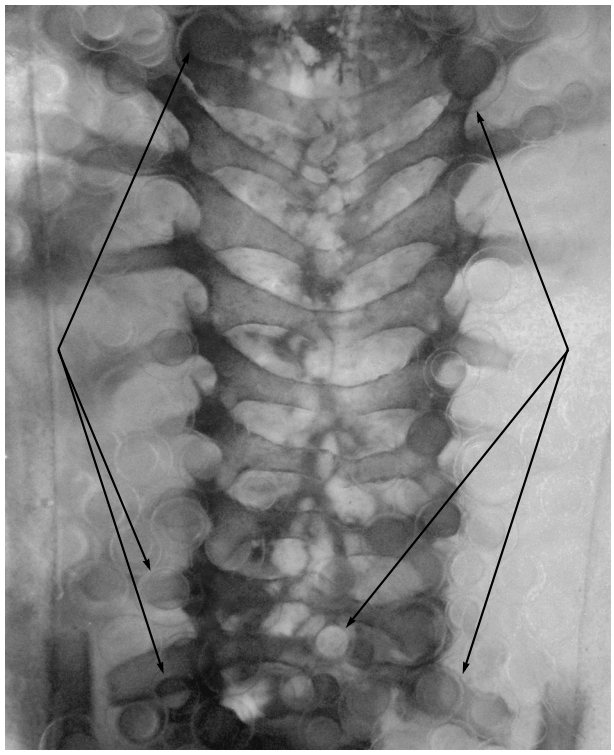

Fig. 1. Abnormal vertebrae (disruption and asymmetry) in the neighborhood of trematode cysts in young-of-the-year *R. arvalis*.

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Table 1. The spectrum and frequency of skeletal abnormalities in junior frogs (%)

Abnormality	II	III	IV	C
Brachycephaly	0	1	0	0
Disruption of vertebral body	21.6	22	23.3	8.5
Asymmetry of vertebra	12.9	20	20.8	5.1
Asymmetry of vertebral processes	2.6	2	0	0
Fragmentation of vertebra	0	0	3.33	0
Fusion of vertebrae	0	0	0.83	0
Deviations of urostyle structure	0	6	2.5	7.6
Incomplete ossification of vertebra	0	0	0.83	0
Ectromelia	0	1	0	0.85
Ectrodactylia	0.86	1	0	0
Oligodactyly	0	0	0.83	0
Thickening of foot phalange	0	0	0	0.85
Asymmetry of feet	0	1	0	0
Thickening of hand phalange	0	0	0	0.85
Deformation of limbs	0	2	0.83	0

Note: here and in the text: (II) multistory buildings, (III) low-rise buildings, (IV) the forest–park zone, (C) suburbs.

adults, in the gills of tadpoles. Both forms give rise to larvae.

Studying the definitive skeleton of young-of-the-
1 year *R. arvalis* demonstrated that the most diverse skeletal abnormalities were in the low-rise building and forest–park zones of the city (table).

In general, the highest incidence of skeletal abnormalities was found in the population from the forest–park zone (IV), 38.3%. Among these, a high proportion of vertebral and urostyle abnormalities was determined (49.09 and 2.5%, respectively). The high incidence of frogs infested with trematode cysts in the forest–park and suburban zones illustrates the extensiveness of cercariosis in them (Fig. 2). The identified cysts belonged to the bigenetic trematode *Holostephanus volgensis*; they were usually located under skin and in some other soft tissues of the host. This parasite uses an amphibian as the secondary intermediate host, whereas fish-eating birds are definitive hosts. In *R. arvalis*, the cysts are located near the 1
cloaca at the rear of the body and in the mesenchyme of the abdominal wall.

The incidence of skeletal abnormalities coincided with the percentage of eosinophils, which serve as marker of parasitic infestations (Fig. 3). We have found

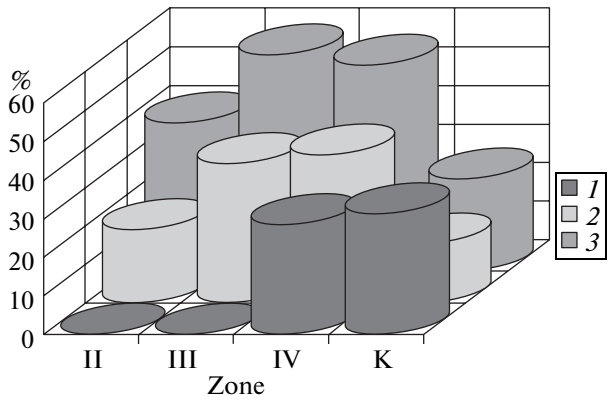

Fig. 2. Frequencies of (*1*) skeletal abnormalities, (*2*) young-of-the-year frogs with skeletal abnormalities, and (*3*) infested individuals in the gradient of urbanization.

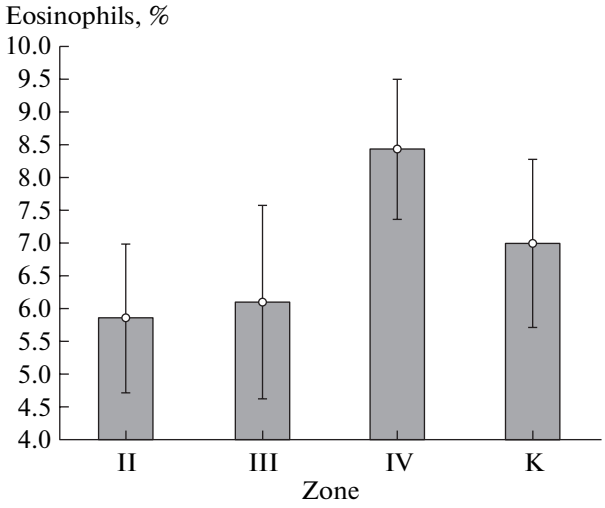

Fig. 3. The proportion of eosinophils in the blood of young-of-the-year *R. alvaris* in the gradient of urbanization.

out an increase in the proportion of eosinophils in the populations of the forest–park zone and in the control population, which is related to permanent invasion and more characteristic of natural amphibian populations than of urban ones [13]; this is expressed in a
2 higher eosinophil percentage in the peripheral blood. A significant level of eosinophils in the forest–park and suburban populations is indicative of the degree of trematode infestation (Fig. 4). The peaks of trematode infestation in specific populations (and accordingly, an increase in the proportion of eosinophils) seem to testify to a higher incidence of individuals of a new generation with deviant forms of the axial skeleton.

It has been demonstrated that a mechanical obstacle, namely the presence of cysts in areas of active morphogenesis, leads to disturbances in these areas and finally results in the formation of deviant forms of the urostyle and other elements of the skeleton [7]. As exemplified by polygenetic trematodes, polymelia facilitates the consumption of infested individuals by the definitive host, a bird or reptile. In the case we have described, infestation also makes the animals more accessible for predators, but, in addition, it often leads to their death, though external abnormalities are not observed as a rule. Trematode infestation was not characteristic of the urban population, but it was found only in the forest–park and control zones. The contribution of parasitic infestation into the formation of the deviant skeletal forms was 69.2 and 29.2% in the forest–park and forest populations, respectively. Hence, despite similar infestation rates of the forest and forest–park populations (31.2 and 28.3%), the ontogeny stability was 2.5 lower in the latter case, which is probably a result of the combined effect of pollutants and encysted metacercariae.

Thus, we have demonstrated that, under Palearctic conditions, infestation with trematode cysts has an
1 effect on the skeletal morphogenesis in *R. arvalis*.

SPELL: 1. arvalis, 2. eosinophil

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REFERENCES

1. Voitkevich, A.A., *Bull. Biol. France Belg.*, 1961, vol. 95, pp. 569–600.
2. Fisher, J.-L., *Bull. Mens Soc. Linn.*, Lyon, 1976, vol. 45, no. 1, pp. 43–46.
3. Baranovskii, A.E. and Kudokotsev, V.P., *Ratsional'noe ispol'zovanie okhrana, vosproizvodstvo biologicheskikh resursov i ekologicheskoe vospitanie* (Rational Use, Protection, and Reproduction of Biological Resources and Ecological Education), Zaporozh'e, 1988, vol. 209.
4. Neff, A.W., Briggs, F., and Chung, H.-M., *J. Exp. Zool.,* 1987, vol. 241, no. 3, pp. 309–316.
5. Okawa, H., *Jap. J. Herpetol*, 1994, vol. 15, no. 4, pp. 147–148.
6. Gendron, A., Hontela, A., Bishop, C.A., and Fortin, R., in *II World Congress of Herpetology,* Adelaide, 1994, pp. 96–97.
7. Hecker, L. and Sessions, S.K., *Bios*, 2001, vol. 72.
8. Dawson, A.B., *Stain. Technol*, 1926, vol. 1, pp. 123–125.
9. Kost, E.A., *Spravochnik po klinicheskim i laboratornym metodam issledovaniya* (Handbook of Clinical and Laboratory Investigation Methods), Moscow: Meditsina, 1975.
10. Voitkevich, A.A., *Priroda*, 1955, no. 12, p. 100.
11. Hebard, W.B. and Brunson, R.B., *Copeia*, 1963, pp. 570–572.
12. Beer, S.A. and Voronin, M.V., *Tserkariozy v urbanizirovannykh ekosistemakh* (Cercarioses in Urbanized Ecosystems), Moscow: Nauka, 2007.
13. Lebedinskii, A.A. and Ryzhkova, N.A., in *Ekologiya i okhrana okruzhayushchei sredy* (Ecology and Environment Protection), Ryazan', 1994, pp. 95–96.