The effect of water extract from bentonite on embryonic and larval development of the green toad (bufoviridis) with different phenotypes

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Abstract. Ontogenesis is a complex process of morphophysiological transformations. Embryonic and postembryonic development is continuously affected by both endogenous and environmental factors, which lead to variability in morphometric parameters. The research was conducted in the laboratory of ecological embryology of the Federal State Budgetary Educational Institution of Higher Education of the North Osetian Federal University (Vladikavkaz). The results showed that under the influence of bentonite extract, the morphological parameters of green toad larvae with a brown phenotype are more variable than in larvae with an olive green phenotype in both the control and experimental groups. Thus, the embryos and larvae of the green toad with the olive green phenotype are more stable than the larvae that had the brown phenotype, both in natural circumstances and under the influence of bentonite clay extracts.

1 Introduction

The emergence of the species was accompanied by the adaptation of organisms to certain environmental conditions, the appearance of useful mutations, fixed by natural selection. Each species is characterized by a certain range of hereditarily fixed variability, that is, the scope of physiological adaptation to environmental changes. The course of evolution creates its own relations to the external environment, its own range of adaptations to changing conditions. As a result of evolution, the relationships of organisms in the environment changed, the patterns of heredity and variability changed, and this complex process is reflected in the diversity of living nature, the peculiarities of variability and heredity in organisms.

Ontogenesis – is a complex process of morphological, physiological and biochemical transformations that an organism undergoes, during which a natural change in phenotypes specific to the given species is manifested. During embryonic development, the connection of the organism with the external environment is not interrupted, and there is a constant exchange of things. In the developing organism, destructive and structural-educational processes are continuously occurring at different levels of the organization. Thus, embryonic and postembryonic morphogenesis and its regulation are in continuous interaction with the environment. Environmental factors, along with internal (endogenous) factors, act as regulators of embryonic processes. Complex interactions between internal and external causes of embryonic development lead to spatio-temporal variability in the morphology of individuals in the population [1, 2].

The variability of embryos and larvae is manifested at all stages of embryogenesis and postembryogenesis. However, the variability of embryonic structures is significantly higher at the initial stages of development and decreases by its completion.

Ecological factors of the environment during embryonic and postembryonic development can cause morphological variability of individuals within the normal response range or lead to disruption of the normal course of development, causing abnormalities in development up to death. In relation to all the necessary conditions for development, we can distinguish a minimum, maximum, and optimum, the value of which may be different for different embryos [1, 2, 3].

One of the environmental factors in the research area is the presence of bentonites which form deposits on the territory of North Ossetia-Alania. Bentonite permeates the rivers, which during spring floods carry part of bentonite clays to the habitats and development of amphibians. Therefore, a single bentonite extract can cause morphological changes in the larvae of the green toad. Early studies have shown that bentonite extract affects the morphometric parameters of amphibian larvae in different directions. Thus, short-term exposure to bentonite extracts has a positive effect on the body parameters of amphibian larvae, while longer exposure has a negative effect on the morphometric parameters of larvae [4, 5, 6].

2 Research material and methods

The objects of the study were embryos and larvae of the green toad, living in a reservoir in the surroundings of Vladikavkaz. At the zygote stage the embryos were divided into two phenotypic groups: olive green (control, experimental) and brown (control, experimental). In the experimental group, the embryos developed in water extract from bentonite, and in the control group, the embryos developed in settled tap water. Water extract from bentonite was prepared as follows: 1 kg of crushed bentonite clay was placed in a glass vessel, then 3,5 liters of standing tap water were added there. The extract for the experiment was used no earlier than 3 days after the clay was placed in the settled tap water.

After leaving the egg shells, the larvae was fixed in a 10% formalin solution. Masonry studies, observation of developmental events and structural features of embryonic and larval development, as well as measurement of larvae were performed using a binocular microscope. The data was analyzed using the Stadia statistical program.

3 Results and discussion

In amphibian larvae that had a brown phenotype, the frequency distribution of occurrence of parameters in the control and experimental group differed from the statistically normal by the tail height at the root (K-S= 0.2108, $P<0.01$; K-S= 0.2008, P) and the body length (K-P=0.143, $P>0.05$; K-P=0.132, $P>0.05$), torso length (K.-S.= 0.1468, $P>0.05$; K.-S.= 0.1493, $P>0.05$) and tail length (K.-S.=0.1225, $P>0.05$; K.-S.=0.1291, $P>0.05$) distribution characteristics differ from normal.

In the comparative analysis of the average values of the parametric Student's t-test and the nonparametric Wilcoxon test, the results showed that the linear dimensions of the larvae in the experimental group significantly become smaller compared with the control group (body length $t= 7.8$, $P<0.001$; the tail length $t=8.5$, $P<0.001$; torso length $t=8.2$, $P<0.001$; the tail height at the root $W=13$, $P<0.001$) (Fig.1).

By the parametric Fisher test and the non-parametric Ansari-Bradley test the analysis of variance found differences in the distributions of the body lengths and the tail length, as for the rest of the studied parameter, no differences were found (body length, F=4.94, P<0.001; tail length F=7.7, P<0.001; torso length F=1.4, P>0.05; the tail height at the root Za=45, P>0,05).

Both in the control and in the experimental group, according to the coefficient of variability, all compared the morphometric parameters are variable to an average degree (Fig. 3).

As a result of the research, 23% of larvae died in the experimental, and 16% of larvae in the control group (Fig. 2).

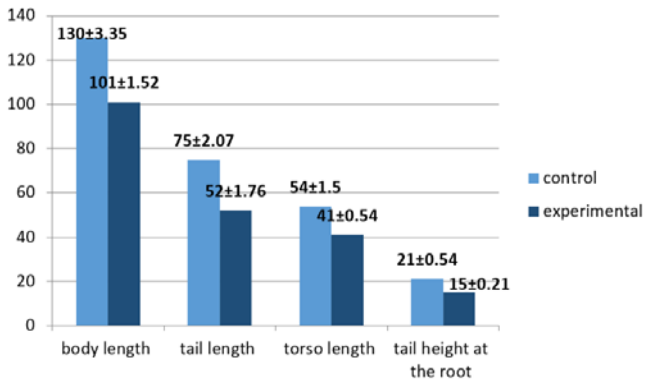

Fig. 1. Average values in the control and experimental group

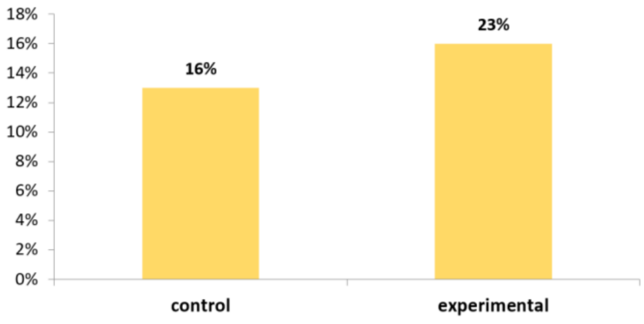

Fig. 2. Larval mortality in control and experimental group

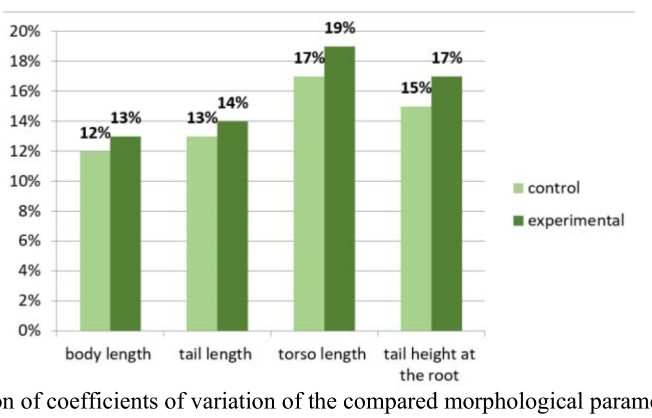

Fig. 3. Comparison of coefficients of variation of the compared morphological parameters

The distribution of frequencies of occurrence of the parameters in the larvae of the green toad, which had *the olive green phenotype,* as in the control and experimental groups do not differ from the normal distribution of all compared morphometric parameters of body length (K.-S.=0.122, P>0.05; K.-S.=0.1124, P>0.05), the tail length (K.-S.=0.156, P>0.05; K.-S.=0.0942, P>0.05), torso length (K.-S.=0.158, P<0.01; K.-S.=0.144, P>0.05), the tail height at the root (K.-S.=0.154, P>0.05; K-P=0.145, P>0.05).

When comparing average values by the Student t-test results did not show differences between the analyzed parameters in the control and experimental groups(body length t=0,9, P>0.05; the tail length t=1.1, P>0.05; torso length t=0.8, P>0.05; the tail height at the root of t= 0.7, P>0.05) (figure 4).

No differences were found between the distributions of morphometric values by the variance analysis by the Fisher test for the compared features both in the control and in the experimental group (body length F=0.92, P>0.05; tail length F=0.9, P>0.05; torso length F=0.82, P>0.05; tail height at the root F=0.9, P>0.05).

According to the coefficient of variability, both in the control and in the experimental group, all the compared parameters are slightly variable (Fig. 6).

After exposure to bentonite extract 13.3% of tadpoles died, and in the control group, 10% of larvae died as a result of the experiment (Fig. 5).

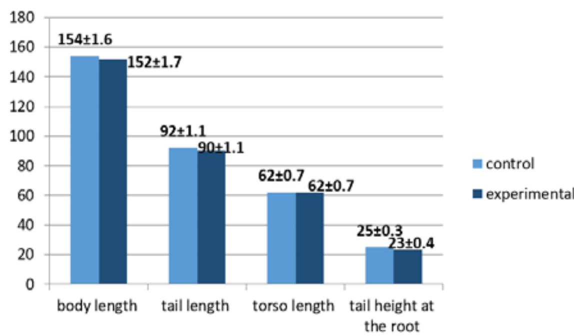

Fig. 4. Average values in the control and experimental group

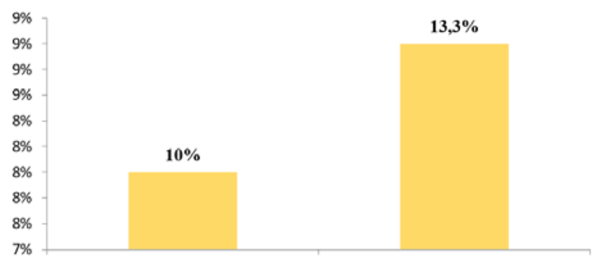

Fig. 5. Larval mortality in control and experimental group

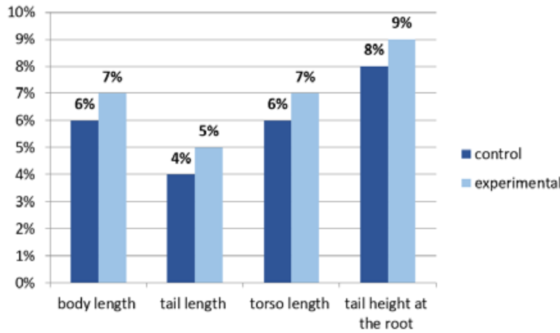

Fig. 6. Comparison of coefficients of variation of the compared morphological parameters

4 Conclusion

The results of the experiment showed that the effect of water extract from bentonite leads to destabilization of the analyzed parameters in larvae with a brown phenotype compared to larvae that, developed in the control group. And when exposed to water extract from bentonite on larvae with an olive green phenotype, the studied parameters remained stable in comparison with the control larvae. According to the coefficient of variation in embryos with a brown phenotype, the compared parameters are moderately variable, and in larvae with an olive green phenotype, the analyzed parameters are slightly variable. The mortality rate also shows that most of the deaths in both the control and experimental groups occured to larvae with a brown phenotype. Therefore, green toad embryos and larvae with an olive green phenotype are more stable than larvae that had a brown phenotype, both in natural conditions and under the influence of bentonite clay extracts. That is, the variability of embryos at the zygote stage may be correlated with the variability of embryos and larvae at later stages of development.

The study showed that the established morphometric changes in the linear parameters of the larvae of the green toad can be linked to the variability of zygotes.

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