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Interpopulation Variability in Growth and Puberty Rates in Male Moor Frogs (*Rana arvalis* Nilsson, 1842)

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Abstract—The age distribution, body size, and annual growth rates in amphibians inhabiting three water reservoirs of different hydrochemical compositions have been analyzed to identify the interpopulation characteristics of the growth and puberty rates in the moor frog males. Altogether, 117 pubescent males at the age of 2 to 6 years old were studied. It has been determined that the ambient surface water salinity in the Middle Urals in the springtime is at the level of 150 mg/dm^3. The interannual variations in salinity in the analyzed water reservoirs are related to the changes in the sulphate concentration. It has been noted that a water salinity of approximately 300 mg/dm^3 during the spawning season is not a negative factor limiting growth of the moor frog. The relatively early pubertal maturation of the male moor frog can be observed under close-to-optimal conditions and is followed by the redistribution of the energy cost for the reproductive process advantages, along with a delay in the growth of the amphibians.

Keywords: amphibians, moor frog, growth, annual growth rate, skeletochronology, water salinity, hydrochemistry

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INTRODUCTION

At present, the problem of the growth rates and, consequently, the variability in an indicator such as the body size in amphibians is studied by many reseachers. The age and seasonal characteristics of the growth rates in the amphibians are reported. Thus, it is noted that current-year juveniles in the southern populations, when compared to those in the northern populations, start their hibernation with a large body size (Krivosheev et al., 1960; Loman, 1978; Lyapkov et al., 2008). The summer weather conditions can define the body sizes of the amphibians born in the current year, before hibernation, exerting both direct effects and indirect effects through the larval feeding-stage period (Kutenkov and Mosiyash, 2013). In natural environments, the current-year juvenils of moor frogs with weight reaching 200 mg appear more viable (Schwarz, 1980). The larger specimens at the stage of matamorphosis preserve advantages in body sizes after hibernation (Ishchenko, 1980), while some other situations also can be observed (Ishchenko, 1999).

Examples of 31 and 81 adult species of anurous amphibians frpm Europe and North America, respectively, can show that the animal average body size increases towards the north (Olalla-Tárraga and Rodríguez, 2007). Some authors consider that the thermal balans plays a key role in this process, while others designate the acceleration of pubertal maturation in amphivbians of the southern populations as the major cause (Lyapkov, 2007). In addition, the possibility of using the warm climate advantages may be limited by local conditions such as the low humidity level (Lyapkov et al., 2008).

In addition, it should be noted that some works pay most attention to the impact of factors such as temperature (Zhitnikov, 2012), salinity, and acidity of water (Kuznetsov and Lobachev, 2007); herbicides (Peskova, 2004); fluctuations in chemism of the environment because of urbanization (Vershinin et al., 2006); etc., on the amphibian growth rates. Thus, the reduction in the body length of the moor frog adult females can occur along with the amplification of anthropogenic transformation of the environment; with regard to current-year juveniles, an increase in the body size is recorded (Vershinin et al., 2006).

Data on the hydrochemical indicators for the water reservoir and the characteristics of the growth rates in the amphibians especially in the natural population are insufficiant. However, an experiment with the great pond snail showed that a daily drop in salinity in the range of 1–3% accelerate its growth rates; the further increase in this indicator is followed by a deterioration in its growth (Konstantinov et al., 2007). A similar result produced by the other variables for the factor was obtained during the experiments to study the effect of salinity on the development of the common newt and the representatives of the anurous amphibians at the embryinic and larval stages. The periodical variations in this factor generally have a positive effect on their development. In addition, the clawed frog and the green toad, as opposed to the common newt, reached the best values assigned to the survey items in the halo regime with the range of 2% (Lobachev, 2008). It is also noted that the higher levels of salinity in the water reservoir can have no effect on the development and the growth rates of newts, while it can decrease their survival rates and modify their behavior (Winkler and Forte, 2011).

Therefore, the characteristics of the amphibian growth rates in association with the variabilities in the local reservoir hydrochemical environments remain incompletely understood.

The objective of this work was to compare the age structure, body size, growth rates, and the the age of puberty for male moor frogs inhabiting three water reservoirs of different hydrochemical composition.

MATERIALS AND METHODS

The moor frogs (117 specimens) were caught in the spring—summer period in two habitats in the area of the Middle Urals and in one habitat located in the Southeast Trans-Urals. The first habitat is located in the territory of the Shartash Forest Park (1) (Yekaterinburg). According to the urban landscape typification (Vershinin et al., 2006), the given locality is identified to the forest—park zone in a city. It should be noted that urban forest parks, as the least disturbant landscapes, play a specific role in supporting the communities in a megalopolis. On the one hand, the similar ecosystems represent the natural habitats of animals, while on the other hand they are exposed to a negative complex of factors accompanying urban agglomerations. Therefore, they can help to ascertain the roles of the factors having a negative effect on the organisms. Information on the characteristics of the growth of the moor frog in similar biotopes is unavailable.

The second habitat (2) is located in the environs of the settlement of Verkhniye Sergi, Nizhniye Sergi raion, Sverdlovsk oblast; it is 91 km from Yekaterinburg. The given biotope may be identified to the weakly transformed territories outwards from the large built-up area boundaries.

The third habitat (3) is a lake in the Southeast Trans-Urals (environs of settlement of Stepnoye, Kurgan oblast). The lake in Kurgan oblast is characterized by high-grade overall mineralization (hydrocarbonate-, cloride-, and sulphate ions prevail among the anions; the cation composition includes ions of sodium, calcium, potassium, and magnesium), which is caused by the influence of the underlaying rock bottom (*The lakes...*, 1998). The parameters of the water reservoirs of such a type may be close to that in the territory of the urban agglomeration.

The morphological measures in the trapped animals were taken with the Kraftool digital caliper with a scale interval of 0.1 mm. The animal age was defined by the method of skeletochronology in the modification proposed by A.V. Ledentsov (1990). In order to do that, the cross sections from the middle of the third phalange of the fourth toe of the hind limb, stained with Ehrlich hematoxylin, were made. Despite the simple count of the glue lines of arrested growth visible in the phalange cross sections, the measurements of their diameters were taken. The values for the duameters of the annual layers and the relative annual growth can indicate the adequacy of the technique for defining the lines of the first and second hibernation. The proposed approach can make it possible to define the age of the moor frogs more precisely. The measurements of the dyameters of the annual layers and the toe phalange areas in the amphibians were carried out with Levenhuk ToupView Software. The relative growth rates in the bone tissue (Y_i) for the hibernation-to-hibernationation period were calculated using the formula:

$$Y_i = \frac{D_{1+1} - D_i}{D_i}.$$

Where D is a bone dyameter limited by one glue line and i is the running number of the visible glue line (numbering from the medullary cavity). The most and least widths of the section were measured, since the cross sections had a form approximate to an oval. The average value between these two measurements was called the layer dyameter in all of the subsequent calculations. The section having the least dyameter was used for measurements.

The point annual (2014 and 2015) water samples from the analyzed reservoirs were taken in the spring period corresponding to the spawning season of amphibians under State Standard 51592—2000: Water; General Requirements for Sampling. The range of the chemical indicators was defined according to the objectives of the survey. The chemical and analytical surveys of water samples were carried out with the certified methods by specialists from the accredited laboratory for physical and chemical analyses, Ural State Mountain University.

The statistical data were processed using Statistica 8.0 (StatSoft) applied software. Fisher's angula transformation was used to compare two samples in the frequency distribution of ages. The analysis of the variablility in the body sizes, phalange areas, and the annual groth rates in bony tissue was conducted using the multifactor analysis of variance. The significance level of 5% was chosen for testing the hypotheses about the factor significance.

Table 1. Major hydrochemical indicators of reservoir water (May)

Indicator	Habitat		
	Shartash (1)	Verkhniye Sergi (2)	Stepnoye (3)
	$M \pm m$ lim (min–max)	$M \pm m$ lim (min–max)	$M \pm m$ lim (min–max)
Hydrogen ion exponent, pH units	6.43 ± 0.19 6.24–6.62	7.41 ± 0.025 7.39–7.44	7.69 ± 0.31 7.38–8.0
Salinity, mg/dm^3	2.37.85 ± 88.55 149.3–326.4	153.85 ± 41.05 112.8–194.9	295.45 ± 12.55 282.9–308.0
Permanganate oxidation susceptibility, mg O_2/dm^3	38.0 ± 30.0 8–68.0	18.88 ± 15.12 3.76–34.0	18.40 ± 11.6 6.8–30
Nitrates, mg/dm^3	1.54 ± 0.62 0.93–2.16	2.0 ± 0.1 1.9–2.10	3.6 ± 2.2 1.48–5.88
Chlorides, mg/dm^3	24.35 ± 2.25 22.1–26.6	30.8 ± 20.2 10.6–51.0	55.95 ± 20.55 76.5–35.4
Sulphates mg/dm^3	114.15 ± 55.55 58.6–169.7	25.4 ± 6.6 18.8–32.9	5.11 ± 1.52 3.59–6.63
Sodium, mg/dm^3	10.91 ± 1.49 9.42–12.4	4.76 ± 0.78 3.98–5.54	53.8 ± 7.3 46.5–61.1
Calcium, mg/dm^3	25.1 ± 1.9 23.2–27.0	27.1 ± 7.6 19.5–34.7	29.5 ± 6.4 23.1–35.9
Potassium, mg/dm^3	9.4 ± 1.71 7.69–11.1	1.21 ± 0.25 0.96–1.46	10.1 ± 1.81 8.29–11.9

RESULTS

Hydrochemical Indicators of Water Reservoirs

The major hydrochemical indicators of the analyzed water reservoirs in the period of spawning season in amphibians are shown in Table 1. It was ascertained that the level of salinity in the water reservoirs in the forest-park territory in different years might be over or lower than the values for that in the water reservoirs in the suburbs. The interannual variations in salinity are related to the variances in the sulphate concentration in the water reservoirs. In large cities, the sources of the sulphates attributed to precipitation and transferred along with the dust into the water reservoirs are the nearest highways and vehicles (Nikitskaya and Makarova, 2012). The distinct characteristics of the water reservoirs in the city territory are permanganate oxidability and low pH values for environments.

In locality 3, the level of salinity in springtime is more stable and comprises 300 mg/dm^3, which is 1.9 times lower than the summer values (Vershinin and Baitimirova, 2012). In habitats 1 and 3, the levels of the total water hardness and the concentrations of chloride, sodium, and potassium are similar. Habitat (2), when compared to the other localities, is characterized by a pH value of 7 and low concentrations of sodium and potassium.

Variability in Body Length

One hundred and seventeen of the moor frog pubescent males at the ages of 2 to 6 years old are totally studied. The average lengths of the animals in each age class are shown in Table 2.

The age distribution dynamics is one of the indicators for rates of involving the specimens into reproduction and their deaths; therfore, this parameter was used (Lyapkov et al., 2007).

The specimen age-frequency distributions in the analyzed populations are displayed in Fig. 1. It is shown that the frequency of occurrence of the 3-year-old animals is maximal at the third site; the significant differences with both the first site ($\varphi^*\text{emp} = 5.381$, $p < 0.05$) and the second site ($\varphi^*\text{emp} = 5.629$, $p < 0.05$) are traced. A small percentage of the two-year specimens (15%) among the spawning animals in the third area should be noted. The principal sample portions from the first and second sites are comprised of the 4-year-old and 5-year-old animals. Therefore, the model spawning age at sites 1 and 2 is 4 years, while it is 3 years for habitat 3. Consequently, the moor frog males in habitat 3, when compared to those in habitat 1 and 2, start spawning approximately a year earlier.

A two-factor model (“habitat” and “age”) was used to analyze the variation in the body size of the moor frog pubescent specimens. It was indicated that

Table 2. Moor-frog body lengths and ages

Age	Habitat		
	Shartash (1)	Verkhniye Sergi (2)	Stepnoye (3)
	M ± *m*	*M* ± *m*	*M* ± *m*
2+	–	–	47 ± 1.03 *N* = 8
3+	48.43 ± 1.44 *N* = 5	51.49 ± 0.67 *N* = 7	50.47 ± 0.70 *N* = 30
4+	53.09 ± 0.44 *N* = 9	55.20 ± 0.46 N = 21	52.85 ± 0.76 *N* = 10
5+	56.36 ± 1.20 *N* = 3	58.18 ± 0.90 *N* = 15	52.93 ± 0.78 *N* = 4
6+	58.69 ± 0.43 *N* = 4	59.27 *N* = 1	–

the average male body length is more in habitat 2, when compared to that in habitat 3 (differences are significant) (Fig. 2).

However, the analysis of the interaction between the age and habitat factors and the subsequent paired comparisons in each age class with the post hoc test (LSD test) indicated the absence of significant differences in the male body length between the habitats. At a trend level, the larger body sizes may be indicated in frogs aged 3 years in habitats 2 and 3; in the older age groups, the average values for the body size of frogs in habitat 2 exceeds those in the other regions (Fig. 3). Therefore, the registered interpopulation dofferences in the male average body size are related to the characteristics of the age structure of animals in the analyzed habitats.

Analysis of the Annual Growth Rates in Amphibians

It is known that the allometric relationship between the toe phalange thickness and the body length is indicated in amphibians (Smirina, 1983). The coefficient of the correlation between the body length and the area of the cross section in the narrowest place of the third phalange is 0.67 in the pubescent males of the analyzed populatons.

The results of the phalange-area analysis conducted with dispersion modeling are present in Fig. 4. The absence of significant differences in the phalange area between the analyzed populations is indicated in each age class. In addition, a delay in growth was observed in animals of each population, which might be related to the start of the reproductive season. The measurements of

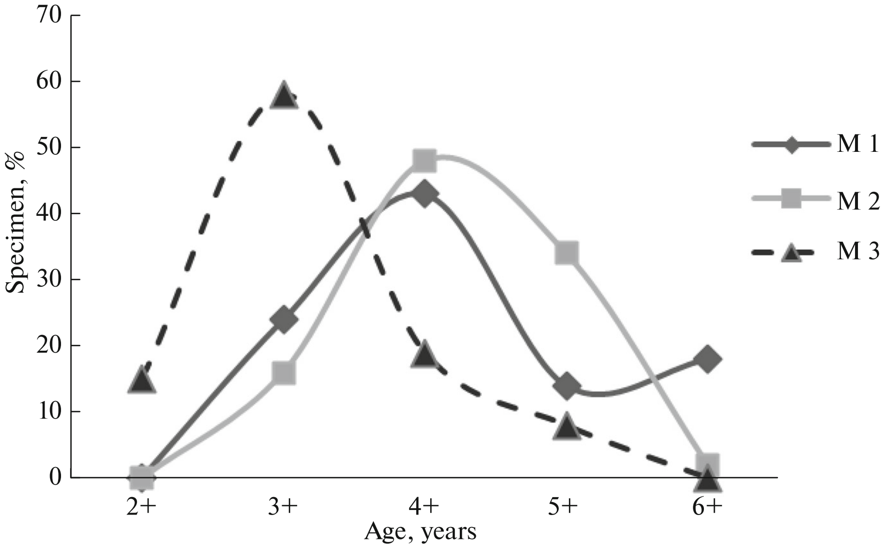

Fig. 1. Age-frequency distribution in male moor frogs in the analyzed populations. M, habitat: (1) Shartash, (2) Verkhniye Sergi, and (3) Stepnoye.

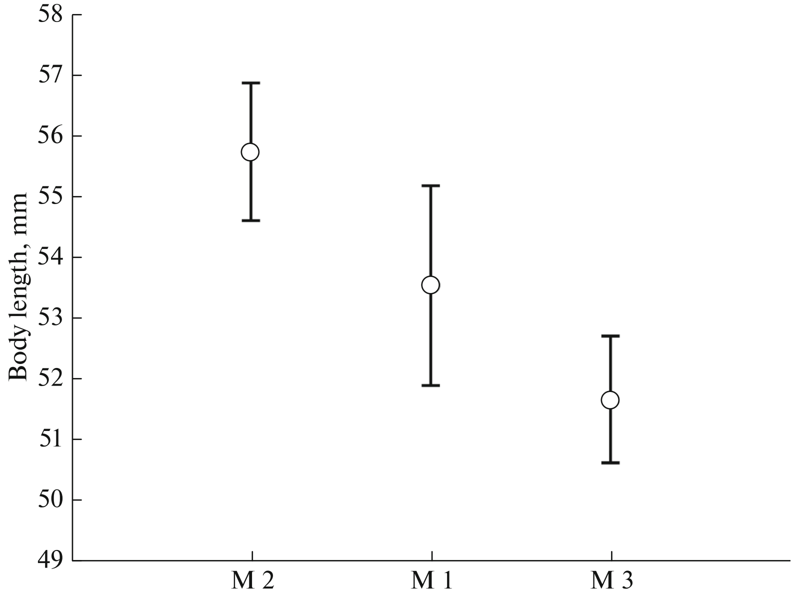

Fig. 2. Moor-frog body length (unweighted average ±0.95 confidence interval) upon the impact of the habitat factor. $F(2, 114) = 13.7$ ($p < 0.001$). M, habitat: (1) Shartash, (2) Verkhniye Sergi, and (3) Stepnoye. Body length, mm

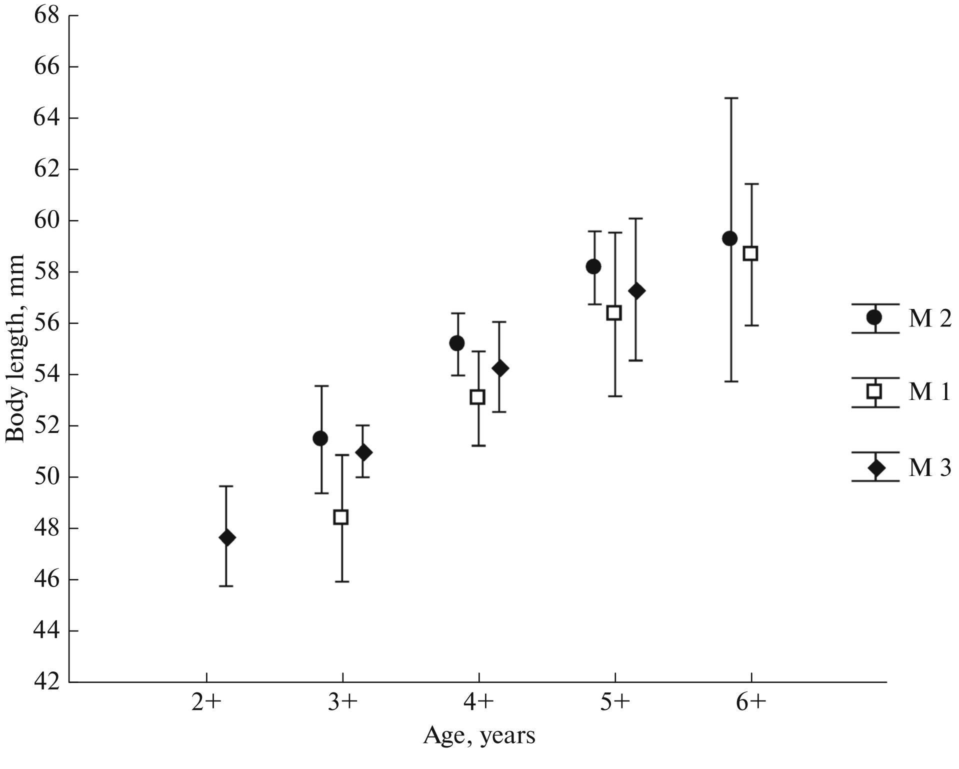

Fig. 3. Moor-frog body length (average ±0.95 confidence interval) at the interaction between the habitat and age factors. $F(5, 105) = 0.2$ ($p <= 0.98$). M, habitat: (1) Shartash, (2) Verkhniye Sergi, and (3) Stepnoye.

the annual growth (Fig. 5) in frogs of the analyzed populations showed that a significant decrease in the growth rates was typical for the animals in the third year of life in habitat 3 (the post hoc test (LSD test), $p < 0.05$); in the other analyzed populations, the active growth continued up to 4–5 years.

Therefore, the amphibian delay of growth occurs along with the pubertal development. It can be observed during the analyses of the annual growth of bone tissue and the total area of the toe phalange cross section. However, the analyzed regular occurrence in the latter case can be evident in order from the level of trends.

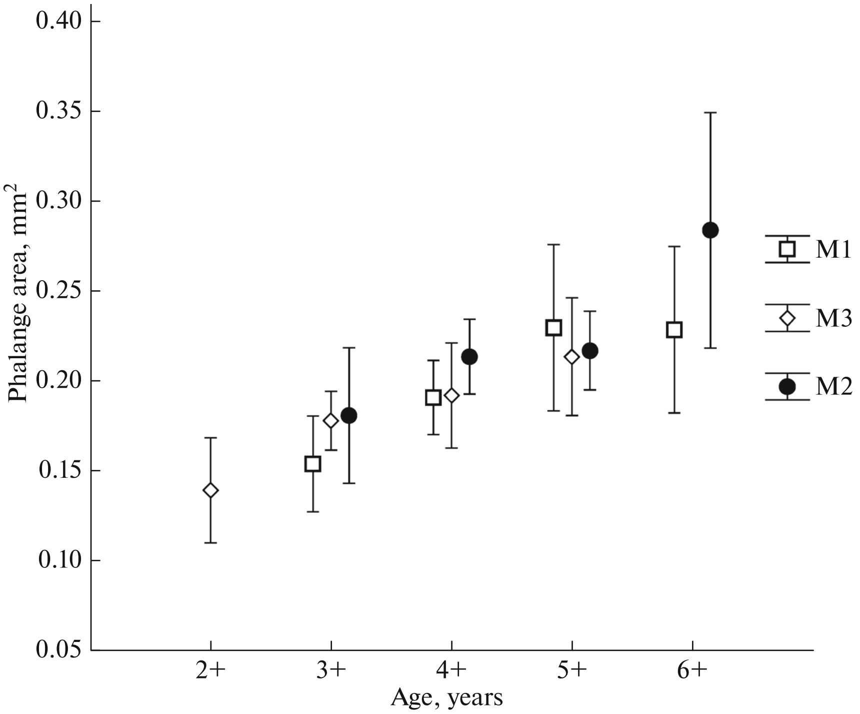

Fig. 4. Phalange cross-section areas in male moor frogs (average ±0.95 confidence interval) at the interaction between the habitat and age factors. $F(5, 105) = 0.72$ ($p = 0.61$). M, habitat: (1) Shartash, (2) Verkhniye Sergi, and (3) Stepnoye.

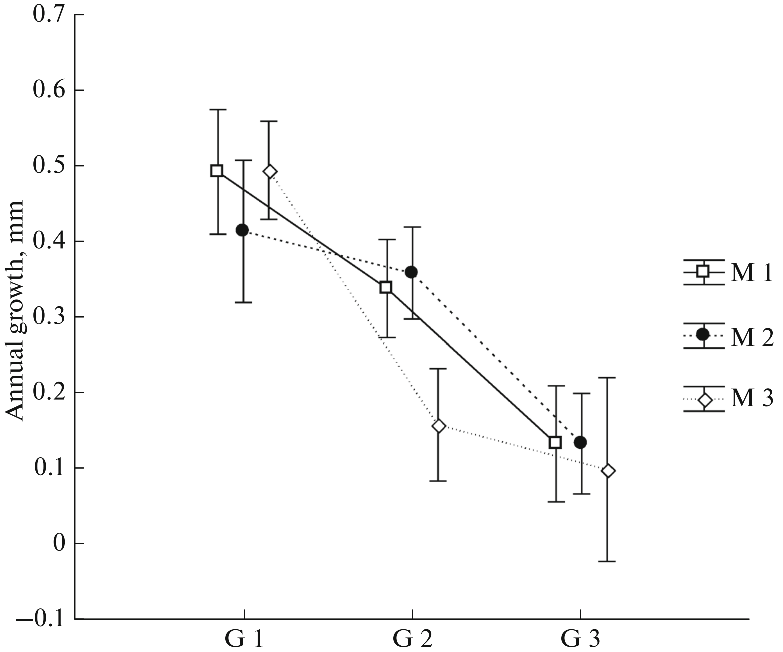

Fig. 5. Annual growths (mm) in moor-frog specimens (average ±0.95 confidence interval) at the interaction between the habitat and age factors. $F(4, 137) = 3.79$ ($p = 0.005$). M, habitat: (1) Shartash, (2) Verkhniye Sergi, and (3) Stepnoye. G, growth: after the first hibernation, (2) after the second hibernation, and (3) after the third hibernation.

DISCUSSION

Within each natural population, there is generally a set of factors so that any observed effect represents a result of interaction of these factors. Thus, the hydrochemical characteristics of the spawning water reservoirs are considered the major factors of the analyzed amphibian habitats within this survey. In addition, the possible geogtaphic variations in the growth rates, which are usually related to climatic differences such as the period duration and the positive average daily air temperature, should not be ignored (Lyapkov et al., 2007). However, the effects of this factor on the linear gtowth rates have not been fully studied yet.

One of the analyzed moor frog habitats (site 3) with natural higher salinity surface waters is located 2° southwards from Yekaterinburg in the Trans-Ural area (Kurgan oblast). This region, when compared to the Middle Ural area, is characteized by higher summer temperatures.

The microclimate-warming trend is one of the well-known urban area effects. As a rule, the temperature in the town is higher than that in the suburbs (Oke, 1973); the variations may reach 10°C (Oke, 1981). Many research works are devoted to the formation of the urban heat island in a metropolitan area (Matveev, 2007; Akimov, 2009; Matveev et al., 2011; Elansky et al., 2012; Gorchakov et al., 2014, Isakov and Shklyaev, 2014). In addition, the reverse situation is possible in arid environments, when the average temperature in the cities with a great deal of the irrigated land (landscaping, parks) may be lower than that in the surround arid regions as in Sacramento, California, United States, for instance (Grimmond et al., 1993). Moreover, it is not indicated whether similar climate changes are able to affect the growth processes in amphibians.

A similar trend in the variability in amphibian body sizes within these populations might be expected, considering both the assumption of the temperature proximity regimes and the certain hydrochemical characteristics of the water reservoirs in habitats 1 and 3. The results make it possible to indicate the specificity of growth in males within each analyzed population.

The lifestyle of mature specimens, which spend most of their time on dry land and return to the water reservoir only during the spawning season (when water desalination can be observed in springtime), results in the fact that mature animals are not affected by the extreme salinity. The smaller body sizes in pubescent animals at site 3, when compared to those at site 2, can be explained by the difference in the age structure of the analyzed populations.

The analysis of the annual growths and the phalange areas in the animals offer the possibility to describe the characteristics of growth in the male moor frog in each of the analyzed populations. According to the "theory of life strategies," organisms are able to redistribute metabolic and energetic costs between life processes (growth, reproduction, etc.), depending on the habitat conditions. The results of our survey offer the possibility to state that an insignificant improvement of the living conditions may be indicated in the suburban population compared with the forest-park population. It results in an insignificant (the differences are not statistically significant) but more intensive growth of males in the suburban population. The complex conditions in habitat 3 may be called optimal, since the active growth and the start of the reproductive season at the first opportunity are observed there. The relatively early pubertal maturation of these males can indicate the redistribution of the energetic costs for the processes of reproduction under the favorable conditions. A similar life strategy is described in detail with the example of daphnids using the "deposit method" (Polishchuk and Faiferberg, 2006). The confirmation of more favorable conditions fit for moor-frog habitation in the surroundings of the settlement of Stepnoye, when compared to habitats 1 and 2, can be the state that puberty with a smaller body sizes can increase the adaptability of a group and lead to increases in the lifetime and number of reproductive periods per specimen. When puberty occurs at relatively small body sizes, the organism can keep its capacity for further growth to implement it during longer time periods (Ishchenko, 1991, 2007).

Therefore, the formation mechanisms of this phenomenon are different, despite the fact that at first sight pubescent animals from sites 1 and 3, when compared to those from site 2, are characterized by smaller body sizes. At site 3, the warmer temperature regime and the gradual salinity change in the water reservoir may exert stimulating effects and stipulate the high growth rates during the first years of life of animals. Later it results in the earlier start of the reproductive season and an appropriate delay in the growth of amphibians.

Neither the urban warmer temperature regime nor the gradual (spring–summer) increase in salinity of water in the reservoir are capable of compensating for the negative impacts of the concomitant conditions.

CONCLUSIONS

At this stage of research, therefore, it may be concluded that the level of salinity of water in the reservoir within 300 mg/dm3 in the spawning season cannot limit the growth of the moor frog. It is more apparent that the given parameter of the water environment exerts a stimulating effect on the growth rates of the bone tissue. The characteristics of the growths of the frogs in the urban population can confirm the assumption that the possibilities of the favorable climate conditions may be limited by the local conditions (Lyapkov et al., 2008).

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