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ZOOLOGY

Growth Characteristics and Rates of the Mash Frog *Pelophylax ridibundus* Pall. Introduced into Water Bodies of the Middle Urals

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Abstract—The characteristics and growth rates of mash frogs introduced upon the completion of metamorphosis into the cooling ponds of heating stations have been studied. The age composition of frog populations has been determined. It has been shown that an extra year must be added to the visible adhesion lines in the tissue of long bones. It has been found that the population of mash frogs inhabiting the Verkhnii Tagil Reservoir is characterized by not only a longer life span than in the Reftinskoe Reservoir, but also by a sufficiently high frequency of occurrence in the samples of older age groups.

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INTRODUCTION

An earlier cycle of studies on the biological characteristics of mash frogs, an invasive species of cooling ponds of the heating station in the Middle Urals, is continued. These frogs appeared in the herpetofauna of the Middle Urals in the 1970s as the reservoirs cooling the heating stations were stocked with fish (Toporkova, 1977; Ivanova, 1995). The source species was mash frogs from Ukraine and Krasnodar krai (the same haplotype by the *ND3* gene) (Bol'shakov and Ivanova, 2016). The reservoirs are located at a distance of 100 km from each other and are similar in hydrochemical parameters, but the Verkhnii Tagil Reservoir does not freeze in winter, and the average temperatures in it are 4–6°C higher than in the Reftinskoe Reservoir.

The habitat conditions in the new ponds turned out to be favorable for mash frogs. Both populations are currently dwelling on a large territory, having spread a dozen kilometers from the area of release (the territory of fish farms) (Bol'shakov and Ivanova, 2016). Their feeding spectra stably include food objects avoided by the aboriginal species of amphibians. Therefore, mash frogs pose no real danger to the latter (Vershinin and Ivanova, 2006).

It was previously shown that animals from these populations have different spawning types, fertility, duration of the larval period, and growth and development rates of larvae (Ivanova, 1995, 2002; Zhigal'skii, 2011). The growth of amphibians is commonly characterized as continuous, but it slows down with sexual maturity. The growth rate is the key ecological feature. Body size is the one of the most important morphophysical features that determines the degree of adaptability of a species and its population (Shvarts et al., 1968; Reminnii, 2007). It has been a common practice to identify the age of amphibians by the annual layers in their long bones. This method is widely used to find the individual age of animals and to describe their growth characteristics. Every annual layer consists of a wide zone of bone tissue and a narrow adhesion line formed during the winter pause in body growth (Smirina, 1972, 1983, 1987; Castanet, 1975; Gogoleva, 1985; Pavlov et al., 2009; Yanchurevich and Novitskii, 2012).

The aim of this study is to evaluate the growth characteristics and rates, as well as the life span of the invasive species (mash frog *Pelophylax ridibundus* Pallas, 1771) inhabiting the cooling ponds of the Middle Urals.

MATERIALS AND METHODS

This study is based on materials collected from 1997 to 2014 from the territory of cooling ponds of the Verkhnii Tagil and Reftinskoe heating stations. Sampling was conducted in spring during the reproductive period and in summer, upon completion of larval development. After wintering, frogs commonly emerge at the end of April and the beginning of May. Depending on the water and air temperature, mating takes place either at the beginning or in the middle of May. The sampled series included frogs of various age groups, from those that had already completed their metamorphosis and yearlings that had overwintered once, up to mature ones beginning reproduction. In the Reftinskoe Reservoir, 456 frog specimens were investigated. In the Verkhnii Tagil Reservoir, 918 specimens were analyzed.

The vegetation period is one of the major environmental characteristics, because it directly coincides with the growth period of animals on land when metamorphosis is completed. In order to compare the growth rates of animals from different populations, it is necessary to take into account the actual period of time during which this growth occurs. The samples considered describe populations dwelling under similar climatic conditions, at a small distance (~100 km), but isolated from each other. No data are available on the exchange of specimens between them despite the high migration ability of frogs (Ivanova, 1995).

For each frog caught, the body length was measured with a caliper to an accuracy of up to 0.1 mm and the fourth toe of the hind limb was amputated. Preparations were made from the third phalanx of the amputated finger to determine individual growth. Sections of all samples of the bone tissue were taken in strictly defined areas of long bones, in the very center of the diaphysis of the toe phalanx. Transverse sections (20–25 μm thick) were stained with Ehrlich's hematoxylin and placed in glycerol. The measurements were carried out with the help of an eye lens and a micrometer (grating period 0.01).

RESULTS AND DISCUSSION

In order to achieve the set aim, the age of frogs must be determined using reliable methods. It has been demonstrated based on many animal species, including amphibians, that resorption of the originally deposited bone tissue from the side of the endosteal cavity occurs in the long bones during the growth of animals, as a result of which the initial layers can partially or completely disappear (Smirina, 1972, Mina and Klevezal', 1976). Therefore, to ensure the accuracy of age determination, it is necessary to establish how many layers were resorbed by the time of capture of the frogs. To determine the resorption rate, the method used by Smirina (1983) on the gray toad *Bufo bufo* was applied: the diameter of the bone marrow cavity and the diameters of the irregular circles formed by the first, second, and subsequent adhesion lines were compared.

For this purpose, yearlings were caught after metamorphosis and before wintering, as were frogs that had survived one winter. In young, after metamorphosis, frogs and those that had overwintered one time, the diameter of the bone was measured; in adults, the bone marrow cavity was also measured, and the mean one of the greatest and smallest diameter of the transverse section was chosen. Previously (Smirina, 1972), it was proven experimentally that, after reaching maturity, when the growth of the animal slows down, the rate of resorption decreases or it disappears. At the same time, the rate of resorption, i.e., the number of adhesion lines that can be resorbed before maturity, should be established not only for each newly researched species, but also for populations of a single species living in different geographic zones, because the growth rates of animals can differ significantly under different climatic conditions. We compared the cross-sectional dimensions of the bone in the yearlings and frogs that overwintered one time with the size of the bone marrow cavity and the bone diameter limited by the first whole visible line of adhesion and subsequent lines in immature and sexually mature individuals.

According to our measurements, the adhesion line corresponding to the first wintering in mash frogs introduced into the territory of the Middle Urals by the time of maturity is mostly resorbed. However, in some animals (from 6.7 to 13.9% in different years) it remains completely or there are signs of the resorbing adhesion line located in the bone marrow cavity corresponding to the first wintering. This is indicated by the comparison of the size of the bone marrow cavity of adult frogs and the size of the bone in the cross section in specimens that had wintered one time. The diameter of the first visible adhesion line in sexually mature animals was 0.31–0.55 mm in the population of the Reftinskoe Reservoir and 0.26–0.52 mm in the Verkhnii Tagil Reservoir, and the width of the bone limited by the adhesion line in the animals after the first wintering was 0.27–0.52 and 0.27–0.49 mm, respectively. The adhesion line corresponding to the second wintering is usually preserved, but in some animals (0.65%) it can be partially or completely resorbed. Thus, it is necessary to add an extra year to the visible adhesion lines in mash frogs inhabiting the Middle Urals in order to determine the number of full years lived.

Although the studied samples belong to the same geographical locality, they are still different. Detailed investigation of the age composition showed that the Reftinskoe population is dominated by specimens of three (from 21.3 to 38.5% in different years) and four (from 23 to 30%) years of age. In the Verkhnii Tagil population, most specimens are older: five (10.6–26% in different years) and six (14.3–24.5%) years.

The studies performed on various species of tailless amphibians demonstrated that the most important consequence of differences in the life span is the different number of reproduction periods per specimen between populations. An increase in the number of reproductive periods throughout the life of a specimen creates a basis for the higher stability of populations even in the case of continuous adverse effects of external factors (Ishchenko, 1999).

In the course of this investigation, a maximum age of 11 winterings was registered in a single frog specimen from the Reftinskoe population with a body length of 95.8 mm and a weight of 74 g. This once again proves the assumption that the maximum age

Long bone width in yearlings after metamorphosis and in overwintered frogs

	n	Body length, mm	Bone width limited by adhesion lines	
			first	second
Reftinskoe population				
Yearlings				
that have completed metamorphosis	27	29.4 ± 0.7 (22.7–37.4)	0.31 ± 0.06 (0.25–0.46)	–
after first wintering	15	40.8 ± 2 (26.2–46)	0.43 ± 0.02 (0.27–0.52)	–
Frogs after second wintering	19	74.9 ± 1.8 (58.5–82.6)	0.44 ± 0.05 (0.34–0.55)	0.67 ± 0.07 (0.42–0.79)
Verkhnii Tagil population				
Yearlings				
that have completed metamorphosis in July–August	25	23.6 ± 2.9 (20–28.7)	0.26 ± 0.03 (0.21–0.31)	–
June	33	34.5 ± 1.8 (21.6–46)	0.33 ± 0.02 (0.25–0.42)	–
after first wintering	14	27.4 ± 3.8 (19.1–31.5)	0.33 ± 0.02 (0.21–0.39)	–
	19	34.7 ± 2 (26.5–46.3)	0.38 ± 0.06 (0.27–0.49)	–
Frogs after second wintering	21	68.6 ± 1.6 (52.8–77.3)	0.34 ± 0.03 (0.3–0.38)	0.57 ± 0.06 (0.45–0.76)

Ranges of values are given in brackets. "–" means no line.

among amphibians is not reached by the most rapidly growing specimens (Gittins et al., 1982; Smirina, 1987). The maximum age of frogs from the Verkhnii Tagil population was nine winterings in females and males with a body length and weight of 108.3 mm and 148 g and 92 mm and 90 g, respectively. These animals account for 2–2.4% of the population in different years. The maximum age of frogs inhabiting the Volga-Kama Reserve is also 11 years (Shaldybin, 1976). In Ukraine (Chernivtsi oblast), the maximum age reached by mash frogs is eight winterings (Savchuk, 2012). This age group accounts for 1.3% of the population. In the Danube River delta, the maximum registered age of frogs is seven years (Matveev, 2010).

During the analysis of variations in the body sizes of specimens of the same age, differences were found. With the same body length of 98.5 mm, frogs from the Verkhnii Tagil population had an age of four and six winterings, while frogs from had lived through three or four winterings. The specimens of the same age from the populations studied differ in the body length ($p < 0.0001$). For example, the mean body length of frogs that had overwintered five times was 104.7 ± 1.97 mm (80–118 mm) in the Reftinskoe population, while frogs from the Verkhnii Tagil population that were five years old were characterized by a significantly smaller body size, i.e., 88.9 ± 1.32 mm (80.3–102 mm). Discrepancies between the body size and age groups have been found in other amphibian species (Smirina, 1972; Ishchenko, 1999; etc.).

The body length values of yearlings that had completed their metamorphosis and after the first wintering are presented in the table. The vegetation period duration, i.e., the period of potential growth of yearlings before wintering, is ~1 month in the Reftinskoe population. According to our data, the body size of yearlings that had completed their metamorphosis varies from year to year (29.4 ± 0.7, 24.6 ± 2, 29.6 ± 0.86 mm). After the first wintering, the body lengths of animals vary as well (39.5 ± 2.4, 36.6 ± 1.8, and 40.8 ± 2 mm). An intensive growth was observed in specimens after the first wintering. Under the conditions of the Middle Urals, the mean body length of frogs that have overwintered two times is 74.9 ± 1.8 mm. In natural water bodies on the territory of Ukraine from where the frogs were introduced into the Middle Urals before wintering, their body length is 30–35 mm. By the time of the second wintering, frogs reach 70–79 mm (Matveev, 2010).

In the Verkhnii Tagil population, the spawning period is long. Mass spawning begins in the first ten-day period of May, then this process wanes, and the last layings were recorded at the beginning of July. The results of observations of larval growth and development showed that the beginning of the vegetation period is marked by the simultaneous presence of tadpoles at the earliest stages of development and with well-developed hind limbs (Ivanova, 2002). Considering that spawning starts during this period, it can be assumed that these tadpoles did not manage to complete their development in the previous year and overwintered. Since the water temperature in the reservoir did not fall below 10°C, they overwintered and continued their development in the spring of the following year. The first yearlings were discovered in the beginning of June. It is known that the minimum larval period takes 75–80 days under natural conditions. Consequently, with a high degree of probability it can be assumed that these were the yearlings of the wintering tadpoles, the larval period lasting almost 12 months. The active yield of yearlings was observed in July and August. Therefore, it can be suggested with a high degree of probability that they were yearlings from overwintered tadpoles, whose larval period lasted almost 12 months. The active emergence of yearlings was observed in July and August.

The average size of yearlings that had completed their metamorphosis from overwintered tadpoles is significantly larger than the size of yearlings from tadpoles of the current year that completed their metamorphosis at the end of July and at the beginning of August (see table). The periods of potential growth until the first wintering differ between yearlings. Thus, their mean body size is 34.5 ± 1.8 and 23.6 ± 2.9 mm before the first wintering or at least at the end of the growth period. The body length of frogs that had overwintered twice is 68.6 ± 1.6 mm, on average. Under the conditions of Krasnodar krai, yearlings before wintering reach a body length of 44 ± 2.2 mm (Kubantsev and Zhukova, 1982).

In the populations studied, some mash frogs become sexually mature after the second wintering, having a body length of 78 and 61 mm in the Reftinskoe and Verkhnii Tatil populations, respectively (Ivanova, 2002). After the third wintering, all females become sexually mature. The age of maturity in mash frogs varies from region to region. Thus, frogs that live in the waters of Northern Greece (Kyriakopoulou-Sklavounou, 1990) reach sexual maturity after the first wintering at a body length of 62–66 mm. In the delta of the Danube River, frogs become sexually mature in their third year of life (Matveev, 2010). In mountain water bodies of Armenia, only a small part of frogs begin reproduction after the third wintering and most of them start after the fourth wintering (Aleksandrovskaya and Kotova, 1986).

Therefore, an extra year must be added to the visible adhesion lines to determine the number of full years lived by mash frogs on the territory of the Middle Urals. Having considered all the data, it was revealed that the population of mash frogs in the Verkhnii Tagil Reservoir is characterized by not only a longer life span compared to the Reftinskoe Reservoir, but also by a sufficiently high frequency of occurrence in samples of specimens from older age groups. This may be evidence of the high survival rate after reaching maturity and differences in the number of reproduction periods per specimen in different populations. The obtained results make it possible to claim that the revealed differences are adaptive and associated with the temperature regime in water bodies.

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