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SHORT COMMUNICATIONS

Development of Specific Features of Marsh Frog (*Pelophylax ridibundus*) Populations in Water Bodies of the Middle Urals

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Invasions of alien species may result both in extinction of native species and in the loss of biodiversity [1]. There also is evidence that regional biodiversity increases due to expansion of new animal and plant species [2]. Therefore, it is important to study alien species that have somehow appeared in the regional fauna, including their effect on the native fauna and ways of adaptation to novel habitat conditions, since it depends on this whether a given species will contribute to regional biodiversity or disappear. Geographically isolated populations formed in new regions may differ from populations within their native range in ecological, morphophysiological, and genetic features [3].

The marsh frog *Pelophylax ridibundus* Pallas, 1771 is an invasive amphibian in the Middle Urals. This species has expanded far beyond the boundaries of its native range and invaded many regions. Its populations have been described in thermal water bodies in the environs of Yakutsk [4], in the Altai [5], Krasnoyarsk krai [6], and Kamchatka [7]. The marsh frog is a highly tolerant and ecologically flexible species capable of living in areas where other amphibian species cannot normally grow, develop, and reproduce [8–10].

Pelophylax ridibundus appeared in the Middle Ural herpetofauna in the 1970, when cooling reservoirs of the Reftinsky and Verkhny Tagil regional power plants (GRES) were stocked with fish fry from water bodies of Krasnodar krai and Ukraine. Marsh frog tadpoles were apparently introduced together with fish stock [11, 12].

This species continues its expansion in the Middle Urals and has already colonized water bodies at considerable distances from the centers of its introduction. Marsh frogs from the Verkhny Tagil Reservoir have migrated along the Tagil River to drinking-water and settling ponds within the Nizhny Tagil city limits and then to the upper reaches of the Neiva River [9]; they have been found in the cooling pond of the Serov GRES, Iset River, and Beloyarka Reservoir. Their expansion occurs due to seasonal changes in the hydrological regime and by way of active migration on land. It should be noted that *P. ridibundus* does not populate shallow shoreline areas where frogs of the genus *Rana* gather for spawning but prefers relatively large water bodies with steep shores and abundant macrophyte vegetation. Food items common in its diet are usually avoided by native amphibians, and therefore *P. ridibundus* poses no risk to them [13].

The purpose of this study was to assess basic ecological characteristics of this invasive species in the Middle Urals, including the size and age composition of populations, timing of breeding, fecundity, and life span in natural and thermal water bodies (the Pyshma River and Reftinsky Reservoir, respectively).

The Reftinsky Reservoir was constructed on the Reft River, a left tributary of the Pyshma River (the Ob–Irtysh basin), in 1968 it serves as a cooling reservoir of the Reftinsky GRES. The reservoir is a flowing water body with a surface area of 2530 ha; its depth reaches 22 m, averaging 5.4 m; the bottom is silt or, in places, gravely sand or rubble. In the period of *P. ridibundus* larval growth and development (July–August), the water temperature in the zone of heated water outlets reaches 24–28°C; in winter, the temperature in the main water area is 4–5°C. Due to high summer temperatures and active mixing, the water is enriched in oxygen and organic and mineral nutrients, which provides favorable conditions for the growth of zoo- and phytoplankton [14]. Marsh frogs from the Reft Reservoir migrated along the Reft River to the Pyshma, where self-reproducing populations of the species were formed during the 1980s. Water temperatures in the Pyshma are lower than in the Reftinsky

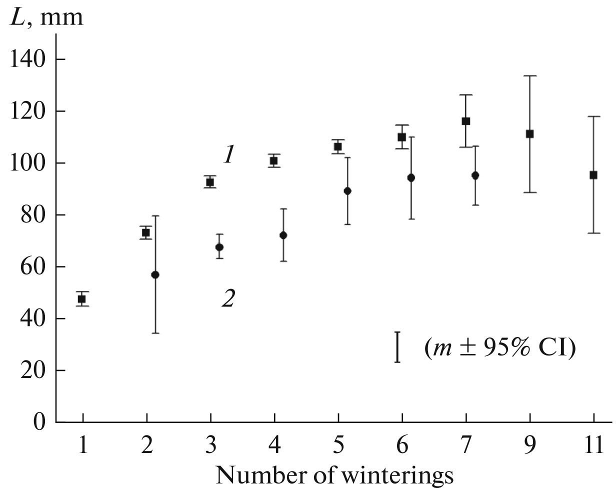

Fig. 1. Body length in *P. ridibundus* frogs of different ages from (*1*) the Reftinsky Reservoir and (*2*) the Pyshma River.

Reservoir and correspond to the temperature regime of natural water bodies in the Middle Urals [14].

The study was performed with marsh frogs from the Reftinsky Reservoir and from the Pyshma River (near the village of Kur'i, 40 km from the reservoir), where stable *P. ridibundus* populations were formed by the early 1990s. The frogs were collected in late May, during the spawning period. The sample consisted of young of the year, subadults (overwintered once), and adult spawners. A total of 452 adult frogs were examined, including 38 ind. from the Pyshma (2017–2018) and 414 ind. from the reservoir (1993–2015).

The most meaningful parameters characterizing the populations of tailless amphibians were evaluated: the size and age composition, timing of breeding, age at maturation, and fecundity (the data on age and fecundity were obtained only for adult frogs).

In each frog, the snout-to-vent length (longitudo corporis) was measured with 0.1 mm accuracy, and the fourth toe was cut off to make preparations for age determination from its third phalanx [15]. Young of the year from the Pyshma ($n = 3$) and Reftinsky Reservoir ($n = 27$) were used to estimate the rate of bone tissue resorption according to Smirina [16]. Cross sections through the center of phalanx diaphysis (20–25 μm thick) were stained with Ehrlich's hematoxylin and embedded in glycerol [16, 17]. Measurements were made using an ocular micrometer with 0.01 mm graduation. The bone cross-section size in young of the year and overwintered yearlings were collated with the size of the bone marrow cavity and the bone diameter delimited by the first continuous adhesion line and subsequent annual growth lines in immature and sexually mature frogs. To calculate complete age in years, one year was added to the number of visible annual growth lines [17]. The length of the spawning period was estimated from the dates when the first and last egg masses appeared, and sexually mature males were identified by well-developed vocal sacs. To estimate female fecundity, the ovaries of adult females were dissected to count the number of mature oocytes under an MBS-10 binocular microscope.

The results were processed statistically using Statistica 6.0 (StatSoft Inc., 2001) (license no. AXXR003A622407-FAN8) Differences between the samples were evaluated by one-way ANOVA and considered significant at $p < 0.05$.

Studies on the Pyshma population are at the initial stage, but the results have already shown that marsh frogs from this river differ in some parameters from those inhabiting the Reftinsky Reservoir. The body size is one of the main morphophysiological characteristics determining the adaptive potential of species and populations [18]. Differentiation in body size is already observed at the early stages of postmetamorphic growth, and larger individuals may mature more rapidly and become involved in reproduction a year earlier [19, 20]. The growth rate in female marsh frogs is 8% higher than in males [21].

Our data provided a basis for a comparative analysis of body size in frogs of different ages (Fig. 1). The results show that visible differences in body size depending on habitat are absent in all age groups ($F(5;43) = 0.70$, $p = 0.62$). However, frogs from the Reftinsky Reservoir show a tendency toward increase in this parameter: their body length after three winterings averages 93.0 ± 1.3 mm, compared to 68.1 ± 1.4 mm in frogs from the Pyshma ($F = (1;12) = 81.88$, $p < 0.01$); after four winterings, 101.2 ± 1.3 compared to 72.5 ± 1.0 mm ($F = (1.87) = 28.40$, $p < 0.01$). An overlap of body sizes in frogs of the same age is observed in all age groups. For example, from the Pyshma may have a body length of 71.4 mm after either three or four winterings.

The longest life span among marsh frogs from the Reftinsky Reservoir (11 winterings) was determined for a female with a body length of 95.8 mm. It is known that the oldest individuals are not necessarily the largest: thus, the greatest body length (132.2 mm) was recorded in a female that overwintered six times [12]. In the sample from the Pyshma, the longest life span (seven winterings) was noted in a female with a body length of greater number of 107.0 mm. Marsh frogs from their native range in Odessa oblast are larger: their body length reaches a maximum of 170 mm, averaging 130.0–139.0 mm [22].

Marsh frogs usually emerge from their wintering grounds in March to April; in southern regions, where winters are warm, they may remain active all year long. Mating starts soon after the end of wintering, the spawning season is more than 3 months long, and part of frogs may spawn repeatedly in the same year [22–24].

In the Middle Urals, overwintered marsh frogs usually emerge in late April to early May. Spawning in the study water bodies starts in mid-May, at a water temperature of 15–18°C, and continues for an average of 3 weeks. The spring of 2018 was delayed and cold, and the first egg masses in the Pyshma appeared as late as early June. According to our data, the period of larval development is 70 to 90 days long [25], and therefore young of the year that complete metamorphosis at the very end of the warm season have not enough time to prepare for wintering.

The age at maturation in *P. ridibundus* depends on environmental conditions [22, 23]. In northern Greece, marsh frogs reach sexual maturity after the first wintering, at a body length of 62.0–66.0 mm [24]. In mountain water bodies of Armenia, a small proportion of marsh frogs start breeding after the third wintering, whereas most of them become reproductive year later [26]. In the Reftinsky Reservoir, part of females start breeding after the second wintering, at a body length of 78.0 to 83.0 mm (2523 to 2448 eggs in the ovaries), with all females reaching sexual maturity after the third wintering [19].

The sample from the Pyshma included 22 females that survived three winterings. Eggs ready for spawning were found in three females with body lengths of 61.1, 75.8, and 76.5 mm (1260, 2417, and 2990 mature oocytes in the ovaries, respectively). The ovaries of females with a body length of 62.7–74.4 mm were a pale sandy color, and oocytes were invisible to a naked eye, indicating that these females were immature. Therefore, female marsh frogs in the Pyshma start breeding a year later than in the Reftinsky Reservoir, i.e., mainly after the fourth wintering, with only few of them reaching sexual maturity after the third wintering.

The absolute female fecundity in the Reftinsky Reservoir averaged 5778 ± 267 eggs, with a minimum of 1199 eggs at a body length 88.3 mm (after two winterings) and a maximum of 13 252 eggs at a body length of 124.0 mm (four winterings). In the Pyshma, female fecundity averaged 4456 ± 1754 eggs, with a maximum of 11 166 eggs at a body length of 107.0 mm (seven winterings) and a minimum of 1260 eggs at a body length of 61.1 mm (three winterings). Marsh frogs from the Pyshma do not differ in this parameter from frogs collected in the Reftinsky Reservoir ($F(1.39) = 0.01$, $p = 0.96$) and from natural *P. ridibundus* populations in Odessa oblast, where the absolute fecundity averages составляет 5400 (850–12 400) eggs [22].

Thus, marsh frogs inhabiting water bodies with different thermal regimes have a specific set of features related primarily to body size and age. In some age generations, frogs from the Reftinsky Reservoir are significantly larger than frogs of the same age from the Pyshma River.

Female frogs in the Pyshma start breeding a year later than in the Reftinsky Reservoir, i.e., mainly after the fourth wintering, with only few of them reaching sexual maturity after the third wintering. Females from the two water bodies do not differ in the numbers of eggs they spawn.

After *P. ridibundus* was discovered in cooling reservoirs, it was included in the 2006 Red Data Book of the Middle Urals [27]. However, our studies showed that this species is invasive, and it was therefore excluded from the list of protected amphibian species in the 2008 edition of the Red Data Book of Sverdlovsk Oblast [28].

The results presented above confirm the high ecological flexibility of *P. ridibundus,* which allows this species to colonize new water bodies and thereby successfully expand its range.

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COMPLIANCE WITH ETHICAL STANDARDS

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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