



DOI: <https://doi.org/10.23859/estr-240305>

EDN: <https://elibrary.ru/bpckjw>

UDC 59.002;591.431.4;591.84

Article

Age structure and growth of the Dybowski's frog (*Rana dybowskii*) from the urban population in the south of Primorsky Krai

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Abstract. The paper presents the study results of the age structure and growth characteristics of *Rana dybowskii* dwelling in the city of Fokino (Primorsky Krai, Russia). We measured a body length of the captured frogs, removed the phalanx of the fourth digit of the hind limb and released the animals in the place of their capture. Age was determined using the method of skeletochronology. A total of 48 specimens (10 immature frogs, 18 females, and 20 males) were studied. The age of sexually mature females and males did not differ statistically and varied within 1–5 years (2.44 years on average) in the first group and 1–4 years (1.95 years on average) in the second. Females were predominantly represented by two-year (33.33%) and three-year-olds (27.78%), while males by yearlings (55.0%). In females, only one-year and three-year-olds statistically significantly differed in body size. In males, such differences were noted between the age 1 and age 3, the age 1 and age 4, as well as between the age 2 and age 4 frogs. Most one-year-olds exhibited the pronounced signs of sexual dimorphism. A significant age dependence of the body length was established for both sexes. In the urban population of *R. dybowskii*, animals reached sexual maturity early, continued to grow after maturation, had a short lifespan and, as a result, most of them participated in reproduction only during the 1–2 season.

Keywords: true frogs, skeletochronology, demography, lifespan, Russian Far East

Funding. The work was supported by the Russian State Agrarian University–Moscow Timiryazev Agricultural Academy Development Programme within the framework of the Strategic Academic Leadership Project “Priority-2030”.

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To cite this article: Kidov, A.A. et al., 2025. Age structure and growth of the Dybowski's frog (*Rana dybowskii*) from the urban population in the south of Primorsky Krai. *Ecosystem Transformation* 8 (3), 138–150. <https://doi.org/10.23859/estr-240305>

Received: 05.03.2024

Accepted: 02.08.2024

Published online: 15.08.2025

DOI: <https://doi.org/10.23859/estr-240305>EDN: <https://elibrary.ru/bpckjw>

УДК 59.002;591.431.4;591.84

Научная статья**Возрастная структура и рост дальневосточной лягушки (*Rana dybowskii*) в городской популяции на юге Приморского края**А.А. Кидов* , Р.А. Иволга , Т.Э. Кондратова *Российский государственный аграрный университет – МСХА имени К.А. Тимирязева, 127550, Россия, г. Москва, ул. Тимирязевская, д. 49*

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Аннотация. В работе представлены результаты изучения возрастной структуры и особенностей роста *Rana dybowskii* в г. Фокино (Приморский край, Россия). У пойманных лягушек измеряли длину тела и отсекали фалангу четвертого пальца задней конечности, после чего животных выпускали в месте поимки. Определение возраста осуществляли методом скелетохронологии. Всего были изучены препараты от 48 особей (10 неполовозрелых лягушек, 18 самок и 20 самцов). Возраст половозрелых самок и самцов не имел статистических различий и варьировал в пределах 1–5 лет (в среднем 2.44 года) для первых и 1–4 лет (в среднем 1.95 года) для вторых. Самки преимущественно были представлены двухлетними (33.33%) и трехлетними особями (27.78%), а самцы – в основном годовиками (55.0%). У самок по длине тела статистически значимо различались только однолетние и трехлетние особи. У самцов различия по длине отмечены между годовальными и трехлетними лягушками, между годовальными и четырехлетними лягушками, между двухлетними и четырехлетними лягушками. Большинство годовалых животных имеют выраженные признаки полового диморфизма. Наблюдается статистически значимая зависимость длины тела лягушек обоего пола от возраста. Отмечается, что в городской популяции *R. dybowskii* животные рано достигают половой зрелости, продолжают расти и после созревания, имеют низкую продолжительность жизни и, вследствие этого, большинство принимают участие в размножении только 1–2 сезона.

Ключевые слова: настоящие лягушки, скелетохронология, демография, продолжительность жизни, Дальний Восток России

Финансирование. Работа выполнена за счет средств Программы развития РГАУ–МСХА имени К.А. Тимирязева в рамках Программы стратегического академического лидерства «Приоритет-2030».

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Для цитирования: Кидов, А.А. и др., 2025. Возрастная структура и рост дальневосточной лягушки (*Rana dybowskii*) в городской популяции на юге Приморского края. *Трансформация экосистем* 8 (3), 138–150. <https://doi.org/10.23859/estr-240305>

Поступила в редакцию: 05.03.2024

Принята к печати: 02.08.2024

Опубликована онлайн: 15.08.2025

Introduction

Of all modern vertebrates, amphibians are most vulnerable to the growing anthropogenic impact (Luedtke et al., 2023). This is due to increased demands for clean fresh water, where reproduction and early ontogenesis of the majority of species take place (Wilbur, 1980). By now, the crisis has affected not only narrow-range amphibian species, but also widespread and previously common ones (Baker, 1997; Beebee et al., 1990). In this regard, ongoing investigations of urbanization influence on the age structure of populations are topical (Cayuela et al., 2022).

Dybowski's frog (*Rana dybowskii* Günther, 1876), is abundant in the southern Russian Far East (Kuzmin, 2012), as well as on the Korean Peninsula and northeastern China (Borzee, 2024). The dried oviducts (so-called "frog fat") obtained from this species are highly valued in traditional Chinese medicine (Fan et al., 2022; Liu et al., 2023; Wang et al., 2023; Xiao and Jiang, 2010) that contributes to animals overhunting throughout its range, including Russia (Kuzmin and Maslova, 2005). Cases of mass mortality of *R. dybowskii* caused by viral infections (Park et al., 2021) are also recorded. Disruption of the genetic structure of natural populations due to transportation and release of animals from different localities by Chinese farmers brings the threat to this species (Xia et al., 2011). However, among major factors of decline in species number are habitat destruction and range fragmentation (Borzee et al., 2021; Othman et al., 2022).

Along with abundance, size and age characteristics are the most important indicators of the state of amphibian populations (Ishchenko, 1978; Lyapkov, 2005). For almost 50 years, skeletochronology (Kleinenberg and Smirina, 1969) has remained the main method for age determination in amphibians. It is based on estimating the lines of arrested growth formed during wintering in tubular bones (Lyapkov et al., 2020, 2021b; Sinsch, 2015; Smirina, 1994). Previously, the specialists studied the age structure of the Dybowski's frog populations (Ishchenko, 1996). Meanwhile, these research were either carried out in the special protected areas or combined the findings on separate species of brown frogs designated as *R. chensinensis*.

The aim of this work was to assess the age structure and growth characteristics of Dybowskii's frog from the urban population in the south of Primorsky Krai.

Material and methods

The material was collected in the third decade of July 2022 in the city of Fokino, Primorsky Krai. *R. dybowskii* was caught in sites where they overwintered, i.e. to the west of Belashev street along the left bank of the Promyslovka (= Kogotun') River (N 42.966° E 132.402°, 7–9 m above sea level). In the city, reproduction of these frogs occurs in puddles on dirt roads and in drainage ditches along them. Within Fokino, the habitats of the Dybowski's frog are not isolated due to adjoining (in the south) weakly transformed forest massif of the Promyslovka River mouth.

Sex determination of the captured animals was implemented by external signs. Body length (SVL) of frogs was measured with a digital caliper according to the standard method (Bannikov et al., 1977), the third phalanx of the fourth digit from the right hind limb was removed, and the animals were released in the place of their capture.

For age determination, we used the standard procedure (Smirina, 1989) of counting the lines of arrested growth on digit phalanges decalcified and stained with Ehrlich's hematoxylin. Since Endosteal resorption often affects the estimation of adhesion lines, we applied the back-calculation method (Hemelaar, 1985). This method involves measuring the minimum and maximum diameters of the first and second visible lines of arrested growth for each individual with further analyzing the overlapped area. If diameters of the first visible line are smaller than the average of the second one, resorption does not

occur. To the contrary, if diameters of the first line are equal or greater than the average of the second line, the first one is resorbed.

The photographs of preparations were taken using a Levenhuk M500 BASE digital camera (Fig. 1). In total, phalangeal sections from 48 individuals (18 females, 20 males, and 10 young animals of indeterminate sex) were examined.

Statistical processing and visualization of the data were performed in STATISTICA 10 and Origin-Pro 2022 programmes. The arithmetic mean and standard deviation ($M \pm SD$), as well as the range of features (min–max) were calculated. Hypotheses about the normality and homogeneity of sample distribution were checked via using the Lilliefors and Levene tests. Data analysis was made based on the one-way ANOVA (F), Tukey's test (Q), Student's t-test (tst), Mann–Whitney U-test (U), and Spearman's rank correlation test (r). The growth of frogs was described by nonlinear estimation (R^2) using the von Bertalanffy equation (Bertalanffy, 1938):

$$SVL_t = SVL_{max} - (SVL_{max} - SVL_{met}) * e^{-k*(t+0.17)}$$

where SVL_t is the average body length at a certain age, SVL_{max} – the maximum body length (may differ from the maximum recorded), SVL_{met} – the body length of metamorphs, k – the growth coefficient, t – the age (in years). The size of young frogs emerging on land (SVL_{met}) was taken as the average body length of yearlings immediately after metamorphosis (16.5 mm) in accordance with the data of S.L. Kuzmin and I.V. Maslova (2005).

The survival rate (S) of sexually mature frogs distributed by age was estimated with the Robson & Chapman formula (1961):

$$S = T/(T + n - 1)$$

where $T = n_{i+1} + 2n_{i+2} + 3n_{i+3} + \dots$; $n = \sum n_i$, n_i is the number of individuals in the age group i (starting with 1+).

The life expectancy (ESP) of frogs was identified due to the Seber formula (Seber, 1973):

$$ESP = 0.5 + 1/(1 - S)$$

Note that ESP may differ from the maximum recorded age of the studied individuals.

Results

In the examined preparations, the first line of arrested growth was partially or completely preserved in 39 individuals (81.25%), while fully resorbed in 4 females (8.33%) and 5 males (10.42%) (Fig. 1).

The age of sexually mature females varied within 1–5 years (on average 2.44 ± 1.149), males – 1–4 years (on average 1.95 ± 1.234), showing no statistical differences between males and females ($t_{st} = -1.27$; $p = 0.211$). Females were mainly represented by two-year (33.33%) and three-year-old individuals (27.78%); the modal age of males made up 1 year (55.0%) (Fig. 2).

Apparently, most *R. dybowskii* from Fokino mature before the second wintering period. Life expectancy for females after reaching sexual maturity is 3.03 ($S = 0.605$), and for males – 2.50 years ($S = 0.500$).

Females of different age groups differed in body size ($F = 4.457$; $df = 3, 13$; $p = 0.023$). At pairwise comparisons of the means, statistically significant differences were noted only between one-year- and three-year-old individuals ($Q = 4.59$; $p = 0.029$). Males of different ages also differed in their length ($F = 15.088$; $df = 3, 16$; $p < 0.001$): one-year-olds were statistically significantly smaller than three-year ($Q = 4.14$; $p = 0.044$) and four-year frogs ($Q = 9.19$; $p < 0.001$), while two-year-olds were inferior in body size only to four-year individuals ($Q = 5.52$; $p = 0.006$) (Table 1).

In the sample, females were generally much larger than males ($t_{st} = 3.19$; $p = 0.003$). However, the pairwise comparison of the body size of frogs of both sexes showed significant differences only between the age 1 females and males ($U = 6.0$; $p = 0.033$). The calculated values of the maximum body length of females (63.94 ± 2.345 ; $p < 0.001$) and the growth coefficient (0.992 ± 0.167 ; $p < 0.001$) were greater than for males (59.14 ± 2.653 ; $p < 0.001$ and 0.918 ± 0.141 ; $p < 0.001$, respectively) (Fig. 3).

Most yearlings exhibit the pronounced signs of sexual dimorphism; their growth slows down, but does not stop. A statistically significant age dependence of body length is observed for animals of both sexes ($r = 0.72$; $p < 0.05$ for females; $r = 0.77$; $p < 0.05$ for males).

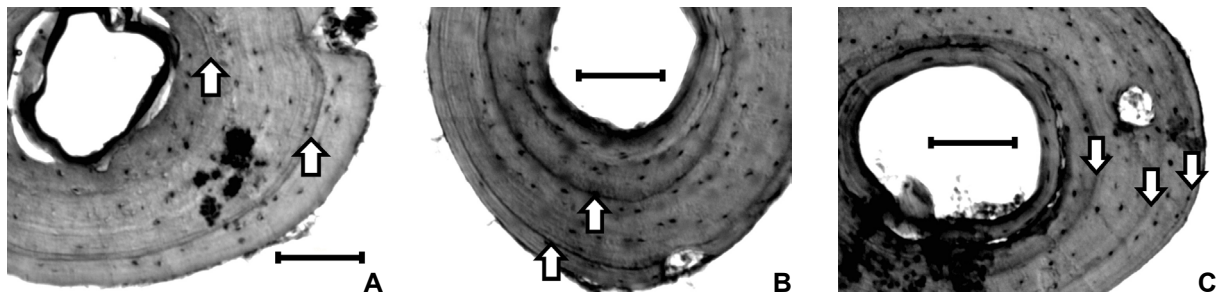


Fig. 1. Cross-sections of *Rana dybowskii* digit phalanges. **A** is a two-year-old female (SVL = 60.63 mm); **B** is a three-year-old male with the first resorbed line of arrested growth (SVL = 55.40 mm); **C** is a four-year-old male with the first resorbed line of arrested growth (SVL = 58.13 mm). White arrows indicate the lines of arrested growth. The 0.1 mm scale bar is used in all photographs.

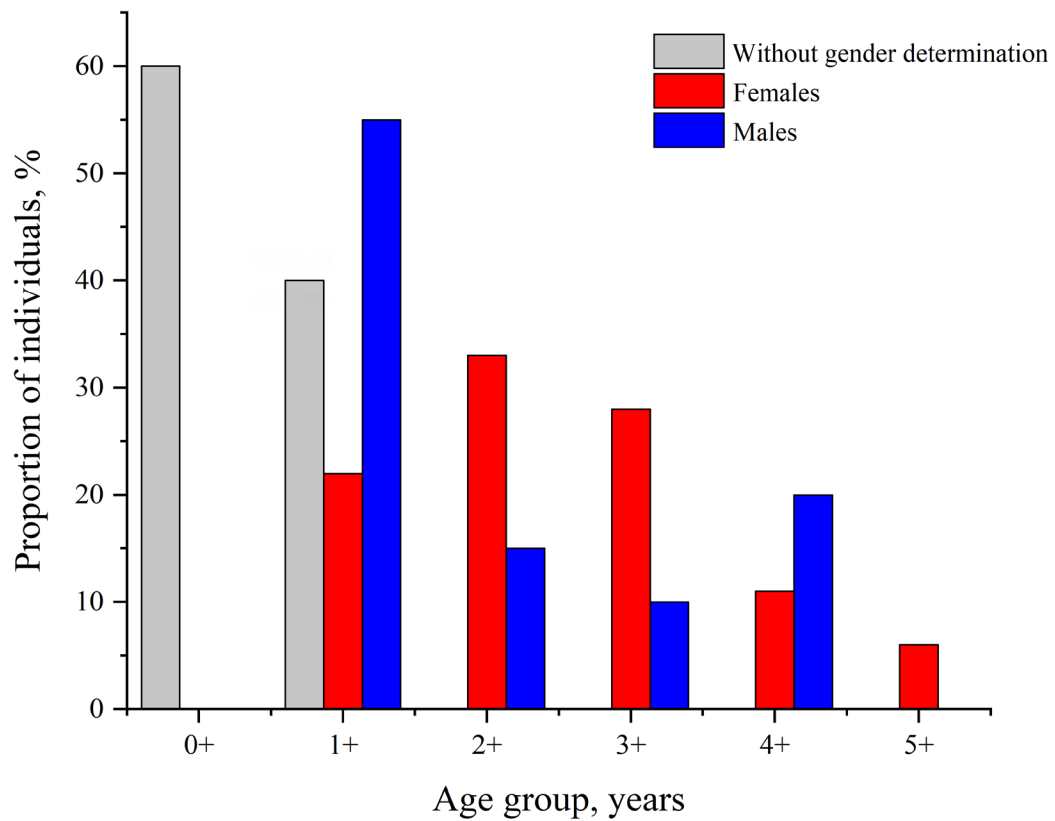
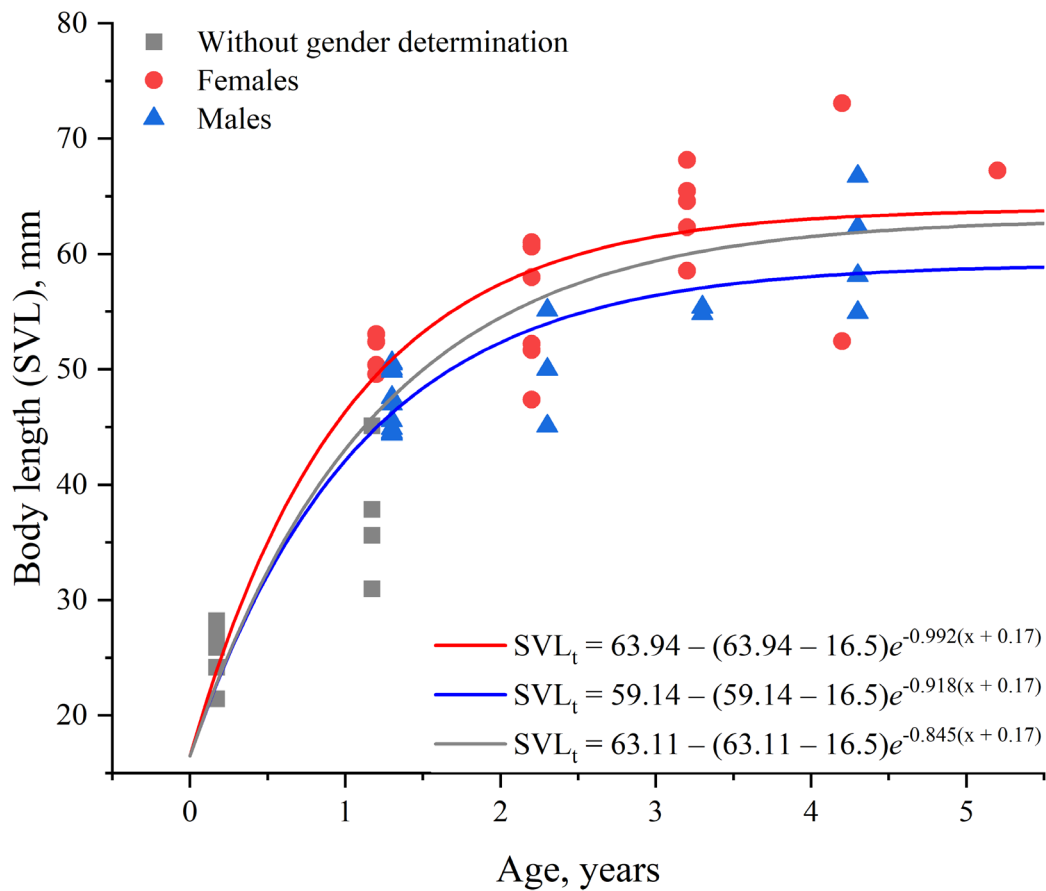


Fig. 2. Age structure of *Rana dybowskii* in the south of Primorsky Krai.

Table 1. Body size of *Rana dybowskii* of different age groups. The arithmetic mean \pm standard deviation is indicated above and the feature range below the line.

Age group	Immature individuals		Females		Males	
	n	SVL, mm	n	SVL, mm	n	SVL, mm
0+	6	$\frac{25.82 \pm 2.624}{21.45-28.24}$	–	–	–	–
1+	4	$\frac{37.40 \pm 5.874}{31.01-45.10}$	4	$\frac{51.35 \pm 1.635}{49.59-53.06}$	11	$\frac{47.21 \pm 2.602}{44.39-50.56}$
2+	–	–	6	$\frac{55.15 \pm 5.539}{47.38-61.01}$	3	$\frac{50.08 \pm 5.030}{45.08-55.14}$
3+	–	–	5	$\frac{63.82 \pm 3.603}{58.56-68.15}$	2	$\frac{55.12 \pm 0.403}{54.83-55.40}$
4+	–	–	2	$\frac{62.76 \pm 14.595}{52.44-73.08}$	4	$\frac{60.54 \pm 5.133}{54.93-66.74}$
5+	–	–	1	67.25	–	–
In total	10	$\frac{30.45 \pm 7.148}{21.45-45.10}$	18	$\frac{58.23 \pm 7.480}{47.38-73.08}$	20	$\frac{51.09 \pm 6.309}{44.39-66.74}$

**Fig. 3.** Growth trends of *Rana dybowskii* in the south of Primorsky Krai.

Discussion

In urban conditions, most males and females of the Far Eastern frog reach sexual maturity after the first wintering. Since the material was collected in July and spawning of frogs in Primorsky Krai occurs in April–May (Kuzmin and Maslova, 2005), we did not register breeding frogs at the age of one year. In this regard, we cannot be sure that *R. dybowskii* begins to breed before the second wintering. The results of our work demonstrate a low threshold of maturation for this species (*R. dybowskii* is believed to reach its sexual maturity only by the third wintering (Ishchenko, 1996)). At the same time, the maximum lifespan of city animals is significantly lower (5 years for females and 4 years for males) as compared to the studied natural populations (up to 5–8 years) (Ishchenko, 1996; Kolobaev, 2000). The reproductive core of the population obviously consists of individuals breeding for the first time. This is probably due to high mortality of adult frogs, primarily, crushed by vehicles during the period of reproductive migration and spawning.

It is interesting that the growth of the Dybowski's frog in the studied population after reaching sexual maturity slows down, but does not stop, unlike many other species (Borkin and Tikhenko, 1979; Kidov et al., 2018; 2023a, b; Lyapkov et al., 2021a). The preservation of stable somatic growth after maturation is also noted for a number of synanthropic amphibians (Stepankova et al., 2023) and rarely for individual natural populations (Kidov et al., 2023). Bearing in mind that fertility of females of many Palearctic amphibians correlates with their size (Matushkina and Kidov, 2013; Stepankova et al., 2020), it can be assumed that accelerated growth rate and, consequently, the greater reproductive potential compensate for high mortality of animals in the urban environment.

It is known that under conditions of high anthropogenic transformation of habitats, older animals become extinct (Ushakov et al., 1982). For example, individuals with a high growth rate, early sexual maturity and a shorter lifespan predominate in the core producers of urban populations of the common frog *R. temporaria* L., 1758 (Vershinin, 1997). Also, in the urbanized areas of cities Kazan (Zamaletdinov, 2003) and Tolyatti (Zamaletdinov et al., 2013), the age of the marsh frog *Pelophylax ridibundus* L., 1758 reaches maximum 6 years, in contrast to populations (with a 7-year lifespan) dwelling in suburban forest parks (Zamaletdinov, 2003).

Conclusion

Thus, the Dybowski's frog from the urban population and populations of other synanthropic amphibians show similar trends in demographic features (Cayueta et al., 2022; Stepankova et al., 2023). For instance, animals reach sexual maturity early, continue to grow after maturation, have a short lifespan, and as a result, most individuals breed only during the 1–2 season.

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