Acta et Commentationes, Exact and Natural Sciences ISSN: 2537-6284
Volume 17(1), 2024, Pages 7–18 E-ISSN: 2587-3644
CZU: 597.6/.8:591.5+597.6/.8:591:9 DOI: 10.36120/2587-3644.v17i1.7-18

The ontogenetic development of *Rana dalmatina* Bonaparte, 1840 species (Amphibia: Ranidae) in the context of climate change

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Abstract. Globally, amphibians represent one of the main objects of study in the context of climate change, because the temperature variations recorded and in the Republic of Moldova also represent a major threat to their development and sustainability.
For this purpose, a complex ecological study was carried out regarding the embryonic and larval development strategies of the *Rana dalmatina* Bonaparte, 1840, species and the main particularities related to its population structure and dynamics were described. This research allowed us to evaluate the degree of ecological plasticity of natural populations of *Rana dalmatina* in relation to the plurality of environmental factors, which allows us to develop suitable and safe measures for monitoring and conservation of the species.
Keywords: *Rana dalmatina*, ecology, climate change, Moldova.

Dezvoltarea ontogenetică a speciei *Rana dalmatina* Bonaparte, 1840 (Amphibia: Ranidae) în contextul schimbărilor climatice

Rezumat. Pe plan mondial, amfibienii reprezintă unul din principalele obiecte de studiu în contextul schimbărilor climatice, deoarece, variațiile temperaturilor înregistrate la ora actuală și în Republica Moldova reprezintă o amenințare majoră asupra dezvoltării și sustenabilității acestora.
În acest scop, a fost efectuat un studiu ecologic complex referitor la strategiile de dezvoltare embrionară și larvară a speciei *Rana dalmatina* Bonaparte, 1840 și s-au descris principalele particularități referitoare la structura și dinamica populațională a acesteia. Aceste cercetări ne-au permis de a evalua gradul de plasticitate ecologică a populaţiilor naturale de *Rana dalmatina* în raport cu influiența complexă a factorilor de mediu, ce ne-a permis de a elaborara unele măsuri efective de monitorizare şi conservare a speciei.
Cuvinte-cheie: *Rana dalmatina*, ecologie, schimbări climatice, Moldova.

1. Introduction

In recent years, amphibians have emerged as a reliable model of biological indicators under the continuous pressure of environmental climate change, their effects on ecosystems, and the degree of evolutionary adaptability resulting from specific ecological relationships.

The sustainability of the species is determined by the population's capacity to produce new generations capable of surviving under conditions of instability and high fluctuations in non-periodic ecological factors. However, this particularity, in turn, distinguishes itself throughout specio-specific evolution, thus representing one of the distinctive criteria of the species.

Reproductive behavior, morpho-physiological adaptive characteristics during the growth and development period in fluctuating environmental conditions, constitute one of the indispensable and extremely important components of the ontogeny of all amphibians for their perpetuation, including the species *Rana dalmatina* Bonaparte, 1840. The specificity of reproduction and development in amphibians is determined by some characteristic adaptations to the environment and their living conditions [4, 6, 7].

2. Materials and Methods

The study area encompasses both natural and anthropized aquatic ecosystems specific to the *Rana dalmatina* species within the Codrii Centrali region. Species identification, including both adult forms and embryonic and larval stages, was conducted using classical deductive methods, focusing on morphometric parameters and body coloration [1, 2, 3].

To obtain scientific results regarding the embryonic and larval developmental characteristics of the *Rana dalmatina* species, its phenology was evaluated in relation to environmental fluctuations across various types of aquatic ecosystems, both natural and anthropized. Additionally, the population structure of the species (age structure, size structure, spatial structure) and 142 egg masses were monitored to assess the numerical abundance of eggs, embryonic and larval developmental stages, as well as to obtain a detailed and comprehensive characterization of these aspects.

Given that the investigations were focused on studying the entire annual life cycle with reference to deciphering the embryonic and larval developmental characteristics (which occur exclusively in aquatic environments), we specifically examined all aquatic basins within the study area. This enabled us to obtain valuable data regarding the specific developmental strategies (which constituted the main objective of our research) of the tailed amphibian species *Rana dalmatina* Bonaparte, 1840.

Climate change represents a global phenomenon that endangers natural, social, and economic systems due to their sensitivity and vulnerability to climatic factors. In the current pedoclimatic conditions of the Republic of Moldova, the animal world is influenced by the degradation of plant associations, food and water deficits, fluctuations in temperature regimes and breeding grounds caused by climate change, as well as anthropogenic impacts. The increased vulnerability of the animal world in the Republic of Moldova is the result of the low functionality of natural ecosystems. Most natural ecosystems are fragmented, degraded, and polluted. Recently, there has been an intensification of water eutrophication processes in river basins, which has a direct negative impact on the development of amphibian embryonic and larval stages, their growth, reproduction, as well as the ontogeny of other groups of invertebrate and vertebrate animals. Deforestation along riverbanks directly leads to increased water evaporation processes and reduces the ecological capacity of aquatic basins, ponds, or streams to maintain a certain degree of ecological balance - favorable for amphibian development, as well as for maintaining rich aquatic fauna diversity.

To assess the state of amphibian adaptation as a biological resource to climate change, a study was conducted on the relationship between the environment with all its characteristics and the growth and development process of the species *Rana dalmatina* Bonaparte, 1840 (Agile frog), encompassing all stages of ontogenetic development in various natural and anthropized, aquatic and terrestrial ecosystems of the Codrii Centrali region in the Republic of Moldova.

Agile frog is a woodland species, inhabiting marshy areas or habitats with increased humidity, forming isolated and sparse populations in the Central and Northern regions of the country.

As an early breeding species, it becomes active when the environmental temperature reaches +5.8°C, migrating from hibernation sites to summer habitats once specific favorable climatic conditions of temperature and humidity are established. Together, these conditions play a crucial role in the reproductive process: spatial distribution within breeding basins, mate attraction and pair formation, egg deposition, and fertilization.

Dependent on the composition of suitable substrates for egg attachment, sunlit areas, zones occupied by dense aquatic-air vegetation, shrubbery, and arboreal vegetation, they exhibit distinct spatial distributions within breeding aquatic basins. Correspondingly, according to our research, freshly deposited egg masses are found along the bottoms of breeding aquatic basins, at depths ranging between 15 and 45 cm. After hydration, the majority of egg masses (65%) rise to the water's surface. Some of these masses are within the water column (27%), while only a relatively small number (8%) remain free on the basin floor.

Following deposition, the egg masses resemble spheres, with diameters ranging between 6-12 cm, depending on the age of the breeding pair. After hydration, most egg masses appear at the water's surface, with their volume increasing by approximately 3-5 times. While at the water's surface, these masses are subject to direct temperature influence, which accelerates their embryonic development.

In recent years, temporary fluctuations in temperature regime have been increasingly observed in the Central region of the country, particularly in the Codrii Centrali, with values falling below the permissible limit for the development of *Rana dalmatina* egg masses, where the surface layer of water or vegetation partially freezes (Figure 1). Nevertheless, due to the transient nature of spring frosts as limiting factors, the egg masses withstand these unfavorable temperature conditions, and once optimal ecological conditions are established, they resume their embryonic development.

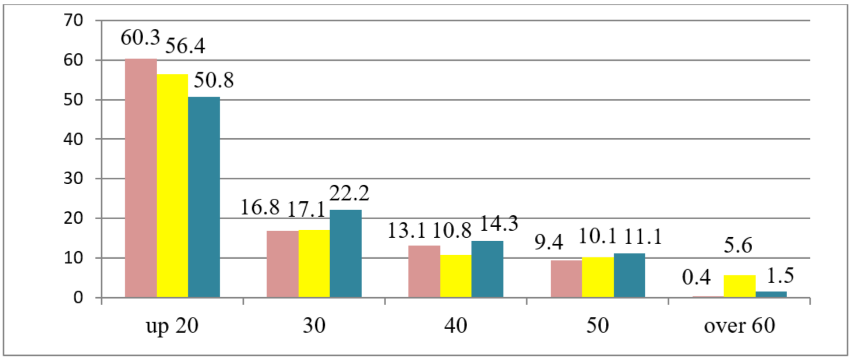
a) b)

Figure 1. The appearance of the temporary aquatic pools for the reproduction of the *Rana dalmatina* species and the phenomenon of short-term freezing in the conditions of the ecosystems of the Forest Codrii.

The spatial distribution pattern of egg masses depends on several natural ecological factors, such as lake size and depth, the presence and distribution of submerged vegetation, solar radiation, water temperature, and the degree of protection of oviposition sites from wind action, among others. These specified ecological factors, through their complex interactions, influence the spatial distribution of egg masses, with their abundance varying from one aquatic basin to another. Following a comprehensive assessment, it was determined that in the ecosystems of the "Codrii" Reserve, the most favorable lakes for species reproduction are lakes No. 4, 5, and 6, where the egg mass density at oviposition stations ranges from 0.7 to 1.4 egg masses/m^2.

According to scientific data from certain authors (Sherbac, Sherbani), in colder zones (mountainous areas) within the species' range, egg masses are deposited in clusters, which, upon full hydration, form true "islands" on the water surface. This grouping strategy of egg masses represents an efficient adaptation to protect the eggs from negative diurnal temperature fluctuations, as it has been established that the temperature within these egg mass clusters is 2-4 °C higher than that of the water in the breeding basins.

As a result of evaluating the placement of egg masses based on water depth in the breeding stations, it was determined that the maximum depth at which egg masses can be found is 0.7 m, with the majority of them (up to 73%) being located at depths of 20-30 cm (Figure 2).



The depth of the ponts

Figure 2. Distribution of ponts of the *Rana dalmatina* depending on the water pool depth.

Among the 142 egg masses of *Rana dalmatina* examined, the number of eggs ranged from 404 to 1560, with the average fecundity of females in the populations from the Codrii Centrali being 784±89.

Given that the population structure of *Rana dalmatina* varies from one aquatic basin to another, as well as the size and age structure of females depositing egg masses in the same breeding aquatic basin, it was observed that the fecundity of females is closely correlated with individual age. Similarly, the numerical quota of egg masses based on the number of eggs contained in them also varies [2].

Therefore, based on the obtained data, it can be noted that based on fecundity, *Rana dalmatina* females are categorized as follows:

(1) females with low fecundity (300-500 eggs) – 29.2%;

(2) females with medium fecundity (501-700) – 37.5%;

(3) females with relatively high fecundity (701-900) – 25.0%;

(4) females with high fecundity (901-1300) – 8.3% (Figure 3).

Subsequently, with the establishment of favorable environmental conditions, when the average water temperature in the aquatic basins ranges between 8.1-9.7 °C, the embryonic development of the *Rana dalmatina* species occurs, but as temperature values increase, larval development also takes place.

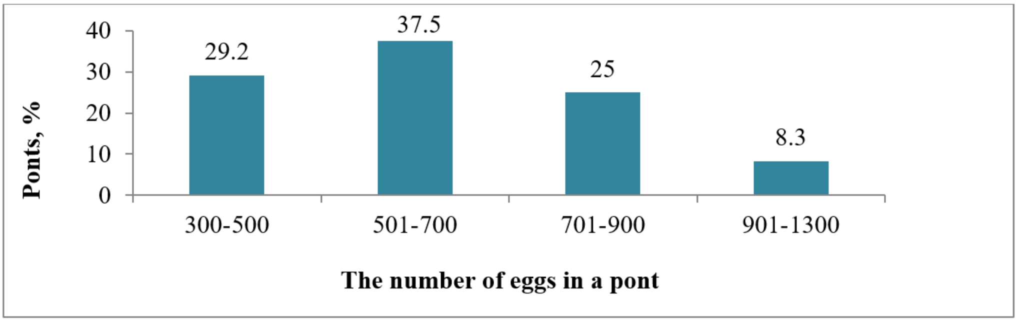


Figure 3. Numerical share (%) of *Rana dalmatina* ponts depending on the number of eggs contained in them (Forest Codrii lake no. 7).

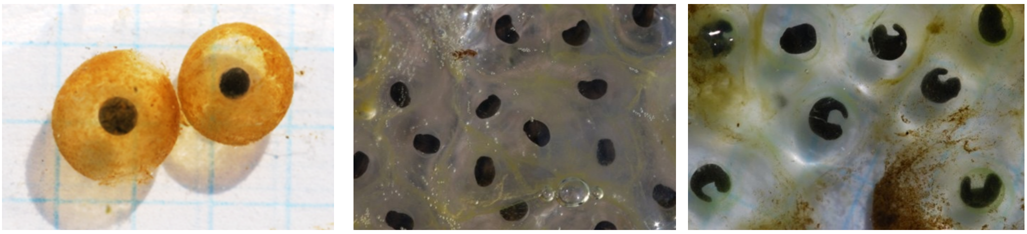
The embryonic development of the *Rana dalmatina* species progresses through the following 3 stages:

(1) **Initial embryonic stage.** At this stage, the eggs are freshly deposited, aged 5-7 hours, and fully hydrated. Morphologically, they have a spherical shape, both the ova themselves and their gelatinous proteinaceous envelope. The diameter of the eggs is 8.3-8.5 mm, including the ova - 2.4 mm, and the thickness of the proteinaceous envelope is 3.0 mm. The eggs are black in color, and their proteinaceous envelope initially appears colorless. However, due to biocenotic relationships within the respective aquatic ecosystem, the proteinaceous envelope of the eggs later becomes covered with microscopic algae, imparting a brownish-green color to them (Figure 4, a).

(2) **Intermediate stage of embryonic development (the "crescent" stage).** Embryos within the eggs are 7-9 days old. As a result of consecutive divisions, the ova elongate slightly and curve, acquiring the appearance of a "crescent." Embryos at this stage have two distinct regions: the head - with small primordia of the gills, and the trunk - with the yolk sac (Figure 4, b).

(3) **3. Final stage of embryonic development (the "pre-hatching" stage).** Embryos at this stage are greatly elongated, curving into a ring shape, so that their tail

tips almost reach their heads. The embryos already exhibit the three distinct characteristic body regions - head, trunk, tail, and are prepared for hatching, which is why this stage is referred to as “pre-hatching.” At this ontogenetic stage, the embryos already perform their first movements within the egg (Figure 4, c).



a) b) c)

Figure 4. The embryonic development stages of the *Rana dalmatina* species in the ecosystems of the Forest Codrii: a – the initial embryonic stage, b – the “crescent” stage, c – the pre-hatching stage.

Depending on the specific microclimatic conditions in the aquatic basins where the egg masses of the *Rana dalmatina* species were deposited, the frequency of embryonic developmental stages was evaluated, and this is depicted in Figure 5.

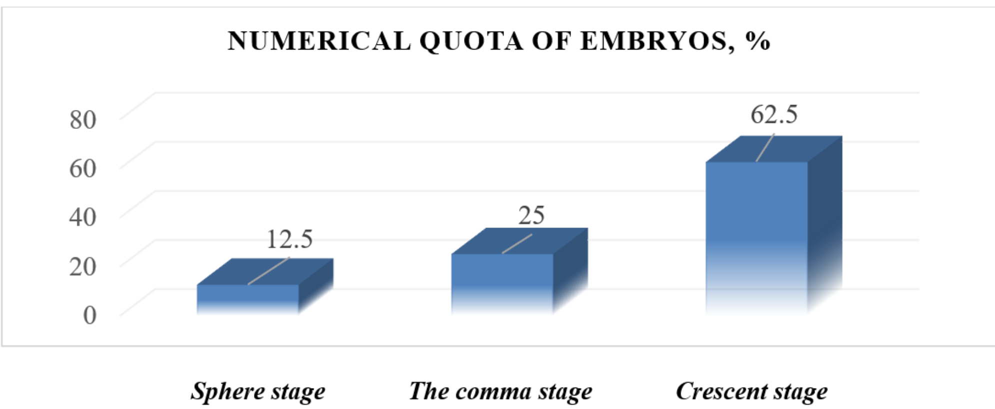


Figure 5. The frequency of embryonic stages of development of the *Rana dalmatina* species in a populations of Codrii Forest.

After the hatching of embryos in the aquatic environment, the larval development process of the species begins, which, under natural conditions, progresses through the following developmental stages:

(1) **Initial larval stage:** Larvae are one day old, with a length ranging from 8.4 to 10 mm (M± m = 9.2 ± 0.3, n = 12). The larval body is distinctly differentiated into head, trunk, and tail, with mean dimensions of 1.1 ± 0.1 (n = 12), 3 ± 0.1 (n = 12), and 5.1 ± 0.3 (n = 12), respectively (Figure 5, a). The percentage ratio of these three body regions is 12%:32.6%:55.4%, indicating that the tail fin is approximately 1.2 times larger than the combined length of the head and trunk. The larvae lack a mouth opening at this stage and are attached to the proteinaceous envelope of the eggs using the prebuccal sucker for nourishment from the yolk sac. Larvae are black in color, while the tail fins appear brown.

The two transparent lobes of the caudal fin are not equal in size: the lower lobe begins near the anal opening, while the upper lobe begins at the posterior end of the head, and is therefore much longer than the lower lobe. This morpho-functional peculiarity gives the larvae a high degree of stability during swimming. The larvae at this stage of development do not yet have a mouth opening, and due to this fact, in the first hours of their existence, the larvae are fixed with the help of the preoral suction cup to the protein coating of the eggs. They feed on the nutrients of the yolk sac. The color of the larvae is black, and that of the caudal lobes – brown.

(2) **Stage of mouth opening and initiation of active life.** The larvae are 2-3 days old post-hatching. Their body length ranges from 11.4 to 12.4 mm (M ± m = 12 ± 0.3, n=12), and the dimensional ratio of "head + trunk : tail" is 29.3%:70.7% or 1:2.4. According to the obtained data, it can be concluded that the length of the tail, crucial for swimming, has significantly increased compared to its length in the initial developmental stage when it was still inactive (Figure 5, b).

(3) **Stage of hind limb appearance:** In this developmental stage, larvae reach their 40th day of existence. Throughout their larval development, their appearance undergoes considerable changes. Their bodies become robust, with large heads and trunks, while the tail fin becomes tall and the caudal trunk very strong, ensuring rapid and maneuverable swimming. Posterior limbs emerge at the anal opening line between the trunk and tail. These limbs are incompletely developed, short, with a length of up to 4 mm, and undifferentiated into thigh, shank, and foot, but with the initial formation of the first three toes. The hind limbs lie flat, oriented along the tail.

The larvae's body length ranges from 40.8 to 41.9 mm (M ± m = 41.6 ± 0.4), with the ratio of the three body regions (**head-trunk-tail**) being 12.6%:24.7%:62.1% (or 1:2:5). In comparison, it is noted that in the initial larval developmental stage, this dimensional ratio was 1:2.7:4.6, indicating an increase in the trunk and tail proportions at the current developmental stage. The color of larvae at this ontogenetic stage becomes gray-silver, and numerous amoeboid-shaped melanin spots appear on the tail fin (Figure 5, c).

(4) **Stage of complete hind limb development:** Larvae at this ontogenetic stage are 62 days old. The hind limbSs have reached full development, possessing all three characteristic regions – **thigh**, **shank**, and **foot**. The shape and orientation of the larvae's limbs are identical to those of mature individuals, differing only in size. The hind limbs are already used for both locomotion (larvae pushing against submerged vegetation, the bottom of the aquatic basin, or other objects on the bottom) and for support on substrates during stationary periods. The larvae's dimensions are 43 mm, with the ratio of the three body regions (head - trunk - tail) being 1:1.4:3.7. On the lateral sides of the larvae's trunk, at the posterior end of the gill chamber, a characteristic concavity appears. In this location, the wall of each gill chamber will subsequently rupture, allowing the release of the fully developed forelimbs, which have completed their development (Figure 5, d).

(5) **Stage of forelimb appearance:** Larvae of the species enter this stage of development on the 63rd day of existence. The length of larvae at this stage coincides with the length of larvae from the previous stage of development ($M \pm m = 43 \pm 0.2$, $n = 13$). However, in addition to the fully developed hind limbs (thigh length = 5 mm, shank length = 6.4 mm, foot length = 9.2 mm), fully developed forelimbs are also present (arm length = 3.1 mm, forearm length = 4 mm, hand length = 2.1 mm). Larvae at this stage of development closely resemble future juveniles: they represent a transitional stage from a true larva (which has a tail) to a true juvenile (which has both pairs of limbs) because, at the same time, they have both a tail and limbs. The dimensional ratio between the three body regions at the stage of forelimb appearance – **head** - **trunk** - **tail**, is 5 mm:10 mm:28 mm or 1:2:5.6. At this ontogenetic stage, individuals already exhibit morphological features specific to adult forms of the species: their color is brownish, the hind limbs are long and graceful with dark oblique stripes on the thigh and shank, on the dorsal side of the trunk, they exhibit the two characteristic dermal folds extending from behind the head to the hind limbs, and on the back, at the level of the forelimbs, there is a characteristic pattern resembling the letter "V" (Figure 5, e).

(6) **The stage of metamorphosis and emergence of juveniles onto land** (Figure 5, f). At this stage, the larvae are 63-65 days old (between June 9-11) and are characterized by radical morpho-physiological transformations associated with

the reorganization of certain larval organs and organ systems into adult ones. Externally, only the reduction of the caudal fin is noted, replaced by the presence of a "caudal bud" measuring 1.5-2.5 mm. Additionally, it is observed that the juveniles already emerge to the water surface and swim freely on its mirror, or they stay on emergent vegetation, indicating their transition from branchial to cutaneous and pulmonary respiration. Gradually, the juveniles concentrate in the shoreline area, where they position themselves at the "water-land" demarcation line or stay on aquatic-aerial vegetation in well-insolated aquatic basin sectors. Juveniles exhibit species-specific coloration, with dimensions ranging from 13.2 to 16.1 mm (M ± m 15.1* ± 0.2, N = 17), depending on the functional state of the given ecosystem.

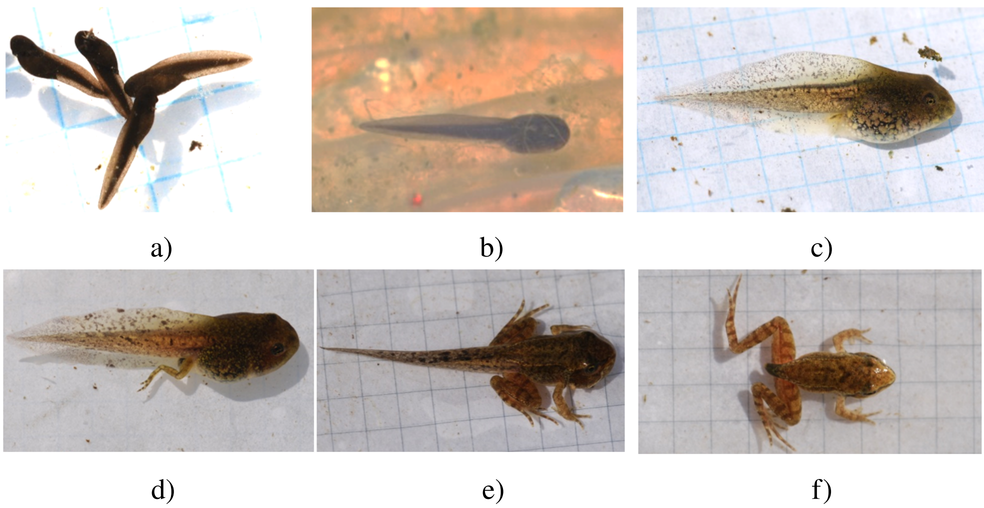


Figure 6. The larval development of the *Rana dalmatina* species in the Forest Codrii area.

Due to the species *Rana dalmatina*'s secure adaptation to environmental conditions, presently within our country, however, its population size is not reduced solely because of temperatures falling below the permissible threshold during oviposition. Additionally, abrupt increases in temperature during larval development directly contribute to the evaporation of water from small habitats crucial for the reproduction and development of the species, or even complete desiccation (Figure 7).

Therefore, the embryonic and larval development of the species *Rana dalmatina* in the conditions of the Republic of Moldova is determined by the direct and continuous influence of inappropriate climatic factors, which represents an increasingly significant

a) b) c)

Figure 7. Temporary breeding pools, the phenomenon of heating and evaporation of water.

risk factor for the population structure of the species and, overall, a threat to amphibian diversity.

3. Conclusions

The swiftness of the direct influence of climatic factors on biodiversity varies greatly in different regions, with amphibians, in particular, demonstrating a high degree of adaptability. Moreover, the impact of these factors is challenging to assess by scholars, although science in recent times is characterized by the most advanced research methodologies and equipment.

The fire-bellied toad exhibits a certain degree of morpho-physiological and eco-ethological specialization, ensuring its existence in various environmental conditions with the most unfavorable fluctuations in temperature and water regime during embryonic and larval development.

In the context of the aforementioned, conducting detailed and comprehensive research on the embryonic and larval development characteristics in various aquatic ecosystems, both natural and anthropogenic, of the species *Rana dalmatina* is crucial for the sustainable management and protection of populations of this amphibian species in relation to the succession of natural ecological factors.

Acknowledgements

The article was carried out within the framework of the project "Evaluation of the structure and functioning of biocoenoses, aquatic and terrestrial habitats under the influence of biotic and abiotic factors in the context of ensuring ecological security and population well-being" 010701 and within the project "Helminth fauna of amphibians (Amphibia), their importance as vectors in *the formation and maintenance of parasitic zoonoses" 23.00208.7007.05/PD II and the project no. 01-23p-096/03-05-2024.*

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Received: April 25, 2024 *Accepted: July 15, 2024*

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