AN OVERVIEW OF THE *RANA ESCULENTA* LIFE CYCLE IN WESTERN ROMANIA

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Abstract. *This study highlights the ecological significance of Rana esculenta as a key bioindicator species, essential for assessing environmental health and understanding the impacts of habitat degradation. The research examines the developmental stages of Rana esculenta tadpoles under controlled laboratory conditions, with a specific focus on crucial environmental factors such as temperature, water quality, and food availability. Eggs were collected from two sites in Romania and maintained in environments that closely simulated natural conditions. Over a seven-week observation period, important milestones were recorded in limb formation, organ maturation, and behavioural changes, providing detailed insights into the influence of environmental conditions on tadpole development. Results indicate that optimal temperature, between 20 and 23°C, along with high water quality, were vital for successful metamorphosis. By day 53, the majority of tadpoles had fully transitioned into juvenile frogs, displaying characteristic adult morphology of Rana esculenta. The findings underscore the critical role of maintaining healthy aquatic habitats, as poor water quality or limited food availability can negatively impact the survival and growth of tadpoles. This research contributes valuable data for conservation efforts, shedding light on the specific ecological requirements for Rana esculenta and emphasizing the species' sensitivity to environmental changes. Moreover, the study reinforces the broader role of amphibians as bioindicators, with their developmental success serving as an indicator of ecosystem health. As amphibians respond acutely to pollution, climate shifts, and habitat alterations, monitoring species like Rana esculenta can aid in detecting environmental stressors early. Ultimately, these findings support informed conservation strategies and the sustainable management of natural habitats, helping to preserve biodiversity and the overall health of ecosystems.*

Keywords*:* frog development stages, tadpole, *Rana esculenta*

INTRODUCTION

Amphibians, particularly frogs, play crucial roles in maintaining ecological balance and are considered key bioindicators of environmental health. The edible frog (*Rana esculenta*), a widespread species in Europe, is of particular interest due to its unique reproductive biology and hybridization mechanisms (Graf & Müller, 1979). As amphibians are highly sensitive to environmental changes, their populations can reflect shifts in habitat quality, water pollution, and climate change (Beebee & Griffiths, 2005). This makes them valuable subjects for environmental monitoring and conservation efforts (D'Amen & Bombi, 2009).

Frogs, including *Rana esculenta*, exhibit a complex life cycle that includes distinct stages: egg, tadpole, juvenile, and adult (Duellman & Trueb, 1986). Each stage is marked by specific physiological and morphological adaptations to aquatic and terrestrial environments (Wells, 2007). The metamorphosis from tadpole to adult frog is a critical process governed by hormonal changes, especially the role of thyroid hormones, which regulate the development of limbs, lungs, and other vital organs necessary for the transition from an aquatic to a terrestrial lifestyle (Kikuyama et al., 1993).

The conservation status of amphibians has become a growing concern, with nearly one-third of species worldwide threatened by habitat loss, climate change, pollution, and disease (Stuart et al., 2004). Recent studies have highlighted the vulnerability of amphibian populations to environmental stressors such as chemical pollution, habitat fragmentation, and the introduction of invasive species (Collins & Storfer, 2003). In particular, *Rana esculenta* populations have been shown to decline in areas with increased water contamination, which can disrupt both reproductive success and larval development (Kiesecker et al., 2001).

Research on *Rana esculenta* also emphasizes its unique reproductive system. This species is part of a hybridogenetic complex involving *Rana lessonae* and *Rana ridibunda*, where hybrids reproduce clonally while maintaining the genetic material of one parental species (Berger et al., 1978; Uzzell et al., 1975). This reproductive strategy offers interesting insights into evolutionary biology and species conservation, as hybridogenesis allows these frogs to adapt to varying environmental conditions while maintaining genetic diversity (Hotz et al., 2001).

The study of amphibian larval development, particularly in *Rana esculenta*, is essential for understanding the impacts of environmental variables on their life cycle. Tadpoles require specific water quality, temperature, and food resources to successfully complete metamorphosis (Alford, 1999). Disruptions in these conditions due to anthropogenic influences, such as agricultural runoff or urban development, can result in abnormal development or increased mortality rates (Blaustein et al., 2003). Thus, understanding the ecological needs of tadpoles and their responses to environmental changes is critical for the development of conservation strategies (Carey & Alexander, 2003).

In this context, the present study aims to investigate the development of *Rana esculenta* tadpoles under controlled laboratory conditions, focusing on the factors influencing their growth and metamorphosis. By examining the influence of temperature, water quality, and food availability, this research seeks to contribute to the broader understanding of amphibian developmental biology and highlight the ecological requirements necessary for the conservation of *Rana esculenta* populations.

MATERIAL AND METHODS

The edible frog (*Rana esculenta*) spawn was collected from two distinct sites in Romania: The first site was an artificial pond located in the courtyard, in the city of Timisoara. The pond is inhabited by both fish and *Rana esculenta* frogs. The second site was a reservoir in the locality of Dumbravita . This reservoir supports a diverse ecosystem, housing a variety of aquatic and terrestrial organisms, including fish, amphibians, and birds.

Figure 1 show the collection process at the Timisoara site, where the spawn was placed in a transparent container and transported to the laboratory.

Figure 2 illustrates the collection of tadpoles from Dumbravita, similarly transported to the lab in suitable containers.

Upon arrival at the laboratory, the collected spawn and tadpoles were housed in controlled environments designed to mimic their natural habitats. Aquatic plants and stones were added to their containers to recreate the pond conditions. The water temperature was maintained between 20-23°C, and non-chlorinated water, mainly rain and spring water, was used to prevent stress or mortality among the tadpoles.

The laboratory containers were of adequate size to ensure tadpole mobility, and the water was regularly changed to maintain cleanliness and quality. The tadpoles were fed a diet of chopped grass and lettuce to facilitate their growth. Throughout the experiment, their well-being was carefully monitored, and all necessary precautions were taken to maintain optimal living conditions.

The experiment lasted for a period of seven weeks, during which the developmental stages of the tadpoles were closely tracked. Key morphological features, such as size, limb development, and behavioral changes, were observed and recorded at various intervals.

Figure 1. The collection of spawn at *Rana esculenta* from Timisoara

Figure 2. The collection of tadpoles from Dumbravita

RESULTS AND DISCUSSIONS

The development of *Rana esculenta* was carefully monitored in a controlled environment, tracking significant morphological and physiological changes throughout the metamorphosis process. The data collected provides insight into the progression from hatchling to juvenile frog, highlighting key stages of growth, the emergence of critical anatomical structures, and physiological adaptations necessary for survival.

On Day 1, the eggs of Rana esculenta hatched**,** releasing tadpoles that closely resembled small fish. These early-stage larvae were aquatic, with no visible limbs and simple internal structures **(**figure 3). The primary focus during this period was on growth and the establishment of basic respiratory functions through gills.

Figure 3: The spawn at *Rana esculenta*

Between days 3 and 6, the early stages of tadpole development begin, marking a critical phase in the transformation from embryo to juvenile frog. During this period, significant internal changes are observed as the tadpoles initiate the development of their internal organs, which are essential for their future growth and survival. By Day 6, the tadpoles have grown to a length of approximately 5 mm, showing noticeable progress in size.

Although they still resemble fish due to their elongated, streamlined bodies and lack of limbs, subtle morphological changes start to become apparent. This includes the initial formation of vital organs, such as the heart and digestive system, which are crucial for sustaining life and supporting the metabolic demands of rapid growth. The respiratory system also begins to take shape as the gills develop, allowing the tadpoles to breathe underwater.

Externally, the tadpoles retain a simple body structure, with a prominent tail used for swimming. However, the first signs of their future transformation begin to emerge. Their overall body shape remains primitive, designed for aquatic life, but the foundations for later, more complex changes are already being laid. As the tadpoles continue to grow, these early morphological and behavioral adaptations will eventually enable them to transition into their adult amphibious form, equipped for survival both in water and on land.

This developmental phase, although still early, is crucial for the successful progression of the tadpoles through their life cycle, setting the stage for the dramatic changes that will occur in the following weeks.

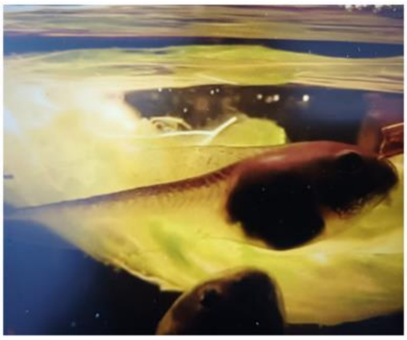
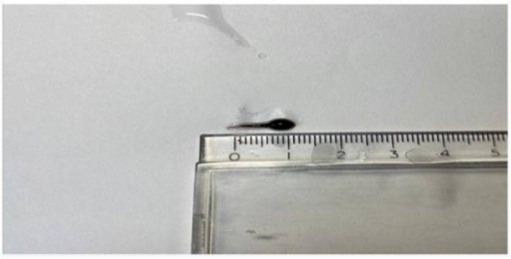


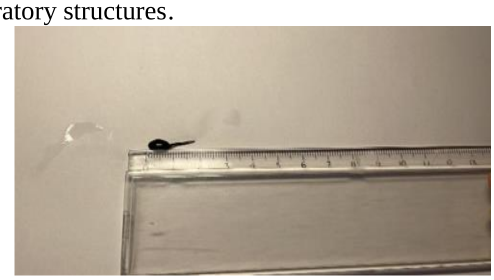
Figure 4. The tadpole is developing its internal organs

By Day 10, the tadpoles had grown to approximately 12-14 mm in length (Figure 5. a), demonstrating significant growth since hatching. During this period, the development of internal organs advanced, including the digestive, respiratory, and nervous systems. The tadpoles continued to actively feed, which supported their rapid growth and organ maturation. Their physical form, although still aquatic, began to show the first signs of differentiation, as they were now distinct from their earlier fish-like appearance.

Between Days 16 and 24, the tadpoles continued to grow, reaching a length of approximately 20 mm (Figure 5.b). The development of hind limb buds became evident, marking the beginning of the morphological transition from a larval form to a juvenile frog. These buds indicated the imminent development of functional hind legs, essential for their eventual adaptation to terrestrial environments.

On Day 26, the tadpoles' lungs began to form, allowing them to prepare for life outside the water. The concurrent development of both hind limbs and lungs represents a crucial point in the tadpoles' metamorphosis. By the end of this period, the tadpoles had not only increased in size but also started the complex process of transitioning to a more amphibious form, with limbs and improved respiratory structures.





a b

Figure 5: The tadpole a) at 10 days and b) at 16 - 24 days

Between Days 32 and 45, the tadpole undergoes significant and visible developmental changes. At this stage, it exhibits clear signs of growth and maturation, as key anatomical features begin to take shape. The mouth, gills, and eyes are now fully visible, reflecting the advancement in the development of its respiratory and sensory systems. These features are crucial for the tadpole's ability to interact with its environment, feed more effectively, and respire efficiently in its aquatic habitat.

One of the most noticeable changes during this period is the formation of the hind limbs, which begin to develop (as shown in figure 6). This is a critical step in the tadpole's transformation, as these limbs will eventually allow it to transition from a purely aquatic life to a more amphibious existence. The appearance of the anal opening further indicates the maturation of the excretory system, showing that the tadpole is progressing towards a more complex and functional physiological state.

These developmental milestones are clear indicators that the tadpole is transitioning from its earlier stages of life. The formation of the limbs, along with the visibility of sensory and respiratory organs, signals the rapid approach of metamorphosis. In the upcoming days, the tadpole will continue to undergo even more dramatic changes, as it prepares to become a fully developed frog capable of thriving both in water and on land.

Figure 6. Highlighting of the mouth, anal openings, eyes, hind limb buds, and gills

At this stage, the tadpoles' overall growth had reached a point where they no longer resembled their earlier aquatic form. The emergence of hind limbs and the beginning of forelimb development marked their nearing metamorphosis into juvenile frogs (figure 7).

On Day 47, the tadpoles underwent a critical phase of metamorphosis, during which the forelimbs became fully developed. At this point, the tadpoles had a more frog-like appearance, with distinct limbs that allowed for movement both in water and on land. The tadpoles also showed a shift in diet, beginning to consume small insects and plants, indicating the transition to a more diverse diet typical of adult frogs.

Figure 7. The development of the hind limbs

By Day 53, the metamorphosis was nearly complete. The tadpoles had transformed into juvenile frogs, with fully developed limbs, lungs, and a significantly shortened tail (Figure 8).

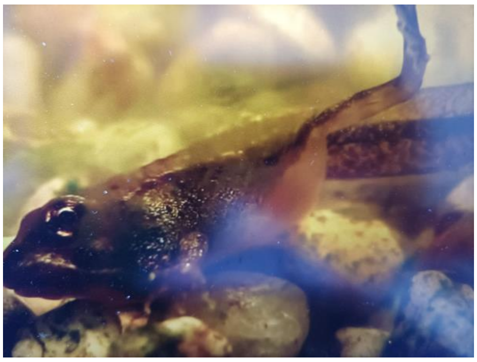


Figure 8: Metamorphosis in *Rana esculenta*

The disappearance of the tail indicated that the transition from an aquatic to a terrestrial environment was nearly complete. The frogs had fully adapted to their new habitat, capable of living both on land and in water environment.

DISCUSSIONS

The results of this study demonstrate the complex and highly regulated process of Rana esculenta metamorphosis. The growth and development of tadpoles, from early organ formation to full limb development, highlight the critical stages required for their successful transition from an aquatic to an amphibious lifestyle. The observation of internal organ development, followed by the formation of hind and forelimbs, aligns with existing literature on amphibian metamorphosis. The development of lungs and the shift in diet are key indicators of the adaptation to a terrestrial life. These findings contribute to our understanding of the physiological demands placed on amphibians during metamorphosis and how these organisms manage the transition between vastly different environmental conditions.

Research could explore the environmental factors, such as temperature and water quality, that might influence the timing and efficiency of these developmental processes. Understanding these factors may provide insights into how amphibians, like *Rana esculenta*, respond to changing ecosystems and what conservation efforts are necessary to protect their habitats.

CONCLUSIONS

The evolutionary cycle lasted 7 weeks for the tadpoles to develop into juveniles. Observing this experiment has led to a better understanding of the biological processes involved, helping to identify factors that can influence their development (temperature, diet, water quality, and environmental/anthropogenic factors). These findings contribute to our knowledge of the vulnerabilities and risks faced by these animals.

Frogs play a crucial role in ecosystems and are often considered bioindicators. This means they can provide valuable information about the health, quality, and pollution of the ecosystems they inhabit. They are sensitive to environmental changes, particularly changes in water quality and habitat, as well as to climatic changes.

RECOMMENDATIONS

The protection and conservation of frogs is essential. Preserving their natural habitats is vital; habitat destruction and human impact must be avoided. Reducing pollution, especially chemical pollution in aquatic environments, is crucial. Protecting breeding sites is particularly important for species survival. Educating the public about the role of frogs in nature and continuing scientific research are key aspects of conservation efforts.

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