

## OBSERVATIONS ON THE LIFE CYCLE AND REPRODUCTIVE BEHAVIOR IN *RANA DALMATINA* F.

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**Key words:** protected species, embryonic, larval and post-larval development, experimental conditions

### INTRODUCTION

Amphibians are a group of unique animals, oviparous and poikylothermic of different sizes. There are organisms that inhabit terrestrial and aquatic ecosystems but being involved in aquatic food chains by both food consumption and the fact that they are food for other animals. There are animals with naked skin, rich in glands and allowing their heavily vascularized aerated water and air exchanges both in the larval stage and as adults. Pulmonary respiration in adult and larval stages brachial complements the skin breath. Avoid areas of excessive dryness and are less active in the days without precipitation.

In the past 20 years scientists have reported global decline of amphibian populations. In 1996, the International Union for Conservation of Nature has made the red list, 156 species of amphibians, and recent data show that currently are listed over 2000 species (about 38% of all species of amphibians).

*Rana dalmatina* F. or agile frog is a relatively abundant species. Common species, mostly in Central Europe, its area of distribution extends from western France to the Balkan peninsula, isolated populations are present in Germany, Denmark and southern Sweden but is also present in northern Turkey. Missing from the Iberian peninsula and the northern countries of the continent.

Although it is often in deciduous forests of the lowlands and hills, their population is declining species being declared a strictly protected - as nomination of Annex II of the law 13/1993 on Romania's accession to the Convention on wildlife and natural habitats of Europe adopted on Bern on 19/09/1979 - primarily due to habitat destruction, this including: deforestation (pronounced in recent years), fragmentation of aquatic and terrestrial habitats; and pollution (including acid rain), climate change and overexploitation.

### Systematic classification:

Kingdom *Animalia*  
Vertebrate phylum  
Class *Amphibia*  
Order *Anura*  
*Ranidae* family

The genus *Rana*

Species *dalmatina* (Fitzinger, 1839 and Bonaparte, 1840)

Synonyms *Rana agilis* (Thomas, 1855)

### Ecology, dispersal, habitat

*Rana dalmatina* - agile frog or toad jumping, has a slender shape, elegant, is a medium sized frog, male (about 6 inches) lower than the female (8-9 cm). Body and elongated snout, large eyes with pupil horizontal eardrum is smaller than the eye and clearly outlined, located behind and below it. Vomer teeth are distributed in two oblique rows. Skin color varies slightly from specimen to specimen but is uniform. Both males and females have a dorsal yellow-gray, yellow-red to dark brown uniform or sprinkled with small black spots. The hind limbs are present broad strokes, transverse darker. Ventral coloration is white uniform except goiter and chest sometimes colored edges.

Sexual dimorphism in this species is not very pronounced. The male has no vocal bags, based on previous thumb has a rounded tubercle, if not present, it is replaced with a small white spot, and during rough gray reproduction.

When the hind leg is stretched forward, tibio-tarsal joint exceeds tip of the snout (Figure 3), all fingers shows large subarticular tubercles very prominent, metatarsial pronounced tuber, smooth skin.

In Romania, *Rana dalmatina* F. is a species widespread in almost all hardwood (such as oak forest, beech, hornbeam, ash, etc...), to an altitude of 900 m, with water. Not found in places such as arable land without trees. Lead a shadowy but during breeding periods are active both during the day and night. More terrestrial species some individuals just entering the water only for coupling. Anura very agile sometimes capable of jumping up to two meters in length, enter hibernation in late October to February-March, when the snow is just beginning to thaw. This species of amphibians being the first oviposition. Find small ponds in the forest or its outskirts. Amplexus is axillary and clock has a pie and attached to tree branches, roots or stems of the plants to a depth of more than 50 cm. Unlike other

brown frogs, *R. dalmatina* rarely sinks to the bottom of the pond if it is deep.

Important link in the food chain, *R. F. dalmatina* feeds on insects on the ground but in turn are food for other predators including humans.

### **Life cycle and reproductive behavior**

*Rana dalmatina* adults out of hibernation in late February early March, immediately after snowmelt and puddles gather reproduction. Mating usually takes 20 days depending on the weather, but if temperatures drop it is interrupted. Males are the first to fall in the water, only females around clutch and leave the pool immediately after submission.

Male voice is not very strong, due to lack vocal sac, the song takes up to 12 seconds and can be reproduced and thus underwater hear a very short distance.

During mating males went to sing in the choir at the water surface to attract females breeding pond.

Sometimes used for breeding pools and temporary ponds that dry very quickly, not to allow larval metamorphosis. Often reproduce its entire population of adults in one go, making clusters of hundreds of individuals.

Amplexus is axillary and clock consists of a single lot of eggs externally protected by a gelatinous mass measuring 10 to 15 cm. Ponta is glued to the branches, roots and / or stems of aquatic plants. A lot can count between 500 and 2,000 eggs they deposited the night.

The diameter of a single egg jelly SC is 1.5 - 2.5 mm.

Tadpoles are large and may reach 3.5 cm before metamorphosis, some specimens being even higher. The anus is located near the insertion point of the median tail. The tail is high and ends in a sharp point. The dorsal color is light brown with brown spots. The belly is white with golden spots that close and sides. The upper portion of the tail are black spots often present.

### **MATERIAL AND METHODS**

The biological material was represented by approximately 200 *Rana dalmatina* F. eggs collected from one breeding ponds Gadinti the commune, Neamt County, with landing net (Figures 1,2,3,4,5). Eggs were collected between March 2014 and then were transported to the laboratory.

In the laboratory eggs were placed in a container of about 5 liters of water station about 48 hours.

Observations were made live on:

- Embryonic development,
- Larvre development,
- Behavior in captive conditions larvae and tadpoles,
- Tadpole metamorphosis.

Throughout the experiment, the observations were noted and there have been numerous photos macroscopic.

Embryo observation observations were made digital microscope magnifying glass and prepared fresh and canned, the zygote and larvae. The images were then processed by computer.

### **RESULTS AND DISCUSSIONS**

#### **Embryonic development**

Many of the eggs collected were already transformed into embryos which shows that the clock was made about a week (Figures 6,7). In the laboratory, the container was sitting near a window and the membrane of the egg jelly-green algae have been invaded by favoring oxygenation.

After about three days a part of embryos began to hatch, they tried to attach to the walls of the container where they were by means of the adhesive body. And other specimens of larvae sought to sit sideways on the substrate. Figures 8, 9 can be seen the emergence of separate protuberances of gills and eyes, and in Figures 8 and 10 are observed neurulei small external gills.

*R. dalmatina* embryos were observed involuntary muscle spasm (Figure 11 A and B). These involuntary contractions occur even when the embryo in the egg.

After the larvae hatch they have a uniform dark color on the back and almost white on the ventral side. You can see the eyes and external gills. Mouth still not formed larva measures about. 9 mm (Figures 12, 13, 14,15,16, 17, 18, 19).

After about 30 hours after hatching, the larvae are completely transformed: no visible external gills appears pigmentation (fig. 20), intestine spiralizeaza (Figures 21, 22, 23), mouth full form (fig. 24) and the larvae begin to feed on vegetation in the water.

To see what specimens grow faster, larvae, approx. 30 hours after hatching were separated into plastic cups 20, one for the first ten cups and two for the next ten (Figure 27). Maintained and population control in containers of 5 liters (Figure 25).

Larvae of plastic cups were fed with fish food ( 33% crude protein, 4% crude fat) from first day of separation (Fig. 26). Population control was left to feed on algae and egg membrane remaining after all larvae hatched, and after completing this type of food was supplemented with fish food.

Larvae were fed every three days and the water was partially changed at one week and three weeks total, so the control specimens and those of glasses. Laboratory temperature was constant between 18 and 25 C.

From observations made during the first two weeks, the increase was relatively constant at all larvae. In the three weeks we have seen an increase in advanced of 1-2 mm larvae separate glasses. Larvae found in glass by two started but have

territorial and cannibalistic, lack of space, killing and consuming smaller ones. Of the 10 glasses with two larvae, in the fourth week there were only one that survived both larvae *R. dalmatina*.

### Metamorphose

After about four weeks transformations occur expressing preparing the transition to terrestrial life. Changes occur in nicel head (Figures 28,29,30) and mouth) Figures 31,32). Hind the larvae occur in the tail that grows continuously for one week (Figures 33, 34). With the emergence of forelimbs frog hurry to dry out so going to pulmonary respiration (Figures 35, 36).

After metamorphosis, the animal is carnivorous and feeds only on living prey. Also the shape of the body is altered, gaining the appearance of frog, the tail is reabsorbed until it disappears completely (Figures 37, 38, 39). Intestine, the larva which was long and twisted spiral, undergoes a reorganization and its length is reduced to one-sixth that of the larva.

### After metamorphosis

Larvae that were metamorphosed were placed in containers in which to put some water and a layer of moss forest (Figures 40, 41). Frogs began to be fed with insects collected from the grass using entomological net. This species of frog lives about 10 years and reach sexual maturity after 3-4 years of life.

Part of metamorphosed specimens of *R. dalmatina* were released into the forest at Gadinti, close the breeding ponds.

### CONCLUSIONS

Embryonic development dalmatina F. *Rana* is a spectacular process. His study in laboratory conditions can provide valuable data about the conditions of their embryonic, larval and post-larval important for understanding and conserving this species of amphibians.

The study of embryonic development in agile frog allowed accurate determination of the length staging, timing and type of conversion factors and their influence on the development of the zygote, embryo and larvae in laboratory conditions

Embryonic development, larval and post-larval (metamorphosis) of frogs of the genus *Rana dalmatina* F. can be traced easily in the laboratory and low consumption of materials.

Habitat destruction, pollution and deforestation, leading to a rapid decline of the species due to its sensitivity, there is danger of extinction. So jumping frogs are protected by law being declared a strictly protected species.

### ABSTRACT

This paper presents a study of embryonic development, larval and post-larval (metamorphosis) of the frogs of the species *Rana dalmatina* F. (agile frog) under laboratory conditions. This species has a shrinking population and is declared strictly protected species. Egg collection was made in one of the breeding ponds Gadinti the commune, Neamt County, March 2014. Study, conducted with low material brings valuable data about the conditions of their embryonic, larval and post-larval and possibility of increasing laboratory frogs important aspects for understanding and conserving this species of amphibians.

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Fig. 1. *Rana dalmatina* F.♂ dorsal seen



Fig. 2. *Rana dalmatina* F.♂ ventral seen



Fig. 3. *Rana dalmatina* ♂ with the hind leg stretched forward



Fig. 4. *R. dalmatina* ♀ in the her life



Fig. 5. Puddle of breeding for *R. dalmatina*



Fig. 6. *R. dalmatina* clock - Stadia different egg development. - embryos and neurula



Fig. 7. *R. dalmatina* egg



Fig. 8. Advanced neurula



Fig. 9. Detail neurula: emergence protuberances in the gills and eyes



Fig. 10. Detail neurula: small external gills observed neurulei.



Fig. 11. A - *R. dalmatina* neurula with involuntary muscle response - stage 1



Fig. 11. B - *R. dalmatina* neurula with involuntary muscle response – stage 2



Fig. 12. *R. dalmatina* tadpoles side view



Fig. 13. *R. dalmatina* - dimensions tadpoles



Fig. 14. External gills of larva



Fig. 15. Detail - Eye larva immediately after hatching

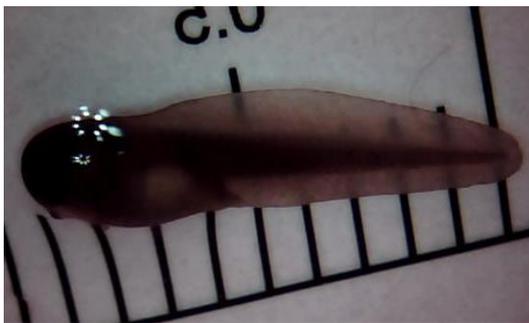


Fig. 16. Larva - overview



Fig. 17. The appearance of the intestine



Fig. 18. Detail - tail of larvae immediately after hatching



Fig. 19. Larva seen dorsal

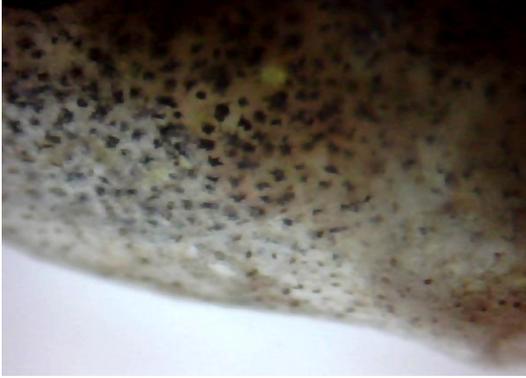


Fig. 20. Larval pigmentation



Fig. 21. Protective membrane formation

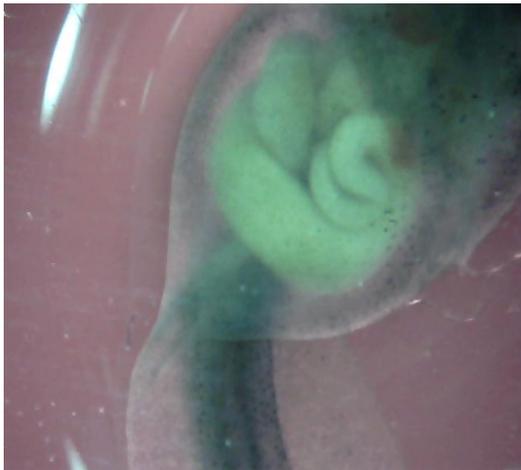


Fig. 22. Formation of the tadpole intestine



Fig. 23. Spiraling intestine

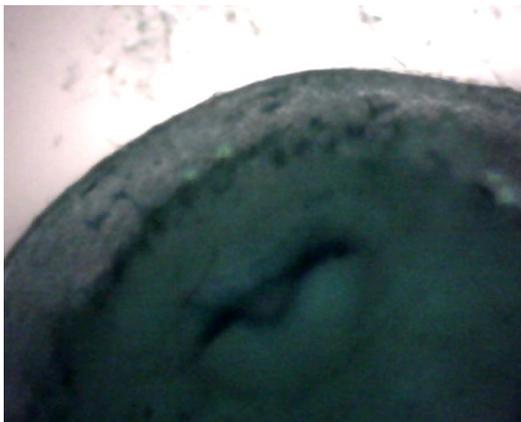


Fig. 24. Training mouth to larva



Fig. 25. Control population; can be observed varying sizes of larvae



Fig. 26. Aspects of feeding



Fig. 27. Glasses with larvae used in the experiment



Fig. 28. Details of the head, head shape, eye



Fig. 29. Head and eye, side view



Fig. 30. Head latero-ventral view

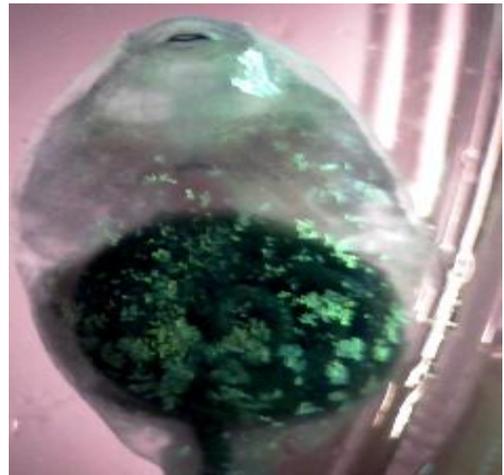


Fig. 31. Detail of spiral-shaped gut and mouth (beak and vomer teeth)

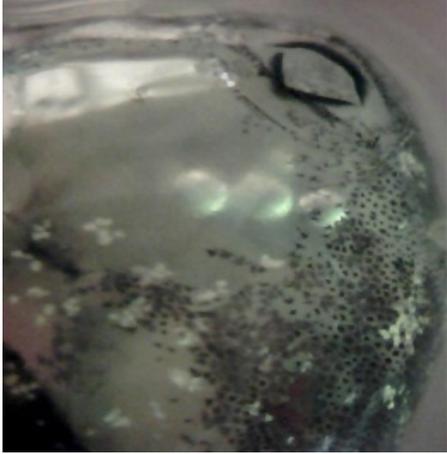


Fig. 32. Mouth (beak and vomer teeth)



Fig. 33 Hindlimb appearance in tadpoles.



Fig. 34. Hindlimb appearance in tadpoles - detail



Fig. 35. Forelimb emergence

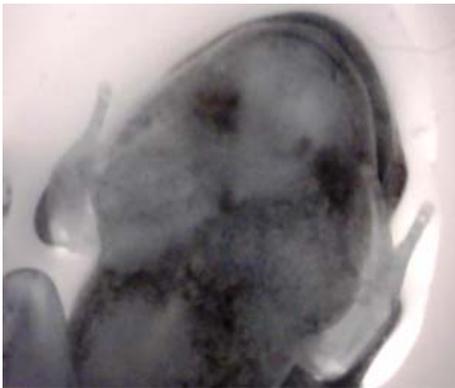


Fig. 36. Forelimbs appearance - more advanced



Fig. 37. Tail resorption – early stage



Fig. 38. Tail resorption - advanced



Fig. 39. Complete metamorphosis



Fig. 40. Young frogs



Fig. 41. *R. dalmatina* ♂ - adult