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THE CONSERVATION AND DECLINE PREVENTION OF SPECIE *HYLA ARBOREA* (LINNEAEUS, 1758) – THE EUROPEAN TREE FROG – THROUGH ITS GROWTH IN CAPTIVITY

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***Key words*:** *Hyla arborea,* embryo development, behaviour in captivity

INTRODUCTION

Hyla arborea is the only member of tropical tree frog family that are indigene in Europe. The specific characteristics of this family are the adhesive discs placed at the finger terminations, which are used when they climb in the trees.

Although he can be found often in the nature, their population decreased in the last years, mainly due to the destruction of their habitat, here being included: deforestation (especially in the last years), fragmentation of terrestrial and aquatic environments, but also due to pollution (inclusive acid rains), climatic modifications and overexploitation.

For survival, like the majority of amphibians, he needs adequate marsh and puddles, surrounded by a terrestrial habitat with good quality.

The study regarding the growth of green frog, *Hyla arborea* in captivity, as well as the type of habitat that he prefers for living and reproduction can contribute substantially to knowledge and conservation of this specie and to the prevention of its decline.

Hyla arborea or *the European tree frog, green frog* is an anure of small dimensions, the length of an adult being of almost 5 am. The head is more large than long, the snout is short and rounded, lateral eyes, visible tympanum. The members are long, with adhesive discs at finger top, the anterior fingers slightly palmate at base, the first one being shorter than the second, the posterior ones palmate. The skin is smooth, dorsal shinny, ventral sabulous (except with the neck at male). A black or brown stripe starts from the nostril until the femure base, in the lombar region it forms a curl orientated up.

The male has a big vocal sac, dark – brown or yellow – brown, under his neck; when is empty it seems like a skin fold. The croaking is loudly, being heard from big distances (almost 5 km). It is formed from a series of "crac", 3-6 per second, during few seconds. The frogs don't have nuptial callosities (fig. 1).

The female doesn't have vocal sac, and despite the male, presents granulations also on its neck, their colour being gray-violet. (fig. 2).

THE BIOLOGIC MATERIAL

Thee biologic material is represented by 2 ♂♂ and 2 ♀♀ of frogs from the specie *Hyla arborea* collected around the village Gâdinți, Neamț district, in the spring of 2009 year.

WORKING METHODS

a) Collection of material

The exemplars of frogs were captured with the hand and introduced in jars adequate for transportation. In the lab the frogs were placed in an aquarium with a maximum capacity of 45 litres, in which we introduced a plastic recipient with water and plants in order to mimic the natural reproduction medium. After reproduction and eggs overlay, the adult exemplars were released in the same place where they were collected.

b) Observations:

In aquariums, we made observations regarding the behaviour of green frog - *Hyla arborea.* We concentrated mainly on the following issues:

- Mating at *Hyla arborea*
- Eggs overlay
- Embryo development
- Larva development
- Behaviour in captivity

For the observation of embryo and larva stages of development we utilised the binocular microscope both for fresh and conserved samples. The most significant images were photographed, the images being than processed with the computer.

RESULTS AND DISCUSSIONS

Growth in captivity

Eggs overlay. The pairs of *Hyla arborea* and mating are presented in fig. 3 and 4.

♀♀ of *Hyla arborea* overlay 10 packs of eggs during one week (some stick to vegetation, other directly in water); (fig. 5 and 6).

The egg is round, surrounded by two gelatinous membranes (fig. 7). When the eggs get in contact with the water, they rapidly bloat, thus offering protection to the newly appeared egg against the mechanical shock. Also, the external membrane is very sticky, the eggs packs being able to fix easily on the vegetation.

After the adults were released the aquarium was washed and 10 l of fuggy water (de-chlorinated) was introduced. A part of the eggs packs was re-introduced in the aquarium while the other part was left in a glass recipient with a 5 l volume. Here took place the embryo development at a medium temperature of water of 20°C (± 1°C), with natural light in a clean, de-chlorinated water.

The eggs packs from the glass recipient developed youngster after almost 9 days from deposal. During this time we monitories the embryo development from zygote to the appearance of larva from egg (fig. 8).

Embryo development. Like the other amphibians, the egg of Hyla is a heterolecit type, with sherules of vitelus disposed after a gradient, small in the region of animal hemisphere and gradually larger toward the vegetative pole. The egg presents, also a pigment gradient, with a larger quantity of pigment in the animal hemisphere, that gave a dark-brown colour to this hemisphere, compared with the vegetative hemisphere which is more yellowish. **The segmentation** is total and unequal. After the third division of segmentation appears the difference between blastomere: micromere in the region of animal hemisphere and macromere in the vegetative hemisphere. At the end of segmentation it results the celoblastule, perfectly spherical, with a pluri-slaty and unequal thick (fig. 9, 10, 11, 12). **Gastrulation** is accomplished through a serious of synchronous processes: delamination or gastrulean cleavage, epibolia and emboli. The first manifestation of gastrulation is delamination, which is visible through a narrow slit that separates the internal macromeres by their external macromeres. The apparition of this slit in the passage area between the micromeres and macromeres expresses the beginning of epibolia. As the epibolia and embolia evolutes, the blastopor appeared in the vegetative hemisphere changes its form, becoming ring-shapped. In this stage the macromeres can not be seen from the exterior but only in the interior, circular blastoporal slit where they form the Rusconi vitelin dope (fig. 13). Gradually, the blastopore elongates and is divided in two orifices: one anterior that becomes the neurenteric canal and a posterior one that became the cloacal orifice. At the end of gastrulation the embryonar form is still spherical. Once the **neurulation** begins the embryo elongates antero-posterior and the ectoblastic thickness starts to form: the medular lama and the transversal cerebral shirr. In a more advanced stage the medular lama and the transversal cerebral shirr became proeminent. Along the medular lama the nervous tube is formed. Concomitant with the forming of central nervous system, complex processes of evolution of embryo foil take place (fig. 14, 15, 16, 17, 18).

Larva development. Immediately after the embryo leaves the eggs envelope, having now the name of larva or tadpole, he tries to fix hymself by the aquatic plants or by the aquarium walls with the help of adhesive organ and does not prefers to stay on the bottom of water, as the larva of other anures.

At its appearance, the larva measures around 4 till 7 mm (fig. 19). A period of 2-3 days the larva stays still and than when the tail muscle became functional, they started to swim actively in the aquarium. Also after 2-3 days the viteline spherules that served as food, started to disappear, the pigmentation appear and also the larva mouth (fig. 20).

The green frog larva is vegetarian feeding with algae and vegetal detritus. As food supplement we used food for fish in granule shape. After appearance, the larva breathes firstly through external branchia, and the through internal branchia. Even at appearance, the larva has almost differentiated eyes, olfactory and auditory organ. 9 days after appearance the larva measures from head to tail 15 mm length, and the long intestine spiral shaped is totally formed (fig. 21, 22, 23, 24, 25, 26).

Metamorphosis. In the second period of larva development (after almost 6 weeks), a series of transformations take place.

These transformations mark the passage to the life in aerial medium: the mezonefros is organised, the development of lungs and circulator apparel, also the members started to appear, first the posterior ones and than the anterior ones. As the anterior members appeared, the green frog hurry to reach the land, thus passing to lung respiration. The growth of members makes the tail to regress (fig. 27, 28).

During the metamorphosis the body shape modifies, becoming more and more similar to the

frog-shape, the tail is regressing until it disappears.

The intestine, which at larva is long and spiral convolute suffers a reorganisation and its length is reduced; the animal becomes carnivore and feed only with live prey.

After metamorphosis (fig. 29 and 30) we collected from the aquariums almost 70 exemplars of *Hyla arborea* that were introduced in the transportation recipients.

Five exemplars were placed in terrariums for their growth in captivity, while the rest were set free in nature, in an area where the green frog disappeared few years ago, more precise in Secuieni village, Neamț.

Aspects of growth in captivity. The exemplars that remained were introduced in terrariums of 10 l in which we deposited a layer of gravel, than plastic folia, a layer of soil of almost 5 cm with grass, an apartment flower, utilised by the frogs for climbing.

For hiding we utilise imitation of galleries from coconut. The scenery was daily watered and after two-three days we introduced a recipient with water for swimming. The terrariums were placed near the windows, so the frogs can have access to natural light (fig. 31, 32, 33, 34).

We also observed that the homocromy at young exemplars is very rapidly, the individual being able to change his colour from yellowish green to brown in few minutes.

Due to the high temperature inside the room, during the winter the frogs did not hibernate.

Feeding. After metamorphose, the animals with a carnivore regime were fed separately in transparent recipients with larva and adults of *Drosophila melanogaster*.

The feeding was accomplished every day for one month, and than we passed to larger prays and the feeding was rarer, at a period of 2-3 days. Along with dipteral larva and adults, the frogs were fed also with spiders. For a calcium supplements we utilised dust from sepia bones sifted over the food (fig. 35 and 36).

Terraristic. Due to its pleasant aspect, colour and relatively small size, *Hyla arborea* L. is a specie that is often met as a companion animal.

Although in Romania the terraristic is only at its beginning more and more animal lovers that the aquariums that was in the past utilised for fishes, to became a house for the amphibians (*Hyla arborea* can live 22 years in captivity).

CONCLUSIONS

The growth in captivity has a great importance in what concern the knowledge and conservation of *Hyla arborea* L. specie. By growing in captivity, in adequate conditions, healthy populations can be obtained that can be then re-introduced in their natural environment, thus contributing to the prevention of its decline.

The embryo, larva and post larva development (metamorphosis) can be easily tracked, in lab conditions with low consumption of materials. The embryo and larva development at *Hyla arborea* L. is a spectacular process. His study imn laboratory conditions as well as his growth in captivity can bring important information concerning the environmental conditions and the modality in which the growth and development of individuals take place.

According with IUCN (*International Union for Conservation of Nature*) and "*The Red Book of Vertebrates from Romania" Bucureşti 2005, Hyla arborea* L. has the statute of vulnerable specie. The populations of puddles with fishs, destryment of habitats through their fragmentation as well as deforrestration, grassland and hay field leads to a rapid decline of this specie due to its sensibility, existingthe danger of its extinction. The collection of individuals for comercialisation as „pets", the low rate reproduction and their natural enemies determine the diminuish of *Hyla arborea* L. population.

ABSTRACT

Two pairs of *Hyla arborea* L.-green frog were bred in captivity in order to discover different aspects regarding: copulation, eggs overlay, embryo development, metamorphosis, feeding, and behaviour. We manage to establish the optimum growth conditions in captivity and we made recommendations regarding the terraristic. The study can contribute to the knowledge and conservation of this endangered specie.

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Fig. 1. ♂ *Hyla arborea*

Fig. 2. ♀ *Hyla arborea*

Fig. 3. Pairs of *Hyla arborea* utilised for the experiment

Fig. 4. Axillary amplex at *Hyla arborea*

Fig. 5. Egg packs fixed on vegetation

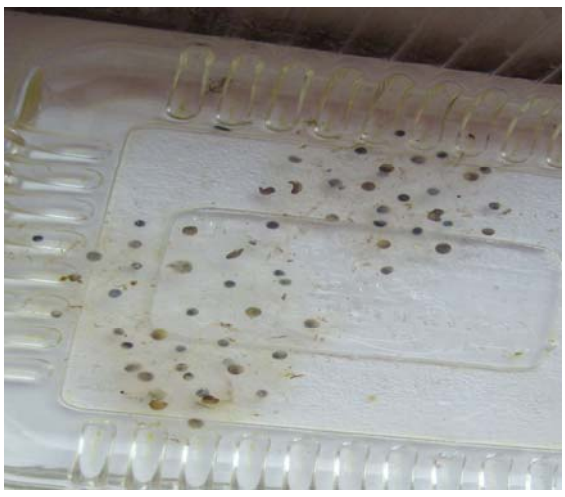

Fig. 6. The deposal of ponta in the aquarium

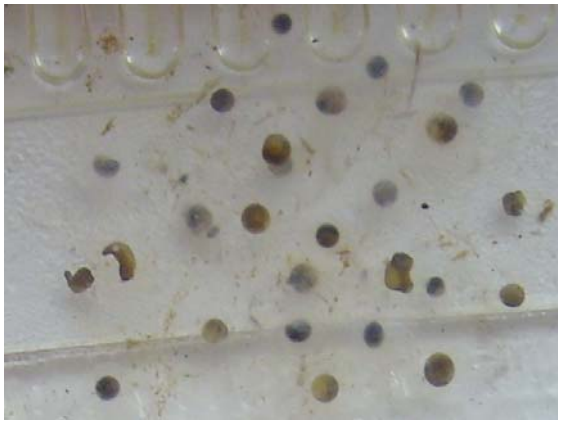

Fig. 7. Eggs in different stages of embryonary development

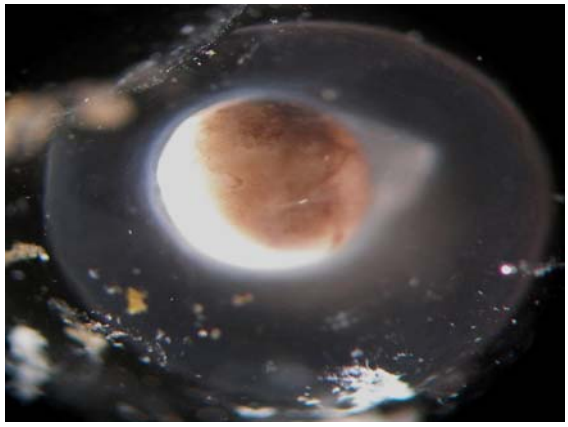

Fig. 8. Egg of Hyla arborea

Fig. 9. Segmentation: stage of 2 cells

Fig.10. Segmentation: stage of 4 cells

Fig 11. Segmentation: stage of 8 cells

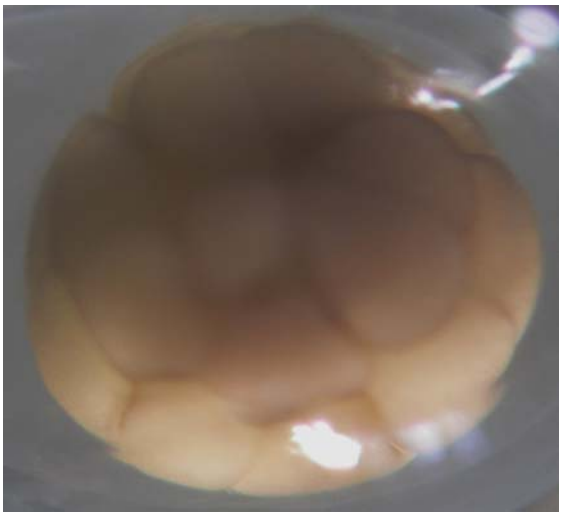

Fig. 12. Segmentation: stage of 16 cells

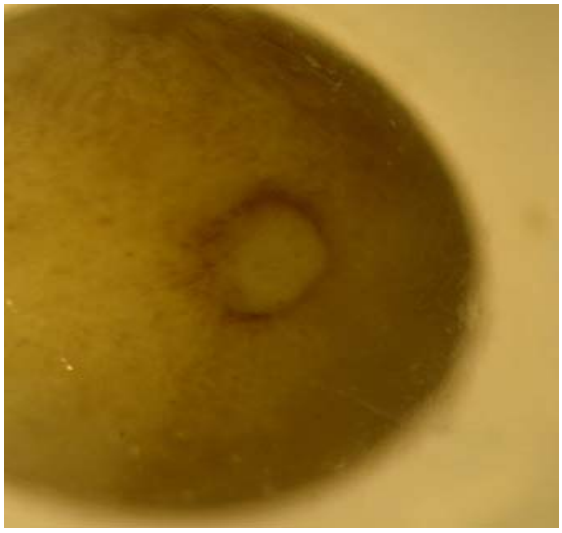

Fig. 13. Gastrulation: Blastule with circular blastopor and viteline cork

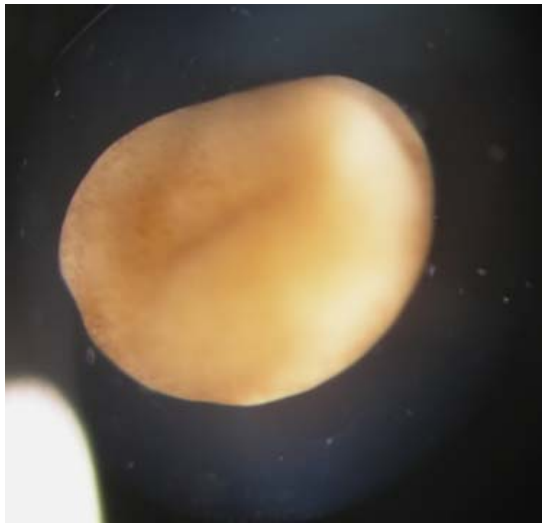

Fig. 14. Elongation of blastopore and beginning of neuraltie

Fig 15. Neurula view from exterior

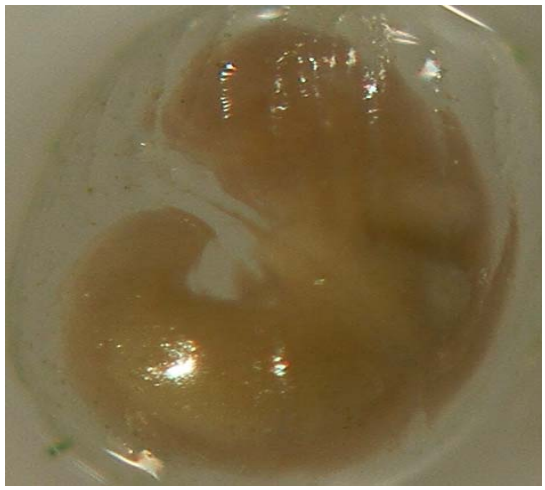

Fig. 16. Advanced neurula with optical vesicles and caudal bud

Fig 17. Advanced neurule

Fig. 18. Neurule before its release from the egg

Fig. 19. Larva of *Hyla arborea* measured after eclosation

Fig. 20. Larva, assemblage; on observe the olfactory nostrils, the adhesive organs, viteline spherule

Fig. 21. Larva after 9 days from eclozation

Fig. 22. Larva with food supplement

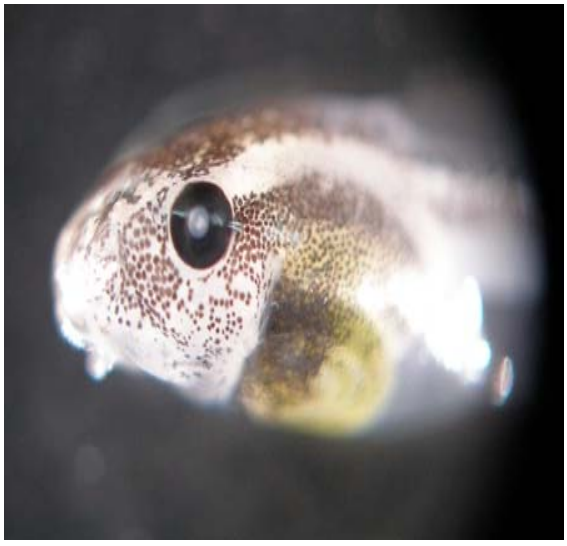

Fig. 23. Detail head area – on observe pigmentation, eyes and mouth

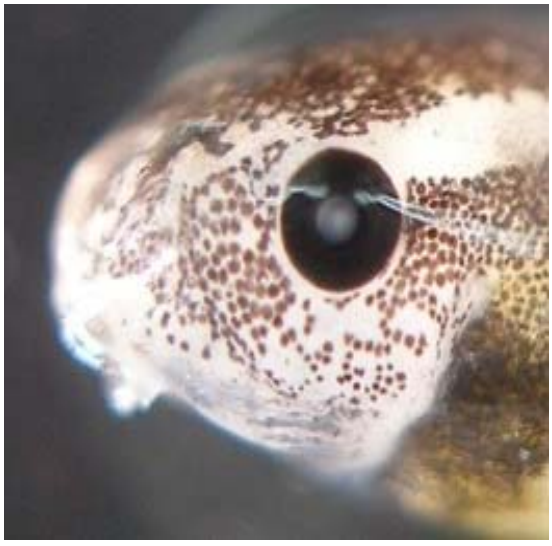

Fig. 24. Larva – detail of the eye

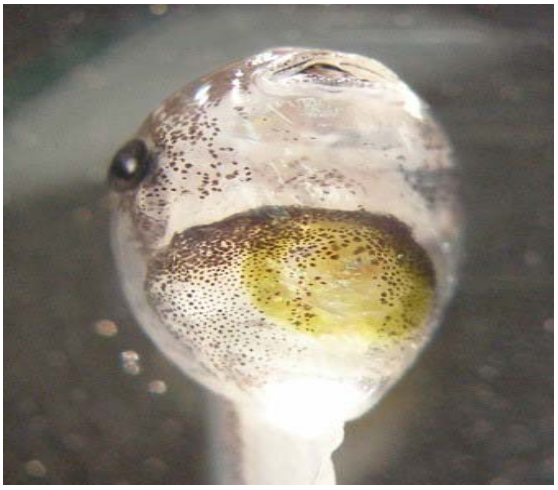

Fig. 25. Detail: the ventral part of larva

Fig. 26. Larva - detail: mouth

Fig. 27. Larva of *Hyla arborea* with posterior limbs

Fig. 28. Larva of *Hyla arborea* with posterior and anterior limbs

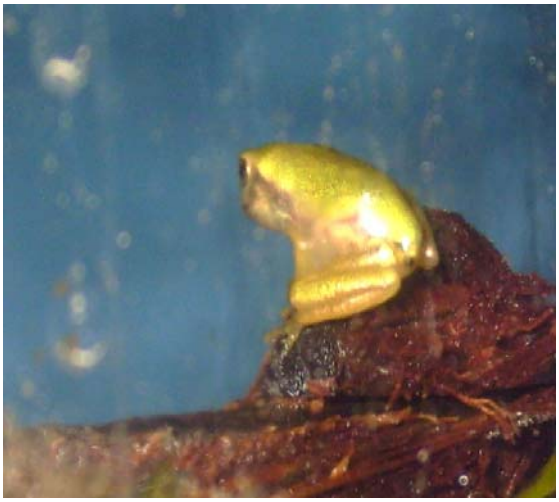

Fig. 29. *Hyla arborea* L. juvenile after metamorphosis

Fig. 30. *Hyla arborea* L. juvenile of *Hyla arborea*

Fig. 31. Terrarium utilised for growing in captivity of green frogs

Fig. 32. Terrarium utilised for growing in captivity of green frogs

Fig. 33. Utilised for growing in captivity of green frogs

Fig. 34. Utilised for growing in captivity of green frogs

Fig. 35.Aspects of feeding at *Hyla arborea* specie

Fig. 36. Aspects of feeding at *Hyla arborea* specie