

OBSERVATION ON GROWTH IN CAPTIVITY OF AMPHIBIAN SPECIES FROM SALAMANDRIDAE FAMILY - *SALAMANDRA SALAMANDRA* SUB-SPECIES *SALAMANDRA* (LINNAEUS, 1758)

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INTRODUCTION

20 amphibian species live in Romania out of the 7,901 species described so far in the world according to www.amphibiaweb.org (Jul 18, 2018).

Lissamphibia	Anura	Caudata	Gymnophiona	Total
Family	55	10	10	75
Genus	452	68	33	553
Species	6977	716	208	7901

The greatest diversity occurs in tropical forests, and the riches of species are generally lower in temperate and arid regions and are entirely absent from the marine environment. However, amphibians are limited to wet habitats because of the need to preserve their moisture.

Fire salamandra (*Salamandra salamandra*) (fig.1) is a colorful species. This species is present in Central, Eastern and Southern Europe and we find it in Romania too. Is a member of the class *Amphibia*, order *Urodela*, family *Salamandridae*, genus *Salamandra*, species *salamandra*. In our country it is spread in the hills and mountains, from 200 m to an altitude of 1800 m. Sporadic observations are reported in caves, during reproduction or hibernation. Species prefer microhabitats covered with thick bedding of leaves and moss.

Several subspecies are present in literature, but for genetic reasons some subspecies have recently been recognized as species such as *S. algira* and *S. corsica*. In our country lives *S. Salamandra* subspecies *salamandra*. The fire salamandra is the largest species in the *Salamandridae* family, the length at maturity ranges from 15 to 25 cm, but there were also 30 cm specimens. His body is black, with irregular yellow or orange spots. The ventral part is usually dark gray with fewer spots. The tail is small compared to the body, and the limbs are thick.

They like to live alone, during the wintering period they can gather in large numbers in the same place. Males and females of this species do not have pronounced sexual dimorphism. However, during the reproduction period, males have a swollen gland around the vent. This gland produces the spermatophore. The male courtship the female on

land, then deposit a sperm that the female takes with the vent. Spermatophore is kept in a „receptaculum seminis”, so fertilization may be delayed, larva deposition may occur in the following spring.

Females give birth to an average of 20, exceptional 40 larvae and the larval stage (fig. 2) lasts from 2 to 5 months depending on the temperature. And they live 15-20 years in the wild and over 50 years in captivity. After 3-4 years they reach sexual maturity.

These animals produce toxins as adults and the glands develop after metamorphosis. The poisoning produced by *Salamandra salamandra* is secreted by the parotid glands around the head region and the glands on the dorsal part of the body. If salamandra is threatened, it has the ability to spray poison with neurotoxic effect and will initially cause local anesthesia followed by muscle seizures, hypertension and respiratory paralysis. The poison is not only for the protection against predators, is to defend itself from microbial infections to. But the poison of this salamander species is not a problem for people as long is not ingested.

The main threats for this specie include the destruction of habitats, unsatisfactory forest management and pollution of breeding sites, commercial purposes (i.e. pet trade), the introduction of predatory species (salmonids and crayfish) in breeding ponds, population fragmentation and adult mortality due to roadkill in some areas of the world but also in Romania during migration periods. Another major danger is the presence of *chitridiomycosis* reported in some salamander populations in Spain for example. *Chitridiomycosis* is a fatal disease associated with the decline and extinction of amphibian populations worldwide.

The present study aims to provide information for a good growth of salamander larvae in order to conserve this species, if we also face in Romania the fungal infection produced by *Batrachochytrium salamandrivorans*.

MATERIAL AND METHODS

The biological material was represented by 40 larvae of *Salamandra salamandra* (fig. 2) received on

December 22, 2017 from a breeder and all larvae come from the same female born and raised in captivity. The larvae are about three centimeters long and have external gills. Body shape is stocky, head is wide, bilateral compressed tail and short limbs. The colors are dark brown with gray spots.

In the laboratory the larvae were placed in an Exo Terra Faunarium (fig.3), 35Lx20Lx15H cm in which about 5 l of water was placed, a stone and a plant to allow the larvae to hide, a sponge for water filtration, connected to an aquarium air pump in order to oxygenate the water. The water used to grow the larvae was tap water left to dechlorinated over 48 hours. Every week the water was totally replaced and the Exo Terra Faunarium was cleaned.

We have made live observations on the evolution and behavior of salamander larvae in the aquatic stage but also on the terrestrial stage and observations on various aspects of faunarium arrangements.

These observations focused on the following aspects:

- ✓ the larval behavior in water,
- ✓ larve development,
- ✓ regeneration,
- ✓ metamorphosis,
- ✓ adapting to conditions in captivity.

Throughout the experiment, the observations were recorded in the workbook and numerous macroscopic photographs were taken with Canon EOS 1300D; Canon PowerShot and Huawei P9 lite 2017.

RESULTS AND DISCUSSIONS

The larvae behavior in water

After the salamander larvae were placed in the Exo Terra Faunarium, they sought a place to shelter (fig. 4). They got accommodated in minutes, however I have noticed a very aggressive behavior (fig. 5) in the community, especially during feeding, each larvae being attracted to any moving object and then attack. Although they were fed daily, the larvae biting one another at the tip of the tail or limbs. And the main method of avoiding attacks was that they were immobile for a very long time.

The larvae in the aquatic stage were fed with frozen live fish food, represented by red mosquito larvae and daphnia, but also with live food represented by *Daphnia* sp. raised in the laboratory (fig. 6.a and 6.b).

Larvae development

Two months later, the larvae measure between 5 and 7 cm, the largest appear to have characteristic yellowish spots on the top, at the base of the limbs (fig. 7). But also on the dorsal side. Although the larvae were fed all with the same type of food and abundantly, had a water temperature approximate of 20°C, however, the 40 salamander larvae did not

have a linear development and the differences after two months were significant.

Regeneration

A few days after their placement in the Exo Terra faunarium, I noticed a specimen lacking a front leg, does not seem to bother it because it was eating and was as lively as the other cohabitated larvae. In two months a total of 4 larvae have missing a front leg (fig. 8.a, b), nearly 20 larvae have the tip of the tail torn and one larvae the external gills were broken.

In about three weeks the missing limbs of these young larvae grew back almost completely (fig. 8.c). But the one larvae with the broken gills die, because gills have not been able to regenerate in time to help breathe.

Metamorphosis

After two months the larger larvae began metamorphosis, the external gills was resorbed, the caudal crest that served as a fin and the larvae were resorbed to and the larvae move to pulmonary respiration and spend more time at the surface of the water trying to go on land (fig. 9) (in other case on the rock).

Adapting to conditions in captivity

From 40 larvae, they completely metamorphosed 36 and 4 larvae died:

- 2 were eaten by larger larvae;
- 1 died because the gills destroyed did not regenerate;
- 1 died immediately after the metamorphosis.

After the metamorphosis, the larvae were moved to terrestrial Faunarium where I laid a soil layer (a mixed soil - flower soil + coconut fiber), a water container and coconut hides. They start to accentuate the color, yellow spots appear on a black background as an adult. Salamander larvae eat well and stay almost all the time in hides, are fed each day with frozen fish food (red mosquito larvae) (fig. 12.a).

I noticed that the substrate was sticking to their skin and they had difficulty getting them to molt correctly. And after a week, I took out all the salamander larvae from the Terrestrial Faunarium, and I placed a new soil (a mixed soil - flower soil + coconut fiber) in top I put forest moss, the coconut shelters were replaced with tree bark and were placed in the Faunarium and some oak leaves to mimic the natural environment (fig. 10).

When I returned the salamanders, they have hide almost immediately under the bark and oak leaves (fig. 11). The Faunarium was sprayed once a day with fresh water to maintain a relatively moderate humidity.

After the first week from metamorphosis, we introduced live food such as tenebrio larvae and earthworms in their diet and in the third week we offered them only live food (fig 12.b).

CONCLUSIONS

Captive breeding have a particular importance in the knowledge of amphibian species, especially *Salamandra salamandra* L. because can bring us precious data on the conditions of larval and post-larval stages that are important for knowing this species.

The growth in captivity can substantially contribute to the conservation of this specie endangered by the reduction of its natural habitat and can also be used for the prevention of its decline due to infection with *Batrachochytrium salamandrivorans*.

Larval and postlarval (metamorphosis) of salamanders from species *Salamandra salamandra* can be traced easily in the laboratory with low consumption of materials.

From the experiment we find that regeneration of limbs and tail is done without problems but if the external gills are destroyed, because they have a major importance in breathing in the larval stage, they do not regenerate fast enough and the larva dies.

Captive growing under the right conditions can provide healthy populations that can be reintroduced into the natural environment, thus contributing to the decline of this species.

ABSTRACT

Finding solutions to cope with the decline and extinction of amphibians is one of the greatest global challenges nowadays. Frogs, toads, salamanders, newts, and gymnophiona according to the International Union for Conservation of Nature (IUCN) estimate that at least one third of the known amphibian species are threatened with extinction, a higher rate than birds and mammals put in one place. Major threats to amphibians include habitat loss or degradation and chytridiomycosis, an infectious disease with devastating effect on the decline or mass extinction of amphibian populations worldwide.

The purpose of this paper is to provide information for a good growth of *Salamandra salamandra* subspecies *salamandra* for the conservation of this species, if we are confronted in Romania with the fungal infection produced by *Batrachochytrium salamandrivorans*, this is a pathogenic fungus what was described in 2013 on the basis of a strain collected from the skin of salamander's skin (*S. salamandra*).

Through these observations we have obtained important data on larval aquarium behavior, larval development, aquarium larvae regeneration, metamorphosis, development of larval metamorphosis and adaptation to captive conditions. The study may contribute to the knowledge and conservation of this species.

REFERENCES

1. BLAUSTEIN LEON et al., 2018 - Compassionate Approaches for the Conservation and Protection of Fire Salamanders, Israel Journal of Ecology & Evolution, 2-9, DOI: 10.1163/22244662-06303001;
2. BOSCH JAIME et al., 2018 - Long-term monitoring of an amphibian community after a climate change- and infectious disease-driven species extirpation, Global Change Biology, 1-10, DOI: 10.1111/gcb.14092;
3. BOZORGI, FARNAZ; KIABI, BAHRAM H.; KAMI, HAJI GHOLI; 2018 - Feeding habits of spot-bellied salamander *Salamandra infraimmaculata* semenovi (NESTEROV, 1916) based on examination of three populations from zagros mountains, western Iran (caudata: Salamandridae), Russian Journal of Herpetology . 2018, Vol. 25 Issue 1, p11-16. 6p.;
4. DEGANI, G., GOLDENBERG, S. & WARBURG, M.R. HYDROBIOLOGIA, 1980 - Cannibalistic phenomena in salamandra salamandra larvae in certain water bodies and under experimental conditions, Volume 75, Issue 2, pp 123–128, DOI - <https://doi.org/10.1007/BF00007425>;
5. DEGANI G. AND M. R. WARBURG, 1978 - Population Structure and Seasonal Activity of the Adult *Salamandra salamandra* (L.) (Amphibia, Urodela, Salamandridae) in Israel, JOURNAL OF HERPETOLOGY 12(4):437-444;
6. IOSOB GABRIEL-ALIN, MARIA PRISECARU, IONUȚ STOICA, 2016 - Contributions concerning the current status of amphibian species in Roman town and its surroundings, Studii și Cercetări Mai 2016 Biologie 25/2 41-50 Universitatea "Vasile Alecsandri" din Bacău, Ed. Alma Mater;
7. IOSOB GABRIEL ALIN, MARIA PRISECARU, 2014 - Observations on the life cycle and reproductive behavior in *Rana dalmatina* F., Studii și Cercetări, Biologie 23/2, p. 50-59 Universitatea "Vasile Alecsandri" din Bacău, Ed. Alma Mater;
8. KRAUSE E. TOBIAS SEBASTIAN STEINFARTZ BARBARA A. CASPERS, 2011 - Poor Nutritional Conditions During the Early Larval Stage Reduce Risk-Taking Activities of Fire Salamander Larvae (*Salamandra salamandra*), Ethology International Journal of Behaveural biology 117 416–421 <https://doi.org/10.1111/j.1439-0310.2011.01886.x>;
9. MANENTI RAOUL Et al., 2009 - Water, stream morphology and landscape: complex habitat determinants for the fire salamander *Salamandra salamandra*, Amphibia-Reptilia, Volume 30,

- Issue 1, pages 7 – 15, DOI: 10.1163/156853809787392766;
10. PRISECARU MARIA, VOICU ROXANA, IOSOB ALIN, 2008 - Observations on the embryonic development of *Triturus* / *Lissotriton vulgaris* L., Studii și Cercetări, Biologie 13, p. 56–67, Universitatea din Bacău, Ed. Alma Mater;
 11. SANCHEZ EUGENIA et al., 2018 - Automatic quantification of colour proportions in dorsal black-and-yellow coloured amphibians, tested on the fire salamander (*Salamandra salamandra*), Herpetology Notes, volume 11: 73-76;
 12. SANCHEZ EUGENIA et al., 2018 - Morphological and transcriptomic analyses reveal three discrete primary stages of postembryonic development in the common fire salamander, *Salamandra salamandra*, J Exp Zool (Mol Dev Evol), 1–13, <https://doi.org/10.1002/jez.b.22792>;
 13. SCHMIDT, B.R., FELDMANN, R., SCHAUB, M., 2005 - Demographic processes underlying population growth and decline in *Salamandra salamandra*. Conserv. Biol. 19:1149-1156;
 14. ZAKRZEWSKI, M., 1987 - Effect of definite temperature ranges on development, metamorphosis and procreation of the spotted salamander larvae, *Salamandra salamandra* (L.). Act. Biol. Cracov. Zool. 29: 77-83;
 15. WATTERS M. AMANDA et al., Larval salamanders are as effective at short-term mosquito predation as mosquitofish, Canadian Journal of Zoology, 1-20, <https://doi.org/10.1139/cjz-2017-0267>;
- *** <https://amphibiaweb.org/lists/index.shtml>
 *** https://en.wikipedia.org/wiki/Fire_salamander
 *** <http://www.iucnredlist.org/details/59467/0>
 *** <http://zoologysp.blogspot.com/2010/03/salamandra-salamandra-sau-salamandra-de.html>

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Fig. 1. Fire salamander - *Salamandra salamandra* – juvenile



Fig. 2. Salamandra salamandra larvae – aquatic stage



Fig. 3. Faunarium exo terra



Fig. 4. *Salamandra salamandra* larvae - hiding



Fig. 5. Aggressive behavior of salamander larvae



a)



b)

Fig. 6. Feeding salamander larvae with frozen food for fish a) and live food (*Daphnia* sp.) b)



Fig. 7. Yellowish spots on salamander larvae

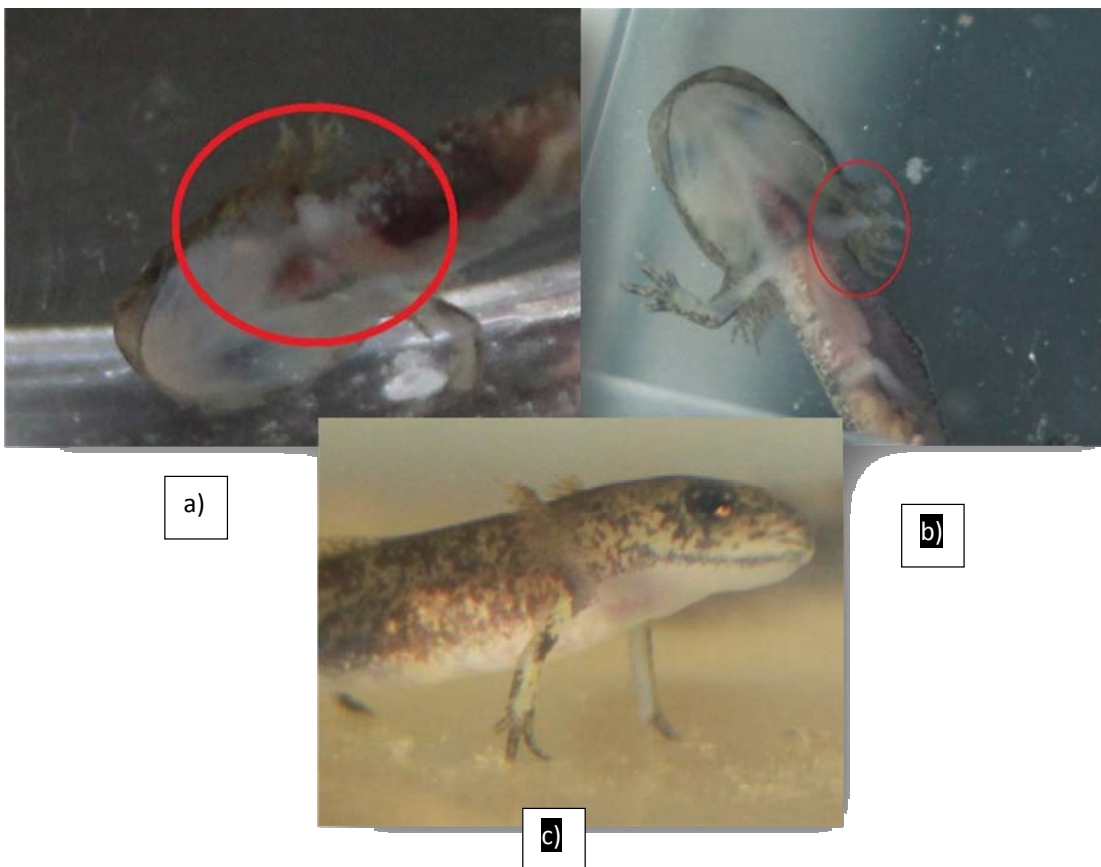


Fig. 8. Regeneration limb on fire salamander – a) and b) missing limb c) limb almost complet regenerated



Fig. 9. Salamandra larvae metamorphosis after two months in the aquatic stage



Fig. 10. Terrestrial Faunarium for salamanders



Fig. 11. Juvenile salamanders hiding



a)



b)

Fig. 12. Feeding the juveniles salamander with a) frozen fish food (red mosquito larvae) and b) with live food