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OBSERVATIONS ON THE EMBRYONIC DEVEPOLMENT OF TRITURUS / *LISSOTRITON VULGARIS L.*

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INTRODUCTION

Triturus vulgaris (common triton) is the most common newt species of the *Triturus* genus of amphibians. In order to survive, just like the other amphibian species, it requires appropriate swamps and ponds, surrounded by a high quality terrestrial habitat.

Changes in agricultural practices over the past decade have had a serious impact on newts life conditions. As a result of arable lands extention, many swamps and ponds have dissapeared. Intensive soil work, irrigation systems extention and chemical substances usage have turned many areas unhospitable for amphibians. The population of some ponds with some predatory fish species and the habit of burning vegetal waste from the fields after harvesting have greatly led to the restraining of favourable areals for triton species. Therefore, some species such as, *Triturus cristatus* (Great Crested Newt), have been declared endangered and protected species and are still threatened to become extinct.

The study of the embryonal development of *Triturus vulgaris*, of the preferred types of habitats and of life and reproduction conditions may largely contribute to the knowledge and preservation of this species.

Triturus vulgaris, smooth or common newt is the smallest newt species, roughly 6-11cm head to tail length. Relatively slender body. The male newt has a single black line running down the centre of the spine, the females have two parallel lines on either side of the centre.

The male (fig.1.) has yellow-brown/olive green or olive brown dorsal surface and flanks. Its back and flanks have black spots and longitudinal stripes. Wavy dorsal crest with black stripes, too. The male's cloaca is very distended, whilst the female's is nearly invisible. The stomach area is pale yellow, with irregular black spots. On its head, there are three pairs of black stripes, along the upper jaw, along the eyes and above them; on the crown there is another unpaired black stripe.

The female (fig.2.)has a similar pale brown to yellow colouration. The female also develops spots, but not on the stomach area, which is paler than the one of males, and theirs are generally smaller. The female does not develop crests. The stomach annd gizzard are also pale yellow with small black spots which are not common.

<u>Triturus vulgaris</u> is a member of the class <u>Amphibia</u>, order <u>Urodela</u>, family <u>Salamandridae</u>, genus <u>Triturus</u>, species <u>vulgaris</u>, (Linnaeus 1758). In Romania there live two species of vulgaris: <u>T.v. vulgari</u> and <u>T.v. ampelensis</u>.

They are one of the commonest amphibians in Europe, spread evreywhere in Romania, from the Danube Delta to altitudes of 1500 m. It is considered a plain species. It breeds in almost any water area (in a variety of habitats sauch as deciduous woodland, wet heathland, bogs, marshes, gardens, parks and farmland). They prefer standing water with plenty of weeds, such as lake margins, ponds and ditches, in which to breed. *T. vulgaris* enters in water early in spring and after milder winters, we meet th first species in mid February and at the beginning of March.

BIOLOGICAL MATERIAL

The biological material has been represented by 20 adult individuals of *Triturus vulgaris*, 10 ♂♂ şi 10 ♀♀, which have been collected from a pond in the vicinity of Boghicea village, Neamț department, between 7 March and 4 April 2008.

The newt samples have been caught by hand, introduced in containers suitable for their transportation and subsequently hosted in lab aquariums. There have been used 2 aquariums with a capacity of 20 l with a gravel bed, 15 l of water, specific plants, leaving enough swimming space for the newts.

RESEARCH METHOD

There have been live observations made in aquariums on the behaviour of common newts: female courtship, egg-laying, embryonic and larval development.

All along the experiment, the observations have been registered in the research log and tave been taken numerous macroscopic photographs.

With a view to observing the embryonal stages, there have been performed observations sing binocular lenses on fresh samples, on zygotes and larvae. The most relevant images have been obtained by using and Olympus camera attached to the binocular lenses and the computer. Thse images have been subsequently processed by the computer.

RESULTS OBTAINED

The newt samples were placed in the aquariums on the 10 of March 2008. The female courtship by males has started the next day, being followed by the ovocites laying and their fertilization.

Each female laid approx. 10 – 15 eggs, individually (each attached to the submersed plants). The egg is round, covered with three gelatinous membranes. These membranes, once in contact with water, become inflated and offer protection to the newly-hatched egg against mechanical shocks. The adhesive membrane fixates the egg onto the plant (fig.3, 4.).

CELL DIVISION

At about 2 hours after fertilisation, the zygote splits along a longitudinal plane (which comprises the animal and the vegetal poles) into two cells (the two-cell stage).

At approx. 1 hour after the first division, the second division is also longitudinal, but at 90 degrees to the plane of the first, advancing towards the same direction (vegetal pole) and dividing the zygote into 4 equal cells. The cells derived from cleavage are called *blastomeres*.

The third division appears 15-20 mins after second division; it is perpendicular to the first two and is equatorial in position (closer to the animal pole). It results in the formation of uneven cells: 4 smaller cells to the animal pole (*micromeres*) and 4 bigger cells to the vegetal pole (*macromeres*).

The next stages of plasmodieresis have split the zygote into smaller and smaller blastomeres and within 4 hours since fertilisation, they formed a compact mass called the *morula.*

At approx. 7-8 hours, after 13-14 division stages, the embryonic form of 8.000 – 16.000 microscopic cells represents the *blastula*.

Depending mostly on the amount of yolk in the egg, the cleavage can be holoblastic (total or entire cleavage) or meroblastic (partial cleavage). For these organisms, the *cleavage* is total. Cleavage ends with a perfectly sferical blastula (celloblastula), with a multi-layered wall which shelters the blastocoel).

Each division is followed by nuclear division preceded by the duplication of the genetic material in the mitotic interphase (fig.5, 6, 7, 8, 9 10, 11, 12, 13).

GASTRULATION

Gastrulation is achieved by a series of simultaneous processes: delamination (the external cells divide, leaving the daughter cells in the cavity), gastrulean cleavage, epiboly (expansion of one cell sheet over other cells) and emboly (the formation of a gastrula from a blastula by invagination). During gastrulation, many of the cells at or near the surface of the embryo move to a new, more interior location. Gastrulation involves changes in cell motility, cell shape, and cell adhesion.

The first manifestation of gastrulation is delamination, which is characterized by a sheet of cells separating the internal macromeres from the exterior ones. In epiboly, a sheet of cells of yellow whitish colour gets to spread by thinning. This reduction of surface is explained by the fact that there begins the process of invagination, too. The cells below the centre of the gray crescent invaginate to form the lip of the future blastopore. The *blastopore* develops in the vegetal hemisphere, until it can be observed as a semi-circular ring of involuting cells (the future dorsal lip of the blastopore which forms due to the contraction of bottle cells). The vegetal plate undergoes invagination to produce the archenteronic cavity (primitive gut).

In subsequent stages, the blastopore changes shape, looks like a horse shoe due to the lateral lips and finally gets a ring shape when the ventral lip forms. At this stage, the macromeres cannot be observed from the exterior anymore, only along the blastopore streak where they form the Rusergni yolk plug.

With the elongation of the archenteron across the blastocoel, where it attaches near the animal pole of the embryo, there appear two holes, corresponding to the neurenteric canal at the front and the cloacal orifice at the back.

After about 15 hours since the blastula stage, gastrulation is almost complete. The blastopore is perfectly circular, the yolk-rich macromeres are completely internalised and form the *endoblast*. The micromeres at the exterior form the *ectoblast*.

The primary layers (*endoderm, mesoderm and ectoderm*) are formed and organized in their proper locations during gastrulation. Endoderm, the most internal layer, forms the lining of the gut and other internal organs. Ectoderm, the most exterior layer, forms skin, brain, the nervous system, and other external tissues. Mesoderm, the middle layer, forms muscle, the skeletal system, and the circulatory system. The archenteron wall will be formed of ventral hypoblast (a tissue type that forms from the inner cell mass; lying beneath the epiblast, it consists of small cuboidal cell) results out of the mass of macromeres inside the gastrula (fig.14, 15, 16).

NEURULATION

At the end of gastrulation, the embryonic form is still spherical. Now there begin transformations that lead to central neural system formation. This complex and dynamic process can be followed by observing sectional images of the surface of the embryonic form.

On the dorsal side of the embryonal form, called *neurula*, on both sides of the anterior-posterior axis, there can be observed two darker stripes which are united in the anterior part by a transversal stripe.

Neurulation begins with the formation of a *neural plate*, a thickening of the ectoderm caused when cuboidal epithelial cells become columnar. Changes in cell shape and cell adhesion cause the edges of the plate fold and rise, meeting in the midline to form a *tube.* The anterior part of the tube becomes the encephalum, whereas the posterior forms the spine.

The cells at the tips of the neural folds come to lie between the neural tube and the overlying epidermis. These cells become the *neural crest cells*, which will differentiate neural ganglions and nerves. Sense organs begin to differentiate in the cephalic region.

When the neural tuber closes, from its lateral-ventral wall there emerge the paired evaginations of the forebrain of the embryo from which the sensory and pigment layers of the retina of the eye develop (*ocular vesicles*). In the formation of sense organs, there are also involved some epiblastic regions called *placodes* (area of thickening in the embryonic epithelial layer) wich may evolve into nasal, olfactory and otic placodes.

Towards the end of the neurula stage, there begins the mesoblast transformation. The mesoblast plates, placed between ectoblast and hypoblast, divide into *somites, intermediary pieces* and *lateral plates*. Somites emerge in a metameric pattern and lateral plates are unique. Somites evolve in *sclerotome* (forming the mesenchymal structure around the axillary zone resulting in the vertebral column), *dermatome* (the part of a mesodermal somite from which the dermis develops) and *myotome* (the segment that differentiates into axial muscles).

Intermediary pieces contribute to excretory organs formation: pronephros, until metamorphosis, mesonephros, at adults; metanephros organized in the second half of the larval period; part of it forms the mesenchym.

Lateral plates become fused in the ventral part, the coelom cavity being bordered by *splanchnopleure* (visceral sheet) and *somatopleure (*parietal sheet). Ventral hypoblast proliferates in the dorsal part surrounding the primitive gut. The mesoblastic plates form the intestinal hypoblast, a double wall called *dorsal mesentery*. The same plates will generate the *mesoblast* (mesoderm germ-layer cells that differentiate into mesothelium, endothelium and myocardium) out of which the medial-ventral blood island is formed and leads to many different parts of the circulatory system (blood vessel and heart.

In the pharyngeal zone, the *hypoblastic epitellium* forms 7 pairs of *pharyngeal pouches*. At the level of the first 5 pouches, there forms a double epiblastic-hypoblastic membrane which will not be resorbed in the direction of the first pocuh (becoming the ear drum membrane). At the other pouches, the obturant membranes are resorbed and establish communication of pharynx with the exterior. The sixth pouch takes part in thyroid formation, whereas the seventh represents the lung primordial, which will become fully functional in the second interval of larval stage (fig.17, 18, 19, 20, 21], 22, 23, 24, 25).

The embryonal development duration is determined, among other factors, by water temperature. For Triturus vulgaris, it takes 7-10 days since the beginning of fecundation, until the embryo emerges from the egg membranes and becomes a *larva*.

Upon eclosion, the larva is 5-7 mm in length. The hatching takes place at approx. 15 days. The caudal bud is well developed and there appears a clearly deliniated caudal region. On the first days, the larva remains stuck to the water plants by means of an adhesive organ which secretes a viscous substance.

The adhesive organ develops early in the embryonic stage on the sides and back of te pharingian membrane. The larva mouth appears by the pharygeal membrane resorption, 3-5 days after hatching.

Until they begin to feed in the external environment and even after that, the main source of plastic substances is represented by vitellus (yolk) sferules. They are in larger quantities in hypoblastic cells which form the ventral multi-layered wall of primitive gut. The vitellus sferules are found in all embryo and larva cells. As the primordial cells have specific metabolism, the vitellus disappears at different speeds. The most rapidly there dissappear the cells of the cerobrospinal axis. After the mouth is formed, the feeding regime is similar to that of adults, the difference being that larvae feed on ciclops and zooplancton, whereas adults feed on living food such as worms and insect larvae, such as mosquito larvae etc.

After eclosion, the larva breathes through its external gills, then through internal gills.

The larva has a high dorsal crest which begins at the point of insertion of fore limbs. The crest ends with a sharp tip. The colour is yellow with dark spots. The hind limbs appear in about 3 weeks after the first limbs. Upon metamorphosis, several changes occur, leading to the animal preparation for live in the air environment.

The epidermis thickens, the cutaneous glands are differentiated and there appears a thin horn-like cover/layer.

As the lungs are fully developed, the gills disappear. Bony teeth appear. The circulary system and the intestine suffer severe trasformations. The head shape changesm (fig.26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37).

ABSTRACT

There have been observations made on the reproductive behaviour of 20 individuals – (10♂ and 10♀) of Triturus vulgaris L., egg-laying, embryo development and larval evolution. A female lays about 10-15 eggs (each attached to a submersed plant). The egg is round, covered with three gelatinous membranes which insure its protection and fixation on the plant.

Cell division begins at appproximately 4 hours after egg-laying and evolves to the stage of blastula in abour 30 hours. *Gastrulation* is marked by the appearance of the blastopore which represents the reference point all along the gastrulation process, differentiating the middle section of the animal pole. At the end of gastrulation, there begin transformations that lead to the formation of the neural system (*neurulation*). At its hatching, the larva has oceli, external gills and pigmentation. Its forelimbs form at 7-10 days after hatching, whereas the hind limbs appear after 1 month. Before its passing on land, the larva suffers a reduction of the gills tissue crests.

CONCLUSIONS

The embryonic, larval and post-larval development (metamorphosis) can be easily observed in lab conditions and few resources.

The embryonic development of *Triturus vulgaris* is a spectacular process. Its study in lab conditions can provide invaluable data on the conditions of development of the embryo, larval and postlarval stages, all important to knowing and preserving this amphibian species.

The morphological value of different organs of adult animals cannot be established unless their embryonic development is studied. Early stages cannot be known and correctly interpreted without macro and micorscopic study of their embryonic development. Careful observation of the development of newt individuals emphasizes the laws of this process.

Embryonal development study of common newt contributed to precisely establishing the duration of the evolutionary stage, the moment and type of transformation, the factors and their influence on the zygote, embryo and larva evolution.

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Fig.1. ♂ Triturus vulgaris

Fig.2. ♀ Triturus vulgaris

Fig.3. Egg spawn

Fig.4. Eggs of newt deposed isolated and fixed on the submerse plant

Fig. 5. Egg of newt (on observe the gelatinous membrane)

Fig.6. Zygote

Fig.7. Segmentation: two cells stage

Fig.8. Segmentation: four cells stage

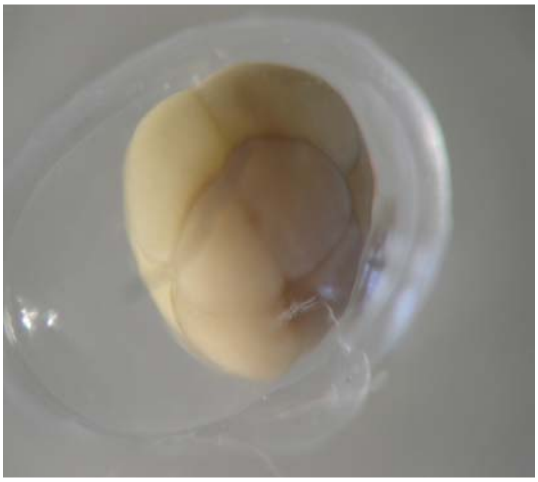

Fig. 9. Segmentation: eight cells stage

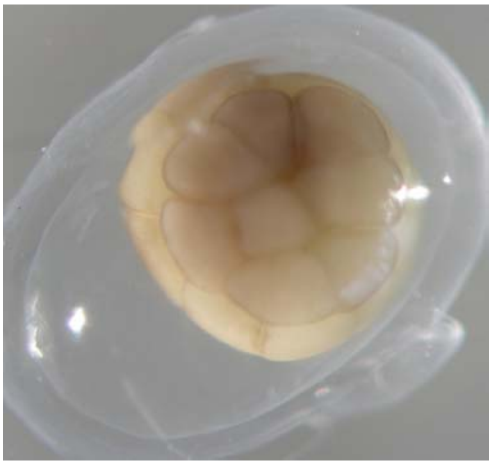

Fig.10. Segmentation: sixteen cells stage

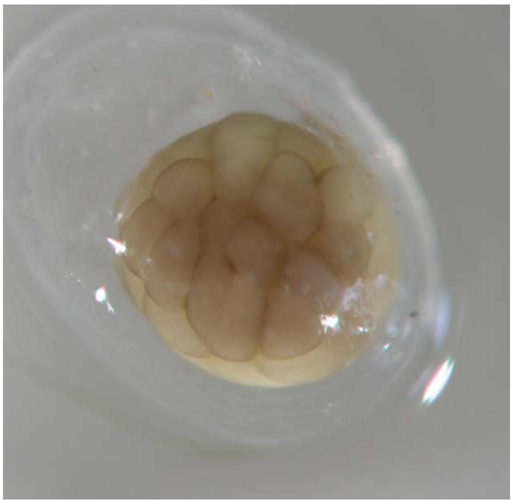

Fig.11. Segmentation: thirty two cells stage

Fig.12. Segmentation: morula

Fig.13. Segmentation blastula

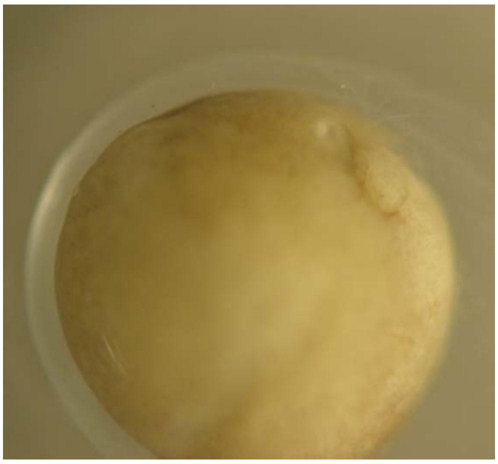

Fig.14. Gastrulating: apparition of blastopor

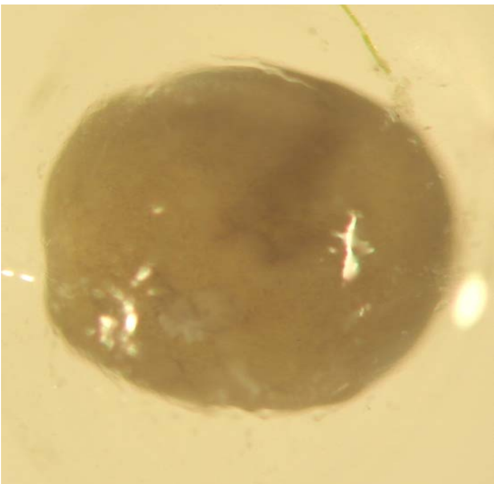

Fig.15. Gastrulating: apparition of antero-posterior median line

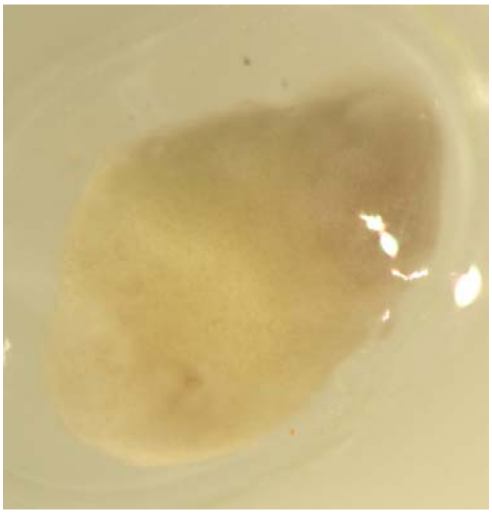

Fig.16. Gastrulating: antero-posterior extension of blastocel with the formation of two orifices

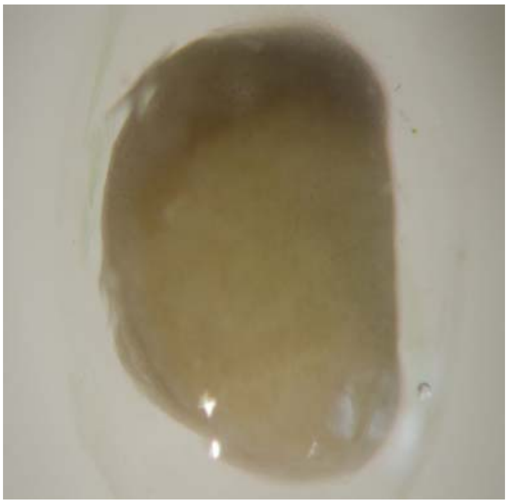

Fig.17. Neurulation: early neurula, forming of medullar lamella

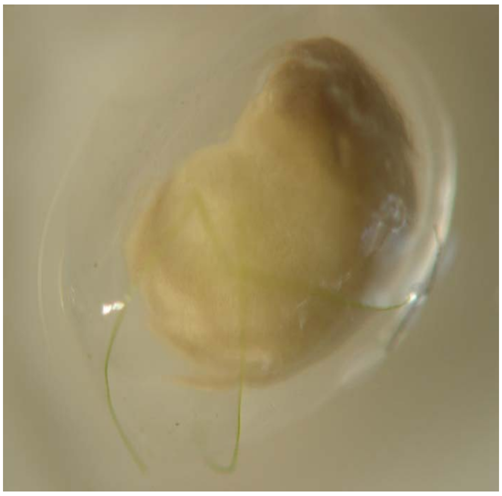

Fig.18. Neurulation: stage of neural plaque;

Fig.19. Neurulation: forming of nervous tube and of cerebral vesicle

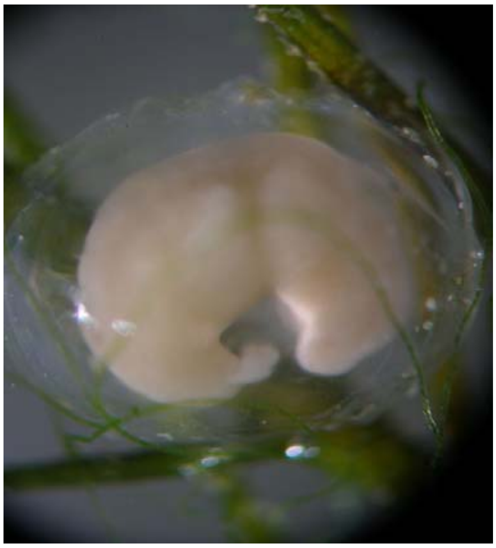

Fig.20. Neurulation: elongation of embryonic form and the division of someite; apparition of tail bud

Fig.21. Neurulation: apparition of distinct protuberances at eye level

Fig.22. Neurulation: advanced neurula, apparition of anterior limb's bud

Fig.23. Neurulation: apparition of external gills and of pigments on trunk and tail

Fig.24. Neurula before eclozation with eternal gills and tegument comb

Fig.25. Advanced neurula: head region (detail); on observe the oral orifice, eyes and external gills

Fig.26. Newt larva pulling out of egg

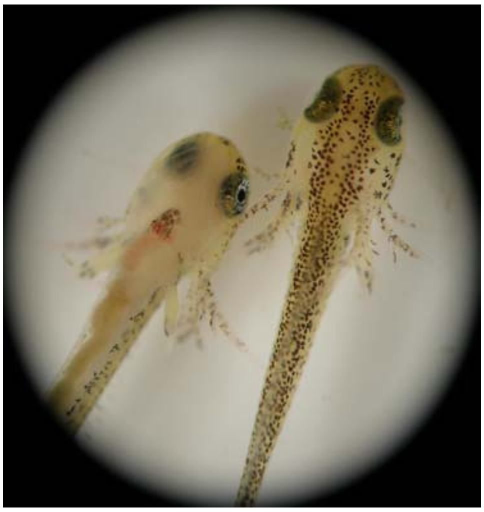

Fig.27. Newt larva immediately after eclozation dorsal and ventral view

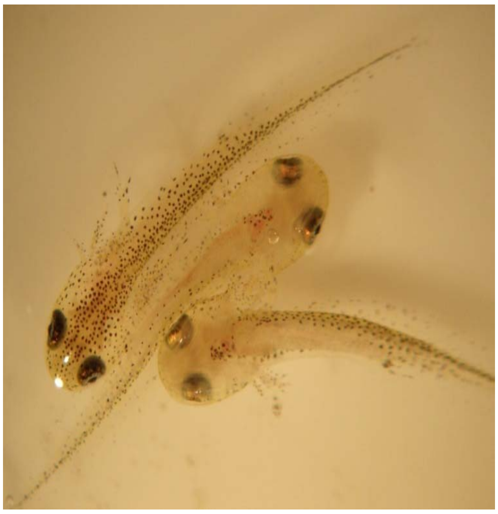

Fig.28. Newt larva with the anterior limbs viewed ventrally

Fig.29. Detail of head: on observe the pigmentation, eyes, extern gills and limbs

Fig.30. Detail of anterior limb: on observe the finger presence

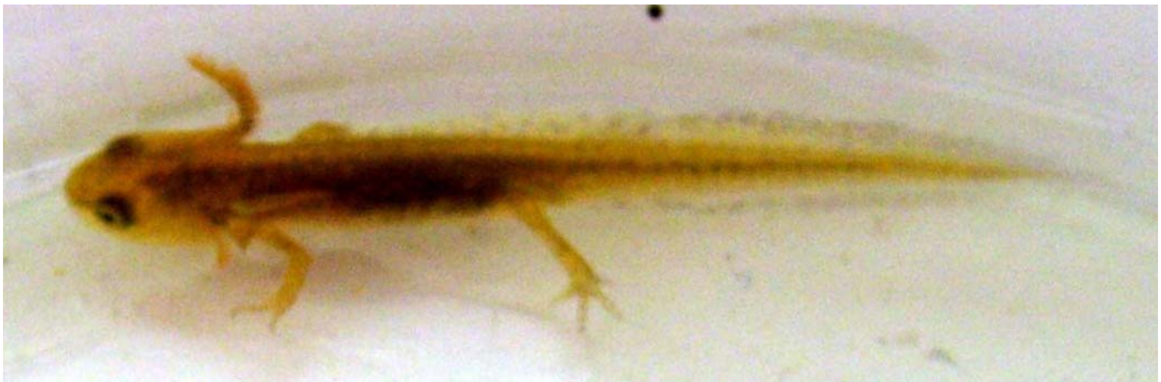

Fig.31. Newt larva with the posterior limb well developed and tegument comb, with limbs completely formed

Fig.32. Newt larva with external gills 2 months old

Fig.33. Newt larva with external gills and tegument comb

Fig.34. Stage of passing to dry land

Fig.35. Larva with pulmonary respiration and without external gills

Fig.36. Triturus vulgaris - junenile larva

Fig.37. Triturus vulgaris - junenile larva