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Reproductive cycles of the Siberian newt *Salamandrella keyserlingii* Dybowsky, 1870

V. N. Kuranova[1], S. V. Saveliev[2]

Abstract. Females of *Salamandrella keyserlingii* with deposited or fertilized eggs from the previous year spawn in the end of April- beginning of May. In females that have not spawned in spring maturing eggs are found in the oviduct by the end of May and copulation takes place in the beginning of July. It seems that both variants of breeding cycle exist side by side. If females could not get spermatozoids during summer then eggs are reserved for the next breeding season.

Introduction

The genitals and the means of fertilization in *Salamandrella keyserlingii* (Hynobiidae, Caudata, Amphibia) are poorly known. There exists an opinion that fertilization in this species is external and precedes courting (Bannicov et al., 1977; Kuzmin, 1999). A number of studies confirm that females of *S. keyserlingii* are polygamous, i.e. copulation with several males takes place at the same time. On the basis of these assumptions the conclusions about primitive nature of reproduction in *S. keyserlingii* are made (Berman et al., 1983; Sytina et al., 1987; Ishchenko et al., 1995; Kuzmin et al., 1995; Borkin, 1999). Studies of the breeding ecology in *S. keyserlingii* have been conducted near Tomsk, south-east of Western Siberia since 1975. Earlier we have described the genitals, internal fertilization and three year sexual cycle of the siberian newt (Kuranova, 1991; Saveliev et al., 1991, 1993). The results of these studies are presented below.

Material and methods

The study caried out in Tomsk Oblast' (West Siberian), in 2003-2005. Totally 97 specimens (46 females and 51 males) were investigated. The snout-vent length (SVL) and total length (TL) of 48 specimens (31 females and 17 males) captured in spawning ponds in first-second decade May was measured to the nearest 1 mm; the length of testicles was measured in 25 sexually mature males captured between May and August 2005. Age and seasonal dynamics of testicles are described by index L. tes/SVL (where L.tes. – length of testicle). The fecundity defined on number of eggs in laying (n = 296). We have studied the reproductive system of 24 Siberian newts (including 15 females) by means of histological and histochemical methods from April till August, 2003. Species were captured during migration before breeding and during spawning (the third decade of April - May) and also during summer. For histological research the urinogenital system was fixed in 10 % formaldehyde, for scanning and transmission microscopy the urinogenital system was fixed in 2 % paraformaldegide. Ovarium, various parts of oviductus, testicles with vas efferens, ren with Ductus Wolfi were separated urinogenital system. Sections were stained according to Mallori method. The spermatozoa in the oviducts of female *S. keyserlingii* were traced indirectly with marked antibodies. The antibodies of 2 classes were used: A (IgA) and G (IgG). The marked material was studied by means of scanning electronic microscope Hitachi S-500 and Jeol 100 In, and a photomicroscope Leitz Ortholux 2 Pol BK. The statistical processing of material was conducted with the spreadsheets MS Excel 7.0 and statistical package STATISTICA 6.0. Differences of means were estimated by criterion of Mann-Whitney (U-test).

Results and discussion

Sexually mature males with SVL of 59.7 ± 0.8 mm (n=17) and females with SVL of 59.6 ± 1.0 mm (n=31) can be found in spawning ponds in spring. The sexual dimorphism express in TL: males (mean ± SE) = 112.5 ± 2.5 mm (range 88-123), females = 106.3 ± 1.8 mm (range 91-126) ($p \leq 0.05$). The average fecundity in Tomsk population varied from 98.0 ± 11.3 to 171.6 ± 16.3 egss in different years. The average long term fecundity is 134.2 ± 2.6 eggs (limit 37-254, n=296).

The sample of *S. keyserlingii* females can be divided into two groups based on histological studies of their genitals. The first group consists of individuals that have spawned in spring. In April-May females spawned eggs which had been already fertilized in the previous breeding season. After spawning ovaries contain evidence left by of disintegration mature unfertilized eggs (Fig. 1). Eggs are subjected to involution in 2-4 weeks time. Disintegration is accompanied by forming the morphogenetic trace of the egg. This trace is comprised of the nucleus that has been subjected to rapid chromatolysis and of the yolk filled cytoplasm which persists for a longer time period. In 7-10 days

1 Department of Vertebrate of Zoology and Ecology, Tomsk State University, Pr. Lenina, 36, Tomsk, 634050, Russia; kuranova49@mail.ru

2 Institute of Human Morphology, Academy of Medical Sciences, St. Tsyurupy, 3, Moscow, 117418, Russia; embrains@mtu -net.ru

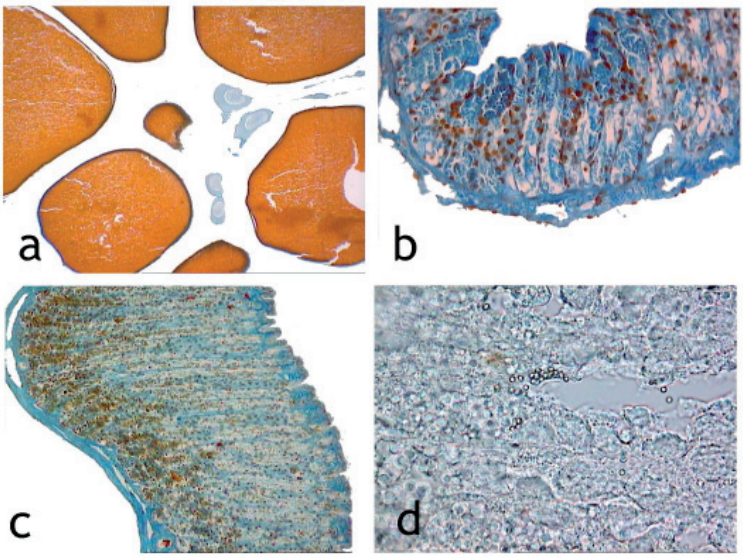

Figure 1. (a) Reproductory system in female *Salamandrella keyserlingii* before spawning, (b) Mature eggs in the ovary, (c) active secretory glands in oviducts, and (d) spermatozoa marked with monoclonal antibodies.

the active degeneration of secretory epithelium starts in oviducts of spawned females (Fig. 2). In spawning females cells of oviduct walls are autolysed and multiple necrosis centers are found. The oviduct is filled with cellular detritus. It consists of disintegrated secretory cells, fragments of nuclei and fibroblasts of degraded epithelium. In Siberian newts this process continues until the end of May. No necrosis of the epithelium is to be found in the oviducts from the beginning of June.

However there is a certain problem. Time is needed to restore the secretory epithelium after degeneration. We did not find females with traces of spawning in ovaries and with undegenerated epithelium of the oviduct. Hence in the end of May their ovary has not been restored after the spring spawning and the epithelium of the oviduct is in the state of morphological regeneration. Females recycle sexual system within 40-50 days. Sexual system's preparation of females takes much more time then the maturation of male spermatozoa.

The second group consists of specimens with other state of ovaries and oviducts. These are the females which have not spawned in the year of collection. There are no fertilized eggs in ovaries. In the middle of May ovaries contain some ovocites of various ages with signs of

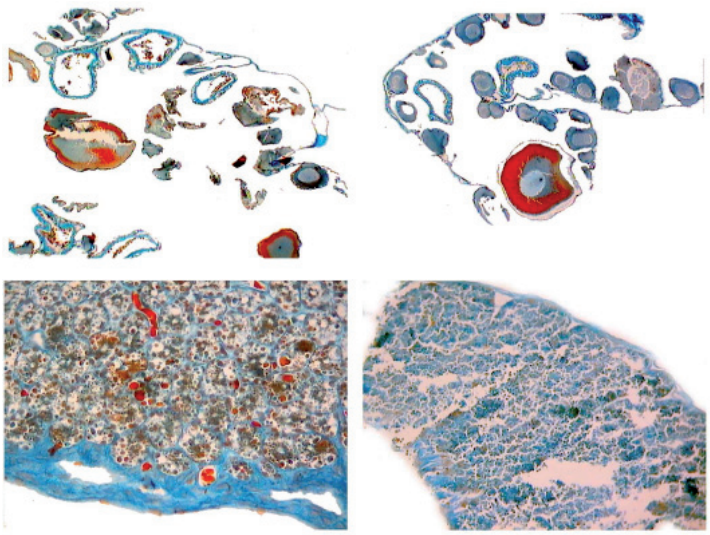

Figure 2. The ovaries and oviducts in females after spawning. Unfertilized eggs are being destructed in the ovaries. Secretory glands of oviducts are subjected to destruction.

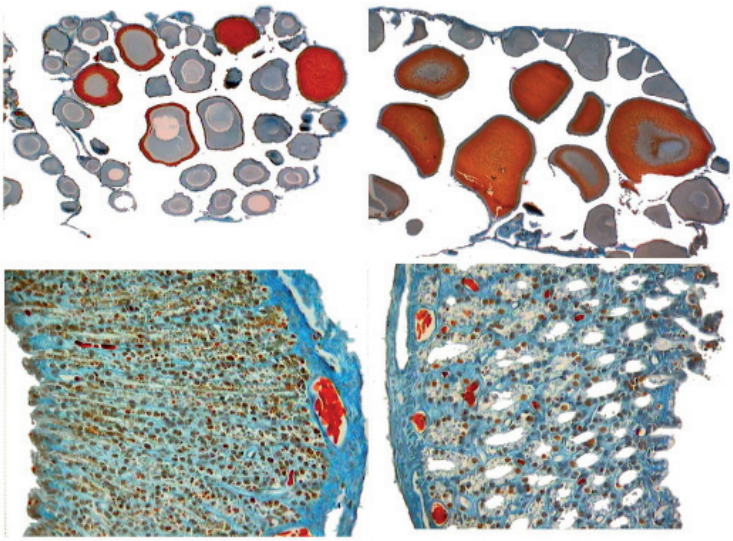

Figure 3. The ovaries and oviducts in the females of *Salamandrella keyserlingii* in July. The maturation of the eggs and the regeneration of oviducts are started.

primary maturing yolk in the cytoplasm. Oviducts are normal but signs of degeneration and necrosis of epithelium are not present. Such situation is typical for females with mature but unfertilized eggs (Fig. 3). In this case the ovary is filled with healthy large eggs, but the epithelium of the oviduct is not degenerated. It indicates that the unspawned females do not abort the unfertilized eggs and the latter are reserved for the next year. Presumably they are able to spawn these mature eggs in the next breeding season. Maturing eggs in this group of females are noticed in the end of June. At the same time the epithelium of the oviducts is well developed and vascularized. In the beginning of July spermatozoa have been found in oviducts of these females using common histochemical methods and antibodies. These spermatozoa loose terminal filament and are deposited in folds of secretory epithelium. From this moment on the maturation of ovaries is accelerated. In the end of July the ovary is filled with maturing eggs by 40-50%.

Research of the reproductive system in males shows the following results: early in May the testicles display sporadic spermatides but mature spermatozoa are absent. The number of spermatides is increased only by the end of May. The mature spermatozoa are found in the beginning of July (Fig.4, 5). Spermatozoa are characterized by a complex morphological structure (Fig. 5). They possess a long head, thick body, the tail

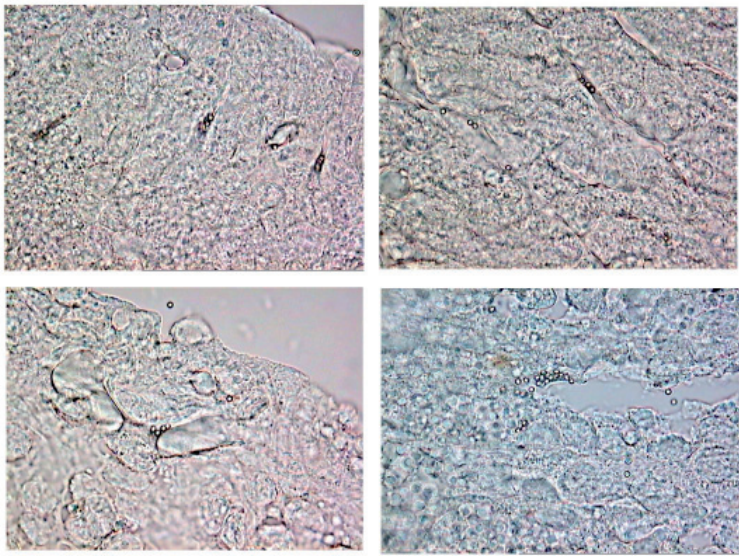

Figure 4. Marking of the spermatozoa in the oviducts of females with monoclonal antibodies. Spermatozoa are marked with antibodies joined to microspheres.

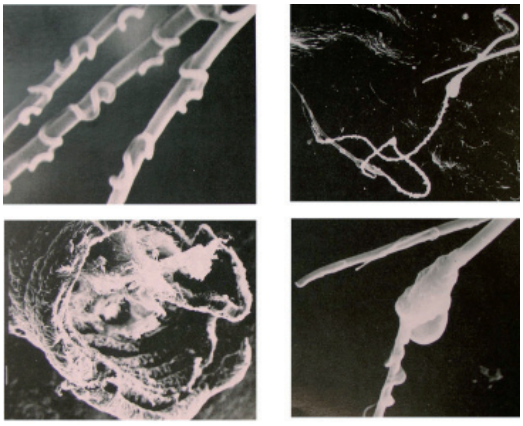

Figure 5. Spermatozoa in *Salamandrella keyserlingii* carry a large reserve of proteins and hydro carbonates which allows them to survive through winter.

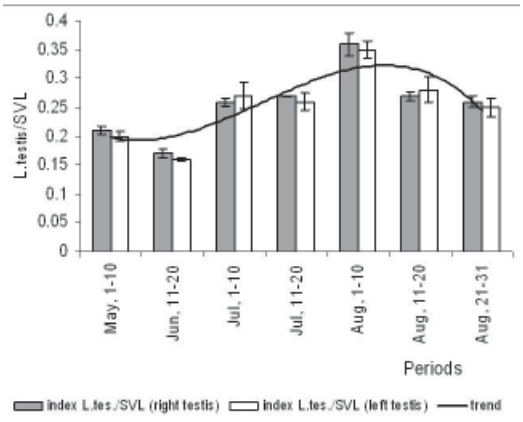

Figure 6. Seasonal dynamics of the testicles length of males in *Salamandrella keyserlingii* (L_{tes} /SVL, n=25; Tomsk area, 2005)

adjacent cylinder and a terminal filament The general length of the sperm cell is 115.0 ± 6.0 mkm (*n*=270). During the active spermatogenesis the size of testicles is increased (Fig. 6). The seasonal dynamic of length in left and right testicles is simular. The size increases in second half of July with maximim in first decade of August.

It is possible to assume the following scheme of egg laying and reproduction in Siberian newts. Females with deposited or fertilized eggs from the previous year spawn between the end of April and the beginning of May. In their ovaries there remain degrading unfertilized eggs. After spawning the epithelium degenerates and can not be restored by June. These females will be ready for reproduction next year. They will restore oviducts and ovaries in the present season. However it can not be excluded that during favorable seasons a female will have a chance to restore quickly and to reserve spermatozoa in the end of July - beginning of August. By this time males have enough mature spermatozoa.

In females that have not spawned in spring the epithelium does not show signs of degeneration and maturing eggs are found in the oviduct by the end of May. They are ready to accept mature spermatozoa in the beginning of July. Copulation takes place in the beginning of July. This fact is proved by presence of spermatozoa in oviducts of females of bylm's time. Females reserve spermatozoa or fertilized eggs until spring. It seems that both variants of breeding cycle exist side by side. If females could not get spermatozoids during summer then eggs are reserved for the next breeding season.

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