



RESEARCH PAPER

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Male reproductive cycle phases (spermatogenesis) of White Chub - *Squalius platyceph* (Actinopterygii: Cyprinidae) from Skadar Lake (Montenegro)

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Abstract

Male reproductive cycle phases of *Squalius platyceph* from Skadar Lake, Montenegro are analysed in an annual cycle. Maturity phases of the reproductive cycle are estimated on the base of macroscopic and histological descriptions of male gonads. Temporal aspect of the maturity phases of male reproductive cycle are presented respectively. Research data for *Squalius platyceph*, as a newly recognized species, are discussed in the relation to the reproductive cycle of other cyprinid species from Skadar Lake.

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Introduction

There is only one work published on the reproductive cycle of White Chub - *Squalius platyceps* (*Leuciscus cephalus albus*, Bonaparte, 1838.) from the Skadar Lake (Sekulovic and Ivanovic 1969), so far. Unfortunately, in that work oogenesis of White Chub from Skadar Lake was analysed on the base of macroscopic descriptions only, so histological descriptions of both gametogenesis, and particularly spermatogenesis, have not been analysed for this newly recognized species, so far.

White Chub - *Squalius platyceps* from Skadar Lake is distinguished from other species of the genus *Squalius* in the Adriatic basin (Zupancic *et al*, 2010). However, analyses of gametogenesis were done for following species / subspecies from Skadar Lake: *Alburnus albidus alborella*, *Alosa falax nilotica*, *Carassius auratus gibelio*, *Chondrostoma nasus ohridanus*, *Cyprinus carpio*, *Pachychilon pictum*, *Rutilus rubilio* and *Scardinius erythrophthalmus scardafa* (Ivanovic, 1964, 1968, 1969, 1977, Sekulovic & Ivanovic 1969, 1971, Ivanovic & Knezevic 1975, Knezevic 1984, Jankovic 1971, Soric 1979, 1984 and Maric 1998).

On the other side, previously initiated works on gametogenesis were completed for *Scardinius erythrophthalmus scardafa* (Knezevic, 1984) and carp - *Cyprinus carpio* (Jankovic, 1971). Ginogenesis, as a specific form of reproduction of *Carassius auratus gibelio* was researched by Maric in 1998.

Materials and methods

In the frame of reproductive study on *Squalius platyceps* that author carried out in Skadar Lake, 119 male samples are collected on following localities in the north-west part of the Lake (Fig. 1.): (i) Vucko blato near to Lesendro island (Fig. 2.), (ii) zone around Cakovica island (Fig. 3) and (iii) zone around Kamenik island (Fig. 4.). Apart from these important spawning places, White Chub is also present in the sublacustrine springs of Skadar Lake such as Radus, Bazagur and Karuc. However, samples from these sublacustrine springs were not collected because of different physical, chemical and biological conditions of their waters. Overall results from the study are presented in authors doctoral thesis (Krivokapic, 2002).

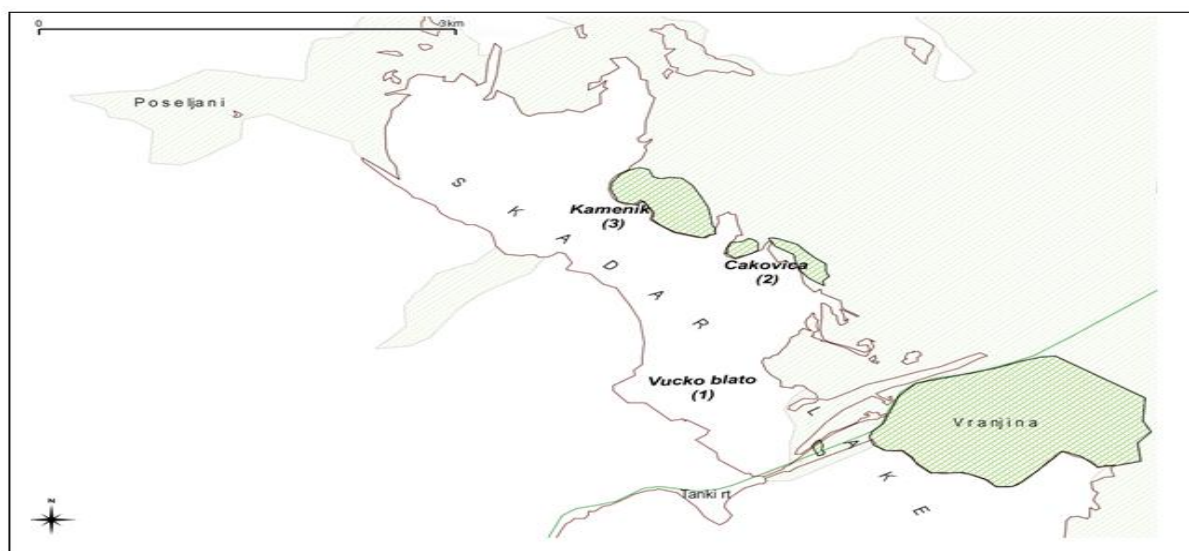


Fig. 1. Sampling locations in north-west part of Skadar Lake.

Macroscopic and histological features of the phases of male reproductive cycle are basically described and determined on the base of Kiseljevic scale (Sekulic and Buckaja, 1963; Jovanovic – Krsljanin, 1982,

Jankovic, 1985) and then terminologically standardized in the reproductive phases given by Brown - Peterson (2011).

Since data on spermatogenesis of White Chub *Squalius platyceph* from other locations in the basins of Skadar, Drin and Adriatic are missing, there was no possibility to compare data that are presented in this paper.

Results

Male reproductive cycle of White Chub from Skadar Lake (*spermatogenesis*) is differentiated on six maturity phases that are described and determined on the base of macroscopic histological features characteristic for the phases in *Kiseljevic scale*:

I phase

Designate juvenile, sexually immature fish. Male gonads are in the form of bands with no differences to ovaries (Fig. 5., Fig. 6.). This phase characterize appearance of spermatogonias and beginning of forming sperm ducts.



Fig. 2. Locality Vucko blato (Photo V. Buskovic).

II phase

gonads are still small, rounded shape in whitish pink colour. Further development of sexual elements is characteristic for this phase. Sperm ducts are formed and spermatogonias circulate.

III phase

size of the gonads is suddenly increased. They are elastic on contact, gray pink or yellow white in colour. This is phase of active spermatogenesis, so in gonads are present spermatogonias and spermatocytes of I and II level with spermatides. Spermatozooids still do not exist and milt doesn't leak out when gonad (testis) cut.

IV phase

Males are sexually mature. They gonads are milky white in colour. If gonad cut, milt will leak out. First appearance of spermatozooids is characteristic for this phase. For time being spermatozooids are present in small groups. Due to their intensive forming, later on, there is a mass presence of the spermatozooids. Remarkable large spermatogonias also characterize this phase.



Fig. 3. Locality Cakovica - island (Photo V. Buskovic).

V phase

Gonads have maximum size. In gonads is also produced liquid for spermatozoid dilution. Sperm ducts are full of mass spermatozooids. This phase could be named as "running ripe" since gentle pressure on gonads zone of abdomen causing milt run out from urogenital papilla.



Fig. 4. Locality Kamenik island (Photo, M. Krivokapic).

VI phase

Comes after spawning phase in which milt is released and sperm ducts are partially or completely emptied. Gonads are of reddish - brown nuance and have a

shape of little empty sacks with small number of unreleased spermatozoids. After a shorter period of time gonads are turned from VI phase back to III phase.

Phases in the male reproductive cycle of White Chub from Skadar Lake can be differentiated from maturity and temporal (seasonal) aspects that easily accommodate to newly standardized terminology (Brown - Peterson, 2011) for the developing phases, as follows:

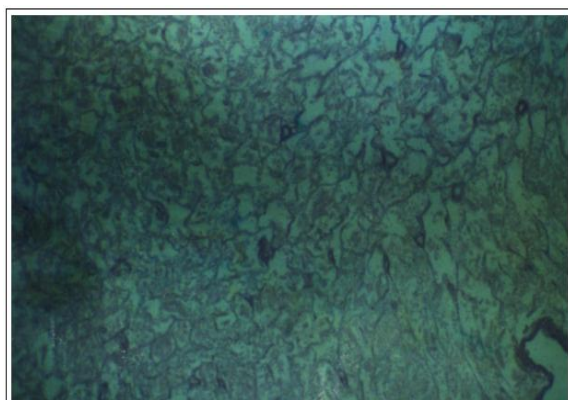


Fig. 5. Histology cut of sexually immature white Chub male gonad, 1⁺ age group (en 100×).

A. Maturity phases:

I. Immature phase

correspond to Kiseljevic I phase and designate juvenile, sexually immature - never spawned fish, testes are small and thin, in them are present spermatogonias.

II. Developing phase

cover a period of *slow spermatogenesis* that take place in winter season, upon short *period before spawning* is finished and lasts three months, in average (December, January and February). Physiological maturing and spermatozoa differentiation begin during this period. Morphologically, in this period male gonads look like stretched bands in poorly visible pink shade / nuance. Slow increase of male gonad characterize this period. Histological analyses done in January and February prove that sperm ducts are full of circulating spermatogonias in distribution, while primary and secondary spermatocytes with spermatides are

present in testes lobules. Spermatozoas are not visible in gonad lobules and sperm ducts.

III. Spawning capable phase is actually *period of spawning* that take place in spring, with portion release of sexual products (batch spawning) and lasts from 2,5 to 3 months (April, May and June). Histological analyses of the gonads prove asynchronic maturing of the sexual elements. Asynchronic maturing and partially i.e. periodically emptied sperm ducts, confirm that males of White Chub are taking part in spawning more than one time. Male gonads are enlarged to their maximum dimensions and occupy most of abdominal cavity. These gonads are full of mass spermatozoids and occupy most of abdominal cavity. After the first portion release of mature spermatozoids, in sperm ducts are present spermatogonias and spermatocytes in different maturity phases. Over the time, they are changed to spermatozoids that are ready for the release in new portions.

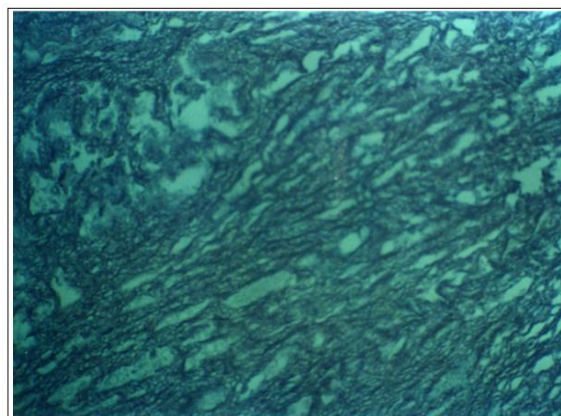


Fig. 6. Histology cut of sexually immature white Chub male gonad, 2⁺ age group (en 100×).

IV. Regressing phase is a post-spawning period that take place in summer and include resorption of remaining sexual products - spermatozoas, as well as for reproduction and regeneration of spermatogonias. This period lasts approximately 2 months (July and August). Stores of spermatozoids in sperm ducts are depleted and their number is decreased in June. Morphologically, male gonads are thin and loose with minimum number of spermatozoids by the end of June and beginning of July. Due to spawning is over, spermatozoids are missing in the male gonads and

resorption process is taking place upon June, in July and August. Spermatogonias are reproducing (proliferating) actively at the periphery of gonads.

V. Regenerating phase characterize small testes even *active spermatogenesis* - proliferation of spermatogonias. This phase take place in autumn and usually last 3 months (September, October and November). Gonads are pale milky colour, shaped like a band and their size is increasing. Histological analyses of the gonads in these moths confirm presence of spermatogonias in the walls of sperm ducts and residual spermatozoas in their lumen.

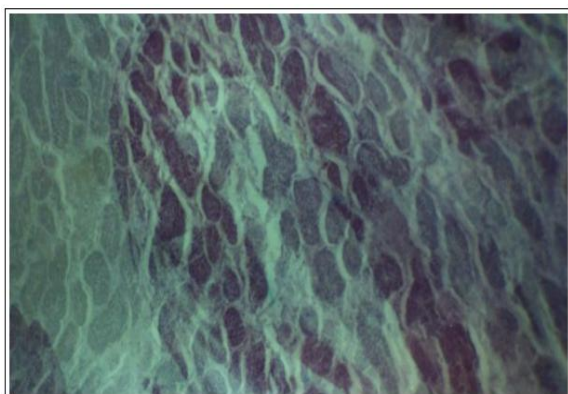


Fig. 7. Histology cut of White Chub male gonad, caught in February (winter period) (en 100×).

B. Temporal phases:

Winter period

Morphologically, in this period testes have a shape of stretched bands in poorly visible pink shade (nuance). Slow increase of male gonads is characteristic for winter season (Fig. 7. and Fig. 8.). Histological analyses of the gonads in January and February confirm that sperm ducts are full of circulating spermatogonias, while primary and secondary spermatocytes and spermatides are present in testes.

Spring period

Testes are enlarged to their maximum size in spring period (Fig. 8.). They are full of mass spermatozoas and occupy most of abdominal cavity. Spermatogonias are also present in the sperm ducts, as well as spermatocytes in different maturity phases. This is a period of full sexual maturing. Asynchronic maturing of sexual elements is identified in the histological analyses. Upon first portion of mature

spermatozoids released, spermatogonias and spermatocytes are passing different maturity phases in testes. Over the time, they are changed to spermatozoas that are ready for the release in new portions.

Summer period

Number of spermatozoas in sperm ducts is decreasing in June. Morphologically, testes are thin and loose with small number of spermatozoas in their lumen in the period end of June - beginning of July. Since spawning is over, spermatozoas are missing in the male gonads and resorption process is taking place upon June, in July and August (Fig. 9.). Spermatogonias are reproducing actively.

Autumn period - Male gonads are pale milky colour and shaped like a band. Size of the gonads is getting to increase in autumn period. Sperm ducts and gonads come into their growth period in September, October and November. Histological analyses of the gonads in these moths confirm presence of active spermatogonias in the sperm ducts that are full of them (Fig. 10.).

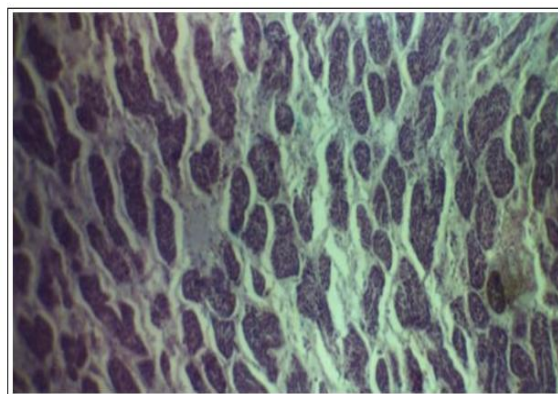


Fig. 8. Histology cut of White Chub male gonad, caught in March (transition period from winter to spring) (en 100×).

Discussion

Results presented above are similar or consistent to data for other cyprinid species from Skadar Lake. In general, reproductive cycle of these species also consist five principal maturity phases in both female and male cycle. Complete gametogenesis was done for smaller number of species and subspecies, on the base of macroscopic descriptions in most of cases.

The first work was done in 1964 by Ivanovic for *Alburnus albidus alborella*. In 1968 this author also provided data on mobility of spermatozoas in different water temperature regimes for following cyprinid species from the Skadar Lake: *Cyprinus carpio*, *Chondrostoma kneri*, *Scardinius erythrophthalmus scardafa*, *Alburnus albidus alborella*, *Pachychilon pictum* and *Rutilus rubilio*. These data are devoted to the lifetime and first phase of spermatozoa mobility, which is in proportion with the increase of water temperature. Ivanovic also worked on gametogenesis of *Pachychilon pictum* in 1969, oogenesis of *Scardinius erythrophthalmus scardafa* in 1975, reproduction cycle of *Alosa falax nilotica* in 1977 and oogenesis of *Chondrostoma nasus ohridanus* in 1978. On the other side, previously initiated works on gametogenesis were continued for *Scardinius erythrophthalmus scardafa* (Knezevic, 1984), *Pachychilon pictum* (Soric, 1949, 1984) and carp - *Cyprinus carpio* (Jankovic, 1971). Ginogenesis, as a specific form of reproduction of *Carassius auratus gibelio* was researched by Maric in 1998.

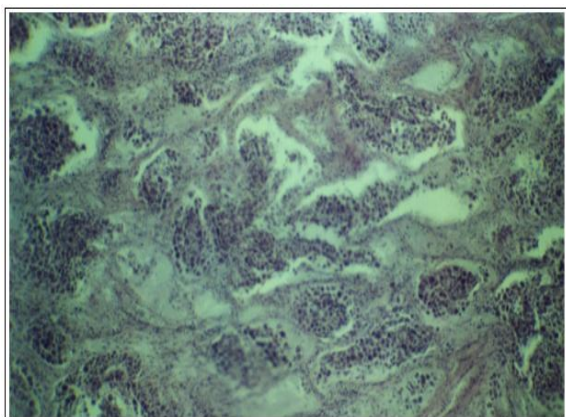


Fig. 9. Histology cut of White Chub male gonad, caught in July (summer period) (en 100×)

Concerning White Chub *Leuciscus cephalus albus* research was done on population structure, general spawning characteristics and relative fecundity (Krivokapic, 2001, 2002, 2004, 2009). Oogenesis was analysed on the base of macroscopic descriptions, only (Sekulovic and Ivanovic 1969).

There is a obvious lack of comparable, species - specific data for male reproduction cycle of White

Chub from Skadar Lake in both, Montenegro and Albania. *Leuciscus cephalus albus* from Skadar Lake and Drin river basins is now recognized as new species *Squalius platyceps* that is distinguished from other species of the genus *Squalius* in the Adriatic basin.

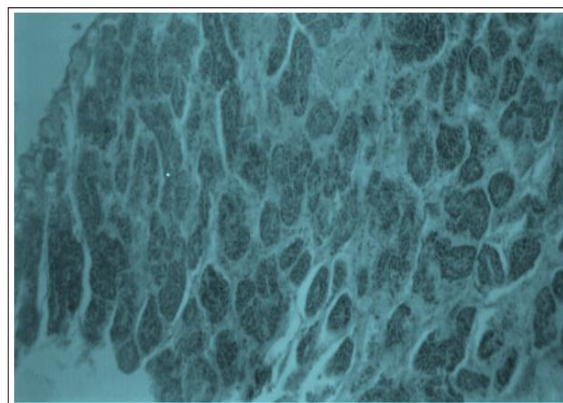


Fig. 10. Histology cut of White Chub male gonad, caught in October (autumn period) (en 100×).

Histological descriptions of the phases of spermatogenesis for this newly recognized species, given in parallel to macroscopic descriptions, above, present a significant contribution to the knowledge of White Chub from Skadar Lake and Drin river basins, but these data couldn't be compared with adequate comparable data on spermatogenesis of White Chub *Squalius platyceps* from other locations in the basins of Skadar Lake and Drin river, as well as widely in the Adriatic basin.

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