CYTOMORPHOLOGICAL CHARACTERISTIC OF THE GONADS OF VYREZUB *Rutilus frisii* (Nordman, 1840) OF DIFFERENT POPULATIONS AFTER SPAWNING

CURCUBET Galina, DOMANCIUC Vasilii, FULGA Nina

Abstract. The research results on the reproductive system of sexually mature females of vyrezub *Rutilus frisii* (Nordman, 1840), the Red Data Book of Republic of Moldova species, caught after spawning from the Dubasari reservoir and the lower reach of the Dniester River have been reflected. The age of puberty was determined as well as the size of protoplasmic growth oocytes, the gonadosomatic index and the type of oocytes development under the conditions of these water bodies. The high rates of growth and the relative mass of the four-year-old female gonads of vyrezub from the Dubasari reservoir contribute to a higher rate of their reproduction. The study of oogenesis revealed asynchrony in the development of germ cells in females from the reservoir.

Keywords: vyrezub, Dubasari reservoir, lower Dniester River, oocytes, gonadosomatic index.

Rezumat. Caracteristica citomorfologică a gonadelor la virezubul *Rutilus frisii* (Nordman, 1840) din diferite populaţii după reproducere. Sunt prezentate rezultatele studierii sistemului reproductiv al femelelor mature de virezub *Rutilus frisii* (Nordman, 1840) capturate în perioada după reproducere din lacul de acumulare Dubăsari și cursul inferior al fluviului Nistru. Au fost determinate vârsta maturării, dimensiunea oocitelor în creșterea protoplasmatică, indicele gonadosomatic și tipul de dezvoltare a oocitelor în condițiile acestor bazine acvatice. Ratele sporite de creștere și greutatea relativă sporite a gonadelor la femelele de virezub cu vârsta de patru ani din lacul Dubăsari favorizează rată mai mare a reproducerii lor. Studiul oogenezei a relevat dezvoltarea asincronică a oocitelor la femele din lacul Dubăsari.

Cuvinte cheie: virezub, lacul de acumulare Dubăsari, Nistru inferior, oocite, indicele gonadosomatic.

INTRODUCTION

The areal of *Rutilus frisii* (Nordman, 1840) is spread in the river basins of the Black Sea and Azov Sea, from Eastern Bulgaria to Western Transcaucasia: Dniester, Southern Bug, Dnieper and other rivers (BURNASHEV, 1962; OPALATENKO, 1978). In the Dubasari reservoir and lower Dniester River this species is semi-anadromous and also forms a freshwater form (MOSHU, TROMBITSKY, 2020). The authors also note that the vyrezub is carried into the middle and lower parts of the Dniester during floods, while in the upper middle section of the Dubasari reservoir it finds favourable spawning conditions. This species has a number of useful qualities, one of which is its ability to feed on molluscs. The active consumption of molluscs, including invasive *Dreissena* spp., occurs even at temperatures of 8-12°C and this is valuable because molluscs currently represent a significant food resource in most reservoirs (KARPEVICH, 1998).

Under the influence of several factors, such as lack of spawning grounds, sharp fluctuations in water level and temperature variations during spawning, the number of vyrezub has decreased so much that it is on the verge of extinction and is included in the Red Book of Russia, Ukraine and Moldova (MOVCHAN, 2001).

The vyrezub's biology have received considerable attention in the ichthyological literature. This includes works by authors such as MYSHKIN (2020), HUDYI (2008) and others. Due to the fact that the vyrezub is listed in the Red Data Books of Republic of Moldova and Ukraine, a small number of literature sources has been devoted to histological analysis of the development of germ cells in various aquatic ecosystems. This paper presents the biological and morpho-functional characteristics of post-spawning ovaries of vyrezub from the Dubasari reservoir and lower Dniester River.

MATERIAL AND METHODS

Gonads of females and males collected from net catches after spawning period in the beginning of May 2020 from Dubasari reservoir and lower Dniester River were used for histological studies. The gonad samples were fixed in 4% formaldehyde, followed by treatment according to generally accepted methods. The maturity stages of gonads were determined according to Meyen with the specifications of SAKUN and BUTSKAIA (1963), the degree of oocyte development was determined according to the classification of KAZANSKII (1949). 7 µm thickn sections were stained using the Mallori method (ROSKIN, LIVENSON, 1957). All individuals of vyrezub were subjected to general biological analysis with the determination of linear weight indicators, age, gonadosomatic index (GSI) and fatness coefficient (FC) according to Fulton and Clark (PRAVDIN, 1966). The GSI was defined as the ratio of gonads weight to carcass weight. Micrographs as well as measurements of oocytes in the completed phase of protoplasmic growth were taken using an Axio Imager A2 microscope. Statistical data processing was performed using Microsoft Excel 2007 and STATISTICA 6.0 for Windows.

RESULTS AND DISCUSSION

In these reservoirs vyrezub becomes sexually matured at the age of three years and its length and body weight are significantly higher than those of the fish from the Dniester reservoir (HUDYI, 2008). The author also notes a higher rate of linear growth of fish from the Dniester reservoir than that of individuals from the Dniester before its regulation by hydropower dams. According to MYSHKIN (2020), the maturation of vyrezub and its ability to natural reproduction, in the conditions of reservoirs in the European part of areal, occurs at the age of 3-5 years.

In the Dubasari reservoir at the beginning of May, the spawned females in the catches included individuals with gonads at stage II of maturity, which contain oocytes of protoplasmic growth and resorbing oocytes of the second generation in the phase of vacuolization (Fig. 1), which indicates asynchrony in their development. However, in the bulk of the spawning broodstock in this water body, the gonads contain only one generation of oocytes in the protoplasmic growth phase. Asynchronous oocyte development and the formation of the second batch of eggs followed by their resorption have also been observed in females of vyrezub from the Dniestrovsky reservoir (HUDIY et al., 2009).

Both asynchronous and synchronous gametogenesis types are characteristic for the female of vyrezub in the Dubasari reservoir, unlike in the Dniester river (Fig. 1).

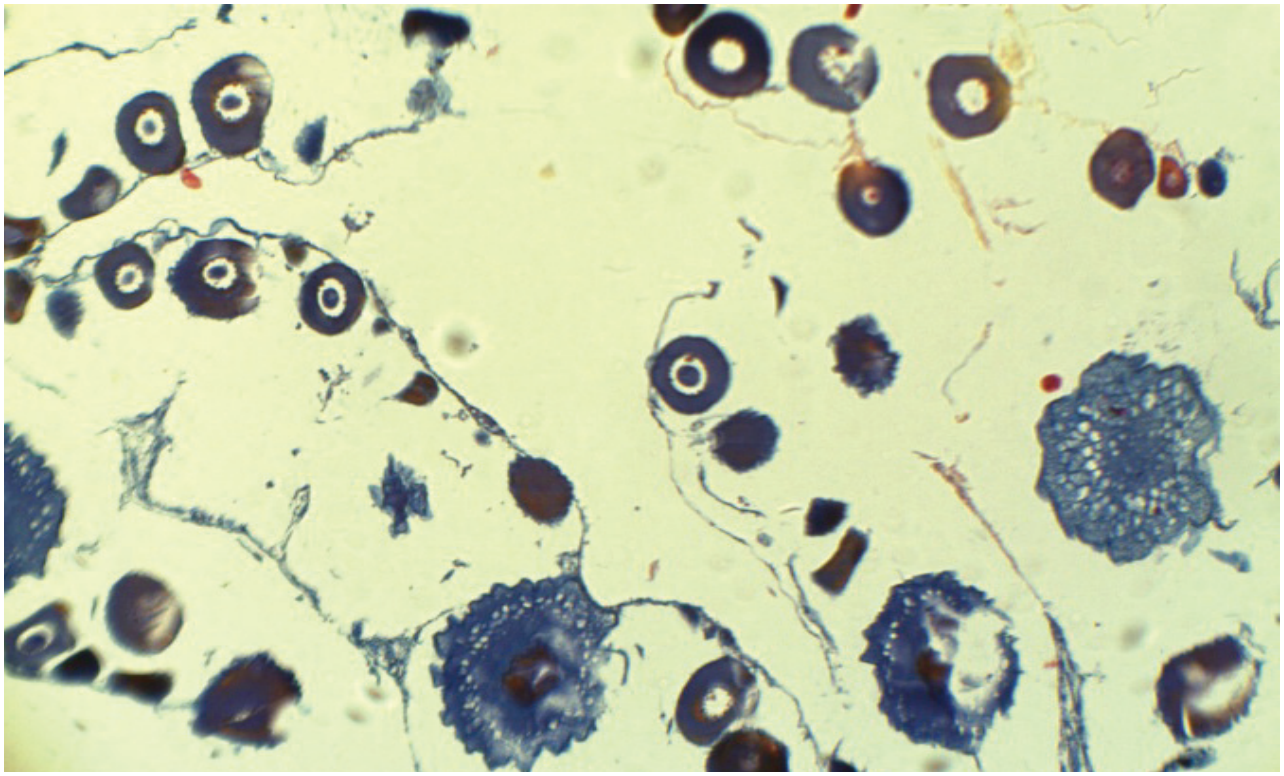

Figure 1. Ovary at II after the spawning stage of maturity.
Second generation oocyte resorption during the vacuolisation phase (original).

A histological study of the gonads in Dniester females during this period indicates the synchronous development of eggs, which is confirmed by the presence in the ovary of spawned fish of released follicular membranes, residual elements from past spawning and oocytes at all phases of protoplasmic growth of the generation of the next year (Fig. 2).

Three-year-old females from the lower Dniester had lower values for all morpho-physiological indicators comparing with females from the Dubasari reservoir (Table 1). As it can be seen from the table, the lowest values of length, body weight, gonad weight and GSI are recorded for fish from the lower Dniester.

In females of different populations, the dependence of the oocyte size of the single-layer follicle (phase 'C') on the age of the fish can clearly be seen (Table 2). The oocyte diameter in four yearlings from the reservoir was significantly higher than $P \geq 0.999$. In the three-year-old females from the Dniester, the mean oocyte diameter is slightly smaller compared to females from the Dubasari reservoir, but these differences are not significant.

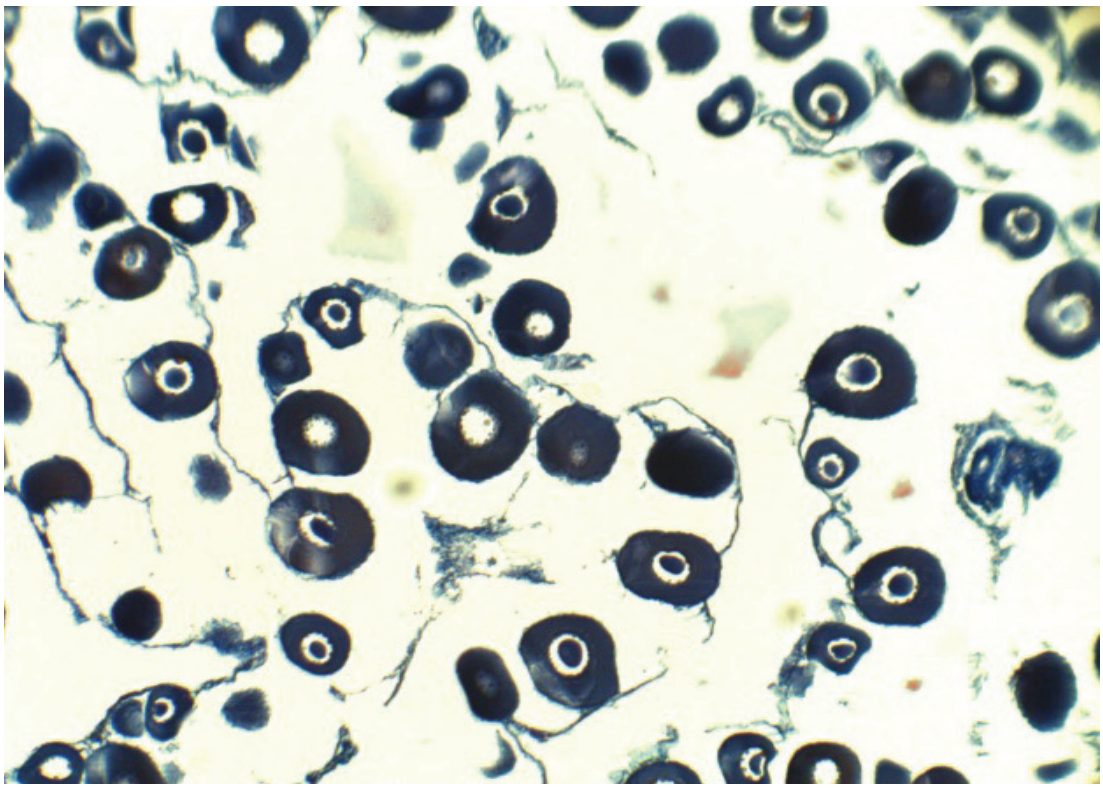

Figure 2. Ovary at II after the spawning stage of maturity of the Dniester females.
The synchronous development of oocytes is observed (original).

Table 1. Biological characteristics of vyrezub of different water bodies.

Fish age, year	Length *l*, cm	Body weight, g	Carcass weight, g	FC according to Clark	FC according to Fulton
Dubasari reservoir					
3 ♂	38,5±0,70	1175±160,0	951±44,0	1,67±0,14	2,06±0,16
4 ♂	42,5±0,35	1500±140,0	1277±98,0	1,71±0,16	2,02±0,18
3 ♀	40,0±0,98	1300±105,0	1020±58,0	1,60±0,09	2,03±0,04
4 ♀	45,7±1,20	1937±95,0	1587±65,0	1,65±0,06	2,04 ± 0,07
Lower Dniester					
jv	-	130	114	1,32	1,50
3♀	34,5±2,9	827±200,0	740±190,0	1,64±0,03	1,87±0,04

Table 2. Reproductive biology of vyrezub female in different water bodies.

Fish age, year	Stage of maturity	Gonads weight, g	GSI, %	Oocytes size at phase "C", µm
Dubasari reservoir				
3	II, after spawning	11,0±0,36	1,07±0,48	123,6 ± 1,39
4		13,25 ±0,48	0,840 ± 0,18	132,8 ± 1,46
Lower Dniester				
3	II, after spawning	2,5 ± 0,50	0,360 ± 0,07	120,8 ± 2,51

Vyrezub is very sensitive to external habitat conditions. It is negatively affected by such factors as turbidity caused by wastewater discharges; especially dangerous are salvo chemical discharges and sudden temperature changes (MYSHKIN, 2020). In females of vyrezub from the lower Dniester, after hatching, the total degeneration of oocytes of the new generation (the protoplasmic growth period) is observed, a sign of which is the destruction of the nucleus, homogeneization of cytoplasm and formation of pyknotic cells, which is caused by adverse environmental conditions in the lower Dniester during this period (Fig. 3).

Signs of resorption in the ovaries during the summer period were also observed in vyrezub inhabiting the Dniester reservoir (HUDYI, 2009).

In females and males of different age in the Dubasari reservoir insignificant fluctuations between indicators of fatness coefficient according to Clark were revealed, which are determined by favourable conditions for fish fattening. It should be noted that fish from this reservoir have a large fat layer on the gonads and in the body cavity, whereas fish from the Dniester this layer is virtually absent, indicating lower values of fatness coefficient according to Fulton in the latter (Tab. 1). After the spawning season in males a small number of resorbed residual sperm cells are observed in the seminal duct. The seminal ampullae are collapsed and cysts with fissile spermatocytes of the first order are arranged in several layers on their periphery (Fig. 4).

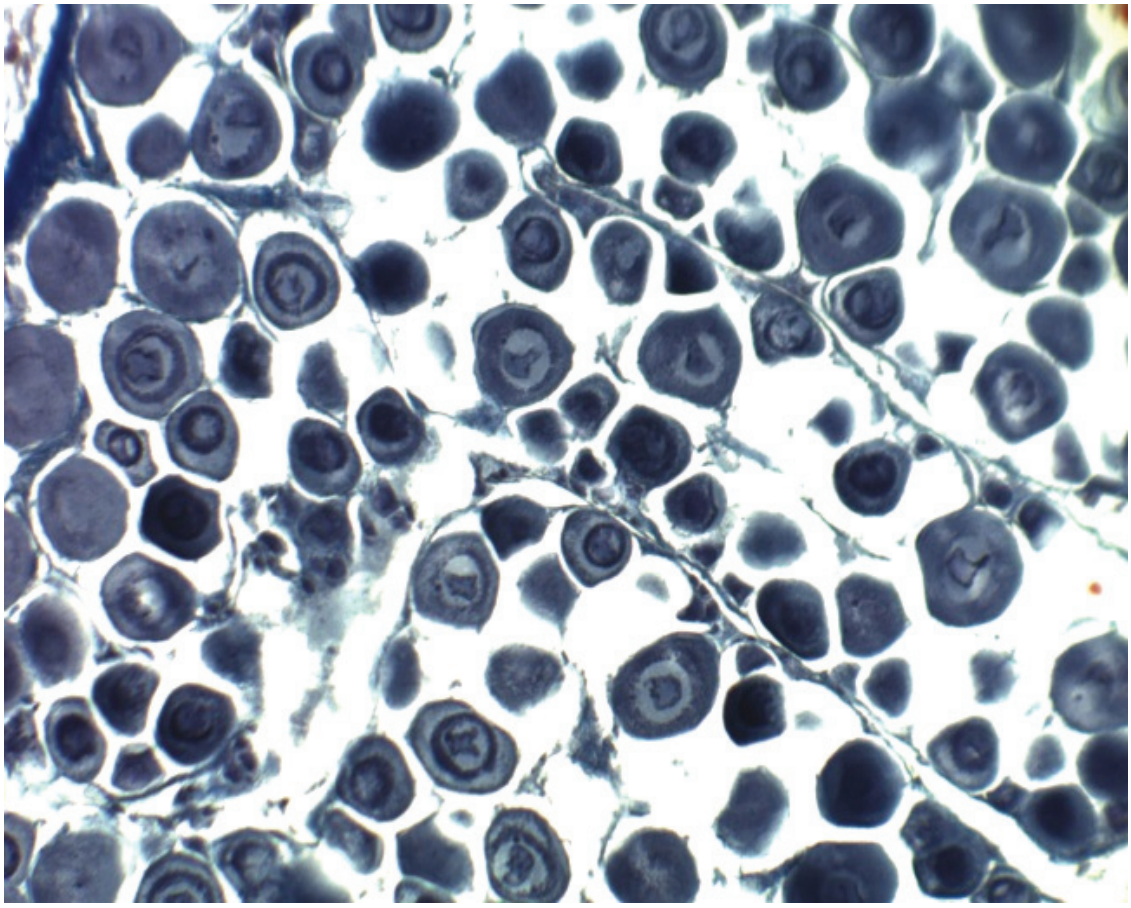

Figure 3. Female ovary from the lower Dniester with protoplasmic growth oocytes in degeneration (original).

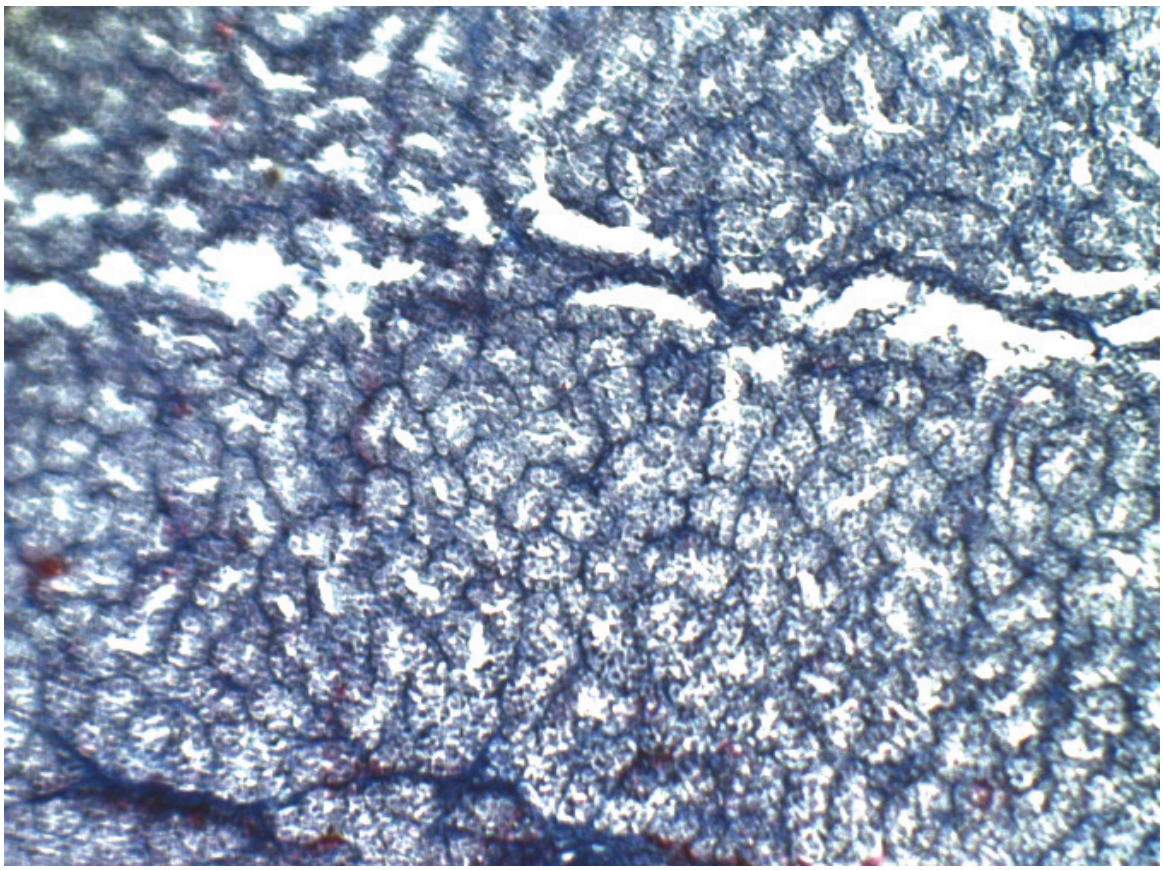

Figure 4. Testes of vyrezub after spawning (original).

CONCLUSIONS

1. The biological analysis of sexually mature specimens of the vyrezub (*Rutilus frisii*) from the Dubasari reservoir and the Lower Dniester revealed differences in linear weight indices, which are determined by the environmental conditions of different populations. Females in both habitats in the Dniester become sexually mature at the age of three years.

2. The physiological state of sexually mature females in the post-spawning period is the most favorable for individuals in the Dubasari reservoir. Higher indicators of growth rate and relative weight of gonads of four-year-old females also contribute to higher reproduction rate of population of vyrezub in the Dubasari reservoir.

3. Some females from the reservoir are characterized by an asynchronous type of oocyte development, but only one generation of oocytes is hatched and oocytes of the second generation (in the vacuolization phase) undergo resorption. The dependence of the protoplasmic growth oocyte size from the age of the fish has been registered in all the females.

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Domanciuc Vasilii, Fulga Nina
Research Center for Genetic Aquatic Resources "AquaGenResurs",
Constantin Tanase str., 6, MD-2005, Chisinau, Republic of Moldova.
E-mail: scsp59@mail.ru

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