BIOLOGICAL AND MORPHO-PHISIOLOGICAL CHARACTERISTICS OF BIGHEAD GOBY *PONTICOLA KESSLERI* FEMALES (GÜNTER 1861) FROM THE LOWER DNIESTER

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Abstract: In this study we present our results on the investigation of reproductive system during spawning season of adult bighead goby *Ponticola kessleri* (Günter, 1861) females from the Lower Dniester. It was determined the size and age of gonadal maturity, size of oocytes during trophoplasmatic growth and terms of the spawning season of the fish. Due to asynchronous development of the oocytes, females of bighead goby produce three clutches during the spawning season, which lasts from April to June. During one reproductive season ovary weight is decreasing after each clutch, which leads to a decreased gonadosomatic index. It was observed that increased water temperatures during the reproductive season in the Lower Dniester, which range from 11 to 24 ºC, negatively influence the formation of a new clutch of oocytes.

Keywords: bighead goby, generation of oocytes, gonadal maturation stages, gonadosomatic index (GSI), *Ponticola kessleri*, portioned spawning

Introduction:

Bighead goby *Ponticola kessleri* (Günter 1861) is a Ponto-Caspian relict goby species, native to Eurasia. This is an invasive species which has expanded its distribution area due to a very high ecological plasticity. So far, it has been recorded from several non-native water systems, reaching the Baltic watershed through Rhine and Elbe Rivers. The native areal of *Ponticola kessleri* includes lower river parts and river mouths of Dniester, Bug, Dnieper and Danube Delta (Bănărescu 1964).

It is known that environmental and ecological effect of biological invasions performed by alien species is possible to occur in case of naturalization of such species. Thus, it integrates successfully onto the new ecosystem and becomes a part of native community of organisms (Elton 1960).

At present, two major factors are affecting especially large rivers, global warming and river fragmentation and flow regulation, leading to transformation of rivers into a chain of lake type reservoirs. Namely, these two circumstances are responsible for

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triggering and maintenance of biological invasions of fishes in the basins of the largest Ponto-Caspian rivers (Slynko and Tereshchenko 2014). A key role in the expansion of the favourable areas suitable for gobies occurrence and existence, is the reduced ecological pressure from predators and the depleted composition of ichthyocenoses. A wide range of adaptations related to reproduction, such as asynchronous oogenesis, portioned spawning, wide range of temperature tolerance required for spawning, male care of offspring contribute to its fast increase in population size and spatial distribution.

Several biological aspects of *Ponticola kessleri* have been studied by many authors (Berg 1949; Burnashev et al. 1955; Kalinina 1976; Gavlena 1977; Kirilenko and Shemonaev 2010). However, there is a lack of knowledge regarding reproductive characteristics (e.g. terms of spawning, amount of eggs per clutch, gonadal development in adult individuals during reproductive cycle) or, rather are scarcely studies. This study is mainly focused on the investigation of oogenesis of bighead goby in order to reveal the peculiarities of oocyte development, amount of clutches per reproductive season, cycles and spawning periods.

Materials and methods:

Ichthyologic samples were collected during 2016-2018 period from the Lower Dniester. Fish specimens were sampled using a 6-meter length beach seine with mesh size of 5×5 mm designed to collect small benthic fishes. Further, samples were analysed using classical ichthyologic and environmental methods (Pravdin 1966; Koblitskaya 1981). The dominance index (D) expresses the following values: D1 insignificant (<1.1%), D2 Minor (1.1% - 2%), D3 Subdominant (2.1% - 5%), D4 Dominant (5.1% -10%), D5 Absolute dominant (>10%) (Gomoiu and Skolka 2001).

For histological sectioning there were isolated gonads from 56 adult females, which were collected during their spawning and reproductive cycles. Small pieces of the ovaries of 5 mm thickness were sampled from each specimen and fixed in Bouin solution followed by the classical histological procedure. Stages of gonadal maturation were determined according to Mayenne (1939) with specifications by Sakun and Butskaya (1968), stages of oocyte growth were determined according to classification of Kazanskiy (1949). Sections with 7 µm thickness were stained following Malori method (Roskin and Levinson 1957). For all collected females were performed several measurements and analyses, including gonado-somatic index (GSI) (Pravdin 1966). The gonado-somatic index was calculated as the gonad weight to the fraction of the whole body weight. Microphotography, as well as the measurement of oocytes in the phase of complete protoplasmic growth and at different phases of trophoplasmic growth were performed using an Axio Imager A2 microscope. The obtained data were processed statistically using the Microsoft Excel-2007 and STATISTICA 6.0.

Results and discussion:

The obtained values of environmental indices for *Ponticola kessleri* from the Lower Dniester over the past three years indicate a gradual increase of their ratio per total structure of ichthyofauna, moving from a subdominant in 2017 - 2.52% (D3), 3.96% (D3) - in 2018, to dominant positions 5.37% (D4) - in 2019. The negative impact caused by the positive dynamics of the abundance of this species in the river is primarily associated with competition for habitat, predation, interference with spawning and competition for food. Moreover, this species inflicts a great negative impact upon hatching success of native fish species by feeding on their eggs and their hatched juveniles.

Age structure of *Ponticola kessleri* is represented by five main groups (Tab. 1). A

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significant part of the catches consists of mature individuals. The sex ratio in all populations is female biased, engendering an index of 64.86%. According to GSI, sexual maturity was estimated to be reached at the age of two years old (1+) featuring a length of 9.0 cm and a weight of 12.46 g. According to previously published data, in other gobies from Black Sea and Azov Sea, sexual maturity was estimated to occur at the same age with a length of 8.4 cm and a mass of 17 g (Kalinina 1976).The size composition of the bighead goby varies between populations from Lower Dniester and Dubasari reservoir, where the mean range of size is 16 – 22 cm (Burnashev et al. 1955). The different age composition in bighead goby populations from the Lower Dniester indicates that this species has been adapted to the environmental conditions of the river having become the new permanent representative of the local ichthyofauna of the Lower Dniester. Similar to other fish species with a relatively short life cycle, this species shows a fast rhythm of growing particularly in the first two years. On the other hand, the greatest gains in body mass were observed at those of and five years old (Tab. 1).

Table no. 1 Age-related dynamics of linear-weight indices in females of *Ponticola kessleri* of the lower Dniester

Age, year (n = 59)	Standard body lenght, cm	Body weight, g
0+	6.56 ± 0.10	4.43 ± 0.26
1+	9.03 ± 0.20	12.46 ± 0.72
2+	11.00 ± 0.17	25.43 ± 1.28
3+	12.94 ± 0.17	41.90 ± 1.81
4+	15.26 ± 0.14	66.63 ± 12.03

The spawning season in individuals of bighead goby in the Lower Dniester begins in the second decade of April at a water temperature of 14 °C. Females lay eggs on rocks, stones, empty shells of molluscs and aquatic plants, while males are guarding the eggs until hatching (Fig. 1).

Bighead goby females in the Dubasari water reservoir are starting their spawning period as it was mentioned above (Burnashev et al. 1955), while in Volgograd reservoir their spawning begins later in June (Gavlena 1977). Also, during this period, among spawning specimens are found females with gonads completing the last stage IV of maturity. In this period, their GSI reaches maximum values (Tab. 2). Follicular epithelial cells in oocytes that have completed the accumulation of yolk show signs of high functional activity, they take a cubic shape and their height reaches 13.1 ± 0.28 μm (Fig. 2). During the oocyte maturation, the yolk vesicles are merge forming a homogeneous mass.

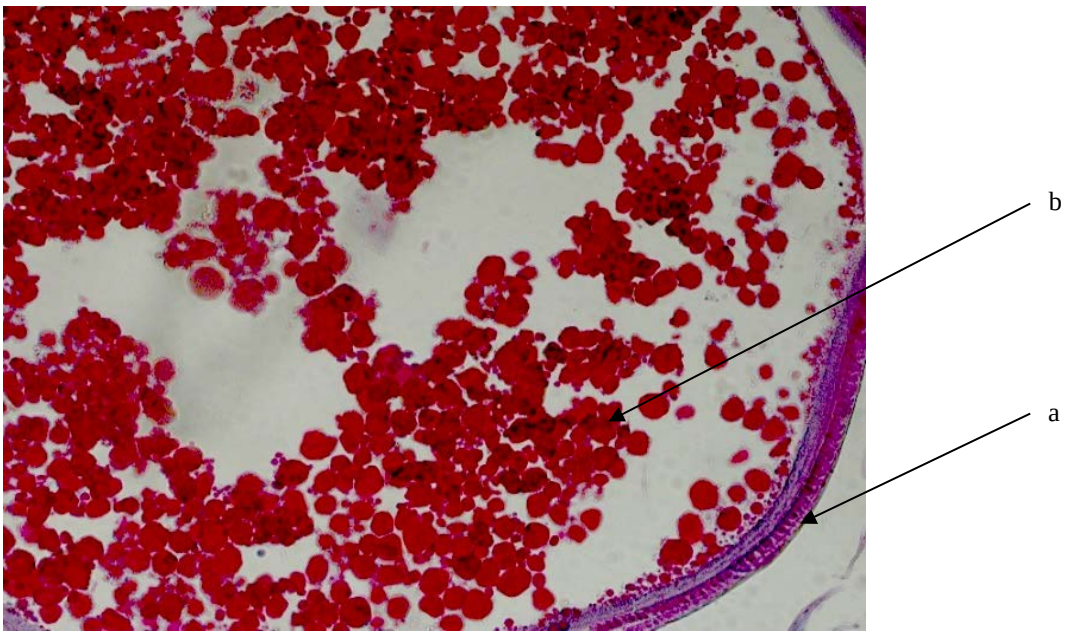
After the first spawning, in gonads with empty follicles and oocytes at different phases of protoplasmic growth, there were also observed oocytes in the vacuolization stage, fat deposits and accumulation of yolk enjoing an average size of 514.66 ± 4.08 microns. Subsequently, with the increasing of water temperature in the later stages of oocyte development, belonging to the next round of spawning, there was observed an intensive accumulation of yolk granules with an average maximum size of 10.50 ± 18.06 μm.

In the second decade of May the second clutch of eggs is laid, after which the gonads are undergoing the VI – III stages of maturity. The rate of GSI decreases and shows relatively low values, in comparison with the first spawning ($P \geq 0.95$), which indicates a decrease in the number of oocytes for the next portion of eggs (Tab. 2). Similar patterns were observed in round goby (*Neogobius*

melanostomus), where the number of eggs in the first clutch was higher than in the subsequent ones (Kulikova and Fandeeva 1975).

Figure no. 1 Egg clutch of *Ponticola kessleri* in an empty shell of a mollusc in the Lower Dniester

Figure no. 2 Section through oocyte yolk



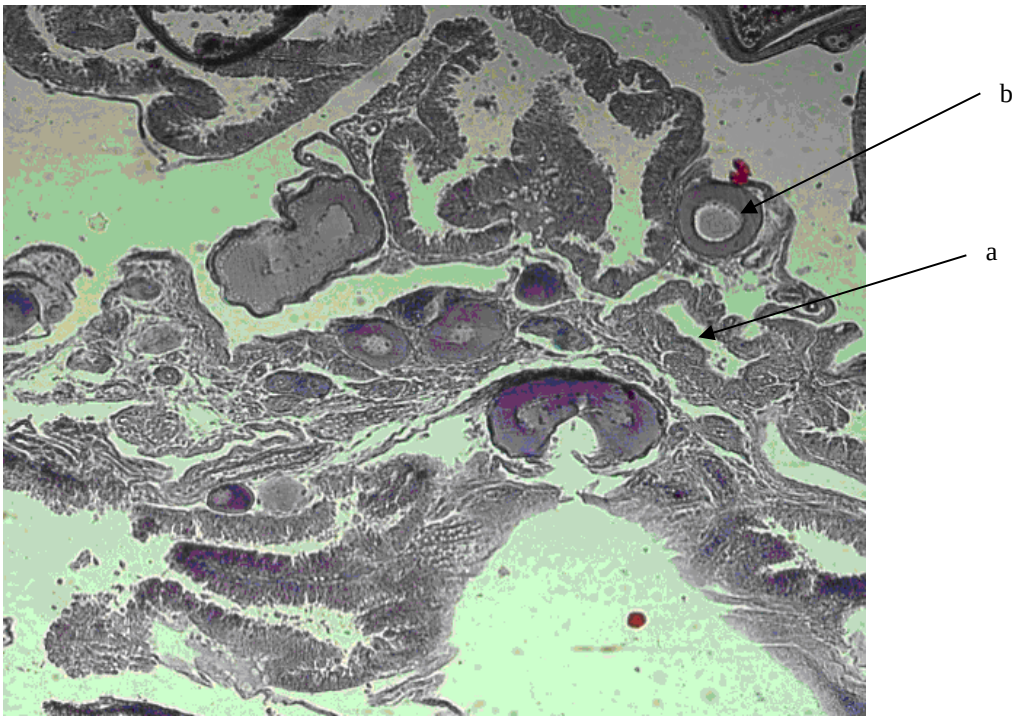
Note: a - Follicular epithelial cells; b - Yolk granules

Table no. 2 Functional characteristics of *Ponticola kessleri* females during spawning period in Lower Dniester

Date	Stage of gonadal growth	Gonadal weight, g	GSL, %	KU by Clark
25 February	IV unfinished	3.15 ± 0.54	13.55 ± 1.13	1.34 ± 0.15
19 April	IV finished	5.47 ± 0.31	18.50 ± 2.08	1.20 ± 0.12
	VI-III$_2$	0.83 ± 0.12	4.03 ± 0.61	1.26 ± 0.09
20 May	VI-III$_3$	0.54 ± 0.13	2.09 ± 0.34	1.62 ± 0.16
6 June	VI-II	0.015 ± 0.002	0.05 ± 0.008	1.58 ± 0.21
14 July	II	0.10 ± 0.02	0.56 ± 0.04	1.64 ± 0.12
20 August	II-III	0.12 ± 0.03	0.71 ± 0.09	1.60 ± 0.07
10 October	III-IV	0.41 ± 0.12	2.71 ± 0.49	1.62 ± 0.11

Eggs of the third clutch/portion, formed at a water temperature of 20-23 ºC, are laid in the first decade of June. Gonads of spawned females are entering VII-II stages of maturity, containing a large number of empty follicles, which indicates a complete release of eggs, as well as the presence of protoplasmic growth oocytes belonging to the next generation of the following year (Fig. 3). Therefore, with the increasing of water temperature in the spring - summer period (May-June), the development of the third portion of eggs is accelerated due to a more intensive metabolic rate. After the end of the spawning season, females display a significant decrease in the weight of gonads, GSI significantly decreasing to the minimum values ($P \geq 0.999$) (Tab. 2).

Figure no. 3 Section through emptied ovary after third round of spawning

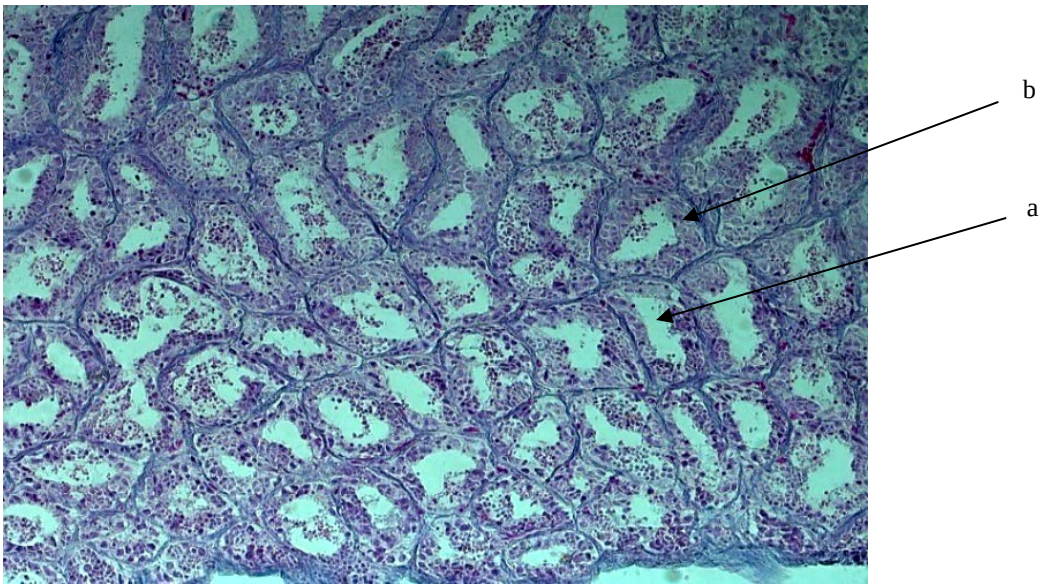


Note: a - Emptied follicular membranes; b - Oocytes at different phases of protoplasmic growth

The lower values of the gonado-somatic index after each spawning are explained by the fact that, after releasing each portion of eggs from the same season, most of the remained oocytes are in the phases of vacuolization and a small number – in the phase of vitellogenesis.

During June, along with spawning females, there are also mature spawning males whose gonads contain empty and partially empty ampoules, indicative of the completion of the breeding season of *Ponticola kessleri*. At the periphery of the seminiferous tubules, dividing germ cells are organised in several layers, pointing to a new round of spermatogenesis (Fig. 4).

Figure no. 4 Section of a male gonad at the end of the spawning season



Note: a - Seminiferous ampoules; b - Cysts of dividing spermatogonial cells

According to our studies, during the entire spawning period, females of *P. kessleri* in the lower Dniester lay three clutches of eggs. Based on the observations of M.S. Burnashev et al. (1955), during the early years of building of the Dubasari reservoir the spawning of this species occurred during April - May. Notably, during the entire spawning season, there were also observed females with oocytes which underwent the yolk resorption (Fig. 5). It is known that, in different types of gobi males spawn once during the breeding season, fertilizing eggs laid by several females (Yankovskiy 1966). Furthermore, if the males that have spawned or are not ready for spawning, unreleased mature eggs from females undergo resorption of yolk.

After spawning, in female gonads after the end of resorption processes, a new wave of oogenesis begins. In July, gonads contain oocytes at all phases of protoplasmic growth, with a maximum size of 108.12 μm (Fig. 6).

In August, when water temperature is usually around 24 °C the oocytes that will be released at the beginning of the following reproductive season in spring, begin the process of vacuolization of the cytoplasm, which corresponds to the transition of the gonads to the II-III stage of maturity. The

oocyte size in the initial phase of cytoplasmic vacuolization corresponds to 211.32 ± 4.08 μm.

Figure no. 5 Section through female ovary with unreleased eggs at the stage of yolk resorption

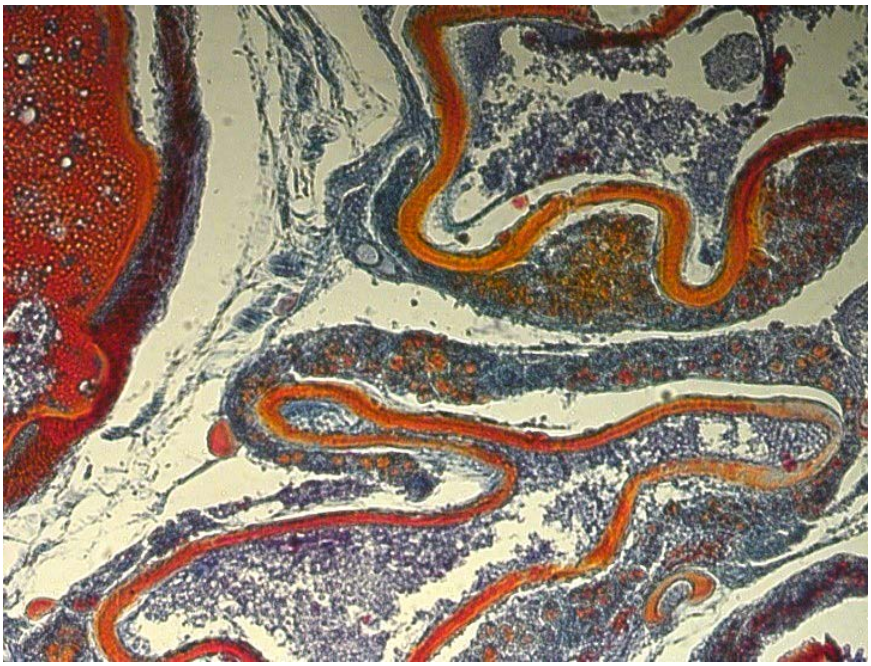
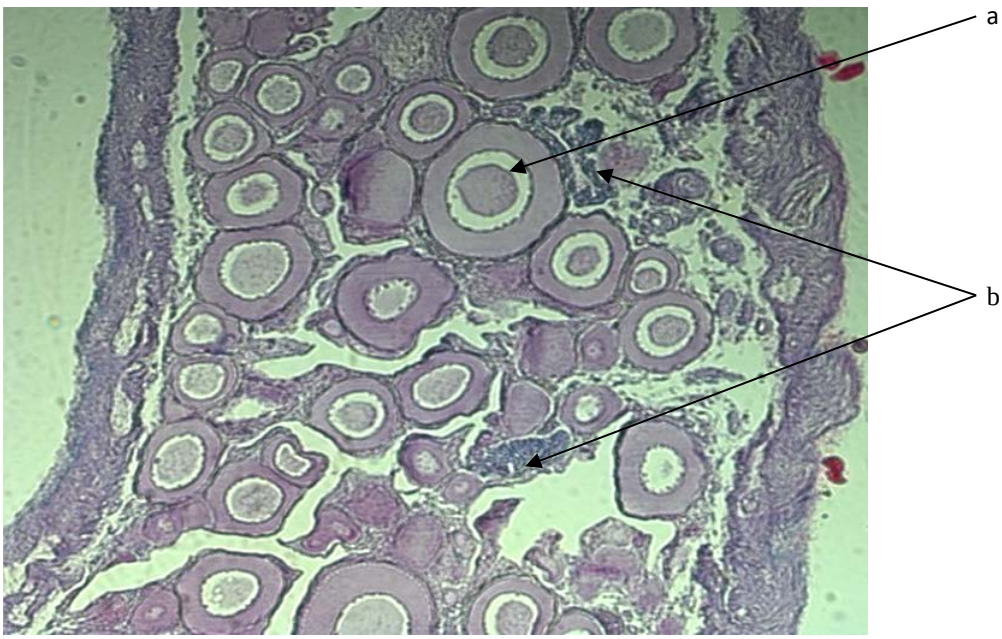


Figure no. 6 Section through female ovary at the second stage of maturity



Note: a - Oocytes during different phases of protoplasmic growth; b - Collapsed follicular membranes

In October, with a decrease of water temperature to 11 °C, the accumulation of yolk granules begins in the eggs, which corresponds to the transition of the gonads to the III-IV stages of maturity. As result, there is a significant increase in GSI (Tab. 2).

During winter there is a constant development of oocytes. In February, at a water temperature of 5 °C, in female ovaries, along with oocytes in protoplasmic growth, are also present oocytes at later phases of growth in different stages of yolk accumulation. Such a diversity of oocytes in different stages indicates the transition of the ovaries into the IV incomplete stage of maturity. It was revealed seasonal dynamics of fatness; females are reaching the maximum value during feeding, while the minimum value – during winter (Tab. 2).

It is known that in fishes with asynchronous vitellogenesis, the growth rate of oocytes during the maturation period of the first and subsequent portions is different (Koshelev 1962). Our studies show that the formation of the first portion of eggs in *Ponticola kessleri* in the lower Dniester takes 7 months (October-February-April) at a water temperature range of 11-5-14 °C. Second spawning occurs a month later after the first spawning, at a water temperature of 14-20 °C, while the third spawning lasts 16 days, at a water temperature during May-June of 20-23 °C. Studies provided by Kulikova (1985) also indicate a reduction in the period of development of the next generation of oocytes correlated with the increase of water temperature in round goby.

Conclusions:

The presence of different age groups in the studied populations suggests that *Ponticola kessleri* has adapted to the local conditions of the Lower Dniester and successfully reproduce.

Females of *P. kessleri* are characterized by an asynchronous type of oocyte development and portioned spawning. During the spawning season, from April to June, females lay three clutches of eggs. From the beginning to the end of spawning, a decrease in the relative mass of gonads and, consequently, GSI is observed.

With an increase of water temperature from 11 °C to 24 °C in the Lower Dniester, the period of oocyte maturation significantly decreases. The development of oocytes of the first generation occurs within 7 months, the second - 1 month and the third 16 days. The absence of ready to spawn males is triggering the resorption of yolky oocytes in mature females.

Rezumat:

CARACTERISTICI BIOLOGICE ȘI MORFO-FIZIOLOGICE ALE FEMELELOR DE GUVID DE BALTĂ *PONTICOLA KESSLERI* (GÜNTER, 1861) DIN NISTRUL DE JOS

În acest studiu prezentăm rezultatele noastre cu privire la investigația sistemului reproductiv în timpul sezonului de depunere a icrelor la femelele de vârstă adultă de *Ponticola kessleri* (Günter, 1861), femele din Nistrul de Jos. S-au determinat mărimea și vârsta maturității gonadale, mărimea ovocitelor în timpul creșterii trofoplasmatice și termenii sezonului de reproducere a peștilor. Datorită dezvoltării asincrone a ovocitelor, femelele de guvid de baltă produc trei ponte în timpul sezonului de reproducere, care durează din aprilie până în iunie. În timpul unui sezon de reproducere, greutatea ovarului scade după fiecare pontă, ceea ce duce la un indice gonadosomatic scăzut. S-a observat că temperaturile crescute ale apei în timpul sezonului de reproducere în Nistrul de Jos, care variază de la 11 la 24 °C, influențează negativ formarea unei noi ponte de ovocite.

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