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Reproductive features of bream *Abramis brama* (Linnaeus, 1758) of Zaporozhian (Dnipro) Reservoir (Dnipropetrovsk, Ukraine)

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ABSTRACT

The research was held in terms of Zaporozhian Reservoir (Dnipropetrovsk, Ukraine) from 2010 till 2014 year. For the first time in the last 50 years complex histological studies of reproductive system of bream from Zaporozhian Reservoir was conducted. The regularities of oogenesis and gonadogenesis, passing of sexual cycles and ecology of spawning of bream in the conditions of ecological transformation of Zaporozhian Reservoir were researched. Bream spawning population included 10 age classes. Absolute fertility of individuals of different ages ranged from 54.31 to 856.0 thousand eggs, and the average fertility was within 148-161 thousand eggs. Histological study of the ovaries showed asynchronous oocyte development in period of vitellogenesis. In conditions of Zaporozhian Reservoir bream generate only one portion of spawn, and 6-10 % of oocytes in phase of vacuolization were resorpted. The current environmental conditions of reservoir bream populations are subjected to natural and forced intra-differentiation, which is the adaptive mechanism of its reproduction.

***Keywords*:** bream; *Abramis brama*; development of gonads; oocyte; Zaporozhian Reservoir

1. INTRODUCTION

The modern existence of continental reservoirs is characterized by increased pressures on components of aquatic ecosystems. Water pollution by flows of technogenic and service-utility origin, which contain mineral and organic substances, pesticides, oil products and radionuclides, change the habitat of aquatic lives, which is reflected in their species composition and dynamics of quantitative indicators. In turn, this leads to the transformation of the species composition of fish fauna, and it reduces the number of valuable fish species (Pike *Exos luceus* Linnaeus, 1758; Russian sturgeon *Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833; Sterlet Acipencer ruthenus Linnaeus, 1758; Pikeperch Sander lucioperca Linnaeus, 1758) and the number eurybiontic short-cycle of fish (Black Sea sprat *Clupeonella cultriventris* Nordmann, 1840; European bitterling *Rhodeus amarus* Bloch, 1782; Pseudorasbora *Pseudorasbora parva* Temminck et Schlegel, 1846; gobies Gobiidae Fleming, 1822) grows up. Also this is contributed by deterioration of the main species reproduction conditions. Everyone knows that the volume of commercial reserve and the level of the reproduction of certain fish species are determined by the efficiency of their breeding. This means that the number of fish populations in natural waters is limited mainly by breeding conditions. Many regulated reservoirs have significant stress factors for natural reproduction of native fish species – violation of level regime in the spring, the adverse condition of spawning grounds, illegal fishing during the spawning season, etc.

The new conditions for fish existence emerged as a result of over-regulation of the Dnieper River runoff and creating cascade of reservoirs; they have caused the significant restructuring of qualitative and quantitative ichthyofaunal content [1-3]. The number of migratory and reophilic fish species (European asp *Aspius aspius* Linnaeus, 1758; Common vimba *Vimba vimba* Linnaeus, 1758; Common barbel *Barbus barbus* Linnaeus, 1758; Pontic-Azov shad *Alosa pontica* Eichwald, 1838; Russian sturgeon *Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833) gradually decreased, while the number of limnophilic fish (Prussian carp *Carassius gibelio* Bloch, 1782; Silver bream *Blicca bjoerkna* Linnaeus, 1758; Common tench *Tinca tinca* Linnaeus, 1758; Common rudd *Scardinius erythrophthalmus* Linnaeus, 1758; European perch *Perca fluviatilis* Linnaeus, 1758; Common bream *Abramis brama* Linnaeus, 1758; Common carp *Cyprinus carpio* Linnaeus, 1758) respectively increased. The latter takes the leading position in fish catches. The runoff over-regulation regime which acts in the reservoir waters over the past 80 years has led to siltation, overgrowth of natural spawning grounds with aquatic vegetation, shoaling shallow coastal areas. As a result, throughout the reservoir water area, rather tense situation with natural reproduction of resource fish species has been formed, and the overall environmental situation of the basin has worsened on the background of intense human impact [4].

Analysis of data on ichthyologic and fish-breeding research obtained in the Dnieper region reservoirs and published in the past 50 years has shown that the reproduction ecology of some valuable commercial fish species is not fully investigated [5-9]. It remains unclear how human activities impact on life cycles of some fish, especially on the state and functioning of their reproductive systems. Recent studies of fish reproductive indexes also indicate some disorder in formation of genital products and in fertility of valuable industrial species in habiting areas with permanent environmental stress [1]. Research of fish gonads has not only theoretical interest, but the significant practical importance in solving a number of issues of industrial and environmental value as well.

In this context, the aim of the work was to carry out the comprehensive studies of reproductive systems of bream *Abramis brama* (Linnaeus, 1758) in conditions of environmental transformation of Zaporozhian Reservoir using the modern histological methods.

2. MATERIALS AND METHODS

Researches were conducted in Zaporozhian Reservoir – the multi-purpose reservoir during 2010-2014. The reservoir is located in the South-west of Ukraine, in the territory of the agro-industrial zones and been under strong anthropogenic influence. According to the results of long-term monitoring researches the bar area of high heavy metals concentration, salinity and eutrophication were found in the reservoir waters [10,11]. Samples of fish were collected in the lower part of the reservoir, where fishery is constantly hold and a graziery of commercial fish species is concentrated. Ichthyologic samplings were carried out during the growing season through research and control catches in the waters of Zaporozhian Reservoir: near the Viyskove village (48°22'30.75" N; 35°20'80.05" E) and in Samara (Bay 48°53'40.21" N; 35°18'73.20" E) (Fig. 1).

Totally, 1480 specimens of bream *Abramis brama* (Linnaeus, 1758) were used for full biological analysis, 410 samples of bream eggs were collected and analyzed to determine the fertility, 120 histological slices of fish eggs were manufactured and analyzed.

Research fish captures were carried out on the basis of catch permits for special use of water biological resources. The captures were committed with a standard set of stationary nets accordingly to the classical ichthyologic methods under the applicable law. Fishing was performed with a standard set of stake nets with mesh step of 30 to 120 mm [12,13].

Biological analysis of fish was carried out in accordance with classical methods in ichthyology [14]. To determine the fish age scales were taken from at least 10 individuals from each class interval of the size range. The fish age was determined by methods of V.L. Briuzgin and I.I. Chugunova [15]. The size-age structure of the fish populations was determined by Morozov-Mayorov method [13]. The physiological state of fish was estimated with fatness coefficients.

In order to study the fish fertility female ovaries were taken at the IV stage of their maturity. The degree of gonad maturity was determined visually. Individual absolute fecundity (IAF) was determined by weight method. Gonad-somatic index (GSI) was calculated as the ratio of gonad weights to the fish body weights shown in percentage [16].

The young fish was caught in the third decade of July – the first decade of August in shallow water at standard control stations. Fry captures were carried out with 10-meter length webbing minnow seine of 1 m height, which is made of mill gas number 7 and with fingerling trawl made of capron net webbing with 4 mm mesh size. All young fish catch was distributed by species, their numbers were counted, and measurements of their length were carried out with the accuracy up to 1 mm and of their individual masses-up to 0.01 g. The number of fingerling per 100 m^2 of catching area was assumed as the abundance of young fish.

With the aim to study the reproductive capacity and peculiarities of fish reproduction the female ovaries were taken at different stages of maturity. Samples were fixed in Buen’s solution with followed treatment in accordance to conventional histological techniques. To produce slices, MS-2 sledge microtome was used. Slices were stained with hematoxylin-eosin and with Mallory's technique. Photos of preparations were made with the *"Sciencelab T500 5.17 M"* digital camera, which was connected to the "Biolam 70" microscope. All designations and terms used under describing periods and phases of growth and development of gametes, and ovarian maturity stages were those adopted in ichthyologic research [1,17-19]. Statistical analysis of the experimental material was performed using *Microsoft Excel* and *STATISTICA 6.0* software packages for personal computers.

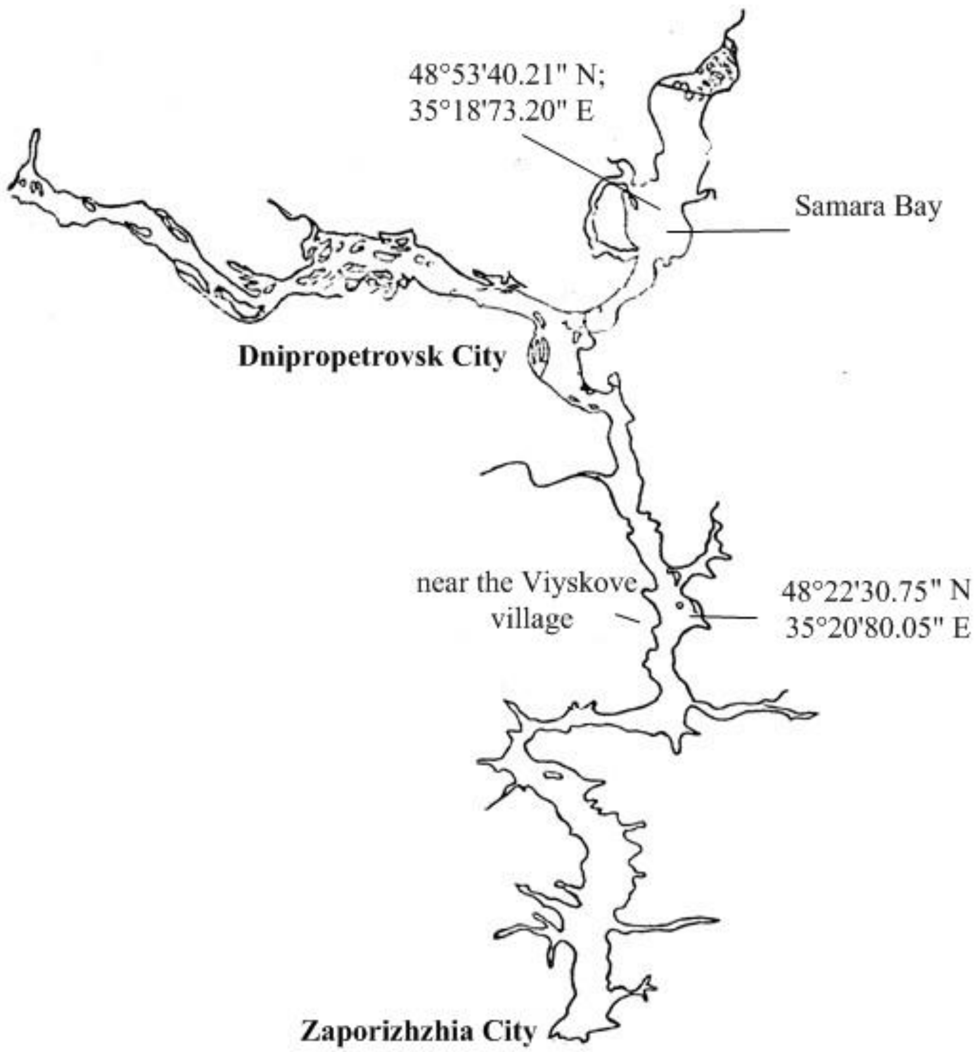

Figure 1. The scheme of Zaporozhian Reservoir and the site of samples location.

3. RESULTS AND DISCUSSION

Recently, the bream share in total catches on the Reservoir increased from 6 % to 9.4 %. The bream age composition in fishing gears of the control order remains limited and includes 10 age classes (Fig. 2). Number of older age groups is less than 2 %.

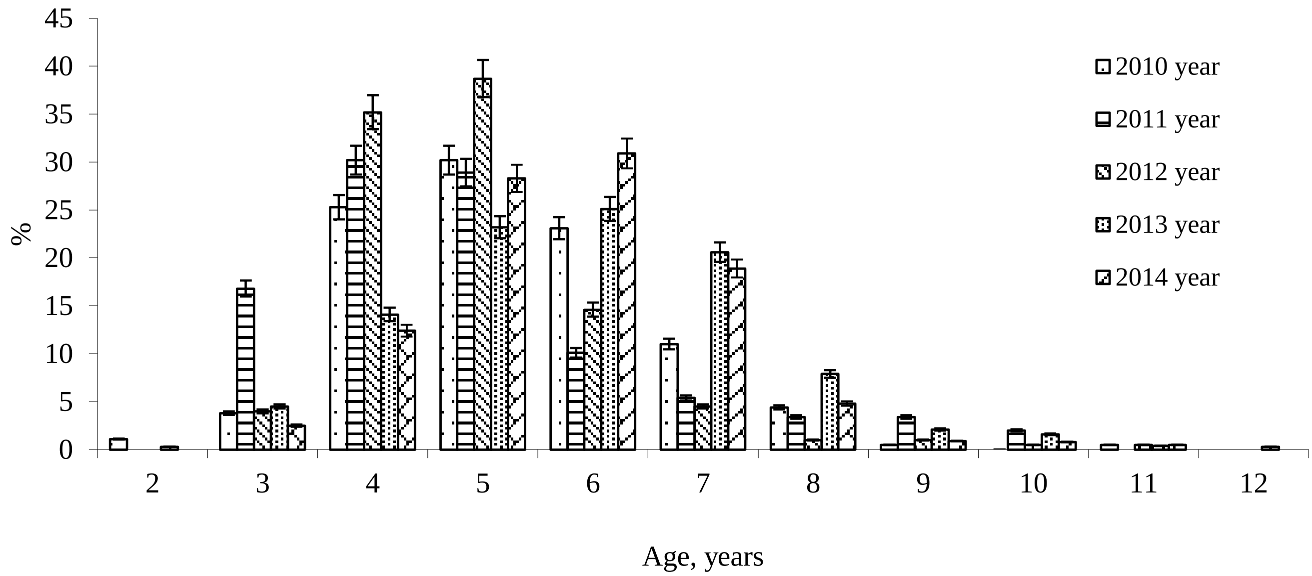

Figure 2. The age structure of the spawning populations of bream, %

Dynamics of the bream length-age parameters is characterized by stability; they remain practically at the same level over the past 20 years: maximum standard length is 35.6±0.9 cm, weight of mature individuals – 1255.5±110.0 g. The middle-aged values of the Fulton's condition factor are stable for several years and amount to 2.33±0.05 units in females and 2.18±0.03 units in males. Thus, the indexes of growth and physiological state show the favorable feeding conditions, sufficient supply with food resources and stable state of the bream population.

Indicators of absolute fecundity of the bream uneven-aged specimens range from 54.31 to 856.0 thousand eggs, and weighed fertility parameter is within 148-161 thousand eggs (Table 1). In conditions of Zaporozhian Reservoir the spawning frequency of the bream females is 8 times. Coefficient of commercial return of eggs is 0.0025 %, this is consistent with the results of investigations of commercial return of the bream eggs in other reservoirs of the Dnieper [2,9].

Table 1. Fertility of bream from Zaporozhian Reservoir

Value	Age of females, years										Average value
	3	4	5	6	7	8	9	10	11	12	
IAF, ths. pcs.	54.3	125.3	136.6	145.7	177.8	211.9	254.3	310.2	542.1	856.0	148.2 – 161.0

The bream of Zaporozhian Reservoir has the longer pre-vitellogenesis period than the roach has. The bream spawning occurs in the last decade of April–the first half of May, and it starts when the water temperature reaches 13-13.5 °C. Mass spawning occurs at the water temperature of 15.5-18 °C (Table. 2).

Table 2. Spawning terms of bream in the Zaporozhian Reservoir

Year	Start of spawning	Water temperature, °C	Availability of spawning	Water temperature, °C	End of spawning	Water temperature, °C
Samara Bay						
2010	23.04.	13.9	27–30.04.	15.2	05.05.	16.4
2011	20.04.	13.5	26–29.04.	15.0	03.05.	16.5
2012	22.04.	12.6	25–28.04.	14.1	05.05.	15.5
2013	21.04.	13.6	24–25.04.	14.2	28.04.	15.0
2014	23.04	12.8	25–27.04	14.3	30.04.	15.8
The lower area of the Zaporozhian Reservoir (near the Viyskove village)						
2010	06.05.	13.3	09–12.05.	14.2	20.05.	15.6
2011	05.05.	13.7	15–20.05.	17.5	25.05.	18.8
2012	06.05.	13.5	11–14.05.	15.5	20.05.	16.6
2013	02.05.	14.0	09–12.05.	16.0	16.05.	18.0
2014	06.05.	14.,1	10–15.05	15.8	19.05.	17.5

By the nature of spawning the bream is characterized with single spawning but it is realized through several approaches of seed fish to the spawning grounds, so this process may take about a month. In early May, the bream females being at the IV stage of maturity had enough high GSI value – 22.1±2.2 %. In the last days before spawning here was the GSI increase to 24.1 %, after which the mass bream spawning had occurred. The spawning period ended after May, 15 (Fig. 3).

After spawning gonads of the bream females switch to VI–II-stage of maturity, containing empty follicular shells and single oocytes that undergo resorption. During this period, the GSI value took the least meanings and amounted to 2.14±0.1 %. Resorption of eggs in the bream ovaries lasts about one month. Then fish ovaries switch to the II stage of maturity, typical for fish species with a single spawning. In mid-summer the bream oocytes are in the period of protoplasmic growth and they reach the size of 120-180 μm (Fig. 4).

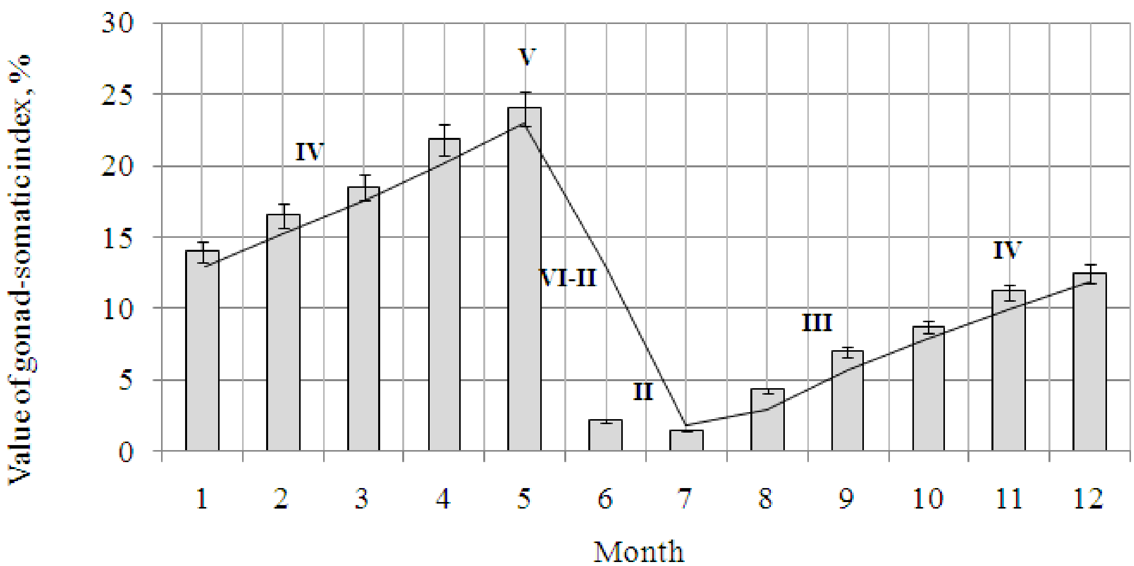

Figure 3. Values of gonad-somatic index of the bream

The first vacuoles appear in the bream oocytes peripherally as a chain in the cortical layer, their number ranges from 28 to 36. During formation of the first row of vacuoles oocytes come into the "D_1" phase and their size reaches 220-270 μm. Then eggs go into the "D_2" phase, and vacuoles become arranged in two or more rows. Before "D_3" phase vacuoles fill in the cytoplasm totally, and cell diameter is 390-420 μm. Later, the cloddy yolk appears between zones with vacuoles. The yolk membrane thickens gradually ("E_1"phase).

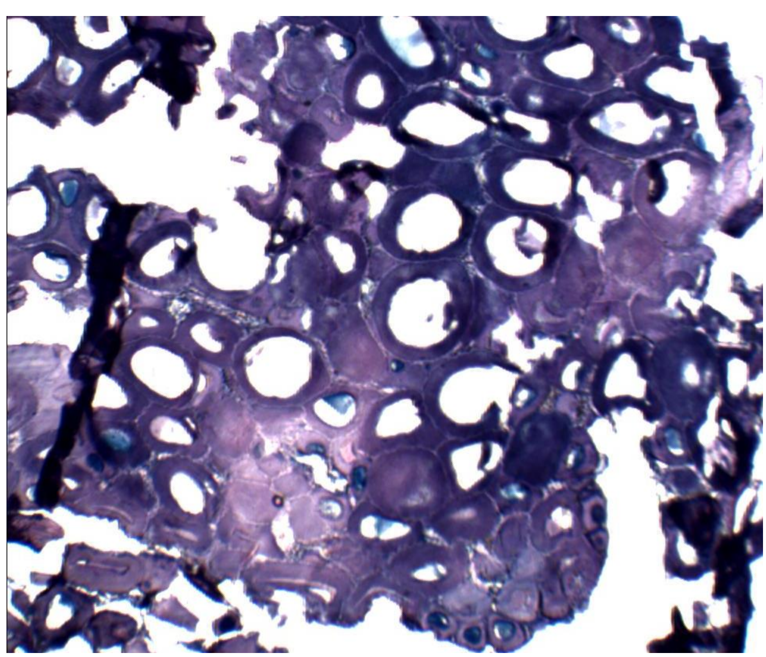

Figure 4. The bream oocytes are in the period-of protoplasmic growth

The yolk continues to fill in the area inside the egg and also the inter-vacuolar space of outer rows of vacuoles, which are pushed to the periphery. When the “E_2” phase has been reached, the yolk takes a half of oocyte and pushes vacuoles to the cortical area. At this time, the oocytes diameter amounts to 730-780 μm. While reaching the “E_3” phase the bream oocytes are completely filled with yolk and 2-3 rows of vacuoles with 20-30 μm diameter remain in the cortical layer (Fig. 5).

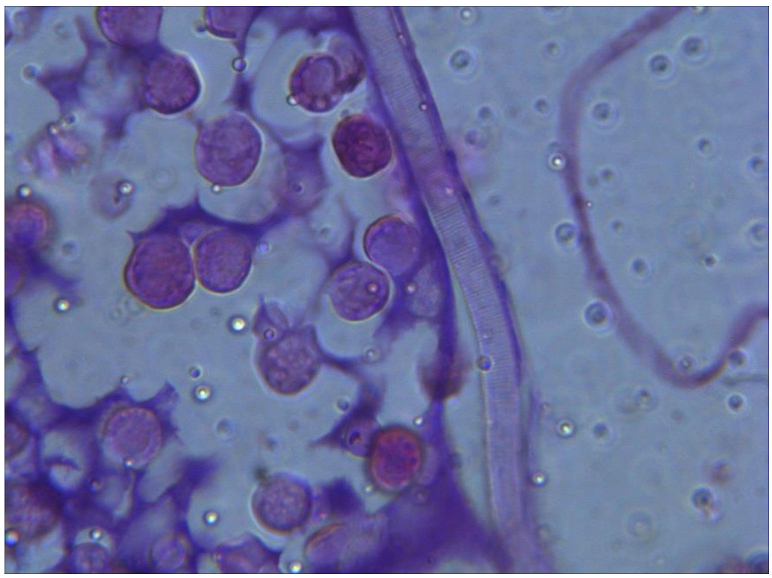

Figure 5. The bream oocytes vacuoles

The bream oocytes coming into the period of maturation reach the size of 800-890 μm. After the process of the yolk accumulation has completed, it is observed that its lumps merger into larger formations being the condensed yolk. The bream oocytes reach the definitive size. In this period cores of eggs have size of 56-126 μm, and they are shifted from the center to the periphery, towards micropyle. In pre-spawning period eggs witch to the “F” phase, reach their maximum size (950-990 μm) and then they are ready for ovulation (Fig. 6).

Histological examinations undertaken with ovaries of the bream from Zaporozhian Reservoir show the noticeable asynchrony in oocyte development within the vitellogenesis period (Fig. 7); that is more typical for the batch spawning fish, however, in conditions of Zaporozhian Reservoir the bream lays its eggs only once and a small amount (about 6-10%) of oocytes being in the phase of vacuolization is resorbed.

Asynchrony in gonadal development of the bream females in the summer can be considered as an example of the adaptation of the fish reproductive system to changes in environmental conditions, especially to the temperature and water regime fluctuations in Zaporozhian Reservoir. Thus, the bream populations living in the environmental conditions of Zaporozhian Reservoir are exposed both to natural and to forced intra-population differentiation and therefore they have the specific adaptation mechanism for the reproduction.

The bream fingerlings were quite common and occurred in all parts of basins. The greater number of the bream inhabited the lower part of Zaporozhian Reservoir. The bream population replenishment with new generations occurred every year, but not to the same extent, the fish yield in coastal areas of Zaporozhian Reservoir declined from 6.50 ind./100-m^2 (2010) to 2.38 ind./100-m^2 (2012) and 0.75-ind./100 m^2 (2013). Biomass, which is created with the bream fingerlings in the intertidal zone of the reservoir, also varies in a wide range from 2.39 g/100 m^2 to 46.80 g/100 m^2. Thus, the bream reproduction in Zaporozhian Reservoir is at an unsatisfactory level.

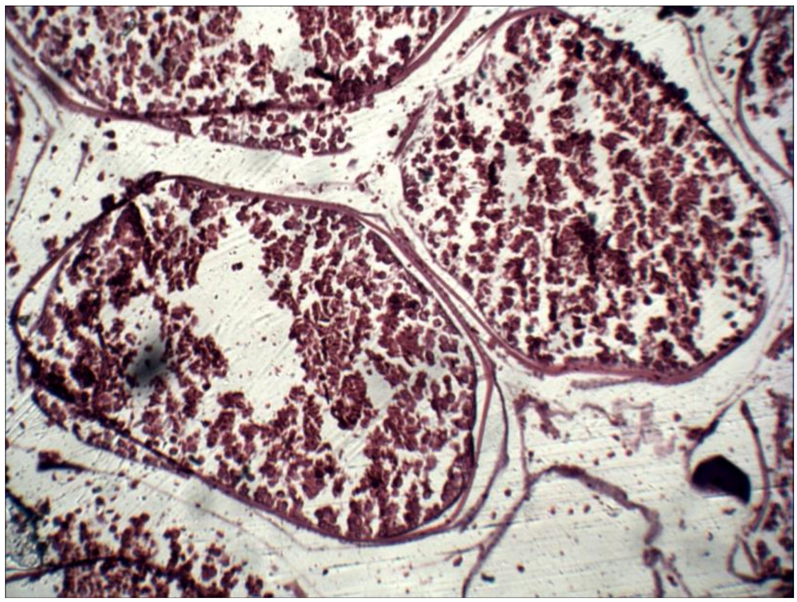

Figure 6. The mature oocyte of bream, the "F" phase

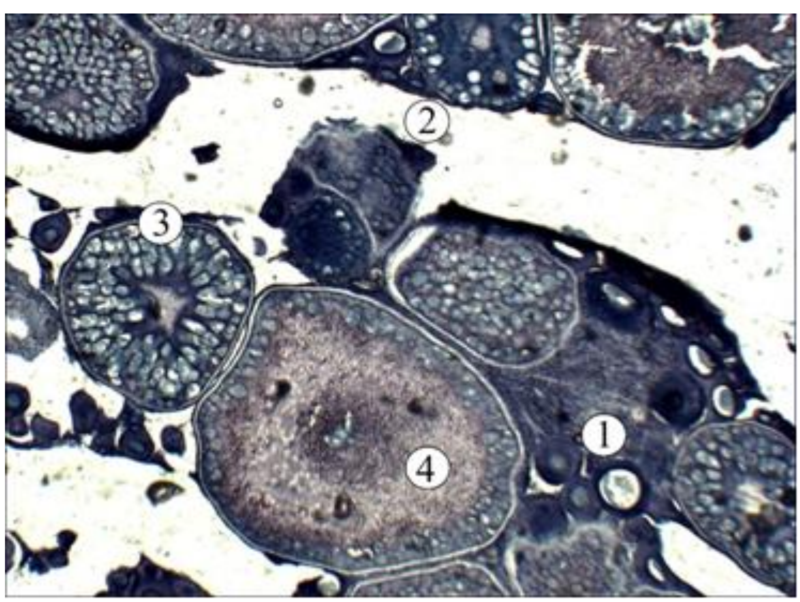

Figure 7. Asynchrony in eggs development of the bream (mid-August): 1 – oocytes in the "C" phase; 2 – oocytes in the "D_1" phase; 3 – oocytes in the stage of vacuolization, "D_3" phase; 4 – oocytes in the late vacuolization stage, the "E_1" phase.

In the waters of the Zaporozhian Reservoir fish breeding and reclamation measures complex is quite standard. In recent years it is the installation of artificial spawning nests. Their number varies from year to year and depends on the funding and efforts of water bioresources users. So, in 2002, it was put up 16.8 thousand nests in 2004-2005 – at about 4.5-4.9 thousand respectively. In 2008, the number of exposed artificial spawning nests reached 8.2 thousand nests [5]. In the years 2010–2013 there were set 4.5 thousand nests per year in two areas of Zaporozhian Reservoir: Samara Bay (500 pcs.) and a lower portion of the Zaporozhian Reservoir (4000 pcs.) [1]. This amount of artificial spawning nests is several times less than the required number of biologically based number of artificial nests, which is recommended to put in Zaporozhian Reservoir.

To compensate the reduction of natural spawning grounds different types of artificial spawning nests are being created. For phytophilic fish (Common bream *Abramis brama* Linnaeus, 1758; Roach *Rutilus rutilus* Linnaeus, 1758; Common carp *Cyprinus carpio* Linnaeus, 1758) in most cases are being created floating spawning nests. They are especially important in the conditions of strong fluctuations of the water level when stationary spawning nests are unsuitable. Artificial spawning nest is strengthened on a metal hoop netting with a mesh size of 12–16 mm with a caproic bristles (substrate), painted in the most attracting colors for one or the other fish in the spawning period (Fig. 8).

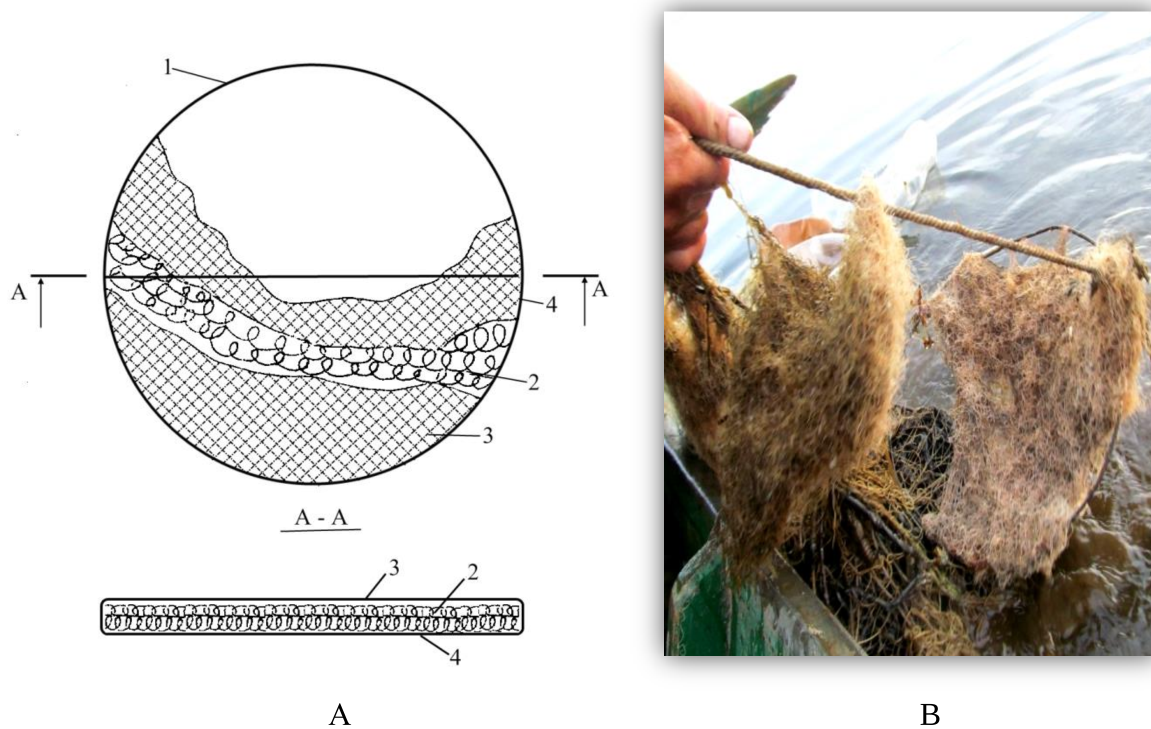

Figure 8. Artificial spawning nest: A – scheme of spawning nest: spawning nest, a top view and a sectional view along A-A: 1 – a metal frame 2 – spawning substrate 3–4 – upper and lower layers of the network fabric; B – general view of the established spawning nest

It is noted that the artificial nests are effectively used by bream. It is necessary to draw the user`s attention to the fact that the installation of spawning substrates improves the reproduction of fish resources, increases the survival rate of eggs and young fish output value (Fig. 9).

Figure 9. Bream eggs laid on artificial spawning substrate

In the spring, there were observed daily fluctuations of the level regime of Zaporozhian Reservoir, which could reach the 0.8 m in its lower area. Spawning nests effectively protected spawn against changes in the water level. As a result of using spawning nests it was managed to get nearly 16.5 million of fish larvae of bream.

To make optimal use of artificial spawning nests, we recommend put spawning nests stepwise, according to heating of the water and the approach of sires of different species of fish (or purposefully put nests to each approach of sires) to the spawning nests. The best time of the installation of spawning nests – before spawning, when the temperature is at 2-3 °C below spawning. This optimizes the use of additional spawning areas. If the substrate is filled with spawn by 75 %, and spawning is still continue, it is recommended the installation of additional spawning nests. To prevent siltation of spawning substrates it is necessary to wash spawning nests every two days, remove foreign objects from them, to check the presence of eggs it them.

To improve the reproduction conditions for fish resources it is necessary to gradually increase the annual number of spawning nests up to their optimal number – 120 th. pcs. including the annual supplement: in 2016 – 40 th. pcs.; in 2017 – 20 th. pcs.; in 2018 – 30 th. pcs.; in 2019 – 30 th. pcs. Use of artificial spawning nests will allow optimizing the natural reproduction and reconditioning 20,000 m^2 of effective spawning grounds by 2019.

Works with spawning nests should be carried out by the users of aquatic resources and the public authorities, but under the control of fisheries and the environment authorities, and also accompanying research organizations.

4. CONCLUSIONS

The state of spawning populations of bream was investigated in conditions of ecological transformation of Zaporozhian Reservoir. Regularities of oogenesis and gonadogenesis of bream were found. The adaptive potential of reproductive systems of fish, which is expressed through the asynchrony of sexual products development and the functional peculiarities of the fish spawning process, was displayed. According to results of the research, the following conclusions might be formulated:

1. Recently, the bream share in total catches within the reservoir increased from 6% to 9.4 %. At what the bream age structure remains limited and includes 10 age classes. Number of older age groups is less than 2%. At the time of the research the dominance of the male number over the female number is marked in the structure of the bream spawning populations that is the result of adverse changes in environmental conditions of Zaporozhian Reservoir. In addition,there is a low level of female migration to the spawning grounds, particularly because of poaching capture during the spawning period.

2. The dynamics of the bream linear-age indicators is characterized by stability over the past 20 years: maximum standard length is 35.6±0.9 cm, weight of mature individuals - 1255.5±110.0 g. The absolute fecundity of the bream individuals of different ages fluctuates from 54.31 to 856.0 thousand eggs, and the average weighted fertility is within 148-161 thousand eggs over the past two years.

3. Histological study of the bream ovaries carried out for Zaporozhian Reservoir, showed the significant asynchrony of oocytes development within the vitellogenesis period; that is more typical for the batch spawning fish, but in the media of Zaporizhzhya Reservoir the bream lays only one portion of eggs, and a small amount (about 6-10 %) of its vacuolization phase oocytes is resorbed.

4. Thus, in the modern environmental conditions of Zaporozhian Reservoir the bream populations are exposed to the natural and forced intra-population differentiation, and therefore they have the specific adaptation mechanism for their reproduction.

5. The bream population replenishment with new generations occurred every year, but not to the same extent, the fish yield in coastal areas of Zaporozhian Reservoir declined from 6.50 ind./100 m^2 (2010) to 2.38 ind./100 m^2 (2012) and 0.75 ind./100 m^2 (2013). Biomass, which is created with the bream fingerlings in the intertidal zone of the reservoir, also varies in a wide range from 2.39 g/100 m^2 to 46.80 g/100 m^2.

6. To improve the reproduction conditions for fish resources it is necessary to gradually increase the annual number of spawning nests up to their optimal number – 120 thousand units including the annual supplement: in 2016 – 40 th. pcs.; in 2017 – 20 th. pcs.; in 2018 – 30 th. pcs.; in 2019 – 30 th. pcs. Use of artificial spawning nests will allow optimizing the natural reproduction and reconditioning 20,000 m^2 of effective spawning grounds by 2019.

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