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Morphofunctional Characteristics of the Ovaries of Pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) from the Kuchurgan Reservoir (Cooling Water Body of the Moldavian Regional Power Plant)

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Abstract—The results of studies of the reproductive system in sexually mature pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) (Centrarchidae) females during spawning in the Kuchurgan Reservoir (liman), the cooling water body of the Moldavian Regional Power Plant, are presented. Asynchronous development of germ cells in the ovary is characteristic of this invasive species. The females spawn three times in May–July, and the relative gonad weight decreases with each generation, so that the gonadosomatic index before the second and third spawning is sequentially reduced. This is confirmed by the decrease of the weight of eggs and the relative gonad weight during the formation of the second and third egg cell portions within a specific breeding season. Females with severe impairments of gonad development were identified.

Keywords: pumpkinseed *Lepomis gibbosus*, oocyte generation, fractional spawning, vitellogenesis, resorption, gonadosomatic index, GSI, ovary maturity stage, sterile females

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INTRODUCTION

The pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) belongs to the genus *Lepomis* of the Centrarchidae family and originates from the upper reaches of the Mississippi River in North America. The pumpkinseed was first introduced in Europe in the early 1980s, mostly as an aquarium fish. Germany was the first country, to which the species was imported (Freyhof, 2003). The species was observed in the Upper Rein and its tributary, the Main River, in 1903 (Bănărescu, 1964). The pumpkinseed subsequently spread to the water bodies of Eastern Europe through the Rein, Oder, and Danube. The species was deliberately introduced in Spain, Italy, Belgium, and the Netherlands directly from the United States for "enrichment of natural stocks and as food for other predators" (Iacob and Petrescu-Mag, 2008, p. 47). The species was seen in Denmark in 2002; it already reached the inland water bodies of Norway in 2005 (Przybylski and Zięba, 2011).

Pumpkinseed of the Danube delta spread throughout the entire northwest runoff area of the Black Sea, including the Dniester River, via the brackish coastal waters (Movchan, 2009). Pumpkinseed from the Danube entered the Prut River, its tributaries, some lakes (Bratesh, Beleu, Manta, Kagul, etc.), and channel ponds (Cozari et al., 2003) of the Republic of Moldova. Pumpkinseed became an absolute dominant in some anthropogenic ecosystems, such as the Kuchurgan liman (reservoir). The species reached a high population size and inflicted enormous damage on the aboriginal fish species, such as rudd *Scardinius erythrophthalmus*, roach *Rutilus rutilus*, bream *Abramis brama*, tench *Tinca tinca*, common ruff *Gymnocephalus cernuus*, and perch *Perca fluviatilis*. Its high competitiveness and feeding on the roe and fry of these species greatly reduced the survival of the offspring, growth rate, and the overall productivity of the water bodies (Krepis et al., 2013).

Few reports concerning the gonad development in pumpkinseed were published until recently. Germ cell morphology throughout the breeding season was reported (Magalhães and Ratton, 2005; Rangel et al., 2012) for fish from water bodies located in Brazil. Data on the number of roe portions deposited by fish of this species in the Mississippi River (the historical range) (Scott and Crossman, 1973) and the water bodies of South America and Europe invaded by the species

Table 1. Functional characteristics of *Lepomis gibbosus* females during the breeding season

Month, decade	Gonad maturity stage	GSI, %	*t*	*P*	Number of fish
May 21–31	IV	$\frac{23.29 \pm 0.92}{27.11–19.36}$	–	–	16
May 21–31	VI–IV_2	$\frac{11.03 \pm 0.45}{13.48–9.51}$	–	–	9
June 1–10	IV_2	$\frac{16.74 \pm 0.56}{18.26–14.48}$	6.06	0.999	13
June 11–20	VI–IV_3	$\frac{6.61 \pm 0.54}{8.48–4.21}$	–	–	10
July 1–10	IV_3	$\frac{12.78 \pm 0.57}{15.72–10.12}$	4.92	0.999	14
October 11–20	II–III	$\frac{1.90 \pm 0.22}{3.07–0.92}$	–	–	12

(Andre et al., 2005; Copp and Fox, 2007) were published.

The aim of our research was to characterize the reproductive biology of pumpkinseed from the Kuchurgan Reservoir, used as the cooling water body of the Moldavian Regional Power Plant, as well as to analyze the pattern of egg cell development and to determine the amount of portions of roe deposited during spawning.

MATERIALS AND METHODS

Ichthyological material was collected in 2010–2014 in the Kuchurgan Reservoir, the cooling water body of the Moldavian Regional Power Plant. The fish were captured by a set of four one-wall stake nets with the following characteristics: (1) mesh size 15 × 15 mm, net height 1.5 m, net length 50 m; (2) mesh size 20 × 20 mm, net height 1.5 m, net length 50 m; (3) mesh size 30 × 30 mm, net height 3 m, net length 75 m; (4) mesh size 30 × 30 mm, net height 3 m, net length 75 m; also a 6-m dragnet (mesh size 5 × 5 mm) was used.

The gonads from 74 four- and five-year-old pumpkinseed females collected during the spawning season were used for the histological studies. The ovary samples were 0.5 cm long and 0.5 cm tall. The samples were fixed in Bouin's liquid and dehydrated in an alcohol series (70° to 100° alcohol). The samples were then transferred into a mixture of 100° alcohol + ether and clarified in celloidin oil for five days. The gonad samples were transferred from the oil to chloroform I, chloroform II, chloroform + paraffin, and paraffin prior to paraffin embedding. Gonad maturity was determined as described by Meien (1939) with refinements proposed by Sakun and Butskaya (1963), and the degree of oocyte development was classified according to the system of Kazanskii (1949). The sections (7 μm thick) were subjected to Mallory staining (Roskin and Levinson, 1957). All females captured were subjected to general biological analysis, and the dimensions, weight, age, and gonadosomatic index (GSI) (Pravdin, 1966) of the fish were determined. The gonadosomatic index was calculated as the ratio (%) of gonad weight to eviscerated body weight. Measurements of oocytes that had completed vitellogenesis and microphotography were performed on an Axio-Imager A2 microscope. Microsoft Excel 2007 and Statistica 6.0 for Windows software packages were used for statistical processing of the data.

RESULTS

The pumpkinseed of the Kuchurgan Reservoir start spawning in the last ten days of May at a water temperature of 20°C, and the spawning period ends in July. Most of the water in this water body comes from the Dniester River through the Turunchuk branch. The reservoir has been used as the cooling water body for the Moldavian Regional Power Plant since 1964. Females captured in the last ten days of May were at the prespawning stage, with gonads at the completed maturity stage IV. The average diameter of oocytes of the oldest generation (with yolk granule accumulation completed) was 621.0 ± 1.24 μm in these fish. The gonadosomatic index of the fish was maximal during this period (Table 1).

Changes related to the preparation for release from the ovary occur in egg cells after the accumulation of nutrients is completed. Each germ cell contains a single lipid droplet immediately before the ovulation, a homogeneous mass of yolk fills the entire egg cell cytoplasm, and the follicular epithelium becomes thin and somewhat separated from the gelatinous shell of the oocyte.

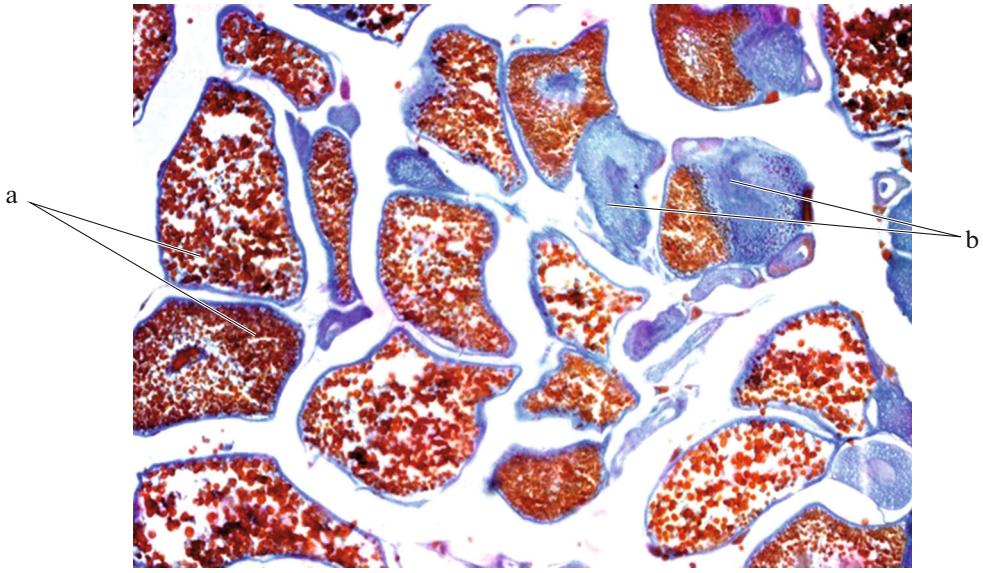

Fig. 1. A fragment of pumpkinseed ovary with third-generation oocytes undergoing resorption: (a) deformation of yolk-containing egg cells, (b) unreleased oocytes undergoing resorption.

Postspawn females with gonads at the VI–IV_2 maturity stage, as evident from the presence of empty follicular shells and oocytes at the phases of intensive vitellogenesis, initial yolk accumulation, and vacuolization, were found in the catches during this study period. As evident from Table 1, the GSI value in the females was the highest before the first spawning, whereas the parameter decreased significantly in the fish before the deposition of the second and third portions of roe (Table 1).

Oocytes at different stages of the trophoplasmic growth period were found along with empty follicular shells in the females after the deposition of the first portion of roe, this being indicative of an asynchronous character of development. Yolk accumulation was completed and the maturation phase started in oocytes of the oldest generation prior to the second spawning of the season during the first days of June. The ovulation of mature germ cells in the females started in the second ten days of June.

Gonads of the females captured at the end of the first ten days of July were at the IV_3 maturity stage. Oocytes of the third generation completed the intensive accumulation of nutrients within a short time, and the fusion of lipids into larger droplets started in these cells. The deposition of egg cells of the third generation by the females from this water body started in the second ten days of July.

Females with degenerating gonads were identified before the season's third spawning in July. Examination of the histological preparations revealed destructive changes in the vitellogenic oocytes, such as the absence of cell turgor, elimination of nuclei, and partial homogenization of the yolk. Reserve egg cells at the cytoplasm vacuolization stage also underwent resorption accompanied by vacuole destruction and fusion of the vacuole contents to form a homogeneous mass (Fig. 1).

One of the females captured (length 11 cm, weight 45 g) had a sterile ovary, this being due to pronounced disturbances in reproductive system development. Germ tissue was replaced by loose connective tissue interspersed with solitary degenerating oocytes at the protoplasmic growth, vacuolization, and vitellogenesis stages (Fig. 2).

Our studies showed that three generations of egg cells are formed and prepared for spawning in May–July (Table 1). A small number of yolk-containing oocytes undergoing resorption and a complex of protoplasmic-growth oocytes is retained in the female gonads after the end of the spawning season. This corresponds to gonad maturity stage II, which persists until early October of the same year.

A new wave of oogenesis starts in the fish after breeding and the end of the resorption processes. A decrease in water temperature by the second ten days of October brings about the transition of oocytes to the trophoplasmic growth stage (the cytoplasm vacuolization stage) and the transition of gonads to the maturity stage II–III. Ovaries of the wintering females are at maturity stage III, with oocytes at various vacuolization stages.

DISCUSSION

The pumpkinseed females of the Kuchurgan liman (the reservoir that serves as the cooling water body for the Moldavian Regional Power Plant) are characterized by a fractional spawning pattern (in three por-

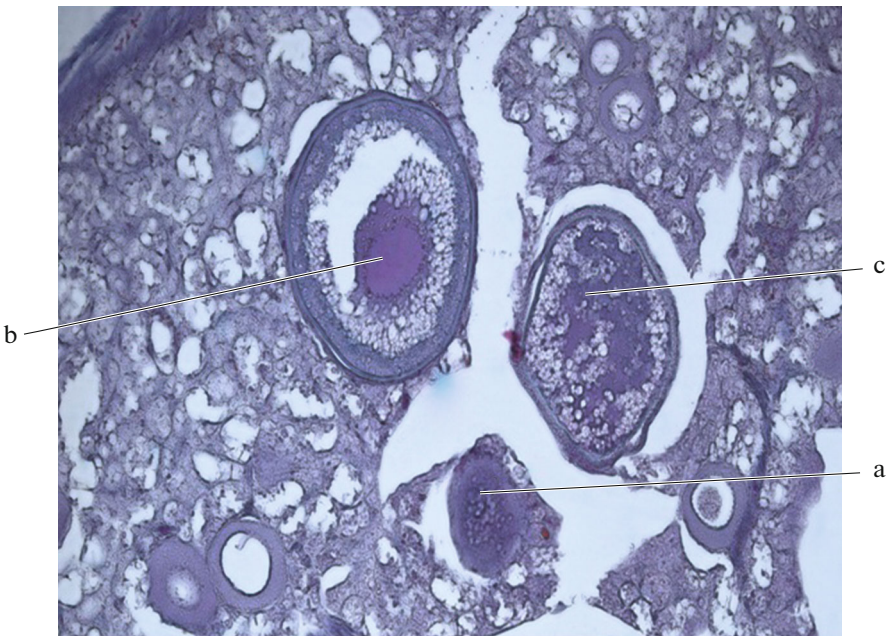

Fig. 2. A gonad fragment from a sterile pumpkinseed female: (a) oocyte at the protoplasmic growth stage, (b) oocyte at the vacuolization stage, (c) oocyte at the vitellogenesis stage.

tions) associated with asynchronous development of germ cells throughout the reproductive cycle. A similar pattern of egg cell development in females of this species was observed in Lake Gamba (Brazil) (Rangel et al., 2012). Fractional spawning is characteristic of *Lepomis gibbosus* in the native range, the Mississippi River (Scott and Crossman, 1973), and in the invasive range, water bodies of South America (Magalhães and Ratton, 2005) and Europe (Copp and Fox, 2007). The time of spawning of females is determined by the temperature regime of the water body. The breeding season of *L. gibbosus* in the Kuchurgan Reservoir starts in the first ten days of May at a water temperature of 20°C and continues through July. The species reproduces throughout the year, from February through March of the next year, at a distance of 20 km from the Lake Gamba (South America) (Magalhães and Ratton, 2005). Females spawn from May to August in the native range of the species, the Wisconsin River (a tributary of the Mississippi) (Becker, 1983). This species reproduces during four months of the spring and summer season in Greece and Spain (Neophytou and Giapis, 1994).

The highest values of the gonadosomatic index in the sexually mature females of the Kuchurgan Reservoir during spawning were observed before the first spawning, and the values observed in the fish preparing for the subsequent instances of spawning during the same breeding season were lower. This is demonstrated by the decrease of the amount of roe and the relative gonad weight (GSI) during the formation of the second and third generations of egg cells within a given breeding season. Lower values of the gonadosomatic index after each instance of spawning are due to the predomination of oocytes at the cytoplasm vacuolization stage, with a small amount of egg cells at the vitellogenesis stage, in the ovaries after a portion of roe is deposited.

Gonad sterility is a consequence of severe disturbances in reproductive system development that apparently occur as a result of contamination of the water body by the wastewater from the Moldavian Regional Power Plant. Excessive development of the ovarian stroma and abundant connective tissue interspersed with solitary oocytes undergoing resorption is observed in such fish. Sterile gonads were found in females of other invasive fish species, such as *Neogobius melanostomus* Pallas, 1814 from the Saratov Reservoir (Mineev, 2009).

CONCLUSIONS

(1) Fractional spawning and asynchronous development of germ cells during the entire breeding period are characteristic of the pumpkinseed, an alien fish species, from the Kuchurgan Reservoir (used as the cooling water body for the Moldavian Regional Power Plant). The spawning season of the pumpkinseed in this water body starts in the last ten days of May and ends in July.

(2) A decrease in the relative gonad mass after the first period of oocyte production is observed in the females that spawn three times during the season, and this leads to a consistent decrease in the gonadosomatic index before the second and third spawning. Lowered values of this parameter after each instance of

spawning are due to the decrease in the number of yolk-containing oocytes from the current generation.

(3) Gonad sterility in pumpkinseed females from the Kuchurgan Reservoir is a consequence of severe disturbances in reproductive system development.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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