Arch. Pol. Fish. (2011) 19: 137-143
DOI 10.2478/v10086-011-0017-4

RESEARCH ARTICLE

State of lake minnow, *Eupallasella percnurus* (Pall.), gonads during pre-spawning season – preliminary results

Received – 05 July 2011/Accepted – 30 August 2011. Published online: 30 September 2011;

Piotr Hliwa, Jacek Wolnicki, Jarosław Król, Justyna Sikorska, Rafał Kamiński, Andrzej Ciereszko

Abstract. Active protection measures towards the population of lake minnow, *Eupallasella percnurus* (Pall.), require comprehensive studies on the reproduction biology of this endangered representative of the native ichthyofauna. One of the key issues is the analysis of the reproduction cycle of sexually mature individuals, which enables the explicit determination of the time and the course of natural spawning. The first attempt at investigating this issue was undertaken in May 2009. Gonads of six *E. percnurus* specimens (3 males and 3 females) were histologically evaluated. The fishes with the mean body length of 4.9 cm (the range 3.3-6.1 cm) and the mean body weight of 3.1 g (the range 0.8-6.2 g) were caught in the reservoir in Zielonka (Mazowieckie Voivodeship). Values of the gonadosomatic index for females ranged from 6.2 to 18.7%, and for males – from 0.7 to 3.1%. The histological analysis of *E. percnurus* ovaries during the pre-spawning season revealed apparent asynchronicity in the maturation of oocytes. A few (3-4) ova fractions were found in gonads, they were heterogeneous in terms of maturity stages. Single oogonia, previtellogenic and vitellogenic oocytes (both the initial and final stage of the trophoplasmatic growth) were found in female gonads. The cross-sections of *E. percnurus* testes revealed the dominance of germ cells characteristic of the final cytological stages of spermatogenesis in the central part of seminal ampullae, i.e. spermatids and spermatozoids. Along their edges – free spaces reflecting the loosened structure of gonads occurred. At the borderline between the adjacent ampullae, single spermatogonia were visible, as well as seminal ampullae filled up with primary and secondary spermatocytes, which potentially ensure the spawning readiness of male *E. percnurus* for a longer period.

Keywords: lake minnow, ovaries, testes, pre-spawning season, histological analysis

P. Hliwa [✉], J. Król
Department of Ichthyology
University of Warmia and Mazury in Olsztyn
Oczapowskiego 5, 10-719 Olsztyn
Tel. +48 89 523 47 87; e-mail: phliwa@uwm.edu.pl

J. Wolnicki, J. Sikorska, R. Kamiński
Pond Fishery Department in Żabieniec
Inland Fisheries Institute in Olsztyn, Poland

A. Ciereszko
Department of Gamete and Embryo Biology
Institute of Animal Reproduction and Food Research of the Polish
Academy of Sciences, Olsztyn, Poland

Introduction

The lake minnow, *Eupallasella percnurus* (Pall.), is one of the most endangered Polish cyprinid fishes. In Poland, fishing of this species was entirely banned already in 1975 (Wolnicki and Radtke 2009). In 1983 *E. percnurus* was included on the list of strictly protected species, and its protection requires active methods. It is a priority species in the European Ecological Natura 2000 Network, which requires designation of special areas of conservation. Moreover, the species was included in the Polish Red Data Book of Animals and the Red List of Threatened Animals in terms of taxa endangered or critically endangered with extinction (Kusznierz 2001, Witkowski et al. 2009). Most of the Polish locations of *E. percnurus*, which demarcate

the western boundary of the species range, are very shallow, small water bodies with the advanced processes of vegetation succession leading to their complete disappearance. All Polish sites (172) are situated within the borders of five Voivodeships, with their largest concentration in the Kashubian Lakeland in Pomorskie Voivodeship (Wolnicki et al. 2011).

Groundwater level lowering, irrational land improvement treatments and unmonitored anthropopressure are the main factors that intensify the extinction of *E. percnurus* habitats and negatively affect the number of species populations. Therefore, protection measures towards this species should be focused both on the preservation of the species habitats and on active measures related to reinforcement of already existing populations and the development of the new ones (Kusznierz 2001, Wolnicki and Radtke 2009). One of the conditions to make these measures successful are comprehensive studies on the reproduction biology of this endangered representative of the native ichthyofauna, including the analysis of the reproduction cycle of sexually mature individuals, which enables the explicit determination of the time and the course of natural spawning. The knowledge about the dynamics of seasonal changes in gametes occurring in ovaries and testes guarantees the optimization of fishing time and selection of spawners to perform the potential artificial reproduction.

In polycyclic multi-spawning fish, such as *E. percnurus* (Karasev 1987), gametes are formed in the processes of oogenesis or spermatogenesis within the annual cycle of the gonads, described according to maturity stages of oocytes or sperm cells present in gonads at a given moment. According to Sakun and Bucka (1968), the annual cycle of gonads in teleosts includes six stages and in each successive stage, different types of oocytes/sperm cells dominate, starting from oogonia/spermatogonia – i.e. first cells of the female or male sex line respectively (stage I) till mature, ready for ovulation eggs or mature spermatozoa (stage V). In the literature there are no comprehensive histological studies on seasonal changes occurring in gonads of *E. percnurus*, which would explicitly document the type and the course of its natural reproduction. The only data of this kind are provided by Zhukov (1965), according to whom two types of oocytes were observed in ovaries of *E. percnurus* females before the reproduction. Oocytes of the first type, with a diameter of 0.9-1.1 mm, were filled up with yolk (probably from the first portion) and oocytes of the second type were cream-white in colour with a diameter of 0.1-0.6 mm (probably from the successive and spare portions). However, these results were not supported by histological examinations.

The aim of the present paper was to analyse the macro- and microscopic views of ovaries and testes in *E. percnurus* during the pre-spawning season, as well as to describe the developmental stages of oocytes and sperm cells characteristic for this time.

Materials and methods

For histological examination of the state of gonads in *E. percnurus* during the pre-spawning season, six sexually mature individuals (3 females and 3 males) were used. Fishes were caught in the small water body in Zielonka (Mazowieckie Voivodeship) in May 2009 by means of baited traps, usually used for this purpose (Wolnicki and Radtke 2009). Measurements of the body length (l.c.) within the accuracy of 0.1 cm and the body weight (W) within the accuracy of 0.1 g were performed in a laboratory. Scales from all specimens were collected in order to determine their age, as well as otoliths – for possible verification of readings from scales. Next, post-mortem examinations were performed on the fish, the sex was determined macroscopically, with attention to the location of gonads, their shape, size, colour, vascularity and possible pathological changes. The isolated gonads (Wg) were weighed within the accuracy of 1 mg used MEDICAT 160M (Medicat Ltd., Switzerland) weigher. Based on the body weight and the weight of gonads, the value of the gonado-somatic index (GSI) was calculated, according to the formula:

$$GSI = (Wg \times W^{-1}) \times 100\%$$

were:
Wg – weight of gonads (g),
W – body weight (g).

The pieces of each gonad were preserved in Bouin's fluid (mixture of picric acid, glacial acetic acid and formaldehyde). After samples were preserved, they were rinsed in a series of ethanol (concentrations from 70% to 95%), dehydrated, over-exposed in xylene and immersed in paraffin blocks. Then, the blocks were cut with the microtome RM 2255 (LEICA Ltd.) into 4-5 μm thick slices, and stained with the topographic method HE (haematoxylin and eosin) and Mallory's method (Zawistowski 1986, modified by Demska-Zakęś). Evaluation of the cross--sections and photographic documentation were prepared using the light microscope LEICA DM 3000 and the software for the image analysis LEICA QWinPro (LEICA Microsystems Ltd., Switzerland). The nomenclature of sex cells and cellular structures was used according to Hliwa et al. (2002) and Schulz et al. (2010), whereas maturity stages of gonads were determined using the scale of Sakun and Bucka (1968).

Figure 1. Macroscopic view of *E. percnurus* gonads in pre-spawning season: a – testes of acinar type; b – ovaries of trough-shaped type.

Results

The sexually mature used in the studies were fish at the age of 1+ and 2+ with the mean body length of 4.9 cm and the mean body weight of 3.1 g. The differences in the size of particular specimens were reflected in the values of the gonadosomatic index, which for females ranged from 6.2 to 18.7%, and for males – from 0.7 to 3.1% (Table 1).

The regular topographic structure of gonads was observed in all specimens; both ovaries and testes filled up most of the body cavity. Macroscopic analysis of testes during the pre-spawning season revealed the presence of milky-white, elongated gonads, embedded laterally in relation to a two-chambered swim bladder (Fig. 1a). Whereas in females, the presence of relatively large ovaries was recorded, with cylindrical and compact structure, filled up with oocytes in yellow shades. Those were trough-shaped gonads, attached to the peritoneum with two mesentery strings – typical for cyprinids (Fig. 1b).

On histological cross-sections of female gonads, 3-4 fractions of oocytes were found, which clearly differ from each other in the developmental stage and the size, probably corresponding to spawning portions of eggs. In two females, previtellogenic and vitellogenic oocytes (both the initial and final stage of the trophoplasmatic growth) were observed. Also single maturing oocytes were visible on cross-sections. This picture corresponded to stage III-IV according to the scale of Sakun and Bucka

Table 1
Materials used to analyze of state of *E. percnurus* gonads in pre-spawning season

	Female		Male	
Parameter	Range	Mean	Range	Mean
Body length (cm)	4.0-6.1	5.2	3.3-5.9	4.6
Body weight (g)	1.8-6.2	3.9	0.8-5.2	2.3
GSI (%)	6.2-18.7	10.7	0.7-3.1	2.2

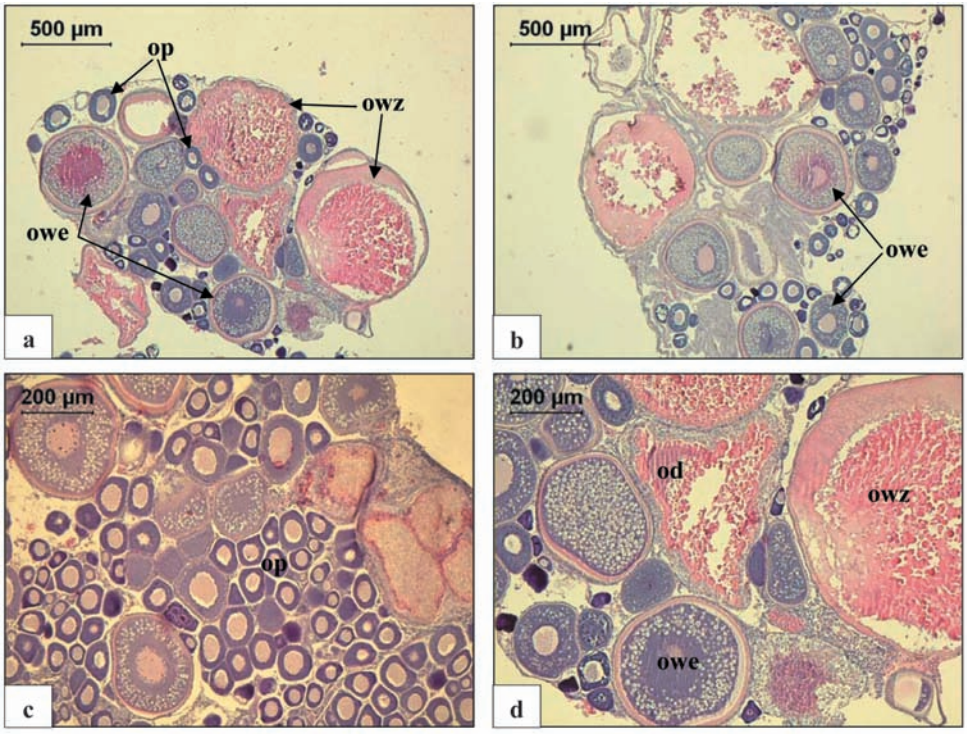

Figure 2. Histological cross-sections of *E. percnurus* ovaries in pre-spawning season (stained HE method): a, b – stage III/IV, c – stage II/III, d – stage III/IV (with single atrophic oocytes). Description: op – previtellogenic oocytes, owe – early vitellogenic oocytes during endogenic growth, owz – vitellogenic oocytes during exogenic growth; od – atrophic oocytes.

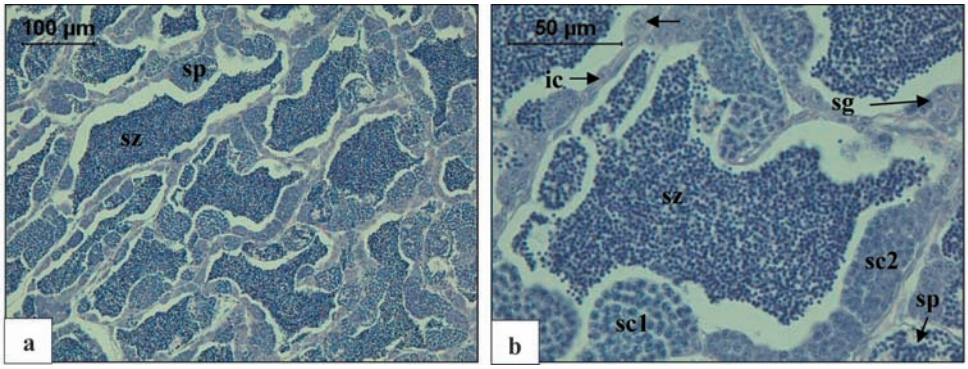

Figure 3. Histological cross-sections of *E. percnurus* testes in pre-spawning season (stained HE method): a – view of testes lobe; b – view of seminal ampullae. Description: ic – interstitial tissue; sg – spermatogonia, sc1 – primary spermatocytes; sc2 – secondary spermatocytes; sp – spermatids; sz – spermatozoids; Sc – Sertoli cells.

(Fig. 2a, b). Vacuoles were clearly visible along the edges of vitellogenic (endogenous) cells, gradually filling more and more space of cytoplasm limited by ovarian shelters. In one female, however, the smallest one, a relatively large quantity of previtellogenic oocytes was found in the microscopic picture, as well as the presence of few oocytes of early vitellogenesis. This picture corresponded to stage II-III the scale of Sakun and Bucka (Fig. 2c). Furthermore, the occurrence of single degenerating oocytes was confirmed on several cross-sections, which were subject to absorption (Fig. 2d).

In males, seminal ampullae in testes were characterized by regular shape. Their central part was filled up with cells representing the most advanced developmental stages of spermatogenesis, i.e. spermatids and spermatozoids (Fig. 3a). Whereas, along the edges of testes' ampullae, cells characteristic of the intensive process of spermatocytogenesis were found, mainly spermatogonia and primary

spermatocytes. At the borderline between the adjacent ampullae, single spermatogonia were observed, as well as seminal ampullae filled with primary and secondary spermatocytes, which differed in the size and staining of the nuclei material. Apart from them, the presence of spermatids was confirmed, embedded slightly further in relation to the central part of seminal lobules (Fig. 3b). On histological cross-sections near walls of ampullae with regular shapes, some free space was observed, which proves that the structure of gonads was loosened and they were ready to release a portion of sperm.

Discussion

Present data on the structure of *E. percnurus* gonads revealed that ovaries of this fish species are trough-shaped and are attached to the peritoneum with two strings. This type of structure of ovaries is known among most of the cyprinids, i.a. vimba, *Vimba vimba* (L.) or chub, *Leuciscus cephalus* (L.) (Hliwa et al. 2003, 2009). The arrangement of female and male gonads in the body cavity, resembles the layout observed in another representative of cyprinids, with which *E. percnurus* sometimes coexists in water bodies, i.e. the invasive topmouth gudgeon, *Pseudorasbora parva* (Temminck & Schlegel). Also values of the gonadosomatic index turned out to be similar for these two species during the pre-spawning season. For *E. percnurus* females, they fluctuated within the range of 6.2 and 18.7%, whereas for females of *P. parva* – from 6.7 to 16.4% (Hliwa 2010). This shows that reproductive strategies and spawning behaviour of these fishes are similar. When analysing the reproductive biology of female specimens related to European minnow, *Phoxinus phoxinus* (L.) from a small, mountain lake called Bray'Pool in Wales, Wootton and Mills (1979) reported that in March-April when the value of GSI was lower than 7%, the distribution of the size of oocytes in ovaries was bimodal. When at the beginning of May the value of the gonadosomatic index for females was higher than 7%, three generations of different-size ova were observed in ovaries. In the case of fish from the site located in Zielonka, with the average GSI value of 10.7%, the presence of 3-4 ova fractions was recorded, most probably corresponding to successive portions of ovulated eggs during spawning.

Macroscopic observations were confirmed by the histological analysis of *E. percnurus* ovaries in the pre-spawning season. The analysis revealed the apparent asynchronicity in the maturation of oocytes. Because previtellogenic and vitellogenic oocytes of different stages of trophoplasmatic growth, as well as single maturing oocytes were found in ovaries. Production of several batches of ova, maturing in different periods and spawned in portions, increases not only the absolute fecundity of fish, but probably also their reproductive success. Usually this behaviour is a response to reproduction conditions unfavourable to the survival of offspring, which may include: a different spawning grounds, the nature of the substrate or fluctuations of the water temperature (Brylińska et al. 1979). In the case of *E. percnurus* living in small, shallow reservoirs, where large and frequent changes in physicochemical water parameters are a typical phenomenon, this reproductive strategy can be of key for the population preservation.

In the male of fish, the sperm is formed in the process of spermatogenesis, and the development of sperm cells proceeds inside lobules or tubules forming spermatogenic cysts, which are surrounded by the interstitial tissue containing Leydig or Sertoli cells (Schulz et al. 2010). The histological picture of testes observed in *E. percnurus* males was characteristic of fish with portioned spawning, breeding during the spring and summer season. In the central part of seminal ampullae, germinal cells typical for the final stage of spermatogenesis dominated, i.e. spermatids and spermatozoids. Near walls of ampullae with regular shapes, free space was observed, which proves that the structure of gonads was loosened and they were ready to release a portion of sperm. At the same time, single spermatogonia and testicular ampullae filled up with primary and secondary spermatocytes were observed along the edge of ampullae, which potentially ensure the spawning readiness of *E. percnurus* males over a longer period of time.

A different histological state of testes in the pre-spawning season (May 2005) was observed in males of *P. parva*. In several studied specimens, at the age of 1+ to 3+ and the body weight ranging from 4.05 to 9.88 g, limits between the adjacent seminal ampullae became blurred and their insides were filled mainly with spermatozoids. Few, younger stages of germinal cells, i.e. spermatogonia and primary spermatocytes, were situated only next to ampullae walls. On the other hand, GSI values recorded in males of both species turned out to be comparable during the analysed period. In the case of *E. percnurus*, they ranged from 0.7 to 3.1%, whereas in *P. parva* – within the range of 0.4-2.2% and were the highest during the whole annual cycle (Hliwa 2010).

The presented results are the first data on the macro- and microscopic structure of gonads in *E. percnurus* during the pre-spawning season. The histological picture of gonads in these small, economically unexploited representatives of ichthyofauna confirms the flexibility of their reproductive strategy. The portioned spawning and related potential prolongation of the reproductive season are the attributes supporting the sustainability of *E. percnurus* populations, often living in extremely unfavourable environmental conditions. These are qualities, which are extremely important elements of the reproductive strategy, significantly increasing the survival rate of juvenile stages (Brylińska et al. 1979). Whereas, the abundance of individuals, different in respect of age or/and the size, on the one hand ensures the spawning readiness over a longer period of time, and protects against too low genetic diversity of the offspring (Katano and Maekawa 1997).

Active forms of nature conservation with the primary objective to restore or preserve valuable populations have to base their assumptions on accurate description of species biology, particularly with reference to the reproductive system. As it is impossible to overlook the issues related to the structure, development or functioning of this system, which is one of the most sensitive indicators of the population condition. When considered comprehensively, it comprises mutually interdependent phenomena and elements of the species biology, which constitute the most important biological act in the fish life, which is spawning and consequently – the species survival (Strüssmann and Nakamura 2002). Therefore in the future, for the authentication and utilitarianism of the presented researches, both the number and frequency of samples should be increased.

Acknowledgments. The project was financed from the funds of the National Science Centre granted on the basis of the decision number DEC-2011/01/D/NZ9/00254.

References

Brylińska M., Bryliński E., Mieczykowski K. 1979 – Influence of individuals features on fecundity of tench (*Tinca tinca*) in several lakes of Masurian and Chełmińsko-Dobrzyński Lake District – Rocz. Nauk Rol. 99: 25-54.

Hliwa P., Demska-Zakęś K., Martyniak A. 2002 – Annual ovarian cycle of *Vimba vimba* (L.) from the Drawienski National Park in northwest Poland – Arch. Pol. Fish. 10: 41-50.

Hliwa P., Demska-Zakęś K., Martyniak A., Król J. 2003 – Gonadal differentiation in *Vimba vimba* (L. 1758) – Czech J. Anim. Sci. 48: 441-448.

Hliwa P., Żyła A., Król J. 2009 – Gonadogenesis in chub *Squalius* (*Leuciscus*) *cephalus* (L. 1758) – Folia Biol. 57: 115-120.

Hliwa P. 2010 – Elements of the reproductive biology of alien invasive fish species, racer goby *Neogobius gymnotrachelus* (Kessler, 1857) and topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846) – Rozprawy i monografie, 156, Wyd. UWM, Olsztyn: 1-97 (in Polish).

Karasev G.L. 1987 – Fish of Transbaikalia – Izd. Nauka, Novosibirsk (in Russian).

Katano O., Maekawa K. 1997 – Reproductive regulation in the Japanese minnow, *Pseudorasbora parva* (Cyprinidae) – Environ. Biol. Fish. 49: 197-205.

Kusznierz J. 2001 – Lake minnow *Eupallasella perenurus* (Pallas, 1814) – In: Polish Red Data Book of Animals (Ed.) Z. Głowaciński, PWRiL, Warszawa: 301-303 (in Polish).

Sakun O.F., Bucka N.A. 1968 – Determinations of maturity stages and study of sexual cycles of fishes – Izd. Min. Ryb. Choz. SSSR, Murmansk: 1-45 (in Russian).

Schulz R.W., de França L.R., Lareyre J.J., Le Gac F., Chiarini-Garcia H., Nobrega R.H., Miura T. 2010 – Spermatogenesis in fish – Gen. Comp. Endocrinol. 165: 390-411.

Strüssmann C.A., Nakamura M. 2002 – Morphology, endocrinology, and environmental modulation of gonadal sex differentiation in teleost fishes – Fish Physiol. Biochem. 26: 13-29.

Witkowski A., Kotusz J., Przybylski M. 2009 – The conservation status of the Polish freshwater fish. Red list of the fish and the lampreys – Chrońmy Przyr. Ojcz. 65: 33-52 (in Polish).

Wolnicki J., Radtke G. 2009 – Assessment of the present state of the occurrence, threats and protection of lake minnow *Eupallasella percnurus* (Pallas, 1814) in Poland – Chrońmy Przyr. Ojcz. 5: 329-340 (in Polish).

Wolnicki J. Kamiński R., Sikorska J. 2011 – Occurrence and protection of the endangered cyprinid fish species, lake minnow *Eupallasella percnurus* (Pallas, 1814), in Poland – In: Water biodiversity assessment and protection (Eds.) M. Jankun, G. Furgała-Selezniow, M. Woźniak, A.M. Wiśniewska, Agencja Wyd. Argi, Wrocław: 35-41.

Wootton R.J., Mills L.A. 1979 – Annual cycle in female minnows *Phoxinus phoxinus* (L.) from an upland Welsh lake – J. Fish Biol. 14: 607-618.

Zawistowski S. 1986 – Histological techniques, histology and the bases of histopathology – PZWL, Warszawa, 548 p. (in Polish).

Zhukov P.I. 1965 – Fish of Byelorussia – Izd. Nauka i Technika, Minsk (in Russian).

Streszczenie

Wstępne wyniki oceny stanu gonad strzebli błotnej *Eupallasella percnurus* (Pall.) w okresie przedtarłowym

W ramach aktywnych działań ochronnych wobec populacji strzebli błotnej, zagrożonego przedstawiciela rodzimej ichtiofauny, podjęto próbę makro- i mikroskopowej oceny stanu jej gonad w okresie przedtarłowym. Gonady badanych osobników były prawidłowo ukształtowane i wypełniały większą część jamy ciała ryb. Makroskopowa analiza jąder w okresie przedtarłowym ujawniła obecność gonad koloru mleczno-białego, wydłużonych i osadzonych bocznie względem dwukomorowego pęcherza pławnego. Z kolei u samic zanotowano występowanie stosunkowo dużych jajników, z obłą i zwartą strukturą, wypełnionych zróżnicowanymi kolorystycznie oocytami w odcieniach żółtych. Były to gonady typu rynienkowatego, podwieszone dwoma niciami krezki do otrzewnej – typowe dla ryb karpiowatych.

Zróżnicowanie wielkościowe osobników obu płci, wykorzystanych w badaniach, miało swe odzwierciedlenie przede wszystkim w zanotowanych wartościach indeksu gonado-somatycznego, które oscylowały u samic w granicach od 6,2 do 18,7%, a u samców od 0,7 do 3,1%. Analiza histologiczna jajników strzebli błotnej w okresie przedtarłowym wykazała wyraźną asynchroniczność dojrzewania oocytów. W jajnikach zanotowano obecność 3-4 frakcji komórek jajowych, zróżnicowanych pod względem stopnia dojrzałości. W gonadach samic stwierdzono pojedyncze oogonia, oocyty prewitellogeniczne i witellogeniczne w początkowej oraz końcowej fazie wzrostu trofoplazmatycznego. Z kolei w obrazie mikroskopowym jąder strzebli błotnej, w centralnej części ampuł nasiennych, obserwowano dominację komórek płciowych charakterystycznych dla końcowych stadiów cytologicznych szlaku spermatogenezy, tj. spermatyd i plemników, zaś na ich obrzeżu wolne przestrzenie świadczące o rozluźnieniu struktury gonad. Na granicy sąsiadujących ze sobą ampuł widoczne były pojedyncze spermatogonia oraz gniazda nasienne wypełnione spermatocytami I i II rzędu, potencjalnie zapewniające samcom strzebli błotnej gotowość tarłową w dłuższym okresie.