Arch. Pol. Fish.	Archives of Polish Fisheries	Vol. 10	Fasc. 1	41-50	2002

ANNUAL OVARIAN CYCLE OF *VIMBA VIMBA* (L.) FROM THE DRAWIEŃSKI NATIONAL PARK IN NORTHWEST POLAND

Piotr Hliwa, Krystyna Demska-Zakęś, Andrzej Martyniak

University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT. Macroscopic and microscopic changes which occur during the annual ovarian cycle of vimba females from the Drawieński National Park (DNP) were studied. The study was carried out in 1997-1998 using 146 fish with an average body length (*longitudo corporis*) of 24.2 cm and an average body weight of 274.9 g. No abnormalities or pathological changes in the ovaries of the investigated fish were detected. The histological picture showed the clear asynchronicity of oocyte maturation. The following ovary maturity stages, characteristic for portion spawners, were distinguished: IV_2; V_2; IV_3; V_3. A relatively short post-spawning stage was also identified (VI/II). It was confirmed that in vimba from the Drawieński National Park (DNP) the longest the maturity stage is III, which starts in October and finishes in March or April of the next year.

Key words: *VIMBA VIMBA*, OVARIAN CYCLE, GONADOSOMATIC INDEX

INTRODUCTION

Seasonal changes in the ovaries of polycyclic fish are described in an annual cycle. In the literature, there are several scales which describe the annual cycle of gonad maturity including Mejen (1939) and Roblin and Bruslé (1983). However, the most commonly used is the six-stage scale by Sakun and Bucka (1968).

The duration of each maturity stage has an impact on when sexual maturity is achieved, the character of spawning and the entirety of the complex sexual cycle. The time it takes to reach sexual maturity depends on the duration of stage I and especially stage II during which there are oogonia and protoplasmatic oocytes in the ovaries. The remaining stages in sexually mature fish determine when and how they spawn. Vimba, *Vimba vimba* (L.) is classified as a spring-summer spawning species. For such fishes, stages II and III occur relatively quickly after spawning, and in fall the ovaries reach maturity stage IV which lasts throughout the winter. It is also possible that maturity stage III is the longest and finishes in spring; thus, stage IV is very short (Bieniarz and Epler 1991).

The aim of this work was to analyze the macroscopic and microscopic changes which occur in vimba ovaries throughout the annual cycle and to identify the characteristic features of this species.

MATERIAL AND METHODS

The fish for the analyses of the ovarian cycle were collected in 1997 and 1998 using gill-nets with a mesh size length ranging from 26 to 55 mm at two stations in Ostrowieckie Lake located in Drawieński National Park in northwest Poland (Fig. 1).

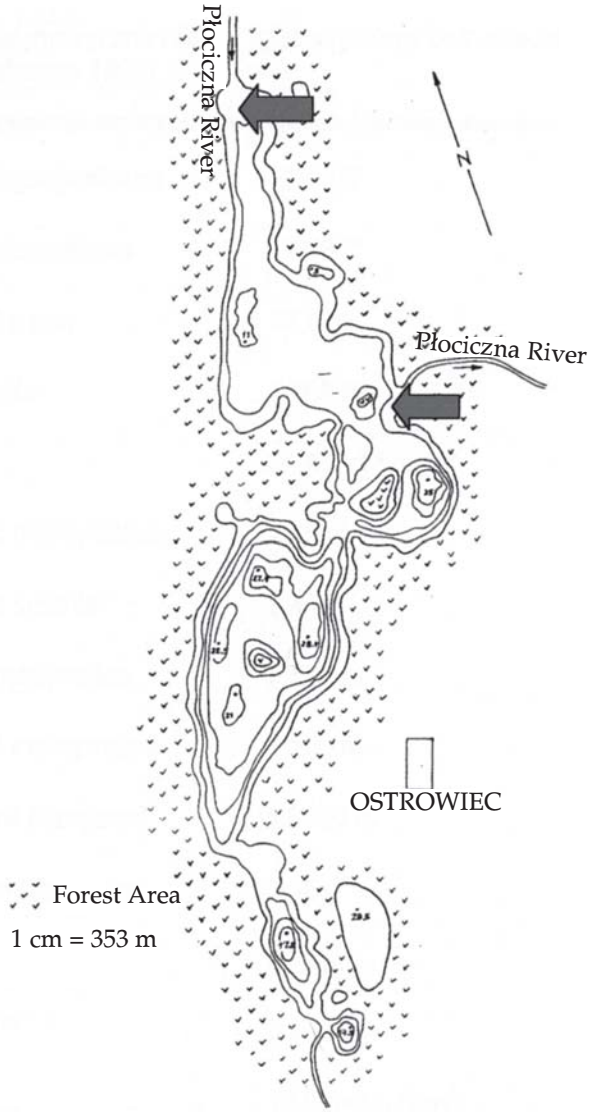

Fig. 1. Sampling stations (arrows) for the study of the ovarian cycle of vimba in Ostrowieckie Lake (Drawieński National Park).

In the laboratory, scales were collected from the specimens in order to determine their age. They were measured (*longitudo corporis* - l.c.) to the nearest 0.1 cm and weighed (W) to the nearest 0.1 g. The sex of each specimen was determined, and the ovaries were prepared and weighed (Wg) to the nearest 1 mg. The body and ovary weight data was used to determine the gonadosomatic index (GSI), which is the ratio of gonad weight (Wg) to body weight (W) expressed in percentages.

A total of 146 females with an average body length ranging from 22.0 to 27.9 cm and an average body weight from 187 to 393 g were studied. The average values of the GSI varied from 1.4 in August 1997 to 14.2% in June 1997 (Table 1).

TABLE 1

Characteristics of study material used to investigate the ovarian cycle of vimba collected in 1997-1998 at the Drawieński National Park

Sample	Number of females	Body length (l.c.) [cm]		Body weight [g]		Gonadosomatic index [%]	
		Range	Average (SD)	Range	Average (SD)	Range	Average (SD)
27 March 1997	15	22.2 - 34.0	26.4 (3.0)	166 - 693	336.4 (135.7)	2.0 - 6.1	4.8 (1.1)
28 April 1997	7	20.6 - 33.4	27.5 (3.9)	144 - 672	391.8 (171.9)	4.1 - 9.9	6.2 (2.0)
3 June 1997	19	22.4 - 30.0	25.7 (2.0)	239 - 499	344.0 (78.7)	11.4 - 18.0	14.2 (2.0)
10 July 1997	5	24.4 - 30.5	27.9 (2.3)	261 - 480	393.0 (90.9)	1.7 - 3.4	2.4 (0.7)
21 August1997	7	20.8 - 28.0	23.6 (2.7)	171 - 392	261.7 (80.9)	0.6 - 2.3	1.4 (0.5)
10 October 1997	5	17.8 - 31.6	23.5 (6.5)	101 - 678	301.4 (149.5)	1.5 - 3.8	2.2 (0.9)
17 March 1998	16	19.2 - 29.1	22.0 (2.6)	121 - 490	202.1 (93.4)	3.5 - 25.9	10.3 (8.7)
30 April 1998	25	20.2 - 25.6	22.4 (1.4)	153 - 297	207.8 (40.9)	5.4 - 9.4	7.3 (1.2)
11 May 1998	18	20.8 - 26.2	22.6 (2.1)	141 - 292	186.8 (38.7)	6.2 - 11.8	8.9 (2.3)
8 July 1998	6	20.5 - 24.4	22.4 (1.6)	154 - 244	205.0 (32.3)	1.2 - 5.4	2.6 (1.6)
7 August 1998	17	21.3 - 23.8	22.7 (1.0)	177 - 279	225.0 (31.2)	1.1 - 2.4	1.9 (0.3)
21 October 1998	6	20.3 - 24.6	23.6 (1.0)	172 - 290	244.4 (43.7)	1.2 - 6.1	3.9 (2.1)

SD – standard deviation

All of the prepared ovaries were evaluated with regards to potential macroscopic changes, external type and blood supply. No distortions in gonad structure were observed. Fragments of the prepared ovaries were preserved in Bouin liquid, then the samples were dehydrated in ethanol of increasing concentrations (from 70 to 95%), over-exposed in xylene and immersed in paraffin blocks. The samples were cut into 5 μm slices using a rotational microtome, and then dyed using the HE (hematoxyline - eosin) topographic method and the Mallory method (Zawistowski 1986). Canada balm was applied to the dyed samples. They were placed on microscopic slides and viewed under a light microscope. These histological samples were used to trace the

developmental stages of the germ cells in the ovaries. The names of the cells and cell structures were taken from Długosz (1986) and Mayer and Shackley (1988). The stages of vimba ovary maturity were determined using the gonad maturity scale developed by Sakun and Bucka (1968).

RESULTS

Females with body lengths from 22.2 to 34.0 cm and body weight from 166 to 693 g and aged from 5+ to 9+ were present in the sample collected in March 1997 (Table 1). All the vimba females caught were sexually mature, and their ovaries were in maturity stage IV. Oogonia, protoplasmatic oocytes and two generations of trophoplasmatic oocytes were observed in the gonads. In some samples cytoplasmic vacuolisation was apparent, while in others the protein accumulation process was occurring (Photo 1A).

The asynchronicity of oocyte maturation in the ovaries clearly began in late April (28.04.1997). At this time, in addition to oogonia and previtellogenetic oocytes, vitellogenetic oocytes and ruptured theca folds and follicles (post-ovulation corpora lutea) were also observed in the histological cross-section of the ovaries. This is characteristic of an ovary in stage IV_2 (Photo 1B). In this sample, there were six females with an average body length of 27.5 cm and an average body weight of 391.8 g, and their GSI oscillated between 4.1 and 9.9% (Table 1). One female with a body length of 20.6 cm and a body weight of 144 g was sexually immature, as only oogonia and previtellogenetic oocytes were observed in its ovaries (Photo 1C).

The ovaries of vimba females were in maturity stages IV_3 - V_3 in early June, and the GSI reached its highest average value of 14.2% at this time (Table 1). Numerous traces of the resorption of unreleased oocytes and post-ovulation corpora lutea were observed in the ovaries. Oogonia, previtellogenetic oocytes and two generations of vitellogenetic oocytes were also observed (Photo 1D). Some of them had completed trophoplasmic growth and were ready for ovulation, while others were beginning to accumulate trophic substances. In the first ten days of July 1997, the ovaries were in maturity stages VI/II. The same situation was observed in ovaries from the sample of seven females with an average body length of 23.6 cm and an average body weight of 261.7 g collected on 21 August 1997 (Photo 1E; Table 1). The post-spawning season is when intensive resorption processes take place and the fecundity of the next spawning season is shaped. For vimba, this season lasted until October, which is confirmed

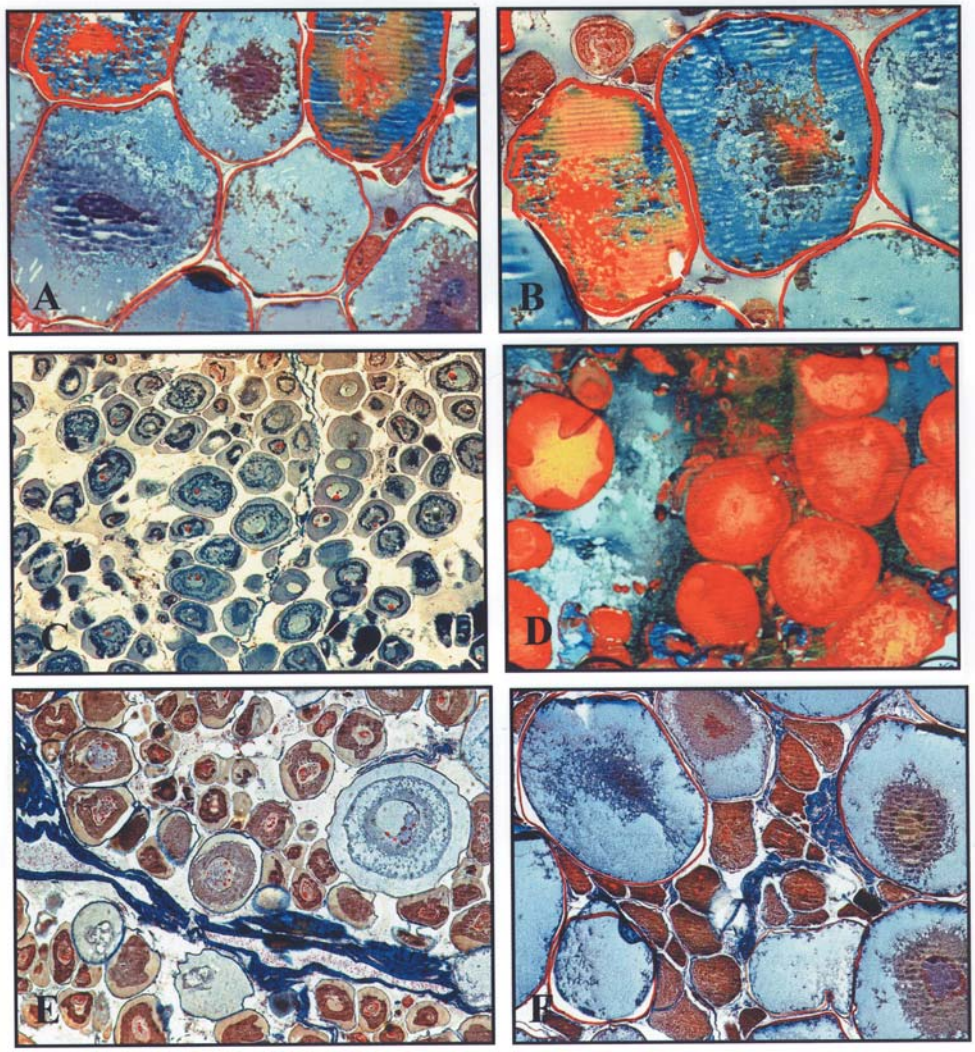

Photo 1. Histological picture of fish ovaries used in the study in 1997 (magn. 120x): A - maturity stage IV; B - maturity stage IV_2; C - maturity stage II (sexually immature female); D - maturity stage IV_3 - V_3; E - maturity stage VI/II (post- spawning – July/August); F - maturity stage III (October).

by the low average values of the GSI (2.4% - July and 1.4% - August). The resorption of corpora lutea pre- and post-ovulation as well as immature oocytes (in the preliminary stage of vitellogenesis), oogonial and two generations of previtellogenetic oocytes were observed in the histological cross-sections of the ovaries. The smaller of these had relatively uniform, slightly basic-absorbing cytoplasm that dyed well, while the

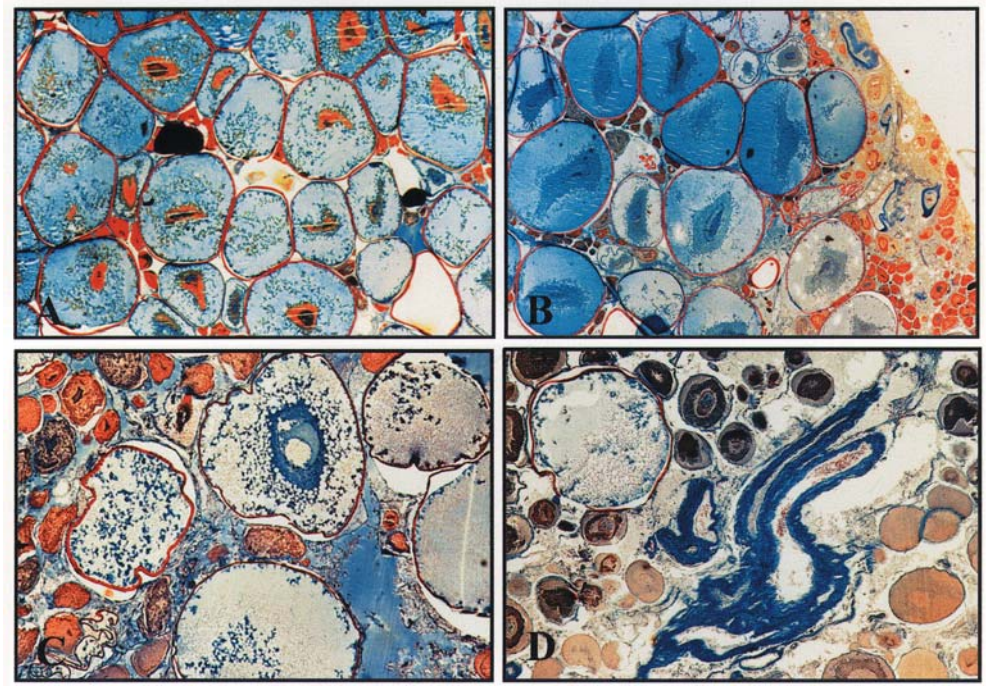

Photo 2. Histological picture of fish ovaries used in the study in 1998 (magn. 120x): A - maturity stage III-IV; B - maturity stage IV_2; C - maturity stage VI/II (May/June); D - maturity stage VI/II (July/August).

larger ones had numerous nucleoli in the nucleus and cytoplasm that dyed unevenly. In some cases, a yolk nucleus (Balbiani structure), was observed at the periphery of the cytoplasm.

In the sample collected on 10 October 1997, the ovaries of vimba females were in maturity stage III. Previtellogenetic oocytes and oocytes in the vacuolisation stage were visible in the histological cross-sections (Photo 1F).

The analysis of the vimba ovarian cycle in 1998 did not produce significant differences in comparison to the results obtained in 1997. Only mature females were present in the sample from 17 March 1998. They ranged in body length from 19.2 to 29.1 cm and body weight from 121 to 490 g (Table 1). Some females had ovaries in stages III - IV and some in stage IV (Photo 2A). The season's highest average value of the GSI at 10.3% was also recorded in this sample (Table 1).

In the next sample, from 30 April 1998, some of the females had ovaries in stage IV_2, which indicated the release of the first eggs portion (Photo 2B). A month later

(30.05.1998), the vimba ovaries were already in post-spawning stage VI/II. Oogonia, previtellogenetic oocytes, oocytes in the early stage of vitellogenesis as well as corpora lutea, both pre- and post-ovulation, were visible in the ovaries (Photo 2C). A similar situation was observed among fish collected in July and August 1998; however, the intensive resorption of corpora lutea and oocytes in the early stage of trophoplasmatic growth were clearly evident (Photo 2D). A large number of protoplasmatic oocytes were observed and they had numerous nucleoli located directly adjacent to the nucleus membrane. As in the previous year, the lowest average values of the GSI were noted for females which were caught in August-October (1.9 and 3.9%) (Table 1).

DISCUSSION

The sexual cycles of fish, whose integral aspects include gonad development, the maturation of oocytes, the ovulation of mature eggs and spawning, are regulated by the hypothalamus. This area of the brain produces an agent that releases gonadotropin hormones from the pituitary gland and regulates the target organs of the gonadotropin, i.e. either the ovaries or testes. This process is controlled by environmental conditions, such as temperature (Epler and Bieniarz 1979, Horoszewicz 1983), light (Fenwick 1970, Epler et al. 1999), salinity or pollution (Epler et al. 1996).

Studies of changes in vimba male gonads were carried out in 1950s by Pliszka (1951), who, while describing the annual cycle of testes in Vistula River specimens, confirmed the very strong impact of temperature on all stages of spermatogenesis and determined a time framework for each stage of the process.

Sakun (1954) analyzed the annual cycle of ovaries of vimba from the Dzwina and Volchov rivers. She confirmed that the female gonads were in maturity stage IV (on the six-stage Sakun and Bucka scale) in winter, while pre-spawning stage V was achieved very quickly in spring. After releasing the first batch of eggs, the maturation of the next batch of oocytes took place. After the last batch of eggs was released, the ovaries moved from stage VI (post-spawning) to stage II. In the latter stage, the primary gonad weight was composed of oocytes undergoing protoplasmatic growth; these comprised the store of germ cells for the next spawning season.

Shishabekov (1979) identified the time framework for the particular maturity stages of ovaries in an annual cycle for a population of *Vimba vimba persa* Pall. which inhabit waters in Dagestan. After spawning in early June and the resorption of

unreleased oocytes, the ovaries moved to stage II, in which they remained for about three months (June-August). In September, the ovaries were in stages II-III, but by late November they had already moved to stage III, in which they remained throughout the winter. In spring the oocytes matured rapidly and the ovaries moved from stage III to V (pre-spawning). Additionally, every year a relatively high percentage of females that did not spawn and resorbed eggs was observed and confirmed by histological studies.

The annual cycle of vimba from the Drawieński National Park appears to be similar to the cycles characteristic of fish which spawn in the spring-summer periods, e.g. stickleback *Gasterosteus aculeatus* L. (Bieniarz and Epler 1991). However, the rate of collecting nutrition substances before winter is slightly different and is probably due to the creation of the stationary form. Preliminary observations (Hliwa et al. 1998) indicated that ovaries of vimba from the DNP are in stage III during winter and only in spring do they move rapidly to maturity stages IV and V. This may result from the lack of spawning migrations or adverse, physico-chemical changes in the environment (temperature, salinity). The results presented in some works on the sexual maturity of migrating populations of vimba (Pliszka 1951, Sakun 1951) indicate that the beginning of the migrations was strictly correlated to gonad maturity stage. The population from the current study has some similarities with that of the *Vimba vimba persa*, for which spring was the season of the most intensive nutrient accumulation and oocyte maturation.

The analysis of the histological preparations confirmed asynchronicity in the accumulation of stores and maturation of oocytes in the studied population of vimba. This was noted because after the ovary reached maturity stage IV not all of the oocytes had simultaneously completed trophoplasmatic growth, reached their full size or were ready for ovulation. After releasing the first batch of eggs, the ovaries did not move to maturity stage VI/II as they do in discontinuous spawners, but rather returned to the so-called stage V_2 through stage IV_2, and then, after releasing the second batch of eggs they moved to maturity stages IV_3 and V_3. The histological picture provided evidence to the fact that in 1997 the first batch of eggs was released in the first ten days of May, but a year later this occurred at the end of April. The definitive end of spawning had a similar pattern. In 1997, the third and final batch of eggs was released in mid-June, while in 1998 this occurred at the end of May. It can be postulated that the timing of reproduction was related to environmental temperature, because the winter of 1996 was longer and spring 1997 cooler than in 1998. In princi-

ple, the vimba ovaries were in maturity stage III at the beginning of October, and thus, fecundity for the next spawning season was set. It must be emphasized that a relatively high percentage of oocytes in the vacuolisation stage did not mature, and their resorption processes were more intensive in summer from July and August. This might have been caused by adverse environmental conditions for spawning such as bad water quality and/or temperature variations or the lack of appropriate spawning grounds. This concurs with the thesis by Kozlovskii (1968) who ascertained that the resorption of germinal cells is a reaction of fish to reproduction hindrances as well as an adaptive behavior in response to the distortion of reproduction conditions, especially by anthropogenic factors. Furthermore, the atresia and resorption of oocytes «relax» heredity and widen the possibilities for a population to adapt to reproducing in completely new ecological conditions.

REFERENCES

Bieniarz K., Epler P. 1991 - The Reproduction of Fish - Wyd. Lettra, Kraków, 202 pp. (in Polish).

Długosz M. 1986 - Oogenesis and the annual cycle of gonad development for selected fish species in reservoirs with different thermal conditions - Acta Acad. Agricult. ac Tech. Olstenensis, Protectio Aquarum et Piscatoria14 (B): 69 pp. (in Polish).

Epler P., Bieniarz K. 1979 - Sexual maturity of fish in heated waters - Pol. Ecol. Studies 5(3): 52-63.

Epler P., Sokołowska-Mikołajczyk M., Popek W., Bieniarz K., Kime D.E., Bartel R. 1996 - Gonadal development and spawning of *Barbus sharpei, Barbus luteus* and *Mugil hishni* in fresh and saltwater lakes of Iraq - Arch. Pol. Fish. 4(1): 113-124.

Epler P., Mikołajczyk T., Chyb J. 1999 - New technologies of carp fry production (hormonal stimulation of reproduction and the impact of environmental conditions) - IRS Olsztyn, Poland, No. 179, 17 pp. (in Polish).

Fenwick J.C. 1970 - The pineal organ: photoperiod and reproductive cycles in the goldfish *Carassius auratus* L. - Journal of Endocrinology 46: 101-111.

Hliwa P., Demska-Zakęś K., Martyniak A., Kozłowski J. 1998 - The annual cycle of the gonads of vimba *Vimba vimba* (L.) from the Drawieński National Park - In: Materiały Ogólnopolskiej Konferencji "Funkcjonowanie i ochrona ekosystemów wodnych na obszarach chronionych", Wigry, 11-13.05.1998 (in Polish).

Horoszewicz L. 1983 - Reproductive rhythm in tench (*Tinca tinca* L.) in fluctuating temperatures - Aquaculture 32: 79-94.

Kozlovskii D.A. 1968 - Resorbtsiya polovykh produktov u ryb kak stimul perestroiki ikh biologii - Vopr. Ikhtiol. 8(6): 1009-1014.

Mayer S., Shackley E. 1988 - Aspects of the reproductive biology of the bass *Dicentrarchus labrax*. I. An histological and histochemical study of oocyte development - J. Fish Biol. 33: 609-622.

Mejen V.H. 1939 - K voprosu o godovom tsikle izmeneniya yayetsnikov kostistykh ryb - Izv. AN SSSR, Biol. 3.

Pliszka F. 1951 - Observations of the biology of vimba *Vimba vimba* (L.) regarding changes in its testes in an annual cycle - Rocz. Nauk Rol. 57: 379-389.

Roblin C., Bruslé J. 1983 - Ontogenése gonadique et différenciation sexuelle du loup (*Dicentrarchus labrax*), en conditions d'élevage - Reprod. Nutr. Dev. 23(1): 115-127.

Sakun O.F. 1951 - Tsyrt riek Latviiskoi SSR kak obekt rybovodstva - Ryb. Khoz. 7: 30-32.

Sakun O.F. 1954 - Analiz funktsii polovykh zhelez u samtsov i samok ryb v svyazi z kharakterom neresta - DAN SSSR 98(3): 505-508.

Sakun O.F., Bucka N.A. 1968 - Opredelenie stadii zrelosti i izuchenie polovykh tsiklov ryb - Ministr. Ryb. Khoz. SSSR, Murmansk, 5-45.

Schischabekov M.M. 1979 - O biologii razmnozheniya kutuma *Rutilus frisii kutum* (Kamensky), zherekha *Aspius aspius* (L.), rybtsa *Vimba vimba persa* (Pallas) i krasnopierki *Scardinius erythrophthalmus* (L.) v vodoemakh Dagestana - Vopr. Ikhtiol. 19(3): 495-502.

Zawistowski S. 1986 - Histological techniques, histology and the foundations of histopathology - PZWL, Warszawa, 548 pp. (in Polish).

STRESZCZENIE

CYKL ROCZNY JAJNIKÓW CERTY *VIMBA VIMBA* (L.) Z DRAWIEŃSKIEGO PARKU NARODOWEGO

Celem badań była analiza zmian makroskopowych i mikroskopowych zachodzących w jajnikach samic certy w cyklu rocznym oraz określenie ewentualnych cech charakterystycznych dla gatunku. Materiał badawczy stanowiło 146 ryb pozyskanych w latach 1997-1998 na terenie Drawieńskiego Parku Narodowego (NW Polska)(rys. 1, tab. 1). U żadnej ze złowionych samic nie stwierdzono nieprawidłowości lub zmian patologicznych w budowie jajników. W obrazie histologicznym gonad, wyróżniono stadia dojrzałości charakterystyczne dla ryb z porcyjnym tarłem: IV_2, V_2, IV_3, V_3 oraz stosunkowo krótkie - stadium potarłowe (VI/II)(fot. 1, 2). Stwierdzono, że u certy z DPN najdłużej trwa III stadium dojrzałości jajnika, rozpoczynające się w październiku, a kończące w marcu/kwietniu roku następnego. Wszystkie badane samice cechowała wyraźna asynchroniczność dojrzewania owocytów, a jednocześnie obserwowano w jajnikach stosunkowo duży procent komórek w fazie wakuolizacji (owocytów witellogenicznych), które nie dojrzewały i były resorbowane.

CORRESPONDING AUTOR:

Dr Piotr Hliwa
Uniwersytet Warmińsko-Mazurski
Zakład Genetyki Ewolucyjnej
ul. Oczapowskiego 5
10-957 Olsztyn
Tel./Fax:+48 89 5234787; e-mail:phliwa@uwm.edu.pl