| Arch. Pol. Fish. | Archives of Polish Fisheries | Vol. 11 | Fasc. 1 | 69-78 | 2003 |
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# HISTOLOGICAL ANALYSES OF GONAD DEVELOPMENT IN FEMALE SPINY-CHEEK CRAYFISH *ORCONECTES LIMOSUS* RAF.

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ABSTRACT. Histological analyses were preformed to investigate the annual cycle of the ovaries of female spiny-cheek crayfish *Orconectes limosus* Raf. (Crustacea). In May, the crayfish ovaries were filled mainly with oocytes in the initial stage of development (previtellogenic oocytes). The histological picture of the ovaries indicated the presence of empty follicles and mature oocytes undergoing resorption. In the summer months, the volume of the oocytes gradually increased and surplus substances material began to be synthesized (phase I or endogenous vitellogenesis). The histological picture of the ovaries in August was varied; some of them contained previtellogenic and vitellogenic oocytes in phase I, while others contained mainly oocytes in phase II of vitellogenesis when the cytoplasm fills with yolk and lipid globules. In fall, winter and spring the ovaries of crayfish *O. limosus* were filled mainly with oocytes in phase II of vitellogenesis; these were gradually increasing in volume. The annual gonadosomatic index (GSI) was calculated in the range of 0.41 to 5.83. The percentage of previtellogenic to vitellogenic oocytes prior to spawning was 23:77, and following spawning it was 70:30.

Key words: SPINY-CHEEK CRAYFISH *(ORCONECTES LIMOSUS)*, REPRODUCTIVE CYCLE, OOGENESIS, GONADOSOMATIC INDEX

## INTRODUCTION

The spiny-cheek crayfish *Orconectes limosus* Raf. occurs in nearly all areas of Poland (Jażdżewski and Konopacka 1995, Strużyński and Śmietana 1998, Mastyński 1999). Since *O. limosus* is of little commercial value, mainly due to its small size, the biology of this species has not been well described in the literature. Among Polish publications, Stypińska's work (1972, 1973, 1978) on the fecundity of this crayfish is important. There are no publications which address the annual cycle of ovarian development, although there is one unpublished work by Punda and Ślusarczyk (1980). Papers which describe other Decapoda species address the hormonal regulation of oogenesis (Kulkarni et al. 1991, Sarojini 1994, Mc Rae and Mitchell 1995), the impact of temperature and photoperiod on this process (Portelance and Dube 1990, Castanon-Cervantes et al. 1995) and the ultrastructure of cells which participate in oogenesis (Adiyodi and Subramoniam 1983, Charniaux-Cotton 1985).

The aim of the present work was to analyze the process of oogenesis and variability in the development of the ovaries of *O. limosus* crayfish during the annual cycle.

## MATERIAL AND METHODS

The material for the study was collected from Lake Staw Płociczno at monthly intervals from September 1999 to April 2001. Lake Staw Płociczno is located in northeastern Poland (54°01.28′ N and 22°59.20′ E) in the Niemen catchment area. It is connected with the Niemen River by an unnamed stream, Lake Wigry and the Czarna Hańcza River.

The crayfish samples were caught in a trap deployed at the outflow of the unnamed stream from Lake Staw Płociczno into Lake Wigry. The ovaries from a total of 96 sexually mature females were collected and analyzed. Female *O. limosus* are sexually mature when they reach a body length of 60 mm (Pieplow 1938). Body length was measured to the nearest 1 mm, and the specimens were weighed to the nearest 0.1 g. The length of the specimens ranged from 63 to 98 mm, and weight ranged from 7.2 to 24.7 g. Following preparation, the ovaries were weighed to the nearest 0.001 g; the range was from 0.048 g (in May) to 0.996 g (in March). The gonadosomatic index (GSI) was derived using the ratio of gonad weight to body weight; the average values by month are presented in Figure 1.

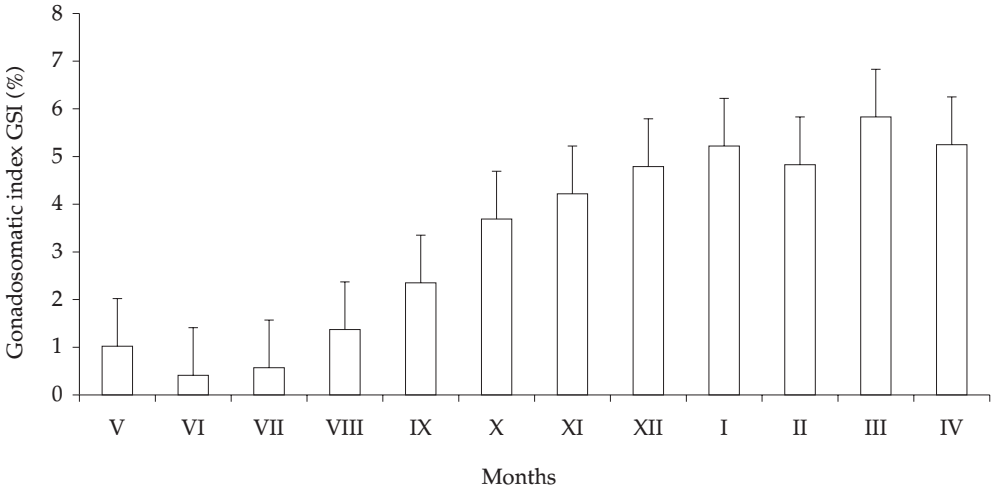


Fig. 1. Gonadosomatic index (GSI) of *O. limosus* females (average values and standard deviation).

Between 3 and 11 ovaries were collected for each analyses and were prepared according to the techniques described by Zawistowski (1986). The ovaries were preserved in either Bouin's fluid or buffered formaldehyde. Thin sections (7 μm) were dyed with Mayer's hematoxylin and eosin (HE). The samples were then analyzed under a Nikon light microscope.

Computer analysis was conducted on the histological samples with the Multiscan program, and the percentage of oocytes in stages I and II of development were determined in the subsequent months of the annual cycle.

## RESULTS AND DISCUSSION

In May, the ovaries of *O. limosus* were mostly small and milky-white in color. Of the nine females collected in May, only one had ovaries containing mature oocytes. The histological structure of the ovaries of the other females indicated that they had already spawned, although not all of them were "in berry". The ovaries of the females which had spawned contained mainly previtellogenic oocytes (Photo 1). The nuclei of these cells were large, oval and filled with loose chromatin. This indicated the beginning of prophase I of meiotic division, during which previtellogenesis (collection of development information) and vitellogenesis process (synthesis of substances) take place. Concentrations of oogonia (the germarium), which give rise to subsequent generations during mitotic division, were also noted (Photo 2). It is not until the beginning of prophase I of meiotic division that oogonia became oocytes. Oogonia were observed in the center of the ovaries near the previtellogenic oocytes. The numerous

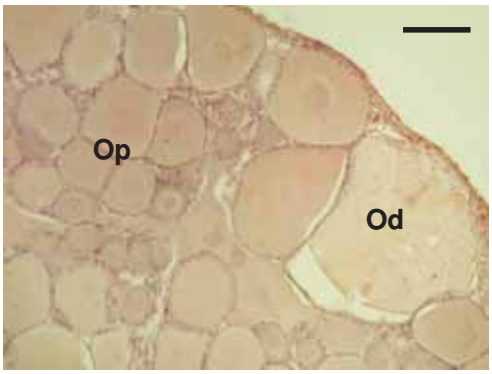


Photo 1. Ovary cross-section of an *O. limosus* specimen in May. Op – previtellogenic oocytes, Od – oocyte during resorption (scale = 0.25 mm).

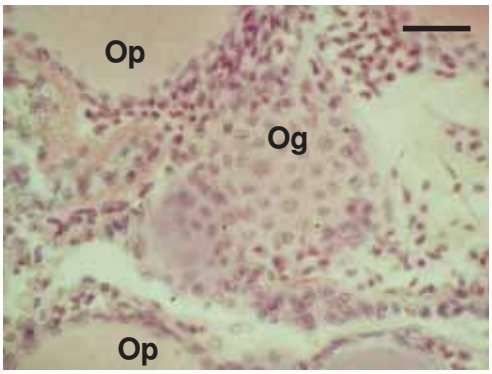


Photo 2. Ovary cross-section of an *O. limosus* specimen in May. Oo – oogonia (germarium), Op – previtellogenic oocytes (scale = 0.05 mm).

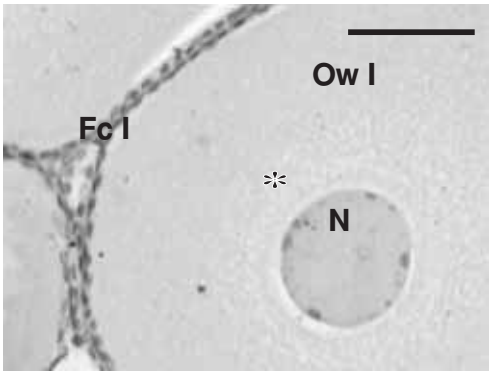


Photo 3. Ovary cross-section of an *O. limosus* specimen in June. Ow I – vitellogenic oocyte in phase I, Fc I – primary follicular epithelium, N – oocyte nucleus, * - bright stria – cell organelles surrounding the oocyte nucleus (scale = 0.5 mm).


Photo 4. Ovary cross-section of an *O. limosus* specimen in July. Od – oocyte during resorption (scale = 0.25 mm).

empty follicles indicated that the *O. limosus* females had already spawned in that reproduction season, i.e. in April or early May. The oocytes which had not been released degenerated and were resorbed (Photo 1).

The GSI index was 1.02 (Fig. 1); this relatively high value resulted from the fact that not all females had spawned. The GSI index which was calculated only for the females which had already spawned was much lower at 0.32.

In June and July, the ovaries were slightly transparent and yellowish. The volume of the oocytes had increased significantly. A bright stria was clearly visible around the nucleus of the oocyte (Photo 3). This was most likely formed by the aggregation of cellular organelles (Ganion and Kessel 1972, Adiyodi and Subramoniam 1983). This phenomenon indicates the completion of development previtellogenesis and the commencement of vitellogenesis, i.e. the deposition of substances in the oocytes (mainly yolk proteins, lipid droplets and polysaccharides) which will be used by the developing embryo. The first phase of the process is vitellogenesis I or endogenous vitellogenesis (Adiyodi and Subramoniam 1983, Charniaux-Cotton 1985), during which substances are deposited in the oocytes. Oocytes in the first phase of vitellogenesis were surrounded by primary follicular cells, which constituted a single-layer epithelium (Photo 3). Nucleoli were visible in the large, oval nucleus under the nucleus membrane. Usually, four large nucleoli and from three to four smaller ones were observed (Photo 3). Empty follicles and oocytes which were being resorbed were still visible in the ovaries (Photo 4). The GSI indexes in June and July were 0.41 and 0.57, respectively (Fig. 1).


Photo 5. Ovary cross-section of an *O. limosus* specimen in August. Op – previtellogenic oocytes, Ow –vitellogenic oocytes in phase I , Fe – empty follicle (scale = 0.5 mm).

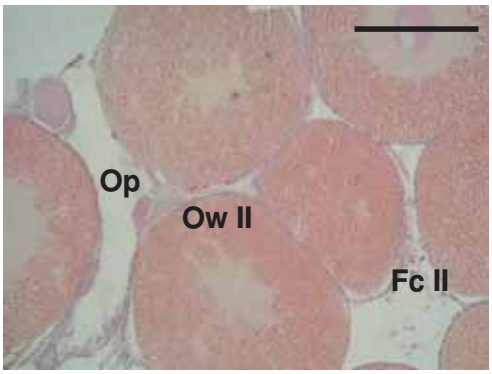


Photo 6. Ovary cross-section of an *O. limosus* specimen in August. Ow II – oocytes in phase II of vitellogenesis, Op – previtellogenic oocytes, Fc II – secondary follicular epithelium (scale = 0.5 mm).

In August, the ovaries were no longer transparent. They were mat and the color had changed from dark yellow to gray-green. There was a definitive increase in the GSI index this month with an average of 1.37 (Fig. 1).

The histological picture of the ovaries in August was not uniform. The histological structure of the ovaries of some of the crayfish studied resembled that from July, i.e. mainly previtellogenic oocytes and phase I of vitellogenesis were observed, and there were still empty follicles. No degenerating oocytes were observed (Photo 5). The ovaries of the second group were filled mainly with oocytes in phase II of vitellogenesis, which are much larger than oocytes in phase I. The center of the ovary was occupied by previtellogenic oocytes. These two groups of *O. limosus* females could not be divided according to size. The cytoplasm of the oocytes in phase II of vitellogenesis was filled with yolk and lipid droplets (Photo 6). The substances taken up by the crayfish oocytes contained both organic and inorganic components. The main organic components were proteins (about 25%) and lipids (about 22%), whereas carbohydrates constituted only about 1% of all the substances deposited (Adiyodi and Subramoniam 1983, Vogt 2002). The oocytes in phase II of vitellogenesis are undergoing exogenous vitellogenesis. During this phase, vitellogenin, the precursor of the egg yolk protein, is produced by the liver and then transported from the blood to the oocyte by endocytosis (Biliński et al. 1995). This is why the majority of initial yolk collection is seen under the oolema. These oocytes develop synchronously and are surrounded by a secondary follicular epithelium (Photo 6). The modifications which the follicular epithelium in crayfish ovaries

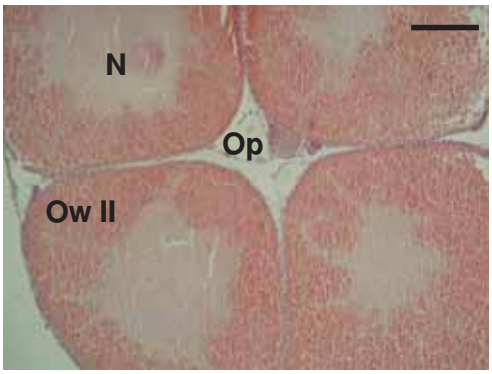


Photo 7. Ovary cross-section of an *O. limosus* specimen in September. Ow II – oocytes in phase II of vitellogenesis, Op – previtellogenic oocytes, N – nucleus of a vitellogenic oocyte (scale = 0.25 mm).

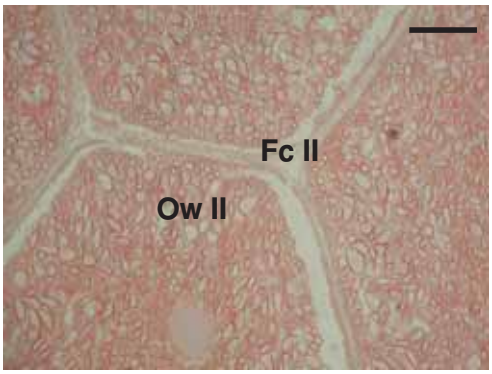


Photo 8. Ovary cross-section of an *O. limosus* specimen in October Ow II – oocytes in phase II of vitellogenesis, Fc II – secondary follicular epithelium (scale = 0.25 mm).

undergoes throughout oogenesis - the appearance of the primary epithelium during endogenous vitellogenesis and the secondary epithelium during exogenous vitellogenesis - has been described by Charniaux-Cotton (1985) as double folliculogenesis.

In September, the ovaries of *O. limosus* females contain previtellogenic oocytes as well as oocytes in phase II of vitellogenesis (Photo 7). The previtellogenic oocytes were located in the central part of the ovaries, while the oocytes in phase II of vitellogenesis were in the cortical. The center of the latter contained a nucleus with nucleoli. In September and October, the ovary color had changed from light brown to dark green. The GSI index in September was 2.35 (Fig. 1).

In October, significantly fewer previtellogenic oocytes were observed than those in phase II of vitellogenesis, and the latter had increased in size greatly. The entire cytoplasm of the oocyte was filled with the yolk and lipid drops (Photo 8). The GSI index was 3.69 (Fig. 1).

In winter from November to March, the histological picture of the ovaries was similar. The ovaries contained oocytes in phase II of vitellogenesis that were increasing in volume. They had small nuclei that were shifted to the edge of the oocyte and the nucleoli were still visible. These oocytes were close together (Photo 9). In April, the follicular epithelium separated from the oocyte membrane as the oocytes finished growing and prepared for ovulation (Photo 10). In winter, the ovaries were opaque and dark green. The GSI index increased from 4.22 in November to a maximum value of 5.83 in March (Fig. 1).

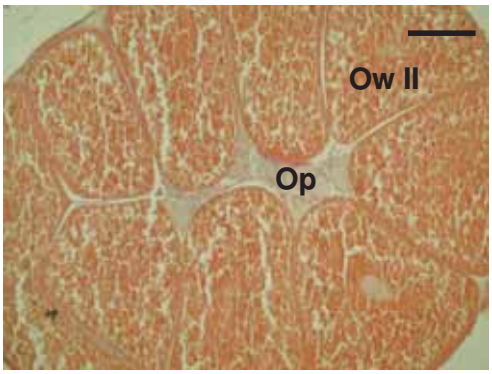


Photo 9. Ovary cross-section of an *O. limosus* specimen in November. Ow II – oocytes in phase II of vitellogenesis, Op – previtellogenic oocytes (scale = 0.5 mm).

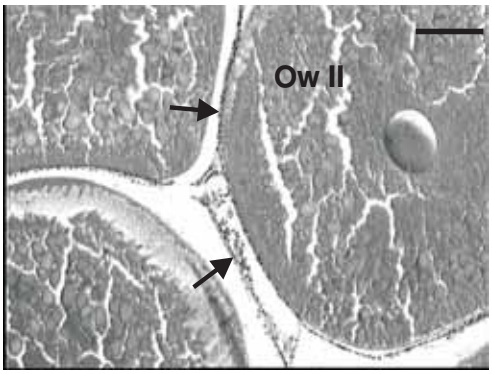


Photo 10. Ovary cross-section of an *O. limosus* specimen in April. Ow II – vitellogenic oocyte in phase II, arrow – separation of the secondary follicular epithelium from the oocyte epithelium (scale = 0.25 mm).

The crayfish *O. limosus* mates in the fall from September to November, when the male transfers sperm to the female's sperm sack. However, unlike the indigenous Polish crayfish species *Astacus astacus* and *A. leptodactylus* which are fertilized and spawn in fall, the *O. limosus* is fertilized in spring when mature eggs are deposited under the female's abdomen. The histological analysis of the ovaries indicated that if this species mated in the fall, fertilization would not be possible since vitellogenesis (the growth stage) is incomplete and the eggs are not yet ready for ovulation. If the females lose the sperm or do not mate in fall, then they can mate again in spring. Spring mating of *O. limosus* was observed by Ulikowski and Borkowska (1999). That this species is able to mate in spring was also confirmed by the work of Chybowski and Juchno (2002) on the development of the male gonads. The authors noted that in spring the testicles of some males contain enough sperm to allow for fertilization.

*O. limosus* females had the largest store of previtellogenic oocytes in the summer months after spawning (May - 70%, August - 44%) (Fig. 2). Prior to spawning in winter and early spring, there is a marked decrease in the percentage of previtellogenic oocytes (to some 30%) in favor of vitellogenic oocytes (about 70%). This percentage distribution of previtellogenic to vitellogenic oocytes is characteristic of aquatic animals which spawn once during the reproduction season, e.g. fish that spawn in single batches (Bieniarz and Epler 1991). When these fish spawn, the proportion of vitellogenic oocytes drops incrementally, and the store of previtellogenic oocytes is used to create a new portion of eggs for the next season.

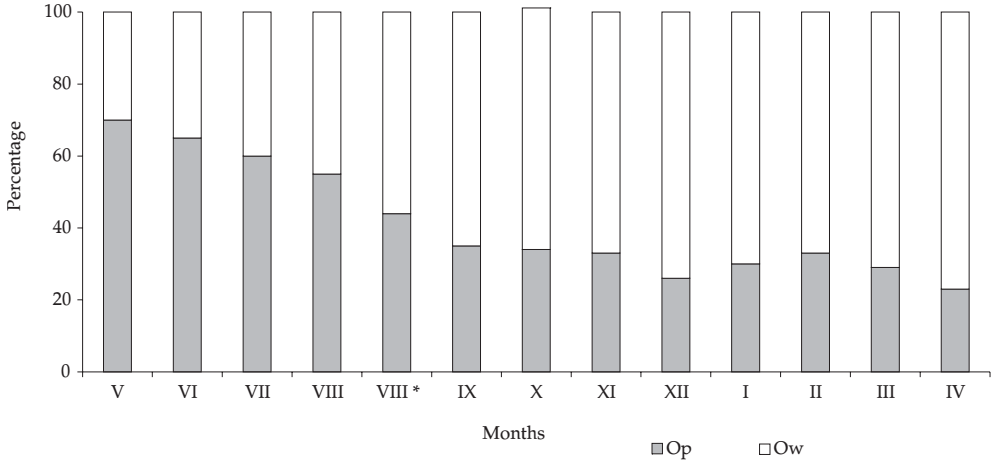


Fig. 2. Annual contribution of previtellogenic (Op) and vitellogenic oocytes (Ow) in the ovaries of *O. limosus* expressed in percentages. VIII* - ovaries containing oocytes in phase II of vitellogenesis (description in text).

## CONCLUSIONS

1. The histological picture of the ovaries of female *O. limosus* changes significantly during the annual cycle. In May, the histological structure of the ovaries of the majority of females indicated that the *O. limosus* females had already spawned in that reproduction season.
2. When crayfish males transfer sperm to the females in fall, fertilization cannot occur because the ovaries contain oocytes which have not yet completed the growth stage and are not ready for ovulation.
3. The gonadosomatic index (GSI) is the highest before spawning (March, April) and is the lowest after it (June).
4. The highest percentage of previtellogenic oocytes occurs after spawning in spring and summer, while it clearly decreases prior to spawning.

### ACKNOWLEDGEMENTS

*The authors would like to thank Andrzej Koryzno for his help with the photography.*

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## STRESZCZENIE

ANALIZA HISTOLOGICZNA ROZWOJU GONAD SAMIC RAKA PRĘGOWATEGO *ORCONECTES LIMOSUS* RAF. W CYKLU ROCZNYM

Celem pracy było zaobserwowanie zmian zachodzących w obrazie histologicznym jajnika raka *O. limosus* w cyklu rocznym. Gonady samic raka *O. limosus* pobierano w comiesięcznych odstępach, od września 1999 do kwietnia 2001 roku. W maju jajniki raków wypełnione były głównie oocytami prewitelogenicznymi (wczesna faza wzrostu oocytów) (fot. 1). W obrazie histologicznym występowały również puste otoczki folikularne i dojrzałe oocyty w trakcie resorpcji. W miesiącach letnich stopniowo zwiększała się objętość oocytów, które rozpoczynają syntezę materiałów zapasowych (witelogeneza I lub endogeniczna) (fot. 3). W sierpniu obraz histologiczny jajników był niejednorodny. U części badanych samic gonady wypełnione były oocytami prewitelogenicznymi i witelogenicznymi I (fot. 5). U drugiej grupy samic w gonadzie znajdowały się głównie oocyty witelogeniczne II, których cytoplazma wypełniona była kulami żółtka i kroplami lipidowymi (fot. 6). Oocyty witelogeniczne II są komórkami w fazie witelogenezy egzogenicznej, w czasie której prekursory tworzącego się żółtka, witelogeniny, wytwarzane są w wątrobotrzustce, a następnie przekazywane z krwi do oocytu. Jesienią, zimą i wiosną jajniki raków *O. limosus* wypełnione były głównie oocytami witelogenicznymi II, które stopniowo zwiększały swoją objętość (fot. 8, 9, 10). Najwyższa wartość współczynnika GSI - 5,83 występuje w marcu, przed złożeniem jaj, a najniższa - 0,41, w czerwcu, po złożeniu jaj (rys. 1). Największy udział procentowy oocytów prewitelogenicznych w jajniku (do 70%), stanowiących zapas komórek płciowych na następny sezon rozrodczy jest latem, po złożeniu jaj. Natomiast zimą i wiosną, przed złożeniem jaj, udział oocytów prewitelogenicznych wyraźnie spada (do 23%) na korzyść wysokiego udziału w jajniku (do 77%) oocytów witelogenicznych (faza intensywnego wzrostu) (rys. 2).

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