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HISTOLOGICAL ANALYSIS OF THE ANNUAL CYCLE OF GONAD DEVELOPMENT IN THE MALE SPINY-CHEEK CRAYFISH *ORCONECTES LIMOSUS* RAF.

Łucjan Chybowski, Dorota Juchno***

*The Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland
** University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT. Histological analyses were conducted of the annual cycle of male gonads of spiny-cheek crayfish *Orconectes limosus* Raf. Although changes in the male gonads throughout the year are clearly evident, they are not uniform in all males. Spiny-cheek crayfish mate mainly in the autumn from September to November. After mating, the gonads of male crayfish can be divided into two groups according to their histological structure. In the first group there is an abundance of sperm in the gonads, while the testicular tubules in the second group are either empty or contain a small amount of sperm. This division remains until May. In May, the histological picture of the gonads is uniform, and there are either very few or no tubules containing sperm. In June *O. limosus* males occur in two forms. The histological pictures of first- and second-form male gonads do not differ. However, beginning in July and also in August and September, the gonads of first- and second-form males do differ. First-form male crayfish gonads contain more sperm than those of second-form males. From October onwards, all of the males are first-form, and primarily spermatids and sperm are visible in the gonads. The possibility that the spiny-cheek crayfish mates twice annually, once in autumn and again in spring, is discussed based on changes observed in the histological picture of the gonads.

Key words: SPINY-CHEEK CRAYFISH (*ORCONECTES LIMOSUS)*, MATING, REPRODUCTION, SPERMATOGENESIS

INTRODUCTION

The spiny-cheek crayfish *Orconectes limosus* Raf. is one of four crayfish species which are currently present in Polish inland waters. The noble crayfish *Astacus astacus* (L.), the narrow-clawed crayfish *Astacus leptodactylus* Esch., and the more recently introduced signal crayfish *Pacifastacus leniusculus* (Dana) are also present (Jażdżewski and Konopacka 1995).

The morphologic structure of the first pair of copulatory pleopods is a characteristic feature of the *O. limosus* male. These limbs occur in two morphologic forms; form I occurs only among reproductively mature crayfish from summer (July – September) until late spring (May– June), while form II occurs among reproductively mature males only after the spring molt and lasts until the subsequent molt. Only form-II copulatory pleopods occur in reproductively immature *O. limosus* males. While form I facilitates female fertilization, form II does not (Pieplow 1938, Kossakowski 1966).

Male *O. limosus* reach reproductive maturity at an age of 1–2 years and a body length of approximately 6 cm (Lehmann and Quiel 1927, Pieplow 1938, Kossakowski 1966).

The process of spermatogenesis in Decapoda, especially at the ultrastructure level, has been widely described (Moraes 1995, Zhao Yunlong et. al. 1997, Yang Wanxi 1998, Hong ShuiGen et. al. 1999, Huang Haixia et. al. 2001). Many papers focus on the structure and creation of sperm (Li Taiwu 1995, Medina 1995, Richer de Forges et. al. 1997, Wang Lan et. al. 1999) and the creation of spermatophores (Bauer and Lin 1993, Subramonian 1993, Wang Lan et. al. 1996, MacDiarmid and Butler 1999). The ultrastructural build of sperm was the basis for phylogenetic considerations regarding the Decapoda order (Storch and Jamieson 1992, Jamieson et. al. 1993, 1995, Medina 1995, Tudge 1995). Crayfish sperm is spherical and flagellated (Photo 1). However, in the available literature there is no description of the annual histological changes which take place in the gonads of reproductively mature *O. limosus* males.

According to Pieplow (1938), Kossakowski (1966) and Brink et. al. (1988), the mating period for *O. limosus* occurs in autumn, just as it does for *A. astacus*, *A. leptodactylus* and *P. leniusculus*. However, observations of *O. limosus* behavior in spring indicate that it also mates at this time (Ulikowski and Borkowska 1999, Chybowski, unpublished data). Autumn and spring mating would indicate that *O. limosus* males are capable of fertilizing eggs for a period of six months or that some of them mature in spring and some in autumn.

The aim of this work is to describe the changes which occur in gonads of reproductively mature spiny-cheek crayfish males in an annual cycle and to discuss the possibility that *O. limosus* mates twice yearly.

MATERIAL AND METHODS

The material for the study was comprised of crayfish *O. limosus* from Lake Staw Płociczno. This lake is located in northeastern Poland at 54°01,28′ N and 22°59,20′ E, in the Niemen River catchment area. The lake is connected with the Niemen River by an unnamed stream, Lake Wigry and the Czarna Hańcza River.

The crayfish for the study were caught with a fish weir located at the outflow of the unnamed stream from Lake Staw Płociczno to Lake Wigry. Catches were made monthly from September 1999 to August 2000. The gonads of a total 56 *O. limosus* males were studied. The crayfish were measured to the nearest mm and weighed to the nearest 0.1 g. The body length of the specimens ranged from 56.0 to 98.0 mm, and they weighed from 5.5 to 29.1 g. The water temperature was recorded during the catches (Table 1).

TABLE 1

Water temperatue (°C) in Lake Staw Płociczno

Depth (m)	Sampling date											
	13 September 99	11 October 99	15 November 99	13 December 99	18 January 00	14 February 00	10 March 00	10 April 00	15 May 00	12 June 00	10 July 00	16 August 00
	Temperature (°C)											
0	18.0	11.5	4.0	3.0	0.2	0.1	3.0	6.0	16.0	20.0	20.0	19.5
1	18.0	11.5	4.0	4.0	1.0	1.5	3.5	6.0	16.0	20.0	18.0	19.0
2	18.0	11.0	4.0	4.0	4.0	4.0	4.0	6.0	16.0	20.0	17.5	18.5
3	17.2	11.0	4.0	4.0	4.5	5.0	5.0	6.0	15.0	19.0	17.5	17.0

The crayfish were categorized as either first- or second-form males based on the morphological structure of the copulatory pleopods.

Three to six gonads were used for each series of histological analyses. Samples were prepared in accordance with techniques described by Zawistowski (1965). The gonads were preserved in Bouin's fluid or buffered formaldehyde, and 7 μm tissue sections were dyed with Mayer hematoxylin and eosin (HE). The samples were then analyzed using a Nikon light microscope.

RESULTS AND DISCUSSION

Based on the morphological structure of the copulatory pleopods, both first- and second-form *O. limosus* male specimens were observed in September. The gonadal tubules in males with form-II copulatory pleopods were filled with early stages of spermatogenesis - spermatogonium and spermatocytes (Photo 2), those with spermatids were less numerous, and with sperm the least numerous. However, the gonads of first-form males were filled with the late stages of spermatogenesis - spermatids and sperm (Photo 3).

In October and November all of the specimens collected were first-form males. Mainly spermatids and sperm were observed in the gonads (Photo 4 and Photo 1).

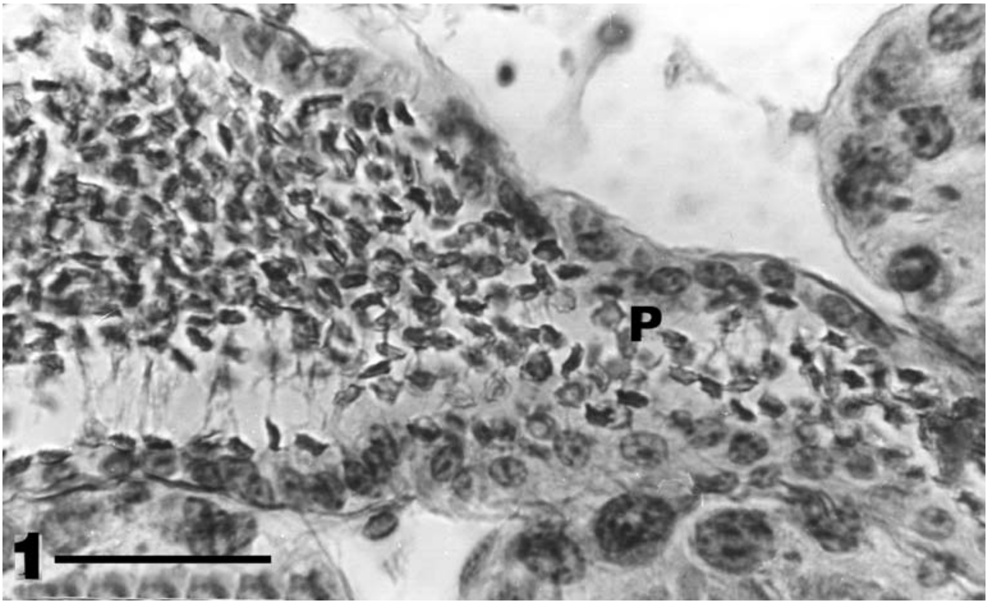

Photo 1. Cross-section of a male *Orconectes limosus* gonad. Sperm is visible inside the tubule (P), scale = 0.25 mm.

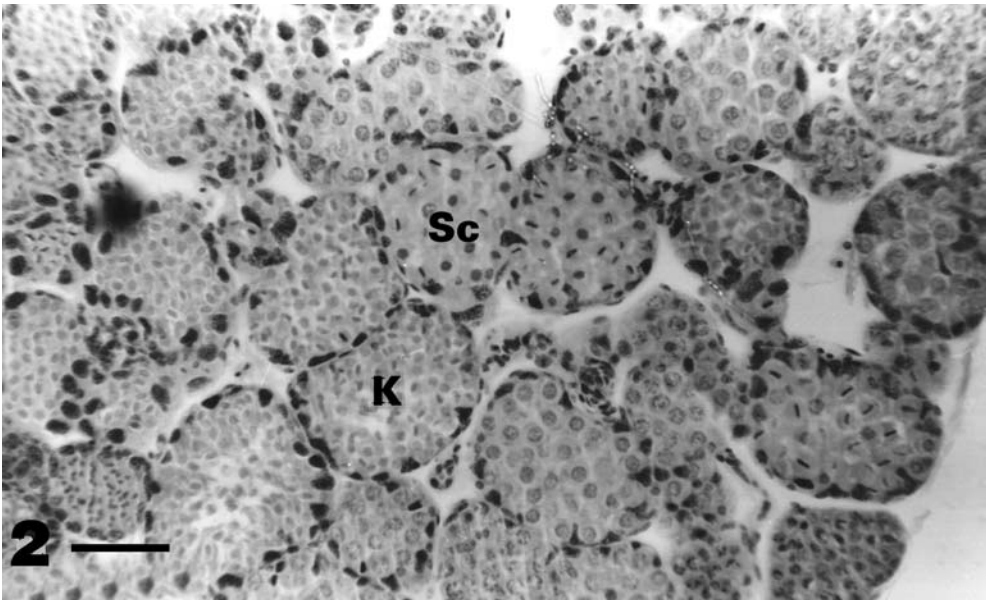

Photo 2. Cross-section of a male *Orconectes limosus* gonad (form II) – September. The greater part of the gonad consists of tubules (K) with spermatocytes (Sc), scale = 0.50 mm.

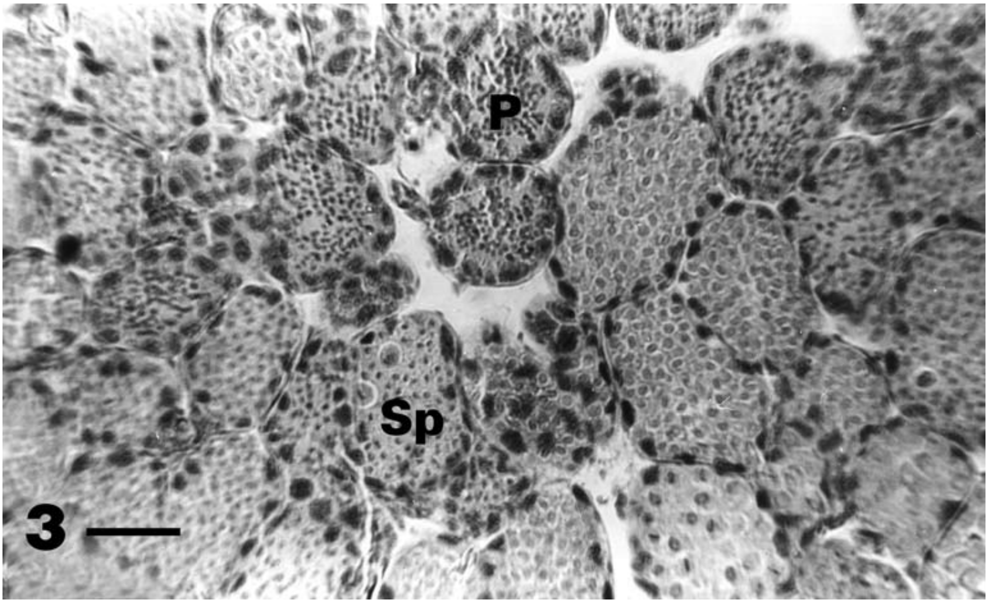

Photo 3. Cross-section of a male of *Orconectes limosus* gonad (form I) – September. The greater part of the gonad consists of tubules with spermatids (Sp) and sperm (P), scale = 0.50 mm.

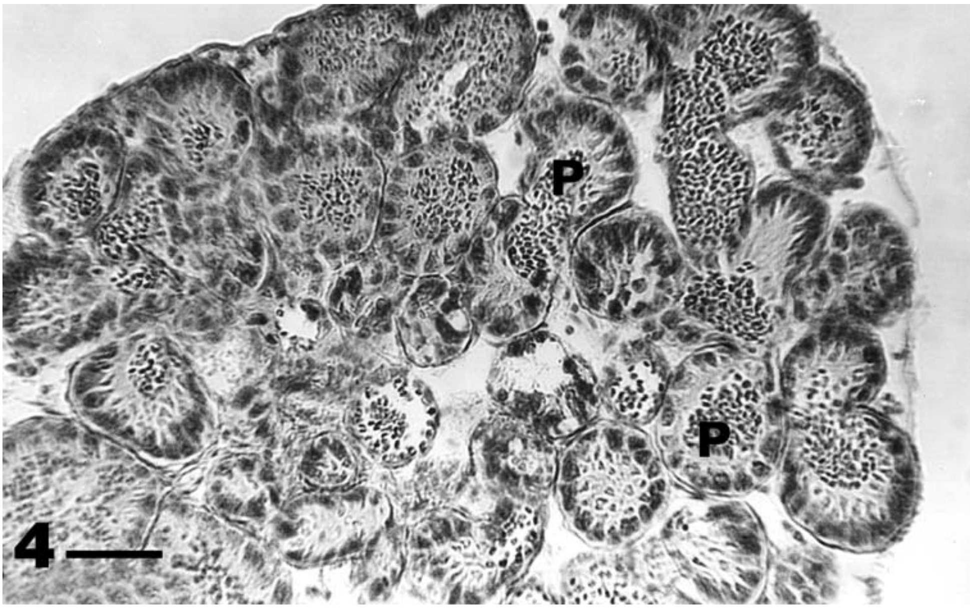

Photo 4. Cross-section of a male *Orconectes limosus* gonad - October. The tubules are filled with sperm (P), scale = 0.50 mm.

The histological picture indicated that a small portion of the gonad exhibited signs of degeneration (Photo 5).

The crayfish mated in the autumn between September and November and at this time the sperm was transferred to the female's *annulus ventralis*. It is unclear whether the sperm deposited into the female in autumn is immediately capable of fertilization or whether this ability is achieved in spring when oviposition occurs, i.e. when the female lays eggs under her abdomen and fertilizes them with the earlier collected sperm which was stored in the *annulus ventralis*.

In the winter between December and February, following the mating period, the male gonads were divided into two groups according to histological structure. The first group of specimens retained a similar amount of sperm in their gonads as was recorded in October and November, and these males probably had not transferred their sperm to the females. In this instance, the sperm can either be resorbed or stored until spring. In some of the specimens, the tubules containing sperm were fairly loose and they occupied the central part of each testicle. The outermost areas of the gonads were filled with early stages of spermatogenesis. In the second group of males, the testicular tubules contained small amounts of sperm or were empty (Photo 6); these specimens had most certainly mated. The histological analyses of this group of males showed that a part of the gonad dyed differently with HE, and here the tubules formed a compact structure. Only the early stages of spermatogenesis, i.e. spermatogonium and spermatocytes, were present in this part (Photo 7). In February, the majority of the gonads examined showed signs of degeneration; this could indicate the increased resorption of sperm collected in the tubules.

In the subsequent months until May, the males remained divided into two groups which differed in histological gonad structure.

In March and April, the degenerated portion of the gonads was decidedly larger. However, there were still many tubules with sperm. Tubules with early stages of spermatogenesis also appeared. The sperm which had not been transferred to the females was probably continuously being resorbed, hence the degenerated part increased in size. A new cycle of spermatogenesis began in the non-degenerated part of the gonads where tubules with spermatogonium and spermatocytes appeared (the so-called "compact part") (Photo 7).

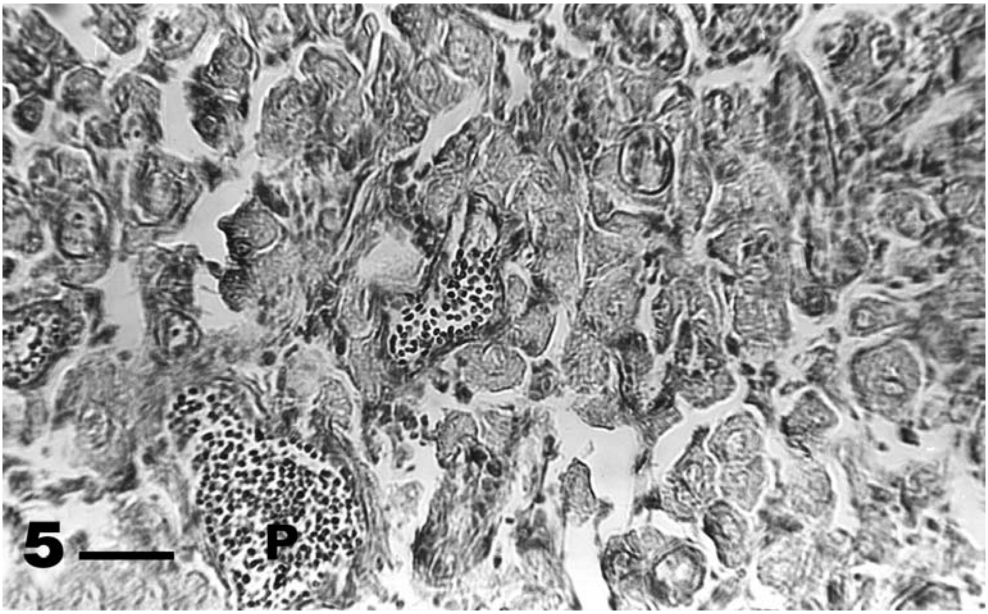

Photo 5. Cross-section of the degenerated part of a male *Orconectes limosus* gonad. The remains of the resorbed sperm are visible (P), scale = 0.50 mm.

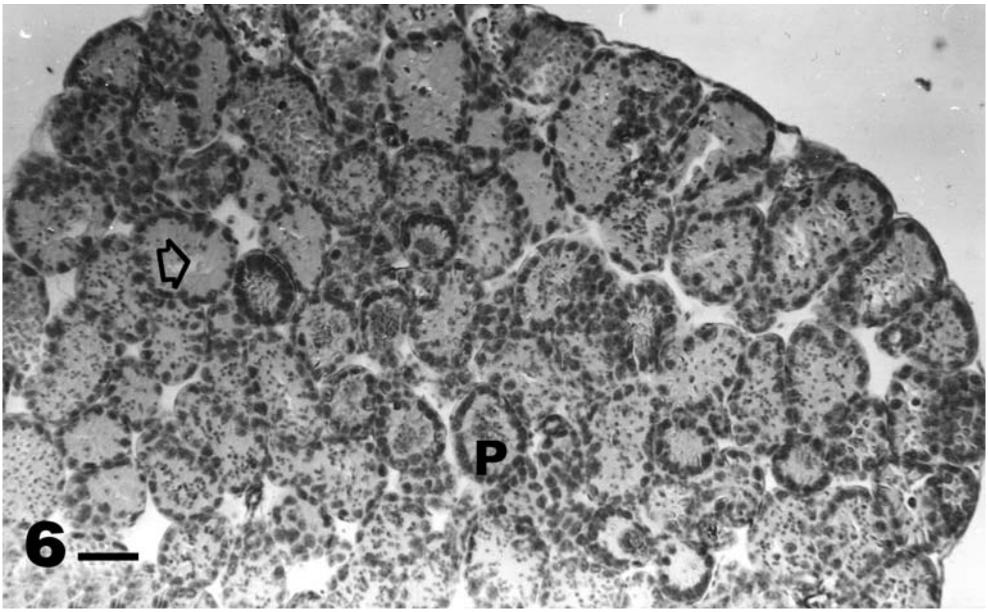

Photo 6. Cross-section of a male *Orconectes limosus* gonad – December. Empty tubules (⇒) with small amounts of sperm (P) are visible, scale = 0.50 mm.

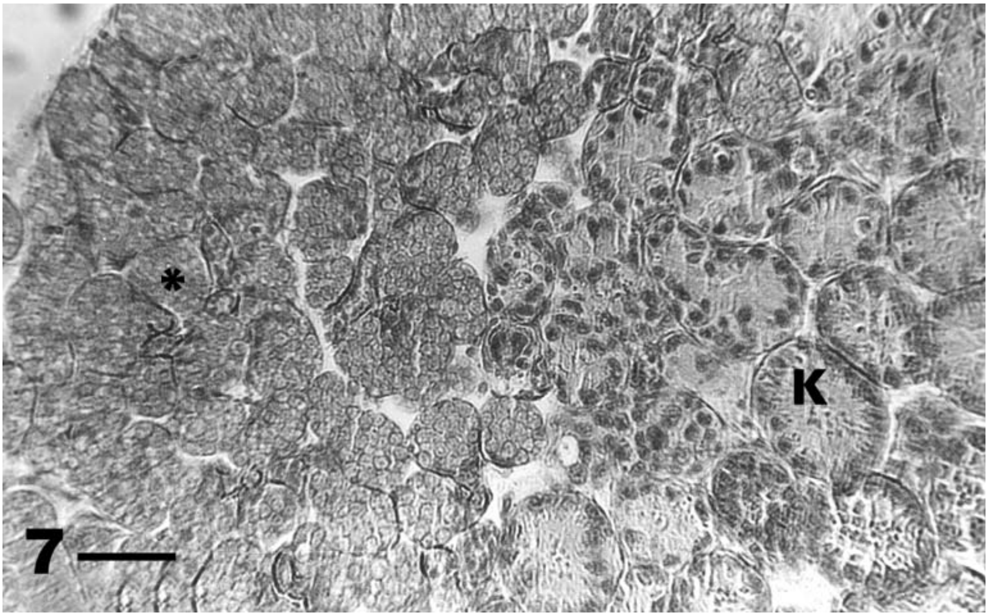

Photo 7. Cross-section of a male *Orconectes limosus* gonad – February. The so-called compact part (✻) with early stages of spermatogenesis is visible next to tubules (K) with sperm, scale = 0.50 mm.

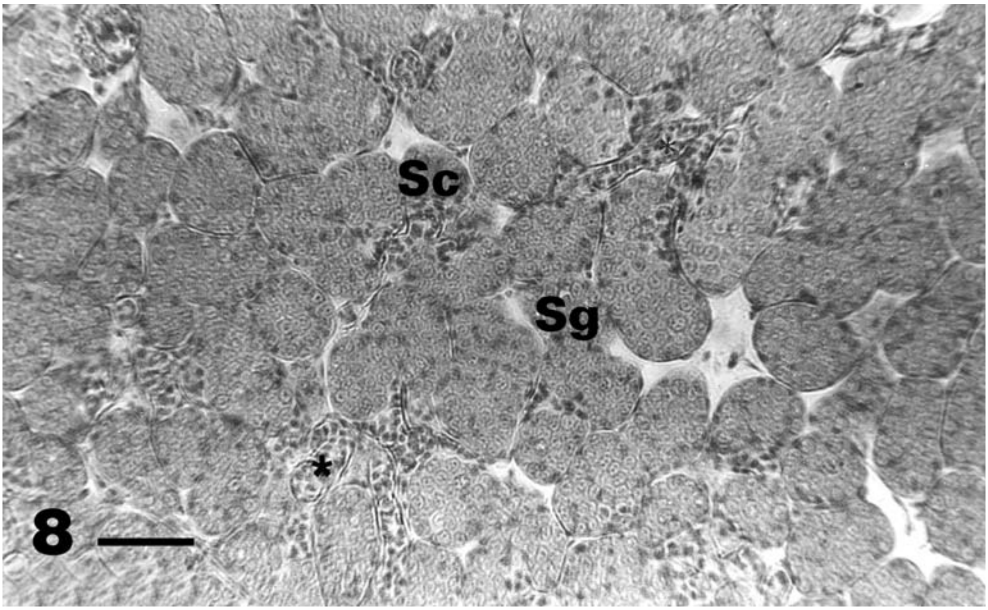

Photo 8. Cross-section of a male *Orconectes limosus* gonad – May. Tubules with early stages of spermatogenesis - spermatogonium (Sg) and spermatocytes (Sc) and tubules with Sertoli cells (✻) are visible, scale = 0.50 mm.

The histological picture was uniform; there was no sperm in the tubules or innumerous tubules with sperm. Large, regular tubules containing early stages of spermatogenesis comprised at least half of the gonads (Photo 8). Among these, there were numerous tubules containing only Sertoli cells. The latter were probably resorbing sperm and new stages of spermatogenesis were not yet visible. This could indicate that the sperm resorption process was complete or that the sperm had been transferred to females during spring mating.

O. limosus males again occurred in two forms in June. The histological picture of the gonads of first- and second-form males did not differ. In all of the males examined there was sperm in the degenerated part of the gonads and early stages of spermatogenesis occurred in the greater part of the testicle (Photo 9). However, by July and August the gonads of first- and second-form males differed from each other. There was more sperm in the gonads of first-from crayfish (Photo 10) than in the gonads of second-form males (Photo 11), although in both cases the greater part of the gonad was occupied by early stages of spermatogenesis. First-form males were ready to mate and by July single mating couples were observed. However, it is unclear whether females which have mated in early summer but lost their *annulus ventralis* during the autumn molt mate again in autumn or wait until the next spring, or whether spring mating is caused by the "loss" of the *annulus ventralis* in autumn.

According to Ulikowski and Borkowska (1999), the mating season is at peak intensity in mid May. The histological picture of *O. limosus* male gonads from Lake Staw Płociczno does not confirm that the mating of this species is possible at this time as the gonads contained early spermatogenesis stages but no sperm. However, spring mating is definitely variable in time. During studies in Lake Staw Płociczno, the author has observed mating crayfish in April. In April, a portion of the specimens examined had gonads which contained an abundance of sperm and the transfer to females was possible. That *O. limosus* is able to mate in spring was confirmed in studies by Strużyński (2000). Female crayfish which did not have sperm in their *annulus ventralis* were fertilized under laboratory conditions and later laid eggs.

Studies of other crustaceans indicate that only some of the mature males begin mating at the same time. Sagi et al. (1988) distinguished three morphotypes of the mature, male freshwater shrimp *Macrobrachium rosenbergii* (de Man), which each exhibited differing degrees of spermatogenesis. These were reproductively active small males, reproductively inactive males with an orange claw and reproductively active males with a blue claw. Although the males with an orange claw were repro-

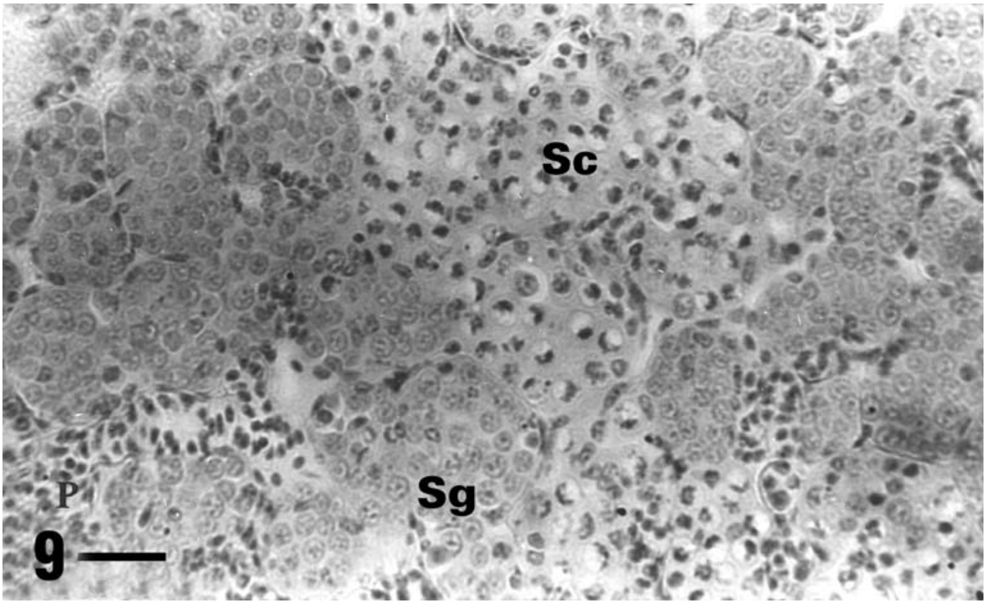

Photo 9. Cross-section of a male *Orconectes limosus* gonad (I and II forms) – June. Tubules containing spermatogonium (Sg) and spermatocytes (Sc); remains of sperm (P), scale = 0.25 mm.

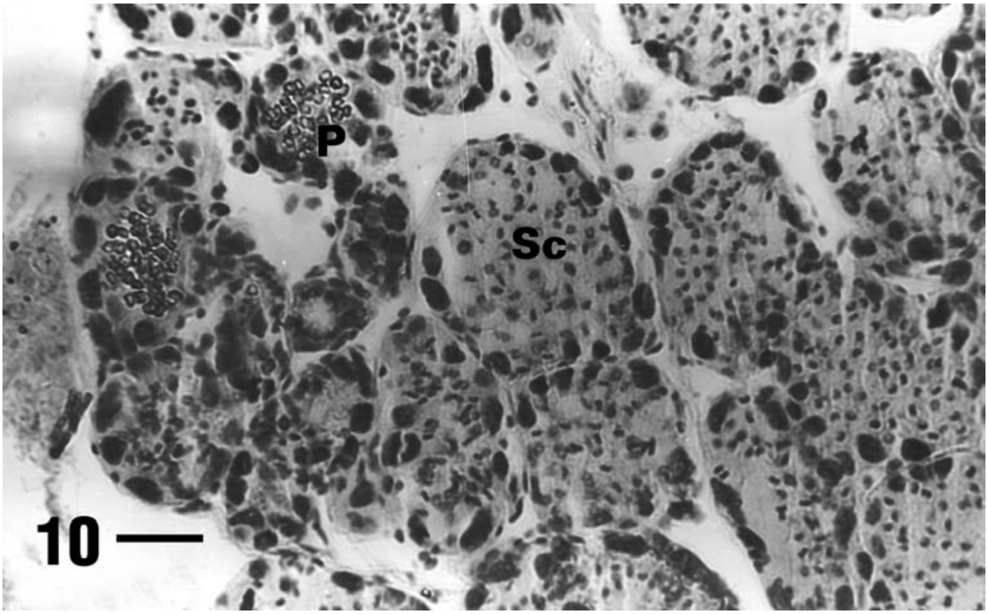

Photo 10. Cross-section of a male *Orconectes limosus* gonad (I form) – July-August. Spermatocytes (Sc) and sperm (P) are in the tubules, scale = 0.25 mm.

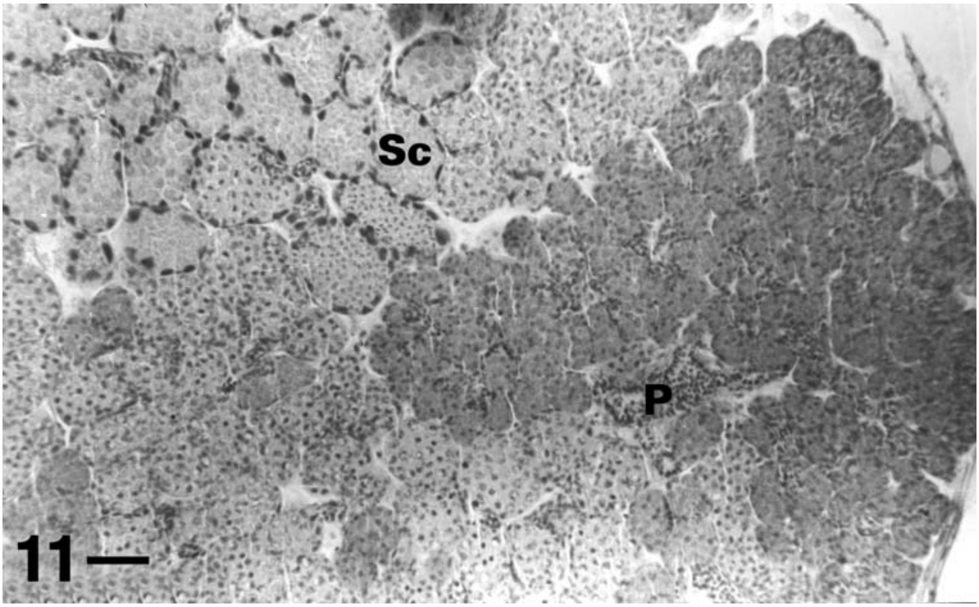

Photo 11. Cross-section of a male *Orconectes limosus* gonad (II form) – July-August. Spermatocytes (Sc) and small amounts of sperm (P) are in the tubules.

ductively inactive, there was a high level of spermatogenesis in their gonads, while there were mainly large quantities of mature sperm in the testes of reproductively active small males and those with a blue claw. These results and those of the current study indicate that the presence of sperm in the male gonads stimulates mating.

CONCLUSIONS

1. The histological picture of *O. limosus* male gonads clearly changes in an annual cycle, although these changes are not uniform. After the autumn mating season, some males had no mature sperm in their gonads while others had large quantities.
2. It is possible that some *O. limosus* males which had an abundance of sperm in their gonads were also reproductively active in spring. Other individuals were active only in autumn.
3. The histological pictures of the gonads of first- and second-form *O. limosus* males did not vary from each other only in June. Starting in July, there is more sperm in the gonads of first-form males than in those of second-form males, thus, the former are ready to fertilize.

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STRESZCZENIE

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

Gonady samców raka *O. limosus* pobierano w comiesięcznych odstępach, od września 1999 do sierpnia 2000 roku. Celem pracy było zaobserwowanie zmian zachodzących w obrazie histologicznym gonad raka *O. limosus* w cyklu rocznym.

Dojrzałe płciowo samce raka pręgowatego (*Orconectes limosus* Raf.) w ciągu roku występują w dwóch formach, różniących się wyglądem odnóży kopulacyjnych (I para odnóży odwłokowych). Forma I umożliwia samcowi przeniesienie nasienia do woreczka nasiennego samicy podczas parzenia się. Natomiast budowa odnóży w formie II uniemożliwia przeniesienie nasienia. Dojrzałe płciowo samce raka *O. limosus* występują w II formie latem, po wiosennej wylince (od maja - czerwca do sierpnia - września).

We wrześniu obraz histologiczny gonad tych dwóch form samców różnił się. Gonada samców występujących w I formie wypełniona była przede wszystkim późnymi stadiami spermatogenezy: spermatydami i plemnikami (fot. 3), natomiast gonada samców występujących w II formie wypełniona była wczesnymi stadiami spermatogenezy: spermatogoniami i spermatocytami (fot. 2). W czerwcu kanaliki gonady I jak i II formy samców wypełnione były jedynie wczesnymi stadiami spermatogenezy (fot. 9).

Na podstawie analizy histologicznej gonad spróbowano odpowiedzieć na pytanie czy rak pręgowaty może parzyć się także wiosną. Wiadomo, że rak *O. limosus*, podobnie jak inne gatunki raków występujących w Polsce parzy się jesienią. Z przeanalizowanego materiału wynika, że u części samców, wiosną, w kanalikach gonady pozostaje jeszcze sporo plemników (fot. 7). Te osobniki mogły przystąpić do wiosennego parzenia się. W drugiej grupie samców gonada zawierała jedynie wczesne stadia spermatogenezy (fot. 6), co zdecydowanie wyklucza możliwość przekazywania nasienia samicy.

CORRESPONDING AUTHOR:
Dr Łucjan Chybowski
Instytut Rybactwa Śródlądowego
Zakład Rybactwa Jeziorowego
ul. Rajska 2
11-500 Giżycko
Tel./Fax: (87) 428 38 81; e-mail: lchybowski@infish.com.pl