STUDIES OF ANNUAL GONADAL DEVELOPMENT AND GONADAL ULTRASTRUCTURE IN SPINY-CHEEK CRAYFISH (*ORCONECTES LIMOSUS*)

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ABSTRACT

The aim of this study was to follow the gonadal development in freshwater crayfish *Orconectes limosus* (Rafinesque) by histological examination of ovaries and testes as well as monthly investigation of the gonadosomatic index over a period of one year. Male gonadosomatic index (I_G) ranged between 0.11 to 0.79% with a minimum in July (0.11%) and maximum in September (0.79%) while in females it ranged between 0.25 to 6.15% with a minimum in July (0.25%) and maximum in April (6.15%). Between April and May, the histological dissection indicated presence of mature oocytes and oocytes undergoing resorption. Egg extrusion took place in mid April. During the summer months, the volume of oocytes gradually decreased. In winter and spring, the volume of oocytes began to increase during preparation for egg extrusion. The size of oocytes in the ovary increased from 0.2 mm at the beginning of the reproductive cycle to 1.8 mm immediately before egg extrusion. Average number of eggs in the ovary was 140.8 ± 51.63 (76-290). The ovary was of brown colour for the majority of the cycle, but white in June and July and orange in August and September. Mating started in October and continued through the winter. A difference was found in the proportions of testis and *vas deferens* in the male reproductive organ during the year. The male gonadosomatic index increased in September during preparation for mating (I_G = 0.79%). During the mating season, the male spermatophores were predominantly filled with spermatozoa and sperm was also noted in the *vas deferens*. Two out of 15 males sampled during the winter were found to be intersex, in which atretic oogonia and oocytes were present at the periphery of testicular tissue and occupied less than 15% of testicular tissue. The evidence of intersex strongly suggested transitional stages of a gradual change of sex, which may be qualified as partial hermaphroditism.

Key-words: Gonadosomatic index, *Orconectes limosus*, ovary, testis, *vas deferens*, intersex.

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ÉTUDE DU CYCLE ANNUEL DE DÉVELOPPEMENT DES GONADES ET DE LEUR ULTRASTRUCTURE CHEZ L'ÉCREVISSE AMÉRICAINE (*ORCONECTES LIMOSUS*)

RÉSUMÉ

Le cycle annuel de reproduction de l'écrevisse américaine (*Orconectes limosus*) a été décrit par l'observation externe des gonades, en enregistrant les changements de l'indice gonadosomatique (I_G), et par l'analyse histologique des organes reproductifs (ovaires et testicules). L'observation et l'analyse histologique ont été realisées mensuellement. Les valeurs suivantes de l'I_G ont été trouvées : 0,11-0,79 % (mâle) et 0,25-6,15 % (femelle). La valeur minimale de l'I_G pour les mâles a été observée au cours du mois de juillet (0,11 %) et la valeur maximale au cours du mois de septembre (0,79 %). Pour les femelles la valeur minimale a été observée en juillet (0,3 %) et la valeur maximale en avril (6,15 %). La formation d'ovocytes mâtures et leur désintégration ont été observées ou cours de la période avril-mai. L'expulsion d'ovocytes mâtures a été observée en avril. Le volume des ovocytes a augmenté principalement en été. Néanmoins les ovocytes ont commencé à grandir au cours de l'hiver et du printemps. Les ovocytes ont varié de 0,2 mm (début de la phase de reproduction) à 1,8 mm (ovocytes mâtures prêts à être expulsés). Le nombre moyen des ovocytes dans les ovaires a été de 140,8 $\pm$ 51,63 (76-290). La couleur des ovaires était principalement brune, mais au cours des mois de juin et juillet, blanche, et au cours des mois d'août et de septembre, orange. La mâturation a commencé en octobre et a continué pendant l'hiver. Des changements morphologiques ont été observés sur les testicules et les *vas deferens* des mâles pendant l'année. L'I_G des mâles a augmenté en septembre quand la préparation pour la mâturation a commencé (I_G = 0,79 %). Pendant la période de mâturation, les spermatophores ont été principalement remplis par des spermatozoïdes, et du sperme a été observé aussi dans les *vas deferens*. Deux individus intersex ont été trouvés pendant l'hiver, avec des oogones et des ovocytes atrétiques à la périphérie des testicules. Les ovocytes occupaient moins de 15 % du tissu testiculaire. L'observation d'intersex suggère la forte probabilité de changement de sexe. On peut appeler ce processus l'hermaphroditisme partiel.

Mots-clés : Indice gonadosomatique, *Orconectes limosus,* ovaires, testicules, *vas deferens*, intersex.

INTRODUCTION

The spiny-cheek crayfish *Orconectes limosus* (Rafinesque) is one of five crayfish species currently in Czech open waters. Noble crayfish *Astacus astacus* (Linnaeus), stone crayfish *Austropotamobius torrentium* (Schrank), narrow-clawed crayfish *Astacus leptodactylus* (Eschscholtz) and signal crayfish *Pacifastacus leniusculus* (Dana) are also present in Czech open water ecosystems (KOZÁK *et al.*, 2002; KOZÁK *et al.*, 2004). *O. limosus* is the most widespread non-native species in the Czech Republic (ĎURIŠ *et al.*, 2006; PETRUSEK *et al*., 2006). This crayfish is of little commercial value, but it is important for open water ecosystems (HOLDICH *et al*., 2002). Although *O. limosus* occurs in the main Czech rivers and is still spreading, its reproductive biology under Central Europe conditions has not been described in detail apart from field study from Switzerland (STUCKI, 2000). Since its first introduction to Europe in 1890, this crayfish has spread naturally or by secondary translocations to over 15 European countries, including the Czech Republic and its neighbours - Poland, Germany and Austria (HENTTONEN *et al.*, 1997; HOLDICH, 2002).

Crayfish have separate male and female sexes (dioecious) and the gonads are located in the dorsal portion of the thorax (MILLER and HARLEY, 1992). In the Cambarid species, mating occurs just after the female has moulted, usually in autumn. The male deposits

sperm near the openings of the female gonoducts (at the base of the 3rd periopods) and uses the two modified pleopods to guide the sperm into the female sperm receptacle. Fertilisation occurs when the eggs, sperm and sticky seminal plasm are released into a protected space created by the female when curling her abdomen beneath. The clutch of several hundreds of eggs remains attached to the female pleopods for several weeks. The young hatch as juvenile instars and remain attached to their mother for several days. The average life span for an *O. limosus* crayfish is two to three years (MILLER and HARLEY, 1992; BAR, 1994).

Several publications have focused on fecundity of this crayfish (STUCKI, 2000; HAMR, 2002; KOZÁK *et al.*, 2006). In Québec populations, mating takes place in September-October and again in March-April (HAMR, 2002). According to BRINK *et al.*, (1988), the mating period for *O. limosus* in the Netherlands occurs in autumn, just as it does for *A. astacus*, *A. leptodactylus* and *P. leniusculus*. However, observations of *O. limosus* behaviour in spring indicate that it also mates at this time. STUCKI (2000) says that in Switzerland mating occurs continuously from late August to April. The process of spermatogenesis and oogenesis in Decapoda has been widely described, especially at the ultrastructure level (MORAES, 1995; YUNLONG *et al.*, 1997, ANDO and MAKIOLA, 1998; HUANG HAXIA *et al.*, 2001). Many papers focus on the structure and production of sperm (LI TAIWU 1995; RICHTER de FORGES *et al.*, 1997; WANG LAN *et al.*, 1999) and the production of spermatophores (WANG LAN *et al.*, 1996; MAC DIARMID and BUTLER, 1999).

Papers on several crayfish species address the hormonal regulation of oogenesis (KULKARNI *et al.*, 1991; SAROJINI *et al.*, 1994; McRAE and MITCHELL, 1995) and the impact of temperature and photoperiod on this process (PORTELANCE and DUBE, 1990; CASTANON-CERVANTES *et al.,* 1995).

The present paper is the result of a field study of more than one year and examines the reproductive biology of spiny-cheek crayfish *Orconectes limosus* with special attention to annual gonadal development and gonadal histology. It aims to: (1) confirm the timing, frequency and duration of the spawning period; (2) monitor changes in the gonado-somatic index of males and females over 1 year; (3) describe the stages of testicular, oocyte and ovarian development; and (4) discuss the possibility that male *O. limosus* can mate twice a year.

MATERIAL AND METHODS

Animals

The entire experiment was carried out at the experimental station of the University of South Bohemia, Research Institute of Fish Culture and Hydrobiology (RIFCH) at Vodňany in the Czech Republic. Mature male and female spiny-cheek crayfish (altogether one hundred crayfish – 72 of them were used for the experiment) were captured in December 2002 from an inundated mining pit at Kličov, Czech Republic, at 3-6 m water depth, and an average water temperature of 4.5 °C (6.0 °C at the bottom). The crayfish were transferred indoors to the research station, where they were kept under controlled conditions in a 100-l aquarium with shelters. The aquarium system was supplied with running water with O_2^- content > 7 mg.l^{-1}, and pH varying between 7-8. The water temperature fluctuated naturally over a range from 3 °C to 25 °C and was monitored throughout the whole period (Figure 1). Water temperature was also measured with a hand thermometer. Crayfish were fed daily with frozen zooplankton and commercial pellets according their weight. Experiments with crayfish were performed in accordance with EU and Czech Republic legal requirements.

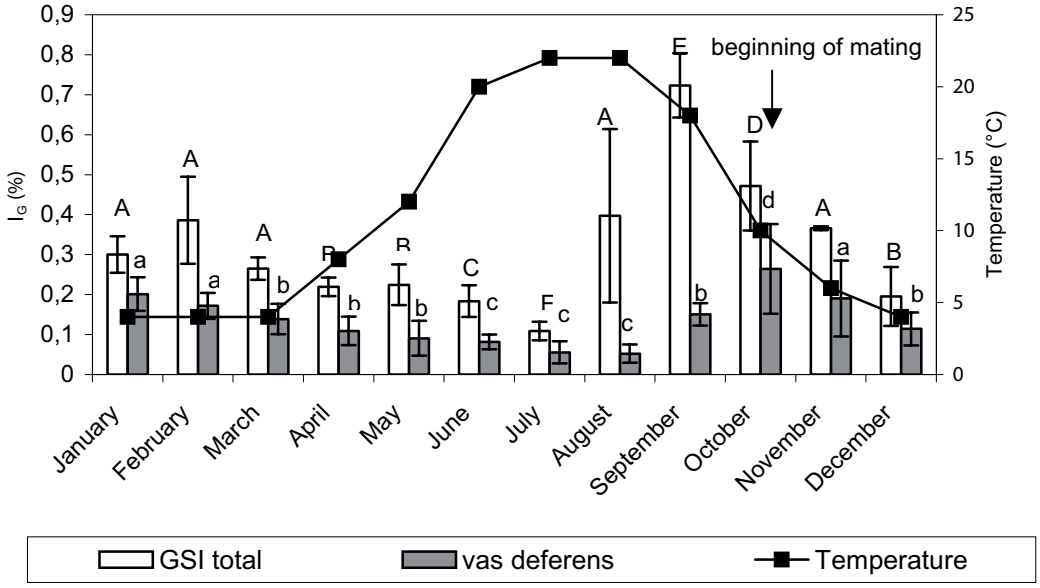

Figure 1

Yearly changes in spiny-cheek crayfish male Ig and water temperature. The total gonadosomatic index, GSI, was marked by capital letter and the vas deferens by lower case. Different letters indicate significant differences between groups at P < 0.05 and groups with common superscript do not differ significantly at P < 0.05. Points and bar represent the mean (± SE) of values obtained from crayfish males and values of temperature during the year.

Figure 1

Évolution annuelle de l´indice gonadosomatique des écrevisses américaines mâles, et de la température de l´eau. La valeur de l'indice gonadosomatique total GSI est indiquée par une lettre majuscule et celle du vas deferens par une lettre minuscule. Le seuil de risque α de première espèce est fixé à P < 0,05 et les valeurs auxquelles sont attribuées des lettres identiques ne diffèrent pas significativement. Toutes les données sont exprimées en moyennes ajustées (± SE, erreur standard) et sont obtenues à partir de mesures faites sur des écrevisses mâles et sur les valeurs de la température de l´eau mesurées pendant l'année.

Laboratory processing

Three pairs of mature crayfish O. limosus, were dissected monthly and their gonads were removed. Total whole body wet weight of each individual was determined to the nearest 0.1 g and the total length and carapace length were measured. Gonads were weighed to the nearest 0.0001 g. Testes and vas deferens were weighed separately. Vas deferens as a proportion of the whole testes wet weight (100 × (ratio of wet weight vas deferens to whole testes wet weight) was calculated. Gonadosomatic index (I_G: 100 × (ratio of wet weight of ovary/testis to whole body wet weight)) was calculated for each crayfish. The number of oocytes at the 2nd stage (previtellogenic) was counted. Size of 10 ova from each ovary was measured using a binocular microscope, to the nearest 0.1 mm [BW1]. Samples of gonads were then fixed in 4% formaldehyde.

Histological processing and staging

Transverse sections from 36 males and 36 females were taken from defined regions along the length of gonad referred to as distal, central, proximal and ductal. Samples were embedded in paraffin wax, sectioned at 5 µm, mounted on slides and stained with Mayer´s

haematoxylin and eosin. Histological preparations were examined with an Olympus BHS microscope, coupled with a 3CCD Sony DXC-9100P colour camera, Sony video monitor and PC-486 computer. Software used was CUE-2 by Galai Inc., Israel. Slides were scored under objective magnification (10x-60x) with oil immersion. Individual objects were recorded and stored using Olympus MicroImage v.4.0 software.

Oocyte measurement and staging

From each slide, the horizontal and vertical diameter of nucleated oocytes was measured under a light microscope with objective micrometer. The size of 40 oocytes from each developmental stage was measured at × 40 magnification. Only oocytes sectioned through the nucleus were measured. Oocytes that were atypical in shape or character were ignored. After measurement, nucleated oocytes were histologically staged into one of the following categories: I: oogonium; II: previtellogenic oocyte (phase I chromatin nucleolus oocyte and phase II, perinucleolar oocyte with strongly basophilic cytoplasm and no or few oil droplets); III: cortical alveolus stage (oocyte with oil droplets in cytoplasm, but not strongly basophilic cytoplasm); IV: early vitellogenic stage (phase I: oocyte containing peripheral yolk granules); V: midvitellogenic stage (phase II containing peripheral yolk granules and central yolk pellets); VI: mature oocytes (initiation of ovulation, indicated by the peripheral movement of the nucleus).

Testes classification and staging

Testes were histologically classified based on the presence or absence, relative abundance and size of spermatogonia, spermatocytes and spermatozoa. The relative amounts of interstitial tissue, size of spermatophores in testicular matrix and presence or absence of spermatozoa in the *vas deferens* were also assessed. The testicular tissue was histologically staged into one of the following categories: I: prespermatogenic stage (spermatophores containing both primary and secondary spermatogonia); II: early spermatogenic stage (spermatophores containing primary and secondary spermatogonia, primary and secondary spermatocytes); III: midspermatogenic stage (spermatophores containing all developmental stages in approximately equal number); IV: late spermatogenic stage (this stage included all stages of development but spermatozoa predominated); V: spent testes (spermatophores contained only spermatogonia and some residual sperm)

Statistical analysis

All data are presented as the mean ± SE. Differences in I_G during the year and between males and female were analysed by one-way ANOVA. Differences between means of males and females were assessed with Tukey's studentized range test. Normality of residuals was assessed with the Shapiro-Wilk test. All data were analysed using the programs STATISTICA for Windows. The significance level considered was $P < 0.05$.

RESULTS

Male gonadosomatic index and proportion of vas deferens

The I_G of males ranged from 0.11 to 0.79% throughout the year. The maximum and statistically the highest I_G (0.79%: ANOVA, $P < 0.05$) occurred in September and the minimum and statistically lowest (0.11%: ANOVA, $P < 0.001$) in July (Figure 1). The I_G began to increase in August (0.40%) and in September (0.79%) during preparation for mating. The increase of the I_G corresponded to a larger size of testis. Remarkable changes in the proportion of *vas deferens* to whole gonad and were observed prior to mating in August (13% of gonad weight) and September (21% of gonad weight) compared to other months (Figure 1). In August and September the testes represented > 80% of the bulk of the gonads. During the rest of the year, *vasa deferentia* represented *c.* 50% of gonads

and ranged between 40-70%. The I_G and size of testes of males gradually decreased from October (0.44%) to July (0.11%) but there were no statistical differences (ANOVA, P = 0.046) over the period from October to July in I_G and testis values.

Female gonadosomatic index and colour of ovary

The female I_G ranged from 0.25-6.15% throughout the year (Figure 2). The smallest I_G occurred from May (0.4%) to July (0.3%). The ovary was white (Figure 3). The average size of oocytes in the 2nd (previtellogenic) developmental stage ranged from 0.2 to 0.3 mm in this time (Figure 3). I_G increased in August (0.5%) and September (0.8%), the ovary became orange, and average size of oocytes at the 2nd (previtellogenic) developmental stage ranged from 0.5-0.7 mm. The differences in I_G from May (0.4%) to September (0.8%) were significant (ANOVA, *P < 0.05*) and statistically smaller than in other months (Figure 2). From October I_G ranged from 3.5% to a maximum of 6.1% in April, just before egg extrusion. The I_G of females in April was significantly higher than in all other months (ANOVA, *P < 0.01*). The ovary was brown and average size of oocytes ranged from 1.4 to 1.8 mm (Figure 3). The number of oocytes at the 2nd (previtellogenic) stage of development correlated positively with carapace length (data not shown). Average number of oocytes at the 2nd (previtellogenic) stage was 140.8 ± 51.63 (76-290) for females of average body length 63.5 ± 7.53 mm (52-82). The weight of gonad and number of oocytes were also positively correlated. Egg extrusion occurred at the end of April.

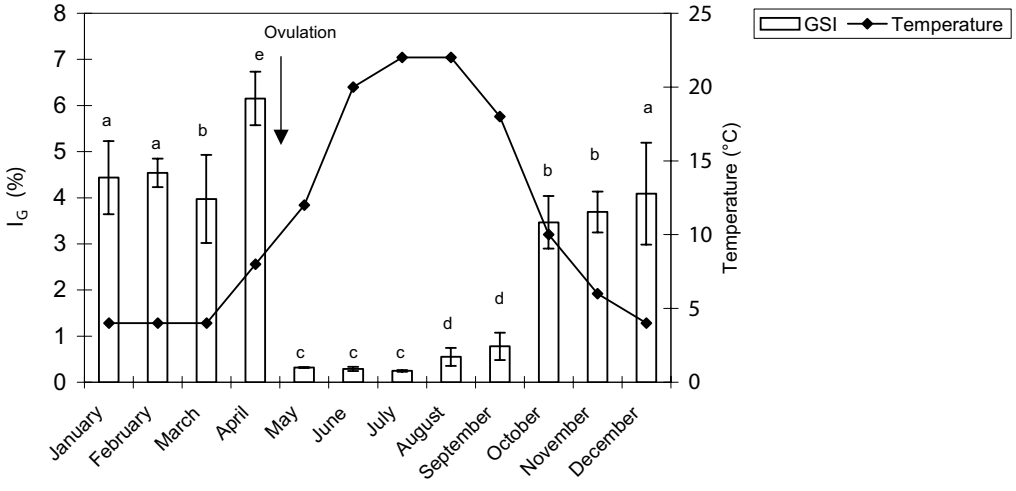

Figure 2

Yearly changes in spiny-cheek crayfish female I_g and water temperature. Different letters indicate significant differences in I_g between groups at P < 0.05 and groups with common superscript do not differ significantly at P < 0.05. Points and bar represent the mean (± SE) of values obtained from crayfish females and values of temperature during the year.

Figure 2

Évolution annuelle de l´indice gonadosomatique des écrevisses américaines femelles, et de la température de l´eau. Les valeurs indiquées avec des lettres identiques ne diffèrent pas significativement (P < 0,05). Toutes les données sont exprimées en moyennes ajustées (± SE, erreur standard) et sont obtenues à partir de mesures faites sur des écrevisses femelles et sur la température de l´eau pendant l'année.

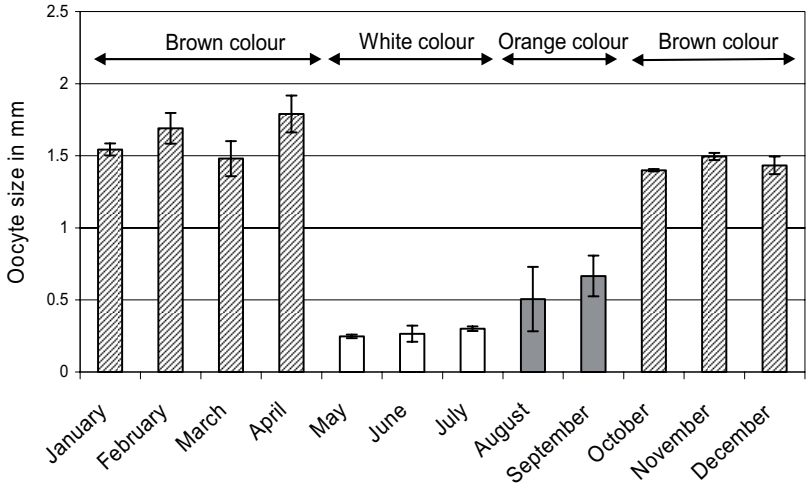

Figure 3

Colour and size of oocytes in ovary of *Orconectes limosus* during the yearly reproductive cycle. Bars represent changes in colour of ovary and sizes of oocytes during the year (see text). Points and bars represent the mean (± SE) of values obtained from crayfish females and values of temperature during the year. The significance level was P < 0.05.

Figure 3

Couleur et taille des ovocytes dans les ovaires d'*Orconectes limosus* pendant une année. Les barres représentent des changements annuels de couleur des ovaires et de taille des ovocytes (voir texte). Les données proviennent d'observations faites sur des écrevisses femelles et sur des mesures de la température de l'eau tout au long de l'année, et sont exprimées en moyennes ajustées (± SE, erreur standard) avec un niveau de significance P < 0,05.

Histological characteristics of annual gonadal development

Female

All 15 females (51-82 mm) collected for histological study from December 2002 to April 2003 had ovaries at the early vitellogenic stage. The oocyte at the beginning of true vitellogenesis (average size: 251.59 ± 13.83 µm) contained deeply eosinophilic protein and yolk globules. Nuclei (average size: 37.59 ± 3.27 µm) were irregular in shape and increased with oocyte size (Figure 4a). Perinucleolar and atretic oocytes generally constituted < 15% of the vitellogenic oocyte. There were no signs of infection or parasites.

Females collected in April had ovaries with mature oocytes. In these cases, the follicular epithelium of vitellogenic oocytes was separated from the oocyte membrane and the oocytes were prepared for ovulation. The perinucleolar oocytes were of irregular shape (average size: 108.87 ± 8.86 µm) with large nuclei (average size: 46.27 ± 1.31 µm) and many nucleoli towards the periphery of the nucleus. Three females collected in May were post-ovulatory. The ovaries of females that had spawned contained mainly previtellogenic oocytes and fragments of primary follicles. Oogonia were observed in the centre of the ovaries near the previtellogenic oocytes. The degenerated oocytes were resorbed and not released.

Histologically, ovaries collected from females between June and September 2003 (n = 12) contained mainly previtellogenic oocytes (average size: 102.65 ± 6.76 µm) and fragments of vitellogenic oocytes. In June, gonads began to develop. Samples of gonads collected from July to August 2003 were not uniform. In July, ovaries were still

filled mainly with previtellogenic oocytes, but in August ovaries were filled with early and mid-vitellogenic oocytes. The percentage of ovaries (in transverse section) occupied by vitellogenic oocytes was as low as 30%. Atretic oocytes constituted < 15% vitellogenic oocytes and *c.* 20-30% from previtellogenic oocytes.

In autumn from September to November 2003, ovaries of *O. limosus* collected from females (n = 12) were similar. The previtellogenic oocytes were located in the central part of the ovaries, while the vitellogenic oocytes in phase II were at the peripheral part of the gonads. Atretic follicles and vacuolisation of oocyte cytoplasm were recorded.

Male

The gonads of 16 males were dissected between August and November 2003. In August, the gonadal tissues of males were in midspermatogenic stage (Figure 4c). Spermatophores were filled with all developmental stages of germ cells. There were small amounts of spermatozoa in the lumen of spermatophores. Lobules were large and separated by interstitial tissue. Spermatophores were filled with spermatogonia (average cell diameter: 14.12 ± 2.8 µm), spermatocytes (average cell diameter 8.64 ± 1.75 µm) and spermatids (average cell diameter 4.545 ± 1.23 µm). Atretic spermatophores occupied < 15% from transverse section.

In September and November 2003, histological analysis showed fully developed spermatozoa in the interior of spermatophore lumina as well as in the anterior and posterior portion of the *vas deferens* (Figure 4b). During this period gonadal tissue was in late spermatogenic stage, but a small portion of the gonad was degenerating.

From December 2002 to February 2003 following the mating season, gonads collected from 9 males were histologically staged as spent testes. Lobules were small and supplied with intersticial tissue and blood capillaries. The lobules of spermatophores were filled mainly with residual sperm and cellular debris. Among these, there were numerous lobules containing only Sertoli cells. Residual spermatozoa and atretic spermatophores were present in small amounts in the *vas deferens.*

Two individuals out of 15 collected at that time were intersexes (Figure 4d). The outermost area (both sides) of dissected gonad was filled with early stages of spermatogenesis and the lobules contained some residual sperm and cellular debris. Atretic oogonia and previtellogenic oocyte were present at the periphery of the testicular tissue and occupied < 15% of it.

In the subsequent months histological pictures were uniform. The gonadal tissues were in prespermatogenic and early spermatogenic stages. Large lobules contained only spermatogonia and spermatocytes. Sperm resorption was completed.

DISCUSSION

European population of *O. limosus* mate in the autumn and the juveniles hatch in May or June, corresponds to the same timing as in native European crayfish species. However, native European crayfish as well as the signal crayfish *P. leniusculus* both spawn and fertilise in the autumn, while in *O. limosus* mating occurs in the autumn and spring but fertilisation appears to occur only in spring when mature eggs are extruded and attached under the female's abdomen (STUCKI, 2000). In the present study, mating occurred during the whole winter season (under laboratory conditions) as in Switzerland (STUCKI, 2000). The testes enlarged in August and September, which corresponded to signal crayfish under similar conditions (KOZÁK *et al.*, 2002). A slight but insignificant increase in male I_G in February was related mainly to enlargement of testes and could relate to a second mating period in March and April referred to by HAMR (2002). However among

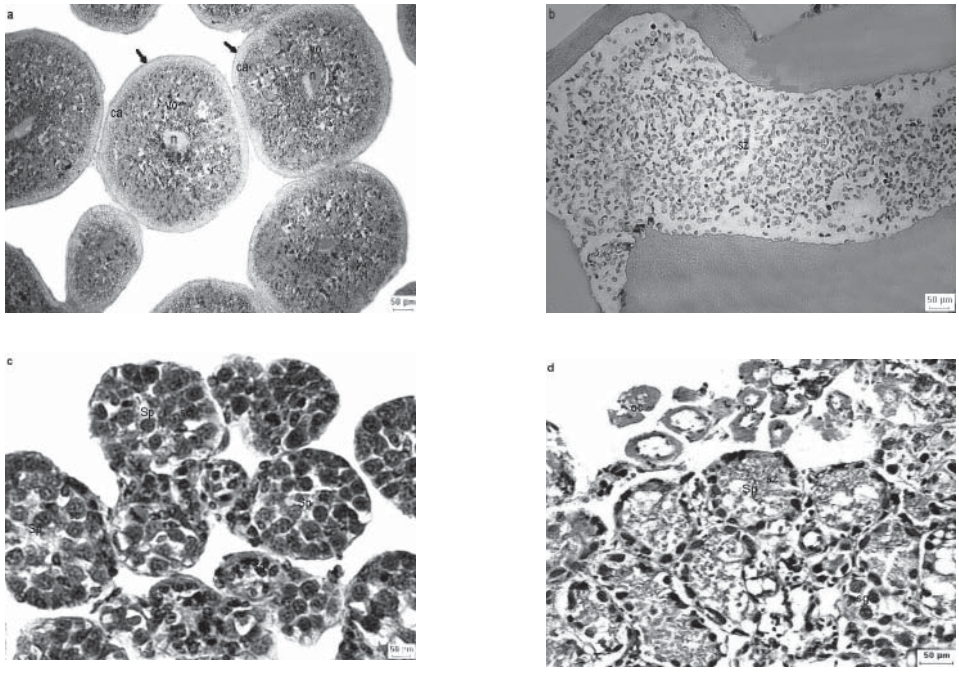

Figure 4

Histological sections of spiny cheek crayfish gonad showing different stages of gonadal differentiation. (a) The oocytes in vitellogenic stage (vo) with nucleus (n) and cortical alveoli (ca) pushed to the periphery of oocytes. The black arrows indicate vitelline envelope (20x). (b) The cross section of the vas deferens with spermatozoa (sz) in lumen (20x). (c) The testicular tissue at mid-spermatogenic stage with spermatophores (Sp) contained spermatocytes (sc) and spermatogonia (sg) (20x). (d) The intersex gonads contained spermatophores (Sp) filled with spermatozoa (sz) spermatogonia (sg) and atretic previtellogenic oocytes (oc) at the periphery of tissue (20x). Bar indicates 50 µm.

Figure 4

L´analyse histologique des gonades d´écrevisses américaines a déterminé des stades différents de mâturité. (a) ovocytes vitellogéniques (vo) avec noyau (n) et cortical alveoli (ca) accumulés en périphérie du cytoplasme. Les flèches noires montrent l'enveloppe vitelline (20x). (b) Coupe en travers de vas deferens avec spermatozoïdes (sz) dans le lumen. (c) Tissu de testicule à mid-spermatogenèse avec spermatophores (Sp) contenant des spermatocytes (sc) et des spermatogonies (sg) (20x). (d) Gonades d'intersex contenant des spermatophores (Sp) pleins de spermatozoïdes (sz), de spermatogonies (sg) et d'ovocytes atrétiques (atrésie préovulatoire) (oc) en périphérie de tissu (20x). Échelle = 50 µm.

others, the histological dissection of male gonads in the present study showed that the lobules of spermatophores were small and supplied with cellular debris and some residual sperm. The sperm resorption process was noted also in the *vas deferens* and new stages of spermatogenesis were not visible. These results strongly indicated that the sperm resorption process was complete or sperm had been transferred to females during the mating. There was no evidence that males of spiny-cheek crayfish can fertilize eggs twice a year. However, the reproductive ability of males in this study was testes only under the influence of controlled laboratory conditions. That could be why the males failed to mate during the spring as noted by HAMR (2002). The influence of habitat was not tested in

the present study because all specimens were sampled from one specific type of habitat. Nevertheless, reproductive ability of spiny-cheek crayfish could be the sum of many factors such as physical size, nutrition, habitat or season (or region ie: latitude).

The histological analysis of male gonads in winter interestingly showed that thirteen had testes and two had ovotestes. The atretic oogonia and oocytes were noted at the periphery of testicular tissue and occupied < 15% of the dissected gonads. Such intersexes strongly suggest transitional stages of a gradual change of sex, which may be qualified as partial hermaphroditism. However such evidence of intersexes in a population of *O. limosus* is not enough to determine which factor or factors would be responsible for selection of this type of sex in this population.

The seasonal pattern and range of I_G enhancement towards the ovulation period (0.25-6.15%) of female *O. limosus* registered in the present results, corresponds to the data noted for several other species of crayfish. HUNER (1993) found I_G-values of *Orconectes rusticus* (Girard) and *Orconectes immunis* (Hagen) females of 4% and 5.9%, respectively. LUCIĆ *et al.* (2006) found I_G-values of *A. astacus* males and females of 0.2-1.53% and 0.47-12.3%, respectively. I_G-values of 0.4-4.0% towards ovulation were found for *P. leniusculus* (KOZÁK *et al.*, 2002) and for *Cherax albidus* (Clark) (McRAE and MITCHELL, 1995; MITCHELL and COLLINS, 1995). By contrast, DANIELS *et al.* (1994) found an initial value of 0.063% for *Procambarus clarkii* (Girard) and a final range of 0.48-1.23% according to temperature and photoperiod. Colour and size of ovary are related to the development stage of oocytes. Seven developmental stages of both the oocytes and the ovary in *Cherax destructor* (Clark) were described by McRAE and MITCHELL (1995). The ovaries of *O. limosus* in the present studies were white in May, June and July. Histological dissection of ovaries at that time showed mainly previtellogenic and some vitellogenic oocytes. In June ovaries began to develop. Then the colour changed from yellow to orange in August and September. From October to spawning in April, the ovary was brown and I_G increased, which is different from astacid species (VOGT, 2002; LUCIĆ *et al.,* 2006).

Average size of *O. limosus* oocytes ranged from 1.4 to 1.8 mm which was comparable with 1.8 mm for *P. clarkii* (ANDO and MAKIOKA, 1998) and 1.2 mm for *P. leniusculus* (KOZÁK *et al.,* 2002). Average number of oocytes at the 2nd (previtellogenic) developmental stage was 140.8 ± 51.63 oocytes (76-290 oocytes) for females 63.5 ± 7.5 mm (52-82 mm) of total lenght. This was low compared to a maximum of 550 pleopodal eggs (21-24 mm of female carapace length) found by STUCKI (2000).

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