Acta Zoologica (Stockholm) **88**: 223–229 (July 2007) doi: 10.1111/j.1463-6395.2007.00269.x

Morphology of the male reproductive system and spermatophore formation in the freshwater 'red claw' crayfish *Cherax quadricarinatus* (Von Martens, 1898) (Decapoda, Parastacidae)

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Accepted for publication:
22 December 2006

Abstract

López Greco, L.S., Vazquez, F. and Rodríguez, E.M. 2007. Sperm production and spermatophore formation in the freshwater 'red claw' crayfish *Cherax quadricarinatus* (Von Martens 1898) (Decapoda, Parastacidae). — *Acta Zoologica* (Stockholm) **88**: 223–229.

The morphology of the male reproductive system was studied in *Cherax quadricarinatus*. The testes and vasa deferentia were dissected, fixed, cut and stained. Testes appear as two parallel and opalescent strands; they present many testicular lobes, each lobe containing cells in the same stage of the spermatogenic cycle. A vas deferens arises from the external side of each testis and three parts were clearly distinguished: proximal vas deferens (PVD), middle vas deferens (MVD) and distal vas deferens (DVD). The PVD is opalescent and highly convoluted, the MVD is pale white in colour and convoluted, but wider in diameter than the PVD, while the DVD shows the widest diameter, is straight and is white in colour. A single-layered epithelium is recognized in the vas deferens; with cylindrical cells in the PVD and cuboid cells in the MVD and DVD. The formation of the spermatophore starts at the PVD, while the secondary layer of the spermatophore seems to be added at the MVD. At the DVD, the highly coiled spermatophore is surrounded by the periodic acid Schiff-positive sticky components of the secondary layer. Many aspects of spermatophore formation in *C. quadricarinatus* differ from those of other Astacida. The applied aspects of this study for aquaculture purposes are discussed.

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Introduction

Freshwater crayfish of the infraorder Astacida are represented by three families, the Cambaridae, Astacidae and Parastacidae, whose phylogenetic relationships have been extensively studied (see Scholtz 2002 for revision). Only the last family is distributed in the southern hemisphere, including the 10 species of South American Parastacidae (Rudolph and Almeida 2000; Retamal and Rudolph 2005) and the conspicuous species distributed in Australia, Tasmania and New Guinea (Scholtz 2002).

Cherax quadricarinatus (Parastacidae) is a large freshwater crayfish; a native of north-west Queensland and the Northern Territory of Australia, it is intensively cultured for economic purposes in Australia and many other countries in southern Asia, North and South America and Africa (Lawrence and Jones 2002; Edgerton 2005).

Many biological aspects related to the culture of *C. quadricarinatus* have been studied, including reproduction, growth and nutrition (e.g. Jones 1995a,b, 1997; Khalaila *et al.* 1999; Lawrence and Jones 2002; García Guerrero *et al.* 2003a,b; Karplus and Barki 2004; Naranjo-Páramo *et al.*

2004; Thompson *et al*. 2005; Vazquez and López Greco 2006), to optimize the culture of this species. However, knowledge of the morphology of the male reproductive system is still scarce in many aspects as it is in other families of the Astacida, a fact that contrasts with the extensive research on male morphology conducted in commercially important species of shrimps (Chow *et al*. 1991; Díaz *et al*. 2001; Akarasanon *et al*. 2004), lobsters (Radha and Subramonian 1985; Kooda-Cisco and Talbot 1986) and crabs (Sainte Marie and Sainte Marie 1999; Benhalima and Moriyasu 2000). Studies on male reproductive morphology and spermatophore formation constitute the previous knowledge used to understand the process of sexual maturation in the species, the cyclic changes throughout the year, and the effect of captivity on sperm production; they are a prerequisite to assay spermatophore preservation for aquaculture purposes and brood-stock management (Jerry 2001; Akarasanon *et al*. 2004). In fact, it has been proven that sperm production and quality and spermatophore formation are important variables in captive male reproduction (Leung-Trujillo and Lawrence 1987; Díaz *et al*. 2001).

The objective of this study is to characterize the morphology of the reproductive system of male *C. quadricarinatus*, including the testes, vasa deferentia and spermatophore formation, from both macroscopic and microscopic points of view, to improve the knowledge about spermatophore formation and extrusion. These aspects are compared with those of other freshwater crayfish species.

Materials and Methods

Eight adult male *C. quadricarinatus* (mean weight: 139.78 ± 6.07 g, maximum carapace length: 79.60 ± 2.46 mm) were purchased from a local dealer (BECRUX farm, Buenos Aires, Argentina). Once in the laboratory, the animals were killed by cold treatment (5 min at –20 °C). After removal of the carapace, both the testes and vasa deferentia were dissected and fixed in Bouin's solution for 4 h at 20 °C. These organs were then sequentially passed through 90% ethanol for 20 min, 96% ethanol for 20 min, 96% ethanol–buthylic alcohol (1 : 1 v/v) for 30 min and buthylic alcohol for 30 min, and then embedded in paraffin. Histological sections, 5–6 μm thick, were cut with a Carl Zeiss ultramicrotome and were stained with haematoxylin & eosin and periodic acid Schiff (PAS).

To determine the morphology of spermatophores transferred to the females, four mating groups (comprising four females with one male per group) were maintained in glass aquaria containing 20 L dechlorinated tap water (pH 7.4, hardness 80 mg/L, as $CaCO_3$ equivalents), under continuous aeration, at a temperature of 27–28 °C with a 14 : 10 h (light : darkness) photoperiod, and they were fed daily on *Elodea* sp. (a freshwater weed widely used as aquarium vegetation) and commercial Tetradiskus granules. Females were checked daily to determine the presence of spermatophores on the sternum. If present, the form and consistency of the spermatophores were recorded.

Results

The testes are paired structures along their complete length, with a total length ranging from 40 to 50 mm. They are circular in transverse section, with a diameter ranging from 2 to 3 mm and are strongly joined to each other at their middle portion by connective tissue. The colour of *C. quadricarinatus* testes varied from opalescent to pale yellow (Fig. 1A,B).

The internal architecture of the testes consists of several testicular lobes and collecting ducts (Fig. 2). The testicular tunica comprises a monolayer of flat to cubic cells. Each testicular lobe always contains a single stage of spermatogenesis, regardless of the stages occurring in the adjacent lobes. Therefore, no regionalization could be detected in the spatial pattern of spermatogenesis within the testes (Fig. 2B). In these mature males (these males were adult according to their size and weight, Jones 1995a), most of the lobes contain spermatids or spermatozoa as well as many non-germinal, Sertoli cells (Hinsch 1993a) found at the base of each testicular lobe. In *C. quadricarinatus*, Sertoli cells are round in shape in the lobes containing spermatogonia or spermatocytes I or II but have a flatter shape, as well as being pycnotic in appearance, at the base of lobes containing spermatids or spermatozoa.

Vasa deferentia are laterally connected with both testes, defining two anterior testicular lobes that extend up to the level of the gastric mill, and two posterior lobes dipping 5–10 mm inside the pleon. Each vas deferens presents three macroscopically distinguishable portions: the proximal (PVD), middle (MVD) and distal (DVD) vasa deferentia. The PVD exhibits a pale, white colour, similar to that of testes, a highly convoluted aspect (Fig. 1B,C), and has a diameter ranging between 0.3 and 0.6 mm. Histologically, the PVD is surrounded by a single-layered epithelium, consisting of high cylindrical cells. Single layers of both circular muscle cells and connective tissue occur internal to the epithelium. A PAS-positive material is observed surrounding the mass of spermatozoa, in the lumen of the PVD, probably conforming to the primary layer of the spermatophore (Fig. 3A,B).

The MVD shows an intense white colour and a highly folded appearance, with its diameter ranging between 1 and 2 mm (Fig. 1B,C). The epithelium surrounding this portion ranges from cubic to flat. The secondary layer of the spermatophore becomes evident (Fig. 3C,D).

The DVD is a straight, intensely white and relatively wide (6 mm) structure, although it becomes narrower from its connection with the fifth periopod coxa, up to reaching to the distal end of each elongate appendix masculine (Fig. 1A–C). A thick muscular layer, five to six times larger than in the MVD epithelium, is observed at this portion (Fig. 3E). At the DVD level, the primary layer of the spermatophore was

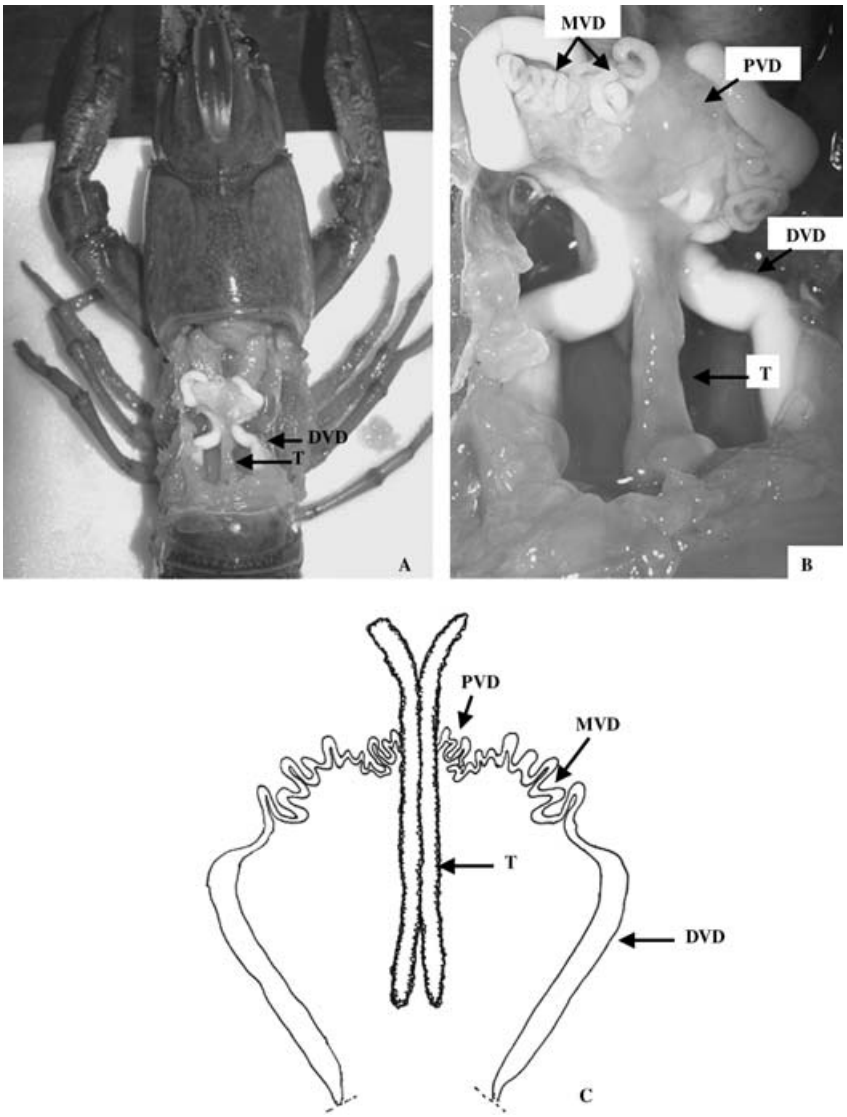

Fig. 1—**A**. General view of the male reproductive system in the red claw *Cherax quadricarinatus.* —**B**. Detail of the reproductive system. —**C**. Schematic diagram of the male reproductive system: T, testes; PVD, proximal vas deferens; MDV, middle vas deferens; DVD, distal vas deferens.

completely surrounded by the acellular material that represents the major component of the secondary layer (Fig. 3F).

At the time of extrusion, the spermatophore of *C. quadricarinatus* presents a soft appearance. Within 24 h after extrusion, it becomes harder and increases its size two to three times (approximately 10 mm long, 5 mm wide and 5 mm high). The spermatophore retains its hardened aspect for up to 3 days after extrusion, becoming clearly dehiscent at the fourth day after extrusion.

Discussion

The macroscopic morphology of the testes of *C. quadricarinatus* showed a similar pattern to that described in *C. albidus* (Talbot and Beach 1989) and presented slight differences with the South American Parastacidae, *Parastacus brasiliensis* (De Almeida and Buckup 1997) and *P. varicosus* (Rudolph *et al.* 2001). In *P. brasilensis*, testes are paired structures but are laterally connected by connective tissue, so that the gonads appear to be an unpaired structure (De Almeida and Buckup 1997), while in *P. varicosus*, testes are two clearly separated parallel structures without any contact between them (Rudolph *et al.* 2001). The pattern observed in *P. varicosus* is similar to that observed in early juveniles of *C. quadricarinatus* (Vazquez and López Greco 2006).

All studied parastacid species have paired testes, which contrast with the pattern described in Astacidae and Cambaridae, which typically present two anterior lobes and only one posterior lobe (Dudenhausen and Talbot 1983; De Almeida and Buckup 1997; Vogt 2002).

The microscopic architecture of a *C. quadricarinatus* testis consists of several testicular lobes (also referred to as

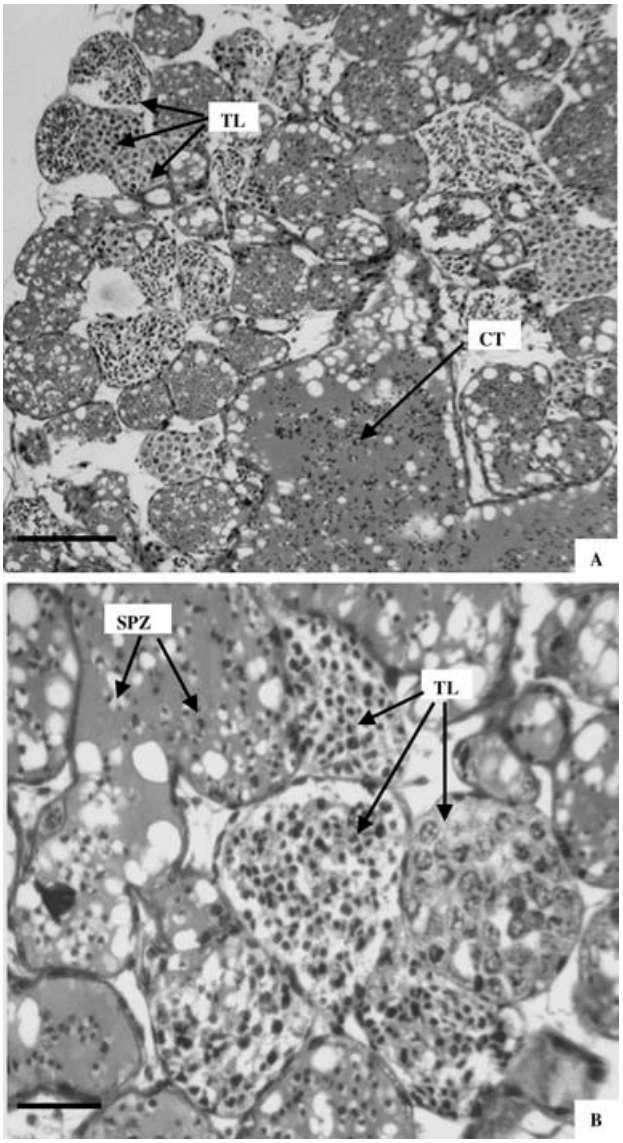

Fig. 2—Histological section of the testes of *Cherex quadricarinatus* (Parastacidae). —**A**. General morphology (bar 200 μm). —**B**. Detail of testicular lobes (bar 100 μm). CT, collecting tubule; SPZ, spermatozoa; TL, testicular lobes.

seminiferous tubules or testicular acini in the literature) and a central collecting duct, which is the general pattern described for decapods (Krol *et al*. 1992; Taketomi *et al*. 1996).

Each testicular lobe contains cells undergoing a single stage of spermatogenesis, regardless of the different stages occurring in the adjacent lobes. This random pattern seen in the *C. quadricarinatus* testis is termed 'unrestricted lobular', according to the classification proposed by Grier (1993). In contrast, the testes of *Procambarus clarkii* (Cambaridae) only present cells in one or two stages of spermatogenesis in any transverse section, which is termed 'synchronic' (Krol *et al*. 1992). Following this latter criterion, the testis of *C. quadricarinatus* would fall in the category of 'asynchronic'. According to the photographs shown in De Almeida and Buckup (1997), Rudolph *et al*. (2001) and Rudolph (2002), the testicular pattern of the Parastacidae *P. brasiliensis*, *P. varicosus* and *Samastacus spinifrons* would be asynchronic too.

The presence of non-germinal cells, referred to as accessory, sustentacular, interstitial, nurse, nutritive or Sertoli-like cells, seems to be widely reported in the Crustacea (see Hinsch 1993c for revision). According to Hinsch (1993b,c), Sertoli cells are strongly involved in the spermatogenesis and spermiogenesis processes in the Decapoda. Changes in the cytoarchitecture of Sertoli cells, related to the synthetic activity of the Golgi apparatus and rough endoplasmic reticulum, have been described for the anomuran crab *Coenobita* and *Procambarus* (Hinsch 1993c). In *Procambarus paeninsulanus*, these cells could have a function of producing and secreting the mucopolysaccharide capsule that surrounds each spermatozoon (Hinsch 1993b,c). This capsule was absent in *C. quadricarinatus* (present study), *C. albidus* and *C. tenuimanus* (Beach and Talbot 1987), is not reported in any previous study of other Parastacidae, but is characteristic of Astacidae and Cambaridae (Dudenhausen and Talbot 1983; Beach and Talbot 1987; Vogt 2002).

In *Procambarus* and *Coenobita*, mature spermatozoa are released into the testicular lumen (collecting ducts), surrounded by a flocculent material that is secreted by the Sertoli cells (Hinsch 1993c). In *C. quadricarinatus*, large quantities of PAS-positive substances were detected in the testes within lobes containing only spermatozoa (Fig. 2A,B). The possible origin of this acellular material is suspected to be the Sertoli cells.

The discrete sections within the vas deferens identified in *C. quadricarinatus* show characteristic form, diameter, colour and histological differentiation in accordance with the results reported for *C. albidus* (Talbot and Beach (1989) and *C. destructor* (Jerry 2001). These characteristics contrast with the short, straight (without coiling) and macroscopically undifferentiated form of the vasa deferentia observed in *P. varicosus* (Rudolph *et al*. 2001) and *P. brasiliensis* (De Almeida and Buckup 1997). In *S. spinifrons*, some degree of proximal to distal regionalization of the vas deferens was observed (Rudolph 2002).

From a microscopic point of view, the vas deferens of *C. quadricarinatus* resembled the one described for *C. albidus*. In particular, the DVD epithelium showed a very disorganized aspect, probably because the apocrine secretion forms the second layer of the spermatophore, as reported in *C. albidus* (Talbot and Beach 1989; Hinsch 1991).

Cherax quadricarinatus presents a pattern of spermatophore formation similar to that of *C. albidus*, with primary and secondary spermatophore layers being added in successive

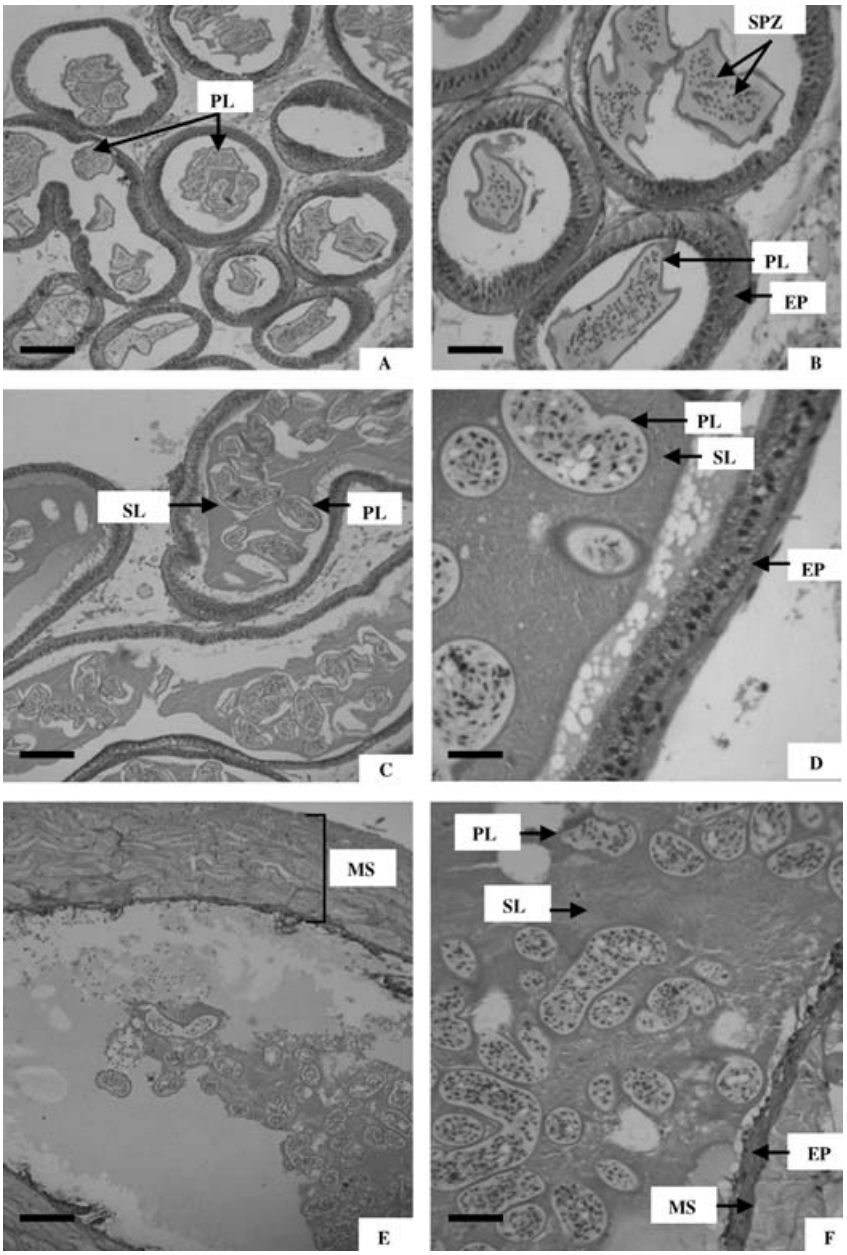

Fig. 3—Transverse section of the vas deferens and spermatophore formation in *Cherex quadricarinatus* (Parastacidae). —**A**. General view of the anterior vas deferens in transverse section (bar 300 μm). —**B**. Detail of proximal vas deferens and the formation of the primary layer of the spermatophore (bar 170 μm). —**C**. General view of the middle vas deferens in transverse section (bar 680 μm). —**D**. Detail of middle vas deferens and the formation of the secondary layer of the spermatophore (bar 300 μm). —**E**. General view of the muscularized distal vas deferens (bar 750 μm). —**F**. Detail of distal vas deferens (bar 350 μm). EP, epithelium; MS, muscular sheath; PL, primary layer of the spermatophore; SL, secondary layer of the spermatophore; SPZ, spermatozoa.

parts of the vas deferens. This pattern contrasts with the structure of the spermatophore of *Pacifastacus leniusculus*, which consists of a central sperm mass and a three-layered spermatophore wall (Dudenhausen and Talbot 1983). The structure and shape of astacid spermatophores clearly contrast with those reported in the Brachyura and Anomura (Hinsch 1991; Krol *et al*. 1992). Within the South American Parastacidae, although not studied in detail, a two-layered spermatophore was not observed in the distal end of the sperm duct of *S. spinifrons* (Rudolph 2002). In this species, the sperm mass was embedded in a dense matrix, the homology of which with the primary and/or secondary layers of the *Cherax* spermatophore has yet to be determined.

The primary and secondary layers of the spermatophore of *C. quadricarinatus* are as highly PAS-positive as the granular component of the secondary spermatophore layer of *Panulirus homarus*, which is reported as chondritin sulphate, a material that is highly adhesive and PAS-positive (Radha and Subramoniam 1985). The hardening process of the spermatophore in *Cherax* occurs in the water, contrasting with the internal hardening verified in the peneid shrimp *Penaeus setiferus* and *P. vannamei* (Chow *et al*. 1991). The chemical transformation

taking place in the secondary layer of the spermatophore, produces a change from a soft to a hard consistency and could be related to processes such as phenolic tanning or calcification, as proposed by Hinsch (1991), or to hydration (Beninger *et al*. 1993). Our laboratory observations would indicate that the hydration process occurring in the first hours after extrusion is one of the initial steps during the attachment of the spermatophore to the sternal surface of the female. This hardened spermatophore, once attached to the sternal surface of a female, had the tubular shape characteristic of Macrura. According to Jones (1995a), females of *C. quadricarinatus* would break the spermatophore using the dactylus of the fifth periopods. For other Parastacidae, no information is available about the mechanisms involved in spermatophore rupture and/or dehiscence or the chemical changes involved in its hardening.

The examination of the testes and the analysis of spermatophore formation within the vas deferens of *C. quadricarinatus* allows the male reproductive potential within the reproductive stock to be evaluated in culture throughout the year, in relation to age and/or different diets, hormone treatments and photoperiod and temperature regimens. In addition, it is a useful tool for the study of electrical stimulation of spermatophore extrusion (e.g. Kooda-Cisco and Talbot 1983; Jerry 2001) and of the effects of such stimulation on quantity and quality of sperm production, spermatophore formation and the recovery of the vas deferens tissue after the electrical injury.

Acknowledgements

This research was funded by Antorchas Foundation (project 4248–138), CONICET (res. number 1106/2004), ANPCYT (PICT 953) and UBACYT 2004–07 (projects X143 and X312).

References

Akarasanon, K., Damrongphol, P. and Poolsanguan, W. 2004. Long-term cryopreservation of spermatophore in the giant freshwater prawn *Macrobrachium rosembergii* (de Man). – *Aquaculture Research* **35**: 1415–1420.

Beach, D. and Talbot, P. 1987. Ultrastructural comparison of sperm from the crayfishes *Cherax tenuimanus* and *Cherax albidus*. – *Journal of Crustacean Biology* **7**: 205–218.

Benhalima, K. and Moriyasu, M. 2000. Structure and function of the posterior *vas deferens* of the snow crab, *Chionoecetes opilio* (Brachyura, Majidae). – *Invertebrate Reproduction and Development* **3**: 11–23.

Beninger, P., Lanteigne, C. and Elner, R. 1993. Reproductive processes revealed by spermatophore dehiscence experiments and by histology, ultrastructure, and histochemistry of the female reproductive system in the snow crab *Chionoectes opilio* (O. Fabricius). – *Journal of Crustacean Biology* **13**: 1–16.

Chow, S., Dougherty, M., Dougherty, W. and Sandifer, P. 1991. Spermatophore formation in the white shrimps *Penaeus setiferus* and *P. vannamei*. – *Journal of Crustacean Biology* **11**: 201–216.

De Almeida, A. and Buckup, L. 1997. Aspectos anatómicos e funcionais do aparelho reprodutor de *Parastacus brasiliensis* (von Martens) (Crustacea, Decapoda, Parastacidae). – *Revista Brasilera de Zoologia* **14**: 497–509.

Díaz, A., Fernández Jiménez, A., Harán, N. and Fenucci, J. 2001. Reproductive performance of male argentine red shrimp *Pleoticus muelleri* Bate (Decapoda, Penaeoidea) in culture conditions. – *Journal of the World Aquaculture Society* **32**: 236–242.

Dudenhausen, E. and Talbot, P. 1983. An ultrastructural comparison of soft and hardened spermatophores from the crayfish *Pacifastacus leniusculus* Dana. – *Canadian Journal of Zoology* **61**: 182–194.

Edgerton, B. 2005. Freshwater crayfish production for poverty alleviation. – *World Aquaculture* **36**: 48–64.

García Guerrero, M., Racotta, I. and Villarreal, H. 2003a. Variation in lipid, protein, and carbohydrate content during the embryonic development of the crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). – *Journal of the Crustacen Biology* **23**: 1–6.

García Guerrero, M., Villarreal, H. and Racotta, I. 2003b. Effect of temperature on lipids, proteins, and carbohydrates levels during development from egg extrusion to juvenile stage of *Cherax quadricarinatus* (Decapoda: Parastacidae). – *Comparative Biochemistry and Physiology A* **135**: 147–154.

Grier, H. 1993. Comparative organization of Sertoli cells including the Sertoli cell barrier. In Rusell, L. and Griswold, M. (Eds): *The Sertoli Cells*, pp. 703–739. Cache River Press, Clearwater FL.

Hinsch, G. 1991. Structure and chemical content of the spermatophores and seminal fluid of reptantian decapods. In Bauer, R. and Martin, J. (Eds): *Crustacean Sexual Biology*, pp. 290–307. – Columbia University Press, New York.

Hinsch, G. 1993a. Ultrastructure of spermatogonia, spermatocytes and Sertoli cells in the testis of the crayfish, *Procambarus paeninsulanus*. – *Tissue and Cell* **25**: 737–742.

Hinsch, G. 1993b. The role of Sertoli cells in spermatid maturation in the testis of the crayfish *Procambarus paeninsulanus*. – *Tissue and Cell* **25**: 743–749.

Hinsch, G. 1993c. Comparative organization and cytology of Sertoli cells in invertebrates. In Rusell, L. and Griswold, M. (Eds): *The Sertoli Cells*, pp. 660–683. Cache River Press, Clearwater FL.

Jerry, D. 2001. Electrical stimulation of spermatophore extrusion in the freshwater yabby (*Cherax destructor*). – *Aquaculture* **200**: 317–322.

Jones, C. 1995a. Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae). I. Development of hatchery and nursery procedures. – *Aquaculture* **138**: 221–238.

Jones, C. 1995b. Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae). II. Juvenile nutrition and habitat. – *Aquaculture* **138**: 239–245.

Jones, C. 1997. The biology and aquaculture potential of the tropical freshwater crayfish *Cherax quadricarinatus.* Department of Primary Industries Queensland, Queensland Government, Information Series Q 190028.

Karplus, I. and Barki, A. 2004. Social control of growth in the redclaw crayfish, *Cherax quadricarinatus:* testing the sensory modalities involved. – *Aquaculture* **242**: 321–333.

Khalaila, I., Weil, S. and Sagi, A. 1999. Endocrine balance between male and female components of the reproductive system in intersex *Cherax quadricarinatus* (Decapoda: Parastacidae). – *Journal of Experimental Zoology* **283**: 286–296.

Kooda-Cisco, M. and Talbot, P. 1983. A technique for electrically stimulating extrusion of spermatophores from the lobster, *Homarus americanus*. – *Aquaculture* **30**: 221–227.

Kooda-Cisco, M. and Talbot, P. 1986. Ultrastructure and role of the lobster vas deferens in spermatophore formation: the proximal segment. – *Journal of Morphology* **188**: 91–103.

Krol, R., Hawkins, W. and Overstreet, R. 1992. Reproductive components. In Harrison, F. W. and Humes, A. G. (Eds): *Microscopic Anatomy of the Invertebrates*, Vol. 10, pp. 295–343. Willey-Liss, New York.

Lawrence, C. and Jones, C. 2002. *Cherax*. In Holdich, D. M. (Ed.): *Biology of Freshwater Crayfish*, pp. 635–669. Blackwell Science, Cambridge.

Leung-Trujillo, J. and Lawrence, A. 1987. Observations on the decline in sperm quality of *Penaeus setiferus* under laboratory conditions. – *Aquaculture* **65**: 363–370.

Naranjo-Páramo, J., Hernández-Llamas, A. and Villarreal, H. 2004. Effect of stocking density on growth, survival and yield of juvenile redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) in gravel-lined commercial nursery ponds. – *Aquaculture* **242**: 197–206.

Radha, T. and Subramonian, T. 1985. Origin and nature of spermatophoric mass of the spiny lobster *Panulirus homarus*. – *Marine Biology* **86**: 13–19.

Retamal, V. and Rudolph, E. 2005. *Ocurrencia de hermafroditismo protándrico en una población del género Virilastacus Hobbs, 1991 (Crustacea: Decapoda: Parastacidae)*. – XI Congreso Latinoamericano de Ciencias del Mar, Viña del Mar, Chile, 16–20 May, 2005.

Rudolph, E. 2002. New records of intersexuality in the freshwater crayfish *Samastacus spinifrons* (Decapoda, Parastacidae). – *Journal of Crustacean Biology* **22**: 377–389.

Rudolph, E. and Almeida, A. 2000. On the sexuality of South American Parastacidae (Crustacea, Decapoda). – *Invertebrate Reproduction and Development* **37**: 249–257.

Rudolph, E., Verdi, A. and Tapia, J. 2001. Intersexuality in the burrowing crayfish *Parastacus varicosus* Faxon, 1898 (Decapoda, Parastacidae). – *Crustaceana* **74**: 27–37.

Sainte Marie, G. and Sainte Marie, B. 1999. Reproductive products in the adult snow crab *(Chionoectes opilio)*. I Observations on spermiogenesis and spermatophore formation in the *vas deferens*. – *Canadian Journal of Zoology* **77**: 440–450.

Scholtz, G. 2002. Phylogeny and evolution. In Holdich, D. M. (Ed.): *Biology of Freshwater Crayfish*, pp. 30–52. Blackwell Science, Cambridge.

Taketomi, Y., Nishikawa, S. and Koga, S. 1996. Testis and androgenic gland during development of external sexual characteristics of the crayfish *Procambarus clarkii*. – *Journal of Crustacean Biology* **16**: 24–34.

Talbot, P. and Beach, D. 1989. Role of the vas deferens in the formation of the spermatophore of the crayfish *(Cherax)*. – *Journal of Crustacean Biology* **9**: 9–24.

Thompson, K., Muzinic, L., Engler, L. and Webster, C. 2005. Evaluation of practical diets containing different protein levels, with or without fish meal, for juvenile Australian red claw crayfish (*Cherax quadricarinatus*). – *Aquaculture* **244**: 241–249.

Vazquez, F. J. and López Greco, L. S. 2006. Sexual differentiation in the freshwater crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae). – *Revista de Biología Tropical* (in press).

Vogt, G. 2002. Functional anatomy. In Holdich, D. M. (Ed.): *Biology of Freshwater Crayfish*, pp. 53–151. Blackwell Science, Cambridge.