Intersex females in the red claw crayfish, *Cherax quadricarinatus* (Decapoda: Parastacidae)

Fernanda J. Vazquez* & Laura S. López Greco**
Lab. Comparative Animal Physiology, Dept. Biodiversity and Experimental Biology, FCEyN, University of Buenos Aires, Cdad. Univ. C1428EHA, Buenos Aires, Argentina; *fvazquez@bg.fcen.uba.ar, **laura@bg.fcen.uba.ar

Received 14-X-2005. Corrected 23-VI-2006. Accepted 16-III-2007.

Abstract: *Cherax quadricarinatus* is a large freshwater crayfish species (Parastacidae) native of north-west Queensland and the Northern Territory of Australia. The species typically exhibits a gonochoristic sexual system, although in cultured populations various types of intersex individuals have been described as functional males. In the present study, the macroscopic morphology and the gonadal histology of one type of intersex are described and discussed. All intersexes having both pairs of genital openings (female and male openings) and lacking both *appendix masculinae* and red patches were functional females with normal ovaries and oviducts. From a histological point of view, they did not differ from normal females having previtellogenic and/or vitellogenic ovaries according to size. Rev. Biol. Trop. 55 (Suppl. 1): 25-32. Epub 2007 June, 29.

Key words: *Cherax quadricarinatus*, intersexuality, intersex females, sexual characters, gonad morphology.

Cherax quadricarinatus (von Martens, 1868) is a large freshwater crayfish native of north-west Queensland and the Northern Territory of Australia which 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). Although many biological aspects related to the culture of *C. quadricarinatus* have been studied, including reproduction, growth and nutrition (Jones 1995a,b, 1997, Khalaila *et al.* 1999, Lawrence and Jones 2002, García Guerrero *et al.* 2003a,b, Karplus and Barki 2004, Shechter *et al.* 2005, among others), the sexual pattern of this species and its hormonal regulation is not fully understood.

C. quadricarinatus is considered a gonochoristic species with a bilaterally symmetrical reproductive system. In females, it consists of a pair of ovaries, oviducts and genital openings at the base of the third pereiopods, while in males a pair of testes, vasa deferentia and genital openings at the base of the fifth pereiopods are observed (Sagi *et al.* 1996). Males also present *appendix masculinae* at the base of the fifth pereiopods. An important male secondary sexual character is the soft red patch which is found on the outer surface of the propodus of the male claw giving to this species the common name of "red claw" crayfish (Thorn and Fielder 1991, Karplus *et al.* 2003). In this species, a variable proportion of several types of intersex animals can be found in which both male and female genital openings occur in different combinations (Thorn and Fielder 1991, Medley and Rouse 1993, Sagi *et al.* 1996). Morphological, biochemical and endocrinological studies of intersexes have demonstrated that these individuals are functionally males with previtellogenic ovaries (e.g., Sagi *et al.* 1996, Khalaila and Sagi 1997, Khalaila *et al.* 1999, Sagi *et al.* 2002, Shechter *et al.* 2005).

Intersexuality, i.e. the presence of male and female primary and/or secondary sexual characters within an individual, is widespread, but relatively uncommon throughout all gonochoristic crustaceans. It has been reported in species which exhibit protogyny or protandry (true hermaphroditic species) and in gonochoristic species (Sagi *et al.* 1996, Bauer and Holt, 1998, Bauer and Newman, 2004, Turra 2004). Among gonochoristic species, intersexuality is observed within Australian Parastacidae of the genus *Engaeus* Erichson, 1846, *Engaewa* Riek, 1967, and *Cherax* Erichson, 1846 (Horwitz 1988, Medley and Rouse 1993, Sagi *et al.* 1996) and in South American Parastacidae of the genus *Parastacus* Huxley, 1879, *Samastacus* (Philippi, 1882) and *Virilastacus* Hobbs, 1991 (Almeida and Buckup 2000, Rudolph and Almeida 2000, Retamal and Rudolph 2005).

According to some investigations done in our laboratory in which we observed some individuals having both pairs of genital openings being ovigerous "females" and hatching normal juveniles, we decided to carry out a macroscopic and histological analysis of the reproductive system of these intersex animals to achieve a better understanding of intersexuality in *C. quadricarinatus.*

MATERIALS AND METHODS

Female (n=33) and intersex (n=41) *C. quadricarinatus* purchased from a local dealer (San Mateo Farm, Buenos Aires, Argentina) selected for this study ranged between 19.6 and 47.3 mm carapace length and between 1.9 and 24 g body weight. All selected females presented their genital openings at the basis of the third pereiopods, while intersex individuals presented both genital openings at the base of the third and fifth pereipods, respectively (Fig. 1). None of them had *appendix masculinae* nor red patches. Crayfish were cold-anaesthetized at -20 °C during 15 min. The carapace was removed and the gonads were inspected recording their size, form and colour. Subsequently, they were quickly dissected and fixed in Bouin's solution for 4h at room temperature. Gonads 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 embedded in paraffin. Sections (5-6 µm thick) were stained with haematoxylin-eosin. At least three slides for each crayfish were inspected under light microscope (López-Greco 1997).

RESULTS

Upon macroscopic and microscopic inspection, the gonads from females and intersex individuals presented an identical pattern. Both females and intersexes showed paired ovaries constituted by a modified H-shaped structure located within the cephalothorax, consisting of a pair of anterior lobes and a pair of posterior ovarian lobes. In the median part of the gonad, both ovarian lobes were very close and surrounded by the ovarian theca resembling an impair structure. A short and straight oviduct extended laterally from each posterior ovarian lobe toward the coxae of the third pereipods (Fig. 2). No evidence was observed of the presence of an atrophied male reproductive system (neither testicular tissue nor vasa deferentia).

According to their colour, immature ovaries were transparent to white, intermediate ovaries appeared as pale-orange, and mature ovaries were dark green, both in females and intersexes. From a histomorphological point of view, the ovary of *C. quadricarinatus* females and intersexes belongs to the "mandibulate type" (Krol *et al.* 1992, Ando and Makioka 1998). It is a sac-like structure where the proliferative zone, containing mainly oogonia, is located in the center of the gonad next to the ovarian lumen, while oocytes are surrounding the nests of oogonias in a more peripheral position. In immature ovaries, only germinative centers containing oogonia surrounded by primary vitellogenic oocytes and follicular

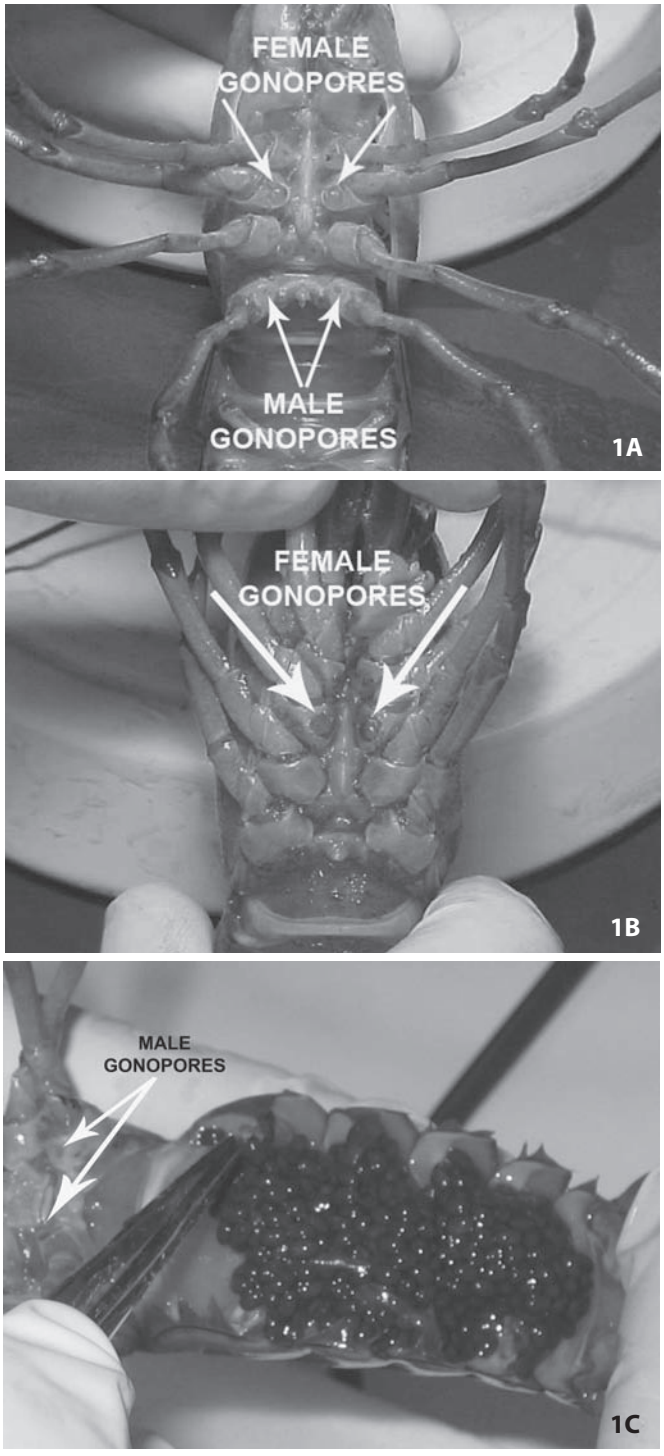

Fig. 1. *Cherax quadricarinatus* in ventral view. **A.** Intersex female with female and male gonopores. **B.** Female with the corresponding gonopores at the basis of the third pereipods. **C.** Intersex female with male gonopores and eggs attached to the pleopod setae.

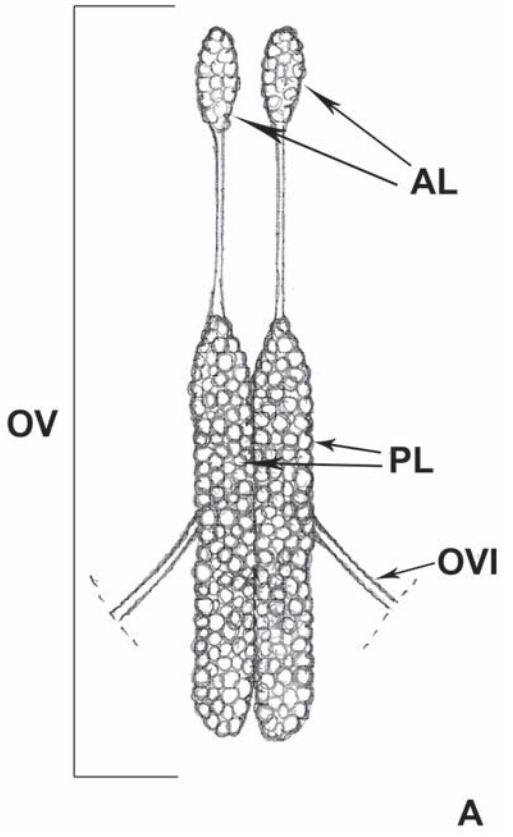

Fig. 2. Reproductive system of intersex female of *Cherax quadricarinatus*. **A.** The intersex reproductive system. **B.** Histological section of the ovary (scale bar: 600 µm). AL= anterior ovarian lobe, PL= posterior ovarian lobe, OV= ovary, OVI= oviduct, PO= primary oocyte, SO= secondary oocyte.

cells were observed in the center, while in maturing and mature ovaries both primary and secondary oocytes were found (Fig. 2). The ovarian lumen was usually seen both in immature and mature ovaries.

DISCUSSION

The present study demonstrates the presence of intersex females in *C. quadricarinatus*, a species in which intersexuality has been described as different types of functional males (Sagi *et al.* 1996, Khalaila *et al.* 1999). These intersex females posses vitellogenic ovaries, being in accordance with the intersex "ovigerous" females observed.

According to the observations in previous and the present studies, we consider that more research is needed to clarify the patterns of intersexuality in *C. quadricarinatus.* In accordance with Sagi *et al.* (1996), we also observed other intersex crayfish in which one or the two *appendix masculinae* occurred, and these individuals were uni- or bilaterally males, although female genital openings were present, too. It seems that for being intersex males there must be at least one *appendix masculina*. Since the development of this secondary sexual character is under the hormonal control of the androgenic gland, it is necessary to present at least a unilateral male reproductive system that allows differentiating as a functional intersex male. Intersex males usually present one or two red claws in accordance with the role of the androgenic gland on the differentiation of this character, too (Sagi *et al.* 1997).

It has been stated that crustacean culture can be improved by selecting monosex stocks (Sagi *et al.* 1986, Curtis and Jones 1995, Lawrence and Morrissy 1997, Lawrence *et al.* 2000). In *C. quadricarinatus*, both males and females have a greater somatic growth in a monosex culture than in a mixed culture; moreover, isolated males have a significant higher growth rate than females (Curtis and Jones 1995). Therefore, the early identification of sex could be important to improve the

growth rate of juvenile stages by means of a monosex culture, as well as to increase the probability of inducing the sexual reversion of both females and the described type of intersex juveniles to males, and the administration of sex-specific inducers of growth and reproduction. Our previous studies have demonstrated that secondary sexual characters in *C. quadricarinatus* appear early during the development, at stages VI-VIII for females, males and intersex females (Vazquez *et al.* 2004).

Besides the importance of these results for aquaculture purposes, another interesting theoretical fact emerged about intersexuality in Parastacidae. The presence of male and female intersexes has been reported for the South American parastacids *Parastacus pugnax* Poeppig, 1835, *P. varicosus* Faxon, 1898, *P. pilimanus* von Martens, 1869, *P. defossus* Faxon, 1898, *P. saffordi* Faxon, 1898, *Samastacus spinifrons* Phillipi, 1882 (Almeida and Buckup 2000, Rudolph and Almeida 2000, Rudolph *et al.* 2001, Rudolph 2002) and *Virilastacus* Hobbs, 1991 (Retamal and Rudolph 2005) including permanent or partial protandric hermaphroditism (Rudolph 1995, Almeida and Buckup 1997, Almeida and Buckup 2000, Rudolph and Almeida 2000, Rudolph 2002, Retamal and Rudolph 2005), but not for the Australian, Madagascar and New Zealand parastacids which are mainly gonochoristic (except for *C. quadricarinatus*).

According to Hobbs (1974, 1988) and Vogt (2002), the present Astacoidean taxa have evolved from a gonochoristic species; for the South American parastacids it has been proposed that intersexuality may be an adaptation for their burrowing life-style (these species dig deep and complex burrows where they live) (Rudolph and Almeida 2000). Burrowing crayfish species were classified by Hobbs (1942) into three general categories (primary, secondary and tertiary burrowers) according to the complexity of the burrow and the habitat where the species are distributed (Gherardi 2002). According to this criterion, South American species can be considered primary burrowers (with the exception of *Samastacus spinifrons*) and *Cherax* species tertiary burrowers. *C. quadricarinatus* burrows simple, not deep caves and they are not permanent occupants of the burrow. For this reason, in the case of *C. quadricarinatus*, we do not consider that intersexuality is related to its burrowing life-style. However, we can not discard that the presence of intersex individuals could be related to culture and/or laboratory conditions (e.g., density, sexual proportion, stress) since no evidence of intersexuality has ever been reported from animals captured from nature.

Further research must be carried out on the development of primary and secondary sexual characters of south hemisphere parastacids to understand the role of the habitat and/or social organization of the species and the underlying mechanism modulating the pattern of sexual differentiation. In addition, more evidence is needed to address the question if intersexuality is an ancestral or derived character among freshwater crayfish.

ACKNOWLEDGEMENTS

This work was supported by Antorchas Foundation (project 4248-138), UBACYT 2004-2007 (project X143) and CONICET (res. number 1106/2004). The authors thank Fabiana L. Lo Nostro from Department of Biodiversity and Experimental Biology, University of Buenos Aires, Argentina, and Leonardo E. Galiñanes, for their support.

RESUMEN

Cherax quadricarinatus, es un astácido dulceacuícola de gran tamaño de la (familia Parastacidae) originario del noroeste de Queensland y del norte de Australia. Presenta un sistema sexual gonocórico, aunque en poblaciones de cultivo se han descrito varios tipos de individuos intersexos como machos funcionales. En el presente estudio se describe y discute la morfología macroscópica y la histología gonadal de un tipo de intersexos. Todos los intersexos que presentan ambos pares de aberturas genitales (femeninas y

masculinas) y carecen de ambos apéndices masculinos y de la mancha roja, fueron hembras funcionales con ovarios y oviductos normales. Desde el punto de vista histológico no difieren de las hembras normales, presentando ovarios previtelogénicos y/o vitelogénicos de acuerdo a su tamaño.

Palabras clave: *Cherax quadricarinatus*, intersexualidad, intersexos hembras, caracteres sexuales, morfología gonadal.

REFERENCIAS

Almeida, A.O. & L. Buckup. 1997. Aspectos anatómicos e funcionais do aparelho reprodutor de *Parastacus brasiliensis* (von Martens) (Crustacea, Decapoda, Parastacidae). Rev. Brasil. Zool. 14: 497-509.

Almeida, A.O. & L. Buckup. 2000. Occurrence of protandric hermaphroditism in a population of the neotropical freshwater crayfish *Parastacus brasiliensis* (Parastacidae). J. Crust. Biol. 20: 224-230.

Ando, H. & T. Makioka. 1998. Structure of the ovary and mode of oogenesis in a freshwater crayfish, *Procambarus clarkii* (Girard). Zool. Sci. 15: 893-901.

Bauer, R.T. & G.J. Holt. 1998. Simultaneous hermaphroditism in the marine shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae): an undescribed sexual system in the decapod Crustacea. Mar. Biol. 132: 223-235.

Bauer, R.T. & W.A. Newman. 2004. Protandric simultaneous hermaphroditism in the marine shrimp *Lysmata californica* (Caridea: Hippolytidae). J. Crust. Biol. 24: 131-139.

Curtis M.C. & C.M. Jones. 1995. Observations on monosex culture of redclaw crayfish *Cherax quadricarinatus* Von Martens (Decapoda: Parastacidae) in earthern ponds. J. World Aquaculture Soc. 26: 154-158.

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

García Guerrero, M., I.S. Racotta & H. Villarreal. 2003a. Variation in lipid, protein, and carbohydrate content during the embryonic development of the crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). J. Crust. Biol 23: 1-6.

García Guerrero, M., H. Villarreal & I.S. Racotta. 2003b. Effect of temperature on lipids, proteins, and carbohydrates levels during development from egg extrusion to juvenile stage of *Cherax quadricarinatus* (Decapoda: Parastacidae). Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 135: 147-154.

Gherardi, F. 2002. Behaviour, p. 259-290. *In* D.M. Holdich (ed.). Biology of Freshwater Crayfish, Blackwell Science, London, England.

Hobbs, H.H. Jr. 1942. The crayfishes of Florida. University of Florida Publications, Biol. Ser. 3: 1-179.

Hobbs, H.H. Jr. 1974. Synopsis of the families and genera of crayfishes (Crustacea, Decapoda). Smithson. Contrib. Zool. 164: 1-31.

Hobbs, H.H. Jr. 1988. Crayfish distribution, adaptative radiation and evolution, p. 52-82. *In* D.M. Holdich & R.S. Lowery (eds.). Freshwater Crayfish: Biology, Management and Exploitation. Croom Helm, London, England.

Horwitz, P. 1988. Secondary sexual characteristics of females of the freshwater crayfish genus *Engaeus* (Decapoda, Parastacidae). Crustaceana 54: 25-32.

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

Jones, C.M. 1997. The biology and aquaculture potential of the tropical freshwater crayfish *Cherax quadricarinatus*. Department of Primary Industries Queensland, Queensland Government.130 p.

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

Karplus, I., A. Sagi, I. Khalaila & A. Barki. 2003. The soft red patch of the Australian freshwater crayfish *Cherax quadricarinatus* (von Martens): a review and prospects for future research. J. Zool. Lond. 259: 375-379.

Khalaila, I. & A. Sagi. 1997. Intersexuality and its control by the androgenic gland in the crayfish *Cherax quadricarinatus*. Invertebr. Reprod. Dev. 43: 69-70.

Khalaila, I., A. Weil & A. Sagi. 1999. Endocrine balance between male and female components of the

reproductive system in intersex *Cherax quadricarinatus* (Decapoda: Parastacidae). J. Exp. Zool. 283: 286-296.

Krol, R.M., W.E. Hawkins & R.M. Overstreet. 1992. Reproductive components, p. 295-343. *In* F.W. Harrison & A.G. Humes (eds.). Microscopic Anatomy of the Invertebrates, Willey-Liss. New York, USA.

Lawrence, C. & C. Jones. 2002. *Cherax*, p. 635-669. *In* D. Holdrich (ed.) Biology of Freshwater Crayfish, Blackwell Science, London, England.

Lawrence, C. & N. Morrissy. 1997. Enhancement of yabbie production from WA farm dams. Freshwater Farmer 4: 6-7.

Lawrence, C.S., W. Cheng, N.M. Morrissy & I.H. Williams. 2000. A comparison of mixed-sex vs. monosex growout and different diets on the growth rate of freshwater crayfish (*Cherax albidus*). Aquaculture 185: 281-289.

López-Greco, L.S. 1997. Adquisición de la madurez sexual y caracterización del patrón de crecimiento y reproducción de *Chasmagnathus granulata* Dana 1851 (Brachyura, Grapsidae, Sesarminae) de Bahía Samborombón, Buenos Aires, Argentina. Tesis Doctoral, Universidad de Buenos Aires, Argentina. 244 p.

Medley, P. & D.B. Rouse.1993. Intersex Australian red claw crayfish (*Cherax quadricarinatus*). J. Shellfish Res.12: 93-94.

Retamal, F.A. & E.H. Rudolph L. 2005. Ocurrencia de hermafroditismo protándrico en una población del género *Virilastacus* Hobbs, 1991 (Crustacea: Decapoda: Parastacidae). Proceedings of XI Congreso Latinoamericano de Ciencias del Mar, mayo del 2005, Viña del Mar, Chile.

Rudolph, E.H. 1995. Partial protandric hermaphroditism in the burrowing crayfish *Parastacus nicoleti* (Philippi, 1882) (Decapoda, Parastacidae). J. Crust. Biol. 15: 720-732.

Rudolph, E. 2002. New records of intersexuality in the freshwater crayfish *Samastacus spinifrons* (Decapoda, Parastacidae). J. Crust. Biol. 22: 377-389.

Rudolph, E. & A. Almeida. 2000. On the sexuality of South American Parastacidae (Crustacea, Decapoda). Invertebr. Reprod. Dev. 37: 249-257.

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

Sagi, A., E. Snir & I. Khalaila. 1997. Sexual differentiation in decapod crustaceans: role of the androgenic gland. Invert. Reprod. Develop. 31: 55-61

Sagi, A., Z. Ra´anan, D. Cohen & I. Wax. 1986. Production of *Macrobrachium rosenbergii* in monosex populations: yield characteristics under intensive monoculture conditions in cages. Aquaculture 51: 265-275.

Sagi, A., I. Khalaila, A. Barki, G. Hulata & I. Karplus. 1996. Intersex red claw crayfish, *Cherax quadricarinatus* (von Martens): functional males with previtellogenic ovaries. Biol. Bull. 190: 16-23.

Sagi, A., R. Manor, C. Segall, C. Davis & I. Khalaila. 2002. On intersexuality in the crayfish *Cherax quadricarinatus*: an inducible sexual plasticity model. Invertebr. Reprod. Dev. 41: 27-33.

Shechter, A., E.D. Aflalo, C. Davis & A. Sagi. 2005. Expression of the reproductive female-specific vitellogenin gene in endocrinologically induced male and intersex *Cherax quadricarinatus* crayfish. Biol. Reprod. 73: 72-79.

Thorn, M.J. & D.R. Fielder. 1991. The red cuticle on the claw of male *Cherax quadricarinatus* (Decapoda: Parastacidae). Memoir. Queensl. Mus. 31: 277.

Turra, A. 2004. Intersexuality in hermit crabs: reproductive role and fate of gonopores in intersex individuals. J. Mar. Biol. Assoc. UK. 84: 757-759.

Vazquez, F.J., A. Chaulet, E. M. Rodríguez & L.S. López Greco 2004. Development of the secondary sexual characters in the Australian freshwater crayfish, *Cherax quadricarinatus* (Parastacidae). Proceedings of the 3rd Brazilian Crustacean Congress and 2004 TCS Summer Meeting, Santinho, Florianópolis, Brasil.

Vogt, G. 2002. Functional anatomy, p.53-151. *In* D.M. Holdrich (ed.). Biology of Freshwater Crayfish, Blackwell Science, London, England.