

REPRODUCTIVE SYSTEM AND SIZE MATURITY OF THE PADDLE CRAB *OVALIPES TRIMACULATUS* (BRACHYURA: PORTUNIDAE) ALONG THE ARGENTINE COAST

Micaela Vallina*, María Paz Sal Moyano, Elena Irene Cuartas, and María Andrea Gavio

Instituto de Investigaciones Marinas y Costeras (IIMyC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Estación Costera J. J. Nágera, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata, Argentina

ABSTRACT

Ovalipes trimaculatus (de Haan, 1833) is a commercial species captured in the Mar del Plata coast, Argentina. However, no studies were conducted in order to study its population features neither its reproductive biology. The morphometric and physiological maturity of both sexes, the histology of the female seminal receptacle and ovary, and the ultrastructure of the male gonopods were studied in the present paper. In females, morphometric maturity (44.6 mm of carapace width, CW) was acquired prior to physiological maturity (54.6 mm CW). By contrast, in males, physiological maturity (46.6 mm CW) was acquired prior to morphometric maturity (52.3 mm CW). The ovary is connected to the dorsal part of the seminal receptacle. Neither Spermatophores, nor sperm packets were found inside the seminal receptacle; instead free spermatozooids were observed indicating that sperm from different males is probably mixed favoring sperm competition. The ultrastructure of the male gonopods was similar to the described for other Brachyura. Different types of setae and the presence of internal rosette glands were described and probable functions were suggested.

KEY WORDS: maturity, Portunidae, reproduction

DOI: 10.1163/1937240X-00002239

INTRODUCTION

The diversity of mating strategies in brachyurans and their reproductive systems has been largely investigated mainly in species of commercial value. An understanding of the functions of the components of the reproductive system is fundamental to the elucidation of reproductive processes in these organisms. Brachyuran reproductive systems show considerable morphological variation in both sexes (Goldstein and Dupré, 2010). Females possess paired ovaries, oviducts, storage organs such as the seminal receptacles derived from the oviduct, vaginas, and vulvas. The seminal receptacles are structures functioning in sperm retention after mating and play a key role in the reproductive behavior of crabs, storing the sperm from one or more matings from several males (Beninger et al., 1988; Diesel, 1989, 1991; Sainte-Marie and Lovrich, 1994; González-Gurriarán et al., 1998; Goldstein and Dupré, 2010). Becker et al. (2011) summarized the most relevant studies using histological data of the female seminal receptacle including five species of Portunoidea.

Males have paired testes, vas deferens, and ejaculatory ducts that discharge on the first pair of pleopods modified as gonopods (Beninger et al., 1991). They are used in the transfer of spermatophores into the female seminal receptacles during mating (Bauer, 1986). They are of particular interest in the brachyuran male reproductive system, because they are morphologically diverse yet taxonomically very conservative, and hence provide valuable taxonomic and phylogenetic information (Beninger et al., 1991; Wee et al., 1995;

Goldstein and Dupré, 2010; Lai et al., 2010; Padate et al., 2010; Sal Moyano et al., 2011a; Guinot et al., 2013). The particular shapes of the gonopods and the presence of microstructures, suggest the hypothesis that they have different roles during copulation (mechanism of insertion, function of the setae) (Sal Moyano et al., 2011a). Differences in the relative size of gonopods would be related to the capacity of the individual to mate successfully (Davidson and Marsden, 1987). The length of the first gonopod can be used as a source of morphometric data for marking morphological sexual maturity (Guinot et al., 2013).

In brachyuran crabs, maturity can be defined according to different criteria such as morphology and physiology. The size at which these reproductive events occur could vary intra- and inter-sex (Comeau and Conan, 1992; Sainte-Marie and Carrière, 1995; Barón et al., 2009). Morphological maturity is based on changes in the relative growth of different body parts to detect changes in levels of allometry (Hartnoll, 1974; Conan et al., 2001; Mura et al., 2005; Corgos and Freire, 2006). Those morphological changes are mostly shown by male chelipeds and female pleon, which characterize growth stages and hence the transition between immature and mature instars is achieved at the puberty molt (Hartnoll, 1974). Morphological sexual maturity is exhibited in males by the acquisition of a disproportional large chelipeds, which plays an important role in mating displays (Donaldson and Adams, 1989; Sainte-Marie et al., 2008). The assessment of physiological maturity involves the study of functional and structural characteristics of

* Corresponding author; e-mail: micaelavallina@conicet.gov.ar

female and male genitalia. In particular, the morphological ones are related to macroscopic analysis such as the shape and color of gonads. Functional features are related, in males, to the production of spermatophores (Comeau and Conan, 1992); while in females they depend on oocytes development (Johnson, 1980).

The portunoid *Ovalipes trimaculatus* (de Haan, 1833) is distributed in the Southern Hemisphere in South Africa (Cape Town and Buena Esperanza Cape), Japan, Australia, Argentina, Uruguay, Brazil, and Perú from Independencia Bay to the Trinidad Canal in Chile, including Juan Fernández Island (Melo, 1996; Alvarez et al., 2009). In Argentina, it is found along the coasts of the Buenos Aires, Rio Negro, and Chubut provinces, and are frequently captured as by-catch of artisanal fisheries using trawl nets at depths of 3–65 m (Boschi, 1964; Fenucci and Boschi, 1975; Spivak, 1997).

In spite of its importance as a potential resource, experimental fishery studies were conducted along the Patagonian coast to provide information on the feasibility of their potential extraction (Narvarte et al., 2007). Given the increasing interest to exploit this species and the scarce biological information available, we investigated key aspects of its reproductive biology and describe here the reproductive system, assess the physiological and morphometric maturity in females and males, and the detailed ultrastructure of the male gonopods using SEM and histological sections.

MATERIALS AND METHODS

Crabs were monthly collected along the coast of Mar del Plata (38°S, 57°33'W), Argentina, using trawl nets (40 mm opening) towed by commercial boats, from March 2006 to June 2007. Carapace width (CW) was measured with digital calipers and used as the reference variable. Data were pooled and no seasonal information was considered. Total size frequency distribution was calculated for both sexes.

Reproductive System and Physiological Maturity

Females ($N = 124$) and males ($N = 26$) were dissected and gonad development was classified according to macroscopic characteristics as the shape and coloration of ovaries and testes and vas deferens, respectively. Different stages of gonad development were described following Armstrong (1988), and Mantelatto and Franzoso (1999). Following Vallina (2007), ovaries and seminal receptacles of females, and the testes and vas deferens of mature males of CW > 30 mm were selected and treated for histological study. Tissue samples were fixed in Bouin's solution during 24 hours, dehydrated through a series of graded alcohol solutions, cleared in xylene agent, and embedded in paraffin. Serial sections (5–7 μm) were cut with a Minot microtome. Sections were mounted on glass slides and stained with standard hematoxylin and eosin for observation under a light microscope.

In females, the content of the seminal receptacle was analyzed to relate their condition (presence or absence of spermatozoa) with gonad development stages. In the case of males, they were considered as immature or mature according to the absence or presence, respectively, of spermatophores inside their vas deferens. The relative size at which females and males reach gonad maturity (CW at which 50% of females and males are physiologically mature, CW_{50}) was calculated using the logistic function $y = 1 / (1 + e^{r(CW - CW_{50})})$, where CW_{50} corresponds to the size at which 50% of the individuals are considered mature and r stands for the slope of the curve (Conan et al., 2001; Castiglioni and Negreiros-Franzoso, 2006; Corgos and Freire, 2006).

Morphometric Maturity

To assess the female morphometric maturity, CW and maximum pleon width (PW) at a level of the fourth pleomere were measured, while in males, CW, cheliped length (ChL), and gonopod length (GL) were measured. Quantitative relationships between morphometric data were described by

least square regressions. Slopes and intercepts of different regression lines were compared by covariance analysis (ANCOVA) to identify the level and significance of allometric growth for each dimension. Slope values were compared to the expected isometric values using Student's t -test, with significance level at $\alpha < 0.05$. When $b = 1$ growth is isometric, whereas when $b < 1$ growth is negatively allometric or $b > 1$ and is positively allometric. The relative size that females and males reach morphometric sexual maturity (CW at which 50% of females and males are morphometrically mature) was estimated by the program 'Mature' (Somerton, 1980; de Lestang et al., 2003; Fumis et al., 2005; Barón et al., 2009), using the variables CW and PW for females and the ChL for males. The upper bound of size ranges for juvenile male and female were 55.8 and 46.5 mm CW respectively, while the lower bound for adult males and females were 50.3 and 40.9 mm CW respectively.

Ultrastructure of Male Gonopods

The gonopods of 5 individuals ranging from 43.2 to 85 mm CW were removed and selected for SEM studies, while those of 8 males ranging from 21.8 to 99.3 mm CW were used for histological sections. Samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2–7.4). Samples were dehydrated in a graded alcohol series, dried in hexamethyldisilazane replacing the critical-point, coated with gold-palladium and viewed with a JEOL 6460 microscope. For histological studies, samples were treated as described above.

RESULTS

Reproductive System and Physiological Maturity

The reproductive system of the females of *O. trimaculatus* is bilateral with regards to ovaries, oviducts, seminal receptacles, vaginas, and gonopores. The ovary extends below the carapace from the frontal spine to the posterior end; both lobes are connected by a central bridge in the region of the heart and behind the stomach, and descend ventrally and towards the 6th thoracomere on both sides. According to the macroscopic characteristics of ovaries, four stages of gonad development were described; two corresponded to immature and two to mature phases. Stage I presented undifferentiated white ovaries, while immature females of stage II a thin, pale-orange and clearly differentiated ovaries. Histological sections of immature ovaries showed the presence of oocytes ($20 \pm 27 \mu\text{m}$) with visible nucleus and nucleolus and absence of vitellogenic droplets in the cytoplasm. Accessory rounded cells were observed surrounding the oocytes. Mature females had conspicuous ovaries dark orange in stage III, and brownish in stage IV. Histological sections of mature ovaries showed large lobes separated by abundant connective fibers containing oocytes ($80 \pm 13 \mu\text{m}$) with visible nucleus and nucleolus, rounded by vitellogenic droplets, or larger oocytes ($100 \pm 21 \mu\text{m}$) where the nucleus was completely covered by vitellogenic droplets. Accessory flattened cells were observed surrounding the oocytes. The seminal receptacles were empty in stages I and II, while they were full of spermatozooids in stages III and IV.

Macroscopically, the seminal receptacles look pouch like in shape, the ovary is observed connected to the dorsal part of the seminal receptacle, opposite to the vagina, although the exact oviduct connection cannot be distinguished (Fig. 1A). In the histological sections, the seminal receptacle showed a dorsal region characterized by stratified epithelium, and a ventral portion by a simple one lined internally by a cuticle layer (Fig. 1B). Inside the seminal receptacles only presence of a unique mass of spermatozooids and seminal fluid was observed (Fig. 1B). A detailed view of the spermatozooids demonstrated that they are characterized by

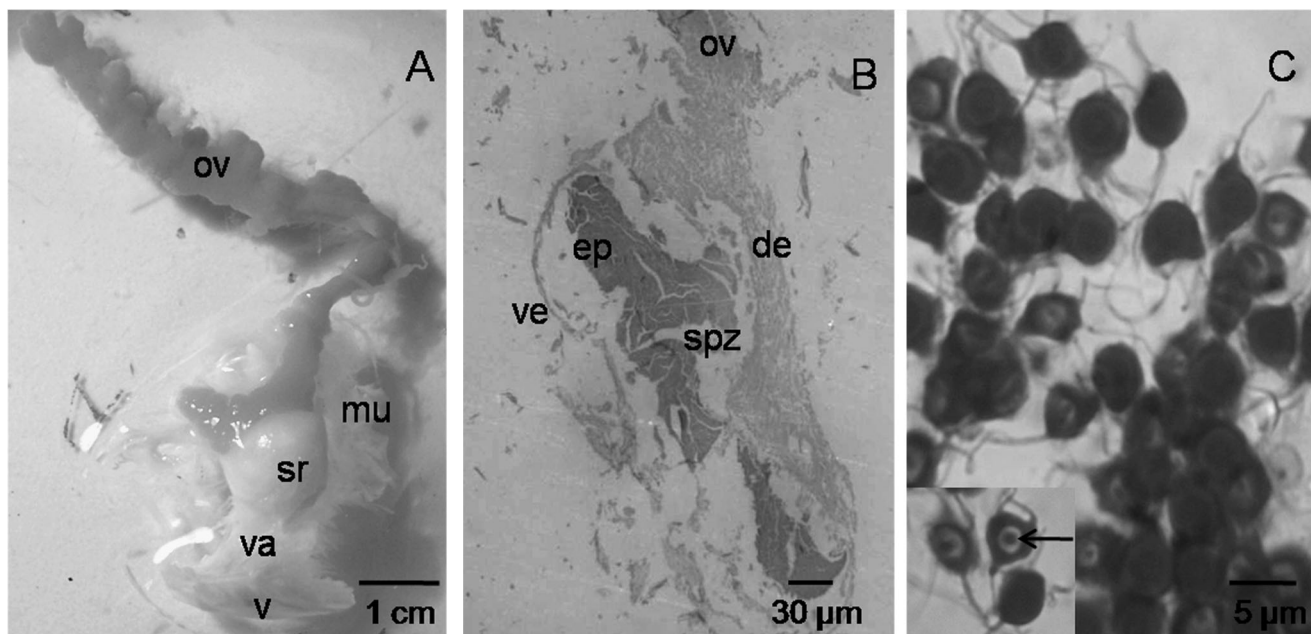


Fig. 1. Seminal receptacle of *Ovalipes trimaculatus*. A, macroscopic view of the connection between the ovary and oviduct (ov) to the dorsal part of the seminal receptacle (sr). The vagina (va) and the vulva (v) are observed in the ventral part. The musculature (mu) that attaches the seminal receptacle to the sternites is also observed. B, histological section showing the dorsal stratified (de) and the ventral simple (ve) epithelium of the seminal receptacle connected to the ovary (ov) and a unique mass of spermatozooids (spz) and seminal fluid occupying the entire seminal receptacle. C, spermatozooids found inside the seminal receptacle, characterized by the presence of 2-4 arms. Inset: detailed view of a spermatozoid showing its acrosomal core (arrow).

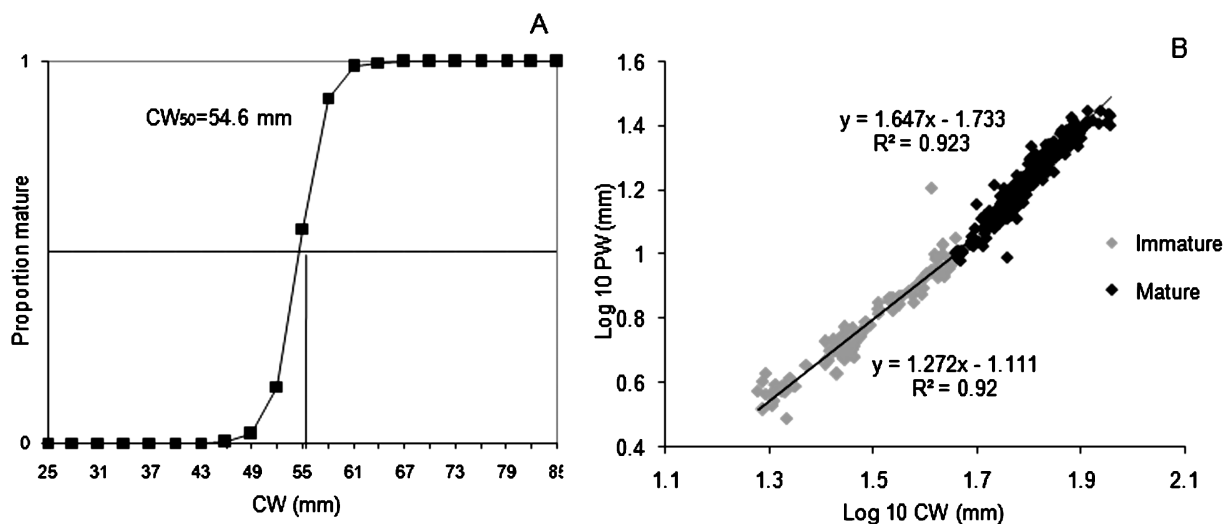


Fig. 2. *Ovalipes trimaculatus*. A, relationships between the proportion of females reaching gonad maturity and carapace width (CW), with fifty per cent of females reaching gonad maturity at 54.6 mm CW; B, log-log relationships between pleon width (PW) and carapace width (CW); the regression lines correspond to immature and mature females ($N = 588$).

the presence of 2-4 arms and an acrosomal core could be easily distinguishable (Fig. 1C).

The size distribution analyses per stages showed that stage I females ranged from 35 to 50 mm CW, females of stage II from 40 to 70 mm, of stage III ranged from 55 to 90 mm CW and females of stage IV from 65 to 90 mm CW. All females larger than 65 mm CW were mature, corresponding to stages III or IV. The logistic equation showed that the size at which 50% of females reached gonad maturity is $CW_{50} = 54.6$ mm (Fig. 2A).

Male reproductive system consists of paired testes, vas deferens, ejaculatory tubes, and paired gonopods. According to the macroscopic characteristics of the testis and vas deferens, two stages of gonad development, immature and mature were described. Immature males were characterized by thin, translucent, and not clearly differentiated testis and vas deferens with no spermatophores. Mature males presented large, convoluted white testis and vas deferens with spermatophores inside. Histological sections of the mature vas deferens showed the presence of an anterior portion with a

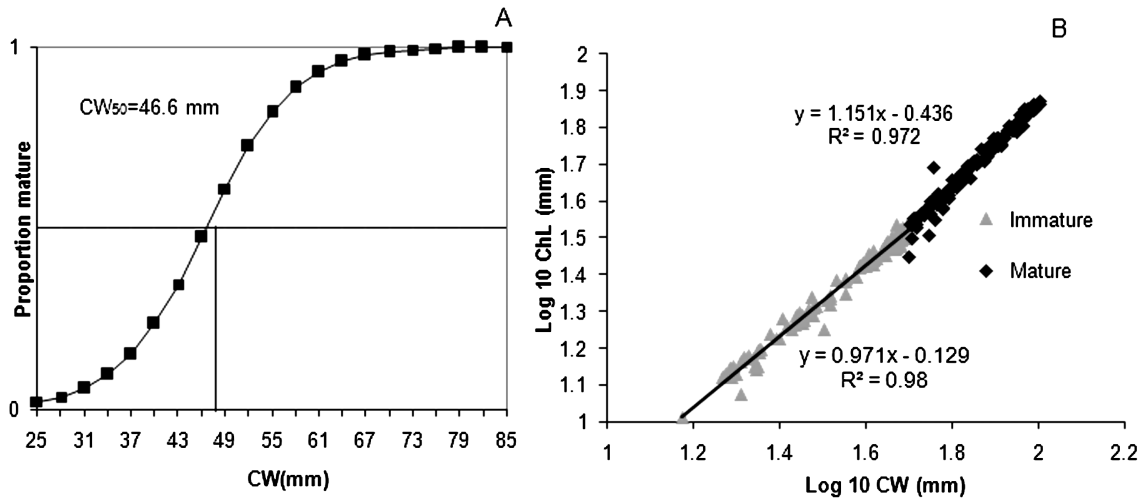


Fig. 3. *Ovalipes trimaculatus*. A, relationships between the proportion of males reaching gonad maturity and carapace width (CW), with fifty percent of males reaching gonad maturity at 46.6 mm CW; B, log-log relationships between cheliped length (ChL) and carapace width (CW); the regression lines correspond to immature and mature males.

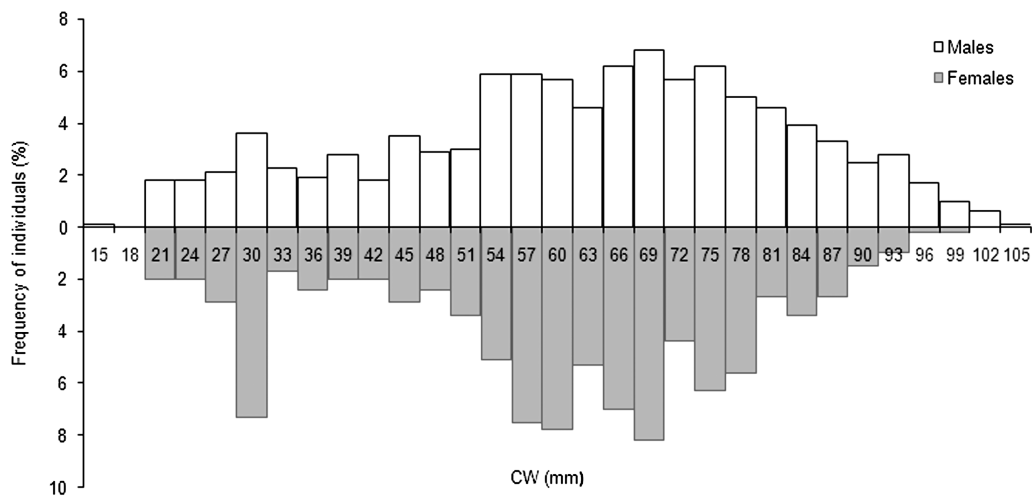


Fig. 4. Overall size-frequency distribution of carapace width (CW) by sex of *Ovalipes trimaculatus* ($N = 1312$).

columnar epithelium containing few spermatophores, a middle portion with a lower epithelium full of spermatophores, and a posterior portion with the lowest epithelium containing secretion substances. All males larger than 55 mm CW were mature, while all males smaller than 35 mm CW were immature. The analysis of the logistic equation showed that the size at which 50% of males reached gonad maturity is $CW_{50} = 46.6$ mm (Fig. 3A).

Morphometric Maturity

A total of 588 females were measured. The carapace width ranged from 18.2 to 98.3 mm in females and from 65.3 to 70.2 mm in ovigerous females ($N = 5$) (Fig. 5). Relative growth analysis in females showed that the growth of PW with respect to CW was positively allometric in immature ($\log PW = 1.111 + 1.272 \log CW$, $R^2 = 0.92$, $t = 3.49$, $P < 0.001$) and mature females ($\log PW = 1.733 + 1.647 \log CW$, $R^2 = 0.923$, $t = 23.68$, $P < 0.001$) (Fig. 2B). The slopes of the $PW \times CW$ relationships differ between female phases (ANCOVA, $F = 52.08$, $P < 0.001$).

The size at which 50% of females were morphometrically mature was 44.6 mm. The smallest mature female measured 39.8 mm CW, and the largest immature female 50.1 mm CW.

A total of 724 males were measured. The carapace width ranged from 14.9 to 104.5 mm (Fig. 4). The relative growth of ChL with respect to CW showed a negative allometric phase in immature males ($\log ChL = 0.129 + 0.971 \log CW$, $R^2 = 0.98$, $t = 2.12$, $P < 0.05$) and a positive allometric phase in mature males ($\log ChL = 0.436 + 1.151 \log CW$, $R^2 = 0.972$, $t = 8.78$, $P < 0.001$) (Fig. 3B). The slopes of the regression lines differed significantly when the $ChL \times CW$ relationship was compared between immature and mature males (ANCOVA, $F = 65.5$, $P < 0.01$). The size at which 50% of males were morphometrically mature was 52.3 mm. The smallest mature male measured 47.9 mm CW, and the largest immature male measured 53.7 mm CW.

The relative growth of the GL with respect to the CW showed two positive allometric phases between immature males ($\log GL = 1.152 + 1.310 \log 10CW$, $R^2 = 0.829$, $t = 3.42$, $P < 0.001$) and mature males ($\log GL =$

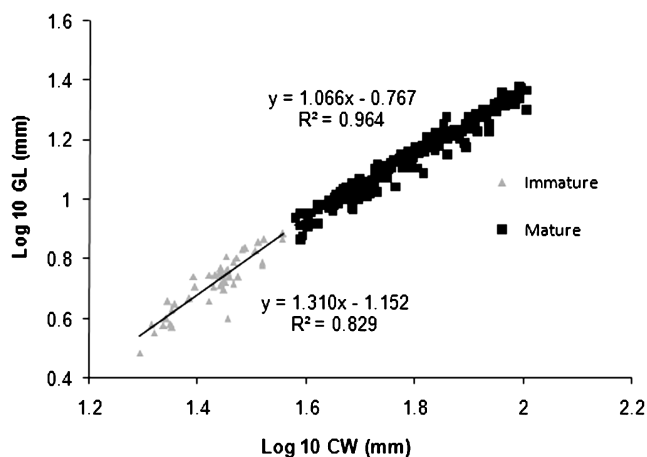


Fig. 5. Log-log relationships between gonopod length (GL) and carapace width (CW); the regression lines correspond to immature and mature males.

$0.767 + 1.066 \log_{10} CW$, $R^2 = 0.964$, $t = 4.44$, $P < 0.001$) (Fig. 5). The slopes of the $GL \times CW$ relationships differed significantly between male phases (ANCOVA, $F = 13.9$, $P < 0.001$).

Ultrastructure of Male Gonopods

The first gonopod (G1) is composed of a basal protopod and the long, slender distal endopod that narrows towards its terminal end, while the second gonopod (G2) is approximately threefold smaller than G1, with the endopod of tubular form (Fig. 6A). In the G1, the cuticle is ventro-laterally infolded forming a tube that functions as an ejaculatory duct, ending in a single tip without setae (Fig. 6B). The G1 mid-region presents two types of setae: the ventral region holds setae type 1 (s1) and the dorsal one holds setae type 2 (s2) (Fig. 6C). Setae type 1 are characterized by a long conical form with infracuticular insertion (Fig. 6C), while setae type 2 are characterized by its short conical form with infracuticular insertion protruding from pits (Fig. 6D, E). The basal region of G1 presents setae type 3 which are pinnate, with pinnules growing randomly along its axis and with infracuticular insertion (Fig. 6F). The G2 ends in two processes, one continues the line of the protopod and lacks setae, while the other is shorter, extended laterally with respect to the protopod, and holds setae type 4 (Fig. 6G). These setae are characterized by a conical form, longer than setae type 2 but and with fused cuticle insertion (Fig. 6H). Regarding the size of morphometric maturity calculated in the presented study, all gonopods analyzed by SEM corresponded to mature individuals.

Internal anatomy of G1 showed the presence of rosette glands along its length (Fig. 7A). Glands are composed of secretory cells with basal nucleus, radially arranged around a central pore cell, and a canal cell that connects to the secretory surface (Fig. 7B). Glandular ducts of the rosette glands are observed through the cuticle (Fig. 7C). Considering the size of morphometric maturity assessed in the presented study, the gonopods analyzed histologically corresponded to mature and immature individuals. However, no differences in their internal anatomy were found between them.

DISCUSSION

Portunids are important components of small-scale coastal fisheries in many countries. In Argentina, however, the paddle crab *O. trimaculatus* is obtained as by-catch of shrimp fisheries (Fenucci and Boschi, 1975), yet it is a potential resource along the Argentinian coast which has been recently started to be exploited in the San Matías Gulf (Narvarte et al., 2007). Biological information on this species is limited to former studies in the Buenos Aires Province on size frequency distributions and sex ratios during one calendar year in the early seventies (Fenucci and Boschi, 1975). Our study is the first one to address the description of the general reproductive anatomy combined with the size at which both sexes undergo sexual maturation.

Regarding the general features of the reproductive system, *O. trimaculatus* is similar to those reported for other portunid species as *Portunus sanguinolentus* (Herbst, 1783) (Ryan, 1967b) and *Callinectes sapidus* Rathbun, 1896 (Johnson, 1980). The macroscopic anatomy and the histological sections of the female ovary described in this study allows assigning four stages of gonad development that show two immature and two mature phases. The stages of the ovaries development described here were similar to the ones characterized by other portunids as *Ovalipes catharus* (White, 1843) studied by Armstrong (1988), and *Callinectes ornatus* Ordway, 1863 studied by Mantelatto and Fransozo (1999). Hartnoll (1968) assigned the dorsal type of seminal receptacles to portunids. Those species as *Carcinus maenas* (Linnaeus, 1758) and *Callinectes sapidus* tend to use the oldest sperm (sperm from the first male to mate) to fertilize the oöcytes (Diesel, 1990, 1991). Experimental and field evidence indicate that sperm competition could occur in *C. sapidus* because females re-mate within several days after their final molt, storing both ejaculates in their entirety such that sperm from both males may have equal access to the oöcytes (Jivoff, 1997). In some majoids, the ejaculate stored inside the seminal receptacle consisted of spermatophores (Diesel, 1989; Sainte-Marie et al., 2000), while in cancrids free sperm was observed in the lumen of seminal receptacles shortly after insemination (Burfitt, 1980; Hankin et al., 1989; Shirley and McNutt, 1989; Jensen et al., 1996). In our study, no spermatophores were observed inside the seminal receptacles of *O. trimaculatus*, but free spermatozooids were encountered embedded in sperm gel. Since the seminal receptacle is the site where eventual sperm competition must necessarily take place (Orensanz et al., 1995), the absence of sperm packets may indicate that females are capable of mixing the ejaculates from different males, thus, favoring sperm competition. Future studies evaluating possible re-mating in *O. trimaculatus* will help to test this hypothesis.

Regarding the morphology of the spermatozooids described here for *O. trimaculatus* consisting on the presence of variable 2-4 arms, reptant decapods have spermatozooids with radiating appendages (arms) extending from the main cell body (Felgenhauer and Abele, 1983). Their morphology and number of radial arms vary among species, and arms appear to originate as extensions of the nucleus or of the cytoplasm (Hinsch, 1986). The presence of radial arms in the spermatozooids was also described for *Ovalipes ocellatus* (Herbst, 1799) (Hinsch, 1986).

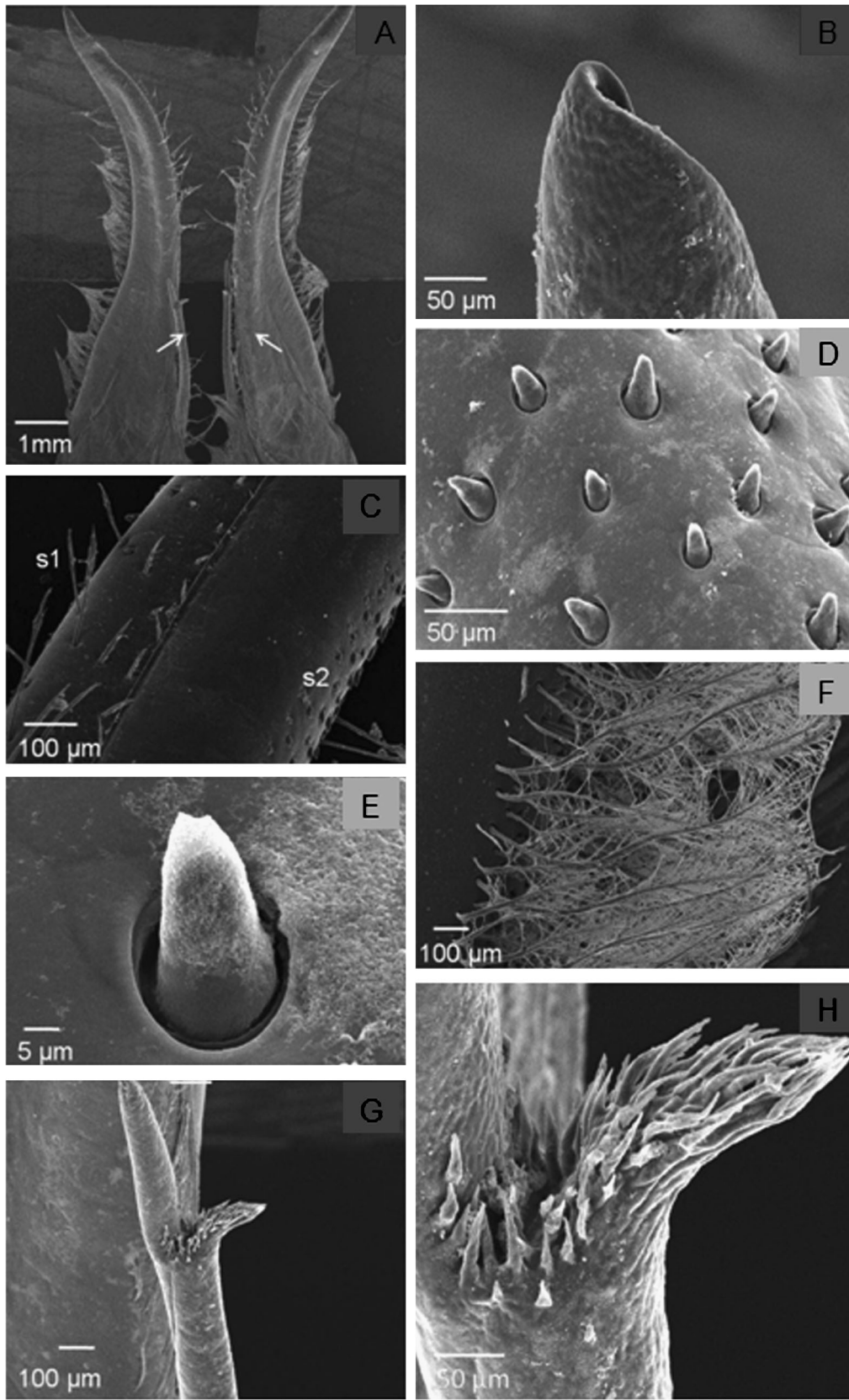


Fig. 6. Gonopods of male *Ovalipes trimaculatus*. A, general view of gonopods 1 and 2 (arrows); B, apical tip of gonopod 1 showing its cuticle in-folded forming the ejaculatory canal; C, apical portion of gonopod 1 showing in its ventral region the setae type 1 (s1) and in its dorsal region the setae type 2 (s2); D, general view of setae type 2; E, detailed view of setae type 2; F, pinnate setae of type 3; G, tip of gonopod 2; H, detailed view of gonopod 2 showing setae type 2 and setae type 4.

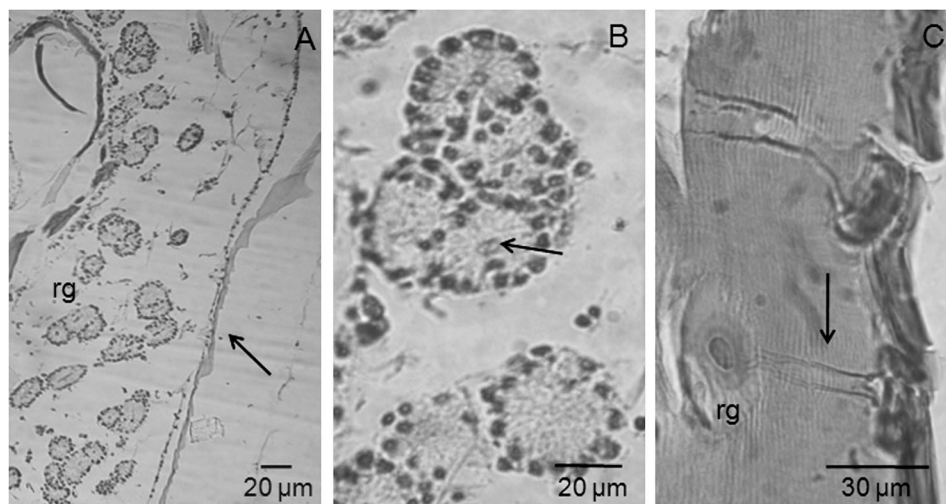


Fig. 7. Histological sections of *Ovalipes trimaculatus* male gonopod I. A, cross-section of the gonopod length showing the external cuticle (arrow) and the rosette glands (rg); B, detailed view of the rosette glands showing the central pore cell (arrow); C, detailed view of the external cuticle showing the cuticle canal (arrow) of the rosette gland (rg).

The size at which sexual maturity is attained and the way it is assessed are important aspects of the crustacean life cycle and it can be determined through the study of reproductive aspects, whereas morphometric techniques can indicate allometric changes in size related to the external morphological maturity (Castiglioni and Negreiros-Fransozo, 2006). According to the sizes at which the individuals acquired morphometric and physiological maturity, we found that the acquisition of morphometric maturity in *O. trimaculatus*-females occur preceding the physiological maturity. While the importance of the enlargement of the pleon is related to its function as an incubation chamber sufficient for carrying the developing eggs (Hartnoll, 1974; Haefner, 1985; Davidson and Marsden, 1987); the maturation of ovaries finally allows females to be functional mature to copulate with males. Other studies have also registered that maturation of ovaries occurs later than morphometric maturity (Hinsch, 1972; Jones and Hartnoll, 1997), or that both types of maturity are coincident at a similar size (Hartnoll, 1963; Bryant and Hartnoll, 1995; Sal Moyano et al., 2011b).

In the case of males, the acquisition of physiological maturity occurred prior to morphometric maturity, considering the length of the cheliped (ChL) as the variable, pattern found in many other male crab species (Hinsch, 1972; Haefner, 1985; Conan and Comeau, 1986; Armstrong, 1988; Beninger et al., 1988; Homola et al., 1991; Sainte-Marie and Carrière, 1995; Rotllant et al., 2000; Corgos and Freire, 2006; Sal Moyano et al., 2011b). It could be proposed that the physiologically mature males of small sizes might be favored by displaying a “pure search” mating strategy, as it was shown to occur in polymorphic shrimp (Bauer and Thiel, 2011). Also, immature majoid males appear to ‘mimic’ females avoiding aggressive behavior from the morphologically mature males, thus they are able to increase their mating opportunities by ‘sneak’ mating (Laufer and Ahl, 1995). The allometry found in the relative growth of the GL versus the CW of *O. trimaculatus* was similar to the portunid *O. catharus* studied by Davidson and Marsden (1987),

and also to other crabs as the parthenopid *Solenolambrus tenellus* Stimpson, 1871 studied by Davidson and Marsden (1987) and the majoid *Libidoclaea granaria* Milne Edwards and Lucas, 1842 studied by Schejter and Spivak (2005). In *O. trimaculatus*, the length of the G1 increases with a slight positive allometric growth in immature individuals but reduces its slope when attain the puberty molt.

The general gonopod morphology of *O. trimaculatus* and the proposed mechanism of insertion during ejaculation are similar to other brachyurans. Male first gonopods of higher brachyurans are characterized by the complete folding of the cuticle, forming a tube-shaped structure (Hartnoll, 1975; Beninger et al., 1991; Neumann, 1996), which is inserted in the female vulvae during copulation to transfer the spermatophores to the female seminal receptacle (Guinot and Quenette, 2005). The G2 is inserted inside G1 and acts as a hydraulic pump to push the seminal fluid through the ejaculatory duct (Beninger et al., 1991). The gonopod tip of *O. trimaculatus* has a single ending similar to the genera *Chionoecetes* studied by Beninger et al. (1991), *Maja* studied by Neumann (1996), and *Inachus* studied by Rorandelli et al. (2008).

Regarding the function of setae, the type 1 described here were similar to those observed in *Chionoecetes opilio* (Fabricius, 1788) by Beninger et al. (1991) and in *Libinia spinosa* Milne Edwards, 1834 by Sal Moyano et al. (2011a). Beninger et al. (1991) suggested a possible mechanoreceptor role, related to their infracuticular insertion, helping to position the gonopod and facilitating the copula. Setae type 2, described here, are similar to the setae present in the G1 of *Maja* (Neumann, 1996) and in the antennae of *Homarus americanus* H. Milne Edwards, 1837 studied by Derby (1982). A similar function could be proposed for *O. trimaculatus*, sensing female condition during the gonopod insertion. Setae type 3 are morphologically similar to those described by Phillips and Macmillan (1987), Beninger et al. (1991), and Garm (2004) for other crustaceans. These setae could rub against the abdomen during copulation (Beninger

et al., 1991). In the case of setae type 4 encountered in G2, they are similar to the “pyramid-like” setae of *Maja* (Neumann, 1996), the “cuspidate” setae of the maxillae and maxillipeds of *Cherax quadricarinatus* (von Martens, 1868) studied by Garm (2004), and the “tooth-like” setae of *Inachus phalangium* (Fabricius, 1775) studied by Rorandelli et al. (2008). Since its fused-cuticle insertion, a mechanical function could be proposed. They may be involved in anchoring the G2 inside the G1 during ejaculation, while the other process lacking setae, could be acting as a hydraulic bomb allowing the seminal fluid to be pushed down the ejaculatory canal of G1.

With respect to internal rosette glands, they were described in several species of Portunoidea, Cancroidea, Grapsoidea (Beninger and Larocque, 1998), and Majoidea (Diesel, 1989; Beninger et al., 1991; Sal Moyano et al., 2011a). Secretions of these glands could play multiple functions: they could participate in the formation of seminal fluid and sperm plugs (Beninger et al., 1991, 1993) or in the molting process (Gorvett, 1946; Talbot and Zao, 1991). The presence of them along the G1 length could indicate their function in the seminal fluid formation, although, the presence of cuticle pores, also may indicate another function during the molting event.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Associate Editor and two anonymous reviewers for their suggestions, comments and for kindly correcting our English text, which highly improved this manuscript. This research was supported by Ideawild, the project EXA 310/05 developed by the Universidad Nacional de Mar del Plata, Argentina, and the project PICTO 2004-00749, FONCYT, to M.A.G., M.V. and M.P.S.M. had a fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

REFERENCES

- Alvarez, J., C. Véliz, and J. Meruane. 2009. Estados del ciclo de muda de la jaiba nadadora *Ovalipes trimaculatus* (de Hann, 1833) basados en observaciones de la morfología externa. *Revista de Biología Marina y Oceanografía* 44: 217-225.
- Armstrong, J. H. 1988. Reproduction in the paddle crab *Ovalipes catharus* (Decapoda: Portunidae) from Blueskin Bay, Otago, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 22: 529-536.
- Barón, P. J., A. P. Quiroga, G. A. Leal, and X. González-Pisani. 2009. Morphological maturity of the knobbed spider crab, *Leurocyclus tuberculatus* (H. Milne Edwards & Lucas, 1842) (Brachyura, Majidae) in the Northern Patagonian Gulfs. *Crustaceana* 82: 267-273.
- Bauer, R. T. 1986. Phylogenetic trends in sperm transfer and storage complexity in decapod crustaceans. *Journal of Crustacean Biology* 6: 313-325.
- , and M. Thiel. 2011. First description of a pure-search mating system and protandry in the shrimp *Rhynchocinetes uritai* (Decapoda: Caridea). *Journal of Crustacean Biology* 31: 286-295.
- Becker, C., D. Brandis, and V. Storch. 2011. Morphology of the female reproductive system of European pea crabs (Crustacea, Decapoda, Brachyura, Pinnotheridae). *Journal of Morphology* 272: 12-26.
- Beninger, P. G., and R. Larocque. 1998. Gonopod tegumental glands: a new accessory sex gland in the Brachyura. *Marine Biology* 132: 435-444.
- , R. W. Elner, T. P. Foyl, and P. H. Odense. 1988. Functional anatomy of the male reproductive system and the female spermatheca in the snow crab *Chionoecetes opilio* (O. Fabricius) (Decapoda: Majidae) and a hypothesis for fertilization. *Journal of Crustacean Biology* 8: 322-332.
- , ———, and Y. Poussart. 1991. The gonopods of the majid crab *Chionoecetes opilio* (O. Fabricius). *Journal of Crustacean Biology* 11: 217-228.
- , C. Laintegne, and R. W. Elner. 1993. Reproductive process revealed by spermatophore dehiscence experiments and by histology, ultrastructure, and histochemistry of the female reproductive system in the snow crab *Chionoecetes opilio* (O. Fabricius). *Journal of Crustacean Biology* 13: 1-16.
- Boschi, E. E. 1964. Los crustáceos decápodos *Brachyura* del litoral bonaerense (R. Argentina). *Boletín del Instituto de Biología Marina* 6: 1-76.
- Bryant, A. D., and R. G. Hartnoll. 1995. Reproductive investment in two spider crabs with different breeding strategies. *Journal of Experimental Marine Biology and Ecology* 188: 261-275.
- Burfitt, A. H. 1980. Glucose phosphate isomerase inheritance in *Cancer pagurus* L. broods as evidence of multiple paternity (Decapoda: Brachyura). *Crustaceana* 39: 306-310.
- Castiglioni, D. da S., and M. L. Negreiros-Fransozo. 2006. Ciclo reproductivo do caranguejo *Uca rapax* (Smith) (Crustacea, Brachyura, Ocypodidae) habitante de um estuário degradado em Paraty, Rio de Janeiro, Brasil. *Revista Brasileira de Zoologia* 23: 331-339.
- Comeau, M., and G. Conan. 1992. Morphometry and gonad maturity of males snow crab, *Chionoecetes opilio*. *Canadian Journal and Fisheries and Aquatic Science* 49: 2460-2468.
- Conan, G., and M. Comeau. 1986. Functional maturity and terminal molt of the male snow crab, *Chionoecetes opilio*. *Canadian Journal of Aquatic Science* 43: 1710-1719.
- , ———, and M. Moriyasu. 2001. Are morphometrical approaches appropriate to establish size at maturity for male american lobster *Homarus americanus*. *Journal of Crustacean Biology* 21: 937-947.
- Corgos, A., and J. Freire. 2006. Morphometric and gonad maturity in the spider crab *Maja brachydactyla*: a comparison of methods for estimating size at maturity in species with determinate growth. *ICES Journal of Marine Science* 63: 851-859.
- Davidson, R. J., and I. D. Marsden. 1987. Size relationships and relative growth of the New Zealand Swimming Crab *Ovalipes catharus* (White, 1843). *Journal of Crustacean Biology* 7: 308-317.
- de Haan, W. 1833-1850. Crustacea. In, P. P. von Siebold (ed.), *Fauna Japonica sive Descriptio Animalium, quae in Itinere per Japoniam, Jussu et Auspiciis Superiorum, qui Summum in India Batavia Imperium Tenent, Suscepit, Annis 1823-1830 Collegit, Notis, Observationibus et Adumbrationibus Illustravit*: 1-8, i-xvii, i-xxxii, 1-224, pis. 1-55, A-Q, 1-2. J. Miller & Sons, Leiden.
- de Lestang, S., N. G. Hall, and I. C. Potter. 2003. Reproductive biology of the blue swimmer crab (*Portunus pelagicus*, Decapoda: Portunidae) in five bodies of water on the west coast of Australia. *Fishery Bulletin* 101: 745-757.
- Derby, C. D. 1982. Structure and function of cuticular sensilla in the lobster *Homarus americanus*. *Journal of Crustacean Biology* 2: 1-21.
- Diesel, R. 1989. Structure and function of the reproductive system of the symbiotic spider crab *Inachus phalangium* (Decapoda: Majidae): observations on sperm transfer, sperm storage and spawning. *Journal of Crustacean Biology* 9: 266-277.
- . 1990. Sperm competition and reproductive success in the decapod *Inachus phalangium* (Majidae) – a male ghost spider crab that seals off rivals sperm. *Journal of Zoology* 220: 213-223.
- . 1991. Sperm competition and the evolution of mating behavior in Brachyura, with special reference to spider crabs (Decapoda, Majidae), pp. 145-163. In, R. T. Bauer and J. W. Martin (eds.), *Crustacean Sexual Biology*. Columbia University Press, New York, NY.
- Donaldson, W. E., and A. E. Adams. 1989. Ethogram of behavior with emphasis on mating for the tanner crab *Chionoecetes bairdi* Rathbun. *Journal of Crustacean Biology* 9: 37-53.
- Fabricius, J. C. 1775. *Sistema Entomologiae, sistens Insectorum Classes, Ordines, Genera, Species, adiectis Synonymis, Locis, Descriptionibus, Observationibus*. Korte: Flensburgi & Lipsiae, xxvii + 832 pp.
- . 1788. *Mantissa Insectorum sistens eorum species nuper detectas adiectis Characteribus generis Differentiis specificis Emendationibus, Observationibus*. I: i-xx, 1-348. Hafniae.
- Felgenhauer, B. E., and L. G. Abele. 1983. Ultrastructure and functional morphology feeding and associated appendages in the tropical freshwater shrimp *Atya innocuous* with notes on its ecology. *Journal of Crustacean Biology* 3: 336-363.
- Fenucci, J. L., and E. E. Boschi. 1975. Contribución al conocimiento biológico del cangrejo comercial de las aguas costeras de la Provincia de Buenos Aires *Ovalipes trimaculatus* (de Haan) (Crustacea, Decapoda, Portunidae). *Physis A* 34: 291-308.

- Fumis, P. B., A. Fransozo, and G. B. Braga. 2005. Morphometry of the crab *Hexapanopeus schmitti* (Decapoda: Xanthoidea) on the northern coast of the state of São Paulo, Brazil. *Revista de Biología Tropical* 55: 163-169.
- Garm, A. 2004. Revising the definition of the crustacean seta and setal classification systems based on examinations of the mouthpart setae of seven species of decapods. *Zoological Journal of the Linnean Society London* 142: 233-252.
- Goldstein, M., and E. Dupré. 2010. Sistema reproductivo de hembras y machos de *Cancer setosus* (Molina, 1872) (Decapoda, Brachyura). *Latin American Journal of Aquatic Research* 38: 274-280.
- González-Gurriarán, E. L., J. F. Fernández, and R. Muiño. 1998. Mating and role of seminal receptacles in the reproductive biology of the spider crab *Maja squinado* (Decapoda: Majidae). *Journal of Experimental Marine Biology and Ecology* 220: 269-285.
- Gorvett, H. 1946. The tegumental glands in the land Isopoda. A. The rosette glands. *Journal of Microscopical Science* 87: 209-235.
- Guinot, D., and G. Quenette. 2005. The spermatheca in podotreme crabs (Crustacea, Decapoda, Brachyura, Podotremata) and its phylogenetic implications. *Zoosystema* 27: 267-342.
- , M. Tavares, and P. Castro. 2013. Significance of the sexual openings and supplementary structures on the phylogeny of brachyuran crabs (Crustacea, Decapoda, Brachyura), with new nomina for higher-ranked podotreme taxa. *Zootaxa* 3665: 1-414.
- Haefner, P. A. Jr. 1985. Morphometry, reproduction, diet, and epizootes of *Ovalipes stephensoni* Williams, 1976 (Decapoda, Brachyura). *Journal of Crustacean Biology* 5: 658-672.
- Hankin, D. G., N. Diamond, M. S. Mohr, and J. Ianelli. 1989. Growth and reproductive dynamics of adult female Dungeness crabs (*Cancer magister*) in northern California. *Journal of Conseil Permanent International pour l'Exploration de la Mer* 46: 94-108.
- Hartnoll, R. G. 1963. The biology of Manx spider crabs. *Proceedings of the Zoological Society of London* 47: 79-300.
- . 1968. Morphology of the genital ducts in female crabs. *Journal of the Linnean Society of London, Zoology* 47: 279-300.
- . 1974. Variation in growth pattern between some secondary sexual characters in crabs (Decapoda: Brachyura). *Crustaceana* 27: 51-156.
- . 1975. Copulatory structure and function in the Dromiacea and their bearing on the evolution of the Brachyura. *Publicazioni della Stazione Zoologica di Napoli* 39: 657-676.
- Herbst, J. F. W. 1782-1804. Versuch einer Naturgeschichte der Krabben und Krebse. Berlin und Stralsund. 3 vols, 72 pls.
- Hinsch, G. W. 1972. Some factors controlling reproduction in the spider crab *Libinia emarginata*. *Biological Bulletin* 143: 358-366.
- . 1986. A comparison of sperm morphologies, transfer and sperm mass storage between two species of crab, *Ovalipes ocellatus* and *Libinia emarginata*. *International Journal of Invertebrate Reproduction and Development* 10: 79-87.
- Homola, E., A. Sagi, and H. Laufer. 1991. Relationship of claw form and exoskeleton condition to reproductive system size and methyl farnesoate in the male spider crab *Libinia emarginata*. *Invertebrate Reproduction and Development* 20: 219-225.
- Jensen, P. C., J. M. Orensanz, and A. Armstrong. 1996. Structure of the female reproductive tract in the Dungeness crab (*Cancer magister*) and implications for the mating system. *Biological Bulletin* 190: 336-349.
- Jivoff, P. 1997. The relative role of predation and sperm competition on the duration of the post-copulatory association between the sexes in the blue crab *Callinectes sapidus*. *Behaviour Ecology and Sociobiology* 40: 175-185.
- Johnson, P. T. 1980. *Histology of the blue crab Callinectes sapidus: a model for the Decapoda*. Praeger, New York, NY.
- Jones, D. R., and R. G. Hartnoll. 1997. Mate selection and mating behavior in spider crabs. *Estuarine, Coastal and Shelf Science* 44: 185-193.
- Lai, J. C. Y., J. C. E. Mendoza, D. Guinot, P. F. Clark, and P. K. L. Ng. 2010. Xanthidae Mac Leay, 1838 (Decapoda: Brachyura: Xanthoidea) systematics: a multi-gene approach with support from adult and zoeal morphology. *Zoologischer Anzeiger* 250: 407-448.
- Laufer, H., and J. S. B. Ahl. 1995. Mating behaviour and methyl farnesoate levels in male morphotypes of the spider crab *Libinia emarginata* (Leach). *Journal of Experimental Marine Biology and Ecology* 193: 15-20.
- Linnaeus, C. 1758. *Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis*. Edition 10, 1: i-iii, 1-824.
- Mantelatto, F. L., and A. Fransozo. 1999. Reproductive biology and molting cycle of the crab *Callinectes ornatus* (Decapoda: Portunidae) from the Ubatuba Region, São Paulo, Brazil. *Crustaceana* 72: 63-76.
- Melo, G. A. S. 1996. *Manual de Identificação dos Brachyura (caranguejos e siris) do Litoral Brasileiro*. Plêiade, São Paulo.
- Milne Edwards, H. 1834-1837. *Histoire naturelle des Crustacés comprenant l'anatomie, la physiologie et la classification de ces animaux*. Libraire Encyclopedique de Roret, Paris. Vol. 1: i-xxxv, 1-468. Vol. 2: 1-531. Atlas, 1837: 1-32, pls. 1-42. Vol. 3: 1840: 1-638.
- , and H. Lucas. 1842-1844. *Crustacés*, pp. 1-39. In: A. d'Orbigny (ed.), *Voyage dans l'Amérique méridionale (le Brésil, la République orientale de l'Uruguay, la République Argentine, la Patagonie, la République du Chili, la République de Bolivie, la République de Pérou)*, exécuté pendant les Années 1826, 1827, 1828, 1829, 1830, 1831, 1832 et 1833. Bertrand, Paris.
- Mura, M., F. Orrùand, and A. Cau. 2005. Size at sexual maturity of the spider crab *Anamathia rissoana* (Decapoda: Majoidea) from the Sardinian Sea. *Journal of Crustacean Biology* 25: 110-115.
- Narvarte, M., R. González, P. Osovnikar, M. Camarero, L. Curtolo, and M. Reinaldo Ocampo. 2007. Experimental trap fishery for the crabs *Platyanthus patagonicus* and *Ovalipes trimaculatus* in the San Matias Gulf, Patagonia, Argentina. *Journal of the Marine Biological Association of the United Kingdom* 87: 1235-1242.
- Neumann, V. 1996. Comparative gonopod morphology of the European spider crabs of the genus *Maja* Lamarck 1801. *Senckenbergiana Biologica* 75: 143-157.
- Ordway, A. 1863. *Monograph of the genus Callinectes*. Boston Journal of Natural History 7: 567-583.
- Orensanz, J. M., A. M. Parma, D. A. Armstrong, J. Armstrong, and P. Wardrup. 1995. The breeding ecology of *Cancer gracilis* (Crustacea: Decapoda: Cancridae) and the mating systems of cancrid crabs. *Journal of Zoology* 235: 411-437.
- Padate, V. P., C. U. Rivonker, A. C. Anil, S. S. Sawant, and V. Krishnamurthy. 2010. A new species of portunid crab of the genus *Charybdis* (De Haan, 1833) (Crustacea: Decapoda: Brachyura) from Goa, India. *Marine Biology Research* 6: 579-590.
- Phillips, B. F., and D. L. Macmillan. 1987. Antennal receptors in puerulus and postpuerulus stages of the rock lobster *Panulirus cygnus* (Decapoda: Palinuridae) and their potential role in puerulus navigation. *Journal of Crustacean Biology* 7: 122-135.
- Rathbun, M. J. 1896. The genus *Callinectes*. *Proceedings of the United States National Museum* 18: 349-375.
- Rorandelli, R., F. Paoli, S. Cannicci, D. Mercati, and F. Giusti. 2008. Characteristics and fate of the spermatozoa of *Inachus phalangium* (Decapoda: Majidae): description of novel sperm structures and evidence for an additional mechanism of sperm competition in Brachyura. *Journal of Morphology* 269: 259-271.
- Rotllant, G., P. Takac, L. Liu, G. L. Scott, and H. Laufer. 2000. Role of ecdysteroids and methyl farnesoate in morphogenesis and terminal moult in polymorphic males of the spider crab *Libinia emarginata*. *Aquaculture* 190: 103-118.
- Ryan, E. P. 1967. Structure and function of the reproductive system of the crab *Portunus sanguinolentus* (Herbst) (Brachyura: Portunidae). II. Female system. *Journal of the Marine Biological Association of India. Symposium Series* 2: 522-544.
- Sainte-Marie, B., and C. Carrière. 1995. Fertilization of the second clutch of eggs of snow crab, *Chionoecetes opilio*, from females mated once or twice after their molt to maturity. *Fishery Bulletin of the United States* 93: 758-763.
- , T. Gosselin, J. M. Sévigny, and N. Urbani. 2008. The snow crab mating system: opportunity for natural and unnatural selection in a changing environment. *Bulletin of Marine Science* 83: 131-161.
- , and G. A. Lovrich. 1994. Delivery and storage of sperm at first mating of female *Chionoecetes opilio* (Brachyura: Majidae) in relation to size and morphometric maturity of male parent. *Journal of Crustacean Biology* 14: 508-521.
- Sainte-Marie, G., B. Sainte-Marie, and J.-M. Sévigny. 2000. Ejaculate-storage patterns and the site of fertilization in female snow crabs (*Chionoecetes opilio*, Brachyura, Majidae). *Canadian Journal of Zoology* 78: 1902-1917.
- Sal Moyano, M. P., M. A. Gavio, and E. I. Cuartas. 2011a. Copulatory system of the spider crab *Libinia spinosa* (Crustacea: Brachyura: Majoidea). *Journal of the Marine Biological Association of the United Kingdom* 91: 1617-1625.

- , ———, and M. Maggi. 2011b. Morphometric and gonad maturity of the spider crab *Libinia spinosa* (Crustacea: Brachyura: Majoidea: Epialtidae) in Argentina. *Journal of the Marine Biological Association of the United Kingdom* 91: 837-844.
- Schejter, L., and E. Spivak. 2005. Morphometry, sexual maturity, fecundity and epibiosis of the South American spider crab *Libidoclea granaria* (Brachyura: Majidae). *Journal of Marine Biology* 85: 857-863.
- Shirley, T. C., and L. McNutt. 1989. Precocious mating and trans-molt sperm retention by female Dungeness crabs. *American Zoologist* 29: 131 A.
- Somerton, D. A. 1980. A computer technique for estimating the size of sexual maturity in crabs. *Canadian Journal of Fisheries and Aquatic Science* 37: 1488-1494.
- Spivak, E. D. 1997. Cangrejos estuariales del Atlántico sudoccidental (25°-41°S) (Crustacea: Decapoda: Brachyura). *Investigaciones Marinas, Valparaíso* 25: 105-120.
- Stephenson, W. 1968. Studies on *Portunus pelagicus* (Linnaeus) and *P. sanguinolentus* (Herbst). *Occasional Papers of the Bernice P. Bishop Museum* 23: 385-399.
- Stimpson, W. 1871. Preliminary report on the Crustacea dredged in the Gulf Stream in the Straits of Florida, by L. P. de Pourtalès, Assist. U. S. Coast Survey. Part I. Brachyura. *Bulletin of the Museum of Comparative Zoology* 2: 109-160.
- Talbot, P., and P. Zao. 1991. Secretions at molting by the pleopod tegumental glands of the lobster *Homarus americanus* (Milne Edwards). *Journal of Crustacean Biology* 11: 1-9.
- Vallina, M. 2007. Estructura poblacional, crecimiento relativo y características morfológicas asociadas a la reproducción del cangrejo comercial *Ovalipes trimaculatus* (Crustacea: Decapoda: Portunidae) en la costa de Mar del Plata. Licentiate Thesis, Universidad Nacional de Mar del Plata, Argentina, 63 pp.
- von Martens, E. 1868. Über eine neue Art und Untergattung der Cyprinoiden, *Homaloptera* (*Octonema*) *rotundicauda*, über einige neue Crustaceen und über die neuholländischen Süßwasserkrebse. *Monatsberichte der Königlichen Preußischen Akademie der Wissenschaften zu Berlin* 1868: 607-620.
- Wee, D. P. C., and P. K. L. Ng. 1995. Swimming crabs of the genera *Charybdis* de Haan, 1833, and *Thalamita* Latreille, 1829 (Crustacea: Decapoda: Brachyura: Portunidae) from Peninsular Malaysia and Singapore. *Raffles Bulletin of Zoology* 1: 1-128.
- White, A., and E. Doubleday. 1843. List of the Annulose animals hitherto recorded as found in New Zealand, with the descriptions of some new species, pp. 265-295. In, E. Dieffenbach (ed.), *Travels in New Zealand; with contributions to the geography, geology, botany, and natural history of that country*. Vol. II. John Murray, London.

RECEIVED: 30 October 2013.

ACCEPTED: 31 March 2014.