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# **Research Article**

# Reproductive biology of the spider crab *Leucippa pentagona* (Decapoda: Epialtidae), in Bustamante Bay, Argentina

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**ABSTRACT.** Sizes at maturity, breeding period, and fecundity of the spider crab *Leucippa pentagona* in Bustamante Bay, Argentina (45.11°S, 66.56°W) were analyzed. Specimens were collected monthly between May 2006 and February 2008. The size at morphometric maturity was determined based on relative changes in cheliped length for males and abdomen width for females. The presence of spermatophores in the male deferent ducts and of eggs in the female abdomens were the criteria used to determine physiological maturity. The temporal variation of the proportion of ovigerous females was analyzed. Oocyte sizes in ovigerous females were measured to evaluate the occurrence of multiple spawnings. In males, the sizes at morphometric and physiological maturity was 18.5 mm CL, whereas size at physiological maturity was 18.25 CL. Ovigerous females were found between June and February in both years. Ovigerous females were found to have oocytes with advanced vitellogenesis. Fecundity was similar in both years and varied between 645 and 7114 eggs. The extended breeding period and the presence of vitellogenic oocytes in ovigerous females suggest the occurrence of two or more spawnings per reproductive season. Finally, the distribution range of this species in the Atlantic Ocean was extended from 43.3° to 45.11°S.

Keywords: spider crab, maturity, fecundity, multiple spawning, Bustamante Bay, Argentina.

# Biología reproductiva del cangrejo araña *Leucippa pentagona* (Decapoda: Epialtidae), en Bahía Bustamante, Argentina

**RESUMEN.** Se analizó las tallas de madurez sexual, el periodo de ocurrencia de hembras ovígeras y la fecundidad del cangrejo araña *Leucippa pentagona* en Bahía Bustamante, Argentina (45,11°S, 66,56°W). Los ejemplares fueron muestreados mensualmente entre mayo 2006 y febrero 2008. La determinación de la talla de madurez morfométrica se basó en cambios relativos en la longitud del quelípedo y el ancho del abdomen para machos y hembras, respectivamente. La presencia de espermatóforos en el conducto deferente de los machos y de huevos en las hembras fueron los criterios empleados para determinar la madurez fisiológica. Se analizó la variación temporal de la proporción de hembras ovígeras. El tamaño de los oocitos de hembras ovígeras fue medido para evaluar la ocurrencia de desoves múltiples. Las tallas de madurez morfométrica y fisiológica en los machos fueron 28,05 y 16,7 mm de longitud de caparazón (LC), respectivamente. En las hembras, la talla de madurez morfométrica fue 18,5 mm de LC y la de madurez fisiológica fue 18,25 mm de LC. Hembras ovígeras ocurren desde junio a febrero, en ambos años. En hembras ovígeras se encontraron oocitos en vitelogenesis avanzada. La fecundidad fue similar en ambos años y varió entre 645 y 7114 huevos. El prolongado periodo en que las hembras portan huevos y la presencia de hembras ovígeras con oocitos vitelogénicos sugieren la ocurrencia de dos o más desoves durante una temporada reproductiva. Finalmente, se amplía el rango de distribución de esta especie en el océano Atlántico desde 43,30° hasta 45,11°S.

Palabras clave: cangrejo araña, madurez sexual, fecundidad, puestas múltiples, Bahia Bustamante, Argentina.

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# INTRODUCTION

The superfamily Majoidea is a major group of brachyurans commonly known as spider crabs, with a diversity of body shapes and wide geographical distribution. Nearly 250 majid species have been reported in coastal waters of America, 11 of which exhibit an amphioceanic distribution. Nine species were recorded in Argentine waters: Eurypodius latreillei Guérin, 1828; Collodes rostratus A. Milne Edwards, 1878; Leurocyclus tuberculosus (H. Milne Edwards & Lucas, 1842), Libinia spinosa H. Milne Edwards, 1834; Libidoclaea granaria H. Milne Edwards & Lucas, 1842; Rochinia gracilipes A. Milne Edwards, 1875; Pelia rotunda A. Milne Edwards, 1875; Pyromaia tuberculata (Lockington, 1877) and Leucippa pentagona Milne Edwards, 1833 (Boschi et al., 1992; Schejter et al., 2002). The spider crab L. pentagona belongs to the family Epialtidae, which is mainly characterized by having the first pereiopods disproportionately longer than the others. This species is the only representantive of the genus (Ng et al., 2008).

Leucippa pentagona is a small amphioceanic species, the maximum adult size recorded is 30 mm carapace length (CL) in Argentina (Boschi et al., 1992) and 20 mm CL in Brazil (Melo, 1996). The species is found on subtidal soft bottoms at depths between 20 and 80 m, often associated with aggregations of benthic algae (Boschi et al., 1992) and mytilid beds (Bremec & Roux, 1997). However, the bathymetric range is higher than reported in previous studies because it can be found from the upper sublitoral (Durbas, pers. comm). This crab is distributed from California to southern Chile in the Pacific Ocean and from Brazil to the Argentine Province of Chubut in the western Atlantic Ocean. According to Boschi et al. (1992), the Rawson harbor  $(43.30^{\circ}\text{S})$  is the southern limit of its distribution in the Atlantic Ocean.

Some majid species have been thoroughly studied due to their high abundance and economic importance, while there is little information on most majids inhabiting the Argentine coast. At the present time, sexual maturity of *Leurocyclus tuberculosus* (Barón *et al.*, 2009) and *Libidoclea granaria* (Schejter & Spivak, 2005) have been studied. This paper analyzed sex ratio, sizes at sexual maturity, reproductive season and fecundity of *L. pentagona* from Bustamante Bay (Argentina) and represents the first contribution to the knowledge of benthic stages of *L. pentagona*, including first data concerning its reproduction.

# MATERIALS AND METHODS

# Study area and sampling

This study was carried out in Bustamante Bay  $(45.11^{\circ}$  S,  $66.56^{\circ}$  W), in the north coast of San Jorge Gulf (Fig. 1) where a well established population of *Leucippa pentagona* was found. It is an enclosed bay with dense beds of the economically important red alga *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine & Farnham growing in the subtidal environment (Martin *et al.*, 2010). The area is influenced by cold-temperate waters of the Patagonian Current and, occasionally, by warmer waters of the Brazil Current during summer.

Sampling was performed in subtidal algae beds of Bustamante Bay between May 2006 and February 2008. Samples were arranged along four transects parallel to the shore from the low tide line to 20 m depth. Each month, a total of 20 sampling units (0.25  $m^2$ ) were collected. All crabs included in the sampling units were collected manually by a scuba diver. Crabs were fixed in 4% saline formaldehyde for further analysis in the laboratory.

### Sizes at sexual maturity

The determination of morphometric maturity was based on changes in relative growth of secondary



Figure 1. Location of Bustamante Bay in northern coasts of San Jorge Gulf, southwestern Atlantic Ocean.

**Figura 1.** Localización de Bahía Bustamante en el norte del Golfo San Jorge, océano Atlántico sudoccidental.

sexual characters. Sizes at the onset of sexual maturity were indicated by changes in the relative growth of the cheliped and abdomen width for males and females respectively. In all crabs collected, carapace length (CL) was measured from the tip of the rostrum to the posterior margin of the carapace. All measures were made with a digital calliper to the nearest 0.01 mm. In males, length (ChL) and height (ChH) of the cheliped propodus were also recorded. Chela symmetry was tested using a Student's t test for paired samples. In females, the maximum abdomen width (AW) was measured. For both sexes, morphometric maturity was evaluated using the computer routine Mature 1 (Somerton, 1980) adapted for Microsoft Office Excel. This routine estimates the reference value (CL) at which 50% of the specimens show a change in the relative growth of a secondary sexual character.

Physiological maturity refers to the CL at which crabs have mature gonads. This was estimated by the presence of spermatophores in the deferent ducts of males and by eggs attached to pleopods in females. Males were dissected and deferent ducts were removed. A smear of the deferent ducts was examined under a compound microscope at 100x magnification. Physiological maturity for both sexes was estimated using a logistic regression at size intervals of 1 mm. The size at physiological maturity was defined as the CL at which 50% of the crabs have mature gonads in males and eggs in females (Wenner *et al.*, 1974).

## Sex ratios and breeding period

Monthly variation in sex proportion was analyzed considering only physiologically mature specimens. To determine whether the sex ratios differed significantly from the 1:1 expected ratio, a goodness of fit  $\chi^2$  test (Yates correction) were used (Zar, 2010).

Reproductive period was analyzed by the temporal variation of ovigerous females. This was determined by the presence of embryonated eggs. The proportion of ovigerous females was calculated in relation to specimens with a CL larger than the mean size at physiological maturity. Analysis of the vitellogenic oocyte size of ovigerous females was conducted to identify possible multiple spawnings during a reproductive season. Oocyte size was measured monthly from a subsample of 10 ovigerous females, whose carapace was removed and the gonads were dissected. The ovary was removed and oocytes were separated on a slide. About 50 oocytes from each ovigerous female were measured to the nearest 0.01 mm. Due to deformation of advanced oocytes, we measured the minimum (D1) and maximum diameter (D2). Then, the mean diameter (D) was estimated as D = (D1 + D2)/2.

#### Eggs stages and realized fecundity

Approximately, forty eggs from each ovigerous individual were separated from pleopods, classified and measured ( $\pm$  0.01 mm). Eggs were classified according to a maturity scale based on the volume occupied by yolk, stage of embryo development and mean egg diameter.

Realized fecundity is defined as the number of eggs within the brood carried by a female (Anger & Moreira, 1989). This analysis was estimated using gravimetric procedures and did not include broods at late developmental stages, since losses during embryo development may lead to an underestimate of fecunddity. The crabs were measured (CL) and the egg mass were detached from pleopodal setae. Eggs were ovendried at 60°C to constant weight, which was determined with an analytical balance to the nearest 0.0001 g. A fraction of no less than 300 eggs was counted under stereoscopic microscope. The relationship between the number of eggs and CL was also tested. An analysis of covariance (ANCOVA) was used to compare the fecundity between the reproductive periods June 2006-February 2007 and June 2007-February 2008 using CL as a covariate. Size-adjusted mean fecundities were estimated for each period. Assumptions of homogeneity of variances using Cochran C test and homogeneity of slopes was tested prior to performing ANCOVA (Zar, 2010)

## RESULTS

#### Sexual maturity

The maximum sizes registered were 34.65 mm CL for males and 26.95 mm CL for females. Males showed symmetry in the length (Paired t test,  $t_{116} = -0.78$ ; P =0.43) and height (Paired t test,  $t_{116}$ = 0.16; P = 0.86) of the cheliped propodus. Morphometric maturity was calculated for 297 males. A change in the relative length of cheliped was observed at 28.05 mm CL for 50% of males. The largest juvenile had a CL of 28.6 mm and the smallest adult a CL of 23.15 mm. The slope of the regression of ChL on the CL for juvenile males was significantly smaller than for mature males  $(F_{(1, 293)} = 124; P < 0.001)$  (Fig. 2a). Size of males at physiological maturity was 16.7 mm CL (Fig. 2b). The deferent ducts were translucent, thin and poorly developed in immature males; opaque and expanded, with spermatophores in intermoult mature males; and translucent and without spermatophores in postmoult mature males. The smallest male with spermatophores in the deferent ducts was 13.85 mm CL.

Morphometric maturity was calculated for 315 females. Mature and immature females are different-



**Figure 2.** Sexual maturity in males of *Leucippa pentagona*. a) Morphological maturity is based in the relationship between carapace length (CL) and the length of right cheliped (ChL), b) physiological maturity, proportion of mature on the basis of the presence of spermatophores in the deferent ducts.

**Figura 2.** Madurez sexual en machos de *Leucippa pentagona*. a) Madurez morfológica basada en la relación entre la longitud del caparazón (CL) y la longitud de la quela derecha (ChL), b) madurez fisiológica, la proporción de machos maduros está determinada por la presencia de espermatóforos en el conducto deferente.

tiated by the morphology and relative width of the abdomen (AW). Size at morphological maturity was 18.5 mm CL. The slope of the regression of AW on the CL for juvenile females was significantly smaller than for mature females ( $F_{(1, 210)} = 28.2$ ; P < 0.001) (Fig. 3a). The size at which 50% of the females reached physiological maturity was 18.25 CL (Fig. 3b). Smallest physiologically mature female was 16.91 mm CL. All the ovigerous females were morphologically mature.

#### Sex ratio and breeding period

During the studied period the female:male proportion was 1: 0.66, differing significantly from the expected 1:1 (Chi-square test  $\chi^2 = 22.07$ ; df = 1; P < 0.01). Sex ratios varied across months and no significant differences were found in some months. A well defined reproductive season was detected at both



**Figure 3.** Sexual maturity in females of *Leucippa pentagona*. a) Morphological maturity is based in the relationship between carapace length (CL) and abdomen width (AW), b) physiological maturity, proportion of mature on the basis of the presence of eggs attached to pleopods

**Figura 3.** Madurez sexual en hembras de *Leucippa pentagona.* a) Madurez morfológica basada en la discontinuidad en la relación lineal entre la longitud del caparazón (CL) y ancho del abdomen (AW), b) madurez fisiológica, la proporción de hembras maduras está determinada por la presencia de huevos adheridos a los pleópodos.

studied years. The breeding period began in June and continued through the following nine months. The proportion of ovigerous females reached a peak in spring and dropped to a minimum at the end of summer. This pattern was similar at both reproductive seasons (Fig. 4).

Oocytes in advanced vitellogenesis were identified by the presence of abundant yolk platelets in the cytoplasm. Oocyte size ranged between 0.19 and 0.56 mm. Ovigerous females with larger oocytes were found in August and in subsequent months until February. In summer, a few females have eggs, most females have their gonads retracted (Fig. 5).

#### Eggs stages and fecundity

Four egg stages could be distinguished and were described in Table 1 In both years, there were abun-



**Figure 4.** Temporal variation of percentages of ovigerous females of *Leucippa pentagona* in Bustamante Bay between May 2006 and February 2008.

**Figura 4.** Variación temporal del porcentaje de hembras ovígeras de *Leucippa pentagona* en Bahía Bustamante entre mayo 2006 y febrero 2008.

dant females with eggs at early stages of development, while females with eggs at late stages of development (IV) were observed from August onwards, but at a low percentage (7.34%). Realized fecundity was analyzed in 123 specimens. The number of eggs carrying by ovigerous females ranged between 645 and 7114 eggs. The log-log relationship of eggs number to CL was linear. Fecundity did not differ significantly between two reproductive seasons studied (ANCOVA, F (1, 119) = 1.07; P = 0.30) (Fig. 6). The size-adjusted mean fecundities were 2618 eggs (SE = 129, N = 55) and 2483 eggs (SE = 63, N = 68) for the first and second reproductive seasons, respectively.

## DISCUSSION

Reproductive biology is an important issue to elucidate the population dynamics of a species and to establish management and conservation plans. Decapods exhibit a variety of reproductive patterns because of the high diversity of the group and the variety of environments they inhabit. The size at sexual maturity, fecundity and duration of the reproductive season are the most common reproductive aspects reported in the literature (e.g. Hartnoll, 1963; Sainte-Marie, 1993; Hernández-Reyes et al., 2001; Cobo, 2002; Vinuesa & Balzi, 2002; Vinuesa, 2007a; Monteiro-Teixeira et al., 2009).

Sexual maturity is an important life history trait of decapods and traditionally has been analyzed from three points of view: a) morphometric maturity (Hartnoll, 1978; Somerton, 1980; Paul, 1992; Corgos & Freire, 2006); b) physiological mature (Paul, 1992;

Sainte-Marie et al., 1995; Sampedro et al., 1999) and c) functional maturity (Conan & Comeau, 1986; Mura et al., 2005). According with Corgos & Freire (2006) physiological maturity in male crabs usually takes place one or two moults before morphometric maturity. Male gonads mature before the terminal moult in many majid species such as Chionoecetes opilio Fabricius, 1788 (Conan & Comeau, 1986); Chionoecetes bairdii Rathbun, 1924 (Paul, 1992); Maja brachydactyla Balss, 1922 (Corgos & Freire, 2006); and Anamathia rissoana (Roux, 1828) (Mura et al., 2005). In males of L. pentagona, the size of morphological maturity is 67% bigger than the size of physiological maturity. Given that in other majoids species, the increased size at the terminal moult was lesser than 40% (Tunberg & Creswell, 1991; González-Gurriarán et al., 1995; Hartnoll & Bryant, 2001), our results suggests that males L. pentagona reach a physiological maturity, two or more moults before morphometric maturity. However, sizes at morphometric and physiology maturity were similar by females of L. pentagona. The synchronization of morphometric and physiological maturity sizes in females is a fact that can be considered normal in spider crabs (Hartnoll, 1963; Mura et al., 2005).

In several Majoidea, the size of morphometric maturity is greater for males than females. This has been reported for *C. bairdii* (Donaldson *et al.*, 1981), *Mithraculus forceps* (A. Milne Edwards, 1875), (Hernández-Reyes *et al.*, 2001) and *Acanthonyx scutiformis* (Dana, 1851) (Monteiro-Texeira *et al.*, 2009). Similarly, the males of *L. pentagona* attained morphological maturity and reached a maximum size at larger CL than females, possibly related to female holding during mating. In addition, it is well known that males expend more energy on growth and development of secondary sexual characters, while females invest more in oogenesis and embryo care.

Skewness in sex ratio has been frequently documented in many marine invertebrate populations, including crustaceans. According to Wenner (1972) this may result from: a) different nutritional limitations, b) activity differences, c) differential movement, d) differential habitat selection, and e) differential mortality rates, among other factors. In addition, differences in maturity size may determine deviations in sex ratio of adults because they would be subject to different predation pressures (Monteiro-Texeira *et al.*, 2009). Little is known about predation in *L. pentagona*, but is expected that females are more vulnerable to predation, given their smaller size. Moreover, dense algae beds and other complex habitat



**Figure 5.** Vitellogenic oocyte size distribution in ovigerous females of *Leucippa pentagona*. **Figura 5.** Distribución de tallas oocitarias en hembras ovígeras de *Leucippa pentagona*.

are known to increase survival during incubation in crab populations (Warner, 1967; Monteiro-Teixeira *et al.*, 2009). Difference in sex ratios of *L. pentagona* at Bustamante Bay could be related with a major

dependence of algae beds that provides shelter to females. However, the study area represents only part of the habitat of the species, which might contribute to the observed deviation between sexes.

Table 1. Eggs stages of *Leucippa pentagona*. The mean diameter and standard deviation are shown.Tabla 1. Estadios de desarrollo de los huevos de *Leucippa pentagona*. Se indica el diámetro medio y la desviación estándar para cada estadio.

Stage	Characteristics of the eggs	Diameter (mm)
Ι	Uniform mass of yolk that occupies the entire vol- ume of the egg. No visible development.	$0.441 \pm 0.067$
II	Embryo visible. The yolk occupies approximately 80% egg volume. Eye spots pigmented with a crescent shape.	$0.498 \pm 0.054$
III	The yolk is approximately 40% of egg volume. The dark eyes are rounded and are visible the cephalic appendages.	$0.522 \pm 0.045$
IV	The yolk is scarce. It has the same features as the Stage III, but also thoracic appendages are observed. The embryo looks like a zoea.	$0.526 \pm 0.039$



**Figure 6.** Relationship between Log number of eggs and Log carpace length (mm). Black and gray lines are the linear fit for the first and second reproductive seasons, respectively.

**Figura 6.** Relación lineal entre el Log del número de huevos y Log de la longitud del caparazón. Las líneas negra y gris son las rectas de regresión para la primera y segunda temporada reproductiva, respectivamente.

In crustaceans, the duration of the reproductive season is strongly influenced by environmental variations. Continuous reproductive patterns are observed in species inhabiting tropical, subtropical or very deep waters, with stable temperatures throughout the year, while seasonal reproductive patterns are associated with variable environmental conditions. Populations subjected to seasonal variations must adjust their life cycle to take advantage of more favourable conditions (Sastry, 1983). The presence of multiple spawnings and ovigerous females all year round have been reported for some low-latitude species of Majoidea, such as *Microphrys bicornutus* Latreille, 1825 (López-Greco *et al.*, 2000), *M. forceps*  (Hernández-Reyes et al., 2001; Cobo, 2002) and Acanthonyx scutiformis (Monteiro-Texeira et al., 2009). Seasonal reproductive patterns have been observed in Chionoecetes opilio (Sainte-Marie, 1993) and C. japonicus (Yosho, 2000). The prolonged reproductive season of L. pentagona in Bustamante Bay contrasts with the much shorter seasonal reproduction pattern of other species in the study area, such as Lithodes santolla Molina 1882 (Vinuesa & Balzi, 2002), Munida gregaria Fabricius, 1793 (Vinuesa, 2007a), or even with the alien crab Carcinus maenas (Vinuesa, 2007b). However, the presence of ovigerous females with advanced embryos in August implies that larvae eclosion occur during austral spring, similar to that observed in other decapods of the gulf. Temperature and food availability may favour larval survival during this period. The wide temporal distribution of ovigerous females is consistent with the fact that L. pentagona is typical of warm-temperate waters of the Argentine biogeographic province, in contrast to the seasonal spawning of both Magellanic species mentioned above. It is expected that in northern populations, the reproductive period and larval support of L. pentagona should be even more prolonged.

Distribution of ovigerous females and the presence of secondary vitellogenic oocytes in ovigerous females suggest the occurrence of two or more spawnings. The occurrence of ovigerous females with advanced eggs embryos and larger oocytes in August suggests that the females can produce a new spawning immediately after the hatching of the embryos of the previous brood. A synchronisation in the oocyte and embryo maturation is also described in *Pisa armata* (Latreille, 1803) (Vernet-Cornubet, 1958). Moreover, embryo and oocyte development in *P. armata* is faster in summer than in winter (Vernet-Cornubet, 1958). On this basis, embryonic development would be expected to have a shorter duration in spring than in winter, thereby allowing females to undergo multiple spawnings during the same reproductive season.

Fecundity is an important parameter for estimating the population replacement rate. A positive relationship between fecundity and size of female has been observed in several decapods species (e.g. Haynes et al., 1976; Vinuesa, 2007a; Cobo & Okamori, 2008). Spider crabs have a lower fecundity compared to other brachyurans, but this is compensated by a large egg size (Hines, 1982). The small spider crabs M. forceps (Hernández-Reyes et al., 2001) and Stenorhynchus seticornis (Herbst, 1788) (Okamori & Cobo, 2003). have a similar eggs size but a minnor fecundity that L. pentagona. An increase in embryonated eggs diameter with advancing embryonic development was registered in this work. According to Wear (1974) egg diameter increase is due to the osmotic absorption of water. This was particularly observed in S. seticornis (Okamori & Cobo 2003), Microphrys bicornutus Latreille, 1825 (López-Greco et al., 2000) and Mithrax forceps (A. Milne Edwards, 1875) (Hernández-Reyes et al., 2001) among other decapods species.

Finally, reproductive traits described here are consistent with the warm temperate origin of *L. pentagona*, but there are some adaptations to the cold temperate environment and reproduction in winter. In addittion, the present study provides the first biological data on benthic stages of *L. pentagona* and reports its first record in Bustamante Bay, San Jorge Gulf, which extends the latitudinal range of its distribution.

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