

Morphometric and gonad maturity of the spider crab *Libinia ferreirae* Brito Capello, 1871 (Decapoda: Majoidea: Epialtidae) on the south-eastern Brazilian coast

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Sexual maturity of the spider crab Libinia ferreirae was assessed for each sex, based on body dimensions and observations of gonad condition. A total of 346 crabs were analysed, of which 68% were females. Immature and adult individuals were recognized based on their allometric growth and gonad development. Abdomen width (AW) vs carapace width (CW) and propodus length (PL) vs CW were the relationships that best separated allometric groups of females and males, respectively. For females, gonad and allometric morphological maturity were, respectively, 38.77 and 39.43 mm of CW, which is close to the carapace size of the smallest ovigerous female (38.08 mm). For males, gonad maturity was 34.86 mm of CW and three allometric phases were observed: immature (IM♂), adolescent (AD♂) and adult morphometrically mature (MM♂). The IM♂ phase showed lower values of CW and PL than the AD♂ phase, without spermatophores inside the vas deferens; the AD♂ phase exhibited higher CW values than IM♂, but lower CW and PL values than the MM♂ phase, and the presence of spermatophores in the vas deferens; the MM♂ phase had higher values of CW and PL than the AD♂ phase and spermatophores in the vas deferens. Therefore, females showed synchronic morphometric, gonadal and functional maturity, while in males, gonadal maturity was attained before morphometric maturity, which probably could be a reproductive strategy for this species.

Keywords: Allometry, gonad maturity, morphometric maturity, morphotypes, spider crab

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INTRODUCTION

The onset of sexual maturity for brachyurans is one of the main criteria used to describe population dynamics and structure (Pinheiro & Fransozo, 1998). Knowledge concerning the legal minimum size for fishing, for both commercially exploited and accidentally captured (by-catch) species, is important for preserving marine biodiversity, allowing animals to reproduce before capture (Conan *et al.*, 2001).

Reproductive maturity in crustaceans may be assessed based on three lines of evidence: morphometric, as changes in relative growth of secondary sexual characters (such as the cheliped and abdomen); by the presence of mature oocytes or spermatozooids/spermatophores; and by the capacity to copulate and carry embryos (Hartnoll, 1969, 1974; López-Greco & Rodríguez, 1999; Viau *et al.*, 2006). For brachyuran crabs, morphological changes in male chelipeds and in the female abdomen commonly characterize the transition from morphologically immature to the morphologically

mature stage, which may be represented by pubertal moult (Hartnoll, 1974).

However, Majoidea crabs present a terminal moult (or pubertal moult, according to some authors – see Teissier, 1935; Hartnoll, 1978) after the prepubertal moult, featuring morphological maturity and the end of their growth (Hartnoll, 1974, 1982; Sampedro *et al.*, 1999; Sal Moyano *et al.*, 2011). As a consequence, this group reveals some reproductive peculiarities that have frequently been the subject of investigation, mainly concerning (1) the number of allometric stages in males, which varies from two (Comeau & Conan, 1992; Corgos & Freire, 2006) to three stages (Hartnoll, 1963, 1974; Carmona-Suárez, 2003), (2) different mating behaviour patterns for male morphotypes, as observed for *Libinia emarginata* Leach, 1815 and *Libinia spinosa* Milne-Edwards, 1834 by, respectively, Laufer & Ahl (1995) and Sal Moyano & Gavio (2012), or (3) the synchronism between gonad and morphometric maturities, which may occur concomitantly or one preceding the other (Corgos & Freire, 2006).

The species *Libinia ferreirae* Brito Capello, 1871 shows a wide distribution in the western Atlantic, occurring from Venezuela to Brazil (from Pará to Santa Catarina State), from the coastal region to depths down to 35 m, preferably in muddy bottoms (Melo, 1996). As a typical brachyuran, it plays an important role in marine food webs, occupying

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several different levels (Rocha *et al.*, 1998). Additionally, this species may present symbioses with algae, sponges and cnidarians, among other animals, using them as camouflage, which may have contributed to its evolutionary success. This characteristic allows these crabs to move over a wider area with a lower predatory rate, besides increasing the distribution of several sedentary or sessile species with which they are associated (Nogueira Junior & Haddad, 2005; Hultgren & Stachowicz, 2011).

Knowledge of the size at onset of sexual maturity in *L. ferreirae* would enable us to better understand and preserve the marine community, since this species has suffered constant indirect exploitation by the shrimp fishing fleet in the south-eastern Brazilian littoral (Graça-Lopes *et al.*, 2002). Thus, this study investigated the sexual maturity of the spider crab *L. ferreirae* in south-eastern Brazil, based on morphometric and gonadal analyses.

MATERIALS AND METHODS

Biological sampling

Crabs were captured monthly from February 2013 to January 2014, in the complex lagoon-estuarine system of Cananéia-Iguape, and its adjacent oceanic area, in São Paulo state, using a shrimp boat fitted with double-rig nets. In order to capture juvenile and adult individuals, sample stations (i.e. trawls) were previously determined at isobaths from 5 to 15 m deep ($25^{\circ}04'43''\text{S}$ $47^{\circ}50'34''\text{W}$).

Captured crabs were frozen and kept in insulated boxes filled with ice until morphometric measurements and gonadal analyses were performed. The specimens were identified according to Melo (1996) and Tavares & Santana (2012), and they were separated by sex, based on the abdomen shape for adults (elongated pattern for males and oval for females) and on the number of pleopods for juvenile individuals (two pairs for males and four pairs for females) (Melo, 1996; Almeida *et al.*, 2013).

Abdomen closure and gonadal maturation

Individuals of both sexes were assessed by abdomen adherence to the thoracic sternite. Individuals considered here as immature presented a sealed abdomen, with cementing substances present in the contour of the abdomen (which do not allow its extension) (Bolla & Negreiros-Fransozo, 2015). Individuals showing an unlocked abdomen were considered here as adults, wherein the abdomen could be easily flexed even with the presence of a press-button mechanism, but without cementing substances. Thus, the adult individuals were considered able to reproduce (Haefner, 1990; Guinot & Bouchard, 1998), as they can display movements which expose their gonopods to females.

After this categorization, a gonad macroscopic analysis was conducted, for both sexes, and development stages were characterized according to shape, colour and size, for ovaries, testes and vas deferens, following Choy (1988), Abelló (1989) and Sal Moyano *et al.* (2011). Thus, the gonad development stages registered, according to the literature above, were: four stages for females, i.e. (1) immature, with sealed abdomen; (2) rudimentary, with thin and whitish-coloured ovaries and unlocked abdomen; (3) developing, with thin

and light orange-coloured ovaries and unlocked abdomen; and (4) developed, with thicker and dark orange-coloured ovaries and unlocked abdomen. Likewise, three gonad development stages were characterized for males: (1) immature, with sealed abdomen and unrecognizable vas deferens; (2) rudimentary, with a translucent and thin vas deferens and unlocked abdomen; and (3) developed, with a thicker and white-coloured vas deferens and unlocked abdomen (for more details see Choy, 1988 and Abelló, 1989). We adopted here the following abbreviations in the analysis and graphs: the individuals in immature gonad stages as immature females (GI♀) or males (GI♂); and individuals in rudimentary, developing or developed gonad stages as mature females (GM♀) or males (GM♂).

The presence of ovigerous females (i.e. bearing embryos retained on the pleopods) was recorded, identifying their functional maturity, as well as providing evidence of the reproductive period (Sal Moyano *et al.*, 2011).

Relative growth and morphometric maturity

The measurements used for the morphometric analyses were based on a study carried out by Sal Moyano *et al.* (2011). For females, carapace width (CW) and abdomen width (AW) (closest to the fifth abdominal segment) were measured. For males, the length (PL), width (PW) and height (PH) of the propod, in addition to the carapace width (CW), were measured (Figure 1). Measurements were made using digital callipers (accuracy 0.01 mm) and, for individuals with a CW less than 5 mm, a microscope/stereoscope (Zeiss® Stemi SV6) was utilized, equipped with an image capture system (Zeiss Stemi 2000-C) (accuracy 0.0001 mm).

Relative growth analyses followed the allometric model proposed by Huxley (1950), in which equations were calculated for both males and females separately. Data were ln-transformed to fit a linear model and allometry (positive, negative or isometry) was verified by Student's *t*-test, at a 5% level of significance ($\alpha = 0.05$) (Sampedro *et al.*, 1999).

In order to separate the morphometric phases, data from separated sexes were submitted to a 'K-means clustering' analysis (Sokal & Rohlf, 1979), commonly used in similar studies (see Sampedro *et al.*, 1999; Corgos & Freire, 2006; Hirose *et al.*, 2010). Such analysis is based on the establishment of predetermined groups, attributing each empirical point to any group by means of an iterative process that minimizes

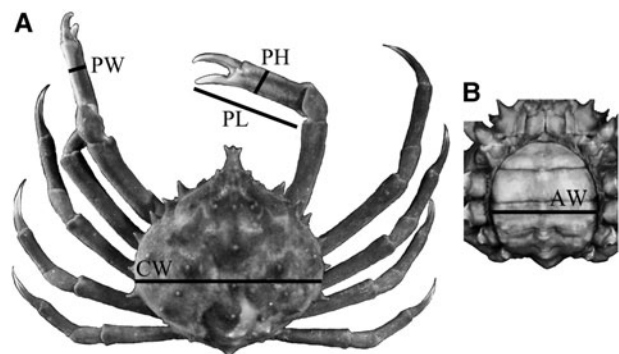


Fig. 1. *Libinia ferreirae* Brito Capello, 1871. Morphological structures measured for morphometric growth analyses. (A) carapace width (CW), length (PL), height (PH) and width (PW) of the propodus; (B) abdomen width (AW).

the variance inside groups and maximizes it among groups. Then, a discriminant analysis is applied in order to discriminate the characteristics of each group, allowing the reallocation of each point to the group that best represents it, classifying each group into distinct categories: immature (MI♂), adolescents (AD♂) and morphometrically mature adults (MM♂) for males; and immature (MI♀) and morphometrically mature adults (MM♀) for females. Subsequently, to verify the accuracy of each group, the slopes (b) and intercepts (a) of the equations of all groups were tested using covariance analysis (ANCOVA), at a 5% level of significance ($\alpha = 0.05$) (Zar, 1999).

It is important to note that, for males, the estimated value of morphometric maturity was based on the relationship, among all tested (PL, PW and PH vs CW), that presented the highest determination coefficient (r^2), for all size groups, since this coefficient indicates the best fit of the equations to the empirical data (Zar, 1999).

When an overlap between size groups was detected in all analyses (both gonadal and morphometric), individuals were grouped into size classes (interval: 3 mm of CW for gonadal analyses; 2 mm of CW for morphometric analyses) and the size at the onset of sexual maturity was determined by means of CW_{50} by fitting the following logistic function: $\%Adults = 1/1 + e^{r(CW-CW_{50})}$, where CW_{50} corresponds to the carapace size in which 50% of the individuals are considered mature and r corresponds to the curve slope (modified from Aguillar *et al.*, 1995; Vazzoler, 1996). For all maturity values, 95% confidence intervals were obtained using the bootstrap interaction method (Macro-supplement for Microsoft Excel®).

Nevertheless, when there was no overlap between size groups, morphometric maturity was determined based on the mean value between the largest individual from a size group and the smallest individual from the next size group.

RESULTS

A total of 346 crabs were collected. The proportion of females (68%) was significantly ($\chi^2 < 0.001$) higher than that of males (32%). For females (non-ovigerous, $N = 97$; ovigerous, $N = 137$), the CW ranged from 3.09 to 71.64 mm; the smallest ovigerous females had a CW of 38.08 mm. A size comparison between sexes showed a greater size variation of CW (6.29 to 77.84 mm) in males ($N = 112$) than in females. However, males showed a lower mean size (CW = 37.81 mm, against 42.19 mm of females) (Mann-Whitney rank sum test, U statistic = 15294; $P = 0.012$).

Gonadal maturity

Based on the logistic curves, gonadal maturity (CW_{50}) for females was a CW of 38.77 mm, with a 95% confidence interval between 37.49 and 40.12 mm (bootstrap interactions: 10 000) (Figure 2A), which is close to the size of the smallest functionally mature female (smallest ovigerous female: CW of 38.08 mm). The largest GI♀ had a CW of 41.3 mm.

For males, gonadal maturity (CW_{50}) was at 34.86 mm, with a 95% confidence interval between 30.91 and 38.02 mm (bootstrap interactions = 10 000) (Figure 2B). The smallest males with rudimentary and developed gonad were, respectively, a

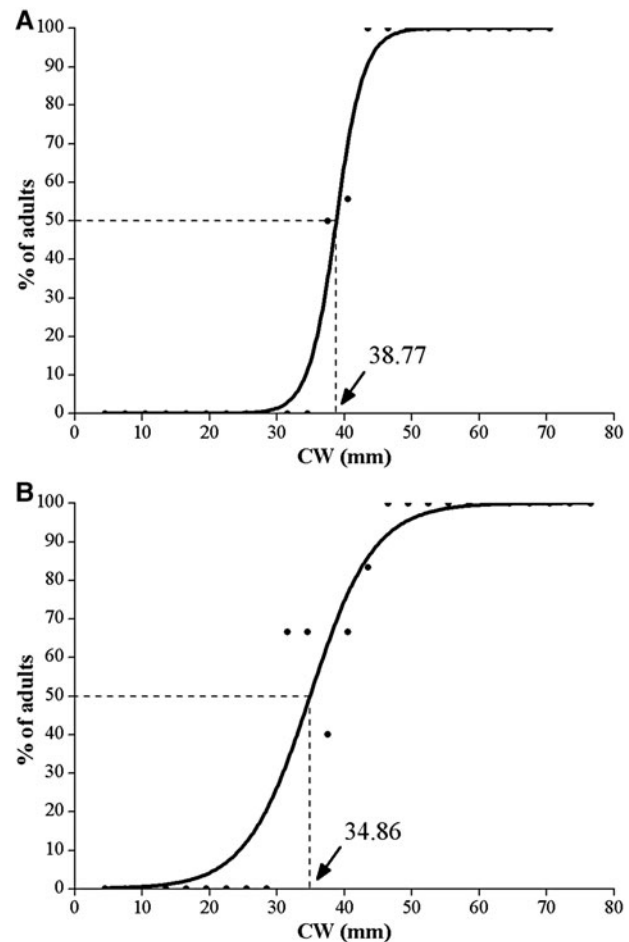


Fig. 2. *Libinia ferreirae* Brito Capello, 1871. Gonadal maturity (CW_{50}) for females (A) and males (B).

CW of 31.83 and a CW of 35.12 mm; the largest GM♂ had a CW of 43.11 mm.

Relative growth and morphometric maturity

For females, the relative growth analysis indicated that the MI♀ group showed positive allometric growth and there was a terminal moult marked by an elevated change in AW and a decreased degree of allometry (ANCOVA, $P < 0.05$) (Table 1). There was an overlap in CW between 38.08 mm (smallest MM♀) and 45.69 mm (largest MI♀). The size increment from MI♀ to MM♀ was 0.9 mm for CW and 8.73 mm for AW (this calculation was performed with the mean values of the overlapping data). The morphometric maturity estimated was CW_{50} 39.43 mm of CW, with a 95% confidence interval between 38.09 and 40.64 mm (bootstrap interactions: 10 000) (Figure 3).

For males, the relative growth of the right propodus length (RPL), in relation to the CW, presented the highest determination coefficient (r^2), among all relationships tested, and revealed three allometric groups: (1) MI♂ that showed isometry, small CW and PL; (2) AD♂ that showed high positive allometry in the chelipeds; and (3) MM♂, which also showed positive allometry and the largest CW and PL (Figure 4). A similar pattern was observed when analysing the left PL and the PH (both left and right), but only partially

Table 1. *Libinia ferreirae* Brito Capello, 1871. Relative growth analysis between males (♂) and females (♀), for all the relationships.

Relationship	Demographic category	N	Linear eq.: $\ln Y = a + b \ln X$		r^2	t	Allometry level	ANCOVA				
			a	b				($b = 1$)	Sex: category	Factor	F	p
CW vs AW	MI♀	55	-1.639	1.198	0.985	9.776	+	♀: MI vs MM	b	49.215	0.0000	*
	MM♀	171	-0.125	0.895	0.867	3.889	-					
CW vs RPL	MI♂	43	-0.645	1.006	0.991	0.445	o	♂: MI vs AD	b	27.642	0.0000	*
	AD♂	30	-2.193	1.467	0.862	4.210	+	♂: MI vs MM	b	14.522	0.0003	*
	MM♂	20	-1.756	1.424	0.885	3.508	+	♂: AD vs MM	b	0.051	0.8219	
CW vs RPW	MI♂	44	-1.950	0.817	0.947	6.156	-	♂: MI vs AD	b	13.790	0.0004	*
	AD♂	34	-3.406	1.278	0.751	2.138	+	♂: MI vs MM	b	9.708	0.0028	*
	MM♂	21	-3.417	1.393	0.840	2.822	+	♂: AD vs MM	b	0.245	0.6230	
									a	40.514	0.0000	*
CW vs RPH	MI♂	43	-2.058	0.990	0.957	0.310	o	♂: MI vs AD	b	3.306	0.0731	
									a	15.887	0.0002	*
	AD♂	34	-2.738	1.217	0.756	1.771	+	♂: MI vs MM	b	7.954	0.0065	*
	MM♂	22	-3.413	1.481	0.873	3.809	+	♂: AD vs MM	b	1.693	0.1990	
CW vs LPL	MI♂	39	-0.643	1.005	0.991	0.320	o	♂: MI vs AD	b	71.653	0.0000	*
	AD♂	35	-1.697	1.324	0.816	2.959	+	♂: MI vs MM	b	13.205	0.0005	*
	MM♂	24	-2.174	1.527	0.933	6.028	+	♂: AD vs MM	b	37.930	0.0000	*
									a	1.415	0.2393	
CW vs LPW	MI♂	40	-1.984	0.827	0.933	4.801	-	♂: MI vs AD	b	69.056	0.0000	*
	AD♂	34	-3.838	1.392	0.797	3.154	+	♂: MI vs MM	b	18.570	0.0001	*
	MM♂	24	-4.567	1.663	0.898	5.540	+	♂: AD vs MM	b	24.389	0.0000	*
									a	1.962	0.1670	
CW vs LPH	MI♂	40	-2.050	0.990	0.976	0.401	o	♂: MI vs AD	b	62.326	0.0000	*
	AD♂	34	-3.198	1.342	0.854	3.492	+	♂: MI vs MM	b	13.056	0.0006	*
	MM♂	24	-3.147	1.418	0.779	2.592	+	♂: AD vs MM	b	8.529	0.0049	*
									a	0.169	0.6824	
								a	67.734	0.0000	*	
								a	5.589	0.0213	*	

CW, carapace width; AW, abdomen width; RPL, RPW, RPH, length, width and height of the right propodus; LPL, LPW, LPH: length, width and height of the left propodus, respectively; MI, immature; AD, adolescent; MM, morphometric mature; N, number of individuals; a , linear coefficient; b , angular coefficient; r^2 , determination coefficient; t , Student's t -test; o, -, +, isometry, negative and positive allometries, respectively; ANCOVA, covariance analysis; sig., significant for $\alpha = 0.05$.

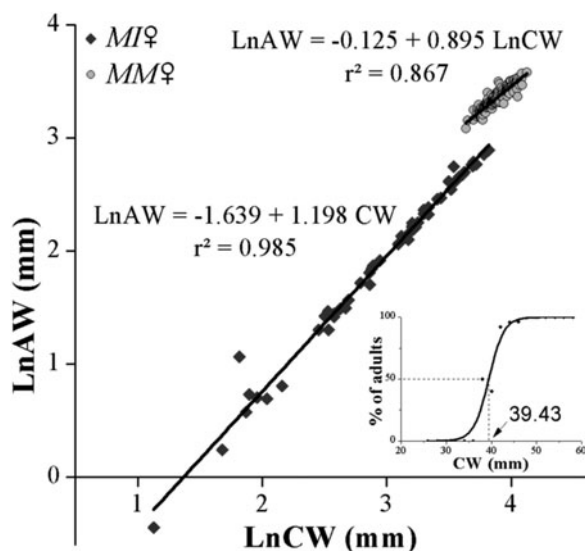


Fig. 3. *Libinia ferreirae* Brito Capello, 1871. Relationship between carapace width (CW) and abdomen width (AW), considering immature (MI♀) and mature (MM♀) females. r^2 = determination coefficient; Inset graph: CW₅₀ results on the overlap between MI♀ and MM♀.

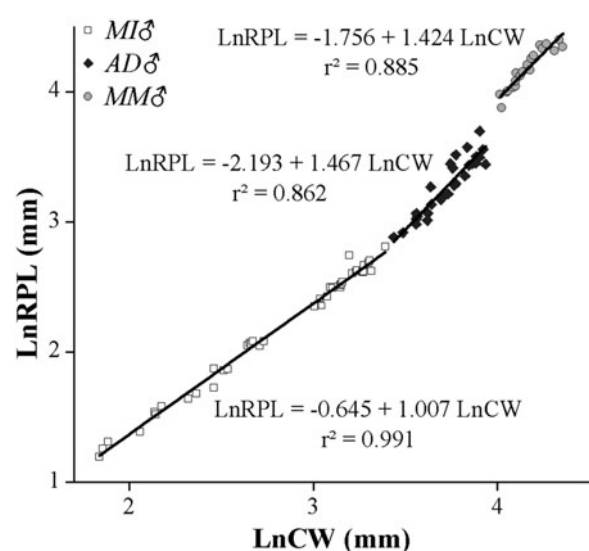


Fig. 4. *Libinia ferreirae* Brito Capello, 1871. Relationship between carapace width (CW) and right propodus length (RPL) for immature (MI♂), adolescent (AD♂) and morphometrically mature (MM♂) males. r^2 = determination coefficient.

found when analysing the PW (both left and right) because the MI♂ group showed negative allometric growth (ANCOVA, $P < 0.05$) (Table 1).

Considering that there was no overlap among the size groups of the males, the morphometric maturity obtained for AD♂ and for MM♂ was, respectively, a CW of 30.41 and 53.25 mm. The covariance analysis (ANCOVA) significantly validated the separation of all groups for both males and females (Table 1).

DISCUSSION

The higher proportion of females, observed in the present results, diverges from previous studies performed for *L. spinosa* and *L. emarginata*, in which the abundance of males was higher (Sal Moyano *et al.*, 2011) or similar to that of females (DeGoursey & Auster, 1992). Therefore, it is likely that either there is a differential occupation of habitats between sexes and the distribution of males was not included in these samples or, indeed, the population has a higher abundance of females. However, reproductive capability may not be, necessarily, injured by the lower abundance of males, since Majoidea females, due to the presence of a seminal receptacle, do not require periodic copulae and can show several spawns with only one spermatid mass from a single copula (González-Gurriarán *et al.*, 1998). Additionally, males can mate with several females in same season (Hartnoll, 1969; Diesel, 1991; Sainte-Marie & Lovrich, 1994).

The sexual dimorphism observed between the largest individuals of each sex of *L. ferreirae* is expected, as females grow to a lesser extent than males in the terminal moult, probably since they allocate a higher amount of energy to egg production (Hartnoll, 1982). Additionally, smaller females could facilitate the mating and post-copulatory guarding behaviour by MM♂, which could offer better protection to females during or after copula (Hartnoll, 1969; Conan & Comeau, 1986).

It was possible to observe that *L. ferreirae* presents different sizes at maturity for both sexes, in addition to the occurrence of the AD♂ group, as already reported for other Majoidea species (see Hartnoll, 1963, 1974; Sampedro *et al.*, 1999; Carmona-Suárez, 2003; Sal Moyano *et al.*, 2011). Sexual maturity of Majoidea crabs is still much discussed, mainly in relation to the sequence in which gonad and morphometric maturity occurs.

Gonad maturity

Studies carried out on the species *Maja squinado* (Herbst, 1788), *Hyas coarctatus* Leach, 1815, *Inachus dorsettensis* (Pennant, 1777) and *L. spinosa* indicated that gonad and morphometric maturity could be coincident with similar body sizes for females (Bryant & Hartnoll, 1995; Sampedro *et al.*, 1999; Sal Moyano *et al.*, 2011), as observed in our investigation. However, this does not seem to be a rule for Majoidea crabs; gonad maturity can occur after morphometric maturity (see Jones & Hartnoll, 1997) or before, as attested by Alunno-Bruscia & Sainte-Marie (1998) studying *Chionoecetes opilio* (O. Fabricius, 1788), in which it was found that females acquired gonad maturity first and subsequently reached morphological maturity with the terminal moult.

For males, gonad maturity was found to occur prior to morphometric maturity. It is proposed that this pattern could favour the reproductive success of AD♂ males, like an opportunistic copula, especially when there is a high abundance of reproductive females in the environment or when MM♂ are rare in the environment, as suggested by Sampedro *et al.* (1999) and Sal Moyano *et al.* (2012). Thus, AD♂ could remain in prepubertal intermoult for a longer time before they perform their terminal moult (Laufer & Ahl, 1995; Sampedro *et al.*, 1999; Sal Moyano & Gavio, 2012). Elner & Beninger (1995) have suggested that *C. opilio* AD♂ bearing spermatophores inside the vas deferens could opportunistically copulate or, according to Laufer & Ahl (1995), increase their mating opportunities by 'sneak' mating. Indeed, this behaviour was reported for *L. spinosa* by Sal Moyano & Gavio (2012), as AD♂ successfully copulated when MM♂ were absent or in agonistic behaviour with other MM♂ males. However, further studies should be performed, under laboratory conditions, to verify if these copulas could produce a viable brood.

Relative growth and morphometric maturity

For *L. ferreirae* females, the relative abdominal growth suggests a clear separation between immature individuals and those that are able to reproduce, a pattern commonly observed for Majoidea, wherein such a morphometric split occurs after the terminal moult, concomitant with sudden alterations in the allometric coefficients. Positive allometry ($b = 1.19$) during the immature phase indicates that the abdomen is being prepared to become an embryo-incubating chamber, a process that will be completed after the terminal moult and consequent beginning of the mature phase (Hartnoll, 1974, 1982; Sampedro *et al.*, 1999).

Regarding *L. ferreirae* males, relative growth relationships revealed the existence of two significantly different groups of allometric growth (the MI♂ and AD♂ groups) and one size allometry group (MM♂) where the increase is the result of males of different sizes undergoing their terminal moult. Morphologically mature males presented a higher allometry level, associated with cheliped size, than MI♂ and AD♂. This fact is related to the terminal moult, which provides high sexual dimorphism in males, as was also found in *M. squinado* and *L. spinosa* by Sampedro *et al.* (1999) and Sal Moyano *et al.* (2011), respectively. Many authors have proposed that the male morphotypes are a result of the ontogenetic process they go through to reach maturity and ensure reproductive success (Hartnoll, 1963; Homola *et al.*, 1991; Laufer & Ahl, 1995). Nevertheless, the terminal moult is not necessarily the maturation moult, because spermatophores can be found inside spermatid ducts even before terminal moult, as assumed by Elner & Beninger (1995), Laufer & Ahl (1995), Rotllant *et al.* (2000) and Sal Moyano *et al.* (2010). It is also important to note that AD♂ are grouped based only on morphology because of their small cheliped and are close to the pubertal moult. However, they can include physiologically mature or immature individuals.

A larger cheliped is of great importance in reproduction, because it is useful for both combat and female protection during mating and may be a decisive factor in the selection of the male by females (Sal Moyano & Gavio, 2012). Therefore, although the occurrence of mating by AD♂ crabs could be an important reproductive strategy in

situations where larger animals (with larger secondary sexual characters) are rare or absent (López-Greco & Rodríguez, 1999), only MM♂ present post-copulatory guarding behaviour, such as embracing and female guarding when other males approach, while AD♂ rapidly depart from the female after mating (Laufer & Ahl, 1995; Sal Moyano & Gavio, 2012). In addition, AD♂ seem to imitate female behaviour when they are close to MM♂, thereby avoiding confrontation and so increasing their mating opportunities in opportunistic cohorts (Laufer & Ahl, 1995; Sal Moyano & Gavio, 2012).

Therefore, further laboratory experiments aiming to observe mating among females and the different male morphotypes are necessary to better understand functional maturity and its relationship with the reproductive process. Furthermore, studies concerning population structure, distribution, growth and longevity should be conducted in order to understand which factors drive the population biology of *L. ferreirae*.

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