



Sexual Maturity of an Endemic Insular Land Crab: Priority Information toward the Conservation of *Johngarthia lagostoma*

Marcio C. A. João^{1,2,*}, Rafael C. Duarte^{3,†}, Letícia S. Bispo da Silva¹, Andrea S. Freire⁴, and Marcelo A. A. Pinheiro^{1,2}

¹ Universidade Estadual Paulista (UNESP), Instituto de Biociências, Campus do Litoral Paulista (IB/CLP), Grupo de Pesquisa em Biologia de Crustáceos (CRUSTA), Laboratório de Biologia da Conservação de Crustáceos e Ambientes Costeiros (LBC), CEP 11330-900, São Vicente, SP, Brazil

² Programa de Pós-Graduação em Ecologia, Evolução e Biodiversidade (UNESP IB/RC), CEP 13506-900, Rio Claro, SP, Brazil

³ Universidade Federal do ABC (UFABC), Centro de Ciências Naturais e Humanas, CEP 09606-045, São Bernardo do Campo, SP, Brazil

⁴ Universidade Federal de Santa Catarina (UFSC), Departamento de Ecologia e Zoologia, Laboratório de Crustáceos e Plâncton, CEP 88010-970, Florianópolis, SC, Brazil

Abstract

*Insular land crabs (Gecarcinidae) can transit between terrestrial and aquatic environments and inhabit vacant ecological niches that other species do not occupy in oceanic islands. During the reproductive period, these crabs migrate between residential and reproductive areas; this is a critical moment because individuals are more vulnerable to stressful conditions, especially species occupying anthropized islands. Currently, many insular crab species are considered threatened; yet few studies have evaluated the biology of this group, especially the size at which individuals reach sexual maturity. Here, we evaluate the size at the onset of morphological, physiological, and functional maturity for the insular land crab *Johngarthia lagostoma* in Trindade Island (Brazil) and assess the chronology of the events underlying those processes. Males and females exhibited the same order of occurrence of the different maturity processes, starting by being morphologically, physiologically, and, finally, functionally mature at similar sizes (about 56 mm carapace width). This value corresponds to at least half of the maximum size that *J. lagostoma* reaches in Trindade Island and is close to the average relative value registered to other Gecarcinidae species. Considering the current decline in the population of insular crabs, such estimates can be used in management programs, mainly for the definition and protection of breeding and recruitment areas. Specifically, our results can be used toward the conservation of *J. lagostoma*, which is currently classified as endangered in Brazil, especially in the isolated population of Trindade Island.*

Introduction

Crab species that migrate between terrestrial and aquatic environments rarely have success in continental areas mainly because of the complex composition of the communities

at these places and also because they compete with local organisms that have an older evolutionary history in these habitats (Vermeij and Dudley, 2000). However, land crabs of the Gecarcinidae family have an adaptive advantage in

Received 27 October 2021; Accepted 25 April 2022; Published online 8 August 2022.

* Corresponding author: marcio.joao@unesp.br.

† Present address: University of Exeter (Penryn Campus), Centre of Ecology and Conservation, Cornwall TR10 9FE, United Kingdom.

Abbreviations: AW, abdomen width; CW, carapace width; DE, developing gonads; GP1L, first gonopod length; GP2L, second gonopod length; IM, immature gonads; MA, mature gonads; PL, major cheliped propodus length.

insular environments, where the absence of many animal groups, especially large predators, provides low predation pressure, reduced interspecific competition (Lindquist *et al.*, 2009), and the possibility of crabs conquering opened niches (Paulay and Starmer, 2011). During the reproductive season, some land crabs perform migrations between highlands and the sea; at such times individuals are more exposed and vulnerable to stressful conditions and predation events (Hicks, 1985; Jeng and Liu, 2005; Hartnoll *et al.*, 2007; Paulay and Starmer, 2011). Studies have been performed to investigate the migratory behavior of the Christmas Island red land crab (*Gecarcoidea natalis*), which is capable of walking kilometers between residential and reproductive areas (Hicks, 1985; Adamczewska and Morris, 2001). However, little is known about the biology and ecology of other insular land crab species, especially basic information concerning population structure and reproduction (Hartnoll *et al.*, 2006, 2009; Turner *et al.*, 2011).

Several body transformations occur during the transition between juvenile and adult phases, considered a critical moment for many species (Eyck *et al.*, 2019) when individual changes result in sexually mature individuals for reproduction (Hartnoll, 1978; Sainte-Marie *et al.*, 1995; Gerhart and Bert, 2008). In crustaceans, both morphological and physiological maturity analyses are frequently used to assess the transition between life phases, with the first identifying changes in growth rates and/or the appearance of some secondary sexual characters (*i.e.*, chelipeds and abdomen) and the second registering the moment when gamete production begins for each sex (Hartnoll, 1978; Somerton, 1980; Conan and Comeau, 1986; Sainte-Marie *et al.*, 1995; Pinheiro and Fransozo, 1998; Corgos and Freire, 2006; Gerhart and Bert, 2008). In addition, although not common for crustaceans, observations about reproductive behaviors (*i.e.*, copulation and/or migration events) can be used to estimate the size at the onset of behavioral maturity (Orensanz *et al.*, 2007; Gerhart and Bert, 2008). However, the sequence in which these maturation processes occur along the species ontogeny is not fixed, varying intra- and interspecifically (Conan and Corneau, 1992; Fernández-Vergaz *et al.*, 2000). Therefore, in order to be considered functionally mature, crabs must meet all of the abovementioned criteria, then go through the process of sexual selection, and finally reproduce (Pinheiro and Fransozo, 1998; Corgos and Freire, 2006; Gerhart and Bert, 2008). Identifying the size at the onset of sexual maturity is one of the most important aspects to be studied in brachyuran crustacean populations (Pinheiro and Fransozo, 1998; López-Greco and Rodríguez, 1999). However, to conduct a robust study on sexual maturity, two fundamental points need to be taken into account, namely: (i) the selection of the most adequate criteria to estimate sexual maturity (*e.g.*, morphological, physiological, functional, or, preferentially, a combination of these), and (ii) the correct use of the statistical methodology inherent for each case, aiming to obtain a reliable size at the onset

of sexual maturity, which is essential information for future studies on species management and conservation strategies (Corgos and Freire, 2006).

The Gecarcinidae family includes the most terrestrial species in the infraorder Brachyura (Burggren and McMahon, 1988), with some taxa inhabiting isolated places such as oceanic islands (Paulay and Starmer, 2011; Guinot *et al.*, 2018). At these places, land crabs are considered key species due to their opportunistic habit, since they can accelerate the decomposition of organic matter and act directly on nutrient cycling (Green *et al.*, 1997; O'Dowd *et al.*, 2003; Glen *et al.*, 2006; Perger *et al.*, 2013). Therefore, any direct impact decreasing the crab population can promote large-scale modifications to the structure of insular communities (O'Dowd *et al.*, 2003; Pitman *et al.*, 2005; Paulay and Starmer, 2011). Indeed, it is already known that the different life phases of some insular crab species, many of them currently classified as endangered (Hicks, 1985; Paulay and Starmer, 2011; Ascension Island Government, 2015; Pinheiro *et al.*, 2016; Perger, 2019), are under risk, with juveniles being preyed on by exotic species (*i.e.*, cats and rats; O'Dowd *et al.*, 2003; Dawson, 2013) and adults threatened mostly by exploitation (Baine *et al.*, 2007). The few studies to date about sexual maturity in Gecarcinidae species have used a single criterion to define the transition size between juvenile and adult phases (Bliss *et al.*, 1978; Hicks, 1985; Jeng and Liu, 2005; Liu and Jeng, 2007; Sanvicente-Añorve *et al.*, 2016; Molina-Ortega and Vázquez-López, 2018; Doi *et al.*, 2019). In this way, estimating the sexual maturity of insular land crabs by considering the different criteria of maturity and using adequate statistical inferences are essential to obtain reliable information about the spatial distribution of juveniles and adults; this will be fundamental in generating future population management practices (Pinheiro *et al.*, 2016).

Johnngarthia lagostoma (H. Milne Edwards, 1837) is an insular land crab endemic of four oceanic islands in the South Atlantic, being classified in Brazil as endangered (EN; according to IUCN, 2012), due to its restricted area of occurrence and being threatened by different anthropogenic impacts, such as habitat suppression and the introduction of exotic species (Pinheiro *et al.*, 2016). Little is known about the biology of this species, with the majority of the studies restricted to populations from Ascension Island, where it was described as a seasonal migration for reproduction. The crabs migrate from the inland areas where they live to the breeding areas on the shore where copulation takes place, and females bury themselves to wait until the moment of larval release (Hartnoll *et al.*, 2009, 2010). According to Hartnoll *et al.* (2010), the size at the onset of sexual maturity for *J. lagostoma* in Ascension Island is attained with 60 and 63 mm carapace width (CW) for males and females, respectively. However, although using different variables to assess the sexual maturity of the species (*i.e.*, the stage of gonadal maturation, the proportion of ovigerous females, and the presence of migratory individuals

in the population), Hartnoll *et al.* (2010) define a single size of maturity for each sex without specifying the statistical method used to obtain these estimates. Still, the published results could be considered biased because only 0.7% of the analyzed crabs were smaller than the estimated size at maturity. In the present study, we statistically determined the size at the onset of morphological, physiological, and functional maturity for *J. lagostoma* in Trindade Island, Brazil. We aimed to understand the chronology of these three events along the species ontogeny and finally discuss sexual maturity estimates among land crab species. This information can help future studies on the management of *J. lagostoma* and other insular land crab populations, especially by providing tools for the conservation and monitoring of breeding and recruitment areas.

Materials and Methods

Study area, crab sampling, and biometry

Individuals of *Johngarthia lagostoma* (H. Milne Edwards, 1837) were sampled in Trindade Island ($20^{\circ}51'09.4''$ S, $29^{\circ}30'82.3''$ W), located in the South Atlantic Ocean, about 1200 km off of the Brazilian coast. This island is permanently inhabited by the Brazilian Navy and researchers, resulting in a fluctuating population of about 40 people. Part of Trindade Island has been included in a large marine protected area (known as Mona–Natural Monument) since 2018 (ICMBio, 2018). Crabs can be found almost all over the island, especially near the shore and in the sand-hill vegetation, as well as occupying the slopes of hills up to 600 m in altitude. Sampling was conducted in two expedi-

tions, between February and April 2019 and between December 2019 and February 2020. These months matched the reproductive season of *J. lagostoma* populations from other islands (December to May in the Rocas Atoll and Ascension Island, according to Teixeira, 1996, and Hartnoll *et al.*, 2010, respectively). Crabs were randomly sampled during the night period in two beaches (Andradas and Tartarugas) and two hills (Príncipe, 136 m, and Desejado, 612 m). All individuals were sexed according to the abdominal dimorphism (males, subtriangular; females, semi-rounded) and the number of pleopod pairs (males, two uniramous pairs; females, four biramous pairs). The CW was measured as the largest distance across the cephalothorax, which was used as body size reference for both sexes (Fig. 1A). Other measured structures were the major cheliped propodus length (PL, measured for both sexes; Fig. 1B), the maximum abdomen width (AW, fifth somite, measured only for females; Fig. 1C), and the first and second gonopod length (GP1L and GP2L, measured in males; Fig. 1D, E, respectively). Large structures (*e.g.*, CW, PL, and AW) were measured with a mechanical precision caliper (0.05 mm) in the field, while both GP1L and GP2L were further measured through a system of image analysis (KS-300, Zeiss) integrated to a stereomicroscope (Axiolab, Zeiss, 0.01 mm). Most of the crabs were measured and sexed in the field, with animals being released after measurement. However, about 10 crabs of each sex and size class (10 mm CW) were individualized in plastic bags, cryo-anesthetized, and kept in a freezer (-10°C) until further processing for gonopod biometry and dissection for physiological and functional maturity estimates (see details below in *Physiological and functional maturity*).

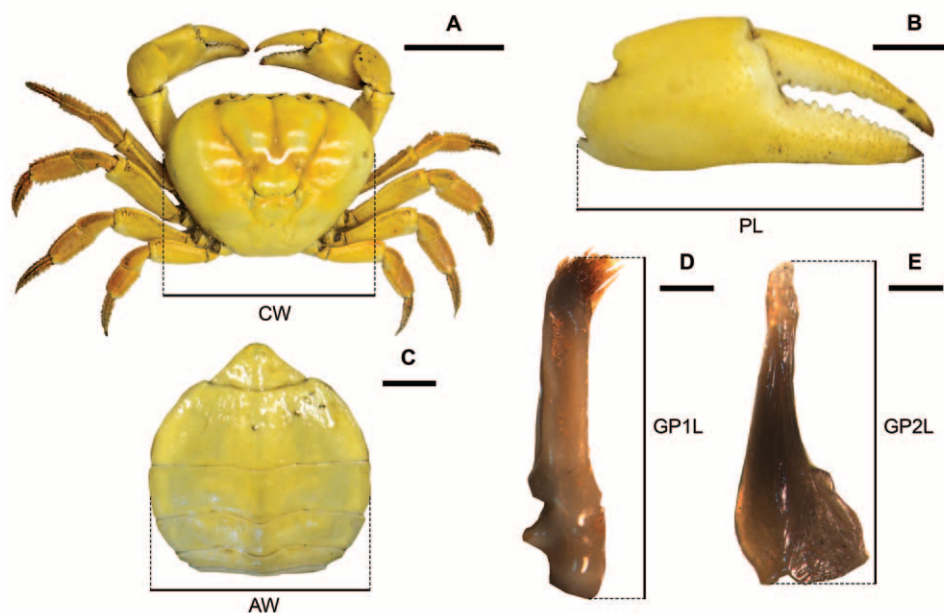


Figure 1. Dorsal view of *Johngarthia lagostoma* crabs and the morphological traits used for the analysis of sexual maturity. (A) Carapace width (CW; scale bar = 50 mm); (B) major cheliped propodus length (PL; scale bar = 10 mm); (C) abdomen width in fifth somite (AW; scale bar = 10 mm); (D) first gonopod length (GP1L; scale bar = 2 mm); and (E) second gonopod length (GP2L; scale bar = 0.5 mm).

Relative growth and morphological maturity

We used the allometric function ($Y = aX^b$) to assess the relative growth of the different secondary sexual characteristics with respect to the carapace width of *J. lagostoma* males and females, following protocols for brachyuran crustaceans established by Hartnoll (1974, 1978, 1982). In order to identify the linear equations and separate each ontogenetic phase (juvenile and adult), data were log transformed and analyzed through the segmented library (Muggeo, 2008) in R (ver. 4.0.0; R Core Team, 2020). This method allows the discrimination of the empiric points for each biometric relationship into two or more groups (*i.e.*, as juveniles and adults, with the latter being represented by their morphotypes); each ontogenetic group is identified by the segmented method (Muggeo, 2008) represented by an equation. We considered the relative growth constant b (model slope) to analyze the growth pattern of each model as isometric ($b = 1$), positive allometric ($b > 1$), or negative allometric ($b < 1$), with its determination coefficients (R^2). Student's *t*-tests for departures from isometry were calculated in all relationships obtained.

The values of morphological maturity were determined for each morphometric relationship following the procedures established by Somerton (1980), using the mature routine in R (Ihaka and Gentleman, 1996). In cases when an abrupt breaking point was observed between growth phases, the size of morphological maturity was considered the interception point. Conversely, when there was an overlap between ontogenetic phases, we adjusted a sigmoidal curve (*i.e.*, Galton's Ogive) to the percentage of adult individuals for each size class (10 mm CW) and estimated the size at which half of the individuals were morphologically mature (CW 50%). Finally, we used separate analysis of covariance (ANCOVA; Zar, 1996) to test for differences in the estimated slope between ontogenetic phases (juvenile and adults), considering each secondary sexual characteristic as the dependent variable (PL, GPL, and AW) and the CW of the crabs as a covariate. In the case of significant interaction between the categorical variable (ontogenetic phase) and CW, Tukey's *post hoc* tests were applied to compare the estimated slopes for the biometric relationships represented by more than two lines (*i.e.*, more than two ontogenetic phases), using the emmeans library (Lenth, 2020) in R.

Physiological and functional maturity

A subset of the sampled crabs was dissected for the evaluation of the macroscopical gonadal maturation stage and estimation of the size at which half of the individuals of each sex were physiologically mature (CW 50%). The stages of gonadal development were defined based on the gonadal coloration and the gonad : hepatopancreas size ratio. All of these aspects were used to classify each individual between three maturation stages (IM, immature; DE, developing; and MA, mature) according to Pinheiro and Fransozo (1998) and Shinozaki-Mendes *et al.* (2012a, b), adapted for *J. lagostoma* (Fig. 2). Immature males and females (Fig. 2A,

B) presented uncolored and invisible gonads to the naked eye. Developing gonads are visible and slender for both sexes (Fig. 2C, D), but with a size equivalent to 1/6 and 1/4 of the hepatopancreas size for males and females, respectively (males, testis are translucent and the vas deferens are translucent to white; females, ovaries exhibit color varying between light and dark orange). Mature gonads are easily visible in both sexes (Fig. 2E, F) and have a size equivalent to 1/4 and 1/1 of the hepatopancreas size in males and females, respectively (males, gonads are white and highly folded mainly in the vas deferens; females, ovaries are voluminous with coloration varying between light and dark brown). Therefore, juveniles were recognized to have IM gonads, while adults were represented by a cluster of DE and MA gonads. The proportion between juveniles and adults was established in each 10-mm size class for each sex; and the adult proportion values were adjusted to a sigmoid function, allowing us to calculate the size at which half of the individuals of each sex were physiologically mature (CW 50%).

Functional maturity was calculated differently for each sex. Despite the inconsistency of how to evaluate this aspect in males, we considered that hard-shelled males are functionally mature when they have the ability to transfer and fertilize female eggs (Elner and Beninger, 1989; Sainte-Marie *et al.*, 2008; González-Pisani *et al.*, 2017). Unfortunately, there are no studies to date reporting mating marks in *J. lagostoma*, which would confirm that males have actually reproduced. Therefore, in our case, males were considered functionally mature when they were larger than the estimated size of morphological maturity and presented DE or MA gonads, adapting the criteria used in previous studies for other species (Pinheiro and Fransozo, 1998). This method does not confirm reproduction but identifies males that are potentially ready to mate. In parallel, the functional maturity of females was evaluated following the same procedures described for males, but we also considered as an additional criterion of maturity females carrying embryos in the abdomen (*i.e.*, ovigerous females) and/or exhibiting the seminal receptacle partially filled by spermatophores (*i.e.*, observed with the naked eye and indicating previous egg release; adapted from González-Pisani *et al.*, 2017). Similarly, the proportion of mature individuals was calculated for each 10-mm size class; and data were adjusted to a sigmoid function, from which the size at which half of the individuals were functionally mature (CW 50%) was estimated for each sex.

Results

Relative growth and morphological maturity

A total of 1658 individuals of *Johngarthia lagostoma* were analyzed, including 1001 males and 657 females. The size (CW) of the sampled crabs varied from 13.1 to 105.5 mm for males (mean \pm SD: 74.4 \pm 17.4 mm) and from 9.2 to

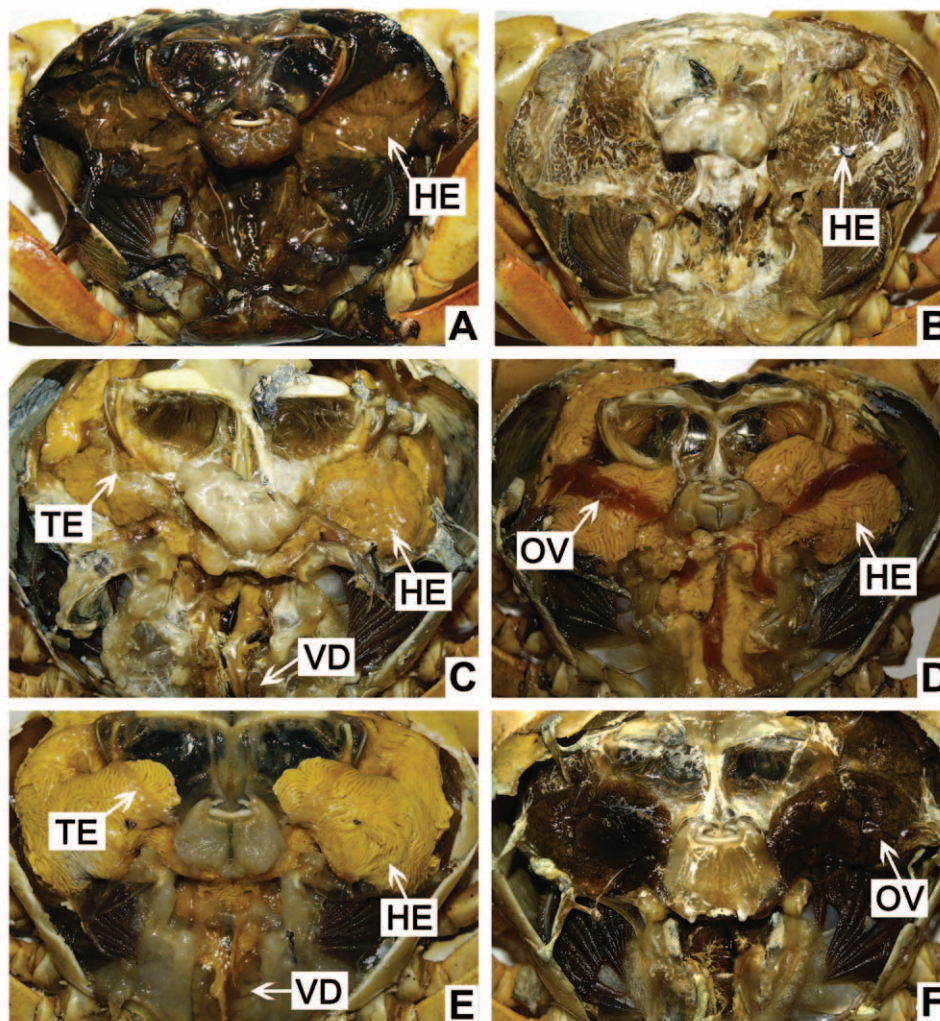


Figure 2. Macroscopic view of the stages of gonadal maturation for *Johngarthia lagostoma* males and females. Immature males (A) and females (B) with gonads invisible to the naked eye and with the hepatopancreas highly developed. Developing gonads visible to the naked eye, with translucent white and light orange coloration and with the gonad : hepatopancreas size ratio close to 1 : 6 and 1 : 4 for males (C) and females (D), respectively. Mature gonads with white and brown coloration and with the gonad : hepatopancreas size ratio close to 1 : 4 and 1 : 1 for males (E) and females (F). HE, hepatopancreas; OV, ovaries (female gonads); TE, testis (male gonads); VD, vas deferens (male gonads).

100.5 mm for females (69.6 ± 16.9 mm). All biometric relationships exhibited two or more phase lines, with differences in the relative growth depending on the ontogenetic phase (Table 1). For both sexes, the PL \times CW relationship presented two breaking points, separating the model into three ontogenetic phases (juvenile, adult-1, and adult-2) (Table 1; Fig. 3A, B), with the level of allometry increasing after the juvenile phase. In males, the breaking points occurred at 52.5 and 64.5 mm CW (Fig. 3A), corresponding, respectively, to the size at the onset of morphological maturity and to a second allometric increment in cheliped growth of adults. The slopes of the regression lines differed between ontogenetic phases (Table 2), with juveniles already exhibiting a positive allometry ($b_{juv} = 1.08$, $P < 0.01$), significantly increasing 30.6% between the two adult phases ($b_{ad-1} = 1.43 < b_{ad-2} = 2.06$, $P < 0.01$). For females, the breaking points occurred at 44.2 and 65.2 mm CW

(Fig. 3B), following the same pattern observed for males, with the first considered as the size at the onset of morphological maturity and the second as another increment in cheliped growth during the adult phase. Although also differing between ontogenetic phases (Table 2), the slope of the regression line for the cheliped growth of juvenile females was isometric ($b_{juv} = 0.99$, $P > 0.05$), while for the adults, two regression lines of positive allometric growth ($b_{ad-1} = 1.20 < b_{ad-2} = 1.40$, $P < 0.01$) were obtained, with an increase of 14.3% between morphotypes (adult-2 in relation to adult-1).

The relative growth of both male gonopods (GP1L \times CW and GP2L \times CW) presented only one breaking point along a crab's ontogeny, separating juvenile (positive allometric) and adult (negative allometric) phases (Table 1). The sizes at the onset of morphological maturity based on both gonopods were very similar, being estimated at 48.9

Table 1

Relative growth of <i>Johngarthia lagostoma</i> from Trindade Island (Brazil)					
Relationship, sex or phase	<i>N</i>	Power function ($Y = aX^b$)	R^2	<i>t</i>	Allometry
PL × CW					
JM	106	PL = 0.38CW ^{1.08}	0.97	3.79*	+
AM1	127	PL = 0.096CW ^{1.43}	0.58	3.94*	+
AM2	733	PL = 0.0069CW ^{2.06}	0.86	34.62*	+
JF	46	PL = 0.48CW ^{1.00}	0.99	0.35 ^{ns}	0
AF1	183	PL = 0.23CW ^{1.20}	0.82	4.83*	+
AF2	380	PL = 0.010CW ^{1.40}	0.91	17.69*	+
GP1L × CW					
JM	29	GP1L = 0.11CW ^{1.19}	0.94	3.19*	+
AM	112	GP1L = 0.51CW ^{0.79}	0.97	15.61*	-
GP2L × CW					
JM	34	GP2L = 0.045CW ^{1.14}	0.92	2.36*	+
AM	107	GP2L = 0.16CW ^{0.81}	0.94	8.93*	-
AW × CW					
JF	62	AW = 0.21CW ^{1.17}	0.98	2.66*	+
AF	573	AW = 0.47CW ^{1.01}	0.95	0.17	0

Mathematic models obtained by regression analysis based on dependent variables (AW, abdomen width of fifth somite; GP1L, first gonopod length; GP2L, second gonopod length; PL, major cheliped propodus length) in function of the independent variable (CW, carapace width). AF, adult female; AM, adult male; JF, juvenile female; JM, juvenile male. R^2 , coefficient of determination; *t*, value of the *t*-test evaluating departure from isometry ($b = 1$), expressed by * $P < 0.05$ and ^{ns}non-significant ($P > 0.05$), indicative of significant allometry degree (+, positive; -, negative; 0, isometry).

and 50.5 mm CW for GP1L and GP2L, respectively (Fig. 4A, B). The relative growth of the female abdomen (AW × CW) exhibited an overlap between the empiric points of juveniles (superior line: positive allometric) and adults (inferior line: isometric) (Fig. 4C). In this case, juvenile fe-

males occurred when $CW \leq 59.8$ mm, while adult females first appeared when individuals had 42.9 mm CW. Within this interval, the size at which half of the females were morphologically mature was 47.8 mm CW. Differences between the slopes of juvenile and adult regression lines were observed for both gonopods and abdominal biometric relationships (Table 2).

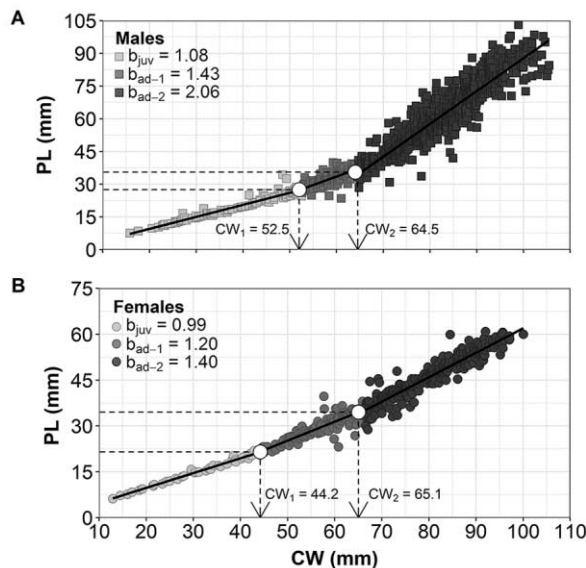


Figure 3. Morphological maturity of *Johngarthia lagostoma*. Biometric relationship between the major cheliped propodus length (PL) of males (A) and females (B) and the carapace width (CW). The half-tone variation of gray squares and points indicates the different ontogenetic phases of males and females, respectively. The dashed arrows indicate the growth changes between ontogenetic phases, with the first mark of each graph (CW_1) indicating the sexual maturity based on the relative growth of PL and the second (CW_2) indicating a posterior allometric increment in cheliped growth of both adult males and females. *b*, slope of the regression model for juveniles (b_{juv}) and adults (b_{ad-1} , b_{ad-2}).

Physiological and functional maturity

A total of 243 crabs (126 males and 117 females) from all size classes were sacrificed and dissected to assess the stage of gonadal maturation. Immature males ($n = 35$, 27.8%) presented a size ranging from 25.7 to 66.9 mm CW, while males with developing ($n = 60$, 47.6%) and mature ($n = 31$, 24.6%) gonads occurred above 44.9 mm CW. Based on that, the size at which half of the males were physiologically mature occurred at 57.9 mm CW (Fig. 5A). However, the size of immature females ranged from 12.9 to 67.8 mm CW ($n = 26$, 22.2%), while those with developing ($n = 84$, 71.8%) and mature ($n = 7$, 6.0%) gonads occurred above 47.8 mm CW. Therefore, the size at the onset of physiological maturity of females was estimated at 51.7 mm CW (Fig. 5B).

In relation to functional maturity, immature males ($n = 40$, 31.7%) had a size varying from 26.7 to 66.9 mm CW, while functionally mature individuals ($n = 86$, 68.3%) were registered above 53.0 mm CW. Therefore, the size at which half of the males reached functional maturity was 56.4 mm CW (Fig. 5C). In the case of females, immature individuals ($n = 27$, 23.1%) had a size ranging from 12.9 to 67.8 mm CW, with the first functionally mature female appearing at 50.4 mm CW. This value corresponds to the small ovigerous

Table 2

Summary results of the analysis of covariance (ANCOVA) testing the effects of ontogenetic phase in the biometric relationship between different secondary sexual characters in function of the independent variable (CW, carapace width) measured in *Johngarthia lagostoma* crabs from Trindade Island (Brazil)

Dependent variable, source of variation	Males			Females		
	df	MS	F	df	MS	F
PL						
CW	1	190.98	25,824.10*	1	87.31	22,235.70*
Phase	2	0.77	104.00*	2	0.14	35.21*
CW × phase	2	2.13	288.30*	2	0.23	57.55*
Residuals	960	0.01		607	0.00	
GP1L						
CW	1	18.39	7513.27*	–	–	–
Phase	1	0.022	9.00*	–	–	–
CW × phase	1	0.26	106.78*	–	–	–
Residuals	138	0.002		–	–	–
GP2L						
CW	1	18.24	4978.60*	–	–	–
Phase	1	0.00	0.00	–	–	–
CW × phase	1	0.20	53.16*	–	–	–
Residuals	137	0.004		–	–	–
AW						
CW	–	–	–	1	82.12	43,140.19*
Phase	–	–	–	1	0.73	383.61*
CW × phase	–	–	–	1	0.16	85.68*
Residuals	–	–	–	630	0.00	

Ontogenetic phases: juvenile, adult-1, and adult-2; secondary sexual characters: AW, abdomen width of fifth somite; GP1L, first gonopod length; GP2L, second gonopod length; and PL, major cheliped propodus length. df, degrees of freedom; MS, mean squares. Values followed by an asterisk indicate statistical significance ($P < 0.05$).

female with mature gonads and is larger than the estimated size of morphological maturity. The small female showing the seminal receptacle partially filled had 54.3 mm CW. Thus, the estimated size at which half of the females were functionally mature was 56.6 mm CW (Fig. 5D).

Discussion

Here, the chronology of the events underlying the sexual maturity of *Johngarthia lagostoma* was statistically evaluated, characterizing one of the few works with this purpose for an insular Gecarcinidae crab. For both sexes, the size at the onset of morphological maturity preceded the size of physiological maturation (see Fig. 6). Also, the estimated sizes of functional and physiological maturity in males were synchronous, with a small difference (1.7 mm CW) between these two criteria, while for females the functional maturity was achieved subsequently to the morphological and physiological maturity criteria. Nevertheless, regardless of the considered criteria, males matured later than females, but the difference between the small and large estimate was close to 10 mm for both sexes (males: 48.9 to 57.9 mm CW, coefficient of variation percentage [CV%] = 7.4%; females: 44.2 to 56.6 mm CW, CV% = 10.6%). Toward more conservative perspectives for the management and conservation of *J. lagostoma*, the size at the onset of sexual maturity for the population in Trindade Island was approximately the same for both sexes (males: 56.4 mm CW;

females: 56.6 mm CW). The present study, together with others about the population structure (MCAJ, RCD, N. Krieglner, UNESP-IB/CLP Brazil, ASF, MAAP, unpubl. data), recruitment (LSBdS, MCAJ, MAAP, unpubl. data), and function of color patterns (MCAJ, RCD, N. Krieglner, UNESP-IB/CLP Brazil, ASF, MAAP, unpubl. data), will increase the knowledge about *J. lagostoma* in Trindade Island and help the management and conservation of this endangered species in Brazilian insular places.

Morphological, physiological, and functional maturity

Regardless of the sex of the individuals, the relative growth of the crab's chelipeds exhibited three different ontogenetic phases (juvenile, adult-1, and adult-2), with a progressive increase in the rate of allometry after maturity. Although males and females invest highly in the growth of chelipeds, the difference in the growth rate of this structure between the adult-2 and juvenile phases was larger for males (47.6%) than females (29.3%). Similar results were observed for a population of *J. lagostoma* from Ascension Island, on which an evident heterochely (*i.e.*, individuals with one cheliped significantly larger than the other) was confirmed for both sexes, including a progressive chelar polymorphism (*i.e.*, the ratio of heterochelous individuals over homochelous in the population increases along the ontogeny) (Hartnoll *et al.*, 2017). Chelipeds are weaponry structures used in territory

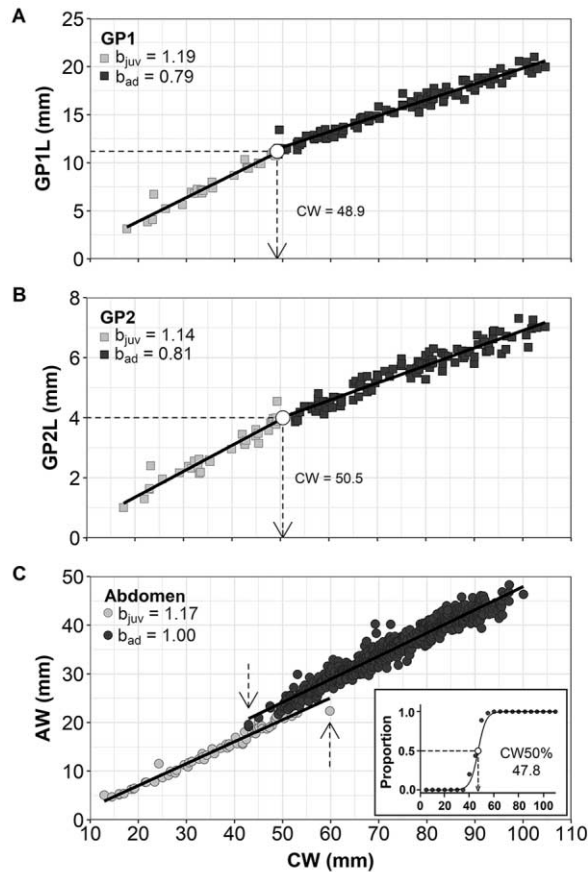


Figure 4. Morphological maturity of *Johngarthia lagostoma*. Biometric relationship between the (A, B) male gonopod length (GP1L and GP2L) and (C) the female abdomen width (AW) with the carapace width (CW). The halftone variation of gray squares and points indicates the different ontogenetic phases of males and females, respectively. The dashed arrows indicate the growth changes marking the sexual maturity in (A) and (B). In (C), the arrows indicate the size interval at which maturity occurs; the inset graph shows the adjustment of the adult proportion along the different size classes to a sigmoidal curve, with the white dot indicating the size at which half of the individuals are mature (CW 50%). b , slope of the regression model for juveniles (b_{juv}) and adults (b_{ad}).

defense and reproductive combat by many crab species (Hartnoll, 1982), being generally larger in males for species in which there is intense male-male competition by receptive females (Baeza and Asorey, 2012). Such sexual dimorphism in cheliped growth is observed for many Gecarcinidae species, with males presenting larger claws that increase even more in size along the ontogeny (Hartnoll, 2012). However, no study to date has assessed the ultimate factors behind the progressive growth of the chelipeds in these crabs even after individuals reach sexual maturity. In the specific case of *J. lagostoma* from Trindade Island, the frequency of heterochelous individuals is lower than homochelous individuals, but the relative contribution of the first to the overall population increases above the 60-mm size class (MCAJ, RCD, N. Kriegler, UNESP-IB/CLP Brazil, ASF, MAAP, unpubl. data). Thus, heterochelous individuals in the population are probably composed from the adult-2 group, above

64.5 and 65.1 mm CW, in males and females, respectively. However, at the same island, males exhibiting similarly or differently sized chelipeds are equally represented in copulating couples (João et al., 2021); therefore, it is unclear whether such differential investment in cheliped growth is regulated by sexual selection or by other life-history traits of the species, requiring future experimental studies.

The relative growth of chelipeds is commonly used for estimating the sexual maturity in many brachyuran species (Hartnoll, 2012). However, in the case of *J. lagostoma*, the use of this structure alone would provide inconclusive results, since two breaking points were observed for the relationship between cheliped and carapace size for both sexes. The transition between the juvenile and adult phases of brachyurans is defined by the occurrence of the puberal ecdysis when different morphological characters change at the same time (Hartnoll, 1978). Based on that, there is an urgent need for studies integrating different aspects of an animal life history to estimate the size at the onset of sexual maturity (Corgos and Freire, 2006; González-Pisani et al., 2017). Our findings showed that near the transition between the juvenile and adult-1 phases, determined by the relative growth of chelipeds, all of the other morphological transformations (i.e., changes in the growth of gonopods and abdomen) also occur. However, the chelipeds are the only structure that keeps growing with a positive allometry after maturity, while the gonopods and abdomen change from a positive allometry when juvenile to a negative allometry and an isometry, respectively, when adult. This indicates a possible change in the invested energy for the growth of gonopods and the abdomen to the growth of chelipeds in the secondary adult phase of both sexes, during which the higher allometric level was observed ($b_{males} = 2.06$; $b_{females} = 1.40$). Unlike the chelipeds, both male gonopods and the female abdomen have only reproductive purposes, functioning for sperm transfer and incubation and protection of eggs, respectively (Hartnoll, 1974; Pinheiro and Fransozo, 1998; Freire et al., 2011; McLay and Becker, 2015; González-Pisani and López-Greco, 2020).

The relative growth of both male gonopod pairs and the female abdomen follows a similar pattern for other brachyuran crabs (see Hartnoll, 1974, 1982 and references therein), with an evident decrease in the energetic investment on these structures after individuals reach the maturity size. Here, we described the relative growth of gonopods for a Gecarcinidae crab; for *J. lagostoma*, the allometric change occurs in crabs larger than 48.9 mm CW, regardless of the gonopod pair. However, future studies about the growth and morphology of gonopods in other gecarcinid species are needed to test whether the same pattern of growth is observed in all land crabs. In parallel, the pattern of abdominal growth change observed for *J. lagostoma* was also observed in other Gecarcinidae species (e.g., *Gecarcinus lateralis*, Bliss et al., 1978; *Gecarcinus ruricola*, Hartnoll et al., 2007; *Cardisoma guanhumi*, Shinozaki-Mendes et al., 2013; and *Epigrapsus politus*,

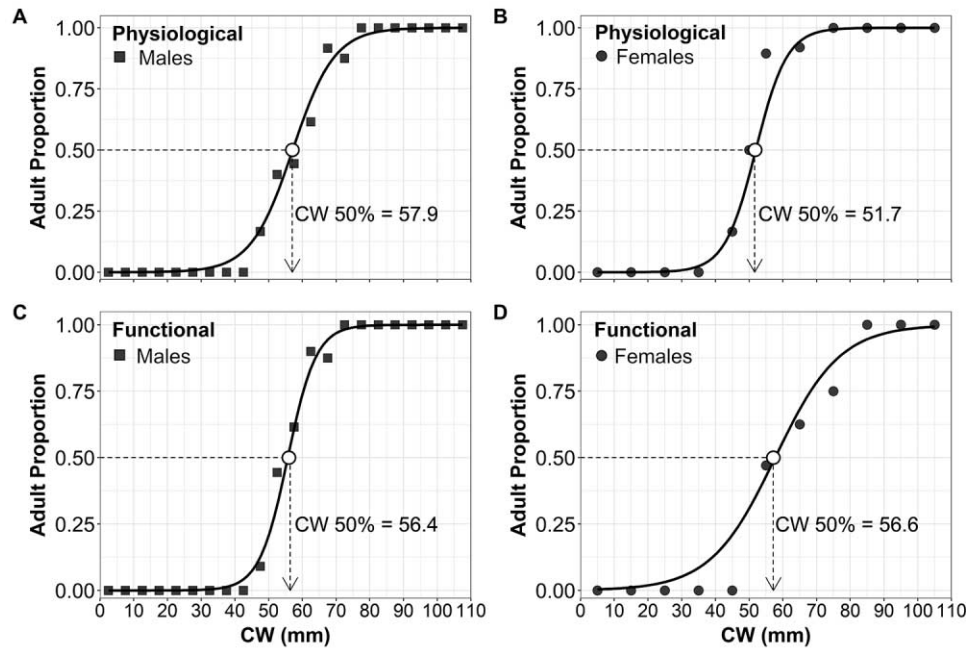


Figure 5. Physiological and functional maturity of *Johngarthia lagostoma*. Representation of sigmoidal curves of physiological (A, B) and functional (C, D) maturity for males and females, respectively, adjusting the empiric points of the adult proportion along the different size classes (CW, carapace width). The white dots indicate the size at which half of the individuals for both sexes are mature (CW 50%).

Doi *et al.*, 2019). Higher investment in the abdominal growth of juvenile females provides a larger chamber for egg incubation after these reach sexual maturity, improving the protection of incubated embryos against attrition and desiccation (Baeza and Fernández, 2002; Davanzo *et al.*, 2016). In this way, offspring viability is increased by both high investment in the abdominal growth and the behavior of ovigerous females to build galleries close to the sea after migration, where they hide and protect their eggs until the moment of larval release (Hicks, 1985; Hartnoll *et al.*, 2007). However, the reproductive output of insular land crabs is probably affected by the number of reproductive migrations that one

female can perform during her lifecycle, since some old and large individuals can die at the end of the process (Hartnoll *et al.*, 2009). Future studies are necessary to understand whether each female can participate in more than one reproductive event and whether the breeding output is changed after subsequent migration seasons.

Regardless of sex, *J. lagostoma* crabs reach morphological maturity at a smaller size than they reach physiological maturity; however, the functional maturity of males was reached at the same size as physiological maturity, and in females functional maturity occurred at a larger size than any other maturity criteria (Fig. 6). It is known that the chronology of the events underpinning the sexual maturity in brachyuran crabs varies intra- and interspecifically, without a common pattern for the entire group (Fernández-Vergaz *et al.*, 2000; Flores *et al.*, 2002; Corgos and Freire, 2006; Gerhart and Bert, 2008; Freire *et al.*, 2011; Shinozaki-Mendes *et al.*, 2013; González-Pisani *et al.*, 2017). Most of the studies about the sexual maturity in Gecarcinidae crabs have indicated identical sizes for the different criteria used or have used a single criterion to define the size at the onset of sexual maturity (Hicks, 1985; Jeng and Liu, 2005; Hartnoll *et al.*, 2007, 2010). Besides our study with *J. lagostoma*, only for *C. guanhumi* (Shinozaki-Mendes *et al.*, 2013) was it used as an integrative approach to estimate the maturity size in a crab species from this family (*i.e.*, using different maturity criteria to obtain a range of size values). Although these two species exhibit similar evolutionary histories, the sequence at which the maturation events occur was distinct, with morphological maturity being the last criterion to be reached in *C. guanhumi*. However, the size interval between the first and last

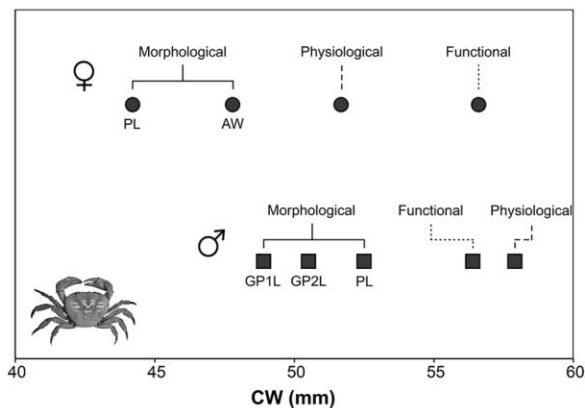


Figure 6. Chronology of the sexual maturity of *Johngarthia lagostoma* crabs from Trindade Island considering the different maturity criteria used in the present study for males and females (morphological, physiological, and functional). AW, abdomen width in fifth somite; CW, carapace width; GP1L and GP2L, first and second gonopod length, respectively; PL, major cheliped propodus length.

maturity criteria that crabs achieved was around 10 mm CW for both *C. guanhumi* (58.7 to 71.0 mm CW) and *J. lagostoma* (44.7 to 57.9 mm CW), which is a short range, considering the maximum size that each species can reach (94.0 mm for *C. guanhumi* and 105.5 mm for *J. lagostoma*). Previous studies have shown that crab species with more terrestrial habits exhibit slower growth patterns compared to less terrestrial species, especially those in which adults perform one ecdysis per year before the reproductive season (Henning, 1975; Pinheiro et al., 2005). Therefore, although evaluated for few gecarcinid species, if the slow growth pattern observed for some insular land crabs is common to other species from this group, the capacity to reach all maturity criteria within a short size interval can be considered a highly adaptive trait.

An overview about the sexual maturity of land crabs

A general pattern about the sexual maturity of Gecarcinidae crabs was first considered by Hartnoll et al. (2007, 2010), who suggested that land crab species become adult with half (50%) of the maximum individual size. To test this assumption, we reviewed the current information about 11 of the 22 Gecarcinidae species (Table 3) and confirmed the pattern presented by Hartnoll et al. (2007, 2010), show-

ing that, on average, the size of maturity was achieved with 55.7% of the maximum size attained by all species. After separating the data between species by the terrestrial degree (e.g., high degree: *Gecarcoidea*, *Gecarcinus*, and *Johngarthia*; low degree: *Cardisoma* and *Epigrapsus*), we found that sexual maturity was achieved with 51.6% and 60.8% of the maximum size registered for species of these groups in the field, respectively, which probably reflects a terrestrial influence on the maturation of these crabs. The first group is composed mainly of insular species that are exposed to a lower predation pressure (Vermeij and Dudley, 2000), and reducing the size of sexual maturity will probably allow smaller individuals to participate in reproductive migrations. If confirmed, this could reduce the negative impacts of this energy-demanding process on populations of crabs since the risks associated with migration can reduce crabs' longevity (López-Victoria and Werding, 2008; Turner et al., 2011). However, reaching sexual maturity earlier can be problematic for populations threatened by the introduction of invasive species, which exerts a strong predation pressure on land crabs from oceanic islands (Paulay and Starmer, 2011).

Based on the literature review presented in this study and the conclusions of Turner et al. (2011), we realized that the sexual maturity in Gecarcinidae crabs has been

Table 3

Size at the onset of sexual maturity in land crabs (Gecarcinidae family) encompassing 11 species from 5 genera available in the literature

Species	Reference	Sex	CW (mm)		
			Max	SM	SM%
<i>Cardisoma crassum</i>	Molina-Ortega and Vázquez-López, 2018	M	94.0	71.0 ^f	73.5
<i>Cardisoma guanhumi</i>	Shinozaki-Mendes et al., 2013	M	94.4	71.0 ^m	73.5
		F	92.2	64.0 ^m	66.4
<i>Epigrapsus notatus</i>	Jeng and Liu, 2005	M	36.4	22.0 ^m	60.4
		F	34.8	16.9 ^b	48.6
<i>Epigrapsus politus</i>	Doi et al., 2019	M	21.9	13.6 ^m	62.1
		F	20.0	14.3 ^m	71.5
<i>Gecarcinus lateralis</i>	Bliss et al., 1978	F	52.0	32.8 ^m	63.1
<i>Gecarcinus ruricola</i>	Hartnoll et al., 2007	M	107.1	50.0 ^p	46.7
		F	102.7	50.0 ^p	48.7
<i>Gecarcoidea lalandii</i>	Liu and Jeng, 2007	F	78.0	42.0 ^f	53.8
<i>Gecarcoidea natalis</i>	Hicks, 1985	M	100.0	45.0 ^f	45.0
		F	100.0	45.0 ^f	45.0
<i>Johngarthia lagostoma</i>	Hartnoll et al., 2010	M	120.0	60.0 ^f	50.0
		F	110.0	63.0 ^f	57.3
	Present study	M	105.5	56.4 ^f	54.9
		F	100.1	56.6 ^f	52.9
<i>Johngarthia malpilensis</i>	López-Victoria and Werding, 2008	M	82.1	<u>35.0</u>	42.6
		F	65.6	<u>35.0</u>	53.4
<i>Johngarthia planata</i>	Sanvicente-Añorve et al., 2016	M	102.7	67.8 ^m	66.0
		F	88.7	57.2 ^m	64.5
Gecarcinidae		M			55.8
		F			57.1
		Total			56.5

M, male; F, female; max, maximum size based on carapace width (CW); SM, size at the onset of sexual maturity estimated by four criteria (m, morphological; p, physiological; b, behavioral; f, functional); and SM%, percentage of the maximum size that each species reaches sexual maturity. Underlined entries indicate that the size of sexual maturity was estimated without explanation about what methods were used.

poorly described, using no standardized analytic methods and preventing replicability and comparisons between the different estimates. For instance, adults of the insular crab from Malpelo Island (*Johngarthia malpilensis*) were supposed to occur above 35 mm CW (López-Victoria and Werding, 2008), without any description of the methodological analysis. Additionally, the maturity of *J. lagostoma* in Ascension Island was based mainly on the sampling of adults (99.3%) (Hartnoll *et al.*, 2010). Considering the urgent need for the management and conservation of Gecarcinidae crabs, especially for endangered species (Paulay and Starmer, 2011; Pinheiro *et al.*, 2016; Perger, 2019), the adoption of a fixed proportional size at which sexual maturity occurs (*i.e.*, 56.5% of the maximum size) can be a good alternative for studies aiming to determine the size at the onset of sexual maturity for isolated insular crab species. Therefore, identifying the size at which sexual maturity occurs is more than basic information for gecarcinid crabs, being essential to a better understanding of the life cycle of these species as well as providing a starting point to identify and manage reproductive and recruitment areas. Specifically for *J. lagostoma* in Trindade Island, size at the onset of sexual maturity is a fundamental tool for local conservation (Pinheiro *et al.*, 2016). However, *J. lagostoma* populations are genetically divided into three evolutionary units (Ascension Island, Trindade, and Atol das Rocas/Fernando de Noronha) (Rodríguez-Rey *et al.*, 2016) within a scenario of very unlikely population connectivity (Freire *et al.*, 2021). Therefore, future studies in other South Atlantic islands, in addition to those conducted in Ascension Island (Hartnoll *et al.*, 2010), should disclose the uniqueness of each crab population, allowing the identification of residential, reproductive, and recruitment areas.

Acknowledgments

We thank the Brazilian Navy (First District), SECIRM (Inter-ministerial Secretariat for Marine Resources), and PROTRINDADE (Programa de Pesquisas Científicas na Ilha da Trindade), under the commanders L. Felipe S. Santos and C. C. Vitória Régia, who guaranteed our presence in Trindade Island and helped with the project's logistics. We thank Isabella Dias, Isis Batistela, Maria Antonia Machado, Nicholas Kriegler, and Vanessa Martins for help during the field sampling and with crab dissections in the laboratory. We are grateful to two anonymous reviewers for their valuable comments on an earlier draft of the manuscript. MCAJ, LSBS, and RCD thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for master (FAPESP 2019/16581-9), scientific-initiation (FAPESP 2020/09968-1), and postdoctoral (FAPESP 2019/01934-3) fellowships, respectively. MAAP thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support provided by Oceanic Island Crabs Project (CNPq 404224-2016), which guaranteed this study,

as well as the Research Productivity Fellowship granted to ASF and MAAP (CNPq 311994/2016-4 and 305957/2019-8, respectively).

Ethical Care Considerations

Field samplings were conducted under permits granted by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and supported by Sistema de Autorização e Informação da Biodiversidade (SISBIO 65446-5). Ethical care of the animals followed established guidelines approved by SISBIO. These included handling animals in the field without any stressful procedures and performing euthanasia of a minimum number of crabs by freezing.

Data Accessibility

The original data of this manuscript are available in https://github.com/marcio-joao/j.lagostoma_maturity.

Literature Cited

- Adamczewska, A. M., and S. Morris. 2001.** Ecology and behavior of *Gecarcoidea natalis*, the Christmas Island red crab, during the annual breeding migration. *Biol. Bull.* **200**: 305–320.
- Ascension Island Government. 2015.** *Johngarthia lagostoma* action plan. Pp. 1–5 in *The Ascension Island Biodiversity Action Plan*. Ascension Island Government Conservation Department, Georgetown, Ascension Island.
- Baeza, J. A., and C. M. Asorey. 2012.** Testing the role of male-male competition in the evolution of sexual dimorphism: a comparison between two species of porcelain crabs. *Biol. J. Linn. Soc.* **105**: 548–558.
- Baeza, J. A., and M. Fernández. 2002.** Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding. *Funct. Ecol.* **16**: 241–251.
- Baine, M., M. Howard, E. Taylor, J. James, A. Velasco, Y. Grandas, and R. Hartnoll. 2007.** The development of management options for the black land crab (*Gecarcinus ruricola*) catchery in the San Andres Archipelago, Colombia. *Ocean Coast. Manag.* **50**: 564–589.
- Bliss, D. E., J. Van Montfrans, M. Van Montfrans, and J. R. Boyer. 1978.** Behavior and growth of the land crab *Gecarcinus lateralis* (Fréminville) in southern Florida. *Bull. Am. Mus. Nat. Hist.* **160**: 112–152.
- Burggren, W. W., and B. R. McMahon. 1988.** *Biology of the Land Crabs*. Cambridge University Press, New York.
- Conan, G. Y., and M. Comeau. 1986.** Functional maturity and terminal molt of male snow crab, *Chionoecetes opilio*. *Can. J. Fish. Aquat. Sci.* **43**: 1710–1719.
- Conan, G. Y., M. Comeau, and G. Robichaud. 1992.** Life history and fishery management of majid crabs: the case

- study of the Bonne Bay (Newfoundland) *Chionoecetes opilio* population. *ICES J. Mar. Sci.* **1992**: 21.
- 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 J. Mar. Sci.* **63**: 851–859.
- Davanso, T. M., F. G. Taddei, G. L. Hirose, and R. C. Costa. 2016.** Sexual maturity, handedness and sexual dimorphism of the freshwater crab *Dilocarcinus pagei* in southeastern Brazil. *Bol. Inst. Pesca* **42**: 269–279.
- Dawson, E. 2013.** The diet and distribution of invasive ship rats (*Rattus rattus*) on Ascension Island. Ph.D. dissertation, University of Exeter, Exeter, United Kingdom.
- Doi, W., S. Kato, D. Itoh, A. Mizutani, and H. Kohno. 2019.** Distribution, size structure, and relative growth of *Epigrapsus politus* (Brachyura: Gecarcinidae) in a subtropical bay in Japan. *Crustac. Res.* **48**: 145–157.
- Elnor, R. W., and P. G. Beninger. 1989.** Comment on functional maturity in small male snow crab (*Chionoecetes opilio*): sizing up the evidence. *Can. J. Fish. Aquat. Sci.* **46**: 2037–2039.
- Eyck, H. J. F., K. L. Buchanan, O. L. Crino, and T. S. Jessop. 2019.** Effects of developmental stress on animal phenotype and performance: a quantitative review. *Biol. Rev.* **94**: 1143–1160.
- Fernández-Vergaz, V., L. López Abellán, and E. Balguerías. 2000.** Morphometric, functional and sexual maturity of the deep-sea red crab *Chaceon affinis* inhabiting Canary Island waters: chronology of maturation. *Mar. Ecol. Prog. Ser.* **204**: 169–178.
- Flores, A. A. V., J. Saraiva, and J. Paula. 2002.** Sexual maturity, reproductive cycles, and juvenile recruitment of *Perisesarma guttatum* (Brachyura, Sesarmidae) at Ponta Rasa mangrove swamp, Inhaca Island, Mozambique. *J. Crustac. Biol.* **22**: 143–156.
- Freire, A. S., M. A. A. Pinheiro, H. Karam-Silva, and M. M. Teschima. 2011.** Biology of *Grapsus grapsus* (Linnaeus, 1758) (Brachyura, Grapsidae) in the Saint Peter and Saint Paul Archipelago, equatorial Atlantic Ocean. *Helgol. Mar. Res.* **65**: 263–273.
- Freire, A. S., M. M. Teschima, M. C. Brandão, T. Iwasa-Arai, F. C. Sobral, D. K. Sasaki, A. O. Agostinis, and M. R. Pie. 2021.** Does the transport of larvae throughout the south Atlantic support the genetic and morphometric diversity of the Sally Lightfoot crabs *Grapsus grapsus* (Linnaeus, 1758) and *Grapsus adscensionis* (Osbeck, 1765) (Decapoda: Grapsidae) among the oceanic islands? *J. Mar. Syst.* **223**: 103614.
- Gerhart, S. D., and T. M. Bert. 2008.** Life-history aspects of stone crabs (genus *Menippe*): size at maturity, growth, and age. *J. Crustac. Biol.* **28**: 252–261.
- Glen, F., A. C. Broderick, B. J. Godley, and G. C. Hays. 2006.** Thermal control of hatchling emergence patterns in marine turtles. *J. Exp. Mar. Biol. Ecol.* **334**: 31–42.
- González-Pisani, X., and L. S. López-Greco. 2020.** Male reproductive strategies in two species of spider crabs, *Leurocyclus tuberculatus* and *Libinia spinosa*. *Zoology* **143**: 125847.
- González-Pisani, X., P. J. Barón, and L. S. López-Greco. 2017.** Integrated analysis of sexual maturation through successive growth instars in the spider crab *Leurocyclus tuberculatus* (Decapoda: Majoidea). *Can. J. Zool.* **95**: 473–483.
- Green, P. T., D. J. O'Dowd, and P. S. Lake. 1997.** Control of seedling recruitment by land crabs in rain forest on a remote oceanic island. *Ecology* **78**: 2474–2486.
- Guinot, D., N. K. Ng, and P. A. R. Moreno. 2018.** Review of grapsoid families for the establishment of a new family for *Leptograpsodes* Montgomery, 1931, and a new genus of Gecarcinidae H. Milne Edwards, 1837 (Crustacea, Decapoda, Brachyura, Grapsoida MacLeay, 1838). *Zoosystema* **40**: 547.
- Hartnoll, R. G. 1974.** Variation in growth pattern between some secondary sexual characters in crabs (Decapoda Brachyura). *Crustaceana* **27**: 131–136.
- Hartnoll, R. G. 1978.** The determination of relative growth in Crustacea. *Crustaceana* **34**: 281–293.
- Hartnoll, R. G. 1982.** Growth. Pp. 111–196 in *The Biology of Crustacea*, Vol. 2, G. Abele, ed. Academic Press, New York.
- Hartnoll, R. G. 2012.** Relative growth: description and analysis. Pp. 365–401 in *Treatise on Zoology: Anatomy, Taxonomy, Biology: The Crustacea, Revised and Updated from the Traité de Zoologie*, J. Forest and J. C. von Vaupel Klein, eds. Brill, Leiden.
- Hartnoll, R. G., M. S. P. Baine, Y. Grandas, J. James, and H. Atkin. 2006.** Population biology of the black land crab, *Gecarcinus ruricola*, in the San Andres Archipelago, western Caribbean. *J. Crustac. Biol.* **26**: 316–325.
- Hartnoll, R. G., M. S. P. Baine, A. Britton, Y. Grandas, J. James, A. Velasco, and M. G. Richmond. 2007.** Reproduction of the black land crab, *Gecarcinus ruricola*, in the San Andres Archipelago, western Caribbean. *J. Crustac. Biol.* **27**: 425–436.
- Hartnoll, R. G., A. C. Broderick, B. J. Godley, and K. E. Saunders. 2009.** Population structure of the land crab *Johngarthia lagostoma* on Ascension Island. *J. Crustac. Biol.* **29**: 57–61.
- Hartnoll, R. G., A. C. Broderick, S. Musick, B. J. Godley, M. Pearson, S. A. Stroud, and K. E. Saunders. 2010.** Reproduction in the land crab *Johngarthia lagostoma* on Ascension Island. *J. Crustac. Biol.* **30**: 83–92.
- Hartnoll, R. G., N. Weber, S. B. Weber, and H. C. Liu. 2017.** Polymorphism in the chelae of mature males

- of the land crabs *Johngarthia lagostoma* and *Epigrapsus* spp. *Crustaceana* **90**: 931–951.
- Henning, H. G. 1975.** Aggressive, reproductive and molting behaviour-growth and maturation of *Cardisoma guanhumi* Latreille (Crustacea, Brachyura). *Forma Funct.* **8**: 463–510.
- Hicks, J. W. 1985.** The breeding behaviour and migrations of the terrestrial crab *Gecarcoidea natalis* (Decapoda: Brachyura). *Aust. J. Zool.* **33**: 101–110.
- ICMBio (Instituto Chico Mendes para Conservação da Biodiversidade). 2018.** Decreto no. 9.312 de 19 de março de 2018. [Online]. Cria a Área de Proteção do Arquipélago de Trindade e Martim Vaz e o Monumento Natural das Ilhas de Trindade e Martim Vaz e do Monte Columbia. Diário Oficial da União, 54, seção 1, pp. 1–3. Available: <https://www2.camara.leg.br/legin/fed/decret/2018/decreto-9312-19-marco-2018-786326-publicacaooriginal-155049-pe.html> [2022, April, 16].
- Ihaka, R., and R. Gentleman. 1996.** R: a language for data analysis and graphics. *J. Comput. Graph. Stat.* **5**: 299–314.
- IUCN (International Union for Conservation of Nature). 2012.** IUCN Red List categories and criteria (version 3.1). [Online]. Available: <https://www.iucnredlist.org/resources/categories-and-criteria> [2022, July 21].
- Jeng, M.-S., and H.-C. Liu. 2005.** Reproduction of *Epigrapsus notatus* (Brachyura: Gecarcinidae) in Taiwan. *J. Crustac. Biol.* **25**: 135–140.
- João, M. C. A., N. Krieglér, A. S. Freire, and M. A. A. Pinheiro. 2021.** Mating strategies of the endangered insular land crab *Johngarthia lagostoma* (H. Milne Edwards, 1837). *Invertebr. Reprod. Dev.* **1**: 1–12.
- Lenth, R. 2020.** emmeans: estimated marginal means, aka least-squares means. R package version 1.4.8. [Online]. Available: <https://CRAN.R-project.org/package=emmeans> [2022, April, 16].
- Lindquist, E. S., K. W. Krauss, P. T. Green, D. J. O’Dowd, P. M. Sherman, and T. J. Smith. 2009.** Land crabs as key drivers in tropical coastal forest recruitment. *Biol. Rev.* **84**: 203–223.
- Liu, H. C., and M. S. Jeng. 2007.** Some reproductive aspects of *Gecarcoidea lalandii* (Brachyura: Gecarcinidae) in Taiwan. *Zool. Stud.* **46**: 347–354.
- López-Greco, L. S., and E. M. Rodríguez. 1999.** Size at the onset of sexual maturity in *Chasmagnathus granulata* (Grapsidae, Sesamidae): a critical overall view about the usual criteria for its determination. Pp. 675–689 in *Crustaceans and the Biodiversity Crisis: Proceedings of the Fourth International Crustacean Congress, Amsterdam, the Netherlands*, F. R. Schram and J. C. von Vaupel Klein, eds. Brill, Leiden.
- López-Victoria, M., and B. Werdling. 2008.** Ecology of the endemic land crab *Johngarthia malpiliensis* (Decapoda: Brachyura: Gecarcinidae), a poorly known species from the tropical eastern Pacific. *Pac. Sci.* **62**: 483–493.
- McLay, C. L., and C. Becker. 2015.** Reproduction in Brachyura. Pp. 185–243 in *Treatise on Zoology: Anatomy, Taxonomy, Biology: The Crustacea: Decapoda: Brachyura*, P. Castro, P. J. F. Davie, D. Guinot, F. R. Schram, and J. C. von Vaupel Klein, eds. Brill, Leiden.
- Molina-Ortega, M. G., and H. Vázquez-López. 2018.** Crecimiento relativo de *Cardisoma crassum* Smith, 1870 (Decapoda: Gecarcinidae) en el estero El Salado, Puerto Vallarta, Jalisco México. *Biol. Cienc. Technol.* **11**: 808–823.
- Muggeo, V. M. 2008.** Segmented: a R package to fit regression models with broken-line relationships. *R News* **8**: 20–25.
- O’Dowd, D. J., P. T. Green, and P. S. Lake. 2003.** Invasional “meltdown” on an oceanic island. *Ecol. Lett.* **6**: 812–817.
- Orensanz, J. M., B. Ernst, and D. A. Armstrong. 2007.** Variation of female size and stage at maturity in snow crab (*Chionoecetes opilio*) (Brachyura: Majidae) from the eastern Bering Sea. *J. Crustac. Biol.* **27**: 576–591.
- Paulay, G., and J. Starmer. 2011.** Evolution, insular restriction, and extinction of oceanic land crabs, exemplified by the loss of an endemic *Geograpsus* in the Hawaiian Islands. *PLoS One* **6**: e19916.
- Perger, R. 2019.** A new species of *Johngarthia* from Clipperton and Socorro Islands in the eastern Pacific Ocean (Crustacea: Decapoda: Gecarcinidae). *Pac. Sci.* **73**: 285–304.
- Perger, R., C. Pacheco, and J. Cortés. 2013.** Closing a distributional gap of over 3000 km and encountering an invisible barrier: new presence/absence data for *Johngarthia planata* Stimpson, 1860 (Decapoda, Brachyura, Gecarcinidae) for Central America and biogeographic notes on East Pacific Gecarcinida. *Crustaceana* **86**: 268–277.
- Pinheiro, M. A. A., and A. Fransozo. 1998.** Sexual maturity of the speckled swimming crab *Arenaeus cribrarius* (Lamarck, 1818) (Decapoda, Brachyura, Portunidae), in the Ubatuba littoral, Sao Paulo State, Brazil. *Crustaceana* **71**: 434–452.
- Pinheiro, M. A. A., A. Fiscarelli, and G. Y. Hattori. 2005.** Growth of the mangrove crab *Ucides cordatus* (Brachyura, Ocypodidae). *J. Crustac. Biol.* **25**: 293–301.
- Pinheiro, M. A. A., W. Santana, E. S. Rodrigues, C. T. C. Ivo, L. C. M. Santos, R. A. Torres, H. Boos, and J. D. Neto. 2016.** Avaliação dos caranguejos gecarcinídeos (Decapoda: Gecarcinidae). Pp. 167–181 in *Livro Vermelho dos Crustáceos do Brasil: Avaliação 2010–2014*, M. A. A. Pinheiro and H. Boos, eds. Sociedade Brasileira de Carcinologia, Porto Alegre, Brazil.
- Pitman, R. L., L. T. Ballance, and C. Bost. 2005.** Clipperton Island: pig sty, rat hole and booby prize. *Mar. Ornithol.* **33**: 193–194.

- R Core Team. 2020.** R: a language and environment for statistical computing. [Online]. R Foundation for Statistical Computing, Vienna. Available: <https://www.r-project.org/> [2022, April 16].
- Rodríguez-Rey, G. T., R. G. Hartnoll, and A. M. Solé-Cava. 2016.** Genetic structure and diversity of the island-restricted endangered land crab, *Johngarthia lagostoma* (H. Milne Edwards, 1837). *J. Exp. Mar. Biol. Ecol.* **474**: 204–209.
- Sainte-Marie, B., S. Raymond, and J. C. Brethes. 1995.** Growth and maturation of the benthic stages of male snow crab, *Chionoecetes opilio* (Brachyura: Majidae). *Can. J. Fish. Aquat. Sci.* **52**: 903–924.
- Sainte-Marie, B., T. Gosselin, J. M. Sévigny, and N. Urbani. 2008.** The snow crab mating system: opportunity for natural and unnatural selection in a changing environment. *Bull. Mar. Sci.* **83**: 131–161.
- Sanvicente-Añorve, L., E. Lemus-Santana, and V. Solís-Weiss. 2016.** Body growth pattern of an isolated land crab species (*Johngarthia planata*) (Decapoda, Gecarcinidae) from the eastern tropical Pacific: an ecological approach. *Crustaceana* **89**: 1525–1539.
- Shinozaki-Mendes, R. A., J. R. F. Silva, L. P. de Sousa, and F. H. V. Hazin. 2012a.** Histochemical study of the ovarian development of the blue land crab *Cardisoma guanhumi* (Crustacea: Gecarcinidae). *Invertebr. Reprod. Dev.* **56**: 191–199.
- Shinozaki-Mendes, R. A., J. R. F. Silva, and F. H. V. Hazin. 2012b.** Development of male reproductive system of the blue land crab *Cardisoma guanhumi* Latreille, 1828 (Decapoda: Gecarcinidae). *Acta Zool.* **93**: 390–399.
- Shinozaki-Mendes, R. A., J. R. F. Silva, J. Santander-Neto, and F. H. V. Hazin. 2013.** Reproductive biology of the land crab *Cardisoma guanhumi* (Decapoda: Gecarcinidae) in north-eastern Brazil. *J. Mar. Biol. Assoc. U.K.* **93**: 761–768.
- Somerton, D. A. 1980.** A computer technique for estimating the size of sexual maturity in crabs. *Can. J. Fish. Aquat. Sci.* **37**: 1488–1494.
- Teixeira, A. L. 1996.** Aspectos biológicos do caranguejo terrestre *Gecarcinus lagostoma* (H. M. Milne Edwards, 1837) no Atol das Rocas–Brasil. Ph.D. dissertation, Universidade Federal de Pernambuco, Recife, Brazil.
- Turner, L. M., J. P. Hallas, and S. Morris. 2011.** Population structure of the Christmas Island blue crab, *Discoplax hirtipes* (Decapoda: Brachyura: Gecarcinidae) on Christmas Island, Indian Ocean. *J. Crustac. Biol.* **31**: 450–457.
- Vermeij, G., and R. Dudley. 2000.** Why are there so few evolutionary transitions between aquatic and terrestrial ecosystems? *Biol. J. Linn. Soc.* **70**: 541–554.
- Zar, J. H. 1996.** *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, NJ.