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Populational structure and sexual maturity of *Aratus pisonii* (H. Milne Edwards, 1837) (Crustacea, Decapoda, Sesarmidae) in the estuarine channels of Mundaú Lagoon, Northeastern Brazil

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Abstract: The aim of this study was to analyse the morphological and physiological maturity of the crab *Aratus pisonii* at Mundaú Lagoon, Alagoas, Northeastern Brazil. A total of 594 specimens were collected, being 212 males and 382 females, of which 150 were ovigerous females. More females than males were found in most months except July/2015. The morphological sexual maturity was estimated observing the morphological characters and the physiological maturity through the stages of gonadal maturation. The relationships MCPL vs. CW for males and AW vs. CW for females were chosen to represent the onset size of morphological maturity (CW50%). The CW varied from 5.13-25.11 mm for males, 6.09–27.07 mm for non ovigerous females and 11.65–23.96 mm for ovigerous females. The sizes that 50% of males reached the morphological and physiological maturity (CW50%) were 20.08 mm and 17.15 mm, respectively, and females, 17.62 mm and 13.51 mm, respectively. In this research, the physiological and morphological maturities were not synchronic for both sexes and the species matures later in the study site than in the other sites that the species was studied and this characteristic may be related to the climatic conditions and the conservation status of the mangroves.

Keywords: Reproduction, mangrove, growth, crab, gonadal development.

Resumo. Estrutura populacional e maturidade sexual de *Aratus pisonii* (H. Milne Edwards, 1837) (Crustacea, Decapoda, Sesarmidae) nos canais estuarinos da laguna Mundaú, NE do Brasil. O objetivo deste estudo foi analisar a maturidade morfológica e fisiológica do caranguejo *Aratus pisonii* na Lagoa Mundaú, Estado de Alagoas situado no Nordeste do Brasil. Um total de 594 indivíduos foram coletados, sendo 212 machos e 382 fêmeas, destas 150 estavam ovígeras. Foram encontradas mais fêmeas que machos na maioria dos meses, exceto em Julho/2015. Para a determinação das maturidades morfológica e fisiológica foram utilizados caracteres morfológicos e estágios de maturação gonadal. A largura da carapaça variou de 5.13 a 25.11 mm para machos, para fêmeas a variação foi de 6.09–27.07 mm e fêmeas ovígeras, 1.65–23.96 mm. O tamanho em que 50% dos indivíduos machos alcançaram a maturidade morfológica e fisiológica (LC50%) foi, 20.08 mm e 17.15 mm de largura de carapaça, respectivamente, e as fêmeas, 17.62 mm e 13.51 mm, respectivamente.

Neste trabalho, as maturidades fisiológica e morfológica não são sincrônicas para ambos os sexos e a espécie amadurece mais tarde no local de estudo que nos outros locais que a espécie foi estudada, e esta característica pode estar ligada as condições climáticas e estados de conservação dos manguezais.

Palavras-Chaves: Reprodução, manguezal, crescimento, caranguejo, desenvolvimento gonadal.

Introduction

The superfamily Grapsoidea MacLeay, 1838 is composed of crabs specially inhabiting trees in mangrove forests; they are distributed in seven families occurring in tropical and subtropical zones (Ng *et al*. 2008). Sesarmidae Dana, 1851 is the most common family in mangroves, including 32 species in Brazil (Ng *et al*. 2008). The "mangrove tree crab" *Aratus pisonii* (H. Milne Edwards, 1837) is one of its main representatives, being generally found over aerial roots and trunks of *Rhizophora mangle*, L. The species lives in salt marshes and nearby shores of Western Atlantic, from Florida to south Brazil (Santa Catarina) including the Caribbean Sea and the Gulf of Mexico (Beever *et al.* 1979, Melo 1996, Thiercelin and Schubart 2014).

The individuals of *A. pisonii* are very agile and the juveniles spend more time over emerged aerial roots, searching for protection against predators, while adults easily reach the top of the trees (Branco 1991, Conde *et al.* 2000). The species has been studied especially in topics such as population ecology, feeding and reproduction (ie. Warner 1967, Beever *et al.* 1979, Díaz & Conde 1989, Conde & Díaz 1989, Leme & Negreiros-Fransozo 1998, Leme 2002,2006, López-Sánchez & Quintero-Torres 2015). However, there are only two studies related to morphological and physiological maturity in Brazilian coast, which Pescinelli *et al*. (2015) determined the size of onset of the morphological maturity from a population from São Paulo State, and Nicolau (2009) from Rio de Janeiro State.

Among the localities on the Brazilian coast where the species occurs, we can highlight the Mundaú-Manguaba estuarine complex, located in the Northeastern Brazil. This area is very important for the development of the human population, providing a great diversity of species of economic importance for fishermen. The biodiversity of Mundaú Lagoon is poorly known, having some authors treated about crustaceans: decapods were observed by Teixeira & Sá (1998), Sousa *et al.* (2000), Calado & Sousa (2003), the biology of *Ucides cordatus* (Linnaeus, 1763) by Araújo & Calado (2008), and *Goniopsis cruentata* (Latreille, 1803) by Lira *et al*. (2012) and Lira e Calado (2013), however, no study has addressed to the biology of *Aratus pisonii*. The aim of this study is to determine the onset of morphological and physiological maturity and aspects of populational biology as sexual proportion, abundance and reproductive period of a population of *Aratus pisonii* living at Mundaú Lagoon, State of Alagoas, Northeastern Brazil.

Materials and Methods

The study was realized at the inlet channel to Mundaú Lagoon, which belongs to Mundaú/Manguaba estuarine complex (9°38'28'' and 9°38'35'' S; 35°44'47'' and 35°57'21'' W). This area is under two well-defined seasons, the rainy season, from March to August, and the dry season, from September to February (Eskinazi-Leça 1976). This estuarine complex has suffering several environmental impacts through the decades such as misuse of marginal areas of rivers, and effluent releases from sugar-alcohol plants, domestic dwelling and paper and fertilizer factories (Marques 1991, Leahy 1995, Melo-Magalhães *et al*. 1998).

Monthly collections were carried out from August/2014 to July/2015, when the individuals of *Aratus pisonii* were captured by hand in the trunks and aerial roots of the mangrove trees. Four sampling stations were determined, in which an effort of 30 minutes in each station was performed by two collectors (Fig. 1). Further, the catch per unit effort (CPUE mg/h) was monthly estimated. During the expeditions, the air temperature (AT) and water temperature (WT) were measured *in situ*. The samples of the water were collected and analyzed in laboratory of Hydrochemistry (LABMAR/UFAL) to obtain the values of pH and salinity. The *Student t* test was applied to compare the seasonal variation of these variables ($\alpha = 0.05$).

The sampled individuals were stored in plastic bags, transported to the laboratory and frozen until the analysis. All individuals were sexed and the following biometric variables were measured with digital calipers (0.01 mm): carapace width (CW) for both sexes, abdomen width (AW) for females, and major cheliped propodus length (MCPL), major

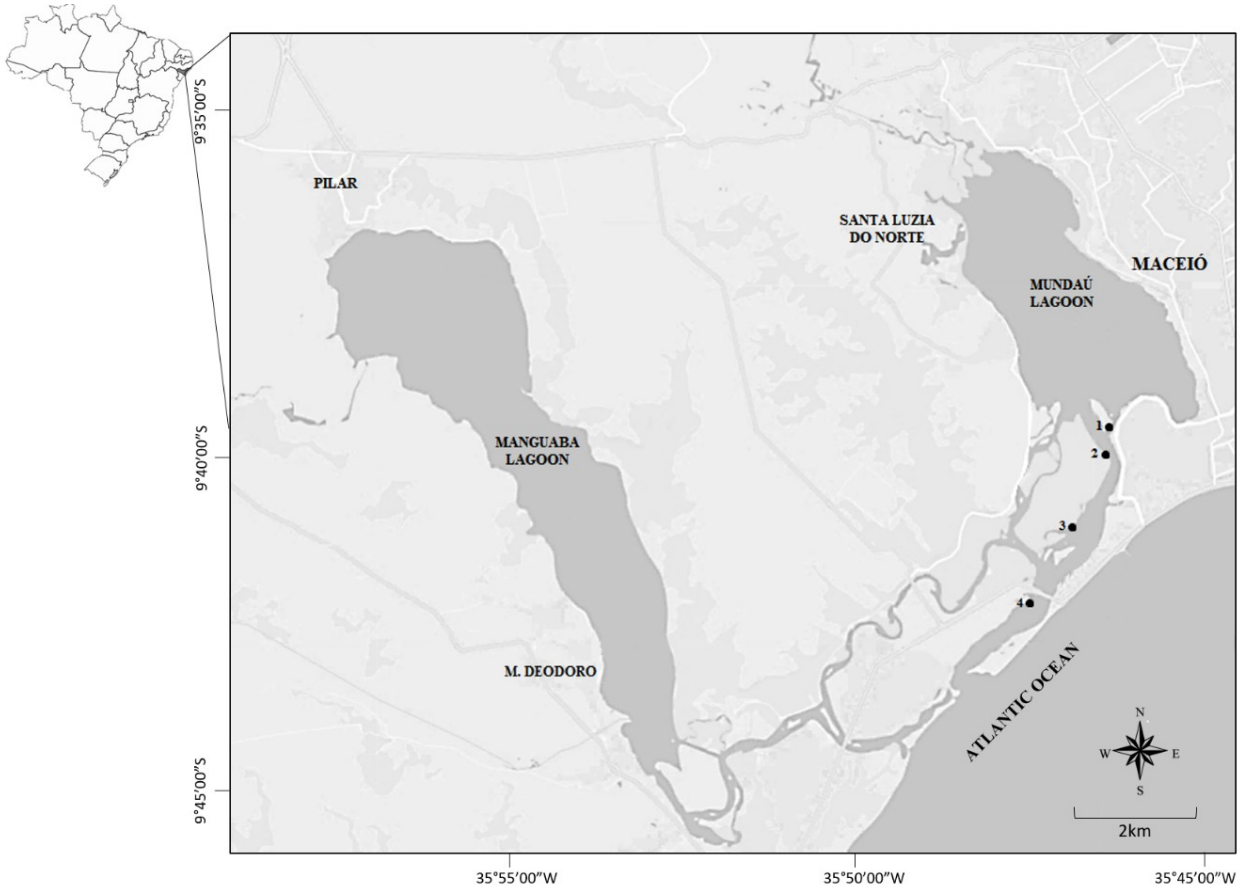

Figure 1. Map of Mundaú/Manguaba estuarine complex (CELMM), state of Alagoas, Brazil, showing the four sampling points at the inlet channel of Mundaú Lagoon.

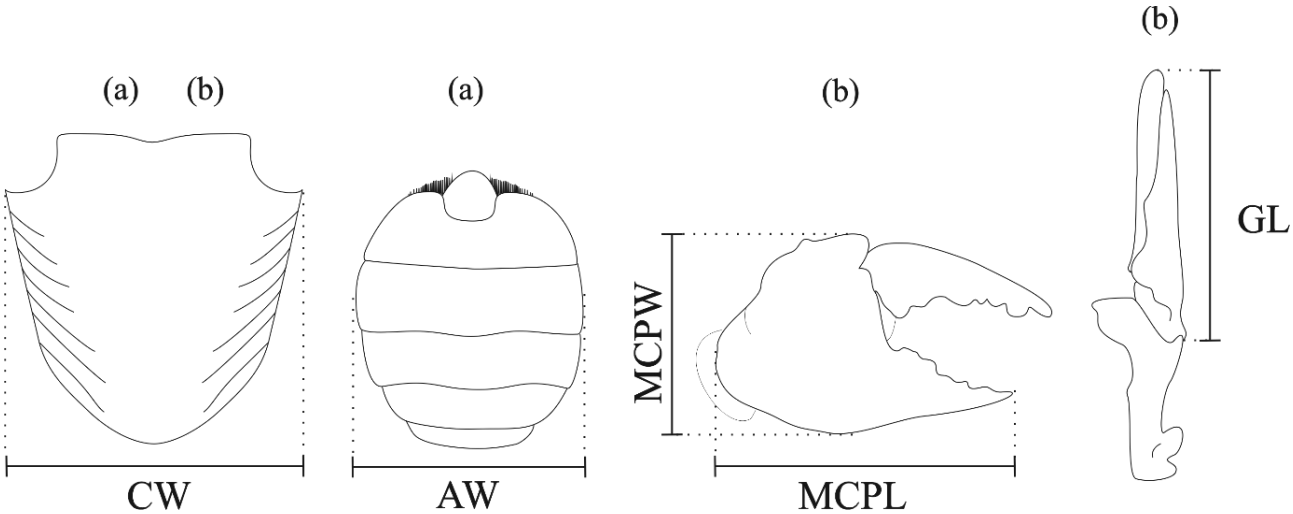

Figure 2. Biometric variables of *Aratus pisonii* from Mundaú Lagoon (state of Alagoas, Brazil) for females (a) and for males (b): carapace width (CW), abdomen width (AW), major chelipod propodus length (MCPL), major chelipod propodus width (MCPW) and gonopod length (GL). Modified from Pescinelli *et al.* (2015).

cheliped propodus width (MCPW) and gonopod length (GL) for males (modified from Silva *et al.* 2007) (Fig. 2). The minimum, mean (± standard deviation) and maximum values of each variable (both abiotic and biometric) were estimated. The individuals were distributed on 10 classes of CW size, that was determined using the software Bioestat 5.0.

The sex ratio of population of *A. pisonii* was established by the total number of males in relation with the females, and also was obtained the monthly sex ratio (Nicolau, 2009). Chi-square test was

applied to verify if the sex ratio deviated significantly from the expected proportion (1:1). All analysis were realized using $\alpha = 0.05$. A correlation matrix with Pearson's coefficient of linear correlation (*r*) was applied to verify the influence of the abiotic factors in the total abundance of *A. pisonii*, as well as in the abundance of ovigerous females. The breeding period was determined by observing the percentage of ovigerous females throughout the year (Vazzoler 1996). The data for these females were monthly distributed and compared with the percentage of non ovigerous females (Benedetto & Masunari 2009).

The empirical points of the relationships MCPL *vs.* CW, MCPW *vs.* CW and GL *vs.* CW (males) and AW *vs.* CW (females) were submitted to the regression analysis. The relationships were adjusted to the power function using an allometric equation ($y = a\ x^{b}$), considering carapace width (CW) as independent variable (Huxley 1950). The power function was linearized (Log y = a + b Log x). Through a non-hierarchical classification (K-means cluster), one model for the young phase and another for the adult phase for each biometric relation were obtained (Corgos & Freire 2006). The morphological sexual maturity was estimated to all relationships, but only the relationships MCPL *vs.* CW for males and AW *vs.* CW for females were chosen to represent the onset size of morphological maturity (CW50%), considering the importance of propodus and abdomen in the reproductive processes of males and females, respectively (Sampedro *et al*. 1999, Moura & Coelho 2004, Araújo *et al.* 2012). The type of growth curve was established by the value of the constant 'b' of the power function, which can be isometric (b = 1), allometrically positive (b > 1) or allometrically negative (b < 1) (Hartnoll 1982) in the relationships MCPL *vs.* CW for males and AW *vs.* CW for females.

After the biometric measurement, a dorsal cut along the margin of carapace was performed and the dorsal surface was turn upwards in all the individuals for macroscopic analysis of gonads. The coloring and the relative size of gonad with viscera and carapace cavity were observed (adapted from Moura & Coelho 2004). Four stages of gonadal maturation were considered for females: Immature - the ovary is slim, pallid and translucent; In development - the ovary is located at the top, orange, pallid and stands out between another viscera; Mature - the ovary present color bright orange and occupies large part of the body cavity; Immature in recovery (post posture) has the same characteristics as the immature ovary, but the spermathecae in females are full of sperm or keeping some vestiges of eggs in pleopods. For males, the gonads were classified only into two stages: Immature - the testicles are slim, pallid and translucent rolled and tied: Mature - the anterior part of the duct spermatic is swollen, diverticula prominent sides and spermatic duct white and opaque (modified from Hartnoll 1965, Sampedro *et al.* 1999). For the analysis of gonadal stages were employed the methods of frequency of occurrence and percentual composition (Hynes 1950). In the estimation of gonadal CW50%, the equation of logistics curve in software Statistica 6.0 was used (Statsoft 2001) as suggested by Barreto *et al*. (2006).

Results

A total of 594 individuals of *Aratus pisonii* were collected, being 212 males (35.70%), 232 females (39.05%) and 150 ovigerous females (25.25%), from August/2014 to July/2015, whose measurements are shown in the Table I. During the collection period, of the population of *Aratus pisonii* were less expressive in CPUE in August and October of 2014 and more expressive in September/2014 and April/2015, apparently, without any correlation with dry or rainy season (Fig. 3). The high abundance in April/15 coincided with increasing temperatures of the air and the water. However, for the total study period, there were not significant influence of the abiotic variables in their abundance (Water Temperature: $t = 0.36$; $p > 0.05$. Air Temperature: $t = 0.74$; $p > 0.05$).

The air temperature varied from 25.75 to 32.12°C (28.93 ± 4.50), the water temperature from 25.75 to 30.30 °C (28.02 ± 3.21), the salinity from 35.1 to 36.7 (35.9 ± 1.13) and the pH from 7.06 to 8.07 (7.56 ± 0.71). Females were more abundant than males of *A. pisonii* in most months (1♂:1.8♀), except in July/2015 (1.19♂:1♀; $\chi^2=0.62$), where the frequency of females was lower (44.83%) than that of males (55.17%) (Figure 3). Females were more frequent in the size classes from 15.1 to 21.0 mm CW, while males dominated the larger classes, from 21.1 to 27.1 mm CW (Table II).

The Pearson's coefficient of linear correlation between the abiotic variables and the abundance of total individuals and the abundance of ovigerous females showed no significance ($p>0.05$) with all the abiotic variables, although positively correlated with both abundances (Table III).

Table I. Minimum, maximum, mean and standard deviation of biometric variables (mm) of males, females and ovigerous females of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil.

Mensures	Males				Non ovigerous Females		Ovigerous Females	
	CW	MCPL	MCPW	GL	CW	AW	CW	AW
Minimum	5.13	2.68	1.00	1.86	6.09	1.66	11.65	10.05
Maximum	25.11	18.24	11.27	9.35	27.07	16.09	23.96	14.25
Mean	19.49	10.96	6.91	6.85	18.49	11.11	19.10	11.54
Stand. dev.	3.42	2.92	2.12	1.22	2.85	1.79	2.04	1.09

Note: CW = carapace width; MCPL = major cheliped propodus length; MCPW = major cheliped propodus width; GL = gonopod length; AW = abdomen width.

Figure 3. Temporal oscillation of the monthly average of CPUE (mg/h) of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil, during the study period from August/2014 to July/2015 with standard error bar.

Two reproductive peaks were observed during the year (Figure 5), the first in March/15 (52,17% of ovigerous females) and the second in June/15, with 60,00% of ovigerous females in the population.

For males, MCPL *vs.* CW (t = 34.50; $p < 0.05$; r=0.92) indicated the best relationship for the maturity test between the variables analyzed, while for females, it was AW *vs.* CW (t = 43.53; $p < 0.05$; r = 0.91). Males showed positive allometric growth (b = 1.37), while females, positive allometric growth with tendency to isometric growth (b=1.07). Changes in the growth rates in the graphs between dependent variables (MCPL for males and AW for females) and the carapace width (CW) showed that the onset size of morphological maturity (CW50%) for males of *Aratus pisonii* is attained at 20.08 mm CW, while for females, at 17.62 mm CW (Figures 6 and 7).

Regarding the gonads, 38.20% of males were physiologically immature and 61.80% mature. In females, 16.24% were immature, 30.26% under development, 34.03% mature and 19.37% in recovery. Males with immature gonads were more abundant in November (65.21%), while those with

Table II. Distribution of sex ratio in size classes of CW for males and females of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil, from August/2014 to July/2015.

Size classes of CW (mm)	Male	Female	Male:Female
5.1-7.0	1	2	1:2*
7.1-9.0	1	0	1:0
9.1-11.0	1	1	1:1
11.1-13.0	2	10	1:5*
13.1-15.0	18	23	1:1.2
15.1-17.0	27	55	1:2*
17.1-19.0	40	129	1:3.2*
19.1-21.0	44	111	1:2.5*
21.1-23.0	50	41	1:0.8*
23.1-27.1	28	10	1:0.3*
Total	**212**	**382**	**1:1.8***

Note: *proportion differs significantly.

mature gonads, in October (88.88%). The mature males occurred in size range of 13.1 – 15.0 mm CW, while the smallest females physiologically matured for reproduction belonged to n the size class 11.1 – 13.0 mm (Figures 8 and 9).

The adjustment of the logistics curve for the physiological maturity resulted in very different values from the morphological maturity, both in males (morphological $CW_{50\%}$ = 20.08 mm; physiological CW50% = 17.15 mm) (Figure 10) and in females (morphological CW50% = 17.62 mm; physiological CW50% = 13.15 mm) (Figure 11).

Discussion

In this study the pH, salinity and water temperature had no influence in the reproductive cycles or sexual ratio. The relation of the number of ovigerous females with the water temperature was not significant, probably the adult individuals to be found in emerged roots, while the juveniles

Table III. Matrix of correlation between the abiotic variables and the abundance of individuals (males, females and ovigerous females) of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil.

	Correlation with the abundance of total individuals				Correlation with the abundance of ovigerous females			
	WT	**AT**	**pH**	**Sal**	**WT**	**AT**	**pH**	**Sal**
r	0.1194	0.2415	0.1022	0.1592	0.3095	0.4211	0.1489	0.4051
t	0.3609	0.7465	0.3081	0.4838	0.9765	1.3929	0.4517	1.3292
p	0.7265	0.4744	0.7650	0.6401	0.3543	0.1970	0.6622	0.2164

Note: WT = water temperature; AT = air temperature; Sal = salinity

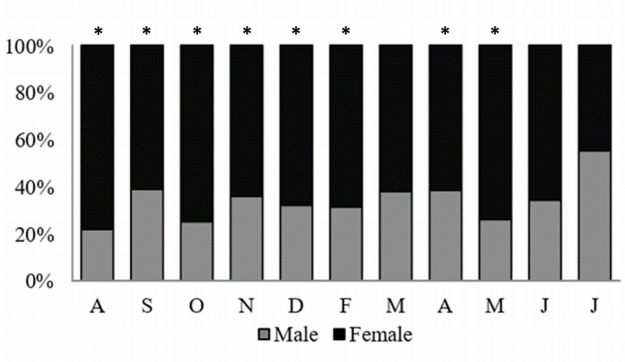

Figure 4. Frequency of occurrence of males and females of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil, from August/2014 to July/2015. * indicates significant differences between the months.

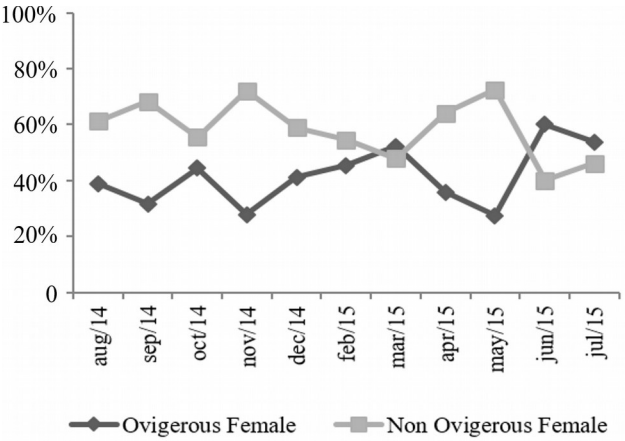

Figure 5. Percentage of ovigerous females and non-ovigerous of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil, in the study period.

immature of *A. pisonii* are found in immersed roots (Conde *et al.* 2000), making the influence of air temperature, although not significant, the positive correlation stronger than the others. However, in this paper the air temperature had significant influence, due at summer the high temperatures it's the ideal moment for many species to reproduce, favouring the larval development shorter, increasing the recruitment in mangrove species (Emmerson, 1994).

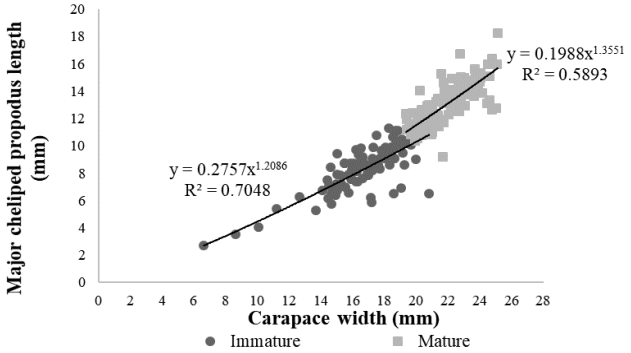

Figure 6. Dispersion of points and growth equation of the relationship between the major cheliped propodus length (MCPL) and carapace width (CW) in males of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil.

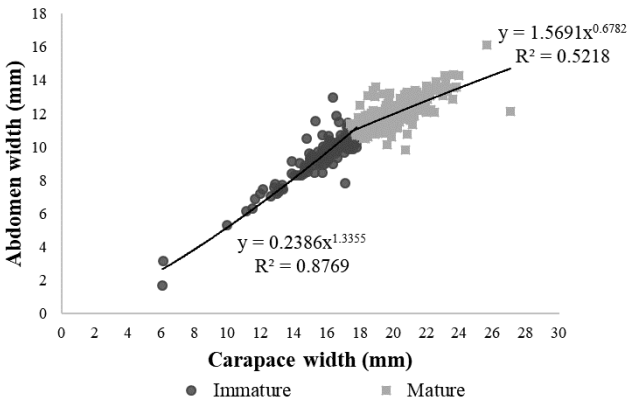

Figure 7. Dispersion of points and growth equation of the relationship between the abdomen width (AW) and the carapace width (CW) in females of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil.

The individuals sampled in Mundaú Lagoon reached higher means of CW when compared to the values for *A. pisonii* found in other studies, such as in Fortaleza Bay by Leme & Negreiros-Fransozo (1998) (16.80 mm for both sexes), in Escuro River mangrove by Leme (2002) (13.60 mm for both sexes) and in the mangrove of the estuarine-lagoon complex of Cananéia-Iguape by Pescinelli *et al.*

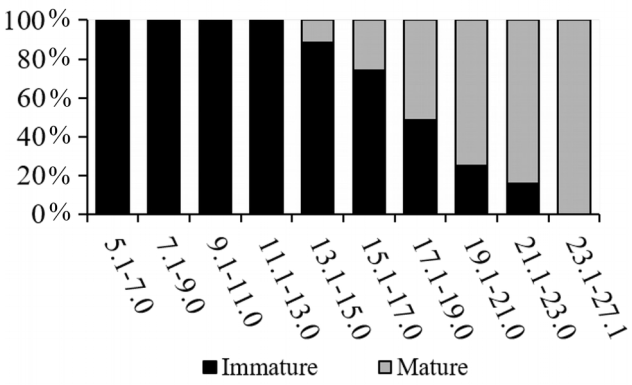

Figure 8. Distribution of the gonadal maturity stages into CW classes (mm) in males of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil.

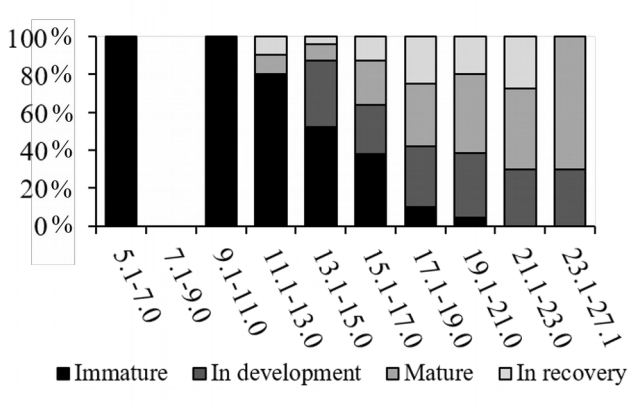

Figure 9. Distribution of the gonadal maturity stages into CW classes (mm) in females of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil.

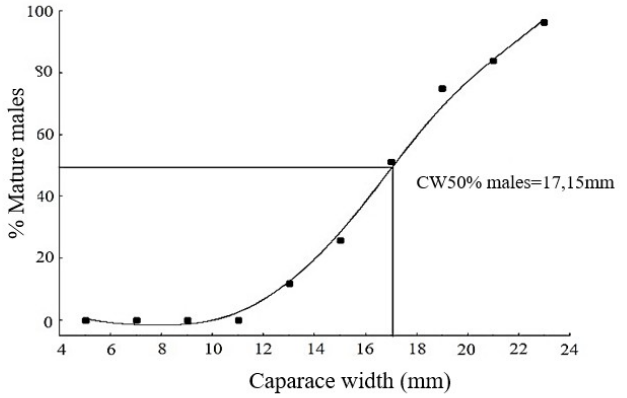

Figure 10. Hypothetical representation of physiological maturity curve in males of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil.

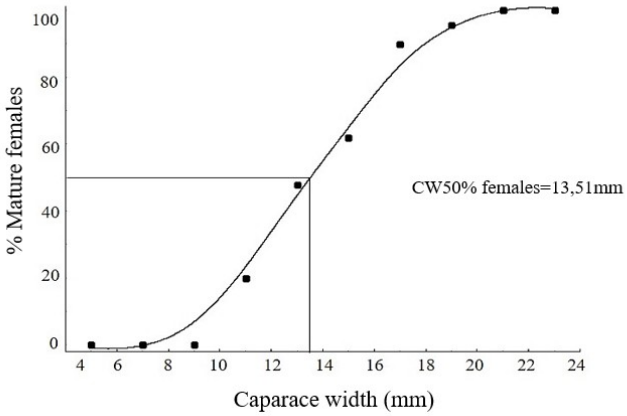

Figure 11. Hypothetical representation estimation of physiological maturity (CW50%) curve in females of *Aratus pisonii* from Mundaú Lagoon, state of Alagoas, Brazil.

(2015) (15.00 mm and 13.51 mm for males and females, respectively), all sampling areas in state of São Paulo, Brazil.

The variation between sex ratio is relative among the species of brachyuran crabs (Góes & Fransozo 2000). For example, Kowalczuk & Masunari (2000), studying the species *Armases angustipes* (Dana, 1852) did not observe significant differences in the sex ratio. In the present study, however, females were more abundant than males (sex ratio = 1♂:1.8♀), a result in accordance with those found for *Aratus pisonii* by Conde & Díaz (1989), Díaz & Conde (1989) in Venezuela, and Leme & Negreiros-Fransozo (2002) in state of São Paulo, which registered sex ratios of 1♂:1.6♀, 1♂:1.3♀ and 1♂:1.2♀, respectively. On the other hand, for same species, Nicolau & Oshiro (2007) observed males more abundant than females (1♂:0.7♀) in a population in Itacuruçá's mangrove, state of São Paulo.

In the present study, the ovigerous females of *A. pisonii* occurred throughout the year, corroborating with results found by Warner (1967), Díaz & Conde (1989) in Venezuela and by Leme & Negreiros-Fransozo (1998) in state of São Paulo, indicating that the reproduction occurs continuously. On the other hand, Nicolau & Oshiro (2002), studying the same species in state of Rio de Janeiro, observed the absence of ovigerous females in months of May and July, suggesting that the species presents a defined reproductive season (Table IV). According to Emmerson (1994), the brachyuran crabs inhabiting tropical region usually reproduce continuously, due to the stability of the environmental variables.

The females reached sexual maturity earlier than males (between 15.1 and 21 mm) and were observed the males dominating the largest size classes (> 21.1 mm). This relationship is associated to the females investing more energy for reproduction, including the production of eggs,

Table IV. Previous studies on CW range and reproductive peaks of *Aratus pisonii*, state of Alagoas, Brazil.

Locality	CW range (mm)	Reprodutive peak	Reference
Venezuela: Laguna of Tacarigua	4.9 – 29.90	November	Conde & Díaz (1989)
Venezuela: Morrocoy National Park	6.5 – 26.80	November	Díaz & Conde (1989)
Brazil: Fortaleza Bay, São Paulo	4.2 – 25.90	March	Leme & Negreiros Fransozo (1998)
Brazil: Escuro River, São Paulo	4.1 – 25.70	March	Leme (2002)
Brazil: Itacuruçá, Rio de Janeiro	5.1 – 25.00	February and March	Nicolau & Oshiro (2002)
Brazil: Cananéia-Iguape, São Paulo	5.2 - 24.39	-	Pescinelli *et al*. (2015)
Brazil: Mundaú Lagoon, Alagoas	5.13 - 27.07	March, June and July	Present study

which demands more energy than the production of sperm, while the males invest energy for the somatic growth, spermatocytes production, fighting for females and territorialism (Hartnoll 2006, Castiglioni & Negreiros-Fransozo 2006, Silva *et al*. 2007, Araújo *et al*. 2012). Physiological maturity was first achieved rather than morphological probably in response to some anthropogenic impact to which the environment may be subjected, which forces individuals to mature faster.

Pescinelli *et al*. (2015) observed for *A. pisonii* smaller sizes at morphological maturity than those observed in the present study (males: 10.47 mm and females: 11.52 mm of CW) and the males reached the sexual maturity earlier than females. This difference can be explained probably due to the different latitude, resulting in higher temperatures in state of Alagoas when compared to state of São Paulo, besides greater solar incidence and food supply (Conde & Díaz 1989). According to Annala *et al*. (1980) and Armitage & Landau (1982) places with high temperature tend to have specimens with late sexual maturity, when compared to places with lower temperatures.

Nicolau & Oshiro (2002) registered the size of first gonadal maturity synchronic with the first morphological maturity for females of *A. pisonii* in the state of Rio de Janeiro, phenomenon recorded for several groups of crustaceans (Santos 1994, Costa 1995, Pinheiro & Fransozo 1998). However, in the present study, the individuals reached physiological maturity before morphological maturity, similar to the results found for *Armases angustipes* by Lima & Oshiro (2006) and for *Callinectes ornatus* by Van Engel (1990). The present study proved to be relevant to the knowledge of the *Aratus pisonii* species, because even though they occur in a mangrove with different anthropogenic impacts, it presented a structured population and in constant development, through the continuous reproduction.

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