

# Population dynamics of the pea crab *Austinixa aidae* (Brachyura, Pinnotheridae): a symbiotic of the ghost shrimp *Callichirus major* (Thalassinidea, Callianassidae) from the southwestern Atlantic

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**ABSTRACT.** The Pinnotheridae family is one of the most diverse and complex groups of brachyuran crabs, many of them symbionts of a wide variety of invertebrates. The present study describes the population dynamics of the pea crab *Austinixa aidae* (Righi, 1967), a symbiont associated with the burrows of the ghost shrimp *Callichirus major* (Say, 1818). Individuals (n = 588) were collected bimonthly from May, 2005 to September, 2006 along a sandy beach in the southwestern Atlantic, state of São Paulo, Brazil. Our data indicated that the population demography of *A. aidae* was characterized by a bimodal size-frequency distribution (between 2.0 and 4.0 mm and between 8.0 and 9.0 mm CW) that remained similar throughout the study period. Sex ratio does not differ significantly from 1:1 ( $p > 0.05$ ), which confirms the pattern observed in other symbiotic pinnotherids. Density values ( $1.72 \pm 1.34$  ind.  $\cdot$  ap.<sup>-1</sup>) are in agreement with those found for other species of the genus. The mean symbiosis incidence (75.6%) was one of the highest among species of the Pinnotheridae family, but it was the lowest among the three studied species of the genus. Recruitment pattern was annual, beginning in May and peaking in July, in both years, after the peak of ovigerous females in the population (from March to May). Our findings describe ecological and biological aspects of *A. aidae* similar to those of other species of this genus, even from different geographic localities.

**KEYWORDS.** Population structure, pinnotherids, intertidal zone, symbiosis, Brazil.

**RESUMO.** Dinâmica populacional do caranguejo *Austinixa aidae* (Brachyura, Pinnotheridae): simbiote de *Callichirus major* (Thalassinidea, Callianassidae) no Atlântico sudoeste. Pinnotheridae constitui um dos mais diversos e complexos grupos de caranguejos, sendo muitos deles simbiotes de uma grande variedade de invertebrados. O presente estudo descreve a dinâmica populacional do caranguejo pinoterídeo *Austinixa aidae* (Righi, 1967), um simbiote que vive associado às galerias do “corrupto” *Callichirus major* (Say, 1818). Os exemplares (n = 588) foram coletados a cada dois meses entre maio de 2005 e setembro de 2006, em uma praia arenosa no litoral de São Paulo, Brasil. Nossos resultados indicaram que a população de *A. aidae* foi caracterizada por uma distribuição bimodal em classes de tamanho (entre 2,0 e 4,0 mm (CC: comprimento da carapaça) e entre 8,0 e 9,0 mm (CC)), padrão constante ao longo do período de estudo. A razão sexual não diferiu significativamente de 1:1 ( $p > 0,05$ ), confirmando o padrão observado para outros pinoterídeos simbiotes. Os valores de densidade desta espécie ( $1,72 \pm 1,34$  indivíduos por toca) estão de acordo com os estabelecidos para o gênero. A incidência média (75,6%) está entre as mais altas das espécies de Pinnotheridae, entretanto é a menor dentre as três espécies do gênero já estudadas. O padrão de recrutamento foi anual (tendo início em maio e atingindo maior proporção em julho, nos dois anos de estudo), e foi registrado após os picos de ocorrência de fêmeas ovígeras na população (de março a maio). Nossos resultados confirmam que *A. aidae* possui padrões ecológicos e biológicos similares aos desenvolvidos pelas espécies do gênero, mesmo em diferentes localidades.

**PALAVRAS-CHAVE.** Estrutura populacional, pinoterídeos, região entremarés, simbiose, Brasil.

Pinnotheridae pea crabs (Brachyura) are typically small and symbiotic of a wide variety of benthonic invertebrate hosts (SCHMITT *et al.*, 1973; WILLIAMS, 1984; HARRISON & HANLEY, 2005). They are endo and/or ectosymbionts found associated with bivalves, gastropods, echinoderms, echinurids, brachiopods, balanoglossids and ascidians, and inside polychaets' tubes and crustacean burrows (SCHMITT *et al.*, 1973; MANNING & MORTON, 1987; CAMPOS, 1990; HARRISON & HANLEY, 2005). Moreover, pinnotherid crabs are widely distributed along the marine coastal habitats of almost all oceans (SCHMITT *et al.*, 1973; MARTINS & D'INCAO, 1996). Despite their ecological importance and their great diversity, the information about the population demography of the pea crabs is still scarce, especially about those species that inhabit the Atlantic coast of the Americas.

*Austinixa* Heard & Manning, 1997 species are found on sandy beaches and own peculiar ecological features, such as the symbiotic life with thalassinoid crustaceans of the *Callichirus major* (Say, 1818) complex

or with few ecologically equivalent species (HEARD & MANNING, 1997; HARRISON, 2004), where they inhabit the upper portion of the host's burrows (MANNING & FELDER, 1989) and shelter near the entrance of these structures (Douglas F. Peiró, J. Antonio Baeza & Fernando L. Mantelatto, unpublished data). Studies that investigate the ecology of the sandy beaches indicate that the pinnotherid crabs from the *Austinixa* genus (= *Pinnixa*) are the frequent dominant infauna of the Americas (MANNING & FELDER, 1989; SOUZA & GIANUCA, 1995). The body feature of *Austinixa* (much wider lengthwise and with the third pair of walking legs more developed) is an adaptation to their symbiotic life style (HEARD & MANNING, 1997). All morphological adaptations facilitate their typical lateral locomotion into the confine narrow pipes and galleries of their hosts (HEARD & MANNING, 1997). Some of these features, shared with *Pinnixa* White, 1846, distinguish them from other brachyuran crabs (MCDERMOTT, 2006).

*Austinixa aidae* (Righi, 1967) has been reported as a symbiont of *C. major*, a callianassid shrimp that inhabits

the intertidal zone of many beaches in Brazil (RODRIGUES & SHIMIZU, 1997). This species owns an Occidental Atlantic geographic distribution, from the state of Amapá to the state of Rio Grande do Sul (Brazil) (MELO, 1996; COELHO, 1997). Recently, its northern edge of distribution has been expanded until Tobago (Caribbean Sea), due to a synonymy with *A. hardyi* Heard & Manning, 1997, as described by HARRISON & HANLEY (2005), who found neither morphological nor molecular significant differences to support the existence of the two species. NG *et al.* (2008) also considered the synonymy of this species, but PALACIOS-THEIL *et al.* (2009) did not agree with HARRISON & HANLEY (2005) about the synonymy of *A. aidae* with *A. hardyi*.

Few studies on the *Austinixa* genus associated with the Callianassidae were reported, some of them on population dynamics (ALVES & PEZZUTO, 1998a), distribution patterns (ALVES & PEZZUTO, 1998b; ALVES & RODRIGUES, 2003), and relative growth (ALVES & PEZZUTO, 1999; ALVES *et al.*, 2005) of *A. (= Pinnixa) patagoniensis* (Rathbun, 1918). Other researches clarify the evolution, biogeography and morphological/molecular systematic of the *Austinixa* genus (HARRISON, 2004; HARRISON & HANLEY, 2005; PALACIOS-THEIL *et al.*, 2009); and one reports on the biology of *A. gorei* (Manning & Felder, 1989) (McDERMOTT, 2006). *Austinixa aidae* is poorly known outside its taxonomy (MANNING & FELDER, 1989; COELHO, 1997; HEARD & MANNING, 1997; HARRISON, 2004; HARRISON & HANLEY, 2005; PALACIOS-THEIL *et al.*, 2009), description of the first zoeal stage (MANTELATTO & CUESTA, 2010), its mating system and relative growth (D. F. Peiró, J. A. Baeza & F. L. Mantelatto, unpublished data) have been recently studied.

Based on this promising scenario for investigation, this study describes the population dynamics of the symbiotic crab *A. aidae* in southeastern Brazil. The specific goals were to know more about size frequency distribution, sex ratio, density and mean incidence (percentage of occupation) in its hosts' galleries. The relation between density and size of the host, and reproductive and recruitment periods were also determined. Considering the morphological and ecological (symbiotic) intra-generic similarity in *Austinixa*, we evaluated these similarities' hypothesis in the population and in the biology of *A. aidae* and compared them to other species of this group using other available studies of the scientific literature -  $H_0$ ; there are differences among the species;  $H_1$ : there are no differences among the species.

## MATERIAL AND METHODS

**Samples and procedures.** The Perequê-açu beach (Ubatuba, northern coast state of São Paulo, Brazil; southwestern Atlantic), is located in the northern extremity of Ubatuba Bay. This beach is semi-protected and dissipative, composed by fine sand. Sampling was conducted bimonthly in the intertidal zone of the beach at daytime and during low tide, from May, 2005 to September, 2006. It was taken from a plot that was ~ 400 m long by ~ 30 m wide, parallel to the waterline, by the right side of river Indaia's mouth (23°24'59"S, 45°03'17"W).

The crustaceans were collected for 2 hours during each sampling visit by two people with a commercial

yabby pump (MANNING, 1975), used for sampling the *C. major* galleries. Once collected, the specimens were carefully washed with seawater and separated from the sand with a 1 mm mesh sieve and were directly placed into plastic bags, then labeled, frozen, and transported to the laboratory to be analyzed. Preliminary attempts permitted us to perfect the protocol above for shrimp and crab collections. We are confident that we were very efficient in capturing all animals from each gallery (D. F. Peiró, J. A. Baeza & F. L. Mantelatto, unpublished data). All material was preserved in ethanol 80% and deposited in the crustacean collection of the Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, (CCDB/FFCLRP/USP) under the catalogue # 2102. In each sampling period, water temperature and salinity were measured before the collection.

The burrows of *C. major* own only one aperture to the sand surface that varies in shape and has a ~ 5 mm diameter. Behind their opening, the burrows resemble a narrow tube that runs perpendicular to the surface up to 20 or 40 cm deep. *Austinixa aidae* inhabits this tubular section of the burrow and not the sections below it (D. F. Peiró, J. A. Baeza & F. L. Mantelatto, unpublished data). From this point below, the diameter of the gallery increases; it directs to the sides and deepens farther. The length and the diameter of this last section are proportional to the size of the shrimp that constructs the gallery and it attains approximately 1.5 m depth and between 10 and 30 mm diameter (RODRIGUES & SHIMIZU, 1997).

During sampling, each burrow opening was pumped only twice, since the sediment collapses due to the fine grains of the sand. However, the rapid collapsing of the sediment during the process of pumping does not affect the ability to catch the crabs, given that these crabs only inhabit the narrower upper part of the pumped burrows. Importantly, the rapid collapse also eliminates the risk of sampling the same burrow more than once (ALVES & PEZZUTO, 1998a). Taking into consideration that the galleries of *C. major* have a single opening to the sand surface (SHIMIZU & ROSSO, 2000), the values reported herein refer always to *A. aidae* per gallery of *C. major* (ind. • ap.<sup>-1</sup>) (ALVES & PEZZUTO, 1998a).

Three ontogenetic stages were considered: recruits, juveniles and adults. The recruits - defined as those individuals that survived the pelagicbenthic transition and became first crab instars - correspond to the smallest individuals collected, which formed the first size class in the size structure. In essence, recruits are the early juveniles or the young of the year (PARDO *et al.*, 2007). Juveniles were all non identified individuals that displayed undifferentiated or not well-developed pleopods, including the recruits. Adults were classified as either males or females according to the analysis of the pleopod. The males have a pair of long and thin modified pleopods on the ventral surface of the first abdominal somite and a pair of short modified pleopods on the second abdominal somite. The females have four pairs of short pleopods from the second to the fifth abdominal somites. The ovigerous females carry an egg mass adhered to the pleopods. The following measurement was performed under a stereomicroscope with the aid of a drawing tube (0.1 mm precision): the

maximum carapace width (CW) of all crabs retrieved from each gallery was measured. The oval area length (OL) of the *Thalassinidea* hosts was measured with a 0.02 mm precision caliper.

Data analysis. In order to evaluate the correlation between the environmental factors (water temperature and salinity) and the abundance of crabs, the Pearson's product moment correlation was utilized. The Normality Test KS (Kolmogorov-Smirnov) was used to evaluate the normality of the population according to the size classes' structure. The number of classes was determined by the STURGES (1926) formula:  $k = 1 + \text{Log}_2 n$ ; "k" is the number of classes and "n" is the size of sample.

The temporal patterns in the mean densities (number of *A. aidae* per gallery) and the incidence (percent of galleries occupied by *A. aidae*) were analyzed. Possible relations between densities and mean sizes of crabs and the size of their hosts were checked by the Pearson's product moment correlation (ALVES & PEZZUTO, 1998a). Differences from the expected 1:1 sex ratio pattern were checked for the total of samples collected and for each sampled month by the  $\chi^2$  test (WENNER, 1972).

Reproductive periods and recruitment were identified by the proportion of ovigerous females in relation to the total of females (PINHEIRO & FRANZOZO, 2002) and to the presence of juveniles in the samples (ALVES & PEZZUTO, 1998a), respectively. All statistical tests were conducted according to ZAR (1996), adopting the significance level  $p < 0.05$ .

## RESULTS

Population structure. During the studied period, a total of 588 specimens of *A. aidae* were collected in 455 galleries of *C. major*; they were distributed as 247 males (42.0%), 265 females (45.1%) (55 ovigerous: 9.4%), and 76 juveniles (non determined individuals) (12.9%) (13 recruits: 2.2%). The total number of individuals collected ranged from 24 (January, 2006) to 121 (July, 2006) (Tab. I). No correlation was found between water temperature ( $23.9 \pm 2.3^\circ\text{C}$ ), salinity ( $27.3 \pm 8.5\%$ ) and the total number of collected individuals, just between males, females and juveniles individually ( $p > 0.05$ ).

The carapace width varied between 2.4 and 10.1 mm (mean  $\pm$  SD =  $6.13 \pm 2.24$ ) for males, between 2.5 and 10.5 mm ( $6.27 \pm 2.03$ ) for females, and between 1.0 and 5.2 ( $2.46 \pm 0.68$ ) for juveniles. No significant differences in CW between the sexes were detected (t-test;  $-0.56$ ;  $p = 0.5791$ ), indicating the absence of sexual dimorphism with respect

to this dimension. Distribution in size classes was slightly bimodal for the total population (KS = 0.087;  $p < 0.05$ ), reflecting a non-normal pattern, except in September, 2005 and 2006 (KS = 0.079 and KS = 0.059;  $p > 0.200$ , respectively). Were detected two size class peaks, between 2.0–4.0 mm (CW) and between 8.0–9.0 mm (CW): the first peak mainly represented by juveniles (2.0–3.0 mm) and females (3.0–4.0 mm); the second by males and females (8.0–9.0 mm) (Fig. 1).

The maximum number of individuals collected per gallery was 14 crabs. The total mean density was  $1.72 \pm 1.34$  ind.  $\cdot$  ap.<sup>-1</sup>. The lowest mean density was registered in September, 2006 ( $1.3 \pm 0.5$  ind.  $\cdot$  ap.<sup>-1</sup>) and the highest in July, 2006 ( $2.52 \pm 1.94$  ind.  $\cdot$  ap.<sup>-1</sup>), with a particularly clear juveniles' variation (Fig. 2) during the sample period. The total mean incidence - percentage of occupation - of crabs in the galleries was 75.6%, with a variation during the sample period (Tab. I; Fig. 3). A significant positive correlation was found between the density (ind.  $\cdot$  ap.<sup>-1</sup>) of *A. aidae* and the size (OL) of their respective hosts *C. major* ( $r = 0.501$ ;  $p < 0.05$ ;  $n = 73$ ), i.e. the larger the hosts, the higher the number of symbiotic crabs in their burrows. On the other hand, no correlation was found between the size of crabs and the size of their respective hosts (OL) ( $r = 0.037$ ;  $p > 0.05$ ).

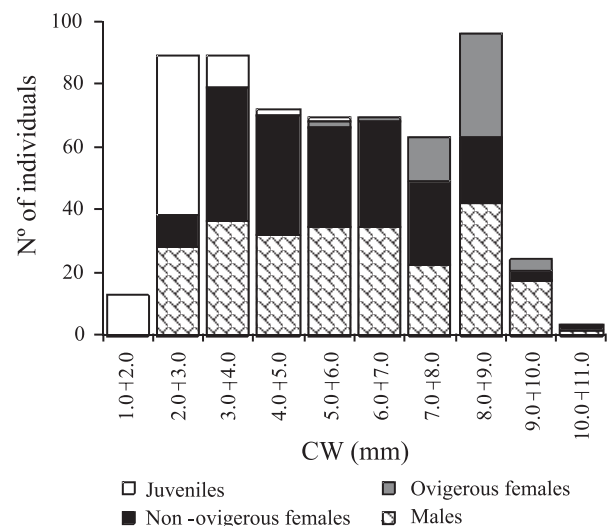


Figure 1. *Austinixa aidae* (Righi, 1967). Distribution frequency in size classes (CW, carapace width) of individuals collected inside *Callichirus major* (Say, 1818) galleries at Perequê-açu beach, Ubatuba, SP, Brazil, from May, 2005 to September, 2006. Recruits are the first size class individuals (1.0–2.0 mm CW).

Table I. Number of individuals of *Austinixa aidae* (Righi, 1967) collected inside the *Callichirus major* (Say, 1818) galleries at Perequê-açu beach, Ubatuba, SP, Brazil (Ovi., ovigerous females; %, percentage of ovigerous females in relation to total females; Juv., juveniles; Rec., recruits; Ratio, sex ratio male/female; % Inc., frequency of galleries occupied by crabs; S. gal., total of sampled galleries).

Sample period	Number of crabs				Juv.	Rec.	Total	Ratio	% Inc.	S. gal.
	Male	Female	Ovi.	%						
May 2005	25	18	9	50	7	1	50	1.39	79.5	39
July	22	24	0	0	18	5	64	0.92	84.8	33
September	24	32	3	9.4	2	0	58	0.75	81.1	53
November	30	34	14	41.2	1	0	65	0.88	64.8	54
January 2006	11	11	2	18.2	2	0	24	1.00	44.4	36
March	29	38	12	31.6	0	0	67	0.76	84.3	51
May	23	27	8	29.6	19	2	69	0.85	75.4	61
July	54	42	3	7.1	25	4	121	1.29	81.4	59
September	29	39	4	10.3	2	1	70	0.74	78.3	69
Total	247	265	55	20.8	76	13	588	0.93	75.6	455

The highest incidence of juveniles followed the months with a higher density of ovigerous females in the population (Figs 2, 3). It started in May and reached a higher proportion in July, in both years. Males and females did not present great peaks of density, the males' ranging from 0.50 to 1.13 ind. • ap.<sup>-1</sup> ( $0.73 \pm 0.20$  ind. • ap.<sup>-1</sup>), and the females' from 0.58 to 0.97 ind. • ap.<sup>-1</sup> ( $0.77 \pm 0.14$  ind. • ap.<sup>-1</sup>). Regarding the males, the tendency of density increase occurred in November, 2005, four months after the recruitment of juveniles; and the second in July, 2006, subsequent bimonthly to the coming of the juveniles to the population. Regarding the females, four light tendencies of density increase (July and November, 2005, and March and July, 2006) occurred. Males and females presented a higher mean of incidence ( $54.5 \pm 18.4\%$  and  $57.7 \pm 14.9\%$ , respectively) than the juveniles ( $17.8 \pm 20.2\%$ ); however the percentages of occupation vary correspondently during the sample period. The values of juvenile density fluctuated from 0 to 0.64 ind. • ap.<sup>-1</sup> ( $0.23 \pm 0.24$  ind. • ap.<sup>-1</sup>).

The total sex ratio was 0.93:1 in favor of females, which does not differ significantly from 1:1 ( $\chi^2 = 0.57$ ;  $p > 0.05$ ). The same pattern was obtained each month. In almost all size classes (7) the ratios do not differ

significantly from 1:1 ( $p > 0.05$ ), except in classes between 2.0–3.0 mm and 9.0–10.0, where males predominate (2.8:1 and 2.4:1, respectively); and between 7.0–8.0 mm, where females predominate (0.5:1) (Fig. 4).

Reproductive period and recruitment. The percentage of ovigerous females in relation to the total of females was 20.8%; the frequency varies from 0 (July, 2005) to 50% (May, 2005) among the samples (Tab. I; Fig. 5). The ovigerous females occurred in almost all sample months, except in July, 2005. Seasonal increases in frequency and abundance of this group were identified, with expressive peaks in 2005 (May and November: 50.0 and 41.2%, respectively), and in 2006 (between March and May: 31.6 and 29.6%, respectively). Due to the presence of ovigerous females throughout the year and the seasonal peaks, the reproductive period was characterized as seasonal continuous. This reproduction system can supply a constant larval input reflecting in continuous recruitment, but not observed in this study. It is important to point out in our study that the recruits were identified only in the months subsequent to the peaks of ovigerous females. The recruitment starts in May and attains major proportion in July, in both years, and it contributes to increase the abundance in the subsequent size classes in the subsequent sample months (Figs 6, 7).

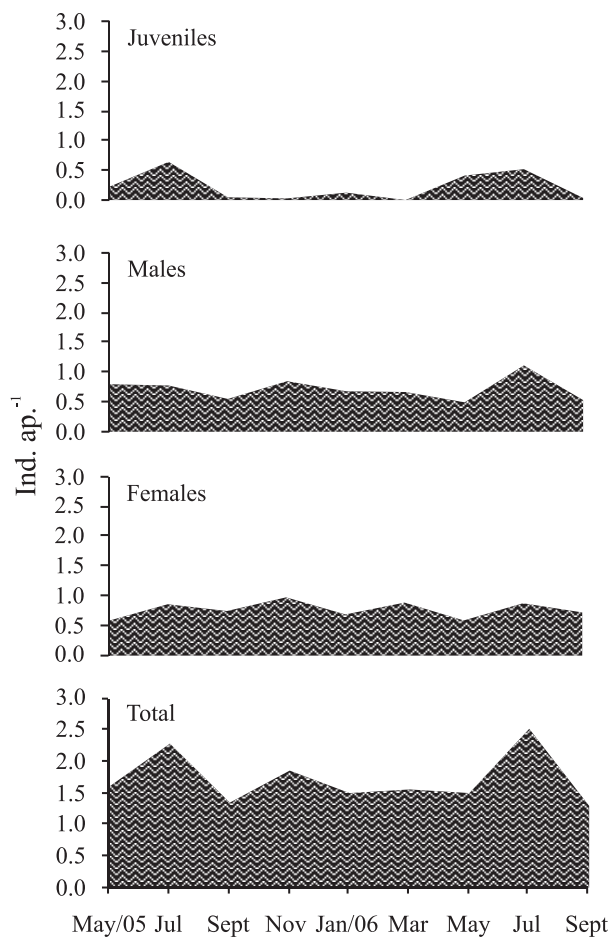


Figure 2. *Austinixa aidae* (Righi, 1967). Bimonthly variation of mean densities (Ind. • ap.<sup>-1</sup>: number of individuals per gallery) of juveniles, males, females and total individuals collected inside *Callichirus major* (Say, 1818) galleries at Perequê-açu beach, Ubatuba, SP, Brazil, from May, 2005 to September, 2006.

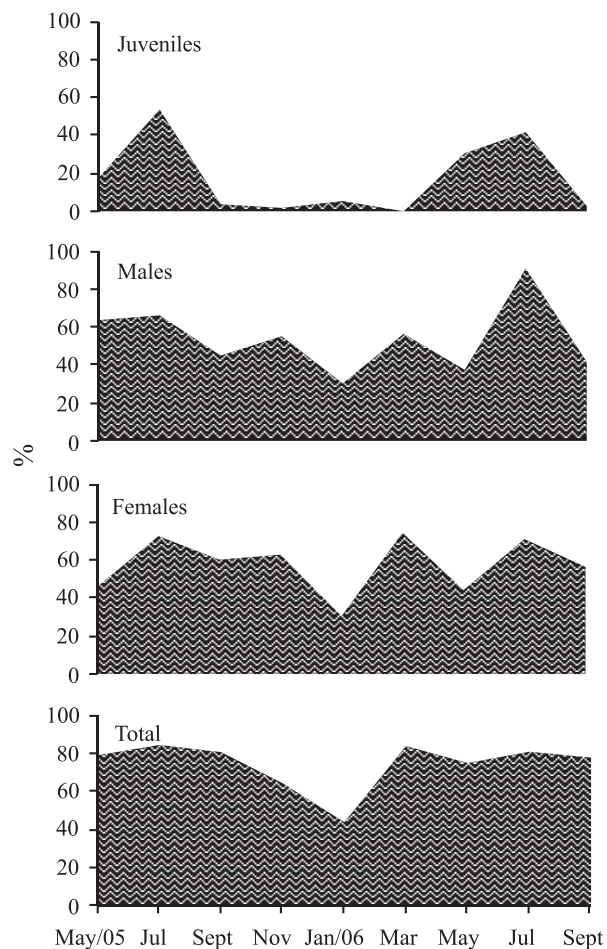


Figure 3. *Austinixa aidae* (Righi, 1967). Bimonthly variation of incidence (%) of galleries inhabited by juveniles, males, females and total of individuals collected inside *Callichirus major* (Say, 1818) galleries at Perequê-açu beach, Ubatuba, SP, Brazil, from May, 2005 to September, 2006.

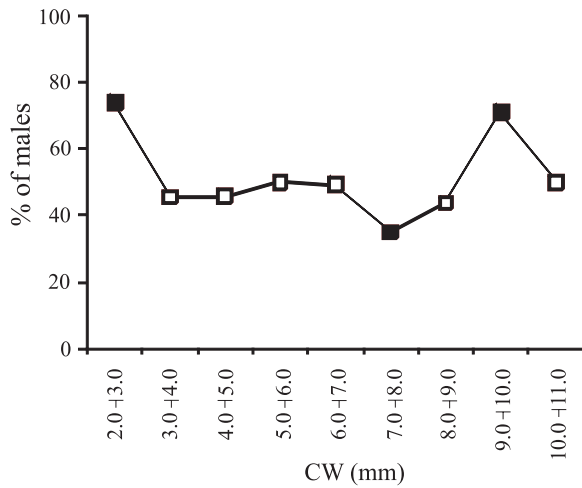


Figure 4. *Austinia aida* (Righi, 1967). Percentage of males in relation to the total of females per size classes collected inside *Callinectes major* (Say, 1818) galleries at Perequê-açu beach, Ubatuba, SP, Brazil, from May, 2005 to September, 2006 (CW, carapace width; black quadrates: sizes with sex ratio different from 1:1).

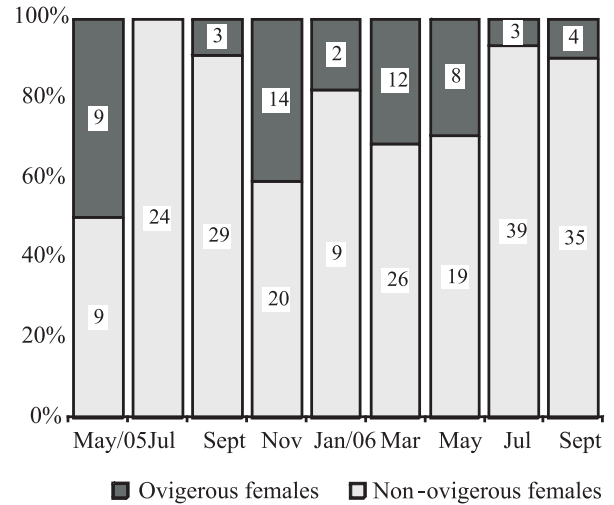


Figure 5. *Austinia aida* (Righi, 1967). Bimonthly frequency of ovigerous females and non-ovigerous females collected inside *Callinectes major* (Say, 1818) galleries at Perequê-açu beach, Ubatuba, SP, Brazil, from May, 2005 to September, 2006. The values inside bars are the number of individuals.

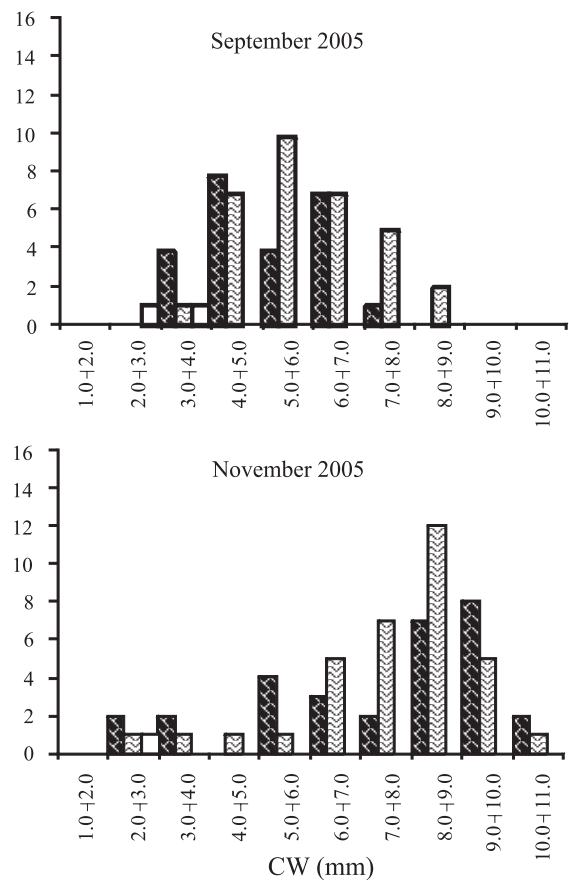
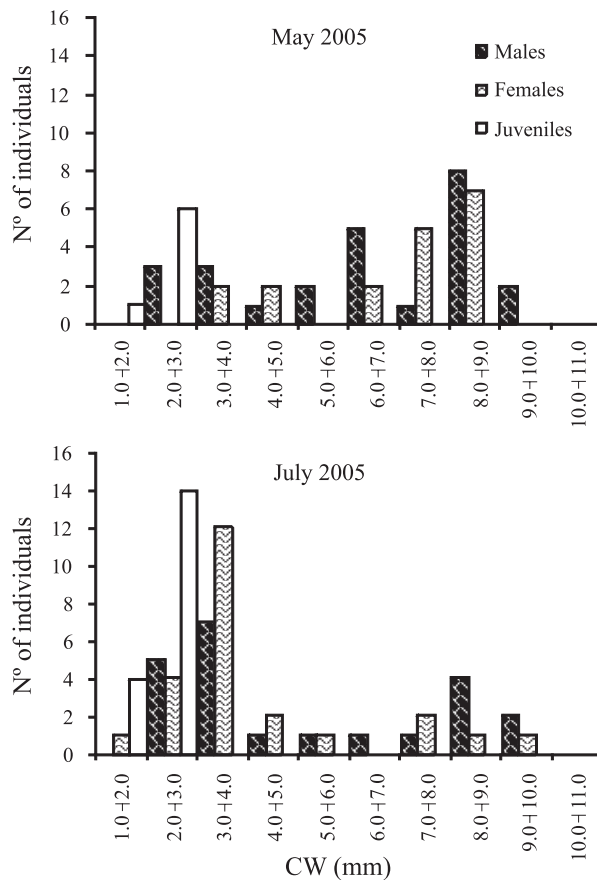


Figure 6. *Austinia aida* (Righi, 1967). Size frequencies' distribution (CW = carapace width) of individuals collected inside *Callinectes major* galleries at Perequê-açu beach, Ubatuba/SP, Brazil, from May, 2005 to November, 2005.

The juveniles were present in almost all samples, except in March, 2006, with seasonal abundance increases. The peaks of juveniles occur in May and July, in both years, in the months subsequent to the ovigerous females' peaks (Fig. 8) (remembering

that the recruits are included in this classification). An exception occurred thereafter November, 2005 ovigerous female peak (the month with higher incidence), but subsequent months there are not a peak of juveniles.

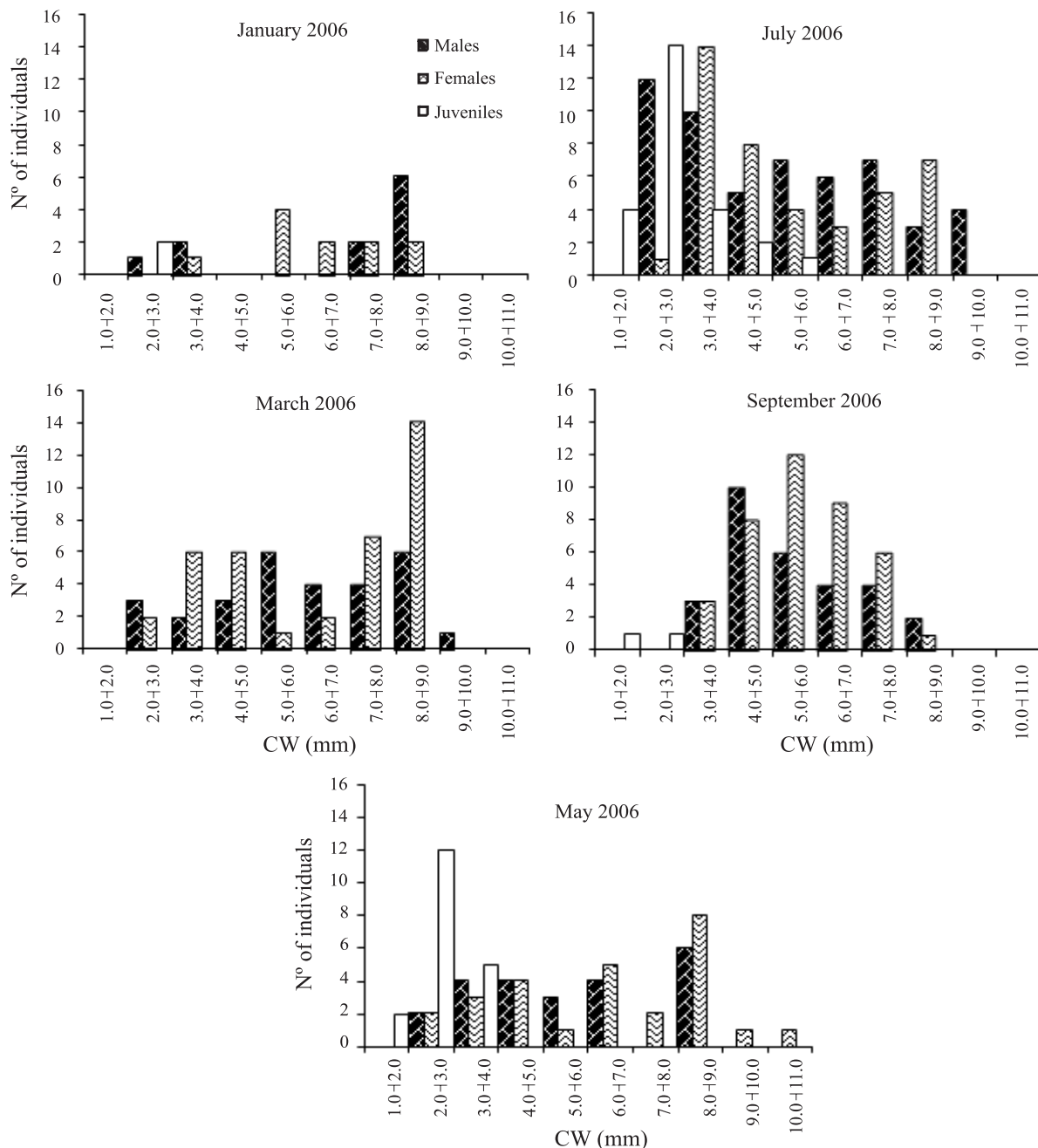


Figure 7. *Austinixa aida* (Righi, 1967). Size frequencies' distribution (CW = carapace width) of individuals collected inside *Callichirus major* galleries at Perequê-açu beach, Ubatuba/SP, Brazil, from May to November, 2006.

The size classes' distribution of juveniles (Figs 6, 7) corroborated the same pattern identified by density (Fig. 2) and incidence (Fig. 3) analyses, with the seasonal continuous reproduction and the input of juveniles occurring throughout the year. Two courts were also identified in some sampled periods (Figs 6, 7).

## DISCUSSION

**Population structure.** It was detected that the dynamics of *Austinixa aida* followed the pattern observed for congeneric species inhabiting different regions and different hosts, with some peculiarities. This profile can be hypothesized as adaptations for living in a

different host and with polygynandrous mating systems (D. F. Peiró, J. A. Baeza & F. L. Mantelatto, unpublished data). The population of *A. aida* shows common symbiotic characteristics that had been observed in a few other pinnotherid of the genus. At Perequê-açu beach, these crabs did not show correlation with the environmental factors. Nor did the ovigerous females separately, which can be more subject to the action of these factors (SASTRY, 1983). The absence of correlation may be related to the water temperature and salinity measurement method, which was performed only before the collections. This method cannot reflect the values that represent the sampled months. Moreover, tropical and subtropical regions present minor seasonal variation

in environmental factors, with fall variations during the year and, in some cases, no correlation can be detected (AYRES-PERES & MANTELATTO, 2008; MIRANDA & MANTELATTO, 2009).

A bimodal distribution frequency of individual size in the *A. aidae* population - with two peaks in the size class structure - reflects two reproductive and settlement pulses per year, one of them more intensive, accumulating individuals in higher size classes (where the growth stops), besides the differentiated growth between males and females. The continuous presence of individuals in all size classes in the bimonthly size-class frequency distribution (except in September, 2005 and 2006) is a strong argument against the hypothesis of catastrophic mortality (DÍAZ & CONDE, 1989). Considering that the present study has provided no information about behavioral and mortality differences between sexes, the bimodal condition could be explained by the intensive settlement in certain periods (MANTELATTO & SOUSA, 2000; MIRANDA & MANTELATTO, 2009). Considering the symbiotic lifestyle and that *A. aidae* is not monogamous (D. F. Peiró, J. A. Baeza & F. L. Mantelatto, unpublished data) we cannot discard the hypothesis about the influence of the host aspects on the frequency distribution pattern observed here.

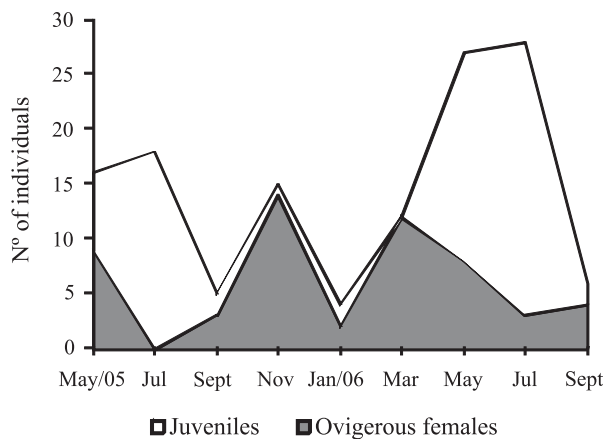


Figure 8. *Austinixa aidae* (Righi, 1967). Bimonthly abundance of juveniles and ovigerous females collected inside *Callichirus major* galleries at Perequê-açu beach, Ubatuba/SP, Brazil, from May, 2005 to September, 2006.

In some studies with Pinnotheridae - due to its typical symbiotic relationship - the main variables were expressed in number of individuals per host, per pipe aperture or per hosts' gallery aperture (McDERMOTT, 1981; ALVES & PEZZUTO, 1998A; McDERMOTT, 2006). Comparing our results with other *Austinixa* species, the number of host gallery apertures is a differential factor to be considered, once they vary among the Callianassidae members (Tab. II).

Space and food availability in the gallery may be the most important factors controlling the Pinnotheridae density. The strong currents and water changes produced by the host provide a high supply of oxygen and food at the burrow entrance, probably in a sufficient rate for many crabs (ALVES & PEZZUTO, 1998a). The maximum and the mean densities of *A. aidae* per gallery were lower than the values found in *A. patagoniensis* from the Southern Brazil (state of Rio Grande do Sul) associated with Callianassidae *Sergio mirim* (Rodrigues, 1971) (ALVES & PEZZUTO, 1998a), and higher than those found in *A. gorei* associated with Callianassidae *Gilvossius setimanus* (DeKay, 1844) from Miami (USA) (McDERMOTT, 2006) (Tab. III). Again, as mentioned above, we cannot discard the hypothesis about the influence of the host aspects on the pattern observed here.

The total density increases were directly influenced by the recruitment and/or entrance of juveniles in the population (mainly in May and in July); the increases of this interest group reflect significantly on the total population. Differently from the Pinnotheridae that live inside the shells, on the echinoderms, or in relatively small polychaets' pipes (MANNING & MORTON, 1987; ALVES & PEZZUTO, 1998A; CAMPOS, 1990), the galleries of *C. major* provide a sufficient space to the coexistence of a higher number of *A. aidae*, as found here ( $1.72 \pm 1.34$  ind. • ap.<sup>-1</sup>). The mean incidence of *Austinixa* is among the highest when compared with other Pinnotheridae species (ALVES & PEZZUTO, 1998a). However, *A. aidae* owns the lowest mean incidence among the three studied species of the genus, 75.6% (Tab. III). This parameter shows strong variation among the Pinnotheridae species, and more studies are necessary for better conclusions. The high incidence in samples from autumn and winter months corresponds to recruitment. The correspondence between

Table II. Number of gallery apertures (n°. ap.) of respective hosts (Thalassinidea) occupied by *Austinixa* Heard & Manning, 1997 species (Pinnotheridae); (Ratio, sex ratio male/female).

Species	Host	n°. ap.	Ratio	Reference
<i>A. patagoniensis</i>	<i>Sergio mirim</i>	> 1	1.00	ALVES & PEZZUTO (1998a)
<i>A. gorei</i>	<i>Gilvossius setimanus</i>	1	0.75	McDERMOTT (2006)
<i>A. aidae</i>	<i>Callichirus major</i>	1	0.93	Present study

Table III. Number of sampled galleries, mean percentage of incidence, mean density and maximum density of *Austinixa* Heard & Manning, 1997 per gallery of respective host (Thalassinidea). Values between parenthesis (minimum and maximum), unavailable data (ud).

Species	Host	Sampled galleries	Mean % of incidence	Mean density (ind. • ap. <sup>-1</sup> )	Maximum density (ind. • ap. <sup>-1</sup> )	Reference
<i>A. patagoniensis</i>	<i>Sergio mirim</i>	510	83.9 (63-98)	3.1	29	ALVES & PEZZUTO (1998a)
<i>A. gorei</i>	<i>Gilvossius setimanus</i>	35	100 (ud)	1.2	5	McDERMOTT (2006)
<i>A. aidae</i>	<i>Callichirus major</i>	442	75.6 (44-85)	2.6	14	Present study

the males' and females' density was more evident after the juveniles' peaks. Decrease in this parameter was observed after the peaks. It is important to register the strong drop in the incidence and in the number of collected individuals in January, 2006, which followed the decrease that started bimonthly before, in November, 2005. That period corresponded to a higher incidence of tourists in the area using suction pumps to capture the host and use it in non-professional fisheries. Although no studies are available to check this matter, and analogously to what was observed in the southern area (SOUZA & BORZONE, 2003), we believe in the influence of those practices on the dynamics of both host and pinnotherid populations in this area during the summer.

The analysis between the density of *A. aidae* and the size of their respective host shows a positive correlation, as opposed to what was found in *P. patagoniensis* when associated with *Sergio mirim* (ALVES & PEZZUTO, 1998a). This result grants a peculiar characteristic to *A. aidae* in the family. The positive correlation is more frequently observed among crabs that are symbiotic of bivalves or polychaets tubes, where the free space in the shell is limited (GRAY, 1961; PEARCE, 1966a, 1966b; JONES, 1977; BIERBAUM & FERSON, 1986).

The sex ratio did not differ significantly from the pattern proposed by FISCHER (1930), where the natural selection supports an equal proportion for both sexes. In most of the sampled months, the sex ratio was in favor of females, yet not significantly different from 1:1 ( $p < 0.05$ ). When a ratio variation occurs, it can be explained by mortality, habitat preferences and alimentary restrictions acting intensively on one sex (TURRA & LEITE, 2000); however, these factors do not evaluated here. In addition, the previous hypothesis that suggested that adult crabs featured a mating system other than monogamy in the studied species (D. F. Peiró, J. A. Baeza & F. L. Mantelatto, unpublished data) should be considered an explanation for the pattern of the sex ratio observed here.

It was not possible to apply any classification proposed by WENNER (1972) to the sex ratio pattern in this population. No significant differences between the male-female ratios in the majority of size classes were found; however, there were significant differences in the 2<sup>nd</sup> and the 9<sup>th</sup> size classes in favor of males and in the 7<sup>th</sup> in favor of females. This probably brings us to a new classification that has been registered here for this group of symbiont and that we named "near pattern". This classification is close to the "pattern" proposed by WENNER (1972), in which there are no differences in the male/female proportion in most size classes, but allows some few significant differences between the sexes, in favor of males in the final size classes.

The sex ratio of *A. aidae* is similar to that found in *A. patagoniensis* ( $\approx 1:1$ ) (ALVES & PEZZUTO, 1998a), however it diverges significantly from *A. gorei* (0.75:1) (McDERMOTT, 2005) (Tab. II). The sex ratio in Pinnotheridae varies considerably among and inside the species (ALVES & PEZZUTO, 1998a): *Pinnixa chaetoptera* Stimpson, 1860, a symbiotic of the Polychaeta *Amphitrite ornata* (LEIDY, 1855) from New Jersey (USA) shows 0.86:1; *Chaetopterus variopedatus* (Renier, 1804) from North Carolina (USA) presents 0.97:1 (McDERMOTT, 2005) and *Pinnixa cylindrica* (Say, 1818), a symbiotic in the pipes

of the Polychaeta *Arenicola cristata* (Stimpson, 1856), has the most unequal sex ratio of 0.25:1 (McDERMOTT, 1981). These traits may reflect adaptations between hosts, species and environmental conditions, demonstrating a high plasticity of the species' sex ratio features in their distribution.

Reproductive period and recruitment. The ovigerous females of *A. aidae* were present throughout the year; however, they were more expressive in May and in November, 2005 and in May, 2006. The reproductive pattern at Perequê-açu beach was classified as Seasonal Continuous (according to PINHEIRO & TERCEIRO, 2000), corroborating the pattern found in the congener *A. patagoniensis* (ALVES & PEZZUTO, 1998a), in which both species occurred in subtropical climate areas. The reproductive pattern registered in this study had been described to other Brachyura from the north shore of the state of São Paulo: *Callinectes danae* Smith, 1869; *Portunus spinimanus* Latreille, 1819; *Callinectes ornatus* Ordway, 1863 and *Arenaeus cribrarius* (Lamarck, 1818) (COSTA & NEGREIROS-FRANZOZO, 1998; SANTOS & NEGREIROS-FRANZOZO, 1999; MANTELATTO & FRANZOZO, 1999; PINHEIRO & TERCEIRO, 2000, respectively). Generally, Brachyura crustaceans from temperate areas present seasonal reproduction, which occurs in the hottest seasons of the year (WARNER, 1977; ASAKURA & KIKUCHI, 1984), while crabs from tropical and subtropical regions have a continuous reproduction throughout the year, usually in approximate rates (SASTRY, 1983).

The recruitment increases occurred predominantly between May, 2005 and July, 2005, and in July, 2006 (Autumn-Winter), which were the sampled months subsequent to the ovigerous females' peaks (except in November, 2005 peak). These patterns reflect seasonal recruitment events with two annual increases, similar to those found in other species of the family, such as *A. patagoniensis* (ALVES & PEZZUTO, 1998a), *Pinnixa chaetoptera* (McDermott, 2005), *P. littoralis* Holmes, 1894 and *P. faba* (Dana, 1851) (PEARCE, 1966a). However, there are records on continuous reproductive periods in *A. cristata* (Rathbun, 1900), *P. cylindrica* and *P. sayana* Stimpson, 1860, based on the presence of zoea in the plankton community in a North Carolina estuary (USA) (DOWDS, 1978).

Comparing this study to others realized with the congeneric *A. patagoniensis* from southern Brazil, and with *A. gorei* from Florida (USA), similar patterns were verified in ecological and biological aspects among the three species, even in cases from far geographical localities, corroborating the postulated by other authors who had referred the similarity of these parameters among the *Austinixa* genus (MANNING & FELDER, 1989; HEARD & MANNING, 1997; ALVES & PEZZUTO, 1998a; McDERMOTT, 2006; D. F. Peiró, J. A. Baeza & F. L. Mantelatto, unpublished data). However, a comparison of the *A. aidae* population dynamics with other species of pinnotherids was not entirely satisfactory due to a lack of information and studies about their geographical distribution limits. The differences regarding the relation with the host size obtained between *A. aidae* and *C. major* inspire new ideas and doubts about this interesting lifestyle that should be evaluated in the future in order to better contribute to the understanding of the symbiosis evolution.



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#### REFERENCES

- ALVES, E. S. & PEZZUTO, P. R. 1998a. Population dynamics of *Pinnixa patagoniensis* Rathbun, 1918 (Brachyura: Pinnotheridae): a symbiotic crab of *Sergio mirim* (Thalassinidea: Callianassidae) in Cassino Beach, Southern Brazil. **Publicazioni della Stazione Zoologica di Napoli: Marine Ecology** 19(1):37-51.
- \_\_\_\_\_. 1998b. Dispersão de *Pinnixa patagoniensis* no mediolitoral da praia do Cassino, RS, Brasil. **Atlântica** 20(1):5-21.
- \_\_\_\_\_. 1999. Crescimento relativo de *Austinixa patagoniensis* (Rathbun, 1918) (Decapoda: Pinnotheridae), simbiote de *Sergio mirim* (Rodrigues, 1971) (Decapoda: Callianassidae) na praia do Cassino, RS. **Nauplius** 7(1):83-92.
- ALVES, E. S. & RODRIGUES, S. A. 2003. Distributional patterns of *Austinixa patagoniensis* (Rathbun, 1918) (Decapoda: Pinnotheridae), a symbiotic crab of *Callichirus major* (Decapoda: Callianassidae) in Balneário Camboriú Beach, Santa Catarina, Brazil. **Journal of Coastal Research** 35:408-417.
- ALVES, E. S.; RODRIGUES, S. A. & PEZZUTO, P. R. 2005. Estudo do crescimento relativo de *Austinixa patagoniensis* (Rathbun) (Decapoda, Pinnotheridae) simbiote de *Callichirus major* (Say) (Decapoda, Callianassidae) no mesolitoral da praia de Balneário Camboriú, Santa Catarina, Brasil. **Revista Brasileira de Zoologia** 22(3):784-792.
- ASAKURA, A. & KIKUCHI, T. 1984. Population ecology of the sand dwelling hermit crab, *Diogenes nitidimanus* Terao. 2. Migration and life history. **Publications from the Amakusa Marine Biological Laboratory** 7(2):109-123.
- AYRES-PERES, L. & MANTELATTO, F. L. 2008. Patterns of distribution of the hermit crab *Loxopagurus loxochelis* (Moreira, 1901) (Decapoda, Diogenidae) in two coastal areas of southern Brazil. **Revista de Biologia Marina y Oceanografía** 43(2):399-411.
- BIERBAUM, R. M. & FERSON, S. 1986. Do symbiotic pea crabs decrease growth rate in mussels? **The Biological Bulletin** 170(1):51-61.
- CAMPOS, E. 1990. *Calyptraeothers*, a new genus of Pinnotheridae for the limpet crab *Fabia granti* Glassell, 1933 (Crustacea, Brachyura). **Proceedings of the Biological Society of Washington** 103(2):364-371.
- COELHO, P. A. 1997. Revisão do gênero *Pinnixa* White, 1846, no Brasil (Crustacea, Decapoda, Pinnotheridae). **Trabalhos Oceanográficos da Universidade Federal de Pernambuco** 25:163-193.
- COSTA, T. M. & NEGREIROS-FRANZOZO, M. L. 1998. The reproductive cycle of *Callinectes danae* Smith, 1869 (Decapoda, Portunidae) in the Ubatuba region, Brazil. **Crustaceana** 71(6): 615-627.
- DÍAZ, H. & CONDE, J. E. 1989. Population dynamics and life history of the mangrove crab *Aratus pisonii* (Brachyura, Grapsidae) in a marine environment. **Bulletin of Marine Science** 45(1):148-163.
- DOWDS, R. E. 1978. Distribution and reproduction of four species of *Pinnixa* (Crustacea: Brachyura) in a North Carolina estuary. **American Zoologist** 18:599.
- FISCHER, R. A. 1930. **The genetical theory of natural selection**. 2. ed. New York, Dover. 291p.
- GRAY, I. E. 1961. Changes in abundance of the commensal crabs of *Chaetopeterus*. **The Biological Bulletin** 120(3):353-359.
- HARRISON, J. S. 2004. Evolution, biogeography, and the utility of mitochondrial 16s and COI genes in phylogenetic analysis of the crab genus *Austinixa* (Decapoda: Pinnotheridae). **Molecular Phylogenetics and Evolution** 30(3):743-754.
- HARRISON, J. S. & HANLEY, P. W. 2005. *Austinixa aidae* Righi, 1967 and *A. hardyi* Heard and Manning, 1997 (Decapoda: Brachyura: Pinnotheridae) synonymized, with comments on molecular and morphometric methods in crustacean taxonomy. **Journal of Natural History** 39(42):3649-3662.
- HEARD, R. W. & MANNING, R. B. 1997. *Austinixa*, a new genus of pinnotherid crab (Crustacea: Decapoda: Brachyura), with the description of *A. hardyi*, a new species from Tobago, West Indies. **Proceedings of the Biological Society of Washington** 110(3):393-398.
- JONES, J. B. 1977. Natural history of the pea crab in Wellington Harbour, New Zealand. **New Zealand Journal of Marine and Freshwater Research** 11(4):667-676.
- MANNING, R. B. 1975. Two methods for collecting decapods in shallow water. **Crustaceana** 29:317-319.
- MANNING, R. B. & FELDER, D. L. 1989. The *Pinnixa cristata* complex in Western Atlantic, with a descriptions of two new species (Crustacea: Decapoda: Pinnotheridae). **Smithsonian Contributions to Zoology** 473:1-26.
- MANNING, R. B. & MORTON, B. 1987. Pinnotherids (Crustacea: Decapoda) and leptonaceans (Mollusca: Bivalvia) associated with sipunculan worms in Hong Kong. **Proceedings of the Biological Society of Washington** 100(3):543-551.
- MANTELATTO, F. L. & CUESTA, J. A. 2010. Morphology of the first zoeal stage of the commensal southwestern Atlantic crab *Austinixa aidae* (Righi, 1967) (Brachyura: Pinnotheridae), hatched in the laboratory. **Helgolander Marine Research** 64(4):343-348.
- MANTELATTO, F. L. & FRANZOZO, A. 1999. Reproductive biology and moulting cycle of the crab *Callinectes ornatus* (Decapoda, Portunidae) from the Ubatuba region, São Paulo, Brazil. **Crustaceana** 72(1):63-76.
- MANTELATTO, F. L. & SOUSA, L. M. 2000. Population biology of the hermit crab *Paguristes tortugae* Schmitt, 1933 (Anomura, Diogenidae) from Anchieta Island, Ubatuba, Brazil. **Nauplius** 8(2):185-193.
- MARTINS, S. T. S. & D'INCAO, F. 1996. Os Pinnotheridae de Santa Catarina e Rio Grande do Sul, Brasil (Decapoda, Brachyura). **Revista Brasileira de Zoologia** 13(1):1-26.
- MCDERMOTT, J. J. 1981. Biology of the symbiotic crab *Pinnixa cylindrical* (Say) (Decapoda, Pinnotheridae). **Proceedings of the Pennsylvania Academy of Science** 55:23-27.
- \_\_\_\_\_. 2005. Biology of the brachyuran crab *Pinnixa chaetoptera* Stimpson (Decapoda: Pinnotheridae) symbiotic with tubicolous polychaets along the Atlantic coast of the United States, with additional notes on other polychaets associations. **Proceedings of the Biological Society of Washington** 118(4):742-764.
- \_\_\_\_\_. 2006. The biology of *Austinixa gorei* (Manning & Felder, 1989) (Decapoda, Brachyura, Pinnotheridae) symbiotic in the burrows of intertidal ghost shrimp (Decapoda, Thalassinidea, Callianassidae) in Miami, Florida. **Crustaceana** 79(3):345-361.
- MELO, G. A. S. 1996. **Manual de identificação dos Brachyura (Caranguejos e Siris) do litoral brasileiro**. São Paulo, Plêiade/FAPESP. 604p.
- MIRANDA, I. & MANTELATTO, F. L. 2009. Estimating population features of the anomuran crab *Petrolisthes armatus* (Porcellanidae) in a remaining and impacted mangrove area of the western Atlantic. **Journal of Natural History** 43(33-34):2027-2039.
- NG, P. K. L.; GUINOT, D. & DAVIE, P. J. F. 2008. Systema brachyurorum: Part 1. An annotated checklist of extant brachyuran crabs of the world. **The Raffles Bulletin of Zoology** 17:1-286.
- PALACIOS-THIEL, E.; CUESTA, J. A.; CAMPOS, E. & FELDER, D. L. 2009. Molecular genetic re-examination of subfamilies and polyphyly in the Family Pinnotheridae (Crustacea: Decapoda). *In*: MARTIN,

- J. W.; CRANDALL, K. A. & FELDER, D. L. eds. **Crustacean Issues: Decapod crustacean phylogenetics**. Boca Raton, Taylor & Francis/CRC. p.423-442.
- PARDO, L. M.; PIRAUD, F.; MANTELATTO, F. L. & OJEDA, F. P. 2007. Ontogenetic pattern of resource use by the tiny hermit crab *Pagurus villosus* (Paguridae) from the temperate Chilean coast. **Journal of Experimental Marine Biology and Ecology** **353**(1):68-79.
- PEARCE, J. B. 1966a. On *Pinnixa faba* and *Pinnixa littoralis* (Decapoda: Pinnotheridae) symbiotic with the clam, *Tresus capax* (Pelecypoda: Maetridae). In: BARNES, H. (ed.) **Some contemporary studies in Marine Science**. Allen Unwin Ltd., London. p.565-589.
- \_\_\_\_\_. 1966b. The biology of the mussel crab, *Fabia subquadrata*, from the waters of San Juan Archipelago, Washington. **Pacific Science** **20**:3-35.
- PINHEIRO, M. A. A. & FRANSOZO A. 2002. Reproduction of the speckled swimming crab *Arenaeus cribrarius* (Brachyura: Portunidae) on the Brazilian coast near 23°30' S. **Journal of Crustacean Biology** **22**(2):416-428.
- PINHEIRO, M. A. A. & TERCEIRO, O. S. L. 2000. Fecundity and reproductive output of the speckled swimming crab *Arenaeus cribrarius* (Lamarck, 1818) (Brachyura, Portunidae). **Crustaceana** **73**(9):1121-1137.
- RODRIGUES, S. A. & SHIMIZU, R. M. 1997. Autoecologia de *Callichirus major* (Say, 1818). **Oecologia Brasiliensis** **3**(1):155-170.
- SANTOS, S. & NEGREIROS-FRANZOZO, M. L. 1999. Reproductive cycle of the swimming crab *Portunus spinimanus* Latreille, 1819 (Crustacea, Decapoda, Brachyura) from Ubatuba, São Paulo, Brazil. **Revista Brasileira de Zoologia** **16**(4):1183-1193.
- SASTRY, A. N. 1983. Ecological aspects of reproduction. In: VERNBERG, F. J. & VERNBERG, W. B. eds. **The Biology of Crustacea VIII: environmental adaptations**. New York, Academic. p.179-270.
- SCHMITT, W. L.; MCCAIN J. C. & DAVIDSON, E. S. 1973. Decapoda I. Brachyura I. Family Pinnotheridae. In: GRUNER, H. E. & HOLTHUIS L. B. eds. **Crustaceorum Catalogus**. The Hague, W. Junk. v.3, p.32-37.
- SHIMIZU, R. M. & ROSSO, S. 2000. Influence of an oil spill on the abundance of *Callichirus major* (Say, 1818) on a sand beach in southeastern Brazil (Crustacea: Decapoda: Thalassinidea). **Nauplius** **8**(1):63-72.
- SOUZA, J. R. B. & BORZONE, C. A. 2003. A extração do corrupto, *Callichirus major* (Say) (Crustacea, Thalassinidea), para uso como isca em praias do litoral do Paraná: as populações exploradas. **Revista Brasileira de Zoologia** **20**(4):625-630.
- SOUZA, J. R. B. & GIANUCA, N. M. 1995. Zonation and seasonal variation of the intertidal macrofauna on a sandy beach of Parana State, Brazil. **Scientia Marina** **59**(2):103-111.
- STURGES, H. A. 1926. The choice of a class interval. **Journal of the American Statistical Association** **21**:65-66.
- TURRA, A. & LEITE, F. P. P. 2000. Population biology and growth of three sympatric species of intertidal hermit crab in south-eastern Brazil. **Journal of the Marine Biological Association of United Kingdom** **80**(6):1061-1069.
- WARNER, G. F. 1977. **The biology of crabs**. London, Elek Science. 202p.
- WENNER, A. M. 1972. Sex ratio as a function of size in marine Crustacea. **The American Naturalist** **106**(949):321-350.
- WILLIAMS, A. B. 1984. **Shrimps, lobsters and crabs of the Atlantic coast of the eastern United States, Maine to Florida**. Washington, Smithsonian Institution. 550p.
- ZAR, J. H. 1996. **Biostatistical Analysis**. New Jersey, Prentice-Hall. 907p.