



Symbiotic reproductive strategies of *Austinixa aidae* in the southwestern Atlantic Ocean, Brazil: sexual maturity of females, fecundity and egg features

Douglas Fernando Peiró^{1,2}, Emerson C. Mossolin³, Fernando L. Mantelatto^{1,*}

¹Laboratory of Bioecology and Crustacean Systematics (LBSC), Postgraduate Program in Comparative Biology, Department of Biology, Faculty of Philosophy, Science and Letters at Ribeirão Preto (FFCLRP), University of São Paulo, Av. Bandeirantes 3900, 14040-901, Ribeirão Preto, São Paulo, Brazil

²Laboratory of Aquatic Biology, Department of Biological Sciences and Health (DCBS), University of Araraquara (Uniar), Rua Carlos Gomes 1338, CEP 14801-340, Araraquara, São Paulo, Brazil

³Department of Biological Sciences of Federal University of Goiás (UFG), Regional Catalão, Av. Lamartine Pinto de Avelar 1120, CEP 75704-020, Catalão, Goiás, Brazil

ABSTRACT: A great variety of reproductive strategies have been observed among brachyurans. Most strategies aim to maximize offspring survival, maintain population stocks, and differentially increase parental genes. We hypothesized that reproductive strategies of a symbiont are not similar to other related but non-symbiotic brachyuran crabs. To test this hypothesis, we analyzed the following parameters of the pea crab *Austinixa aidae*, symbiont to ghost shrimp *Callichirus* species, from the southeastern coast of Brazil: fecundity and its seasonal variation, egg size and volume, number of developing eggs, and the relationship between fecundity and crab size and weight. Reproductive parameters of *A. aidae* were strongly related to the mean size of individuals. Females reached sexual maturity at 5.1 mm carapace width, corroborating previous allometric research. A positive correlation between number of eggs with body size and weight was found. The reproductive period of this species was classified as seasonally continuous. Many early stage eggs were found in all reproductive periods, indicating high reproductive potential. Additionally, the proportion of early stage eggs found during the period of the study indicates a rapid embryonic development. The effects of latitude, environmental conditions, and host mesohabitat characteristics in combination influence reproduction in symbiotic species.

KEY WORDS: Brachyura · Callianassidae · *Callichirus* · Fitness · Pinnotherid · Pinnotheridae · Reproduction · Sandy beach

INTRODUCTION

The Brachyura is a highly diverse group of crustaceans distributed worldwide that have a great variety of reproductive strategies to maximize offspring survival and population stock maintenance (Hartnoll & Gould 1988), and differentially increase parental genes (R. Bauer pers. comm.). Growth, molt cycles, gonadal development, reproductive patterns, morphologic maturity, and fecundity are some of the

main aspects of the reproductive biology studied in these crustaceans (Pinheiro & Terceiro 2000).

Fecundity (number of eggs per clutch) is used to determine the reproductive potential of a species and/or the input into the population. Study of fecundity enables inferences to be made about the adaptations and reproductive strategies of a species with respect to environmental conditions and the possible influences of habitat, intra/inter-specific resources, and lifestyle (see Sastry 1983 for review).

*Corresponding author: flmantel@usp.br

It is also possible to analyze differences between non-symbiotic species and symbiotic species regarding reproductive strategies. The reproduction of non-symbiotic crabs, like that of most crustaceans, is directly related to factors such as temperature, salinity, and food availability, and these 3 factors can also be related to latitude. For symbiotic crabs, there are factors in addition to the ones mentioned above that are of extreme importance, such as the size of the host, its burrow (if it is a burrowing species), and their behavior within this burrow. For example, McDermott (2005) examined pinnixid crabs living with *Amphitrite* and *Chaetopterus* (tubicolous polychaetes) and found differences between individuals of the same species in different hosts and latitudes, mainly concerning size, breeding season, brood size and the number of broods per season, and Ocampo et al. (2012) found a close relationship between female crab and host bivalve body size for *Calyptraeotheres garthi*.

Egg size is another important life cycle parameter that affects reproductive performance, and sometimes it is the only way to quantitatively determine the contribution of a generation to progeny (Timofeev & Sklyar 2001). Egg volume is considered an indicator of energy input to embryonic development, since the quantity of yolk decreases as the embryo grows and develops. Thus, estimating egg volume is important in order to understand population mechanisms used to face the environmental challenges affecting survival and reproduction (Hernández & Palma 2003).

The period and level of reproduction may be estimated from the occurrence and proportion of ovigerous females and/or females with complete gonadal development throughout the year (Pinheiro & Terceiro 2000, Turra & Leite 2000). Determination of these ecological features and other factors controlling reproduction allows a better understanding of the life history and reproductive strategies of a species (Sastry 1983).

Pinnotherid crabs are typically small and form symbiotic associations with a wide variety of benthonic invertebrate hosts (Schmitt et al. 1973, Williams 1984, Harrison & Hanley 2005). *Austinixa* species are symbionts of ghost shrimps (axiidean crustaceans or ecologically equivalent species) and are found on sandy beaches (Heard & Manning 1997, Harrison 2004, Peiró & Mantelatto 2011). The body characteristics of *Austinixa* (much wider than long and with the third pair of walking legs more developed than the other pairs) are an adaptation to their symbiotic lifestyle inside the narrow axiidean burrows (Heard & Manning 1997). *A. aidae*, the target species, has a western Atlantic geographic distribution, from the state of

Amapá to the state of Rio Grande do Sul in Brazil (Peiró & Mantelatto 2011).

Few studies were found in the literature which addressed the reproductive strategies of the genus *Austinixa*. Some reproductive parameters have been referred to in publications primarily focused on population dynamics, biology, or mating systems of *A. patagoniensis* (Rathbun, 1918) (Alves & Pezzuto 1998), *A. gorei* (Manning & Felder, 1989) (McDermott 2006) and *A. aidae* (Righi, 1967) (Peiró & Mantelatto 2011, Peiró et al. 2011, 2013). Additionally, studies of reproduction within pinnotherid symbiotic relationships are scarce, e.g. *Dissodactylus mellitae* (Rathbun, 1900) (Bell & Stancyk 1983) and *Calyptraeotheres garthi* (Fenucci, 1975) (Ocampo et al. 2012). Other relevant studies concerning reproduction of pinnotherids are: Hines (1992), who investigated general patterns of reproductive output, and Lardies & Castilla (2001), who examined the effects of latitudinal variation on the reproductive biology of *Pinnaxodes chilensis*.

Because there is little information regarding this topic for pinnotherids, we chose to investigate the reproductive strategies of the symbiont *A. aidae*, a burrow-living pea crab in relationship with intertidal ghost shrimps of the genus *Callichirus*. We hypothesized that the strategies of *A. aidae* are not similar to other related but non-symbiotic brachyuran crabs. To test this hypothesis, we analyzed fecundity and its seasonal variation, egg size and volume, number of developing eggs, the relationship between fecundity and crab size and weight, and the relationship among reproductive parameters and environmental factors in a population from the northern coast of the State of São Paulo.

MATERIALS AND METHODS

Pea crabs *Austinixa aidae* were collected from the galleries of their host, a ghost shrimp species of the genus *Callichirus*. Samples were obtained during the day, at low tide, and in the intertidal zone (in a plot ~400 m long by ~30 m wide). Samples were collected bimonthly from May 2005 through September 2006 at Perequê-açu Beach, Ubatuba, State of São Paulo, Brazil (23° 24' 59" S, 45° 03' 17" W). The study site is a semi-protected, dissipative sandy beach. Both crustaceans were collected using commercial suction pumps (100 cm long and 5 cm in diameter) developed by Rodrigues (1966) and were similar to those described by Manning (1975). The surface water temperature and salinity were measured before sampling.

Pea crab individuals were separated from sand using a 1 mm mesh sieve, placed in individual plastic bags, labeled, frozen, and transported to the laboratory for analysis. We are confident that our sampling procedures avoided the loss of eggs. All materials were preserved in ethanol (80%) until further examination. In the laboratory, crabs were sexed by their pleopods. Male crabs have 1 pair of long and thin pleopods (gonopods) on the ventral surface of the first abdominal somite and 1 pair of short pleopods on the second abdominal somite. Female crabs have 4 pairs of long setose pleopods from the second to the fifth abdominal somites. Specimens with undifferentiated pleopods or undeveloped pleopods were considered juveniles (Peiró et al. 2011, 2013). The carapace width (CW) and abdominal width (AW) (between the fourth and fifth abdominal somites) were measured under a stereomicroscope with a drawing tube (0.1 mm precision). The wet weight (WW) was measured with an electronic analytical scale (0.0001 g precision) to assess its possible correlation with the number of eggs. Individuals were drained using paper towels before taking measurements. Among decapods, fecundity is generally related to the size or weight of ovigerous females (Ogawa & Rocha 1976, Mantelatto et al. 2002, Peiró et al. 2013). The crabs were deposited in the Crustacean Collection of the Department of Biology, Faculty of Philosophy, Science and Letters at Ribeirão Preto, University of São Paulo (CCDB/FFCLRP/USP) under the catalogue no. 2102.

A previously studied dataset of females (Peiró & Mantelatto 2011, Peiró et al. 2011) were utilized here, in order to compare different approaches used to determine their maturity. Females were classified into the following 3 groups using the size of the smallest ovigerous female as a reference (see Peiró et al. 2011): (1) Immature, females <5.1 mm CW; (2) Mature non-ovigerous, females ≥ 5.1 mm CW without eggs adhered to pleopods; (3) Ovigerous, females ≥ 5.1 mm CW with eggs adhered to pleopods. Ovigerous females were also classified according to the following stages of egg development, based on Boolootian et al. (1959) and modified by Mantelatto & Garcia (1999): Early (Stages 1 to 4), yolk occupying all to 3/4 of the egg, eyes of larvae not visible; Intermediate (Stages 5 to 8), yolk reduced to 1/4 of the egg, eyes visible; Final (Stages 9 to 10), yolk absent, zoea becomes visible. During development, egg volume increases due to embryonic growth. The thickness and resistance of the membrane prevents egg enlargement (Lardies & Wehrmann 1996).

Only crabs with early stage embryos were selected for the fecundity and egg volume analyses. Use of

recently spawned eggs is preferable for fecundity studies because this reduces error related to egg loss throughout development due to predation, parasitism (Mantelatto & Fransozo 1997), and unidentified sources of embryo mortality (e.g. displacement of increasingly larger embryos within a small space; mechanical displacement when the female is moving about in a narrow space) (Corey & Reid 1991, Bauer 2004).

The eggs were carefully removed from the pleopods and counted under a stereomicroscope. Females with intermediate and final stage eggs were used to estimate egg loss and differences in egg volume during embryonic development.

The average volume (mm^3) of each egg was calculated using Jones & Simons' (1983) formula $1/6\pi I^3$ (where I is the mean of the minimum and maximum diameters of these ellipsoid eggs) with a subsample of 15 eggs per female. This calculation was applied to eggs at all developmental stages to evaluate changes in volume during the incubation period (ANOVA). The minimum and maximum diameters of each egg were measured under a stereomicroscope with a drawing tube (0.1 mm precision).

The normality of the size distribution of ovigerous females was assessed with the Kolmogorov-Smirnov (K-S) test. The number of size classes was determined according to Sturges (1926) as follows: $k = 1 + \log_2 n$, where k is the number of classes and n is the size of the sample.

The Pearson product moment correlation was used to determine the relationship of the CW and the WW of ovigerous females with the number of eggs. The same coefficient was used to investigate the correlation between environmental factors (water temperature and salinity) and ovigerous females. The mean fecundity per size class was also determined. To better visualize seasonal fecundity, bimonthly samples were grouped into Summer (January), Autumn (March and May), Winter (July), and Spring (September and November), according to Mantelatto & Garcia (1999). Results were considered statistically significant when $p < 0.05$ (Zar 1996). Statistics and graphics were performed using the programs SPSS Statistics and Microsoft Office Excel, respectively.

RESULTS

Abundance and ovigerous females

A total of 588 crabs were collected during the study: 247 males (42.0%), 265 females (45.1%), and 76 juve-

niles (12.9%). There were 55 ovigerous females (9.4% of all individuals and 20.8% of all females). Ovigerous females occurred throughout the study, except in July 2005, and ranged from 7.1 to 50.0% of all females; the highest proportion was found between November and May (warmest months) (e.g. May [50.0%] and November 2005 [41.2%]; March [31.6%] and May 2006 [29.6%]). Immature females made up a greater proportion between March and September (Fig. 1). There was no correlation between water temperature ($23.9 \pm 2.3^\circ\text{C}$, $r = -0.125$, $p = 0.789$) or salinity ($27.3 \pm 8.5\%$, $r = -0.237$, $p = 0.608$) and the total number of ovigerous females.

The mean size of ovigerous females (CW) was 8.1 ± 0.9 mm, ranging from 5.1 to 10.5 mm. Only 3 ovigerous females were smaller than 7 mm, and most were between 7 and 9 mm CW (Fig. 2). The size frequency distribution of ovigerous females was unimodal (K-S test, $p = 0.092$).

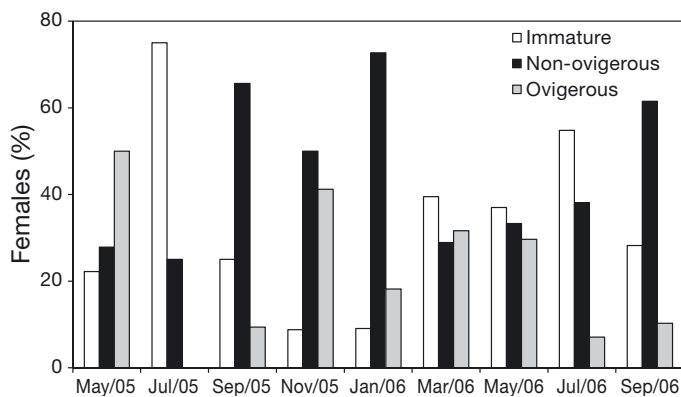


Fig. 1. Percentage of immature, non-ovigerous and ovigerous *Austinixa aidae* females collected in *Callichirus* galleries at Perequê-açu beach, Ubatuba, São Paulo, Brazil, from May 2005 to September 2006

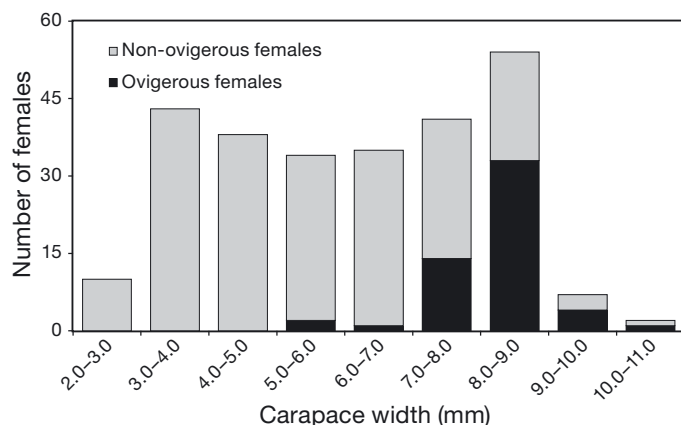


Fig. 2. Size-frequency distribution (carapace width) of *Austinixa aidae* females collected in *Callichirus* galleries at Perequê-açu beach, Ubatuba, São Paulo, Brazil, from May 2005 to September 2006

The relationship between the AW/CW ratios versus the CW in addition to the minimum size of ovigerous females (5.1 mm CW) revealed that mature females had AW/CW ratios ≥ 0.41 (Fig. 3), approximately corresponding to 5.1 mm CW. We observed a gap in dispersion points at AW/CW ratios ≥ 0.41 and CW ≥ 5.1 mm (Fig. 3) and an inflection around 7 mm CW.

Ovigerous females carrying early stage eggs (78.2%) were found during the entire study period, with frequencies over 40.0% (except July 2005; and 100% in September 2005 and January, July, and September 2006) (Fig. 4). Females carrying intermediate and final stage eggs comprised 14.5 and 7.3% of all females, respectively, and were found only in months with higher reproductive activity.

Fecundity and egg features

The mean fecundity of 25 ovigerous females carrying early stage eggs was 1024 ± 441 eggs, ranging from 204 (5.1 mm CW) to 2039 eggs (8.1 mm). The early stage eggs were orange or whitish and rounded and had a mean diameter of 0.23 ± 0.02 mm and a mean volume of 0.0061 ± 0.0012 mm³. Mean fecundity of ovigerous females carrying intermediate stage eggs was 965 ± 244 eggs and that of females carrying final stage eggs was 732 ± 369 eggs. This is an indicator of the reduction in the number of eggs through development. The mean volume was 0.0074 ± 0.0016 and 0.0078 ± 0.0025 mm³ for the intermediate and final stages, respectively; the eggs were also rounded.

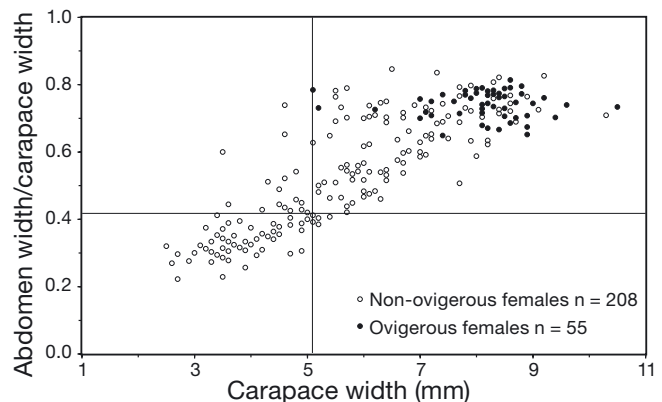


Fig. 3. Maturation of *Austinixa aidae* females collected in *Callichirus* galleries at Perequê-açu beach, Ubatuba, São Paulo, Brazil, from May 2005 to September 2006. Ratios between abdominal width and carapace width versus carapace width. The upper right quadrant represents morphologically mature females

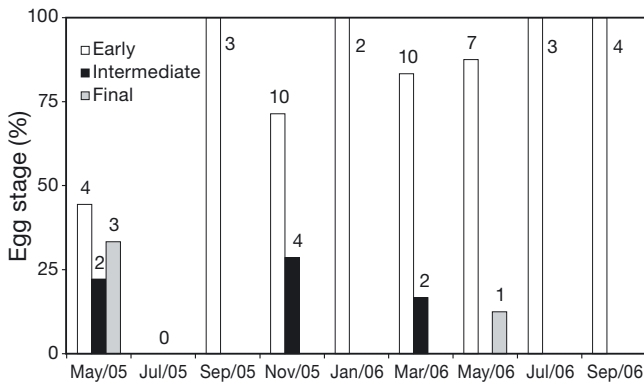


Fig. 4. Egg stage frequency of *Austinixa aidae* collected in *Callichirus* galleries at Perequê-açu beach, Ubatuba, São Paulo, Brazil, from May 2005 to September 2006. The numbers above the bars indicate the total number of individuals per stage

Egg volume tends to increase during embryogenesis; the average egg volume of all 3 developmental stages was significantly different (ANOVA, $F = 17.00$, $p < 0.001$). Egg number (early stage) was correlated with the CW ($r = 0.85$) and WW of females ($r = 0.80$) ($p < 0.05$) (Fig. 5). The mean number of eggs increased with CW and also with size class (Fig. 6).

Differences in the seasonal means of the number of eggs were evident during the sampling period. The lowest means occurred in Spring 2005 and 2006 (611 ± 370 and 782 ± 504 eggs, respectively). Non-ovigerous females were collected in Winter 2005 (Fig. 7). The highest mean number of eggs occurred in Autumn 2005 (1276 ± 386 eggs). The smallest ovigerous females occurred in the low fecundity season (Spring 2005 and 2006).

DISCUSSION

Our results indicate that *Austinixa aidae* have some reproductive traits that might be influenced by the symbiotic relationship with the ghost shrimp, but many others follow the general family characteristics. Differences in ovigerous female size in the genus *Austinixa* can be related to environmental characteristics such as burrow size of the host and host size (Peiró et al. 2013). In general, differences in latitude affect environmental conditions (such as temperature and salinity) and availability of resources (such as food and shelter) (Mantelatto & Garcia 1999, Mantelatto et al. 2002), and consequently, also act on the mesohabitat of the host. The combination of all these factors influences reproduction in symbiotic species, as discussed below.

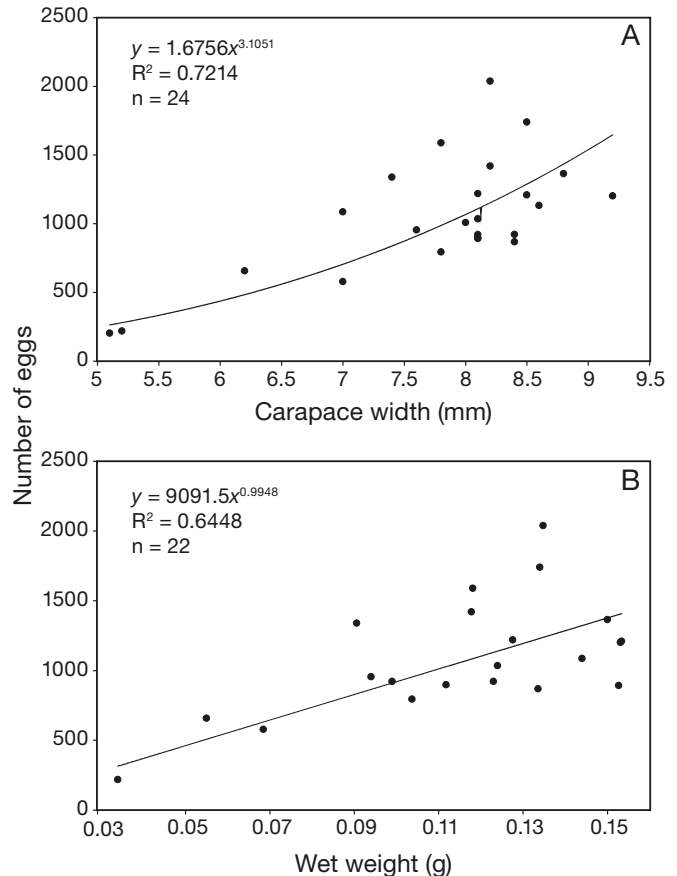


Fig. 5. Relationship between fecundity (number of eggs) and (A) carapace width and (B) wet weight of *Austinixa aidae* ovigerous females collected in *Callichirus* galleries at Perequê-açu beach, Ubatuba, São Paulo, Brazil, from May 2005 to September 2006

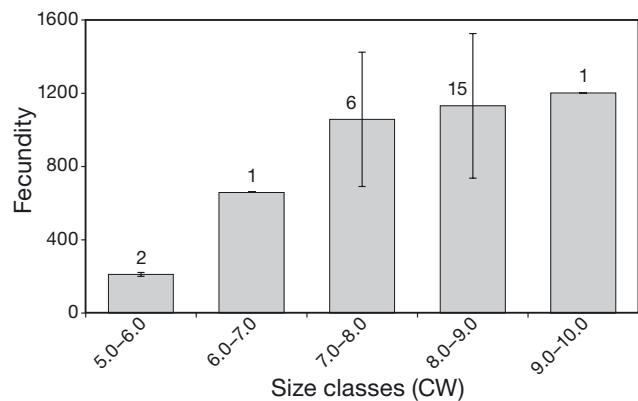


Fig. 6. Mean fecundity (number of eggs) by size class (CW = carapace width in mm) in *Austinixa aidae* ovigerous females collected at Perequê-açu beach, Ubatuba, São Paulo, Brazil, from May 2005 to September 2006. Lines represent standard deviations. The numbers above the bars indicate the total number of individuals per stage

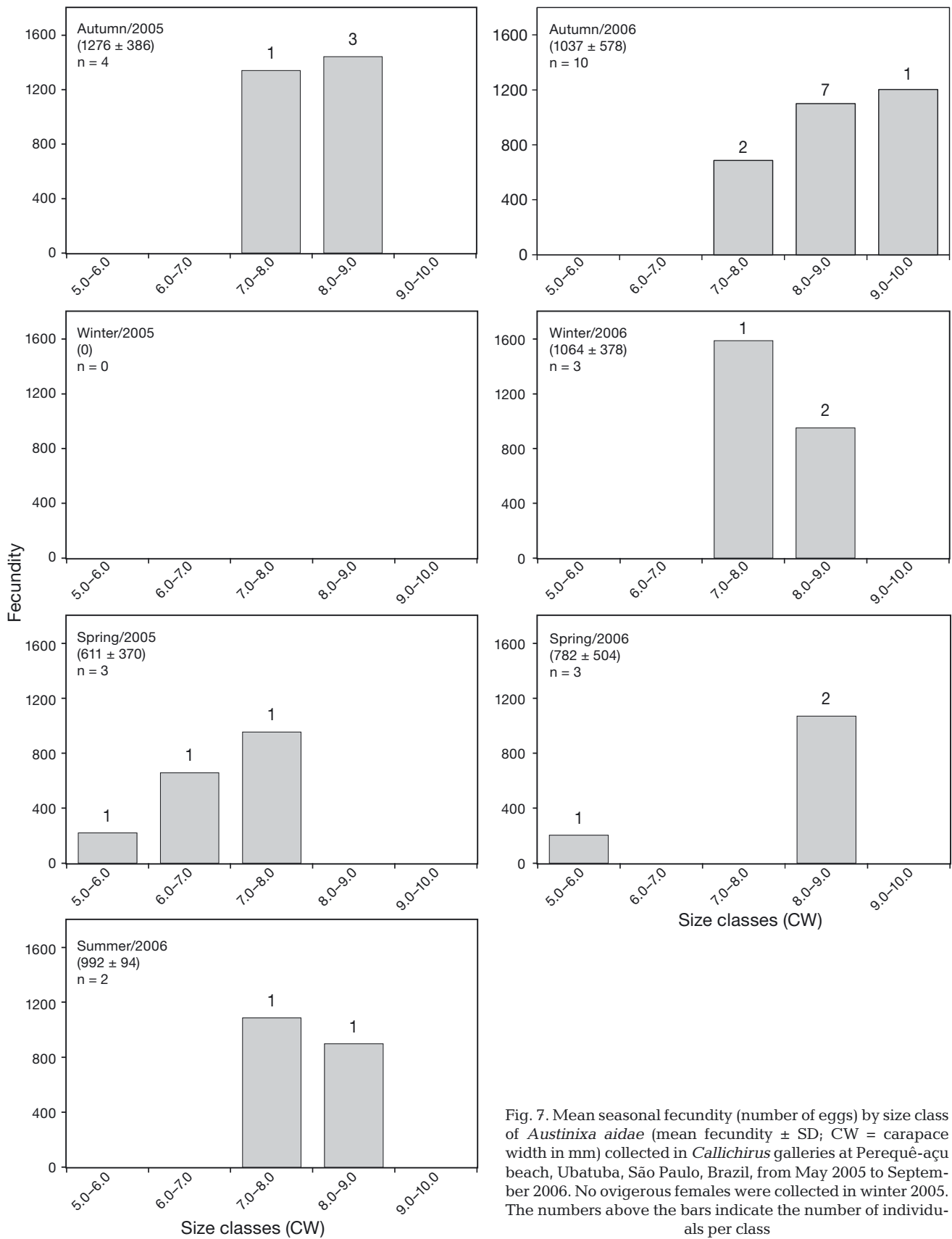


Fig. 7. Mean seasonal fecundity (number of eggs) by size class of *Austinixa aidae* (mean fecundity ± SD; CW = carapace width in mm) collected in *Callichirus* galleries at Perequê-açu beach, Ubatuba, São Paulo, Brazil, from May 2005 to September 2006. No ovigerous females were collected in winter 2005. The numbers above the bars indicate the number of individuals per class

The host body plan or host body size might influence differences in ovigerous female body size of the symbiotic species. The larger the host size, the larger the size and/or the number of symbiotic individuals. This rule has been observed in several parasitic species, including pinnotherid crabs (see Kane & Farley 2006, pea crab *Tumidotheres maculatus*). The same pattern has also been observed with the symbiotic *A. aidae* (Peiró et al. 2013). Our results showed that ovigerous females of *A. aidae* have a broader size range than other species in the genus (from 5.1 to 10.5 mm CW). For instance, *A. patagoniensis* ranges in size from 8.0 to 11.2 mm, a size that is associated with *Sergio mirim* (Rodrigues, 1971) (Alves & Pezzuto 1998) and *Callichirus* sp. (Alves et al. 2005); *A. gorei* ranges in size from 6.3 to 8.3 mm, a size that is associated with *Gilvossius setimanus* (DeKay, 1844) (McDermott 2006). Each pinnotherid host cited above is from the family Callianassidae. Probable differences in burrow structure, water availability promoted by the host (offering oxygenation and food), and geographical location (latitude) may influence the differences among the cited symbiotic pinnotherids.

Our results regarding the relationship between the AW/CW ratios and CW corroborate the previous allometric study by Peiró et al. (2011). Sexual maturity was reached at the size of the smallest ovigerous female (5.1 mm CW). According to Hartnoll (1982), an enlarged abdomen is evidence of sexual maturity. This feature is the most effective means of determining physiological and morphological maturation because the incubation and protection of eggs depends on this structure. In many cases, morphological maturity is also associated with gonadal maturity,

allowing one to infer that morphologically mature individuals are also functionally mature (Pinheiro & Terceiro 2000, Peiró et al. 2011).

Among decapods, reproductive traits have been observed to vary with temperature and salinity (Giese 1959, Pinheiro & Fransozo 1995). Nevertheless, fecundity in *A. aidae* was not correlated with temperature and salinity (Peiró & Mantelatto 2011); ovigerous females, which are more susceptible to these factors (Sastry 1983), were also not influenced by seasonality. We have 2 non-exclusive explanations for the lack of match between biological traits and the environmental parameters: (1) It may be caused by the methodology used to obtain the temperature parameter. This was made during the day of sampling, thus not offering a general overview of the periodic variation. (2) It may be due to the relative stability of abiotic (environmental) factors inside the burrow. However, these hypotheses must be tested in further studies.

The frequency of ovigerous females indicates a variably continuous reproduction with increases between November and May (Figs. 1 & 4), a period associated with elevation of temperature and a photoperiod with longer daylight hours (Bauer 1989, Müller & Carpes 1991, Pavanelli et al. 2008). The reproductive period was classified as seasonally continuous (according Pinheiro & Terceiro 2000) and was similar to that found for *A. patagoniensis* from southern Brazil by Alves & Pezzuto (1998) (Table 1); both species occur in a subtropical climate.

Early stage eggs were the most frequent in all reproductive periods, an indicator of high reproductive performance (Fig. 4). This suggests a continuous reproductive cycle with seasonal variation (season-

Table 1. Minimum size (carapace width) of ovigerous females, mean fecundity, reproductive period, and hosts of *Austinixa* species from sandy beaches in the southeastern USA and southern and southeastern Brazil. na: not available

Species	Locality (Latitude)	Min. size of ovigerous females (mm)	Mean number of eggs (min.–max.)	Callianassidae host	Reproductive period	References
<i>Austinixa gorei</i>	Miami, USA (25° 43' N)	6.3	na (195–525)	<i>Gilvossius setimanus</i>	na	McDermott (2006)
<i>A. aidae</i>	Ubatuba, Brazil (23° 24' S)	5.1	1124 (204–2039)	<i>Callichirus</i> sp.	Seasonal-continuous	Present study Peiró & Mantelatto (2011), Peiró et al. (2011), Peiró et al. (2013)
<i>A. patagoniensis</i>	Balneário Camboriú, Brazil (26° 59' S)	7.9	1719 (933–2747)	<i>Callichirus</i> sp.	Continuous	Alves et al. (2005)
<i>A. patagoniensis</i>	Balneário Cassino, Brazil (32° 13' S)	8.0	1529 (830–2472)	<i>Sergio mirim</i>	Seasonal (October to March)	Alves & Pezzuto (1998)

ally continuous reproduction) and rapid embryonic development. The proportion of the different egg stages found during our study period indicated quick embryonic development, although we did not determine a precise time of incubation based on our observations. Further investigations are recommended to obtain this information.

Fecundity in *A. aidae* is intermediate when compared to *A. patagoniensis* and *A. gorei* (Table 1). Egg production is strongly correlated with female body dimension (size), and the number of eggs tends to increase as the width and/or length of the carapace increases, a relationship commonly observed in diverse groups of decapods (Hines 1988, Corey & Reid 1991, Anger & Moreira 1998, Mantelatto et al. 2002). Despite some exceptions, in most decapods, fecundity and the most representative body size dimension (carapace width and/or weight) are positively correlated (Sastry 1983). This correlation has been observed in *A. aidae* (present study), *A. patagoniensis* (Alves & Pezzuto 1998) and *A. gorei* (McDermott 2006), indicating that the pea crabs of the genus *Austitixia* also follow the decapod pattern, as in many symbiotic pinnotherid crabs (Hines 1992).

An important factor that affects fecundity is the primiparous or multiparous spawning condition of females. Little variation in egg number was observed within individual size classes. Considering body size, the lowest fecundities were observed in females who most likely were spawning for the first time. Primiparous females produce fewer eggs than multiparous females, but they still contribute to an increase in population size (Somerton & Meyers 1983, Mantelatto & Garcia 1999). According to Somerton & Meyers (1983), primiparous female Alaskan tanner crabs *Chionoecetes bairdi* allocate most of their energy to growth, consequently reducing the amount of energy used for egg production, whereas multiparous females divert most of their energy toward egg production. The same pattern was found in this study, with a species living in very different environmental conditions.

Environmental conditions must also be considered in fecundity analysis because variation in fecundity is observed even within the same genus (Mantelatto & Garcia 1999), as reported here (Table 1). These intraspecific variations are related to environmental adaptations, variations in body size and geographic location (Mantelatto & Fransozo 1997), and latitudinal variations (Lardies & Wehrmann 2011), with greater egg production in higher latitude than in lower latitude populations/species (Jones & Simons 1983). Egg size of *A. aidae* is similar to that of *A. gorei*

from Miami, USA, a similar latitude in a different hemisphere (McDermott 2006). Studies with more species are recommended to determine the relationship between fecundity and latitude. However, differences in egg size may partly be attributed to differences in reproductive strategies (Mantelatto & Garcia 1999). Most likely, the reproductive performance in *A. aidae* is related to the size of the host and the polygynandrous mating system of *A. aidae*, where males move between burrows in search of receptive females (Peiró et al. 2013).

The behavior, maturity, fecundity, breeding season, spawning, and egg hatching in Decapoda result from the coordinated action of endogenous and exogenous factors (Sastry 1983, Pinheiro & Terceiro 2000), and the host may influence these reproductive parameters (i.e. host size, Peiró et al. 2013). In *A. aidae*, reproductive aspects may be related to the mean size of the species. In fact, we have shown a dependence (limitation) of the 'guest' (Pinnotheridae) host burrow size (protection and refuge for growth and reproduction), which itself depends on the size of the host. These crabs are among the smallest symbiotic Brachyura, and the body size of the host shelter has a positive relationship with fecundity (Peiró et al. 2013).

Other parameters must be studied to understand the reproductive complexity of this symbiotic species. For instance, the existence of larval migration/settlement is a key factor determining whether larvae settle in the same place where they live as mature individuals (like some pinnotherid species). When compared with other species of the genus, *A. aidae* has similar ecological and biological patterns with some unique features related to the region where they are found and to the host-use pattern (see Peiró et al. 2013 for review). The next step to study the effect of larval migration/settlement on the *A. aidae* life cycle may involve experimental studies to observe Pinnotheridae behavior with their hosts.

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