

Testing the resource economic monopolization hypothesis and its consequences for the mating system of *Alpheus estuariensis* (Decapoda, Caridea, Alpheidae)

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*The aim of the present study is to test the resource economic monopolization hypothesis and the hypothesis of monogamy using the shrimp *Alpheus estuariensis* as a model. The shrimps were collected in two areas in the Vaza-Barriz estuary, north-east Brazil, from August to November 2016. The average abundance of refuges was obtained through 30 random replicates. The shrimp presented a random distribution in both areas. Males and females found together showed a weak relation between their sizes, with males being larger than females. In addition, the cheliped of males grows proportionally more than that of females. The great abundance of refuges present in the environment, added to the aforementioned results, do not support the idea of refuge-guarding behaviour or monogamy. These results, which are in disagreement with those already found for some shrimps of the same family, genus, and even species, reinforce the idea that Alpheidae can be used as a model in the study of how environmental conditions are capable of shaping the social behaviour of a species.*

Keywords: Population distribution, snapping-shrimp, refuge, monopolization, guarding behaviour

Submitted 22 September 2017; accepted 7 June 2018; first published online 23 July 2018

INTRODUCTION

The ability to monopolize resources by an individual is an important feature that influences population distribution, and such ability can determine reproductive success within the population (Wilson, 1975; Emlen & Oring, 1977; Baeza & Thiel, 2007). Some individuals make use of territorial or guarding behaviour against competitors belonging to the same species or not in order to monopolize shelter, food and sexual partners (Foster, 1985; Huber, 1987). Such behaviour directed toward any resource is expected whenever the monopolization of these resources is ‘economic’ – that is, whenever the benefits acquired in this behaviour exceed the costs of it (Brown, 1964; Emlen & Oring, 1977; Thiel *et al.*, 2003a, b). According to Baeza & Thiel (2003), the probability of success in the monopolization of a resource, such as a host (refuge), decreases with increases in abundance, complexity and relative size due to the increase in the energy cost. Baeza & Thiel (2007) proposed a model for crustaceans living in symbiosis, in which the host is commonly used as a

refuge (often discrete and discontinuous). This model considers that ecological (abundance and distribution) and morphological (relative size and structural complexity) characteristics of the host (refuge), associated with the risk of predation (of the guests outside their refuges) are jointly able to predict the host use pattern (refuge), as well as the adoption of different reproductive strategies. In this way, variations in such predictive characteristics affect the monopolization capacity of the refuge by its guests, as well as the adoption of different mating systems (Pfaller *et al.*, 2014).

Mating systems can be extremely diverse (Wilson & Pianka, 1963; Emlen & Oring, 1977; Arnold & Duvall, 1994; Bauer & Abdalla, 2001; Baeza & Thiel, 2007). In many species, monogamy is the mating strategy (Baeza, 2008, 2010; Baeza *et al.*, 2016a), while many others are polygamous (Shuster & Wade, 2003). Also, it is possible to find promiscuous species in which the males are constantly roaming in search of receptive females (Bauer & Abdalla, 2001). Although knowledge about reproductive biology and mating strategies has grown considerably (Parker, 1970; Bradbury & Vehrencamp, 1976; Wickler & Seibt, 1981; Grafen & Ridley, 1983; Shuster & Wade, 2003), the mating systems of many invertebrates remain unknown (Baeza *et al.*, 2016b).

Crustaceans display wide diversity, in terms of morphology, behaviour, lifestyle and mating systems in marine

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habitats (Christy, 1987; Jormalainen, 1998; Correa & Thiel, 2003; Bruyn *et al.*, 2009; Peiró *et al.*, 2013). During the last decade, some studies have proposed explanations for the variety of refuge-use patterns and mating systems in organisms adapted to live in discrete habitats (Baeza & Thiel, 2003, 2007; Thiel *et al.*, 2003a, b; Baeza, 2008; Baeza & Piantoni, 2010). Taking into account the theoretical assumptions mentioned above, it is expected, for example, that symbiotic crustaceans are monogamous when the predation risk away from hosts is high and when their hosts are relatively rare in the environment and large enough to house a few (e.g. two) co-specific individuals (Baeza & Thiel, 2007; Baeza, 2008). In addition, it is expected to find little or no sexual dimorphism in body size and weaponry (e.g. chelipeds used for intra-sexual aggression) due to the low intensity of sexual selection (Shuster & Wade, 2003; Baeza & Thiel, 2007; Baeza, 2008). Although the model has been proposed for symbiotic species, it is based on the use of discontinuous habitats (hosts) and can therefore be applied to other aquatic or terrestrial organisms that live in discrete and discontinuous refuges (Baeza & Thiel, 2007). Studies concerning the mating system of crustacean symbionts have confirmed the theoretical premises of this model (Baeza & Thiel, 2003, 2007, 2008; Baeza, 2008); however, more studies are necessary to test the generalities and the consistency of these theoretical considerations.

Among the Caridea, Alpheidae forms a group with more than 600 species known as snapping shrimp, found in waters of tropical and subtropical regions. In addition to being found in association with several other organisms (Castro, 1971; Criales, 1984; Silliman *et al.*, 2003; Anker *et al.*, 2008), a characteristic of many species of this family is the formation of monogamous pairs which share and maintain the same refuge for a period longer than their reproductive cycles (Mathews, 2002a, b; Correa & Thiel, 2003). Alpheid shrimps can also be territorial, with males and females able to share the defence of a territory or occupied burrow (Mathews, 2002b). *Alpheus estuariensis* Christoffersen, 1984 is commonly found in estuarine environments in the mud, in burrows or under rocks between herbaceous vegetation or *Rhizophora* roots, from the intertidal region down to a depth of 22 m (Almeida *et al.*, 2012). They occur in the Western Atlantic from Florida to the State of Santa Catarina, southern Brazil (Almeida & Mantelatto, 2013). Taking into account the aforementioned, the present study has two objectives, using the shrimp *A. estuariensis* as a model: (1) to test whether or not shrimp monopolize their refuges; and (2) to test the hypothesis of monogamy in two populations from north-east Brazil. We expected that species displaying resource monopolization would show a uniform distribution pattern and there would be a limited supply of a given resource that is not complex from a structural point of view. Also, if *A. estuariensis* is monogamous, we expect that (1) the population distribution of this species is non-random with paired shrimps found more often than expected by chance alone; (2) the sex distribution of shrimps in pairs is non-random with male-female pairs being found more often than expected by chance alone; (3) males pair with females regardless of their reproductive state; (4) male-female pairs display size assortative pairing; and lastly, (5) shrimps should display little or no sexual dimorphism in terms of body size and weaponry.

MATERIALS AND METHODS

Shrimp collection

The shrimps were collected in two muddy flat areas named A1 (11°05'47"S 37°09'30"W) and A2 (11°07'32.08"S 37°09'22"W), both located in one of the mean estuarine systems of Sergipe State, Brazil, the estuary of the Vaza-Barris River, during August to November 2016. The region is characterized by a quasi-uniform seasonal thermal regime, in which rainfall constitutes one of the main elements of climate (Amâncio-Martinielli *et al.*, 2013). In this mixohaline ecosystem, mangroves occur in a low tide plain environment occupying an area of 59.37 km² (Carvalho & Fontes, 2007). In each sample area, 30 replicates were performed, in which the numbers of refuges present within the delimited area were counted. In addition, in each area, during the daytime tidal period, 100 randomly selected burrows were investigated. The collection was done manually with the aid of a tube (PVC tube) of 230 mm in diameter and 400 mm in length, which was buried in the sediment so as to isolate a burrow from the others. All the burrow openings contained in the PVC tube were considered to belong to the same burrow. The sediment contained within the PVC tube was manually excavated until there were no further shrimp to be collected. The captured individuals were packed in plastic bags, transported to the laboratory, and the occurrence and occupation rates of refuge were counted. Additionally, the average abundance of refuges was determined by the counting of shrimp burrows openings by the square method, using a square frame of 1.20 m, totalling 1.44 m² of the sample area.

In the laboratory, identification of *Alpheus estuariensis* was performed according to Soledade & Almeida (2013). The males were identified according to the presence of the male appendix located in the second pleopod and the females according to the absence of this structure or according to the presence of eggs adhered to the pleopods in the abdomen chamber and classified in two categories: ovigerous and non-ovigerous, according to the presence or absence of eggs. The eggs of the ovigerous females were classified according to the stage of development of the embryos, with the following characteristics: stage I (embryos containing well-distributed yolk and absence of eyes), stage II (embryos containing agglomerated yolk and visible eyes, but not well developed) and stage III (embryos with well-developed eyes, free abdomen and thoracic appendages) (Wehrtmann, 1990). In both sexes of *A. estuariensis*, morphometric measurements of the following body structures were taken with the aid of a digital calliper to the nearest 0.01 mm: carapace length (CL), which comprises the length along the mid-dorsal line from the tip of the rostrum to the posterior margin of the carapace; propodus cheliped length (PL), which refers to the length of the largest cheliped from the top of the fixed finger to the distal end of this structure; and pleuron width (PW) of the second abdominal segment (maximum lateral width of the right pleuron).

Determination of size at onset of maturity (SOM)

Taking into consideration that only adult individuals participate in the mating process in a population, the size at the onset

of sexual maturity was determined with the purpose of excluding juvenile individuals from the analyses used to determine the mating system of *Alpheus estuariensis*. The structures used for the determination of SOM were propodus cheliped length (PL) and pleura width (PW) for males and females, respectively. Once these structures were determined, a 'K-means clustering' analysis was used on the logarithmic data matrix. This analysis was used to separate age groups (juvenile and adult) to minimize variances within groups and to maximize variances between groups. The result of the classification of the K-means analysis was refined using a discriminant analysis (AD) for a reclassification of the age groups. This statistical methodology was based on the work of Sampedro *et al.* (1999) and Corgos & Freire (2006). After separation of age groups, individuals were divided into 0.5-mm size classes and the proportions of juveniles and adults in each class were calculated. The data obtained was adjusted to a logistic equation ($y = a/(1 + be^{-cx})$) allowing the calculation of SOM, the size in which 50% (CL_{50%}) of the specimens exhibited morphometric relationships that characterize the adult condition.

Burrowing experiment

In order to investigate the structural complexity of refuges of *A. estuariensis*, we designed an experiment. We placed 30 shrimps (7.83 ± 0.53 mm), of which 16 were male and 14 were female (7 ovigerous), in 1.5-L plastic containers containing 1 kg of sediment and 0.5 L of water from the collection site. For a period of 24 h, the shrimp were allowed to excavate the sediment and construct their refuges. Following this period, we counted the number of openings in the sediment and used this as an estimate of the complexity of the refuges of *A. estuariensis*. The greater the number of burrow openings, the greater the structural complexity of the refuge and, consequently, the lower monopolization potential of this resource. We performed a Mann–Whitney test ($\alpha = 0.05$) to verify if the number of openings produced varied between the sexes.

Refuge-use pattern of *Alpheus estuariensis*

To examine the presence or intensity of refuge-resource monopolization as well as to test for monogamy, we examined the refuge-use pattern by shrimp, which includes the description of its population distribution as well as the pattern of association between males and females. Initially, it was determined whether the shrimps live alone, form aggregations, or live in pairs within their refuges. For this, the observed frequencies of occurrence of refuges with or without different numbers of shrimp were compared with the frequencies expected from a random Poisson distribution (Elliot, 1983). A Chi-square test was used to inspect for significant differences between the observed and expected distributions (Sokal & Rohlf, 2012). Once these differences were found, specific frequencies between both distributions were compared by subdividing the Chi-square test, using a Bonferroni sequential correction (Rice, 1989; Sokal & Rohlf, 2012). When found in pairs in the same refuge, it was determined whether the sexes were randomly distributed by comparing the observed distribution with a binomial distribution.

A linear regression analysis was used to examine if there is a significant relationship between the size of males and females found in heterosexual pairs in the same refuge. We

examined whether the presence of eggs and their stage of development affected the frequency of male presence. A Chi-square test was used to inspect for significant differences between the frequency of occurrence of males and the presence of ovigerous females as well as between the frequency of males and the presence of females carrying eggs at different stages of development (Sokal & Rohlf, 2012). Finally, a binomial test was used to test for statistical differences in the expected proportion (1:1) between the sexes in both areas.

Sexual dimorphism in *Alpheus estuariensis*

To examine the existence of significant differences between the body size of males and females of both populations studied, a Student's *t*-test ($\alpha = 0.05$) was used. In addition, we analysed whether the size of the cheliped grows linearly in relation to the body size in males and females of *A. estuariensis*. These structures function as weapons during intra-sexual interactions or are used for communication between the sexes (Hartnoll, 1978). The relationship between cheliped length (PL) and carapace length (CL) was verified using the allometric model $y = ax^b$ (Hartnoll, 1978). The slope *b* of the linear regression represents the rate of increase ($b > 1$) or decrease ($b < 1$) of the cheliped, with an increase unit in the body size of the shrimp (Huxley, 1950). To verify if the relation deviates from linearity, the value of *b* was subjected to a *t*-test. If the cheliped grows more or less relative to a unit in body size, the value of *b* should be greater or less than 1, respectively (Hartnoll, 1978). To test the equality of slopes and line intercepts for each morphometric variable studied among sex, an analysis of covariance was performed (ANCOVA) (Zar, 2010).

RESULTS

Determination of size at onset of maturity (SOM)

A total of 97 shrimps (45 males, 51 females, 33 of which were ovigerous and 1 unidentified) and 168 shrimps (84 males, 83 females, 48 of which were ovigerous) were sampled at A1 and A2, respectively. The individuals ranged from 3.00 to 9.23 mm (6.52 ± 1.50) in A1 and from 3.40 to 10.23 mm of CL (7.01 ± 1.56) in A2. *Alpheus estuariensis* was found in 74 and 79% of the burrows from A1 and A2, respectively. The number of burrows ranged from 73 to 193 (133.33 ± 32.35) and from 204 to 368 (291 ± 39.35) at A1 and A2 areas; the differences were statistically significant ($t = -16.99$, $df = 58$, $P < 0.05$). The estimated size at the onset of maturity was 6.25 and 6.43 mm (A1 and A2, respectively) for males and 6.0 and 6.64 mm (A1 and A2, respectively) for females.

Burrowing experiment

After 24 h, 14 of the 30 shrimps used in this experiment built burrows. Of these, one built a burrow with two openings, three built burrows with three openings, eight built burrows with four openings, and two other shrimps built burrows with five openings. The remaining 16 shrimps remained on the surface of the sediment without performing any excavation activity. There was no statistical difference in the number of

openings produced between males and females (Mann-Whitney test, $U = 31.00$; $P > 0.05$).

Refuge-use pattern of *A. estuariensis*

The number of shrimps found per burrow ranged from 0 to 2 (0.97 ± 0.70) and from 0 to 4 (1.15 ± 0.99) at A1 and A2, respectively. Taking into account only adult individuals, that is, those with sizes equal to or greater than the estimated value of sexual maturity, 45% and 28% of the burrows did not contain any shrimp. A total of 44% and 31% of the burrows contained a single shrimp (21 and 12 males, 23 and 19 females, of which 18 and 18 had eggs at different stages of development) at A1 and A2, respectively. A total of 11% and 29% burrows contained two shrimps. The pairs of shrimps found consisted of associations between males and males, females and females, and males and females (8 males-males (A2 only), 2 and 3 females-females, and 9 and 18 males-females in A1 and A2, respectively). At A2 alone, a total of 10 and 2 burrows housed 3 and 4 shrimps, respectively. Of these 10 burrows, a trio was formed by 2 males and 1 ovigerous female and another one by 3 males. Three trios were formed by 2 males and 1 female and another three trios by 1 male, 1 female and 1 ovigerous female. Finally, two trios were composed of 1 male and 2 ovigerous females. Of the two burrows containing 4 shrimps, both were formed by 2 males and 2 ovigerous females.

The population distribution of *Alpheus estuariensis* did not present a random pattern in A1 ($\chi^2 = 4.45$, $df = 1$, $P < 0.05$). However, after the sequential Bonferroni correction, in which the P value was adjusted, no statistical difference was observed between the expected and observed frequencies ($P > 0.05$), which was a random distribution. Concerning A2, the population distribution also presented a random pattern ($\chi^2 = 3.52$, $df = 2$, $P > 0.05$). In general, the frequency of individuals present in the burrows was similar to that expected by chance alone (Figure 1A and B). Taking into account the binomial distribution, the number of burrows housing heterosexual couples at random should be 5.31 and 14.06 for A1 and A2. Therefore, the number of shrimp found to form heterosexual pairs was higher than expected by chance alone in

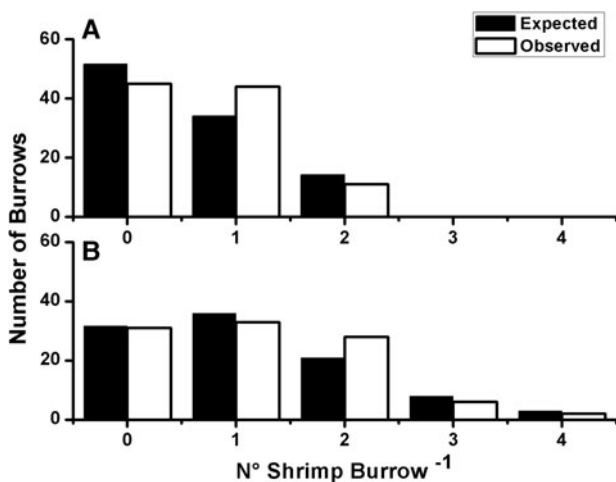


Fig. 1. Population distribution of the shrimp *Alpheus estuariensis* in study areas A1 and A2 (A and B, respectively). No statistical differences were observed between the expected and observed frequencies in both areas.

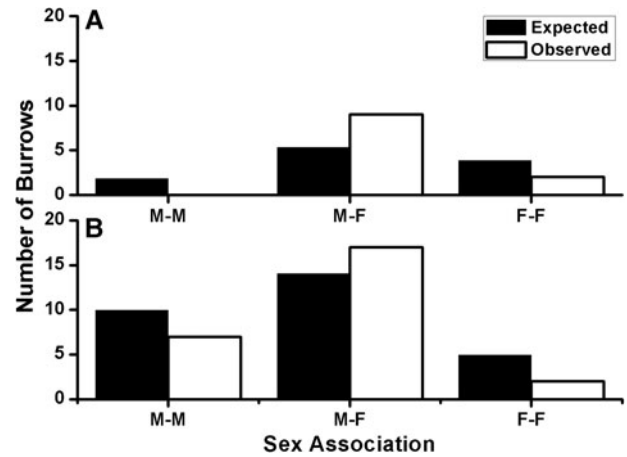


Fig. 2. Patterns of association between males and females of *A. estuariensis* found in pairs in study areas A1 and A2 (A and B, respectively).

both areas (Figure 2A and B). The number of heterosexual pairs found was 9 and 18 in the areas A1 and A2, respectively.

During the study period, a low, but positive relationship was observed between body size (CL) of males and females found in pairs ($F = 4.48$, $df = 1$, $P < 0.05$; $F = 6.48$, $df = 1$, $P < 0.05$, for A1 and A2 respectively): only 14.85% and 19.95% of the variation in the CL of the females was explained by the CL of the males ($R^2 = 0.1485$; $R^2 = 0.1995$; Figure 3A and B). In relation to females found in the same burrow with a male, 100% ($N = 9$) and 77.77% ($N = 14$) were ovigerous in areas A1 and A2. Of these, 7, 1 and 1 carried eggs in stage I, II and III, respectively, in A1 and 7, 3 and 4 in stages I, II and III, respectively, in A2. Males were preferentially associated with females in ovigerous condition in both areas ($\chi^2 = 9.00$, $df = 1$, $P < 0.05$; $\chi^2 = 7.11$, $df = 1$, $P < 0.05$, for A1 and A2, respectively). The proportion of females with embryos at different stages of development showed a significant difference in A1 ($\chi^2 = 8.00$, $df = 2$, $P < 0.05$), but the same difference was not found in A2 ($\chi^2 = 1.85$, $df = 2$, $P > 0.05$). Thus, males of A1 are preferentially associated with females harbouring eggs at stage I, while in A2, although a larger number of males were observed together with females harbouring eggs at stage I, apparently the association of males with females is independent of the egg developmental stage. The sex ratio did not differ from the theoretical ratio predicted (1:1) in both areas (binomial test, $P > 0.05$).

Sexual dimorphism in *Alpheus estuariensis*

The mean CL of males was statistically larger than that of females in both areas ($t = 2.25$, $df = 150$, $P < 0.05$; $t = 2.14$, $df = 165$, $P < 0.05$, A1 and A2, respectively), indicating sexual dimorphism in *A. estuariensis* (males > females) in relation to body size. A positive relationship was also observed between CL and the PL of the largest cheliped in both sexes in both areas (Figure 4A and B) (Table 1). In males, the slope (b) of the relation CL vs PL was significantly higher than 1 ($b = 1.39$, $P < 0.05$; $b = 1.10$, $P < 0.05$, for A1 and A2, respectively), characterizing the growth of the cheliped as a positive allometry in relation to the body size. In females, the value of b for the same relation was significantly lower than 1 ($b = 0.84$, $P < 0.05$; $b = 0.57$, $P < 0.05$, for A1 and A2, respectively), characterizing the growth of the cheliped as

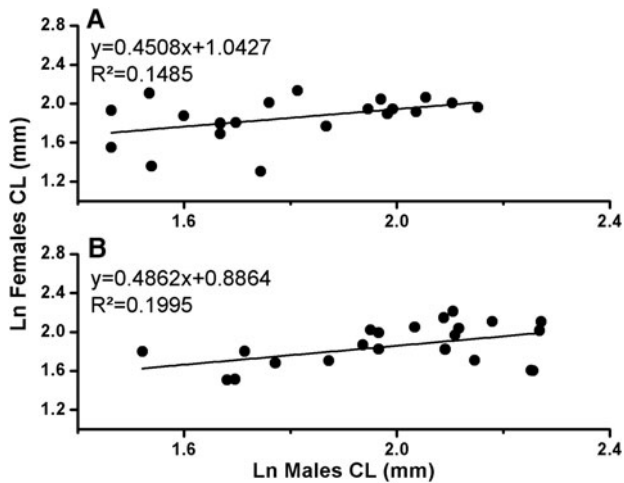


Fig. 3. Relationship between carapace length (CL) of heterosexual pairs of *Alpheus estuariensis* found in the same burrow in the two studied areas A1 (A) and A2 (B).

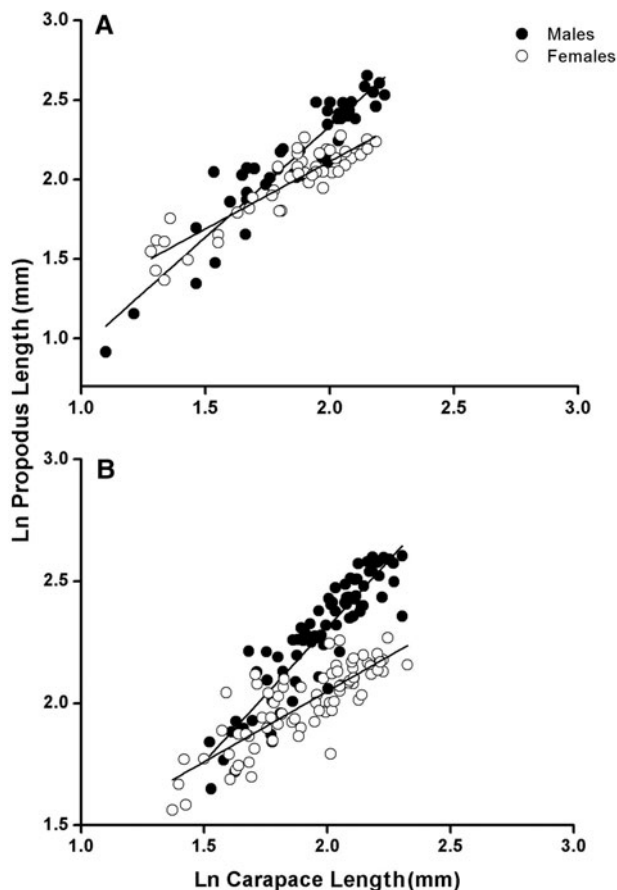


Fig. 4. Relative growth of the cheliped as a function of carapace length in males and females of *Alpheus estuariensis* in the studied areas A1 and A2 (A and B, respectively).

negative allometric. An analysis of covariance (ANCOVA) indicated a significant effect of sex ($F = 24.35$, $df = 1$, $P < 0.01$; $F = 38.21$, $df = 1$, $P < 0.01$, A1 and A2 respectively) and CL ($F = 559.32$, $df = 1$, $P < 0.01$; $F = 576.48$, $df = 1$, $P < 0.01$, A1 and A2 respectively) in propodus length (Table 2).

Table 1. Relationship between carapace length (CL) and propodus cheliped length (PL) of males and female *Alpheus estuariensis* in both studied areas.

Sex	Area	y	x	Regression	R ²	P value
Males	A1	PL	CL	$y = 1.3932x - 0.4524$	0.8818	<0.05
Females	A1	PL	CL	$y = 0.8466 + 0.4200$	0.8340	<0.05
Males	A2	PL	CL	$y = 1.1015x + 0.1070$	0.8341	<0.05
Females	A2	PL	CL	$y = 0.5779x + 0.8931$	0.6717	<0.05

DISCUSSION

The results of the present study do not support the idea that *Alpheus estuariensis* monopolizes its refuge. Initially, a random distribution pattern was verified for this species in both studied areas. This result contrasts with theoretical predictions in which species that monopolize their refuges should have a uniform distribution pattern over an aggregate or random distribution (Baeza & Thiel, 2003). One of the main selective pressures responsible for the development of a guard resource behaviour, including territoriality, is the limitation of a resource in the environment, as already verified for different vertebrate and invertebrate taxa (Wilson, 1975; Barash, 1982; Grant, 1993; Chapman & Kramer, 1996; Duffy *et al.*, 2000). In this sense, the refuge used by *A. estuariensis* does not seem to be a limiting resource in the environment and, therefore, it is not expected to display monopolization behaviour. Similar results have already been found for other species of crustaceans, such as *Liopetrolisthes mitra* (Baeza & Thiel, 2003). These results are also in contrast with the hypothesis of monogamy. If *A. estuariensis* were a monogamous species, we would expect a non-random population distribution – essentially, pairs of individuals would cohabit the same refuge more often than expected by chance alone, and that, among these pairs, the number of heterosexual couples would also be greater than expected by chance alone, as already evidenced in other crustaceans that present monogamy as mating system (Baeza, 2008; Baeza *et al.*, 2011, 2013, 2016a). Although the number of pairs formed by heterosexual couples was greater than expected, the distribution of the populations as a whole did not differ from a random distribution, and the number of shrimp inhabiting the burrows in pairs did not differ from that expected at random. Similar results have already been found in other species of non-monogamous crustaceans (Baeza *et al.*, 2015, 2016b; Baeza & Hernández, 2015).

Another line of evidence that suggests the absence of refuge-guarding behaviour in *A. estuariensis* is the structural complexity of its refuges, which may, according to the results of the burrowing experiment, present up to five openings. According to Thiel & Baeza (2001), a high structural complexity in hosts harbouring symbiotic crustaceans makes it inefficient to supervise (guard) the entire host by the symbiont. Consequently, symbionts can migrate freely between hosts without any impediment on the part of their co-species. The same principle can be applied to *A. estuariensis* and its refuges. Other studies show that burrows of other species belonging to the genus, such as *A. heterochaelis*, *A. floridanus* and *A. migrans* among others, are highly complex and formed by U-shaped tunnels with up to eight surface openings (Dworschak & Ott, 1993; Stieglitz *et al.*, 2000; Dworschak & Pervesler, 2002). Overall, the characteristics of the refuge

Table 2. Allometric analysis using carapace length as independent variable and analysis of covariance (ANCOVA) between male and female *Alpheus estuariensis* for the relationship between carapace length and the length of the propodus.

Relationship	Area	Sex	N	ln(a)	(b)	R ²	t (b = 1)	P	Allometry	ANCOVA P value	
										A	B
CL vs PL	A1	M	44	-0.45	1.39	0.88	5.063	<0.05	+	-	<0.01
		F	47	0.42	0.84	0.83	2.761	<0.05	-	-	<0.01
	A2	M	81	0.10	1.10	0.83	1.850	<0.05	+	-	<0.01
		F	77	0.89	0.57	0.67	9.139	<0.05	-	-	<0.01

CL, carapace length; PL, propodus length; A1, Area 1; A2, Area 2; +, positive allometry; -, negative.

restrict the development of guarding the resource due to the high energy expenditure, in terms of both time and energy, in relation to the alternative activities by a single or a small group (pairs) of shrimps (Baeza & Thiel, 2003).

If *A. estuariensis* lived in heterosexual pairs in their refuges (hosts) for a period longer than a reproductive cycle, a tight correlation between the sizes of males and females would be expected (Adams *et al.*, 1985; Baeza, 1999; Mathews, 2002b), due to the growth restrictions imposed by the host they are associated with (Adams *et al.*, 1985; Baeza, 1999, 2008). In the case of *A. estuariensis*, growth restriction of associated males and females would be imposed by the size of the burrow in which they live. Although a significant relationship was found between associated males and females of *A. estuariensis*, this relationship does not suggest that the pairing between them is long-lasting. In other species of monogamous symbiotic crustaceans, such as *Pinnixa transversalis* and *Pontonia margarita*, male body size explains 77.65 and 63.8% of the variation in female body size, respectively (Baeza, 1999, 2008). In the present study, less than 20% of the variation in male body size explained the variation of the size of the females in both areas. This weak relationship between males and females found together does not support the idea of monogamy.

The pattern of sexual dimorphism found in *A. estuariensis* argues in favour of territoriality in males (but not females) due to the positive allometry in cheliped size. This pattern has already been reported in the porcelain crab *Allopetrolisthes spinifrons* (Baeza *et al.*, 2001; Baeza *et al.*, 2002). Moreover, sexual dimorphism in relation to the size of the chelipeds does not support the notion of monogamy. Theory suggests that monogamous species should exhibit low or no sexual dimorphism in structures that function as weapons (Baeza & Thiel, 2007). In contrast, the higher relative growth of chelipeds in males compared with those in females suggests that sexual selection may be an important factor for *A. estuariensis*. In addition, the presence of dimorphism in relation to this structure indicates that both intra-sexual competition and sexual selection are more intense among males than among females of this species (Shuster & Wade, 2003). Males of polygamous shrimp invest more energy resources in this structure to compete with other males through agonistic interactions when in search of receptive females (Bauer, 2004; Baeza & Thiel, 2007), and this pattern has already been verified for other crustaceans (Bauer, 2004; Biagi & Mantelatto, 2006; Baeza & Thiel, 2007; Peiró *et al.*, 2013; Baeza *et al.*, 2015). Thus, if the males of *A. estuariensis* change their burrows in search of females, the size of the

cheliped possibly determines the winner of the agonistic interaction and access to the female. Furthermore, reverse sexual dimorphism (in relation to body size) is expected in monogamous species, in which males present lower sizes in relation to females due to the low intensity of intra-sexual competition present in this type of mating system (Thiel & Baeza, 2001; Shuster & Wade, 2003), as already verified in other species of monogamous Caridean shrimps, such as *Pontonia manningi* (Baeza *et al.*, 2016a) and *P. margarita* (Baeza, 2008).

Implications for the mating system of *A. estuariensis*

Mating systems can be characterized by the mating strategies used by males in the population (Shuster & Wade, 2003). In turn, male mating strategies strongly depend on the environmental potential for monopolization of females and/or resources (refuge) to attract them (Shuster & Wade, 2003; Baeza & Thiel, 2007). In this study, refuge abundance and structural complexity were expected to favour group living and constrain territoriality and monogamy in *A. estuariensis*. Given the low potential of refuge monopolization, according to the theoretical expectations, males would increase their mating opportunities by using pure-searching rather than territorial mating tactics (Bauer & Abdalla, 2001; Bauer, 2004; Baeza & Thiel, 2007). Other results, in addition to those presented above, agree with the idea that *A. estuariensis* features a pure-search mating system.

Although males were found preferentially with ovigerous females at stage I at A1 and independent of the stage of development at A2, the presence of solitary ovigerous females carrying eggs at different developmental stages reinforces the idea that this species is not monogamous. Caridean shrimp females do not have the capacity to store sperm and, therefore, need to be inseminated by males shortly after moulting for new eggs to be fertilized (Bauer, 2004). In this way, the presence of solitary ovigerous females in the burrows indicates that the males transit between the burrows in search of receptive females. This result is not in agreement with what has already been recorded for other species of monogamous carideans in which solitary females do not harbour eggs, such as *Paranchistus pycnodontae* (Baeza *et al.*, 2013), and agrees with what has already been verified for non-monogamous carideans, such as *Odontonia katoi* (Baeza *et al.*, 2015). In symbiotic species as well as free-living crustaceans, in which males often transit between their hosts or refuges in search of females, it is common to find a deviant sex ratio toward

females (Nakashima, 1987; Baeza & Díaz-Valdés, 2011). However, we hypothesize that the great availability of refuges in the environment allows males of *A. estuariensis* to migrate frequently with low risks associated with this movement; therefore, they do not suffer changes in the sexual ratio of the population.

CONCLUSION

The results of the present study are in agreement with the hypothesis of refuge-guarding behaviour and monogamy in *A. estuariensis*. The establishment of guarding of the refuge as well as long-lasting heterosexual couples does not seem to be an adaptive strategy, given the environmental conditions in which the species is found in the studied areas: abundant and complex refuges from the structural point of view where the predation risk becomes low. The importance of the characteristics of the refuge, such as abundance and distribution, in the determination of the social behaviour of terrestrial and marine organisms is already well established in the literature (Emlen & Oring, 1977; Shuster & Wade, 2003; Baeza & Thiel, 2007, 2003). The guarding behaviours as well as the mating system verified for *A. estuariensis* go against other species belonging to the same family, the same genus (Mathews, 2002a, b; Correa & Thiel, 2003), and even the same species in another area of Brazil (Costa-Souza *et al.*, 2014). This result reinforces the idea that Alpheidae can be used as an excellent model in the study of the effect of environmental conditions in determining the social behaviour of a species.

ACKNOWLEDGEMENTS

We would like to thank all of our colleagues at the Laboratory of Carcinology of the Federal University of Sergipe for all of the help provided during the study period.

COMPLIANCE WITH ETHICAL STANDARDS

There are no conflicts of interest. All applicable national guidelines for the care and use of animals were followed (SISBIO license #24097-1).

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