Refuge size, group living and symbiosis: testing the "resource economic monopolization" hypothesis with the shrimp *Betaeus lilianae* and description of its partnership with the crab *Platyxanthus crenulatus*

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ABSTRACT

Theory predicts that refuge size is most relevant in driving the population distribution of marine organisms. Relatively small refuges are expected to harbor single or pairs of conspecifics because defense against intruders is energetically inexpensive. Relatively large shelters should harbor aggregations because guarding behaviors turn energetically expensive. Here, we used the intertidal shrimp *Betaeus lilianae* to test the hypothesis that species inhabiting large refuges live in aggregations and not solitarily or in pairs. Also, we provided information on the lifestyle of this species, specifically regarding a newly discovered partnership with the crab *Platyxanthus crenulatus*. In agreement with theoretical expectations, *Betaeus lilianae* was found living in aggregations in rock pools, characterized by their large size. Shrimp aggregations featured female-biased sex ratios more frequently than expected by chance alone and had no particular complex social structure. There was no effect of pool size and shrimp group size on sex ratio and no significant relationship between the difference in body size of the largest and second largest male and shrimp group size was observed. Relative growth analyses showed that the major claw had positive allometry in males and females but relative claw growth was greater in males. The information above permitted rejecting several alternative hypotheses on the mating system of *B. lilianae*: it is neither socially monogamous nor features a promiscuous pure-search mating system. Additional studies are needed to reveal the mating behavior of the studied species. Field observations and laboratory experiments demonstrated that *B. lilianae* associate preferentially with the crab *P. crenulatus*.

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1. Introduction

Among the Decapoda, shrimps from the Infraorder Caridea are recognized for their remarkable anatomical, ecological, and behavioral diversity (Bauer, 2004). Recent studies have uncovered an evolutionary history and phylogenetic relationships within the group more complex than originally thought (Braken et al., 2009). Furthermore, some studies on shrimps combining systematic, physiological, behavioral and/or ecological observations, have exposed most peculiar behaviors and the conditions favoring them: multiple independent origins of eusociality (Duffy, 1996; Duffy et al., 2000; Morrison et al., 2004); condition-dependent alternative mating tactics (Correa et al., 2000); extreme sex allocation in sequential-simultaneous hermaphrodites (Baeza, 2006, 2007); advertising and

cleaning behavior in tropical environments (Becker and Grutter, 2004, 2005; Becker et al., 2005); and social monogamy in discrete refuges (Knowlton, 1980; Mathews, 2003; Rahman et al., 2003; Baeza, 2008). While our knowledge of the evolutionary history and diversity of caridean shrimps has increased substantially, the behavior and ecology of many species belonging to numerous genera and families still remains unknown.

Among carideans from the family Alpheidae, one of the most species-rich clades of shrimps (Anker et al., 2006), the genus *Betaeus* is of particular interest. Shrimps from this genus demonstrate a considerable diversity of lifestyles, habitats, and coloration. The 13 recognized species are distributed worldwide inhabiting intertidal and shallow subtidal rocky and soft bottom environments in temperate and subtropical coasts. Some species live in aggregations (*B. emarginatus* — Antezana et al., 1965), whereas, others remain solitarily within shelters (*B. setosus* — Jensen, 1986). Several species with cryptic coloration dwell under rocks or in crevices in the intertidal (*B. truncatus* — Antezana et al., 1965), but other more

colorful species, are associated with sea urchins (*B. macginitae* — Hart, 1964) or abalones (*B. hardfordi* — Hart, 1964) in shallow subtidal rocky reefs. The ecological diversity of the genus suggests that these shrimps are ideal model systems to explore the role of the environment in favoring evolutionary innovations. However, our understanding about most remarkable evolutionary innovations in these and other shrimp genera is constrained due to the lack of detailed phylogenetic information and the paucity of studies on their life history and autoecology.

In this study, we are particularly interested in understanding the conditions driving the population distribution and intra-specific association patterns of these shrimp as well as other marine invertebrates using refuges in the intertidal. The ability of individuals to monopolize resources is of major importance in determining population distribution and resource-holding power determines to a large extent the reproductive success of individuals within a population (see Wilson, 1975; Emlen and Oring, 1977; Shuster and Wade, 2003; Baeza and Thiel, 2007). Territoriality (shelter defense) or guarding of any other resource (e.g., space, food or members of the opposite sex) is predicted to evolve when their monopolization is "economic"; when the benefits of monopolization exceed the costs of defense (Brown, 1964; Emlen and Oring, 1977). Recent theoretical considerations suggest that refuge size (relative to the species using a shelter) is most relevant in modulating the costs and benefits of refuge monopolization, and thus, the population distribution of shrimps and other small invertebrates using refuges (Baeza, 2008; Baeza and Thiel, 2003, 2007). In relatively large refuges, defense against intruders might be difficult (i.e., energetically costly), whereas relatively small refuges should be relatively easy to defend against intruders (Thiel and Baeza, 2001; Baeza and Thiel, 2007; Baeza, 2008). Considering the above, small refuges are expected to harbor single or pairs of conspecifics while relatively large refuges should shelter large groups or aggregations because guarding behaviors turn energetically expensive (Baeza and Thiel, 2003, 2007). Manipulative and observational experiments in several species of symbiotic crustaceans support the expectations above (see review in Baeza and Thiel, 2007). Also, the available information in shrimps from the genus *Betaeus* seems to fit with these expectations. Small crevices used as refuge by *Pachycheles* crabs and also harboring single *B. setosus* shrimp are much smaller than intertidal pools from which aggregations of *B. truncatus* are retrieved. Additionally, these intertidal pools are structurally much more heterogeneous than the small crevices inhabited by *Pachycheles* crabs. We expect a solitary (or pair living) lifestyle in other species of *Betaeus* using small refuges, whereas, species inhabiting large refuges should occur in aggregations (Baeza and Thiel, 2007).

The aim of this study is testing the "resource economic monopolization" hypothesis above, which predicts that species inhabiting large refuges live in aggregations and not solitarily or in pairs using the shrimp *Betaeus lilianae* as a model. The relatively large size of intertidal rocky pools is expected to prevent their monopolization by a single or a small group (pair) of shrimp. *Betaeus lilianae* inhabits the southwestern Atlantic coast, from Rio Grande do Sur, Brazil to Rio Negro, Argentina (Spivak, 1997). Little is known about its life history and ecology other than its occurrence in rocky intertidal environments (Boschi et al., 1992; Spivak, 1997). Thus, our second goal was providing information on the lifestyle of this species, including the possibility of a symbiotic partnership with the crab *Platyxanthus crenulatus* (Platyxanthidae). The latter is a predatory species, endemic of the Southwestern Atlantic, found in crevices at intertidal and subtidal rocky shores (Spivak, 1997, NEF unpublished data). The adoption of a symbiotic lifestyle (symbiosis here defined sensu de Bary (1865) quoted by Vermeij (1983) as dissimilar organisms living together) is a remarkable adaptation among caridean shrimps. Shrimp species are described as developing partnerships with fish, crabs, thalasinid prawns as well as other species of caridean shrimps (e.g., Vannini, 1985; Silliman et al., 2003; Gillikin et al., 2001; Boltaña and Thiel, 2001). Unfortunately, little is known about the details of these partnerships. Ecological studies such as the present will help to set the stage for future comparative studies in this outstanding clade of shrimps.

2. Materials and methods

2.1. Collection of shrimps

Individuals of *B. lilianae* were collected from the rocky intertidal at Estafeta Postal beach (S 38° 09′, W 57° 38′), 16 km south of Mar del Plata city, Argentina from December 2008 to April 2009. The sampling site was characterized by large sedimentary rocky outcrops with interspersed pools. During low tides, randomly selected pools were examined for the presence of shrimp. Usually one but occasionally two or three persons drained a particular pool using small plastic containers. While water was being drained, any observed shrimp was collected and placed in small plastic bags for transport to the laboratory. Also, after pools were completely drawn off, rocks or pebbles at the bottom of these pools were discarded and any other shrimp observed was collected. We took special care of examining crevices found at the walls and bottom of these pools so to retrieve all shrimp from them. After shrimp collection, a total of 26 pools were photographed and their area was measured (to the nearest centimeter) using the software Skaletti 2.5 (1999). Also, we recorded the presence or absence of the crab *P. crenulatus* in 25 out of these 26 studied pools.

In the laboratory, the carapace length (CL, mm), the length of the largest of the first pair of claws, and the right second abdominal segment (maximum lateral length of the pleura) of all shrimps were measured under the stereomicroscope to the nearest 0.168 mm. Also, the sex of each shrimp was determined based on external characters; in males a pair of gonopores (located on the coxae of the fifth pair of walking legs), cincinnuli on the base of the endopod of the first pleopods, and appendices masculinae on the base of the endopod of the second pleopods. Each female shrimp was classified according to the presence or absence of embryos (ovigerous or non-ovigerous).

2.2. Population distribution of Betaeus lilianae

We examined whether the population distribution of shrimps in pools differed significantly from a random distribution by comparing the observed distribution with the truncated Poisson distribution (Elliot, 1983). A Chi-square test of goodness of fit was used to test for significant differences between the distributions (Sokal and Rohlf, 1995). When significant differences were observed, we compared specific frequencies between the observed and expected distributions by subdivision of the Chi-square test using the sequential Bonferroni correction (Rice, 1989; Sokal and Rohlf, 1995).

Poisson regression was used to test for a significant effect of pool size on shrimp number (SAS, 2004). Before the test, the assumption of over dispersion was examined and found to be satisfactory.

2.3. Sexual dimorphism in Betaeus lilianae

In alpheids, including shrimps from the genus *Betaeus*, the first pair of thoracic appendages bears the largest of the two pairs of claws. These structures serve as weapons during intra-sexual interactions or for inter-sexual communication (Shuster and Wade, 2003). In turn, the pleura of the second abdominal segment are greatly enlarged and help protect the embryos (i.e., from physical abrasion) carried by females beneath their abdomen (Bauer, 2004).

We examined whether the claw in the second pair of pereiopods and the pleura of the second abdominal segment increases linearly with body size in males and females of *B. lilianae*. The relationship

between the length of the merus of the largest second claw or the length of the pleura of the second abdominal segment and body size of shrimps (CL, mm) was examined using the allometric model $y = ax^b$ (Hartnoll, 1978, 1982). The slope b of the log–log least-squares linear regression represents the rate of exponential increase ($b > 1$) or decrease ($b < 1$) of the claw and abdominal segment with a unit of increase in body size of shrimps. To determine if the relationship deviates from linearity, a t-test was used to test if the estimated slope b deviates from the expected slope of unity. If the claw or the abdominal pleura grow more or less than proportionally with a unit increase in body size of shrimps, then the slope should be greater or smaller than the unity, respectively (Hartnoll, 1978).

2.4. Crab-dependent shelter selection by Betaeus lilianae

We tested the null hypothesis of no overt preference of shrimp for refuges harboring individuals of the xanthoid crab *P. crenulatus* by allowing shrimp to colonize shelters harboring or not crabs in the laboratory. Experiments were conducted in white plastic containers (30×40 cm, 18 L volume) containing aerated seawater. In each container, two plastic shelters (hemi-cylinders, 11 cm radius, 14 cm length, opened in one side) separated by a distance of 20 cm each one from another were provided for shrimp and crab colonization. Each refuge was open at one side and these openings did not face each other but opposed one another at an angle of 90°. At the start of the experiment, one adult crab was placed at the center of each aquarium and was left free to choose one of the available shelters for ~30 min. Next, an individual of *B. lilianae* was placed into a white plastic cylinder that was maintained inverted for at least 20 min at the bottom of the aquaria equidistant between the shelters. After this initial period of acclimatization, the plastic cylinder was gently lifted from the container and the presence/absence of the crab and shrimp on each available shelter was recorded immediately (15 min), 1, 2, 3, 4, 5 and 10 h after the start of the experiment. Each replicate ($N = 30$ in total) was conducted with different shrimps (mean $\pm$ s.d.: 8.44 ± 1.65 mm CL) and crabs (53.83 ± 8.03 cm CW). To determine preferences by shrimps for shelters harboring crabs, we tested for significant differences between the observed distribution of shrimp in shelters with or without crabs and the expected random binomial distribution (1:1) using a binomial test (Zar, 1999). The power of each test was calculated as explained in Zar (1999).

3. Results

3.1. Population distribution of Betaeus lilianae

A total of 295 shrimps (115 males and 180 females) were retrieved from all pools examined at Estafeta Postal beach. Considering only pools in which shrimp were found ($N = 55$), the number of shrimps per pool varied between 1 and 17 with a mean ($\pm$ s.d.) of 5.47 (± 3.43). The population distribution of *B. lilianae* on intertidal pools did not display a random pattern (Chi-square test of goodness of fit, $\chi^2_4 = 12.95$, $P = 0.0115$). However, the discrepancy between observed and expected distributions was mostly explained by the observed number of pools harboring six shrimp that was lower than expected by chance alone (Fig. 1a). Pool size varied between 0.09 and 0.44 m² with a mean $\pm$ s.d. of 0.27 ± 0.086. No statistically significant correlation between pool size (area) and shrimp number was found ($N = 24$ pools, Poisson regression; $P = 0.0933$).

Of the five shrimp found solitarily in pools, four were females (all brooding embryos) and one was a male. In pools with two or more shrimp, 32, 10 and 8 harbored shrimp with a female-biased, male-biased or equal sex ratio (Fig. 1b). Taking into consideration pools harboring only two or more shrimp, shrimp aggregations featured female-biased sex ratios more frequently than expected by chance alone (32:10 vs 21:21, binomial test, $P < 0.0001$).
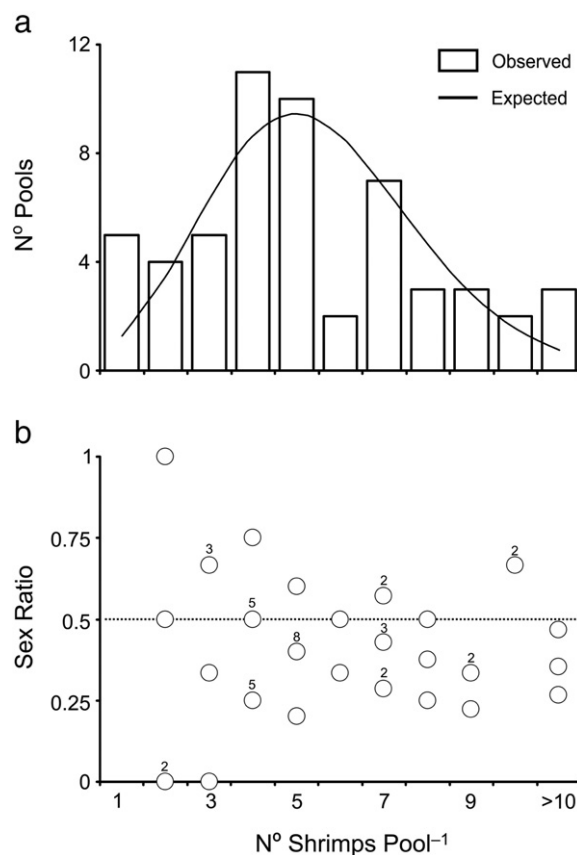

Fig. 1. (a) Population distribution of the shrimp *B. lilianae*, inhabiting intertidal pools at Estafeta Beach, Mar del Plata, Argentina. Observed frequency of shrimps on pools differs significantly from the expected Poisson random distribution. (b) Sex ratio in pools harboring different numbers of shrimp. Small numbers indicate number of pools represented by data point when more than one.

Visual examination of the data suggested that small aggregations (<4 shrimps) presented much more variability in sex ratio than pools harboring moderate numbers of shrimps ($4 <$ shrimp per pool < 9) and that sex ratio became more female-biased with increasing group size (Fig. 1b). Nevertheless, logistic regression indicated no effect of shrimp group size on sex ratio (Poisson regression; $P = 0.65$).

We examined the data in search of any indication of female monopolization by males given that the female-biased sex ratio of most aggregations suggests a harem-like group structure. We compared the body sizes of shrimps among groups with different sex ratios because we found no effect of pool size and shrimp group size on sex ratio (see above). The size of the largest male in a group did not vary significantly among groups with different sex ratios (Kruskal Wallis: K–W $= 2.37$, $P = 0.30$). In turn, females were, on average, smaller in male-biased than in groups with female- or non-biased sex ratios (Kruskal Wallis: K–W $= 7.56$, $P = 0.02$) (Fig. 2). Examination of shrimp size structure in each group did not reveal any indication of female monopolization by males (Fig. 3). For instance, considering only groups with a female-biased sex ratio, males were the largest shrimp in the group only in 15 out of 30 aggregations. When two males cohabited in the same pool, the difference in body size between the largest and second largest male was either minimal (e.g., aggregations on pools no. 12, 21, 22, 25 and 32 in Fig. 3) or large (e.g., aggregations on pools no. 10, 11, 13, 14, 19, and 24 in Fig. 3). Also, no significant relationship between the difference in body size of the largest and second largest male and shrimp group size was observed (Poisson regression, $P = 0.11$). The information above suggests that shrimp aggregations do not have a particular structure.
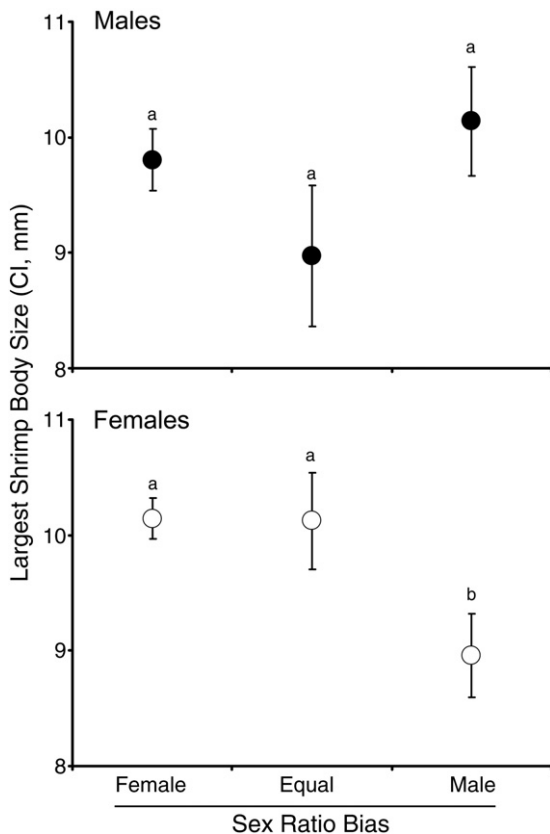
Fig. 2. Carapace length of the largest male (a) and female (b) shrimp in pools harboring aggregations with equal or male/female-biased sex ratios. Shrimp body size did not vary among aggregations with different sex ratios but females were smaller in groups with a male-biased sex ratio. See Materials and methods for further details.

3.2. Sexual dimorphism in Betaeus lilianae

The carapace length of male and female individuals varied respectively between 4.96 and 12.58 mm (mean ± s.d.; 8.74 ± 1.74) and between 5.21 and 11.86 mm (8.66 ± 1.44). No significant differences in CL between the sexes were detected (Kruskal–Wallis test; K–W = 0.39, P = 0.53) Thus, there is no marked sexual dimorphism with respect to body size in this species (Fig. 4).

A positive correlation between body size of shrimps and the length of the merus of the major claw and the length of the pleura of the second abdominal segment was detected for shrimps of both sexes (P < 0.001 in all cases, Fig. 4). Nevertheless, the different structures differed with respect to the status and degree of allometry depending on the sex of the shrimps. In males and females, the major claw presented positive allometry; the slope of the relationship between shrimp body size and major claw size was significantly greater than unity (Table 1; Fig. 4). Nevertheless, relative claw growth was greater in males than in females (comparison of slopes: F = 4.61, df = 1.24, P = 0.032). In turn, the second abdominal segment presented negative allometry in males but the same structure presented positive allometry in females (Fig. 4; Table 1).

3.3. The effect of crab presence on refuge preference in Betaeus lilianae

A total of 88% (n = 22) out of 25 ponds in which the presence/absence of P. crenulatus crabs was recorded also harbored at least one shrimp individual, a proportion that differs significantly from the number of ponds expected by chance alone (i.e., binomial distribution, mean: 0.88 c.l.: 0.68–0. 97) and suggests that either shrimp or crab are associating preferentially with the other species. Laboratory experiments demonstrated that shrimps colonize artificial refuges

harboring crabs more frequently than expected by chance alone (binomial test: P < 0.05 in 4 out of 5 observation periods) (Fig. 5). At the start of the experiment, shrimps colonized shelters in numbers that did not differ significantly from an expected random distribution. However, the number of shrimps found in the same shelter with a crab was more frequently than expected by chance alone during 4 out of the 5 other different times in which observations were conducted. During the experiments, shrimps were observed crawling around the experimental container and examining the two refuges before selecting one. Shrimps were seen in between the claws or above the carapace, most of the time in very close contact with the crab. In one occasion, we observed the resident crab repelling the shrimp immediately after the latter went inside the refuge.

4. Discussion

4.1. Group living in Betaeus lilianae

Here, refuge relative size was predicted to drive group living in B. lilianae. Supporting this prediction, shrimp aggregations rather than pairs or solitary individuals were found in intertidal pools more frequently than expected by chance alone. These large shelters most probably represent a resource that is too expensive, in terms of energy and time, for monopolization by shrimp individuals. Concomitantly with shelter size, other refuge characteristics (structural complexity) and predation pressure might be considered important in driving gregarious behavior in the studied shrimps and other marine invertebrates (Wilson, 1975; Baeza and Thiel, 2007). Refuge structural complexity is expected to increase the costs of refuge monopolization by solitary or pair of conspecifics, a notion supported by studies on the host use pattern of symbiotic crustaceans. For instance, symbiotic crustaceans living in aggregations use host species with greater structural complexity than that of symbiotic species occurring either solitarily or in pairs in/on hosts (Baeza and Thiel, 2003, 2007). Also, group living is predicted to spread mortality risk among individuals composing a group when attacked e.g., by predators or parasites (Hamilton, 1971; see also Mooring and Hart, 1992). In crustaceans, this idea is partially supported by at least one study in the fiddler crab Uca pugilator (McLain et al., 2005). During sampling, we observed four potential predators of B. lilianae: the fishes Bovichthys argentinus and Ribeiroclinus eigenmanni, the octopus Octopus tehuelchus and the crab P. crenulatus. However, the fishes and octopus were rarely observed at the study site and our laboratory observations suggest that P. crenulatus is not a predator but a symbiotic partner of B. lilianae (see below). The information so far available does not allow determining whether or not predation pressure (together with other refuge characteristics) is relevant in favoring gregariousness in the studied species. Betaeus lilianae might be used as a model to explore the relative importance of shelter attributes and predation risk in marine invertebrates from temperate environments.

4.2. Resource monopolization: implications for the mating system of Betaeus lilianae

The shrimp B. lilianae is gregarious, inhabiting intertidal rocky pools as large aggregations or groups of moderate size. It could be argued that each shrimp aggregation is comprised by many male–female (socially monogamous) pairs that use small crevices in the pools as refuge, as reported for several other Alpheidae shrimp (Mathews, 2003; Anker et al., 2006). Nonetheless, the female-biased sex ratio observed for most pools does not support this notion. If pair living was the actual smallest social unit in this shrimp, we would have expected equal or nearly equal sex ratios in most or all pools. Also, pools harboring only two shrimp at the study site were comprised equally likely by two males, two females or heterosexual pairs. If B. lilianae was socially monogamous, we would have expected
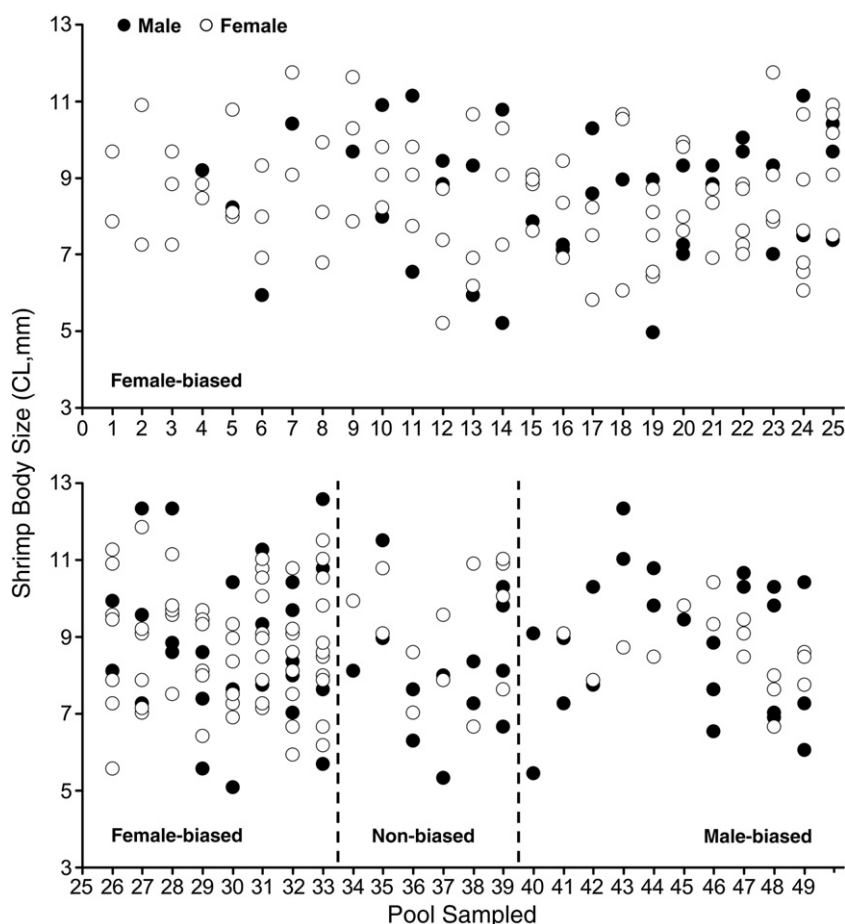
Fig. 3. Carapace length of shrimp within groups for shrimp inhabiting intertidal pools at Estafeta Postal beach, Mar del Plata, Argentina.

to find more heterosexual pairs in pools harboring only two shrimp. Furthermore, in socially monogamous species sexual dimorphism is reduced: males are similar in size or slightly smaller than females, there is no major differences in claw size between the sexes, and usually, there is no positive allometric growth of claws or any other structure that might be used as weapon during intra-sexual agonistic interactions (e.g., maxillipeds — Correa et al., 2003) both in males and females (Baeza, 2008; Baeza and Thiel, 2007; Correa and Thiel, 2003). Although, males of *B. lilianae* were similar in body size to females, claws were considerably more developed in males than females, and in the two sexes, claws grew more than proportionally with body size. The information above does not support the idea that aggregations of *B. lilianae* at the study site are comprised by long-term heterosexual pairs.

Betaeus lilianae is not socially monogamous, in contrast to that reported for some congeneric species (*B. macginitae* and *B. hardfordi* — Hart, 1964; Ache and Davenport, 1972) and for several other members of the family Alpheidae (Mathews, 2003; Boltaña and Thiel, 2001; Thiel and Baeza, 2001 and references therein). Given the impossibility of shelter monopolization by male shrimp, and thus, the constrained opportunities for males to attract females to shelters and resources present on it, males are expected to attempt mating by using exploitative "pure-search" strategies (Bauer, 2004; Baeza and Thiel, 2007). In pure-search promiscuous species, males are continuously searching for females. Once a receptive female is found, there is no evident courtship, insemination takes place rapidly and males depart immediately after copula in search for other receptive females (Bauer and Abdalla, 2001). Because males do not compete aggressively for females, their claws are not well developed. Instead, males are more active, agile and smaller than females because this set of

characters renders mate searching more efficiently (Correa and Thiel, 2003). Also, sex ratios are female-biased in some of these species, a phenomenon explained by the greater exposure to predators and mortality suffered by roaming males relative to more sedentary females (Baeza and Thiel, 2007). Supporting the idea of a pure-search mating system in the studied species, sex ratios were female-biased in *B. lilianae*. However, males were not, on average, smaller than females, and these males feature well developed claws that could be used as weapons to fight for females or resources to attract them. This developed weaponry suggests that males are competing by means of agonistic behavior for receptive females and not by exploitation (i.e., pure-searching). Thus, the information so far available disagrees with the idea of *B. lilianae* featuring a promiscuous pure-search mating system.

The structural complexity of rock pools should also constrain the evolution of harem polygamy in *B. lilianae*. In harems, males defend not a refuge but female groups directly (Wilson, 1975). Males are larger than females, have stronger weaponry, and adult sex ratio within a group is highly female-biased given that a single male monopolize several females (Wilson, 1975). In some species, increases in male size translate into increasing female group size and when "sneaker" males are present within a group, they are usually young and small (Shuster, 1987; Shuster and Wade, 2003). At first glance, the female-biased sex ratio predominantly observed in aggregations and the sexual dimorphism with respect to body size suggests that this species might be featuring harem polygamy. However, in disagreement with the idea of harem polygamy in *B. lilianae*, the size of the largest male within a pool did not varied significantly among groups with different sex ratios. Also, when groups with a female-biased sex ratio were considered alone, males
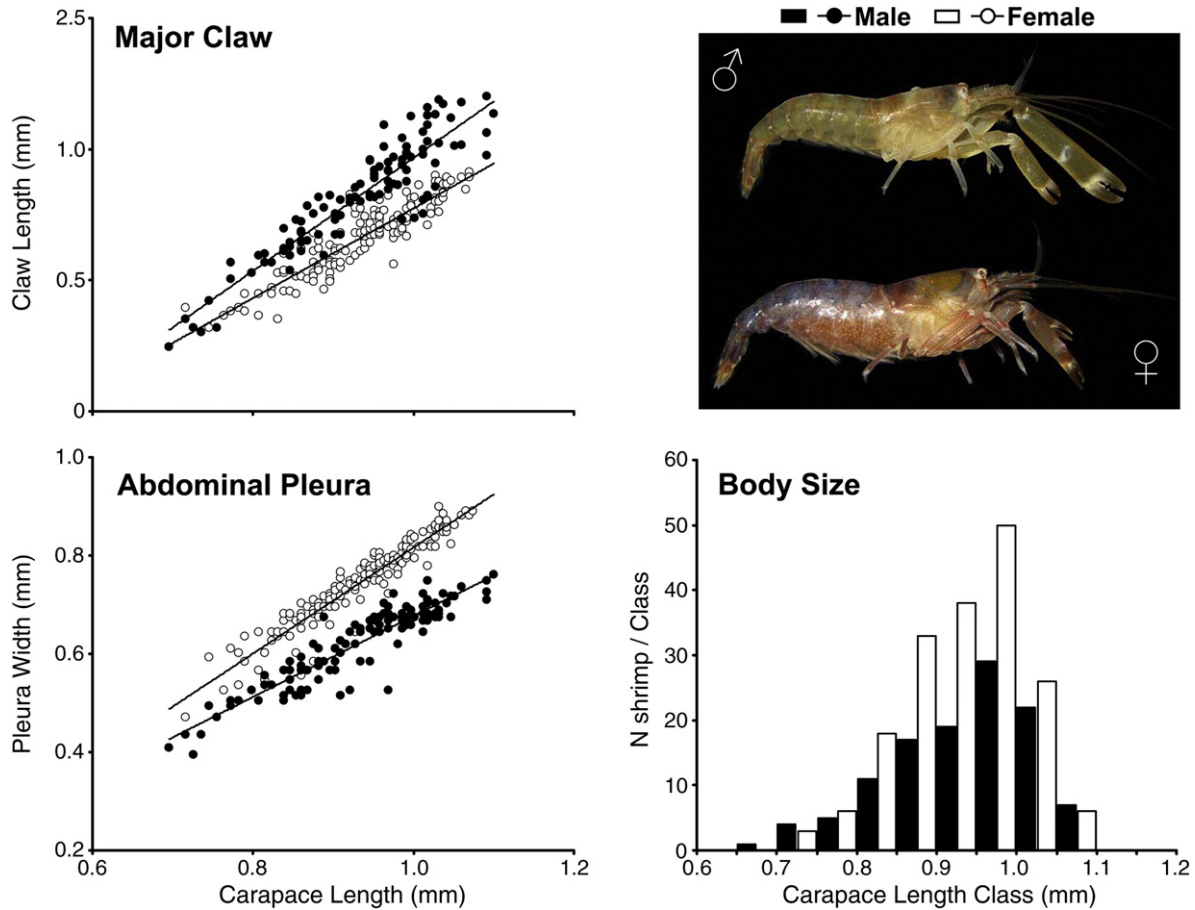
Fig. 4. Relative growth of major claw length (top left) and pleura of second abdominal segment (bottom left) as a function of carapace length in males and females of *B. lilianae*. Size frequency distribution for males and females of *B. lilianae* (bottom right). Measurements are in mm. Lineal regression equation obtained previous log–log transformation of the data are shown for each sex on Table 1.

were the largest shrimp in the group only in 15 out 30 aggregations. Lastly, when two males cohabited in the same pool, the difference in body size between the largest and second largest male was either minimal or large. The information available does not support harem polygamy as the mating system of *B. lilianae*.

Overall, the information so far available neither allows clearly identify the mating system of *B. lilianae* nor revealing details about the reproductive behavior of males and females within the population. For instance, we do not know if males search for females actively, if females are guarded by males before and after copulation and if males abandon females immediately after insemination. Field observations on the movement pattern of shrimp might reveal relevant details about the reproductive tactics of males and females in the population. Also, future studies should focus on describing the mating behavior of

Table 1
Relative growth of selected structures in males and females of *Betaeus lilianae*. The regression equations, correlation coefficients, standard errors of the slopes (SE$_s$), and the allometric status of each studied variable are shown (CL, ClaL, and AbdL = carapace length, length of the merus of the major chelae, and length of the second abdominal pleura, respectively).

y	x	Regression	r^2	SE$_s$	t_s	P	Allometry
Males							
ClaL	CL	$y = 2.156x - 1.189$	0.842	0.0856	8.88	<0.001	+
AbdL	CL	$y = 0.824x - 0.147$	0.849	0.0313	5.39	<0.001	−
Females							
ClaL	CL	$y = 1.667x - 0.894$	0.825	0.0576	4.82	<0.001	+
AbdL	CL	$y = 1.058x - 0.242$	0.892	0.0276	2.09	0.03798	+

B. lilianae under different social conditions to reveal the possibility of alternative mating tactics in this species.

4.3. Association between Betaeus lilianae and Platyxanthus crenulatus

Betaeus lilianae is associated preferentially with the brachyuran crab *P. crenulatus* as indicated by our field observations and laboratory experiments. Partnerships involving shrimps, including other members from the Alpheidae, and other invertebrates, including brachyuran crabs (*Alpheus lottini* — Vannini, 1985; *Alpheus heterochaelis* — Silliman et al., 2003; *Merguia oligodon* — Gillikin et al., 2001), are common in tropical environments, but much less frequent at temperate latitudes (Boltaña and Thiel, 2001; Silliman et al., 2003). For most of these partnerships, little is known about the cost and benefits experienced by the symbiotic partners. That *B. lilianae* shrimps were observed on the carapace, among the claws and near the mouth of *P. crenulatus* during the laboratory experiments suggests that two of the main benefits for shrimps are food acquisition and protection against predators. *B. lilianae* might found protection against predators such as the blennid fishes observed at the study site. The large claws of *P. crenulatus* and the close contact among shrimp and their crab partners most probably decrease the chances of attack by fish predators. Also, *B. lilianae* might feed on particles suspended during the crab foraging activities. *P. crenulatus* is a specialist shell-breaking crab, but it also consumes carrion as well almost anything that they can catch (e.g. ascidians and polychaetes) (NEF unpublished data). Shrimps might follow crabs during their night foraging excursions, as suggested by our laboratory observations, and consume particles suspended in the water as a result of prey dislodgment by crabs. Shrimp might also consume *P. crenulatus* feces
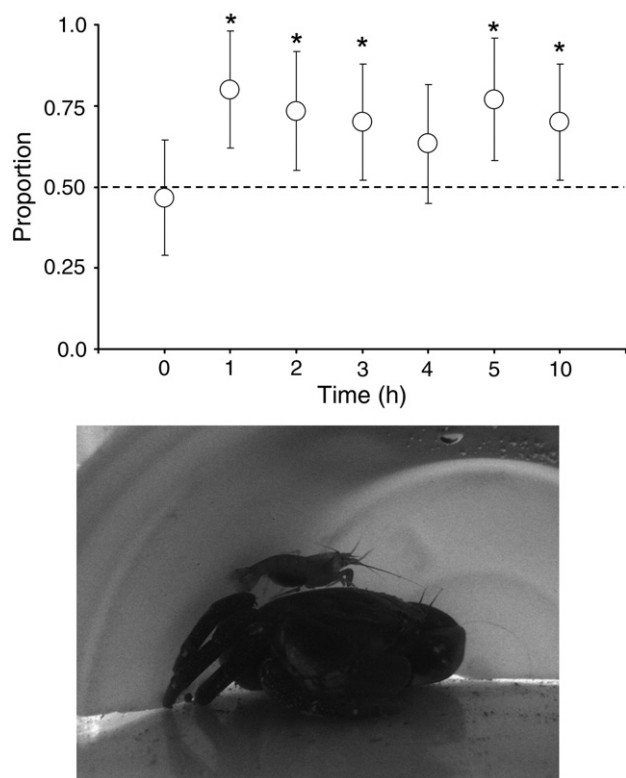
Fig. 5. Preference of *Betaeus lilianae* shrimp for shelters harbouring or not one individual of *Platyxanthus patagonicus*. The position of the shrimp and crab within two containers provided as shelter during the experiment was monitored immediately or various hours after the experiment was initiated.

as reported before for other shrimp associated to crabs (i.e., the hippolytid *Merguia oligodon* inhabiting the lairs of *Neosarmatium smithi* — Gillikin et al., 2001).

Lastly, *B. lilianae* might ingest fouling organisms directly from the carapace of *P. crenulatus* (e.g., larvae, barnacle recruits, bryozoans and algae, among others) actually providing cleaning services to crabs. Cleaning symbioses were thought to be restricted only to the tropics. Nonetheless a recent study has demonstrated cleaning of large hosts (e.g. fish) by shrimp from temperate environments (Östlund-Nilsson et al., 2005; Jensen 2006). Most interestingly, we rarely observed crabs with their carapace covered by barnacles or any other fouling organism commonly found among rocks and mollusk shells at the study site. Fouling is not uncommon in large and old brachyuran crabs from temperate habitats (in *Romaleon setosus* and *Homalaspis plana* from the temperate southeastern Pacific — JAB, personal observations). Large crabs are prone to suffer severe fouling as they experience decelerated growth and prolonged molting cycles. Whether shrimp behavior is responsible for the rarity of fouling on the crab's carapace remains to be experimentally addressed.

Benefits for *P. crenulatus* from the association with *B. lilianae* might include energy savings. A clean carapace is lighter than a heavily fouled one and might imply diminished energetic costs to crabs during e.g., foraging or mate searching excursions. These energy savings might be relocated to sperm production or agonistic behaviors related to female monopolization. It is difficult to foresee additional advantages for crabs due to their association with shrimp. Shrimps from the related genus *Alpheus* successfully defend hosts from predatory attacks or cooperate with hosts in defending refuges against common enemies using their powerful snapping claw (Anker et al., 2006 and references therein). However, shrimps from the genus *Betaeus* lack a snapping claw (Anker et al., 2006). Thus, benefits for shrimp might be greater than for crabs, and such benefit asymmetry between these two species might imply conflict of interest between

symbiotic individuals. Putative conflicts might explain aggression from crabs towards shrimp as observed during our experiment. The partnership between *B. lilianae* and *P. crenulatus* might be used as model to study cooperation dynamics and conflict resolution among marine symbiotic relationships.

4.4. Outlook

The present study supports the hypothesis that large shelters favor group living. *Betaeus lilianae* lives in aggregations in intertidal rocky pools as reported for other two congeneric species (*B. truncatus* and *B. emarginatus* — Antezana et al., 1965). This gregarious behavior contrasts with the solitary habit or pair-living behavior reported for other species from the genus (solitary habit: *B. setosus* — Jensen, 1986; pair-living habit: *B. macginitiae* and *B. hardfordi* — Hart, 1964). Similarly, the association between *B. lilianae* and the xanthoid crab represents the fourth symbiotic relationship reported for the genus. *Betaeus setosus*, *B. macginity* and *B. hardfodi* are reported to be symbiotic with porcelain crabs, sea urchins, and abalones, respectively (Jensen, 1986; Ache and Davenport, 1972). Shrimps from this genus demonstrate a considerable diversity of lifestyles. A molecular phylogeny for the group currently under development together with studies on the natural history of these shrimp will allow in the near future exploring the role of the environment in favoring evolutionary innovations in the marine environment.

Acknowledgments

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