Journal of Experimental Marine Biology and Ecology 429 (2012) 36–46

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Host-specific reproductive benefits, host selection behavior and host use pattern of the pinnotherid crab *Calyptraeotheres garthi*

Emiliano H. Ocampo [a,b,c,*], Jesús D. Nuñez [a,b,c], Maximiliano Cledón [a,b,c], J. Antonio Baeza [d,e]

[a] EGEM—Laboratorio de Ecología y Genética de Especies Marinas, Dpto. de Cs. Marinas, FCEyN, Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata, CP 7600, Argentina
[b] Instituto de Investigaciones Marinas y Costeras (IIMyC), FCEyN, Universidad Nacional de Mar del Plata, Consejo Nacional de Investigaciones Científicas y Técnicas, Funes 3350, Mar del Plata, CP 7600, Argentina
[c] Aquarium Mar del Plata, Av. Martínez de Hoz 5600, Mar del Plata, CP 7600, Argentina
[d] Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949, USA
[e] Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile

ARTICLE INFO

Article history:
Received 30 January 2012
Received in revised form 11 June 2012
Accepted 13 June 2012
Available online 13 July 2012

Keywords:
Fitness
Habitat use
Host selection
Pinnotheridae
Preference
Symbiosis

ABSTRACT

In organisms using a wide variety of refuges, both the cost and benefits to the users might be refuge-specific. Under these circumstances, users using mechanisms (e.g., behavioral preference) that allow them to find and colonize refuges in which net benefits are maximized are expected to be selected by the environment. Herein, we tested the hypothesis that resource generalists display strong preferences for resources that provide the greatest reproductive benefits using *Calyptraeotheres garthi*, a crab that inhabits the limpets *Crepidula cachimilla* and *Bostrycapulus odites* in the south-western Atlantic. In the field, female crabs inhabiting *C. cachimilla* featured larger average body size, and subsequently, larger average fecundity and brood weight than those inhabiting *B. odites*. Thus, *C. cachimilla* is a higher quality host for *C. garthi* compared to *B. odites*. In contrast to expectations, host selection experiments revealed that female crabs were attracted to the host species from which they were collected and not to the host species that provided the greatest reproductive benefits (*C. cachimilla*). Host chemical and/or visual cues imprinted in crabs early during ontogeny (soon after colonization of their first host), rather than genetic differences, may explain the source-host fidelity of *C. garthi*. In the two hosts, females of *C. garthi* lead a solitary lifestyle and appear to inhabit the same host individual for long periods of time as indicated by the close relationship between female crab and host body size. The frequency of male–female pairs within host individuals was lower than expected by chance alone. Also, males were, on average, smaller than females and did not appear to inhabit the same host individual for long periods of time. The above suggests that males might be using a pure-search mating strategy, continuously roaming among host individuals in search of receptive females. The details of the mating system of *C. garthi* need to be further investigated.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Refuges (defined as a spatial zone or physical heterogeneity of the ambient or biological entities that enable organisms to reduce environmental stress or escape predators — Woodin, 1978) represent an important resource for vertebrate and invertebrates from both terrestrial and aquatic environments. Refuges may be non-living structures (e.g., crevices in rocks), parts of or whole death organisms (e.g., litter or fallen trees in temperate or tropical forests) or parts of living organisms (Woodin, 1978). In the latter case, the living organism represents a host used by smaller symbiotic partners (symbiosis herein defined sensu de Bary (1879) as dissimilar organisms living together).

Refuges provide important benefits to their dwellers, including protection against predators (Maynard-Smith, 1974; McNair, 1986; Persson and Eklöv, 1995), protection against stressful abiotic conditions (e.g., desiccation — Schwarzkopf and Alford, 1996; waves —Gabel et al., 2008), a direct or indirect food source (Gosselin and Chia, 1995; Stauber, 1945), and/or access to sexual partners (Christy, 1987). Refuges, on the other hand, also result in costs to their users, including energy allocated to their defense when refuges are scarce (Christy, 1987) and/or diminished opportunities to acquire extra food or sexual partners when refuges become depleted of the resources above and when mobility among refuges is constrained (Martín et al., 2003; Persson and Eklöv, 1995; Werner et al., 1983). Organisms might be refuge-specialists, i.e., a single type of refuge with particular characteristics is inhabited by all ontogenetic stages of a "user" species, or refuge-generalists, i.e., a species uses a wide diversity of refuges with dissimilar characteristics (Krebs and Davies, 1993).

* Corresponding author at: EGEM—Laboratorio de Ecología y Genética de Especies Marinas, Dpto. de Cs. Marinas, FCEyN, Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata, CP 7600, Argentina.
E-mail address: eocampo@mdp.edu.ar (E.H. Ocampo).

0022-0981/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2012.06.009

For organisms that use a wide variety of refuges, both the cost and benefits are expected to be refuge-specific (Levins, 1968). Some refuges with particular features might provide greater net benefits to users than other refuges with other set of characteristics. Even some type of refuges might convey net costs to their users. Therefore, users displaying mechanisms that allow them to find and colonize particular types of refuges in which the net benefits are maximized should be favored by natural selection (Jaenike, 1978; Levins and MacArthur, 1969). Overall, resource generalists are expected to display strong preferences for those resources that provide the greatest net benefits to them.

The hypothesis of refuge generalists showing active preferences for refuges that provide the greatest net benefits has been explored before in only a few groups of organisms (e.g., insects — Bergström et al., 2006, reptiles — Wilson, 1998; Kolbe and Janzen, 2002). Among marine invertebrates, most studies on the relationship between resource quality and behavioral preference by users have been conducted in hermit crabs that use empty gastropod shells as refuges (Bertness, 1980, 1981a, 1981b; El-Kareem Ismail, 2010; Garcia and Mantelatto, 2001). In these anomuran crabs, the size and architecture of the different shell species that serve as refuge largely influence different components of the hermit crab fitness that include growth rate, fecundity, vulnerability to predators, and protection against physical stress (Bertness, 1981a; Garcia and Mantelatto, 2001). Importantly, some species have been reported to exhibit particular preferences for specific shells in which fitness is maximized (Bertness, 1981a, 1981b; Garcia and Mantelatto, 2001). Host-generalist symbiotic crustaceans are an ideal model system to further explore the effect of host characteristics on the fitness of their associates and to test the hypothesis that host specific reproductive benefits should correlate with host preferences.

Within the large order Decapoda, one of the most species-rich clade of crustaceans (De Grave et al., 2009), the family Pinnotheridae, is recognized for its symbiotic lifestyle and remarkable morphological, ecological, and behavioral diversity (Alves and Pezzuto, 1998; Bell, 1984; Kruczynski, 1973; Ross, 1983). The 302 currently described species of Pinnotheridae (De Grave et al., 2009) are collectively known as "pea crabs" due to their rounded shape, smooth external appearance and small size. Pea crabs inhabit a wide diversity of host species, including gastropods, bivalves, ascidians, holothurians and sea urchins (among others) (Schmitt et al., 1973). Pinnotherid crabs also use burrows constructed by echiuran worms, annelid worms, and other crustaceans as a refuge (Schmitt et al., 1973). This wide ecological and morphological diversity matches the diversity of mating systems within the Pinnotheridae (Baeza, 1999; De Bruyn et al., 2009; Hamel et al., 1999; Møller Christensen and McDermott, 1958). Some species have been reported to inhabit their hosts as socially monogamous pairs (e.g., *Pinnixa transversalis* — Baeza, 1999). Other species are polygynandrous; in *Dissodactylus primitivus* both male and female pea crabs move among host individuals in search of food and/or mating partners (De Bruyn et al., 2009). Also other species appear to be highly promiscuous; in *Tumidotheres maculatus* and *Fabia subquadrata*, male and female crabs form mating swarms in open water. Then, females (but not necessarily males) colonize host individuals for long periods of time and continuously produce embryos during the rest of their life (Pearce, 1964, 1966). Although our knowledge of the mating behavior in the Pinnotheridae has increased during the last years, the mating system of most pinnotherid species still remains overlooked.

Some pinnotherid crabs colonize for the first time their hosts, and thus, establish a symbiotic association early during their life, either during the first post-larval stage (megalopae) or during the first-stage crab phase (Møller Christensen and McDermott, 1958; Pearce, 1964; Pohle and Telford, 1981). However, in some species, mature crabs can and do leave their hosts (e.g., to mate in open waters or to acquire food in other hosts individuals) during adulthood and (re)colonize the same or a different host individual more than once during their lifetime (Møller Christensen and McDermott, 1958). Some species of pea crabs inhabit a wide number of different host species with diverse body plans, body sizes, and ecology. For instance, *T. maculatus* is reported from at least 20 different host species pertaining to 3 different invertebrate phyla (Bierbaum and Ferson, 1986; Derby and Atema, 1980; McDermott, 1962). Early experimental studies have already demonstrated the ability of pinnotherid crabs to detect chemical cues produced by their respective hosts and to colonize them based on these cues (Davenport et al., 1960; Derby and Atema, 1980; Gray et al., 1968; Stevens, 1990a). In some species, host choice experiments have shown that crabs prefer source hosts (those from where crabs are collected) over non source hosts (e.g., *T. maculatus* — Derby and Atema, 1980, *Dissodactylus mellitae* — Gray et al., 1968, *Pinnotheres atrinicola* and *Nepinnotheres novaezelandiae* — Stevens, 1990a). However, the relationship between refuge quality and host preference is ill explored in most pea crabs.

Calyptraeotheres garthi is a pinnotherid crab which inhabits the mantle cavity of limpets from the family Calyptraeidae in the southwestern Atlantic (Campos, 1999). In Argentina, this pinnotherid is ubiquitous, being present in almost all naturally occurring calyptraeid limpets (i.e., *Crepidula argentina*, *Crepidula cachimilla*, *Crepidula plana*, *Bostrycapulus odites*, and *Trochita pileus* — Ocampo et al., 2012). The occurrence of *C. garthi* varies markedly from one host to other, which might be considered a preliminary indication of preference for particular limpets (Ocampo et al., 2012). Nothing is known about the effect of the host on the reproductive biology of this symbiotic crab. If the host species indeed affects the reproductive parameters of this symbiotic crab, we expect preferences by crabs for the host species in which reproductive benefits are the greatest.

In this study we first explored whether reproductive parameters of *C. garthi* differed between two host species, *C. cachimilla* and *C. odites*. Next we conducted laboratory experiments to test the hypothesis of active preferences for the host species that provides the greatest reproductive potential as suggested by evolutionary theory. Given that the socioecology of this species is poorly known, we also report on the host use pattern of *C. garthi*, including the description of its population distribution, male–female association pattern and host–crab body size relationships.

2. Material and methods

2.1. Collection of hosts and symbiotic crabs

The two limpets *C. cachimilla* and *C. odites* were collected during February 2010 and February 2011 in San Matías Gulf (North Patagonia, Argentina). *Crepidula cachimilla* inhabits the subtidal zone (10–40 m) and is found associated to banks of the mussel *Mytilus edulis* (Cledón et al., 2004) while *B. odites* occurs both in the subtidal and intertidal zones, mainly attached to rocks. *Crepidula cachimilla* were obtained by SCUBA diving at ~12 m depth near Piedras Coloradas (S 40° 57′, W 65° 06′). Specimens of *B. odites* were collected by hand during low tides from the intertidal and shallow subtidal zones (0.5–1 m deep) at San Antonio Oeste (S 40° 43′, W 64° 55′). The two limpets form stacks of two or more individuals in the field.

Host and crab specimens collected during 2010 were immediately fixed (formaldehyde 4%) and stored until measurements were taken. Specimens collected during 2011 were quickly placed in containers with aerated sea water and then transported alive to the Laboratory of Marine Ecology and Genetics (National University of Mar del Plata, UNMdP, Argentina). During transportation, no crab was observed leaving its host. In the laboratory, the shell length (SL) of each limpet collected during 2010 and 2011 was measured with a digital caliper to the nearest 0.1 mm. Next, each limpet was detached from its substratum and carefully inspected for symbiotic crabs. The carapace width (CW) of each crab retrieved from limpets was

measured under the stereomicroscope equipped with a calibrated ocular micrometer (precision = 0.01 mm). Crabs were sexed based on the presence or absence of gonopods (McLaughlin, 1980). Limpets were also sexed on the basis of the presence or absence of a penis (Cledón et al., 2004). Lastly, taking into account the morphology of their abdomen and pleopods, each female crab was classified either as immature (= prehard and II–IV stages — Pearce, 1964; Jones, 1977) or mature (= stage V — Pearce, 1964; Jones, 1977).

After being measured and sexed, most crabs collected during 2011 were returned to the same limpets from which they were originally extracted and each limpet was reattached to a new substrate consisting of a small piece of translucent glass. These hosts and crabs were used for the host selection experiments (see below).

2.2. Host use pattern of Calyptraeotheres garthi in the two hosts species

We examined the host use pattern of *C. garthi*, which includes a description of its population distribution, male–female association pattern and host–crab body size relationships. First, we examined whether *C. garthi* crabs live solitarily, aggregated, or in pairs inside the two host species. For this purpose, we examined whether the distribution of *C. garthi* in each one of the two host species (i.e., the frequency of occurrence of hosts without crabs and with different numbers of crabs) differed from a random distribution. We compared the observed distribution (frequency of occurrence of hosts with zero, one, two, three or more crabs) with the Poisson random distribution (Elliot, 1983). Significant differences between the distributions were examined using a Chi-square test of goodness-of-fit (Sokal and Rohlf, 1981). When significant differences were observed, specific frequencies between the observed and expected distributions were compared by subdivision of the Chi-square test and using the sequential Bonferroni correction to control for false discovery rate (Rice, 1989). To estimate the frequency of occurrence of *C. garthi* crabs, we considered only limpet larger than 11.3 and 12.3 mm of SL for *C. cachimilla* and *B. odites*, respectively. Limpets smaller than the above do not harbor symbiotic crabs.

Next, we tested whether the body size of solitary males and females differed significantly from that of paired males and females, respectively. For this purpose, two different Student's *t*-tests were conducted (Sokal and Rohlf, 1981). Prior to the Student's *t*-tests, the data were tested for normality (Shapiro–Wilks test) and for homogeneity of variances (Cochran's test) and were transformed (if necessary) to meet statistical assumptions (Underwood, 1997; Zar, 1999).

Lastly, we calculated the sex-ratio as the number of males divided by the total number of crabs. For each host, the observed proportion of males to females was tested for deviations from a 1:1 sex ratio using a binomial test (Wilson and Hardy, 2002).

2.3. Reproductive biology of Calyptraeotheres garthi in the two hosts species

A total of 21 and 14 ovigerous females carrying early embryos retrieved from *C. cachimilla* and *B. odites*, respectively, were used to examine the effect of host species on the reproductive biology of *C. garthi*. The totality of the embryos carried by each female was carefully extracted with forceps from beneath the abdomen and counted under the stereomicroscope using a millimeter-subdivided camera. Then, the length (L) and width (W) of twenty randomly selected embryos per female were measured under a microscope equipped with a calibrated ocular micrometer (precision = 0.01 mm). Next, embryo volume (EV) was calculated for each measured embryo using the formula for the volume of an ellipsoid ($V = \pi LW^2/6$). Finally, the studied females and their embryo masses were dried for 48 h at 60 °C and then weighed with an analytical balance (precision = 0.01 mg).

With the measurements above, we calculated the fecundity (n° embryos female^{-1}) and reproductive output (RO = brood dry weight/female body dry weight — Clarke et al., 1991) of each female. We determined whether brood dry weight increased linearly (isometrically) with female body dry weight and whether fecundity and EV increased linearly with CW. For this purpose, the relationship between brood weight and body weight, fecundity and CW, and EV and CW of females 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 change of each reproductive parameter with an increase in body weight or CW of female crabs. The relationship between brood dry weight and female body dry weight and that between fecundity and CW were considered isometric when b was = 1, positively allometric when $b > 1$ and negatively allometric when $b < 1$. In turn, the relationship between EV and CW was considered isometric when b was = 3, positively allometric when $b > 3$ and negatively allometric when $b < 3$ (Somers, 1991). Departures from isometry were tested using a Student t–test (Sokal and Rohlf, 1981).

Host features can affect reproductive traits of their symbiotic associates either directly or indirectly; via its effect in other phenotypic traits (e.g., body size) that in turn drive reproductive traits (Tsai et al., 2001). In the latter case, differences in e.g., fecundity of *C. garthi* between the two studied limpet species might be explained only by differences in body size of this crab between the host species. The importance of body size in determining fecundity, egg size, and/or reproductive output in marine invertebrates, including crustaceans, is well recognized (Bertness, 1981a; Hernáez et al., 2008; Hines, 1992; Lardies and Castilla, 2001). Herein, we conducted different analyses of covariance (ANCOVAs) and one–way analyses of variance (ANOVAs) to test whether host species has a direct or indirect effect on the different reproductive parameters measured above for females of *C. garthi*. In these analyses, either body weight or CW was used as a covariate and host species as a main factor to estimate differences between host species in brood weight, fecundity, and egg size. In each ANCOVA, we assumed homogeneity of slopes if there was no significant interaction between the main categorical factor (host species) and the covariate (body weight or CW) (Sokal and Rohlf, 1981). If the ANCOVAs detected a significant effect of host species in the different reproductive parameters herein studied, then we concluded that host species directly affects reproductive parameters in *C. garthi*. On the other hand, if the ANCOVAs did not detect a significant effect of host species but the ANOVA did detect an effect of host species in reproductive parameters, then we predicted that the effect of host species in the reproductive parameters of *C. garthi* was indirect. In the latter case, we attributed any difference in reproductive parameters of *C. garthi* between host species to the effect of crab body size (see Results section).

2.4. Laboratory experiments on host preference

All crabs and limpets used during the experiment were maintained and acclimatized in a container of 300 L with flowing natural sea water (6–10 L min^{-1}) for at least 2 weeks before the start of the experiment. The experiment was conducted in white plastic cylindrical aquarium (3 L) that contained aerated seawater. In each aquarium, two limpets, one from *C. cachimilla* and one from *B. odites*, matched in size (difference less than 3 mm SL) were placed opposite to each other at the periphery of the aquarium (~14–15 cm apart). Prior to the start of the experiment, each test crab was extracted from its original limpet (source host) and maintained isolated in 1 L container for 24 h. After these first 24 h, each crab was moved and placed at the center of the experimental aquarium within a small inverted plastic chamber. After a period of acclimatization of ≥15 min, the inverted plastic chamber was gently lifted and the reaction and host selection behavior of the female crab was recorded immediately (3 min) and after 15 min, 1 h, 3 h and 8 h. Crabs either remained motionless at the center of the aquarium, explored the surroundings rather erratically, or colonized limpets. We recorded these three behavioral categories for each

female during each experimental period. The clear glass to which limpets have been reattached before the experiment (see above) allowed us to observe the presence/absence and position of the experimental crabs on the paleal cavity of the limpets. A total of 82 females (replicates), 43 retrieved from *C. cachimilla* and 39 from *B. odites*, respectively, were used during the experiment. We selected only female crabs similar in size for our experiments. The average body size of those crabs was 4.55 ± 0.70 mm CW (from *C. cachimilla*) and 4.27 ± 0.66 mm CW (from *B. odites*). No significant differences in CW of crabs from the two host species were detected (*t* test, $t = 1.857$, $df = 80$, $P = 0.067$). Also, the average body size of *C. cachimilla* and *B. odites* was 21.9 ± 4.05 and 19.4 ± 3.8 mm SL, respectively. No significant differences in SL of the two host species used during the experiment were detected (*t* test, $t = 1.911$, $df = 80$, $P = 0.062$).

In order to test whether crabs choose one host over another, we compared the observed distribution of crabs on the two offered hosts during the experiment with the binomial random distribution, which, in the case of no difference, indicated no preference by crabs for a particular host species (Sokal and Rohlf, 1981). The significance level was established with a two-tailed binomial test (Zar, 1999).

3. Results

3.1. Host use pattern of Calyptraeotheres garthi on the two species of host limpets

A total of 161 *C. cachimilla* (90 and 71 sampled during 2010 and 2011, respectively) and 1834 *B. odites* (339 and 1495 sampled during 2010 and 2011, respectively) were collected from San Matías Gulf, Argentina. Pooling the specimens collected during 2010 and 2011, a total of 154 and 60 crabs of *C. garthi* were obtained from within *C. cachimilla* and *B. odites*, respectively. In the two hosts species, crabs positioned themselves between the ventral side of the head and an anterior fold of each limpet's foot (Fig. 1).

The body size (SL) of the limpets varied between 9 and 44.8 mm (31 ± 6.9), and between 4.7 and 26.2 mm (17.1 ± 4.4), in *C. cachimilla* and *B. odites*, respectively (Fig. 2). The frequency of occurrence of crabs in *C. cachimilla* (72.3%, 115 out of 159 collected hosts > 11.3 mm SL was considerably higher than that in *B. odites* (4%, 58 out of 1433 collected hosts > 12.3 mm SL) (Chi-square test of independence, $\chi^2 = 171.8$, $df = 1$, $P < 0.0001$). The smallest limpets harboring crabs were a male of 11.3 mm SL in *C. cachimilla* and a male of 12.3 mm SL in *B. odites*. Thus, during their protandric life, both male and female limpets from the two species and from all size classes harbor pea crabs (Fig. 2).

The number of individuals of *C. garthi* per host varied between 0 and 3 (average ± s.d. = 0.97 ± 0.74) in *C. cachimilla*. The population distribution of *C. garthi* on limpets of *C. cachimilla* did not display a random pattern (observed versus expected Poisson distribution, Chi-square test of goodness of fit, $\chi 2 = 76.53$, $df = 3$, $P < 0.0001$) (Fig. 3). More limpets of *C. cachimilla* harbored one crab compared to the number expected by chance alone and fewer limpets with zero or more than one crab were observed compared to the number expected by chance alone ($P < 0.05$). In turn, the number of symbiotic crabs per host varied between 0 and 2 (0.04 ± 0.21). In *B. odites*, the population distribution of *C. garthi* on limpets display a random pattern (Chi-square test of goodness of fit, $\chi 2 = 1.16$, $df = 2$, $P > 0.05$).

In *C. cachimilla*, a total of 78 (49.1%) hosts harbored a single crab, and most of these solitary crabs (81%, n = 63) were mature females. Other crabs occurring solitarily in their hosts included 5 males

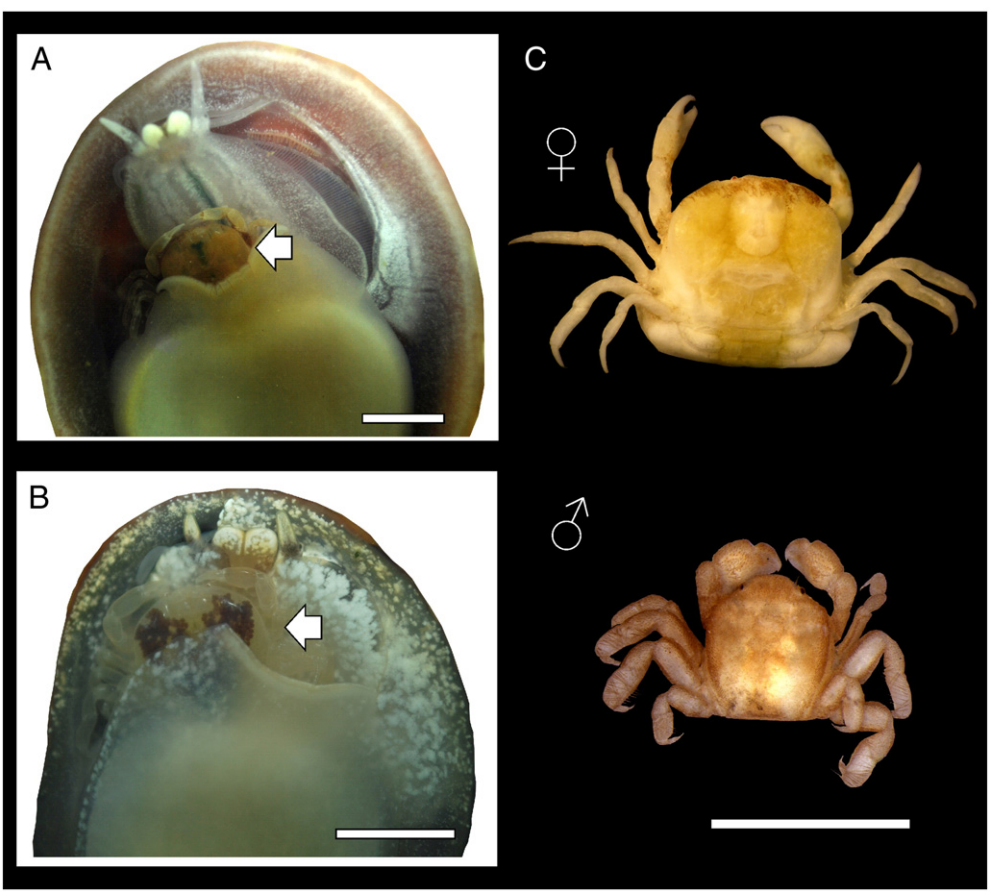

Fig. 1. Ventral view of the limpet hosts (A) *Crepidula cachimilla* and (B) *Bostrycapulus odites*, showing female crabs of *Calyptraeotheres garthi* (arrows) inside of the limpet paleal cavity and covered in part by a fold of the foot. (C) Detail of female and male of *C. garthi*. Scale bar = 5 mm.

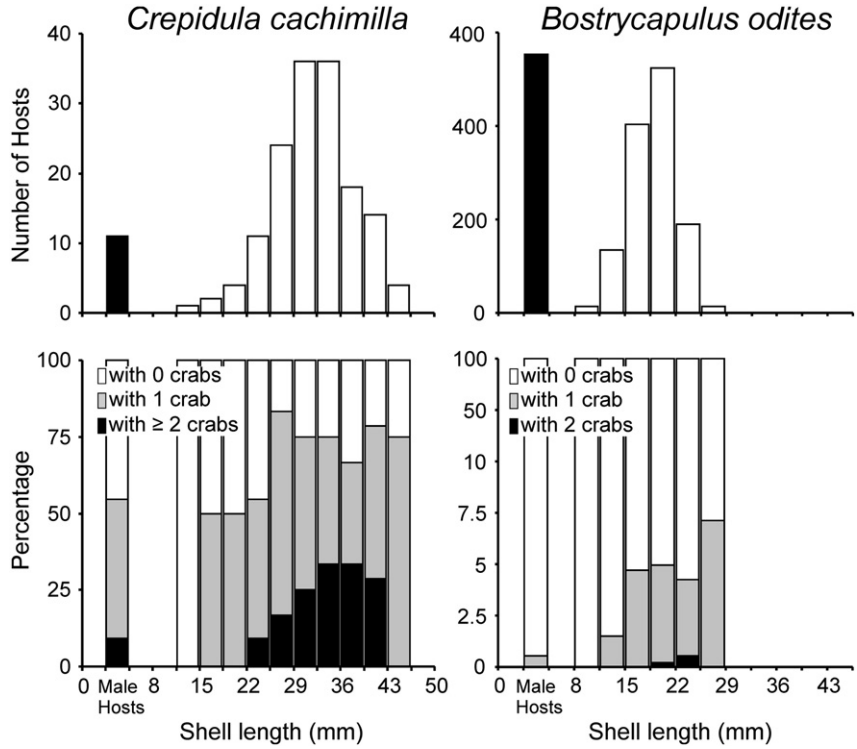

Fig. 2. Population structure of the host species *Crepidula cachimilla* and *Bostrycapulus odites* (open bars = female limpets; black bars = male limpets). Below, percentage of occurrence of crabs in each host species from different size classes. The data from both collecting dates (2010–2011) were pooled.

(6.1%), 8 immature females (8.9%), and 3 small crabs whose sex was not possible to determine (3.8%). Also, a total of 35 *C. cachimilla* harbored pair of crabs in their mantle cavity, and most of these pairs (80%, n = 28) comprised one male and one female. Among these heterosexual pairs, 21 (75%) comprised one mature female and one male, 5 (17.9%) comprised one immature female and one male, and 2 (7.1%) comprised one small crabs whose sex was not possible to determine and one male. Limpets harboring two crabs in their mantle cavity were among the largest collected from the field (Fig. 2). Five limpets of *C. cachimilla* harbored homosexual pairs and four of these pairs comprised one mature female and one non-mature female. In one host, two mature females shared the same host individual. Lastly, one out of the 2 hosts harboring three crabs contained one mature female, one immature female and one male. The other triplet consisted of one mature female plus two males.

In *B. odites*, a total of 56 (3.9%) hosts harbored a single crab. Most of these solitary crabs (73.2%, n = 41) were mature females. Additionally, 11 males and 4 immature female crabs were also found living solitarily in this host species. Only two limpets of *B. odites* harbored a pair of symbiotic crabs. These two pairs consisted of one immature female with one male and one mature female with one small, probably immature, male.

In *C. cachimilla*, there was no significant difference in CW between solitary mature females and mature females that shared their host individuals with a male (t test, $t = 0.015$, $df = 76$, $P = 0.988$). Similarly, no significant differences in CW between solitary males and males

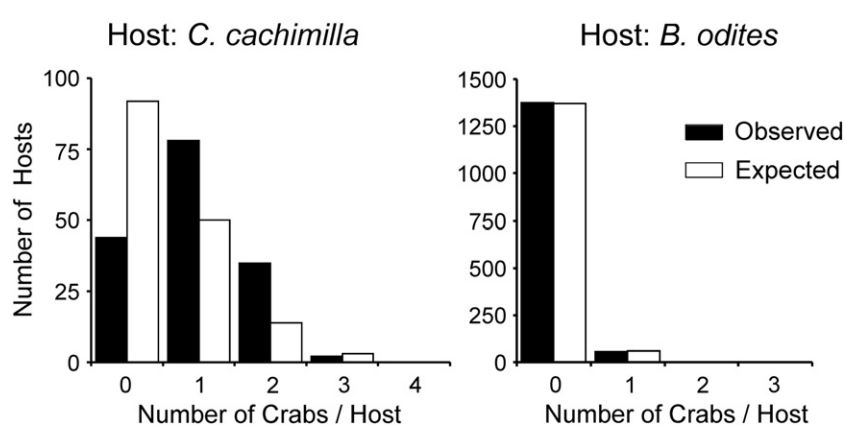

Fig. 3. Population distribution of the pinnotherid crab *Calyptraeotheres garthi* in two host species. Observed frequency of occurrence of crabs on *Crepidula cachimilla* differs significantly from the expected Poisson random distribution, while the observed frequency of occurrence of crabs on *Bostrycapulus odites* displays a random pattern. The data from both collecting date (2010–2011) were pooled for this analysis.

paired with mature females were observed (t test, $t=0.444$, $df=24$, $P=0.667$). Lastly, the presence of a male was independent of the presence of embryos in females inhabiting *C. cachimilla* (Chi-square test of independence, $\chi^2=0.86$, $df=1$, $P=0.354$).

A positive, statistically significant correlation between host size and crab size was recorded for females inhabiting the two host species (Pearson test, $R=0.61$, $P<0.001$ and $R=0.60$, $P<0.001$ for females retrieved from *C. cachimilla* and *B. odites*, respectively). However, male CW was not correlated with limpet SL (Pearson test, $R=0.08$, $P=0.811$ and $R=0.03$, $P=0.935$ for *C. cachimilla* and *B. odites*, respectively) (Fig. 4).

Sex-ratio varied between 0.27 and 0.21 in *C. cachimilla* and *B. odites*, respectively. Sex-ratio departed significantly from an expected 1:1 ratio and was skewed toward female crabs in the two host species (binomial test: $P<0.001$ in the two hosts).

3.2. Reproductive biology of Calyptraeotheres garthi in the two host species

Females of *C. garthi* ranged in size from 2.9 to 7.4 mm CW (4.93 ± 1.00, n = 86) and from 3 to 5.3 mm CW (4.27 ± 0.64, n = 41) in *C. cachimilla* and *B. odites*, respectively. Females of *C. garthi* inhabiting *C. cachimilla* were significantly larger than those inhabiting *B. odites* (t test, $t=3.864$, $df=125$, $P=0.0002$). In turn, the CW of male crabs inhabiting *C. cachimilla* and *B. odites* varied, respectively, between 1.5 and 3.3 mm (2.32 ± 0.40, n = 36) and between 1.7 and 2.9 mm (2.28 ± 0.49, n = 12) (Fig. 3). No significant differences in body size between males retrieved from the two host species was detected (t test, $t=0.128$, $df=46$, $P=0.898$).

The minimum and maximum body size of ovigerous females retrieved from *C. cahimilla* and *B. odites* were 3.1 and 7.1 mm CW and 3.2 and 5.6 mm CW, respectively. Fecundity of *C. garthi* inhabiting *C. cachimilla* and *B. odites* varied, respectively, between 201 and 3838 embryos female^{-1} (1794.3 ± 1071.2, n = 21) and between 296 and 1819 embryos female^{-1} (935.5 ± 420.5, n = 14) (Fig. 3). A positive, statistically significant correlation between carapace width (CW) and fecundity was detected for females dwelling in the two host species (Pearson correlation test; *C. cachimilla*: $R=0.945$, $P<0.0001$; *B. odites*: $R=0.918$, $P<0.0001$). The slope of the log–log relationship between CW and fecundity was not significantly different from the three (t test; *C. cachimilla*: $t=1.379$, $df=19$, $P=0.184$; *B. odites*: $t=-1.225$, $df=12$, $P=0.244$), which means that, in the two hosts species, fecundity was isometric (Fig. 5). The ANCOVA demonstrated no significant effect of host species in crab fecundity (host species term: $F=2.55$, $df=1$, $P=0.12$) and, as expected, the ANCOVA detected an effect of crab size in fecundity ($F=184.5$, $df=1$, $P<0.0001$) (Table 1). The interaction term was not significant ($F=3.010$, $df=1$, $P=0.092$). The one-way ANOVA detected a significant difference of host size in fecundity ($F=5.870$, $df=1$, $P=0.021$) (Table 1). Therefore, host identity affects fecundity indirectly in females of *C. garthi*. Given that the average body size of female crabs was greater in *C. cachimilla*, than in *B. odites*, females from the former host produced more eggs than females from the latter host.

Reproductive output (RO) was 0.55 (±20.5, range: 0.28–0.89) and 0.44 (±0.12, range: 0.29–0.66) in females collected from *C. cachimilla* and *B. odites*, respectively. A positive, statistically significant relationship between brood weight and body weight was observed for females inhabiting the two host species (Pearson correlation test. *C. cachimilla*: $R=0.858$, $P<0.0001$; *B. odites*: $R=0.851$, $P<0.0001$). The slope of the log–log relationship between brood weight and body weight was not significantly different from unity (*C. cachimilla*: $t=1.317$, $df=19$, $P=0.203$; *B. odites*: $t=-0.316$, $df=12$, $P=0.758$), indicating that, in the two host species, female crabs increased allocation to brood mass proportionally with a unit of increase in body weight (Fig. 5). The ANCOVA showed no significant effect of host species in crab brood weight (host species term: $F=0.600$, $df=1$, $P=0.446$) (Table 1). The ANCOVA detected an effect of crab size in brood weight ($F=74.15$, $df=1$, $P<0.0001$). The interaction term was not significant ($F=1.180$, $df=1$, $P=0.286$). The one-way ANOVA demonstrated a significant difference of host size in fecundity ($F=8.850$, $df=1$, $P=0.005$) (Table 1). Therefore, host species affected brood weight indirectly in females of *C. garthi*.

Egg volume of *C. garthi* females inhabiting *C. cachimilla* and *B. odites* varied, respectively, between 0.010 and 0.019 mm^3 (0.014 ± 0.003, n = 21) and between 0.010 and 0.015 mm^3 (0.013 ± 0.002, n = 14) (Fig. 3). The correlation between egg volume and body weight of female crabs was not significant regardless of the host species from which these females were retrieved (*C. cachimilla*, Pearson correlation test: $R=0.175$, $P=0.448$, *B. odites*, $R=0.391$, $P=0.167$) (Fig. 5). The absence of a relationship between the two variables above did not allow us to conduct an ANCOVA to determine the effect of host identity on egg volume. There were no significant differences in egg volume between female crabs inhabiting the two host species (ANOVA, $F=1.16$, $df=1$, $P=0.289$).

3.3. Host preference by females of Calyptraeotheres garthi

Laboratory experiments demonstrated that females do exhibit a preference for particular limpet species and that the host species from which crabs were collected affect their host-choice behavior. At the start of the experiment, between 3 and 15 min, female crabs of *C. garthi* either remained inactive (motionless at the center of the arena) or active (explored their surroundings erratically) (Fig. 6). During this initial period, females rarely colonized limpets of the two offered host species. The few females colonizing hosts did it in numbers that did not differ significantly from an expected random distribution (binomial test: $P<0.05$ in these two observation periods). Over time, however, the erratic movement of these females

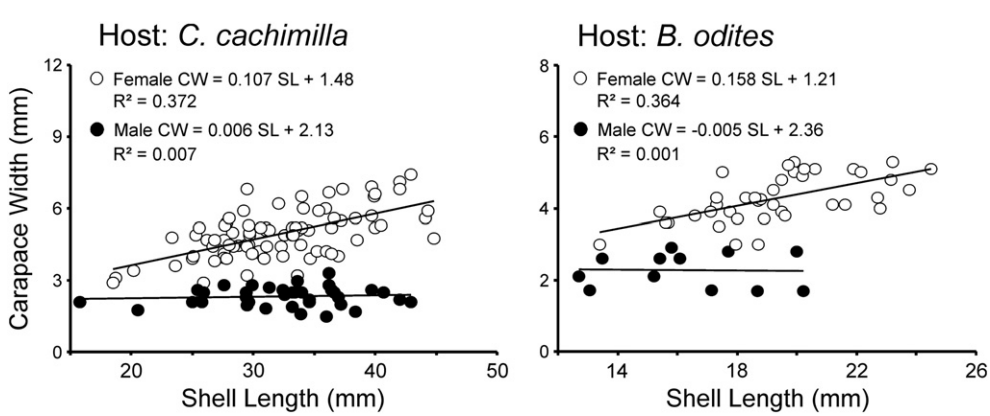

Fig. 4. Relationship between carapace width of female and male of the pinnotherid crab *Calyptraeotheres garthi* and the shell length of their respective hosts: *Crepidula cachimilla* and *Bostrycapulus odites*. The data from both sampling date (2010–2011) were pooled.

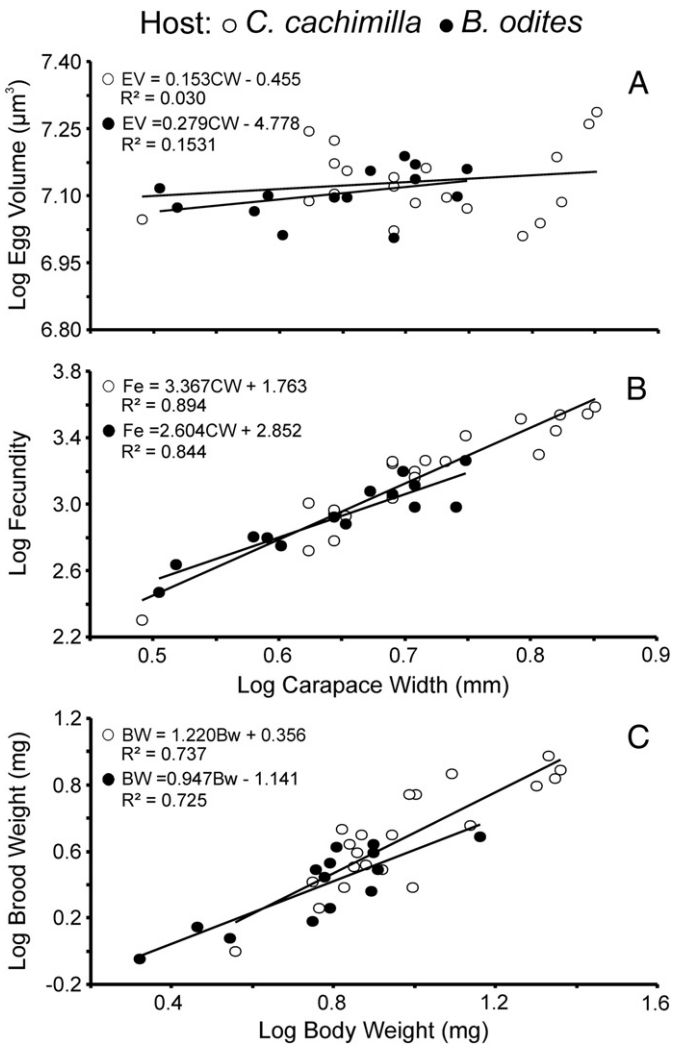

Fig. 5. Relationship among (A) egg volume (EV) and (B) fecundity (Fe) (n° embryos per female) with carapace width (CW); and (C) brood weight (BW) with body weight (Bw) of female crabs of *Calyptraeotheres garthi* sourced from *Crepidula cachimilla* and *Bostrycapulus odites*. The number of crabs used is 21 ovigerous female from *C. cachimilla* and 14 ovigerous female from *B. odites*. Lineal regression equations were obtained previous log–log transformation of the data.

diminished and they started colonizing host individuals. Later during the experiment, females colonized limpets of the same species from which they had been originally collected with more frequency than expected by chance alone (binomial tests for females collected from *C. cachimilla*: $P=0.016$ and $P=0.005$ at 3 and 8 h after the start point of experiment, respectively; binomial tests for females collected from *B. odites*: $P=0.036$ and $P=0.005$, at 3 and 8 h after the start point of experiment, respectively) (Fig. 6). At the end of the experiment, crabs which were classified as colonizing hosts were observed in close contact with the limpet (besides or above of it), trying to enter into the paleal cavity of the limpets, or, in occasions, already within the paleal cavity of the limpets. These last observations confirm the notion that adult crabs can re-colonize host individuals after leaving hosts either voluntarily or accidentally.

4. Discussion

4.1. Reproductive biology and behavioral preference of Calyptraeotheres garthi

Female crabs of *C. garthi* inhabiting the limpet *C. cachimilla* were, on average, larger and attain larger final body sizes than females inhabiting the limpet *B. odites*. Furthermore, female crabs retrieved from the former host attained greater average fecundity and brood weight (but similar egg size) than females retrieved from *B. odites*. However, when the body size of female crabs was taken into account (ANCOVAs, see Results section), no differences in size-specific fecundity, brood weight and egg size were found between females inhabiting the two host species. We interpret these results as limpet species affecting the reproductive biology of the female symbiotic crabs indirectly, rather than directly, via its effect on growth rate and body size of the associated crabs, as discussed below.

There is often a strong correlation between host size and pea crab size (Haines et al., 1994; McLay, 1988; Miller et al., 2008; Pregenzer, 1978; Tablado and López Gappa, 1995) and space availability within host individuals appears to be most relevant in constraining growth rate and in determining the average and final body size of their symbiotic crabs (e.g., in the pea crab *N. novaezelandiae* — Miller et al., 2008; in other crustaceans: hermit crabs — Bertness, 1981a; parasite isopods — Tsai et al., 2001). A strong indication of space constraints within hosts for crab growth (and also for long-term host fidelity, see below) in females of *C. garthi* is the close correlation between female crab and host body size found in this study. If female crabs of *C. garthi* would not spend long periods of time within the same host individual and if their growth rate would not be limited by the host individual, then the positive correlation between female crab and host body size herein observed would be difficult to explain. The information above suggests that host individuals and the pea crabs they harbor grow together and that the body size attained by symbiotic crabs within a host individual appears to be directly driven by host body size. Importantly, various species of pea crabs appear to depend strongly from their hosts not only for refuge but also for nutrition (Kruczynski, 1975; McLay, 1988; Telford, 1982), and *C. garthi* does not appear to be the exception. Laboratory observations have shown that female crabs of *C. garthi* feed directly from mucus strings rich on filtered phytoplankton on the gills of the two studied host species (Ocampo, unpublished results). A similar feeding behavior has been observed before in other congeneric species (e.g., *Calyptraeotheres* sp. — Chaparro et al., 2001), in other pea crabs/host systems (*T. maculatus* — Kruczynski, 1975; *Dissodactylus* spp. — Telford, 1982; *Nepinnotheres novaezelandie* — McLay, 1988) and in other symbiotic crustaceans (*Trapezia ferruginea* — Adams et al., 1985). Overall, the laboratory and field information above suggests that space constraints, food availability within hosts or both limit growth and body size of symbionts. Given that

Table 1
Result of ANCOVA and ANOVA of the comparison of reproductive parameters of the pinnotherid crab *Calyptraeotheres garthi* sourced from the limpet hosts *C. cachimilla* and *B. odites*. See Results section for further details.

Reproductive parameters	*SS*	*df*	*MS*	*F*	*P*
ANCOVA					
Fecundity					
Carapace width (covariate)	1.872	1	1.872	184.5	< 0.001*
Slope (interaction)	0.030	1	0.030	3.010	0.092
Elevation (host species term)	0.026	1	0.026	2.550	0.120
Residual	0.314	31	0.010		
Brood weight					
Body weight (covariate)	1.673	1	1.673	74.150	< 0.001*
Slope (interaction)	0.027	1	0.027	1.180	0.286
Elevation (host species term)	0.013	1	0.013	0.600	0.446
Residual	0.699	31	0.022		
ANOVA					
Fecundity	0.476	1	0.476	5.870	0.021*
Residuals	2.674	33	0.081		
Brood weight	0.705	1	0.705	8.850	0.005*
Residuals	2.628	33	0.079		
Egg volume	0.006	1	0.006	1.160	0.289
Residuals	0.163	33	0.005		

Significant *P* values ($P<0.05$) are marked by asterisks.

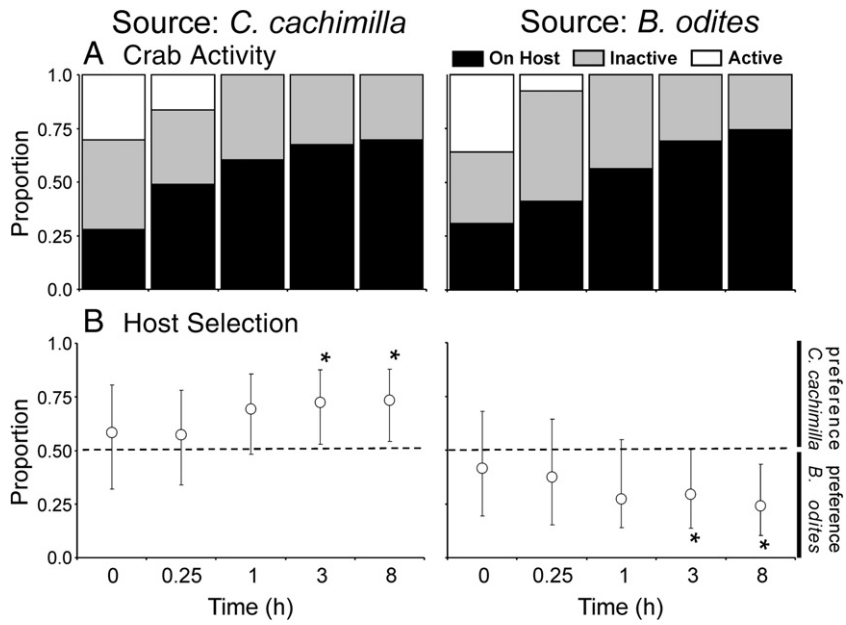

Fig. 6. Preference of female crabs of *Calyptraeotheres garthi* tested by selection experiments in which crabs sourced from *Crepidula cachimilla* and *Bostrycapulus odites* were offered a choice between those two hosts. (A) Female crab activity through time in selection experiment. (B) Proportion of female crabs that prefer to colonize *C. cachimilla* or *B. odites*. See Results section for further details. * indicates significant differences ($P<0.05$) between the observed distribution of crab on the host and the expected binomial random distribution.

C. cachimilla reaches almost twice the size of *B. odites*, female crabs inhabiting the former host attain larger average and final body sizes. Given the strong correlation between crab body size and fecundity and brood weight shown in this study, crabs growing in *C. cachimilla* most probably have greater lifetime fecundity and reproductive output than conspecific crabs inhabiting *B. odites*.

Taking into account theoretical considerations, we expected that females of *C. garthi* showed active behavioral preferences for *C. cachimilla* than for *B. odites*. Given a choice between the two studied host limpets, female crabs colonizing and remaining for long periods of time in *C. cachimilla* are expected to attain larger body sizes and augment their fecundity and reproductive output in comparison to females colonizing *B. odites*. At a first glance, the disproportionately high occurrence of *C. garthi* in *C. cachimilla* compared to *B. odites* suggests that *C. garthi* indeed naturally prefers to inhabit the most beneficial host (*C. cachimilla*). The natural abundance of *B. odites* is greater than that of *C. cachimilla* at the study site (Ocampo, unpublished results). Thus, this higher frequency of occurrence in the least common host species does appear to be a strong signal for preference of *C. garthi* for the host that offers the greatest benefits. However, in contrast to our expectations, we did not observe the predicted host preference during laboratory experiments in which we controlled for host source and host offer. Given a choice between two host species, female crabs chose hosts from where they were retrieved with higher frequency than expected by chance alone. A similar effect of source host on host selection behavior has been demonstrated before in other crustaceans, including pinnotherids crabs (Pinnotheridae: *T. maculatus* — Derby and Atema, 1980, *D. mellitae* — Gray et al., 1968, *P. atrinicola* and *N. novaezelandiae* — Stevens, 1990a, non Pinnotheridae: *Allopetrolistes spinifrons* — Baeza and Stotz, 2003). What mechanism might be controlling the host selection behavior of *C. garthi*?

The fidelity herein reported by females of the pea crab *C. garthi* for their source host might reflect the existence of two host races in *C. garthi* with individuals from each host having different host preferences. In *N. novaezelandiae*, population genetic analyses have demonstrated the existence of two host races, each restricted to one out of two host species (Stevens, 1990a). Crabs from the different host races show a strong preference for their own host (Stevens, 1990b). The existence of host populations that are genetically differentiated and specialized in a particular host has been demonstrated before in several other terrestrial insect–host and marine crustacean–host symbiotic interrelationships (Bush and Smith, 1998; Jaenike, 1990; Sotka, 2005). Interestingly, in *C. garthi*, a preliminary population genetics study using the mitochondrial marker cytochrome oxidase I (COI) has failed to demonstrate genetic differentiation among crabs retrieved from *C. cachimilla* and *B. odites* (Ocampo, unpublished results). This preliminary result argues against the notion of genetic determination of host selection behavior in *C. garthi*.

Alternatively, preference of *C. garthi* females for host species that have been previously inhabited might be explained by the phenomenon of habituation. Symbiotic crustaceans, including pea crabs, are known to use chemicals and/or visual cues to detect and choose among different host species (Brooks and Rittschof, 1995; Davenport et al., 1960; Derby and Atema, 1980; Guo et al., 1996; Stevens, 1990a). After colonization, host cues might be imprinted in symbiotic individuals resulting in the conditioning of future host choices (De Bruyn et al., 2010; Derby and Atema, 1980). Imprinting, however, is not irreversible. In *T. maculatus* and *D. mellitae*, crabs extracted from a host species and forced to live in another species develop an affinity for the new host species (Derby and Atema, 1980; Gray et al., 1968). Particularly interesting is the case of *D. primitivus*, for which juveniles do not display any original preference for the host species in which they naturally occur. However, adults living in a particular host species for a relatively long period of time develop a preference and are attracted to that particular host species (De Bruyn et al., 2010). In the case of *C. garthi*, crabs originally colonizing and living within *C. cachimilla* or *B. odites* in the field may have become chemically conditioned to their hosts. These cues might be imprinted in crabs during their early life (i.e. when megalopae or first crabs colonize a host individual for the first time) and continue driving their choices for hosts of different species during their later life. Given the lack of genetic differences (mitochondrial COI) between crabs from *C. cachimilla* and *B. odites* (see above) we believe that habituation most probably explains the host selection behavior of *C. garthi*. Additional host selection experiments in which crabs are forced to live in a novel host, or alternatively, using crabs in early stages of development (megalopae or first

decapodites that have not been in contact with hosts), accompanied with population genetic analysis using more sensitive molecular markers (e.g. micro satellites) are perhaps the necessary step to clarify this matter. As for now, it is clear that female crabs of *C. garthi* prefer to return to their original host species rather than to choose a novel host species given the opportunity, regardless of the benefits that each host species might offer.

4.2. Mating system in Calyptraeotheres garthi

Females of *C. garthi* most commonly lead a solitary lifestyle in the paleal cavity of the two studied host limpets, *C. cachimilla* and *B. odites*. A solitary lifestyle has been reported before for various other symbiotic crustaceans, including other members of the family Pinnotheridae (*Zaops ostreoum* — Møller Christensen and McDermott, 1958; *T. maculatus* — Pearce 1964, — Tablado and López Gappa, 1995; *Pinnotheres pisum* — Seed, 1969; *Arcotheres sinensis* — Asama and Kosaku, 2002) and other crustaceans (the porcelain crab *A. spinifrons* Baeza et al., 2001, the caridean shrimp *Ascidonia flavomaculata* — Baeza and Díaz-Valdés, 2011). Agonistic behavior among conspecifics to secure host individuals has been demonstrated before for other symbiotic crustaceans with solitary adult lifestyles (e.g., the porcelain crab *A. spinifrons*: Baeza et al., 2002; the spider crab *Inachus phalangium*: Wirtz and Diesel, 1983). Thus, females of *C. garthi* might feature a territorial behavior defending their host individuals against conspecific intruders, as suggested before for another pinnoterid crab that inhabits their hosts solitarily (e.g., *P. pisum* in *M. edulis* — Haines et al., 1994). Controlled field or laboratory experiments are needed to examine whether females of *C. garthi* are territorial as well as to reveal the details of the ontogenetic development of such territorial tactics that might start early in life, short after settlement and host initial colonization, given that small non reproductive females were also found inhabiting host individuals solitarily. The solitary lifestyle of *C. garthi* might be the function of host traits, in particular, host small body size (relative to crab size), and the host low structural complexity. Such host characteristics have been suggested before to favor territoriality and a solitary lifestyle in symbiotic species (Baeza and Thiel, 2003).

The solitary lifestyle of females of *C. garthi* in the two species of limpets most probably is due to agonistic behavior that expresses early during their symbiotic life. Males of *C. garthi* were also found either solitarily or sharing a host with a reproductive female most of the time. Heterosexual pairing is not uncommon in symbiotic marine invertebrates and male–female pairs represent the most frequent "social unit" in some symbiotic crustaceans, including other pinnotherid crabs (Pinnotheridae: *P. transversalis* — Baeza, 1999; *Pinnixa chaetopterana* — McDermott, 2005) and in non Pinnotheridae (*Pontonia margarita* — Baeza, 2008; *Pontonia mexicana* — Baeza et al., 2011). At a first glance, the frequent pairing of males and females of *C. garthi* within host limpets suggests a monogamous mating system for this species (monogamy here defined sensu Wickler and Seibt (1981) as pairs of conspecifics spending extensive periods of time together). Nonetheless, there are at least two lines of evidence in our data suggesting that the mating system of *C. garthi* is not monogamous but rather promiscuous. First, in contrast to that reported for monogamous symbiotic crustaceans, male–female pairs of *C. garthi* were not observed inhabiting their host individuals with more frequency than the expected by chance alone. If males and females of *C. garthi* shared host individuals for a long period of time, we would have expected male–female pairs to be the most common "social unit" in this species, as reported before for monogamous symbiotic crustaceans (*P. transversalis* — Baeza, 1999, the shrimp *P. margarita* — Baeza, 2008). Indeed, the high frequency of solitary individuals, both males and females, fits with that reported for non monogamous symbiotic crustaceans in which pairing last a short period of time and in which males abandon host–resident females soon after insemination in search of additional mating opportunities (Diesel, 1988; Knowlton, 1980).

A second line of reasoning suggesting that *C. garthi* is not monogamous but promiscuous is the absence of a relationship between the body size of host individuals and male symbiotic crabs. If males were staying for long periods of time in the same host individual with the same female, as it would be expected for a monogamous species, both host and symbiotic crab would have grown together, and in turn, such synchronous growth pattern would have determined a close correlation between host and symbiotic crab and between males and females establishing putatively long-term pairs. Such tight correlations between hosts and associates and between males and females have been reported before for other symbiotic crustaceans that appear to establish themselves as long term male–female pairs within a single host individual (e.g., *P. transversalis* — Baeza, 1999; *P. margarita* — Baeza, 2008). Indeed, the absence of a correlation between host and male crab body size argues in favor of males roaming among host individuals in search of sedentary receptive females, as demonstrated before for other symbiotic crustaceans in which males appear to play a promiscuous mating strategy (e.g., *Athanas kominatoensis* — Nakashima, 1987; see also Baeza and Díaz-Valdés, 2011). This last observation does not agree with what would have been expected for monogamous males. Moreover, the positive correlation between female crab body size and host body size suggests female fidelity for its host, at least to an extent greater than that of the male (see above).

Lastly, males of *C. garthi* have been observed in the laboratory to leave their host individuals and to re-enter them later or to colonize another limpet different from that originally harboring these males (Ocampo, unpublished results). These observations additionally argue in favor of the propensity of males of *C. garthi* to leave hosts.

In general, the observations above support the idea that *C. garthi* is not a monogamous species and further suggest that males of *C. garthi* might be frequently roaming among hosts in search of receptive females. Such male mating strategy might explain why the sex ratio was biased toward females during our study. Males roaming among hosts might suffer greater mortality by predation than do sedentary females (see Baeza and Díaz-Valdés, 2011). Sex specific differences in mortality rates by predators, in turn driven by the greater propensity of males (compared to females) to switch among host individuals could lead to the observed sex ratio bias towards females during the study period.

Unfortunately, our observations do not allow further conclusions about other details of the reproductive behavior of *C. garthi*. For instance, we do not know if males abandon females immediately after insemination, or if females are guarded by males for a relatively short period of time after copulation. Laboratory experiments on the movement pattern of crabs from the two sexes might help in revealing more details about the mating system of *C. garthi*. The existence of different morphotypes in *C. garthi* also needs to be addressed in future studies. In a few species of pinnoterid crabs, both males and females experience considerable morphological changes during ontogeny (Jones, 1977; Møller Christensen and McDermott, 1958; Pearce, 1964). Short after settlement and colonization of their first host individual, both males and females adopt a swimming morphology characterized by a hard and flat carapace and robust appendages covered with swimming setae (Jones, 1977; Møller Christensen and McDermott, 1958). Later, after re-colonization of the same or another host individual, females but not males grow larger, their carapace turns soft, and their legs become rudimentary. Thus, detailed observations on the morphology of the two sexes during ontogeny should help to further advance our understanding of the mating system of this cryptic symbiotic species.

Acknowledgments

We are grateful to Dr. Enrique Morsan and colleagues of the IBMP for their hospitality during the travels to San Matías Gulf. We also

thank fisherman Mr. Samuel Silva for helping us in collecting *C. cachimilla*. Sincere thanks are extended to two anonymous reviewers for their suggestions and criticism that clearly improved the manuscript. The present work was partially supported by the project EXA 453/09 of Universidad Nacional de Mar del Plata. J.A.B. is most grateful to Tomas A. Luppi who made possible his visit to Argentina in 2009 and this collaboration. Additional support to E.O. was provided through a doctoral scholarship funded by CONICET—Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina. The present results are part of the PhD dissertation of E.O. This is the contribution number 885 of the Smithsonian Marine Station at Fort Pierce. [**SS**]

References

Adams, J., Edwards, A.J., Emberton, H., 1985. Sexual size dimorphism and assortative mating in the obligate coral commensal *Trapezia ferruginea* Latreille (Decapoda, Xanthidae). Crustaceana 48, 188–194.

Alves, E., Pezzuto, P.R., 1998. Population dynamics of *Pinnixa patagoniensis* Rathbun 1918 (Brachyura: Pinnotheridae) a symbiotic crab of *Sergio mirim* (Thalassinidea: Callianassidae) in Cassino Beach, Southern Brazil. Mar. Ecol. 19, 37–51.

Asama, H., Kosaku, Y., 2002. Life history of the pea crab, *Arcotheres sinensis*, in terms of infestation in the bivalve mollusk, *Septifer virgatus*. J. Mar. Biol. Assoc. U. K. 2, 1–5.

Baeza, J.A., 1999. Indicators of monogamy in the commensal crab *Pinnixa transversalis* (Milne Edwards & Lucas) (Decapoda: Brachyura: Pinnoteridae): population distribution, male–female association and sexual dimorphism (in Spanish). Rev. Biol. Mar. Oceanogr. 34, 303–313.

Baeza, J.A., 2008. Social monogamy in the shrimp *Pontonia margarita*, a symbiont of *Pinctada mazatlantica*, in the tropical eastern Pacific coast. Mar. Biol. 153, 387–395.

Baeza, J.A., Díaz-Valdés, M., 2011. The symbiotic shrimp *Ascidonia flavomaculata* lives solitarily in the tunicate *Ascidia mentula*; implications for its mating system. Invert. Biol. 130, 351–361.

Baeza, J.A., Stotz, W., 2003. Host-use and selection of differently-colored sea anemones by the symbiotic crab *Allopetrolisthes spinifrons*. J. Exp. Mar. Biol. Ecol. 284, 25–39.

Baeza, J.A., Thiel, M., 2003. Predicting territorial behaviour in symbiotic crabs using host characteristics: a comparative study and proposal of a model. Mar. Biol. 142, 93–100.

Baeza, J.A., Thiel, M., Stotz, W.B., 2001. The life history of *Allopetrolisthes spinifrons*, a crab associate of the sea anemone *Phymactis clematis*. J. Mar. Biol. Assoc. U. K. 81, 69–76.

Baeza, J.A., Stotz, W., Thiel, M., 2002. The agonistic behaviour and development of territoriality during ontogeny of the sea anemone dwelling crab *Allopetrolisthes spinifrons*. Mar. Freshw. Behav. Physiol. 35, 189–202.

Baeza, J.A., Bolaños, J.A., Hernandez, J.E., Lira, C., López, R., 2011. Monogamy does not last long in *Pontonia mexicana*, a symbiotic shrimp of the Amber pen-shell *Pinna carnea* from the southeastern Caribbean Sea. J. Exp. Mar. Biol. Ecol. 407, 41–47.

Bell, J.L., 1984. Changing residence: dynamics of the symbiotic relationship between *Dyssodactylus mellitae* Rathbun (Pinnotheridae) and *Mellita quinquiesperforata* (leske) (Echinodermata). J. Exp. Mar. Biol. Ecol. 82, 101–115.

Bergström, A., Janz, N., Nylin, S., 2006. Putting more eggs in the best basket: clutch-size regulation in the comma butterfly. Ecol. Entomol. 31, 255–260.

Bertness, M.D., 1980. Shell preference and utilization patterns in littoral hermit crabs of the bay of Panama. J. Exp. Mar. Biol. Ecol. 48, 1–16.

Bertness, M.D., 1981a. The influence of shell-type on hermit crab growth rate and clutch size (Decapoda, Anomura). Crustaceana 40, 197–205.

Bertness, M.D., 1981b. Predation, physical stress, and the organization of a tropical rocky intertidal hermit crab community. Ecology 62, 411–425.

Bierbaum, R.M., Ferson, S., 1986. Do symbiotic pea crabs decrease growth rate in mussels? Biol. Bull. 170, 51–61.

Brooks, W.R., Rittschof, D., 1995. Chemical detection and host selection by the symbiotic crab *Porcellana sayana*. Invertebr. Biol. 114, 180–185.

Bush, G.L., Smith, J.J., 1998. The genetics and ecology of sympatric speciation: a case study. Res. Popul. Ecol. 40, 175–187.

Campos, E., 1999. Inclusion of the austral species *Pinnotheres politus* (Smith, 1869) and *Pinnotheres garthi* Fennuci, 1975 within the genus *Calyptraeotheres* Campos, 1990 (Crustacea: Brachyura: Pinnotheridae). Proc. Biol. Soc. Wash. 112, 536–540.

Chaparro, O.R., Saldivia, C.L., Paschke, K.A., 2001. Regulatory aspects of the brood capacity of *Crepidula fecunda*, Gallardo 1979 (Gastropoda: Calyptraeidae). J. Exp. Mar. Biol. Ecol. 266, 97–108.

Christy, J.H., 1987. Competitive mating, mate choice and mating associations of brachyuran crabs. Bull. Mar. Sci. 41, 177–191.

Clarke, A., Hopkins, C.C., Nilssen, E.M., 1991. Egg size and reproductive output in the deep water prawn *Pandalus borealis* Krøyer, 1838. Funct. Ecol. 5, 724–730.

Cledón, M., Simone, L.R., Penchaszadeh, P.E., 2004. *Crepidula cachimilla* (Mollusca: Gastropoda): a new species from Patagonia, Argentina. Malacologia 46, 185–202.

Davenport, D., Camougis, G., Hickok, I.F., 1960. Analysis of the behaviour of commensals in host-factor. 1. A hesioned polychaete and a pinnotherid crab. Anim. Behav. 8, 209–218.

De Bary, A., 1879. Die Erscheinung der Symbios (The phenomenon of symbiosis). Privately printed in Strasburg.

De Bruyn, C., Rigaud, T., David, B., De Ridder, C., 2009. Symbiosis between the pea crab *Dissodactylus primitivus* and its echinoid host *Meoma ventricosa*: potential consequences for the crab mating system. Mar. Ecol. Prog. Ser. 375, 173–183.

De Bruyn, C., David, B., De Ridder, C., Rigaud, T., 2010. Asymmetric exploitation of two echinoid host species by a parasitic pea crab and its consequences for the parasitic life cycle. Mar. Ecol. Prog. Ser. 398, 183–191.

De Grave, S., Pentcheff, N.D., Ahyong, S.T., et al., 2009. A classification of living and fossil genera of Decapod crustaceans. Raffles Bull. Zool. 21, 1–109.

Derby, C.D., Atema, J., 1980. Induced host odor attraction in the pea crab *Pinnotheres maculatus*. Biol. Bull. 158, 26–33.

Diesel, R., 1988. Male–female association in the spider crab *Inachus phalangium*: the influence of female reproductive stage and size. J. Crustacean Biol. 8, 63–69.

El-Kareem Ismail, T.G., 2010. Distribution and shell selection by two hermit crabs in different habitats on Egyptian Red Sea Coast. J. Exp. Mar. Biol. Ecol. 36, 314–324.

Elliot, J.M., 1983. Some methods for the statistical analysis of samples of benthic invertebrates, Freshwater Biological Association, Scientific Publication n 25, 3rd Edition.

Gabel, F., Garcia, X.F., Brauns, M., Sukhodolov, A., Leszinski, M., Pusch, M.T., 2008. Resistance to ship-induced waves of benthic invertebrates in various littoral habitats. Freshw. Biol. 53, 1567–1578.

Garcia, R.B., Mantelatto, F.L.M., 2001. Shell selection by the tropical hermit crab *Calcinus tibicen* (Herbst, 1791) (Anomura, Diogenidae) from Southern Brazil. J. Exp. Mar. Biol. Ecol. 265, 1–14.

Gosselin, L.A., Chia, F.S., 1995. Distribution and dispersal of early juvenile snails: effectiveness of intertidal microhabitats as refuges and food sources. Mar. Ecol. Prog. Ser. 128, 213–223.

Gray, I.E., McCloskey, L.R., Weihe, S.C., 1968. The commensal crab *Dissodactylus mellitae* and it reaction to sand dollar host factor. J. Elisha Mitchell Sci. Soc. 84, 472–481.

Guo, C.C., Hwang, J.S., Fautin, D.G., 1996. Host selection by shrimps symbiotic with sea anemones: a field survey and experimental laboratory analysis. J. Exp. Mar. Biol. Ecol. 202, 165–176.

Haines, C.M.C., Edmunds, M., Pewsey, A.R., 1994. The pea crab, *Pinnotheres pisum* (Linnaeus, 1767), and its association with the common mussel, *Mytilus edulis* (Linnaeus, 1758), in the Solent (UK). J. Shellfish. Res. 13, 5–10.

Hamel, J.F., Ng, P.K.L., Mercier, A., 1999. Life cycle of the pea crab *Pinnotheres halingi* sp. nov., an obligate symbiont of the sea cucumber *Holothuria scahra* Jaeger. Ophelia 50, 149–175.

Hartnoll, R.G., 1978. The determination of relative growth in crustacean. Crustaceana 34, 281–292.

Hartnoll, R.G., 1982. Growth. In: Abele, L.G. (Ed.), The Biology of Crustacea 2. Academic, New York, pp. 111–196.

Hernáez, P., Palma, S., Wehrtmann, I.S., 2008. Egg production of the burrowing shrimp *Callichirus seilacheri* (Bott 1955) (Decapoda, Callianassidae) in northern Chile. Helgol. Mar. Res. 62, 351–356.

Hines, A.H., 1992. Constraint on reproductive output in brachyuran crabs: pinnotherids test the rule. Am. Zool. 32, 503–511.

Jaenike, J., 1978. On optimal oviposition behaviour by phytophagous insects. Theor. Popul. Biol. 14, 350–356.

Jaenike, J., 1990. Host specialization in phytophagous insects. Annu. Rev. Ecol. Syst. 21, 243–273.

Jones, J.B., 1977. Post-planktonic stages of *pinnotheres novaezelandiae* filhol, 1886 (Brachyura: Pinnotheridae). J. Mar. Freshw. Res. 11, 145–158.

Knowlton, N., 1980. Sexual selection and dimorphism in two demes of a symbiotic, pair-bonding snapping shrimp. Evolution 34, 161–173.

Kolbe, J.J., Janzen, F.J., 2002. Impact of nest-site selection on nest success and nest temperature in natural and disturbed habitats. Ecology 83, 269–281.

Krebs, J.R., Davies, N.B., 1993. An Introduction to Behavioural Ecology. Wiley-Blackwell.

Kruczynski, W.L., 1973. Distribution and abundance of *Pinnotheres maculatus* Say in Bogue Sound, North Carolina. Biol. Bull. 145, 482–491.

Kruczynski, W.L., 1975. A radioactive tracer study of food uptake by *Pinnotheres maculatus* in molluscan hosts. Biol. Bull. 148, 60–67.

Lardies, M.A., Castilla, J.C., 2001. Latitudinal variation in the reproductive biology of the commensal crab *Pinnaxodes chilensis* (Decapoda: Pinnotheridae) along the Chilean coast. Mar. Biol. 139, 1125–1133.

Levins, R., 1968. Evolution in Changing Environments. Princeton Univ. Press, Princeton, NJ.

Levins, R., MacArthur, R., 1969. An hypothesis to explain the incidence of monophagy. Ecology 50, 910–911.

Martín, J., López, P., Cooper, W.E., 2003. Loss of mating opportunities influences refuge use in the Iberian rock lizard, *Lacerta monticola*. Behav. Ecol. Sociobiol. 54, 505–510.

Maynard-Smith, J., 1974. Models in Ecology. Cambridge University Press.

McDermott, J.J., 1962. The incidence and host–parasite relations of pinnotherid crabs (Decapoda, Pinnotheridae). Coast. Shall. Wat. Res. Conf., 1, pp. 162–164.

McDermott, J.J., 2005. Biology of the brachyuran crab *Pinnixa chaetopterana* Stimpson (Decapoda: Pinnotheridae) symbiotic with tubicolous polychaetes along the Atlantic coast of the United States, with additional notes on other polychaete association. Proc. Biol. Soc. Wash. 118, 742–764.

McLaughlin, P.A., 1980. Comparative Morphology of Recent Crustacea. W.H. Freeman and Co., San Francisco.

McLay, C.L., 1988. Brachyura and crab-like anomura of New Zealand. Leigh Laboratory Bulletin, 22. University of Auckland. 463 pp.

McNair, J.N., 1986. The effects of refuges on predator–prey interactions: a reconsideration. Theor. Popul. Biol. 29, 38–63.

Miller, A., Inglis, G.J., Poulin, R., 2008. Use of the introduced bivalve, *Musculista senhousia*, by generalist parasites of native New Zealand bivalves. N.Z. J. Mar. Freshw. Res. 42, 143–151.

Møller Christensen, A.M., McDermott, J.J., 1958. Life-history and biology of the oyster crab, *Pinnotheres ostreum* Say. Biol. Bull. 114, 146–179.

Nakashima, Y., 1987. Reproductive strategies in a partially protandrous shrimp, *Athanas kominatoensis* (Decapoda: Alpheidae): sex change as the best of a bad situation for subordinates. J. Ethol. 5, 145–159.
Ocampo, E.H., Nuñez, J.D., Cledón, M., Robles, R., 2012. New record of calyptraeid hosts for the pea crab Calyptraeotheres garthi (Fenucci, 1975) (Decapoda, Pinnotheridae) in Argentina. Crustaceana. http://dx.doi.org/10.1163/156854012X651682.
Pearce, J.B., 1964. On reproduction in *Pinnotheres maculatus* (Decapoda: Pinnotheridae). Biol. Bull. 127, 384.
Pearce, J.B., 1966. The biology of the mussel crab, *Fubia subquadrata* from the water of the San Juan Archipelago, Washington. Pac. Sci. 20, 3–35.
Persson, L., Eklöv, P., 1995. Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. Ecology 76, 70–81.
Pohle, G., Telford, M., 1981. The larval development of *Dissodactylus crinitichelis* Moreira, 1901 (Brachyura: Pinnotheridae) in laboratory culture. Biol. Bull. 31, 753–773.
Pregenzer Jr., C., 1978. *Pinnotheres hickmani* (Guiler) in wild and cultured *Mytilus edulis* (mussels) in Port Phillip Bay, Victoria. Aust. J. Mar. Freshw. Res. 29, 127–139.
Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.
Ross, D.M., 1983. Symbiotic relations. In: Abele, L.G. (Ed.), The Biology of Crustacea 7. Academic Press, New York, pp. 163–212.
Schmitt, W.L., McCain, J.C., Davidson, E.S., 1973. Decapoda I, Brachyura I, family Pinnotheridae. In: Gruner, H.E., Holthuis, L.B. (Eds.), Crustaceorum Catalogus. Dr. W. Junk BV, Den Haag, The Netherlands.
Schwarzkopf, L., Alford, R.A., 1996. Desiccation and shelter-site use in a tropical amphibian: comparing toads with physical models. Funct. Ecol. 10, 193–200.
Seed, R., 1969. The incidence of the pea crab, *Pinnotheres pisum* in the two types of *Mytilus* (Mollusca: Bivalvia) from Padstow, south-west England. J. Zool. 158, 413–420.
Sokal, R.R., Rohlf, F.J., 1981. Biometry. Freeman, San Francisco.
Somers, K.M., 1991. Characterizing size-specific fecundity in crustaceans. In: Wenner, A., Kuris, A. (Eds.), Crustacean Egg Production: Crust. Issues, 1, pp. 357–378.
Sotka, E.E., 2005. Local adaptation in host use among marine invertebrates. Ecol. Lett. 8, 448–459.
Stauber, L.A., 1945. *Pinnotheres ostreum*, parasitic on the American oyster, *Ostrea* (*Gryphaea*) *virginica*. Biol. Bull. 88, 269–291.
Stevens, P.M., 1990a. Specificity of host recognition of individuals from different host races of symbiotic pea crabs (Decapoda: Pinnotheridae). J. Exp. Mar. Biol. Ecol. 143, 193–207.
Stevens, P.M., 1990b. A genetic analysis of the pea crabs (Decapoda: Pinnotheridae) of New Zealand. I. Patterns of spatial and host-associated genetic structuring in *Pinnotheres novaezelandiae* Filhol. J. Exp. Mar. Biol. Ecol. 141, 195–212.
Tablado, A., López Gappa, J., 1995. Host–parasite relationships between the Mussel, *Mytilus edulis*, and the pea crab, *Tumidotheres maculatus* (Say), in the Southwestern Atlantic. J. Shellfish. Res. 14, 417–423.
Telford, M., 1982. Echinoderm spine structure, feeding and host relationship of four species of *Dissodactylus* (Brachyura: Pinnotheridae). Bull. Mar. Sci. 32, 584–594.
Tsai, M.L., Li, J.J., Dai, C.F., 2001. How host size may constrain the evolution of parasite body size and clutch size. The parasitic isopod *Ichthyoxenus fushanensis* and its host fish, *Varicorhinus bacbatulus*, as an example. Oikos 92, 13–19.
Underwood, A.J., 1997. Experiments in Ecology. Cambridge University Press, Cambridge.
Werner, E.E., Gilliam, J.F., Hall, D.J., Mittelbach, G.G., 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology 64, 1540–1548.
Wickler, W., Seibt, U., 1981. Monogamy in crustacea and man. Z. Tierpsychol. 57, 215–234.
Wilson, D.S., 1998. Nest-site selection: microhabitat variation and its effects on the survival of turtle embryos. Ecology 79, 1884–1892.
Wilson, K., Hardy, I.C.W., 2002. Statistical analysis of sex ratios: an introduction. In: Hardy, I.C.W. (Ed.), Sex Ratios: Concepts and Research Methods. Cambridge University Press, Cambridge, pp. 48–92.
Wirtz, P., Diesel, R., 1983. The social structure of *Inachus phalangium*, a spider crab associated with the sea anemone *Anemonia sulcata*. Z. Tierpsychol. 62, 209–234.
Woodin, S.A., 1978. Refuges, disturbance, and community structure: a marine soft-bottom example. Ecology 59, 274–284.
Zar, J.H., 1999. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.