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# The symbiotic lifestyle and its evolutionary consequences: social monogamy and sex allocation in the hermaphroditic shrimp *Lysmata pederseni*

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**Abstract** Sex allocation theory predicts female-biased sex allocation for simultaneous hermaphrodites with a monogamous mating system. Mating systems theory predicts that monogamy is advantageous in environments where refuges are discrete, scarce, relatively small, and when predation risk is high outside of these refuges. These predictions were tested with the Caribbean shrimp *Lysmata pederseni*, a simultaneous hermaphrodite which has an early male phase and lives inside tubes of the sponge *Callyspongia vaginalis*. This host sponge is a scarce resource that, together with the high predation risk typical of tropical environments, should favor monogamy in the shrimp. Field observations demonstrated that shrimps were frequently encountered as pairs within these tube sponges. Pairs were equally likely to comprise two hermaphrodites or one hermaphrodite and one male. Several of these pairs were observed for long periods of time in the field. Experiments demonstrated that

hermaphrodites tolerated other hermaphrodites but not males in their host sponge. These results suggest that pairs of hermaphroditic *L. pederseni* are socially monogamous; they share the same host individual and might reproduce exclusively with their host partners for long periods of time. Nevertheless, males appeared less likely to establish long-term associations with hermaphrodites as indicated by the rate of their disappearance from their hosts (greater than that of hermaphrodites). Sex allocation was female biased in monogamous hermaphrodites. On average, hermaphrodites invested 34 times more to female than to male reproductive structures. Monogamy and female-biased sex allocation seem to be evolutionary consequences of adopting a symbiotic lifestyle in simultaneous hermaphrodites.

**Keywords** Sex allocation · Monogamy · Symbiosis · Hermaphrodite · Caribbean · Sponge · *Lysmata*

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## Introduction

Simultaneous hermaphroditism occurs in species in which individuals allocate resources to both male and female function and simultaneously reproduce as both sexes (Charnov 1982). Sex allocation theory, a robust branch of evolutionary biology, aims to understand allocation of the optimal amount of resources to male and female function in simultaneous hermaphrodites (hereafter hermaphrodites) (Klinkhamer et al. 1997; Schärer 2009). Various hypotheses have been formulated to understand the conditions driving sex allocation in hermaphrodites. Most arguments assume environmental conditions affect the relationship between investment to a specific function and the fitness resulting from that investment (Charnov 1982; Klinkhamer et al. 1997). The optimal sex allocation is that which maximizes

the sum of the fitness gains derived from both male and female investments (Charnov 1982).

Conditions thought to drive sex allocation of hermaphrodites include brooding constraints (Heath 1979), local resource competition (Maynard-Smith 1978), resource budget effects (Klinkhamer et al. 1997), and sexual selection (Baeza 2007a). Among the latter, sperm competition is considered most relevant in shaping sex allocation (Petersen 1991). Initial considerations suggested that hermaphrodites should invest up to 50% of total reproductive resources to the male function when sperm competition was intense because fitness increases concomitantly with male allocation (Charnov 1982). As eggs become available for fertilization, many hermaphrodites in the population attempt to fertilize them. Thus, individuals that produce large amounts of sperm profit from increased paternity (compared to that obtained when producing small amounts of sperm) (Baeza 2007a). In contrast, when sperm competition is weak, there is low risk of losing paternity due to the infrequent multi-male copulation behavior of female-role hermaphrodites. Thus, hermaphrodites investing large amounts of resources in sperm are wasting energy compared to hermaphrodites producing few sperm but numerous eggs (Petersen 1991). Later theoretical treatments argued that sperm displacement mechanisms were important in driving sex allocation of hermaphrodites storing sperm from mating partners. Sex allocation is expected to be female- or male-biased if sperm displacement shows diminishing returns or follows an S-shaped rule, respectively (Pen and Weissing 1999; but see Charnov 1996). Most recently, mathematical models suggest sex allocation might also be male-biased with cryptic female choice (when female-role individuals ingest sperm [Greeff and Michiels 1999] or remove a fixed amount of sperm [van Velzen et al. 2009]; see also van Velzen et al. 2009 and Michiels et al. 2009).

Considering these arguments, sex allocation should be female-biased in socially monogamous hermaphrodites that do not store sperm from mating partners (social monogamy here defined sensu Wickler and Seibt (1983) as pairs of conspecifics spending extensive periods of time together). As sperm competition is absent or weak in this mating system, the risk of losing paternity due to multi-male mating by female-role hermaphrodites is trivial (Shuster and Wade 2003; Baeza and Thiel 2007). Thus, socially monogamous hermaphrodites should produce the smallest amount of sperm necessary to fertilize their partner's eggs. The remaining energy should be invested in eggs to optimize lifetime reproductive success (Baeza 2007a). The predictions of sex allocation theory have received empirical support from various studies in non-monogamous species (e.g., Fischer 1984; Petersen 1991; Schärer and Ladurner 2003). In contrast, sex allocation has rarely been studied

among monogamous hermaphrodites (e.g., in the fish *Serranus tigrinus* [Petersen 1991], *Hypoplectrus nigricans* [Fischer 1981], and the polychaete *Ophryotrocha diadema* [Sella 1990]). Studying monogamous hermaphrodites from disparate evolutionary origins may prove most useful to test the generality of predictions fundamental to sex allocation theory.

Monogamy is widespread in animals and has evolved multiple independent times in species with or without parental care (the fish *Amphiprion ocellaris*—Fricke and Fricke 1977; the dwarf antelope *Madoqua kirkii*—Brotherton and Rhodes 1996). In species with biparental care, the benefits arising from shared parental duties explain its adaptive value (Lack 1968). In the absence of biparental care, various other arguments have been proposed to account for the adaptive significance of monogamy (e.g., “territorial cooperation” hypothesis—Wickler and Seibt 1981; “mate-guarding” hypothesis—Grafen and Ridley 1983; among others). Most recently, the “environmental constraints” hypothesis (Baeza and Thiel 2007) predicts that monogamy is advantageous in environments where refuges are small and support few (e.g., two) individuals, when these refuges are scarce and when predation risk away from refuges is high (see also Baeza 2008a). Under these circumstances, movement among refuges is constrained and their monopolization is favored due to refuge scarcity as well as the large value in offering protection against predators (Baeza and Thiel 2007). Because spatial limitation allows only a few reproductive individuals to cohabit the same refuge, both males and females (e.g., in species with separate sexes) should maximize their reproductive success by sharing “their” dwelling with a member of the opposite sex (Shuster and Wade 2003; Baeza and Thiel 2007; Baeza 2008a).

Following this logic, monogamy should be common among symbiotic invertebrates from low latitudes (symbiosis here defined sensu deBary 1865 [quoted in Vermeij 1983] as “dissimilar organisms living together”). Tropical environments are characterized by diverse but scarce biotic (e.g., sessile macro-invertebrates) or abiotic refuges (e.g., crevices) that serve as hosts to resource-specialized organisms (Baeza and Thiel 2007). Gonochoric and simultaneous hermaphroditic species from low latitudes that are symbiotic commonly form pairs (fish: *Amblygobius nocturnes*—Mazzoldi 2001; crabs: *Trapezia ferruginea*—Adams et al. 1985; shrimps: *Alpheus armatus*—Knowlton 1980; among others). Hosts used by these symbiotic species that form pairs are usually smaller than those of species that form aggregations in/on hosts (Baeza and Thiel 2007). If these monogamous symbiotic species inhabiting small and scarce refuges are simultaneous hermaphrodites, then paired individuals are expected to invest most of their resources to female gametes (Charnov 1982; Petersen 1991). There-



fore, symbiotic hermaphrodites are ideal candidates to test predictions that are fundamental for sex allocation and mating systems theories. Few studies have described mating systems of symbiotic crustaceans (Knowlton 1980; Baeza 2008a) or sex allocation of simultaneous hermaphrodites (Sella 1990; Petersen 1991; Petersen and Fischer 1996; Locher and Baur 2000; Baeza 2007a).

In this study, I determined the mating system and sex allocation of a simultaneously hermaphroditic symbiotic marine invertebrate, the shrimp *Lysmata pederseni*. First, field collections and experimental manipulations were used to describe population distribution and movement patterns, both of which are required to determine the mating system of symbiotic organisms (Baeza and Thiel 2003, 2007). Second, I tested the prediction that female-biased sex allocation occurs in monogamous mating systems.

## Materials and methods

### The study organism

*L. pederseni* (Caridea: Hippolytidae) belongs to a clade of shrimp with a peculiar sexual system: simultaneous hermaphroditism with an adolescent male phase (Bauer and Holt 1998; Bauer 2000; Baeza 2006, 2007a, 2009; Baeza et al. 2009). In these protandric simultaneous hermaphrodites (sensu Bauer and Holt 1998), juveniles invariably mature first as males bearing typical caridean male characteristics (i.e., appendix masculina on pleopod 2). Later, they become functional simultaneous hermaphrodites (Bauer 2000). After maturation, hermaphrodites resemble females of caridean gonochoric species brooding embryos under the abdomen. However, hermaphrodites retain testicular tissue, male ducts, and gonopores and can reproduce as both male and female (unpublished data). After becoming hermaphrodites, individuals probably do not revert to males and no self-fertilization has been observed (unpublished data).

### Study sites

*L. pederseni* were collected from within the tubes of the sponge *Callyspongia vaginalis* by free diving at various locations near Nargana Island, Kunayala, Panama (9°26' N, 78°34' W) or by SCUBA diving at various reefs near Long Key, Florida Keys, USA (24°49' N, 80°48' W) and Carrie Bow Cay, Belize (16°48' N, 88°04' W), between July 2007 and April 2008. At shallow depths (6–15 m) of each locality, the sea fan *Gorgonia flabellum*, the brown tube sponge *Agelas conifera*, and colonies of varying size of brain and lettuce corals *Diploria* spp. and *Agaricia* spp., respectively, were common.

Individuals of *C. vaginalis* were interspersed among other members of the community, either projecting from small coral or rocky outcrops or from the sea floor. Importantly, many of the observed host sponges were occupied by a diverse assemblage of vertebrate and invertebrates (e.g., an unidentified stomatopod shrimp, the stenopodidean shrimp *Stenopus hispidus*, and the caridean shrimps *Brachycarpus biunguiculatus* and *Synalpheus* sp.). All these sympatric associates are recognized for their developed weaponry (e.g., claws).

Several species of omnivorous/predatory fish, including various species of damselfish (*Stegastes* spp.), hamlets (*Hypoplectrus* spp.), wrasses (*Thalassoma bifasciatum* and *Halichoeres* spp.), and sit-and-wait predators (the sand diver *Synodus intermedius* and the scorpionfish *Scorpaena plumieri*) were observed at all locations.

### Collection of *L. pederseni*

At each location, *C. vaginalis* were haphazardly selected and examined for symbiotic shrimp. All shrimps found in a sponge tube were collected. Slow and gentle squeezing of each tube (from their base to the tip) forced shrimps towards the distal end where they were trapped in Ziploc bags. This procedure was repeated until at least 100 sponges were sampled at each location. After removal of the shrimp(s), the total number of tubes and the length of the largest tube of each sponge were recorded. Shrimps were transported to the laboratories of the Caribbean Coral Reef Ecosystems (CCRE), in Belize, the Keys Marine Laboratories in Florida, and the Naos Marine Laboratories, Smithsonian Tropical Research Institute (STRI) in Panama.

In the laboratory, the number of symbiotic shrimps per host was counted. Carapace length (CL, millimeter) of each shrimp was measured under a stereomicroscope to the nearest 0.168 mm. Shrimps were considered male if they had male appendages on the endopods of the second pleopods and hermaphroditic if they did not, following Baeza (2008b). Finally, hermaphroditic shrimps were classified by the presence or absence (brooding or non-brooding) and developmental stage (I, II, and III) of embryos under the abdomen. The mass of embryos was inspected under the stereomicroscope to allow classification of the embryo developmental stages by the following characteristics: stage I, embryo with uniformly distributed yolk and absence of eyes; stage II, embryo with yolk clustered and visible but not well-developed eyes; and stage III, embryo with well developed eyes, free abdomen, and thoracic appendages.

### Host-use and population distribution of *L. pederseni*

I examined whether the distribution of *L. pederseni* in *C. vaginalis* differed from a random distribution by comparing

the observed distribution with the Poisson random distribution (Elliott 1983). At each locality, a large number of sponges sampled had no shrimp so the statistical power of the chi-square goodness-of-fit tests was expected to be low. Also, shrimps were expected to be socially monogamous. Thus, to increase the power of the tests and because shrimps were predicted to occur more frequently than expected by chance as pairs within tubes (if they were socially monogamous), one-tailed tests were conducted (SAS Institute 2004). When found as pairs in the same host, the two sexual phases (males and hermaphrodites) were tested for a random distribution by comparison with the binomial distribution using the chi-square goodness-of-fit test (SAS Institute 2004).

At Carrie Bow, shrimps were frequently found in pairs within tube sponges (e.g., male–hermaphrodite or hermaphrodite–hermaphrodite pairs; see “Results”). I tested whether there was a correlation between the carapace length of paired shrimps. Importantly, in the case of hermaphrodite–hermaphrodite pairs, an a priori assignment of partners to a specific axis is not possible (Vreys and Michiels 1997). This means the product-moment correlation coefficient cannot be used to correlate traits of paired hermaphrodites. Thus, I used the intra-class correlation coefficient to measure the relationship between the studied variables as proposed by Vreys and Michiels (1997). Briefly, a one-way ANOVA was first employed with pair-number as the classification factor. Next, the intra-class correlation coefficient  $r_i$  was calculated as the proportion of the total variance explained by the variance between groups (Vreys and Michiels 1997).

#### Mark and recapture experiments

The fidelity of shrimps toward their partners and host individuals was examined at Carrie Bow Cay, Belize because shrimps were relatively common and socially monogamous at that location (see “Results”). Forty shrimps from 20 different pairs (10 hermaphrodite–male and 10 hermaphrodite–hermaphrodite pairs) naturally occurring in sponges in different small patch reefs were tagged soon after their collection (<3 h). Shrimps were tagged using an elastomer tag injected into their abdomen (Northwest Marine Technology, Inc., USA) as in Baeza (2007b, c). CL of each tagged shrimp was measured prior to their return to their host. A combination of tag colors (four in total), placement in the abdomen (left or right), and carapace length permitted individual shrimp identification and pair recognition. Two shrimps that appeared to be negatively affected by the tagging and/or sampling procedure were discarded from the experiment (together with its partner). Other than these two shrimps, all individuals used in this experiment behaved normally (crawling and swim-

ming around within containers) immediately after tagging and before/during transportation to the field. Tagged shrimps were returned the next day to their respective host individuals. In the field, the hosts were marked using PVC stacks, small plastic buoys (red-white, 4 cm in diameter) or both. PVC stacks were placed >1 m apart from the focal host. Similarly, plastic buoys were anchored to the bottom >1 m apart from the focal host and floated approximately 2 m above the tallest tube of the focal host. Presence of the shrimps in the tagged host was followed for five consecutive days immediately and 49 days after tagging and the distance from the focal host to its closest conspecific (nearest neighbor) was measured with a ruler to the nearest 1 cm at the end of the experiment.

I tested the null hypothesis of no differences in host fidelity between males and hermaphrodites. Comparisons in the time-to-disappearance of tagged shrimps from hosts between sex phases were conducted with a modified version of Cox’s maximum partial likelihood regression, a type of survival (time-to-failure) analysis (Lee et al. 1992). For each tagged shrimp, the time from the start of the experiment until disappearance was measured during a maximum period of 54 days. Individuals that remained after that time comprise right-censored data. Curves were plotted for each sex phase (treatment) using estimates of the proportion of tagged shrimp remaining in their hosts obtained with the procedure PHREG in the software SAS (SAS Institute 2004). I tested the hypothesis of differences in time-to-disappearance (time-to-failure) curves between males and hermaphrodites by testing for homogeneity of treatment (sex phase) curves with the Wald chi-square method (Allison 1995). In survival analysis, failure-time (time-to-disappearance) observations on members of the same experimental unit (e.g., two shrimps sharing the same host) may be correlated (dependent) (Lee et al. 1992). Hence, time-to-disappearance of tagged shrimp from the same host was not treated as independent. The possible dependence among failure-time observations was included in the modified Cox’s regression analysis by clustering failure-time observations of tagged shrimps from the same host individual. In addition, I employed the COVSANDWICH option of PHREG to test for homogeneity of time-to-failure because it generates robust standard errors for non-independent observations (Lee et al. 1992).

#### Host sharing and resource-holding power of shrimp

Resource (host) sharing and holding power was experimentally compared between the two sex phases in Belize. For this experiment, shrimps were collected from patch reefs around Carrie Bow Cay and transported to the laboratories of the CCRE. Shrimps were maintained in 20–40-L recirculating aquaria at 30°C, 34–35 ppt salinity,

and natural light/dark cycle of 13 h:11 h. They were fed daily with small pieces of fresh clams or food pellets (Wardley® shrimp pellets). No shrimp was used more than once during the experiments.

I tested the null hypothesis of no differences in resource (host) sharing between shrimps of the different sex phases (and body sizes, see below) while recording the behavior of shrimps “competing” for a single host individual in the laboratory. One small male and one large hermaphrodite (experiment 1) or one small hermaphrodite and one large hermaphrodite (experiment 2) were placed in the same aquarium (10 L) containing a single host *C. vaginalis*. The host sponges had a single tube and were relatively short (<15 cm tube length). The objective of using a host sponge size that was among the smallest observed in the field was to encourage agonistic and resource monopolization behaviors. After an initial acclimatization period of 30 min in 1 L containers, the two experimental shrimps were gently placed in the aquarium at matched distances from the host individual and in the vicinity (8–10 cm) of the sponge. Most commonly, shrimps moved towards the sponge soon after being placed in the aquarium. Behavioral interactions (i.e., approach, striking at opponent with maxillipeds and pereopods, retreat) between shrimps for the host individuals were noted for the first 5 min for each of the 19 and 16 replicates in experiments 1 and 2, respectively. All experiments were conducted in dim light and began in early evening (8:00–9:00 p.m.). The shrimps observed dwelling inside the tube sponge (e.g., possessing the host) the next morning (after 10–11 h of darkness) were declared the winner. The null hypothesis of no association between shrimp size/sex phase and interaction outcome (winner vs. loser) categories was tested with an exact chi-square goodness-of-fit test (SAS Institute 2004). If host resource-holding potential differs with the sex phase/size of shrimps, then individuals of one of the sex phases will monopolize their hosts more frequently than expected by chance alone.

There was no significant difference in body size (CL, millimeter) of large hermaphrodites used in the two different experiments (body size of hermaphrodites in the first and second experiments was  $9.44 \pm 2.01$  ( $N=19$ ) and  $10.03 \pm 1.66$  ( $N=16$ ), respectively;  $t$  test:  $t=0.94$ ,  $P=0.3522$ ). The body size of small males ( $6.19 \pm 0.61$ ,  $N=19$ ) and small hermaphrodites ( $8.66 \pm 1.77$ ,  $N=16$ ) was smaller than that of large hermaphrodites in each experiment (small males vs. large hermaphrodites  $t$  test:  $t=6.75$ ,  $P<0.001$ ; small vs. large hermaphrodites  $t$  test:  $t=2.26$ ,  $P=0.031$ ). However, males in the first experiment were smaller than small hermaphrodites in the second experiment ( $t$  test:  $t=5.73$ ,  $P<0.001$ ). For the second experiment, it was not possible to obtain hermaphrodites as small as the smallest males used in the first experiment given the sexual system of this species.

## Sex allocation in hermaphroditic shrimp

Male vs. female gonad mass has been used as a proxy for sex allocation in shrimp (Baeza 2007a). Although gonad biomass is relatively easy to measure, using it as a proxy for sex allocation estimates presents some problems: the energetic and temporal costs of sperm and oocytes might differ (Schärer and Robertson 1999), allocation to male or female function may take the form of investments in behaviors (i.e. mate searching—Baeza and Thiel 2007) or body structures (i.e., incubation space in brooding hermaphrodites—Heath 1979), and static biomass might not represent the overall sex-specific expenditure of an hermaphrodite during its lifetime (see Schärer 2009 and references therein). Although the accuracy of sex allocation estimates might improve considerably if investments other than gonad biomass are measured, static biomass measurements still represent the most efficient cost/benefit method. Here, I estimated the biomass of static male (testes and stored sperm) and female (ovaries) reproductive structures assuming these measurements correlate well with overall resource allocation to the male and female function, respectively.

To quantify sex allocation, I collected 15 hermaphroditic shrimps from patch reefs around Carrie Bow Cay and transported them to the laboratories of the CCRE. All were found in pairs in tube sponges, had mature ovaries, and included the range in body sizes reported for the species. In the laboratory, sperm mass, testes mass, and ovaries mass were measured. Sperm mass contained in the ejaculatory ducts (that serve as reservoirs in shrimps) was collected by applying short electric shocks (10–12 V and 1.5 A) near the male gonopores following Baeza (2006). Then, each shrimp was dissected to extract the ovarian and testicular portions of its ovotestes. Finally, the remaining shrimp body and the ovaries, sperm mass, and testes were dried for at least 48 h at 60°C in an oven, placed in centrifuge tubes, transported to the Smithsonian Marine Research Station at Fort Pierce (Florida, USA), dried again for 48 h at 70°C, and weighed to the nearest 0.01 mg on a microbalance (Sartorius Microbalance CP2P).

Reproductive allocation was estimated in four ways. Allocation to female and male function was estimated respectively as the ovaries mass and testes mass plus sperm mass in the ejaculatory ducts (stored sperm), respectively. Sex allocation was estimated as (1) the ratio of female to male allocation and (2) the quotient between male allocation and total allocation (ovaries plus testes plus stored sperm mass). These two descriptors of sex allocation above have been used by different authors (e.g., Petersen and Fischer 1996; Baeza 2007a) and represent the relative proportion of resources that individuals invest in function in each sex. Total reproductive investment was estimated as the sum of the male and female allocations.

I tested whether sex allocation varied with body size in hermaphrodites by examining the relationship between sex allocation and body dry mass of hermaphrodites. To test for size dependency of sex allocation, a *t* test was used to determine if the slope of the relationship was significantly different from zero (SAS Institute 2004). If sex allocation is size-dependent, then the slope between the two variables should be greater or smaller than zero.

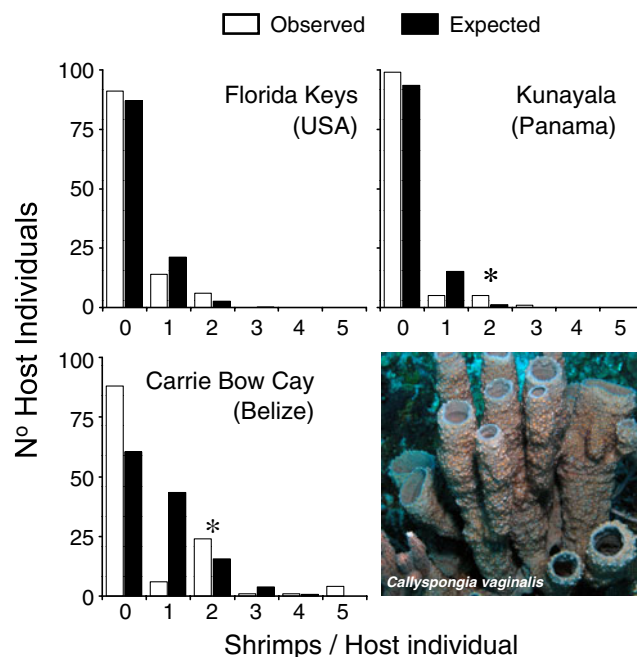
Finally, if sex allocation was found to depend on body size, I examined the contribution of male and female reproductive tissues in explaining this dependency as in Baeza (2007a). I tested whether total reproductive investment in simultaneous hermaphrodites increases linearly with body size. The relationship between total reproductive investment and body dry mass of hermaphrodites was examined using the allometric model  $y = ax^b$  (Klinkhamer et al. 1997). The slope *b* of the log-log least-squares linear regression is the rate of exponential increase ( $b > 1$ ) or decrease ( $b < 1$ ) of the total reproductive investment with shrimp dry mass (Schärer et al. 2001; Baeza 2007a). An *F* test was used to test whether the estimated slope *b* deviates from the expected slope of unity (SAS Institute 2004). If total reproductive investment per body mass neither increases nor decreases with body size in *L. pedersenii*, then the slope should not differ significantly from unity. The same approach was used to determine if the relationship between male allocation (sperm plus testes dry mass) or female allocation (ovaries dry mass) and body dry mass were all linear. Evaluations of the assumptions of normality and homogeneity of variances were checked and found to be satisfactory for each independent test.

## Results

### Host-use and population distribution of *L. pedersenii*

*L. pedersenii* occurs at very low prevalence and abundance within hosts at the three sites (see Supplementary Online Material, Table S1). At each site, solitary shrimps were found in tube sponges at low frequencies. Also, two shrimps sharing the same host were observed more often than predicted at random (Fig. 1). The maximum number of shrimps observed in the same host was five, but rarely more than two shrimps cohabited in the same sponge (Fig. 1). Only in Florida did the distribution of the shrimp within tube sponges not differ significantly from the expected Poisson frequency (one-tailed chi-square test:  $\chi_1^2 = 3.08$ ,  $P = 0.129$ ; Table S1). The relatively large number of hosts harboring two shrimps in Belize permitted description of pairing associations.

In Belize, pairs of shrimp in tube sponges usually comprised two hermaphrodites or one hermaphrodite and

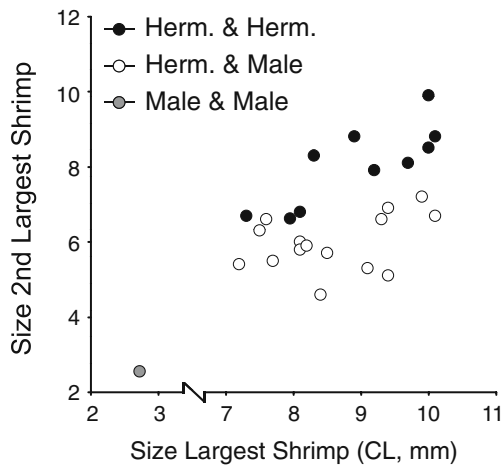


**Fig. 1** Population distribution of the shrimp *Lyasmata pedersenii*, symbiotic with the tube sponge *Callyspongia vaginalis* at different localities in the Caribbean Sea. The observed frequency of occurrence of shrimps on hosts (white bars) was compared with an expected Poisson random distribution (black bars) (see Table S1 for further details). The inset on the bottom left shows a tube sponge at Carrie Bow Key, Belize (photograph by R. Ritson-Williams)

one male ( $n = 10$  and 15 pairs, respectively). Once (out of 26 sponges harboring pairs) two male shrimps shared the same host. Size of paired shrimp was correlated in hermaphrodite–hermaphrodite pairs (intra-class correlation coefficient:  $r_i = 0.53$ ,  $F_{9, 10} = 3.22$ ,  $P = 0.041$ ; Fig. 2). On average ( $\pm$ SD), the smaller of the pair was only  $11.28 \pm 6.34\%$  (or  $0.91 \pm 0.64$  mm CL) smaller than the larger. In contrast, no significant correlation was observed for hermaphrodite–male pairs ( $F_{1, 13} = 2.07$ ,  $P = 0.174$ ; Fig. 2). Males were invariably smaller than hermaphrodites within pairs (paired *t* test:  $t_{14} = 10.75$ ,  $P < 0.001$ ). The body size of the two males comprising the only pair observed was very small ( $< 3.0$  mm CL, Fig. 2).

Most hermaphrodites in hermaphrodite–hermaphrodite and hermaphrodite–male pairs were brooding embryos (16 out of 20 and 11 out of 15 hermaphrodites, respectively). The four non-brooding hermaphrodites in hermaphrodite–hermaphrodite pairs were close to spawning as their ovaries were full of vitellogenic oocytes visible through the carapace. Similarly, three of the four hermaphrodites in hermaphrodite–male pairs were also close to spawning. The 27 brooding hermaphrodites in pairs had embryos at different stages of development (5, 11, and 11 hermaphrodites brooded embryos in stage I, II, and III, respectively). The proportion of hermaphrodites with embryos at different





**Fig. 2** Comparison between carapace length (CL, mm) of shrimps found as pairs inside host sponges of *Callyspongia vaginalis*. For hermaphrodite–hermaphrodite pairs, the largest individual within a pair was plotted on the x-axis only for visual and not for statistical purposes (see “Materials and methods” for further details)

developmental stages did not differ significantly from a random binomial distribution ( $P > 0.05$ ).

The total number of tubes and the length of the tallest tube comprising a host individual ( $N=26$ ) varied between 2 and 28 tubes with an average ( $\pm$ SD) of 12 ( $\pm 7$ ) tubes and between 28 and 80 cm with an average ( $\pm$ SD) of 55.33 ( $\pm 15.17$ ) cm, respectively. Host size (estimated as total number of tubes or length of the longest tube) was not correlated with shrimp mean size for either hermaphrodite–hermaphrodite or hermaphrodite–male pairs ( $P > 0.05$ ). However, hermaphrodite–hermaphrodite pairs inhabited sponges with more tubes than did hermaphrodite–male pairs. The number of tubes [mean  $\pm$  SD] of sponges with hermaphrodite–hermaphrodite and male–hermaphrodite pairs was  $17 \pm 7$  and  $8 \pm 4$  tubes, respectively (Kruskal–Wallis test [variances were not homoscedastic]:  $K-W=1.66$ ,  $P=0.008$ ).

#### Mark and recapture experiments

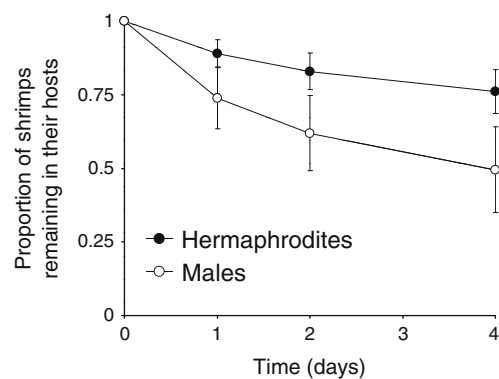
Fourteen of the 20 original pairs were observed 5 days after initiation of the experiment. Only five of the original pairs (three hermaphrodite–hermaphrodite and two hermaphrodite–male pairs) were present at the end of the experiment (after 54 days). Pairs of shrimps shared the same host for 1 to 54 days. Disappearance of shrimp from hosts depended on the availability of other sponges. The distance between a focal host (where shrimps were initially observed and tagged) and its nearest neighbor was greater for hosts in which both shrimp stayed together compared to that of hosts in which one or the two tagged shrimps disappeared, during the first and second recording periods (Kruskal–Wallis test, after 5 days:  $K-W=1.61$ ,  $P=0.011$ ; after

54 days:  $K-W=1.42$ ,  $P=0.035$ ; Fig. S1). Finally, host fidelity was greater in hermaphrodites than males (Wald test:  $\chi_1^2=5.47$ ,  $P=0.019$ ; Fig. 3).

#### Host sharing and resource-holding power of shrimp

When one small male and one large hermaphrodite were presented with a single host individual, solitary shrimps were found more often than expected by chance in the sponge after 10 h of darkness (17 out of 20 replicates,  $\chi_1^2=10.32$ ,  $N=19$ ,  $P=0.001$ ; Fig. S2). In 15 of these 17 replicates, the solitary shrimp was a large hermaphrodite. This latter frequency differed significantly from chance alone (observed vs. binomial distribution:  $\chi_1^2=8.48$ ,  $N=17$ ,  $P=0.004$ ). During this experiment, agonistic behaviors between small males and large hermaphrodites were occasionally observed. For instance, when the small male was the first to colonize the host, the large “intruder” hermaphrodite entered the host and repelled the “resident” male by repeatedly striking at its body with the third maxillipeds and/or the small (but clawed) first pereopods. Overall, agonistic interactions usually lasted only a few seconds and did not appear to cause any harm to either individual.

In contrast to the first experiment, in 11 of 16 replicates (69%) in which two hermaphrodites (one small and one large) were presented with one host individual, the two hermaphrodites were cohabiting in the same host sponge after 10 h of darkness as expected by chance alone ( $\chi_1^2=1.56$ ,  $N=16$ ,  $P=0.212$ ). It is worth noting that three of the five hermaphrodites found outside the host sponge in this second experiment molted during the night. After molting,



**Fig. 3** Host fidelity of *Lyssmata pedersenii*. Curves denote the proportion of hermaphrodites and males remaining in their hosts over time (days). For each sex phase (males and hermaphrodites), points on curves were estimated with the Cox’s maximum partial likelihood regression method using the software SAS (see “Materials and methods” for details). It is not possible to estimate the proportion of shrimps remaining in their hosts at the end of the experimental period because all data pertaining to that particular point in time are right-censored (see SAS Institute (2004) for details)



shrimps attain a soft body condition that impairs fighting and self-defense. If these three replicates are not considered in the analysis, then hermaphrodites were sharing host sponges more often than expected by chance alone ( $\chi_1^2 = 4.92$ ,  $N=13$ ,  $P=0.027$ ).

### Sex allocation in hermaphroditic shrimp

When the effect of shrimp body size was not taken into account, the ratio of female (ovaries) to male (testes plus stored sperm) dry reproductive tissue mass varied between 8.73 and 68.34 with an average ( $\pm$ SD) of 34.43 ( $\pm$ 18.55). This is much greater than 1:1 (sign test: statistic=3.61,  $P < 0.001$ ). Therefore, on average, hermaphrodites invested about 34 times more in female than male function when measured as ovaries and testes plus sperm dry mass.

Sex allocation is also size-dependent in *L. pederseni* with small hermaphrodites allocating proportionally more resources to male function compared to large hermaphrodites (Fig. 4a, b). The slope of the relationship between shrimp dry mass and sex allocation was greater than zero ( $b=0.95$ ,  $P=0.027$ ). Even though overall sex allocation is highly female-biased, smaller hermaphrodites invested a comparatively larger amount of resources to male reproduction than larger hermaphrodites.

Size-dependent sex allocation in *L. pederseni* was explained by the disproportional increase in allocation to the female function and not by the disproportional decrease in allocation to male reproductive structures with body size.

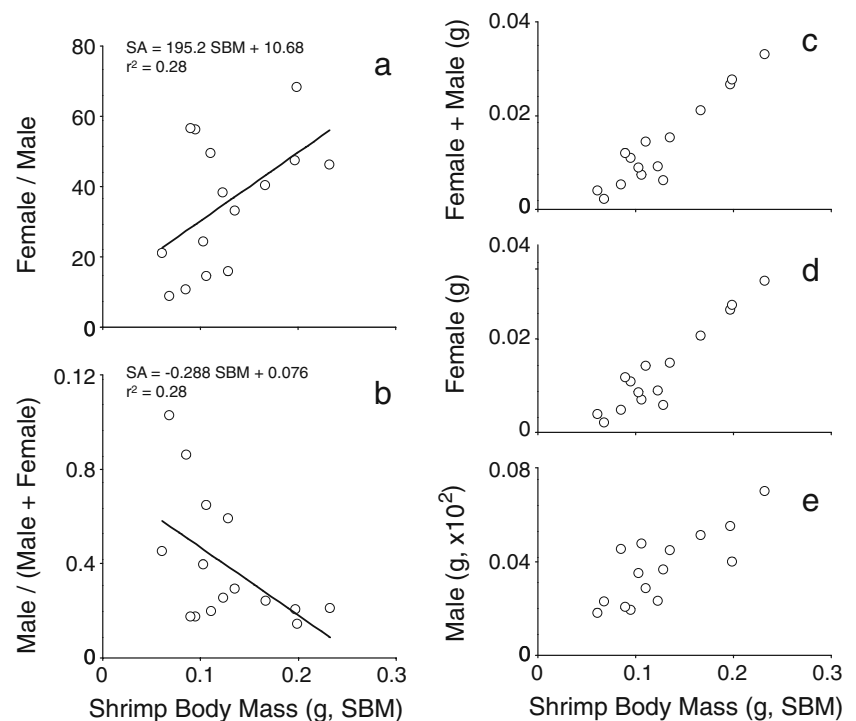
Hermaphrodites invested proportionately more to reproduction (ovaries, stored sperm, and testes dry mass) with increasing body size; the slope of the relationship between shrimp dry mass and total reproductive investment dry mass was always  $>1$  ( $b=1.73$ ,  $P=0.011$ ; Fig. 4c, Table S2). Also, hermaphrodites allocated proportionately more to female reproductive structures with increasing body size as the slope of the relationship between shrimp dry mass and ovaries dry mass was  $>1$  ( $b=1.77$ ,  $P=0.01$ ; Fig. 4d, Table S2). In contrast, hermaphrodites allocation to male reproductive structures (testes plus stored sperm) is proportional to body size ( $b=0.57$ ,  $P=0.389$ ; Fig. 4e, Table S2).

## Discussion

### Social monogamy in *L. pederseni*

*L. pederseni* was predicted to be socially monogamous given host characteristics and environmental conditions. Supporting this prediction, pairs of shrimps were found in tube sponges more frequently than expected by chance alone in two of three sites (Kunayala and Belize). The large number of tube sponges with no shrimp in Florida ( $>87\%$ ) most probably affected the likelihood of detecting real differences between observed and predicted random distributions at this site. Three lines of reasoning suggest that paired shrimps, especially hermaphrodites, remain together for long time intervals.

**Fig. 4** Reproductive measurements and their variation with body size in *Lysmata pederseni*. **a** Sex allocation depicted as the quotient between ovaries and testes plus stored sperm mass. **b** Sex allocation depicted as the quotient between testes plus store sperm mass and total allocation (ovaries plus testes plus stored sperm). **c** Total reproductive investment (male plus female allocation). **d** Female allocation. **e** Male allocation (see Table S2 for statistics and details)



First, each hermaphrodite in a pair in the same host was in a different reproductive state than the other (not brooding or brooding embryos at different stages of development). Pairing is also observed in promiscuous and polygamous species. However, male-role individuals quickly abandon female-role individuals after mating in those species (Diesel 1988; van der Meeren 1994). Thus, male-role individuals are usually found only with female-role individuals during molting and when spawning a new batch of unfertilized eggs (with late stage or no embryos but mature ovaries). These patterns of promiscuous and polygamous species contrast with those observed in *L. pedersenii*.

Second, the size relationship between paired shrimps in hermaphrodite–hermaphrodite pairs suggests each individual has a long-lasting association with its partner. If hermaphrodites frequently switched hosts and partners, the sizes of the pairs should be poorly or not correlated, as in symbiotic species in which individuals swap repeatedly among hosts (Baeza and Thiel 2000). Size-assortive pairing, on the other hand, is common among long-term monogamous symbiotic species (fish: Mazzoldi 2001; crabs: Adams et al. 1985; shrimp: Knowlton 1980; Baeza 2008a).

Lastly, mark and recapture information indicates shrimps in hermaphrodite–hermaphrodite pairs often shared the same sponge for long periods of time and certainly would have exceeded the experimental maximum of 54 days if allowed to do so. Because *L. pedersenii* hermaphrodites mate as females immediately after molting and inter-molt periods last ~10 days (unpublished results), shrimps staying together for 54 days probably mated and reproduced (maybe exclusively) with each other for six or more female reproductive cycles. So far, information suggests that hermaphrodites of *L. pedersenii* inhabit sponges with only one other hermaphrodite for long time intervals and during several reproductive cycles. Therefore, the mating system of *L. pedersenii* can be classified as social monogamy. It remains to be addressed if individuals in these socially monogamous pairs reproduce exclusively with each other during much longer periods of time (lifetime).

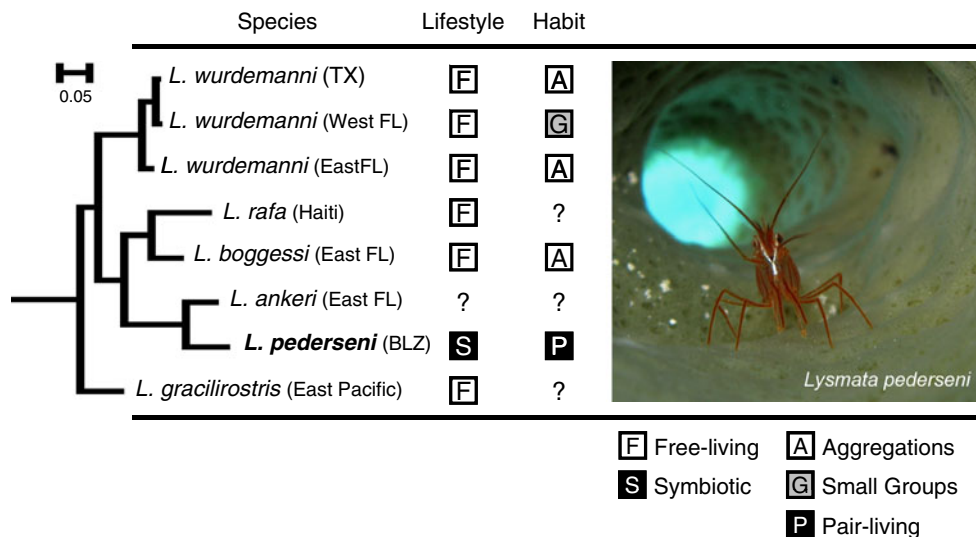
Social monogamy in *L. pedersenii* was suggested to be a function of high risk of predation away from hosts, host scarcity, and small host size (relative to symbiont size). Supporting the ideas above, omnivorous and/or predatory fishes from several families were observed during sampling at all localities. Although predation risk at the study sites was not quantified, predation is known to be relevant in tropical environments, including coral reefs, and the importance of fish as predators of small tropical marine invertebrates, including shrimp, is well established (Randall 1967). On the other hand, in partial disagreement with the ideas above, the majority of hosts did not harbor any *L. pedersenii*. This actually suggests that sponges were not

scarce (as expected) but abundant. Alternatively, the diverse assemblage of crustaceans inhabiting *C. vaginalis*, and the well-developed weaponry of most of these species (e.g., large claws that improve competitive ability), might explain the exceptionally low prevalence of *L. pedersenii* at all sites. The presence of individuals from these other symbiotic species might make hosts unsuitable (and scarce) for *L. pedersenii*. Shrimps might end up inhabiting (in pairs) the few hosts that are available and suitable. Similarly, the diverse assemblage of crustaceans inhabiting *C. vaginalis* might explain pairs of shrimps in structurally complex sponges, e.g., composed of many tall tubes. The presence of other crustaceans with developed weaponry might restrict space available within hosts to *L. pedersenii*. This “enemy pressure” might favor social monogamy by turning relatively complex and large host individuals into small and simple refuges for other symbiotic species with poorly developed weapons (e.g., *L. pedersenii*).

Overall, *L. pedersenii* is socially monogamous. But the notion that host scarcity and small size favor social monogamy in symbiotic crustaceans is only partially supported by *C. vaginalis* characteristics (e.g., many sponges contained no shrimps and several sponges with shrimps had numerous interconnected tall tubes). Clearly, the importance of inter-specific competition (expected to be high in diverse assemblages as those inhabiting tube sponges) as a condition favoring pair living in *L. pedersenii* deserves more attention. The pair living habit of *L. pedersenii* contrasts to that reported for all other free-living relatives of this species (pertaining to the Neotropical clade—Baeza 2009) that form aggregations or small groups and use relatively abundant and structurally complex crevices or space under stones as shelter (Fig. 5). Field and laboratory experiments are needed to understand the conditions favoring diversity of mating systems in shrimp from the genus *Lysmata*.

#### Alternative mating tactics in *L. pedersenii*?

Hermaphrodites of *L. pedersenii* are socially monogamous. This contrasts with less host fidelity in males during the field experiment. Alternative non-exclusive explanations for the greater rate of disappearance of males from host sponges are (1) males are less prone to form pairs, (2) hermaphrodites discharge males as partners (as observed in the laboratory), and/or (3) hermaphrodite–male pairs last shorter than hermaphrodite–hermaphrodite pairs. The fact that the size of male–hermaphrodite pairs was not correlated fits the first and/or second explanation and might also suggest that some males in the population are switching hosts more frequently than hermaphrodites. No correlation between size of individuals sharing a host is commonly reported in symbiotic species with low host fidelity (Baeza



**Fig. 5** Phylogeny and ecological diversity of the Neotropical clade of peppermint shrimps to which *Lysmata pederseni* belongs (adapted from Baeza et al. 2009). The figure shows the adoption of a symbiotic lifestyle by *L. pederseni* within a monophyletic group of free-living species that live in aggregations. Tree topology suggests that the most

common recent ancestor of this Neotropical clade was free living. Monogamy appears to be the consequence of this symbiotic lifestyle. Information on the lifestyle and habit of the different species was taken from Baeza (2009) and Baeza et al. (2009). The photograph shows the symbiotic shrimp inside a tube sponge at Carrie Bow Key, Belize

and Thiel 2000; Baeza and Thiel 2007). On the other hand, some males remain with hermaphrodites (many of them brooding embryos at different stages of development) for as long a time period as hermaphrodite pairs. This suggests the propensity of other males in the same population for long-term pairing. These conflicting results might be explained if some males are monogamous while others are promiscuous (swapping often among hosts in search of receptive female-role hermaphrodites). This latter strategy might be advantageous when sponge density is greater, as suggested by the tagging experiment. Sponge availability in the surroundings is expected to diminish costs of traveling among hosts if males search for sexual partners (Baeza and Thiel 2007). Alternative mating tactics have been described for several invertebrates, including shrimp (Correa et al. 2003). In the free-living relative *Lysmata wurdemanni*, males roam around more than hermaphrodites and are more successful in inseminating female-role hermaphrodites than hermaphrodites acting as males (Baeza 2007b, c and unpublished observations). Studies describing movement patterns of shrimp and investigation of conditions (e.g., host density) favoring or constraining host-switching behavior are needed to demonstrate variable mating tactics in this species.

In the field, *L. pederseni* pairs were equally likely to comprise two hermaphrodites or one hermaphrodite and one male. It could be argued that hermaphrodites should select other hermaphrodites but not males as long-term sexual partners. Hermaphrodites paired with other hermaphrodites would be expected to double their reproductive output (by producing embryos and inseminating “their”

sexual partner) in comparison to hermaphrodites paired with males (with no immediate opportunities to produce offspring other than their own). That most hermaphrodites tolerated other hermaphrodites but rejected males in the laboratory agrees with this idea. One possible explanation of the prevalence of hermaphrodite–male pairs in the field is limited food supply in host sponges. If food availability is insufficient to support two female-role shrimps in a single host, hermaphrodites should pair with small males instead of large hermaphrodites. That hermaphrodite–hermaphrodite pairs inhabited (larger) host sponges with more tubes than hermaphrodite–male pairs fits with this idea (e.g., limited food in small hosts). Unfortunately, little is known about the foraging ecology of *L. pederseni* and costs and benefits for symbiotic partners. Studies examining the cost and benefits experienced by these symbiotic species and the foraging behavior of *L. pederseni* are warranted to understand the conditions driving the prevalence of mating strategies in the field. Also, the importance of food availability derived from hosts in driving the mating system of symbiotic organisms deserves more attention.

#### Sex allocation in *L. pederseni*

In *L. pederseni*, sex allocation was predicted to be female-biased when shrimps are monogamous. Supporting this prediction, hermaphrodites in pairs in the field invested, on average, 34 times more to ovaries than to testes plus stored sperm. This female-biased sex allocation is greater than that of other hermaphroditic species (Sella 1990; Schärer et al.



2001) but not as extreme as that reported for the congeneric shrimp *L. wurdemanni* (e.g., hermaphrodites allocate 118 times more to female than to male function—Baeza 2007a). In *L. wurdemanni*, sperm competition is absent due to the monoandrous behavior of female-role shrimp (shrimps copulate only once and with a single other shrimp immediately after molting—Baeza 2007a, c). This complete absence of sperm competition seems to explain such extreme female-biased sex allocation (Baeza 2007a). The less strongly female-biased sex allocation in *L. pedersenii* suggests that sperm competition is more important in this species than in the free-living *L. wurdemanni* (Charnov 1982, Petersen 1991). In *L. pedersenii*, sperm competition might occur if some males are playing a promiscuous pure search mating strategy, as discussed above. Comparative studies in the genus *Lysmata* might shed light on the role of sperm competition in driving sex allocation in simultaneous hermaphrodites.

Hermaphrodites of *L. wurdemanni* do allocate more energy to the female than the male function (see Schärer 2009). However, future studies should measure other components of sex allocation in hermaphrodites, including behavioral, physiological, and morphological investments.

In *L. pedersenii*, sex allocation is also size-dependent. Shrimps invested proportionally more in ovaries than testes plus stored sperm with increasing body size. A similar shift in reproductive investment has been demonstrated in a few other simultaneous hermaphrodites (Petersen and Fischer 1996; Schärer et al. 2001; Baeza 2007a). In the symbiotic *L. pedersenii*, size-dependent sex allocation might be explained if resources are abundant for large but limited for small hermaphrodites (Klinkhamer et al. 1997). Body size seems to be important in determining resource-holding power in this species as suggested by the outcome of fights between large hermaphrodites (winners) and small males (losers) in the laboratory although an effect due only to sex phase in determining the outcome of these interactions cannot be discounted. If food supply is limited within hosts (as proposed before), conflict over food resources may exist between monogamous hermaphrodites. If this is the case, even small differences in body size (~12% as reported here) might result in one hermaphrodite monopolizing most food available within a host. Small hermaphrodites, with less access to food resources, should invest more in the cheaper (male) of the sex functions (Klinkhamer et al. 1997). Yet, because little is known about the foraging behavior of this species, other processes might also be important in favoring size-specific reproductive investment in *L. pedersenii* (e.g., brood constraints—Heath 1979: male mating ability and female mate choice—Baeza 2007a). *L. pedersenii* might also be used as a model for understanding the selective forces that drive size-dependent sex allocation in monogamous hermaphrodites.

## Outlook

The hermaphroditic shrimp *L. pedersenii* with its social monogamy and female-biased sex allocation support in general terms predictions of mating systems and sex allocation theories (Charnov 1982; Klinkhamer et al. 1997; Shuster and Wade 2003; Baeza and Thiel 2007) (Fig. 5). Nevertheless, additional studies on various conditions that might favor social monogamy and sex allocation in symbiotic crustaceans deserve more attention (e.g., inter-specific competition, food availability within hosts). Shrimps from the genus *Lysmata* constitute a peculiar monophyletic clade because of their unusual sexual system (protandric simultaneous hermaphroditism) and diverse socioecology (Baeza 2009, 2010). Some species live in aggregations, others in small groups, whereas some species are monogamous in this group (e.g., *L. pedersenii*) (Baeza 2008b, 2009; Baeza et al. 2009; Baeza and Anker 2008; present study) (Fig. 5). Previous studies of sex allocation theory have focused on the free-living shrimp *L. wurdemanni* (Bauer 2002; Baeza and Bauer 2004; Baeza 2006, 2007a, b, c). Recent studies have elucidated the phylogenetic relationships of the species in the genus (Baeza 2009, 2010; Baeza et al. 2009). These findings are setting the stage for comparative studies that will permit understanding the evolutionary origins of protandric sequential hermaphroditism and the role of sexual selection in driving the mating system and optimal sex allocation of hermaphrodites (see Schärer 2009).

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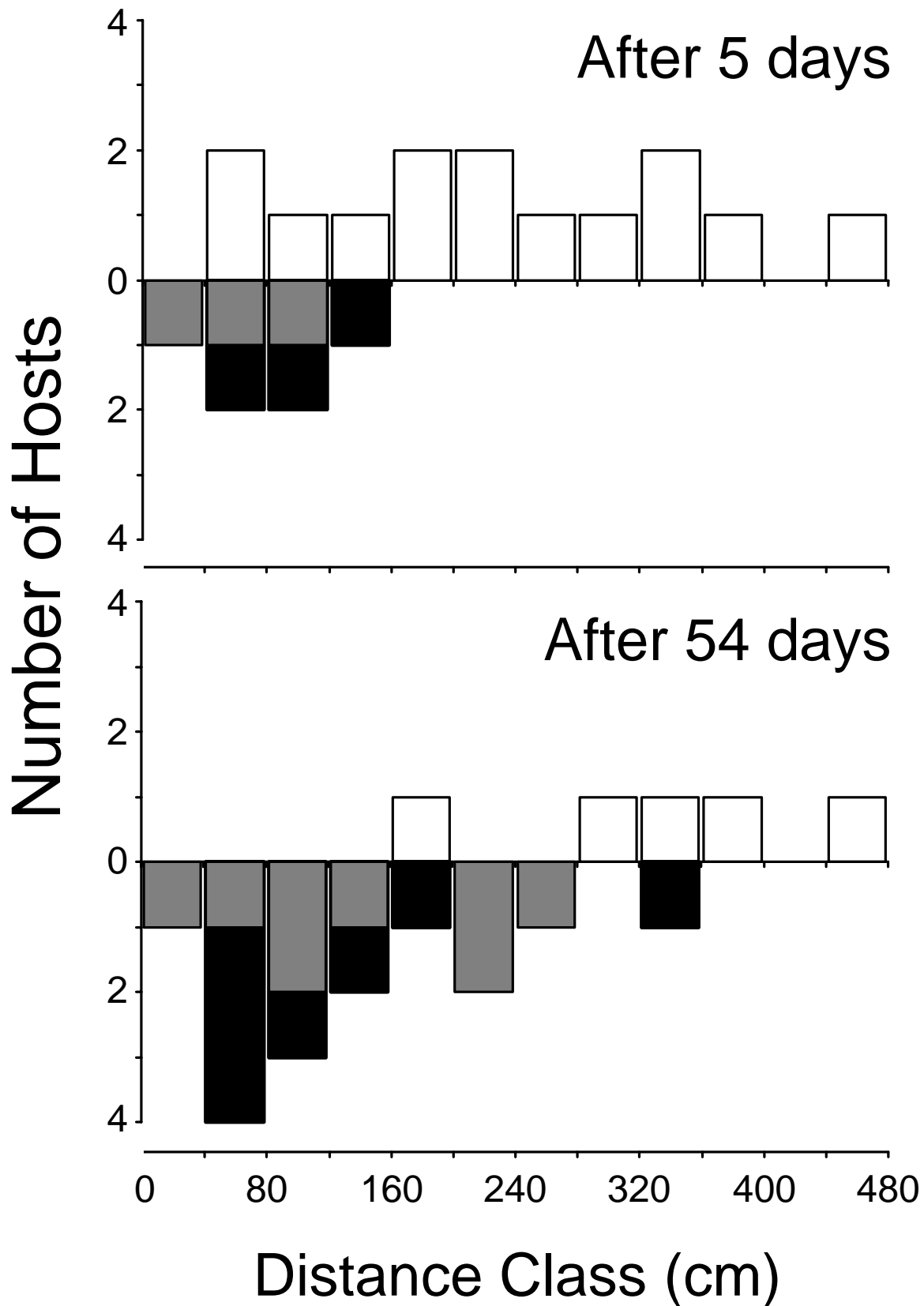
Table S1. General description of the symbiotic association between *Lysmata pederseni* and the host tube sponge *Callyspongia vaginalis* at different localities in the Caribbean Sea. Shown are: the frequency of occurrence of shrimp on hosts (%), the total number of hosts sampled and shrimp collected per locality ( $S_n$  and  $S_s$  respectively), the density of shrimp (number of shrimps per host: mean  $[X] \pm$  standard deviation  $[sd]$ , and range) and the results of specific Chi-square test of goodness-of-fit ( $\chi^2$  statistic and one-tailed  $P$  values) conducted to detect significant differences between the observed frequency of hosts harboring pairs of shrimps and that predicted by a random Poisson distribution. For all Chi-square tests, degrees of freedom = 1.

Locality	%	$S_n$	$S_s$	Density		Poisson	
				$X \pm sd$	range	$\chi^2$	$P$
Florida Keys, FL, USA	12.61	111	26	$0.24 \pm 0.54$	0 – 2	3.08	0.129
Carrie Bow Caye, BLZ	29.03	124	81	$0.72 \pm 1.34$	0 – 5	4.59	0.043
Kunayala, Panama	10.00	110	18	$0.16 \pm 0.54$	0 – 3	16.15	0.003

Table S2. Reproductive measurements and their variation with body size in *Lysmata pederseni*. The adjusted coefficient of determination ( $r^2$ ), the slope (b) of the curve denoting the relationship between a particular measurement and body size of shrimp, and the standard error (S.E.) of the estimated slope are provided for each specific reproductive measure. Also, the F-statistic and the corresponding  $P$  value obtained when testing for a positive correlation and for linearity between a particular reproductive measurement and body size are presented.

Dep. Variable	Model			Test $H_0: b = 0$		Test $H_0: b = 1$	
	$r^2$	b	S.E.	$t_{1,13}$	$P$	$t_{1,13}$	$P$
Repro. investment	0.79	1.73	0.1573	7.00	<0.0001	2.97	0.0109
Female allocation	0.78	1.77	0.262	6.78	<0.0001	2.96	0.0110
Male allocation	0.57	0.82	0.197	4.19	0.0011	-0.89	0.3894

Two Shrimps    One Shrimp    No Shrimp





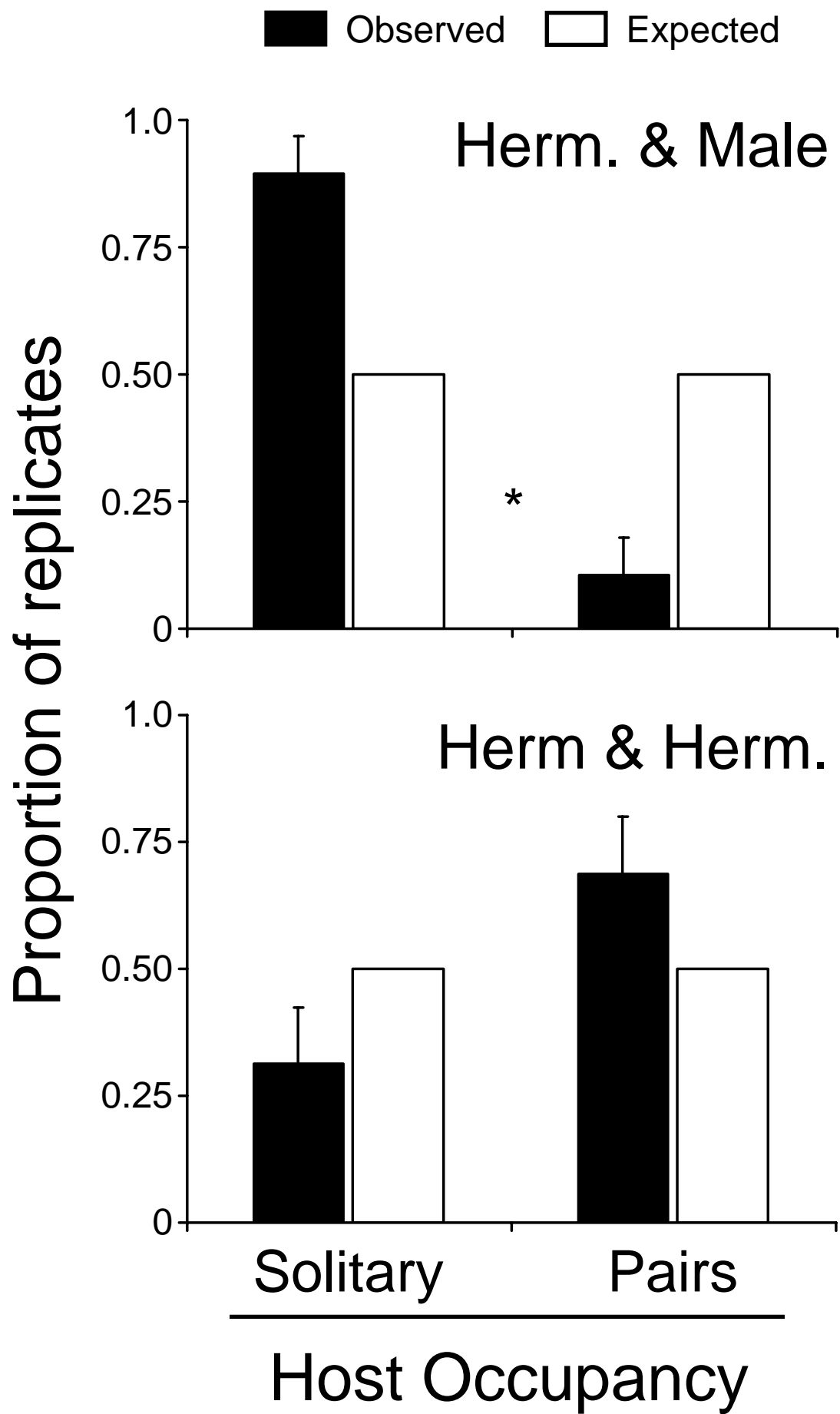


Fig. S2