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ORIGINAL PAPER

Mating system and reproductive performance in the isopod *Parabopyrella lata*, a parasitic castrator of the 'peppermint' shrimp *Lysmata boggessi*

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Abstract

Knowledge of marine host–parasite relationships has been increasing, but little is known about the reproductive strategies of the parasites. Theoretically, parasitic crustaceans inhabiting relatively small and scarce hosts should be monogamous. Parasites are also expected to exhibit higher reproductive performance (RP) than their free-living relatives, a strategy thought to have evolved to increase host-to-host transmission, or to be the outcome of living in a 'stable, nutrient-rich environment'. Here, the parasitic isopod *Parabopyrella lata* and its shrimp host *Lysmata boggessi* were used to test for monogamy and augmented RP, and to examine whether or not *P. lata* castrates its host. Prevalence, population distribution, and reproductive performance were examined in *P. lata* over 1 year (2012–2013) off Homosassa Springs, Florida USA (28.8037° N, 82.5761° W). Prevalence of *P. lata* on *L. boggessi* was greater during the warmer months of the year and infested shrimps were almost exclusively males. *P. lata* lives in male–female pairs within the gill chamber of infested shrimps more frequently than expected by chance alone. The sizes of paired males and females were tightly and positively correlated with each other and with host size. Average fecundity (3660 ± 1146 eggs female^{-1}) and reproductive output (61 ± 19%) in *P. lata*, as with other bopyrid parasites, was much greater than those reported for free-living isopods. This evidence supports the hypotheses that parasites such as *P. lata* are adapted to be monogamous, display augmented RP, and reproductively castrate their host.

Introduction

The Crustacea is a speciose clade of marine invertebrates (Bauer 2004), many have evolved symbiotic relationships [symbiosis herein defined sensu De Bary (1879) as dissimilar organisms living together] with a wide variety of host species, and several of these relationships are parasitic (Baeza 2015; Shields et al. 2015). Our knowledge about

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parasitic partnerships has increased substantially over the last few decades (Rohde 2005; Baeza 2015; Shields et al. 2015), but most remain poorly characterized. Furthermore, little is known about the reproductive strategies exhibited by parasites. Our aim in this study was to focus on the parasite and improve our understanding of its mating system and reproductive performance.

Monogamy [defined as pairs of conspecifics spending extensive periods of time together sensu Wickler and Seibt (1981, 1983)] is common in marine organisms (Knowlton 1980; Vincent and Sadler 1995). Various hypotheses have been proposed to explain the adaptive value of monogamy [e.g., "territorial cooperation" hypothesis (Wickler and Seibt 1981); "mate guarding" hypothesis (Parker 1974)]. According to Baeza and Thiel (2007), monogamy is adaptive in symbiotic crustaceans inhabiting scarce and morphologically simple hosts that attain a small size (relative to the symbiont) in tropical environments where predation risk away from hosts is high. Under these conditions, movement among hosts is constrained by predation risk and host-monopolization is favored in both sexes. Thus, both sexes optimize their reproductive success by sharing a host for long periods. Under monogamy, little sexual dimorphism in body size or weaponry should exist due to the low intensity of sexual selection (Shuster and Wade 2003; Baeza and Thiel 2007). However, if these species depend strongly upon the host for nourishment, natural selection might favor extreme sexual dimorphism in size. Small size in male parasites is favored in this case, because it diverts host-derived nutrients to female partners for offspring production (i.e., a form of indirect paternal care; Andersson 1994; Shuster and Wade 2003), potentially resulting in 'giant' females and 'dwarf' males. Various studies have reported monogamous symbiotic crustaceans inhabiting relatively small and scarce hosts in environments in which off-host predation risk is high (e.g., Baeza 1999, 2008; Baeza et al. 2013). On the contrary, other studies have shown a degree of promiscuity in symbiotic crustaceans living in environments that should favor monogamy (Baeza et al. 2016 and references therein). Certainly, more studies on symbiotic crustaceans are needed before the evolutionary drivers behind their mating systems can be generalized.

Parasites are also predicted to exhibit augmented reproductive performance (= increased fecundity and reproductive output) compared to free-living relatives (Jennings and Calow 1975; Poulin 1996; Whittington 1997). Increased fecundity in parasites is thought to be a strategy for increasing transmission from host-to-host or a benefit of the stable, nutrient rich environment provided by the host (Jennings and Calow 1975; Poulin 1996; Whittington 1997). In marine parasites with indirect development, small larval stages often experience high mortality (Morgan 1995), so augmented fecundity can increase the chances of finding a host individual, as previously suggested for terrestrial trematodes with complex life cycles (Poulin 1996; Trouvé and Morand 1998). Furthermore, some marine parasite clades exhibit complex life cycles using intermediate hosts before infesting final hosts in which reproduction takes place (Sherman and Curran 2014). The use of multiple host species further decreases the chances of locating a suitable final host if that host is not abundant. Thus, increased fecundity and reproductive output would seem selectively advantageous if reproductive opportunities (i.e., hosts) are uncommon. There are few empirical studies exploring these hypotheses (Poulin 1995a, b), but in agreement with the theoretical considerations, parasitic female mites of *Macrocheles subbadius* have been shown to have 2.4 times the lifetime fecundity of females in a closely related free-living species (Luong and Subasinghe 2017). Similarly, endoparasitic flatworms (Platyhelminthes) experience heightened fecundity compared to their free-living relatives (Whittington 1997). In general, we expect parasites to exhibit augmented fecundity and reproductive output compared to free-living relatives.

We used the isopod *Parabopyrella lata* (Epicaridea: Bopyridae) and its shrimp host *Lysmata boggessi* (Caridea, Lysmatidae) to test our hypotheses of monogamy and heightened reproductive performance. The 'peppermint' shrimp *L. boggessi* lives in the sub-tropical eastern Gulf of Mexico and is heavily targeted by the marine aquarium 'ornamental' industry (Baeza et al. 2014; Baeza and Behringer 2017). The genus *Lysmata* is also of significant biological interest because of its unusual sexual system: protandric simultaneous hermaphroditism (Bauer 2000, 2004; Baeza 2013). In this sexual system, juvenile shrimps first develop as functional males and later develop female gonads, in addition to their male gonads (Bauer 2000).

Lysmata boggessi often harbors the parasitic isopod *P. lata* within its branchial chamber (Boyko 2006). The family Bopyridae, to which *P. lata* belongs, contains over 500 species, all of which are considered parasites (Markham and Dworschak 2005; Williams and Boyko 2012; Boyko et al. 2013). Due to the small size of the branchial chamber of *L. boggessi* and the low abundance of hosts in the natural environment (10–80 shrimp km^{-2}, Baeza et al. 2014), we expect *P. lata* to live in monogamous pairs (see Baeza and Thiel 2007). To test this, we examined the population distribution, male–female association pattern, and parasite–host body size relationship. We also expected *P. lata* to exhibit high fecundity and reproductive output compared to free-living isopod species.

Bopyrid isopods exhibit a complex life cycle in which calanoid copepods serve as intermediate hosts and decapod crustaceans as the final hosts (Sherman and Curran 2014). This complex life cycle likely further decreases the chances of locating a suitable final host, especially if that host is not abundant. We compared fecundity and reproductive output estimates for *P. lata* with those from other closely related free-living isopod species to evaluate whether or not a parasitic lifestyle increased reproductive performance. Lastly, nothing is known about the effect of *P. lata* on *L. boggessi* reproduction, so we also tested whether or not *P. lata* was a parasitic castrator (a parasite capable of partially or completely halting host reproduction) as reported previously in other bopyrid isopods.

Materials and methods

Study site, sampling protocol, and shrimp measurements

Lysmata boggessi were collected at night and as by-catch once per month from December 2012 through November 2013 via roller-frame bait-shrimp trawlers in a shallow subtidal region off Homosassa Springs, west coast of Florida. During sampling, the trawler simultaneously deployed two roller-frame trawls (4.27 m height × 0.61 m width) 8–10 times between sunset and 02:30 h. Trawl durations were 30–45 min. A mesh size of 2.54 cm was used near the mouth of the trawl net and throughout the tapered body, with a finer mesh catch bag (1.91 cm) woven into the tailing end. The number of *L. boggessi* landed during each deployment was counted, and ~ 30 individuals from alternating deployments were haphazardly sub-sampled for further laboratory processing. The sub-sampled shrimps were fixed onboard the fishing vessel in 10% neutral buffered formalin and after 48 h, they were washed with tap water and transferred to 70% ethanol for preservation.

In the laboratory, preserved specimens were measured under a stereomicroscope (Leica MC 170 HD) using a reticule scale (carapace length, CL, precision = 0.1 mm), and sex phase and embryo development were assessed. Sex phase was determined by removing the second pair of pleopods and determining the presence (in males) or absence (in hermaphrodites) of appendices masculinae (Bauer and Holt 1998). Hermaphrodites were considered gravid if found carrying embryos underneath their abdomen. Lastly, the presence or absence of the parasite *P. lata* was determined by looking at the branchial chamber of each shrimp through the semi-translucent carapace. In total, 1–23 shrimps month^{-1} were preserved for further study of the host–parasite relationship.

Population distribution of *Parabopyrella lata* on its host *Lysmata boggessi*

A sub-sample of 52 infested shrimps was used to describe the use pattern of *P. lata* on its host, which includes a description of its population distribution, male–female association pattern, and host shrimp size relationships.

For this purpose, we first removed all of the parasites observed in the gill chamber of each infested shrimp and placed them in a separate petri dish for closer inspection. The gill chamber (left versus right) from which the parasites were extracted was not recorded. Female parasites were identified by their large, asymmetrical bodies that take up most of the branchial chamber of the host. The presence or absence of a male (or multiple males) was then determined for each female parasite by inspecting the female body for attached males. Male parasites were identified by their smaller, symmetrical bodies (Boyko 2006). Lastly, each host shrimp was also inspected for the presence of any additional parasites, including other much smaller recently settled juvenile males. Next, the total length of each parasite (from the tip of the cephalon to the end of the telson) was measured using a stage micrometer to the nearest 0.01 mm. Using the information above, we determined if the frequency distribution of *P. lata* among the shrimp population differed from a random distribution. We compared the observed distribution with a truncated Poisson random distribution (Elliott 1983).

Several shrimp hosts contained pairs of isopods (see "Results"). To determine whether the sexes were randomly distributed among pairs inhabiting the same host individual, the observed distribution was compared with the Binomial distribution. The expected random frequencies of the different sexes were calculated based on the proportion of males and females recorded in the entire population. A Chi square test of goodness of fit was used to inspect for significant differences between observed and expected (binomial) distributions (Sokal and Rohlf 1981).

Reproductive performance in *Parabopyrella lata*

A sub-sample of 11 female parasites carrying early-stage eggs (without eyes or obvious development of appendages) was used to describe individual-level reproductive parameters in *P. lata*: fecundity, egg size, and reproductive output. The presence or absence of eggs in the brooding chamber of each female was noted. If present, the total egg mass was gently removed with fine-tipped forceps from the parasitic brooding chamber and placed in a petri dish with tap water. Next, photographs were taken of a sample of 10 eggs from each brood. A stage micrometer (precision = 0.01 mm) was used to measure the maximum length (long axis length) and width (short axis length) of each egg. Egg volume was estimated with the formula for the volume of an ellipsoid (see Baeza et al. 2014), $EV = 1/6(L\ S2\ \pi)$, where L = long axis and S = short axis. Next, all eggs were counted and moved to a separate aluminum weighing boat. The total body length of each brooding female parasite (from the longest portion) was measured using a stage micrometer to the nearest 0.01 mm. The female parasite was then moved to a separate weighing boat, after all the eggs were removed from the brooding chamber. The female isopods and their corresponding embryo mass were then dried at 60 °C in a VWR Gravity Convection Oven (Model 414005-112) for a minimum of 72 h and weighed separately using an analytical balance scale (VWR-164AC) to the nearest 0.0001 g to determine reproductive output (see below).

Reproductive output was estimated as the ratio between dry weight of embryos and dry weight of the females carrying the embryos. We tested whether reproductive output increased linearly (i.e., isometric allocation) with female size. The relationship between egg dry mass and female body dry mass was examined using the allometric model $y = a * xb$ (Hartnoll 1982). The slope b of the log–log least-squares linear regression represents the rate of power increase ($b > 1$) or decrease ($b < 1$) of the estimate of reproductive allocation with a unit of increase in female dry mass. A t test was used to determine if the estimated slope b deviated from the expected slope of unity. Before conducting the analysis, assumptions of normality and homogeneity of variances were checked and found to be satisfactory (Zar 1999).

Comparison of reproductive performance between free-living and parasitic isopods

Lastly, we conducted searches in Google Scholar (https://scholar.google.com/), Aquatic Sciences and Fisheries Abstracts (ASFA, Aquatic Sciences and Fisheries Information System [ASFIS], http://www.fao.org/fishery/asfa/faq/en), and Web of Knowledge (https://webofknowledge.com/) using all of the following term combinations: ‘bopyrid’ or ‘Bopyridae’ or ‘isopod’ or ‘Isopoda’ and ‘fecundity’ or ‘egg production’ or ‘reproduction’ or ‘clutch size’. During our search, we retrieved information only from marine isopods to compare reproductive performance between free-living isopods (regardless of the family to which they belong) and those belonging to the family Bopyridae (see Supplementary Tables 1 and 2). For each manuscript, we recorded the following information: isopod size (minimum, maximum, and average, estimated as total length [mm]), fecundity (minimum, maximum, and average, estimated as number of eggs female^{-1}), egg size (minimum, maximum, and average, estimated as maximum diameter or volume) and reproductive output (RO, minimum, maximum, and average [%]). For symbiotic species, we also recorded the identity of the host species and host size (minimum, maximum, and average, estimated as CL, CW [carapace width], and SL [shield length], in shrimps, brachyuran crabs, and hermit crabs, respectively). Additional details can be found in Supplementary Tables 1 and 2. After completion of the data matrix, we used a general linear model (GLM) to examine the effect of lifestyle (free-living vs symbiotic [parasitic]) on the reproductive performance of isopods using fecundity (no. of eggs per female) as a proxy for reproductive performance. During the GLM analysis, fecundity was the dependent variable, lifestyle was the categorical independent variable, and female size was also included as the covariate in the model to control for female body size. Unfortunately, the lack of a detailed and well-resolved molecular phylogeny of the Isopoda, including the family Bopyridae and allies (see Boyko et al. 2013), precluded us from correcting for phylogenetic effects in our analysis (Pennell and Harmon 2013).

Results

Prevalence of *Parabopyrella lata* on its host *Lysmata boggessi*

During the sampling period, 73 of 1477 (4.94%) *L. boggessi* hosted at least one *P. lata* within their gill chambers, and when there were *P. lat*a, they were invariably within the same gill chamber. The number of *L. boggessi* collected was 110–122 month^{-1}. Prevalence varied during the year with no parasites observed from November 2012 to February 2013. Prevalence increased from March to June 2013, reaching its peak in June (19%) and then decreased to < 1% in November 2013 (Fig. 1a).

Males ($N = 654$) and hermaphrodites ($N = 823$) of *L. boggessi* ranged between 3.5–10.1 ($X \pm \mathrm{SD} = 6.62 \pm 1.24$) mm CL and 4.0–11.3 (8.62 ± 1.12) mm CL, respectively (Fig. 1b). Males accounted for 70 of the 73 infested shrimps and the remaining 3 were hermaphrodites. Only 1 of 3 infested hermaphrodites was ovigerous (CL = 79 mm, fecundity = 611 early-stage eggs). Taking into account the number of males and hermaphrodites in the population, isopods clearly infested males more frequently than expected by chance alone (Chi square test of independence: $\chi^2 = 82.9$, $df = 1$, $P < 0.001$). No isopods were found in shrimps < 40 mm CL. Prevalences in shrimps with CL 40–100 mm were similar, with slightly greater prevalence among shrimps 60–80 mm CL than in the other size classes. No shrimp > 100 mm CL harbored a parasite (Fig. 1c).

Population distribution of *Parabopyrella lata* on its host *Lysmata boggessi*

There were 0–5 isopods host shrimp^{-1} with a mean of 2 ± 0.62 in our sub-sample of 52 infested shrimps. The population distribution of *P. lata* was not random (observed versus truncated Poisson distribution: Chi square test of goodness of fit, $\chi^2_2 = 59.29$, $P = 0.0001$). This was explained by the larger number of hosts harboring two parasites compared to the number expected

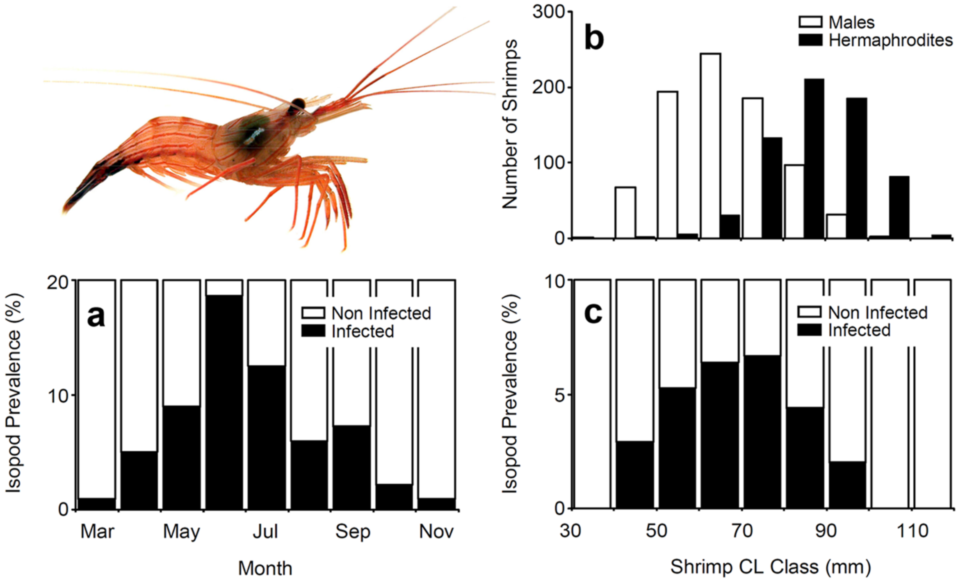

Fig. 1 Parasite prevalence and size-frequency distribution of its host. **a** Prevalence of isopod parasite *Parabopyrella lata* in monthly samples of *Lysmata boggessi*. **b** Size-frequency distribution of male ($N = 654$) and hermaphrodite ($N = 823$) *L. boggessi*. **c** Size-frequency distribution of *L. boggessi* with or without *P. lata*. Top left lateral view of *L. boggessi* (Photo credit: Arthur Anker)

by chance alone (decomposition of the Chi square test of goodness of fit; $\chi_1^2 = 56.83$, $P = 0.0001$, sequential Bonferroni $\alpha = 0.025$) and due to the smaller number of hosts harboring one parasite compared to the number expected by chance alone ($\chi_1^2 = 11.24$, $P < 0.0001$, sequential Bonferroni $\alpha = 0.05$) (Fig. 2a).

A total of 51 male and 52 female isopods were retrieved from the 52 infested shrimps. The isopod sex ratio was not biased toward either sex (considering only sexed isopods: sex ratio = 0.49; Fisher's exact test, $P = 0.539$). A total of 6 out of 52 infested shrimps harbored a single, adult female isopod. All singleton females were found brooding embryos ($N = 4$ and 2 carried early [without ocular development] and late [with well-developed eyes and appendages] embryos, respectively).

A total of 43 (82.7%) of 52 infested shrimps harbored a pair of parasites. Invariably, pairs of parasites comprised one adult male and female and inhabited the same branchial chamber (Fig. 2a). All paired females were brooding embryos ($N = 35$ and 8 carried early and late stage embryos,

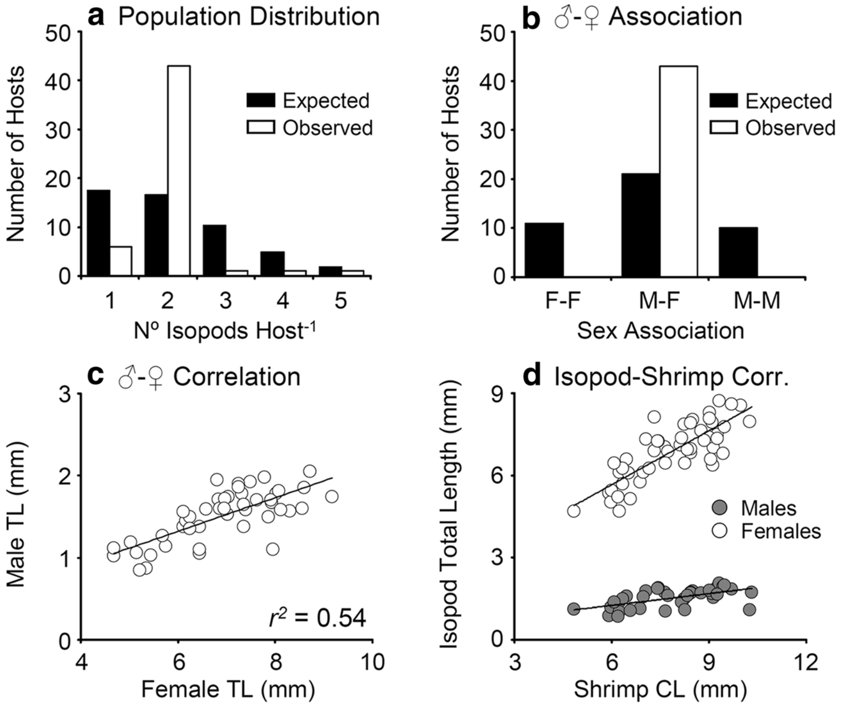

Fig. 2 Population distribution and host-use pattern of *Parabopyrella lata*. **a** Observed and expected (Poisson) parasite frequency distribution on shrimps. **b** Observed and expected (binomial) distribution of male and female parasites. **c** Relationship between paired male and female parasite TL (mm). **d** Relationship between male and female parasite TL and host shrimp CL (mm)

respectively). No shrimp host harbored two adult males or two adult females (Fig. 2b). Taking into account the binomial distribution and the relative abundance of males and females in the studied population, the number of shrimp hosts harboring parasite heterosexual pairs expected by chance alone would have been 21. Therefore, paired shrimps were found to be heterosexual more frequently than expected by chance alone (Fig. 2b).

Paired males were 0.85–2.05 mm TL and females 4.68–9.17 mm. There was a positive correlation in TL between males and females of *P. lata* living in pairs ($R^2 = 0.54$, $F_{1,43} = 50.11$, $P < 0.0001$) (Fig. 2c). Also, there was a significant, positive relationship between male isopod TL and host CL ($R^2 = 0.36$, $F_{1,43} = 24.72$, $P = 0.0001$) and female isopod TL and host CL ($R^2 = 0.72$, $F_{1,49} = 98.85$, $P < 0.0001$) (Fig. 2d).

A total of 3 of 52 infested shrimps harbored more than two isopods. Invariably, these isopods were found in the same branchial chamber. A single shrimp hosted three isopods: one adult female, one adult male, and one juvenile male. A single shrimp hosted four isopods: one adult female, one adult male, and two juvenile males. Lastly, a single shrimp hosted five isopods: one adult female, two adult males, and two juvenile males (Fig. 2a).

Reproductive performance in *Parabopyrella lata*

Fecundity in *P. lata* ranged between 2181 eggs female^{-1} in a specimen with TL = 5.71 mm and 5998 eggs female^{-1} in a specimen with TL = 6.83 mm with an average of 3660 (± 1146) eggs female^{-1}. There was a significant, positive correlation between female TL and fecundity in *P. lata* ($R^2 = 0.62$, $P = 0.0022$) (Fig. 3a). Average egg volume was 0.0311 mm^3 (± 0.0048). The relationship between egg volume and female TL was not statistically significant ($R^2 = 0.071$, $P = 0.401$) (Fig. 3b). Average female mass and egg mass were 2.10 mg (± 0.899) and 1.21 mg (± 0.413), respectively. A significant, positive correlation was found between female and egg mass (dry weight) (after log–log transformation, $R^2 = 0.42$, $P = 0.0228$) (Fig. 3c). The average reproductive output was 0.61 ± 0.19, 44.4–83.8%. Reproductive output decreased with female mass, i.e., RO exhibited negative allometry, as the slope ($b = 0.56$, $SE_b = 0.14$) of the line describing the relationship between these two variables (after log–log transformation) was significantly < 1 (*t* test: $t = 4.2$, $df = 1.10$, $P < 0.002$; Fig. 3c).

Comparison of reproductive performance between free-living and parasitic isopods

A GLM using fecundity as the dependent variable, lifestyle (free-living vs symbiotic [parasitic]) as the categorical independent variable, and female TL as the covariate detected a significant effect of lifestyle on fecundity after controlling for female TL ($F = 23.47$, $df = 1.21$, $P < 0.0001$). The GLM also detected a significant positive effect of female TL on fecundity ($F = 17.17$, $df = 1.21$, $P < 0.0001$). In this analysis, the interaction term was significant ($F = 16.71$, $df = 1.21$, $P < 0.0001$) (Fig. 4). In turn, while RO was limited to 44–84% (average = 61%) of body weight in *P. lata*, in those free-living isopods in which this reproductive trait has been reported, RO is limited to about 11–17% of body weight (Supplementary Tables 1 and 2).

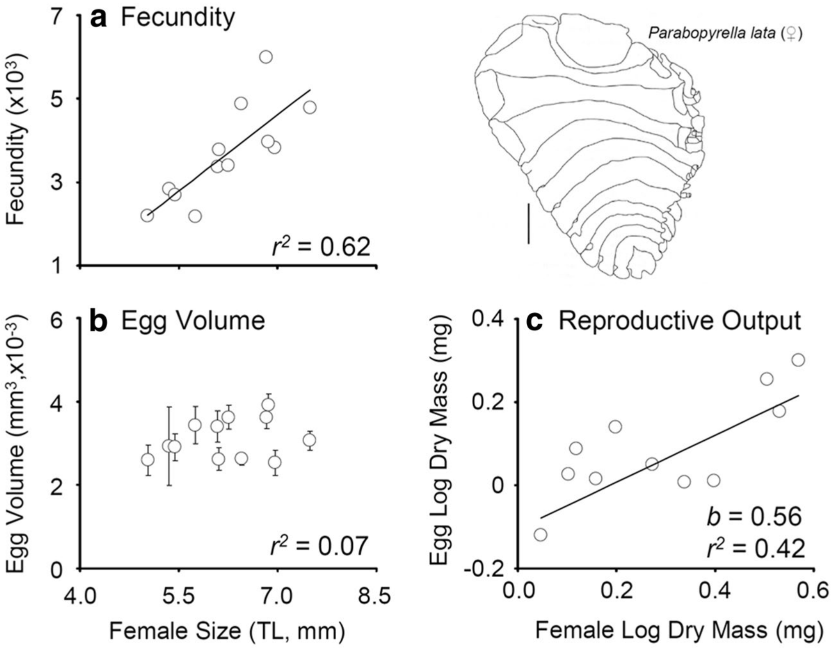

Fig. 3 Reproductive parameters of *Parabopyrella lata*. **a** Female size (TL, mm) and fecundity (egg number female^{-1}) relationship. **b** Female size and egg volume relationship. **c** Female log dry mass (mg) and egg log dry mass relationship (after log-log transformation). Top right, view of female *P. lata* (scale bar = 2 mm, modified from Boyko 2006)

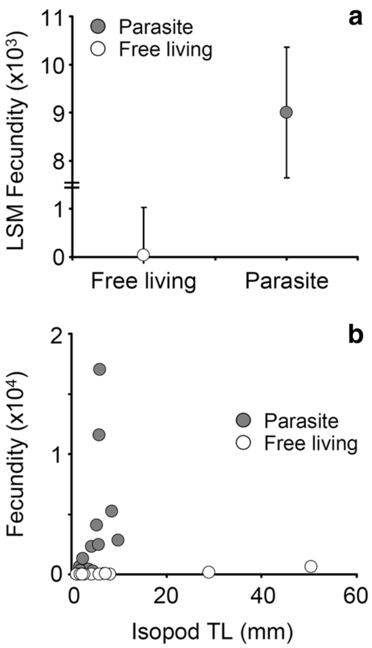

Fig. 4 Fecundity in free-living and parasitic (Bopyridae) marine isopods. **a** Least square mean (LSM) fecundity (± SE) of parasitic ($N = 13$) and free-living ($N = 12$) isopods. **b** Female TL (mm) and fecundity relationship in parasitic ($N = 13$) and free-living ($N = 12$) isopods. See Supplementary Tables 1 and 2 for details

Discussion

We hypothesized that the isopod *P. lata* was socially monogamous, given the host characteristics, and our results confirm this. Paired isopods shared the same host gill chamber with a frequency greater than expected by chance alone and pairs were invariably heterosexual. These results agree with previous reports for other socially monogamous symbiotic crustaceans (other bopyrid isopods: Roccatagliata and Lovrich 1999; Griffen 2009; Cericola and Williams 2015; Romero-Rodríguez et al. 2016, non-bopyrid isopods: Baeza 1999, 2008; Baeza et al. 2013, among others). Below, we discuss the association between *P. lata* and *L. boggessi*, and offer evidence for (and against) the notion of long-lasting monogamy in *P. lata*.

First, the significant and tight relationship found between isopod TL and host shrimp CL and between the TLs of paired male and female isopods sharing the same host individual argue in favor of long-term male–female pairing in *P. lata*. The conditions determining size-assortative heterosexual pairing and symbiont-host pairing in symbiotic species are not fully understood (Baeza and Thiel 2007; Baeza 2008). However, growth restrictions imposed by the host on long-term symbionts often explains such host-symbiont and male–female parasite body size relationships (Cash and Bauer 1993; Baeza 1999, 2008, Baeza et al. 2013). For instance, in the symbiotic and monogamous brachyuran crab *Pinnixa transversalis* and caridean shrimp *Pontonia margarita*, male body size explains 63.8 and 77.6% of variation in female body size, respectively (Baeza 1999, 2008). The values above are similar to those observed in female but not male *P. lata* inhabiting *L. boggessi*. The tight, size-assortative isopod pairing and relationship between host CL and isopod TL in *P. lata* suggest that the association between shrimp and host individuals and between male and female isopod pairs is temporally stable. If male and female isopods were not sharing the same host individuals over long periods of time, the tight correlations above would be difficult to explain.

Second, female *P. lata* were found cohabiting with males in the same host individual regardless of the stage of development of the eggs they were brooding, which also argues in favor of long-term male–female pairing. In promiscuous or polygamous species, heterosexual pairing is also observed (e.g., Diesel 1988; Van Der Meeren 1994), but pairing is short-term, males abandon females shortly after mating, and males are often found with females close to molting and ready to spawn (Baeza 2010). By contrast, male *P. lata* associate with females at all times during their brooding and reproductive cycle, regardless of egg stage. If males were not monogamous and were roaming among host individuals in search of extra-pair copulations, females paired with males should only be brooding late embryos or no embryos. The absence of a relationship between female brood stage and the presence of males further supports the theory that *P. lata* exhibits a monogamous mating system in which the males remain with their sexual partners.

Third, *P. lata* exhibits 'reverse' sexual dimorphism in TL where males attain average and maximum TLs considerably (~ 80%) smaller than females. In symbiotic and free-living crustaceans with polygamous mating systems, males often attain a much larger size than females, because size often determines competitive superiority (Shuster and Wade 2003). By contrast, the lower intensity of male competition characteristic of monogamous mating systems does not drive major size differentiation between the sexes (Shuster and Wade 2003; Baeza and Thiel 2007; Baeza 2008). The observation of reverse sexual dimorphism agrees with the notion that *P. lata* is a monogamous species in which males engage in 'indirect parental care', freeing resources for females to increase reproductive output by constraining their own growth (Andersson 1994; Shuster and Wade 2003; Baeza and Thiel 2007).

While most characteristics of the association between *P. lata* and *L. boggessi* support monogamy in *P. lata*, solitary females were also found brooding eggs. This suggests that some males may exhibit a promiscuous behavior, roaming among hosts in search of extra-pair mating opportunities. Presumably, if males were not switching hosts, solitary females would not have been observed brooding eggs, as reported for other monogamous crustaceans (e.g., Baeza et al. 2013). However, male *P. lata* lack swimming setae or well-developed pereopods for swimming or crawling efficiently between host individuals, in contrast to other promiscuous symbiotic species (see Ocampo et al. 2017). If not moving between hosts, the presence of solitary brooding females could also be explained by small, male *P. lata* suffering higher sex-specific mortality.

Environmental sex determination or sex change could play an important role in the establishment of monogamous pairs in *P. lata*, as shown for other bopyrid isopods (Tsai et al.1999; Cericola and Williams 2015). For an example of the former, cryptoniscus larvae colonizing a non-infested *L. boggessi* host might mature as female, while larvae colonizing a host infested with female bopyridium might develop as male, as suggested for *Athelges takanoshimensis* (McDermott 1991; Cericola and Williams 2015; see also Reinhard 1956). Alternatively, if *P. lata* undergoes sex change (e.g., sequential protandric hermaphroditism), the pioneering larva might mature as male but transition to female, if the colonized host has not been previously infested by a conspecific, while a larva infesting a host individual already harboring a female might mature and remain a male. The latter has been suggested for the protandric cymothoid *Ichthyoxenus fushanensis* (Tsai et al. 1999). It must be noted that some bopyrids appear to exhibit early sex determination (Hiraiwa 1936), and we do not know with certainty the mode of sex determination for *P. lata*. The sexual system, the possibility of environmental sex determination, and the cues used by *P. lata* to find host individuals and decide gender expression during and after host colonization remain to be experimentally addressed.

Fecundity, but not egg size, increased with TL in *P. lata* as reported before in every species of bopyrid isopod in which fecundity and egg size have been studied (see Supplementary Table 1). Bopyrid isopods exhibit moderate to high variability in both fecundity and egg size. The average fecundity we report for *P. lata* is similar to that reported for other species. The mean reproductive output (RO) of *P. lata* was also similar, albeit slightly, greater than that reported for the mud-shrimp *Orthione griffenis* (Smith et al. 2008), the only other bopyrid for which this has been measured. Fecundity and RO in *P. lata* and other bopyrid isopods is considerably higher than that reported for free-living isopods (Supplementary Table 2 and references therein). These comparisons support the notion that parasites are more fecund than their free-living counterparts. However, we argue in favor of additional studies of fecundity, size, and lifestyle coupled with the development of robust phylogenetic hypotheses in closely related free-living and parasitic isopods to improve our understanding of those intrinsic (e.g., size) and extrinsic (e.g., ecological) conditions favoring and constraining fecundity and egg size in parasitic and other symbiotic marine organisms. Whether augmented reproductive performance, as we observed in *P. lata*, is a strategy for increasing transmission from host-to-host (Poulin 1996; Trouvé and Morand 1998) or results from the 'stable, nutrient rich environment' provided by the host (Jennings and Calow 1975; Poulin 1996; Whittington 1997) remains to be examined.

Reproductive output and female body mass allometry scaled negatively in *P. lata*, suggesting female isopods did not proportionally increase allocation to their brood mass with increase in body mass. The relationship between reproductive output and size is poorly explored in isopods, but in other crustaceans (e.g., brachyuran crabs, caridean shrimps), brood mass is isometric or nearly isometric to female body mass. The negative scaling of reproductive output with size in *P. lata* could be explained by limitations on space available for yolk accumulation in the body cavity of large female isopods, as suggested for some brachyuran crabs (Hines 1992). The importance of food availability and quality for brood production is also well-known in crustaceans (Calado 2008). Food limitation can constrain the relationship with increasing female size (see Bolaños et al. 2012). If nutrition derived from the host or the ability of a *P. lata* female to acquire food from its host diminishes with increased parasite size, small but not large females might have access to enough resources to produce and accumulate yolk entirely filling their body cavities. Unfortunately, little is known about how bopyrid isopods derive nourishment from their hosts (Walker 1977; Astete-Espinoza and Caceres 2000; Neves et al. 2004), so future studies are needed to understand if nutrition becomes limiting for large females.

Parabopyrella lata was found infesting males with greater frequency than expected by chance and the few infested hermaphrodites did not brood embryos (with one exception). Furthermore, a tight relationship between host and isopod size was also observed. This suggests that *P. lata* preferentially infests males and is a special type of parasite: a parasitic castrator that halts transition from the male to hermaphroditic phase in *L. boggessi*. Several life history traits common to other macro-parasitic castrators were also observed in *P. lata*, supporting the contention that *P. lata* is a parasitic castrator. Our results show that *P. lata* establish long-term associations with their host individuals, as occurs in many other parasitic castrators (Kuris 1974). Castration of the host shrimp was 'intensity-independent', meaning that a single female isopod (and its diminutive male) completely hampered female reproduction of its shrimp host, as occurs in many other parasitic castrators (Kuris 1974; Lafferty and Kuris 2009; Williams and Boyko 2012). *P. lata* also attains a large size relative to the host, grows concomitantly with the host, and allocates a large percentage of its energy (61%) to reproduction, as noted for many other macro-parasitic castrators (Kuris and Lafferty 2000).

Bopyrids also negatively impact other host life history traits, including reproductive performance (Reinhard 1956; Oliveira and Masunari 1998). They either increase or decrease growth rate and condition of host individuals (Van Wyk 1982; Somers and Kirkwood 1991; Astete-Espinoza and Caceres 2000; Lester 2005; Hall et al. 2007), alter morphology (i.e., carapace shape) and pigmentation (Williams and Brown 1972; Roccatagliata and Jordá 2002), impact gonad development, molting frequency, and various secondary sexual characters (Beck 1979, 1980; Wenner and Windsor 1979; Courtney 1991; McDermott 1991; Raibaut and Trilles 1993; Oliveira and Masunari 1998; Jarrin and Shanks 2008; Smith et al. 2008). Interestingly, suppression of ovarian development by bopyrid castrators is not permanent (Roccatagliata and Lovrich 1999). For example, after experimental extraction of the bopyrid parasite *Pseudione tuberculata*, regeneration and maturation of the ovary was observed in the king crab *Paralomis granulosa* (Roccatagliata and Lovrich 1999). However, the mechanism by which bopyrid isopods affect host traits is far from understood.

Conclusion

Our results support the hypotheses that many symbiotic crustaceans inhabiting relatively small and scarce host species are monogamous (Baeza and Thiel 2007) and that parasitic species have higher fecundity than their free-living close relatives (Hines 1982; Jay 1989; Trouvé and Morand 1998; Salas-Moya et al. 2014). We have also shown that *P. lata* is a parasitic castrator of *L. boggessi*. Our findings highlight the importance of integrating studies of host and symbiont life history to fully understand how their interdependence drives their evolution.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Andersson MB (1994) Sexual selection. Princeton University Press, Princeton

Astete-Espinoza LP, Caceres CW (2000) Efecto del parasitismo del isópodo bopírido *Ionella agassizi* (Isopoda: Epicaridea) (Bonnier, 1900) sobre la fisiología nutricional del nape *Neotrypaea uncinata* (M. Edwards, 1837) (Decapoda: Thalassinidea). Rev Chil Hist Nat 73:243–252

Baeza JA (1999) Indicadores de monogamia en el cangrejo comensal *Pinnixa transversalis* (Milne Edwards & Lucas) (Decapoda: Brachyura: Pinnotheridae): distribución poblacional, asociación macho-hembra y dimorfismo sexual. Rev Biol Mar Oceanogr 34:303–313

Baeza JA (2008) Social monogamy in the shrimp *Pontonia margarita*, a symbiont of *Pinctada mazatlanica*, off the Pacific coast of Panama. Mar Biol 153:387–395

Baeza JA (2010) The symbiotic lifestyle and its evolutionary consequences: social monogamy and sex allocation in the hermaphroditic shrimp *Lysmata pederseni*. Sci Nat 97:729–741

Baeza JA (2013) Molecular phylogeny of broken-back shrimps (genus *Lysmata* and allies): a test of the 'Tomlinson–Ghiselin' hypothesis explaining the evolution of hermaphroditism. Mol Phylogenet Evol 69:46–62

Baeza JA (2015) Crustaceans as symbionts: an overview of their diversity, host use and life styles. In: Watling L, Thiel M (eds) The life styles and feeding biology of the Crustacea. Oxford University Press, Oxford, pp 163–189

Baeza JA, Behringer DC (2017) Integrative taxonomy of the ornamental 'peppermint' shrimp public market and population genetics of *Lysmata boggessi*, the most heavily traded species worldwide. PeerJ 5:e3786. https://doi.org/10.7717/peerj.3786

Baeza JA, Thiel M (2007) The mating system of symbiotic crustaceans. A conceptual model based on optimality and ecological constraints. In: Duffy JE, Thiel M (eds) Reproductive and social behavior: crustaceans as model systems. Oxford University Press, Oxford, pp 245–255

Baeza JA, Ritson-Williams R, Fuentes MS (2013) Sexual and mating system in a caridean shrimp symbiotic with the winged pearl oyster in the Coral Triangle. J Zool 289:172–181

Baeza JA, Behringer DC, Hart RJ, Dickson MD, Anderson JR (2014) Reproductive biology of the marine ornamental shrimp *Lysmata boggessi* in the south-eastern Gulf of Mexico. J Mar Biol Assoc UK 94:141–149

Baeza JA, Simpson L, Ambrosio LJ, Guéron R, Mora N (2016) Monogamy in a hyper-symbiotic shrimp. PLoS ONE 11:e0149797

Bauer RT (2000) Simultaneous hermaphroditism in caridean shrimps: a unique and puzzling sexual system in the Decapoda. J Crustac Biol 20:116–128

Bauer RT (2004) Remarkable shrimps: adaptations and natural history of the carideans. University of Oklahoma Press, Norman

Bauer RT, Holt GJ (1998) Simultaneous hermaphroditism in the marine shrimp *Lysmata wurdemanni*: an undescribed sexual system in the decapod Crustacea. Mar Biol 132:223–235

Beck JT (1979) Population interaction between a parasitic castrator, *Probopyrus pandalicola* (Isopoda: Bopyridae), and one of its freshwater shrimp host, *Palemonetes paludosus* (Decapoda: Caridae). Parasitology 79:431–449

Beck JT (1980) The effects of an isopod castrator, *Probopyrus pandalicola*, on the sex characters of one of its caridean shrimp hosts, *Palaemonetes paludosus*. Biol Bull 158:1–15

Bolaños JA, Baeza JA, Hernandez JE, Lira C, López R (2012) Population dynamics and reproductive output of the non-indigenous crab *Charybdis hellerii* in the south-eastern Caribbean Sea. J Mar Biol Assoc UK 92:469–474

Boyko CB (2006) A new shrimp host for *Parabopyrella lata* (Nierstrasz and Brender a Brandis, 1929) (Crustacea: Isopoda: Bopyridae) from the Florida gulf coast: a novel host–parasite relationship or a case of mistaken identity? Gulf Mex Sci 24:41–44

Boyko CB, Moss J, Williams JD, Shields JD (2013) A molecular phylogeny of Bopyroidea and Cryptoniscoidea (Crustacea: Isopoda). Syst Biodivers 11:495–506

Calado R (2008) Marine ornamental shrimp: biology, aquaculture and conservation. Wiley and Blackwell, New York

Cash CE, Bauer RT (1993) Adaptations of the branchial ectoparasite *Probopyrus pandalicola* (Isopoda: Bopyridae) for survival and reproduction related to ecdysis of the host, *Palaemonetes pugio* (Caridea: Palaemonidae). J Crustac Biol 13:111–124

Cericola MJ, Williams JD (2015) Prevalence, reproduction and morphology of the parasitic isopod *Athelges takanoshimensis* Ishii, 1914 (Isopoda: Bopyridae) from Hong Kong hermit crabs. Mar Biol Res 11:236–252

Courtney AJ (1991) Occurrence and influence of bopyrid parasites on female penaeid prawns from coastal waters of central Queensland (Australia). Aust J Mar Freshw Res 42:615–623

De Bary A (1879) Die Erscheinung der Symbiose: Vortrag gehalten auf der Versammlung Deutscher Naturforscher und Aerzte zu Cassel. Verlag von Karl J. Trübner, Strassburg **(Privately printed in Strasburg)**

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

Elliott JM (1983) Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Association, Westmorland

Griffen BD (2009) Effects of a newly invasive parasite on the burrowing mud shrimp, a widespread ecosystem engineer. Mar Ecol Prog Ser 391:73–78

Hall SR, Becker C, Caceres CE (2007) Parasitic castration: a perspective from a model of dynamic energy budgets. Integr Comp Biol 47:295–309

Hartnoll RG (1982) Growth. In: Abele LG (ed) The biology of Crustacea: 2. Embryology, morphology and genetics. Academic Press, New York, pp 111–196

Hines AH (1982) Allometric constraints and variables of reproductive effort in brachyuran crabs. Marine Biol 69:309–320

Hines AH (1992) Constraint on reproductive output in brachyuran crabs: pinnotherids test the rule. Am Zool 32:503–511

Hiraiwa YK (1936) Studies on a bopyrid, *Epipenaeon japonica* Thielemann. III. Development and life-cycle, with special reference to the sex differentiation in the bopyrid. J Sci Hiroshima Univ (Zool) 4:101–141

Jarrin JRM, Shanks AL (2008) Ecology of a population of *Lissocrangon stylirostris* (Caridea: Crangonidae), with notes on the occurrence and biology of its parasite, *Argeia pugettensis* (Isopoda: Bopyridae). J Crustac Biol 28:613–621

Jay CV (1989) Prevalence, size and fecundity of the parasitic isopod *Argeia pugettensis* on its host shrimp *Crangon francisorum*. Am Midl Nat 121:68–77

Jennings JB, Calow P (1975) The relationship between high fecundity and the evolution of entoparasitism. Oecologia 21:109–115

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

Kuris AM (1974) Trophic interactions: similarity of parasitic castrators to parasitoids. Q Rev Biol 49:129–148

Kuris AM, Lafferty KD (2000) Parasite–host modeling meets reality: adaptive peaks and their ecological attributes. In: Poulin R (ed) Evolutionary biology of host–parasite relationships: theory meets reality. Elsevier, Amsterdam, pp 9–26

Lafferty KD, Kuris AM (2009) Parasitic castration: the evolution and ecology of body snatchers. Trends Parasitol 25:564–572

Lester RJG (2005) Isopoda (isopods). In: Rohde K (ed) Marine parasitology. CSIRO Publishing, Clayton, pp 138–144

Luong LT, Subasinghe D (2017) A facultative ectoparasite attains higher reproductive success as a parasite than its free-living conspecifics. Exp Appl Acarol 71:63–70

Markham JC, Dworschak PC (2005) A new species of *Entophilus* Richardson, 1903 (Isopoda: Bopyridae: Entophilnae) from the gulf of Aqaba, Jordan. J Crustac Biol 25:413–419

McDermott JJ (1991) Incidence and host parasite relationship of *Leidya bimini* (Crustacea, Isopoda, Bopyridae) in the brachyuran crab *Pachygrapsus transversus* from Bermuda. Ophelia 33:71–95

Morgan SG (1995) Life and death in the plankton: Larval mortality and adaptation. In: McEdward L (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton, pp 279–322

Neves CA, Sampedro MP, Maia LE, Santos EA (2004) Effects of the parasite *Probopyrus ringueleti* (Isopoda) on glucose, glycogen and lipid concentration in starved *Palaemonetes argentinus* (Decapoda). Mar Ecol Prog Ser 58:209–213

Ocampo EH, Spivak ED, Baeza JA, Luppi TA (2017) Ontogenetic changes in the external anatomy of the parasitic castrator crab *Calyptraeotheres garthi*: implications for the timing of host colonization and sexual behavior. Biol J Linn Soc 120:54–74

Oliveira E, Masunari S (1998) Population relationships between the parasite *Aprobopyrus curtatus* (Richardson, 1904) (Isopoda: Bopyridae) and one of its porcelain crab hosts *Petrolisthes armatus* (Gibbes, 1850) (Decapoda: Porcellanidae) from Farol Island, southern Brazil. J Nat Hist 32:1707–1717

Parker GA (1974) Courtship persistence and female-guarding as male time investment strategies. Behaviour 48:157–183

Pennell MW, Harmon LJ (2013) An integrative view of phylogenetic comparative methods: connections to population genetics, community ecology, and paleobiology. Ann NY Acad Sci 1289:90–105

Poulin R (1995a) Evolutionary influences on body size in free-living and parasitic isopods. Biol J Linn Soc 54:231–244

Poulin R (1995b) Clutch size and egg size in free-living and parasitic copepods: a comparative analysis. Evolution 49:325–336

Poulin R (1996) The evolution of life history strategies in parasitic animals. Adv Parasitol 37:107–129

Raibaut A, Trilles JP (1993) The sexuality of parasitic crustaceans. In: Baker JR, Muller R (eds) Advances in parasitology. Academic Press, Cambridge, pp 367–444

Reinhard EG (1956) Experiments on the determination and differentiation of sex in the bopyrid *Stegophryxus hyptis* Thompson. Biol Bull Woods Hole 96:13–31

Roccatagliata D, Jordá MT (2002) Infestation of the fiddler crab *Uca uruguayensis* by *Leidya distorta* (Isopoda, Bopyridae) from the rio de la Plata estuary, Argentina. J Crustac Biol 22:69–82

Roccatagliata D, Lovrich GA (1999) Infestation of the false king crab *Paralomis granulosa* (Decapoda: Lithodidae) by *Pseudione tuberculata* (Isopoda: Bopyridae) in the Beagle Channel, Argentina. J Crustac Biol 19:720–729

Rohde K (2005) Marine parasitology. CSIRO Publishing, Clayton

Romero-Rodríguez J, Román-Contreras R, Cházaro-Olvera S, Martínez-Muñoz MA (2016) Growth of individuals within the parasite–host association *Bopyrina abbreviata* (Isopoda, Bopyridae) and *Hippolyte zostericola* (Decapoda, Caridea), and variations in parasite morphology. Invertebr Rep Dev 60:39–48

Salas-Moya C, Mena S, Wehrtmann IS (2014) Reproductive traits of the symbiotic pea crab *Austinotheres angelicus* (Crustacea, Pinnotheridae) living in *Saccostrea palmula* (Bivalvia, Ostreidae), Pacific coast of Costa Rica. ZooKeys 457:239–252

Sherman MB, Curran MC (2014) Sexual sterilization of the daggerblade grass shrimp *Palaemonetes pugio* (Decapoda: Palaemonidae) by the bopyrid isopod *Probopyrus pandalicola* (Isopoda: Bopyridae). J Parasitol 101:1–5

Shields JD, Boyko CB, Williams JD (2015) Parasites and pathogens of Brachyura. In: Castro P, Davie PJF, Guinot D, Schram FR, von

Vaupel Klein JC (eds) The Crustacea. Treatise on Zoology/Traite de Zoologie. Brill, Leiden, pp 639–774

Shuster SM, Wade MJ (2003) Mating systems and strategies. Princeton University Press, Princeton

Smith AE, Chapman JW, Dumbauld BR (2008) Population structure and energetics of the bopyrid isopod parasite *Orthione griffenis* in mud shrimp *Upogebia pugettensis*. J Crustac Biol 28:228–233

Sokal RR, Rohlf FJ (1981) Biometry. WH Freeman and Company, San Francisco

Somers IF, Kirkwood GP (1991) Population ecology of the grooved tiger prawn, *Penaeus semisulcatus*, in the north-western Gulf of Carpentaria, Australia: growth, movement, age structure and infestation by the bopyrid parasite *Epipenaeon ingens*. Mar Freshw Res 42:349–367

Trouvé S, Morand S (1998) Evolution of parasites' fecundity. Int J Parasitol 28:1817–1819

Tsai ML, Li JJ, Dai CF (1999) Why selection favors protandrous sex change for the parasitic isopod, *Ichthyoxenus fushanensis* (Isopoda: Cymothoidae). Evol Ecol 13:327–338

Van Der Meeren GI (1994) Sex- and size-dependent mating tactics in a natural population of shore crabs *Carcinus maenas* (Crustacea: Brachyura). J Anim Ecol 63:307–314

Van Wyk PM (1982) Inhibition of the growth and reproduction of the porcellanid crab *Pachycheles rudis* by the bopyrid isopod, *Aporobopyrus muguensis*. Parasitology 85:459–473

Vincent ACJ, Sadler LM (1995) Faithful bonds in wild seahorses, *Hippocampus whitei*. Anim Behav 50:1557–1569

Walker SP (1977) *Probopyrus pandalicola*: discontinuous ingestion of shrimp hemolymph. Exp Parasitol 41:198–205

Wenner EL, Windsor NT (1979) Parasitism of galatheid crustaceans from the Norfolk Canyon and Middle Atlantic Bight by bopyrid isopods. Crustaceana 37:293–303

Whittington ID (1997) Reproduction and host-location among the parasitic platyhelminths. Int J Parasitol 27:705–714

Wickler W, Seibt U (1981) Monogamy in Crustacea and man. Ethology 57:215–234

Wickler W, Seibt U (1983) Monogamy: an ambiguous concept. In: Bateson PPG (ed) Mate choice. Cambridge University Press, Cambridge, pp 33–50

Williams JD, Boyko CB (2012) The global diversity of parasitic isopods associated with crustaceans hosts (Isopoda: Bopyroidea and Cryptoniscoidea). PLoS ONE 7:e35350

Williams AB, Brown WS (1972) Notes on structure and parasitism of *Munida iris* A. Milne Edwards (Decapoda, Galatheidae) from North Carolina, USA. J Crustac Biol 22:303–308

Zar JH (1999) Biostatistical analysis. Prentice-Hall, Upper Saddle River