Protandric simultaneous hermaphroditism in the shrimps

*Lysmata bahia* and *Lysmata intermedia*

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Abstract. The sexual system of two peppermint shrimps, *Lysmata bahia* and *Lysmata intermedia*, inhabiting intertidal fossil coral terraces at Bocas del Toro, on the Caribbean coast of Panama, was examined. Dissections suggested that the population of each species consisted of functional males and functional simultaneous hermaphrodites. Males have cincinulli and appendices masculinae on the first and second pair of pleopods, respectively, gonopores located at the coxae of the third pair of walking legs, and ovotestes with a well-developed male portion full of sperm, but an undeveloped female portion. Hermaphrodites lacked appendices masculinae and cincinulli. However, they have male gonopores and ovotestes with well-developed ovaries full of mature oocytes and testes with sperm. When hermaphrodites were maintained in pairs, both molted and spawned eggs (to beneath abdomen) that continued developing after 3 d, demonstrating that hermaphrodites can reproduce as males and inseminate other hermaphrodites acting as females. The possibility of self-fertilization or parthenogenetic reproduction was tested and disregarded, because hermaphrodites reared in isolation spawned oocytes that failed to develop, disappearing from the abdomen after 2 d. Males reared in pairs mature as hermaphrodites in <50 d, showing the ability of males to mature as hermaphrodites. These results demonstrate that *L. bahia* and *L. intermedia* are protandric simultaneous hermaphrodites, as reported for all species of this genus whose sexual system has been examined. However, the studied species featured a lifestyle, termed “tropical-low abundance,” here not recognized previously for the genus; they occur in low abundances in tropical environments, they do not develop symbiotic associations with sessile invertebrates, and they are not conspicuously colored. Information on the sexual systems and lifestyles of more species needs to be examined before these observations can be placed into a comparative context within the genus.

Additional key words: hermaphrodite, protandry, sex allocation, Crustacea, Hippolytidae

Sexual systems vary considerably among caridean shrimps. Most species appear to be gonochoric, with individuals in a population reproducing either as a male or as a female during their entire life (e.g., many shrimps from the diverse family Alpheidae: Correa & Thiel 2003; Bauer 2004). Other species are sequential protandric hermaphrodites, with individuals consistently maturing and reproducing first as males to then, later in life, change sex to females (e.g., various species of *Pandalus*: Butler 1964, 1980). Although several variants of protandry have been described (see reviews by Bauer 2000; Correa & Thiel 2003), no study has reported protogyny (female first) among caridean shrimps. Lastly, a particular form of simultaneous hermaphroditism (adolescent protandry *sensu* Ghiselin 1974, protandricosexuality *sensu* Policansky 1982, or protandric simultaneous hermaphroditism *sensu* Bauer 2000) has been described recently for shrimps from the genus *Lysmata* (Wirtz 1997; Fiedler 1998; Bauer & Holt 1998; d’Acoz 2003; Bauer & Newman 2004).

In protandric simultaneous hermaphrodites, juvenile shrimps invariably mature and reproduce as males. These males have typical caridean male external characters, including gonopores located at the coxae of the third pair of walking legs (pereiopods), cincinulli (coupling hooks) on the endopod of the first pair of pleopods (abdominal swimming...
appendages), and appendices masculinae on the endopods of the second pair of pleopods (Bauer & Holt 1998). Males are capable of reproducing only as males, although their gonads are ovotestes with an undeveloped ovarian portion (Bauer & Holt 1998).

Later in life, males attain the female sexual function, developing into functional simultaneous hermaphrodites (Baeza 2007). Externally, these hermaphrodites resemble females of gonochoric shrimp species, having female gonopores on the coxae of the third pair of walking legs, expanded flanges on the basipod of the pleopods, and well-developed lateral abdominal plates that form an abdominal chamber in which embryos are brooded (Högland 1943). Although simultaneous hermaphrodites lose the appendices masculinae and cincinni, they retain testicular tissue, male ducts, and gonopores, and thus have the ability to reproduce as both males and females (Bauer & Holt 1998). After becoming hermaphrodites, individuals do not revert to their initial sexual condition (Baeza 2007) and no self-fertilization has been observed (Bauer & Holt 1998; Baeza & Anker 2008).

So far, the various studies on the sexual biology of shrimps from the genus *Lysmata* suggest that all the species are simultaneous hermaphrodites with an early male phase (*Lysmata grabhami* Gordon 1935 (Wirtz 1997): *Lysmata amboinensis* De MANN 1888 (Fiedler 1998); *Lysmata wurdemanni* Gibbes 1850 (Bauer & Holt 1998); *Lysmata seticaudata* Risso 1816 and *Lysmata nitida* DOHRN & HOLTJUS 1950 (d’Acoz 2003); *Lysmata californica* STIMPSON 1866 (Bauer & Newman 2004); and *Lysmata hochi* Baeza & Anker 2008 (Baeza & Anker 2008). While this sexual system does not vary within the genus, other life-history features do differ. For instance, two distinct lifestyles have been recognized ("crowd" and "pair" species *sensu* Bauer 2000). "Crowd" species mostly occur in warm temperate environments, inhabit refuges (e.g., rocky crevices and caves) as dense aggregations, and do not exhibit any specialized fish-cleaning behavior (i.e., *L. californica*: Bauer & Newman 2004; and *L. wurdemanni*: Baeza 2006). "Pair" species are mostly tropical, occur at low densities in the subtidal, and dwell as socially monogamous pairs on hosts, usually sea anemones, which are used as vantage points for cleaning fish clients attracted by colorful displays and specialized behaviors (i.e., *L. grabhami*: Wirtz 1997; *L. amboinensis*: Fiedler 1998; see Bauer 2000).

Taking this diversity of lifestyles into consideration, Bauer (2000) proposed a historical contingency hypothesis to explain the evolution of protandric simultaneous hermaphroditism in *Lysmata*. According to this author, protandric simultaneous hermaphroditism evolved in a strictly protandric tropical species of *Lysmata* that became a specialized fish cleaner. Restricted mobility of individuals due to their association with the host, and hence, reduced probability of encountering mating partners, would have favored simultaneous hermaphroditism over protandry or gonochorism (Bauer 2000). In this scenario, the "crowd" warm-temperate species that do not exhibit specialized cleaning behaviors would have evolved from tropical species with specialized cleaning behaviors and more complex mating systems. The evolution of these simple characters from more complex ones appeared unlikely to Bauer (2000). Overall, studies on the reproductive biology, life history, and ecology of several other species of *Lysmata* should improve our understanding of the conditions that in the past favored protandric simultaneous hermaphroditism in the genus.

In this study, I demonstrate, through a series of anatomical observations and experiments, that two tropical shrimps, *Lysmata intermedia* KINGSLEY 1878 and *Lysmata bahia* RHYN & LIN 2006, are protandric simultaneous hermaphrodites. Information on their general ecology and life history is provided.

**Methods**

**Collection and maintenance**

Shrimps were collected at night during low tide with aquarium nets from fossilized coral reef terraces near Playa Bluff (9°21'46"N, 82°1'22"W), Isla Colon, Bocas del Toro, Caribbean coast of Panama, between October and December 2006. The terrace extended along ~100 m of coast and when fully exposed (during low tides) was ~10-m wide. Immediately after collection, shrimps were transported to the laboratory at the Bocas del Toro Marine Research Station of the Smithsonian Tropical Research Institute (STRI). Shrimps were maintained in 15-L aquaria at a water temperature of 26–31°C and 34–36‰ salinity (ambient temperature and salinity at the study site) and were fed every other day with chopped frozen clams (thawed minutes before they were offered to shrimps) before being selected for dissections or experiments.

**Dissections**

Observations on reproductive anatomy were conducted in a total of four specimens of each species, four probable males (5.5 and 8.91 mm CL [carapace length]) in *Lysmata bahia* and 8.03 and 8.91 mm CL in

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Lysmata intermedia) and four purported hermaphrodites that carried embryos beneath the abdomen (7.92 and 8.8 mm CL in L. bahia and 8.25 and 8.8 mm CL in L. intermedia). Before dissection, the presence or absence of male gonopores on the coxae of the third pereiopods was recorded for each shrimp. When shrimps had male gonopores, the sperm mass contained in their ejaculatory ducts was retrieved by electro-ejaculation. Short electric shocks (at 10–12 V and 1.5 A) were applied for ~2–5 s near the male gonopores. This procedure resulted in the ejaculation of spermatophores by shrimps. Next, each shrimp was dissected to extract the gonad, which was then examined under a stereomicroscope. Finally, their first and second pleopods were dissected, and the presence or absence of appendices internae and masculinae, respectively, was recorded. Specimens were defined as males or hermaphrodites following Fiedler (1998) and Bauer & Holt (1998), by the presence (males) or absence (hermaphrodites) of coupling hooks (cin cinuli) and appendices masculinae on the endopods of the first and second pleopods, respectively.

Male mating activity of hermaphrodites

To determine whether hermaphroditic shrimps can function as males, three pairs of brooding hermaphrodites (which had reproduced as females) of each species were maintained separately in 15-L aquaria. Daily observations were conducted on the occurrence of embryo hatching, the presence of exuvia from molting, spawning of a new batch of eggs to beneath the abdomen, and development of the newly spawned embryos after 4 d. If brooding hermaphrodites molted and successfully brooded embryos that remained attached to the abdomen and continued developing after 4 d, I inferred that (1) the second brooding hermaphrodite in the aquaria acted as a male inseminating its partner, or that (2) the hermaphroditic shrimp reproducing as a female was capable of self-fertilization or parthenogenetic reproduction.

Self-fertilization by hermaphrodites

To determine whether hermaphrodites can fertilize themselves or can produce viable embryos by parthenogenesis, each of six brooding shrimps of the two studied species were maintained in isolation from conspecifics and observed through one breeding cycle for spawning and brooding of developing embryos. Daily observations were conducted on the hatching of brooded embryos, molting, spawning of a new batch of eggs, and the presence or absence of embryos in the brooding chamber after 4 d of spawning. If shrimps in isolation failed to brood a batch of eggs successfully, I concluded that brooding shrimps cannot self-fertilize themselves and cannot produce viable embryos by parthenogenesis. However, the low number of replicates conducted during this experiment may not have detected low levels of self-compatibility and/or infrequent reproduction by parthenogenesis in the present study.

In caridean shrimps, the production of a new brood by female-acting shrimps requires a new copulation event minutes or hours after molting because they do not have sperm receptacles (Bauer 2004). Therefore, any failure of isolated hermaphrodites to produce a new fertilized brood cannot be attributed to the lack of sperm available from a previous mating (in the wild or in the maintenance aquaria occurring before the experiments took place).

Maturation of males as hermaphrodites

To determine whether males do mature as hermaphrodites later in life, three pairs of males of each studied species were maintained separately in 15-L aquaria for 50 d. Daily observations were conducted on the presence of exuvia from molting in the aquaria, development of mature oocytes in the gonad (visible through the carapace), spawning of a first batch of eggs to the abdomen, and development of the first clutch of embryos after 4 d. If males started developing a female gonad and spawned a batch of eggs to the abdomen, I inferred that male shrimps have the ability to mature as hermaphrodites later in their lives.

Population parameters

Information on the abundance, population structure, and sex ratio (males to hermaphrodites) of each species was obtained from the field. The CL and number of shrimps of each sexual phase and species captured per sampling night were recorded. The sampling effort (total number of hours spent collecting shrimps) was calculated for each sampling night (n = 15 nights). The abundance of shrimps was estimated by dividing the sample abundance (number of shrimps captured) by the sampling effort.

Results

Dissections

Dissections demonstrated that all examined shrimps (brooding or non-brooding) from the two species had male gonopores located at the coxae of the fifth pair of pereiopods (Fig. 1A). From all
specimens, including those brooding embryos, sperm was retrieved from the gonopores by applying electro-shocks (Fig. 1B). Sperm cells retrieved from the gonopores were shaped in the form of inverted umbrellas (Fig. 1C). No obvious differences in the shape of the spermatozoa were observed between the studied species. Dissection of the gonads of brooding (purportedly) hermaphroditic shrimps confirmed the presence of ovotestes, with a relatively large anterior female portion full of green vitellogenic oocytes and a relatively small posterior male gonad portion with sperm (Fig. 1D,E). The gonad dissected from purportedly male shrimps was an ovotestis (Fig. 1F), but with an undeveloped anterior female portion (full of small immature oocytes lacking coloration) (Fig. 1G) and a posterior male gonad containing sperm (as in Fig. 1C). In both brooding and non-brooding shrimps, vasa deferentia and oviducts extended laterally from the male and female gonad portions, respectively (Fig. 1D–F). Shrimps brooding embryos invariably lacked cincinuli and appendices masculinae on the endopod of the first and second pereiopods, respectively (Fig. 1H,I). In contrast, appendices masculinae bearing large spines and numerous cincinuli were observed in the second and first pleopod of non-brooding shrimps, respectively (Fig. 1J–L). Remarkably, the appendix masculina of *Lysmata intermedia* was extremely long compared with that of *Lysmata bahia* (compare the length of the appendix masculina relative with the length of the endopod between the two species in Fig. 1K,L). All these different anatomical characters between brooding and non-brooding shrimps indicate that the populations of *L. bahia* and *L. intermedia* at the study site (Playa Bluff) are indeed composed of males and hermaphrodites.

**Male mating and self-fertilization**

In the experiment conducted to determine whether hermaphrodites were also capable of functioning as males, all shrimps from the two studied species successfully hatched their embryos as larvae, molted, and spawned a new batch of eggs to beneath the abdomen. The eggs remained attached to the pleopods and continued developing after 4 d, suggesting the ability of the other hermaphrodite in the same aquarium to reproduce as a male, or the possibility of self-fertilization by the hermaphrodite acting as a female. However, none of the 12 hermaphrodites (six per species), maintained in isolation, successfully reared their embryos to larvae. Invariably, shrimps reared in isolation molted and spawned eggs to the abdomen. However, the eggs disappeared from the pleopods within a few days after spawning.

In *L. intermedia*, spawning of the eggs contained in the ovarian part of the ovotestis was complete. In contrast, a small visible portion of the oocytes was retained in the ovaries of *L. bahia*. These retained oocytes started losing their coloration 3 d after molting, giving the impression of being reabsorbed. Overall, these observations indicate that hermaphrodites reproducing as females do not self-fertilize nor do they reproduce parthenogenetically, and hence, brooding shrimps (hermaphrodites) maintained in pairs indeed acted as males and fertilized eggs when their partners molted and reproduced as females.

**Maturation of males as hermaphrodites**

In this experiment, 11 out of the 12 experimental males (six per species) showed signs of maturation of the female portion of the gonad in <34 d. The single male in which no maturation of the female portion of the ovotestis was observed was a 6.3-mm CL shrimp of *L. intermedia*. Most probably, this male should have matured as a hermaphrodite if the experiment had lasted longer. All 11 males that matured as hermaphrodites molted when the gonad was full of green vitellogenic oocytes, and spawned a batch of eggs that developed successfully after 4 d. This indicates that males do mature as hermaphrodites later in life and also demonstrates that small male shrimps are
functional; they are capable of inseminating other shrimps reproducing as females.

Overall, taking into consideration all of the preceding information, it is possible to conclude that *L. bahia* and *L. intermedia* are protandric simultaneous hermaphrodites, incapable of self-fertilization and asexual reproduction through parthenogenesis.

**Population parameters**

The abundances of *L. bahia* and *L. intermedia* at the study site were low, with a mean (± standard deviation) of 0.59 (± 0.47) and 0.43 (± 0.41) individuals collected per hour per sampling night, respectively. In both species, the population was biased toward males. The ratio of males to total shrimps collected during the sampling period was 0.77 and 0.60 in *L. bahia* and *L. intermedia*, respectively. Males ranged in size 2.97–5.72 mm CL and 4.95–8.91 mm CL in *L. bahia* and *L. intermedia*, respectively. Hermaphrodites ranged in size 5.5–5.94 mm CL and 7.15–8.8 mm CL in *L. bahia* and *L. intermedia*, respectively (Fig. 2).

Most commonly, shrimps were spotted either solitarily or in small groups (up to four individuals) dwelling in small crevices in the reef terraces. The two species studied seem to have a patchy distribution in the terraces because shrimps were collected from some areas but not from others.

**Discussion**

In *Lysmata intermedia* and *Lysmata bahia*, both large brooding and small non-brooding shrimps have ovotestes and male gonopores full of sperm. However, the female portion of the ovotestis is underdeveloped in small non-brooding shrimps. These small non-brooding shrimps have cincinilli and appendices masculinae in the first and second pair of pleopods, respectively, both diagnostic characters for recognizing males in many gonochoric and protandric caridean shrimps (Bauer 2004). Behavioral experiments confirmed that non-brooding shrimps with external male diagnostic characters as well as large brooding shrimps lacking these male diagnostic characters have the ability to inseminate other shrimps reproducing as females. Therefore, all these anatomical observations and behavioral experiments demonstrate that *L. bahia* and *L. intermedia* are protandric simultaneous hermaphrodites, as reported for every other shrimp in the genus whose sexual biology has been studied (e.g., Bauer & Holt 1998; Fiedler 1998; d’Acoz 2003; Bauer & Newman 2004; Baeza & Anker 2008).

Nonetheless, it must be noted that the limited number of replicates conducted during the first and second experiments might have failed to detect low levels of self-compatibility and infrequent reproduction by parthenogenesis in the species studied. Similarly, due to the low number of replicates in the third experiment, the possibility that a small proportion of males in the field might never switch to hermaphrodites cannot be excluded. Further laboratory experimentation and field studies are necessary to expose less common aspects of variation in the sexual system of the species studied.

In general, the information available from the present study reinforces the idea that the sexual system in the genus *Lysmata* does not vary. Conversely, the diversity of lifestyles in the genus appears to be greater than recognized previously; the two species studied have a lifestyle not described previously for any other species from the genus. *Lysmata intermedia* and *L. bahia* cannot be classified as “crowd” species because they are not abundant and do not live in
large aggregations in warm-temperate environments. In the two species studied, less than one individual was collected per hour of sampling (at night) compared with ≥40 shrimps observed for the “crowd” species *Lysmata wurdemanni* (unpubl. data). Furthermore, the two species studied cannot be classified as forming a “pair” because, although they are tropical, they do not dwell as monogamous pairs on other macro-invertebrate hosts. Also, *L. intermedia* and *L. bahia* are not conspicuous in coloration as reported for “pair” species (Bauer 2000). Instead, the color pattern of the two species resembles that of the “crowd” species *L. wurdemanni* (Rhynes & Lin 2006).

This new lifestyle featured by *L. intermedia* and *L. bahia*, not reported before for any other species from the genus, is termed the “tropical-low abundance” lifestyle herein. Another shrimp that appears to belong to this new socioecological group is *Lysmata hochi*, a recently described species that also inhabits intertidal fossil coral terraces in the southern Caribbean (Baeza & Anker 2008). Like the two species studied here, members of *L. hochi* reach low abundances in the intertidal but do not live as socially monogamous pairs, and have neither a conspicuous coloration nor a specialized fish-cleaning behavior.

Further validating the idea that the lifestyles in *Lysmata* are more diverse than recognized previously, two other species of *Lysmata* from the eastern tropical Pacific, *Lysmata nayaritensis* and *Lysmata cf. trisetacea* Heller 1861, do not exhibit any of the now three recognized lifestyles. The protandric simultaneous hermaphrodites *Lysmata nayaritensis* and *Lysmata cf. trisetacea* are found at high densities in the tropical intertidal from the eastern Pacific (unpubl. data). These latter species inhabit the tropics as reported for other species of the “pair” group (Bauer 2000). However, they are not conspicuous in coloration, and can be found in large aggregations as reported for the “crowd” species from warm-temperate environments. I propose to name this fourth lifestyle as the “tropical-crowd” (Table 1).

This newly recognized diversity of lifestyles suggests that shrimps from the genus *Lysmata* may be ideal candidates for studying the role of environmental conditions in driving socioecological innovations in marine shrimps. Studies attempting to resolve the natural relationships among species of *Lysmata* and potential sister taxa (e.g., *Exhippoolysmata, Lysmatella, Parahippolyte*) and examining in detail their sexual systems and socioecologies should consider whether ecological conditions drive the mating behavior of these shrimps. Also, the phylogenetic relationships between *Lysmata* species, and among the genera of the family Hippolytidae, must be known before a sound hypothesis on the evolution of the sexual system and social characteristics can be developed.

In addition to shrimps, protandric simultaneous hermaphroditism has been confirmed in a polychaete worm (*Ophryotrocha diadema*: Premoli & Sella 1995), a land snail (*Achatina fulica*: Tomiyama 1996), a tunicate (*Manríquez & Castilla* 2005), and a symbiotic barnacle (*Chelonibia patula*: Crisp 1983; see also Weeks et al. 2006). Protandric simultaneous hermaphroditism is also suspected in various other species originally described as strict simultaneous hermaphrodites (e.g., fishes, snails, barnacles) (Ghiselin 1974; Policansky 1982; Crisp 1983). Protandric simultaneous hermaphroditism may be more common than originally noticed in nature. Several species of marine invertebrates currently described as strict simultaneous hermaphrodites may prove to

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be protandric simultaneous hermaphrodites with further research.

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