Mar Biol
DOI 10.1007/s00227-008-1059-z

ORIGINAL PAPER

Is *Hippolyte williamsi* gonochoric or hermaphroditic? A multi-approach study and a review of sexual systems in *Hippolyte* shrimps

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Received: 12 March 2008 / Accepted: 29 August 2008

Abstract Sexual systems vary considerably among caridean shrimps and while most species are gonochoric, others are described as sequential protandric hermaphrodites or simultaneous hermaphrodites with an early male phase. At present, there is confusion about the sexual system exhibited by several species mostly because those studies attempting to reveal their sexual system draw inferences solely from the distribution of the sexes across size classes. Here we investigated the sexual system of the shrimp *Hippolyte williamsi* from Chile to determine if the species is protandric or gonochoric with sexual dimorphism (males smaller than females). Morphological identification and size frequency distributions indicated that the population comprised small males, small immature females, and large mature females, which was confirmed by dissections. No transitional individuals were found. Males maintained in the laboratory molted 1–8 times, and many grew up to reach sizes observed in only a small fraction of males in the field. No indication of sex change was recorded. Our results indicate that *H. williamsi* is a sexually dimorphic gonochoric species and emphasizes the importance of using several kinds of evidence (size measurements, growth experiments, morphological dissections, and histological studies) to reveal the sexual system of *Hippolyte* species. Whether the observed size dimorphism between males and females in many species of *Hippolyte* is expression of contrasting sexual and natural selection, and whether divergent sexual fitness functions can contribute to the evolution of hermaphroditism remains to be revealed in future studies.

Communicated by B. Notes.

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Introduction

Sexual systems vary considerably in the Caridea. Most shrimps are gonochoric, with individuals reproducing as male or female during their entire life (Bauer 2001, 2004). Examples include *Rhynchocinetes typus* (Thiel and Hinojosa 2003), *Macrobrachium rosenbergii* (Karplus 2005), *Hippolyte obliquimanus* (Terossi et al. 2008), and most species from the diverse genus *Alpheus* (Correa and Thiel 2003; Anker et al. 2006). Other species are protandric hermaphrodites, in which individuals change from male to female (e.g., *Pandalus*—Butler 1964, 1980; Hoffman 1972, see also review by Bergström 2000). Several variants of protandry have been reported, such as mixed protandry with primary females in *Processa edulis* (Noël 1976) and *Crangon crangon* (Boddeke et al. 1991; Schatte and Saborowski 2006), or with primary males in *Thor manningi*

(Bauer and VanHoy 1996). No study so far has reported protogyny (female first) among shrimps (e.g., as in the isopod *Gnorimosphaeroma oregonense*—Brook et al. 1994). Finally, simultaneous hermaphroditism with an early protandric phase, i.e. adolescent protandry sensu Ghiselin (1974) or protandric simultaneous hermaphroditism sensu Bauer and Holt (1998), where individuals initially mature as males and later become simultaneous hermaphrodites, has been described for various species of *Lysmata* and might be more common than previously assumed (Baeza 2008a).

Interestingly, within the Caridea there is also considerable variation of sexual systems at the generic level. For instance, while *Thor manningi* has been recognized as a protandric species (Bauer 1986), studies of its congeners, *T. dobkini* and *T. floridanus,* indicated that they are gonochoric (Bauer and VanHoy 1996; Bauer 2004). This variability in gender expression among closely related species suggests that caridean shrimps might be used as models to test the role of the environment in explaining evolutionary innovations in gender in marine invertebrates. However, at present, comparative studies are precluded due to the scarcity of information about the sexual system of many species in diverse families.

While the sexual system of most shrimp species is well known, for some species the distribution of the sexes among individuals is not clear. For several species there is controversy with respect to their sexual system. For instance, initial studies on the population structure of *Crangon franciscorum* indicated that this species was gonochoric (Siegfried 1980, 1989). The body size of the males was almost invariably smaller than that of the females, a condition explained at that time by sex-specific mortality or migratory behavior (op. cit.). The sexual dimorphism reported for this species might also indicate strong selective pressures among males (but not females) favoring a small male condition (see review in Baeza and Thiel 2007). On the other hand, recent studies combining population dynamics, gonad dissections, and rearing of shrimps in captivity have demonstrated that *C. franciscorum* is actually protandric (Gavio et al. 2006). A similar sexual system (i.e., facultative protandry) has been reported for the congener *Crangon crangon*, where a small proportion of males in the population change sex later in life (Boddeke et al. 1991; Schatte and Saborowski 2006).

To the best of our knowledge, the caridean family with the highest diversity of sexual systems is the Hippolytidae. It contains species that are gonochoric, protandric hermaphrodites and simultaneous hermaphrodites (Bauer 2004; Bauer and VanHoy 1996; Baeza 2006; Terossi et al. 2008). Furthermore, there are species whose sexual system remains controversial such as *Hippolyte inermis*, which is reported either as protandric (Zupo 2000, 2001; Zupo and Buttino 2001; Zupo and Messina 2007) or gonochoric (Cobos et al. 2005). Because the two studies examining the sexual system of this species were conducted in localities hundreds of kilometers apart (i.e., Italy and Spain), this controversy might well be explained if the two sampled populations pertain to two different cryptic species, each one with a different sexual system. Data on gender size frequency, gonad anatomy, secondary sexual characters and development in laboratory culture are necessary to determine the sexual system of marine shrimps (e.g. Terossi et al. 2008).

The aim of the present study was to elucidate the sexual system of *Hippolyte williamsi* Schmitt 1924, a small species that inhabits seaweed meadows and seagrass beds of *Heterozostera chilensis* (0–8 m depth) in northern-central Chile (González 1992; Stotz and González 1997). Studies on the general ecology of this shrimp are lacking. However, preliminary observations indicated that males were smaller than females. Although this size difference between the sexes is suggestive of protandry, as reported for its congener *Hippolyte inermis* (Zupo and Messina 2007; but see Cobos et al. 2005), gonochorism cannot be ruled out as an alternative pattern of gender expression (see Terossi et al. 2008). To properly examine the sexual system of *H. williamsi*, we sampled in different seasons to document the population structure and sexual characters. Additionally, we reared males in the laboratory in three different social environments (in isolation, together with females, or in presence of other males), recording possible sex change and death.

Materials and methods

Collection and maintenance of shrimps

Shrimps were collected with kick nets at low tides from a shallow subtidal sea grass bed of *Heterozostera chilensis* at Puerto Aldea (S 30° 17′, W 71° 36′), Tongoy, Coquimbo, Chile, during December 2005, October 2006 and January 2007. The species was identified using d'Udekem d'Acoz (1996, 1997, 2007) and the key of Wicksten (1990), and our original identification was kindly confirmed by Sammy De Grave (Oxford University Museum of Natural History, accession number of the specimen deposited at the museum: OUMNH 2007-12-0002). Immediately after collection, shrimp were either preserved in 5% formalin or kept alive and placed in large plastic buckets and/or ice chests (only for samples from 2006 and 2007) to be transported to the seawater laboratories of Universidad Católica del Norte, Coquimbo, Chile.

Shrimps were maintained in 55 L plastic containers with circulating filtered seawater at water temperatures ranging

from 12 to 20°C (14–18°C during October–December 2006; 17–20°C in January–February 2007; 14–18°C in March-April 2007; 12–16°C May–June 2007) and 34.3–34.7 ppt salinity and were fed daily with planktonic microalgae (*Chaetoceros* spp.), benthic diatoms (*Grammatophora marina, Melosira nummuloides, Navicula* sp. *Nitzschia longissima*) scraped from the surface of large tanks maintained in the laboratory, fish food (TretraFin®), and/or chopped fresh *Emerita analoga*, before being selected for experiments. Previous studies had highlighted the importance of food in development of hippolytid shrimp (see e.g. Zupo 2001; Zupo and Messina 2007). Thus, in order to avoid potential biases caused by a highly specific diet (e.g. only diatoms, or only particular strains of microalgae), we provided a diet of microalgae and animal food representative of the diet of *H. williamsi* in its natural environment.

Measurements and morphological examination of shrimps

Preserved shrimps were used for the examination of reproductive anatomy and measurements of body length. Under the dissecting microscope, the carapace length (CL, measured from the orbital margin to the posterior border of the carapace) of each preserved shrimp was measured. Before measuring the shrimps, all preserved specimens were identified as males, ovigerous and non-ovigerous females, using the presence or absence of embryos beneath the abdomen and of male gonopores on the coxae of the fifth pereiopods. The examination of these characters permitted rapid identification of males and females. A total of 50 males (CL = 2.1–4.2 mm) and 50 females (CL = 3.1–4.4 mm) were selected for a more detailed examination of their sexual anatomy to confirm gender identity based on external characters. Mature specimens were defined as males or females by the presence (females) or absence (males) of brooded embryos and a large rostrum (rostrum long, reaching beyond the first third of the scaphocerite in females, rostrum shorter than the eyes in males—Wicksten 1990), and by the presence (males) or absence (females) of a gonopore on the coxae of the fifth pair of legs, vas deferentia connected to the gonads, and an appendix masculina on the endopod of the second pleopod.

The sex of 306 shrimps could not be reliably identified as either male or female by using only external morphological characters. These 306 individuals were dissected (together with the other 50 males and 50 females above) to reveal the presence or absence of male and female internal characters. Gonads were extracted and examined for the presence or absence of testes, ovaries, vas deferens, and oviducts. Then, the first and second pleopods of each one of these shrimps were dissected and the presence or absence of appendices masculinae was recorded. During dissections, we were particularly interested in recognizing "intermediate" individuals, i.e. males with maturing ovaries and/or shrimps with both male and female characteristics. For instance, individuals carrying embryos beneath the abdomen (female character) but with appendices masculinae on the second pleopods (male characters) would have been considered intermediate shrimps. The existence of intermediates has been reported before for protandric and protandric-simultaneous hermaphroditic shrimps (Bauer and Holt 1998; Bauer 2004; Gavio et al. 2006).

An ideal character denoting "transitional" individuals in a protandric species is the presence of maturing ovaries that can be observed through the carapace in a certain proportion of the males. Unfortunately, it was not possible to look for such a character before dissections in our preserved specimens because their carapace became white and opaque. This color change caused by preservation impedes any observation of internal organs (including the gonads).

In order to characterize the external and internal characteristics of males and females, a sample was collected in October 2007 and immature and mature shrimps were dissected and prepared for scanning electron microscopy. The samples were fixed in 3% glutaraldehyde in filtered sea water (1.0 µm, Millepore®) at room temperature for 10 hours, rinsed 3 times, dehydrated using an ethanol series of 50, 80 and 100%, critical-point dried with CO_2 in a Samdri-780-A (Tousimis, USA), sputter-coated with gold and analyzed on a JEOL T-300 scanning electron microscope (SEM).

Experimental test of sex change

To determine whether males change to females later in life, two different experiments were conducted with shrimps collected in October 2006 and January 2007. In the first experiment, 20 "focal" males ($1.9 < CL < 3.5$ mm) were maintained in isolation in plastic containers (3.5 L) until death or for a maximum time period of 6 months. They were fed with benthic diatoms and fish food (TretraFin®). In the second experiment, pairs of focal males were maintained in plastic containers (3.5 L) with either two other males (MO for Male-Only treatment) or with two females (MF for Male–Female treatment) ($n = 10$ replicates per treatment with 40 different focal shrimps). The experiment lasted until death or for a maximum time period of 4 months. They were fed fresh chopped *Emerita analoga*. The rationale for the second experiment was to subject focal male shrimp to different social environments that might promote sex change (as reported before for *Lysmata wurdemanni*—Baeza and Bauer 2004; Baeza 2007a).

All experiments and treatments were examined daily for the presence of exuvia from molting shrimps. The possibility of sex change by focal males was determined every

seventh day when gently placing and manipulating the focal males under the dissecting microscope to examine for the presence of newly developed female characters (e.g., enlargement of the rostrum, second abdominal pleura).

Growth rates of shrimps in experiment 1 were measured to compare the maximum size of males retrieved from the field to that attained by males in the laboratory. This comparison allowed us to confirm whether laboratory conditions were appropriate for maintenance and growth of male shrimps. The body size of the experimental male shrimps was measured from digital images taken (Canon® PowerShot G3) at the start of the experiment and immediately after every molt. The total length of each shrimp (TL, mm) was measured from the tip of the scaphocerite to the tip of the uropods using the software Image-Pro Plus 4.5 for Windows. Next, TL was transformed to CL using the equation: TL = 6.905 (CL) – 1.859, or correspondingly CL = (TL + 1.859)/6.905 ($r^2 = 0.844$), obtained by measuring the TL and CL of 20 male shrimps. This transformation permitted us to compare the size of males attained in the field and laboratory using the same proxy (CL).

Results

Population structure and dissections

A total of 3,540 shrimps were collected during the study; 1,762 shrimps were classified as males under the dissecting microscope while the remaining 1,778 shrimps were classified as females. Of these 1,778 females, 978 individuals (55%) carried embryos beneath the abdomen. Sex ratio (male to females) did not deviate significantly from a random distribution of 1:1 for each of the sampling dates (December 2005; 0.46:0.54, $\chi^2_1 = 0.32$, $P = 0.5712$, October 2006; 0.41:0.59, $\chi^2_1 = 1.633$, $P = 0.2012$, January 2007; 0.56:0.44, $\chi^2_1 = 0.723$, $P = 0.3952$). No intermediate shrimps with both male and female external or internal characteristics were observed at either sampling date.

Dissections and SEM images demonstrated that all shrimps classified as males based on external characters had male gonopores on the coxae of the fifth pair of pereiopods and the absence of female gonopores on the coxae of the third pair of legs (Fig. 1a–c). Similarly, although the female gonopore was not visible under the dissecting microscope, all shrimps classified as females based on their external features had female but no male gonopores (Fig. 2a–c). In males, the gonopore was clearly defined and the posterior border protruded greatly, forming a rounded lip (Fig. 1c). In females, the gonopores were not clearly delimited and consisted of a slit-like opening closed by a thickened valve-like flap or cover (Fig. 2a–d). In large brooding (mature) females, the section of the gonopore closer to the coxae was more clearly marked than the section distal to the coxae (Fig. 2a, b). The gonopore of small non-brooding (immature) females was smaller and with thinner borders than that of brooding females (Fig. 2c, d). Various setae surrounding the gonopores were visible in both mature and immature females (Fig. 2a–d). In contrast, the gonopores of males were not flanked by these setae (Fig. 1a–c; Fig. 2a, b). In one of the studied females, the gonopore appeared open because the valve-like flap was folded inward in contrast to that observed in the other studied females. The inward position of this cover suggests that this single female was close to molting and spawning (Fig. 2e, f).

In males, the exopods from the first pair of pleopods was approximately twice the length of the endopods (Fig. 1d; Fig. 3a). The endopods from the first pair of pleopods had no appendix interna and no cincinulli (Fig. 3a). In contrast, the endopods from the second pair of pleopods had an appendix interna with numerous cincinulli and an appendix masculina with straight setae at the distal end that were about twice as long as the appendix interna itself (Fig. 1d–g; Fig. 3c). In small, most probably immature males, the appendix masculina was reduced in size, not even reaching half the length of the appendix interna, and had a reduced number of distal setae (Fig. 1g). In females, the endopods from the first pair of pleopods had no appendix interna and cincinulli (Fig. 3b). The endopods of the second pair of pleopods had an appendix interna with numerous cincinulli but lack an appendix masculina (Fig. 3d).

Dissection of the gonads of both ovigerous and non-ovigerous shrimps classified as females confirmed the presence of paired ovaries lying above the hepatopancreas, below the heart and extending into the first abdominal somite (Fig. 2b, c; Fig. 4a–c). The gonads dissected from male shrimps were paired testes located above the hepatopancreas and below the heart (Fig. 4d).

The carapace length of male and female shrimps varied between 1.6 and 4.8 mm (mean ± SD; 2.8 ± 0.57) and between 1.7 and 5.0 mm (3.7 ± 0.59), respectively (Fig. 5). Significant differences in CL between the sexes were detected for each sampling date (Mann–Whitney Rank Sum Test; December 2005, $T = 13{,}276$, $n_{(\text{males})} = 150$, $n_{(\text{females})} = 173$, $P < 0.001$, October 2006; $T = 195094.5$, $n_{(\text{males})} = 547$, $n_{(\text{females})} = 705$, $P < 0.001$, January 2007: $T = 1189779.5$, $n_{(\text{males})} = 877$, $n_{(\text{females})} = 1{,}096$, $P < 0.001$), indicating sexual dimorphism with respect to body size (Fig. 5). Also, the carapace length of (mature) females carrying embryos beneath the abdomen was significantly larger than that of (immature) females with no eggs (4.1 ± 0.29 and 3.3 ± 0.52 mm, for ovigerous and

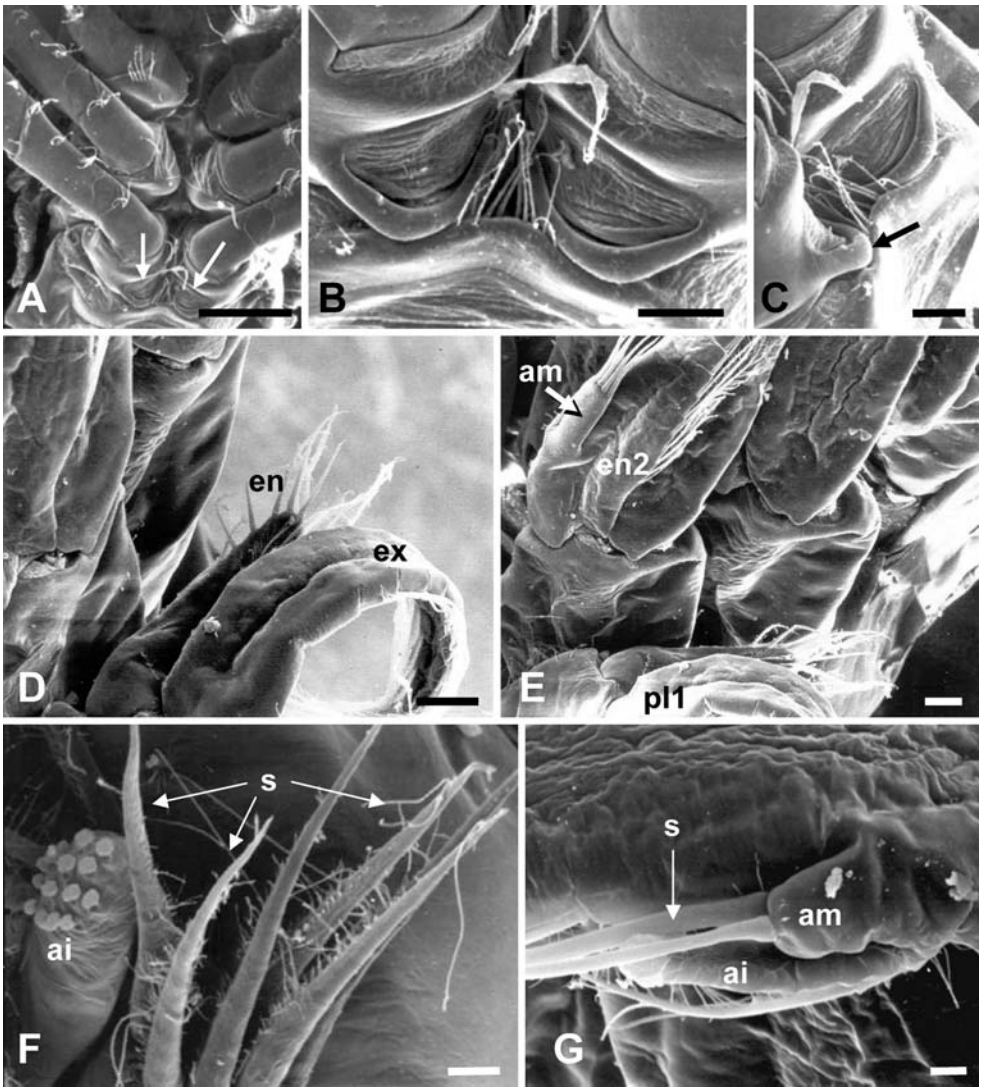

Fig. 1 Scanning electron microscopy (SEM) view of male *Hippolyte williamsi*. **a** Ventral view of the pereiopods; gonopores (*arrows*). **b** Detail of the gonopores. **c** Detail of the posterior lip (*arrow*) of the gonopore. **d–g** First and second pleopods. **d** Exopod (*ex*) and endopod (*en*) of the first pleopod. **e** Endopod of the second pleopod (*en2*), with appendix masculinae (*am*) in the inner border. **f** Distal end of the appendix interna (*ai*) and setae (s) of the appendix masculina. **g** Short appendix masculina (*am*) of immature male. *Scale bar* **a** 0.5 mm, **b–e** 100 μm, **f** 20 μm, **g** 10 μm

non-ovigerous females, respectively; Mann–Whitney Rank Sum Test; $T = 364505$, $n_{(\text{non-ovigerous})} = 800$, $n_{(\text{ovigerous})} = 877$, $P < 0.001$).

Experimental test of sex change

None of the males maintained in isolation in experiment 1 changed to females nor did they acquire any "intermediate" morphology; no widening of the second abdominal pleura or enlargement of the rostrum was noticed. Males molted between 1 and 8 times during the first experiment (3.5 ± 1.9 times during the experiment; 2.5 ± 1.2 times during 3 months, considering all shrimps alive until the third month; 5.0 ± 1.9 times during 6 months considering only those shrimps alive until the sixth month) (Fig. 6). The final body size attained by these focal males (3.7 ± 0.38 mm CL) was greater than that observed for males collected from the field (2.8 ± 0.57 mm CL) (Mann–Whitney Rank Sum Test; $T = 31855.5$, $n_{(\text{experiment males})} = 20$, $n_{(\text{field males})} = 1{,}762$; $P \leq 0.001$) (Fig. 6). These data suggest that males in the laboratory were in good condition and that the absence of sex change was not due to any experimental artifact such as detrimental food conditions.

In the second experiment, males molted several times (number of molts not recorded during this experiment) before either dying or until the experiment was terminated.

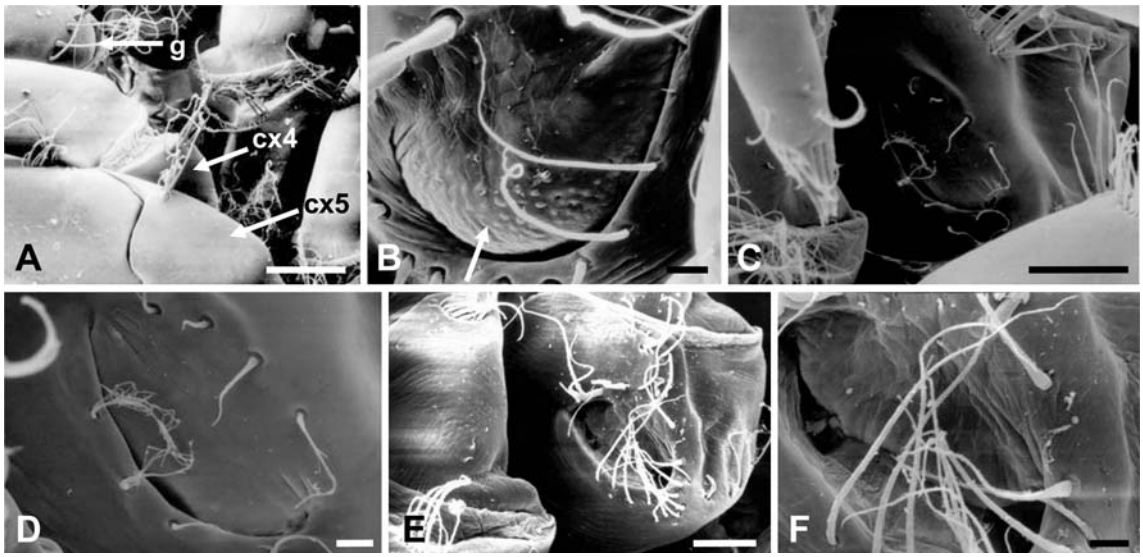

Fig. 2 SEM view of female *Hippolyte williamsi*. **a** Mature female gonopore (*g*), fourth (*cx4*) and fifth (*cx5*) coxae showing no sign of male gonopore. **b** Detail of the gonopore of mature females (*arrow* points to the gonopore). **c** Gonopore of immature female. **d** Detail of **c**. **e** Open gonopore of purportedly spawning female. **f** Detail of **e**. *Scale bar* **a** 200 µm, **b**, **d** and **f** 20 µm, **c**–**e** 100 µm

In this last experiment, insemination of females by males was occasionally observed in the MF treatment, confirming the good condition of male shrimp. The time to death registered for focal males in the second experiment was similar to that of males in the first experiment: eight out of 20 (40%) focal males were alive at the end of the fourth month in the first experiment while 12 out of 60 (20%) of the focal males were alive at the end of the second experiment (this experiment lasted 4 months) (Chi-square of independence; $\chi_1^2 = 3.841$, $P > 0.05$). The somewhat elevated mortality observed in the second experiment was due to cannibalism, mostly observed in the MO treatment. On more than one occasion, some individuals were observed feasting on the flesh of another shrimp that had recently molted.

Discussion

In *Hippolyte williamsi*, the population is composed of both males and females. On average, females were larger than the males, and ovigerous females were larger than non-ovigerous females. Although this size frequency distribution of the sexes agrees with expectations for protandric hermaphrodites (Bauer 2004), no transitional individuals were collected in the field. Also, laboratory experiments demonstrated that males might live and grow steadily for months before dying without experiencing any morphological change that might denote sex change. All this information represents strong evidence that the studied species is not a hermaphrodite but a gonochorist in contrast to that reported for other congeners (Zupo and Messina 2007; but see Cobos et al. 2005).

Sex change may depend on extrinsic factors such as food (Zupo and Messina 2007) or the social environment (Baeza and Bauer 2004; Baeza 2007b). It could be argued that males in the first experiment might not have changed to females due to the absence of environmental stimuli, including social cues, which trigger sex change. The need for such cues triggering changes between ontogenetic phase is recognized for many marine invertebrates, including shrimps (e.g., during settlement—do Santos et al. 2004). On the other hand, experiments conducted in the sequential-simultaneous hermaphroditic shrimp *Lysmata wurdemanni* have demonstrated that males invariably change to the terminal sex phase (hermaphrodites) before dying, even when not exposed to any social cues (Baeza 2007b). The results from our second experiment where males were exposed to different social environments confirmed that none of the males changed to females. Also, many of the males in our first rearing experiment attained sizes larger than that reported for males from the field. That males lived for a long time and grew steadily in the laboratory further supports the idea that *Hippolyte williamsi* is gonochoric.

On the other hand, sex change in *H. williamsi* might not be obligatory but facultative, as suggested for *C. crangon* where less than 2% of the males in the population turn to females (laboratory observations extending over 8 months—Schatte and Saborowski 2006). Also, in the putatively obligatory sex changing *C. franciscorum,* laboratory experiments have demonstrated morphological evidence of sex change only in 2 out of 40 experimental males (Gavio et al.

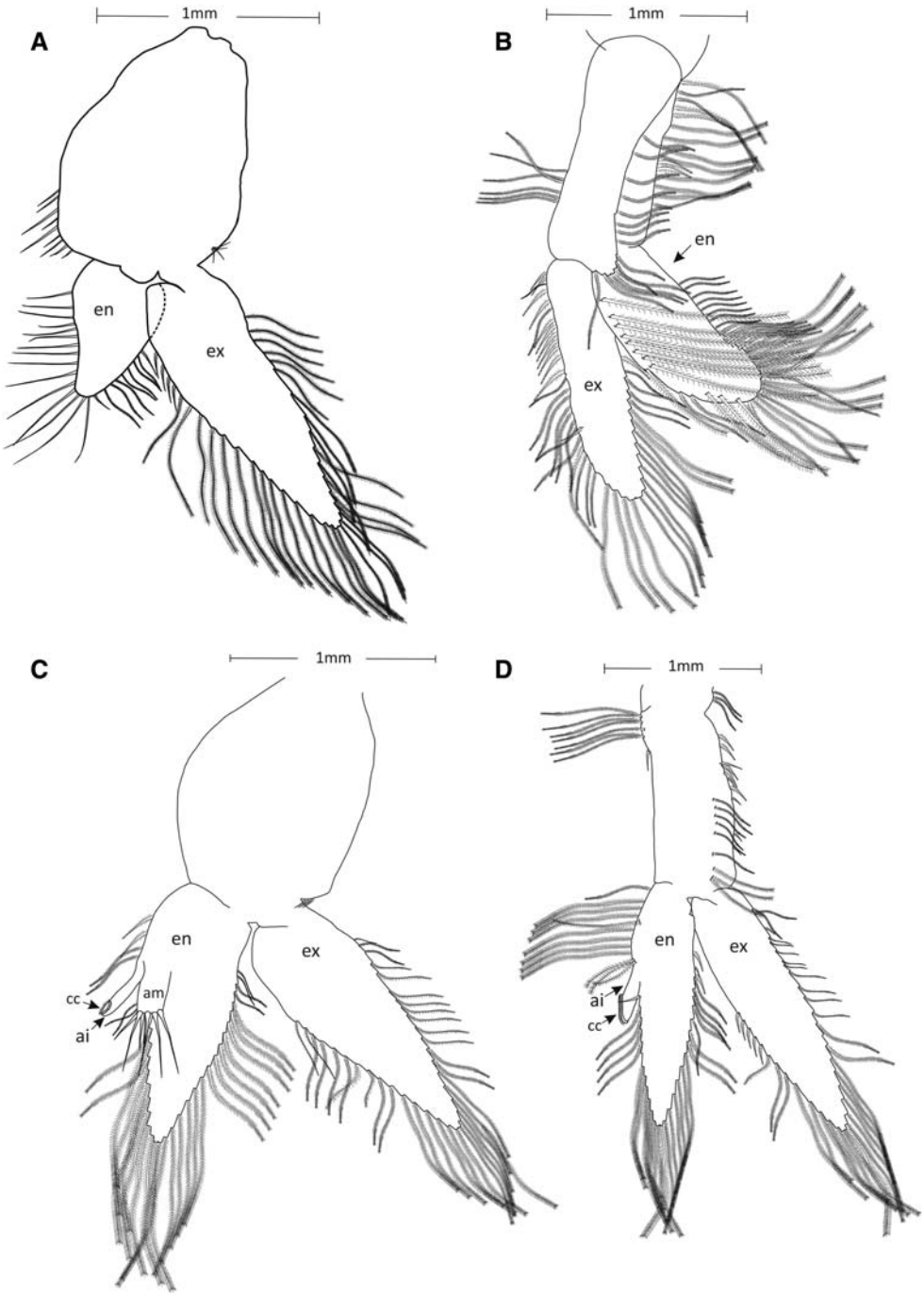

Fig. 3 Pleopods of *Hippolyte williamsi*. **a** First pleopod of male showing exopod (*ex*) and endopod (*en*). **b** First pleopod of female. **c** Second pleopod of male with appendix masculina (*am*) and appendix interna (*ai*) bearing cincinulli (*cc*). **d** Second pleopod of ovigerous female (eggs removed) with appendix interna (*ai*)

2006). If sex change in the studied species is facultative rather than obligatory and the probability of males changing sex in *H. williamsi* is as low as in *C. crangon* or *C. franciscorum*, then our low number of replicates and males (a total of 20 and 60 males in the first and second experiment, respectively) could easily lead to sex change in this species going undetected. Furthermore, although mortality did not differ between our first and second experiment, the chances of detecting facultative sex change in the second experiment might have also been reduced because several recently molted males were consumed by conspecifics. Despite the frequency of sex change in *Crangon* species was extremely low, careful morphological and anatomical examination in *C. crangon* and *C. franciscorum* had confirmed the existence of intermediate individuals with both male and female characteristics (either males with developing oocytes or females with atrophied vas deferens—Boddeke et al. 1991; Gavio et al. 2006; Schatte and Saborowski 2006).

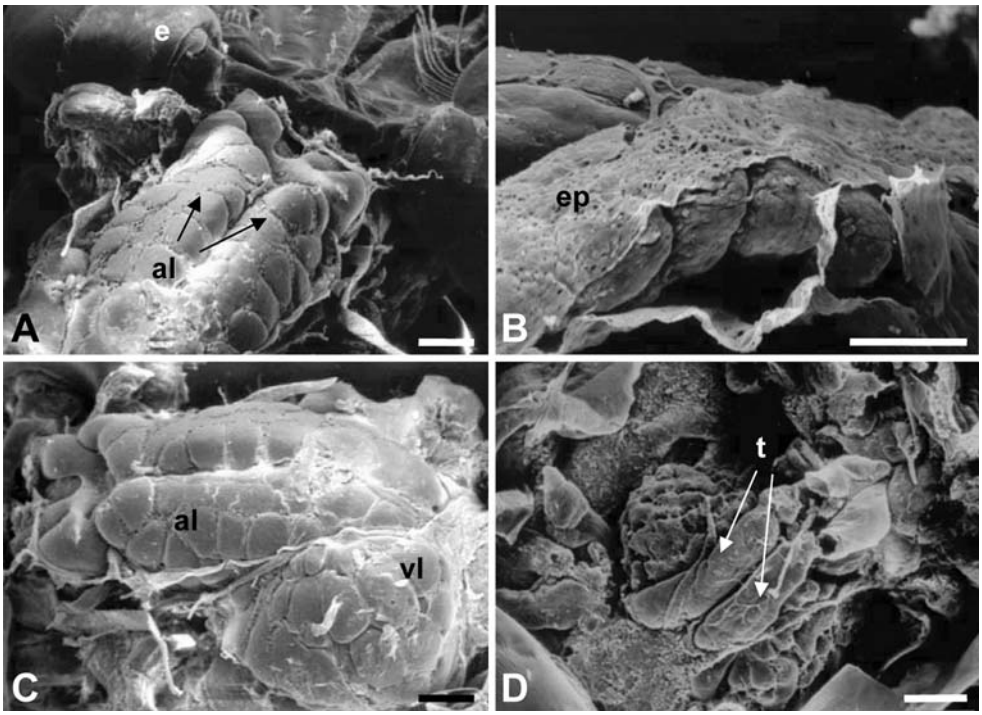

Fig. 4 SEM view of the male and female gonads of *Hippolyte williamsi*. The dorsal carapace was removed to show gonad morphology in each micrograph. **a** Ovary of mature female; anterior lobes (*al*), eye (*e*). **b** Ovary covered with epithelium (*ep*). **c** Lateral view of mature ovary; anterior lobe (*al*), ventral lobe (*vl*). **d** Testes (*t*) of mature male. All *scale bars* = 200 μm

In *H. williamsi*, no intermediate shrimps were recorded from relatively large samples (more than 1,000 shrimps in two out of three samples) taken at three different occasions in two different years. Furthermore, careful dissections of selected individuals offered no indication of intermediate stages based on internal organs. Although future studies need to examine the possibility of facultative sex change in the studied species, all presently available information suggests that *Hippolyte williamsi* is gonochoric.

Hippolyte williamsi features marked sexual dimorphism: on average, females from the natural environment were larger than males. This size difference between the sexes contrasts with that reported for gonochoric shrimps that live in socially monogamous heterosexual pairs (e.g., in *Pontonia margarita* males are only slightly smaller than females – Baeza 2008b) or featuring mating systems in which female monopolization by males during mating interactions is common (e.g., in *Rhynchocinetes typus* males are larger than females – Correa and Thiel 2003). In socially monogamous shrimps, where sexual selection is weak, body size differences between the sexes are absent or are comparatively small (Baeza 2008b). In species with strong hierarchical dominances, males defend females, and thus, sexual dimorphism in body size and structures that may be potentially used as weapons should be favored by intrasexual competition (Baeza and Thiel 2007). In *Hippolyte williamsi*, shrimp were observed as loose dense aggregations in seagrass beds, a very heterogeneous habitat that together with high population density might impede successful monopolization of receptive females by a single dominant male. Under these circumstances, it might be advantageous for males to roam and constantly search for receptive females rather than attempting to monopolize them. This behavior might favor small body size because that leads to an increase in agility and encounter rates with receptive females (Shuster and Wade 2003; Baeza and Thiel 2007). The fact that many males in the laboratory reached sizes found in only a small proportion of the males in the field suggests that males have the potential to grow to larger sizes under suitable conditions. Possibly, natural selection (due to fish predation) eliminates many males in the field before reaching their maximum size. Larger males might be better in defending mates against other males, but might simultaneously be more exposed to visual predators, which are common in seagrass beds (Woods 2002). The actual reasons for the small male size of *H. williamsi* in the field is presently unknown, but this system seems to offer a unique opportunity to examine the interaction between natural and sexual selection, and to test whether small males have higher mating possibilities than large males in environments with high predation pressure (see also Wellborn and Cothran 2007).

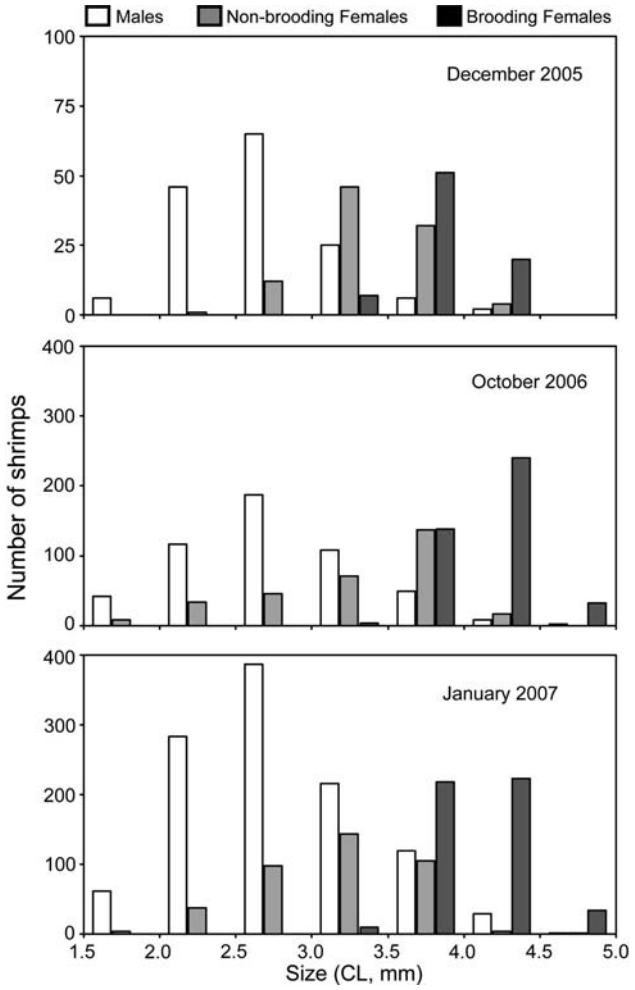

Fig. 5 Population structure of *Hippolyte williamsi* at Puerto Aldea, Coquimbo, Chile, during December 2005 ($n = 323$), October 2006 ($n = 1{,}244$), and March 2007 ($n = 1{,}973$)

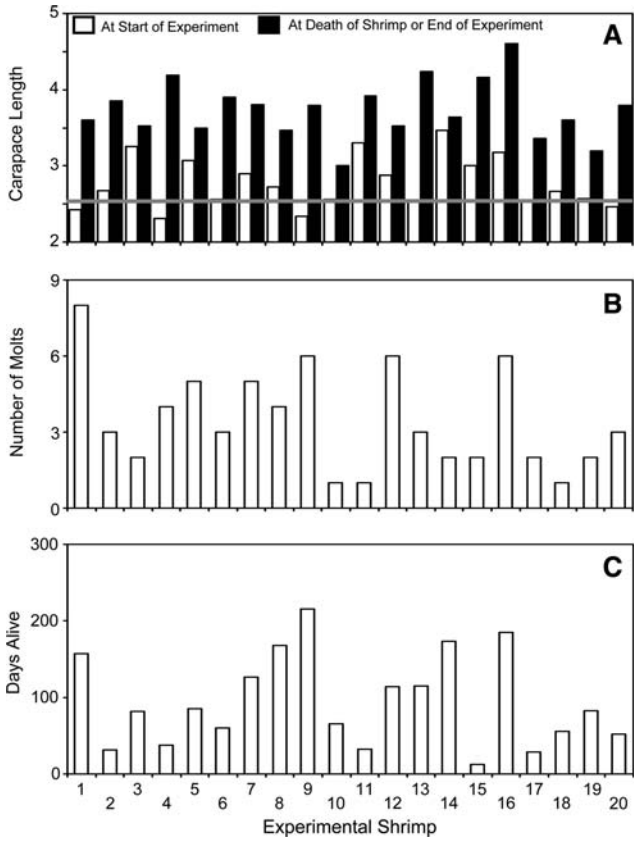

Fig. 6 Experimental test for sex change in *Hippolyte williamsi*. **a** Carapace length at the start and at the end of the first laboratory experiment in a total of 20 experimental male shrimps. **b** The number of molts experienced by shrimps during the experimental period. **c** The number of days that shrimps remained alive during the experiment. The *horizontal grey line* indicates the average size of males (2.5 mm CL) collected in their natural environment ($n = 1{,}762$ males collected in December 2005, October 2006, and January 2007)

Overall, our study represents an example in which a combination of size measurements, growth experiments, examination of external morphology and internal anatomy assisted in characterization of the sexual system of a marine shrimp. This approach will assist in establishing shrimps, in particular the highly diverse family Hippolytidae with the diverse genera *Thor*, *Lysmata* and *Hippolyte*, as model systems to investigate the evolution of sexual systems. A comparative framework might prove most useful to accomplish this task; after correcting for shared ancestry, it should be possible to reveal the environmental (including social) conditions favoring or constraining particular sexual systems (as well as other traits) (see Harvey and Pagel 1991). In particular, the genus *Hippolyte* might be a worthwhile study system because most species live in seagrass and algae beds, i.e. they face similar habitat factors, including food and structural protection from predators (Table 1). An initial comparison indicates substantial size differences between males and females of the species from this genus (Table 1). Future studies might show to which degree sexual selection and natural selection influence the reproductive success of the sexes and their optimum body size. It remains to be shown whether divergent sexual fitness functions favor the evolution of protandric hermaphroditism in the species from this genus.

At present only three species from the genus *Hippolyte* have been carefully examined for their sexual system, using more than one kind of evidence (i.e., *H. inermis*, *H. obliquimanus* and *H. williamsi*—Table 1). Protandric hermaphroditism has been suggested for a number of other *Hippolyte* species, primarily based on sexual size dimorphism. However, two recent studies demonstrated that size dimorphism is not necessarily a reliable indicator for the sexual system of the shrimp from this genus (Terossi et al. 2008; this study). Collaborative efforts involving evolutionary biologists, physiologists and molecular ecologists from different continents and countries will not only help to (re)confirm the sexual system of the species from the genus *Hippolyte*

Table 1 Socioecology of shrimps from the genus *Hippolyte*

Species	Distribution	WD	H[a]	RP[b]	TBL	MBL	MCL	FBL	FCL	SR	SS[c]	EVI[d]	References
H. acuta	S Japan												d'Udekem d'Acoz (1996)
H. australiensis	SE Australia	0–15	Al		25								d'Udekem d'Acoz (1996, 2001)
H. bifidirostris	New Zealand												d'Udekem d'Acoz (1996)
H. californiensis	NE Pacific	0–10	Sg										d'Udekem d'Acoz (1996) and Wicksten (1990)
H. caradina	Australia, New Caledonia												d'Udekem d'Acoz (1996)
H. catagrapha	South Africa	6–8	Sy		22								d'Udekem d'Acoz (2007)
H. clarki	NE Pacific	0–10	Al										d'Udekem d'Acoz (1996) and Wicksten (1990)
H. commensalis	Indo-Pacific												d'Udekem d'Acoz (1996)
H. coerulescens	Central Atlantic	0–1	Al		16.5	13		12.8					Williams (1984), d'Udekem d'Acoz (1996) and Hacker and Madin (1991)
H. edmondsoni	Hawaii												d'Udekem d'Acoz (1996)
H. garciarasoi	NE Atlantic, Mediterranean	0–15	Sg, Al	Su, Fa			0.9	15	1.6	1.8			d'Udekem d'Acoz (1996) and Koukouras and Anastasiadou, (2002)
H. holthuisi	Mediterranean	7–50	Al	Fa	19		1.4		3.5	2.5			d'Udekem d'Acoz (1995) and Koukouras and Anastasiadou, (2002)
H. huntii	E Atlantic, Mediterranean		Sy		17								Ceidigh and McGrath (1978)
H. inermis	NE Atlantic, Mediterranean	0–30	Sg	Sp-Fa		29	4.1	45	7	1.6	PH	S, G, M, H	d'Udekem d'Acoz (1996) and Koukouras and Anastasiadou, (2002)
H. jarvinensis	Central Pacific, Indo-Pacific												d'Udekem d'Acoz (1996)
H. kraussiana	S Africa, Madagascar		Sg				0.9		1.8	2.0	(PH)	S	d'Udekem d'Acoz (1996) and Torres et al. (2007)
H. lagarderei	E Atlantic	0–1	Al		22	14		16.5		1.2			d'Udekem d'Acoz (1995, 1996)
H. leptocerus	NE Atlantic, Mediterranean, Black Sea	0–30	Sg, Al	Sp-Fa		17	2.5	22	6.1	1.3			d'Udekem d'Acoz (1996) and Koukouras and Anastasiadou, (2002)
H. leptometrae	NE Atlantic, Mediterranean	95–130	Sy		18								d'Udekem d'Acoz (1996, 2007)
H. longiallex	Tropical West Africa	35–40	Sy		8								d'Udekem d'Acoz (2007)
H. longirostris	NE Atlantic, Mediterranean		Sg	Su-Fa		17		22		1.3	(PH)	S	Céidigh et al. (1982) and Schaffmeister et al. (2006)
H. multicolorata	New Zealand												d'Udekem d'Acoz (1996)
H. nicholsoni	Caribbean, West Indies	0–28	Al	Var			1.26		1.84	1.5		S	Spotte et al. (1995), Spotte and Bubucis (1996) and d'Udekem d'Acoz (1996, 2007)
H. niezabitowskii	Mediterranean	0.5–5	Sg, Al	Fa		10	2.9	20	4	2.0	(PH)	S	d'Udekem d'Acoz (1996), Koukouras and Anastasiadou (2002) and García Raso et al. (1998)
H. obliquimanus	W Atlantic, Caribbean, Brazil	0–2	Sg, Al		15		2.5		3.2	1.3	GC	S, G, M	d'Udekem d'Acoz (1996) and Terossi et al. (2008)
H. palliola	E Atlantic	0–25	Al					10					d'Udekem d'Acoz (1996)
H. pleuracanthus	NW Atlantic		Sg, Al	Sp-Fa		<♀		18					Shield (1978), Williams (1984) and d'Udekem d'Acoz (1996)

Table 1 continued

Species	Distribution	WD	H[a]	RP[b]	TBL	MBL	MCL	FBL	FCL	SR	SS[c]	EVI[d]	References
H. prideauxiana	E Atlantic, Mediterranean	0–60	SY			10		21		2.1			d'Udekem d'Acoz (1996)
H. proteus	Red Sea				13								d'Udekem d'Acoz (1996)
H. sapphica	Mediterranean	0–1.5	Sg, Al	Sp-Su		12	1.5	27	3.2	2.3			d'Udekem d'Acoz (1996) and Koukouras and Anastasiadou, (2002)
H. varians	NE Atlantic, Mediterranean	0–60	Al	YR	31.5	20	3.4	32	5	1.6	(PH)	S	d'Udekem d'Acoz (1995, 1996) and García Raso et al. (1998)
H. ventricosa	Red Sea, India	0–5	Sg, Al	Sp-Wi	17		2.5		4.5	1.9	(PH)	S	d'Udekem d'Acoz (1999) and Yanagawa and Watanabe (1988)
H. williamsi	E Pacific (Mexico-Chile)	0–10	Sg, Al			24	4.8	28	5	1.2	GC	S, G, M, H	This study
H. zostericola	NW Atlantic, Bermuda, E Pacific		Sg	Sp-Fa		14	4.75	15.5	6	1.1		S	d'Udekem d'Acoz (1996) and Zupo and Nelson (1999)

For each species, distribution, water depth (WD, m), habitat (H), reproductive period (RP), total body length (TBL, mm), male body length (MBL), male carapace length (MCL), female body length (FBL), female carapace length (FCL), size ratio (SR, female to male), sexual system (SS), evidence used to determine the sexual system (EVI) and references are shown

[a] Habitat: *al* algae, *sg* seagrass, *sy* symbiont

[b] Reproductive period: *fa* fall, *sp* spring, *su* summer, *var* variable, *wi* Winter, *yr* year round

[c] Sexual system: *gc* gonochorism, *ph* protandric hermaphroditism, () suggested by authors but more information is needed

[d] Evidence: *s* size measurements, *g* growth experiments, *m* morphology and/or dissections, *h* histological studies

but also contribute to a better understanding of the evolution of hermaphroditism in caridean shrimps.

Acknowledgments JAB thanks for the support from a Smithsonian Tropical Research Institute (STRI) Postdoctoral Fellowship and a Smithsonian Marine Station at Fort Pierce (SMSFP) Postdoctoral Fellowship. JAB specially acknowledges support from Valerie Paul and the SMSFP for funding a field trip to Chile during which most of this manuscript was written. The constructive comments from three anonymous reviewers substantially improved the manuscript. This is contribution number 740 from the Smithsonian Marine Station at Fort Pierce.

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