

***Synalpheus paraneptunus* (Crustacea: Decapoda: Caridea) populations with intersex gonopores: a sexual enigma among sponge-dwelling snapping shrimps**

E. TÓTH¹* and R.T. BAUER²

¹*Smithsonian Tropical Research Institute, PO Box 0848-03092, Balboa, Ancón, Republic of Panama
Tel. +507 212-8831; Fax: +507 212-8790; email: evatoth2004@yahoo.com*

²*Department of Biology, University of Louisiana at Lafayette, Lafayette, LA 70504-2451, USA*

Received 18 December 2007; Accepted 17 April 2008

Summary

Caridean shrimp populations are usually gonochoric, i.e., composed of separate sexes; however, some carideans are protandric sex changers or simultaneous hermaphrodites. In this contribution we investigated the sexual composition and its determination in two sponge-dwelling *Synalpheus* species with different degrees of sociality. Because the usual external sexing methods (presence or absence of appendices masculinae in males) can not be used in *Synalpheus* spp., we sexed individuals by observation of male and female gonopores using scanning electron microscopy (SEM). We found that populations of two species of the *Synalpheus paraneptunus* complex consist almost entirely of individuals with both male and female gonopores (termed herein “intersexes”). Such a high incidence of intersexes has not been previously reported in other decapods. We performed morphological and behavioral observations to test various hypotheses explaining the presence of intersex gonopores. Whereas protandry and simultaneous hermaphroditism are not likely to occur in these two species, capacity of immature helpers to mature as either sex, or developmental anomaly without adaptive significance could explain the presence of both female and male gonopores in these shrimps.

Key words: Caridean shrimp, eusociality, gonochory, intersex, SEM, sexual development, *Synalpheus*

Introduction

Caridean shrimps (Decapoda) form a very diverse group of crustaceans known for variation in sexual systems (Bauer, 2000; 2004). The most prevalent sexual development is gonochory (separate sexes), in which sex is fixed and already determined in a young age. Nevertheless, about 40 species of shrimps are protandric hermaphrodites: individuals develop as males when small, but change into females as they grow larger (e.g.

Bauer, 2000, 2004). Some studies also suggest that it is possible for sex-changed females to revert back to male (Gherardi and Calloni, 1993). Similarly, in some other species individuals start out as males, but after sex change to “females” retain their male ducts and function as simultaneous hermaphrodites (*Lysmata* spp., Bauer, 2000, 2006). In some protandric species, all individuals change sex whereas in others, some individuals are sex changers while others are not, i.e., are either primary

*Corresponding author.

males or females (Bauer, 2000). The precise mechanism of sex change is largely unknown; however in *Hippolyte inermis*, a diet rich in certain species of diatoms early in development may trigger cell death of male gonadal tissue (Zupo and Messina, 2007). The influence of social environment on sex change has been proposed and/or tested in several caridean species (Bergström, 1997; Baeza and Bauer, 2004). Data on the sexual system of many caridean shrimps are available as methods for sexing of individuals using external characters have long been established (Bauer, 2004). However, identifying the sexes in the sponge-dwelling snapping shrimp *Synalpheus* (Decapods, Alpheidae) has been difficult until recently (Tóth and Bauer, 2007).

Snapping shrimps of the genus *Synalpheus* (Caridea: Alpheidae) are among the most abundant mobile crypto-fauna in tropical reefs (Bruce, 1976; Duffy, 1996a). As in another alpheid genus (*Alpheus*), the major (larger) first chela is modified into a “snapping claw” which shoots forward a powerful jet of water, resulting in an audible popping or snapping sound (Ritzmann 1974; Versluis et al., 2000). In *Alpheus* and *Synalpheus*, the snapping claw is used as a weapon in agonistic behavior, both intraspecific (territorial disputes, mate choice, e.g. Nolan and Salmon, 1970; Duffy et al., 2002) and interspecific (defense). In *Alpheus*, sexual pairs are size matched, with females generally having a slightly larger body (Schein, 1975; Hughes, 1996; Rahman et al., 2002) and relatively smaller major chela than males do (e.g. Dawes, 1934; Nolan and Salmon, 1970; Schein, 1975, 1977; Knowlton, 1980; Boltana and Thiel, 2001; Mathews, 2002).

The genus *Synalpheus* Bate, 1888 is one of the largest genera of marine crustaceans in shallow tropical waters, with over hundred species described (Chace, 1989). Most species are characterized by their association with other marine invertebrates. In the Caribbean many species belong to the *gambarelloides* group [recently proposed as a separate genus *Zuzalpheus* by Rios and Duffy, 2007; however, because of cladistic problems (Anker and De Grave, in press) in that paper, we will continue to use the genus name *Synalpheus* in the present manuscript]. Species in this group live inside sponges (Duffy, 1992, 2003), and the group is characterized by a distinctive dense setal brush on the minor first chela (Coutiere, 1909; Dardeau, 1984). Most species live in heterosexual pairs (pair-living), but a few species of this group are eusocial (Duffy, 1996b, 2003): they have overlapping generations, cooperative brood care, and reproductive division of labor as social insects (ants, termites, and some bees, wasps, thrips and aphids), one beetle species, and some mole rats do (Wilson, 1971; Choe and Crespi, 1997; Alexander et al.,

1991). Such shrimp colonies consist of one or a few reproductive females (queens) and many helpers (Duffy, 2003).

An accurate identification of the sexes is not possible in *Synalpheus* using the standard techniques of examination of the abdominal appendages, because males of this group lack an appendix masculina on the second pleopods (Banner and Banner, 1975; Felder, 1982; Dardeau, 1984). Other methods suggested for sexing *Synalpheus*, such as the presence of embryos and developed ovaries in females and their absence in males, or the rounded shape of the abdominal pleurae in females versus their hooked shape in males (e.g. Banner and Banner, 1975), are helpful in the sexing in large reproductive individuals found in strict pairs, but not when more than two individuals inhabit a sponge including non-reproductive individuals. This is a problem in the eusocial species, in which the colonies have only one or a few queens (females with the embryos). The other individuals in the colony, from several to many, are non-reproductive (“helpers” or “workers”) and have no visibly developed gonads. Previously, these helpers were assumed to be males and/or juveniles (e.g. Chace, 1972; Duffy, 1996b, 2003; Duffy et al., 2002; Tóth and Duffy, 2005; Didderen et al., 2006). This very skewed sex ratio within colonies was explained by a possible protandric sex change from male to female as occurs in some other caridean shrimps (Suzuki, 1970; Nakashima, 1987; Gherardi and Calloni, 1993; 1995; Baeza and Bauer, 2004). Unfortunately, gonadal tissue in *Synalpheus* except for reproductive females is very thin and nondescript, and thus finding these organs through dissections is unreliable (pers. obs.).

Recently, a reliable method to distinguish males, non-reproductive females, and juveniles was described (Tóth and Bauer, 2007). Scanning electron microscopy (SEM) was utilized to observe the position of female and male gonopores of helpers in the colony. In carideans, the female gonopores are located on the third pair of pereopods (first walking legs) while male gonopores are found on the coxae of the fifth pair of pereopods (third or last pair of walking legs). Sexing with SEM showed that colony helpers consist of males and females in equal sex ratios (Tóth and Bauer, 2007). We also confirmed that in pair-living species, one individual (with embryos) was a typical caridean female with respect to gonopore position, and the other non-ovigerous individual was the male (gonopores on the fifth pereopod coxae; Tóth and Bauer, in prep).

There is one sponge-dwelling group, the *S. paraneptunus* complex, which deviates from others in the *gambarelloides* group by having only a sparse group of setae on the smaller first chela. In some species of this

group, individuals are characterized by rounded abdominal pleura (female character) not only in individuals carrying embryos or with developed ovaries, but also in individuals lacking those features (presumably males or non-reproductive females). The purpose of the study is to analyze the sexual system (gonochory vs. protandry etc.) of two species (Anker and Tóth, submitted). One is an apparently pair-living species and the other is eusocial (conditionally termed here *S. paraneptunus* “A” and *S. paraneptunus* “small,” respectively; Duffy, 2003). In this contribution we analyze, using gonopore sexing by SEM, the sexual composition of colony members and their queens in the eusocial species and of large individuals originating from three sponges in the other species. To further explore sexuality in these species, we dissected another set of individuals to look for male ejaculatory ducts or sperm. We tested for an indication of sex change (i.e. sequential or simultaneous hermaphroditism) by examining whether smaller individuals function as males while larger ones function as females. We also performed field experiments with *S. paraneptunus* “A” to test the hypothesis of simultaneous hermaphroditism. Finally, we investigated possible sexual dimorphism in body size and chela (weapon) size, found in many other alpheids (e.g. Schein, 1975; Tóth and Duffy, in press).

Material and Methods

Sample collection

Samples were collected using SCUBA and snorkeling at two sites in Panama during 2003 and 2006. *S. paraneptunus* “A” was collected from Bocas del Toro (09°21' N, 82°15' E) and *S. paraneptunus* “small” from the Isla Grande region (09°38' N, 79°34' E). These two species do not overlap in their habitat nor in their sponge hosts; moreover, they differ in an array of morphological characters (Anker and Tóth, submitted). The larger shrimp inhabits the sponge *Xestospongia rosariensis*, Zea and Rützler, 1983, and depending on sponge size, they can be found as a series of adults, with (presumptive females), or without embryos (presumptive males), as well as a few smaller individuals (presumptive subadults and juveniles) in the same host. Usually, the ratio of larger individuals with embryos to individuals without embryos originating from the same sponge, are very close to each other, suggesting an aggregation of a few sexual pairs (we refer to them as “paired” species in the rest of the manuscript). The smaller eusocial species, *S. paraneptunus* “small”, inhabits a sponge tentatively identified as *Calyx podatypa*, de Laubenfels, 1934; individual sponges are

usually inhabited by a single colony, ranging from 17–80 individuals, with only one reproductive female (queen).

Morphometry and sexing

To assess sexual dimorphism, morphometric data were collected by measuring, for each shrimp, chela length and body length using a dissecting microscope equipped with a camera lucida. The body dimension to be measured was drawn and then measured from the tracing using a micrometer scale drawn at the same magnification. We recorded body length (BL) as following the body curvature of the individuals from the tip of rostrum to the posterior margin of the telson. Chela length (ChL) was measured as a straight line from the tip of the fixed (immovable) finger to the proximal edge of the chela (Tóth and Bauer, 2007; Tóth and Duffy, in press). We then prepared individuals for scanning electron microscopy (SEM). Shrimp were initially preserved in 95% ethanol and were taken through the solution series: 95% ETOH to 100% ETOH, then 50:50 ETOH: hexamethyldisilazane (HMDS), and finally 100% HMDS, with at least 1 h in each solution. Specimens were drained of HMDS and air dried. Several dried specimens (up to 26, depending on size) were attached with adhesive to round stubs of 25 mm in diameter for sputter-coating with 40–80 nm gold prior to observation with a Hitachi S-3000-N scanning electron microscope. The presence or absence of gonopores on the coxae of the third and fifth pereopods was recorded for each specimen.

We analyzed 44 individuals from *S. paraneptunus* “A” in a range of body sizes, from 7.3 to 18.2 mm, and additionally three very small (possible juvenile) individuals (BL: 5.0, 5.0, and 6.4 mm). From the eusocial *S. paraneptunus* “small” we sexed one whole colony of 46 helpers with their one queen.

Dissections

In an additional set of individuals we dissected out the fifth pair of coxae of *S. paraneptunus* “A.” We selected and dissected out the coxae of 20 larger individuals of that did not show developed ovaries and carried no eggs (“presumptive males”), ranging from 7.7–10.9 mm BL) and from 20 individuals that had a variety of degrees of ovarian development or eggs (“presumptive females,” 7.85–5.7 mm BL), as well as 20 smaller individuals (4.3–6.6 mm BL). We dissected the left coxa out of each individual and pulled out tissue of the coxa where the ejaculatory duct should be and placed the macerated tissue on a microscope slide,

stained it with acid fuchsin dissolved in low viscosity CMC (Polysciences) and searched for ejaculatory ducts or sperm cells. We used the same procedure for an entire colony of 24 *S. paraneptunus* “small” individuals.

Pairing experiment

Given that individuals of *S. paraneptunus* “A” (paired species) have both male and female gonopores (see Results), we conducted experiments with living animals to investigate if individuals with ovarian development were capable of functioning as male as well as female, i.e., are simultaneous hermaphrodites. We prepared nine pairs of individuals reproductively active as females (with embryos or obvious ovarian development) and introduced them into empty sponges from which the previous shrimp inhabitants had been expelled. We paired one larger individual that carried late stage embryos (eyes present and yolk minimal) and that would release the embryos and spawn again within a few to several days, with another smaller individual that carried no embryos, but had some visible ovarian development (young female). These artificially paired individuals were individually recognizable because one of them was bigger than the other; moreover, they carried the large claw on opposite sides of their bodies. We offered these pairs a piece of their host sponge (10–15 cm length and 8–17 cm in diameter, from which we expelled the resident shrimps by enlarging the exit holes after which we dipped the sponge pieces repeatedly into fresh water. We introduced the experimental pairs and then placed these sponges into separate aquaria. We allowed these pairs to be together for 21–29 days, after which the sponges were destroyed and the shrimp pairs were collected. We examined the shrimps to see if one of the individuals was carrying newly spawned embryos, which would show that a recent mating event had occurred successfully, i.e., one individual had functioned as a male and one as a female.

Statistical analysis

We used Chi-square goodness of fit analyses ($df=1$) to test whether the observed ratios differed from expected ratios among the different size classes in these two species.

Results

Sexing

In *S. paraneptunus* “small,” female gonopores were similar to those found in other *Synalpheus* species sexed with SEM (Tóth and Bauer, 2007). Situated on the coxae of the third pereopods, the female gonopores are

semicircular slits with “egg-guiding” setae just distal to them (Fig. 1A–C). Male gonopores are openings borne on gentle protuberances on the medial sides of the coxae on the fifth pereopods (Fig. 1A,E,F). In the *S. paraneptunus* “small” colony examined, all individuals including the queen were “intersexes” with both male and female gonopores except for two small individuals in which female gonopores were not visible or open (Fig. 2A, Fig. 1D).

Similarly, SEM sexing revealed that all individuals of *S. paraneptunus* “A” were intersexes (Fig. 3A) with both female (Fig. 4A,B) and male (Fig. 4A,C) gonopores, including the three smallest individuals (body length: 5.9, 6.1, 6.2 mm). In one individual, the male gonopores were not clearly open (Fig. 4D,E), and in another the male gonopores were rudimentary (Fig. 4F).

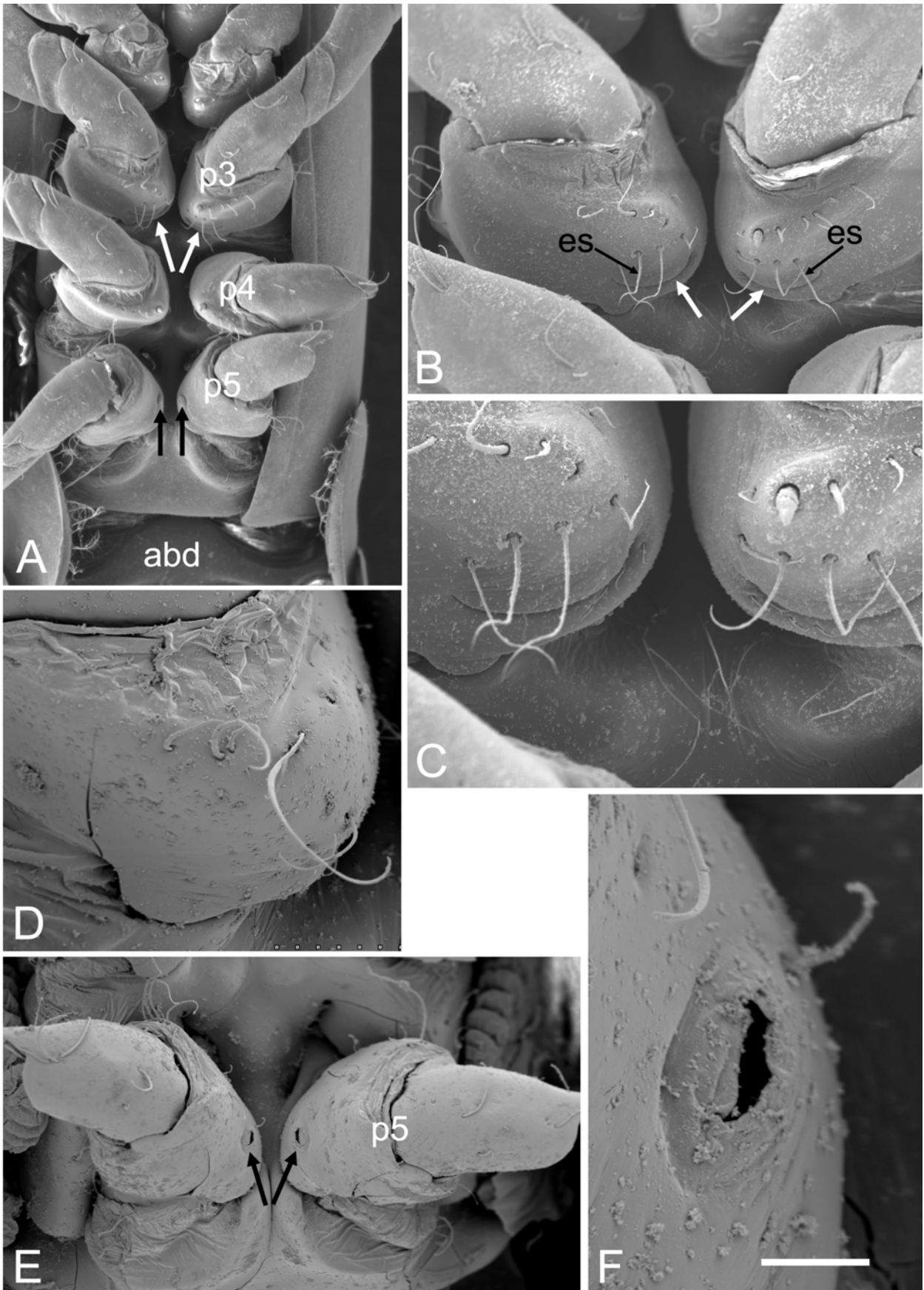
Morphometry

Morphometrical measurements of all individuals in two *S. paraneptunus* “small” colonies show that the queen is the largest individual but does not have the largest chela (Fig. 2A,B). Visual sexing of *S. paraneptunus* “A” individuals (Fig. 3B) based on the presence or absence of developed ovaries/embryos, shows that functional females (queens) in general are larger than animals without developed ovaries, confirming the trend found by others (Tóth and Duffy, in press).

Dissections

Although we were able to distinguish females with egg-filled ovaries from other individuals, the details of gonad morphology were not discernible upon dissection of preserved material. We found sperm in only three of the 24 *S. paraneptunus* “small” individuals investigated.

Fig. 1. Gonopores of *Synalpheus paraneptunus* “small.” A: Ventral view of thoracic area, showing position of posterior pereopods. B, C: Lower and higher magnifications of coxae of the third pereopods, showing the female gonopore slits and “egg-guiding” setae. D: Right coxa from third pereopod of one individual lacking female gonopores. E: Basal segments of the fifth pereopods, with open male gonopores on the coxal segments. F: High magnification of male gonopore from right fifth coxa shown in E. abd, underside of first abdominal segment; es, “egg-guiding” setae; p3, p4, p5, the basal segments (coxa, basis) of the 3rd, 4th, and 5th pereopods (walking legs 1, 2, and 3, respectively); unmarked white arrows indicate female gonopores (u-shaped slits on coxae of pereopods 3); unmarked black arrows indicate male gonopores on the coxae of pereopods 5; scale bar in F represents 360 μm in A, 170 μm in B, 70 μm in C, 50 μm in D, 65 μm in E, and 25 μm in F.



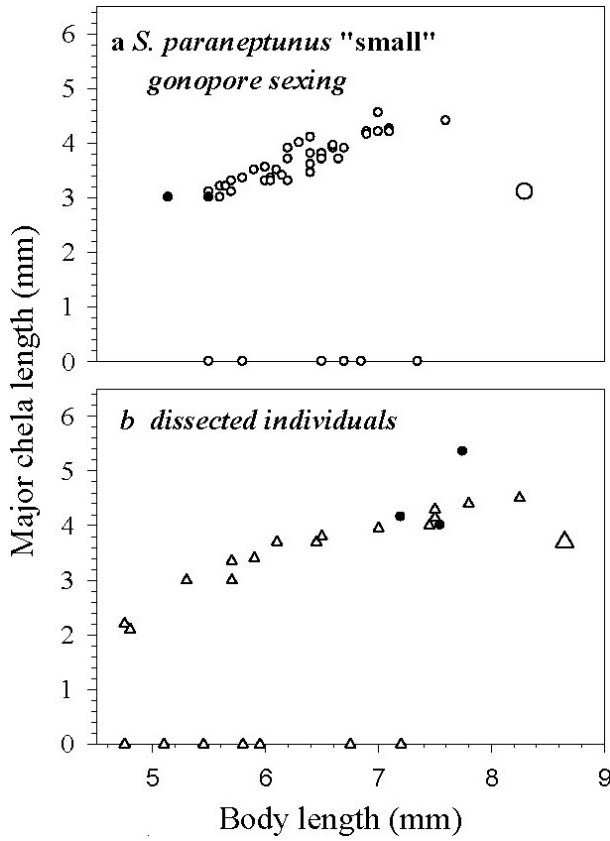


Fig. 2. Size relationships (body length and major chela length) for two *Synalpheus paranepentunus* "small" colonies, helpers (smaller symbols) and queens (larger symbol). Chela sizes of 0 mm indicate individuals that lost their large claw during collection. A: Results for individuals sexed with SEM. Open symbols denote individuals with both female and male gonopores present. Filled symbols denote individuals where the female gonopores were not visible or open. B: Individuals with their fifth gonopores dissected. Open symbols indicate sperm absent, closed symbols indicate sperm present.

Assuming a 50% sex ratio of a gonochoristic species (Tóth and Bauer, 2007), we would have expected to find sperm in a larger number of individuals ($\chi^2 = 13.5$, $P = 0.0002$). Assuming protandric sex change, in contrast, we would have expected to find sperms in the majority (more than 50%) of investigated individuals ($\chi^2 = 18.4$, $P < 0.0001$). All three individuals with sperm were in the largest third of the measured individuals, one of them had the largest chela, and the other two individuals were among upper third of the individuals by chela size (see Fig. 2B).

In the paired species *S. paranepentunus* "A," we found sperm in 15 of the 20 presumptive males, i.e., individuals that did not show ovarian development, which is not significant from what expected assuming that those individuals were all males ($\chi^2 = 1.25$, $P = 0.26$). Sperm

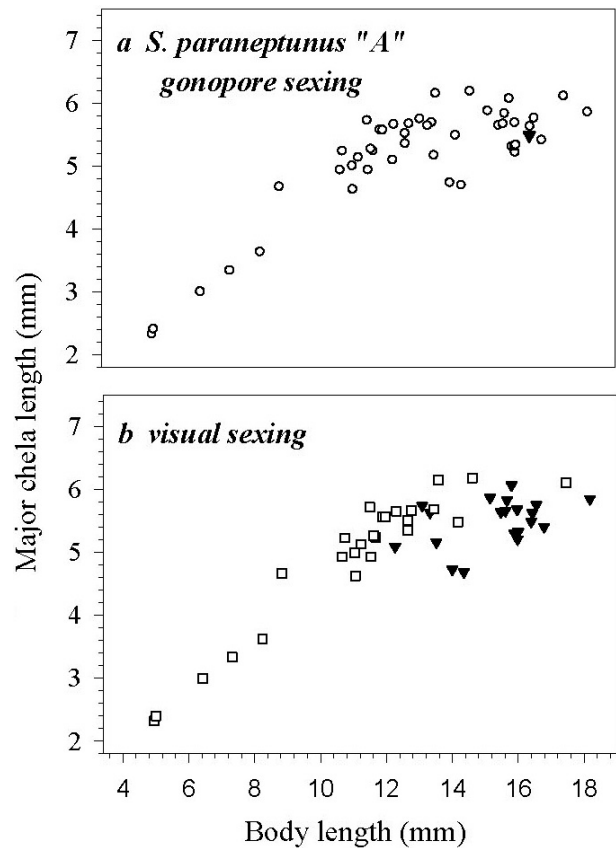
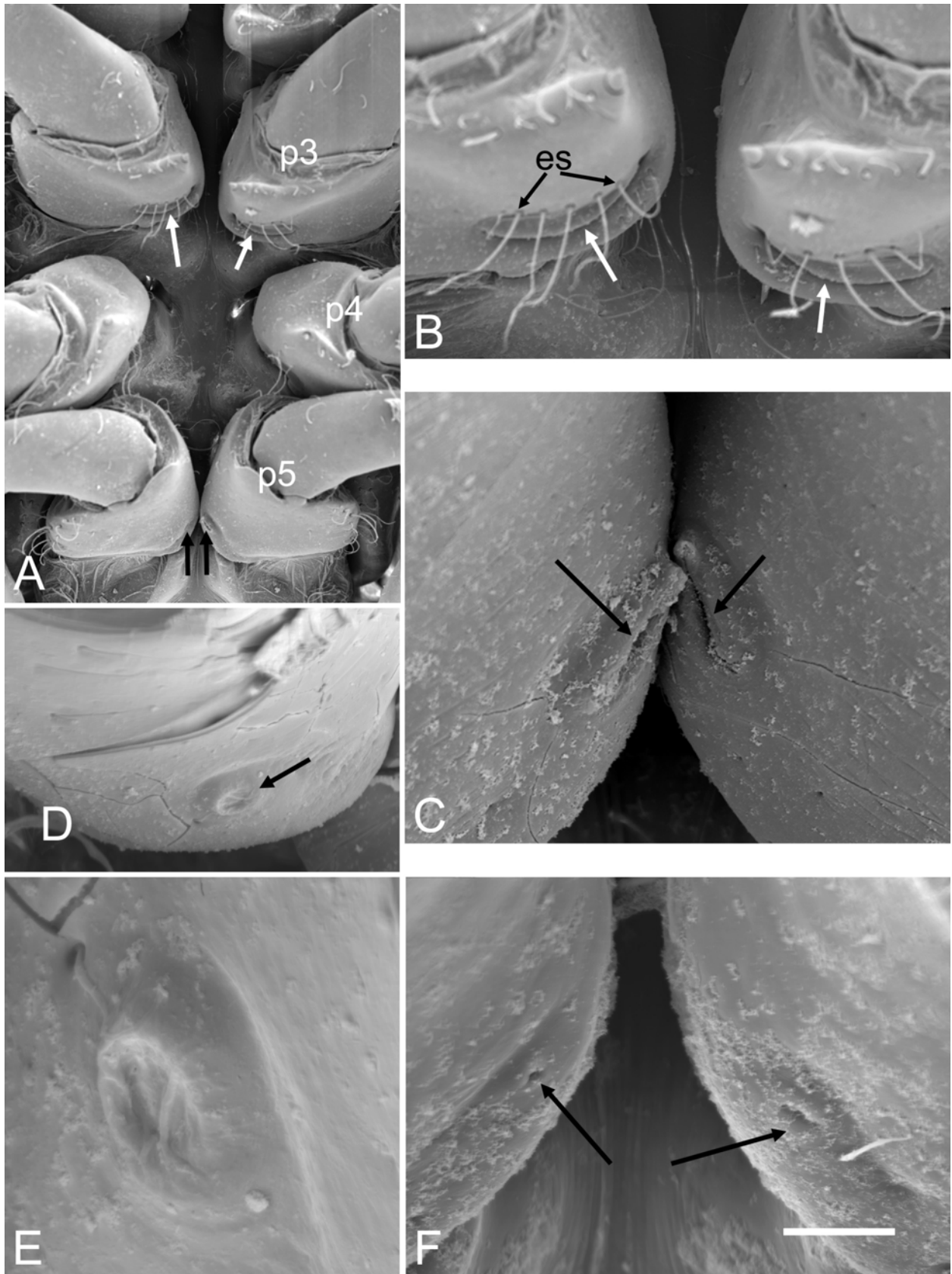


Fig. 3. Size relationships (body length and major chela length) for 44 individuals of *S. paranepentunus* "A". A: Results based on sexing with SEM. Open symbols indicate individuals possessing both female and male gonopores, closed symbol indicate individual with rudimentary male gonopores. B: Results based on visual sexing of individuals shown in A. Open symbols indicate individuals with no ovarian development or embryos; closed symbols denote individuals with ovarian development and/or embryos.

Fig. 4. (opposite). Gonopores of intersexes, *Synalpheus paranepentunus* "A." A: Ventral view of thoracic area and basal segments from pereopod 3 (first walking leg) to pereopod 5 (fifth or last walking leg). B: Coxae of pereopod 3, with female gonopores and line of "egg-guiding" setae just above the gonopore. C: Male gonopores on the coxae of pereopods 5. D: Coxa and apparently closed male gonopore of pereopod 5 in one individual. E: Higher magnification of male gonopore in D. F: Rudimentary male gonopores from the p5 coxae of one of the three smallest individuals collected (5.9–6.2 mm CL). es, "egg-guiding" setae; p3, p4, p5, the basal segments (coxa, basis) of the 3rd, 4th, and 5th pereopods (walking legs 1, 2, and 3, respectively); unmarked white arrows indicate female gonopores (u-shaped slits on coxae of pereopods 3); unmarked black arrows indicate male gonopores on the coxae of pereopods 5; scale bar in F represents 370 μm in A, 140 μm in B, 55 μm in C, 80 μm in D, 13 μm in E, and 23 μm in F.



were not found in any of the 20 individuals examined that had ovarian development and/or carried embryos (“presumptive females”) which is different from expected according to a hypothesis that females are simultaneously hermaphroditic. Only two of the 20 smaller individuals dissected were carrying sperm, which is significantly different from that expected under a hypothesis of protandric sex change, in which most (>50%) of these individuals would be functioning as males ($\chi^2 = 12.8$, $P < 0.0001$).

Pairing experiment

We tested the hypothesis of simultaneous hermaphroditism in *S. paraneptunus* “A,” i.e. to determine if individuals reproductively active as females (“females”) might also be able to function as males. However, the results did not confirm such activity. In eight of the nine experiments with two individuals with developing ovaries placed in the same sponge, no individuals produced embryos, indicating no successful insemination and fertilization. In one case, the larger individual did have recently spawned embryos; however, an additional shrimp (a larger *S. paraneptunus* “A” without ovarian development; a presumptive male) was found in the sponge which was apparently not expelled from the sponge prior to introduction of the experimental pair as was assumed).

Discussion

Our results show that, based on the presence of female and male gonopores, almost all individuals of the two species of *S. paraneptunus* examined in this study were intersexes. Although parastacid crayfishes often have high percentages of individuals with both male and female gonopores (Rudolph, 1995; Rudolph et al., 2007), the nearly 100% incidence of gonopore intersexes found in the two *Synalpheus* spp. is striking. In shrimps of the caridean genus *Lysmata*, in which both male and female gonopores are present, the sexual system is protandric simultaneous hermaphroditism (PSH). In PSH, individuals first develop in a male phase (only male function) and then later change to an apparent female phase in which individuals function as simultaneous hermaphrodites (Bauer and Holt, 1998; Bauer, 2006). In parastacid crayfish, “intersex” individuals (with both male and female gonopores) have been found to be protandric hermaphrodites (Rudolph, 1995; Rudolph et al., 2007).

Despite the fact that almost all individuals examined of *S. paraneptunus* “small” and *S. paraneptunus* “A” had both male and female gonopores, our results do not

support sex change or simultaneous hermaphroditism for the following reasons. First, even smaller individuals in both species possess both gonopores, whereas in the simple protandrous sex change of other sex-changing caridean shrimps, smaller individuals are males (male gonopores only) and larger individuals are predominantly female (female gonopores only) (Bauer, 2000). In the colony of the eusocial species (*S. paraneptunus* “small”) examined by SEM, the two smallest individuals had their female gonopores closed (although their shape was developed), which could be an indication for maleness. However, sperm was not found in small individuals dissected from the other colony, suggesting that such individuals do not function as males. These individuals may thus be juvenile (non-reproductive) individuals whose sexual fate, male or female, is still undetermined.

Second, dissections of smaller individuals of *S. paraneptunus* “A” revealed that only a small percentage of those carry sperm, and the two individuals that carried sperm were the largest in their cohort. These results do not support the sex change theory, which predicts most of the smaller animals functioning as males. If the intersexes of *S. paraneptunus* “A” were simultaneous hermaphrodites, we would have found sperm in at least a few individuals with ovarian development (“presumptive females”). However, no sperm was found in such individuals upon dissection, but most of the larger individuals that did not have developed ovaries did carry sperm (functional males). These observations indicate a gonochoristic (separate sex) system in *S. paraneptunus* “A.”

Of the 24 dissected individuals of a *S. paraneptunus* “small”, only three had sperm, but those were larger and not the smallest individuals as one would expect based if sex change (protandry) occurred in this species. The queen of the dissected colony did not have sperm, what we would expect if she functioned as a simultaneous hermaphrodite.

Third, pairing experiments with *S. paraneptunus* “A” using two presumptive females showed that neither smaller (younger) nor larger (older) females can be stimulated to function as males to fertilize the eggs of other spawning females. All these results indicate that in both *S. paraneptunus* species the sexes are genetically fixed and not plastic, again suggesting a gonochoristic sex determination and that perhaps the presence of intersex gonopores is a developmental anomaly of this species without particular adaptive significance (see below).

Another hypothesis to consider is that the presence of both gonopores indicates that smaller, sexually inactive individuals could potentially develop either into

females or males depending on the circumstances. In bopyrid isopods the first individual who settles on a host develops as a female and the second one develops as a small dwarf male (O'Brien and Van Wyk, 1985). We have shown in a previous paper (Tóth and Bauer, 2007) that larger (older) helpers of *S. rathbunae* can disperse away from the home colony and colonize unoccupied sponges. Perhaps in the *Synalpheus* spp. in this study, the presence of both sets of gonopores allows helpers to mature as either sex given the right ecological or social situation, e.g., dispersion and colonization of an unoccupied sponge or mating opportunity for one sex, respectively. However, in the case of *S. paraneptunus* virtually all sponges have shrimp occupants, indicating that sponges can be found easily. Colonization data of sponges suggest that many small individuals find the same host (pers obs, ET), finding the opposite sex would thus not be a limiting factor. Behavioral observations on social interactions, as well as information on sex ratios and differential investment in body growth would be essential to make predictions on helper opportunities for sex change (St. Mary, 1997).

How common is the possession of both gonopores in *Synalpheus* shrimps? We previously found, in other *Synalpheus* species, some individuals with both male and female gonopores; in two (out of five) eusocial species 8–19% of the investigated individuals possessed both gonopores, although the male gonopore was often rudimentary (Tóth and Bauer, 2007), and in six pair-living species we found intersexes in only one species in very low frequencies (Tóth and Bauer, in prep). It is very possible, as explained above, that intersex gonopores is a non-functional character in *Synalpheus*, perhaps a developmental anomaly as it has been proposed for some crustaceans (Sagi et al., 1997; Ford et al., 2004). Developmental anomalies such as deformations of the rostrum are not uncommon in sponge-dwelling *Synalpheus* shrimp (e.g. Didderen et al., 2006), and it also occurs frequently in the eusocial *S. paraneptunus* “small,” (Anker and Tóth, in press). Because of the patchy distribution of sponges and possibly limited dispersal in *Synalpheus* (Knowlton and Keller, 1986; Duffy, 2003), anomalies such as intersex gonopores might be due to genetic phenomena such as genetic drift and/or inbreeding. Environmental factors, such as pollution (e.g. Moore and Stevenson, 1994; Ford et al., 2004, 2007), as well as reproductive parasites, may increase/be the cause for the incidence of intersex gonopores in *Synalpheus* spp. Many microbial organisms induce a variety of effects on the sexual development of their crustacean hosts, including reduced reproductive capacity (e.g. Ginsburger-Vogel, 1991; Kelly et al., 2004). Intersexuality, however, does

not apparently affect the two *S. paraneptunus* species in a negative way. These two species are quite abundant and reproductive females carry a full load of embryos. We have to stress that our study only focused on the external anatomy of these species; dissection of the gonads of these species, unlike done those on species of similar body size, such as the hippolytid *Thor manningi* (Bauer, 1986), were unsuccessful. Undoubtedly, both histological and hormonal studies would be desirable and might reveal more about sexual development and sexual systems in these shrimps.

Despite the fact that all individuals possess both gonopores, the eusocial species *S. paraneptunus* “small” shows similar morphometrical colony organization as other eusocial shrimp species (Fig. 2, Tóth and Bauer, 2007; Tóth and Duffy, in press). The queen is the largest individual but does not have the largest chela in the colony, suggesting no active participation in the colony defense (Tóth and Duffy, in press). Only a few individuals in the colony carried sperm and no individuals other than the queen showed ovarian development. Assuming the equal sex ratio found in other eusocial species (Tóth and Bauer, 2007), the fact that we only found sperm in three individuals suggests that many helpers do not develop sexually while in the colony, which is also known in other eusocial societies (Wilson, 1971; Choe and Crespi, 1997; Alexander et al., 1991).

In summary, our study showed that in the two species of *S. paraneptunus*, most individuals can be termed intersexes, possessing both female and male gonopores. To our knowledge this is the first report of a crustacean species in which nearly 100% of individuals in the population are intersexes based on external characteristics. Nevertheless, we could not confirm that these shrimp are protandric or simultaneous hermaphrodites. Another hypothesis proposed here is that helpers are sexually immature individuals capable of sex change to male or female, depending on the socio-ecological opportunities to do. Finally, it is possible that these two species are gonochoristic after all and that the presence of both male and female gonopores is a developmental anomaly without particular adaptive significance.

Acknowledgements

We are very grateful to Dr. Thomas Pesacreta and his assistant Anne Hume for allowing us to use the electron microscopy facilities of the University of Louisiana at Lafayette (ULL). This research was supported by the Marine Science Network Fellowship of the Smithsonian Institution, and the Smithsonian Tropical Research Institute (to ET). This is contribution number 121 of the

ULL Laboratory of Crustacean Research. Sample collection complies with the current laws of Panama, collection permits were obtained from ANAM.

References

- Alexander, R.D., Noonan, K.M. and Crespi, B.J., The evolution of eusociality. In: The biology of the Naked Mole-rat, Sherman, P.W., Jarvis, J.U.M. and Alexander, R.D. (eds.), Princeton University Press, Princeton, NJ, 1991, pp. 3–44.
- Anker, A. and De Grave, S., *Zuzalpheus* Rios and Duffy, 2007: a junior synonym of *Synalpheus* Bate, 1888 (Crustacea: Decapoda: Alpheidae). *J. Crust. Biol.*, in press.
- Anker, A. and Tóth, E., A preliminary revision of the *Synalpheus paranepuntus* Coutiere, 1909 species complex (Crustacea: Decapoda: Alpheidae). *Zootaxa*, in press.
- Baeza, J.A. and Bauer, R.T., Experimental test of socially mediated sex change in a protandric simultaneous hermaphrodite, the marine shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae). *Behav. Ecol. Sociobiol.*, 55 (2004) 544–550.
- Banner, D.M. and Banner, A.H., The alpheid shrimp of Australia. Part 2: The genus *Synalpheus*. *Rec. Aust. Mus.*, 29 (1975) 267–389.
- Bauer, R.T., Sex change and life history pattern in the shrimp *Thor manningi* (Decapoda: Caridea): a novel case of protandric hermaphroditism. *Biol. Bull.* 170 (1986) 11–31.
- Bauer, R.T., Simultaneous hermaphroditism in caridean shrimps: a unique and puzzling sexual system in the Decapoda. *J. Crust. Biol.*, 20(2) (2000) 116–128.
- Bauer, R.T., Remarkable Shrimps: Natural History and Adaptations of the Carideans. University of Oklahoma Press, Norman, OK, 2004.
- Bauer, R.T., Same sexual system but variable sociobiology: evolution of protandric simultaneous hermaphroditism in *Lysmata* shrimps. *Integ. Comp. Biol.*, 46 (2006) 430–438.
- Bergström, B.I., Do protandric pandalid shrimp have environmental sex determination? *Mar. Biol.*, 128 (1997) 397–407.
- Boltana, S. and Thiel, M., Associations between two species of snapping shrimp, *Alpheus inca* and *Alpheopsis chilensis* (Decapoda: Carida: Alpheidae). *J. Mar. Biol. Assoc. UK*, 81 (2001) 633–638.
- Chace, F.A., The shrimps of the Smithsonian–Bredin Caribbean expeditions with a summary of the West Indian shallow-water species (Crustacea: Decapoda: Natantia). *Smithsonian Contributions Zool.*, 98 (1972) 103.
- Choe, J.C. and Crespi, B.J., *Social Behavior in Insects and Arachnids*. Harvard University Press, Cambridge, MA, 1997.
- Coutiere, H., The American species of snapping shrimps of the genus *Synalpheus*. *Proc. USNM*, 36, 1659 (1909) 1–93.
- Dardeau, M.R., *Synalpheus* shrimps (Crustacea: Decapoda: Alpheidae). I. The gambarelloides group, with a description of a new species. *Mem. Hourglass Cruises* 7, part 2 (1984) 1–125.
- Dawes, B.D., A study of normal and regenerative growth in the pistol-crab, *Alpheus dentipes* (Guer.), with special reference to the phenomenon of chela reversal. *W Roux Archiv. F. Entwicklungsmechanik*, 131 (1934) 543–574.
- Duffy, J.E., Eusociality in coral reef shrimp. *Nature*, 381 (1996) 512–514.
- Duffy, J.E., The ecology and evolution of eusociality in sponge-dwelling snapping shrimp. In: Kikuchi, T., Azuma, N. and Higashi, S. (eds.), *Genes, Behavior and Evolution in Social Insects*. Hokkaido University Press, Sapporo, 2003, pp. 217–252.
- Duffy, J.E., Morrison, C.L. and Macdonald, K.S., Colony defense and behavioral differentiation in the eusocial shrimp *Synalpheus regalis*. *Behav. Ecol. Sociobiol.*, 51 (2002) 488–495.
- Ford, A.C., Fernandes T.F., Read, P.A., Robinson, C.D. and Davies, I.M., The costs of intersexuality: a crustacean perspective. *Mar. Biol.*, 145 (2004) 951–957.
- Ford, A.C., Martins, I. and Fernandes, T.F., Population level effects of intersexuality in the marine environment. *Sci. Total Environ.*, 1 (2007) 102–111.
- Ginsburger-Vogel, T., Intersexuality in *Orchesia mediterranea* Costa, 1893, and *Orchesia aesturaensis* Wildish, 1987 (Amphipoda): a consequence of hybridization or parasitic infestation? *J. Crust. Biol.*, 11 (1991) 530–539.
- Hughes, M., Size assessment via a visual signal in snapping shrimp. *Beh. Ecol. Sociobiol.*, 38 (1996) 51–57.
- Kelly, A., Hatcher, M. and Dunn, A., Intersexuality in the amphipod *Gammarus duebeni* results from incomplete feminization by the vertically transmitted parasitic sex ratio distorter *Nosema granulosis*. *Evol. Ecol.*, 18 (2004) 121–132.
- Knowlton, N., Sexual selection and dimorphism in two demes of a symbiotic, pair-bonding snapping shrimp. *Evolution*, 34 (1980) 161–173.
- Knowlton, N., and Keller, B.D., Symmetric fights as measure of escalation potential in a symbiotic, territorial snapping shrimp. *Beh. Ecol. Sociobiol.* 10 (1982) 289–292.
- Knowlton, N. and Keller, B.D., Larvae which fall short of their potential: highly localized recruitment of alpheid shrimp with extended larval development. *Bull. Mar. Sci.*, 39 (1986) 213–223.
- Mathews, L.M., Territorial cooperation and social monogamy: factors affecting intersexual behaviours in pair-living snapping shrimp. *Anim. Beh.*, 63 (2002) 767–777.
- Moore, C.G. and Stevenson, J.M., Intersexuality in benthic harpacticoid copepods in the Firth of Forth, Scotland. *J. Nat. Hist.*, 18 (1994) 1213–1230.
- Nakashima, Y., Reproductive strategies in a partially protandric shrimp, *Athanas kominatoensis* (Decapoda: Alpheidae): Sex change as the best of a bad situation for subordinates. *J. Ethol.*, 5 (1987) 145–159.
- Nolan, B.A. and Salmon, M., The behavior and ecology of snapping shrimp (Crustacea: *Alpheus heterochaelis* and *Alpheus normanni*). *Form. Funct.*, 2 (1970) 289–335.

- O'Brien, J. and Van Wyk, P., Effects of crustacean parasitic castrators (epicaridean isopods and rhizocephalan barnacles) on growth of crustacean hosts. *Crust. Issues*, 3 (1985) 191–198.
- Rahman, N., Dunham, D.W. and Govind, C.K., Size-assortative pairing in the big-clawed shrimp, *Alpheus heterochaelis*. *Behaviour*, 139 (2002) 1443–1468.
- Rios, R. and Duffy, J.E., A review of the sponge dwelling snapping shrimp from Carrie Bow Cay, Belize, with description of *Zuzalpheus*, new genus and six new species (Crustacea, Decapoda, Alpheidae), *Zootaxa*, 1602 (2007) 1–89.
- Ritzmann, R., Mechanisms for the snapping behavior of two alpheid shrimps, *Alpheus californiensis* and *Alpheus heterochaelis*. *J. Comp. Physiol.*, 95 (1974) 217–236.
- Rudolph, E., Partial protandric hermaphroditism in the burrowing crayfish *Parastacus nicoletti* (Philippi, 1982) (Decapoda: Parastacidae). *J. Crust. Biol.*, 15 (1995) 720–732.
- Rudolph, E.H., Retamal, F.A. and Martínez, A.W., Partial protandric hermaphroditism in the burrowing crayfish *Virilastacus rucapihuelensis* Rudolph and Crandall, 2005 (Decapoda, Parastacidae). *J. Crust. Biol.*, 27 (2007) 229–241.
- Sagi, A., Snir, E. and Khahaila, I., Sexual differentiation in Decapod crustaceans: role of the androgenic gland. *Invert. Reprod. Develop.*, 31 (1997) 55–61.
- Schein, H., Aspects of the aggressive and sexual behaviour of *Alpheus heterochaelis* Say. *Mar. Beh. Physiol.*, 3 (1975) 83–96.
- Schein, H., The role of snapping in *Alpheus heterochaelis* Say 1918, the big-clawed snapping shrimp, *Crustaceana*, 33 (1977) 182–188.
- St. Mary, C. Sequential patterns of sex allocation in simultaneous hermaphrodites: do we need models that specifically incorporate this complexity? *Am. Nat.*, 150 (1997) 73–97.
- Suzuki, H., Taxonomic review of four alpheid shrimps belonging to the genus *Athanas* with reference to their sexual phenomena. *Sci. Rep. Yokohama Natl. Univ. Sect 2*, 17 (1970) 1–37.
- Tóth, E. and Duffy, J.E., Coordinated group response to nest intruders in social shrimp. *Biol. Lett., Royal Soc. London*, 1 (2005) 49–52.
- Tóth, E. and Duffy, J.E., Influence of sociality on allometric growth and morphological differentiation in sponge-dwelling alpheid shrimp. *Biol. J. Linn. Soc.*, in press.
- Tóth, E. and Bauer, R.T., Gonopore sexing technique allows determination of sex ratios and helper composition in social shrimp. *Mar. Biol.*, 151 (2007) 1875–1886.
- Versluis, M., Schmitz, B., von der Heydt, A. and Lohse, D., How snapping shrimp snap: through cavitation bubbles. *Science*, 289 (2000) 2114–2117.
- Wilson, E.O., *The Insect Societies*. Harvard University Press, Cambridge, MA, 1971.
- Zupo, V. and Messina, P., How do dietary diatoms cause the sex reversal of the shrimp *Hippolyte inermis* Leach (Crustacea, Decapoda). *Mar. Biol.*, 151 (2007) 907–917.