

# Bopyrid parasite infestation affects activity levels and morphology of the eusocial snapping shrimp *Synalpheus elizabethae*

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**ABSTRACT:** Sponge-dwelling snapping shrimps are often more heavily parasitized by bopyrid isopods than their free-living relatives, but little is known about how parasitism influences the behavior and morphology of these shrimps. In this study, we measured parasitism rates in the eusocial snapping shrimp *Synalpheus elizabethae* and tested whether parasitism by bopyrid isopods (*Bopyri- one* sp.) affects shrimp activity levels and morphology. Using field surveys, we found that *S. eliza- bethae* suffer higher parasitism rates when inhabiting the sponge *Lissodendoryx colombiensis* than do co-occurring pair-forming congeners (*S. yano* and *S. dardeauii*) in the same host. In laboratory behavioral assays, parasitized *S. elizabethae* showed 50% lower activity levels than unparasitized colony members, suggesting that infection by bopyrids decreases shrimp activity. Finally, parasitism influenced shrimp morphology; parasitized *S. elizabethae* individuals were larger, but had relatively smaller major chelae (25% reduction) than unparasitized individuals. Parasites comprised ~5% of the entire body weight of an individual shrimp. Together these data suggest that parasitism by bopyrid isopods may incur significant energetic costs for commensal sponge-dwelling shrimps such as *Synalpheus*, and may be especially severe for social species such as *S. elizabethae*.

**KEY WORDS:** Parasitism · *Synalpheus elizabethae* · Eusociality · Bopyridae · Morphology · Behavior

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## INTRODUCTION

Coral reefs are some of the most diverse marine ecosystems, and much of that diversity consists of species involved in specialized relationships, e.g. smaller associate species living in larger 'host' species (Banner & Banner 1975, Bruce 1976). Such close physical associations can have strong morphological and population consequences for both partners in the relationship, with important ecological and evolutionary implications. Snapping shrimps in the genus *Synalpheus* (Decapoda: Caridea: Alpheidae) are a prime example; in the Caribbean, most species belong to the *S. gambarelloides* species group, a monophyletic group of >40 species that dwell exclusively in the internal canals of sponges, and are thought to gain most of their

nutrition from their host sponge (Duffy 1996b, Macdonald et al. 2006, Rios & Duffy 2007). Body size in these shrimps is closely correlated with the size of the interior canals of the sponges they inhabit (Duffy 1992, Hultgren & Duffy 2010). Appropriate sponge hosts are a limiting resource in the field (Macdonald et al. 2006), and living in sponges likely affords shrimps protection from generalist predators (Duffy 1996b, 2007).

Inhabiting a long-lived, valuable resource (the sponge host) is thought to be one of many factors facilitating the evolution of different types of reproductive strategies in sponge-dwelling *Synalpheus*—specifically, the evolution of eusocial breeding strategies. Members of the *S. gambarelloides* species group are unique in that they include the first described case of eusociality in marine invertebrates (Duffy 1996a).

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Eusocial *Synalpheus* live in colonies of 10s to 1000s of individuals, typically with reproduction limited to 1 (or a few) female 'queen(s);' non-breeding colony members serve an important, albeit indirect, role of enhancing colony fitness by using their enlarged snapping claw to aggressively fend off intruders and defend their host sponge (Duffy et al. 2002, Toth & Duffy 2005, Duffy 2007). In *S. regalis*, there is evidence for further morphological differentiation among non-breeders; larger, more aggressive individuals with proportionally larger fighting chelae spend more time near the periphery of the sponge (Duffy 2007, Toth & Duffy 2008).

Although most work on *Synalpheus* has focused on the relative benefits of the sponge-dwelling and group-living lifestyles, early work on *Synalpheus* documented one potential disadvantage to commensal living in sponges. Duffy (1992) showed that sponge-dwelling *Synalpheus* had higher rates of parasitism by bopyrid isopods (Isopoda: Bopyridae) than did non-sponge-dwelling species, possibly due to the high population densities in sponge-dwelling populations. Bopyrid isopods have a complex life cycle. Larvae first attach to an intermediate host (typically a calanoid copepod) and metamorphose into microniscus larvae (O'Brien & Van Wyk 1985, Cash & Bauer 1993); microniscus larvae molt into free-swimming cryptoniscus larvae (detached from the copepod) that seek out and infect the definitive hosts, i.e. decapod crustaceans (Markham 1985, 2003). As adults, bopyrids feed on the hemolymph of their hosts (O'Brien & Van Wyk 1985), decreasing host growth (Anderson 1977, Jay 1989, Somers & Kirkwood 1991) and negatively affecting host metabolism (Anderson 1975). In caridean shrimps, bopyrids typically occur either in the abdominal region (hereafter 'abdominal bopyrids'), or on the right or left side of the branchial cavity ('branchial bopyrids'). Abdominal bopyrids often have strong effects on their host's reproductive system, reducing fecundity (Pike & Manton 1960, Hernandez et al. 2010) or causing partial to full castration of their hosts (Allen 1966, O'Brien & Van Wyk 1985, Nakashima 1995, Calado et al. 2005). Branchial bopyrids are thought to affect metabolism and activity levels through their effects on respiration (Anderson 1975, Bass & Weis 1999, Chaplin-Ebanks & Curran 2005), but have also been shown to inhibit reproduction (Pike & Manton 1960, Choi et al. 2004) and to reduce the size of secondary sex characters in their shrimp hosts (Beck 1980). Mature branchial bopyrids are easily identified because they cause a characteristic bulge in the branchial region of the carapace of the host. As such, branchial bopyrids may impede movement of sponge-dwelling *Synalpheus*, whose streamlined bodies closely match the canal dimensions of their sponge hosts (Hultgren & Duffy 2010).

Here we examine effects of parasitism by branchial bopyrid isopods on *Synalpheus elizabethae*, a eusocial species in the *S. gambarelloides* species group. *S. elizabethae* often co-occur in individual sponges with 2 congeneric species, *S. yano* and *S. dardeau*, that typically live in heterosexual pairs or in larger groups with equal sex ratios (Macdonald et al. 2006). All 3 can be infected by branchial and abdominal bopyrid isopods. Despite relatively high incidences of parasitism in sponge-dwelling *Synalpheus* (up to 30% of a population parasitized; Duffy 1992), few studies have examined the effects of parasitism on *Synalpheus*. Hernandez et al. (2010) found that abdominal parasite infestation decreased fecundity in *S. yano* (but did not inhibit reproduction completely), and ovigerous female individuals of *Synalpheus* with abdominal or branchial parasites are occasionally found in the field (K. M. Hultgren pers. obs.). Together these data suggest that these parasites do not completely castrate their hosts. However, little is known about how abdominal bopyrids affect eusocial species of *Synalpheus*, where reproduction is limited to a few individuals (queen shrimps are rarely parasitized; K. M. Hultgren unpubl. data), or how branchial bopyrids affect shrimp activity levels or morphology. In this study, we focused on 3 questions: (1) do *S. elizabethae* experience different parasitism rates compared to cohabiting non-social species in the sponge *Lissodendoryx colombiensis*; (2) do branchial bopyrids (*Bopyrione* sp.) affect *S. elizabethae* activity levels; and (3) do branchial bopyrids affect *S. elizabethae* morphology?

## MATERIALS AND METHODS

**Field surveys.** We measured parasitism rates of *Synalpheus elizabethae*, *S. yano*, and *S. dardeau* in the Bocas del Toro region of Caribbean Panama (2007 to 2009). *S. elizabethae* typically occurs in 2 different sponge host species in Bocas del Toro (*Hymeniacidon caerulea* and *Lissodendoryx colombiensis*), while *S. yano* and *S. dardeau* occur in *L. colombiensis*. *H. caerulea* was collected at Boca del Drago (9° 25.28' N, 82° 20.56' W), Isla San Cristobal (9° 18.03' N, 82° 16.48' W), Isla Pastores (9° 13.104' N, 82° 19.485' W), and Swan Cay (9° 27.08' N, 82° 18.00' W); *L. colombiensis* was collected at Crawl Cay (9° 14.71' N, 82° 8.60' W) and in seagrass shallows north and south of STRI point (9° 20.91' N, 82° 15.74' W). These collections were part of a set of standardized regional taxonomic surveys of sponge-dwelling *Synalpheus* (Macdonald et al. 2006, 2009, Hultgren et al. 2010). Sponges were collected at 2 to 19 m depth via snorkeling or SCUBA, kept in running seawater for 1 to 4 h until processed, and individually dissected until all macrofauna had been

removed. Live *Synalpheus* were identified and listed as ovigerous (possessing visible ovaries and/or embryos) or non-ovigerous; non-ovigerous individuals include both males and immature females, which cannot be distinguished without electron microscopy (Toth & Bauer 2007). In most cases, while processing we also noted whether live shrimps were parasitized, and preserved parasitized and unparasitized individuals separately. Shrimps were preserved in 95% ethanol, and all individuals were later measured under a dissecting scope (see below) and checked again for parasites.

We calculated mean prevalence of parasitism (mean proportion of conspecific individuals in an individual sponge that were parasitized) of the eusocial species *Synalpheus elizabethae* and the 2 pair-forming species (*S. dardeai* and *S. yano*). A subset of the bopyrid parasites from these 3 species was sent to a specialist for identification. In these sponges, both *S. elizabethae* and *S. yano* were infected by a single branchial bopyrid species (*Bopyrione* sp.); *S. dardeai* was never infected with branchial bopyrids. A range of abdominal bopyrid species infected *S. elizabethae* (new bopyrid species A), *S. dardeai* (*Eophrixus* sp.), and *S. yano* (*Eophrixus* sp. and *Hemiarthus* sp.). Parasite prevalence data were expressed in proportions (and were non-normal) so we used non-parametric tests (JMP 5.1, SAS Institute). First, we used a paired Wilcoxon test to examine whether shrimp species differed in prevalence of abdominal versus branchial bopyrids in each sponge host species. Second, in the sponge *Lissodendoryx colombiensis* (where all 3 species co-occurred), we used Kruskal-Wallis tests to test whether the 3 shrimp species differed in overall parasite prevalence (whether they had a branchial or abdominal parasite), and used individual Mann-Whitney *U*-tests (with Bonferroni-corrected  $\alpha$  values) for pairwise species comparisons. Finally, we compared mean group size (number of individuals of a species living in a sponge) among shrimp species in the sponge *L. colombiensis* using Kruskal-Wallis and Mann-Whitney *U*-tests, and tested whether branchial or abdominal parasite prevalence increased with group size using regression analysis in both sponge hosts and Bonferroni-corrected  $\alpha$  values. Because we were testing correlations between 2 related variables (number of parasitized individuals versus total number of individuals in a sponge), we also tested whether our results might be influenced by spurious correlations (e.g. a correlation between number of parasitized individuals and number of unparasitized individuals in a sponge). All *p*-values given in the results are 2-tailed.

**Effects of parasitism on shrimp activity levels.** Using laboratory assays, we tested whether infection by branchial bopyrids affected activity levels of *Synalpheus elizabethae* in experimental colonies ( $n = 14$

colonies) using laboratory behavioral assays (June 2009). We collected *S. elizabethae* colonies from the sponge *Lissodendoryx colombiensis*, removed all individuals from each sponge, and randomly chose 10 co-occurring shrimps for the experimental colony (5 parasitized and 5 unparasitized shrimps); this colony-wide parasite prevalence (50%) approached maximum parasite prevalence seen in the field. For 10 experimental colonies, we also included the colony's queen (always unparasitized). We confirmed that all individuals were from the same colony (multiple colonies can exist in the same sponge) by observing all individuals in a 50 ml container of seawater; after 15 min without snapping we assumed that all individuals were from the same colony (Duffy et al. 2002). Individual shrimp were briefly dried and their carapaces were marked with a permanent marker so they could be tracked throughout the experiment. All behavioral observations of experimental colonies were made in small 'ant farms' (modified from Duffy et al. 2002); briefly, a 7 mm thick slice of living *L. colombiensis* sponge was sandwiched between 2 transparent plexiglass plates (10 × 10 cm), separated by 7 mm thick strips of plexiglass to prevent shrimps from escaping (shrimps typically preferred to stay in contact with their host sponge). Experimental chambers were clamped against the inside wall of a glass aquarium and supplied with a continuous flow of unfiltered seawater. Shrimps were haphazardly distributed in each sponge farm immediately before it was clamped to the inside of the aquarium, and allowed to acclimatize for 6 h before the first observation.

Following acclimatization, behavioral observations (described below) were taken over a 24 h experimental trial (= block). Three to 4 experimental colonies were observed over 5 temporal blocks ( $n = 14$  colonies in total). Within each block, we recorded activities of all colony members during four 15 min observation periods (00:00, 06:00, 12:00, and 18:00 h), and randomized the order in which colonies were observed. The first experimental block began on 18 June 2009, with subsequent experimental blocks run with 2 to 3 d separating them. We used an event recorder on Microsoft Excel to log the exact time that specific activities occurred.

Shrimp behaviors were classified into 8 categories: (1) picking, (2) moving, (3) grooming, (4) interacting, (5) pleopod fanning, (6) falling, (7) other, or (8) resting. 'Picking' was defined as a shrimp picking or plucking at the inner layer of the sponge canal with its minor chela or first pereopods. 'Moving' was defined as a shrimp moving forward, backward, or to either side. 'Grooming' shrimps engaged in cleaning their head and body with the minor chela or second pereopods. 'Interacting' was noted when shrimps engaged in physical attacks (snaps), harmless contact, or antennal

contact with another shrimp. 'Pleopod fanning' was defined as rapid fanning of the pleopods (paired abdominal appendages used for carrying eggs/swimming). 'Falling' was defined as a discrete event in which a shrimp lost contact with the host sponge, fell on its back or side, and recovered back onto its front side; this activity was only reliably recorded for the last 10 colonies tested. If a shrimp's behavior could not be grouped into 1 of the above categories, its activity was labeled as 'other,' and a brief description of the behavior was noted. Shrimps exhibiting complete absence of motion or activity (by far the most common observation) were said to be 'resting.' For behavioral comparisons between parasitized and unparasitized shrimps, we focused on the 4 most common activities: moving, grooming, pleopod fanning, and falling. Picking, interacting, and 'other' did not occur frequently enough for statistical analysis (shrimps spent  $\ll 1\%$  of their time engaged in these activities).

Because we were primarily interested in the effects of parasitism on various behaviors, we used paired comparisons to compare mean activity levels between parasitized and unparasitized shrimps, using shrimp colonies as the unit of replication ( $n = 14$  colonies). Data were non-normally distributed, so we pooled the mean rates of each activity separately for parasitized and unparasitized individuals in a colony (mean proportion of time doing an activity) and used paired Wilcoxon signed-rank tests with Bonferroni-corrected  $\alpha$  values ( $\alpha = 0.0125$ ) to test for significant differences in activity between parasitized and unparasitized shrimps. For colonies that included queens ( $n = 10$ ), activity rates of queens were not included in the analysis (queens were generally inactive).

We also tested to ensure that body size or the presence of a queen did not influence our results. Although parasitized and unparasitized individuals were chosen haphazardly from each colony, parasitized individuals were on average 6% larger than unparasitized individuals ( $t$ -test,  $p < 0.0001$ ), so we examined whether body size influenced activity using 2 tests. First, we pooled together individuals from all colonies and regressed individual activity rate against individual size (carapace length, CL, in mm) separately for parasitized and unparasitized treatment groups. We also tested whether differences in size between parasitized/unparasitized individuals influenced differences in activity on a per-colony basis by regressing the difference in activity rates ( $UP_{rate} - P_{rate}$ , where  $P_{rate}$  and  $UP_{rate}$  = mean activity rate for all parasitized and unparasitized individuals, respectively, in a colony) against mean difference in size ( $UP_{size} - P_{size}$ , where  $P_{size}$  and  $UP_{size}$  = mean size of all parasitized or unparasitized individuals, respectively, in a colony). Finally, we tested whether presence of a queen (queens were

absent in the first 4 colonies tested) influenced differences in mean activity between treatments ( $UP_{rate} - P_{rate}$ ) using a Wilcoxon test. P-values for all analyses listed in the results are 2-tailed.

**Effects of parasitism on shrimp morphology.** We examined whether parasitized and unparasitized *Synalpheus elizabethae* differed in morphology by comparing major chela size, corrected by overall body size. For these analyses, we measured parasitized (abdominal and branchial bopyrids) and unparasitized non-ovigerous individuals from all *S. elizabethae* colonies collected in the field. Using an ocular micrometer, we measured CL using the distance from the posterior margin of the carapace to the base of the rostrum, and measured major chela length (MCL) using the distance from the tip of the fixed finger to the most proximal part of the chela (see Toth & Duffy 2008). We separately pooled mean values of MCL and CL for parasitized and unparasitized shrimp in each sponge, and used an analysis of covariance (ANCOVA; between-subjects factor, parasitism; covariate, CL) to examine whether there were differences in MCL for individuals with branchial bopyrids compared to unparasitized individuals. We also calculated mean chela size for individuals with abdominal bopyrids, but abdominal bopyrids were generally uncommon, and we lacked sufficient replication ( $n = 4$  sponges) to compare these means to individuals with branchial bopyrids or unparasitized individuals. We recorded shrimp handedness and side parasitized (for shrimps with branchial bopyrids), and measured total body length (TBL) of all abdominal and branchial bopyrids on shrimps. We tested whether there were positive relationships between shrimp size (CL) and parasite size (TBL) using regression analyses. We used chi-squared tests to examine whether shrimp handedness differed significantly from random (a 50:50 ratio), and whether branchial bopyrids tended to preferentially attach to the same side of the branchial cavity that the major chela was on (or the opposite side) in individual shrimps. Finally, for a subsample of *S. elizabethae* with branchial bopyrids, we dried and individually weighed shrimps and their mature female parasites and egg sacs ( $n = 15$ ) to calculate the mean body mass of parasites relative to their shrimp hosts. p-values for all analyses listed in the results are 2-tailed.

## RESULTS

### Field parasitism rates

In the sponge *Lissodendoryx colombiensis*, we found a significant difference in parasitism rates between the eusocial shrimp species *Synalpheus elizabethae* and

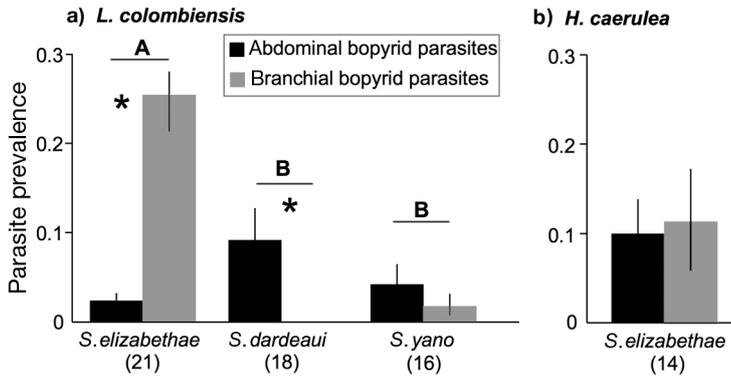


Fig. 1. *Synalpheus* spp. Parasite prevalence (proportion of individuals parasitized per individual sponge, mean  $\pm$  SE) in sponges (A) *Lissodendoryx colombiensis* and (B) *Hymeniacion caerulea*. Numbers noted beneath shrimp species represent the number of individual sponges surveyed. \*Significant differences between branchial parasitism and abdominal parasitism rates (Wilcoxon signed-ranks test, Bonferroni corrected  $\alpha < 0.0167$ ). In (A), shrimp species with different letters have significantly different overall parasitism rates (Mann-Whitney *U*-tests, Bonferroni corrected  $p < 0.0167$ )

pair-forming species *S. dardeai* and *S. yano* (Fig. 1a, Kruskal-Wallis  $\chi^2 = 17.280$ ,  $df = 2$ ,  $p = 0.0002$ ); *S. elizabethae* were more parasitized than *S. dardeai* ( $\chi^2 = 10.694$ ,  $p = 0.001$ ) and *S. yano* ( $\chi^2 = 12.742$ ,  $p = 0.0004$ ), while *S. dardeai* and *S. yano* did not differ ( $\chi^2 = 0.125$ ,  $p = 0.724$ ). When broken down further, we found that branchial parasitism rates were higher than abdominal parasitism rates for *S. elizabethae* in *L.*

*colombiensis* (Fig. 1a,  $n = 21$ ,  $p > 0.0001$ , Wilcoxon signed-ranks test), but not in *Hymeniacion caerulea* (Fig. 1b,  $n = 14$ ,  $p = 0.914$ ). In *L. colombiensis*, *S. dardeai* were infected with only abdominal bopyrids, and there were no differences between abdominal versus branchial parasite prevalence for *S. yano* ( $n = 16$ ,  $p = 0.3750$ ). There were significant differences in group size (mean number of conspecific individuals living in a sponge;  $\chi^2 = 18.996$ ,  $p < 0.0001$ ). *S. elizabethae* group size in *L. colombiensis* (mean  $\pm$  SEM =  $35.14 \pm 6.88$  inds. sponge<sup>-1</sup>) was significantly higher than that of *S. dardeai* (mean  $\pm$  SEM =  $9.78 \pm 2.05$ ,  $p < 0.0001$ ) and *S. yano* (mean  $\pm$  SEM =  $13.00 \pm 5.62$ ,  $p = 0.0012$ , data not shown). Group size did not differ between *S. dardeai* and *S. yano* (Mann-Whitney *U*-test,  $p = 0.756$ ). For all shrimp species/sponge species combinations we tested, shrimps living in larger groups in a sponge did not have significantly higher rates of parasitism than shrimps living

in smaller groups (Fig. 2, Table 1: regression analysis,  $p \geq 0.024$ , Bonferroni  $\alpha = 0.0125$ ).

**Effects of parasitism on shrimp activity levels**

Parasitized and unparasitized *Synalpheus elizabethae* differed in activity levels (Fig. 3). Unparasitized shrimps,

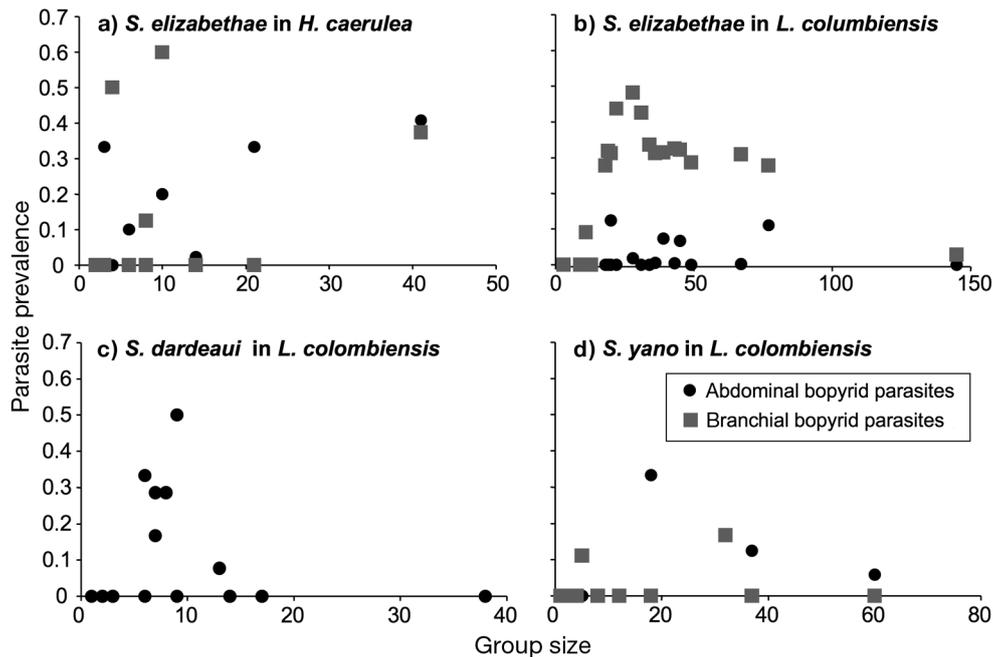


Fig. 2. *Synalpheus* spp. Abdominal and branchial parasite prevalence (proportion of individuals parasitized per individual sponge) as a function of group size (mean number of conspecific individuals living in a sponge) for different shrimp species living in different sponges: *H.*, *Hymeniacion*; *L.*, *Lissodendoryx*

Table 1. *Synalpheus* spp. Parasite prevalence (proportion of total individuals parasitized per sponge) as a function of group size (number of individuals of a *Synalpheus* species living in a sponge) for different shrimp species, types of parasites, and sponge host. N: number of individual sponges assessed; under sponge species, LC: *Lissodendoryx colombiensis*, HC: *Hymeniacion caerulea*. We evaluated significance using Bonferroni-corrected  $\alpha$  values ( $\alpha = 0.0125$ )

<i>Synalpheus</i> species	Sponge species	N	Abdominal parasites			Branchial parasites		
			F-ratio	p	Slope	F-ratio	p	Slope
<i>S. dardeau</i>	LC	18	0.3123	0.584	-0.0025	–	–	–
<i>S. yano</i>	LC	16	3.7824	0.072	0.0026	0.5208	0.4824	0.0006
<i>S. elizabethae</i>	LC	21	0.061	0.8075	0.00001	0.015	0.9036	0.0001
	HC	11	7.3399	0.024	0.00947	0.6556	0.439	0.00525

Table 2. *Synalpheus elizabethae*. Effects of (a,b) body size and (c) presence of queen on activity rates of captive shrimp using regression analyses (a,b) and Wilcoxon 2-sample tests (c). We evaluated significance using Bonferroni-corrected  $\alpha$  values (provided in table)

Treatment	Activity	p	F-ratio	R <sup>2</sup>	Slope	df
<b>(a) Individual regressions of activities as a function of size (<math>\alpha = 0.0063</math>)</b>						
Parasitized	Moving	0.9718	0.0013	0.000021	0.00123	1, 59
	Grooming	0.4314	0.6277	0.01527	0.01227	1, 59
	Pleopod fanning	0.2141	1.5771	0.026	0.01141	1, 59
	No. of falls	0.2872	1.1535	0.01977	0.0139	1, 59
Unparasitized	Moving	0.0219	5.5279	0.0818	6.16523	1, 59
	Grooming	0.9058	0.0141	0.000228	0.09635	1, 62
	Pleopod fanning	0.4238	0.6484	0.0139	0.00232	1, 62
	No. of falls	0.4023	0.7112	0.01134	0.00729	1, 62
<b>(b) Differences in activity as a function of differences in size (<math>\alpha = 0.0125</math>)</b>						
	Moving	0.2964	1.1917	0.0903	0.0941	1, 12
	Grooming	0.0659	4.0943	0.2544	0.052	1, 12
	Pleopod fanning	0.487	0.5143	0.041	-0.0199	1, 12
	No. of falls	0.8416	0.0416	0.003455	-0.1266	1, 12
<b>(c) Differences in activity due to presence of queen (<math>\alpha = 0.0167</math>)</b>						
		Z-value	Prob <  Z			
	Grooming	-1.202	0.2293			
	Moving	-1.202	0.2293			
	Pleopod fanning	0.777	0.4367			

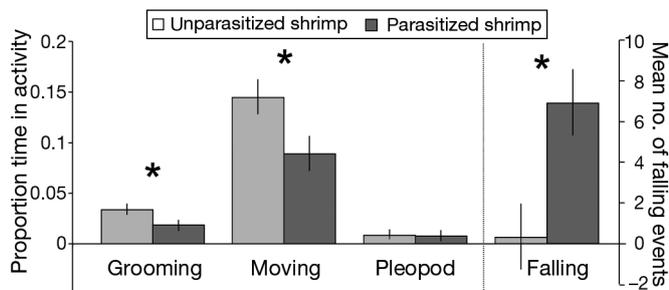


Fig. 3. *Synalpheus elizabethae*. Activity levels of parasitized and unparasitized shrimps (mean  $\pm$  SE). Left panel, mean proportion of total time spent doing that activity ( $n = 14$  colonies); right panel, mean number of times fallen ( $n = 10$  colonies). Pleopod: pleopod fanning. \*Significant differences between unparasitized and parasitized shrimps (Wilcoxon signed-ranks test, Bonferroni corrected  $p < 0.0125$ )

on average, spent a significantly greater proportion of time moving than did parasitized shrimps (Fig. 3: 62% more time; signed-ranks test  $p = 0.009$ ). Unparasitized shrimps also spent 79% more time grooming ( $p = 0.005$ ), although there was no difference in proportion of time spent fanning pleopods ( $p = 0.787$ ). Parasitized shrimps fell significantly more frequently than did unparasitized shrimps (Fig. 3;  $n = 10$  colonies,  $p = 0.002$ ). There were no significant effects of body size on the 4 activities we tested, either when we regressed individual activity rate against individual size (Table 2,  $p > 0.022$ , Bonferroni  $\alpha = 0.0063$ ), or on a per-colony basis ( $p > 0.0659$ , Bonferroni  $\alpha = 0.0125$ ). Presence of a queen did not affect differences in mean activity between treatments (Table 2, Wilcoxon test,  $p > 0.229$ , Bonferroni  $\alpha = 0.0167$ ).

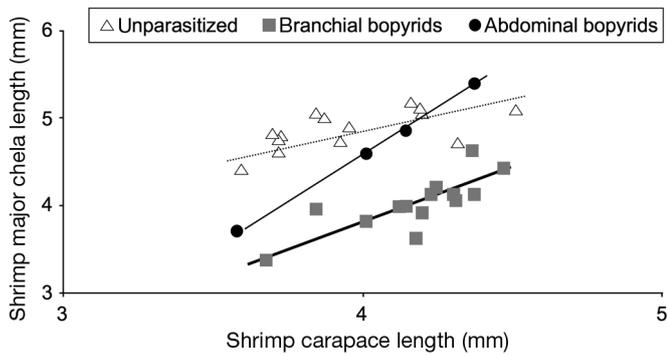


Fig. 4. *Synalpheus elizabethae*. Relationship between shrimp body size (carapace length, CL, in mm) and shrimp major chela length (MCL, mm) for unparasitized shrimp and shrimp infected with abdominal and branchial bopyrids. Individual points indicate pooled mean values of CL and MCL for each treatment group (unparasitized shrimp and shrimp infected with branchial or abdominal bopyrids) within an individual sponge. Note that axes do not originate at zero. Dashed line: line of best fit for unparasitized shrimp; thin solid line: line of best fit for abdominal bopyrids; thick solid line: line of best fit for branchial bopyrids

**Effects of parasitism on shrimp morphology**

*Synalpheus elizabethae* with branchial bopyrids had smaller major chelae (relative to body size) than did unparasitized individuals living in the same sponge host (ANCOVA: between-subjects factor, parasitism; covariate, CL; Fig. 4). There were significant effects of parasitism ( $F_{1,25} = 148.977, p = 0.0$ ) and significant effects of CL ( $F_{1,25} = 21.024, p = 0.0, R^2$  full model = 0.857), but no significant interactions between parasitism and CL ( $F_{1,24} = 3.991, p = 0.057$ ). We also found a significant positive correlation between parasite

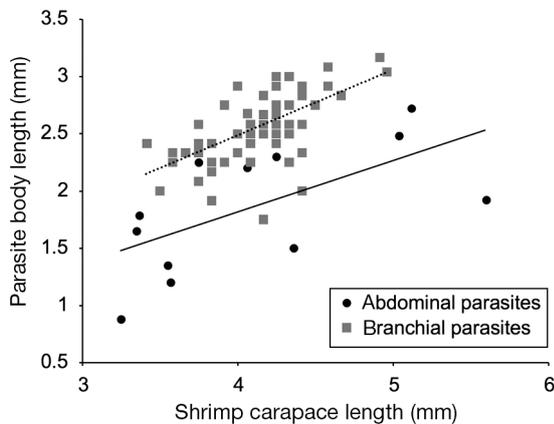


Fig. 5. *Synalpheus elizabethae*. Relationship between shrimp body length (mm) and parasite body length (mm) for abdominal and branchial bopyrids infecting *S. elizabethae*. Note that axes do not originate at zero. Dashed line: line of best fit for branchial bopyrids; solid line: line of best fit for abdominal bopyrids

length and shrimp body length both for branchial bopyrids ( $n = 62, F_{1,61} = 36.186, R^2 = 0.372, p < 0.0001$ ) and for abdominal bopyrids ( $n = 11, F_{1,10} = 6.679, R^2 = 0.400, p = 0.0272$ ; Fig. 5). Proportions of right-handed (0.523) and left-handed (0.476) shrimps did not differ from a 50:50 ratio ( $\chi^2 = 0.2814, p = 0.596$ ), and there was no propensity for individuals to have branchial bopyrids on the same side as their major chela (0.490) or the opposite side of their major chela (0.510;  $\chi^2 = 0.0204, p = 0.8864$ ). Mean bopyrid body mass (expressed as a percentage of host shrimp body mass) was  $5.37 \pm 0.98\%$  SEM ( $n = 15$ ).

**DISCUSSION**

In this study we demonstrated that parasitized individuals of the eusocial shrimp *Synalpheus elizabethae* were less active and had proportionally smaller chelae than did unparasitized individuals. Chela size is a crucial determinant of fighting ability in alpheid shrimps (Schein 1977, Hughes 1996), and decreases in activity and chela size likely have significant effects on the ability of non-reproductive *S. elizabethae* to defend the host sponge. Along with previous work on parasitism in *Synalpheus* (Duffy 1992, Hernandez et al. 2010), our study suggests that the relatively high rates of parasitism documented in *S. elizabethae* and other sponge-dwelling *Synalpheus* species may have strong negative consequences for the shrimp colony. We also found significant differences in parasitism rates among different *Synalpheus* species inhabiting the same sponge host, and among the same species occupying different sponges.

Our behavioral surveys of experimental colonies indicated that parasitized shrimps moved less and groomed less than did unparasitized shrimps. This decrease in activity levels among parasitized shrimps is not surprising, given that other studies have shown that branchial bopyrids decrease metabolic rates (Anderson 1975, 1977; but see Nelson et al. 1986). However, the few experiments that have actually quantified activity have had mixed results; Bass & Weis (1999) found that branchial parasite infection led to reduced shrimp activity levels, while Chaplin-Ebanks & Curran (2005) found no effects of branchial parasitism on activity levels. Although parasitized individuals were slightly larger (6%) than unparasitized individuals, it seems unlikely that these differences had much effect on differences in observed activity, as body size within treatment had no significant effects on any of the movement rates we measured (Table 2). The only marginally significant trend we detected—a positive relationship between movement rates and size in unparasitized shrimps—would tend to increase

mean movement rates for larger (parasitized) shrimps relative to smaller (unparasitized shrimps), opposing the pattern we saw of lower movement rates for parasitized individuals. Positive correlations between movement rates and body size for unparasitized individuals could be indicative of intra-colony division of labor; for example, in the closely related eusocial species *Synalpheus regalis*, larger individuals spent more time moving around the sponge and engaging in aggressive behavior related to colony defense (Duffy 2007, Toth & Duffy 2008). If larger individuals are similarly important in colony defense in *S. elizabethae*, the reduced activity rates (and decreased chela size) seen in larger, parasitized individuals could exert high costs on the ability of a shrimp colony to hold and defend its host sponge. Other than differences in activity, we noticed few obvious differences in spatial segregation in our experimental colonies (i.e. parasitized individuals were not isolated to one section of the sponge). Future experiments are needed to more rigorously quantify whether parasitized individuals withdraw from defense of the host sponge, and what effects that may have on colony success (e.g. ability to repel invasions by non-colony members).

Along with reductions in overall movement rates and grooming rates, we observed higher rates of 'falling' in parasitized individuals—loss of contact with the sponge wall as they moved through the sponge canals. Although this falling behavior has not been noted in previous work on sponge-dwelling *Synalpheus* (Duffy et al. 2002), our observations suggest it is a natural movement, rather than a consequence of dwelling in artificial laboratory colonies. Individuals always 'fell' directly from the side of the host sponge (rather than the plexiglass that formed the front of their experimental colony), and parasitized shrimps generally fell onto their parasitized side, although it is unknown whether the extra mass of the parasite may have precipitated falling.

Parasitism also had strong effects on the morphology of infected shrimps: *Synalpheus elizabethae* with branchial bopyrids had on average 25% smaller major chelae than did unparasitized individuals. Although *S. elizabethae* individuals infected with abdominal bopyrids also appeared to have proportionally smaller major chelae than did unparasitized individuals, we did not have enough sponges ( $n = 4$ ) to make any significant conclusions about the effect of abdominal bopyrids on shrimp morphology. Previous studies have also shown that parasites affect reproductive morphology; branchial bopyrids have been shown to reduce the size of secondary sex characters in their shrimp hosts (Beck 1980), and abdominal bopyrids can cause partial or full castration of their host (O'Brien & Van Wyk 1985, Calado et al. 2005, Marin Jarrin & Shanks

2008, Smith et al. 2008, Hernáez et al. 2010). The mechanism for these changes is thought to be nutritional drain or disruption of endocrine systems (Beck 1980, Calado et al. 2005). Although parasites cannot affect reproductive morphology in *S. elizabethae* workers—as breeding is limited to the queen—parasitism does appear to have effects on major chela size, possibly because of nutritional drain. Interestingly, queens in some eusocial *Synalpheus* colonies also have smaller major chelae (or 2 minor chelae in the case of *S. filidigitus*; (Duffy & Macdonald 1999). Infection by parasites may in effect convert non-reproductive *S. elizabethae* workers into 'reproducers,' albeit propagating bopyrid (rather than shrimp) offspring.

We also examined several morphological patterns that may hint at the factors influencing transmission and parasitism rate. Bopyrid isopods have a complex life cycle, living first on intermediate hosts (calanoid copepods) then molting to free-swimming cryptoniscus larvae that infect the definitive decapod crustacean host (Markham 1985). They are thought to remain until the host dies (Beck 1980), although loss of branchial bopyrids can occur (Pike & Manton 1960). In our study, we found a significant positive correlation between the size of the host shrimp and the size of the mature female bopyrid for both abdominal and branchial bopyrids, suggesting that these parasites remain and grow with their host; this pattern has been seen in many other shrimps parasitized by bopyrids (Pike & Manton 1960, Allen 1966, Jay 1989, Cash & Bauer 1993, Choi et al. 2004) and is generally well-documented for a variety of epicaridean parasites (Kuris 1974, Lafferty & Kuris 2009). Shrimp handedness (whether the major chela was on the right or left side) had no apparent effect on the location of branchial bopyrids (whether they were in the right or left branchial cavity), and side parasitized did not appear to affect grooming patterns (i.e. they did not preferentially groom the inside of their infected gill region). Finally, as previously mentioned, parasitized shrimps tended to be larger than unparasitized shrimps. As shrimp are the secondary host for bopyrids, direct shrimp-to-shrimp transmission is unlikely (assuming the standard 2-host life cycle). One interpretation of the pattern we found—that larger individuals were more likely to be parasitized—suggests that larger individuals are more likely to be parasitized because larger (and presumably older) individuals may have had more time to be infected by free-swimming bopyrid cryptoniscus larvae (O'Brien & Van Wyk 1985). Alternatively, parasitized shrimps may have increased longevity, or may grow rapidly to larger sizes, e.g. due to greater allocation of host energy towards growth (Kuris 1974, O'Brien & Van Wyk 1985, Hechinger 2010). The trend of higher parasitism rates with larger

body size has been seen in many other studies (Schuldt & Capitulo 1985, Choi et al. 2004, Smith et al. 2008).

This study is the first to demonstrate strong effects of parasitism by branchial bopyrid isopods on the behavior and morphology of a eusocial snapping shrimp. Previous studies on *Synalpheus* have focused primarily on the benefits that accompany colony-living and sponge-dwelling (Toth & Duffy 2005, Macdonald et al. 2006, Duffy & Macdonald 2010), and our results highlight parasite infection as a potential cost of group living in *Synalpheus* snapping shrimps, similar to trends noted for other group-living animals (Brown & Brown 1986, Ezenwa 2004). Although our field sample is limited, we did find higher rates of parasitism in the eusocial species *S. elizabethae*, compared to the pair-forming species *S. yano* and *S. dardeai* living in the same sponge host. However, there were no significant correlations between group size and overall parasite prevalence in individual sponges. The lack of correlation between any one factor and parasitism suggests that parasitism rates are likely driven by a number of factors (Duffy 1992). Future large-scale studies are needed to more rigorously test whether eusocial *Synalpheus* species are more parasitized than pair-forming species, and to investigate the potential mechanisms driving variation in parasitism rates—and their fitness implications—in *Synalpheus*.

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#### LITERATURE CITED

- Allen JA (1966) Notes on the relationship of the bopyrid parasite *Hemiarthrus abdominalis* (Kroyer) with its hosts. *Crustaceana* 10:1–6
- Anderson G (1975) Metabolic response of the caridean shrimp *Palaemonetes pugio* to infection by the adult epibranchial isopod parasite *Probopyrus pandalicola*. *Comp Biochem Physiol A Mol Integr Physiol* 52:201–207
- Anderson G (1977) The effects of parasitism on energy flow through laboratory shrimp populations. *Mar Biol* 42: 239–251
- Banner DM, Banner AH (1975) The alpheid shrimp of Australia, Part 2: the genus *Synalpheus*. *Rec Aust Mus* 29: 267–389
- Bass CS, Weis JS (1999) Behavioral changes in the grass shrimp, *Palaemonetes pugio* (Holthuis), induced by the parasitic isopod, *Probopyrus pandalicola* (Packard). *J Exp Mar Biol Ecol* 241:223–233
- Beck JT (1980) The effects of an isopod castrator, *Probopyrus pandalicola*, on the sex characters of one of its caridean shrimp hosts, *Palaemonetes paludosus*. *Biol Bull (Woods Hole)* 158:1–15
- Brown CR, Brown MB (1986) Ectoparasitism as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). *Ecology* 67:1206–1218
- Bruce AJ (1976) Coral reef Caridea and 'commensalism'. *Micronesica* 12:83–98
- Calado R, Bartilotti C, Narciso L (2005) Short report on the effect of a parasitic isopod on the reproductive performance of a shrimp. *J Exp Mar Biol Ecol* 321:13–18
- Cash CE, Bauer RT (1993) Adaptations of the branchial ectoparasite *Probopyrus pandalicola* (Isopoda: Bopyridae) for survival and reproduction related to ecdysis of the host, *Palaemonetes pugio* (Caridea: Palaemonidae). *J Crustac Biol* 13:111–124
- Chaplin-Ebanks SA, Curran MC (2005) The effect of the parasitic isopod, *Probopyrus pandalicola* (Packard, 1879), on tidal activity patterns of the grass shrimp, *Palaemonetes pugio* Holthuis, 1949. *Crustaceana* 78:1053–1061
- Choi JH, Jamieson G, Han KH, Hong SY (2004) *Parapenaeon consolidatum* (Isopoda: Bopyridae) and the relative growth and reproduction of *Metapenaeopsis dalei* (Decapoda: Penaeidae) in South Korea. *J Shellfish Res* 23:237–242
- Duffy JE (1992) Host use patterns and demography in a guild of tropical sponge-dwelling shrimps. *Mar Ecol Prog Ser* 90:127–138
- Duffy JE (1996a) Eusociality in a coral-reef shrimp. *Nature* 381:512–514
- Duffy JE (1996b) Species boundaries, specialization, and the radiation of sponge-dwelling alpheid shrimp. *Biol J Linn Soc* 58:307–324
- Duffy JE (2007) Ecology and evolution of eusociality in sponge-dwelling shrimp. In: Duffy JE, Thiel M (eds) *Evolutionary ecology of social and sexual systems: crustaceans as model organisms*. Oxford University Press, Oxford, p 387–409
- Duffy JE, Macdonald KS (1999) Colony structure of the social snapping shrimp *Synalpheus filidigitus* in Belize. *J Crustac Biol* 19:283–292
- Duffy JE, Morrison CL, Macdonald KS (2002) Colony defense and behavioral differentiation in the eusocial shrimp *Synalpheus regalis*. *Behav Ecol Sociobiol* 51:488–495
- Duffy JE, Macdonald KS (2010) Kin structure, ecology and the evolution of social organization in shrimp: a comparative analysis. *Proc R Soc B* 277:575–584
- Ezenwa VO (2004) Host social behavior and parasitic infection: a multifactorial approach. *Behav Ecol* 15:446–454
- Hechinger RF (2010) Mortality affects adaptive allocation to growth and reproduction: field evidence from a guild of body snatchers. *BMC Evol Biol* 10:136, doi: 10.1186/1471-2148-10-136
- Hernández P, Martínez-Guerrero B, Anker A, Wehrmann IS (2010) Fecundity and effects of bopyrid infestation on egg production in the Caribbean sponge-dwelling snapping shrimp *Synalpheus yano* (Decapoda: Alpheididae). *J Mar Biol Assoc UK* 90:691–698
- Hughes M (1996) Size assessment via a visual signal in snapping shrimp. *Behav Ecol Sociobiol* 38:51–57
- Hultgren KM, Duffy JE (2010) Sponge host characteristics shape the community structure of their shrimp associates. *Mar Ecol Prog Ser* 407:1–12

- Hultgren KM, Macdonald KS, Duffy JE (2010) Sponge-dwelling snapping shrimps of Curaçao, with descriptions of three new species. *Zootaxa* 2372:221–262
- Jay CV (1989) Prevalence, size and fecundity of the parasitic isopod *Argeia pugettensis* on its host shrimp *Crangon francisorum*. *Am Midl Nat* 121:68–77
- Kuris AM (1974) Trophic interactions: similarity of parasitic castrators to parasitoids. *Q Rev Biol* 49:129–148
- Lafferty KD, Kuris AM (2009) Parasitic castration: the evolution and ecology of body snatchers. *Trends Parasitol* 25: 564–572
- Macdonald KS, Rios R, Duffy JE (2006) Biodiversity, host specificity, and dominance by eusocial species among sponge-dwelling alpheid shrimp on the Belize Barrier Reef. *Divers Distrib* 12:165–178
- Macdonald KS, Hultgren KM, Duffy JE (2009) The sponge-dwelling snapping shrimps (Crustacea, Decapoda, Alpheidae, *Synalpheus*) of Discovery Bay, Jamaica, with descriptions of four new species. *Zootaxa* 2199:1–57
- Marin Jarrin JR, Shanks AL (2008) Ecology of a population of *Lissocrangon stylirostris* (Caridea: Crangonidae), with notes on the occurrence and biology of its parasite, *Argeia pugettensis* (Isopoda: Bopyridae). *J Crustac Biol* 28: 613–621
- Markham JC (1985) A review of the bopyrid isopods infesting caridean shrimps in the northwestern Atlantic Ocean, with special reference to those collected during the Hourglass Cruises in the Gulf of Mexico. *Memoirs of the Hourglass Cruises, Vol 7*. St. Petersburg Department of Natural Resources, Bureau of Marine Research, St. Petersburg, FL
- Markham JC (2003) A worldwide list of hermit crabs and their relatives (Anomura: Paguroidea) reported as hosts of Isopoda Bopyridae. *Mem Mus Vic* 60:71–77
- Nakashima Y (1995) Can small male shrimps achieve copulation in the presence of larger ones? *J Ethol* 13:9–16
- Nelson SG, Simmons MA, Knight AW (1986) The energy burden of the bopyrid parasite *Argeia pauperata* (Crustacea, Isopoda) on the grass shrimp *Crangon francisorum* (Crustacea, Crangonidae). *Comp Biochem Physiol A* 83:121–124
- O'Brien J, Van Wyk P (1985) Effects of crustacean parasitic castrators (epicaridean isopods and rhizocephalan barnacles) on growth of crustacean hosts. *Crustac Issues* 3: 191–218
- Pike RB, Manton SM (1960) The biology and post-larval development of the bopyrid parasites *Pseudione affinis* G.O. Sars and *Hemiarthrus abdominalis* (Kröyer) [= *Phryxus abdominalis* Kröyer]. *Zool J Linn Soc* 44:239–251
- Rios R, Duffy JE (2007) 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* 1602:3–89
- Schein H (1977) The role of snapping in *Alpheus heterochaelis* Say, 1818, the big-clawed snapping shrimp. *Crustaceana* 33:182–188
- Schuldt M, Capitulo R (1985) Biological and pathological aspects of parasitism in the branchial chamber of *Palaeomonetes argentinus* (Crustacea: Decapoda) by infestation with *Probopyrus* cf. *oviformis* (Crustacea: Isopoda). *J Invertebr Pathol* 45:139–146
- Smith AE, Chapman JW, Dumbauld BR (2008) Population structure and energetics of the bopyrid isopod parasite *Orthonione griffenis* in mud shrimp *Upogebia pugettensis*. *J Crustac Biol* 28:228–233
- Somers IF, Kirkwood GP (1991) Population ecology of the grooved tiger prawn, *Penaeus semisulcatus*, in the northwestern Gulf of Carpentaria, Australia: growth, movement, age structure and infestation by the bopyrid parasite *Epipenaeon ingens*. *Mar Freshw Res* 42:349–367
- Toth E, Bauer RT (2007) Gonopore sexing technique allows determination of sex ratios and helper composition in eusocial shrimps. *Mar Biol* 151:1875–1886
- Toth E, Duffy JE (2005) Coordinated group response to nest intruders in social shrimp. *Biol Lett* 1:49–52
- Toth E, Duffy JE (2008) Influence of sociality on allometric growth and morphological differentiation in sponge-dwelling alpheid shrimp. *Biol J Linn Soc* 94:527–540

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