Reproductive strategies of the crinoid-associated shrimps Cristimenes commensalis (Borradaile, 1915) and Pontoniopsis comanthi Borradaile, 1915 (Crustacea: Palaemonidae)

Sancia E. T. van der Meij, Magnus L. Johnson, Sammy De Grave

Abstract.— Tropical shallow-water crinoids offer shelter to a diversity of marine life, including various symbiotic palaemonid shrimp species. In this study, the reproductive features of the shrimps Cristimenes commensalis and Pontoniopsis comanthi inhabiting comatulid crinoids are studied and compared. Ovigerous females were collected from the crinoids Anneissia bennetti and Comaster nobilis from Iriomote-jima, Japan. All specimens were examined for the following traits: carapace length, fecundity, embryo volume, brood mass volume and dry weight of the females and the eggs. Cristimenes commensalis produced 15–117 eggs with a volume of $0.04 \text{ mm}^3 \pm 0.00$, whereas the smaller-sized species $P$. comanthi produced 12–57 eggs with a volume of $0.02 \text{ mm}^3 \pm 0.01$. The reproductive output (RO) of $C$. commensalis was $0.16 \pm 0.03$, considerably lower than that of $P$. comanthi at $0.31 \pm 0.05$. Data is beginning to emerge on the fecundity and reproductive output of symbiotic palaemonid shrimps, although comparisons remain hampered by the general lack of knowledge on their biology in general. Pontoniopsis comanthi has the highest recorded reproductive output so far, despite being the smallest species studied. Our results confirm that inhabiting a sheltered habitat allows symbiotic shrimps to allocate more energy to embryo production than free-living species. Different reproductive strategies are, however, apparent for these species inhabiting the same host species, implying the existence of differential selective forces.

Key words: Caridea, co-occurrence, egg volume, fecundity, reproductive output, symbiosis

■ Introduction

Coral reef ecosystems are teeming with symbiotic relationships. All major sessile reef taxa have distinct associated faunas, and many of these symbiont species are specifically adapted to their host organism. Crinoids host numerous symbionts, de facto sustaining a microcosm ruled by crinoid-symbiont as well as symbiont-symbiont interactions (Potts, 1915; Fishelson, 1974; Fabricius & Dale, 1993). These associations are known from modern and fossil faunas, providing evidence for one of the longest-ranging host-symbiont interactions documented thus far (Feldman & Brett, 1998).

The high diversity of crinoid dwellers is likely related to their complex external morphology, which allows for co-occurrence of various associated species. Crinoids are filter-feeders; they concentrate food with their feather-like arms, which they then transport from the pinnales to the mouth along the ambulacral groove, making the food particles easily accessible to symbionts. The crinoid family Comatulidae Fleming, 1828 (previously Comasteridae A.H. Clark, 1908) lacks protective lappets over the groove, and appears to be particularly attractive to symbiotic organisms (Deheyn et al.,
Rates of up to 92% inhabitation of crinoids by symbionts have been reported, with the associated fauna mainly consisting of myzostomids, shrimps, ophiuroids and polychaetes (Fabricius & Dale, 1993; Deheyn et al., 2006).

The caridean family Palaemonidae encompasses species with a diverse range of lifestyles, including free-living and symbiotic species (De Grave, 2002). Studies on the symbiotic relationships between palaemonid shrimps and their invertebrate hosts are numerous, and include research on cleaning behaviour (Horká et al., 2018), host use (e.g., Guo et al., 1996; Ďuriš et al., 2011; Levitt-Barmats & Shenkar, 2018), and host switching from an evolutionary viewpoint (Horká et al., 2016).

Although several studies have been carried out detailing reproductive output, embryo volume and mass of caridean species across several shrimp families (Palaemonidae, Hippolytidae, Alpheidae; Corey & Reid, 1991; Kim & Hong, 2004; Terassi et al., 2010; Soledade et al., 2017), only two such studies have so far been carried out on symbiotic Palaemonidae. The fecundity of the sea-anemone symbiont Periclimenes rathbunae Schmitt, 1924 was studied in Costa Rica (Azofeifa-Solano et al., 2014), whilst the jelly-fish symbiont, Periclimenes paivai Chace, 1969 was studied in Brazil (de Moraes et al., 2017), only two such studies have so far been carried out on symbiotic Palaemonidae. The fecundity of the sea-anemone symbiont Periclimenes rathbunae Schmitt, 1924 was studied in Costa Rica (Azofeifa-Solano et al., 2014), whilst the jelly-fish symbiont, Periclimenes paivai Chace, 1969 was studied in Brazil (de Moraes et al., 2017). This level of detail is also available for two free-living species of a body size similar to the majority of symbiotic species, Cuapetes americanus (Kingsley, 1878) (Negri et al., 2017) and Phycocenes siankaanensis (Martinez-Mayén & Román-Contreras, 2006) (Martinez-Mayén & Román-Contreras, 2009), as Periclimenes siankaanensis. More information is available on the number of eggs produced in symbiotic Palaemonidae, with this being available for several sea-anemone symbionts (Omori et al., 1994; McKeon & O’Donnell, 2015), a fish-cleaner also inhabiting sea-anenomes (Spotte, 1999), a gorgonian associate (Heard & Spotte, 1991), bivalve associates (Johnson & Liang, 1966; Corey & Reid, 1991; Bruce, 2000), as well as several free-living species (Corey & Reid, 1991) of similar body size to the symbiotic species. All these studies have, however, only concentrated on a single species, with no sympatric or host-sharing taxa documented, in contrast to studies in other families (Soledade et al., 2017). This is largely due to the fact that most palaemonid species are highly host specific and it is relatively rare that more than one shrimp species occurs in high population densities on a given host, with crinoid symbionts being a notable exception to this rule (Huang et al., 2005).

Here we report on the fecundity and reproductive output of the symbiotic shrimps Cristimenes commensalis (Borradaile, 1915) and Pontoniopsis comanthi Borradaile, 1915, collected from the comatulid crinoid species Anneissia bennetti (Müller, 1841) (previously known as Oxycomanthus bennetti (Summers et al., 2014)) and Comaster nobilis (Carpenter, 1884). Cristimenes commensalis is widespread across the entire Indo-West Pacific, from Kenya through to Japan and the Marshall Islands (De Grave, 2002), and is associated with a variety of species in the crinoid families Comasteridae, Zygometridae and Himerometridae; generally occupying the distal part of the crinoid arms and occurring in groups of several heterosexual pairs and additional juveniles (Zmarzly, 1984). Pontoniopsis comanthi is equally widespread (De Grave, 2002), primarily associated with crinoids in the families Comasteridae and Himerometridae; generally occurring in relatively large groups, sharing the crinoid ecospace with other species, like said C. commensalis. The co-occurrence on the same host species allows us to compare the reproductive strategies of these crinoid-associated shrimps and study whether the same selective pressures govern their respective reproductive strategies.
Material and Methods

Crinoids were collected during two consecutive days (9–10 June 2014) from Baras Island, Iriomote-jima, Yaeyama Islands, Japan (24°26′ 25.9″N, 123°48′48.4″E). The collecting site was a relatively barren rock/canyon area, with high current velocities and an approximate depth of 15–20 meters. Crinoids were individually bagged into re-sealable zipper storage bags (Umbrella Corp.) and transported in cooler boxes to the laboratory, where the individual crinoids were placed in shallow trays. All visible shrimp symbionts were removed from their host, aided by the occasional application of small quantities of diluted formalin by pipette. After removal of the symbionts, the crinoids were washed with jets of water over a 1 mm sieve to dislodge remaining symbionts, and subsequently preserved in 70% ethanol. The shrimp *C. commensalis* was collected from both *A. bennetti* and *C. nobilis*, whereas *P. comanthi* was only collected from *A. bennetti*.

Female carapace length (CL) was measured from the post-orbital margin to the posterior border of the carapace. The egg masses of gravid *C. commensalis* and *P. comanthi* were counted and classified into two stages (Zimmermann et al., 2015): stage I (early development, no eyespots visible) and stage II (late development, with visible eyespots). Fecundity was determined as the total number of eggs attached to the female’s abdomen. The methods for egg and brood volume measurements were adopted from literature (Zimmermann et al., 2015). Egg samples and females were weighed separately with a KERN ABT 120–5DM analytical balance, with an accuracy of 0.01 mg. Female weight was quantified after removing all the eggs from the abdomen. All weight measurements are dry weight values after 24 hours of oven drying at 60°C in a standard drying cabinet. Brood weight was determined as the total weight of the egg batch. The reproductive output (RO) is defined as the fraction of the total energy budget diverted to reproduction. To obtain the RO values the brood dry weight was divided by the female’s dry weight.

The statistical analyses were performed using R (R Core Team, 2018). Data were assessed for normality using the Shapiro test. For non-parametric data, analysis of covariance was examined using the fANCOVA package (Wang, 2010). To test for any possible effects of host crinoid species on the fecundity of *C. commensalis* a Wilcoxon signed-rank test was used (which in R is identical to the Mann-Whitney U test; Ennos & Johnson, 2018). A significance level of 0.05 was adopted for the statistical analyses and as the data were not normally distributed, results are displayed as medians and 95% confidence interval (CI) to cross compare both species. CIs were calculated using the groupwise Median function (Mangiafico, 2018) with the bootstrap level set to 5000. Although in order to compare with other species, means ± SD are also used, as only those values are reported in literature.

Results

Inhabitation of crinoids by *C. commensalis* was 86.4%, compared to 50% by *P. comanthi*. The group size of *C. commensalis* ranged from 2–13 specimens (median 6), and from 1–17 specimens (median 5) for *P. comanthi*. The groups consisted of a mix of male, female (ovigerous and non-ovigerous) and juvenile specimens, of which only the ovigerous females were examined herein. Of the 32 studied females of *C. commensalis*, the majority (n = 17) carried stage I eggs, with 15 individuals carrying stage II eggs. Of the 25 *P. comanthi* females, 13 carried stage I eggs and 12 carried stage II eggs. There is no significant difference in the number of eggs between stages I and II for *C. commensalis* (W = 159, df = 30, p = 0.24) or *P. comanthi* (W = 60.5, df = 23, p = 0.35). The number of eggs ranged between 15 and 117 for *C. commensalis* and between 12 and 57...
Crustacean Research 47

for *P. comanthis*, with medians (95% CI) of 49.5 (38.0–58.0) and 27 (19.0–32.0), respectively (Table 1).

Carapace length of *C. commensalis* was significantly larger than for *P. comanthis* (*W* = 800, *df* = 55, *p* < 0.001) as was female dry weight (*W* = 798, *df* = 55, *p* < 0.001) (Table 1). There is no significant difference in female dry

Table 1. Reproductive traits (median ± 95% CI) of *Cristimenes commensalis* and *Pontoniopsis comanthis* for all specimens combined, as well as separated according to embryo development stage.

<table>
<thead>
<tr>
<th>Means</th>
<th>CL (mm)</th>
<th>Embryo number</th>
<th>Embryo</th>
<th>Embryo volume (mm³)</th>
<th>Brood mass volume (mm³)</th>
<th>Dry weight-female (mg)</th>
<th>Dry weight-eggs (mg)</th>
<th>RO</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. commensalis</em> (<em>n</em> = 32)</td>
<td>2.03 (1.95–2.10)</td>
<td>15–117</td>
<td>49.5 (38.0–58.0)</td>
<td>0.04 (0.04–0.05)</td>
<td>1.98 (1.57–2.50)</td>
<td>2.83 (2.59–3.30)</td>
<td>0.47 (0.33–0.56)</td>
<td>0.17</td>
</tr>
<tr>
<td>Stage I (<em>n</em> = 17)</td>
<td>2.05 (2.00–2.25)</td>
<td>23–104</td>
<td>53 (38–67)</td>
<td>0.04 (0.03–0.04)</td>
<td>1.82 (1.57–2.33)</td>
<td>2.79 (2.34–3.83)</td>
<td>0.50 (0.30–0.62)</td>
<td>0.18</td>
</tr>
<tr>
<td>Stage II (<em>n</em> = 15)</td>
<td>2.00 (1.75–2.10)</td>
<td>15–117</td>
<td>45 (28–60)</td>
<td>0.05 (0.05–0.06)</td>
<td>2.59 (1.35–2.81)</td>
<td>2.86 (2.30–3.64)</td>
<td>0.43 (0.28–0.58)</td>
<td>0.14</td>
</tr>
<tr>
<td><em>P. comanthis</em> (<em>n</em> = 25)</td>
<td>1.10 (1.10–1.20)</td>
<td>12–57</td>
<td>27.0 (19.0–32.0)</td>
<td>0.02 (0.02–0.03)</td>
<td>0.55 (0.44–0.75)</td>
<td>1.18 (1.04–1.29)</td>
<td>0.38 (0.30–0.43)</td>
<td>0.32</td>
</tr>
<tr>
<td>Stage I (<em>n</em> = 13)</td>
<td>1.10 (1.10–1.25)</td>
<td>12–57</td>
<td>21.0 (18.0–31.0)</td>
<td>0.02 (0.02–0.02)</td>
<td>0.44 (0.28–0.45)</td>
<td>1.04 (0.96–1.24)</td>
<td>0.36 (0.29–0.41)</td>
<td>0.35</td>
</tr>
<tr>
<td>Stage II (<em>n</em> = 12)</td>
<td>1.15 (1.10–1.20)</td>
<td>12–37</td>
<td>29.5 (23.5–32.5)</td>
<td>0.03 (0.02–0.03)</td>
<td>0.81 (0.63–0.97)</td>
<td>1.31 (1.18–1.40)</td>
<td>0.4 (0.30–0.47)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Fig. 1. Weight in relation to carapace length for a) *C. commensalis* and b) *P. comanthis*. ANCOVA indicated no significant difference in the relationships with egg stage as a factor (*p* > 0.05). Stage I eggs = open circles, stage II eggs = closed circles.
weight between egg stages I and II for *C. commensalis* (W = 117, df = 30, p = 0.706), but there is for *P. comanthi* (W = 32.5, df = 23, p < 0.05), with stage II bearing females being heavier. A significant relationship was found between female dry weight and carapace length (Fig. 1). There was also a positive relationship between the number of eggs carried by an individual shrimp and its carapace length (Fig. 2). The embryo volume (Table 1, Fig. 3) of *C.
commensalis was significantly larger than that of P. comanthi (W = 766.5, df = 55, p < 0.001), with a significant increase from stage I to stage II embryos in P. comanthi (W = 22, d.f = 23, p < 0.05) but not for C. commensalis (W = 91, df = 30, p > 0.05).

The mean reproductive output (RO) of C. commensalis over the two stages combined had a median value of 17%, with the values per reproductive event ranging from 9% (stage II
eggs, CL 1.75 mm) to 23% (stage I eggs, CL 2.10 mm) of egg weight relative to female weight. The RO of *P. comanthi* was higher with a median value of 32% over both stages combined, with the values per reproductive event ranging from 23% (stage II eggs, CL 1.20 mm) to 40% (stage II eggs, CL 1.15 mm) (Table 2, Fig. 4).

Possible effects of host crinoid species on the fecundity or reproductive output of *C. commensalis*, which occurred on two host species (*n* = 20 on *A. bennetti*, *n* = 12 on *C. nobilis*) were tested using a Wilcoxon signed-rank test, but no significant differences were found between the two host species (*p* > 0.05).

### Discussion

The crinoids in this study show high levels of inhabitation by the shrimps *C. commensalis* and *P. comanthi*, which is in line with earlier studies (Deheyn *et al*., 2006). Both species show a lack of embryo loss between egg stages (Table 1). The jellyfish symbiont *P. paivai* had a mean percentage of embryo loss between egg stages of 16.6% (de Moraes *et al*., 2017), and for the sea-anemone symbiont, *P. rathbunae* embryo mortality was reported to be 24% (Azofeifa-Solano *et al*., 2014). This species exhibited similar levels of brood loss to free living species, *e.g.* the similar sized *C. americanus* (Negri *et al*., 2017). Notwithstanding the relatively small sample size in the present study, we speculate that this difference is caused by the more enclosed and thus protected ecospace in crinoids, enabling higher levels of parental care and potentially less predation, as opposed to the more open ecospace in sea anemones.

As is generally known for caridean shrimps (Corey & Reid, 1991), fecundity is closely related to female size (Azofeifa *et al*., 2014, and references therein). This trend is herein confirmed once again for the two species studied. Although the minimum number of eggs produced in both *C. commensalis* and *P. comanthi* is broadly comparable to other symbiotic Palaemonidae (Azofeifa-Solano *et al*., 2014), the maximum number is generally lower. Only *Periclimenes patae* Heard & Spotte, 1991, a gorgonian commensal, produces fewer eggs (10–35 (Heard & Spotte, 1991)), with the majority of species having maximal values of 200 or more. A meaningful comparison in this regard is, however, hampered by the fact that our samples originated from a single event, and seasonal variation has been noted in *Actinimenes ornatus* (Bruce, 1969) [(Omori *et al*., 1994), as *Periclimenes ornatus*]. Nevertheless, low fecundity in these crinoid-inhabiting species is likely offset by successive spawning, once sexual maturity is reached, often in relatively quick succession (Bauer, 1991).

The embryo volume (mm$^3$) of *C. commensalis* is approximately in the same range as the other species of symbiotic Palaemonidae for which this is known (Azofeifa-Solano *et al*., 2014). In contrast, the embryo volume of *P. comanthi* is the smallest recorded to date (Table 2). Although this is clearly linked to the size of the species, which is the smallest studied so far, it can be speculated that combined with the lower number of eggs produced that the species would undergo a form of abbreviated larval development compared to *C. commensalis* and other species. Although in shrimps, abbreviated larval development is generally linked to a larger egg size (Strathmann, 1977; Wowor *et al*., 2009), we postulate that the physical size limitations of the pleon in *P. comanthi* do not allow for enlarged eggs. In common with the majority of symbiotic Palaemonidae, no information is available on larval biology, so this remains somewhat speculative.

The area available for egg attachment physically limits the brood mass volume (mm$^3$) of a species, and is strongly linked to carapace volume (Corey & Reid, 1991). When looking at the average brood mass volume, *C. commensalis* has a brood mass volume of 2.15 ±
0.53 mm³, averaged over both egg stages (stage I: 1.80 ± 0.68 mm³, stage II: 2.54 ± 1.60 mm³), compared to 0.59 ± 0.27 mm³ for *P. comanthi* (stage I: 0.42 ± 0.19 mm³, stage II: 0.76 ± 0.24 mm³). These numbers are much lower than the estimated brood mass volumes of *P. paivai* and *P. rathbunae*. In general, ovigerous females of Palaemonidae display a linear relationship between carapace length and the size of the second abdominal pleura (Baeza *et al.*, 2013, 2015), the main structural component of the brood pouch. It follows that larger species, such as the two *Periclimenes* have a larger brood pouch than the herein studied species, which are smaller in body size. Logically this allows for fewer eggs (see above) and thus a smaller total brood mass volume.

Reproductive Output (RO) is a widely used value to compare inter- and intraspecific variation in the fraction of the total energy budget diverted to reproduction, allowing for a cross comparison of the reproductive strategies between species, although this value has only been reported for two species of symbiotic Palaemonidae studies so far (Table 2; Azofeifa-Solano *et al.*, 2014; de Moraes *et al.*, 2017). These studies and the values reported herein show that the RO values of symbiont species are higher than those of free-living species. Commensal caridean shrimp species are thus seemingly able to allocate more energy in brood production than free-living species, potentially linked to shorter larval duration. Despite being the smallest species, the RO of *P. comanthi* is the highest recorded so far for symbiotic palaemonids (Table 2). A large RO may be adaptive to a commensal lifestyle, as larvae may suffer greater mortality during their search for a specific settlement site (Hines, 1992). Moreover, low survival rates have been reported for juvenile symbiotic palaemonids (Omori *et al.*, 1994). An increased RO will help to compensate for larval and juvenile mortality.

The smallest of symbiotic crabs (Cryptochiridae and Pinnotheridae) are able to produce proportionately large broods by specific morphological features (extension of ovaries into the abdomen and reduced calcification of their exoskeleton) (Vehof *et al.*, 2016). Their RO values are even higher than those of the symbiotic shrimp, with investment values of 59% for the cryptochirids and 66% and 97% for the pinnotherids. The pea crabs (*Zaops ostreus* (Say, 1817) [as *Pinnotheres ostreum*] and *Fabia subquadrata* Dana, 1851) have short reproductive seasons bringing their annual investment up to 99% and 146%, respectively (Hines, 1992). The tropical cryptochir crab species *Hapalocarcinus marsupialis* Stimpson, 1859 has an estimated annual investment of 472%, based on a conservative estimate of 8 broods per year (Kotb & Hartnoll, 2002). A study on the temperate sea anemone-associated species *A. ornatus* indicates year-round breeding, although with a drop in the number of ovigerous females in the colder months. In the warmest month the mean interval of two successive egg layings was 10 days (Omori *et al.*, 1994). In tropical species year-round breeding has been reported for symbiotic Palaemonidae (Johnson & Liang, 1966; Bruce, 2000), with embryo incubation periods lasting 5–10 days in several sea grass inhabiting caridean shrimp (Bauer, 1991). Such short embryo incubation periods in tropical species may be a life history adaptation for increasing the number of broods spawned per unit time, or a metabolic response to elevated water temperatures (Bauer, 1991). The annual investment of the tropical species *C. commensalis* and *P. comanthi* could therefore be as high as 384% and 744%, respectively, based on an estimated 24 broods per year (estimated embryo incubation of 10 days, plus ca. 5 days for moulting and spawning of new eggs (derived from Bauer, (1991) and Omori *et al.*, (1994)). These annual investment rates, including those of the cryptochir and pinnotherids, do not take female maturation time and longevity into account and true values are
therefore likely somewhat lower.

A study on *Periclimenes yucatanicus* (Ives, 1891) collected from two morphologically different sea anemone hosts (*Stichodactyla helianthus* (Ellis, 1768) and *Bartholomea annulata* (Le Sueur, 1817)) found that larger sized hosts were capable of supporting larger and more fecund shrimp irrespective of the host species (McKeon & O’Donnell, 2015). The authors suggested that host preference might be determined by host size, via its effect on fecundity in *P. yucatanicus*. In this study, *C. commensalis* was collected from two morphologically different crinoid species; *A. bennetti* is generally larger in size and has more arms than *C. nobilis*. We found, however, no significant effects of host species on fecundity or reproductive output of *C commensalis*.

Comparison of the reproductive strategies of *C. commensalis* and *P. comanthi* reveals a different reproductive approach for each species. *Cristimenes commensalis* specimens are larger in size and produce a higher number of eggs, yet the RO of *P. comanthi* is higher than that of *C. commensalis*. Our results indicate there are different reproductive strategies between symbiotic species inhabiting the same host species, linked to differential evolutionary pressures despite equal environmental forces. In the present case, this is clearly linked to the size difference between both symbionts.

■ Acknowledgements

Funding for the fieldwork was provided by Oxford University’s John Fell Fund and hosted by the Iriomote Station of the Tropical Biosphere Research Center (University of the Ryukyus), facilitated by T. Naruse. Crinoid species were identified by Y. Fujita for which we are grateful. We thank Fabricio Carvalho and an anonymous reviewer for their constructive comments.

■ Literature Cited


de Moraes, I. R. R., Wolf, M. R., Gonçalves, G.


Address

(SETVDM) Groningen Institute for Evolutionary Life Sciences, University of Groningen, PO Box 11103, 9700 CC Groningen, the Netherlands; Naturalis Biodiversity Center, PO Box 9517, 2300 RA Leiden, the Netherlands

(MLJ) School of Environmental Sciences, Faculty of Science and Engineering, University of Hull, HU6 7RX, United Kingdom

(SDG) Oxford University Museum of Natural History, University of Oxford, Parks Road, Oxford OX1 3PW, United Kingdom

E-mail addresses

(SETVDM)* Sancia.van.der.Meij@rug.nl

(MLJ) M.Johnson@hull.ac.uk

(SDG) Sammy.DeGrave@oumnh.ox.ac.uk

* Corresponding author