**ABSTRACT.** Symbiosis between decapods and mollusks provides a unique opportunity to examine some of the evolutionary strategies employed by marine invertebrates. We describe the sexual and reproductive traits of the pearl oyster shrimp, *Pontonia margarita* Verril, 1869, found symbiotically inhabiting the mantle cavity of the rugose pen shell, *Pinna rugosa* Sowerby, 1835. Solitary males and females (ovigerous and non-ovigerous) and heterosexual pairs (with ovigerous and non-ovigerous females) were found in a total of 47 rugose pen shells collected from a sandy area with seagrass meadows on the southeastern coast of the Gulf of California, Mexico. The body length (BL) of female *P. margarita* was correlated with the shell volume of their rugose pen shell host. The sex ratio was female-biased (0.85M:1F). Female *P. margarita* were larger than their male counterparts in terms of BL, cephalothorax length (CL), and the maximum chelae length of the second pereopod (MCL). The CL and MCL were more strongly correlated for males ($r = 0.70$, $p = 0.01$). The number and volume of eggs per ovigerous female varied from 95 to 1,571 and from $5.46 \pm 0.48$ to $8.85 \pm 0.97$ mm$^3$, respectively. Our results indicate polygamous behavior and social monogamy among *P. margarita*, and a short-term pairing system for their association with *P. rugosa*.

**KEY WORDS.** Monogamy, promiscuity, reproduction, sex ratio, shrimp-pen shell association.
guest organisms in each mollusk species (Rada and Milat 2009). Some researchers argue that symbiotic crustaceans have gonochoric sexual dimorphism (Baeza et al. 2013); that is, they are born, reproduce, and remain either male or female throughout their life. However, other investigators have shown that some shrimp species develop reproductive strategies in response to social behaviors and environmental conditions, including partial changes in sex (Nakashima 1987), strictly protandry (i.e., first males and then females) (Baeza and Piantoni 2010), and simultaneous protandric hermaphroditism (Baeza 2010b).

Specifically, a variety of reproductive behaviors have been observed among symbiotic pontoniids. Pacheco et al. (2014) observed Pontonia pinnophylax in the mantle cavity of P. rudis, and Baeza et al. (2011) discovered the shrimp P. mexicana Guérin-Méneville, 1855 in the amber pen shell Pinna carnea Gmelin, 1797. In these studies, both sexual pairs and solitary individuals were reported. The male-female pairs were exclusively heterosexual, suggesting a monogamous relationship (Baeza et al. 2011); nevertheless, the presence of solitary ovigerous females suggests that males abandon the host to look for more females to fertilize (Pacheco et al. 2014). Recently, Góngora-Gómez et al. (2015) described the association between the pen shell Atrina tuberculosa Sowerby, 1835 and P. margarita Verril, 1869 in the southeastern Gulf of California, concluding that both organisms maintain a long-lasting relationship and a mating system based on monogamy. In this study, we describe the sexual and reproductive traits of P. margarita found symbiotically inhabiting the mantle cavity of Pinna rugosa Sowerby, 1835, reporting new morphological and reproductive indicators to better explain this example of symbiosis as an evolutionary adaptation in the marine environment.

**MATERIAL AND METHODS**

A total of 47 rugose pen shell (P. rugosa) specimens were collected by free-diving during diurnal low tides (< 1 m) in the waters surrounding a fishery in Altata Bay (24°38′00″N; 107°55′00″W), Navolato, Sinaloa, Mexico. The location is a sandy area with Caulerpa sertularioides Gmelin, 1768 alga growing in seagrass meadows. Samples were collected between October and December 2017. Once out of the sand but still underwater, each specimen was placed in a separate plastic bag to preserve both host and guest and transported to the Laboratory of Malacology at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional at the Instituto Politécnico Nacional (CIIDIR-IPN), Unidad Sinaloa.

Morphometric measurements of the rugose pen shells were obtained using a caliper (± 0.01 mm). The shell height (SH) was measured as the longest point along a line perpendicular from the umbo, the shell length (SL) was the longest point along a line parallel to the umbo, and the shell width (SW) was recorded as the distance from the left to the right side (Figs 1–3).

In order to better understand a possible relationship between the available space of the pen shell for the shrimp (alone or paired, ovigerous or non-ovigerous females), the approximate volume of each rugose pen shell (SV) was calculated multiplying the three morphometric variables according to Salas-Moya et al. (2014) and Góngora-Gómez et al. (2015). The rugose pen shells were sacrificed by cutting the posterior adductor muscle and the mantle cavity was examined for guest organisms.

The shrimp P. margarita was identified based on Fransen (2002). The number of guests per host was recorded and all
Symbiosis between *Pontonia margarita* and *Pinna rugosa*

Shrimp specimens were fixed in 70% ethanol. The total body length (BL, from the tip of the rostrum to the tip of the telson), cephalothorax length (CL, from the tip of the rostrum to the posterior end of the carapace), and the maximum chela length of the second pereopod (MCL, from the base of the dactyl and propodus to the tip of the claw) of each shrimp were measured using a caliper (± 0.01 mm) with the aid of a stereomicroscope. The shrimp specimens were stored in the Laboratory of Malacology at the CIIDIR-IPN, Unidad Sinaloa, and coded as: IPN-CIIDIR-SINALOA: Malacología/simbiosis/2017(47).

Several measurements on the reproductive anatomy of each *P. margarita* were recorded (CL and MCL) in order to examine the sexual system. The sex of each specimen was determined based on the position of the genital pore (base of the third and fifth pair of pleopods for females and males, respectively) (Baaza et al. 2013), and the presence or absence of eggs beneath the abdomen was registered. The entire brood mass was detached from ovigerous females (n = 10) and distributed in a Petri dish; the total number of eggs was individually counted under microscopic observation. Egg volume, or EV, (n = 15 eggs_ovigerous female) was estimated following the formula proposed by Corey and Reid (1991): 
\[ EV = \frac{(b/2)^2 \times \pi \times a}{2}, \]
where *a* = major diameter, and *b* = perpendicular diameter.

The normality and homoscedasticity of data were confirmed using the Lilliefors and Bartlett’s tests, respectively (Sokal and Rohlf 1995). Morphometric relationships between rugose pen shell length and total shrimp length, between shrimp measurements (BL, CL, and MCL, by sexes), and between berried female measurements (BL, number of eggs, and EV) were studied using Pearson’s correlation (Bhujel 2008). Tukey’s test and ANOVA were used to detect possible differences in the EV of ovigerous females. Significant differences in the BL, CL, and MCL by sex were identified using the Student’s t-test (p ≤ 0.05). Data were analyzed using Statgraphics Centurion software for Windows (ver. 14.0).

**RESULTS**

The rugose pen shell SH varied from 197 to 290 mm and averaged 251.38 ± 22.07 mm. The presence of *P. margarita* in the mantle cavity of rugose pen shells is shown in Table 1.

Table 1. Distribution of *Pontonia margarita* in the mantle cavity of rugose pen shell (*Pinna rugosa*).

<table>
<thead>
<tr>
<th>Shrimp presence and reproductive condition</th>
<th>Number of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>No shrimp</td>
<td>15</td>
</tr>
<tr>
<td>Ovigerous female + male</td>
<td>6</td>
</tr>
<tr>
<td>Non-ovigerous female + male</td>
<td>8</td>
</tr>
<tr>
<td>Ovigerous female only</td>
<td>4</td>
</tr>
<tr>
<td>Non-ovigerous female only</td>
<td>10</td>
</tr>
<tr>
<td>Male only</td>
<td>4</td>
</tr>
</tbody>
</table>

Shrimp specimens were semi-transparent and pale yellow to orange in color (Fig. 4). The pearl oyster shrimp was found in 68% of the total rugose pen shells, inhabiting hosts either in heterosexual pairs (n = 14), as solitary females (n = 14, Fig. 5), or as solitary males (n = 4), with a mean of 0.98 ± 0.01 specimens per rugose pen shell. No rugose pen shells harbored homosexual pairs with two male or two female *P. margarita*. Of the 39 shrimp specimens obtained, 18 were males and 21 females, for a sex ratio of 0.85M:1F.

The smallest rugose pen shell with shrimp had a SH of 198 mm. The BL of female *P. margarita* was the only measure correlated (r = 0.51, p = 0.005) with the shell volume of *P. rugosa* (1.26 ± 0.26 dm³) (Table 2). There was no correlation between the shrimp body length and host shell height (r = 0.32, p = 0.08 for females; r = 0.18, p = 0.46 for males) (Fig. 7).

Table 2. Regression equations, correlation coefficients (r, adjusted for degree of freedom, df), and standard errors of the slopes (SE) between *Pontonia margarita* body length (BL) and *Pinna rugosa* shell volume (SV).

<table>
<thead>
<tr>
<th>Regression</th>
<th>R</th>
<th>SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL = -0.399+0.052 SV</td>
<td>0.51</td>
<td>0.27</td>
<td>0.005</td>
</tr>
<tr>
<td>CL = 1.229+0.008 SV</td>
<td>0.11</td>
<td>0.31</td>
<td>0.560</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL = 1.635-0.015 SV</td>
<td>-0.19</td>
<td>0.32</td>
<td>0.444</td>
</tr>
<tr>
<td>CL = 1.409-0.021 SV</td>
<td>-0.22</td>
<td>0.33</td>
<td>0.427</td>
</tr>
</tbody>
</table>

The mean BL and CL of female and male *P. margarita* were 25.15 ± 3.71 and 20.81 ± 7.95 mm, and 9.84 ± 0.16 and 5.05 ± 0.76 mm, respectively. There was no correlation between the BL of males and that of females (r = 0.19, p = 0.49) in the 14 heterosexual pairs. The BL (t_{14} = 13.03, p = 0.001) and CL (t_{14} = -4.65, p = 0.0001) were significantly longer in female *P. margarita* than in their male counterparts. The CL and MCL were more highly correlated for males (r = 0.70, p = 0.01). The maximum chela length of the second pereopod of females (15.61 ± 1.94 mm) was longer (t_{14} = 3.06, p = 0.006) than that of their male counterparts (12.65 ± 3.03 mm).

Of the 21 total females collected, 10 (solitary or paired) were ovigerous. Of the females found with a male in the same rugose pen shell, four were brooding eggs (Fig. 6). The number of eggs per ovigerous female ranged from 95 to 1,571. There was no correlation between the number of eggs and the BL of ovigerous females (r = 0.52, p = 0.12) or the EV (r = 0.50, p = 0.11). There were significant differences (F = 51.31, p = 0.0001) in the EV of different ovigerous females (Table 3). The EV varied from 5.46 ± 0.48 to 8.85 ± 0.97 mm³.

**DISCUSSION**

Several studies emphasize the importance of the host-guest size relationship between pontoniid shrimps and different bivalve mollusk species. In the present study, shrimps were found...
inhabiting pen shells with an SH varying from 198 to 271 mm, with no correlation between shrimp size and host shell size or any other indication of a size-host preference exhibited by male or female *P. margarita*. Baeza et al. (2013) did not find the shrimp *Paranchistus pycnodontae* Bruce, 1978 in the pearl oyster *Pteria penguin* Röding, 1798 when the bivalve SL was < 175 mm; they concluded that an oyster shell length < 175 mm is too small for the symbiont shrimp. A similar conclusion was reached by Aucoin and Himmelman (2010) when studying the pen shell *P. carnea* as they reported proportional growth between *Ponto-nia* sp. and its host. The average approximate volume of each rugose pen shell (1.26 ± 0.26 dm$^3$) obtained in this study was smaller than that reported by Góngora-Gómez et al. (2015) for *A. tuberculosa* (3.55 ± 0.76 dm$^3$) using the same morphometric calculations. Our result suggests that the SV of the *P. rugosa* shell is large enough to harbor even a male-female *P. margarita* pair inside, the same shrimp species reported by Góngora-Gómez et al. (2015) inhabiting *A. tuberculosa*. Distinctions between our conclusions and those of the aforementioned studies may be partially explained by the different species of bivalves and molulks examined. Future studies should report the host volume, SH, and SL as indices of association in order to better evaluate the host-guest relationship.

In this study, shrimps showed an average CL of 9.48 ± 0.16 and 5.05 ± 0.76 mm for females and males, respectively, being similar to the CL average values (females = 8.21 ± 2.46 mm; males 6.39 ± 2.02 mm) reported by Cabrera-Peña and Solano-López (1996) for the same shrimp species reported by Góngora-Gómez et al. (2015) inhabiting *A. tuberculosa*. Distinctions between our conclusions and those of the aforementioned studies may be partially explained by the different species of bivalves and molulks examined. Future studies should report the host volume, SH, and SL as indices of association in order to better evaluate the host-guest relationship.

In this study, shrimps showed an average CL of 9.48 ± 0.16 and 5.05 ± 0.76 mm for females and males, respectively, being similar to the CL average values (females = 8.21 ± 2.46 mm; males 6.39 ± 2.02 mm) reported by Cabrera-Peña and Solano-López (1996) for the same shrimp species inhabiting in the paleal cavity of the pearl oyster *Pinctada mazatlanica* (Hanley, 1856) in Costa Rica, but higher to the mean CL values obtained by Bruce (1980) in *Cainonia medipacifica* (Edmondson, 1935) (reported as *Pontonia medipacifica*) (6.1 and 3.4 mm for females and males, respectively) in the central Pacific (Midway

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**Figures 4–6.** *Pontonia margarita*: (4) male (small and whitish) and female (large and orange); (5) female (circled) in the mantle cavity of *P. rugosa*; (6) ovigerous female. Scale bar = 1 cm.

**Figure 7.** Relationship between *Pontonia margarita* total body length (mm) and *Pinna rugosa* shell height (mm). Regressions for females and males were $r = 0.32$ (p = 0.08) and $r = 0.18$ (p = 0.46), respectively.

**Table 3.** Comparison of average egg volume (EV, n = 15 eggs per ovigerous female) for ovigerous female *Pontonia margarita* (n = 10) in the mantle cavity of *Pinna rugosa*. BL = body length (mm).

<table>
<thead>
<tr>
<th>BL of ovigerous female (mm)</th>
<th>Mean EV (mm$^3$)</th>
<th>Standard deviation</th>
<th>Coefficient of variation (%)</th>
<th>Total number of eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.59</td>
<td>8.13$^a$</td>
<td>0.55</td>
<td>6.77</td>
<td>95</td>
</tr>
<tr>
<td>29.3</td>
<td>8.68$^{bc}$</td>
<td>0.71</td>
<td>8.21</td>
<td>849</td>
</tr>
<tr>
<td>31.15</td>
<td>6.16$^a$</td>
<td>0.50</td>
<td>8.15</td>
<td>901</td>
</tr>
<tr>
<td>31.94</td>
<td>8.85$^a$</td>
<td>0.97</td>
<td>11.05</td>
<td>1,450</td>
</tr>
<tr>
<td>32.59</td>
<td>7.06$^c$</td>
<td>1.08</td>
<td>15.27</td>
<td>104</td>
</tr>
<tr>
<td>34.52</td>
<td>9.21$^c$</td>
<td>0.84</td>
<td>9.18</td>
<td>335</td>
</tr>
<tr>
<td>34.9</td>
<td>5.51$^a$</td>
<td>0.81</td>
<td>14.81</td>
<td>1,244</td>
</tr>
<tr>
<td>35.08</td>
<td>5.46$^b$</td>
<td>0.48</td>
<td>8.89</td>
<td>687</td>
</tr>
<tr>
<td>35.8</td>
<td>6.23$^c$</td>
<td>0.46</td>
<td>7.52</td>
<td>1,234</td>
</tr>
<tr>
<td>37.54</td>
<td>6.48$^b$</td>
<td>1.49</td>
<td>21.87</td>
<td>1,571</td>
</tr>
</tbody>
</table>

$^*$Significant differences in the mean EV are denoted with different superscript letters (p < 0.05).
Symbiosis between Pontonia margarita and Pinna rugosa

we recorded at the sample

June 6, 2019

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the high water temperature (28.7 °C), On the contrary to the results reported by Góngora-Gómez et al. (2017), in captivity.

It is well documented in crustaceans that brood size increases with female BL. (Hines 1992, Lardies and Castilla 2001, Salas-Moya et al. 2014). However in this study, no correlation was found between the number of eggs or the EV and BL of ovigerous female shrimps (r = 0.52, p = 0.118 and r = 0.50, p = 0.109, respectively). For berried females > 319.4 mm, the number of eggs per female ranged from 687 to 1,450, suggesting brood loss. Also, the egg development stage should be considered to explain the BL of ovigerous female shrimps and the number and volume of eggs. Li et al. (2011) concluded that possible reasons for brood loss of sand shrimp Crangon uritai Hayashi & Kim, 1999, include the increasing egg size, which results in reduced area for eggs to attach. Commonly, brood loss is low because females of symbiotic decapods remain in the host during embryonic development (Lardies and Castilla 2001). The hypothesis that a reduction in food available to females may force them to consume their own eggs should be evaluated by future research. In our study, the apparent short-term monogamous relationship that implies the displacement of male and female shrimps outside of their host could explain the brood loss we observed. Egg volume is an index of energy content transferred from the female (McEdward and Coulter 1987). The EV obtained for P. margarita showed a wide range (5.15 to 8.85 mm³); however, no comparisons with the EV of other pontoniid shrimps are possible due to lack of studies on fecundity.

This is the first report of the symbiosis between Pontonia margarita and Pinna rugosa. Our study provides new information on the sexual and reproductive traits of P. margarita inhabiting the mantle cavity of P. rugosa and how this symbiosis shapes the mating system and social behavior of both species. Also important is the inference about the apparent short-term monogamous relationship of this shrimp species inhabiting bivalves as shelters.

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Author Contributions: VLJ, AMGG, and MGU designed the experiment and wrote the paper. DGU performed the statistical analysis. AMGG, DGU, and JAHS conducted the samplings in the field and biometric analysis in the laboratory.
Competing Interests: The authors have declared that no competing interests exist.
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