Fecundity, embryo size and embryo loss in the estuarine shrimp *Salmoneus carvachoi* Anker, 2007 (Crustacea: Alpheidae) from a tidal mudflat in northeastern Brazil

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Abstract. Studies on fecundity and embryo size have been frequently used to infer about the reproductive potential, embryogenesis, and the energy investment in embryo production in crustaceans. These parameters are relevant to the knowledge of life-history and diversity of reproductive strategies developed by this group. This study addressed the embryo production by the estuarine shrimp *Salmoneus carvachoi* Anker, 2007, a poorly known caridean shrimp. We investigated whether there was (1) a correlation between fecundity and carapace length, (2) an increase in embryo volume along the embryonic development, and (3) loss of embryos along development. In addition, we investigated whether the sternite length and height and width of the pleura of the second abdominal somite were good predictors of fecundity. A total of 53 embryo-carrying individuals were collected in December 2016 and February 2017 in a tidal mudflat from the estuary of the Paripe River, Ilha de Itamaracá, Pernambuco, Brazil. The carapace length ranged from 3.23 to 4.64 mm (3.78 ± 0.26 mm). Among those, 25 individuals had embryos in initial stage, 5 in intermediate stage and 15 in final stage. Fecundity ranged from 14 to 67 (33.65 ± 12.51 embryos) and was weakly correlated with carapace length. However, fecundity was strongly correlated with the width of the second sternite and width of the pleura of the second abdominal somite. Embryo volume increased markedly (93%) from the initial to the final stage of development. This increase might be due to water uptake to facilitate the membrane rupture at the onset of larval hatching. There was significant loss of embryos only between the initial and intermediate stage. Future studies on the embryo production by other species of *Salmoneus* Holthuis, 1955 are necessary to understand and compare these aspects of reproductive biology.

Key-Words. Caridea; Abdominal space; Embryogenesis; Reproductive biology; Reproductive potential.

INTRODUCTION

Among the several reproductive aspects studied in crustaceans, fecundity and embryo size are important indicators of the reproductive potential, embryogenesis, and energy investment in embryo production. These aspects are relevant to the knowledge of life-history and type of reproductive strategy adopted by each species (Corey & Reid, 1991; Anger & Moreira, 1998; Hattori & Pinheiro, 2003). Generally, the reproductive strategy can be affected by genetic and environmental factors (especially temperature and food availability) (Sastry, 1983; Lardies & Wehrmann, 1997; Bazán *et al.*, 2009). In caridean shrimps, these strategies may vary intraspecifically, between populations from different habitats, and interspecifically, between species living in the same habitat (Corey & Reid, 1991; Anger & Moreira, 1998; Pavanelli *et al.*, 2010).

Among the various approaches in the study of crustacean reproductive biology, linear and non-linear regression models are frequently used to describe the relationships between fecundity, embryo volume and mass, ovary size, body size and mass, among others (Corey & Reid, 1991; Anger & Moreira, 1998). Positive correlations between fecundity and carapace length have been reported by most studies on shrimps of the infraorder Caridea, indicating that fecundity increases as females increase in size. This relationship demonstrates that the ability to produce oocytes is positively related to female growth. In addition, the larger the female the larger the abdominal space (where the embryos are carried) (Balasundaram & Pandian, 1982; Bauer, 1991; Anger & Moreira,
1998). The increase in embryo volume and the concomitant embryo loss along the embryo development have also been observed in carideans (Balasundaram & Pandian, 1982; Corey & Reid, 1991; Anger & Moreira, 1998). Within the family Alpheidae, this has been seen in the genus *Alpheus* Fabricius, 1798 (Fernández-Muñoz & García-Raso, 1987; Bauer, 1991; Corey & Reid, 1991; Wehrtmann & Greaves, 1998; Atkinson et al., 2003; Pavanelli et al., 2008, 2010; Harikrishnan et al., 2010; Costa-Souza et al., 2014; Pescinelli et al., 2016; Soledade et al., 2017), *Betaeus* Dana, 1852 (Lardies & Wehrtmann, 1997, 2001) and *Synalpheus* Spence Bate, 1888 (Corey & Reid, 1991; Hernández et al., 2010; Rebollo et al., 2014).

*Salmoeneus* Hultensis, 1955 is the third richest genus among alpheids, with more than 50 species distributed worldwide (De Grave & Fransen, 2011; Anker, 2011a, b; Komai & Anker, 2012; Anker et al., 2014; Anker & Lazarus, 2015; Komai et al., 2015; Düris & Horká, 2016). *Salmoeneus carvachoi* Anker, 2007 is distributed in the Western Atlantic and can be found in Mexico, Guadeloupe, Venezuela, and Brazil (from Paraíba to São Paulo) (Anker, 2007; Vera-Caripe et al., 2015). This estuarine shrimp is commonly found in burrows in muddy sediments, mangroves, mud flats, and under rocks and debris (Christoffersen, 1982; Anker, 2007, 2010; Almeida et al., 2012). Studies about this species are scarce and limited to taxonomy and distribution ([e.g., Ramos-Porto et al., 1994, as *S. ortmanni* (Rankin, 1898); Anker, 2007, 2010; Almeida et al., 2012]), life-history and population structure, symbiotic relationships with other crustaceans, and possible mating and sexual systems (Oliveira et al., 2015). The occurrence of simultaneous hermaphroditism in *S. carvachoi* was suggested based on the observation of the appendix masculina in all individuals, including those carrying embryos, as well as the observation of pairs formed by two brooding individuals (Oliveira et al., 2015). *Salmoeneus carvachoi* has been recorded in the burrows of the snapping shrimps *Alpheus estuariensis* Christoffersen, 1984 and *A. chacei* Carvacho, 1979, as well as in the burrows of the mud shrimp *Axianassa australis* Rodrigues & Shimizu, 1992 (Ramos-Porto et al., 1994, as *S. ortmanni*; Almeida et al., 2012; Oliveira et al., 2015).

Up to the present, there is no information on any aspect of embryo production by *Salmoeneus*. Therefore, the aim of this study was to investigate the embryo production in *S. carvachoi* and whether (1) there was a correlation between fecundity and carapace length; (2) if there was an increase in embryo volume along development; and (3) if there was loss of embryos along the development, as seen in many other caridean shrimps.

In caridean shrimps, the first three abdominal somites provide the abdominal space for embryo incubation (Bauer, 2004). The pleurae of the second abdominal somite in carideans overlaps the pleurae of first and third somites (Bauer, 2004). As this is the largest pleura, this structure may contribute to create more space for the brood and protect the embryos of *S. carvachoi*. In addition, the size of the second abdominal sternite may also indicate more space for embryo incubation, and be independent of the existence of correlation between female size and fecundity. For this purpose, we investigated whether the sternite width, and the height and width of pleura of second abdominal somite are correlated with fecundity.

**MATERIAL AND METHODS**

Embryo-carrying individuals were collected in December 2016 and February 2017 in a tidal mudflat in the estuary of the Paripe River, Ilha de Itamaracá, Pernambuco, Brazil (07°48’38.0”S, 34°51’22.1”W). The specimens were captured by stepping on the mud surface where burrow openings of the alpheid *A. estuariensis* (where *S. carvachoi* is commonly found in association) were present. Due to the stepping, the shrimps leave the burrows and swim in the water film where they can be captured with small hand nets and examined. Individuals with embryos in the abdomen were placed into plastic flasks filled with water from the site, transported to the laboratory, anesthetized on ice, and fixed in formalin 5%.

The individuals were photographed in a stereomicroscope with an image capture system. The software ImageJ 1.45 s (Rasband, 2006) was used to measure the following variables: carapace length (CL) (distance from the tip of the rostrum to the posterior margin of the carapace); sternite width (SW) (distance between the lower limits of two pleurae of the second abdominal somite); height of the pleura of the second abdominal somite (PH) (distance between the lower and upper limits of the pleura); and width of the pleura of the second abdominal somite (PW) (distance between the posterior and anterior limits of the pleura) (Fig. 1). Except for CL, which was measured in all embryo-carrying individuals, the other variables were measured only in individuals carrying embryos at the initial stage of development.

The embryos were removed from the pleopods with the use of a brush, placed in a Petri dish, counted and separated in three stages of development, according to Wehrtmann’s (1990) classification: initial stage (I) embryos with no evidence of eyes; yolk occupying 75-100% of embryo volume; intermediate stage (II) embryos with small and elongated eyes; yolk occupying about 50-75% of embryo volume; final stage (III) embryos with well-developed eyes; yolk occupying 25-50% of embryo volume. In some individuals it was not possible to determine the embryo development stage as they had a whitish appearance and the eyes and the amount of yolk were not visible.

To calculate the embryo volume, the longest and shortest diameters of all embryos were measured (with the same procedure as for the other variables explained above). These values were used in the formula proposed by Bauer (1991): \[ V = \frac{n}{6} \times d_1^2 \times d_2 \], where \( V \) = volume (mm\(^3\)), \( d_1 \) = shortest diameter and \( d_2 \) = longest diameter. The mean embryo volume in each stage of development was calculated. Mean fecundity was obtained through the total number of embryos at the initial stage only, in order to avoid possible bias due to embryo loss in stages II and III (Balasundaram & Pandian, 1982).
The relationship between fecundity vs. CL, fecundity vs. PH, fecundity vs. PW, and fecundity vs. SW were verified using linear regressions. The best adjusted model was the linear function $y = bx + a$, where $y$ is the fecundity, the dependent variable, and $x$ is one of the biometric measures (CL, PH, PW or SW), the independent variable. An Analysis of Covariance (ANCOVA) was used to verify if there are differences between these relationships. An ANCOVA with a posteriori Student’s t-test was used to verify the variation in fecundity between development stages, i.e., to verify loss of embryos along the development. For the embryo volume, a one-way Analysis of Variance (ANOVA) with Student’s t-test a posteriori was used to test differences between stages. The level of significance used in the tests was $\alpha = 0.05$.

RESULTS

We collected a total of 53 embryo-carrying individuals, including eight where the embryo masses could not be classified. Overall, CL ranged from 3.23 to 4.64 mm (3.78 ± 0.26 mm). In individuals carrying embryos in stage I, SW ranged from 1.7 to 2.11 mm (1.85 ± 0.11 mm), PW ranged from 1.65 to 2.3 mm (1.95 ± 0.17 mm), and PH ranged from 0.78 to 1.6 (1.09 ± 0.21 mm).

There was a weak positive correlation between fecundity and CL ($F = 4.56$, d.f. = 1, $p < 0.05$) (Fig. 2A). Positive correlations were observed between fecundity and PW ($F = 17.86$, d.f. = 1, $p < 0.05$), and fecundity and SW ($F = 10.52$, d.f. = 1, $p < 0.05$), indicating that the larger these dimensions, the larger the number of embryos.
carried (Fig. 2B, C). There was no correlation between fecundity and PH (F = 0.08, d.f. = 1, p > 0.05) (Fig. 2D). The analysis of covariance demonstrated that there were no differences between the slopes describing the relationship between fecundity vs. CL, PW and SW (F = 1.644, p > 0.05), indicating that these three structures are good predictors of fecundity.

The volume of stage I embryos varied from 0.03 to 0.06 mm³ (0.04 ± 0.005 mm³), from 0.04 to 0.05 mm³ (0.05 ± 0.003 mm³) in stage II, and from 0.05 to 0.09 mm³ (0.07 ± 0.008) in stage III (Fig. 3). There was a significant difference in embryo mean volume between stages I and II, and between stages I and III, but between stages II and III there was no significant difference (F = 73.36, d.f. = 34.31, p < 0.005) (Table 1), indicating that there was a significant increase in volume from stage I to II, but it remained the same from stage II to III. We observed a total increase of 93% in embryo mean volume from stage I to III (Table 1).

We obtained 25 individuals with embryos in stages I, II, and III. The mean fecundity (based on females carrying embryos in stage I) was 33.65 ± 12.51 embryos per individual, ranging from 14 to 67. There was a significant decrease (F = 52.35, d.f. = 2, p < 0.05) in mean fecundity between stages I and II (t test a posteriori, p < 0.05), and between I and III (t test a posteriori, p < 0.05), due to embryo loss from the initial to the intermediate stage. The fecundity did not change between the intermediate and final stage (Table 1).

**DISCUSSION**

In *S. carvachoi*, a weak positive correlation was observed between fecundity and female size (CL). This result differs from what has been observed in most of studies on caridean shrimps (Balasundaram & Pandian, 1982; Bauer, 1991; Corey & Reid, 1991; Anger & Moreira, 1998, Pavanelli et al., 2008, 2010; Costa-Souza et al., 2014; Rebolledo et al., 2014; Pescinelli et al., 2016). A positive correlation between fecundity and SW (stermite width), and between fecundity and PW (width of the pleura of the second abdominal somite) was observed. These positive correlations were expected, since the larger the female, the larger the size of such body parts, providing more space in the abdomen to harbor the embryo mass (Corey & Reid, 1991; Lardies & Wehrtmann, 1997; Anger & Moreira, 1998). However, there was no positive correlation between fecundity and pleura height (height of the pleura of the second abdominal somite), a variable that could also indicate an increased abdominal space to carry more embryos (Fig. 2D).

The mean embryo volume in *S. carvachoi* was relatively small compared to other alpheids of the genus *Alpheus, Betaeus* and *Synalpheus*, with few exceptions (see Soledade et al., 2017). Only *A. normanni* Kingsley, 1878, a species of similar size (mean CL of 4.5 mm and mean embryo volume of 0.03 mm³) (Bauer, 1991), has an embryo volume similar to *S. carvachoi*. The embryo size in crustaceans may be determined by genetic and environmental factors, which can influence in the type/duration of the embryonic development of each species (Raven, 1961; Sastry, 1983; Bauer, 1991). Unlike most species of the three genera mentioned above, which have a larger CL and embryo volume, the relatively small embryo size of *S. carvachoi* may be explained by its smaller body size. On the other hand, although some species of *Synalpheus*, such as *S. brooksi* Coutière, 1909, *S. herrickii* Coutière, 1909, *S. idios* Rios & Duffy, 2007, *S. pectiniger* Coutière, 1907, and *S. agelas* Pequegnat & Heard, 1979, are similar to *S. carvachoi* in CL (Table 2), their embryo volume are higher. Species of *Alpheus* and *Betaeus*, larger than *S. carvachoi*, also have higher embryo volume. This may be related to the developmental strategy adopted by these species of *Synalpheus*, where there is a higher investment in embryonic volume and a small number of eggs. This pattern is observed in species with direct or abbreviated development (Wehrtmann & Albornoz, 2002), as for example, the eusocial species of *Synalpheus*, which have an abbreviated larval development (Duffy, 1996).

Considerable increases in embryo volume throughout development have been often observed in caridean shrimps (Corey & Reid, 1991; Mossolin et al., 2006; Pavanelli et al., 2008, 2010). This is mainly due to water uptake, which facilitates the membrane rupture at the time of larvae hatching (Wear, 1974; Lardies & Wehrtmann, 1996, 1997). Although the mean volume of embryos in stage III was relatively higher than in stage II, this difference was not statistically significant. The embryos increased 93% in volume, which may be considered a relatively high increase compared to the observed in some species of *Alpheus*, such as *A. carlae* Anker, 2012 [as *A. armillatus* (H. Milne Edwards, 1834)] (64.3%; see Pavanelli et al., 2008), *A. estuariensis* (45.4%; see Costa-Souza et al., 2014), *A. brasileiro* Anker, 2012 (35.4%; see Pescinelli et al., 2016), and *Synalpheus apioceros* Coutière, 1909 (77.2%, see Rebolledo et al., 2014). On the other hand, the increase in embryo size is relatively smaller compared to *Betaeus emarginatus* H. Milne Edwards, 1837 (116.2%; see Lardies & Wehrtmann, 1997) and *B. truncatus* from the Guanaqueros, Chile (94.4%; see Lardies & Wehrtmann, 2001).
Table 1. Mean fecundity, mean embryo volume (mm³), and % increase in embryo volume between three stages of embryo development, measured in ovigerous individuals of *S. carvachoi* obtained in December 2016 and February 2017 in the Paripé River estuary, Pernambuco, northeastern Brazil.

<table>
<thead>
<tr>
<th>Stages</th>
<th>N (number of Individuals)</th>
<th>Mean fecundity ± SD</th>
<th>Mean embryo volume (mm³) ± SD</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25</td>
<td>33.65 ± 12.51 a</td>
<td>0.043 ± 0.005 a</td>
<td>20.0% (I–III)</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>2.2 ± 2.58 b</td>
<td>0.052 ± 0.003 b</td>
<td>72.1% (II–III)</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>2.53 ± 1.92 b</td>
<td>0.071 ± 0.008 b</td>
<td>93.0% (I–III)</td>
</tr>
</tbody>
</table>

Values with at least one same small letter in each column did not differ between stages (p = 0.05).

Table 2. Mean carapace length, fecundity and embryo volume of six species of *Synalpheus* with carapace length (CL) similar to *Salmoneus carvachoi*. Min = Minimum and Max = Maximum.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Mean CL (mm) (Min-Max)</th>
<th>Fecundity Mean (Min-Max)</th>
<th>Mean embryo volume (mm³) (Min-Max)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Synalpheus agelas</em></td>
<td>5</td>
<td>5.0 (4.2 – 5.6)</td>
<td>42.4 (16 – 65)</td>
<td>0.23 (0.03 – 0.64)</td>
<td>Corey &amp; Reid (1991)</td>
</tr>
<tr>
<td><em>S. brooksi</em></td>
<td>10</td>
<td>3.9 (3.4 – 4.5)</td>
<td>5.8 (3 – 11)</td>
<td>0.50 (0.03 – 0.11)</td>
<td></td>
</tr>
<tr>
<td><em>S. herricki</em></td>
<td>4</td>
<td>4.5 (3.5 – 5.12)</td>
<td>45.8 (11 – 81)</td>
<td>0.22 (0.03 – 0.11)</td>
<td></td>
</tr>
<tr>
<td><em>S. pectiniger</em></td>
<td>31</td>
<td>4.2 (3.5 – 4.6)</td>
<td>9.9 (4 – 17)</td>
<td>0.75 (0.03 – 0.11)</td>
<td></td>
</tr>
<tr>
<td><em>S. chacei</em></td>
<td>2</td>
<td>3.7 (3.7)</td>
<td>16 (3)</td>
<td>0.15 (0.03 – 0.11)</td>
<td>Hemández et al. (2010)</td>
</tr>
<tr>
<td><em>S. idios</em></td>
<td>4</td>
<td>3.9 ± 0.2 (3.7 – 4.2)</td>
<td>12 ± 4.3 (9 – 18)</td>
<td>1.04 ± 0.23 (0.2 – 1.2)</td>
<td></td>
</tr>
<tr>
<td><em>Salmoneus carvachoi</em></td>
<td>53</td>
<td>3.78 ± 0.26 (3.23 – 4.64)</td>
<td>33.65 ± 12.51 (14 – 67)</td>
<td>0.043 ± 0.005 (0.03 – 0.06)</td>
<td>Present study</td>
</tr>
</tbody>
</table>

The mean fecundity of *S. carvachoi* was higher than that of four species of *Synalpheus* of similar size: *S. brooksi*, *S. pectiniger*, *S. chacei* Duffy, 1998 and *S. idios* (Table 2). A similar fecundity was observed in two other species of *Synalpheus*: *S. agelas* and *S. herricki* (Table 2). The low fecundity of *S. carvachoi*, as compared to most species of *Synalpheus*, *Alpheus*, and *Betaeus* (Corey & Reid, 1991; Rebolledo et al., 2014; Soledade et al., 2017), is probably related to its small body size, which offers less space in the body cavity to accommodate oocyte growth during ovarian maturation, and less space in the abdomen to carry a large embryo mass (Bauer, 1991; Corey & Reid, 1991).

In caridean shrimps there is usually an inverse relationship between fecundity and egg volume (Corey & Reid, 1991; Lardies & Wehrtmann, 1997, 2001; Harikrishnan et al., 2010). A species may invest in a small number of embryos of a larger volume or in a higher number of embryos of smaller volume (Lardies & Wehrtmann, 1997, 2001; Pavanelli et al., 2008, 2010). *Salmoneus carvachoi* apparently produces larger but less numerous embryos.

The embryo loss during incubation observed in most caridean shrimps occurs mainly due to the space limitation in the female’s abdomen as the embryos increase in volume (Corey & Reid, 1991; Wehrtmann & Lardies, 1999). In addition, other factors may influence this process such as the presence of parasites, maternal cannibalism, mechanical stress, and substrate abrasion, among others (Balasundaram & Pandian, 1982). The significant decrease in fecundity from the initial to the intermediate stage was accompanied by a significant increase in embryo volume. In the same way, there was no embryo loss and no increase in embryo volume between the intermediate and final stages. The significant increase in embryo volume during the embryogenesis might explain the embryo loss in *S. carvachoi*, as is commonly observed in other species of caridean shrimps (Balasundaram & Pandian, 1982; Corey & Reid, 1991; Anger & Moreira, 1998).

**CONCLUSIONS**

The present study provides the first information on embryo production by the poorly known *S. carvachoi*, which is the first species of the genus to have its reproductive biology studied in more detail. Fecundity was weakly positively correlated with CL, and SW and PW were better proxies of fecundity. Embryo volume undergoes a significant increase (93%) from the initial to the final stage of development, which may explain the embryo loss seen during development. Future studies on the embryo production by other species of *Salmoneus* are necessary to compare these aspects of their reproductive biology.

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