Reproductive strategies of a population of a freshwater amphipod (Crustacea, Amhipoda, Hyalellidae) from southern Brazil

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Abstract: The aim of this study was to characterize the reproductive strategies of a population of *Hyalella bonariensis* from southern Brazil. Pairing success, reproductive period of males and females, fecundity, and body size at the onset of reproduction were evaluated. Animals were sampled four times (August 2012, October 2012, January 2013, and April 2013) with the 250 µm-mesh dip net during 20 minutes by only one person. In the field, precopulatory pairs and ovigerous females were individualized. In the laboratory, cephalothorax length (CL) were measured and eggs and juveniles were removed from the females’ marsupium and counted. The mean CL of paired males and females was significantly higher than that of non-paired males and females. A sexual dimorphism in body size was observed in the population - both paired and non-paired males were larger than females. Probably larger males have a higher probability of losing females during precopulatory behavior. A significant correlation was observed between the size of paired males and females - larger males often paired with larger females and smaller males paired with smaller females (r = 0.81). The pairing success of males increased with body size and we can assume that males from all size classes are able to find mates. The pairing success of females was independent of body size - paired and non-paired females had similar mean CL. The reproductive success increased with body size in males and females, and was more evident in males. Females from the largest size classes had null reproductive success. The idea that larger females can produce more eggs but might have a lower probability of finding a mate than smaller females was corroborated by our results. Therefore, smaller females have higher pairing success because they are capable of mating with a higher percentage of males. The estimated mean fecundity of *H. bonariensis* was 17.4 (± 3.89) eggs/juveniles. Our results are similar to those of other species of *Hyalella* from Brazil.

Keywords: fecundity, body size, *Hyalella bonariensis*, reproductive success, pairing success.

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Estratégias reprodutivas de uma população de um anfípodo de água doce (Crustacea, Amhipoda, Hyalellidae) do sul do Brasil

Resumo: Este trabalho teve por objetivo caracterizar as estratégias reprodutivas de uma população de *Hyalella bonariensis* no sul do Brasil, avaliando o sucesso de pareamento, o período reprodutivo de machos e fêmeas, a fecundidade e o tamanho corporal dos indivíduos durante a reprodução. Para isso foram realizadas quatro amostragens (Agosto/2012, Outubro/2012, Janeiro/2013 e Abril/2013) utilizando-se uma rede de mão, com malha de 250 µm, durante 20 minutos por um coletor. Em campo, os casais e as fêmeas ovígeras foram individualizados e em laboratório os mesmos foram mensurados quanto ao comprimento do cefalotórax (CC) (mm) e os ovos ou juvenis encontrados dentro do marsúpio foram contados. O CC médio dos machos e fêmeas pareados foi significativamente superior ao dos machos e fêmeas não pareados. Um dimorfismo sexual no tamanho corpóreo foi observado na população - ambos machos pareados e não pareados foram maiores do que as fêmeas. Aparentemente os machos maiores têm uma maior probabilidade de perder fêmeas durante o comportamento precopulatório. Foi observada correlação positiva entre o tamanho dos machos e fêmeas que foram encontrados formando casais - machos
maiores geralmente parecem com fêmeas maiores e machos menores com fêmeas menores (r=0,81). O sucesso de pareamento dos machos aumentou com o tamanho corpóreo e podemos assumir que os machos de todas as classes de tamanho são capazes de encontrar parceiras sexuais; entretanto, o sucesso de pareamento das fêmeas não depende do tamanho das mesmas, sendo que as fêmeas pareadas e não pareadas apresentaram CC médio semelhante. O sucesso reprodutivo da espécie aumentou com o tamanho corpóreo em machos e fêmeas, sendo mais pronunciado nos machos. As fêmeas pertencentes às maiores classes de tamanho apresentaram sucesso reprodutivo nulo. Os resultados suportam a ideia que as fêmeas grandes poderiam ter uma baixa probabilidade de encontrar parceiro para formar par pré-copulatório, quando comparada às fêmeas de tamanhos inferiores, apesar das fêmeas maiores serem mais fecundas. Neste sentido, as menores fêmeas possuem um sucesso de pareamento maior, pois elas são capazes de copular com uma elevada proporção de machos. A fecundidade média da espécie foi estimada em 17,4 (± 3,89) ovos/juvenis. Os resultados observados no presente trabalho assemelham-se aos encontrados para outras espécies do gênero já analisadas no sul do Brasil.

**Palavras-chave:** fecundidade, *Hyalella*, sucesso de pareamento, sucesso reprodutivo, tamanho corpóreo.

**Introduction**

In general, the reproduction of Amphipoda is well known (Strong 1973; Hartnell 1982; Sastry 1983; Wellborn 1995; Cothran et al. 2015). The sequential events of reproduction can be summarized in four stages: stage I, location of a mate mediated by pheromones; stage II, pairing initiates through contact stimuli; stage III, male and female pairing persists until the female molts; and stage IV, end of mating shortly after the molt (Borowsky 1991).

The precopulatory behavior is a common feature in some amphipod species. Males carry females on their ventral surface during several days prior female’s molting, which signals mating availability (Hynes 1955; Wellborn 1995; Castiglioni & Bond-Buckup 2007; Castiglioni & Bond-Buckup 2008b). The precopulatory behavior probably ensures that the male is near when female reaches its short sexual period (Strong 1973; Borowsky 1984). It is also likely that the male protects the female against predators and from other males during the precopulatory period (Strong 1973; Lewbel 1978; Borowsky 1984; Dick et al. 1990). After mating, the development of amphipod crustaceans occurs within the marsupium and can be divided into two stages: 1) from ovulation to hatching (embryonic period) and 2) from hatching to emergence of the litter (Borowsky 1980). The amphipods present a direct development, with the pups hatching with the body shape similar to that of the adult (Strong 1972; Borowsky 1991; Steele & Steele 1991; Morr & Spicer 1996; Aoki 1997; Thiel 1999).

*Hyalella* Smith, 187 is a genus of freshwater crustacean distributed throughout the American continent, and in this genus, pairing, i.e., precopulatory behavior, occurs before copulation and several factors influence its success. Male and female body size, mate selection, and intrinsic factors, such as water temperature, might influence the reproductive success and fecundity of *Hyalella* (Wellborn 1995; Castiglioni & Bond-Buckup 2008a).

In Brazil, the studies about reproduction concerning *Hyalella* are restricted to species from south and southeast regions. In southern Brazil, reproductive features of the sympatric species *H. castroi* Bond-Buckup, Araujo & Santos, 2008, and *H. pleoacuta* Bond-Buckup, Araujo & Santos, 2008 were described (Castiglioni & Bond-Buckup 2007; Castiglioni & Bond-Buckup 2008a). In southeastern Brazil, where dry and wet seasons are well-defined, reproduction and egg production of *H. carstica* Bastos-Pereira & Bueno, 2012 and *H. longistilta* (Faxon 1876) were evaluated (Torres et al. 2015; Bastos-Pereira & Bueno 2016).

The species *Hyalella bonariensis* Bond-Buckup, Araujo & Santos, 2008 is often found among macrophytes in water courses from southern Brazil and Argentina (Santos et al. 2008; Bueno et al. 2014). Only one study was recently developed on the population dynamics of the species in the municipality of Silveira Martins, central region of the state of Rio Grande do Sul, and it was found that ovigerous females and couples in pre-copulatory behavior were more frequent in the winter (Castiglioni et al. 2016).

The aim of this study is to characterize the reproductive strategy of *H. bonariensis* from a headwater stream from southern Brazil. Pairing success, reproductive period of males and females, fecundity, and body size onset reproduction were evaluated.

**Material and Methods**

The studied population is located in a headwater stream in a private rural property called Portal do Roio (29°39′25.14″S, 53°37′33.53″W), in Silveira Martins municipality, central region of state of Rio Grande do Sul, in the southermmost Brazil. Four samplings were conducted in August and October 2012 and January and April 2013. In this region, the weather is, as the Köppen classification system, subtropical “Cfa”, with average annual relative humidity of the air 82% (Isaia 1992). The central region of the state of Rio Grande do Sul is located in the transition area of the geomorphological compartments called Depressão Central e Planalto. According to Moraes & Bezzi (2009) the Depressão Central is associated with sedimentary rocks such as sandstones, clays and river plains and displays altitude around 90 meters above sea level. The Planalto slope has irregular relief associated with basaltic rocks and the altitude ranges from 500 to 100 meters above sea level (Dantas et al. 2010).

Amphipods use macrophytes as shelters and food so plants from each sample site were collected with a 250 μm-mesh dip net during 20 minutes by only one person. Afterwards, the macrophytes were stored in plastic bags and transferred to the laboratory in thermic boxes. Ovigerous females and precopulatory pairs were individualized and stored into microtubes with 70% ethanol.

In the laboratory, all individuals were separated from the macrophytes, identified, separated into to four categories (juveniles, males, females and ovigerous females) (Borowsky 1991; Castiglioni et al. 2016) and measured (cephalothorax length, CL in mm) under the micrometer ocular of a stereomicroscope.
The mean body size (cephalothorax length in mm) of unpaired and paired males were compared with the mean body size of unpaired and paired females, by means of a t test, after checking for the normality and homoscedasticity of the data (α = 0.05) (Zar, 1996). Moreover, the mean body size of paired and unpaired amphipods was compared, to assess whether pairing success was influenced by body size (t test; α = 0.05) (Zar, 1996).

To evaluate if H. bonariensis have mate preferences, the correlation between CL of paired males and females was estimated with a Person correlation coefficient (r). To evaluate if pairing is influenced by mate size, the mean CL of paired and non-paired males and paired and non-paired females were compared by a t test (α = 0.05). The Pairing Success Index (PSI) was used to evaluate the relationship between pairing success and male and female size. The PSI was adapted by Wellborn (1995) from the relative feeding index (Manly 1974) and have already been used in other studies about Hyalella reproduction (Castiglioni & Bond-Buckup 2008b), and it is given by

\[ \beta_i = \frac{(r_i/n_i)}{\sum r_i/n_j} \]

where,

- \( \beta_i \) is the relative pairing success in size class i;
- \( r_i/n_i \) are the proportion of paired and non-paired individuals, respectively.
- The denominator is the sum of the proportion of paired individuals from all size classes. The minimum CL used for the size classes was the size of the smaller male and female found in precopulatory behavior. The size class intervals were based on 1/2 of the CL standard deviation value (Markus 1971). Considering that the reproductive success also depends on aspects such as the number of fertilized eggs, male reproductive success \( (RM, i) \) was estimated by the following formula:

\[ RM, i = \beta_i e_i / \sum \beta_i e_i \]

where,

- \( RM, i \) is the number of eggs fertilized by males from size class i (\( e_i \)) was estimated through the calculation of the expected fecundity of each paired female using the regression of the number of eggs versus the CL of ovigerous females (\( F = 40.668CL - 4.9461 \)). The denominator refers to the sum of the fecundity of all size classes.
- Female reproductive success \( (RF, i) \) was derived from egg production, as follows:

\[ RF, i = f_i / \sum f_j \]

where,

- \( f_i \) is the observed mean egg production (number of eggs inside the marsupium) of ovigerous females from size class i, and the denominator refers to the sum of the fecundity of all size classes. The minimum CL of the size classes was the size of the smallest precopulatory pair.

To estimate fecundity, the marsupial content was categorized into three stages of embryonic development and one post-embryonic stage according to Hynes (1955), Dick et al. (1998), Castiglioni & Bond-Buckup (2007, 2009). The developmental stages were easily distinguished under stereomicroscope and were characterized as follows: stage I (initial) — orange eggs, completely filled with yolk, and with discrete or no cleavage; stage II (intermediary) — beginning of cell cleavage with well-defined body parts absent; stage III (final) — yolk completely absorbed and reduced to a small portion used for embryonic supply, body somites and eyes visible; and stage IV (juveniles) — newly hatched juveniles attached inside the marsupium.

All eggs and juveniles were removed from the marsupium and counted under a stereomicroscope. The minimum, maximum, and mean number of eggs (from each stage of embryonic development) and juveniles were estimated. The comparison between the mean number of eggs of each stage of embryonic development and juveniles was performed with an analysis of variance (ANOVA), complemented by a Bonferroni test (F; α = 0.05). The minimum, maximum, and mean fecundity of each season was estimated and compared among seasons with ANOVA, followed by a Bonferroni test (F; α = 0.05) (Zar 1996).

All ANOVA tests were preceded by assessments of normality and homocedasticity of the data (Zar 1996).

The relationship between ovigerous females CL (x) and the number of eggs per embryonic stage and number of juveniles (y) was estimated through a regression analysis. Then, a Pearson correlation \( (r) \) was calculated for each relationship (α = 0.05) (Zar 1996).

**Results**

A total of 5,269 individuals of H. bonariensis were captured, 1,315 juveniles, 1,879 males, 2,075 females (240 ovigerous females). Of that total, 54 pairs were found in precopulatory behavior.

The mean CL of paired males and females was significantly larger than that of unpaired males and females (males \( t = 10.5 \); females \( t = 8.67 \); p < 0.05; Table 1). A sexual dimorphism in body size was observed in the population; both paired and non-paired males were larger than females (paired males \( t = 5.46 \); non-paired males \( t = 9.80 \); p < 0.05; Table 1). A positive correlation between the size of males and females found in precopulatory behavior was observed: larger males often paired with larger females and smaller males paired with smaller females (\( r = 0.81 \); Figure 1). Male success in finding a mate increased with body size. Males in larger size classes were approximately 2.5 times more likely of finding a mate than males from smaller size classes (Figure 2). Pairing success also increased with female body size; females from smaller size classes had null pairing success (Figure 2). Additionally, the reproductive success also increased with body size increment in both males and females (Figure 3). However, it seemed that extremely large males had a decreased reproductive and pairing success when compared with intermediate size males (Figure 3).

The cephalothorax length of ovigerous females ranged from 0.32 to 0.74 mm (mean ± standard deviation = 0.55 ± 0.07 mm). Females bearing eggs in different stages of embryonic development and those bearing juveniles had similar mean CL (p > 0.05; Table 2).

Female egg/juvenile production ranged from 8 to 26 (17.4 ± 3.89 eggs/juveniles). The number of eggs produced in each stage of embryonic development and the number of juveniles found inside the marsupium are shown in Table 3. There was a decrease of egg production throughout the embryonic development; the mean number of eggs in stages I, II, and III was significantly higher than that of juveniles inside the marsupium (p < 0.05) (Table 3). In all stages of embryonic development, there was positive linear correlation between the number of eggs produced and the CL of ovigerous females (p < 0.05). The number of juveniles inside the marsupium also showed positive correlation with female size (p < 0.05) (Table 3).
Table 1. Minimum, maximum, and mean cephalothorax length (mm) (± standard deviation) of *Hyalella bonariensis* paired and unpaired males and females from Silveira Martins municipality, state of Rio Grande do Sul, Brazil.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paired</td>
<td>Unpaired</td>
</tr>
<tr>
<td>CL min – max</td>
<td>0.40 – 0.74</td>
<td>0.30 – 0.92</td>
</tr>
<tr>
<td>CL mean ± sd</td>
<td>0.63 ± 0.08 a</td>
<td>0.49 ± 0.10 b</td>
</tr>
<tr>
<td>N</td>
<td>54</td>
<td>1825</td>
</tr>
</tbody>
</table>

Note: Different letters indicate significant difference (t test) between paired and non-paired animals of each sex (p < 0.05); min = minimum; max = maximum; sd = standard deviation; N = number of individuals.

Figure 1. Assortative mating evaluated by Person’s correlation between body size (cephalothorax length, in mm) of *Hyalella bonariensis* paired and non-paired males and females from Silveira Martins municipality, state of Rio Grande do Sul, Brazil.

Figure 2. Relationship between pairing success and size (cephalothorax length, in mm) of *Hyalella bonariensis* males from Silveira Martins municipality, state of Rio Grande do Sul, Brazil.

Figure 3. Reproductive success, based on size (cephalothorax length, in mm) of *Hyalella bonariensis* males (RM,i) and females (RF,i) from Silveira Martins municipality, state of Rio Grande do Sul, Brazil.

Table 2. Minimum, maximum, and mean cephalothorax length (mm) of *Hyalella bonariensis* females bearing eggs in different stages of embryonic development. Animals collected in Silveira Martins municipality, state of Rio Grande do Sul, Brazil.

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>Mean ± sd</th>
<th>Min – Max</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>57</td>
<td>0.54 ± 0.07 a</td>
<td>0.32 – 0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Intermediary</td>
<td>49</td>
<td>0.55 ± 0.08 a</td>
<td>0.32 – 0.74</td>
<td>0.79</td>
</tr>
<tr>
<td>Final</td>
<td>16</td>
<td>0.55 ± 0.09 a</td>
<td>0.52 – 0.67</td>
<td>0.87</td>
</tr>
<tr>
<td>Juvenile</td>
<td>12</td>
<td>0.55 ± 0.09 a</td>
<td>0.32 – 0.74</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Note: N = number of females bearing eggs; min = minimum cephalothorax length; max = maximum cephalothorax length. Values with at least one letter in common are not significantly different (ANOVA and Bonferroni; α = 0.05).

Table 3. Minimum, maximum, and mean (± standard deviation) number of eggs in each stage of embryonic development and juveniles found in the marsupium of *Hyalella bonariensis* females from Silveira Martins municipality, state of Rio Grande do Sul, Brazil.

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>Mean ± sd</th>
<th>Min – Max</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>57</td>
<td>16.8 ± 3.5 a</td>
<td>8 – 26</td>
<td>0.75</td>
</tr>
<tr>
<td>Intermediary</td>
<td>49</td>
<td>17.4 ± 4.0 a</td>
<td>9 – 25</td>
<td>0.79</td>
</tr>
<tr>
<td>Final</td>
<td>16</td>
<td>17.2 ± 4.9 a</td>
<td>14 – 26</td>
<td>0.87</td>
</tr>
<tr>
<td>Juvenile</td>
<td>12</td>
<td>14.7 ± 0.09 b</td>
<td>12 – 17</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Note: N = number of females bearing eggs; min = minimum number of eggs; max = maximum number of eggs. Values with at least one letter in common are not significantly different (ANOVA and Bonferroni, α = 0.05).

There was a significant reduction of the number of juveniles in the marsupium compared with the number of eggs (p > 0.05). Considering the seasonal mean fecundity, egg production was higher in winter and lower in autumn (p < 0.05; Table 4; juveniles were not included in this analysis). In all seasons, there was a positive correlation between female size and the number of eggs produced, meaning that egg production increased with female size (p < 0.05; Table 4).

Discussion

Our observations revealed that paired males and females are larger than non-paired individuals. This finding is also true in populations of the *H. azteca* (Saussure, 1858) species complex from North America (Wellborn & Bartholf 2005) and in the Brazilian species *H. pleoacuta*,...
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Table 4. Minimum, maximum, and mean (± standard deviation) number of eggs inside the marsupium of *Hyalella bonariensis* females in each season in Silveira Martins municipality, state of Rio Grande do Sul, Brazil.

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>Mean ± s.d</th>
<th>Min – Max</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>31</td>
<td>14.1 ± 2.6c</td>
<td>9 – 20</td>
<td>0.74</td>
</tr>
<tr>
<td>Winter</td>
<td>41</td>
<td>19.3 ± 3.1a</td>
<td>12 – 26</td>
<td>0.68</td>
</tr>
<tr>
<td>Spring</td>
<td>20</td>
<td>17.9 ± 4.6b</td>
<td>8 – 23</td>
<td>0.90</td>
</tr>
<tr>
<td>Summer</td>
<td>24</td>
<td>17.1 ± 3.3b</td>
<td>13 – 25</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Note: N = number of females bearing eggs; min = minimum number of eggs; max = maximum number of eggs. Values with at least one letter in common are not significantly different (ANOVA and Bonferroni, α = 0.05).

*H. castroi* (Castiglioni & Bond-Buckup 2007, 2008b), and *H. longistila* (Bastos-Pereira & Bueno 2016). According to Ward (1983) and Dick & Elwood (1990), larger males have a lower probability of losing females during precopulatory behavior. This statement would explain why paired males were significantly larger than non-paired males in our study; similar results were also observed in populations of *H. pleoacuta* and *H. castroi* (Castiglioni & Bond-Buckup 2008b), and *H. longistila* (Bastos-Pereira & Bueno 2016).

The number of pairs in precopulatory behavior in the population of *H. bonariensis* was low, when compared to unpaired adults. The same result was observed in *H. castroi* and *H. pleoacuta* by Castiglioni & Bond-Buckup (2008b) and H. carstica by Torres et al. (2010). Probably the low occurrence of pairs can be related to the fact that the males and females stay in precopulatory behavior for approximately 3 days, a short time (personal observation), since this reproductive behavior can make individuals more vulnerable to predation (Wellborn, 1995). Moreover, the paired amphipods swim lower than unpaired ones (Cothran, 2004), or sampled methods may have led to the separation of males and females (Wellborn 1995; Castiglioni & Bond-Buckup 2008b).

The studied population of *H. bonariensis* reached sexual maturity at a quite small size. Individuals found in precopulatory behavior were relatively small (males: 0.40 mm of CL; females: 0.38 mm of CL) in comparison with the maximum size found in general population (male: 0.92 mm of CL; females: 0.74 mm of CL). Based on these results we can assume that *H. bonariensis* is able to reproduce early in life. Amphipod species which ensure progeny early in their life cycles may have developed a reproductive strategy aimed at reducing predation risk, since larger individuals are more easily seen by predators (Wellborn 1994, 1995, 2002). Species living in environments with unstable conditions, such as prolonged drought or floods can also reach early sexual maturity to ensure reproduction (Wellborn 1994, 1995, 2002; Appadoo & Myers 2004). We observed that paired and non-paired males were larger than paired and non-paired females; similar results were found in populations of *H. pleoacuta* and *H. castroi* (Castiglioni & Bond-Buckup 2008a, b). Amphipod males often direct energy towards copulation, while females allocate time and energy to offspring care, especially in the production of large gametes. It is important to point out that females do not molt during incubation, creating a sexual dimorphism in body size (Wen 1992; Cardoso & Veloso 1996). As a consequence of this reproductive system, females reach a smaller body size compared with most amphipods - e.g., species of *Hyalella* such as *H. azteca* (Geisler 1944; Wen 1992; Othman & Pascoe 2001), *H. pleoacuta* and *H. castroi* (Castiglioni & Bond-Buckup 2008a); and other amphipods such as Gammarus pulex (Linnaeus, 1758) (Adams & Greenwood 1983), Pseudorchestoidea brasiliensis (Dana, 1853) (Cardoso & Veloso 1996), Gammarus leopolinensis (Jazdzewski & Konopacka, 1989) (Zielinski 1998), G. locusta Linnaeus, 1758 (Costa & Costa 1999), and Echinogammarus longisetas Pinkster, 1973 (Guerra 2003).

A positive size assortative mating was observed in the population of *H. bonariensis*, i.e., large males paired with large females. This size correlation was also observed in populations of *H. pleoacuta* and *H. castroi* (Castiglioni & Bond-Buckup 2009), *H. azteca* (Wellborn 1995), and *H. longistila* (Bastos-Pereira & Bueno 2016). Several hypotheses have been proposed to explain this size correlation in mating: loading constraint (Adams & Greenwood 1983); spatial segregation (Birkhead & Clarkson 1980); sexual selection (Ward 1984); guarding time (Elwood et al. 1987); microhabitat segregation (Ward & Porter 1993); and physical constraints (Adams & Greenwood 1987; Adams et al. 1989; Hatcher & Dunn 1997). The most accepted hypothesis is male-male competition, which postulates that large males have competitive advantages in pairing with larger and more fecund females (Ward 1983; Ward 1986; Ward 1988; Elwood et al. 1987; Crespi 1989; Dick & Elwood 1996; Bollache et al. 2000).

The pairing success of *H. bonariensis* increased with male body size and a similar pattern was also recorded in *H. pleoacuta*, *H. castroi* (Castiglioni & Bond-Buckup 2008b), *H. azteca* (Wellborn 1995), *H. longistila* (Bastos-Pereira & Bueno 2016), and Gammarus pulex (Ward 1988). Therefore, we can assume that males from all size classes are able to find mates. However, although larger females of *H. bonariensis* are more fecund than smaller females, they may have a lower probability of finding an available mate, because males have difficulty carrying them during pre-copulatory behavior. Because of that, larger females pair with less males, while smaller females can find more males to copulate with (Wellborn 1995). Wellborn (1995) observed that pairing and reproductive success of *H. azteca* is higher in females from intermediate size classes. These results demonstrate that the pairing system of *H. bonariensis* is explained by the loading constrain hypothesis, as observed for *H. pleoacuta* and *H. castroi* (Castiglioni & Bond-Buckup 2007).

In several crustacean’s groups, eggs produced by females in a single brood have synchronized embryonic development, reinforcing the assumption that fertilization occurs in one unique event after pair separation (Green 1965; Kevrekidis 2005). This pattern was recorded here in *H. bonariensis* and in populations of *H. pleoacuta*, *H. castroi* (Castiglioni & Bond-Buckup 2007, 2009), *H. longistila* (Bastos-Pereira & Bueno 2016), Gammarus duebeni Lilljeborg, 1852 (Sheader 1983), *G. insensibilis* Stock, 1666 (Sheader 1996), Pseudorchestoidea brasiliensis (Cardoso & Veloso 2001), Corophium insidiosum (Crawford, 1937) (Kevrekidis 2004), and *C. orientale* Schellenberg, 1928 (Kevrekidis 2005).

During the embryonic development of amphipods, it is common to observe a reduction of the embryo number per brood (Moore 1981; Williams 1978; Dick et al. 1998; Dick et al. 2002; Kevrekidis 2004; Castiglioni & Bond-Buckup 2009; Bastos-Pereira & Bueno 2016); this was the case for *H. bonariensis*. According to Koch (1990),
As the embryo volume increases, the space inside the marsupium decreases, leading to a premature loss of a few embryos. Apparently, this occurs due to a higher embryo production than the marsupium can hold during the final stages of development. Also, the reduction of eggs/juveniles may be a consequence of the presence of parasites (Sheader 1983; Kuris 1991) or maternal cannibalism, which may occur when food resources are scarce (Sheader 1983; Castiglioni & Bond-Buckup 2009).

A positive correlation between the female size and the number of eggs/juveniles was observed in *H. bonariensis*. Several authors observed an association between females’ size/weight and the number of embryos in other *Hyalella* species (Strong 1972; Othman & Pascoe 2001; Alcocer et al. 2002; Castiglioni & Bond-Buckup 2007, 2009; Torres et al. 2015; Bastos-Pereira & Bueno 2016). According to Hines (1988), this association may be linked to the fact that the egg mass is restricted by the space available for energetic accumulation and gonadal development. In each species, the wide variability of the carapace shape may interfere with the volume available for gonadal development and, consequently, in brood size. Therefore, egg mass and body cavity volume have similar allometry (Hines 1982).

Production of many small eggs in the summer and few large eggs in the winter is a common amphipod reproductive strategy (Steele and Steele 1969; Sheader 1978; Kolding and Fenchel 1981; Powell 1992), reflecting both seasonal change in the reproductive investment of females that may be related to seasonal changes in food availability, and female and offspring mortality (Price 1974; Smith and Fretwell 1974). However, *H. bonariensis* showed a contrasting reproductive pattern to most species, producing fewer eggs during the summer and many in the winter. Probably, these fluctuations in number of eggs appear to be related to the macrophyte cover, which is much depleted during the warmer months (personal observation), reducing the food and shelter available for breeding females, but may also contributing to increased mortality of recruits. A similar reproductive strategy, characterized by the production of small broods during summer, has been observed in other species of amphipods, such as *H. pleocuta* and *H. castroi* (Castiglioni & Bond-Buckup 2009), *Bathypterois pilosa* Lindström, 1855 (Powell 1992) and *Gammarus locusta* (Linnæus, 1758 (Costa and Costa 1999).

The number of eggs produced by *H. bonariensis* is lower than in other *Hyalella* species from southern Brazil (*H. castroi* and *H. pleocuta*) (Table 5). *Hyalella castroi* and *H. pleocuta* are found in aquaculture trout ponds and have constant food availability, so females have sufficient energetic input to produce a high number of eggs (Castiglioni & Bond-Buckup 2009). However, *H. bonariensis* has a higher fecundity in comparison with other tropical species from southeast Brazil and with the North American species *H. azteca*. Distinct fecundities between species might be a consequence of differences in habitats and the microclimate in which they live, as it has been already observed in other amphipod species (Powell 1992; Appadoo & Myers 2004). The high number of eggs produced by *H. bonariensis* might be an attempt to optimize juvenile survival since the mortality rate is elevated during this stage. However, fecundity data from species raised in laboratory would be needed to confirm this assumption.

### Table 5. Comparative fecundity different species of *Hyalella* (data obtained from literature).

<table>
<thead>
<tr>
<th>Species</th>
<th>NE</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hyalella azteca</em></td>
<td>1-50*</td>
<td>Cooper (1965)</td>
</tr>
<tr>
<td><em>Hyalella sp.</em></td>
<td>1-6*</td>
<td>Sampaio (1988)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em> (= <em>H. curvispina</em>)</td>
<td>18.0*</td>
<td>Pennak (1953)</td>
</tr>
<tr>
<td><em>Hyalella pernix</em></td>
<td>13.0*</td>
<td>Severo (1997)</td>
</tr>
<tr>
<td><em>Hyalella sp.</em></td>
<td>31.3*</td>
<td>Morelli (2001)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>1-38*</td>
<td>Alcocer et al. (2002)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>9.0*</td>
<td>Othman &amp; Pascoe (2001)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>5.2*</td>
<td>Wellborn et al. (2005)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>5.3*</td>
<td>Wellborn et al. (2005)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>6.2*</td>
<td>Wellborn et al. (2005)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>5.2*</td>
<td>Wellborn et al. (2005)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>19.2*</td>
<td>Wellborn et al. (2005)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>14.7*</td>
<td>Wellborn et al. (2005)</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>10.3*</td>
<td>Wellborn et al. (2005)</td>
</tr>
<tr>
<td><em>Hyalella pleocuta</em></td>
<td>36.1*</td>
<td>Castiglioni and Bond-Buckup (2009)</td>
</tr>
<tr>
<td><em>Hyalella castroi</em></td>
<td>31.4*</td>
<td>Castiglioni and Bond-Buckup (2009)</td>
</tr>
<tr>
<td><em>Hyalella carstica</em></td>
<td>12.6*</td>
<td>Torres et al. (2015)</td>
</tr>
<tr>
<td><em>Hyalella longistila</em></td>
<td>12.8*</td>
<td>Bastos-Pereira and Bueno (2016)</td>
</tr>
<tr>
<td><em>Hyalella bonariensis</em></td>
<td>17.4*</td>
<td>Present study</td>
</tr>
</tbody>
</table>

Note: NE = number of eggs; • indicates minimum and maximum fecundity; * indicates mean fecundity.

### Conclusions

The present paper demonstrated the importance of male body size as a determinant of reproductive success of *Hyalella bonariensis* similar to other species of genus. Understanding the interactive effects of partner selection, what stimuli are involved, and the evolution of these choices within the genre, including the formulation of predictive models, will be needed to complement studies on reproduction of this crustacean.

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### Author Contributions

Daniela da Silva Castiglioni: Contribution in the concept and design of the study.
Morgana Tais Streck: Contribution to data collection.
Stella Gomes Rodrigues: Contribution to manuscript preparation.
Alessandra Angélica de Padua Bueno: Contribution to critical revision, adding intelectual contente.
Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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