Ecological aspects of natural populations of *Hyalella pampeana* (Crustacea, Amphipoda, Hyalellidae) from the Natural Reserve Island of Martín García (Río de La Plata, Argentina)

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Abstract: Freshwater amphipods of the genus *Hyalella* have a great importance in aquatic ecosystems due to their role in matter and energy cycling and its utilization as bioindicators of environmental health. The aim of this work was to analyze relevant population parameters of *Hyalella pampeana*, such as population density and structure, individual body size, sex ratio, fecundity and recruitment, and to study the possible relation of these parameters to variations in environmental variables. Samples were taken monthly during a one-year period (2006) at three small ponds in the Natural Reserve Island of Martín García, Argentina. The inland aquatic environments showed distinctive physicochemical characteristics, mainly related to pH and conductivity values. Amphipod population density varied among sites and throughout the year, with population peaks during spring, positively correlating with pH values. The operational sex ratio of the total population was almost equal to 1 male: 1 female, displaying seasonal variation. Mean fecundity and mean size of ovigerous females were higher during winter and early spring months than in summer months. The presence of ovigerous females and juveniles in all months indicated that reproduction is continuous during the year for *Hyalella*, in its natural environment.

Key words: freshwater amphipoda, body size, fecundity, sex ratio.

INTRODUCTION

Amphipods inhabit a wide variety of aquatic environments and have a great ecological importance, since they facilitate the matter and energy transference from inferior levels of the food web to consumers (other macroinvertebrates, fish, amphibians and birds). Besides, due to their ubiquity, small body size, short life cycles and easy culturing in laboratory, amphipods are used as water quality bioindicators and as test organisms in toxicology studies (Pilgrim and Burt 1993, Ingersoll et al. 1998, Borgmann et al. 2005, Lasier and Urich 2014, Javidmehr et al. 2015).
The distribution of the freshwater amphipod genus *Hyalella* Smith 1874 is restricted to the Western Hemisphere, with most species found in South America (Väinölä et al. 2008). Most studies in amphipod populations classically focus on *Hyalella azteca* Saussure 1858, a widely distributed species in aquatic ecosystems of North America, and analyze the population density and its temporal fluctuations, age and size, sex proportion, reproduction and recruitment periods (Lindeman and Momot 1983, Edwards and Cowel 1992, Wen 1992, 1993, Moore and Farrar 1996). The evaluation of these population parameters is of crucial importance since they allow to obtain information about the biology of a species, the stability of the population in a given habitat, its adaptability, reproductive success and persistence (probability of leaving offspring for prolonged periods), among others (Odum and Warrett 2005).

South American ecological studies on the genus are mainly focused on the sympatric species *Hyalella castroi* González, Bond-Buckup and Araujo 2006 and *Hyalella pleoacuta* González, Bond-Buckup and Araujo 2006 from Brazil (Castiglioni and Bond-Buckup 2008a, b, 2009, Garcia-Schroeder and Araujo 2009). Other population studies concentrate on *Hyalella longistila* Faxon 1876 (Bastos-Pereira and Bueno 2016) and *Hyalella bonariensis* Bond-Buckup, Araujo and Santos 2008 (Castiglioni et al. 2016), whereas important aspects of the reproductive biology have been recently studied in *Hyalella carstica* Bastos-Pereira and Bueno 2012 (Torres et al. 2015), *Hyalella georginae* Streck et al. 2017 and *Hyalella gauchensis* Streck et al. 2017 (Ozga and Castiglioni 2017). In Argentina, the biology and ecology of the *Hyalella* species are poorly known aspects. Studies on the subject are almost exclusively related to *Hyalella curvispina* Shoemaker 1942, and they analyze the relations between population dynamics and aquatic vegetation (Casset et al. 2001), body chemical composition and population dynamics in pampasic streams (Poretti et al. 2003), biomass variations in littoral ponds (Galassi et al. 2006), and feeding habits (Saigo et al. 2009). Few studies deal with other *Hyalella* species, like *H. pampeana* Cavalieri 1968 (Lopretto 1982, 1983), and *H. pseudoazteca* González and Watling 2003 (Giusto and Ferrari 2008, Carusela et al. 2009).

Due to the lack of ecological studies in natural populations of *H. pampeana*, the objective of this work was to contribute to the knowledge of the population structure and dynamics of this species in pleustonic communities of three water bodies on the Martín García Island. For this purpose, we analyzed relevant population parameters such as amphipods density, body size, recruitment, fecundity and sex ratio, and their monthly and seasonal variation. We also aim to relate some of the population parameters of *H. pampeana* to relevant variables on the aquatic habitat in order to better understand the natural environment where this species lives.

**MATERIALS AND METHODS**

**STUDY AREA**

The Island of Martín García (34° 11’ S and 58° 15’ W) is located at the confluence of the Paraná and Uruguay rivers, Río de La Plata Superior, Argentina (Figure 1). Geologically, the island is a raised and fractured block of the crystalline basement of the mass of Brasilia, later covered by Holocene and Pleistocene quaternary deposits (Ravizza 1984). In different sectors of the island, there are aquatic environments that differ in their origin and substrate (natural sandy ponds or quarries excavated on the rocky bottom), hydrological regimen (permanent or temporal) and water inputs (precipitations and/or flows from the Río de La Plata River). These environments develop carpets of floating vegetation and a wide diversity of aquatic invertebrates inhabits the pleustonic habitat. Some of them include mollusks, annelids, platyhelminthes, crustaceans and insects (Viana...
and placed in white trays where ovigerous females were individualized in labeled plastic vials before transportation to the laboratory.

Physicochemical parameters (air and water temperature, pH, dissolved oxygen, conductivity and total dissolved solids) were registered in situ with a digital multiparametric equipment (Water Quality Meter Sper Sc. LTD).

Since 1998, the island is a Natural Reserve, with a stable human population and frequented by tourists throughout the year, especially during spring and summer (CITAB 2011).

Sampling campaigns were conducted monthly between February and December 2006. Triplicate samples of pleuston were taken with a hand net (0.09 m² surface area and 150 μm mesh size) at three study sites. Site 1 (“Cantera La Gata”) and site 2 (“Cantera Tanque”) are both abandoned quarries, and site 3 (“Laguna Arenalcito”) is a small natural sandy pond. While sites 1 and 3 are temporary, site 2 is a permanent habitat (Figure 1). Samples were fixed in situ with 10% v/v formalin and placed in white trays where ovigerous females were individualized in labeled plastic vials before transportation to the laboratory.

Physicochemical parameters (air and water temperature, pH, dissolved oxygen, conductivity and total dissolved solids) were registered in situ with a digital multiparametric equipment (Water Quality Meter Sper Sc. LTD).

In seasonal analysis, the sampling day date of each month was considered for selecting the corresponding season: February and March (summer); April and May, (autumn); June, July, August and September (winter); October, November and December (spring).

LABORATORY ANALYSIS

Pleuston samples were placed in plastic trays and the aquatic vegetation was gently washed with current
water over a sieve (125 µm) and removed for further identification analysis. Once the vegetation was separated, the remnant material was analyzed under a stereoscopic microscope, and 20 amphipods of each sample (10 males and 10 females) were selected for dissection. The identification of the amphipods was made by observing the anatomical pieces placed in semipermanent slides under microscope and following the specialized taxonomic literature (Cavalieri 1968, Grosso and Peralta 1999).

*H. pampeana* individuals were classified into four categories: males (individuals with enlarged second pair of gnathopods), females (individuals with visible oostegites), ovigerous females (females with eggs or juveniles inside the marsupium) and juveniles (individuals without recognizable sexual characters) (Castiglioni and Bond-Buckup 2008a). All specimens were counted, and total population density and density by categories (ind/m$^2$) were calculated.

In order to obtain the cephalothorax length (CL, in mm), all individuals were measured from the front margin of the head to the posterior margin of the head, in lateral view (Castiglioni and Bond-Buckup 2008a). For measurements, a stereoscopic microscope complemented with a 0.1 mm precision micrometer was used. The relationship between CL and total length (TL, distance between the front margin of the head and the posterior margin of the telson, in lateral view) was analyzed in 25 females, 25 males and 25 juveniles of *H. pampeana*.

Pleustonic vegetal species were identified according to Lahitte and Hurrel (1996). To obtain the plant dry weight, the material was dried during 48hs at 105 °C and weighed using a precision digital scale (Dahus, Explorer).

**DATA ANALYSIS**

**Environmental variables**

An Analysis of Variance (ANOVA) complemented with a posteriori Bonferroni test (α=0.05) was conducted to evaluate significant differences in the total mean values of the environmental variables between the three study sites. The environmental variables were also analyzed seasonally in order to account for differences along the studied period.

A Principal Component Analysis (PCA) was performed in order to analyze the relationship between the environmental variables measured, and to characterize the study sites. The variables included in the analysis were water temperature (°C), pH, dissolved oxygen (DO, mg/l), conductivity (µS/ cm), and plant dry weight (g/m$^2$). Air temperature and total dissolved solids were removed from the analysis since both variables are correlated with water temperature and conductivity, respectively. PCA was conducted using the statistic program XLSTAT 2014.

**Population dynamics**

Differences in total population density of *H. pampeana* (ind/m$^2$) between sites were analyzed by means of ANOVA and a posteriori Bonferroni test (α=0.05). Data were previously log-transformed in order to meet the requirements of the analysis. In order to evaluate the representation of males, females, ovigerous females and juveniles in the population, we also analyzed the population density by demographic categories (Wen 1992, Poi de Neiff and Carignan 1997, Galassi et al. 2006).

Minimum, maximum, mean, and standard deviation (SD) of CL were estimated for males, females (including ovigerous females) and juveniles at each study site. Significant differences between annual mean body size of each amphipod category were compared using a one-way ANOVA (factor: sites) complemented with a posteriori Bonferroni test (α=0.05).

A t-test was conducted in order to determine body size differences between males and females at each sampling site (α=0.05; Sokal and Rohlf 1979) (Castiglioni and Bond-Buckup 2008a). The
relationship between CL and TL of each category of *H. pampeana* was studied by means of a simple regression analysis.

To determine temporal variations in the frequency of each category of *H. pampeana* during the analyzed period, the study site with higher population density (and, consequently, with higher probabilities for each size category to be represented) was selected to conduct a size-frequency distribution analysis. *H. pampeana* individuals were grouped into size classes, and relative frequency histograms were constructed. The number of size classes was determined according to the value of one quarter of the CL standard deviation measurements (Castiglioni and Bond-Buckup 2008a).

A monthly proportion analysis of juveniles and adults of *H. pampeana* was performed at each site. This analysis was conducted to identify reproduction and recruitment periods. Differences in the proportion 1 juvenile: 1 adult were studied by means of a Chi squared test ($X^2; \alpha=0.05$) using the statistic software XLSTAT 2014.

Sex ratio of *H. pampeana* was calculated as the abundance of males/abundance of females (M: F, except ovigerous females) at each site. This ratio is called “operational” (OSR, operational sex ratio), and it considers the mean number of fertilizable females per sexually active male, in a given moment (Emlen and Oring 1977). OSR was also calculated seasonally and by size classes at each study site. A Chi squared test ($X^2; \alpha=0.05$) was conducted in all the cases to test the null hypothesis of 1M: 1F (Sokal and Rohlf 1979).

Fecundity was determined as the number of eggs or juveniles present in the marsupium of each ovigerous female. At each study site, the minimum and maximum egg production was estimated, and the mean fecundity among the sites was compared by means of ANOVA, complemented with a Bonferroni test ($\alpha=0.05$). The monthly variations in mean fecundity and size of the ovigerous females were analyzed by a Pearson correlation analysis in order to determine the relation between both parameters. Frequencies between ovigerous females and adult non-ovigerous females were analyzed seasonally in each study site using a $k$-proportions test ($p<0.05$) with the statistic software XLSTAT 2014.

**Population parameters and environmental variables**

In order to assess if the variation in the population parameters (population density, OSR, recruitment, fecundity) can be related to the variation in the environmental variables measured, a stepwise multiple regression analysis was performed using the SPSS v. 22 software. Variables included in the analysis were water temperature, pH, dissolved oxygen, conductivity and plant dry weight, at all sites sampled. The selection criterion adopted for environmental variables was the $p$-value associated with Student’s $t$-statistic (probability of entry of the variable to the equation, $p <0.01$) (Lepš and Šmilauer 2003).

**RESULTS**

**ENVIRONMENTAL CHARACTERIZATION OF STUDY SITES**

A summary of the seasonal environmental data is shown in Table I. Mean water temperature ranged between 12.3 °C in winter and 26 °C in summer, while pH values were close to neutrality at sites 1 and 2, and slightly acidic at site 3. Dissolved oxygen values showed great variation, with a minimum of 2.17 mg/l and a maximum of 9.17 mg/l. Water conductivity and total dissolved solids (TDS) mean values oscillated between a minimum of 15 µS/cm and 10.5 ppm, and a maximum of 447 µS/cm and 295 ppm, respectively. Mean plant dry weight ranged between 27 and 85.1 g/m². The most frequent vegetal species were *Lemna minuta* Humb. Bonpl. et Kunth 1815 and *Azolla filiculoides* Lam.
1783 at site 1, *Wolffia columbiana* H. Karst 1865 and *Spirodella intermedia* W. Koch 1932 at site 2, and *L. minuta* and *S. intermedia* at site 3. The ANOVA results showed environmental differences between the sites. The lowest pH values were registered at site 3, while the highest conductivity, TDS and plant dry weight values were registered at site 2 (pH: F=86.11, p<0.01; conductivity: F=546.17, p<0.01; TDS: F=439.4, p<0.01 and plant dry weight: F=6.02, p<0.01) (Table I).

According to the PCA results, components 1 and 2 explained 71.77% of the data variability (C1: 48.21% and C2: 23.56%, Figure 2). Water conductivity, pH and plant dry weight account for 85.6% of component 1 variation, while water temperature and dissolved oxygen content account for 82.9% of component 2 variation. Samples from site 2 were located at the positive sector of component 1, related to higher values of conductivity and pH, while samples from sites 1 and 3 were located at the opposite sector, related to lower values of these variables. The exception was site 1 in October, which presented a maximum value of plant dry weight of 106.3 g/m². Component 2 ordered the sites according to water temperature and dissolved oxygen content. Sites located at the positive sector of the component represent the samples taken during warmer months and with higher values of dissolved oxygen.

**POPULATION DENSITY**

Higher mean population density values were registered at site 2 (5,326 ± 3,500 ind/m²). The ANOVA results showed significant differences in annual mean population density (log₁₀ density) between the three study sites (F=21.78; p<0.01) (Figure 3). Figure 4a, b and c shows monthly variations in mean density of *H. pampeana* for each category (males, females, ovigerous females and juveniles).
### Table I

Main environmental parameters (seasonal mean ± standard deviation) of three inland aquatic sites on the Island of Martín García (year 2006). The total mean values (N=11) that showed significant differences between sites are indicated with letters of the same type (capital, small, capital bold and small bold). Values with at least one letter in common did not differ statistically (p > 0.05).

<table>
<thead>
<tr>
<th>Sites</th>
<th>Seasons</th>
<th>Air Temperature (°C)</th>
<th>Water Temperature (°C)</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>TDS (ppm)</th>
<th>DO (mg/l)</th>
<th>Plant Dry Weight (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summer</td>
<td>25.5±0.7</td>
<td>24.0±0</td>
<td>6.17±0.06</td>
<td>42.5±27.6</td>
<td>23.0±11.3</td>
<td>7.05±1.8</td>
<td>47.4±32.2</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>21.8±4.8</td>
<td>18.5±5.8</td>
<td>6.24±0.06</td>
<td>59.3±1.2</td>
<td>36.3±4.04</td>
<td>9.17±1.0</td>
<td>46.3±15.5</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>16.3±3.7</td>
<td>12.7±2.1</td>
<td>6.25±0.07</td>
<td>27.0±13.0</td>
<td>18.07±8.6</td>
<td>5.10±2.9</td>
<td>44.9±11.0</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>20.7±1.6</td>
<td>17.8±2.5</td>
<td>6.16±0.1</td>
<td>15.0±1.0</td>
<td>10.5±0.5</td>
<td>3.20±0.2</td>
<td>52.6±13.5</td>
</tr>
<tr>
<td></td>
<td>Total mean</td>
<td>20.6±4.3</td>
<td>17.7±4.94</td>
<td>6.3±0.2</td>
<td>35.36±20.9</td>
<td>21.9±11.7</td>
<td>6.04±2.8</td>
<td>51.5±22.7</td>
</tr>
<tr>
<td>2</td>
<td>Summer</td>
<td>27.0±2.8</td>
<td>25.1±2.3</td>
<td>6.92±0.02</td>
<td>346.5±37.5</td>
<td>226.5±17.7</td>
<td>3.65±0.92</td>
<td>83.7±6.2</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>24.4±6.6</td>
<td>21.0±4.6</td>
<td>6.82±0.1</td>
<td>388.0±16.1</td>
<td>249.3±4.0</td>
<td>6.90±1.8</td>
<td>62.7±20.0</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>15.0±4.5</td>
<td>12.3±0.6</td>
<td>6.81±0.07</td>
<td>447.0±21.6</td>
<td>295±21</td>
<td>4.53±4.49</td>
<td>70.3±7.7</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>20.7±5.1</td>
<td>19.5±4.0</td>
<td>7.03±0.1</td>
<td>426.3±30.1</td>
<td>281±22.7</td>
<td>2.17±0.49</td>
<td>85.1±11.6</td>
</tr>
<tr>
<td></td>
<td>Total mean</td>
<td>21.2±6.3</td>
<td>19.0±5.5</td>
<td>6.9±0.1</td>
<td>407±43.5</td>
<td>266.3±30.7</td>
<td>4.4±2.9</td>
<td>76.6±14.1</td>
</tr>
<tr>
<td>3</td>
<td>Summer</td>
<td>25.9±1.3</td>
<td>26.0±0</td>
<td>6.07±0.22</td>
<td>49.0±19.8</td>
<td>32±14.1</td>
<td>4.0±2.3</td>
<td>27.0±8.3</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>21.5±4.9</td>
<td>20.8±5.5</td>
<td>5.7±0.15</td>
<td>42.3±22.5</td>
<td>36.4±30.8</td>
<td>8.17±0.8</td>
<td>61.6±26.5</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>15.8±3.3</td>
<td>13.8±2.0</td>
<td>5.65±0.06</td>
<td>29.7±11.6</td>
<td>20.3±8.1</td>
<td>3.53±2.2</td>
<td>60.3±18.6</td>
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<tr>
<td></td>
<td>Spring</td>
<td>22.2±2.8</td>
<td>21.7±5.3</td>
<td>5.55±0.04</td>
<td>16.0±1.0</td>
<td>10.7±2.1</td>
<td>5.80±0.1</td>
<td>62.8±16.1</td>
</tr>
<tr>
<td></td>
<td>Total mean</td>
<td>21.4±5.03</td>
<td>20.6±5.6</td>
<td>5.7±0.22</td>
<td>34.2±18.7</td>
<td>25.3±19.0</td>
<td>5.3±2.7</td>
<td>52.0±19.7</td>
</tr>
</tbody>
</table>


at sites 1, 2 and 3, respectively. *H. pampeana* was registered throughout the whole year at the three study sites, with the exception of site 3 in May, where no amphipods were collected. A population peak occurs during spring months, with maximum values in November at sites 1 and 2 (8,163 ind/m² and 14,244 ind/m², respectively), and in December at site 3 (1,859 ind/m²).

**BODY SIZE**

The correlation between cephalothorax length (CL) and total length (TL) of males, females and juveniles of *H. pampeana* was positive and statistically significant in all cases (males: r=0.93, p<0.01; females: r=0.86, p<0.01 and juveniles: r=0.72, p<0.01).

Mean body size, standard deviation (SD) and range (CL minimum-CL maximum) of each category of *H. pampeana* per site are indicated in Table II. At all sites, the mean body size of males was significantly greater than that of females (site 1: F=150.8, p<0.01; site 2: F=135.6, p<0.01; site 3: F=35.6, p<0.01). The ANOVA results showed significant differences in mean size of males, females and juveniles between study sites (Table II).

**POPULATION STRUCTURE AND RECRUITMENT**

The study site with the highest population density was selected (site 2), and individuals of *H. pampeana* were grouped into 23 size classes of 0.02 mm. All the categories of *H. pampeana* were represented during the year (Figure 5).

The size-classes distribution analysis revealed polymodality in most of the months, with different modes in juveniles and adults. Adults predominated for most of the year. Even though recruitment of juveniles occurs throughout the year, there were two periods when this event had greater intensity. During March and April, a moderate increase of recruitment was observed, with juveniles representing frequencies of 54 and 57%. Finally,
from October to December (spring), the second peak of recruitment occurs, with juvenile frequencies of 55 and 66%.

Table III shows monthly variations in the proportions of juveniles and adults at each study site. A similar trend was observed in each environment: during most of the year, the proportion was 1 adult: 1 juvenile or adults predominated. Juveniles instead, predominated during spring months. At site 3, the only site of natural origin analyzed, juveniles density increased also in April and August. No amphipods were collected during May at this site.

SEX RATIO

The operational sex ratio (OSR) of *H. pampeana* was close to 1 male: 1 female at the three study sites: site 1, 1.16:1 ($X^2=1.13; p=0.29$), site 2, 1.47:1 ($X^2=1.95; p=0.16$) and site 3, 1.31:1 ($X^2=1.46; p=0.23$). Sex ratio analyzed seasonally showed the same proportion (1M: 1F), except for sites 1 (1.87:1; $X^2=4.26; p<0.05$) and 3 (3.8:1; $X^2=22.7; p<0.05$) in autumn, where males predominated (Figure 6a, b and c).

Sex ratio was also analyzed by size classes at the three study sites (Figure 7a, b and c). At all sites, a greater number of females occurred in the smaller size classes in relation to males (until size class: 0.35-0.37 mm at site 1, and 0.41-0.43 mm at sites 2 and 3), although this ratio was not significantly different from 1M: 1F ($p>0.05$). However, in greater size classes (from 0.44-0.46 mm at site 1, and 0.47-0.49 mm at sites 2 and 3), sex ratio was skewed towards males at all sites ($p<0.05$).

FECUNDITY

No significant differences were found in total mean fecundity of *H. pampeana* between sites ($F=2.94; p>0.05$). At site 1, fecundity was 9.97±3.7 eggs/female (range 6-26 eggs/female); at site 2, 10.03±3.6 (range 6-27 eggs/female); and at site 3, 10.9±4.4 (range 6-27 eggs/female). Regarding monthly variation, mean fecundity and size of ovigerous

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**TABLE II**

Summary of body size data of males, females and juveniles of *H. pampeana* and results of the Analysis of Variance (ANOVA) and a posteriori Bonferroni test ($\alpha=0.05$) for the CL mean data recorded at sites 1 to 3. Annual mean CL (mm), standard deviation (SD), number of individuals (N) and CL range (minimum-maximum, in mm). Capital, small and bold letters show differences between mean CL of males and females at sites 1, 2 and 3, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Sites</th>
<th>ANOVA</th>
<th>Bonferroni</th>
</tr>
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<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CL (SD)</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0.44 (A)</td>
<td>0.47 (a)</td>
<td>0.47 (a)</td>
</tr>
<tr>
<td>N</td>
<td>948</td>
<td>2923</td>
<td>264</td>
</tr>
<tr>
<td>CL Range</td>
<td>0.24-0.75</td>
<td>0.27-0.75</td>
<td>0.3-0.69</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CL (SD)</td>
<td>0.4 (B)</td>
<td>0.45 (b)</td>
<td>0.43 (b)</td>
</tr>
<tr>
<td>N</td>
<td>1360</td>
<td>3314</td>
<td>357</td>
</tr>
<tr>
<td>CL Range</td>
<td>0.21-0.63</td>
<td>0.27-0.66</td>
<td>0.33-0.75</td>
</tr>
<tr>
<td><strong>Juveniles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CL (SD)</td>
<td>0.22</td>
<td>0.23</td>
<td>0.25</td>
</tr>
<tr>
<td>N</td>
<td>4352</td>
<td>4876</td>
<td>500</td>
</tr>
<tr>
<td>CL Range</td>
<td>0.09-0.3</td>
<td>0.12-0.3</td>
<td>0.15-0.3</td>
</tr>
</tbody>
</table>
Figure 5 - Monthly size-frequency distribution of *H. pampeana* at site 2 (year 2006). The numbers in the right corner of each graph represent the number of juveniles (J), males (M), females (F) and ovigerous females (OF).
females of *H. pampeana* increased during winter and early spring and decreased during the summer months (Figure 8a, b and c). Both parameters correlated positive and significantly at all sites (site 1: R²=0.44, p<0.05; site 2: R²=0.53, p<0.05 and site 3: R²=0.59, p<0.05). Ovigerous females were collected in all seasons in the three study sites. The seasonal variation indicates a greater frequency (p<0.05) of ovigerous females during autumn and spring compared to winter in site 1, and during autumn, spring and summer in site 3. In site 2, the ovigerous females were more abundant in winter (51.2% of females were ovigerous) (Figure 9a, b and c).

### TABLE III

Monthly variation in the proportion of juveniles and adults of *H. pampeana* at each site with respective results of Chi squared test for goodness of fit (X²). Asterisks indicate significant differences in the proportion 1 adult: 1 juvenile (p<0.01).

<table>
<thead>
<tr>
<th>Months</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Juveniles</td>
<td>Adults</td>
<td>X²</td>
</tr>
<tr>
<td>F</td>
<td>0.09</td>
<td>0.91</td>
<td>168.20*</td>
</tr>
<tr>
<td></td>
<td>(74)</td>
<td>(715)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.43</td>
<td>0.57</td>
<td>1.54</td>
</tr>
<tr>
<td></td>
<td>(281)</td>
<td>(378)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.47</td>
<td>0.53</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>(663)</td>
<td>(759)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.43</td>
<td>0.57</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>(33)</td>
<td>(44)</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>0.52</td>
<td>0.48</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>(196)</td>
<td>(181)</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>0.28</td>
<td>0.72</td>
<td>8.21*</td>
</tr>
<tr>
<td></td>
<td>(126)</td>
<td>(318)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.47</td>
<td>0.5</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>(678)</td>
<td>(766)</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.29</td>
<td>0.71</td>
<td>7.84*</td>
</tr>
<tr>
<td></td>
<td>(115)</td>
<td>(285)</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>0.48</td>
<td>0.52</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>(2,740)</td>
<td>(2,966)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.74</td>
<td>0.26</td>
<td>10.44*</td>
</tr>
<tr>
<td></td>
<td>(6,004)</td>
<td>(2,319)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.94</td>
<td>0.06</td>
<td>392.60*</td>
</tr>
<tr>
<td></td>
<td>(5,207)</td>
<td>(359)</td>
<td></td>
</tr>
</tbody>
</table>

**POPULATION PARAMETERS AND ENVIRONMENTAL VARIABLES**

The results of the multiple stepwise regression analysis of the complete environmental dataset show that pH is the variable that better describes the variations in population density of *H. pampeana* at the study sites. About 61% of the variability in population density (log 10 amphipod density) can be explained by its lineal relation to pH (R² = 0.62; p<0.01). The resulting model corresponded to a simple regression: Log₁₀ amphipod density = 1.23pH - 4.84 (Figure 10).

No significant correlations were found between other population parameters, such as...
Figure 6 - Seasonal sex ratio of *H. pampeana* in (a) site 1, (b) site 2 and (c) site 3. The asterisk indicates significant differences in the proportion 1M: 1F (p<0.05).

Figure 6 - Seasonal sex ratio of *H. pampeana* in (a) site 1, (b) site 2 and (c) site 3. The asterisk indicates significant differences in the proportion 1M: 1F (p<0.05).

DISCUSSION

STUDY SITES

The inland aquatic environments analyzed in this work differ in their biological and physicochemical characteristics. The main differences between study sites were related to water conductivity and pH values. According to Drago and Quirós (1996), one of the most important mechanisms governing Argentinean inland water chemistry is rock dominance. Sites 1 and 2 have a common origin; both are quarries drilled in the granite bottom of the island and later covered with organic matter. Nevertheless, site 1 has a temporary hydrological regimen, with droughts in some months of the year, although it never dried during the study period. Water inputs at this site come from precipitation and floods of the Río de La Plata River (César et al. 2009), where the mean conductivity values are around 75-80 μS/cm (Jaime et al. 2001). Site 1 registered lower values of water conductivity than those reported by these authors; this could be explained by the combined water input (precipitations and flooding) in this environment. On the other hand, site 2 registered the highest values of conductivity, pH and plant dry weight. According to Bini et al. (1999), the occurrence of free-floating plant species such as Lemnaceae is strongly associated with high nutrient conditions (total phosphorus and conductivity), since this species predominantly obtains its nutrient requirements from the water column. In addition, TDS values were also higher at site 2 compared to the other study sites. As the TDS concentration in natural waters is determined by the geology of the drainage, atmospheric precipitation and water balance (Weber-Scannell and Duffy 2007), the permanent hydrological regime of this pond, together with the water input only from precipitation, could explain the high TDS, conductivity and pH values registered. Finally, site 3, the only natural small pond studied, registered slightly acidic pH values, high content of dissolved oxygen, and intermediate values of plant dry weight. Since this temporary pond is located at the base of a sandbank, the water’s physicochemical characteristics could be related to its sandy substrate.

POPULATION DENSITY

*Hyalella pampeana* is a common component of the freshwater biota in lotic and lentic environments of Argentina (Lopretto 1983, Poi de Neiff and Carignan 1997, Poi de Neiff and Neiff 2006). Populations of *H. pampeana* developed during the year with...
wide variability at the three study sites. The high-density values recorded at site 2 (14,244 ind/m² in November) could be related to the permanent hydrological regime of this pond, which allows the amphipod population to grow and stabilize. Galassi et al. (2006) studied the population dynamics of *H. curvispina* in lagoons of the margins of the Paraná River and report 1,000 ind/m² in temporary ponds of 60 cm of depth and low values of dissolved oxygen. In permanent ponds with a depth of up to 130 cm and intermediate values of conductivity, population density values of *H. curvispina* were 6,587 ind/m².

Population peaks were registered during spring at the three sites analyzed. According to Kruschwitz (1978), increments in water temperature reduced the time required for ovarian maturation in cultures of *H. azteca*, shortened the intervals between ovipositions, and increased reproductive rates. This could explain the high densities in natural populations of *Hyalella* during warmer months, as has been reported by other authors (Casset et al. 2001, Poretti et al. 2003). In our study, *H. pampeana* density peaks occur together with an increment on the floating vegetal biomass. Similar results were reported by Giorgi et al. (2005) in a Pampean stream, where amphipods increased their numbers together with the macrophyte development in spring and summer. According to Waterkeyn et al. (2008), aquatic vegetation cover is an important factor influencing invertebrate community structure, presumably because the vegetation creates structural heterogeneity and it can provide refuges and food resources.

**BODY SIZE**

Males of *H. pampeana* were larger than females. This difference in size between sexes has been reported in other *Hyalella* species, such as *H. pleoacuta*, *H. castroi* (Castiglioni and Bond-Buckup 2008a), *H. bonariensis* (Castiglioni et al. 2016), *H. longistila* (Bastos-Pereira and Bueno 2016), *H. georginae* and *H. gauchensis* (Ozga and Castiglioni 2017). According to Low (1978), crustacean growth is similar between sexes until reproductive maturity. After that, males and females present different ecological or reproductive demands. Males invest most of their energy in reproduction, especially in searching for a female and copulation, while in females energy effort is invested in gamete production and parental care. This results in different growth rates, which are probably the main cause of size differences between sexes (Wen 1993). According to Lopretto

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**Figure 7** - Sex ratio of *H. pampeana* by size classes of cephalothorax length (mm) in (a) site 1, (b) site 2 and (c) site 3. Asterisks indicate significant differences in the proportion 1male:1female (p<0.05).
molting has different functions for males and females of *H. pampeana*. For females, it is an event mostly related to reproduction, since its occurrence is indispensable for achieving egg fertilization. Males, on the other hand, exhibit a lower number of molt occurrences, but intervals between molts are longer, allowing considerable increments in size.

In this study, a smaller mean body size of *H. pampeana* was registered at site 1, when compared to the other sites. Strong (1972) found that different populations of *H. azteca* might have different adult body sizes, according to the selective pressure of the habitat. This includes seasonal changes in growth and reproduction and characteristics of the aquatic environment, such as substrate type, productivity, food availability and presence of predators. Although these parameters were not analyzed in the present study, the great variability in water level at site 1, the only pond studied that receives the flooding of the Río de La Plata River, could affect habitat stability and explain the smaller body size of *H. pampeana* registered in this environment.

Positive correlations between cephalothorax and total length like those found in this work are indicated in other *Hyalella* species, like *H. azteca* (Strong 1972, Kokkotis and McLaughlin 2002) and *H. longistila* (Bastos-Pereira and Bueno 2016). Measurement of cephalothorax length is an effective and easy method for obtaining information about body size, and it can be used to replace the measurement of total length.

**POPULATION STRUCTURE AND RECRUITMENT**

Population structure of *H. pampeana* showed several modes in different size categories of males, females and juveniles. Polymodality has been reported in other South American *Hyalella* species, such as *H. castroi*, *H. pleoacuta* (Castiglioni and Bond-Buckup 2008a), *H. bonariensis* (Castiglioni et al. 2016) and *H. longistila* (Bastos-Pereira and Bueno 2016). According to Conde and Díaz (1989), bi- or polymodality in size classes frequency distribution generally reflects recruitment pulses, differential mortality or behavioral differences, while unimodality is associated with a stable population structure with continuous recruitment and constant mortality rate. In this work, juveniles were registered in all months, indicating a continuous moderate recruitment but with more intense pulses in some periods, where this category exceeded 50% of the total population abundance.

Ovigerous females of *H. pampeana* were collected in most of the months, indicating that reproduction is continuous in this species throughout the year, in agreement with data reported by Lopretto (1983) in ponds of the province of Buenos Aires, Argentina. Continuous reproduction has been reported in other *Hyalella* species that inhabit natural populations in tropical and subtropical temperature regimes (Edwards and Cowell 1992, Castiglioni and Bond-Buckup 2009, Bastos-Pereira and Bueno 2016, Ozga and Castiglioni 2017, Torres et al. 2015, Castiglioni et al. 2016). According to Sainte-Marie (1991), gammaridean populations exhibit reproductive patterns that can be associated with latitude. High latitude species generally present biannual or perennial life cycles, large body size, delayed maturity, and single or few broods with many relatively large embryos, while the opposite set of characters occurs in low latitude populations. The continuous food availability in tropical and subtropical climates appears to be one of the main factors allowing all year reproduction and brooding in *Hyalella* populations (Steele and Steele 1991, Edwards and Cowell 1992).

**SEX RATIO**

Operational sex ratio of *H. pampeana* was close to 1:1 when considering the annual data set per study site. However, regarding seasonal variation, OSR was skewed towards males during autumn at
sites 1 and 3. According to Moore (1981), the sex ratio of some amphipods may vary during the life cycle, with male dominance during colder months and female dominance or proportions close to 1:1 during warmer months of the year. In Amphipoda, females are available for reproduction only for a brief period during its molting cycle (Wen 1993). According to Emlen and Oring (1977), the degree of spatial and temporal clumping of the limiting sex is a main factor that produces asymmetries in the sex ratio. For example, continuous long periods of sexual activity by males, coupled with brief and asynchronous periods of receptivity by females, will produce a strong skew in the OSR. When the OSR is skewed toward males, as we observed in *H. pampeana* during autumn in sites 1 and 3, polygyny (individual males frequently gain access to multiple females) is expected; when the skew is toward females, polyandry (individual females frequently gain access to multiple males) should occur. Seasonal fluctuation of OSR was also recorded in *H. longistila* (Bastos-Pereira and Bueno 2016) and *H. bonariensis* (Castiglioni et al. 2016).

Sex ratio analyzed by size classes showed a statistically significant predominance of males over females in larger size classes of *H. pampeana*. Similar results were reported for *H. castroi*, *H. pleoacuta* (Castiglioni and Bond-Buckup 2008a), *H. longistila* (Bastos-Pereira and Bueno 2016) and *H. bonariensis* (Castiglioni et al. 2016), which could be related to differences in energy consumption between sexes. Female amphipods invest most of their time and reproductive effort in egg production, while males do so in pairing. Besides, taking into account that ovigerous females do not molt, males can reach larger sizes than females. This reproductive difference may probably generate differences in adult size of both sexes (Wen 1993), and influence sex ratio in animal populations (Székely et al. 2014).

**FECUNDITY**

Total mean fecundity values of *H. pampeana* at each site were similar to those reported by Lopretto (1983) in laboratory cultures (14 and 12 eggs/female at thermal regimes of 10-22 °C and 23-26 °C, respectively). Fecundity of *H. pampeana* was also comparable to mean fecundity values estimated for *H. carstica*; 12.6 eggs/female (Torres et al. 2015). However, when compared with other south American *Hyalella* species, females of *H. pampeana* produce a lower mean number of eggs (*H. pleoacuta*: 36.1 eggs; *H. castroi*: 31.4 eggs; *H. longistila*: 20.5 eggs; *H. carstica*: 20.0 eggs; *H. pleoacuta*: 17.0 eggs).

![Figure 8](image_url) - Cephalothorax length (CL, in mm) of the ovigerous females of *H. pampeana* and monthly mean fecundity (eggs/female) during 2006 in (a) site 1, (b) site 2 and (c) site 3.
Both mean fecundity and size of ovigerous females increased during winter and early spring, and decreased during summer months. A reduction in fecundity together with a smaller body size of ovigerous females during warmer months of the year have also been reported in *H. pleoacuta* and *H. castroi* (Castiglioni and Bond-Buckup 2009). Temperature is considered to be the main factor in the generation of size and fecundity differences in winter and summer amphipod populations. Villarroel et al. (2000) analyzed the effect of temperature on some population parameters of *H. azteca* in laboratory, and reported a decrease in mean body size, fecundity, incubation time and duration of sexual amplexus with a 6 °C increase in water temperature (24 to 30 °C). Reduction in fecundity and size of amphipods during summer months could be attributed to a metabolic effect. Higher temperatures result in higher costs for females in metabolic maintenance, which could result in a lower amount of energy invested in the production of eggs during warmer months (Nelson 1980, Ward 1986).

Monthly mean size of ovigerous females of *H. pampeana* was positively correlated with mean fecundity. This result agrees with those reported by other authors for several *Hyalella* species, like *H. azteca* (Strong 1972, France 1992, Othman and Pascoe 2001) *H. castroi*, *H. pleoacuta* (Castiglioni and Bond-Buckup 2009), *H. carstica* (Torres et al. 2015), *H. bonariensis* (Castiglioni et al. 2016), *H. gauchensis* and *H. georginae* (Ozga and Castiglioni 2017).

Although ovigerous females of *H. pampeana* were collected in all months of the year, their frequency varied seasonally at each site. In the permanent pond (site 2), the higher frequencies were found during colder months. According to data reported for populations of *H. pleoacuta* and *H. carstica* (Castiglioni et al. 2008a) and *H. bonariensis* (Castiglioni et al. 2016), this greatest occurrence of ovigerous females could be associated with greater food availability during winter (macrophytes). On the other hand, ovigerous females were more frequent during warmer months in the two temporary ponds (sites 1 and 3). Although these ponds never dried completely during the study period, they are more exposed to water level fluctuations than site 2 (site 1 receives...
water during floods of the Río de La Plata River, and site 3 is a small pond in the base of a sand bank that only receives water for precipitation). Unlike permanent ponds, temporary habitats provide less stable physicochemical conditions and, consequently, less time is available for invertebrates to complete their life cycles, colonization and community development (Waterkeyn et al. 2008). We suggest that the seasonal changes in occurrence of ovigerous females among the different ponds could be related to the environmental stochasticity to which they are exposed. Therefore, future studies about the influence of hydroperiod and other related parameters can be important for better understanding the dynamics of amphipods populations in these habitats.

POPULATION PARAMETERS AND ENVIRONMENTAL VARIABLES

In this study, significant correlations between monthly amphipod population density and water pH were found. There are a few studies on the effect of water pH on amphipod populations. Grapentine and Rosemberg (1992) examined the distribution of *H. azteca* in thirty Ontario lakes and reported higher abundances of this amphipod in waters with higher pH values. Besides, with pH values below 6, no individuals of *H. azteca* were registered. Glazier et al. (1992) found similar results in population studies of the continental amphipod *Gammarus minus*. In seven of ten springs analyzed by these authors, the population density of *G. minus* was positively correlated with pH and conductivity. This is in accordance with the results obtained in this study, where the linear relation with water pH can explain 48% of the variability in the population density of *H. pampeana*. Other variables not analyzed in this work could explain the low abundance of amphipods in waters with low pH values. For example, according to the last mentioned authors, slightly acidic environments present a low ionic content that could not meet the calcium requirements needed for the crustacean exoskeleton formation. According to Kestrup and

![Figure 10 - Simple regression model. Variables: pH, and log10 amphipod density. Regression equation and value of the coefficient of determination (R², p<0.01) in the text. Samples references as in Figure 2.](image-url)
Ricciardi (2010), water conductivity influences the growth and survival of gammaridean amphipods. When exposed to conductivity or calcium levels at the lower limit of their tolerance range, crustaceans may suffer reduced growth, lower reproduction, and increased mortality by osmotic stress (Zehmer et al. 2002). This hard water requirement could explain the preference of *Hyalella* species for waters with elevated conductivity, as has been reported in other studies (Miserendino 2001, Miserendino and Pizzolón 2000, Galassi et al. 2006).

**CONCLUSIONS**

The results of this work suggest that although *Hyalella* is a ubiquitous genus, the environmental characteristics of each aquatic microhabitat are important for the establishment and persistence of its populations. *Hyalella pampeana* was registered at the three study sites, showing variable abundance throughout the year. Among the variables analyzed in the aquatic environment, pH was an important factor affecting amphipod density. Population peaks occur during spring, as in other *Hyalella* species. Males were significantly larger than females, and positive correlations between cephalothorax and total length were found in all the demographic categories (males, females and juveniles). Ovigerous females and juveniles were registered in all months, indicating continuous reproduction and recruitment; both events have more intense pulses in some periods of the year. Sex ratio of the total population was close to equality, but, when analyzed seasonally, males predominated in autumn. Fecundity was lower compared to another *Hyalella* species and it fluctuated seasonally, smaller ovigerous females with fewer eggs were found during summer months. Future studies might increase the knowledge about the relations between natural populations of *H. pampeana* with their environment, as well as their possible use as test organisms in ecotoxicological tests.

**ACKNOWLEDGMENTS**

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**AUTHOR CONTRIBUTIONS**

I.I.C designed and performed the field campaigns, collected the data and supervised the project. M.F.C. analyzed the data, performed the statistical analysis and wrote the manuscript. Both authors discussed the results and contributed to the final manuscript.

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