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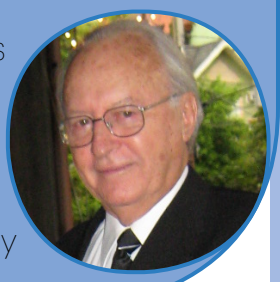

ORIGINAL ARTICLE

Population structure of two freshwater amphipods (Crustacea: Peracarida: Hyalellidae) from southern Brazil

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ABSTRACT

Two recently described amphipods species from southern Brazil, *Hyalella georginae* Streck and Castiglioni, 2017 and *Hyalella gauchensis* Streck and Castiglioni, 2017, had their population structures characterized by sex, females' ovigerous condition, cephalothorax length (mm), size-class frequency distribution, sex-ratio, reproductive period, and recruitment. The specimens were collected with a dip net from a stream source (*H. georginae*) and from a water reservoir (*H. gauchensis*) in the Palmeira das Missões municipality, state of Rio Grande do Sul, Brazil, from August 2012 to July 2013 (12 months). Both species showed a bimodal frequency distribution for total and seasonal size classes, with males larger than females. Overall, the sex ratio favored females when analyzed monthly and seasonally. Ovigerous females were recorded throughout the year, with higher frequency in spring (*H. georginae*) and summer (*H. gauchensis*), characterizing a seasonal reproduction. Both species showed continuous recruitment, with greater intensity in the spring. The population structure of these two *Hyalella* species had similar features,

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showing continuous recruitment and seasonal reproduction related to their life-history strategies, which promote adaptations to their habitat.

Key words

body size, *Hyalella*, recruitment, reproductive period, sex ratio.

Introduction

The study of population structure provides information about the ecological structure of natural populations (Hutchinson, 1981; Santos *et al.*, 1995) such as its stability, productivity, and function within the trophic chain (Cooper, 1965). Understanding the dynamics of a population is useful for providing species conservation measures. Since growth, birth, reproduction, and mortality rates can be inferred, it is possible to predict if a population is expanding or declining to extinction (Cooper, 1965; Muskó, 1992). Population structure of amphipods has been studied through the analysis of density, size and age class distribution, sex-ratio, recruitment, and growth (Guerao, 2003; Appadoo and Myers, 2004; Kevrekidis, 2004, 2005; Subida *et al.*, 2005; Castiglioni and Bond-Buckup, 2008a).

The genus *Hyalella* Smith, 1874 is distributed in the Nearctic and Neotropical biogeographic regions; it is endemic from the Americas, and its species have a restricted distribution (Bousfield, 1996). The genus includes approximately 72 species (Baldinger, 2004; Colla and César, 2015; Streck *et al.*, 2017; Bastos-Pereira *et al.*, 2018.). *Hyalella* is found in a variety of freshwater environments, attached to aquatic macrophytes, swimming in the water column, or buried in the sediment (Kruschwiyz, 1978; Wellborn, 1995; Grosso and Peralta, 1999). Depending on the species, *Hyalella* has herbivorous, carnivorous, omnivorous, or detritivore feeding habits (Cooper, 1965, Witt and Hebert, 2000; Vainola *et al.*, 2008). Individuals of this genus also act as important links in the transfer of matter and energy in their ecosystems (Moore, 1981; Casset *et al.*, 2011), being the prey of animals such as fishes and birds (Musko, 1992; Casset *et al.*, 2011).

Information concerning ecological features of freshwater crustaceans are scarce, especially those with the order Amphipoda. In the last few years, understanding of American's *Hyalella* has risen with an increasing number of taxonomic publications, indicating that there is still a large number of species unknown to science (González and Watling, 2002a, 2002b, 2003a, 2003b; Baldinger, 2004; Pereira, 2004; González *et al.*, 2006; Cardoso *et al.*, 2011; Bueno *et al.*, 2013; Rodrigues *et al.*, 2014; Cardoso *et al.*, 2014; Colla and César, 2015). In Brazil, most studies of *Hyalella* concern new species descriptions (Bastos-Pereira and Bueno, 2013; Bueno *et al.*, 2013; Rodrigues *et al.*, 2014; Cardoso *et al.*, 2014; Bueno *et al.*, 2014; Streck *et al.*, 2017), however, knowledge on their life cycles, population biology e reproductive biology are limited by the studies of Castiglioni and Bond-Buckup (2008a) (*Hyalella castroi* González, Bond-Buckup and Araujo, 2006 and *Hyalella pleoacuta* González, Bond-Buckup and Araujo, 2006), Castiglioni *et al.* (2016) (*Hyalella bonariensis* Bond-Buckup, Araujo and Santos, 2008) and Ozga and Castiglioni (2017) (*Hyalella gauchensis* Streck and Castiglioni, 2017 and *Hyalella georginae* Streck and Castiglioni, 2017). Most of the remaining studies published on population, reproductive, systematic, and taxonomic aspects of *Hyalella* species were performed with the North American species *H. azteca* (Cooper, 1965; Wellborn, 1994; Grosso and Peralta, 1999; Wellborn *et al.*, 2005).

The aim of this study was to increase the knowledge on the population ecology of Brazilian *Hyalella* species. To achieve this aim we made estimates of body size, size-class frequency distribution, sex-ratio, reproductive period, and recruitment of *Hyalella georginae* and *Hyalella gauchensis*. Species were collected from Palmeira das Missões municipality, in the northeast region of state of Rio Grande do Sul, Brazil. The species studied in this work were described recently by Streck *et al.* (2017).

Materials and Methods

Study sites. To study the population structure, individuals were collected monthly from August 2012

until July 2013 (spring: September to November; summer: December to February; fall: March to May; winter: June to August), at "Sítio Taqui" in Palmeira das Missões municipality, state of Rio Grande do Sul (27°53′56″ S, 53°18′ 50″ W). *Hyalella georginae* was sampled from a stream source, while *H. gauchensis* was sampled from a water reservoir. Palmeira das Missões' county is located in the Brazilian southern region, at the Rio Grande do Sul's North Plateau, and it is of great importance to the state's agriculture and farming. The state of Rio Grande do Sul has a climate classified as temperate Cfa according to the classification of Köppen-Geiger (Peel *et al.*, 2007) and is characterized as mild mesothermic, experiencing periods of drought during spring and summer (IBGE, 2013). The monthly sampling was taken at two sites. Site 1 (S1) consisted of a headwater shaded by small trees and surrounded by Gramineae and Pteridophytae. Although macrophytes of the genus *Salvinia* were present in the waterbody, *H. georginae* was only found and collected in the sediment. Site 2 (S2) was a shallow water reservoir (≈30 cm) with a large amount of aquatic *Salvinia*, which was used as shelter by *H. gauchensis*. At Site 2 was observed fishes and frogs that may be predators of *Hyalella* The distance between Site 1 and Site 2 is approximately 20 meters.

Sampling of Hyalella *individuals*. At each site, macrophytes and sediment were collected with a 250 µm mesh dip net during 20 min, placed into plastic bags, and taken to the laboratory. In the field, ovigerous females (with eggs or juveniles in the marsupium) and couples in precopulatory behavior were separated individually into microtubes containing 70% ethanol.

In the laboratory, macrophytes and sediment were sieved (0.177 mm mesh) and washed in running water in order to retain the amphipods.

Hyalella georginae and *H. gauchensis* were grouped into four categories: juveniles – individuals with undeveloped secondary sexual character; males – individuals with a well-developed gnathopod 2; females – individuals with oostegites and a small gnathopod 2; and ovigerous females – females carrying eggs or juveniles in the marsupium (Borowsky, 1991; Castiglioni and Bond-Buckup, 2008a).

All specimens were measured (cephalothorax length, CL in mm) on the ocular micrometer of a stereomicroscope. The CL was taken from the anterior margin of the rostrum to the posterior margin of the cephalothorax. According to Castiglioni and Bond-Buckup (2008a), the CL is correlated with total body length; hence, this measure may represent the actual body size of *Hyalella* individuals.

Data analyses. The minimum, mean, and maximum CL values of males and females were estimated to each species. The mean sizes are given with their standard deviations. The means were compared between sexes and between species using the *t* test ($\alpha = 0.05$) (Zar, 1996).

To compare the temporal variation of the population age–frequency distribution, we estimated total and seasonal frequency distributions of male and female size classes for each species population. We also analyzed seasonality in the species recruitment processes. The class ranges were determined through ¼ of the average standard deviation of the CL of sampled individuals (Markus, 1971). Normality of frequency distributions were analyzed through the Shapiro-Wilk test ($\alpha = 0.05$) (Zar, 1996).

The sex ratio (males:females) was calculated. The total, monthly, seasonal, and size-class (CL) sex proportions were expressed as the total number of males divided by the total number of females. The Chi-Square test (χ^2) with a significance level of 5% (Zar, 1996), was used to verify if the sex-ratio followed the 1:1 ratio.

The frequency of ovigerous females in relation to that of adult females of each species was estimated in order to analyze the seasonal and monthly reproductive period. The proportion of ovigerous females was compared between months and seasons using the multinomial proportions test (MANAP; $\alpha = 0.05$) (Curi and Moraes, 1981). Those *H. georginae* females with CL > 0.40 mm and *H. gauchensis* with CL > 0.35 mm were considered adult females.

For the recruitment analyses, juvenile proportions were compared between months using the multinomial proportion test (MANAP; $\alpha = 0.05$) (Curi and Moraes, 1981).

Results

A total of 2,708 specimens of *H. georginae* were collected; 1,117 males, 1,247 females (213 ovigerous females), and 344 juveniles (Tab. 1) sampled from a stream source. *Hyalella gauchensis* showed 18,953 individuals; 6,403 males, 6,706 females (1,488 ovigerous females), and 5,844 juveniles (Tab. 2) sampled from a

Table 1. Number of specimens sampled monthly for a year, monthly sex ratio and goodness of fit analysis (χ^2) of *Hyalella georginae*, Palmeira das Missões, state of Rio Grande do Sul, Brazil.

	Males	Females	Ovigerous Females	Juveniles	Total	M:F	χ^2
Aug/12	33	30	41	0	104	0.46:1	13.88*
Sep/12	201	179	21	89	490	1.01:1	0.00
Oct/12	157	128	8	0	293	1.15:1	1.51
Nov/12	172	62	68	4	306	1.32:1	5.84*
Dec/12	103	146	20	161	430	0.62:1	14.75*
Jan/13	165	234	29	54	482	0.63:1	22.44*
Feb/13	77	69	16	13	175	0.91:1	0.40
Mar/13	38	34	3	0	75	1.03:1	0.01
Apr/13	59	49	0	1	109	1.20:1	0.93
May/13	38	33	0	9	80	1.15:1	0.35
Jun/13	30	30	1	0	61	0.97:1	0.02
Jul/13	44	40	6	13	103	0.96:1	0.04
Total	1117	1034	213	344	2708	0.90:1	7.17*

Note: * indicates a significant difference in the proportion of males and females ($p < 0.05$).

Table 2. Number of specimens sampled monthly for a year, monthly sex ratio and goodness of fit analysis (χ^2) of *Hyalella gauchensis*, Palmeira das Missões, state of Rio Grande do Sul Brazil.

	Males	Females	Ovigerous Females	Juveniles	Total	M:F	χ^2
Aug/12	286	193	46	233	758	1.20:1	4.21*
Sep/12	1931	1644	37	1515	5127	1.15:1	17.30*
Oct/12	1476	1110	123	2469	5178	1.20:1	21.80*
Nov/12	314	299	27	984	1624	0.96:1	0.23
Dec/12	110	188	11	59	368	0.55:1	25.63*
Jan/13	514	242	349	27	1132	0.87:1	5.37*
Feb/13	761	844	283	111	1999	0.68:1	70.95*
Mar/13	223	110	155	110	598	0.84:1	3.61
Apr/13	342	185	226	222	975	0.83:1	6.32*
May/13	135	173	72	63	443	0.55:1	31.84*
Jun/13	197	162	47	37	443	0.94:1	0.35
Jul/13	114	68	112	14	308	0.63:1	14.82*
Total	6403	5218	1488	5844	18953	0.95:1	7.00

Note: * indicates a significant difference in the proportion of males and females ($p < 0.05$).

water reservoir. The two species were not found together at any of the collection sites.

Males and females had non-normal total distribution frequencies for both *Hyalella* species ($P < 0.05$) (*H. georginae*: males $W = 0.97$ and females $W = 0.95$; *H. gauchensis*: males $W = 0.92$ and females $W = 0.94$). Both species presented bimodal distribution. The populations were divided into groups of juveniles and adults (Figs. 1A, B) and by seasons (*H. georginae* Fig. 2 and *H. gauchensis* Fig. 3).

Hyalella georginae males had CL ranging from 0.40 mm to 1.61 mm; females' CL varied from 0.37 mm to 1.61 mm. For this species, males presented average CL (0.89 ± 0.22 mm) larger than females' (0.79 ± 0.19 mm) ($t = 11.92$, $p < 0.05$). *Hyalella gauchensis* males had CL ranging from 0.35 mm to 1.12 mm; females' CL varied from 0.35 mm to 0.97 mm. Males (0.52 ± 0.11 mm) were significantly larger than females (0.48 ± 0.09 mm) ($t = 18.88$, $p < 0.05$). *Hyalella georginae* males and females had average CL larger than *H. gauchensis* (males: $t = 87.54$, females: $t = 87.16$; $p < 0.05$).

Total sex-ratio for either *H. georginae* and *H. gauchensis* favored females (*H. georginae* 0.90:1; $\chi^2 = 7.17$; $p < 0.05$) (*H. gauchensis* 0.95: 1; $\chi^2 = 7.00$; $p < 0.05$). For *H. georginae*, females were more frequent than males in August and December 2012 and in January 2013 ($p < 0.05$) (Tab. 1). Males were more frequent than females only in November 2012 ($p < 0.05$) (Tab. 1). For *H. gauchensis*, females were more frequent than males in most months — December 2012, January, February, Abril, May, and July 2013 ($p < 0.05$) (Tab. 2). Males were more frequent than females only in August, September, and October 2012 ($p < 0.05$) (Tab. 2).

For the seasonal sex-ratio analysis, *H. georginae* females were more frequent than males only in summer ($\chi^2 = 16.58$; $p < 0.05$) (Fig. 4A). For *H. gauchensis*, females

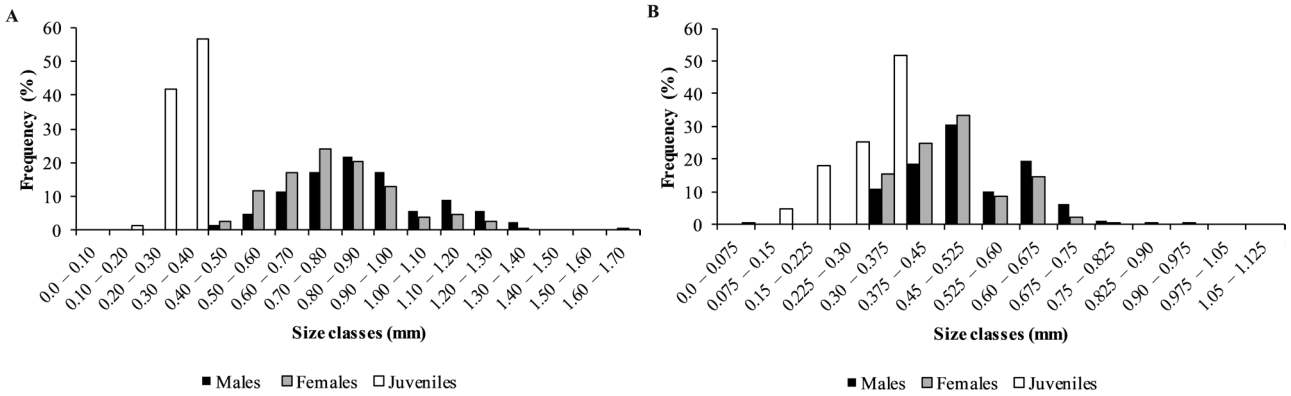

Figure 1. Size-frequency distribution of males, females and juveniles of *Hyalella georginae* (A) e *Hyalella gauchensis* (B), Palmeira das Missões, state of Rio Grande do Sul. Brazil. All sizes shown in the graph have at least one measured individual.

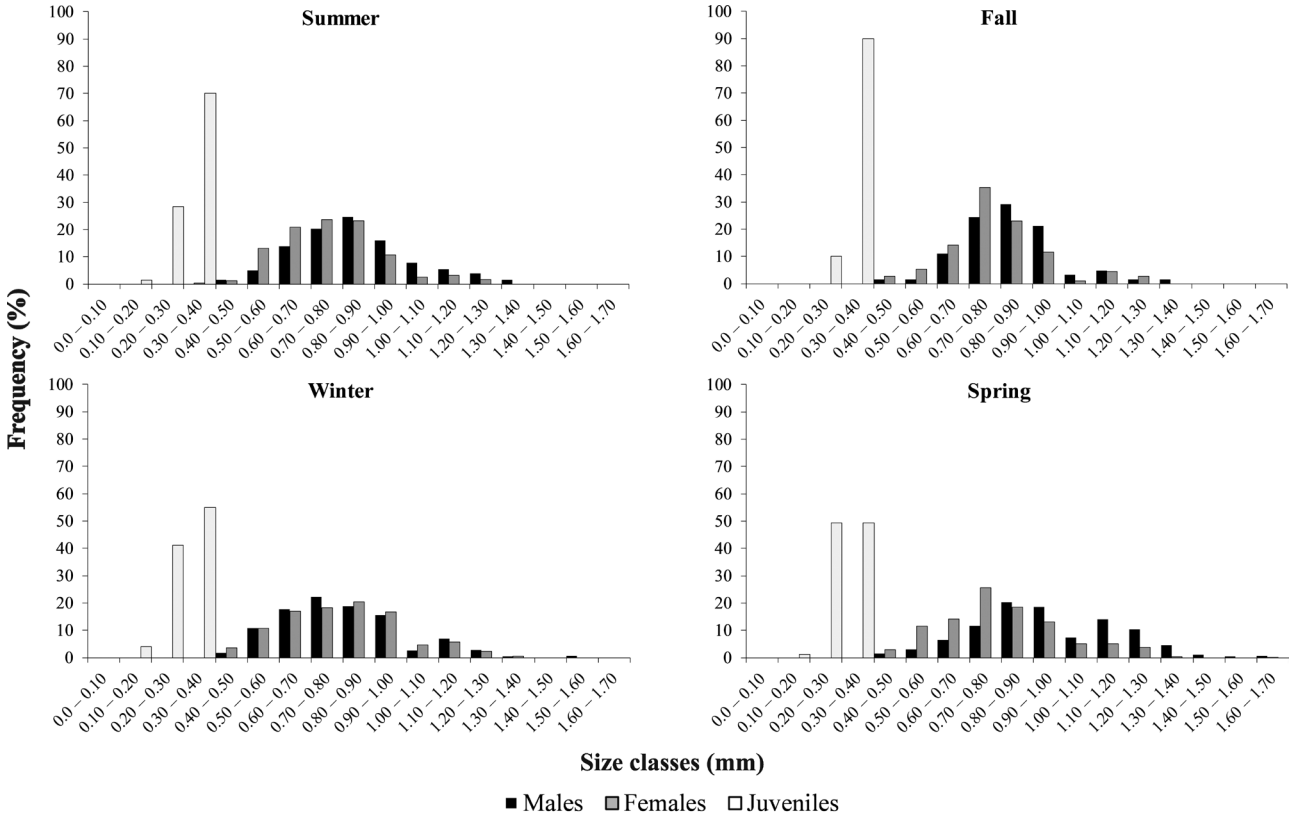

Figure 2. Seasonal size-frequency distribution of males, females and juveniles of *Hyalella georginae*, Palmeira das Missões, state of Rio Grande do Sul. Brazil. All sizes shown in the graph have at least one measured individual.

were more frequent in the fall ($\chi^2 = 23.70$; $p < 0.05$) and summer ($\chi^2 = 67.59$; $p < 0.05$). Males were more frequent in winter ($\chi^2 = 12.04$; $p < 0.05$) and spring ($\chi^2 = 5.51$; $p < 0.05$) (Fig. 4B). Considering the sex-ratio by size classes, females were more frequent in the intermediate size classes, and males in the upper size classes, in both species (Figs. 5A, B).

Ovigerous females of *H. georginae* were captured every month except April and May 2013 (Fig. 6). For *H. gauchensis*, ovigerous females were found across the entire year, and showed a higher frequency fluctuation throughout the year compared with *H. georginae* (Fig. 6). The higher reproductive intensity of *H. georginae* occurred in spring (September, 22th to December, 21th;

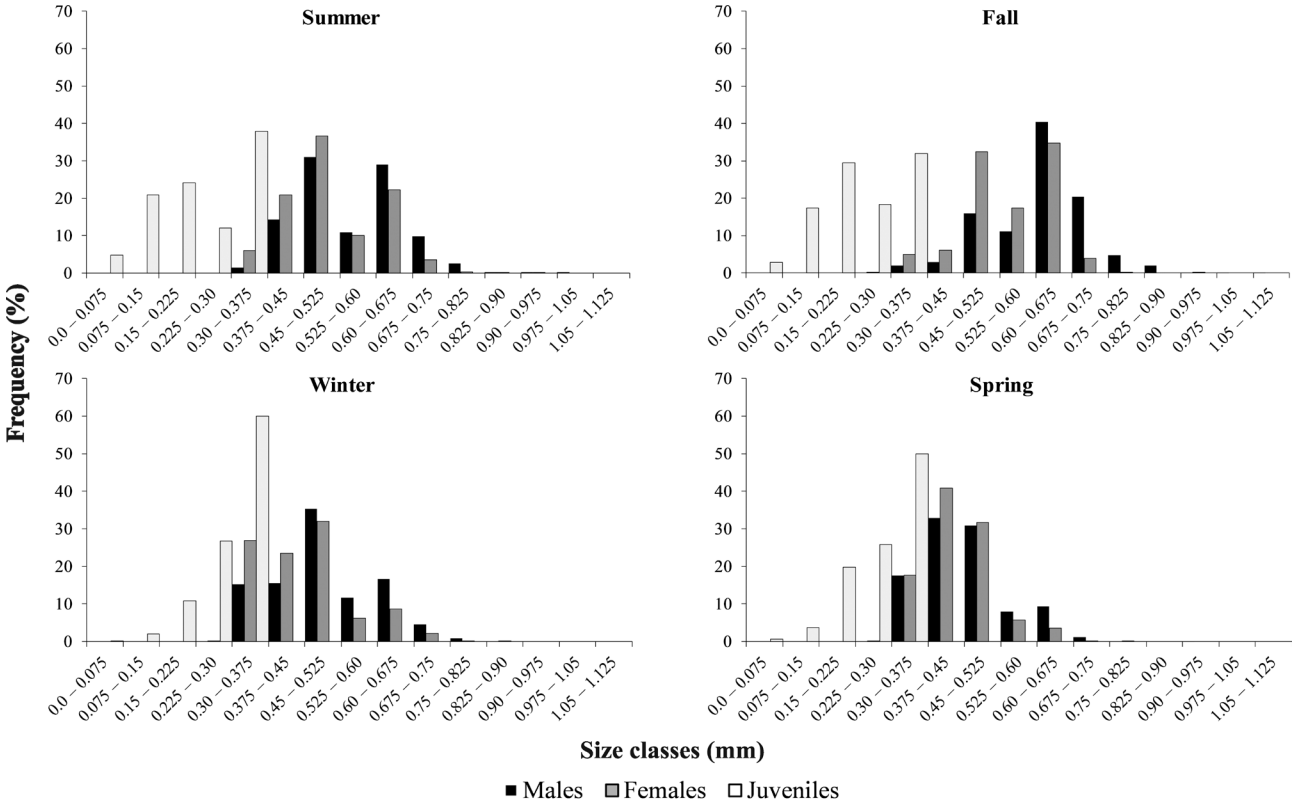

Figure 3. Seasonal size-frequency distribution of males, females and juveniles of *Hyalella gauchensis*, Palmeira das Missões, state of Rio Grande do Sul. Brazil. All sizes shown in the graph have at least one measured individual.

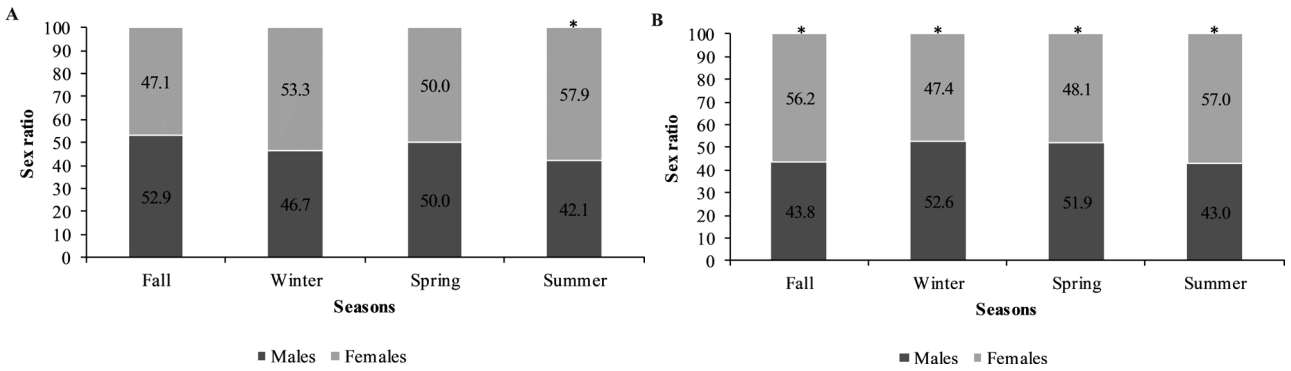

Figure 4. Seasonal sex-ratio in *Hyalella georginae* (A) and *Hyalella gauchensis* (B), Palmeira das Missões, state of Rio Grande do Sul, Brazil. Asterisks above the columns indicate significant diferences between the proportions of males and females ($p < 0.05$).

$\chi^2 = 213.82$; $p < 0.05$); for *H. gauchensis*, it occurred in the summer ($\chi^2 = 67.57$; $p < 0.05$) (Fig. 7).

Recruitment peaked in December 2012 for *H. georginae*, and in October 2012 for *H. gauchensis* (Figs. 8A, B). Recruitment was continuous during the seasons, with higher intensity in spring for both species (Fig. 9), when rainfall and minimum and maximum temperatures in the study area were above the climatological average of the state of Rio Grande do Sul (INMET, 2012).

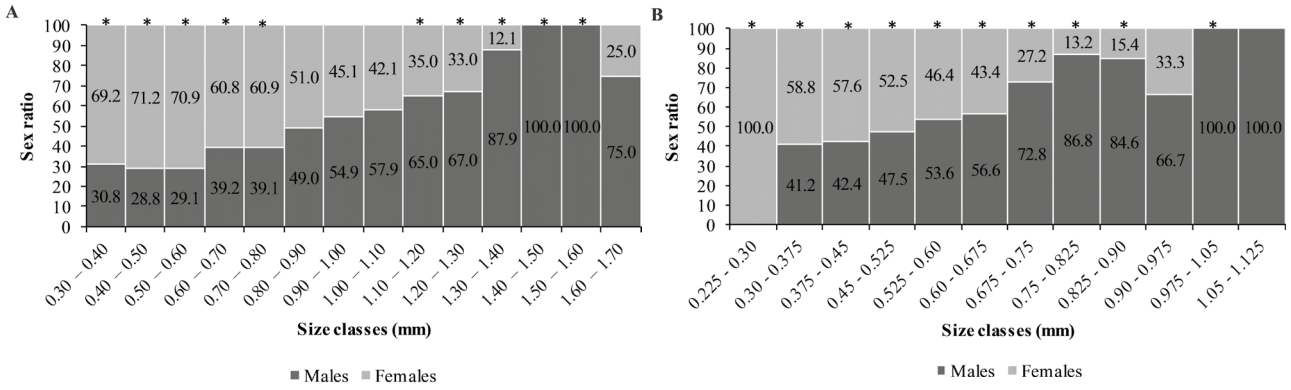

Figure 5. Sex-ratio by size classes in *Hyalella georginae* (A) and *Hyalella gauchensis* (B), Palmeira das Missões, state of Rio Grande do Sul. Brazil. Asterisks above the columns indicate significant diferences between the proportions of males and females ($p < 0.05$). All sizes shown in the graph have at least one measured individual.

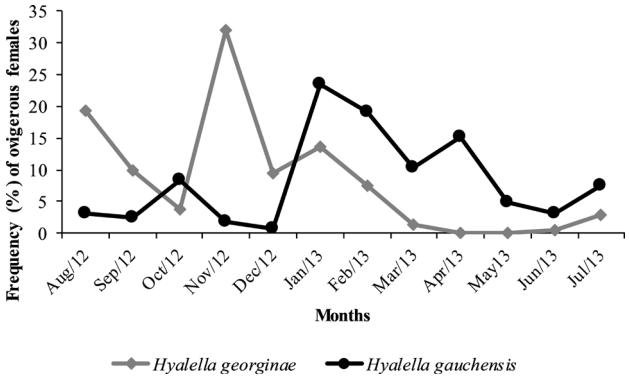

Figure 6. Frequency of ovigerou females of *Hyalella georginae* and *Hyalella gauchensis* carrying eggs or juveniles in the brood pouch along year of study, Palmeira das Missões, state of Rio Grande do Sul, Brazil.

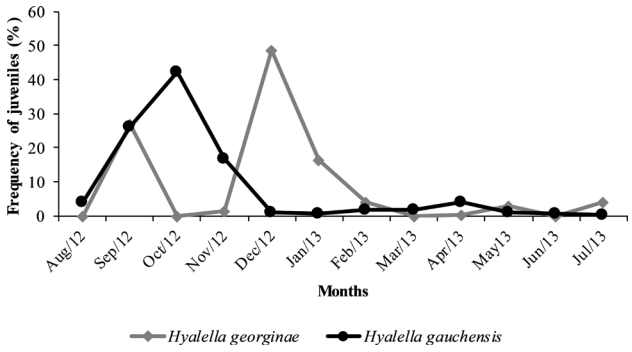

Figure 8. Relative frequency (%) of juveniles of *Hyalella georginae* and *Hyalella gauchensis* along year study, Palmeira das Missões, state of Rio Grande do Sul, Brazil.

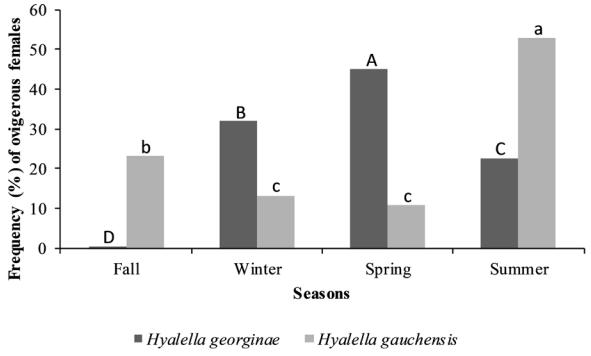

Figure 7. Frequency of ovigerous females of *Hyalella georginae* and *Hyalella gauchensis* carrying eggs or juveniles in the brood pouch along season of study, Palmeira das Missões, state of Rio Grande do Sul, Brazil. Capital letters correspond to the comparisons of the frequency of ovigerous females of *Hyalella georginae* among the seasons of the year and the small letters correspond to the comparisons of the frequency of ovigerous females of *Hyalella gauchensis* among the seasons of the year. Collumns with at least one letter in common did not differ statistically ($p < 0.05$).

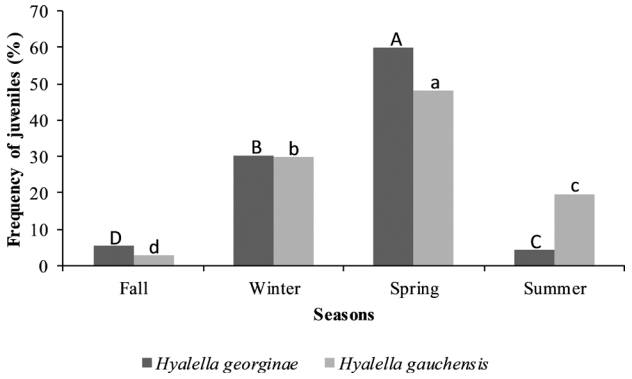

Figure 9. Relative frequency (%) of juveniles of *Hyalella georginae* and *Hyalella gauchensis* by season of the year, Palmeira das Missões, state of Rio Grande do Sul, Brazil. Capital letters correspond to the comparisons of the frequency of juveniles of *Hyalella georginae* among the seasons of the year and the small letters correspond to the comparisons of the frequency of juveniles of *Hyalella gauchensis* among the seasons of the year. Collumns with at least one letter in common did not differ statistically ($p < 0.05$).

Discussion

In order to understand the differences in body size and abundance found in this study, life-history features and habitat characteristics may be considered, as noted by Castiglioni and Bond-Buckup (2008a) for two other *Hyalella* species of the "Campos de Cima da Serra" region in state of Rio Grande do Sul, Brazil. Differences between the study sites were observed and they may be related to differences in the species' life-history strategies, such as food availability and microhabitat: *H. georginae* was captured in the headwater sediment, while *H. gauchensis* was attached to macrophytes or swimming in the water column.

According to Kruschwitz (1978), small-sized individuals reach sexual maturity faster and may reproduce earlier than larger individuals, which may reflect in a population formed by large-sized individuals. *Hyalella gauchensis* was collected at a water reservoir susceptible to environmental perturbations caused by rainfall oscillations, which were indicated by the large numbers of specimens collected in months with warmer temperatures and lower rainfall, respectively in spring and summer (INMET, 2012). According to Boschi *et al.* (2011), state of Rio Grande do Sul regularly experiences periods of drought during spring and summer. During those seasons, there were increases in food availability at the study sites, reflected by large amounts of macrophytes and algae at the shelters where *H. gauchensis* was collected. Some species may be successful in environments subject to periodic disturbance when their populations have a high reproductive output and the progeny are of small size (Townsend *et al.*, 2010). The water regime may influence the macroinvertebrate community structure, mainly those species that have their entire life cycle in the water, such as crustaceans (Wellborn *et al.*, 1996).

The characteristic bimodal frequency distribution of *H. georginae* and *H. gauchensis* populations was marked by the presence of two distinct groups, juveniles and adults. This feature may be related to the seasonal reproduction and, consequently, to the recruitment peaks of the two populations. Bimodal distributions are apparently advantageous since recruitment occurs in warmer months, when food availability is higher, increasing the survival rates (Appadoo and Myers, 2004). This pattern of population frequency distribution is common to amphipod species such as *Corophium multisetosum* Stock, 1952 (Cunha *et al.*, 2000), *Cymadusa filosa* Savigny, 1816, *Mallacoota schellenbergi* Ledoyer, 1984 (Appadoo and Myers, 2004), and *Gammarus chevreuxi* Sexton, 1913 (Subida *et al.*, 2005).

The seasonal frequency distribution of *H. georginae* and *H. gauchensis* was bimodal for most seasons, and probably reflects the seasonal reproduction of both species. Castiglioni and Bond-Buckup (2008a) studying the ecological characteristics of two sympatric species of *Hyalella* also found bimodal size-class frequency distribution for *H. pleocuta*. The authors concluded that either the reproduction of this species is probably more intense during a few months or there is differential mortality throughout the year.

Hyalella georginae males and females had average CL larger than *H. gauchensis* and *H. georginae* and *H. gauchensis* males were significantly larger than females. The body size is considered one of the most significant ecological features and is crucial for the ecological success of the genus *Hyalella* (Wellborn, 2002). Male and female body sizes may vary according to latitude and environmental conditions (Panov and Macqueen, 1998; Xinqing *et al.*, 2013) or to ecological interactions such as completion and predation (Wellborn, 2002). The larger size of males compared with females has also been reported as a sexual dimorphism in other species of *Hyalella*, such as *H. pleocuta*, *H. castroi* (Castiglioni and Bond-Buckup, 2008a), and *H. azteca* Saussure, 1858 (Geisler, 1944; Wellborn *et al.*, 2005). Males and females often have similar growth until maturity is reached, then males grow more (Low, 1978). Females grow less due to egg production and incubation (Hartnoll, 1982) while males continue to grow during the mating period, reaching larger body sizes (Wen, 1992). During incubation, females do not molt, which also hampers them from growing at the same rate as males (Cardoso and Veloso, 1996). Besides, during the precopulatory period, males carry the females in their thorax until ovulation and fertilization (Borowsky, 1991). Smaller females are easily carried by males (Adams and Greenwood, 1983; Adams *et al.*, 1985; Castiglioni and Bond-Buckup, 2008b), and larger males outcompete smaller ones during the mating process (Ward, 1983).

Total sex-ratio for either *H. georginae* and *H. gauchensis* favored females (Tab. 1). Amphipods may have populations with a sex-ratio of 1:1, or it can vary depending on the season: males may be more abundant on colder months and females in warmer months (Moore,

1981). As it is known for amphipod populations, the sex-ratio fluctuates seasonally, and females are often more abundant than males (Cardoso and Veloso, 1996; Appadoo and Myers, 2004; Kevrekidis, 2004). The sex-ratio favoring females seen in this study probably reflects the males' behavior of choosing and guarding females, making them more susceptible to predators (Moore, 1981; Kevrekidis, 2005). A higher proportion of females was also found for *H. pleocuta*, *H. castroi* (Castiglioni and Bond-Buckup, 2008a), and *H. azteca* (Strong, 1972). The sex-ratio of *H. georginae* and *H. gauchensis* were different depending on the size class considered. It favored females in intermediary classes and males in the upper size classes, which characterizes an anomalous sex ratio pattern. Similar results were found by Wenner (1972) and Castiglioni and Bond-Buckup (2008a). The predominance of males in upper size classes is presumably influenced by the females' prolonged parental behavior, in which they carry the offspring attached to their bodies (Borowski, 1991; Thiel, 2003; Castiglioni and Bond-Buckup, 2007). Due to this behavior, females direct their energetic budget towards offspring care instead of molting; therefore, the molt is delayed, limiting the females' body size (Thiel, 2003).

For the seasonal sex-ratio analysis, *H. georginae* females were more frequent than males only in summer. For *H. gauchensis*, females were more frequent in the autumn and summer. The seasonal reproduction observed in both species of this study may be related to environmental factors of the study sites such as temperature and rainfall. Temperature is known to be an important factor influencing the life-history of aquatic invertebrates (Panov and Macqueen, 1998). Cooper (1965) and Kruschwitz (1978) reported that for *H. azteca*, only adult individuals survive winter, since reproduction ceases or is considerably lower then. Differently, in most studies on amphipods, reproduction is considered a continuous event, *e.g.*, *Gammarus troglophilus* Hubricht and Mackin de 1940 (Jenio, 1980) and *H. azteca* (Alcocer *et al.*, 2002). Besides, Castiglioni and Bond-Buckup (2008a) observed a continuous reproduction for *H. pleoacuta* and *H. castroi*, but more intense in winter and fall, respectively. Food availability (Xinqing *et al.*, 2013) and quality (Dutra *et al.*, 2011) influenced the reproductive capacity of individuals, causing abundance fluctuations. In those months that the temperature was higher, there was a decrease in water volume and an increase in macrophyte abundance (personal observation), providing a suitable environment for the species reproduction.

Food availability for adults and for the development of the complete life cycle may be the most important factor influencing reproduction (Sastry, 1983). In the study by Castiglioni and Bond-Buckup (2009) with *H. castroi* and *H. pleocuta* from the "Campos de Cima da Serra", fluctuations in reproductive intensities were related to macrophyte cover. Macrophytes are food and shelter for ovigerous females and juveniles and contribute for the species' reproductive success.

The continuous recruitment, with peaks in some months, was also observed in *H. pleocuta* and *H. castroi* (Castiglioni and Bond-Buckup, 2008a). The recruitment peaked in December 2012 for *H. georginae*, and in October 2012 for *H. gauchensis*. The hypothesis raised by the authors is that reproductive and recruitment peaks can occur in the same season. Considering the embryonic period and parental care duration, females are capable of becoming ovigerous and release the offspring within the same season. The embryonic development, from ovulation to hatching, may last 10–25 days. *Hyalella pleocuta* and *H. castroi* have an embryonic period of approximately 12 days (Castiglioni and Bond-Buckup, 2007), and in *H. azteca* hatching can occur in 9.3–21 days (Geisler, 1944; Cooper, 1965). The parental care in *Hyalella* is known as the period where juveniles remain in the female's marsupium. The parental care may last roughly six days, as in *H. pleocuta* and *H. castroi* (Castiglioni and Bond-Buckup, 2007), or three days as in *H. azteca* (Geisler, 1944).

The population structure of *H. georginae* and *H. gauchensis* had similar features. Frequency distributions were similar, males were larger than females, the sex-ratio favored females, and both species showed seasonal reproduction and continuous recruitment. Those similarities and differences may be related to both species' life-history strategies, which promote adaptations to their habitat variations. The information gathered in this study about *H. georginae* and *H. gauchensis* populations may help to understand the ecological stability of these species at the studied site. Also, the results may lead to a better understanding of the species biology in the future.

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