Population Estimates of Hyalella curvispina Shoemaker (Amphipoda) in Aquatic Vegetation of Northeastern Argentinian Ponds.

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ABSTRACT: Population Estimates of Hyalella curvispina Shoemaker (Amphipoda) in Aquatic Vegetation of Northeastern Argentinian Ponds. Quantitative comparisons were made of the accuracy of different sampling nets ($962\ cm^2$, $707\ cm^2$ and $380\ cm^2$) and two techniques for extracting amphipods from plants. To test the spatial distribution of Hyalella curvispina, eight sites with different environmental conditions were sampled between October and December 1999. Temporal variations in abundance and biomass were quantified from January to December 1999 in one of these sites covered by Pistia stratiotes. Because smallest number of replicate samples for a given level of precision was required using a net of $962\ cm^2$ and Berlese funnels, three replicate samples were taken each month with this sampling size and extraction techniques. High densities populations were found in permanent ponds with electrical conductivity between 65 and 260 $\mu S.cm^{-1}$ and dominance of Pistia stratiotes. Multiple regression analysis revealed that population density in different pond of the Northeastern Argentinean was significantly related to conductivity and this variable explained 36 % of the variability in abundance. Mean monthly abundance of H. curvispina varied between 338 and 3200 per m^2 and its biomass between 0.20 and 1.95 $g.m^{-2}$. The population peak of H. curvispina occurred during October, in which there was no increase in plant biomass.
Key-words: Hyalella curvispina, aquatic macrophytes, subtropical wetlands.

RESUMO: Estimativas da população de Hyalella curvispina Shoemaker (Amphipoda) na vegetação aquática de lagoas do Nordeste da Argentina. Comparações quantitativas foram feitas com a finalidade de verificar a acuracidade de diferentes redes de amostragem ($962\ cm^2$, $707\ cm^2$ e $380\ cm^2$) e de duas técnicas para extração dos anfípodas das plantas. Para testar a distribuição espacial de Hyalella curvispina, oito locais com diferentes condições ambientais foram amostrados entre Outubro e Dezembro de 1999. Variações temporais na abundância e biomassa foram quantificadas entre Janeiro e Dezembro de 1999 em um desses locais com cobertura de Pistia stratiotes. Por causa do menor número de réplicas foi necessário usar uma rede de $962\ cm2$ e funis de Berlese e três amostras foram tomadas em cada mês com este tamanho de amostragem e técnicas de extração. Elevadas densidades da população foram encontradas em lagoas permanentes com condutividade elétrica entre 65 e 200 $uS.cm^{-1}$ e dominância de Pistia stratiotes. A análise de regressão múltipla revelou que a densidade populacional em diferentes lagoas do Nordeste da Argentina era significativamente relacionada à condutividade e esta variável explicava 36 % da variabilidade da abundância. A abundância média mensal de H curvispina variou entre 338 e 3200 por m^2 e sua biomassa entre 0,20 e 1,96 $g.m^{-2}$. O pico da população de H. curvispina ocorreu durante o mês de Outubro, durante o qual não houve aumento na biomassa da planta.
Palavras-chave: Hyalella curvispina, macrófitas aquáticas, áreas úmidas subtropicais.

Introduction

Species of the genus Hyalella integrate different aquatic communities such as benthos (Bechara & Andreani, 1986), pleuston or perizoo (Poi de Neiff, 2003). Some of these have been used as bioindicator in toxicity tests to evaluate sediment and water pollution (Di Marzio et al., 1994; Di Marzio et al., 1999; Mozeto et al., 2001; Rovedatti et al., 2002).

Hyalella curvispina Shoemaker (1942) has a wide distribution in Argentina (Grosso & Peralta, 1999; Lopretto & Tell, 1995). Its population dynamics is only known for streams of the province of Buenos Aires where its abundance was related to phytobenthos (Casset et al., 2001) or submerged plant biomass (Giorgi et al. 2005). This species acts as herbivorous on the phytobenthos (Giorgi & Tiraboschi, 1999)

and also process the fine particulate organic matter deposited among plants so that it would belong to the trophic category of collectors (Poi de Neiff & Carignan, 1997).

Although epiphytic invertebrates are thought to be ecologically important, few studies have been made to improve sampling techniques or lower costs. Invertebrate populations associated with vegetation have been quantified using the number of individuals per unit area (Downing & Cyr, 1985; Poi de Neiff & Neiff, 1977), but in most studies the number of replicates is low or non-existent due to the time required to separate organisms from plants and sediments (Cyr & Downing, 1988). Even if biomass of aquatic plants is positively correlated with abundance of invertebrates associated to vegetation (Cyr & Downing, 1988), very few estimations including vegetation dry weight as a reference unit have been carried out until now (Blanco-Belmonte et al., 1998; Poi de Neiff & Carignan, 1997).

The objectives of this study were: 1) to determine the efficiency of sampling nets of different sizes and different techniques to separate amphipods from aquatic plants, 2) to compare the abundance of the population of this species in eight water bodies with different environmental conditions 3) to estimate the temporal variability of Hyalella curvispina abundance and biomass in a permanent pond during an annual cycle.

We tested the hypothesis that 1- biomass of aquatic macrophytes is determinant factor on the temporal changes in abundance of H. curvispina population and 2- the physical and chemical conditions of different water bodies influence population density.

Material and methods

This study was conducted in eight natural ponds located on both banks of the Paraná Rivers (Fig. 1) near Corrientes city (Argentina). Temporary and permanent ponds are densely vegetated by different species of free floating plants and rooted emergent plants and have different depth, electrical conductivity and dissolved oxygen content (Bonetto et al., 1978; Poi de Neiff & Neiff, 1977).

Figure 1: Location of the sampling sites in Chaco and Corrientes provinces.

At one of this site, comparisons of the precision of samplers of different sizes and the accuracy of two techniques for extracting Hyalella curvispina were made. Replicate samples (n=3) were collected in October 1998 (Site 4) by enclosing Pistia stratiotes L. stands with a 225 mm mesh net with different diameters (962 cm², 707 cm² and 380 cm²) with a handle from variable length. Half of the samples were carried out to the laboratory with no fixing and placed in large Berlese funnels (Southwood, 1995) during 72 h to extract animals from plant material. The rest of the samples was placed in plastic bags and preserved in 5% formalin. Plants were thoroughly washed to detach animals and detritus. Suspensions obtained were filtered through 500 mm and 250 mm sieves. The material retained in each sieve was placed in clear bottom trays and individuals were separated and counted under a stereoscopic microscope. The same procedure was used with the material collected in Berlese funnels. The equation proposed by Downing & Cry (1985):
$$\tilde{n} = 67{,}76 \cdot c^{-0,360} \cdot A^{-0,435} \cdot p^{-2},$$ where ñ is the number of samples, x is the population mean, A is the sampler area and p is the error level adopted (p= 0.2 = 20%), was used to determine the number of replicates needed for different sampler sizes and extraction techniques. The Wilcoxon and Friedman test were used to test the significance of differences among ñ values obtained with each extraction technique (Steel & Torrie, 1985).

Between October and December 1999 eight sites were sampled using a net of 962 cm² and Berlese funnel to test the spatial distribution of Hyalella curvispina.

All sites were sampled in the shortest time possible (during spring) to minimize seasonal variability in population abundance.

Temporal variations in abundance and biomass were quantified from January to December 1999 in a pond covered by Pistia stratiotes (Site 1, 27° 30´S; 58°57´W). Three replicate samples were taken each month with the same net and extraction techniques. After separating the animals, plants were oven dried at 105°C for 72 h to obtain a constant dry weight with a balance Mettler H54 of 0.01 mg of precision. The abundance of populations was referred to the number of individuals per m² covered by Pistia stratiotes and per 1000 g of plant biomass. Specimens of Hyalella curvispina were oven dried at 60°C to obtain biomass $(g.m^{-2})$.

Temperature, electric conductivity and dissolved oxygen were measured with a conductimeter and a polarographic oxygen meter at each collecting date and site. The data of pH were registered in the field with a digital pH meter.

To test the relationship between environmental factors (temperature, electric conductivity and dissolved oxygen), sites, substrata, and depth with population abundance at the different sampling sites it was used simple regression and multiple regression analysis. If necessary the variables were log transformed.

Results

Smallest number of replicate samples for a given level of precision was required using a net of 962 cm² and Berlese funnels (Fig. 2). The variation coefficient using
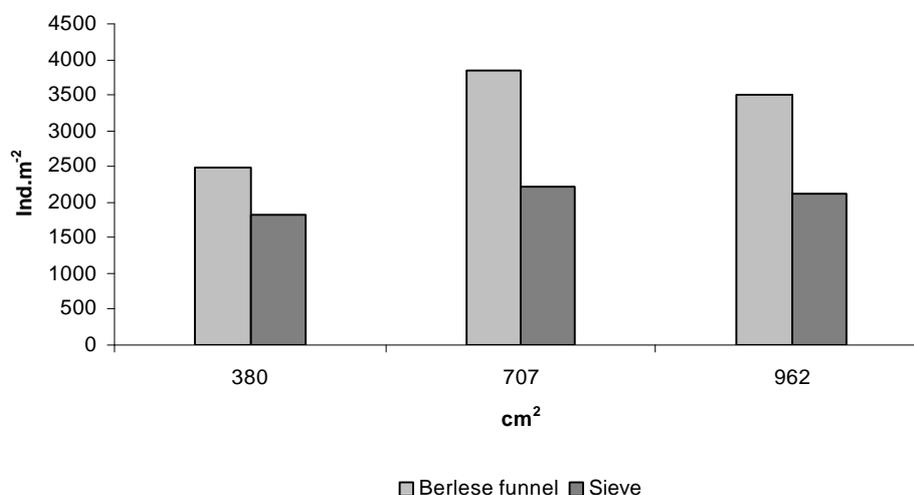
Figure 2: Mean abundance of Hyalella curvispina population collected with different net sizes and extraction techniques from plant material. SD is less 4 than 18%.

Berlese funnels was lower than 4.07 %. If we desire an error of 20 %, seven, five and four samples were needed for 380, 707 and 962 cm² nets respectively. Using the separation by sieves the variation coefficient ranged from 11 to 18 % and the number of samples estimated according to the Downing & Cyr (1985) equation varied between 8 and 5, depending on net size. Moreover, the efficiency of the first technique was much higher than that of the second one if we consider the time that the researcher took in each process. In the first case, 72 h after placing the plants in the funnels the individuals for the recount were obtained. In the second case, the separation of individuals from detritus retained in each sieve took a considerable time (up to two days per sample), depending on the operator training.

Significant differences (0.0020, p‹=0.05)

were found when comparing the number of individuals per m² obtained with the two extracting methods and the SD fluctuated between 4 and 18 %.

Spatial variations of H. curvispina

The studied ponds were temporary (depth less than 60 cm) or permanent with depth until to 130 cm (Tab. I). Sites 3 and 5 exhibited a marked increase in conductivity and temperature during November, and lower oxygen concentration than the other sites. Azolla caroliniana Willd covers the water surface of both sites (Tab. I). Sites 1, 2 and 8 had moderate conductivity and were dominated by Pistia stratiotes. Site 4 (Tab. I) was colonized by floating (P. stratiotes) and rooted macrophytes (Ludwigia sp and Eichhornia azurea (Sw.) Kunth).

In these sites the abundance of H.

Table I: Water conditions in the sampling sites: T: temperature (°C), DO: dissolved oxygen (mg.L⁻¹), EC: electrical conductivity (mS.cm⁻¹) and Depth (cm).

Sites	Aquatic plants	Geographic coordinates	Date	T	DO	EC	pH	Depth
1	Pistia stratiotes L.	27°30'S; 58°57'W	October	25.5	2.7	238	6.6	130
			November	26.0	6.5	260	7.2	130
2	Pistia stratiotes L.	27°20′S; 57°30′W	November	22.5	6.0	90	7.0	90
3	Azolla caroliniana Willd	27°27′S; 58°55′W	October	20.0	5.1	205	7.2	60
			November	31.5	3.2	750	7.7	67
4	Pistia stratiotes L., Ludwigia sp. and Eichhornia azurea (Sw.) Kunth	27°30'S; 58°45'W	October	21.0	5.2	65	6.6	90
5	Azolla caroliniana Willd	27°27′S; 58°55′W	October	19.5	2.7	200	7.0	20
			November	30.1	3.2	400	7.2	20
6	Salvinia biloba Raddi	27°23′S; 58°35′W	October	18.9	7.8	94	7.0	60
			November	23.9	6.9	77	7.0	60
7	Salvinia biloba Raddi	27°29′S; 58°41′W	October	18.9	7.8	94	7.1	90
			November	23.9	6.9	77	7.1	90
8	Pistia stratiotes L.	27°27′S; 58°57′W	October	19.5	5.4	170	6.8	90
			December	20.7	6.3	177	7.0	120

curvispina varied between 125 and 6587 ind. m⁻² during spring (Fig. 3). Even given the significant relationship of sites, substrata, depth and electric conductivity with abundance of H. curvispina we used stepwise multiple regression. This analysis revealed that population density was significantly related to conductivity and this variable explained 36 % (adjusted R^2) of the variability in abundance. Others variables

(depth and substrata) were selected but no significant statistical relationship (p ‹ 0.05).

Temporal variations in abundance and biomass of H. curvispina at site 1

At Site 1, the air temperature was high and monthly mean temperatures varied between 8.4-19.3 °C in winter and 19.8-31.2 °C in summer (Tab. II). Photoperiod varied between 11.2 and 14.7 h and effective
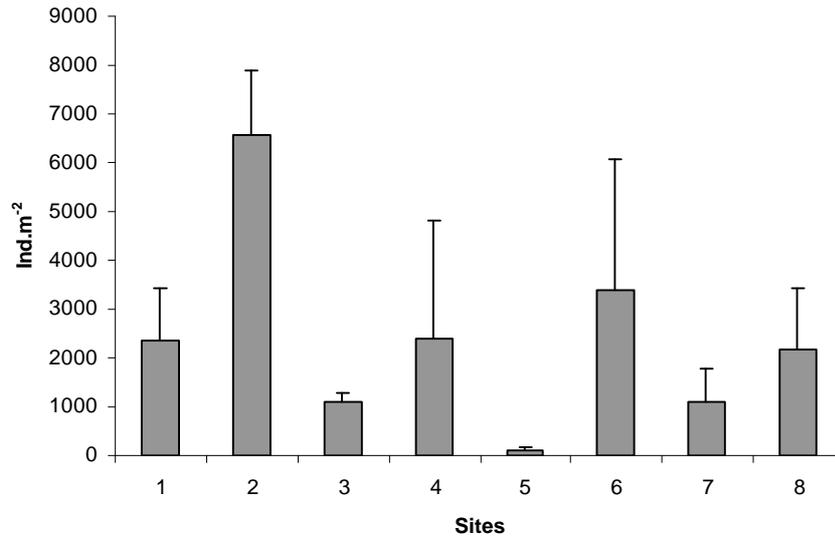
Figure 3: Mean abundance of *Hyalella curvispina* at different sites: 1- Barranquera, 2- Los Gitanos, 3- Cora, 4- Pampín, 5- Antequera, 6- El Tajamar, 7- Brava and 8- La Cava.

heliophany between 4.6 and 9.6 h, which indicates that the studied pond receives high sun radiation.

During the sampling period the water was acid or neutral, temperature fluctuated between 12.5°C and 27.9°C and electric conductivity varied between 154 mS.cm^{-1} and 308 mS.cm^{-1} (Tab. II). Oxygen concentration and the percentage of saturation were very variable during the study period (Tab. II).

Table II: Environmental conditions at site 1.

Variables	Month									
	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Oct.	Dec.
Air maximun temperature (°C)	31.2	31.5	28.5	25.7	23.2	21.9	19.3	20.1	27.6	30.8
Air minimun temperature (°C)	19.8	20.4	19.4	16.8	19.8	13.5	8.4	11.3	20.6	19.4
Air relative humidy	77	75	83	83	82	83	82	70	67	68
Photoperiod (hs.)	14.4	13.8	13.0	12.2	11.6	11.2	11.4	12.0	14.2	14.7
Effective heliophany (hs.)	9.4	9.5	6.8	5.4	6.6	4.9	4.6	6.9	9.6	8.0
Water temperature (°C)	26.5	26.5	25.5	23.5	22.3	19.5	12.5	18.0	25.5	27.9
Conductivity (μS. cm^{-1})	270	193	154	197	203	188	169	210	238	308
pH	6.1	6.9	6.8	7.0	5.9	5.5	6.5	6.8	6.6	7.1
Oxygen (mg.l^{-1})	4.5	4.1	3.5	4.6	4.6	4.4	6.7	5.6	2.7	5.2
% of saturation O$_2$	57	52	43	55	54	49	65	61	33	67

Mean abundance of *H. curvispina* (n=3) in the pond vegetated by *Pistia stratiotes* varied between 338 and 3200 ind.m^{-2}, while when considering the number of individuals per plant biomass values oscillated between 1641 and 18785 per 1000 g (Fig. 4). Plant biomass varied between 92.33 and 218.33 g.m^{-2} and biomass of *H. curvispina* between 0.20 and 1.95 g.m^{-2} (Fig. 5). During October and December, there was an increase in the population and numerous females with eggs and broods were observed in the samples. During September and November, the samples transported alive for the separation in Berlese funnels were damaged, and as consequence both months were not considered.

In July there were differences between estimations of the number of individuals per unit area and per plant biomass due to a lower number of plants per m². This fact could be due to a deficient net collection or to the wind action that can frequently produce dispersion of plants in free floating formations.

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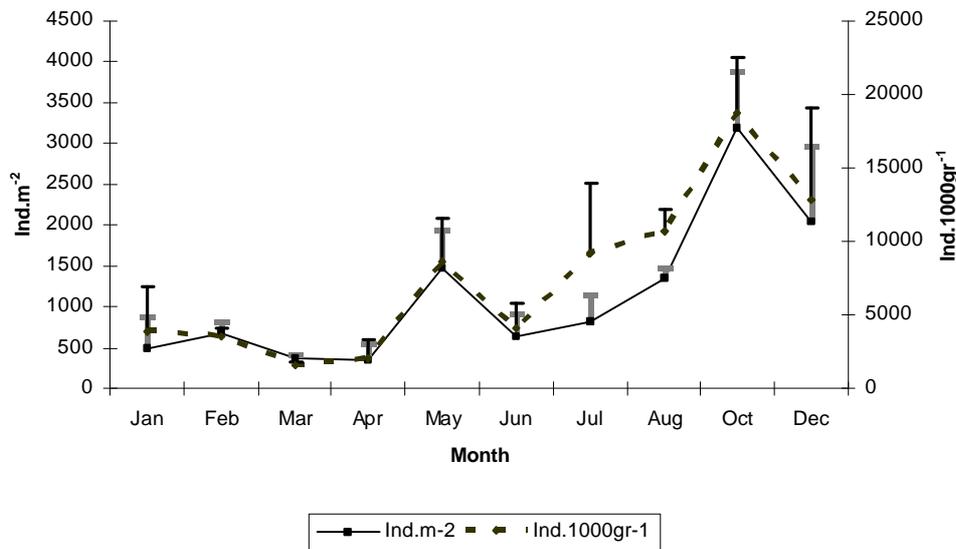
Figure 3: Monthly variations of Hyalella curvispina abundance in number of individuals per area and per plant biomass (dry weight) between January and December 1999.
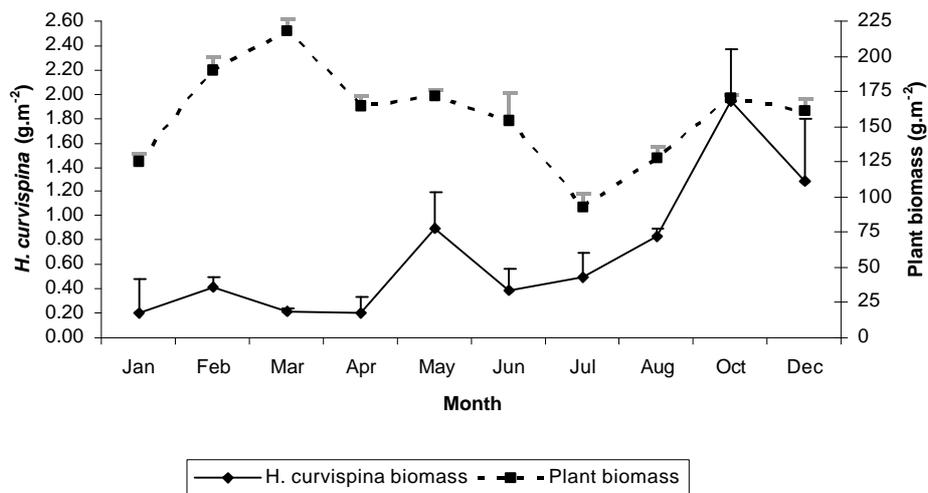
Figure 5: Monthly variations of Hyalella curvispina and Pistia stratiotes biomass between January and December 1999. Vertical bars represent standard deviation.

Discussion

Our observations agree with those of Downing & Cyr (1985), who found that between 3 and 9 replicate samples are necessary for an area of 991 cm² when the number of ind.m^{-2} varies between 10000 and 1000, using different populations of invertebrates associated to vegetation for the calculation. The extraction method by Berlese funnels markedly decreases separation time and it is effective in the case of the population studied since amphipods were not found in the remaining dry plant material after exposure to light and heat. Other invertebrates, as for example, Diptera larvae, moult during the exposure time and their populations are underestimated by this method.

High densies populations were found in permanent ponds with electrical conductivity between 65 and 260 mS.cm^{-1} and dominance of Pistia stratiotes. Although depth, substrate and conductivity were

significantly related to the abundance of H. curvispina, the two first variables were selected with p value ›0.05 in the stepwise multiple regression analysis. These results support the hypothesis that the abundance of H curvispina in different sites of Northeastern Argentinian was positively related to physical and chemical characteristics of the water, especially to conductivity. The low predictive value indicates that other variables could be affecting the abundance of this population. The preference of H. curvispina by waters with high conductivity was indicated by Miserendino & Pizzolón (2000) for Patagonian stream.

Our results indicate that population peak of H. curvispina occurred during October in the site 1, period in which there was no increase in plant biomass. The strong relation between both units of reference used for measured abundance (g. per square meter and per plant biomass), sustain this result. Other authors (Giorgi et al., 2005) found a positive correlation between submerged macrophyte biomass and the abundance of H. curvispina.

The higher density of H. curvispina populations in a stream populated with submerged macrophytes was also registered between October and December (Casset et al., 2001). Spring peaks were also observed in Hyalella azteca Saussure (Wen, 1992) which population increases with temperatures between 20 to 25°C (Wetzel, 1983). In our study, population peaks of October occurred with a monthly mean air temperature between 20.6 and 27.6 $^{\circ}$C and a high photoperiod (between 14.2 and 14.7 hours of light). The combination of a high air temperature and a long photoperiod shortens the periods between moults in experiments carried out with H. azteca (Kruschwitz, 1978) and H. pampeana Cavalieri (Lopretto, 1983) in the laboratory.

Acknowledgments

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