



## Is the biomass of water hyacinth lost through herbivory in native areas important?

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### ABSTRACT

The lamina area damaged and biomass per leaves removed by invertebrate herbivores were measured across seasons on water hyacinth, *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae). The amount of the leaf biomass per meter square lost through herbivory was also assessed in different sampling dates in the plant population. Ten leaves of water hyacinth were sampled in each of 18 site-habitat-date combinations. Sampling dates were chosen to follow the plant phenology. The lamina area damaged (surface abrasions and holes) was measured with the visual estimation method; biomass removed by herbivores (surface abrasions and holes) was calculated indirectly from the damaged lamina area. Significant differences in total damaged area and removed biomass per lamina were found between sampling dates at each site, with highest values in March (end of growth period). Total damaged area per lamina (surface abrasions + holes) varied between 11% in March and 6% in July (decay period). Total removed biomass (surface abrasions + holes) varied between 27% in March and 13% in July. Significant differences in biomass removed by herbivory were found between sampling dates at each site. Biomass of lamina removed by herbivores per m<sup>-2</sup> varied between 26 and 13% in different seasons. The herbivore damage of discrete samples and the indirect method to calculate the biomass removed is useful in sites with aquatic free floating plants, where experimental exclusion of insects may be difficult to carry out.

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### 1. Introduction

Consumption by herbivores of living macrophytes may reduce 30% of the plant abundance (Lodge et al., 1998), but insects reduce a mean of 10% of biomass and the damage by snail are practically lacking in these macrophytes. The aquatic herbivores consume more per unit of biomass compared with terrestrial systems (Cyr and Pace, 1993). However, Kalf (2002) points out that the higher structural carbon content of the macrophytes and their lower growth rate in nature appears to be responsible for the lower proportion consumed by herbivores in comparison with phytoplankton or periphyton. Other studies suggest that plant death contributes substantially to food webs in aquatic habitats (Brock and Van der Velde, 1996; Wetzel, 1983), and most below-water macrophyte biomass is consumed as detritus, but herbivory on above-waterline parts of wetland plants is probably more important than previously expected (Batzer et al., 1999).

Quantification of the amount of herbivore damage to living macrophytes in tropical waters has been rare (Newman, 1991), as have studies that determine the effect of herbivory on aboveground primary production (Medeiros dos Santos and Esteves, 2002).

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms, constitutes the principal species of floating meadows in the large river

floodplains of South America (Blanco Belmonte et al., 1998; Carignan and Neiff, 1992), mainly in the Paraná floodplain downstream from the confluence with the Paraguay River (Neiff et al., 2001). In this area, *E. crassipes* biomass increases from August to March, declining again to about 50% of the annual maximum during winter (Neiff and Poi de Neiff, 1984), with growth limited mainly by the inorganic nitrogen content of the water (Carignan et al., 1994).

In the Paraná floodplain lakes, invertebrates are the most important herbivorous consumers of *E. crassipes* floating meadows (Poi de Neiff et al., 1977; Poi de Neiff and Casco, 2003) as the lakes do not contain herbivorous fishes, for which macrophytes are an important dietary component (Neiff et al., 2009). Leaves of *E. crassipes* are mainly attacked by the snail *Omalonyx unguis* D'Orbigny, adults of two weevils (*Neochetina bruchi* Hustache and *Neochetina eichhorniae* Warner), and adults and nymphs of the semi-aquatic grasshopper *Cornops aquaticum* Bruner (Poi de Neiff et al., 1977; Poi de Neiff and Casco, 2003; Casco and Poi de Neiff, 1998; Franceschini, 2008; Franceschini et al., 2008). These invertebrate herbivores are well studied because they have been used as biological control agents of water hyacinth or their release in non-native areas is still being evaluated (Center et al., 2002; Cordo, 1999; Julien, 2001; Julien et al., 2001; Perkins, 1974; Poi de Neiff et al., 1977).

Enclosure and exclosure experiments have been successfully used to assess herbivore damage (Rogers and Siemann, 2004; Schmitz, 2004). These experiments cannot easily be reproduced in

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the *E. crassipes* floating meadows because the insects may lay eggs within petioles, have endophagous larva, or take refuge among the ligules near the base of the plants. In addition, because the lakes are connected with the Paraná River, the floating meadows move within the lakes and the incoming flood pulses threatened to submerge the enclosures within 24 h (Carignan et al., 1994). In these sites, herbivore damage of leaves, estimated by means of discrete samples across seasons, gives a valuable measure of herbivory, and such estimates are easy to carry out.

Despite the wide distribution of *E. crassipes* in South America (Gopal, 1987) and its high biomass in the Paraná River floodplain (Lallana, 1980; Neiff, 1990; Neiff and Poi de Neiff, 1984), the biomass consumed by invertebrate herbivores across seasons and the impact of this damage on the biomass of the plant population have not yet been evaluated. Green leaves biomass, which constitute the food resource for invertebrate herbivores (Neiff and Poi de Neiff, 1984; Neiff et al., 2008), and abundance of herbivorous populations (Franceschini, 2008; Franceschini et al., 2008; Casco and Poi de Neiff, 1998) varied according to the seasonality in native *E. crassipes* floating meadows. The magnitude of the damage and its temporal variation are key issues for developing strategies of plant management and biological control in ecosystems invaded by this weed (Sanders et al., 1982; Cilliers and Hill, 1996). We did not find data on water hyacinth biomass consumption by herbivores in non-native areas. Center and Spencer (1981) only determined the number of leaves damaged by *Arzama densa* (Lepidoptera: Noctuidae), but the authors did not estimate consumed biomass. Data from Moran (2004) only refer to leaf area removed by *Neochetina*.

The aims of this paper are: (1) to measure the area damaged and biomass of leaves removed by herbivores of *E. crassipes* during different seasons and (2) to assess the amount of the leaf biomass in plant populations lost through invertebrate herbivory across seasons.

Our hypotheses are, first, that herbivorous damage of *E. crassipes* varies according to the seasonality, and second, that the amount of leaf biomass removed per m<sup>-2</sup> by herbivores is high in native floating meadows of this macrophyte.

## 2. Material and methods

### 2.1. Study site

The study area is located within the RAMSAR Site of the Chaco Wetlands (Argentina) on the west bank of the Paraná River, 30 km downstream from its confluence with the Paraguay River. We selected two oxbow lakes where *E. crassipes* was found in monospecific stands that covered up to 70% of the water surface. Plants with elongated leaves (large biotype), which occur in dense mats, were dominant. The short inflated petiole (small biotype), which occurs in more open conditions, was rarely found during the study period. Both oxbow lakes are small (200 m × 2000 m), shallow (0.4–2 m) and separated by alluvial levees (50 m wide, 1–2 m high) occupied by a gallery forest dominated by *Nectandra angustifolia* (Schrader) Nees et Mart., *Peltophorum dubium* (Spreng) Tanb. and *Banara arguta* Briq. San Nicolas Lake (Site A, 27° 27' S, 58° 55' W) is connected to the Paraná River three times per year when the water level at Puerto de Corrientes exceeds 4.85 m. El Puente Lake (Site B, 26° 26' S, 58° 51' W) is more frequently inundated with long-lasting floods. During the sampling period, both wetlands were predominantly in low water conditions and were connected with the river for only a very short time.

The climate of this area is classified as subtropical: summers are warm and prolonged, while winters are shorter and soft, with occasional frost days (frequency of 0.25 days/year) and temperatures less than -5 °C (Bruniard, 1981, 1996).

### 2.2. Experimental design and analyses

To measure herbivorous damage to leaves, we sampled a total of 18 site-habitat-date combinations (2 lakes × 3 habitats × 3 sampling dates) and collected 10 leaves of different water hyacinth plants from each sampling combination, for a total of 180 sampled leaves. In both lakes, the habitats sampled were the littoral area, the centre and the edge of the floating meadows. Sampling dates were chosen to follow the phenology of *E. crassipes* (Neiff and Poi de Neiff, 1984): the period of maximum biomass (December 2002), the end of the growth period (March 2003) and the decay period (July 2003) or spring, summer and winter.

We measured the herbivore damage found on the lamina of mature green leaves. Although the leaf of *E. crassipes* has a pseudolamina (Sculthorpe, 1967), for practical reasons in this paper we use the term lamina. The development of an *E. crassipes* leaf from expansion to senescence takes 5–6 weeks in a Florida lake (Center and Spencer, 1981) and approximately 4 weeks in the study area (Neiff, personal comm.). Because herbivory is frequently related to the nitrogen content of leaves and nitrogen varies according to leaf maturity in macrophytes (Boyd, 1978), we only selected mature green leaves and quantified nitrogen content. Leaves were dried at 60 °C and the content of this nutrient was determined in the different growth periods of *E. crassipes* by employing the Kjeldahl method (AOAC, 1990).

The number of leaves with feeding damage were counted and expressed as a percentage. We also measured lamina size (cm<sup>2</sup>) of these leaves. Following Labandeira (1998), two categories of damage were distinguished and separately measured on the lamina:

- Surface abrasions are caused by epidermis and mesophyll feeding. Tissues are not completely removed, and the most basal tissue persists in the affected areas of leaves. This type of damage is produced in *E. crassipes* by adults of *Neochetina* spp. and *O. unguis* and minor nymphs of Acrididae, mainly *C. aquaticum*. Spots made by *N. bruchi* and *N. eichhorniae* were usually indistinguishable (De Loach and Cordo, 1976).
- Holes involve complete removal of tissues. This damage in *E. crassipes* is caused mostly by adults and major nymphs of Acrididae, mainly *C. aquaticum*, and may be located in the centre or on the margin.

This damage was expressed as damaged area (cm<sup>2</sup> per lamina and percentage of lamina area damaged) and removed biomass (g per lamina and percentage of removed lamina biomass). The lamina area damaged (surface abrasions and holes) was measured with the visual estimation method by overlapping each leaf with a clear grid and counting the number of grid cells (1 mm × 1 mm) covering the damage (Cronin et al., 1998). This visual estimation method allows rapid and accurate measurement of leaf damage in comparison to digitised methods (Dorn et al., 2001). Because *Neochetina* spots have a regular size and shape, the average spot area was calculated with the grid ( $n = 1414$ ), and this value was then multiplied by the total number of spots counted per lamina. The total area of the leaf lamina was also measured with the grid.

Biomass removed by herbivores (surface abrasions and holes) was calculated indirectly using the damaged lamina area data. Surface abrasion was assessed by the difference between the area with this type of damage and the same size area without damage. The biomass was calculated on the basis of the mean weight of 30 squares of 0.1 cm<sup>2</sup> with surface abrasion and the same number of squares of the same size from undamaged areas using the following equation

$$b_s = \frac{\sum ad_s(Wn - Wd)/as}{N} \quad (1)$$

where  $b_s$  is surface abrasion biomass (g),  $ad_s$  is the damaged area by surface abrasion ( $\text{cm}^2$ ),  $as$  is area of a square ( $\text{cm}^2$ ),  $Wn$  is the mean weight of undamaged squares (g),  $Wd$  is the mean weight of damaged squares with surface abrasion (g), and  $N$  is the total number of leaves.

The average weight of undamaged squares was used to calculate the biomass removed by holes because tissues are removed completely in the affected areas; the following equation was used to calculate the biomass removed by this damage

$$b_h = \frac{\sum ad_h Wn/as}{N} \quad (2)$$

where  $b_h$  is hole biomass,  $ad_h$  is the area damaged by holes ( $\text{cm}^2$ ),  $as$  is the area of a square ( $\text{cm}^2$ ),  $Wn$  is the mean weight of undamaged squares (g), and  $N$  is the total number of leaves.

Because in herbivore damage of discrete samples is not possible to measure directly the weight of lamina without damage, to calculate this parameter we also use the indirect method on the basis of undamaged squares of  $0.1 \text{ cm}^2$ , employing the following equation

$$b_l = \frac{\sum as Wn/al}{N} \quad (3)$$

where  $b_l$  the corrected lamina biomass,  $as$  is the area of a square ( $\text{cm}^2$ ),  $al$  is lamina area ( $\text{cm}^2$ ),  $Wn$  is the mean weight of undamaged squares (g), and  $N$  is the total number of leaves.

Removed biomass (holes, surface abrasions and total) and corrected lamina biomass (without damage) were used to calculate the percentage of lamina removed. Leaf squares were dried at  $60^\circ\text{C}$  to stabilise their weight.

To assess the importance of the leaf biomass lost through herbivory, samples of green leaves were taken within a  $0.30 \text{ m}^2$  ring in each of the 18 site-habitat-date combinations; we considered as green leaves those that had 50% or more live tissues estimated visually. Leaves were cut off, separated and counted to estimate leaf density (number per  $\text{m}^{-2}$ ) and lamina including damage, were directly weighed to obtain the “uncorrected lamina biomass” per  $\text{m}^{-2}$  (g). Dry weight was obtained at  $105^\circ\text{C}$ . Removed lamina biomass by herbivores per  $\text{m}^{-2}$  (g) was calculated by multiplying the total removed biomass per lamina (Eq. (1) + Eq. (2)) by the leaf densities of the different growth periods at both sites. Because with

herbivore damage of discrete samples it is not possible to measure directly undamaged lamina biomass per  $\text{m}^{-2}$ , we added to the uncorrected lamina biomass (g per  $\text{m}^{-2}$ ) the values of removed lamina biomass per  $\text{m}^2$  to obtain the corrected lamina biomass per  $\text{m}^2$  (g). Lamina biomass removed by herbivores per  $\text{m}^{-2}$  was expressed as a percentage using values of corrected lamina biomass per  $\text{m}^{-2}$  (g).

The differences in sampling dates between lamina size, herbivorous damage per lamina, leaf density, uncorrected and corrected lamina biomass and lamina biomass removed by herbivores per  $\text{m}^{-2}$  were assessed with ANOVA with post hoc Tukey tests. All variables were log transformed to normalize distributions and stabilise variances. Differences were considered significant at  $p$ -values  $< 0.05$ . InfoStat version 1.1 software (2002) was used for these analyses.

### 3. Results

#### 3.1. Damage to *E. crassipes* leaves across seasons

The number of leaves exhibiting feeding damage was high in all three sampling dates. All sampled leaves had scarring on mature green leaves in December (the period of maximum biomass) and in March (end of growth period). During July (the decay period), the percentages of attacked leaves were 100% at Site B and 97% at Site A. Comparing the lamina areas of these leaves, significant differences were found between sampling dates at both sites (ANOVA,  $p < 0.0001$ ). Lamina area was smallest in July (Site A:  $89.7 \text{ cm}^2 \pm 32.7$ ; Site B:  $97.6 \text{ cm}^2 \pm 27.8$ ) and greatest in December (Site A:  $107.3 \text{ cm}^2 \pm 26$ ; Site B:  $132.2 \text{ cm}^2 \pm 17.4$ ) and March (Site A:  $126.7 \text{ cm}^2 \pm 31.4$ ; Site B:  $143.4 \text{ cm}^2 \pm 37$ ). Nitrogen content of *E. crassipes* leaves was highest in July (Site A:  $1.7\% \pm 0.35$ ; Site B:  $2\% \pm 0.02$ ), intermediate in March (Site A:  $1.2\% \pm 0.02$ ; Site B:  $1.4\% \pm 0.10$ ) and low in December (Site A:  $1\% \pm 0.06$ ; Site B:  $1.3\% \pm 0.05$ ).

The lamina area damaged by invertebrates varied across the seasons at both lakes (Table 1). Surface abrasion made by *Neochetina* ranged from small nicks of  $0.5\text{--}4 \text{ mm}^2$ , with an average of spot area of  $2.77 \text{ mm}^2 (\pm 1.75)$ . Abrasions made by *O. unguis* and *C. aquaticum* nymphs had extended longitudinal or irregular shapes,

**Table 1**  
Lamina area ( $\text{cm}^2$ ), surface abrasions, holes and total damage (surface abrasions + holes) produced by herbivores per lamina expressed as biomass removed (g per lamina) and damaged area ( $\text{cm}^2$  per lamina) across the seasons at sites A and B: mean  $\pm$  standard deviation. %: % removed. Different letters indicate means statistically different between sampling dates (Tukey's test,  $p < 0.05$ ).

Sampling dates	Lamina area		Surface abrasions					
	Site A	Site B	Biomass removed		Damaged area			
			Site A	Site B	Site A	Site B		
December	107.3 <sup>b</sup> $\pm$ 26	132.2 <sup>b</sup> $\pm$ 17	0.122 $\pm$ 0.073 16%	0.180 $\pm$ 0.074 20%	7.04 $\pm$ 4.21 7%	10.39 $\pm$ 4.30 8%		
March	126.7 <sup>b</sup> $\pm$ 31	143.4 <sup>b</sup> $\pm$ 37	0.233 $\pm$ 0.071 21%	0.271 $\pm$ 0.091 20%	13.48 $\pm$ 4.13 11%	15.64 $\pm$ 5.28 11%		
July	89.7 <sup>a</sup> $\pm$ 33	97.5 <sup>a</sup> $\pm$ 28	0.106 $\pm$ 0.096 17%	0.099 $\pm$ 0.063 12%	6.13 $\pm$ 5.58 7%	5.72 $\pm$ 3.67 6%		
Sampling dates	Holes				Total damage (surface abrasions + holes)			
	Biomass removed		Damaged area		Biomass removed		Damaged area	
	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B
December	0.035 $\pm$ 0.094 5%	0.023 $\pm$ 0.055 2.50%	0.47 $\pm$ 1.24 0.40%	0.31 $\pm$ 0.73 0.20%	0.157 <sup>a</sup> $\pm$ 0.120 21%	0.203 <sup>b</sup> $\pm$ 0.094 22%	7.51 <sup>a</sup> $\pm$ 4.41 7%	10.69 <sup>b</sup> $\pm$ 4.37 8%
March	0.057 $\pm$ 1.245 5%	0.055 $\pm$ 0.109 4%	0.75 $\pm$ 1.64 0.60%	0.72 $\pm$ 1.44 0.50%	0.290 <sup>b</sup> $\pm$ 0.128 27%	0.352 <sup>c</sup> $\pm$ 0.159 24%	14.23 <sup>b</sup> $\pm$ 4.06 11%	16.36 <sup>c</sup> $\pm$ 5.80 11%
July	0.035 $\pm$ 0.082 6%	0.009 $\pm$ 0.031 1%	0.46 $\pm$ 1.07 0.50%	0.12 $\pm$ 0.41 0.10%	0.141 <sup>a</sup> $\pm$ 0.120 23%	0.108 <sup>a</sup> $\pm$ 0.069 13%	6.59 <sup>a</sup> $\pm$ 5.57 7%	5.84 <sup>a</sup> $\pm$ 3.67 6%

**Table 2**

Leaf parameters (leaf density, uncorrected lamina biomass, corrected lamina biomass) and lost lamina biomass (g per m<sup>-2</sup>) removed by herbivory (surface abrasions + holes), expressed as average (± represent standard deviation). The uncorrected lamina biomass is the weight of the lamina measured directly, without including herbivorous damage. The corrected lamina biomass is the weight of the lamina including herbivorous damage. Different letters indicate means statistically different between sampling dates (Tukey's test,  $p < 0.05$ ).

	Site A			Site B		
	December	March	July	December	March	July
Leaf density (number per m <sup>-2</sup> )	236.87 <sup>a</sup> ± 5.01	201 <sup>a</sup> ± 66.92	199.76 <sup>a</sup> ± 33.25	174.95 <sup>a</sup> ± 64.74	195.61 <sup>a</sup> ± 40.86	206.81 <sup>a</sup> ± 76.09
Uncorrected lamina biomass (g m <sup>-2</sup> )	141.45 <sup>a</sup> ± 22.04	161.32 <sup>a</sup> ± 19.95	96.61 <sup>a</sup> ± 15.02	123.79 <sup>a</sup> ± 42.52	201.68 <sup>a</sup> ± 22.81	145.89 <sup>a</sup> ± 38.90
Lamina biomass removed (g m <sup>-2</sup> )	37.28 <sup>a,b</sup> ± 0.79	58.36 <sup>b</sup> ± 19.43	28.21 <sup>a</sup> ± 4.70	35.51 <sup>a,b</sup> ± 13.14	63.66 <sup>b</sup> ± 13.30	22.34 <sup>a</sup> ± 8.22
% Lamina biomass removed per m <sup>-2</sup>	21	26	23	23	24	13
Corrected lamina biomass (g m <sup>-2</sup> )	178.73 <sup>a,b</sup> ± 22.52	219.68 <sup>b</sup> ± 39.13	124.82 <sup>a</sup> ± 15.90	159.30 <sup>a</sup> ± 42.03	265.34 <sup>a</sup> ± 33.85	168.24 <sup>a</sup> ± 47.12

with sizes varying between 13 and 427 mm<sup>2</sup>. Surface abrasion per lamina represented on average between 7 and 11% of the lamina area; this type of damage was greatest in March at both sites.

Holes had irregular shapes. While the individual holes on lamina in general were of a considerable size (37–3329 mm<sup>2</sup>), only few leaves had this type of damage. Thus, the average surface area of holes damage represented 0.1–0.6% of the laminar area and had a high standard deviation in comparison with the surface abrasion damage. Like surface abrasion, the amount of this damage was greatest in March.

Total damaged area per lamina (surface abrasions + holes) varied between 11% in March and 6% in July. Significant differences in total damaged area were found between sampling dates in each site (ANOVA,  $p < 0.0001$ ).

Removed biomass per lamina by surface abrasions (Table 1) varied from 12% in July to 21% in March. Biomass lost by holes was considerably lower (<6% of the lamina biomass, Table 1) than that removed by surface abrasions. Total removed biomass (surface abrasions + holes) varied between 27% in March and 13% in July. Significant differences in total biomass removed by herbivory were found between sampling dates in each site (ANOVA,  $p < 0.0001$ ). Post hoc Tukey test to tests for significant differences between sampling dates are indicated in Table 1.

### 3.2. Evaluating the amount of the leaf biomass lost through herbivory to the *E. crassipes* plant population

Leaf density and uncorrected lamina biomass per m<sup>-2</sup> (not including damage) were not significantly different between sampling dates at both lakes (ANOVA,  $p > 0.05$ ). Values of these parameters in each date are presented in Table 2.

Biomass of lamina removed by herbivores per m<sup>-2</sup> (g and percentage) was high at both lakes (Table 2); significant differences between sampling dates were found at Site A (ANOVA,  $p = 0.029$ ) and Site B (ANOVA,  $p = 0.016$ ) being highest in March.

The corrected lamina biomass per m<sup>-2</sup> (calculated by adding the uncorrected lamina biomass to the lamina biomass removed by herbivores) was significantly different between sampling dates at Site A (ANOVA,  $p = 0.01$ ) and not significantly different at Site B (ANOVA,  $p > 0.05$ ). Post hoc Tukey test to tests for significant difference between sampling date are indicated in Table 2.

## 4. Discussion

### 4.1. Damage to *E. crassipes* leaves across seasons

Almost all of the leaves examined from the *E. crassipes* floating meadows exhibited feeding damage in all three sampling dates. A high proportion of attacked leaves (more than 81%) seems to be common in the floating meadows of the Paraná River floodplain, which is dominated by the large biotype of *E. crassipes* (Poi de Neiff and Casco, 2003). Sanders et al. (1982) mentioned that herbivory is

more frequent on elongated leaves relative to inflated petiole leaves.

The damage caused by *Neochetina* weevils as biological control agent in non-native area at the Texas lakes (Moran, 2004) was greater than the surface abrasion area obtained in our study, which includes damage of *Neochetina* sp. and other herbivores. The total damaged area per lamina found in this study in March was less than the 16% of damaged area per lamina (2–3 weeks) that has been found for leaves of *Nuphar variegata* and *Nymphaea odorata* during summer (Cronin et al., 1998). Consumption and damage caused by herbivores were responsible for the disappearance of approximately 22% of the leaf area produced annually by *Nymphoides peltata* (Van der Velde et al., 1982). In amphibious stream plants, mean grazing loss of the oldest leaves in aquatic populations was less than 14% of the leaf area in *Berula erecta* and greater than 14% in *Mentha aquatica* and *Myosotis palustris*, whereas in all terrestrial populations, grazing loss was less than 14% (Sand-Jensen and Jacobsen, 2002).

Underestimation of the herbivore damaged area on lamina and, subsequently, of the biomass removed per m<sup>-2</sup> may occur when herbivores completely eat the leaves so that nothing remains for field sampling (Coley and Barone, 1996; Cronin et al., 1998; García-Robledo, 2005). However, in many studies carried out since 1977, we have not observed leaves of *E. crassipes* completely eaten in the Paraná River floodplain.

Damaged area and biomass removed by herbivory per lamina varied across seasons and was highest in March (end of growth period) at both lakes. The relatively low herbivore damage in July (decay period) is related to the high amount of standing dead leaves and litter produced by *E. crassipes* floating meadows in this period (Neiff and Poi de Neiff, 1984). The growth periods of *E. crassipes* are related to seasonality, and according to Cilliers and Hill (1996), this factor has a direct relationship with the stress inflicted on plants by biocontrol agents of water hyacinth in a non-native area.

Small values of damaged area and biomass removed per lamina in July (decay period) were coincident with the highest level of nitrogen content in the tissues and minor lamina area of *E. crassipes* leaves. High levels of nitrogen content during decay were also found in *E. crassipes* floating meadows in Florida (Tucker and Debusk, 1981) and Texas (Moran, 2004).

### 4.2. Evaluating the amount of the leaf biomass lost through herbivory to the *E. crassipes* plant population

We found that the values of leaf density and uncorrected lamina biomass per m<sup>-2</sup> obtained on three sampling dates corresponding to the growth periods of *E. crassipes* are coincident with those obtained by Neiff et al. (2008) during an annual cycle of this macrophyte in the same lakes.

Small values of lamina biomass removed by herbivory per m<sup>-2</sup> in July was coincident with higher nitrogen content in the leaf



tissue and low and intermediate values of corrected lamina biomass. Therefore, we can suppose that during the period of maximum biomass and end of growth periods (December and March), when herbivores are more active, low levels of nitrogen in the *E. crassipes* plant population may be overcompensated for by the large quantity of biomass available to herbivores. Obermaier and Zwölfer (1999) have also found that food quality and plant biomass in Asteraceae show an inverse trend during the seasons in terrestrial ecosystems, and low nitrogen content in the tissue of the host plant was overcompensated for by large quantities of available food for Chrysomelidae herbivores.

The percentage of lamina biomass of *E. crassipes* removed by herbivory per  $\text{m}^{-2}$  is higher than the mean percentage reduction of standing crops by snails and insects estimated by Lodge et al. (1998) for different taxa of macrophytes. Unlike Lodge et al. (1998), we only evaluated damage with respect to lamina biomass and not including petioles. The petiole base up to a height lower than 14% of the total height of the leaves is attacked by *Thrypticus* larvae, the most abundant herbivore of the *E. crassipes* petioles. Biomass loss due to this herbivore is low, because the larvae damage large-sized leaves (more senescent mature leaves) at the end of the growth periods (Poi de Neiff and Casco, 2003).

Previous works on the herbivory of aquatic macrophytes have shown that invertebrate damage accounts for the seasonal decay of the plants and that herbivores have an even greater effect on detrital inputs and nutrient cycling than the consumption of plants in wetlands (Hunt-Joshi et al., 2004; Newman, 1991; Poi de Neiff et al., 1977; Poi de Neiff and Casco, 2003; Poi de Neiff and Chiozza, 1994). In our study, we found that the lamina biomass removed per  $\text{m}^{-2}$  was high and variable according to the sampling dates, and as a consequence, damage due to herbivory must be taken into account in estimations of the plant biomass and productivity of this macrophyte. In addition, we found that the uncorrected lamina biomass per  $\text{m}^{-2}$  (normally used in estimations of plant biomass and productivity) was similar across seasons, whereas the corrected lamina biomass varied considerably at Site A, showing the importance of including herbivorous damage in these estimates.

Conversely, Gutierrez et al. (2001) mentioned that estimations of *E. crassipes* productivity and biomass need not include herbivorous damage because it is considered unimportant; similar results have found by Medeiros dos Santos and Esteves (2002) with other species of macrophytes. Herbivory on macrophytes has been measured in relation to production, age and turnover of leaves (Brock and Van der Velde, 1996; Jacobsen and Sand-Jensen, 1994; Sand-Jensen and Jacobsen, 2002; Sand-Jensen et al., 1994). Grazing of 10% of leaf production was found for *N. peltata* by Brock and Van der Velde (1996). According to Sand-Jensen and Jacobsen (2002), in amphibious macrophytes, terrestrial populations experience a lower percentage of grazing loss of leaf production (1–5%) than do aquatic populations (3–17%), because leaf production is higher in populations living in terrestrial habitats. In submerged macrophytes, trichopterans may consume a maximum of 25% of standing plant biomass of *Potamogeton perfoliatus*, but the proportion of annual plant production loss through these herbivores was low because consumption was low when plant production peaked (Jacobsen and Sand-Jensen, 1994).

Measuring leaf production is a key point in evaluating herbivore damage, because plant populations that have the same values of leaf consumption per  $\text{m}^{-2}$  may have different values of leaf production, and, as a consequence, suffer a different percentage of grazing loss of leaf production (Sand-Jensen and Jacobsen, 2002). Future studies of production and turnover of *E. crassipes* leaves in the different growth periods of the floating meadows across seasons would be account for assessing herbivory impact as a dynamic process in a context of plant growth. The turnover of

floating meadows biomass of 64%, calculated from Neiff and Poi de Neiff (1984), and its half-life in the litter of 31.5 days (Poi de Neiff et al., 2006), indicate fast production and decomposition processes at the study site.

Our results suggest that the lamina biomass removed by herbivory on water hyacinth was high in native areas, and despite we have not measured the effect of herbivory on the coverage of this macrophyte, the impact on plant population seems to be low. Adis and Junk (2003) also found that the highly specific *C. aquaticum* produces severe damage during the decay period of *E. crassipes* (low water) in the Amazon floodplain; however, it is not enough to prevent subsequent plant biomass development. Gutierrez et al. (2001) have also mentioned that reproduction of *E. crassipes* occurs more rapidly than *Neochetina* can inflict damage. On the other hand, in some invaded ecosystems (Lake Victoria and Papua New Guinea), *Neochetina* caused a significant reduction of water hyacinth coverage, achieving successful biological control in a relatively short time (Center et al., 2002; Hill and Olckers, 2001; Murphy, personal comm.). According to the enemy release hypothesis, in absence of predators, parasites and parasitoids, herbivorous population may overexploit their host plant in non-native areas (Colautti et al., 2004; Hufbauer and Torchin, 2007); however, there are many cases in which biological control have failed because generalist natural enemies of the release areas limited the size of herbivore population (Smith, 2004). Climate, weather, habitat manipulation, interspecific competition and host plant quality are clearly important in regulating the control agent populations (Newman et al., 1998) and they also would explain the contrast between the low effect of herbivorous in native areas and the successful of biological control of some invaded areas. In addition to biotic and climatic factors, density-dependent factors cause more severely upon the herbivorous population, especially when its density is high (Cappuccino, 1995).

Difficulties in the application of experimental designs with exclusion of foliar herbivores and its effect on the evaluation of plants' performance were also mentioned for a temperate herbaceous community by Couple and Cahill (2003) and Schmitz (2004).

Because the greatest damage (damaged area and removed biomass per lamina) was found on leaves in March, our first hypothesis that the herbivorous damage in *E. crassipes* varies according to the seasonality has to be accepted. The damage of lamina biomass per  $\text{m}^{-2}$  of the *E. crassipes* floating meadows found in our study is high in all sampling dates in comparison with a mean of 10% reduction of biomass in macrophytes mentioned in the revision study of Lodge et al. (1998), and as a consequence, we also accept the second hypothesis. We suggest the use of herbivore damage of discrete samples and the indirect method to calculate the biomass removed in sites with aquatic free floating plants, where experimental exclusion of insects may be difficult to carry out. Moreover, we suggest the use of corrected lamina biomass instead of uncorrected lamina biomass in estimations of productivity in standing crops of this macrophyte. More studies are needed to understand the role that invertebrate herbivores play in the physiological and ecological processes of *E. crassipes* floating meadows in native areas.

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