



Invertebrate herbivory on floating-leaf macrophytes at the northeast of Argentina: should the damage be taken into account in estimations of plant biomass?

FEDRA S. MARTÍNEZ and CELESTE FRANCESCHINI

Centro de Ecología Aplicada del Litoral – CECOAL(CCT Nordeste-CONICET-UNNE), Ruta 5, Km 2.5, (3400) Corrientes, Argentina

Manuscript received on May 30, 2016; accepted for publication on September 6, 2017

ABSTRACT

We assessed the damage produced by invertebrate herbivores per leaf lamina and per m² of populations floating-leaf macrophytes of Neotropical wetlands in the growth and decay periods, and assessed if the damage produced by the herbivores should be taken into account in the estimations of plant biomass of these macrophytes or not. The biomass removed per lamina and per m² was higher during the growth period than in decay period in *Nymphoides indica* and *Hydrocleys nymphoides*, while *Nymphaea prolifera* had low values of herbivory in growth period. During decay period this plant is only present as vegetative propagules. According to the values of biomass removed per m² of *N. indica*, underestimation up to 17.69% should be produced in cases that herbivory do not should be taking account to evaluate these plant parameters on this macrophyte. Therefore, for the study of biomass and productivity in the study area, we suggest the use of corrected lamina biomass after estimating the biomass removed by herbivores on *N. indica*. The values of damage in *N. indica* emphasize the importance of this macrophyte as a food resource for invertebrate herbivores in the trophic networks of the Neotropical wetlands.

Key words: plant damage, herbivores, *Nymphoides*, *Nymphaea*.

INTRODUCTION

Recent studies from wetlands in the Northern Hemisphere, mainly in temperate regions show that the herbivory in freshwater macrophytes is significant and can reduce the plant biomass (Bakker et al. 2016) and plant abundance (Wood et al. 2017) by 44-48%. However, these generalizations do not consider the effect and the role of invertebrate herbivores on macrophytes of Neotropical wetlands, because there is little

evidence of herbivory in these environments (Esteves 2011). The Neotropical region contains the greatest concentration of species richness and biodiversity (Wantzen et al. 2006, Nunes et al. 2016) and a great amount of aquatic environments compared to other regions (Footitt and Adler 2009). Thus, the effect of invertebrate herbivores on freshwater macrophytes in Neotropical wetlands could be quite different from the pattern described for wetlands in the Northern Hemisphere.

In the Neotropical wetlands, quantifications of the amount of herbivore damage and the effect on macrophyte populations are scarce and only refer

Correspondence to: Fedra Solange Martínez
E-mail: fedrasolange@yahoo.com.ar

to free floating macrophytes (Adis and Junk 2003, Poi de Neiff and Casco 2003, Sosa et al. 2007, Franceschini et al. 2010, 2013, Braga et al. 2013, Martinez et al. 2013). These studies show that invertebrate herbivores can also feed high amounts of freshwater macrophyte biomass and that the abundance and magnitude of the damage caused by herbivores vary significantly between the growth and decay periods of the macrophyte populations (Braga et al. 2013, Franceschini et al. 2010, 2013).

Among macrophytes, floating-leaf macrophytes can reach high coverage and abundance in freshwater environments across the world (Wiersema 1987, Brock and Van Der Velde 1996). Among the floating-leaf macrophyte species, *Hydrocleys nymphoides* Willd. Buchenau (Alismataceae), *Nymphoides indica* (L.) Kuntze (Menyanthaceae) and *Nymphaea prolifera* Wiersema (Nymphaeaceae) have high abundance and coverage in Neotropical wetlands (Wiersema 1987, Palma-Silva et al. 2008). While *N. indica* is native to tropical and subtropical America, Asia and Australia (Oenduff 1969), *H. nymphoides* and *Nymphaea prolifera* are native only to the Neotropic areas and they were introduced in wetlands of the United States, Australia and New Zealand (Wiersema 1987, Haynes 2000). *H. nymphoides* and *N. indica* have been mentioned as weeds in the irrigation channels and cultivated rice paddies (Lallana 2005).

One of the special features of floating-leaf macrophytes is that their leaves develop first below the water line and then grow up to the water surface, as a result, they can be consumed by aquatic and semi-aquatic invertebrate herbivores (Cronin et al. 1998). Despite the taxa of herbivores, the level of damage and the role played by floating-leaf macrophyte herbivores in wetlands of the Northern Hemisphere are well studied, especially during the growth period (Van Der Velde et al. 1982, Cronin et al. 1998, Nachtrieb et al. 2011), there is little information in this regard for the Neotropical wetlands. In these wetlands, floating-

leaf macrophytes are known to account for 87% of the diet of Orthopterans (Capello et al. 2012), and they can also be consumed by Curculionidae (Wibmer 1989). However, the impact of herbivory damage on the biomass of the floating-leaf macrophyte populations, the biomass consumed by invertebrate herbivores in the growth and decay periods, and therefore the role of these herbivores in wetlands inhabited by these macrophytes, remains unclear. Recent investigations in the study area show that herbivores can reach up to 60 % of the total invertebrates in floating-leaf macrophytes, especially in the growth period (Martínez F.S. et al., unpublished data, Franceschini et al. 2016). Thus, it is important to determine if invertebrate herbivores remove a low amount of leaf biomass of these macrophytes (Hunt-Joshi et al. 2004) or if the leaf biomass removed is high, which could imply that herbivores are important consumers of the living plant tissue in the wetland food webs (Franceschini et al. 2010, Braga et al. 2013). For this reason, the herbivory damage should be taken into account in the estimations of plant biomass and productivity. Abundance and composition of invertebrate herbivores vary according to the temperatures and hydroperiod in the Neotropical wetlands, obtaining the higher herbivore abundances in the growth period of the plant populations (Capello et al. 2013). Likewise, the plant biomass of macrophyte species varies significantly between the growth and decay periods, reducing the biomass of macrophytes in the decay period by up to 50% (Neiff et al. 2008). These differences in plant biomass imply that the food resource available to herbivores also differs in quantity and quality (Peeters et al. 2007), whereby, it would be expected that herbivory would also differ between these periods. In addition, most studies of herbivory in floating-leaf macrophytes focus only on the leaf lamina and give information about the biomass or area damaged by herbivores per leaf lamina (Van Der Velde et al. 1982, 1985, Cronin et al. 1998). However, as leaf densities vary

considerably between growth and decay periods and macrophyte species, damage produced by herbivores per square meter is an indispensable tool to achieve a complete understanding of the herbivory process in macrophyte populations.

The aim of this paper is to: 1) quantify and compare the damage produced by invertebrate herbivores per leaf lamina and per m² of floating-leaf macrophytes in the growth and decay periods, and 2) assess if the damage produced by the herbivores should be taken into account in the estimations of plant biomass of these macrophytes or not.

In relation to the total of the invertebrates found in floating-leaf macrophytes of the study area, herbivores can reach a high abundance, mainly during the growth period. Therefore, our hypothesis is that invertebrate herbivory is significant in floating-leaf macrophytes and that the damage by herbivores should be taken into account in estimations of plant biomass of these macrophytes.

MATERIALS AND METHODS

STUDY SITES

Samplings were carried out in two wetlands in Northeastern Argentina: Site A (27°29'1.43"S, 58°45'0.95"W) and Site B (27°22'0.95"S, 58°20'3.79" W), in the growth period and in the decay period of plant populations. Since the abundance of herbivorous insects can vary significantly according to the successional stage of the vegetation (Silva et al. 2012), these wetlands were selected because the studied macrophytes were present in undisturbed populations, at a late successional stage and with a coverage of 30 to 40% of the water surface. Site A covers approximately 70 ha and supports populations of *H. nymphoides* and *N. prolifera*. Site B covers an extension of 4.91 ha and supports populations of *N. indica*; *N. prolifera* was also present in this site but it was very scarce. The climate of this

area is subtropical, with warm summers and mild winters, occasional frost days (frequency of 0.25 days/year) and temperatures not dropping lower than -5°C (Bruniard 1996). Sampling dates were chosen to follow the phenology of the floating-leaf macrophytes in the study area: the decay period, in winter (June to August) and the growth period, in summer (February to March). In the period under study, *N. prolifera* had no leaves during the decay period, but vegetative propagules were recorded in this period, which coincides with that mentioned by Wiersema (1987). Leaves of *H. nymphoides* and *N. indica* were present in both periods.

SAMPLING DESIGN TO ASSESS HERBIVOROUS DAMAGE PER LAMINA AND PER M²

To measure herbivorous damage on leaves, we sampled a total of 15 macrophyte species- habitat-growth period combinations (2 macrophyte species x 3 habitats x 2 growth periods + 1 macrophyte x 3 habitats x 1 growth period), and collected 10 leaves of different plants from each sampling combination, for a total of 150 sampled leaves. The habitats sampled were the proximal edge, the centre and the distal edges of the floating matt, which were separated by a distance of 100 m, to ensure the randomness of the samples. We chose these three habitats because they represent three heterogeneous zones in floating-leaf macrophyte populations, due to the coexisting vegetation and the location in the wetland.

The herbivore damage on leaves estimated in discrete samples, gives a valuable measure of herbivory, and such estimates are easy to carry out (Franceschini et al. 2010).

According to Labandeira (1998), two categories of damage, surface abrasion and holes, were distinguished on the lamina of floating-leaf macrophytes. The surface abrasions are caused when tissues are not completely removed, and the most basal tissue persists in the affected areas of

leaves. The holes involve complete removal of tissues.

Herbivory was measured at the level of leaf lamina and per square meter (m^2) of vegetation. To assess herbivory at the level of leaf lamina, digital images of the sampled leaves were taken in the laboratory. The area of leaf lamina was measured and the area damaged by herbivores per leaf lamina was quantified (cm^2), considering holes and surface abrasion separately. The software used for processing the digital images was ImageJ 1.44 (Rasband 2016).

Biomass removed by herbivores (surface abrasions and holes) was calculated indirectly using the damaged lamina area data (Franceschini et al. 2010). 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 circles of 0.196 cm^2 with surface abrasion and the same number of circles of the same size from undamaged areas using the following equation

$$b_s = \frac{\sum ad.(Wn - Wd)}{\frac{a_s}{N}} \quad (1)$$

where b_s is surface abrasion biomass (g), ad is the damaged area by surface abrasion (cm^2), a_s is area of a circle (cm^2), Wn is the mean weight of undamaged circles (g), Wd is the mean weight of damaged circles with surface abrasion (g), and N is the total number of leaves.

The average weight of undamaged circles 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

$$b_h = \frac{\sum ad.Wn}{\frac{a_s}{N}} \quad (2)$$

where b_h is hole biomass, ad is the area damaged by holes (cm^2), a_s is the area of a circle (cm^2), Wn is

the mean weight of undamaged circles (g), and N is the total number of leaves.

We assessed the total biomass removed per lamina (holes + abrasion). The dry weight per lamina was obtained by drying at $105\text{ }^\circ\text{C}$ for 72 hours and then laminas were individually weighed to obtain the “uncorrected lamina biomass”. Since herbivore damage to discrete samples makes it impossible to measure undamaged lamina biomass directly (Franceschini et al. 2010), we added the total biomass removed per lamina (holes + abrasion) to the “uncorrected lamina biomass” to obtain the “corrected lamina biomass” (Fig. 1a, b).

In order to quantify leaf density and the biomass removed by herbivores per m^2 in floating-leaf macrophyte populations, samples of green leaves were also taken within a 0.33 m^2 aluminium ring in each of the 15 macrophyte species-habitat-growth period combinations. Three replications were taken and to ensure random samples, each ring was separated by a distance of 100 m. Within the rings, we quantified leaf density (number of leaves per m^2). Leaves were then removed by cutting off petioles at ground level at the bottom of the lakes.

In the laboratory, laminas from the field including damage were dried and were directly weighed to obtain “the uncorrected lamina biomass per m^2 ” (g per m^2). Dry weight was obtained at $105\text{ }^\circ\text{C}$. Removed lamina biomass by herbivores per m^2 (g) was calculated by multiplying the total removed biomass per lamina (equation 1 + equation 2) by the leaf densities of the different growth periods in each macrophyte species. Because with herbivore damage of discrete samples it is not possible to measure directly undamaged lamina biomass per m^2 (Franceschini et al. 2010), we added to the uncorrected lamina biomass (g per m^2) the values of removed lamina biomass per m^2 to obtain the corrected lamina biomass per m^2 (g) (Fig. 1c, d).

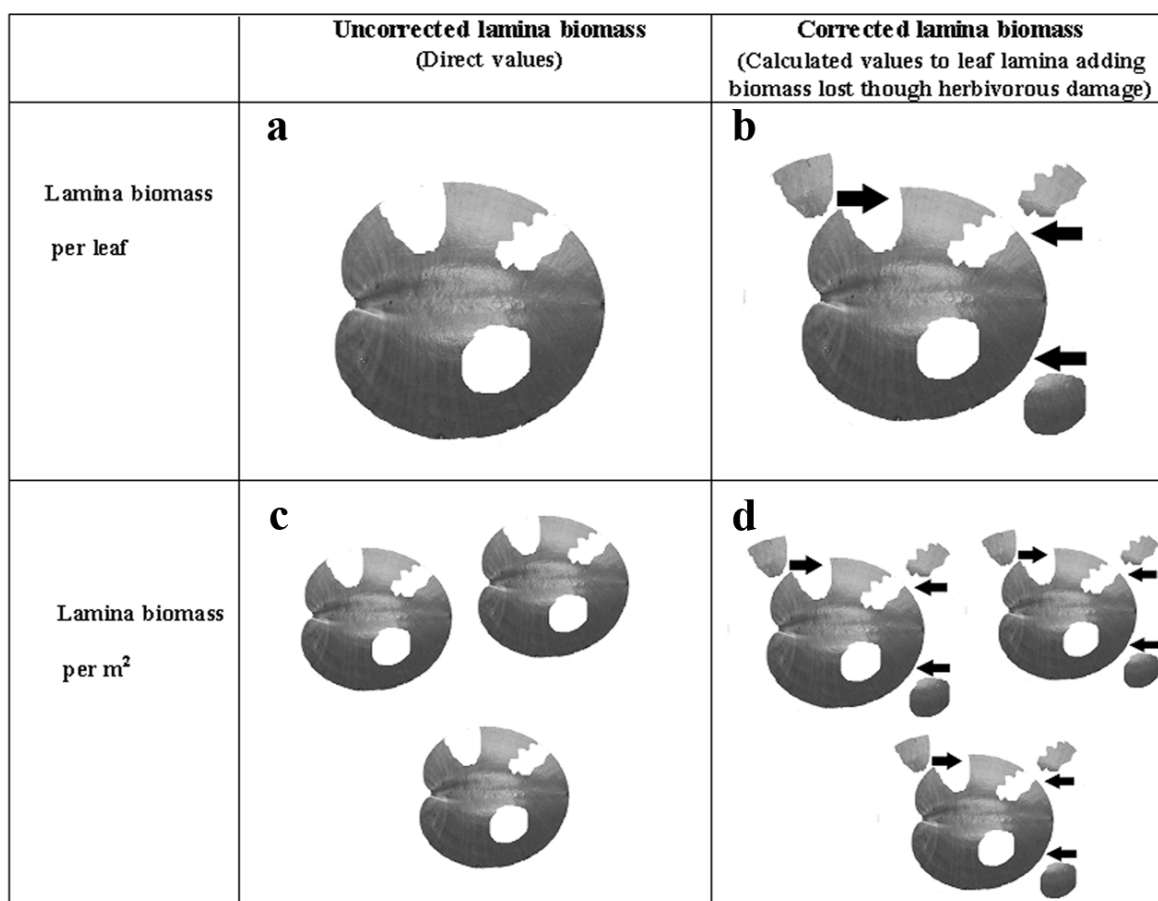


Figure 1 - Uncorrected and corrected lamina biomass in freshwater macrophytes. The uncorrected lamina biomass is the weight of the lamina measured directly, without including herbivorous damage. The corrected lamina biomass is the weight of the lamina adding herbivorous damage. **a**: Uncorrected lamina biomass per leaf; **b**: Corrected lamina biomass per leaf; **c**: Uncorrected lamina biomass per m²; **d**: Corrected lamina biomass per m².

Lamina biomass removed by herbivores per m² was expressed as percentage using values of corrected lamina biomass per m². Leaf area, leaf biomass and leaf density were measured as indicators of food resource available for invertebrate herbivores in different macrophyte species and growth periods.

STATISTICAL PROCEDURE

All data were tested for normality (Shapiro-Wilk tests) and homogeneity of variance (Levene's test). Data that failed to meet the requirements of normality and homogeneity after transformation were analysed using non-parametric statistics. The differences

between sampling dates in the total damaged lamina by herbivores, total biomass removed per lamina and per m², uncorrected and corrected lamina biomass and lamina biomass removed by herbivores per m² were tested by one-way ANOVAs with post hoc Tukey tests. The differences in sampling dates between types of damage produced by herbivores, lamina areas, leaf density were tested by Kruskal-Wallis tests, with post hoc multiple comparison tests. To assess percentage of lamina biomass removed by herbivores per leaf and per m² between species of macrophytes we used one-way ANOVAs with post hoc Tukey tests. To meet the assumptions of normality of dates, the percentages of herbivory were transformed an arcsine of the square root of

the proportion. All analyses were conducted using software Infostat version 2016 (Di Rienzo et al. 2016).

RESULTS

HERBIVORY PER LEAF LAMINA AND M² OF VEGETATION IN GROWTH AND DECAY PERIODS

Considering the food resource available for herbivores per leaf, the lamina area was significantly higher in the growth period than in the decay period on *H. nymphoides* ($H= 32.06$, $p < 0.0001$) and *N. indica* ($H= 30.90$, $p < 0.0001$). *H. nymphoides* offered to the herbivores significantly more leaf biomass in the growth period ($H= 32.06$, $p < 0.0001$) than in the decay period, while *N. indica* showed no significant differences between these periods ($H= 3.2$, $p= 0.0734$). In the growth period, *N. prolifera* offered more food resources to herbivores as it showed 8 to 15 times more of the leaf biomass and twice as much of the leaf lamina area than the other two macrophyte species.

The total area damaged by herbivores per lamina (surface abrasions + holes) on *H. nymphoides* was significantly higher ($H= 11.56$, $p= 0.0005$) during the growth period than in the decay period and the damage represented up to 3.84% of the leaf lamina area. Similarly, on *N. indica* significant differences were found between periods ($H= 19.02$, $p < 0.0001$) in the total area damaged per lamina (surface abrasions + holes). The total area damaged per lamina was 4.5 times higher in the growth period than in the decay period (Table I).

The total biomass removed by herbivores per lamina (surface abrasions + holes) was significantly higher during the growth period than in the decay period on *H. nymphoides* ($H= 10.34$, $p= 0.0011$) and *N. indica* ($H= 19.54$, $p < 0.0001$). Total biomass removed per lamina on *H. nymphoides* was three times more in the growth period than in the decay period, while on *N. indica* it was more than double in the growth period than in the decay period.

Herbivory on *N. prolifera* leaves was low, values of lamina area damaged and biomass removed per lamina were lower than for *N. indica* and *H. nymphoides* in the growth period (Table I).

Considering the food resource available for herbivores in plant populations, leaf density per m² of *H. nymphoides* did not vary significantly ($H= 0.43$, $p= 0.700$) between periods, while on *N. indica* it did vary significantly ($H= 41.82$, $p= 0.0029$) between periods. In the growth period, leaf density per m² of *N. indica* was eight times higher than in the decay period. In the growth period, *N. prolifera* showed an intermediate leaf density per m² in comparison to the other two macrophytes. The percentage of attacked leaves was high in the three macrophyte species, especially in growth period (Table I).

Lamina biomass removed per m² (g) by herbivores was significantly higher in the growth period than in the decay period on *H. nymphoides* ($F_{1,4} = 18.56$, $p= 0.013$) and *N. indica* ($F_{1,4} = 14.91$, $p= 0.018$) plant populations. On *H. nymphoides* herbivores removed four times more biomass per m² (g) in the growth period than in the decay period, whereas on *N. indica*, herbivores removed forty six times more biomass per m² (g) in the growth period than in the decay period. In the growth period, herbivory values per m² on *N. prolifera* were intermediate in relation to the other two species (Table I).

Considering the types of damage produced by herbivores on leaves in the growth period, biomass removed by surface abrasion was significantly higher than holes ($H= 17.14$, $p= 0.0001$) on *H. nymphoides*, whereas on *N. indica* and *N. prolifera* holes were significantly higher ($H= 28.64$, $p < 0.0001$; $H= 11.36$, $p= 0.0007$, respectively) than surface abrasion (Fig. 2). In the decay period significant differences were only observed ($H= 6.35$, $p= 0.0113$) between types of damage for *N. indica*, that is, damage by holes was ten times higher than damage by surface

TABLE I

Area (cm²) and biomass (g) of the leaf lamina and total damage (surface abrasions + holes) produced by invertebrate herbivores per lamina in leaves of *Hydrocleys nymphoides* and *Nymphoides indica* in growth and decay period, and *Nymphaea prolifera* in growth period. Values of herbivory are expressed as damaged area (cm² per lamina and per m²) and biomass removed (g per lamina and per m²): mean ± standard deviation. % removed per lamina. Different letters indicate means statistically different between sampling dates.

	Growth periods	Leaf lamina				Plant population		
		Area	Biomass	Damaged area	Biomass removed	Leaf density (number per m ²)	Damaged leaves (%)	Lamina biomass removed per m ² (g)
<i>Hydrocleys nymphoides</i>	Growth	58.28 ^a ±17.66	0.356 ^a ±0.108	2.24 ^b ±2.22 3.84% ^a	0.009 ^b ±0.011 2.54% ^a	54.25 ^a ±15.95	86.67 ^a ±5.77	0.50 ^a ±0.15 3.62% ^a
	Decay	29.08 ^b ±10.59	0.178 ^b ±0.065	0.73 ^a ±1.11 2.50% ^b	0.003 ^a ±0.004 1.65% ^a	45.21 ^a ±0	50 ^b ±26.46	0.14 ^b ±0 2.62%
<i>Nymphoides indica</i>	Growth	66.72 ^a ±20.11	0.184 ^a ±0.071	9.06 ^b ±7.83 13.59% ^b	0.034 ^b ±0.031 15.60% ^b	176.81 ^a ±77.6	100 ^a ±0.00	6.01 ^a ±2.64 17.69% ^b
	Decay	37.4 ^b ±7.73	0.150 ^a ±0.040	2.03 ^a ±2.74 5.43% ^a	0.007 ^a ±0.010 4.18% ^a	20.09 ^b ±3.48	90.00 ^a ±17.32	0.13 ^b ±0.02 3.25%
<i>Nymphaea prolifera</i>	Growth	136.82 ±46.07	2.900 ±0.977	1.83 ±1.55 1.34% ^a	0.032 ±0.030 1.10% ^a	86.40 ^a ±46.82	93.33 ^a ±5.77	2.80 ^a ±1.52 6.52% ^a

abrasions (Fig. 3). Biomass removed by surface abrasions were produced by adults of *Lixini* sp (Coleoptera: Curculionidae), nymphs of *Marellia remipes* (Orthoptera: Acrididae) and *Omalonyx* sp (Gasteropoda: Succineidae). Holes were produced by adults of *M. remipes*, larvae of Pyralidae and Noctuidae (Lepidoptera) and *Pomacea canaliculata* (Gasteropoda: Ampullaridae). Other herbivores such as *Rhopalosiphum nymphaeae* (Hemiptera: Aphididae) were also observed on *H. nymphoides*.

HERBIVORE DAMAGE, THE UNCORRECTED LAMINA BIOMASS AND THE CORRECTED LAMINA BIOMASS

Herbivory in leaf lamina and in plant population was notably high on *N. indica*, mainly in the growth

period. In fact, *N. indica* had a significantly higher percentage of biomass removed per lamina ($F_{2,87} = 36.32, p < 0.0001$) and percentage of lamina biomass removed per m² ($F_{2,6} = 20.53, p = 0.0021$) than the other floating-leaf macrophyte species (Table I). According to the values of biomass removed per lamina and lamina biomass removed per m² of *N. indica*, subestimations up to 15.60% of the leaf lamina biomass and 17.69% of the leaf biomass per m² should be considered in cases in which herbivory is not taking into account the evaluation of these plant parameters on this macrophyte.

Herbivory per leaf lamina on *H. nymphoides* and *N. prolifera* was low, so subestimations of the leaf lamina biomass should not exceed 2.54%. According to the values of lamina biomass removed

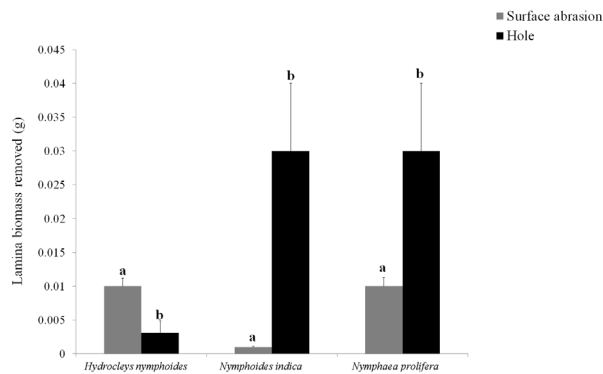


Figure 2 - The lamina biomass removed (g) by surface abrasion and holes per leaf lamina on floating-leaf macrophytes in the growth period. Different letters indicate means statistically different between sampling dates.

per m², up to 6.52% of the leaf biomass per m² should be subestimated in *N. prolifera*, whereas on *H. nymphoides* these values should not exceed 3.62% (Table I).

On *H. nymphoides*, which had low herbivorous damage, significant differences were observed between periods in the uncorrected lamina biomass ($H=32.06, p<0.0001$), which is the biomass measured directly, without including herbivorous damage. Similar results were obtained when the comparison was made using the corrected lamina biomass ($H=32.65, p<0.0001$), which is the biomass that includes herbivorous damage (Figs. 1 and 4).

On *N. indica*, the uncorrected lamina biomass did not vary significantly between periods ($H=3.17, p=0.0749$). Results were different when the comparison was made using the corrected lamina biomass, which includes herbivorous damage (Figs. 1 and 4). In fact, corrected lamina biomass of *N. indica* showed significant differences between periods ($H=11.02, p=0.0009$). The reason why different results are shown, whether the uncorrected or corrected lamina biomass is used in the comparison, is that a considerably high biomass of this macrophyte is removed by herbivores.

Estimations of leaf biomass during the growth period may be notably underestimated for *N. indica*

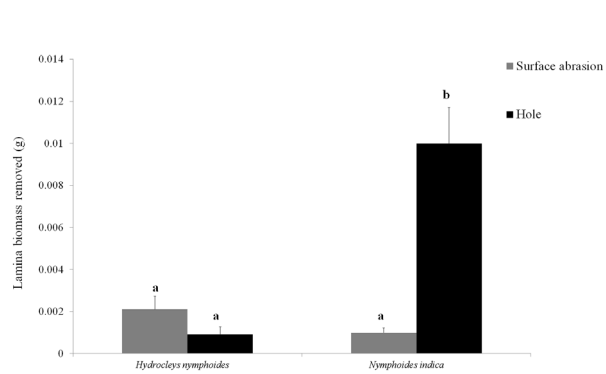


Figure 3 - The lamina biomass removed (g) by surface abrasion and holes per leaf lamina on floating-leaf macrophytes in decay period. Different letters indicate means statistically different between sampling dates.

(15.60%) in cases that the uncorrected lamina biomass is used instead of the corrected lamina biomass (Figure 5).

DISCUSSION

HERBIVORY ON FLOATING-LEAF MACROPHYTES IN GROWTH AND DECAY PERIODS

The great damage produced by herbivores in *H. nymphoides* and *N. indica* in the growth period could be related to the highest availability of resources per leaf and per m² in these macrophytes (Coley et al. 1985). In addition, abundance of invertebrate herbivores is higher in growth than in decay period in the study area (Franceschini et al. 2011, 2013, Martínez F.S. et al., unpublished data). Higher food resource in the growth period than in the decay period is consistent with the results found for *Nymphoides peltata* in the Northern Hemisphere (Van Der Velde et al. 1979).

Higher biomass removed per lamina and per m² in the growth period found in *H. nymphoides* and *N. indica* is consistent with the results found for *E. crassipes* (Franceschini et al. 2010). In fact, *E. crassipes* biomass removed per lamina and per m² was 3.5 and 2.8 times higher in the growth period than in decay period, respectively.

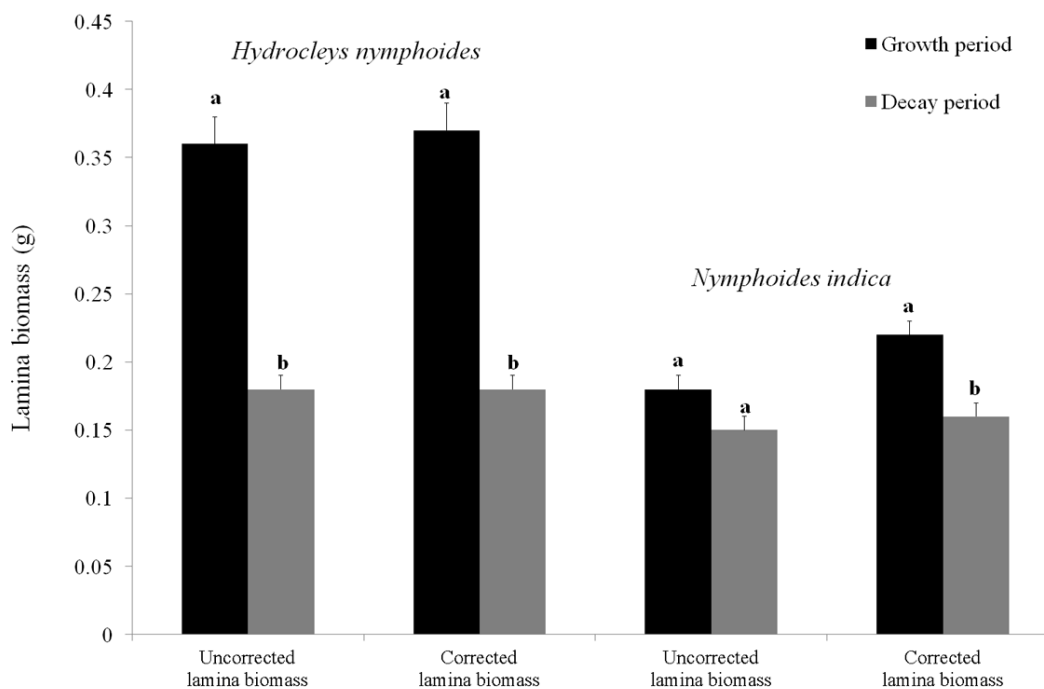


Figure 4 - The uncorrected lamina biomass and the corrected lamina per leaf lamina among floating-leaf macrophyte species in growth period y decay period. The uncorrected lamina biomass is the weight of the lamina measured directly, without including herbivorous damage. The corrected lamina biomass is the weight of the lamina adding herbivorous damage. Different letters indicate means statistically different between sampling dates.

On the other hand, the area damaged in the leaf lamina of *H. nymphoides*, *N. prolifera* and *N. indica* is lower than 26% and 16% of the damaged lamina area for *Eichhornia crassipes* and *E. azurea* leaves in the study area (Franceschini et al. 2010, 2011). This could be related to the anatomic features of leaf tissues. Gonzalez (2005) points out that, whereas floating-leaf macrophytes have star-shaped sclereids and columnar sclereids, distributed in the mesophyll and the palisade parenchyma of the leaves, *E. crassipes* and *E. azurea* leaves have fewer lignified elements. These elements are directly involved in the lower palatability of plant tissues for invertebrate herbivores (Bernays and Chapman 1994).

Several floating-leaf macrophytes are known for producing alkaloids and tannins, including the *Nuphar*, *Nymphaea* and *Nelumbo* species (Kok et al. 1992). Lower damage registered to leaves of *H. nymphoides* and *N. prolifera*, might be the result of the high level

of tannin content in these species, 3.67% and 5.40%, respectively; whereas the least concentrations were found in the leaves of *N. indica* (0.61%) in the study area (Martínez F.S. et al., unpublished data). This phenolic polymer is known for significantly reducing the palatability for herbivorous insects (Rosenthal and Berenbaum 1991).

Differences in the magnitude of herbivory such as the type of damage recorded for floating-leaf macrophyte species, holes and surface abrasions, could be the result of the different taxa of herbivores present in each macrophyte as well as the relative size and activity of the taxa of herbivores, as stated by Cronin et al. (1998). In fact, the highest values of damage by holes in relation to the surface abrasion found on *N. indica* and *N. prolifera* in the growth and decay periods may be related to the predominance of chewing herbivores with high body biomass, like Lepidoptera and Acridoidea, which were confirmed on these macrophytes

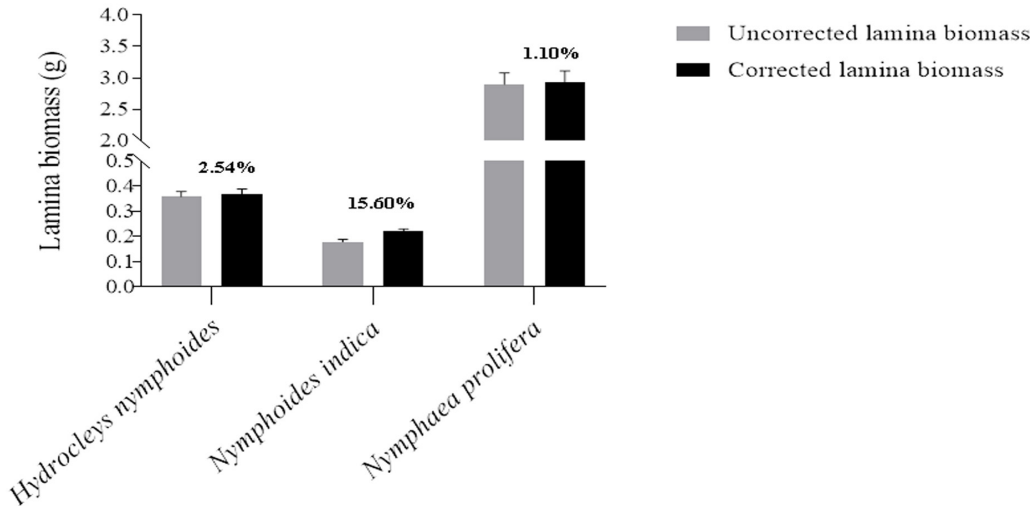


Figure 5 - The uncorrected lamina biomass and the correct lamina biomass per leaf among floating leaf macrophytes species in growth period. The uncorrected lamina biomass is the weight of the lamina measured directly, without including herbivorous damage. The corrected lamina biomass is the weight of the lamina adding herbivorous damage. % indicates biomass removed by herbivory; therefore it indicates underestimation of the leaf lamina biomass in each macrophyte species when the uncorrected lamina biomass is used.

during the sampling. In contrast, on *H. nymphoides*, higher damage by surface abrasion in relation to holes in both growth periods may be related to the predominance of Curculionidae and aphids, which are herbivores with low body biomass that were observed in this macrophyte during sampling and in another study (Martínez F.S. et al., unpublished data, Franceschini et al. 2016).

CORRECTED VERSUS UNCORRECTED LAMINA BIOMASS: DO ECOLOGISTS NEED A CORRECTION FACTOR TO CALCULATE THE LEAF BIOMASS OF FLOATING-LEAF MACROPHYTES?

There is no difference in the results for *H. nymphoides* when the uncorrected lamina biomass and the corrected lamina biomass are compared throughout periods indicating that leaf biomass can be obtained through the direct dry weight of leaves; there is no need either to calculate the biomass removed by herbivores and the corrected lamina biomass. The opposite occurs with *N. indica*, where it is recommended to consider the biomass removed by herbivores to calculate plant biomass and productivity, and to use the corrected lamina

biomass instead of the uncorrected lamina biomass. In fact, differences in results when the uncorrected lamina biomass and the corrected lamina biomass are compared between growth periods support this statement. In addition, herbivorous damage of *N. indica* is significant because up to 15.60% of the leaf lamina biomass and 17.69% of the lamina biomass were removed per m² in the plant population. The importance of considering the values of corrected lamina biomass and biomass removed by herbivores in *N. indica* is consistent with the findings in *E. crassipes* in the study area (Franceschini et al. 2010). Therefore, for the study of biomass and productivity in the study area, we suggest the use of uncorrected lamina biomass on *H. nymphoides*, while in *N. indica* we recommend the use of corrected lamina biomass after estimating the biomass removed by herbivores.

The values of damage per lamina in *N. indica* emphasize the importance of this macrophyte as a food resource for invertebrate herbivores in the tropic webs of the studied wetlands and the role as consumer of live tissue of these invertebrate

herbivores feeding and processing macrophyte biomass in the wetlands. The area damaged per leaf lamina in *N. indica* represents 15.60% of the foliar area, but the effect could be much higher. In fact, the area damaged by herbivores can cause alterations in the photosynthetic and productivity patterns of the leaves in an area 6 to 7 times greater than the area damaged (Zangerl et al. 2002). The low values of damage per lamina and per m² in *H. nymphoides* and *N. prolifera* indicate that the herbivores act mainly as shredders of the living plant tissue, contributing to the decay of the leaves, which would be consumed as detritus mainly by the shredder invertebrate detritivores at the bottom of the wetlands. As *H. nymphoides* and *N. indica* are present throughout the year, it should be pointed out that they are available food resources for invertebrate herbivores during the decay period, when climatic conditions are not ideal and there is a decrease in the plant biomass and leaf density which produce a shortage of resources.

Our hypothesis that invertebrate herbivory is significant in floating-leaf macrophytes, mainly in the growth period and that the damage by herbivores should be taken into account in estimations of plant biomass of these macrophytes, must be partially accepted. Although consumption was higher in the growth period for the macrophyte species analyzed, the damage by herbivores was only significant for *N. indica* in relation to the values estimated. In addition, as high damage per lamina and per m² of vegetation was found only on *N. indica*, the damage by herbivores should only be taken into account in estimations of plant biomass of this macrophyte species.

ACKNOWLEDGMENTS

This study was funded by the PICT 2011-2160 and PICT-2015-1910 projects FONCYT (Argentina), PI: Q001-11 project SEGECYT-UNNE (Argentina). We thank Dr. Martin Videla for the critical reading

of our manuscript and Dr. Analia Lanteri for the identification of Curculionidae.

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