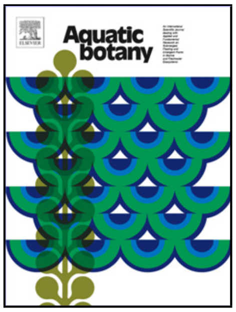

Title: Selection of macrophytes by a generalist invertebrate herbivore and potential impacts for stream rehabilitation

Authors: Laura Estefanía Paz, Ana Clara Ferreira, Máximo A. Simonetti, Alberto Rodrigues Capítulo

PII: S0304-3770(18)30193-1
DOI: https://doi.org/10.1016/j.aquabot.2019.05.006
Reference: AQBOT 3121

To appear in: *Aquatic Botany*

Received date: 16 July 2018
Revised date: 30 May 2019
Accepted date: 31 May 2019

Please cite this article as: Paz LE, Ferreira AC, Simonetti MA, Capítulo AR, Selection of macrophytes by a generalist invertebrate herbivore and potential impacts for stream rehabilitation, *Aquatic Botany* (2019), https://doi.org/10.1016/j.aquabot.2019.05.006

Selection of macrophytes by a generalist invertebrate herbivore and potential impacts for stream rehabilitation.

Laura Estefanía Paz[1,2*], Ana Clara Ferreira[1,2], Máximo A. Simonetti[1], Alberto Rodrigues Capítulo [1,2]

[1-] Instituto de Limnología, Dr.-Raúl A. Ringuelet (ILPLA) (CONICET La Plata-UNLP) La Plata, Buenos Aires, Argentina.

[2]-Facultad de Ciencias Naturales y Museo (UNLP). La Plata, Buenos Aires, Argentina.

Corresponding autor at: Calle 67 y 15, La Plata, Buenos Aires, Argentina. C.P.:1900; estefypaz@hotmail.com

Highlights

- Higher RCR of *P. canaliculata* on *G. spilanthoides* than *E. densa* and *L. peploides*.
- High DMC in macrophytes was related to a low RCR.
- Snails had a selective use of the transplanted beds based on the resources offered.
- *L. peploides* would be a suitable option for transplantation in lowland streams.

Abstract

Macrophytes play an important role as resource for herbivores. When they are introduced into streams by, rehabilitation projects herbivores might be attracted to this new food source. Thus, it is important to know the palatability of macrophytes, especially when high densities of herbivores with a high relative consumption rate (RCR) are present. Plant traits influencing herbivory could be an indicator for macrophyte selection in rehabilitation projects. In the laboratory, we assessed the RCR and preference of *Pomacea canaliculata* for *Ludwigia peploides, Gymnocoronis spilanthoides,* and *Egeria densa,* and linked it to plant chemical and structural traits. We also analysed the use of these macrophytes by the snail in plants transplanted into a recently dredged stream. The macrophyte beds transplanted were the only plants present in the stream. Plants and snails were sampled and snail egg masses quantified. In the laboratory, the RCR of *E. densa* was significantly lower than that of the other species, and the snails did not show any preference among macrophytes. The only trait related to palatability was dry matter content. The abundance of snails in transplanted beds was higher in *E. densa* and *G. spilanthoides,* and lower on *L. peploides* despite its highest coverage. The abundance of egg masses was the highest on *G. spilanthoides*. This indicates that *Pomacea canaliculata* exhibited active selection and differential use of transplanted macrophytes. In conclusion, *L. peploides* would be less affected by a generalist invertebrate herbivore and a suitable option to transplant into stream channels for rehabilitation projects.

1. Introduction

The removal of in-stream vegetation and habitat simplification caused by human activities such as dredging and canalisation causes the loss of the ecological services that streams provide (Suren, 2009). In addition, these practices cause a reduction in the provision of resources such as food (Bakker et al., 2016), the availability of suitable oviposition sites (Reich and Downes, 2003), and protection against possible predators (Schneider and Winemiller, 2008).

Because of the numerous benefits they provide, the transplant of macrophytes into stream channels is a useful technique in recovering the loss of ecological services due to anthropogenic activities (Paice et al., 2015; Paz et al., 2018). Macrophytes play an important role in providing structural heterogeneity and an increase in resource availability (food, protection, refuge and oviposition sites) for aquatic biota (Taniguchi et al., 2003; Burks et al., 2010; Ocon et al., 2013). Furthermore, they affect sediment and nutrient dynamics, are able to modify current velocity, and are highly efficient at removing a variety of contaminants, including trace metal elements (Feijoó and Lombardo, 2007; Guittonny-Philippe et al., 2015; Bonanno et al., 2017).

However, efforts to restore aquatic ecosystems to plant-dominated states in order to improve water quality require a detailed understanding of how plant communities respond to multiple stressors in space and time, and herbivory must be considered as one such of these stressors (Wood et al., 2017; O'Hare et al. 2018). Aquatic plants reintroduced by rehabilitation projects attract herbivores as they provide a new and abundant food source (Noordhuis et al., 2002), preventing macrophytes from increasing their coverage and hampering the re-colonisation process (Sondergaard et al., 2008; Bakker et al., 2013).

Herbivory is highly dependent on plant nutrient quality, as well as on structural and chemical plant traits (Bakker et al., 2016). Therefore, it is important to identify which of these characteristics are related to lower consumption in order to select species that have the highest probability of survival and growth after they are transplanted into streams. Using less palatable species is a way to reduce losses in coverage by grazing and optimise the rehabilitation process, particularly when high densities of herbivores with a high relative consumption rate (RCR) of macrophytes are already present in the stream.

The aquatic apple snail, *Pomacea canaliculata* (Lamarck, 1822) (Gastropoda: Ampulariidae), is native to *del Plata* basin of South America, which encompasses a big area of Argentina and Paraguay, eastern Uruguay and southern Bolivia and Brazil (Thiengo et al., 1993), but was introduced to several countries in Asia in the early 1980s, later becoming an invasive species. Currently, its distribution has spread to Europe (delta of the Ebro River), North America (Mexico and the United States, mainly Hawaii and the southern states of Alabama, Florida, Arizona, Texas and California; Rawlings et al., 2007), and other countries of South America such as Ecuador and Chile (Horgan et al., 2014 a). The introduction of this species has caused severe economic and ecological impacts (Carlsson and Lacoursie, 2005; Wong et al., 2010; Morrinson and Hay, 2011; Horgan et al., 2014 b). For this reason, it is now listed among the worst 100 invasive species (Lowe et al., 2000). This situation has led to intensive research on its feeding habits, although most studies were carried out in the countries it has invaded. Thus, the information reported in our research is relevant to the ecological role and impact of this snail in its native distribution range. *Pomacea canaliculata* feeds mainly on vascular plants (macrophytophagous) (López van Oosterom et al., 2016), so this snail is a real threat to rehabilitation projects that involve macrophytes, both in its native range as well as in invaded freshwater ecosystems (Yam et al., 2016). Nevertheless, its potential effects on transplanted beds or other rehabilitation measures in streams of its native range have not previously been assessed.

We assessed the consumption rate and preference of *P. canaliculata* for three macrophyte species, *Ludwigia peploides* (Kunth) P. H. Raven (Onagraceae), *Gymnocoronis spilanthoides* (Don) DC. (Asteraceae), and *Egeria densa* Planch (Hydrocharitaceae), in relation to some of their most important chemical and structural traits in order to identify which of them make these aquatic plants more palatable and, therefore, less suitable for rehabilitation projects. In a stream that was affected by dredging and canalisation, we analysed the selection and use of these three macrophyte species by *P. canaliculata* in relation to the resources of habitat and oviposition sites offered. Our hypotheses were: 1) *P. canaliculata* has a higher consumption rate on high food quality macrophytes, based on nutrient content, defensive compounds (phenolics) and dry matter content (DMC); 2) this higher consumption rate and preference would be related to active selection and differential use of the transplanted macrophyte beds in the stream.

This study complements a previous study carried out on a lowland stream to analyse the feasibility of the reintroduction of macrophytes for recovery of the structure and complexity of the stream (Paz et al. 2018). Our results will be useful in determining less affected macrophyte species by a generalist herbivore. This information contributes to the improvement of future plant transplant practices in streams based on an integrated vision of resource use.

2. **Methods**

2.1 Laboratory

Two experiments were performed in order to assess the relative consumption rate (RCR, g g^{-1} day $^{-1}$) and host plant preferences of *P. canaliculata* for *L. peploides, G. spilanthoides*, and *E. densa.* The experiments were carried out with macrophytes and juvenile snails collected from the Martín Stream, Buenos Aires, Argentina (34°51'58.62"S; 58°4'7.39"W). Plant species were presented individually to snails in a no-choice feeding experiment in 3 L containers (six replicates for each species and six controls). To test food preference, a choice feeding experiment was performed in which fragments of the three macrophyte species were offered to a single snail at the same time in 3 L containers (sixteen replicates and eight controls).

The snails were acclimated in the laboratory in controlled conditions, providing *ad libitum* food (lettuce: *Lactuca sativa*), aeration, temperature (23 ± 1 °C) and a 12 h light: 12 h dark photoperiod for 15 days. The snails remained without food for 48 h before the experiments, and 34 individuals of 18 - 20 mm shell length were randomly selected for the tests (Baker et al., 2010). Fragments of each plant species with a length of 30 cm (young shoots) were collected in the field on the day when the experiments were performed. The length and weight of all fragments used was the same for all the treatments and replicates (one way ANOVA, $p < 0.05$). These fragments were weighed to the nearest 0.01 mg, using an analytical balance (OHAUS Explorer®) after blotting them with a paper towel (WM0t) and then fed to the snails. The location of the fragments in each containers was randomly set, as well as the location of the containers in the laboratory.

During both experiments, the conditions were the same as those during the acclimation period. Snails were removed after 24 h of grazing and their shell length was measured to the nearest 0.01 mm using a digital calliper. The remaining plant fragments were dried (48 h at 70 °C) to determine their dry mass (DM1t).

The controls of both experiments consisted in containers with fragments of plants without snails. The wet mass of each control fragment was weighed after blotting them with a paper towel at the beginning of the experiment (WM0c), and after 24 h the plant fragments were dried (48 h at 70 °C) to determine their dry mass (DM1c). The control containers were used to estimate what would have been the final dry mass of the plants subjected to herbivory in the absence of grazing, using a linear regression. The regression model between DM1c and WM0c was fixed separately for each plant species following the equations: $\text{DM1c} = a \times \text{WM0c} + b$.

The dry mass consumed was determined by the difference between the final dry mass without grazing (calculated with the estimated coefficients from the regressions of the controls) and their DM1t. The RCR was calculated by dividing the dry mass consumed by the dry mass of the snail (Elger and Barrat- Segretain, 2004). To calculate the dry mass of each snail used in the experiments, a length-mass relationship between the total length of the snail and the shell free dry mass was established. This relationship was assessed assuming that weight and length are related by the general equation $DM = aL^b$, or equivalently, $\log DM = \log a + b \log L$ (where: *a* and *b* are coefficients, DM = dry mass, and L = length). For this purpose, 213 snails were collected from the field and the total shell length of each snail was measured to the nearest 0.01 mm using a digital calliper. Then, the shell was removed and the tissue was dried (48 h at 70 °C) to determine the dry mass to the nearest 0.01 mg. All calculations were made with 'R' (version 3.4.4; R Development Core Team, 2018).

2.2 Structural and chemical plant traits

The macrophyte traits analysed were dry matter content (DMC), nutrient content (C, N and P) and phenolics. For this purpose, fragments (young shoots) of each plant were collected in the field at the same time as the feeding experiments were performed and transported (at 4 °C) to the laboratory. Due to inconveniences during processing and analysis of samples, another set of plant fragments was collected at the same sampling site at the same month (February) and these were used for nutrient content analysis.

Seven fragments of *L. peploides* and *E. densa,* and nine fragments of *G. spilanthoides* were dried (48 h at 70 °C) and then ground and homogenised in an agate mortar. The N content was analysed following the Kjeldahl-N method (APHA, 1998). The concentration of P was determined following Andersen (1976) and then the ascorbic - acid - method (APHA, 1998). The Apollo 9000 Total Organic Carbon (TOC) analyser was used to C content, but in this case, only three samples of each macrophyte could be analysed (n = 9). The C:N, C:P and N:P molar ratios were also calculated (n = 9). Total phenolic content of five fragment of each macrophyte were analysed using the Folin-Ciocalteu Method (Bärlocher and Graça, 2005). To calculate the DMC, nine fragments of each macrophyte were collected in the field. In the laboratory, for each fragment, the wet mass and the dry mass after 48 h at 70 °C was determined to the nearest 0.01 mg, then the DMC was calculated as the ratio between their fresh and dry mass following Burlakova et al. (2008).

2.3 Field sampling

Field sampling was performed in the Martín stream (34°52'5.38"S; 58° 4'9.80"W), a second order lowland watercourse located in the Buenos Aires province of Argentina, which was dredged and channelized before the experiment began. Three beds of the study macrophyte species were transplanted into a section of the Martín stream immediately after dredging and channelization (Paz et al., 2018) so the only macrophytes present in that section at the time of the experiment were the transplanted beds from where the samples were taken. The three beds of each macrophyte were identified and numbered. The first sampling date (T1) was three days after the transplant, then other samples were taken every 25 days (T1: 10th February T2: 7th March T3: 29th March, and T4: 26th April). On each sampling occasion three samples were collected from the beds of the three macrophyte species with a hand net (0.018 m^2; 500 µm pore size) with the aim of collecting *P. canaliculata* individuals. The samples were fixed in situ with 5% formaldehyde and taken to the laboratory where *P. canaliculata* individuals were sorted and counted. At the same time, the number of egg masses deposited by *P. canaliculata* on the aerial parts of the transplanted beds of *G. spilanthoides* and *L. peploides* was recorded in the field.

2.4 Data analysis

The differences between *P. canaliculata's* RCR, host plant preferences, and differential use of each macrophyte were examined using generalised linear model (GLM) and generalised linear mixed model (GLMM). All models were first fitted with a Gaussian error distribution and identity link function. Model residuals were tested for normality using a Shapiro–Wilk test. Since, all model residuals were not normally distributed (Shapiro test: $p < 0.01$), they were refitted using alternative distributions more suited to the response data. Specifically, the gamma error distribution for RCR and preference (link: inversal) and negative binomial error distribution (link = log) was used for count data.

2.4.1 Models with Gamma distribution

The no-choice experiment was analysed using a GLM with RCR as response variable and macrophyte species with three levels as the fixed variable: *L. peploides, G. spilanthoides,* and *E. densa* (formula A). A GLMM was used for the preference experiment; the response variable was RCR with random effect "container" (intercept) because of the lack of independence of the data (each container had one fragment of each macrophyte) and the fixed effect was the macrophyte species, where the three plant species were the levels (formula B). The notation of the models were as follows:

(A)

$$yi = \beta 0 + \beta 1 macrophytes + \varepsilon$$

(B)

$$yi = \beta 0 + \beta 1 macrophytes + b1 container + \varepsilon$$

Where yi is the response variable (RCR), β0 is the intercept, β1 is the coefficient associated with the macrophyte species and $b1$ in the coefficient of the random effect.

2.4.1 Models with negative binomial distribution

The abundance of *P. canaliculata* in the three transplanted macrophyte beds and the egg masses laid in the aerial parts of *L. peploides* and *G. spilanthoides* throughout the experiment were examined using GLMM. The fixed variable was macrophyte species, with three levels: *L. peploides, G. spilanthoides,* and *E. densa* (for the egg masses, the submerged species *E. dens*a was excluded from the analysis). Time was set as random effect (intercept), since knowing the effect on the response variable was not part of the objectives. Considering the changes in the bed size of each species through the experiment due to plant growth (see Paz et al., 2018 for more details), the value of coverage of each beds was added to the model as an "offset" variable (i.e. a variable which affects "y" but the magnitude of its effect is not of interest, Zuur et al., 2009). The notation of the model was as follows:

$$yi = \beta 0 + \beta 1 macrophytes + b1\ time + \varepsilon$$

Where yi is the response variable (abundance of snails or number of egg masses), β0 is the intercept, β1 is the coefficient associated with the macrophyte species and $b1$ in the coefficient of the random effect "time" (T1, T2, T3, T4).

The null model that included no explanatory variables and the random effect was established for each model for further comparison. The comparison between the null and the full model was made with a likelihood ratio test; this was useful for assessing the significance of the model containing predictors of interest (Burnham and Anderson, 2010). When significant differences were found, a Fisher's least significant difference test was used to determine differences between levels of the fixed variable. All the analyses were carried out with 'R' version 3.4.4 (R Development Core Team, 2018) with the packages MASS (Venables and Ripley 2002), MuMIn (Barton, 2013), and lme4 (Bates and Maechler, 2010).

The structural and chemical plant traits of the macrophyte species were compared using a one-way-ANOVA followed by a Tukey post hoc test (Underwood, 1997).

3. **Results**

The linear regression model performed between the DM1c and WM0c for each macrophyte was highly significant and explained a high proportion of variation of the dependent variable, as expressed by the coefficient of determination (*L. peploides:* $p < 0.0001$; $r^2 = 0.94$; *E. densa*: $p < 0.0001$; $r^2 = 0.91$; *G. spilanthoides:* $p < 0.0001$, $r^2 = 0.92$).

Similar results were obtained from the length-mass relationship for the snails ($p < 0.01$; $r^2 = 0.90$). The coefficients are shown in Table 1 and were used to estimate the WM0t, what would have been the final dry mass of the plants subjected to herbivory in the absence of grazing. In the case of the length-mass relationship for the snails, the coefficients were used to estimate the dry mass of the snail from its length (Table 1).

Table 1: Results from the regression models for the dry mass of the three macrophyte species from wet mass and for the dry mass - shell length relationship of *P. canaliculata*. N: number of individual plants or snails used to calculate each regression. Significant results ($p < 0.05$) are shown in bold.

Regressions	Coefficients		r^2	p	n
	a	b			
Ludwigia peploides	0.02	0.13	0.94	**<0.0001**	14
Egeria densa	0.05	0.12	0.91	**<0.0001**	14
Gymnocoronis spilanthoides	-0.07	0.14	0.92	**<0.0001**	14
Pomacea canaliculata	-4.27	2.65	0.90	**0.01**	213

3.1 Laboratory experiments

The results from the GLM analyses showed differences in *P. canaliculata's* RCR of the three macrophytes in the no-choice experiment ($F_{2,15}$ = 18.37; $p < 0.0001$; Fig. 1 a). The least consumed species was *E. densa*, with a RCR of 0.10 ± 0.03 g g^{-1} day^{-1} (mean ± SD) and the coefficient was 9.84 ± 0.76 (β; mean ± SE). The RCR of *G. spilanthoides*, in contrast, showed that it was the most consumed species: 0.22 ± 0.04 g g^{-1} day^{-1} (mean ± SD; β = -5.08; SE = 0.84). Such values, however, were not statistically different from the RCR values recorded for *L. peploides,* 0.17 ± 0.03 g g^{-1}day^{-1} (mean ± SD; β = -4.14; SE = 0.87).

On the other hand, the choice feeding experiment did not show selective consumption among the plant species when they were presented to the snail at the same time ($F_{2,36}$ = 0.47; $p < 0.63$; Fig. 1 b). *Egeria densa* presented a RCR of 0.28 ± 0.09 g g^{-1}day^{-1} (mean ± SD) and the coefficient was 3.59 ± 1.17 (β; mean ± SE). Also, the RCR of *G. spilanthoides* was 0.23 ± 0.08 g g^{-1} day^{-1} (mean ± SD; β = 0.73; SE = 1.92) and the RCR of *L. peploides* was 0.37 ± 0.12 g g^{-1}day^{-1} (mean ± SD; β = - 0.88; SE = 1.48).

The results of nutrients analysis and the structural traits are shown in Table 2. Only the C:P molar ratio showed significant differences, being lower in *E. densa* (mean = 93.30;

SD = 14.17). The DMC, in contrast, showed significant differences among the macrophyte species, with the lowest value being recorded for *G. spilanthoides* (mean = 0.07; SD = 0.01; $p < 0.001$), and the highest concentrations of phenolics (mean = 28.24; SD = 6.60 mg g^{-1}; $p = 0.003$, $F_{2,8} = 22.63$) were recorded in *L. peploides.*

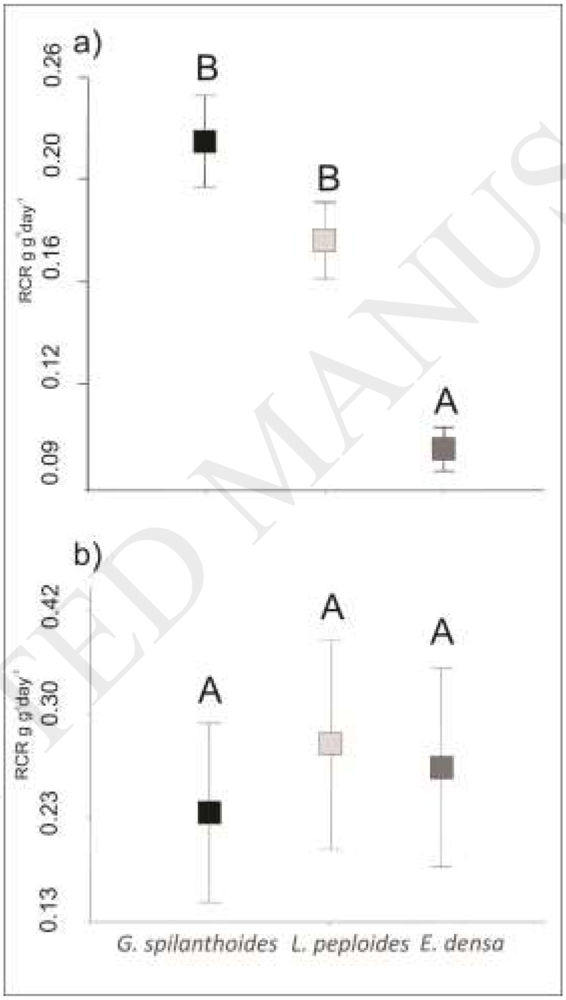

Figure 1: Results from the laboratory experiments. RCR (means ± SD) on the three macrophytes by *Pomacea canaliculata.* a) in the no-choice trial (n = 18); b) in choice feeding trial (n = 16). The same letters indicate membership in homogeneous groups according to

post hoc comparison ($p < 0.05$).

Table 2. Nitrogen (N), carbon (C), phosphorus (P), phenolic, and dry matter content (DMC) as well as C:N, C:P and N:P molar ratios of study macrophytes used in the experiments and field sampling. Data are means ± SD, the same letters (a, b or c) indicate membership in homogeneous groups according to post hoc comparison ($p < 0.05$).

Plant Species	DMC dm:wm	Phenolics mg g^{-1}	P %dm	N % dm	C % dm	C:N Molar ratio	C:P Molar ratio	N:P Molar ratio
Ludwigia peploides	0.07 (± 0.01)^{b}	28.24 (± 6.60)^{c}	0.30 (± 0.04)^{a}	1.91 (± 0.74)^{a}	22.74 (± 2.91)^{a}	20.23 (± 7.16)^{a}	202.77 (± 30.75)^{b}	10.91 (± 3.70)^{a}
Egeria densa	0.08 (± 0.01)^{c}	9.12 (± 1.73)^{a}	0.39 (± 0.11)^{a}	1.30 (± 0.41)^{a}	17.85 (± 3.13)^{a}	16.09 (± 4.64)^{a}	93.30 (± 14.17)^{a}	6.02 (± 1.54)^{a}
Gymnocoronis spilanthoides	0.06 (± 0.01)^{a}	9.41 (± 0.72)^{b}	0.34 (± 0.14)^{a}	1.16 (± 0.17)^{a}	22.47 (± 0.23)^{a}	36.08 (± 17.94)^{a}	207.13 (± 45.80)^{b}	7.04 (± 3.83)^{a}

3.2 Field sampling

The GLMMs with negative binomial error distribution created were significant compared to its corresponding null model based on likelihood ratio tests (abundance of snails: $X^2 = 7.78$; $p < 0.02$; number of egg masses: $X^2 = 6.17$; $p = 0.012$). The abundance of apple snails in *L. peploides* was significantly lower than the abundance found in the other two macrophyte species, *E. densa* ($\beta = 3.41$; SE = 0.93; $Z_{31} = 2.38$; $p = 0.04$) and *G. spilanthoides* ($\beta = - 0.021$;

SE = 0.62; Z_{31} = 2.79; p = 0.01), which showed no difference in abundance between them. The coefficient calculated for *L. peploides* was -1.40 ± 0.58 (β; mean ± SE).

The number of egg masses laid on the aerial parts of the macrophytes was significantly higher for *G. spilanthoides* (52.10 ± 30.00; mean ± SD) than for *L. peploides* (30.80 ± 16.00; mean ± SD).

Details of the densities of the snails (ind m^{-2}) in the transplanted beds over time are shown in Fig. 2. At the beginning of the transplant experiment, the density of the *P. canaliculata* was the highest in *E. densa* beds. However, it decreased during the experiment because of the loss of coverage suffered by this macrophyte, related to a decrease in the water level produced by engineering works (see Paz et al., 2018 for more details). Despite its higher cover during the experiment, the density of snails in *L. peploides* was the lowest. Conversely, relatively high densities of snails were found in beds of *G. spilanthoides* despite its lower cover.

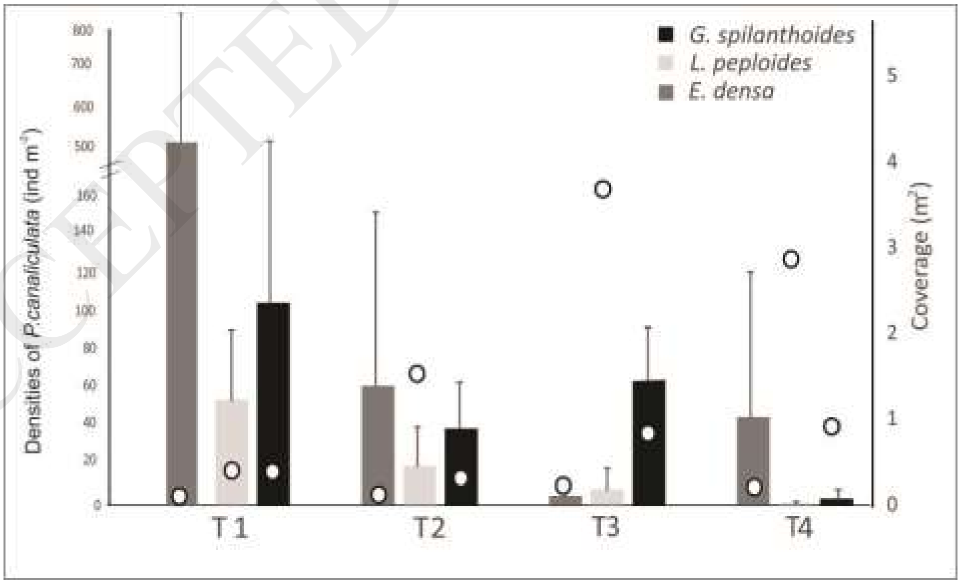

Figure 2: Densities of *P. canaliculata* in beds of the three transplanted macrophyte beds

(mean ± SD; n = 36). The coverage of each macrophyte bed (white circles) during the experiment is shown to relate it to snail abundance (Paz et al. 2018). T1: 10th February T2: 7th March T3: 29th March, and T4: 26th April.

4. **Discussion**

Herbivory is an important biotic process in aquatic ecosystems because it induces changes in plant abundance; higher herbivore density leads to a stronger reduction of plant biomass (Bakker et al., 2013; Wood et al., 2017). Thus, grazing can slow down colonisation or the establishment of transplanted beds (Sondergaard et al., 2008), compromising the success of the rehabilitation technique. Hilt (2006) found more than 90% reduction of *Potamogeton pectinatus* in recently transplanted beds through grazing. Similarly, Lauridsen et al. (2003) lost all their transplanted beds due to herbivory and comparable effects were reported by Hauxwell et al. (2004). Therefore, transplanting species with low palatability, especially when high densities of herbivores with a high RCR are present in target streams, would be an important consideration for improving the success of the technique. The structural and chemical traits analysed would suggest that only the DMC is an indicator of palatability, albeit the consumption rate was highly variable within the study macrophytes. Nonetheless, the field results suggested that *P. canaliculata* displayed selective use of the transplanted bed, but it was not related with a higher consumption rate or a preference for the transplanted macrophyte beds in the stream.

During the no-choice trial, the RCR could be related to the DMC of the study plants. The most consumed species, *G. spilanthoides*, had the lowest DMC, the intermediate value of DMC was associated with intermediate consumption (*L. peploides*), and a high DMC was associated with the lowest consumption (*E. densa*). Our results are consistent with Elger and Willby (2003) and Manara et al. (2018), who pointed out that palatability was related to physical defences. Thus, the DMC could be a suitable indicator of palatability; at it has been previously demonstrated for *P. canaliculata* as well as other invertebrates (Elger and Willby, 2003; Burlakova et al., 2008). Nonetheless, other authors reported that grazing might also be affected by high phenolic content (Wong et al., 2010; Grutters et al., 2017 a) but this was not reflected in this study, since *L. peploides* showed significantly higher phenolic content and its RCR was not lower than the other two species tested. In addition, the emergent species (*G. spilanthoides*) presented intermediate values of phenolic content. Contrary to the findings of Smolders et al. (2000), who reported that emergent species invest more energy in chemical defences than submerged species.

In terms of the nutrient concentration, the values of N and P found during this study were similar to those reported by Güsewell and Koerselman (2001) and Demars et al. (2007). The concentrations of C in plant tissue were lower than values reported by other authors, such as Li et al. (2013) and Grutters et al. (2017 b). Nonetheless, the low values recorded in our study lie within the ranges reported by Duarte (1992) after analyzing 11 species of freshwater angiosperms (between 12 and 55% dw). Spencer and Ksander (1999) also found low values of carbon content in *Ranunculus aquatilis*, which had a mean tissue C content of 26%.

The levels of C, N and P in plant tissue did not show a relationship with RCR, probably because the concentration of these nutrients were similar for the three study macrophytes and thus, these traits were not a good indicator of palatability. Conversely, Wong et al. (2010) found a positive relationship between *P. canaliculata* consumption rate and the N in macrophytes and Yam et al. (2016) reported similar results. However, the nutrient concentration of the macrophytes analysed by these authors were significantly different from one another. In addition, emergent macrophyte species, which present relatively higher C:N and C:P molar ratios because they invest more C in their structural components, present lower consumption rates while, submerged species, with fewer structural components and a consequently lower values in these molar ratios, show higher rates of herbivory (Bakker et al., 2016). Our results indicate that *G. spilanthoides*, an emergent species, showed the highest C:N and C:P molar ratios, but this trait did not seem to have an effect on its palatability, since this plant showed the highest RCR in the no-choice trial. To summarise, the nutrient concentration value of the macrophytes assessed in this study were not related to palatability, in accordance with the results of Manara et al. (2018), but further research is required to clarify this relationship.

Although significant differences were found in phenolics content among macrophytes during this study, these chemical defences did not seem to affect consumption by *P. canaliculata*. Conversely, other authors found a relationship between the RCR and lower concentrations of this compound in plant tissue (Qiu and Kwong, 2009; Wong et al., 2010). Moreover, Yam et al. (2016) found that the growth rate and reproductive output of *P. canaliculata* could be affected by phenolic concentrations in plant tissues, being the values of phenolics reported by those authors similar to those registered in this study.

The choice feeding experiment did not show differences in consumption of the three species of macrophytes offered, although Estebenet (1995), Lach et al. (2000) and Manara et al. (2018) reported a preference by *P. canaliculata* for certain species of aquatic plants. Thus, further research with a greater number of observation and a longer period of exposure may be necessary to confirm that *P. canaliculata* does not show a preference for these macrophytes.

Despite having a low consumption rate in the no-choice trial, the abundance of snails on *E. densa* in the stream was similar to those found on *G. spilanthoides.* Such a preference for the beds of *E. densa* in the stream could be related to this plant species offering a different type of resource to apple snails, for example detritus, another important component of this snail's diet (Lopez van Oosterom et al., 2016). The high complexity of the architecture of this species (Ferreiro et al., 2011) appeared to be the main factor explaining the amount of trapped detritus in the macrophyte bed (Rovira et al., 2016). Additionally, macrophytes with a complex architecture offered more refuge from predators (Schneider and Winemiller, 2008), which also contribute to a greater abundance of snails in transplanted beds of *E. densa*. In the case of *G. spilanthoides*, the snails could be using the transplanted bed not only as a food resource, but also as a refuge and oviposition site. This was in accordance with the results observed by Burks et al. (2010), who found that *Pomacea insularum* actively selected oviposition site compared to its availability. This active selection has also been documented in other invertebrates (Reich and Downes, 2003; Binckley and Resetarits, 2005). *Egeria densa* and *G. spilanthoides* had relatively small beds, the disproportionate use of these resources observed from field sampling, compared with its low availability in the environment is considered a selective use (Beyer et al., 2010).

Despite the information about the feeding habits of *P. canaliculata* in its native distribution given by Estebenet (1995), information about the RCR and preference of *P. canaliculata* on the macrophytes studied here and other native macrophyte species is still scarce (Tamburi and Martín, 2009; Manara et al., 2018). This study is therefore valuable in increasing understanding of the RCR of *P. canaliculata* in its native range. It is also noteworthy that, to date, neither the RCR nor the preference for *G. spilanthoides* by apple snails described above, or by other herbivores have been documented.

On the other hand, Manara et al. (2018) reported that the biomass and coverage of *L. peploides* was not affected by the grazing of *P. canaliculata* in their experiment. In addition, *L. peploides* survived the transplant and could be established and grow in the conditions of the site affected by river engineering works (Paz et al., 2018). Therefore, this species could be a suitable option to transplant into stream channels with the aim of rehabilitating the stream.

Our findings broaden the knowledge base regarding plant transplant techniques with the aim of developing suitable management tools to improve the ecological services of streams. The selection of plant species is relevant to achieving a successful transplant. *Pomacea canaliculata* did not show a clear preference among the three species in the choice feeding trial, therefore that experiment did not help us to select between macrophytes. However, field sampling and other studies suggested that *L. peploides* offers important resources to invertebrates (habitat, refuge, oviposition sites, and periphyton) and it was not affected by the presence of *P. canaliculata*. In addition, this species showed the highest DMC, trait that could be a related to a lower palatability. Therefore, *L. peploides* would be a suitable option for transplantation to lowland streams in rehabilitation projects. Further research is necessary to continue the improvement of the technique and to understand how other invertebrates respond to the reintroduction of aquatic plants in lowland streams.

Acknowledgements

This paper is the Scientific Contribution No. 1139 of the Instituto de Linmología- Dr. Raúl

A. Ringuelet (ILPLA, CONICET La Plata, UNLP). This work was supported by National council research and technology (Argentine) under the Grant PICT 2013- 0810 and the Faculty of Natural Science and Museum (FCNyM, UNLP) under the Grant 738.

References

1. Andersen J M (1976) An ignition method for the determination of total phosphorus in lake sediments. Wat. Res. 10, 329-331.
2. APHA (1998) Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health Association, American Water Works Association and Water Environmental Federation, Washington DC.
3. Baker P, Zimmanck F, Baker S M (2010) Feeding rates of an introduced freshwater gastropod *Pomacea insularum* on native and nonindigenous aquatic plants in Florida. J. Mollus. Stud. 76, 138–143 Doi:10.1093/mollus/eyp050.
4. Bakker E S, Sarneel J M, Gulati R D, Liu Z, van Donk, E (2013) Restoring macrophyte diversity in shallow temperate lakes : biotic versus abiotic constraints. Hydrobiologia 710, 23–37 Doi: 10.1007/s10750-012-1142-9.
5. Bakker E S, Wood K A, Pagès J F, Veen G F (Ciska), Christianen M J A, Santamaría L, Hilt S (2016) Herbivory on freshwater and marine macrophytes: A review and perspective. Aquat. Bot. 135, 18–36 Doi: 10.1016/j.aquabot.2016.04.008.
6. Bärlocher F, Graça MAS (2005) Total phenolics. In Graça MAS, Bärlocher F, Gessner MO (Eds), Methods to Study Litter Decomposition: A Practical Guide. Springer: Dordrecht, The Netherlands, pp. 97–100.
7. Barton K (2013) MuMIn: multi-model inference. R package version 1:11.

8. Bates D, Maechler M (2009) lme4: Linear Mixed-Effects Models Using S4 Classes. R package version 0.999375-32.
9. Beyer H L, Haydon D T, Morales J M, Frair J L, Hebblewhite M, Mitchell M, Matthiopoulos J (2010) The interpretation of habitat preference metrics under use-availability designs. Philos. T. R. Soc. B, Biol. Sci. 365: 2245–2254 Doi: 10.1098/rstb.2010.0083
10. Binckley C A, Resetarits W J (2005) Habitat selection determines abundance, richness and species composition of beetles in aquatic communities. Biol. Lett. 1, 370–374 Doi: 10.1098/rsbl.2005.0310
11. Bonanno G, Borg J A, Di Martino V (2017) Levels of heavy metals in wetland and marine vascular plants and their biomonitoring potential: A comparative assessment. Sci. Total Envirom. 576, 796–806 Doi:10.1016/j.scitotenv.2016.10.17
12. Burks R L, Kyle C H, Trawick M K (2010) Pink eggs and snails: field oviposition patterns of an invasive snail, *Pomacea insularum*, indicate a preference for an invasive macrophyte. Hydrobiologia 646, 243-251.
13. Burlakova L E, Karatayev A Y, Padilla D K, Cartwright L D, Hollas D N (2008) Wetland Restoration and Invasive Species: Apple snail (*Pomacea insularum*) Feeding on Native and Invasive Aquatic Plants. Restor. Ecol. 2, 1–8 Doi: 10.1111/j.1526-100X.2008.00429.x.
14. Burnham K P, Anderson D R (2010) Model selection and multi-model inference. A Practical Information-Theoretic Approach, (2nd edn). Springer, New York.
15. Carlsson N O L, Lacoursière J O (2005) Herbivory on aquatic vascular plants by the introduced golden apple snail (*Pomacea canaliculata*) in Lao PDR. Biol. Invasions

7, 233–241 Doi: 10.1007/s10530-004-0741-4.

16. Demars B O, Edwards A C (2007) Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. Freshwater Biol. 52, 2073–2086. Doi: 10.1111/j.1365-2427.2007.01817.x
17. Duarte C M (1992) Nutrient concentration of aquatic plants: patterns across species. Limnol. Oceanogr. 37, 882-889.
18. Elger A, Willby N J (2003) Leaf dry matter content as an integrative expression of plant palatability: The case of freshwater macrophytes. Funct. Ecol. 17, 58–65 Doi: 10.1046/j.1365-2435.2003.00700.x.
19. Elger A, Barrat-Segretain M H (2004) Plant palatability can be inferred from a single-date feeding trial. Funct. Ecol. 18, 483–488 Doi:10.1111/j.0269-8463.2004.00846.x.
20. Estebenet A L (1995) Food and feeding in *Pomacea canaliculata*. Veliger 38, 277 - 283.
21. Feijoó C S, Lombardo R J (2007) Baseline water quality and macrophyte assemblages in Pampean streams: A regional approach. Wat. Res. 41, 1399–1410 Doi: 10.1016/j.watres.2006.08.026.
22. Ferreiro N, Feijoó, Giorgi A, Leggieri L (2011) Effects of macrophyte heterogeneity and food availability on structural parameters of the macroinvertebrate community in a Pampean stream. Hydrobiologia 664, 199–211 Doi: 10.1007/s10750-010-0599-7.
23. Guittonny-Philippe A, Petit M E, Masotti V, Monnier Y, Malleret L, Coulomb B, Combroux I, …Laffont-Schwob I (2015) Selection of wild macrophytes for use in constructed wetlands for phytoremediation of contaminant mixtures. J Enviro

Manage 147, 108–123 Doi:10.1016/j.jenvman.2014.09.009.

24. Güsewell S, Koerselman W (2002) Variation in nitrogen and phosphorus concentrations of wetland plants. Perspect. Plant. Ecol. 5, 37–61.
25. Grutters B M C, Roijendijk Y O A, Verberk W C E P, Bakker E S (2017 a) Plant traits and plant biogeography control the biotic resistance provided by generalist herbivores. Funct. Ecol. 31, 1184–1192 Doi: 10.1111/1365-2435.12835.
26. Grutters B M C, Saccomanno B, Gross E M, Van de Waal D B, van Donk E, Bakker E S (2017 b) Growth strategy, phylogeny and stoichiometry determine the allelopathic potential of native and non-native plants. Oikos 126, 1770–1779 Doi: 10.1111/oik.03956.
27. Hauxwell J, Frazer T K, Osenberg C W (2004) Grazing by Manatees excludes both new and established Wild Celery transplants: implications for restoration in Kings Bay, FL, USA. J. Aquat. Plant. Manage. 42, 49–53.
28. Hilt S (2006) Recovery of *Potamogeton pectinatus* L. stands in a shallow eutrophic lake under extreme grazing pressure. Hydrobiologia 570, 95–99 Doi 10.1007/s10750-006-0167-3.
29. Horgan F G, Felix M I, Portalanza D E, Sánchez L, Moya Rios W M, Farah S E, Espin E B (2014 a) Responses by farmers to the apple snail invasion of Ecuador’s rice fields and attitudes toward predatory snail kites. Crop. Protection 62, 135–143 Doi: 10.1016/j.cropro.2014.04.019.
30. Horgan F G, Stuart A M, Kudavidanage E P (2014 b) Impact of invasive apple snails on the functioning and services of natural and managed wetlands. Acta Oecol. 54, 90–100. Doi:10.1016/j.actao.2012.10.002

31. Lach L, Britton D, Rundell R, Cowie R (2000) Food preference and reproductive plasticity in an invasivi freshwater snail. Biol. Invasions 2, 279–288.
32. Lauridsen T L, Sandsten H, Møller P H (2003) The restoration of a shallow lake by introducing *Potamogeton spp.*: The impact of waterfowl grazing. Lake Reserv. Manage. 8, 177–188 Doi: 10.1111/j.1440-1770.2003.00224.x.
33. Li W, Cao T, Ni L, Zhang X, Zhu G, Xie P (2013) Effects of water depth on carbon, nitrogen and phosphorus stoichiometry of five submersed macrophytes in an in situ experiment. Ecol. Eng. 61, 358–365 Doi:10.1016/j.ecoleng.2013.09.028
34. López-van Oosterom M V, Ocon C, Ferreira A C, Rodrigues-Capítulo A (2016) The diet of *Pomacea canaliculata* (Gastropoda : ampullariidae) in its native habitat based on gut content and stable isotopes analysis. Intropica 11, 73–83 Doi: 10.21676/23897864.1864.
35. Lowe S, Browne M, Boudjelas S, de Poorter M (2000) 100 of the World's Worst Invasive Alien Species. The Invasive Species Specialist Group, IUCN, Auckland.
36. Manara E, Maldonado M A, Martín P R (2018) The role of an invader in its native range: could differential grazing by apple snails structure the submersed macrophytes assemblages in Southern Pampas (Argentina). Hydrobiologia 828, 229–242 Doi: 10.1007/s10750-018-3815-5.
37. Morrison W E, Hay M E (2011) Feeding and growth of native, invasive and non-invasive alien apple snails (Ampullariidae) in the United States: Invasives eat more and grow more. Biol. Invasions 13, 945–955.
38. Noordhuis R, van der Molen D T, van den Berg M S (2002) Response of herbivorous water-birds to the return of *Chara* in Lake Veluwemeer, the Netherlands. Aquat. Bot.

72, 349–367 Doi: 10.1016/S0304-3770(01)00210-8.

39. O'Hare M T, Aguiar F C, Asaeda T, Bakker E S, Chambers P A, Clayton J S., ...Wood K A (2018) Plants in aquatic ecosystems: current trends and future directions. Hydrobiologia 812, 1-11.
40. Ocon C, López van Oosterom MV, Muñoz MI, Rodrigues Capítulo A. (2013) Macroinvertebrate trophic responses to nutrient addition in a temperate stream in South America. Fund. Appl. Limnol. 182,17-30.
41. Paice R L, Chambers JM, Robson B J (2015) Outcomes of submerged macrophyte restoration in a shallow impounded, eutrophic river. Hydrobiologia 778, 179–192 Doi: 10.1007/s10750-015-2441-8.
42. Paz L E, Nicolosi Gelis M M, Licursi M, Gómez N, Rodrigues Capítulo A (2018) Use of native macrophytes for recovery of the habitat structure and complexity of a lowland stream affected by river engineering works: Implications for management. River Res. Applic. 34, 575-585 Doi: 10.1002/rra.3280.
43. Qiu J W, Kwong K L (2009) Effects of macrophytes on feeding and life- history traits of the invasive apple snail *Pomacea canaliculata*. Freshwater Biol. 54, 1720-1730.
44. R Development Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.
45. Rawlings T A, Hayes K A, Cowie R H, Collins T M (2007) The identity, distribution, and impacts of non-native apple snails in the continental United States. BMC Evol. Biol. 7, 1471–2148 Doi: 10.1186/1471-2148-7-97.

46. Reich P, Downes B J (2003) The distribution of aquatic invertebrate egg masses in relation to physical characteristics of oviposition sites at two Victorian upland streams. Freshwater Biol. 48, 1497-1513 Doi:10.1046/j.1365-2427.2003.01101.x.
47. Rovira A, Alcaraz C, Trobajo R (2016) Effects of plant architecture and water velocity on sediment retention by submerged macrophytes. Freshwater Biol. 61, 758 – 768. doi:10.1111/fwb.12746.
48. Schneider K N, Winemiller K O (2008) Structural complexity of woody debris patches influences fish and macroinvertebrate species richness in a temperate floodplain-river system. Hydrobiologia 610, 235–244. Doi:10.1007/s10750-008-9438-5.
49. Smolders A J P, Vergeer L H T, van der Velde G, Roelofs J G M (2000) Phenolic contents of submerged, emergent and floating leaves of aquatic and semi-aquatic macrophyte species: why do they differ?. Oikos 91, 307–310 Doi: 10.1034/j.1600-0706.2000.910211.x.
50. Sondergaard M, Liboriussen L, Pedersen A R, Jeppesen E (2008) Lake restoration by fish removal: short and long term effects in 36 Danish lakes. Ecosystems 11, 1291–1305 Doi:10.1007/s10021-008-9193-5.
51. Spencer D F, Ksander G G (1999) Phenolic acids and nutrient content for aquatic macrophytes from Fall River, California. J. Freshwater Ecol. 14, 197-209.
52. Suren A M (2009) Using macrophytes in urban stream rehabilitation: A cautionary tale. Restor Ecol 17: 873–883 Doi: 10.1111/j.1526-100X.2008.00446.x.
53. Tamburi N E, Martín P R (2009) Feeding rates and food conversion efficiencies in the apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae).

Malacologia 51, 221–232 Doi:10.4002/040.051.0201.

54. Taniguchi H, Nakano S, Tokeshi M (2003) Influences of habitat complexity on the diversity and abundance of epiphytic invertebrates on plants. Freshwat Biol. 48, 718 – 728 Doi: 10.1046/j.1365-2427.2003.01047.x.
55. Thiengo S C, Borda C E, Araújo J L (1993) On *Pomacea canaliculata* (Lamarck, 1822) (Mollusca; Pilidae: Ampullariidae). Mem. I. Oswaldo Cruz 88, 67-71.
56. Underwood A J (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge: Cambridge University Press Doi: 10.1017/S0025315400072064.
57. Venables W N, Ripley B D (2002) Modern applied statistics with S. Springer, New York.
58. Wong P K, Liang Y, Liu N Y, Qiu JW (2010) Palatability of macrophytes to the invasive freshwater snail *Pomacea canaliculata*: differential effects of multiple plant traits. Freshwater Biol. 55, 2023–2031 Doi: 10.1111/j.1365-2427.2010.02458.x
59. Wood K A, O'Hare M T, Mcdonald C, Searle K R, Daunt F, Stillman R A (2017). Herbivore regulation of plant abundance in aquatic ecosystems. Biol. Rev. 92, 1128 - 1141 Doi:10.1111/brv.12272.
60. Yam R S W, Fan Y T, Wang T T (2016) Importance of macrophyte quality in determining life-history traits of the apple snail *Pomacea canaliculata*: Implications for bottom-up management of an invasive herbivorous pest in constructed wetlands. Int. J. Env. Res. Pub. He. 13, 1–17 Doi: 10.3390/ijerph13030248.
61. Zuur A F, Ieno E N, Walker N J, Saveliev A A, Smith G M (2009) Mixed Effects Models and Extensions in Ecology with R. Gail M, Krickeberg K, Samet J M, Tsiatis

A, Wong W, (Eds). Spring Science and Business Media, New York.