Native snails choose an invasive over a native macrophyte as a food resource

Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2018-0116.R2
Manuscript Type:	Article
Date Submitted by the Author:	10-Oct-2018
Complete List of Authors:	Oliveira, Marcelo; Universidade Estadual de Maringa, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura – Nupélia Dainez, Mario ; Universidade Estadual de Maringa, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura – Nupélia Bertoncin, Ana Paula; Universidade Estadual de Maringa, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura – Nupélia Muniz, Carolina; Universidade Estadual de Maringa, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura – Nupélia Meurer, Thamís; Universidade Federal do Paraná, Departamento de Biodiversidade Figueiredo, Bruno; Universidade Estadual de Maringa, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura – Nupélia Thomaz, Sidnei; Universidade Estadual de Maringa, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura – Nupélia Fávaro, Silvia; Universidade Estadual de Maringa Mormul, Roger; Universidade Estadual de Maringa, Biology
Is your manuscript invited for consideration in a Special Issue?:	Not applicable (regular submission)
Keyword:	Floodplain, Grazing, Biotic resistance, Non-native macrophyte, Hydrilla verticillata, Egeria najas, Pomacea canaliculata

SCHOLARONE™
Manuscripts

Native snails choose an invasive over a native macrophyte as a food resource

Marcelo V. C. Oliveira^{1*}, Mario S. Dainez-Filho¹, Ana P. S. Bertoncin¹, Carolina M. Muniz¹, Thamís Meurer^{1,2}, Bruno R. S. Figueiredo¹, Sidinei M. Thomaz¹, Silvia Luciana Fávaro³ and Roger Paulo Mormul¹

¹ Programa de Pós-graduação em Ecologia de Ambientes Aquáticos Continentais, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura – Nupélia, Universidade Estadual de Maringá – UEM, Bloco H90, Av. Colombo, 5790, CEP 87020-900, Maringá, Paraná, Brazil.

² Departamento de Biodiversidade, Universidade Federal do Paraná, Rua Pioneiro 2153, CEP 85950-000, Palotina, Paraná, Brazil.

³ Departamento de Engenharia Mecânica, Universidade Estadual de Maringá, Av. Colombo, 5790, CEP 87020-900, Maringá, Paraná, Brazil.

*Corresponding author: marvolta@hotmail.com

Phone: 55(44)98834-4272

ABSTRACT

Invasive species cause ecological and economic impacts on invaded ecosystems, although the presence of native species hampers the propagation of invasive species due to biotic resistance. We tested the effects of grazing by the native snail *Pomacea canaliculata* (Lamarck, 1828) over the invasive macrophyte *Hydrilla verticillata* (L. f.) Royle and the native macrophyte *Egeria najas* Planch to evaluate the potential of herbivory as a mechanism to resist invasion. Both macrophyte species were offered, individually and combined, as food resources to the snail to evaluate its feeding preference. The macrophyte energy content and stiffness were also measured. The results indicate a higher *H. verticillata* biomass consumption by *P. canaliculata* when this macrophyte was available both individually and combined with *E. najas*, suggesting that *H. verticillata* is more palatable to the snail, despite this macrophyte being stiffer than the native one. Therefore, *P. canaliculata* may offer resistance to *H. verticillata* invasion. The feeding preference and high rates of herbivory by this snail on *H. verticillata* likely are associated with the higher energy content of the invasive, compared to the native, macrophyte. Experiments combining different food availabilities and snail densities are necessary to evaluate the biological control capacity of *P. canaliculata* under different scenarios.

Keywords: Floodplain. Grazing. Biotic resistance. Non-native macrophyte. *Hydrilla verticillata*. *Egeria najas*. *Pomacea canaliculata*.

INTRODUCTION

Herbivores may prevent the establishment of invasive species and it is one of the mechanisms involved in the biotic resistance hypothesis; (Elton 1958; Catford et al. 2009). Herbivory is an important mechanism in controlling aquatic macrophytes in freshwater ecosystems (Xiong et al. 2008; Li et al. 2009; Schooler et al. 2011; Bakker et al. 2016; Wood et al. 2017; Mormul et al. 2018; O'Hare et al. 2018; Strange et al. 2018), in which snails may negatively impact the macrophytes (Carlsson and Lacousière 2005; Gross and Lombardo 2018). Herbivores may also reduce populations of native macrophyte species, however, thus facilitating colonization by invasive species, which become established due to less regulation by the herbivore than the native species in the invaded habitat (i.e., enemy release hypothesis; Keane and Crawley 2002). Therefore, herbivores choosing between native and invasive macrophyte species may have consequences for the success of the invasive species.

The likelihood of a herbivore choosing a native or invasive macrophyte species as a food item may be related to the randomness of the resource localization (Maiorana 1981; Figueiredo et al. 2015). Food choice is a complex process, involving a balance among resource abundance, nutritional quality, and available energy (Berner et al. 2005; Schallhart et al. 2012). Nutrition quality and available energy could be related to resource palatability. Palatability is not an easy concept to define in relation to biological processes that involve resource selection (Molyneux and Ralphs 1992). The palatability of a food resource is usually associated with features of the tissue, such as its chemical characteristics and structural properties, which are essential to food choice (e.g., Molyneux and Ralphs 1992; Ho and Pennings 2013; Redekop et al. 2018). Moreover, food choice may also be related to the shared evolutionary history of the consumer and its resources (Siemann and Rogers 2003; Lankau et al. 2004). The co-

occurrence of herbivores and plants over evolutionary timescales may favor a defensive advantage for native plants through natural selection, which would decrease the herbivory rate (Hay et al. 1994; Hartley and Jones 1997; Hanley et al. 2007). If so, then it is possible that invasive plant species are chosen by herbivores because the plants do not have sufficient forms of defence to inhibit them (Hokkanen and Pimentel 1989; Colautti et al. 2004). Therefore, understanding which factors drive herbivore food choices could help in determining whether a herbivore will consume both native and invasive submerged macrophytes, and whether the origin of the species matters, as opposed to the palatability of the food.

Pomacea canaliculata (Lamarck 1819) is a herbivore native to South America that also occurs outside its native range in regions such as Asia, where they were introduced for aquaculture purposes (Naylor 1996; Carlsson and Brönmark 2006). In the native range of *P. canaliculata*, there is also the native submerged macrophyte *Egeria najas* Planch, which co-occurs with the invasive submerged macrophyte *Hydrilla verticillata* (L. f.) Royle (Sousa 2011), which is native to Asia and Australia (Cook and Luond 1982). Both macrophytes belong to the same family (Hydrocharitaceae), and have similar ecological and morphological characteristics (e.g., Mormul et al. 2010), but *H. verticillata* has higher invasiveness potential than *E. najas* (Sousa 2011). *Hydrilla verticillata* (L. f.) Royle has spread rapidly in the upper Paraná River in Brazil, but this macrophyte has not successfully colonized floodplain lakes, where *E. najas* occurs at high frequency and with high biomass (Sousa 2011). Some studies have associated the lack of *H. verticillata* in these floodplain lakes to the high concentrations of organic matter in the lake floor sediments (Silveira and Thomaz 2015). On the other hand, the failure of *H. verticillata* to establish in floodplain lakes may also be explained by intense herbivory by *P. canaliculata*, since this snail is found

in high densities in this habitat (R.P.M. pers. obs. 2017). A previous experiment has provided evidence that this snail, along with herbivorous and shredder fish, has the potential to increase resistance against *H. verticillata* invasion (Ribas et al. 2017); however, the mechanisms that explain herbivory (e.g., plant palatability) are less well understood.

Here, we first experimentally evaluated the consumption capacity of *P. canaliculata*, in relation to the native and invasive submerged macrophytes, to assess whether this snail would consume both macrophyte species or not. Then, we measured the stiffness of the macrophytes (which reflects mouthfeel), and the available energy (associated with nutritional quality), of both submerged macrophytes. Finally, we carried out a food choice experiment to determine whether these two facets of palatability could be more important than the life history between consumer and resource, in order to explain the choice of a specific macrophyte.

METHODS

Sampling and acclimation phase

We collected the invasive *H. verticillata* and the native *E. najas* from a floodplain lake connected to the upper Paraná River, Brazil (22°44'56.63"S, 53°15'34.63"W). Before starting the experiment, we acclimated the macrophyte fragments using 24 white polythene containers (diameter = 50 cm) that contained a sediment layer (50% organic matter, 50% sand) 10 cm thick at the bottom, with 80 cm of water-column (60 L of tap water) above. In 12 containers, we planted 24 fragments of *H. verticillata* (15 cm long, two per container), while the other 12 containers received 24 fragments of *E. najas* (15 cm long, two per container). The fragments were planted in the sediment which was collected at the same site of the macrophytes collection and

kept in a greenhouse for 35 days. During this period, there were weekly water exchanges, and dissolved nutrients (nitrogen, phosphorus, and potassium) were added to water of the containers to boost plant growth prior to the experimentation. We added nutrients (N, P, K) with proportions of 4 – 14 – 8, respectively. We carried out the acclimation phase to provide a similar environment for both macrophyte species, regarding nutrient availability in the water and sediment. Also, we checked all of the containers daily to remove potential herbivores.

We surveyed the same floodplain lake where we collected the submerged macrophyte fragments from to obtain *P. canaliculata* individuals. We selected individuals with similar weight (25.05 ± 4.93 g; mean $\pm$ SD), operculum width (36.19 ± 2.54 mm), and shell length (47.46 ± 2.80 mm). These were also acclimated in the greenhouse in 200 L aquaria for 35 days, in which they were free-feeding on *H. verticillata* and *E. najas*, resembling the natural conditions of food availability for the snails and the co-occurrence between submerged macrophytes and the snails.

Consumption experiment

To verify whether *P. canaliculata* would consume both *H. verticillata* and *E. najas* under experimental conditions, we carried out a fast assay inside a greenhouse with stable temperature and light distribution, using 24 rectangular aquaria (width = 22.5 cm, length = 25 cm, height = 35 cm) filled with 12 L of tap water. In this experiment, we measured the amount of macrophyte biomass *P. canaliculata* could consume, with respect to the invasive and native food resources.

All of the food-free aquaria received two individuals of *P. canaliculata*, 48 h prior to the experiment. Then, all of the aquaria received two macrophyte patches containing apical portions (10 cm long) of the macrophyte species taken from the

acclimation containers. Half of the aquaria received *H. verticillata* and the other half *E. najas*. The macrophytes were attached to a plastic net (10 cm x 10 cm) by their lower portions in order to form a monospecific macrophyte patch inside the aquaria. Because the *H. verticillata* apical portions were lighter than the *E. najas* apical portions, we adjusted the number of apical portions in each macrophyte patch, depending on the macrophyte species, to achieve similar macrophyte biomass availability as a food resource to *P. canaliculata*. Each of the *H. verticillata* patches received 12 apical portions (mean fresh weight of $2.24 \text{ g} \pm 0.17 \text{ SD}$), while each of the *E. najas* patches received six apical portions (mean fresh weight of $2.77 \text{ g} \pm 0.18 \text{ SD}$).

We positioned the macrophyte patches and *P. canaliculata* individuals randomly on opposite sides of the aquaria and we monitored the *P. canaliculata* herbivory hourly. We followed the recommendation of Båmstedt et al. (2000) to avoid a decrease in resource availability below 50% because, below this threshold, the consumer could reduce its food intake to keep the food available for an extended period, generating another effect besides the treatment effect *per se*. Therefore, as the macrophyte patches approached resource availability close to 50% (based on visual assessment of the number of apical portions in the aquaria), we removed the macrophyte patches from the experimental unit, and recorded the length of time the macrophyte had been available for *P. canaliculata* herbivory. Using the Båmstedt et al. (2000) approach, ca. 12 h was enough time to achieve fragment availability of around 50% in most of the aquaria, at least regarding one of the macrophyte species, and then, the experiment was over.

We used manual centrifugation to remove the surplus water from all the remaining macrophyte fragments, and thus obtained the remaining macrophyte fresh weight. Because of the variation in initial macrophyte biomass between the experimental units, and due to different experiment durations among the experimental

units, we calculated the percentage of macrophyte biomass consumed (C) in each unit, in relation to the length of time given for consumption in each experimental unit, as follows:

$$C = \frac{IB - FB \times \frac{100}{IB}}{t}$$

Where IB represents the initial macrophyte biomass in the experimental unit, FB represents the final macrophyte biomass in the experimental unit, and t is the length of time (mainly expressed in hours) given for consumption in the experimental unit.

Food choice experiment

After the consumption experiment, we kept *P. canaliculata* individuals without food for 48 h to avoid using satiated individuals in the food choice experiment. We used the same 24 aquaria from the consumption experiment, filled with 12 L of tap water. In the food choice experiment, all aquaria received one macrophyte patch of each macrophyte species, containing the same amount and biomass of fragments used in the consumption experiment. Also, the preparation of the macrophyte patches followed the protocol described previously in the consumption experiment; however, differently from the consumption experiment, both macrophyte patches were added simultaneously, on the same side of the aquaria, while only one *P. canaliculata* individual was added per aquarium in the side opposite to the macrophytes.

The experiment was carried out inside a greenhouse in order to have stable temperature and light distribution. The duration of the experiment, the positions of the macrophyte patches and *P. canaliculata* individuals, and the acquisition of the response variable (percentage of macrophyte biomass consumed) followed the same protocol used in the consumption experiment.

Palatability (stiffness and available energy) assay

We assessed macrophyte stiffness indirectly, using a traction assay carried out in a texturometer (Stable Micro Systems, Surrey, England). Measurements were made on 10 apical fragments of each species (mean diameter: *H. verticillata* = 1.29 ± 0.299 mm; *E. najas* = 1.55 ± 0.105 mm) from each container, obtained at the end of the acclimation phase. All fragments were 15 cm long, and were kept under water until the assay. For each assessment, we dried out the fragments using a hand centrifuge, turning it 30 times. We held the fragment in clamp probes by the apex and the base, so that the extremities were maintained three cm inside the probe, and the fragment remained stretched (without any tensile force, i.e., at rest). We used the texturometer in 'measure force in tension' mode (50 kg load cell) to obtain a relation between stress and strain, in order to determine the elastic modulus of each fragment, following the Callister and Rethwisch (2010) criteria. Elastic deformation is the deformation in which stress and strain are proportional (i.e., when stress and strain have a linear relationship; Callister and Rethwisch 2010). Plotting stress (x axis) versus strain (y axis) results in a linear relationship, the slope of which (angular coefficient) corresponds to the modulus of elasticity (E ; Callister and Rethwisch 2010). Thus, for each stress-strain curve, we took the values between the beginning of stress and the rupture point so as to obtain a linear relationship with less non-explanatory noise. Then, we extracted the angular coefficient (modulus of elasticity) of each plant fragment curve for *E. najas* and *H. verticillata*. The higher the modulus, the stiffer the plant. This approach assumes that the rigidity of the food resource can be considered to be an essential attribute of its palatability to herbivores (e.g., Canhoto and Graça 1999; Yeates and Barmuta 1999; Graça et al. 2001). Considering that hard tissues, such as those containing lignin and cellulose, are

less palatable and digestible (Niklas 1992; Lambers et al. 2008), a high content of hard tissue (and higher *E* values) indicates a less flexible, and so less palatable, plant (Niklas 1992).

To analyze the available energy, we estimated the caloric content of both macrophyte species. We took 12 macrophyte apical portions from each container (12 containers with *E. najas* and 12 containers with *H. verticillata*) at the end of the acclimation phase. The fragments were dried in an oven (48 h at 60 °C), and macerated using a ball-mill until they formed a fine and homogeneous powder, from which we took 0.5 g of each replicate (i.e., from the acclimation container) for calorimetric analysis. We measured the energy density as calories per gram of macrophyte dry weight (cal. g DW⁻¹), using an adiabatic calorimeter (Model Parr 6100).

Data analyses

We used *t*-tests for independent samples to compare the response variables between macrophyte species (*H. verticillata* and *E. najas*) from the consumption experiment (percentage of macrophyte biomass consumed), and the stiffness (modulus of elasticity) and available energy (energy density) assays. We applied a pairwise *t*-test to the percentage of macrophyte biomass consumed to verify whether *P. canaliculata* individuals consumed more *H. verticillata* or *E. najas*. All analyses were performed in Statistica 7.0 software, considering *p* values of < 0.05 as significant results. We used the Levene test and the Shapiro-Wilk test to test data normality and homoscedasticity, respectively. All the data reached the assumptions of normality and homoscedasticity.

RESULTS

Our consumption experiment showed that *P. canaliculata* consumed more biomass of

the invasive *H. verticillata* than of the native *E. najas* ($t_{(d.f. = 14)} = 2.56$, $P = 0.02$; Fig. 1A). *Hydrilla verticillata* (L. f.) Royle fragments had a higher modulus of elasticity than the native *E. najas* fragments ($t_{(d.f. = 25)} = -2.46$, $P = 0.03$; Fig. 1B), indicating that the former species is stiffer than the latter one. Also, we recorded a higher energy density for the invasive *H. verticillata* than for the native *E. najas* ($t_{(d.f. = 22)} = 5.76$, $P < 0.001$; Fig. 1C). Finally, in the food choice experiment, we found results consistent with the consumption experiment. *Pomacea canaliculata* (Lamarck, 1828) chose the invasive *H. verticillata* more often than the native *E. najas* ($t_{(d.f. = 7)} = 3.52$, $P < 0.01$; Fig. 2).

>>> Figure 1

>>>Figure 2

DISCUSSION

Our consumption experiment revealed the generalist character of *P. canaliculata* food intake because the herbivore consumed both the invasive *H. verticillata* and the native *E. najas*. Although being a generalist herbivore, in the consumption experiment, *P. canaliculata* consumed more *H. verticillata*, despite this macrophyte being stiffer than *E. najas*. Also in the food choice experiment, we verified that the consumption by *P. canaliculata* was not only related to random encounters with food; instead, the herbivore freely consumed more of the invasive *H. verticillata*. These results, along with the higher energy content found in *H. verticillata* than in *E. najas*, lead us to assume that the invasive *H. verticillata* is more palatable than *E. najas* to *P. canaliculata*; however, from our study, it seems the palatability of this invasive macrophyte is related only to the higher level of available energy, since *H. verticillata* was shown to be stiffer than *E. najas*. It is likely that the benefits of high energy availability overcame the negative effects of stiffness (unpalatable texture due to high

stiffness demands higher energy spent in consumption), making *H. verticillata* a better resource to *P. canaliculata* than the native *E. najas*. In fact, it is difficult to determine which facet (or combination of facets) of palatability explains the herbivore preference (Redekop et al. 2018). As we evaluated only the stiffness and energy availability, our conclusions about palatability are based on the energy content of *H. verticillata*. Finally, our findings contradict the enemy release hypothesis which suggest that native enemies (herbivores) would provide less pressure (herbivory) on the invasive species arriving in a new habitat compared to native well-established species (e.g., Keane and Crawley 2002), and indicate that nutritional characteristics of the food resource can overcome the lack of life history between the native herbivore and the invasive resource.

The importance of the nutritional characteristics of the resource, as opposed to the life history of the consumer and resource, has been highlighted for other species (e.g., Parker and Hay 2005; Morrison and Hay 2011). From the invasion perspective, our findings indicate that freshwater environments with *P. canaliculata* populations could be less susceptible to *H. verticillata* invasion because *P. canaliculata* has the potential to decrease the density of *H. verticillata*, thereby enhancing resistance against invasion.

The native macrophyte *E. najas* shares a longer life history with *P. canaliculata* than does the invasive *H. verticillata*, the first record of which dates from 2005 in the upper Paraná (Sousa 2011). We observed that *P. canaliculata* feeds on both macrophyte species, but we expected that the shared evolutionary history of the herbivore and *E. najas* would result in higher consumption of the native macrophyte. Our expectation has support in a global meta-analysis about the interaction between herbivores and primary producers, in which Wood et al. (2017) showed that native herbivores can decrease more the abundance of native than invasive plants. However, in our results *P.*

canaliculata more intensively consumed the invasive *H. verticillata*. Thus, an alternative explanation for our findings is that the longer time of co-occurrence of these herbivores and plants may have promoted a defensive advantage for the native plants. For example, plants may present reduced nitrogen concentrations in their tissues, and they may accumulate toxins in response to higher herbivory, which decreases the herbivory rate (Hay et al. 1994; Hartley and Jones 1997; Hanley et al. 2007). Thus, it is possible that invasive plant species are chosen because they do not have sufficient forms of defense to inhibit herbivory, since these species are in a new environment with a new consumer (Hokkanen and Pimentel 1989; Colautti et al. 2004). In addition, *P. canaliculata* found high availability of *H. verticillata*, and probably learned that the consumption of this macrophyte was energetically compensatory.

In the upper Paraná River basin, the population size of *H. verticillata* has increased (Sousa 2011), but this invasive species has not become established in the river's floodplain lakes. One of the main reasons for the failure of *H. verticillata* colonization in this area is likely the high concentration of organic matter in the lake sediments (Silveira and Thomaz 2015); however, *P. canaliculata* does inhabit these lakes, and could be controlling the population size of *H. verticillata* (Ribas et al. 2017), since snail herbivory is reported to control aquatic macrophyte populations (e.g., Sheldon 1987; Pieczynska 2003; Carlsson and Lacousière 2005; Xiong et al. 2008; Li et al. 2009). Indeed, *H. verticillata* has started to colonize these lakes, but only a few young plants have been observed, and they never reach maturity (M.S.D.-F., unpublished data 2017). Furthermore, from the biological invasion perspective, herbivory is one of the biological interactions that can reduce the invasion success (e.g., Rivera et al. 2005; Parker and Hay 2005). Therefore, we suggest that factors other than abiotic characteristics, such as *P. canaliculata* herbivory, are helping to reduce the

success of *H. verticillata* in the shallow floodplain lakes of the upper Paraná River basin.

It is worth noting that the herbivory potential of *P. canaliculata* has also been recognized outside of its native range. In areas where *P. canaliculata* is considered invasive, it causes negative impacts on native populations of the plants it uses as a food resource, resulting in a significant decline in aquatic macrophytes (e.g., Carlsson and Lacousière 2005). In Southeast Asia, *P. canaliculata* is invasive, and its herbivory pressure has caused the devastation of certain rice crops (Halwart 1994), negatively impacting the native semi-aquatic macrophyte species (Carlsson and Lacousière 2005). *Hydrilla verticillata* is a native species from Asia, where *P. canaliculata* was introduced into. Therefore, if *P. canaliculata* was to exert a similar herbivory pressure on *H. verticillata* to that observed in this study, outside of its native range, then we would expect that native populations of *H. verticillata* in Asia could be negatively affected by the herbivory of this snail.

We highlight that our results came from a short-term experiment, with herbivore density and food availability used as a fixed factor, and that food consumption may change with different food availability (e.g., Båmstedt et al. 2000). It also could change in response to different herbivore densities. In addition, we used only two Hydrocharitaceae taxa as a food resource, whilst other macrophyte taxa may present microstructures or secondary compounds that would help to prevent herbivory, and could potentially affect the strength of the consumer–resource interaction (Prusak et al. 2005; Gross and Bakker 2012; Bakker et al. 2016). Hydrocharitaceae species present secondary compounds indeed (e.g., Erhard and Gross 2006; Hilt and Gross 2008) and *E. najas* and *H. verticillata* compounds could be responsible for different epiphyton species attached on them *in situ* (e.g., Mormul et al. 2010). However, we did not find in

the literature, records of secondary compounds from *E. najas* and *H. verticillata* affecting palatability or herbivory. Therefore, we suggest that interpretations of our experimental results, and extrapolations from these for understanding natural interactions *in situ*, should be made with caution.

In summary, our findings indicate that the capacity of plant-herbivore interactions to offer resistance against invasive species would depend, at least in part, on the nutritional characteristics of the different plant species available to the herbivore. Also, in its native range, *P. canaliculata* prefers the invasive *H. verticillata* over the native *E. najas*. This finding suggests that this snail may play a key role in controlling the invasion of *H. verticillata*, promoting resistance and decreasing invasibility. We suggest that, at least for *P. canaliculata*, this control is possible because the nutritional quality of the food resource is more important than life history in the consumer–resource interaction, likely making this snail prefer the invasive over the native macrophyte. Moreover, we hypothesize that, outside of its native range, *P. canaliculata* could negatively impact native macrophyte populations; however, the target populations most affected would be the macrophyte species with the highest energy content. Finally, a wide range of abiotic and biotic interactions can regulate plant communities (Wood et al. 2012; O’Hare et al. 2018). Therefore, we suggest that future studies use gradients of herbivore density, food availability and abiotic factors such as temperature to establish thresholds in which a given herbivory can control the invasive species.

ACKNOWLEDGEMENTS

We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for the continuous funding, the Energetic Ecology Laboratory for supporting the calorimetry procedures, and the Complex of Research Support Centers (COMCAP)

for providing the structure for carrying out the tests in the texturometer. Also, SMT and RPM thank the Brazilian Council for Scientific and Technological Development (CNPq) for the continuing grants. Finally, we thank all who helped in the experiment.

REFERENCES

- Båmstedt, U., Gifford, D.J., Irigoien, X., Atkinson, A., and Roman, M. 2000. Feeding. *In* ICES Zooplankton Methodology Manual. *Edited by* R.P. Harris, P.H. Wiebe, J. Lenz, H.R. Skjoldal, and M. Huntley. New York, Academic Press. pp. 297-399
- Bakker, E.S., Wood, K.A., Pagès, J.F., Veen, G.F., Christianen, M.J.A., Santamaría, L., Nolet, B.A., and 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
- Berner, D., Blanckenhorn, W.U., and Körner, C. 2005. Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. *Oikos*, **111**(3): 525-533. doi:10.1111/j.1600-0706.2005.14144.x
- Callister Jr, W. D., and Rethwisch, D.G. 2010. Materials Science and Engineering, (eight ed.), John Wiley and Sons., New York. 817p.
- Canhoto, C., and Graça, M.A.S. 1999. Leaf barriers to fungal colonization and shredders (*Tipula lateralis*) consumption of decomposing *Eucalyptus globulus*. *Microb. Ecol.* **37**(3):163-172. doi:10.1007/s002489900140
- Carlsson, N.O.L., and Brönmark, C. 2006. Size-dependent effects of an invasive herbivorous snail (*Pomacea canaliculata*) on macrophytes and periphyton in Asian wetlands. *Freshw. Biol.* **51**(4):695-704. doi:10.1111/j.1365-2427.2006.01523.x
- Carlsson, N.O.L., and Lacoursière, J.O. 2005. Herbivory on aquatic vascular plants by the introduced golden apple snail (*Pomacea canaliculata*) in Lao PDR. *Biol.*

- Invasions, **7**(2): 233-241. doi:10.1007/s10530-004-0741-4
- Catford, J.A., Jansson, R., and Nilsson, C. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Divers. Distrib.* **15**: 22-40. doi: 10.1111/j.1472-4642.2008.00521.x
- Colautti, R.I., Ricciardi, A., Grigorovich, I.A., and MacIsaac, H.J. 2004. Is invasion success explained by the enemy release hypothesis? *Ecol. Lett.* **7**(8): 721-733. doi:10.1111/j.1461-0248.2004.00616.x
- Cruz, C., Silva, A.F., Venturini, F.P., Garlich, N., Pitelli, R.L.C.M., and Pitelli, R.A. 2015. Food preference and consumption of aquatic macrophytes submerged by snail *Pomacea canaliculata*. *Planta Daninha*, **33**(3), 433-439. doi:10.1590/S0100-83582015000300006
- Elton, C.S. 1958. The ecology of invasions by animal and plants. Methuen. University of Chicago Press, Chicago and London.
- Erhard, D., and Gross, E.M. 2006. Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. *Aquat. Bot.* **85**: 203-211. doi:10.1016/j.aquabot.2006.04.002
- Ernandes-Silva, J., Ragonha, F.H., Rodrigues, L.C., and Mormul, R.P. 2016. Freshwater invasibility level depends on the population age structure of the invading mussel species. *Biol. Invasions*, **18**(5): 1421-1430. doi:10.1007/s10530-016-1091-8
- Figueiredo, B.R.S., Mormul, R.P., and Benedito, E. 2015. Structural complexity and turbidity do not interact to influence predation rate and prey selectivity by a small visually feeding fish. *Mar. Freshw. Res.* **66**: 170-176. doi:10.1071/MF14030
- Graça, M.A.S., Cressa C., Gessner M.O., Feio, M.J., Callies, K.A., and Barrios, C. 2001. Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshw. Biol.* **46**(7): 947-957. doi:10.1046/j.1365-

2427.2001.00729.x

- Gross, E.M., and Bakker, E.S. 2012. The role of plant secondary metabolites in freshwater macrophyte-herbivore interactions: Limited or unexplored chemical defences? *In* The Ecology of Plant Secondary Metabolites: From Genes to Global Processes. *Edited by* G.R. Iason, M. Dicke and S.E. Hartley. Cambridge University Press. pp.154-169 doi: 10.1017/CBO9780511675751.009
- Gross, E.M., and Lombardo, P. 2018. Limited effect of gizzard sand on consumption of the macrophyte *Myriophyllum spicatum* by the great pond snail *Lymnaea stagnalis*. *Hydrobiologia*, **812**: 131-145. doi:10.1007/s10750-016-2890-8
- Halwart, M. 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *Int. J. Pest Manage.* **40**(2):199-206. doi:10.1080/09670879409371882
- Hanley, M.E., Lamont, B.B., Fairbanks, M.M., and Rafferty, C.M. 2007. Plant structural traits and their role in anti-herbivore defence. *Perspect. Plant Ecol.* **8**(4): 157-178. doi: 10.1016/j.ppees.2007.01.001
- Hartley, S.E., and Jones, C.G. 1997. Plant chemistry and herbivory, or why is the World green? *In* Plant Ecology. *Edited by* M.J. Crawley. Blackwell, Oxford, pp. 284-324.
- Hay, M.E., Kappel, Q.E., and Fenical, W. 1994. Synergisms in plant defenses against herbivores: interactions of chemistry, calcification, and plant quality. *Ecology*, **75**(6): 1714-1726. doi:10.2307/1939631
- Hilt, S., and Gross, E.M. 2008. Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic Appl. Ecol.* **9**: 422-432. doi: 10.1016/j.baae.2007.04.003
- Ho, C.K., and Pennings, S.C. 2013. Preference and performance in plant-herbivore

- interactions across latitude-A study in U.S. Atlantic salt marshes. *Plos One*, **8**(3): 1-11. doi:10.1371/journal.pone.0059829
- Hokkanen, H.M.T., and Pimentel, D. 1989. New associations in biological control: theory and practice. *Can. Entomol.* **121**(10): 829-840. doi:10.4039/Ent121829-10
- Keane, R.M., and Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.* **17**(4): 164-170. doi:10.1016/S0169-5347(02)02499-0
- Lambers, H., Chapin, F.S., and Pons, T.L. 2008. *Plant physiological ecology*. Springer-Verlag New York.
- Lankau, R.A., Rogers, W.E., and Siemann, E. 2004. Constraints on the utilisation of the invasive Chinese tallow tree *Sapium sebiferum* by generalist native herbivores in coastal prairies. *Ecol. Entomol.* **29**(1): 66-75. doi:10.1111/j.0307-6946.2004.00575.x
- Li, K.Y., Liu, Z.W., Hu, Y.H., and Yang, H.W. 2009. Snail herbivory on submerged macrophytes and nutrient release: Implications for macrophyte management. *Ecol. Eng.* **35**(11): 1664-1667. doi:10.1016/j.ecoleng.2008.05.009
- Maiorana, V. C. 1981. Prey Selection by Sight: Random or Economic? *Am. Nat.* **118**(3): 450-451. Available from <https://www.journals.uchicago.edu/doi/abs/10.1086/283838?journalCode=an> [accessed 23 March 2018].
- Molyneux, R., and Ralphs, M. 1992. Plant toxins and palatability to herbivores. *J. Range Manage.* **45**(1): 13-18. Available from <http://www.jstor.org/stable/10.2307/4002519> [accessed 20 March 2018]
- Mormul, R.P., Thomaz, S.M., Silveira, M.J., and Rodrigues, L. 2010. Epiphyton or macrophyte: Which primary producer attracts the snail *Hebetancylus moricandi*? *Am. Malacol. Bull.* **28**: 127-133. doi:10.4003/006.028.0205

- Mormul, R.P., Ahlgren, J., and Brönmark, C. 2018. Snails have stronger indirect positive effects on submerged macrophyte growth attributes than zooplankton. *Hydrobiologia*, **807**(1):165-173. doi:10.1007/s10750-017-3391-0
- Morrison, W.E., and Hay, M.E. 2011. Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *Plos One*, **6**(3): e17227. doi:10.1371/journal.pone.0017227.
- Naylor, R. 1996. Invasions in agriculture: assessing the cost of the golden apple snail in Asia. *Ambio*, **25**(7): 443-448. Available from <http://www.jstor.org/stable/4314515> [accessed 20 March 2018].
- Niklas, K.J. 1992. *Plant Biomechanics: An engineering approach to plant form and function*. University of Chicago Press, Chicago.
- O'Hare, M.T., Aguiar, F.C., Asaeda, T., Bakker, E.S., Chambers, P.A., Clayton, J.S., Elger, A., Ferreira, T.M., Gross, E.M., Gunn, I.D.M., Gurnell, A.M., Hellsten, S., Hofstra, D.E., Li, W., Mohr, S., Puijalón, S., Szoszkiewicz, K., Willby, N.J., and Wood, K.A. 2018. Plants in aquatic ecosystems: current trends and future directions. *Hydrobiologia*, **812**: 1-11. doi: 10.1007/s10750-016-2890-8
- Parker, J.D., and Hay, M.E. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecol. Lett.* **8**(9): 959-967. doi:10.1111/j.1461-0248.2005.00799.x
- Pieczynska, E. 2003. Effect of damage by the snail *Lymnaea (Lymnaea) stagnalis* (L.) on the growth of *Elodea canadensis* Michx. *Aquat. Bot.* **75**(2): 137-145. doi:10.1016/S0304-3770(02)00170-5
- Prusak, A.C., O'Neal, J., and Kubanek, J. 2005. Prevalence of chemical defenses among freshwater plants. *J. Chem. Ecol.* **31**: 1145-1160. doi:10.1007/s10886-005-4253-1
- Qiu, J.W., Chan, M.T., Kwong, K.L., and Sun, J. 2011. Consumption, survival and

- growth in the invasive freshwater snail *Pomacea canaliculata*: does food freshness matter? J. Mollus. Stud. **77**(2): 189-195. doi:10.1093/mollus/eyr005
- Redekop, P., Gross, E.M., Nuttens, A., Hofstra, D.E., Clayton, J.S., and Hussner, A. 2018. *Hydraula nitens*, the only native aquatic caterpillar in New Zealand, prefers feeding on an alien submerged plant. Hydrobiologia, **812**(1): 13-25. doi:10.1007/s10750-016-2709-7
- Ribas, L.G.S., Cunha, E.R., Vitule, J.R.S., Mormul, R.P., Thomaz, S.M., and Padial, A.A. 2017. Biotic resistance by snails and fish to an exotic invasive aquatic plant. Freshw. Biol. **62**(7): 1266-1275. doi:10.1111/fwbi.12943
- Rivera, C.E., Ruiz, G.M., Hines, A.H., and Jivoff, P. 2005. Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. Ecology, **86**(12): 3364-3376. doi:10.1890/05-0479
- Schallhart, N., Tusch, M.J., Wallinger, C., Staudacher, K., and Traugott, M. 2012. Effects of plant identity and diversity on the dietary choice of a soil-living insect herbivore. Ecology, **93**(12): 2650-2657. doi:10.1890/11-2067.1
- Schooler, S.S., Salau, B., Julien, M. H., and Ives, A.R. 2011. Alternative stable states explain unpredictable biological control of *Salvinia molesta* in Kakadu. Nature, **470**(7332): 86-89. doi:10.1038/nature09735
- Sheldon, S.P. 1987. The effects of herbivorous snails on submerged macrophyte communities in Minnesota lakes. Ecology, **68**(6):1920-1931. doi:10.2307/1939883
- Siemann, E., and Rogers, W.E. 2003. Herbivory, disease, recruitment limitation, and success of alien and native tree species. Ecology, **84**(6): 1489-1505. doi:10.1890/0012-9658(2003)084[1489:HDRLAS]2.0.CO;2
- Silveira, M.J., and Thomaz, S.M. 2015. Growth of a native versus an invasive submerged aquatic macrophyte differs in relation to mud and organic matter

- concentrations in sediment. *Aquat. Bot.* **124**: 85-91.
doi:10.1016/j.aquabot.2015.03.004
- Sousa W.T.Z. 2011. *Hydrilla verticillata* (Hydrocharitaceae), a recent invader threatening Brazil's freshwater environments: a review of the extent of the problem. *Hydrobiologia*, **669**: 1-20. doi:10.1007/s10750-011-0696-2
- Strange, E.F., Hill, J.M., and Coetzee, J.A. 2018. Evidence for a new regime shift between floating and submerged invasive plant dominance in South Africa. *Hydrobiologia*, **817**(1): 349-362. doi:10.1007/s10750-018-3506-2
- Xiong, W., Yu D., Wang, Q., Liu, C., and Wang, L. 2008. A snail prefers native over exotic freshwater plants: Implications for the enemy release hypotheses. *Freshw. Biol.* **53**(11): 2256-2263. doi:10.1111/j.1365-2427.2008.02058.x
- Yeates, L.V., and Barmuta, L.A. 1999. The effects of willow and eucalypt leaves on feeding preference and growth of some Australian aquatic macroinvertebrates. *Aust. J. Ecol.* **24**(6): 593-598. doi:10.1046/j.1442-9993.1999.01008.x
- Wood, K.A., O'Hare M.T., McDonald C., Searle, K.R., Daunt F., and Stillman, R.A. 2017. Herbivore regulation of plant abundance in aquatic ecosystems. *Biol. Rev. Camb. Philos. Soc.* **92**: 1128-1141. doi:10.1111/brv.12272
- Wood, K.A., Stillman, R. A., Clarke, R.T., Daunt, F., and O'Hare, M.T. 2012. Understanding plant community responses to combinations of biotic and abiotic factors in different phases of the plant growth cycle. *Plos One*, **7**(11): e49824. doi:10.1371/journal.pone.0049824

Figure captions

Figure 1. Mean and standard errors for the percentage of macrophyte biomass consumed in the consumption experiment (corrected for experiment duration) (A; $n = 12$ aquaria for each macrophyte). Mean and standard errors for the modulus of elasticity (B; $n = 10$ fragments of each macrophyte). Mean and standard errors for the energy density (C), obtained from the stiffness and available energy assays ($n = 12$ containers for each macrophyte).

Figure 2. Mean and standard errors for the percentage of macrophyte biomass consumed in the food choice experiment (corrected for experiment duration) ($n = 24$ aquaria with both macrophytes).

Draft

Figure 1

Figure 2

Draft