

JUVENILE SNAILS, ADULT APPETITES: CONTRASTING RESOURCE CONSUMPTION BETWEEN TWO SPECIES OF APPLESNAILS (*POMACEA*)

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ABSTRACT

Research on aquatic snails usually examines consumption of periphyton, but emergence of large, invasive aquatic snails that prefer macrophytes has necessitated a new understanding about snail herbivory. Ample research exists detailing invasive potential of certain species of applesnails, such as *Pomacea canaliculata*, to successfully invade aquatic ecosystems. However, very few studies examine differences in resource utilization between different size-classes within species, or between closely-related species. To quantify these potential differences, we compared per mass resource consumption at two life history stages by *P. canaliculata* and a lesser-known species recently identified in Texas (USA), *Pomacea insularum*. We presented adult and juvenile snails with whole and reconstituted forms of *Lactuca sativa longifolia* (romaine lettuce), *Myriophyllum* spp. (watermilfoil), and *Eichhornia crassipes* (water hyacinth). In addition, we added chemical extracts to reconstituted watermilfoil and water hyacinth to test if extracts deterred consumption. Addition of periphyton to reconstituted watermilfoil allowed us to examine supplementary structure and chemistry. Juveniles seemed to prefer reconstituted resources. All snails, regardless of life-history stage, avoided water hyacinth in either form. Chemical extracts from both water hyacinth and watermilfoil deterred consumption by all snails. When presented with reconstituted watermilfoil containing additional periphyton, juvenile *P. insularum* consumed more resource with additional periphyton. In contrast, periphyton presence did not produce a noticeable effect on *P. canaliculata* consumption. Overall, juveniles of both species consumed considerably more by mass than their respective adult counterparts. Through increased numbers and difficult detection, juvenile applesnails could feasibly consume a greater proportion of plant biomass than adult applesnails and this may partially underlie the success of global applesnail invasions.

INTRODUCTION

Advantages and disadvantages of size often determine the outcome of ecological interactions (Emmerson & Raffaelli, 2004; Layman *et al.*, 2005). However, relying on size alone to estimate the potential of herbivores to impact aquatic communities can yield mixed results. For example, Lodge *et al.* (1998) found that intermediate-sized consumers, including fish and crayfish, resulted in the largest reduction of macrophyte biomass. Their analysis further suggests that the smallest herbivores, snails and insects, mainly feed on periphyton and not aquatic plants. Recent mollusc invasions, however, tell a different story. Numerous recent studies show large-scale negative ecological and economic effects from snail herbivory on aquatic plants in invaded areas (Kerans *et al.*, 2005; Hall, Dybdahl & VanderLoop, 2006). Fist-sized, *Pomacea canaliculata* have demonstrated voracious consumption of both weeds and crops as invaders of Asian rice fields (Howells *et al.*, 2006).

Beyond size differences between species, larger size of adult snails compared to their juvenile counterparts suggests they represent a greater threat to aquatic vegetation. Reliable predictions of consumption rates of herbivores become more difficult if ontogenetic diet shifts occur, but this is not a problem in gastropods (Dillon, 2000), including *P. canaliculata* (Carlsson & Brönmark, 2006). Comparing adults versus juveniles provides

a surrogate for examining the impact of an established population versus a population in the early state of invasion. Although adult gastropods may seem a larger threat, greater consumption by juveniles could produce more damage to aquatic vegetation. Therefore, the life-history stage of a snail by itself may not encompass the breadth of its impact and may depend on species.

Though large for a freshwater snail, populations of *P. canaliculata* in their native range of Argentina and Uruguay (Rawlings *et al.*, 2007) coexist with an abundance of aquatic vegetation (Meerhoff, personal observation). However, with extensive exotic ranges (Rawlings *et al.*, 2007; e.g. Southeast Asia, Sri Lanka, Guam, Hawaii, Papua New Guinea, the Dominican Republic, California, Arizona and possibly Australia), *P. canaliculata* constitutes a serious agricultural and ecological pest, recently earning dubious honours as one of the 100 worst exotic species globally (Lowe *et al.*, 2004). In comparison, the much less well-known but larger invasive *P. insularum* may pose a considerable threat to agriculture and shallow areas in which the species recently established exotic populations in the USA (i.e. Texas, Georgia, Alabama, Florida) (Howells *et al.*, 2006). Most introduced *P. insularum* specimens indicate a native habitat near Buenos Aires (Río Uruguay), Argentina, but this species probably also occurs in Brazil, Bolivia, Uruguay and Paraguay (Rawlings *et al.*, 2007).

High fecundity coupled with a big appetite for macrophytes contributes to the danger presented by *P. insularum*. Of the two

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species *P. canaliculata* and *P. insularum*, substantially more research exists on *P. canaliculata*; studies on *P. insularum* are virtually nonexistent. Discrepancies in reproductive strategies (M.A. Barnes, unpubl.) between the two species could outweigh greater consumption observed in *P. canaliculata*. Our research investigates if contrasting trends occur in consumption by different size classes of the same species, or between two different *Pomacea* species. Both species exhibit impressive fecundity (Cowie, 2002; Cowie, Hayes & Thiengo, 2006). Laboratory observations suggest that *P. insularum*'s reproductive capacity exceeds that of *P. canaliculata*. Clutches laid by *P. insularum* contain substantially more eggs (~1,600 per clutch) than *P. canaliculata* clutches (~158 per clutch; Estoy *et al.*, 2002). Smaller egg size in *P. insularum* compared to larger eggs in *P. canaliculata* partially explains differences in clutch size (M.A. Barnes, unpubl.). Field hatching rates of around 70% by *P. insularum* (R.L. Burks, unpubl.) suggest that consumption alone may not illuminate the whole threat posed by *P. insularum*.

Numerous biotic and abiotic factors influence the feeding activity of an herbivore. For example, Dorn, Cronin & Lodge (2001) found that chemical cues derived from waterlilies (*Brasenia*) and previous feeding experience promoted consumption by the semi-aquatic lepidopteran, *Munroessa gyralis*. Compared to vascular plants, the absence of lignin, folicles, and other structurally deterrent factors may make periphyton a more accessible resource. However, in an enclosed environment, the generalist snail *P. canaliculata* reduced biomass of both macrophytes and periphyton (Carlsson & Brönmark, 2006). When tough structure was removed from the plant, Cronin *et al.* (2002) found that crayfish (*Procambarus clarkii*) changed their preference to favour nutrient rich resources over less structurally defended, nutrient-poor resources. Research on aquatic snails has often focused on consumption of periphyton (Brönmark, 1990), but emergence of invasive aquatic snails that prefer macrophytes has necessitated a new understanding about snail herbivory.

We conducted three experiments (Table 1) that examined resource consumption of adult and juvenile *P. insularum* in its

exotic range (Texas) and *P. canaliculata* in its native range (Uruguay). The use of three plants that varied in their structural and chemical defences, allowed us to investigate mechanisms behind feeding of juveniles and adults. To determine if plant structure deterred consumption, we modified a method (Hay *et al.*, 1998) to remove plant structure and provided snails with only the chemical plant components (i.e. reconstituted) versus whole plant structure. To access potential inhibitory effects of plant chemistry on consumption, we provided reconstituted plants with or without additional plant metabolites. We also tested for the influence of additional periphyton on juvenile applesnail consumption. Research focused on mechanisms underlying consumption may provide means to mitigate the damage caused by invasive applesnails and alter management plans depending on the prevalence of juvenile snails.

MATERIAL AND METHODS

Field collection of snails

We performed laboratory experiments with exotic *Pomacea insularum* at Southwestern University, Texas (USA) and with native *P. canaliculata* at Facultad de Ciencias, Universidad de República, in Montevideo, Uruguay. We collected adult *P. insularum* from Armand Bayou (29.563°N, 95.071°W) near Houston, TX (USA) during August 2005. We found adult snails primarily at the base of elephant ear (*Colocasia* spp.) stands during the cooler temperatures of late afternoon. The laboratory maintained a population of exotic, adult *P. insularum* at Southwestern University in a 750-l flowthrough system. Four 250-W aquarium heaters kept the water between 21.1°C and 22.8°C.

Many researchers use shell height as an indicator of adult size in applesnails (Cazzigna, 2006; Youens & Burks, 2008), but others (Youens & Burks, 2008) have found that operculum width and weight exhibit the best predictive relationship of each other ($R^2 = 0.978$). In addition, no published studies exist regarding size at first reproduction for *P. insularum* to use as a guideline for determining when snails should be considered

Table 1. Summary of the experiments performed showing snail attributes, the resource species (L = *Lactuca sativa longifolia*; M = *Myriophyllum* spp.; E = *Eichhornia crassipes*), form (whole or reconstituted), and quantity (plant mass or strip size) for each experiment is indicated below mass ranges.

	<i>P. insularum</i>		<i>P. canaliculata</i>	
	Adults	Juveniles	Adults	Juveniles
Structure				
Average snail mass (g ± 1 SD)	106.8 ± 23.8	5.94 ± 2.88	24.56 ± 7.30	2.38 ± 0.66
Mass range (g)	62.56–153.47	1.35–12.74	16.5–49.6	1.4–3.6
Plant species	L, M, E	L, M, E	L, M, E	L, M, E
Plant form	Whole vs. Reconstituted	Whole vs. Reconstituted	Whole vs. Reconstituted	Whole vs. Reconstituted
Resource quantity	8 g vs. 1 strip	8 g vs. 1/2 strip	2–5 g vs. 1/2 strip	1–2 g vs. 1/3 strip
Chemistry				
Average snail mass (g ± 1 SD)	100.54 ± 26.93	6.97 ± 2.94	21.35 ± 7.72	5.91 ± 1.93
Mass range (g)	46.06–163.82	2.16–13.31	10.2–37.5	3.1–10.7
Plant species	M, E	M, E	M, E	M, E
Plant form	Reconstituted	Reconstituted	Reconstituted	Reconstituted
Resource quantity	1 strip	1/2 strip	1/2 strip	1/3 strip
Periphyton				
Average snail mass (g ± 1 SD)	Experiment not conducted	6.26 ± 3.07	21.17 ± 7.92	3.06 ± 1.36
Mass range (g)		1.71–14.18	11.1–33.7	1.4–6.8
Plant species		M (US & UR)	M (US & UR)	M (US & UR)
Plant form		Reconstituted	Reconstituted	Reconstituted
Resource quantity		1/2 strip	1/2 strip	1/2 strip

as adults. Using age as a surrogate for size, we used *P. insularum* between 6 months and 1 year of age as our juveniles (Table 1). We grew these snails from egg clutches because we could not establish a consistent method for location of juvenile *P. insularum* in the field.

We collected adult and juvenile *P. canaliculata* from shallow areas on submerged rocks and vegetation at two lakes (Diario and Sauce) near Maldonado, Uruguay (34.900°S, 54.940°W) during December of 2005 (summer). We maintained snail cultures in two 200-l drums at Facultad de Ciencias in Montevideo, Uruguay (34.883°S, 56.183°W). We also separated *P. canaliculata* adults and juveniles based on mass, with adults weighing greater than 6 g and juveniles less than 6 g (fresh weight including shell, Table 1).

Field collection of plants

We used three plants in our study to allow for consistent comparison between snail species. Romaine lettuce provided little physical defence and laboratory observations suggest few, if any, chemical defences to snail herbivory. Watermilfoil offered limited physical defence but has noted chemical defences against herbivory (Gross, 2003; Burks *et al.*, 2006). Water hyacinth, a freely-floating macrophyte with broad, lignin-rich leaves, provided substantial physical defence (Casco *et al.*, 2003). We collected *Myriophyllum* (watermilfoil) and *Eichhornia crassipes* (water hyacinth) from the field, and obtained *Lactuca sativa longifolia* (romaine lettuce) from the local market in both the US and Uruguay. From Lake Austin in Texas (30.329°N, 97.838°W), we collected a known invasive and chemically defended species of *Myriophyllum*, *M. spicatum* (Eurasian watermilfoil) (Burks *et al.*, 2006). The water hyacinth we used in Texas came from Armand Bayou. However, although we could obtain the same species of both water hyacinth and romaine lettuce in Uruguay and Texas, we could not determine the exact species of watermilfoil we collected in Uruguay. Therefore, we refer to watermilfoil collected in Uruguay as *Myriophyllum* sp. All snails consumed a regular diet of romaine lettuce.

We used *M. spicatum* for all experiments done with *P. insularum*, except when we also offered juveniles unidentified watermilfoil from Uruguay (*Myriophyllum* sp.) with or without periphyton (see 'Influence of periphyton' below). When we tested the influence of structure and chemistry in Uruguay, juvenile and adult *P. canaliculata* received whole *Myriophyllum* sp. or whole *E. crassipes* from Uruguay, but reconstituted *M. spicatum* or *E. crassipes* from Texas. We could not use reconstituted watermilfoil or water hyacinth directly from Uruguay in our *P. canaliculata* experiments due to the time required to make powder (see next section). To summarize, the same species of water hyacinth occurs in Texas and Uruguay, but not *Myriophyllum*. However, many species of *Myriophyllum* harbour secondary chemicals that deter herbivory or act in an allelopathic manner (Gross, 2003; Burks *et al.*, 2006). To address this use of different species, we tested whether the origin of the *Myriophyllum* altered consumption of reconstitute by juveniles of both applesnail species.

Influence of plant structure

To determine how plant structure might alter *P. insularum* consumption (Fig. 1), we modified a procedure that Hay *et al.* (1998) developed to test herbivory in marine and freshwater invertebrates. We ground whole plant for 20 s in a food processor, evaporated bulk water at 60°C for 1 h, and lyophilized material for 24 h to remove trace moisture. We used the greener tips of *L. sativa longifolia*, and predominantly leaves of

M. spicatum and *E. crassipes* due to higher nutrient and lower water content (Almeida-Cortez, Shipley & Arnason, 1999). Lyophilization produced a 9.24% per gram yield for *L. sativa longifolia*, 13.9% for *M. spicatum*, and 7.47% for *E. crassipes*. Following lyophilization, we powdered the material in a coffee grinder for 1 min and then mixed the powder with sodium alginate (details of quantities below) and 15 ml distilled water to create reconstitute. We applied reconstitute paste to aluminum window screening (12 × 12 cm; 1.5 mm² per hole) and pressed this into the screen to adhere. Two ceramic tiles pressed on both sides produced a uniform reconstitute depth of approximately 1.5 mm while wax paper over the reconstitute prevented sticking. Reconstitute dried for 24 h prior to experimentation. Using a microscope slide as a stencil, we then cut reconstitute into 7.6 × 2.3 cm sized strips to produce three replicates. We found variations in consistency and dissimilar whole plant to powder yields among plant species. To compensate, we used a different ratio of sodium alginate to plant powder for each plant species to allow consistent reconstitute structure across plant species [ratio of sodium alginate (g) to plant powder (g) of 3:2 (1 g plant powder) for *L. sativa longifolia* and *E. crassipes* and 3:1 (0.5 g plant powder) for *M. spicatum*]. This resulted in 3.57 g of plant material per strip for *L. sativa longifolia*, 4.42 g for *E. crassipes* and 1.20 g for *M. spicatum* [(grams of plant powder/strip size)/(whole plant per gram yield) = grams of whole plant].

Feeding experiments in Texas with *P. insularum* served as the template for our experiments in Uruguay with *P. canaliculata*. To test the influence of plant structure on *P. insularum* consumption, we employed a 2 × 3 factorial design ($N = 5$) consisting of plant form (either whole or reconstituted) and plant species (*L. sativa longifolia*, *M. spicatum*, and *E. crassipes*). For all experiments with *P. insularum*, we pre-weighed snails and placed individual adults (>6 g) in 10-l aerated containers with 7 l dechlorinated tap water and individual juveniles (<6 g) in 1-l plastic bowls with 800 ml water. Due to the size of snails, we varied the amount offered in different experiments (Table 1), although all treatments received equivalent amounts based on snail size. In all experiments, snails fed for 24 h (12 light/12 dark) under consistent temperature conditions. After this period, we removed snails and either weighed remaining whole plant (after drying) or calculated percent consumption per mass of reconstituted plant matter by counting the number of visible screen holes after reconstitute consumption to determine percent consumed (one strip contains 636 holes). We used percent consumption per mass in all experiments to compensate for mass differences between whole and reconstituted resources (Figs 1–3). Although a nontraditional expression of consumption, we chose this unit because the nature of the recovered reconstitute prevented reliable mass measurements (i.e. mg), but allowed clear visual assessment of percentage (%) of resource consumed by placing it on a standard screen. This also eliminated discrepancy between snail masses.

We tested the influence of plant structure on *P. canaliculata* with the same design used for *P. insularum*. Due to limited experiment time in Uruguay, we brought all plant powders for reconstitution from Texas. All powders remained vacuum sealed until use. For all experiments with *P. canaliculata*, we pre-weighed snails and placed adults in 20-l aerated buckets with 20 l water and juveniles in 1-l plastic bowls with 800 ml water. The smaller size of *P. canaliculata* compared to *P. insularum* resulted in adult *P. canaliculata* receiving less resource (Table 1). We removed snails after the 24 h feeding period and calculated percent consumption per mass as described above. After employing a KS-test to assure normality, we employed two-way ANOVAs for both experiments to determine if plant species or form affected consumption for each size class. A Tukey's

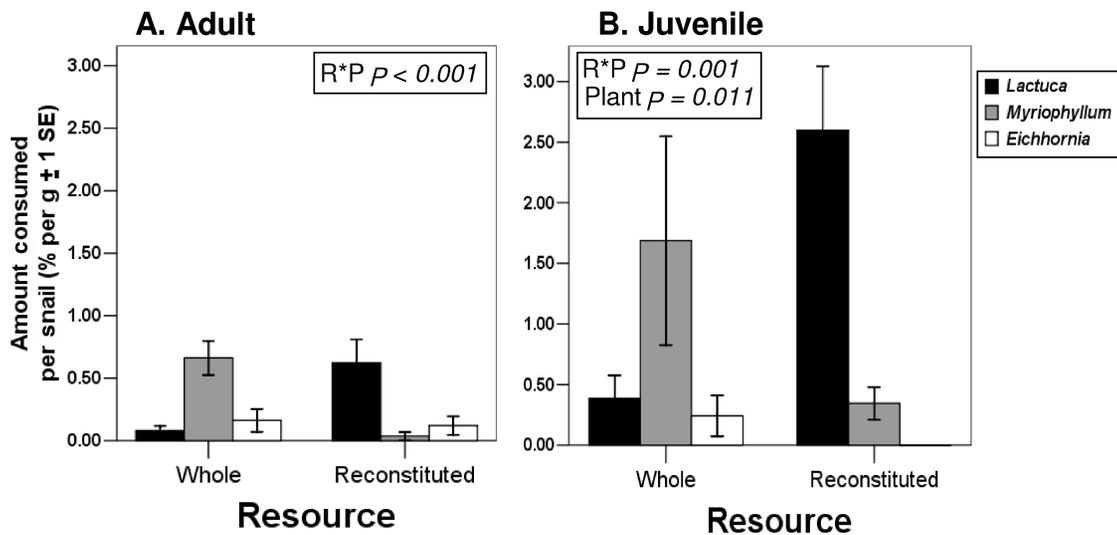
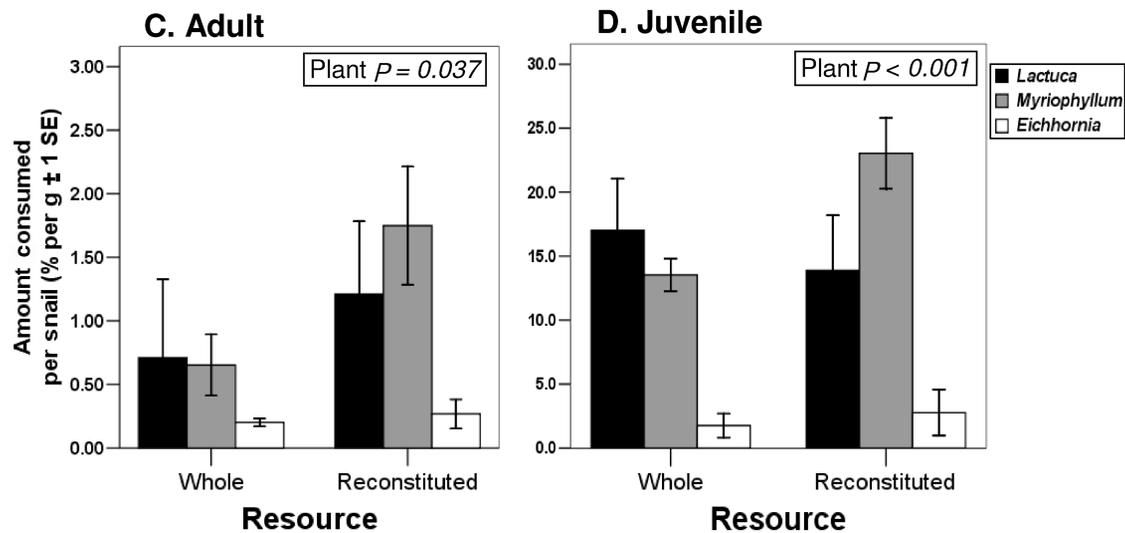
P. insularum*P. canaliculata*

Figure 1. **A.** Per mass consumption by adult *P. insularum* of whole and reconstituted resources suggested that plant species (see legend; P), combined with resource type (R), mediated consumption. R*P interaction comes from two-way ANOVA. **B.** Juvenile *P. insularum* consumption of similar resources suggested a parallel trend. **C.** Adult *P. canaliculata* consumption of whole and reconstituted resources suggested that plant species affected consumption. **D.** Juvenile *P. canaliculata* consumption of similar resources suggested a parallel trend with adults, although with substantially more consumption. Error bars represent one standard error (SE). Please note higher scale on axis for juvenile *P. canaliculata*.

post-hoc test investigated consumption differences between plant species.

Influence of plant chemical compounds

We extracted secondary metabolites of *E. crassipes* and *M. spicatum* based on methods used by Cronin *et al.* (1995) and Choi *et al.* (2002) to examine the effects of plant chemistry on consumption. The procedure extracted all soluble compounds, thus yielding bulk metabolites (alkaloids, phenols, terpenes, etc.). After reducing whole *E. crassipes* and *M. spicatum* to powder as described above, we extracted soluble compounds

in tubes using 10 ml of 95% acetone per gram of powder. Each extraction used 5 g of powder to yield enough metabolites for five reconstitute strips. We extracted powder for 2 h under constant stirring. Following extraction, we centrifuged tubes at 3,000 rpm for 5 min and decanted the supernatant. We re-extracted the original powder under identical conditions and combined supernatants. A constant stream of inert nitrogen gas evaporated the supernatant leaving an oily residue containing metabolites. We covered tubes with parafilm to prevent contamination and vacuum sealed all perishables for transport to Uruguay.

To test the influence of plant chemistry on *P. insularum* consumption (Fig. 2A, B), we employed a 2x2 factorial design

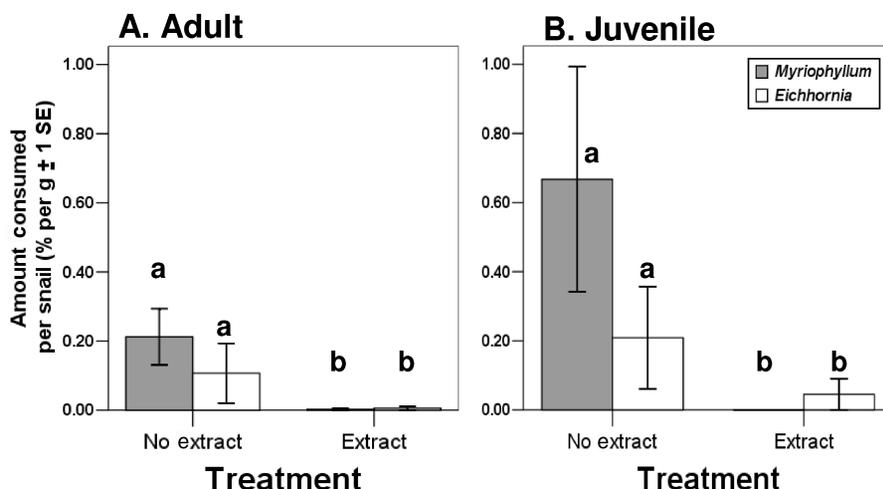
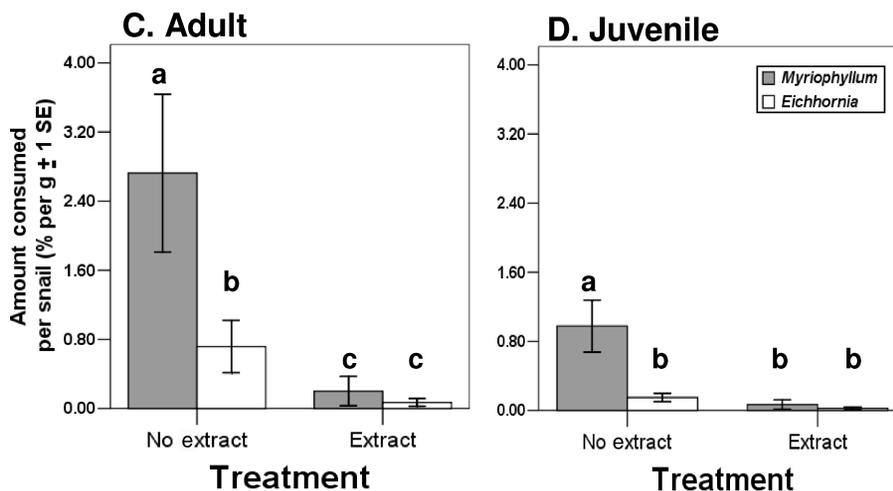
P. insularum*P. canaliculata*

Figure 2. **A.** Per mass consumption by adult *P. insularum* of chemically modified *E. crassipes* and *M. spicatum* provided evidence for deterrence to chemical extracts and *E. crassipes*. **B.** Juvenile *P. insularum* consumption of the same resources produced the same trend. **C.** Adult *P. canaliculata* consumption of chemically modified *E. crassipes* and *M. spicatum* also suggested deterrence to chemical extracts and *E. crassipes*. **D.** Juvenile *P. canaliculata* consumption of the resources showed actual statistical significance at standard confidence levels ($P \leq 0.05$). For **A**, **B** and **C**, different small letters above bars indicate differences at $P \leq 0.07$. Note the larger scale for *P. canaliculata* (**C**, **D**) versus *P. insularum* (**A**, **B**). Error bars represent 1 SE.

($N=5$) consisting of additional plant metabolites (present or absent) and plant species (*M. spicatum* or *E. crassipes*). We created replicates without additional chemistry by reconstituting only plant powder. We made replicates with additional metabolites by mixing sodium alginate, water and plant powder in the tube containing extract. The mass of extract obtained proved difficult to measure. Therefore, we divided one extraction among five replicates to give an extract proportional to 1 g of powder per replicate. Our original reconstitution method created enough reconstitute for three replicates so we increased our measurements and mixed all components in the same tube. We divided paste mass to determine the amount of reconstitute necessary for three replicates. Adult *P. insularum* received one whole strip; juveniles received half a strip (Table 1) due to smaller size.

We employed a similar design with *P. canaliculata* (Fig. 2C, D). Again, *P. canaliculata* received less resource than *P. insularum*,

with adults receiving more than juveniles (Table 1). For both species, we removed snails following the feeding period and calculated percent consumption per mass. Because KS-tests for normality indicated that all plant chemistry data did not fit a normal distribution, we performed non-parametric Kruskal–Wallis analyses to determine the relative ranking of the treatments and a Tukey's post hoc test to analyse the differences between treatments.

Influence of periphyton

Based on field observations of large periphyton mats in Uruguayan lakes, we tested if periphyton promoted grazing in juvenile *P. canaliculata* and *P. insularum* (Fig. 3). Addition of periphyton to resources allowed an alteration of both the structural and chemical constitution. We employed a 2×2 factorial design ($N=5$) consisting of periphyton (present or absent) and origin

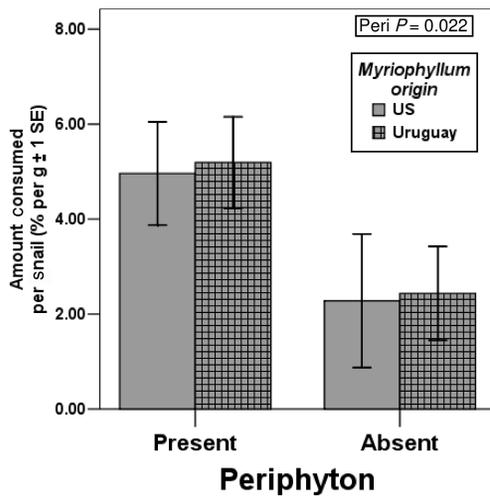


Figure 3. Per mass consumption by juvenile *P. insularum* of reconstituted watermilfoil from either Texas or Uruguay, both with or without additional periphyton. Periphyton statistic comes from 2×2 ANOVA. Error bars represent 1 SE.

of watermilfoil (from Texas or Uruguay). This first allowed us to test if any difference occurred among Texan versus Uruguayan watermilfoil. We brought dry periphyton collected in Uruguay back to the US to use in the experiment with *P. insularum* juveniles. Dried periphyton combined from both Uruguayan lake sites underwent manual removal of any debris, detritus, and small organisms followed by careful rinsing to ensure that the only source of additional structure or chemistry came from periphyton. Replicates with additional periphyton contained 0.23 g of dried periphyton (Table 1). After the feeding period, we removed snails and calculated percent consumption per mass. To compensate for the addition of mass from periphyton, we multiplied percent consumption by 1.4 (total periphyton present in three whole strips). We performed 2×2 factorial ANOVAs to determine effects of *Myriophyllum* type and the addition of periphyton on each species.

RESULTS

Influence of plant structure

We observed no consumption of sodium alginate alone, verifying that plant content mediates consumption. Juvenile *Pomacea insularum* consumed approximately four times more resource by mass than adult *P. insularum*. Neither plant species (Fig. 1A; 2-way ANOVA; $F = 2.511$, $P = 0.102$) nor plant form (Fig. 1A; 2-way ANOVA; $F = 0.225$, $P = 0.639$) alone significantly affected adult consumption. However, we observed a significant (Fig. 1A; 2-way ANOVA; $F = 14.686$, $P < 0.001$) interaction between the two variables for adults. Besides increased consumption of whole *M. spicatum* (Fig. 1B), higher consumption of reconstituted romaine lettuce compared to other reconstituted plants also produced a significant interaction between plant form and species with juveniles (Fig. 1B; 2-way ANOVA; $F = 9.003$, $P = 0.001$). Plant species also significantly affected juvenile consumption (Fig. 1B; 2-way ANOVA; $F = 5.275$, $P = 0.011$), as snails overwhelmingly preferred reconstituted romaine or whole *M. spicatum* and tended to avoid water hyacinth in either form.

Both adult and juvenile *P. canaliculata* (Fig. 1C, D) consumed more than their respective *P. insularum* counterparts (Fig. 1A, B), with adults consuming less resource by mass than juveniles. Juvenile *P. canaliculata* consumed approximately 12 times more

resource by mass than adults. Plant species significantly affected both adult (Fig. 1C; 2-way ANOVA; $F = 3.86$, $P = 0.037$) and juvenile (Fig. 1D; 2-way ANOVA; $F = 18.22$, $P < 0.001$) consumption as *P. canaliculata* also tended to avoid water hyacinth in either form. No significant difference occurred between whole and reconstituted plants and we did not find a significant plant species by form interaction.

Influence of plant chemical compounds

Pomacea insularum demonstrated markedly decreased consumption when presented with reconstituted resource with additional defence compounds (Fig. 2A, B). Consistent with other experiments, juveniles consumed considerably more than adults. In the absence of additional defence compounds, juvenile *P. insularum* consumed nearly six times more by mass than adults. In the presence of defence compounds, juveniles only consumed three times more by mass than adults. Considered perhaps as marginally significant, the presence of additional defence compounds decreased consumption by both adult and juvenile *P. insularum* (Fig. 2A, B; Kruskal–Wallis tests, $H_3 = 6.990$, 7.295 , $P = 0.072$, 0.063 , respectively). Regardless of plant species, all treatments without additional extract ranked higher than those with extract. In addition, plant species also had an effect on adults and juveniles. *Myriophyllum spicatum* ranked higher than *E. crassipes* regardless of the presence of extract, although juveniles consumed slightly more *E. crassipes* than *M. spicatum* with extract.

We observed similar feeding trends with *P. canaliculata* with a few notable exceptions (Fig. 2C, D). Like *P. insularum* (Fig. 2A, B), snails showed less consumption of resources with additional defence compounds. We found significant reductions in juvenile consumption (Fig. 2C; Kruskal–Wallis test, $H_3 = 13.273$, $P = 0.004$) and similar trends in adult consumption (Fig. 2D; Kruskal–Wallis test, $H_3 = 7.091$, $P = 0.069$). In the presence of extra defence compounds, juveniles consumed approximately seven times less by mass than adults. However, in the absence of these compounds, juveniles consumed approximately three times less by mass than adults (Fig. 2D). In agreement with our *P. insularum* results, Kruskal–Wallis rankings showed that, regardless of plant species, all treatments without additional extract ranked higher than those with extract. We observed more pronounced avoidance of water hyacinth and an overall preference for watermilfoil, except with occasional water hyacinth consumption by juveniles. Rankings showed that in the absence of extract adults and juveniles preferred watermilfoil to water hyacinth. In contrast, in the presence of extract, similar rankings between plant species occurred.

Influence of periphyton

The origin of *Myriophyllum* (i.e. Texas versus Uruguay) did not significantly affect consumption of reconstituted resource by either juvenile *P. insularum* or *P. canaliculata* (2×2 ANOVA; all P 's > 0.05). In contrast, juvenile *P. insularum* and *P. canaliculata* showed different responses to resources with additional periphyton. In the presence of additional periphyton, juvenile *P. insularum* consumed nearly 30 times more by mass than juvenile *P. canaliculata*. In the absence of periphyton, juvenile *P. insularum* consumed only about eleven times more by mass than juvenile *P. canaliculata*. The presence of periphyton had a significant positive effect on juvenile *P. insularum* consumption (Fig. 3; 2×2 ANOVA; $F = 5.61$; $P = 0.022$), though it did not influence consumption by *P. canaliculata*.

DISCUSSION

Regardless of species, juvenile *Pomacea* consumed more by mass overall than their adult counterparts. Our results agree with Carlsson & Brönmark (2006) who found that smaller *P. canaliculata* (shell height 1.0 ± 0.2 cm) had a greater relative foraging capacity on macrophytes than adults. These results suggest that juvenile applesnails at high densities may pose a greater threat to aquatic vegetation than adults. Although applesnail diet seems to remain consistent throughout their lifespan, dissimilarity in resource preference (in particular watermilfoil) between applesnail species suggests substantial feeding flexibility in *P. insularum*.

Several key similarities and differences occurred in consumption between species. First, little consumption of water hyacinth occurred whether whole or reconstituted, by either species or life stage. Second, *P. insularum* consumed much more whole watermilfoil, but little reconstituted, whereas *P. canaliculata* consumed similar amounts of both types. Third, we found that both species avoided watermilfoil with additional defensive compounds, but consumed this resource by itself. Compared to other aquatic macrophytes, *M. spicatum* is at the high end of allelopathic activity, with other watermilfoil species (e.g. *M. brasiliense*, *M. verticillatum*) ranked closely below (Burks *et al.*, 2006). We note that the species of *Myriophyllum* used in experiments did not influence consumption, thus providing support for our comparisons between applesnail species. Choi *et al.* (2002) observed diminished growth rates in larval *Acentria ephemerella* fed solely on *M. spicatum* and attributed this observation to the high concentration of hydrolysable tannins (5–9% dry mass) present in the plant. Therefore, greater consumption of *M. spicatum* by *P. insularum* may indicate a greater capacity to feed indiscriminately on macrophyte tissue.

Besides some structure, we found that additional chemical extracts deterred consumption in all snails, suggesting that abundance of secondary metabolites may reduce the palatability of resources. Though we did not identify or quantify the extracted chemicals, our results provide the first evidence for chemically mediated consumption in applesnails. Tough plants with established chemical defences, such as *E. crassipes*, should deter feeding even further. The removal of the structural defences of water hyacinth did not increase consumption, so chemical defences appear important. Lach *et al.* (2000) found that snails could survive if fed only water hyacinth, but did not have higher growth rates than unfed snails. Furthermore, we only offered snails the leafy portion of *E. crassipes*. Carlsson & Lacoursiere (2005) found that only when *P. canaliculata* exhausted other macrophyte resources did snails turn to *E. crassipes*. However, observations during that experiment found that snails consumed the roots and air-filled bulbs of *E. crassipes* first, neither of which we offered to our snails. Although including lettuce in our chemistry experiments would have made our study more comprehensive, we did not find any ecological literature indicating the existence of such deterrent compounds in lettuce. We instead chose to focus our time on plants with known chemical defences. Clearly, both macrophyte structure and chemistry play an important role in applesnail consumption and the addition of resources that alter both factors should provide further insight into underlying feeding mechanisms.

Snails did not consume reconstituted without the addition of plant powder, suggesting that the snails did respond directly to the presence of macrophytes. Reconstituted food cannot help but lose some of the inherent quality of the original resource and we have applied caution to extrapolating our results. However, reconstitution does serve as an accepted method of standardization (Hay *et al.*, 1998; Cronin *et al.*, 2002). In addition, because we used different ratios of whole plant to create reconstituted, greater strip consumption does not

necessarily imply greater whole plant consumption. Our results best reflect consumption of a physically uniform resource and a whole plant correction would specify actual macrophyte content. In addition, the different ratios used mimicked the physical structure of the plants, with *Myriophyllum* having much finer leaves and less bulk than either lettuce or water hyacinth. Furthermore, both sizes of *P. insularum* preferred whole *M. spicatum* to reconstituted, thereby providing further evidence that resource structure mediates preference in generalist feeders (see Cronin, Wissing & Lodge, 1998).

By providing additional periphyton along with reconstituted resources, we hoped to find evidence supporting the feeding versatility of both species of *Pomacea*. Carlsson & Brönmark (2006) found that regardless of size, *P. canaliculata* had a significant negative effect on both macrophyte and periphyton biomass. Our study showed that periphyton did not influence consumption by native *P. canaliculata* juveniles, although we observed decreased consumption by *P. canaliculata* regardless of treatment. This could have resulted from prolonged enclosure in the drum environment which did not have flow-through water. In contrast, the presence of periphyton increased consumption by *P. insularum* juveniles. We could not perform this experiment with adults due to Customs issues. Nevertheless, our results further support our hypothesis that *P. insularum* may exhibit greater feeding versatility than *P. canaliculata*.

The well-documented generalist appetite and remarkable fecundity of *P. canaliculata* (Joshi & Sebastian, 2006) necessitates more research surrounding the growing threat from invasive applesnails. In a field experiment, high densities of *P. canaliculata* produced an absence of macrophytes, greater nutrient concentrations, and increased phytoplankton biomass (Carlsson, Brönmark & Hansson, 2004). Collectively, these impacts push an ecosystem from a clear state to a turbid state (Carlsson *et al.*, 2004). Similarities of the new invader, *P. insularum*, with the established invader, *P. canaliculata*, warrant further research on this emergent invasive species. Our results of stronger impacts on macrophytes by smaller snails could mean that invasion of a new species produces stronger effects at the colonization front and this may have substantial implications for management and control methods for invasive applesnails.

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