

Neuston: A relevant trophic resource for apple snails?



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ABSTRACT

Apple snails are known for the strong impacts they provoke in wetlands and aquatic crops by their macrophytophagous habits. Interestingly, they are able to persist after they have eradicated most palatable aquatic macrophytes in the invaded wetlands. Pedal surface collecting is a distinctive mechanism that apple snails use to capture materials in the water surface. We were interested in knowing the relevance of neuston, the organisms associated with the air–water interface, as an alternative trophic resource. Using the invasive *Pomacea canaliculata* as a model, our experiments with simulated trophic resources in the laboratory showed that neuston is highly consumed even in the presence of abundant palatable macrophytes. *P. canaliculata* was able to grow efficiently using neuston as an alternative trophic resource both under laboratory and natural conditions. Neuston probably plays a relevant role specially when other trophic resources are inedible or absent or have been depleted by apple snails. Pedal surface collecting probably evolved in apple snails as an adaptation to cope with fluctuating trophic resources.

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Introduction

Grazing by apple snails (family Ampullariidae), especially those in the Neotropical genus *Pomacea*, has resulted in changes of great magnitude in the wetlands they have invaded (Carlsson et al., 2004; Fang et al., 2010; Horgan et al., 2014), eradicating most of the submersed and floating macrophytes and consequently increasing the water turbidity due to the enrichment of water and the resuspension of sediments. A striking feature of some of these cases is that apple snails are able to persist (Pointier et al., 1988; Pointier and Jourdan, 2000) and even to maintain high densities (Carlsson et al., 2004) after the catastrophic changes they have provoked in the aquatic vegetation. For some apple snails, like the globally invasive *Pomacea canaliculata* (Hayes et al., 2012), it is probably partly due to the high plasticity of their life history traits relative to the food availability (Estoy et al., 2002a,b; Tamburi and Martín, 2009, 2011), but it is also due to the use of other trophic resources after they have eradicated the palatable macrophytes. Even if post impact densities are low, apple snails seem to be able to prevent the recovery or recolonization of some freshwater macrophytes (Pointier et al., 1988).

The ability to thrive in spite of the catastrophic changes provoked by their voracious consumption of macrophytes is important to the medium and long term impacts that invasive apple snails produce (EFSA 2014; Horgan et al., 2014), since the persistence of their populations at even moderate densities would imply that the changes may be almost irreversible. If apple snails are able to maintain moderate or low densities using alternative trophic resources after most of the macrophyte biomass has been eradicated, they would impede macrophyte regrowth from inedible body parts, and eventually they will deplete the seed bank and also deter recolonization from other sources. They will probably also be able to counteract efforts to restore aquatic vegetation (Burlakova et al., 2009) and to enhance the dominance of unpalatable macrophytes through differential grazing (Horgan et al., 2014).

Even though most of their infamous reputation as pests and drivers of ecosystem changes is related to their voracious consumption of aquatic macrophytes by shredding–scraping in invaded wetlands, apple snails also show other food capture mechanisms that enable them to profit from a wide spectrum of trophic resources (Saveanu and Martín, 2013, 2014). Among these, pedal surface collecting appears to be quite a flexible mechanism that is almost exclusive of apple snails (Dillon, 2000): they form a funnel with the foremost part of the foot, gathering almost any material present on the water surface by ciliary action and then ingesting it (Fig. 1). This mechanism allows them to capture a wide spectrum of trophic resources, such as organic remains (petals, leaves, insect exuviae and corpses, etc.) and neuston (Saveanu and Martín, 2013).

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Fig. 1. An individual of *Pomacea canaliculata* performing pedal surface collecting to capture duckweed in a Southern Pampas' stream using submersed macrophytes as substrate.

Marshall and Gladyshev (2009) defined neuston as all the organisms associated with the air–water interface in aquatic habitats, including small vascular plants commonly known as duckweed and water ferns (e.g. *Lemna*, *Wolffia* and *Azolla*) and also inactive life stages of other organisms (e.g. pollen grains, seeds, spores, etc.). On the other hand, floating macrophytes, such as *Eichornia crassipes*, *Pistia stratiotes* and *Ipomoea aquatica*, are not usually considered as part of the neuston but belong to a different community (i.e. pleuston). These floating macrophytes cannot be attracted by ciliary action of the foot of apple snails (pers. obs.) and they gradually consume their underwater portions (e.g. leaves, roots and petioles of *E. crassipes*) by shredding–scrapping whereas duckweed is ingested in one piece (Carlsson and Lacoursière, 2005; Fang et al., 2010). The air–water interface also contains significantly higher amounts of particulate and dissolved organic matter than the sub-surface water (Södergren, 1987; Maki and Hermansson, 1994) that can also be gathered and ingested by *Pomacea* snails (Cheesman, 1956; Saveanu and Martín, 2013). *Pomacea* snails present a dual respiratory system and routinely visit the water surface to ventilate the lung (McClary, 1964; Seuffert and Martín, 2009, 2010) so they are probably able to test for the presence of food on surface without any additional effort.

In the present study we assessed the relevance of neuston and organic remains associated with the water surface as an alternative or complementary trophic resource for apple snails when macrophytes are absent or unpalatable. In this context we tried to answer the following questions using *P. canaliculata* as a model: do apple snails consume neuston even when the preferred trophic resources (macrophytes) are available? How does the consumption of neuston and macrophytes depend on their relative availability? How do the growth parameters of apple snails consuming neuston compare to those consuming macrophytes? Are apple snails able to grow in waterbodies without macrophytes, feeding only on neuston and associated organic remains?

Materials and methods

In order to answer the questions that prompted our study we performed three sets of behavioural experiments in the lab

(Experiments 1.1, 1.2 and 1.3) and also two growth experiments, both in the lab (Experiment 2) and in the field (Experiment 3).

Origin of snails and maintenance conditions

Adult *P. canaliculata* used in the three parts of Experiment 1 were collected from El Huáscar stream (36°55'50" S, 61°35'48" W; Buenos Aires Province, Argentina). Juvenile snails (Experiments 2 and 3) were obtained from one egg mass laid in the laboratory by adults from an excavated channel that links the Pigué and Venado streams (37°09'59" S, 62°40'28" W; Buenos Aires province, Argentina). Adults were reared collectively in 20 L aquaria and hatchlings individually in 200 mL aquaria until they were used in the experiments. They were all maintained in a rearing room at 25 ± 2 °C, under a photoperiod of 12 h light/12 h dark, with CaCO₃-saturated tap water and fed on fresh lettuce; the aquaria were cleaned and the water was changed once a week.

Preparation of simulated trophic resources in the laboratory

Different trophic resources were simulated using dried lettuce as the raw material in order to maintain the nutritional quality and palatability constant. Dried lettuce was presented as whole leaves floating on the water surface, as whole leaves attached to the bottom and as particulate leaves floating on the water surface, to simulate floating and submersed macrophytes, and neuston respectively.

Whole lettuce leaves were dried at 70 °C for 48 h and were processed with an electric grinder to obtain particles less than 5 mm in diameter. Drying allows both the particulate and whole lettuce leaves to remain floating for far more than a week and also reduces water fouling, especially in the case of particulate lettuce.

Experiment 1: differential use of different trophic resources

To evaluate the differential use of different trophic resources according to their availability, behavioural observations of single snails were performed in 20 L glass aquaria (20 cm wide, 40 cm long, 25 cm deep). One day prior to each trial the snails were randomly chosen from the collective aquaria and put in 3 L aquaria with fresh lettuce to ensure a similar level of satiety. Five aquaria with only one snail each, with tap water at 23–25 °C and natural illumination (complemented with artificial light), were observed simultaneously. Adult snails (32–50 mm of shell length (SL), measured from the apex to the furthest point of the aperture; Estebenet and Martín, 2003) were used. The snails were used only once in each of the three behavioural experiments and the number of males and females used was the same in each one.

The observation period lasted for 2 h. As an indirect way to determine the differential use of the different trophic resources, the time invested in consuming each of them was calculated relative to the total time in activity of each snail: the percentage of time consuming simulated neuston (CN, %) by pedal surface collecting and the percentage of time consuming simulated floating macrophytes (CFM, %) or simulated submersed macrophytes (CSM, %) by shredding–scrapping.

1.1. To test if there is any differential use of neuston and submersed macrophytes according to their availability, observations on 50 isolated snails were performed with particulate lettuce on the surface (neuston) and whole lettuce leaves at the bottom (submersed macrophytes, see "Results" section, Fig. 3A). Five treatments with the same total amount of food per aquarium (0.4 g) but with different relative availabilities of simulated neuston on the surface (NA%: 0, 25, 50, 75 and 100, corresponding to 0, 0.1, 0.2, 0.3 and 0.4 g of particulate lettuce scattered on the water surface) were carried out. The corresponding amount of simulated

submersed macrophytes was supplied in five pieces held by a plastic mesh at the bottom. Each level of relative neuston availability was replicated 10 times.

To avoid a bias due to the habit of the recently activated snails going first to the surface to breathe air, each aquarium was initially half filled. When the snail's lung ventilation was recorded, the aquarium was filled completely and so the experiment started with the snail equidistant between the surface and the bottom. The observation period started in each aquarium when it was completely full. To determine the use of each of the resources offered, the time consuming neuston (CN) and the time consuming submersed macrophytes (CSM) were calculated. Differences in CN and CSM between different treatments were tested with one-way ANOVAs.

1.2. To determine whether there is any differential use of floating macrophytes and neuston when both are equally available, behavioural observations were performed on 20 isolated snails in aquaria with both whole leaves and particulate lettuce on the water surface (see "Results" section, Fig. 3B). In order to equalize the probabilities of encounter with each resource when the snails crawl to the surface, each food type was distributed so that it occupied half of the waterline along the aquarium perimeter: whole leaves were attached to the aquarium walls at intervals and particulate lettuce was scattered on the surface. The experiment started with snail activation at the bottom of the aquarium. Time consuming neuston (CN) and time consuming floating macrophytes (CFM) were calculated and compared by paired-samples *t* test.

1.3. To test if there is any differential use of floating and submersed macrophytes when both are equally available, whole leaves were offered both on the surface and on the bottom. Observations on 20 isolated snails were performed in aquaria with five whole leaves of lettuce on the water surface, attached to the aquarium walls, and five at the bottom, held by a plastic grid (see "Results" section, Fig. 3C). The experiment started in the same way as in Experiment 1.1. The time consuming floating macrophytes (CFM) and submersed macrophytes (CSM) were estimated and compared by paired-samples *t* test.

Experiment 2: effects of different trophic resources and their availability on snail growth

To evaluate the effect of the consumption of macrophytes and neuston at different availabilities on snail growth, 50 juvenile snails (17–20 mm SL) were maintained during 5 weeks under five levels of relative trophic availability (TA%: 100, 75, 50, 25 and 0%) of two trophic resources (TR): simulated neuston and simulated macrophytes. Each combination of TA% and TR was replicated 10 times.

Each TA% was calculated using the following equation for ad libitum daily ingestion of fresh lettuce (equivalent to 100% of trophic availability; Tamburi and Martín, 2009): $TA\% = 0.0033 \cdot SL^{1.9322}$, using the mean SL of the previous week for each TR and TA% combination. The weekly amount of fresh lettuce for each TA% level was dried and offered as whole leaves to simulate macrophytes; to obtain simulated neuston the dried leaves were grown as described above.

Juveniles were reared individually in 3 L glass aquaria with a 1 cm × 1 cm plastic grid on the bottom to prevent coprophagy. Rearing conditions were the same as those used in maintaining the snails collected in the field. Shell length was measured each week when the water was changed. Live weight (LW, g) was recorded at the beginning and end of the experiment with a digital scale (± 0.1 mg) after the snail was allowed to crawl onto a dry plastic surface to drain the water from the mantle cavity. Daily specific growth rates of shell length and live weight (SLR, mm mm⁻¹ day⁻¹ and LWR, g g⁻¹ day⁻¹) were calculated as the difference between the

final and initial values, divided by the initial value and multiplied by the duration of the experiment (35 days). At the end of the experiment the snails were sacrificed by immersion in water at 100 °C, the soft parts were extracted from the shell and dried at 70 °C for 48 h and the shell free dry weight (SFDW, g) was estimated.

The specific growth rates (SLR and LWR) and the shell free dry weight (SFDW) were compared by two-way ANOVAs using trophic availability (TA, %) and trophic resources (TR) as the main factors; some variables were transformed (see "Results" section) after the rejection of Levene's test of homoscedasticity ($p < 0.05$).

Experiment 3: growth in the field feeding on neuston and associated organic remains

To evaluate the significance of neuston and surface particulate organic material as trophic resources for maintenance and growth an experiment was performed in a natural pond where juvenile apple snails were maintained in enclosures without access to macrophytes and bottom sediments. Los Chilenos pond (38°1'50" S, 62°27'17" W, Southern Pampas, Argentina) is a shallow pond with a sandy–muddy bottom, naturally devoid of submersed and floating macrophytes. The aquatic vegetation was mostly composed of a marginal belt of reeds (*Schoenoplectus californicus*) and cattails (*Typha* sp.). The bush *Baccharis junceum* was abundant along the shore during our experiment. *P. canaliculata* had been recorded in Los Chilenos pond as part of a study on the factors determining its distribution (Martín et al., 2001); the water of this pond is quite turbid (total and volatile suspended matter: 0.234 and 0.204 g L⁻¹, respectively), alkaline (pH 8.6) and with low electrolytic content (conductivity = 0.51 mS cm⁻¹).

To estimate the carbon content of neuston and organic remains associated with the water surface the latter was sampled using a modification of the glass plate method (Marshall and Burchardt, 2005; Cunliffe et al., 2009). An acetate blade 11 cm × 15 cm was submersed, gently retrieved and the material adhered to the blade was washed five times with distilled water, which was collected in a plastic container; the procedure was repeated five times to obtain a sample. The total particulate organic matter content (POM (mgC), including neuston and organic remains) of each sample ($n = 5$) was estimated following Strickland and Parsons' procedure (1968). To estimate the POM density at the water surface (mgC m⁻²) of the pond we took into account that our sampling method overestimated the surface density by 20%, which had been estimated in the laboratory in a series of trials with known densities of grown lettuce on the surface.

Twenty juvenile snails (18–24 mm SL) were put into individual floating cylindrical enclosures made of a plastic grid with a mesh size of 1 cm × 1 cm (Fig. 2A). Half of the enclosures had a cylindrical acetate band (3.5 cm above and 3.5 below water surface level) to impede the horizontal renewal of neuston and organic remains (limited renewal of neuston, LRN). The remaining enclosures (unlimited renewal of neuston and organic remains, URN) had an acetate band 3.5 cm-wide located 3.5 cm below the surface to control the consumption by shredding–scraping of periphyton or detritus adhered to the submersed part of the band in the LRN treatment. The enclosures were provided with three external buoys to maintain a constant floating level; the top 3.5 cm of the enclosure remained out of water to allow the snails to breathe air. The LRN and URN enclosures were distributed in pairs in the marginal belt of reeds of the pond in places where the water depth was 50–60 cm to avoid contact with the bottom. The floating enclosures were tethered to the reeds with plastic seals (Fig. 2B) between 23 December 2011 and 15 January 2012.

Shell length and live weight were recorded at the beginning and end of the experiment. Daily specific growth rates of shell length (SLR) and live weight (LWR) and the shell free dry weight (SFDW)

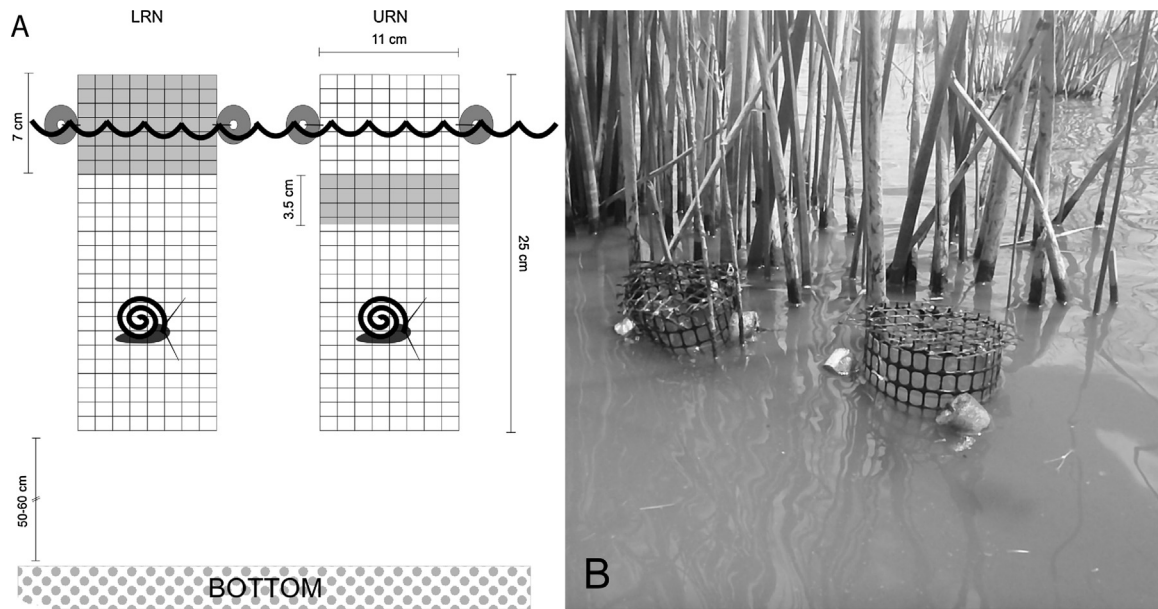


Fig. 2. Enclosures with limited renewal (LRN) and unlimited renewal of neuston and organic remains (URN). (A) Diagram of LRN and URN enclosures. (B) Paired enclosures tethered to the reeds in Los Chilenos pond.

were calculated as in Experiment 2. The differences in these variables between treatments were analyzed by paired-samples *t* tests.

Results

Experiment 1: differential use of different trophic resources

1.1. In the treatment without any simulated submersed macrophytes (100% NA) all the active snails ($n=9$) moved first to the surface whereas in those with simulated submersed macrophytes

there was no difference in the frequency of snails that first chose the surface or the bottom ($X^2_1 = 0.231$, $p = 0.631$). The percentage of time consuming neuston showed no trend relative to the neuston availability between 25 and 100% ($F_{3,36} = 0.506$, $p > 0.680$); when neuston was unavailable only one snail performed pedal surface collecting for a few minutes (Fig. 3D). The percentage of time taken shredding–scrapping on submersed macrophytes decreased significantly, from 41% to 6.8%, with an increasing level of neuston availability ($X^2_4 = 16.837$, $p = 0.002$), resulting in an important decrease in the total time spent feeding (from 41 to 17%).

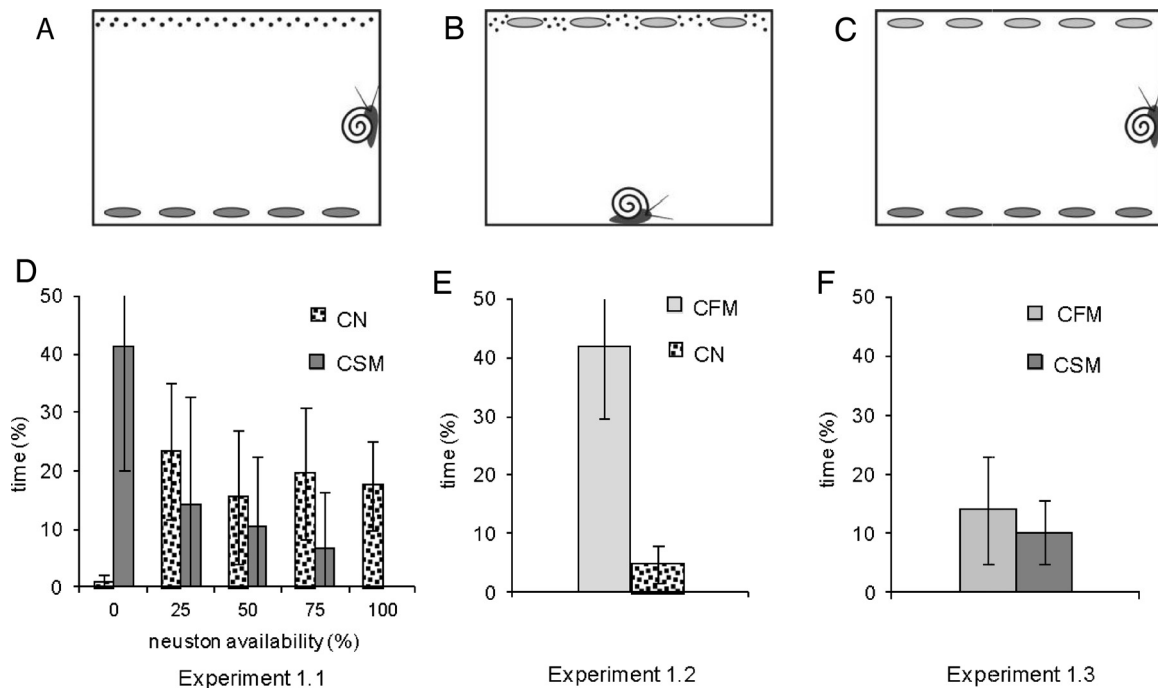


Fig. 3. Diagram of the aquaria with each pair of trophic resources offered to *Pomacea canaliculata* and percentage of time using each of them (mean \pm 95% CI). (A) Experiment 1.1; (B) Experiment 1.2; (C) Experiment 1.3. Black dots represent neuston; light grey ovals on the surface represent floating macrophytes and dark grey ovals on the bottom represent submersed macrophytes. (D–F) CN: time consuming simulated neuston; CFM: time consuming simulated floating macrophytes; CSM: time consuming simulated submersed macrophytes.

Table 1

Summary of two-way ANOVAs for growth variables of *Pomacea canaliculata*. Fixed factors: trophic availability (TA, excluding TA 0%), trophic resources (TR) and its interaction (TA × TR). SLR: shell length growth rate; LWR: live weight growth rate; SFDW: shell free dried weight. SFDW was reciprocally transformed.

Variable	TA	TR	TA × TR
SLR (mm mm ⁻¹ day ⁻¹)	$F_{3,32} = 57.953, p < 0.001$	$F_{1,32} = 21.124, p < 0.001$	$F_{3,32} = 3.597, p < 0.025$
LWR (g g ⁻¹ day ⁻¹)	$F_{3,32} = 76.335, p < 0.001$	$F_{1,32} = 46.213, p < 0.001$	$F_{3,32} = 7.793, p < 0.001$
SFDW (g)	$F_{3,32} = 77.491, p < 0.001$	$F_{1,32} = 24.391, p < 0.001$	$F_{3,32} = 0.859, p > 0.470$

1.2. When simulated floating macrophytes and neuston were supplied at the same availability, the percentage of time shredding–scraping on macrophytes (41%) was significantly higher than the time spent on pedal surface collecting to consume neuston (4%; paired-samples *t* test, $t_{19} = 5.00, p < 0.001$, Fig. 3E). The frequency of snails that first contacted floating macrophytes was not significantly different from the frequency of those that contacted neuston ($X^2_1 = 3.200, p = 0.074$).

1.3. When simulated floating and submersed macrophytes were equally available, no significant differences were found between the percentages of time taken to consume each of them (14 and 10%, respectively; paired-samples *t* test, $t_{19} = 0.682, p > 0.500$, Fig. 3F). The frequency of snails that went to the surface as the first movement was not significantly different from the frequency of those that moved to the bottom ($X^2_1 = 0.474, p = 0.491$).

Experiment 2: effects of different trophic resources and their availability on snail growth

No significant differences were found in initial SL and LW among the snails assigned to the different feeding regimes (two-way ANOVAs, $p > 0.640$ and $p > 0.310$, respectively); none of the snails died during the experiment. Considering only the treatments with food (i.e. excluding TA 0%), SLR, LWR and SFDW increased significantly with an increase in trophic availability and were higher for the snails consuming macrophytes than those consuming neuston (Table 1; Fig. 4). A significant interaction term between the main factors was detected for both daily rates: the difference between the two trophic resources increased with trophic availability but the CI 95% intervals show that they were only significant for the higher TA level; at this level snails that consumed macrophytes grew 50% (SLR) and 80% (LWR) more on average than snails that consumed neuston. The snails reared at TA 25% with both trophic resources grew significantly more than starved ones in the three variables studied (*t* tests, $p < 0.01$).

Experiment 3: growth in the field feeding on neuston and associated organic remains

There were no initial differences in SL and LW (one-way ANOVAs $p = 0.323$ and $p = 0.059$, respectively) between the snails with unlimited or limited renewal of neuston and associated organic remains (URN and LRN treatments, respectively). No deaths were recorded during the experimental period but one snail escaped from the enclosure. At the end of 23 days experimental period all the snails had grown in LW and SL (Fig. 5A and B). Growth rates (SLR and LWR) did not show any significant differences between the snails under URN and LRN treatments. However, SFDW resulted 22% lower for the snails with limited renewal of neuston (Table 2, Fig. 5C).

The mean content of particulate organic matter on the surface of Los Chilenos pond was estimated as 1.825 mgC m⁻² (ranging from 0.437 to 3.710 mgC m⁻²). *In situ* observations on the water surface showed high availability of different materials, some of which were identified as petals, seeds and pappi of *B. junceum*, and parts of inflorescences of *Typha* sp. Surface biofilms were also evident among the reeds.

Table 2

Results and means of growth variables of *Pomacea canaliculata* with unlimited (URN) and limited renewal (LRN) of neuston and associated organic remains in Los Chilenos pond.

Variable	<i>t</i> test	<i>p</i> -Value	Means	
			URN	LRN
SLR (mm mm ⁻¹ day ⁻¹)	$t_8 = 1.379$	$p = 0.205$	0.007	0.006
LWR (g g ⁻¹ day ⁻¹)	$t_8 = 0.965$	$p = 0.363$	0.026	0.020
SFDW (g)	$t_8 = 2.662$	$p = 0.029$	0.284	0.222

Discussion

Our behavioural experiments showed that *Pomacea canaliculata* uses neuston even when other trophic resources such as macrophytes are available within their short term action range. The consumption of neuston by pedal surface collecting when submersed macrophytes are abundant has also been observed in the field (Fig. 1; Saveanu and Martín, 2013). *P. canaliculata* uses neuston even when submersed macrophytes were three times more abundant. Interestingly, the percentage of time consuming neuston remained constant in a 4-fold density range of availability, probably due to the increase in specific capture rates as neuston density increases (Saveanu and Martín, 2013).

There was no difference in the time that snails spent feeding on simulated neuston and macrophytes when the latter were submersed, but when macrophytes were also available at the surface the snails spent 10 times more time consuming the macrophytes than neuston. This last result seems to indicate that the snails preferred to eat macrophytes instead of neuston when they are equally palatable, nutritious and both are located in the same position. However, the ingestion rate of simulated neuston is 16-fold the ingestion rate on simulated macrophytes for an adult snail (Tamburi and Martín, 2009; Saveanu and Martín, 2013) and hence, even if they spent less time consuming neuston, probably they obtain a higher profit consuming neuston of similar nutritional quality.

Several species of macrophytes, especially submersed ones, have proved to be unpalatable and even noxious for *P. canaliculata* due to a combination of toxic secondary compounds, low nutrient and high dry matter content (e.g. Qiu and Kwong, 2009; Wong et al., 2010; Morrison and Hay, 2011). On the other hand, emergent macrophytes are generally unpalatable to *P. canaliculata* and other apple snails due to their toughness (Carlsson et al., 2004; Burlakova et al., 2009). Duckweed and water ferns are readily eaten by *P. canaliculata* through pedal surface collecting (Saveanu and Martín, 2013) and, although their palatability has rarely been assessed quantitatively, some may be strongly preferred over floating macrophytes (Carlsson and Lacoursière, 2005). The differences in palatability would lead to the replacement of palatable species by unpalatable ones in the short and mid-term (Tamburi and Martín, 2009; EFSA 2014; Horgan et al., 2014). So, even if floating and submersed macrophytes are abundant, neuston (including duckweed and water ferns) may constitute an important and valuable trophic resource for apple snail populations.

When simulated floating and submersed macrophytes were equally available the snails did not use floating macrophytes any more intensely. *P. canaliculata* snails have both a functional aquatic

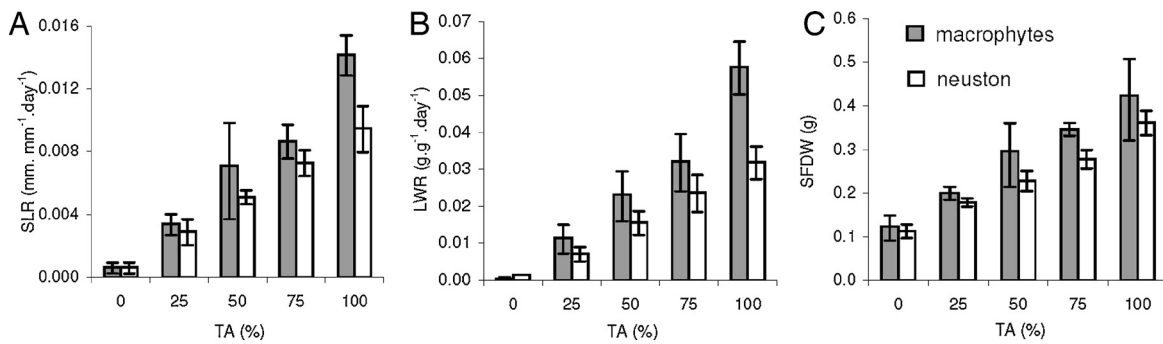


Fig. 4. Growth variables of *Pomacea canaliculata* under five trophic availabilities (TA) and two trophic resources (TR: simulated macrophytes and simulated neuston). (A) Shell length growth rate (SLR). (B) Live weight growth rate (LWR). (C) Shell free dry weight (SFDW). SLR and LWR were square-root transformed and SFDW was log₁₀-transformed. Bars represent back-transformed means ± 95% CI.

gill and an aerial lung and they show certain dependence on atmospheric air, especially when the water is warm and fouled (Seuffert and Martín, 2010). At 25 °C adult snails routinely crawl to the water surface to breathe air (every 25 min on average; Seuffert and Martín, 2009). However, apparently this dependence did not influence their choice of these trophic resources.

Our growth experiment in the laboratory showed that snails were able to grow using pedal surface collecting when only neuston was available, even if the availability of this trophic resource was very low. However, the growth rates and final weight were lower than those attained when feeding on macrophytes by shredding–scraping. On the whole the growth of snails feeding on neuston was similar to those feeding on macrophytes at 25% lower availability. The difference could be attributed to a higher energetic cost of gathering food with the pedal funnel by ciliary action, especially when the access to macrophytes is easy as in our laboratory growth experiment.

The differences in growth between snails fed on neuston and macrophytes decreased as the availability of food decreased in our experiment. The snails probably consume all the neuston in a shorter lapse of time than that required to consume the macrophytes by shredding–scraping, due to the high capture rates attained using pedal surface collecting (Saveanu and Martín, 2013), and after this they become inactive which saves energy until new food is added. This indicates that neuston may be especially important when the availability of palatable submersed macrophytes is low.

In our experimental setting simulated neuston and simulated macrophytes had equal nutritional values and apple snail growth efficiency was lower in the former. However, in natural waterbodies this lower efficiency may be counteracted and even overpowered by the generally higher protein content of some components (especially duckweed and water ferns) of natural neuston

communities as compared to macrophytes (Hasan and Chakrabarti, 2009). In natural waterbodies the consumption of neuston by surface pedal collecting would also permit to capture of other highly nutritive items such as insect corpses (Saveanu and Martín, 2013) and protein monolayers (Cheesman, 1956).

Snails with a limited renewal of neuston and associated organic remains attained a final weight 22% lower than unlimited ones. However, all the snails in the enclosures grew during the trial, probably due to the consumption of neuston but also of periphyton and detritus adhered to the submersed part of the enclosures. The evidence from our field experiment showed that *P. canaliculata* snails are able to grow in waterbodies without any floating and submersed macrophytes. This indicates that the presence of edible macrophytes may be not essential for these apple snails to establish in a waterbody and that alternative trophic resources as neuston and periphyton (Carlsson and Lacoursière, 2005; Fang et al., 2010) may allow their populations to persist after the eradication of macrophytes. It is worth mentioning that in Los Chilenos pond neuston was composed mostly of microscopic organisms (without any small floating vascular plants, e.g. *Lemna* and *Wolffia*) and even so, the apple snail grew at rates comparable to those observed in the laboratory. The growth of the snails with an unlimited renewal of neuston was similar to those in the 100–75% level of trophic availability, whereas those with limited renewal grew at rates that were similar to the trophic availability levels of 75–50%. If the relative trophic availability remains constant at these high levels during the summer and the temperature is around 25 °C, the apple snails would be able to reach maturity in 100 days (Tamburi and Martín, 2009) and their fecundity would not be significantly affected (Tamburi and Martín, 2011).

The daily ingestion rate of an average sized apple snail in the field experiment (21.3 mm SL) is 1.217 g of fresh lettuce (Tamburi and Martín, 2009). The total amount of POM (neuston

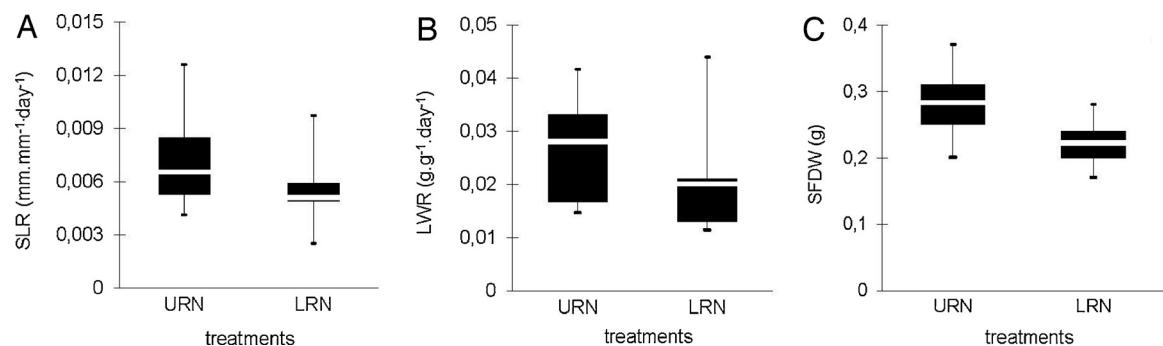


Fig. 5. Growth variables of *Pomacea canaliculata* with unlimited (URN) or limited renewal (LRN) of neuston in Los Chilenos pond (white line: median; black box: interquartile range; bar: extreme values). (A) Shell length growth rate (SLR). (B) Live weight growth rate (LWR). (C) Shell free dry weight (SFDW).

and associated organic remains) in a square metre of the pond was estimated as 1.825 mgC which represents ca. 7% of the daily ingestion rate (taking into account the carbon content of a gram of fresh lettuce; Mota et al., 2011). Even though this amount in a square metre seems very low it probably remains constant in the short term independently of consumption, as neuston and other surface materials tend to redistribute and replenish quickly from adjacent areas due to surface tension. This may be one of the great advantages of pedal surface collecting: in the short term the amount of food on the surface is continuously replenished and the snail does not need to search for any more or move to new trophic sources as they are depleted.

The cut off of the horizontal renewal of neuston and associated organic remains in the enclosures had a significant effect in only one out of three growth parameters tested. This probably indicates that there was an important aerial input of allochthonous organic material, mainly from the emergent macrophytes and riparian vegetation, as well as an input from the water column and bottom of the pond. As there are no deciduous forests in the surrounding area of the pond, the allochthonous input (pollen, floral parts, seeds, drowned insects, etc.) probably reached its maximum just when we performed our field experiment (summer), but the trophic availability of neuston and associated organic remains would be lower in other seasons of the year. Pollen pulses have marked seasonal patterns in different species and may produce pulses of secondary production in neuston food webs (Masclaux et al., 2013).

The massive consumption of submersed and floating macrophytes by *P. canaliculata* shifts the state of wetlands from a “clear water” state dominated by macrophytes to a “turbid water” one dominated by phytoplankton (Carlsson et al., 2004). During this process the abundance of submersed macrophytes decreases to very low levels and even to eradication due to apple snail grazing, light attenuation by dense phytoplankton and the continuous resuspension of fine sediments. On the contrary, the availability of neuston would increase due to the increase in the density of planktonic organisms (Södergren, 1987; Marshall and Gladyshev, 2009) and to the increased water fertility that would especially favour the small, tolerant and rapidly growing water ferns and duckweed (Hasan and Chakrabarti, 2009; Kutschera and Niklas, 2014). Apple snails are probably able to cope better with these ecosystem changes than other freshwater organisms and this ability of using alternative feeding mechanisms and resources may explain their persistence in waterbodies heavily impacted. As pointed out above, our results also indicate that apple snails are probably able to establish populations in waterbodies without submersed and floating macrophytes. However, the ability to exploit extensively neuston and surface materials would depend also on habitat structure, especially the presence of emergent substrate that the snails need for performing pedal surface collecting (Saveanu and Martín, 2013). The presence of emergent macrophytes, even though they are generally unpalatable (Carlsson et al., 2004; Burlakova et al., 2009), has already been considered an important habitat feature for *Pomacea* spp. as a substrate from where to ventilate the lung and also for egg laying (Karunaratne et al., 2006; Seuffert and Martín, 2010; Kyle et al., 2011), but under certain conditions it would be also important from a trophic viewpoint.

Neuston and organic matter associated with water surfaces are highly variable in abundance and composition due to their multiple allochthonous and autochthonous sources (Marshall and Gladyshev, 2009), but at the same time they are ubiquitous. Tadpoles (Goldacre, 1949; Kenny, 1969) and birds make use of neuston and associated materials when they are locally or temporarily abundant (DiGiacomo et al., 2002) and some spiders are specialized predators on the animal components of the neuston community (Zimmermann and Spence, 1989). The importance that these resources represent to apple snails in unpredictable trophic

contexts is reflected in the fact that they have evolved pedal surface collecting as a very peculiar and specialized behaviour for exploiting them (Dillon, 2000). The generalist feeding habits of *P. canaliculata* have been considered a key trait for the success as an invader (Lach et al., 2000).

Viviparids are another lineage of freshwater Caenogastropods in which a complementary feeding mechanism has also evolved but with a very different anatomical basis: the ttenidium has added the capture of particulate organic matter and plankton by filtration to its respiratory function and a ciliated groove to transport food near the head (Dillon, 2000). Among freshwater gastropods, viviparids are only second to ampullariids in size and perhaps the independent evolution of complementary feeding mechanisms in both lineages has allowed them to reach their great sizes. Interestingly, the use of these alternative feeding mechanisms also increases ontogenetically in both the viviparid *Bellamya* (Olden et al., 2013) and in *Pomacea* (Saveanu and Martín, 2013).

The evidence gathered in this study shows that neuston is a relevant trophic resource for *P. canaliculata* that may either substitute or complement the consumption of aquatic macrophytes. Consumption by apple snails can produce significant changes in aquatic vegetation and periphyton (Carlsson et al., 2004; Carlsson and Brönmark, 2006; Fang et al., 2010). Taking into account the ever spreading range of distribution, the high densities and the high feeding rates of *P. canaliculata* the consumption of neuston and associated organic remains by this invasive apple snail may also have wide scale effects on this particular community and biotope.

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