

EFFECT OF FOOD AVAILABILITY ON MORPHOMETRIC AND
SOMATIC INDICES OF THE APPLE SNAIL *POMACEA CANALICULATA*
(CAENOGASTROPODA, AMPULLARIIDAE)

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ABSTRACT

Pomacea canaliculata is a freshwater snail native to South America that together with some congeners has invaded natural wetlands and paddy fields in several continents, especially in Southern Asia. The high variability in shape, color and thickness of *Pomacea* shells and the sexual dimorphism in many traits blurs the species limits and hampers taxonomic identification. Ecological characterization of habitat productivity based on shells was previously proposed for *P. canaliculata* but was never methodically explored. Using full siblings of *P. canaliculata*, we studied the effects of different chronic levels of food availability (from 100% to 20% of daily ingestion rate) on shell shape, somatic indices and sexual dimorphism at maturity. The eight specific morphometric and somatic indices investigated showed different combinations of the effects of food availability and sex: changes related to food availability but independent of sex (relative aperture width), sexual dimorphism independent of food availability (shell globosity and relative aperture expansion), and changes related to food availability and sex, without a noticeable interaction (organic density); a significant interaction that increases the intersexual differences when food availability increases was detected in some indices (relative operculum weight, overall shell density and relative shell investment). The organic density can be used as a condition index to indicate the actual trophic availability in the field, although it should be estimated separately for males and females. The relative aperture width and the overall shell density can be used as paleo-environmental indicators of productivity, as they can be measured on empty shells. The effect of water alkalinity should be taken into account should the latter be used.

Key words: freshwater snail, shape, shell, invasive, organic density.

INTRODUCTION

Apple snails have invaded freshwater habitats of Southeast Asia, North America and the Pacific islands (Cowie, 2002; Hayes et al., 2008; Rawlings et al., 2007), causing economic losses in aquatic crops of several billion dollars (Joshi & Sebastian, 2006); one of the major pests in this group seems to be *Pomacea canaliculata* (Lamarck, 1822) (Cowie et al., 2006; Hayes et al., 2008). The shell of *P. canaliculata* is highly variable in shape, thickness, color and banding pattern among populations and also between sexes (Cazzaniga, 1990; Estebenet, 1998; Estebenet & Martín, 2003; Estebenet et al., 2006), which has contributed to taxonomic confusion in this genus (Cazzaniga, 2002; Cowie et al., 2006).

The inter-population variation in shell shape in *P. canaliculata* has both genetic and environmental causes (Estebenet & Martín, 2003; Estebenet et al., 2006), but the response to specific environmental factors has not been experimentally investigated. Food availability is an environmental factor that shows a strong effect on several life history traits of this species, including growth rates and age and size at maturity (Estoy et al., 2002a, b; Tamburi & Martín, 2009a, b). In other snails (Urduy et al., 2010), theoretical models and empirical evidence have suggested that growth rates can interact with allometric growth patterns to produce different shell shapes.

Adults of different populations of *P. canaliculata* have significant sexual dimorphism in size and shell shape (Cazzaniga, 1990; Estebenet,

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1998; Estebenet et al., 2006), with a variable degree of expression (Estebenet et al., 2006). The dimorphism in aperture shape in this species has been attributed to the development of the penis sheath (Cazzaniga, 1990; Estebenet, 1998), a voluminous ancillary copulatory organ on the mantle edge of males (Gamarra-Luques et al., 2006). *Pomacea canaliculata* males always mature at the same age under a gradient of food availability, but size at maturity decreases with deprivation (Estoy et al., 2002a; Tamburi & Martín, 2009a). Therefore, it is likely that food availability could explain a significant fraction of the variation in sexual dimorphism in shell shape through the effect on male size at maturity.

Estebenet & Martín (2003) suggested that *P. canaliculata* individuals growing at high rates develop thinner shells than those growing slower. The large investment in CaCO_3 to build egg shells (Cazzaniga, 1990) and to provide the embryos with storage for initial shell growth (Turner & McCabe, 1990) leads to the prediction that females should develop thinner shells than males, as previously observed by Cazzaniga (1990). Intersexual differences in shell thickness should increase with food availability due to the higher reproductive output in better fed females (Tamburi & Martín, 2011).

On account of the effects of *P. canaliculata* and congeners on natural wetlands (Carlsson et al., 2004; Cowie et al., 2006; Rawlings et al., 2007), it is important to know the actual trophic availability these apple snails have in the field, since it would be helpful to predict population growth rates on the basis of age at maturity and fecundity (Estoy et al., 2002b; Tamburi & Martín, 2009, 2011). However, it would be misleading to estimate such availability from aquatic vegetation biomass or cover, due to the different palatabilities of aquatic plants (e.g., Boland et al., 2008; Estebenet, 1995; Morrison & Hay, 2010; Qiu & Kwong, 2009) and the diverse feeding mechanisms (Cazzaniga & Estebenet, 1984) of this species. Consequently, an index of nutritional condition that reflects the food availability actually experienced by apple snails would allow a better prediction of their future impact on the aquatic vegetation of recently invaded water bodies. On the other hand, morphometric indices or marks that are measurable or recordable in apple snails when the shell is the only structure available for study have been considered of potential interest for paleo-environmental reconstruction (Martín & De Francesco, 2006; Van Damme & Pickford, 1995).

The main aim of this study was to test experimentally if food availability has an effect on shell shape and relative weight of *P. canaliculata* at maturity, through the response of several morphometric and somatic indices to different levels of food availability in each sex.

MATERIALS AND METHODS

The experimental design has been described in previous studies, in which the effect of food availability on age and size at maturity (Tamburi & Martín, 2009a), growth rates (Tamburi & Martín, 2009b) and reproductive output (Tamburi & Martín, 2011) of *Pomacea canaliculata* was studied. In order to minimize genetic variation, and to focus on environmental effects, snails used in the study were full siblings that came from a single egg mass laid by a laboratory reared female that only had contact with one male, both from the Curamalal Grande stream (36°57'36"S, 62°9'31"W, Buenos Aires Province, Argentina). All the hatchlings from the egg mass were reared during three weeks in a 20 liter collective aquarium. After this initial period, 72 of these snails were randomly selected (mean shell length \pm SE: 4.96 mm \pm 0.056, n = 25), and reared thereafter in isolation in 3 liter aquaria. Aquaria were filled with tap water saturated with CaCO_3 and placed in a rearing chamber that maintained homogeneous conditions of $25 \pm 3^\circ\text{C}$ and a 14 h : 10 h (light:dark) photoperiod; the snails were fed only with fresh lettuce.

The snails were randomly assigned to seven different levels of relative food availability (FA%). An empirical equation with shell length (SL) as the predictive variable (i.e., $\text{FA100\%} = 0.0033 \cdot \text{SL}^{1.9322}$; Tamburi & Martín, 2009b) was used to predict the daily amount of drained fresh lettuce ingested by a snail of a given size (FA100%, g) and the other levels of relative food availability (FA%: 87, 73, 60, 47, 33 and 20%) were calculated accordingly. The weekly ration was calculated according to the mean shell length of all snails assigned to each FA% level in a given week, and it was provided in two half-doses on Tuesdays and Fridays. Once a week the water was changed, aquaria were cleaned, and their position within the rearing chamber was changed randomly; the total shell length of each snail (TL, Fig. 1) was measured using a micrometric lens up to the sixth week or with a caliper thereafter. On each occasion, snails were inspected to detect their sex as

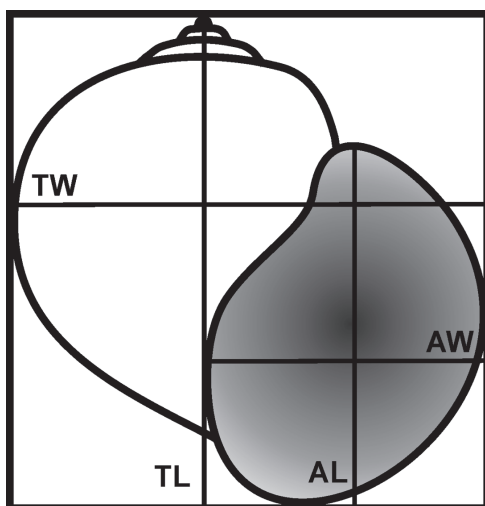


FIG. 1. Morphological measurements on the shells of *P. canaliculata*: total length (TL), aperture length (AL), total width (TW) and aperture width (AW).

early as possible through observation of the testicle through the shell or by the humping of the operculum in males. The number of male snails was equal (five) for each food availability level but, due to the random assignment of hatchlings to treatments, the number of females fluctuated, being 2, 3, 4, 4, 6, 6 and 3 from 100% to 20% FA respectively.

An external set of mature consort snails (three months older and fed with lettuce *ad libitum*) was used to detect the maturity of the experimental snails (i.e., copulatory behavior and egg laying). The randomly assigned consort snail was put into the experimental snail's aquarium for only 24 h a week (after the water

change), to minimize trophic interferences and the experimental snail was fed after the consort was retrieved.

Four weeks after the experimental snails reached maturity (first egg mass laid by experimental or consort females) they were sacrificed by immersion in water at 80°C and stored at -16°C for later dissection. The body was extracted from the shell and dried at 80°C for 48 h, weighed and then calcinated at 600°C for 4 h and the ash weight was obtained using an analytical scale (± 0.0001 g). The organic weight was calculated by the difference between the dry and ash weight. The shell and the operculum were weighed after 48 h at 80°C; the internal volume (V , mm^3) of the shell was estimated by the difference between the weight of the empty shell and its weight after filling it carefully with distilled water (assuming a density of 1 g/cm^3); this procedure of volume estimation was repeated twice and the results averaged. Digital images of the shell in apertural view were obtained and four linear dimensions of the shell (Fig. 1; Estebenet & Martín, 2003) were measured on the digital photographs with SigmascanPro® software.

Four indices of shell morphology were generated for studying the food availability and sexually related changes. The shell globosity (SHG) was defined as the total width (TW) relative to the total length (TL); the relative aperture expansion (RAE) as the aperture width (AW) relative to the total width (TW); the relative aperture width (RAW) as the aperture width (AW) relative to total length (TL) and the relative aperture shape (RAS) as the aperture width (AW) relative to the aperture length (AL).

Four somatic indices based on different combinations of volume, weight and linear measurements of the snails were calculated:

TABLE 1. Tests (p values) for morphometric and somatic indices at maturity in males and females of *P. canaliculata* reared under different food availabilities (x: Cuzick test was not performed if the effect of food availability was not significant). Shell globosity (SHG), relative aperture expansion (RAE), relative opercular weight (ROW), relative overall shell density (OSD), organic density (OD).

	Variable	SHG	RAE	ROW	OSD	OD
Kruskal-Wallis Test	Males	0.064	0.239	< 0.001	< 0.005	0.035
	Females	0.768	0.273	0.614	< 0.005	0.025
Cuzick Test	Males	x	x	< 0.001	< 0.001	< 0.001
	Females	x	x	x	< 0.001	< 0.001
t-Test between sexes		< 0.001	< 0.001	< 0.001	0.313	< 0.001

TABLE 2. Parametric tests (p values of two and one way ANOVAs) for morphometric and somatic indices at maturity in *P. canaliculata* for males and females reared under different food availabilities (FA%) (x: not performed). Relative aperture width (RAW), relative aperture shape (RAS), relative shell investment (RSI).

Variable	RAW	RAS	RSI	RSI (Males)	RSI (Females)
Transformation	None	None	ArcSin	ArcSin	Ln
Sex	0.851	0.095	0.069	x	x
FA%	< 0.005	0.112	< 0.001	0.451	< 0.01
Sex x FA%	0.657	0.259	< 0.005	x	x

the relative shell investment (RSI) was defined as the shell dry weight relative to the body ash weight, the relative opercular weight (ROW) as the operculum dry weight relative to the aperture product (AL x AW); the overall shell density (OSD) was defined as the shell dry weight relative to the internal volume of the shell (V) and the organic density (OD) was defined as the body organic weight relative to the internal volume of the shell (V).

The statistical analyses were performed through two-way ANOVAs when the homogeneity of variances was not rejected, or when it was attained after transformation (as suggested by the Box & Cox method). In the case that this assumption was not fulfilled, one-way non-parametric Kruskal-Wallis tests were carried out for each sex, since two-way Kruskal-Wallis tests could not be performed due to unequal cell sizes (Zar, 1984). In these instances, t-tests for unequal variances for the average of each sex and Cuzick's test for trends (Cuzick, 1985) were calculated to obtain more detailed information on the interaction between sex and food availability.

Voucher specimens have been deposited in Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN, Buenos Aires, Argentina). The specimens were juvenile (full-siblings of experimental snails) and adults snails (collected at the Curamalal Grande stream) registered under the collection numbers MACN-In 39436 and MACN-In 39437, respectively.

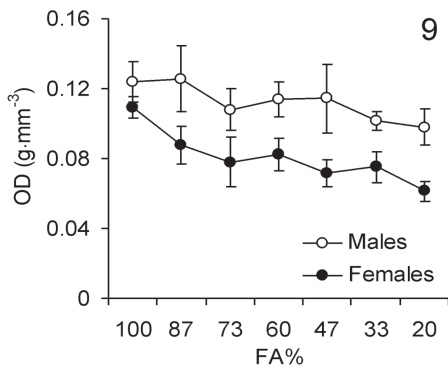
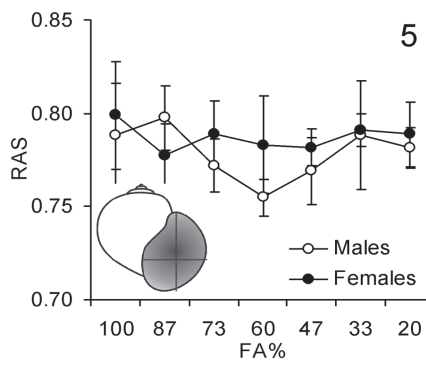
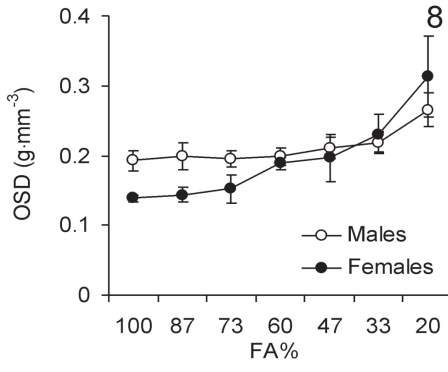
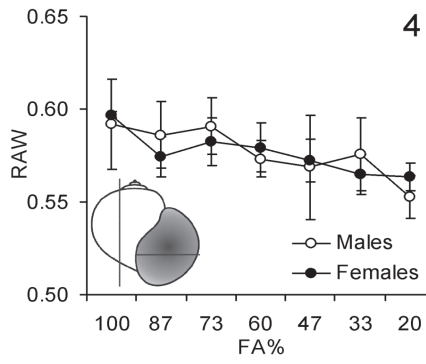
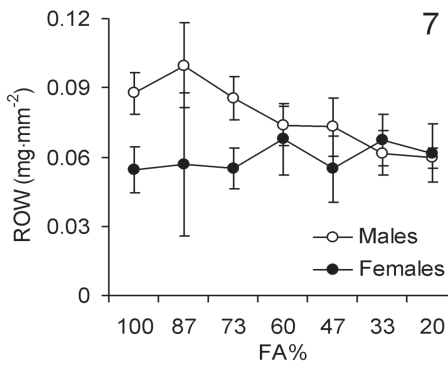
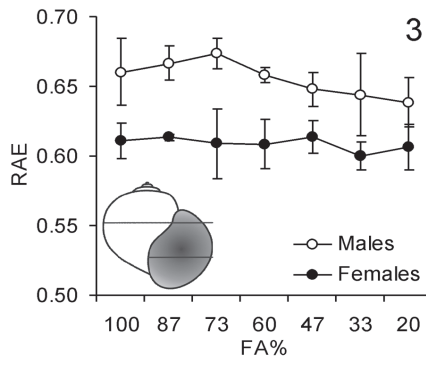
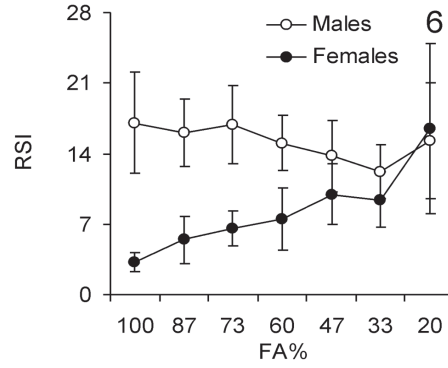
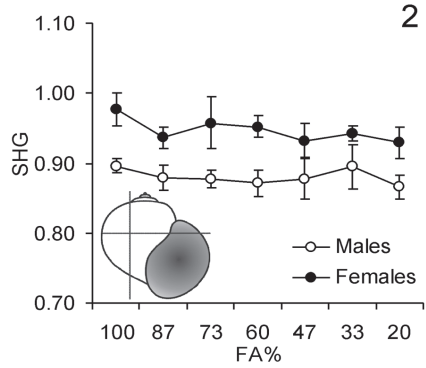
RESULTS

Pomacea canaliculata females showed a more globose shell (higher SHG; Fig. 2) and a relatively smaller aperture expansion (lower RAE) than males, irrespective of food availability (Figs. 3, 4; Table 1). The relative aperture width (RAW) did not show any sexual dimorphism but increased with food availability in both sexes (Fig. 5; Table 2), while the relative aperture shape (RAS) did not show sexual dimorphism or any effect related to food availability (Fig. 6; Table 2).

The relative shell investment (RSI) was independent of food availability in males and on the whole was higher than in females, in which this somatic index decreased with the increase in food availability (Fig. 6; Table 2). Conversely, in females the relative opercular weight (ROW) was independent of food availability and smaller on average than in males, in which it increased with food availability (Fig. 7; Table 1); the differences between sexes increased with food availability. The overall shell density (OSD) increased with food availability without significant sexual dimorphism in the grand mean of all treatments (Fig. 8; Table 1). Nevertheless, some degree of interaction between sex and food availability was apparent, so t-tests for unequal variances for comparing sexes at each food availability level were performed; the three higher food availability levels (100, 87, 73% FA) showed significant differences (global error corrected by Dunn-

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FIGS. 2–9. Morphometric and somatic indices (mean \pm SD) for *P. canaliculata* males and females at maturity depending on the food availability. FIG. 2: Shell globosity (SHG), FIG. 3: Relative apertural expansion (RAE), FIG. 4: Relative apertural width (RAW), FIG. 5: Relative apertural shape (RAS), FIG. 6: Relative shell investment (RSI), FIG. 7: Relative opercular weight (ROW), FIG. 8: Overall shell density (OSD), FIG. 9: Organic density (OD).



Sidak $p = 0.016$) while the remaining four (60, 47, 33, 20% FA) were not significantly different ($p > 0.05$ in each comparison). The organic density (OD) increased with food availability in both sexes, on average being higher in males than in females (Fig. 9; Table 1).

DISCUSSION

Our study showed different combinations of the effect of food availability and sex (single or double, additive or non additive) on eight morphometric and somatic indices for recently matured *Pomacea canaliculata* snails. Among morphometric indices, only the relative aperture width (RAW) was affected significantly by food availability. Several studies have shown a relationship between shell shape and growth rates in different snails (e.g., Chapman, 1997; Kemp & Bertness, 1984; Vermeij, 1980), which may be related to the interplay with allometric growth patterns (Urdu et al., 2010). Growth rates for the experimental snails studied here were strongly affected by food availability: the time required to attain a shell length of 22 mm increased more than three fold with a decrease of 80% in trophic availability (Tamburi & Martín, 2009b). Estebenet (1998) investigated the allometric growth in several dimensions of the shell of *P. canaliculata* and the reported deviations from isometry were mostly small (1.5% to 7%), probably accounting for the slight overall effect of growth rates on shell shape.

The relative opercular weight (ROW) showed no significant effect of food availability in females, probably indicating that the operculum area is proportional to that of the shell aperture. In the case of males, this index increases with food availability, without changes in aperture shape (RAS), probably reflecting an accelerated humping (Estebenet et al., 2006) or thickening of the operculum in males at high growth rates. The values of relative opercular weight for males in the two worst fed treatments were similar to those of females.

The pattern of overall shell density (OSD) relative to food availability indicates that the snails growing at higher rates have thinner shells. This inverse relationship between shell thickness and growth rate in *P. canaliculata* has been attributed to faster progress of the mantle edge (the zone of more intense deposition of CaCO_3) as the growth rate increases (Estebenet & Martín, 2003) and is apparently common among

snails (Urdu et al., 2010). In *P. canaliculata* females, the albumen gland complex stores large amounts of CaCO_3 (Catalán et al., 2006) and this constitutes most of the female's body ash content (Estebenet & Martín, 2003). The overall shell density only showed differences between sexes at the highest food availabilities, suggesting that the growth of the albumen gland complex is only detrimental to shell growth in these treatments, probably due to limitation in the calcium absorption rate (even in CaCO_3 saturated water). The higher levels of shell CaCO_3 relative to those of the soft parts (RSI) of females at low food availabilities are probably related to the smaller albumen glands in females deprived of food (Estoy et al., 2002b).

Youens & Burks (2008) stressed the need for standardized and reliable measurements of size and weight in order to obtain comparable estimates of individual and population biomass in *Pomacea*. In addition, the effects of environmental factors need to be considered in order to reduce, or to understand, the variability in these parameters. For instance, Estebenet & Martín (2003) suggested that the organic weight of the soft parts (adjusted by shell length) of *P. canaliculata* females increases with the productivity of the water bodies in which they live. Similarly, our results indicate that OD (organic weight relative to shell volume) of both females and males increases with food availability. These results suggest that the growth of shell volume is less affected by food availability than the overall growth of soft parts, probably combining lower amounts of stored reserves and muscle or an atrophied digestive gland. The smaller values for females are probably due to the large portion of their internal volume that is occupied by the albumen gland complex, which adds little to organic weight due to its high concentration of calcium spherules (Catalán et al., 2006).

Based on SHG, RAE and RAW indices, females were relatively more globous with less expanded apertures but with a similar aperture width relative to males; this sexual dimorphism can also be summarized as differences in the width of the last whorl (total width – aperture width), which was higher in females. This pattern of sexual dimorphism was not affected by the trophic availability. So, although sexual dimorphism probably increases the overall morphological variability of the shell and causes difficulties in taxonomic identification (Cowie, 2002), this effect is not worsened by interactions with food availability.

Previous researchers found sexual dimorphism in field collected adult snails of *P. canaliculata*, specifically in the opercular width to length ratio (Cazzaniga, 1990) and in the aperture width to shell length ratio (Estebenet, 1998). However, they did not find any differences in shell globosity, a sexually dimorphic variable in the present study, whereas we did not find any sexual dimorphism in shell aperture shape (as shown in the RAS index). These authors worked with lentic populations (two different ponds located near to the La Plata river, about 450 km to the NE of our study site), while in the present study the snails came from a lotic population in the Curamalal Grande stream, which belongs to a closed drainage basin (Encadenadas del Oeste basin; Martín et al., 2001), and hence this inter-population variation in sexual dimorphism could be due both to environmental and genetic differences. On the other hand, the differences with the previous studies may be related to the size of the snails analyzed. The males studied here were in general small to medium in size (24 to 40 mm; Tamburi & Martín, 2009a), while in previous studies snails had been obtained from the field and mostly large sizes had been selected: all the males were between 39 and 54 mm in Cazzaniga's study (1990) and 80% between 40 and 60 mm in the study by Estebenet (1998). Probably aperture-related variables are better for differentiating sex in large snails in which the development of the penis sheath deformed the aperture to a greater extent, while our criterion for sex determination did not need snails near to their asymptotic size.

The organic density, a somatic index interpretable as a nutritional or condition parameter, can be an adequate indicator of actual trophic availability in the field, although it must be estimated separately for males and females. Condition indices used frequently in marine mollusks use the relationship between body weight and shell weight (or total weight including the shell) to determine nutritional state (e.g., De la Hoz Aristizábal, 2010; Mouneyrac et al., 2008). However, in freshwater environments, water calcium and pH constitute strong sources of interpopulation variation in shell weight (e.g., in *Pomacea paludosa* (Say 1829); Glass & Darby, 2008) that have potential confounding effects on the interpretation of such indices. Condition indices, including shell weight, can also be affected by preservation procedures, especially when shell weight is estimated

indirectly (Martín, 2001). The organic density, calculated here as the ratio of body weight to shell volume, avoids this source of variation associated with shell weight, allowing better interpopulation comparisons of nutritional state. Furthermore, our results for this variable indicate that the meat profit obtained by such specialized predators as the snail kite *Rosthamus sociabilis* (Vieillot, 1987) (see Snyder & Snyder, 1969; Sykes et al., 1995) from an apple snail of a given volume would be lower in habitats with low trophic availability (from the snail's point of view).

The euryoecius character of *P. canaliculata* indicates that the presence of fossil shells is probably not a good indicator of past habitat types (Martín & De Francesco, 2006), although the plasticity of this apple snail indicates that other traits would be useful in paleo-environmental reconstruction. The overall shell density (OSD) and the relative aperture width (RAW) can be used as paleo-environmental indicators of productivity, as they show a significant effect of food availability and can be measured on empty shells of *P. canaliculata*. However, the relative shell thickness of *Pomacea* probably undergoes changes due to the water pH and calcium concentration (Glass & Darby, 2008) that may hamper the interpretation. Another factor that must be considered is that some part of the morphological variation among populations may have a genetic component (Estebenet & Martín, 2003).

On the whole our study showed, for the first time for an apple snail, the multiple and diverse effects of a particular environmental factor on morphometric and somatic indices. Food availability, the factor selected in our study, has also been found to produce plastic responses in life history traits (e.g., age and size at maturity or fecundity) of *P. canaliculata* (Estoy et al., 2002a, b; Tamburi & Martín, 2009a, b, 2011). Some of this reaction norms to food availability have been considered as adaptive for a snail living in environments where trophic resources are highly variable and unpredictable (Tamburi & Martín, 2009a, 2011). None of the effects of food availability showed here on morphometric and somatic indices are clearly interpretable as an adaptive developmental plasticity. Instead, most of them are probably the collateral outcome of physiological changes that are either adaptive or strongly influenced by the environment, or a combination of both.

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