



Temperature-induced plasticity in morphology and relative shell weight in the invasive apple snail *Pomacea canaliculata*

Nicolás E. Tamburi^{a,b}, María E. Seuffert^{a,c}, Pablo R. Martín^{a,c,*}

^a GECEMAC (Grupo de Ecología, Comportamiento y Evolución de Moluscos de Aguas Continentales), INBIOSUR (UNS-CONICET), San Juan 671, 8000 Bahía Blanca, Argentina

^b Departamento de Matemática, Universidad Nacional del Sur, Av. Alem 1253, 8000 Bahía Blanca, Argentina

^c Laboratorio de Ecología, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, 8000 Bahía Blanca, Argentina

ARTICLE INFO

Keywords:

Geometric morphometrics
Growth
Shell shape
Shell thickness
Ampullariidae

ABSTRACT

Temperature has a great influence on the life-history traits of freshwater snails. In this study we investigated the long term effects of a range of temperatures on shell morphology of the apple snail *Pomacea canaliculata*, a highly invasive species and an important pest of rice. Analysis of shells using geometric morphometrics showed that the main source of morphological variation was allometry, which was detected in males but not in females. This intersexual divergence in allometric trajectories generates much of the morphological variation evidenced. In females, the monotonic relationship with temperature produced narrower shells in the snails reared at lower temperatures, and more expanded apertures, relatively bigger than the body whorl, at higher temperatures. We also found an inverse relationship between relative shell weight, a proxy for shell thickness, and temperature. The differences in shape and relative shell weight are attributable to the different growth rates associated with different temperatures. Temperature fluctuation around a mean of 23.2 °C seemed to have no influence in shell shape and relative weight when is compared with a constant temperature of 25 °C. Information on the influence of temperature on freshwater snails is important for understanding and predicting changes in the face of global climatic change, especially in traits exhibiting great plasticity, such as shell shape and thickness. This work showed that higher temperatures could result in a relatively thinner shell, implying a greater significance of corrosion in flowing waters and a lower resistance to crushing by predators, especially in low latitude areas.

1. Introduction

Temperature has a great influence on most aspects of the biology and ecology of ectotherms in general (Angilletta, 2009), and on the life-history traits of freshwater snails in particular (Costil and Bailey, 1998; Hoefnagel and Verberk, 2017). Among these traits, the snail shell is an appropriate model to study phenotypic plasticity since variation in shape, color or thickness could be induced by variation in abiotic factors (e.g. temperature, Zdelar et al., 2017), or in resources (e.g. food availability, Tamburi and Martín, 2013). Information on the influence of temperature on freshwater snails is important for understanding and predicting changes in their distribution and performance in the face of global climate change (Tomanek, 2008), especially in traits exhibiting great plasticity.

Pomacea canaliculata (Lamarck 1822) is a freshwater snail in the family Ampullariidae, commonly known as apple snails. The native range of this South American apple snail extends throughout de la Plata

river basin from the subtropical Chaco (Paraguay and northern Argentina) to temperate Southern Pampas (37°S, central Argentina; Hayes et al., 2012; Martín et al., 2001). In the southernmost habitats of its natural distribution, low water temperatures during the coldest part of the year impose up to six months of inactivity and an iteroparous life cycle (Estebenet and Martín, 2002; Seuffert et al., 2010), while in more tropical areas activity is continuous and the life cycle is semelparous (Estebenet and Martín, 2002; Gilioli et al., 2017). The temperature range in which this species dwells has increased in the last three decades because it has been introduced into different regions around the world and has become a successful invader and a serious rice pest, especially in equatorial regions (Ecuador, Philippines, and Thailand) (Cowie, 2002; Horgan et al., 2014; Joshi et al., 2017).

Water temperature is an environmental factor that influences crawling, feeding, growth and reproduction of *P. canaliculata* (e.g. Estebenet and Cazzaniga, 1992; Estebenet and Martín, 2002; Ito, 2002; Matsukura and Wada, 2007; Seuffert and Martín, 2013, 2017; Seuffert

* Corresponding author at: GECEMAC (Grupo de Ecología, Comportamiento y Evolución de Moluscos de Aguas Continentales), INBIOSUR (UNS-CONICET), San Juan 671, 8000 Bahía Blanca, Argentina.

E-mail address: pmartin@criba.edu.ar (P.R. Martín).

<https://doi.org/10.1016/j.jtherbio.2018.04.008>

Received 13 December 2017; Received in revised form 15 March 2018; Accepted 21 April 2018

Available online 26 April 2018

0306-4565/ © 2018 Elsevier Ltd. All rights reserved.

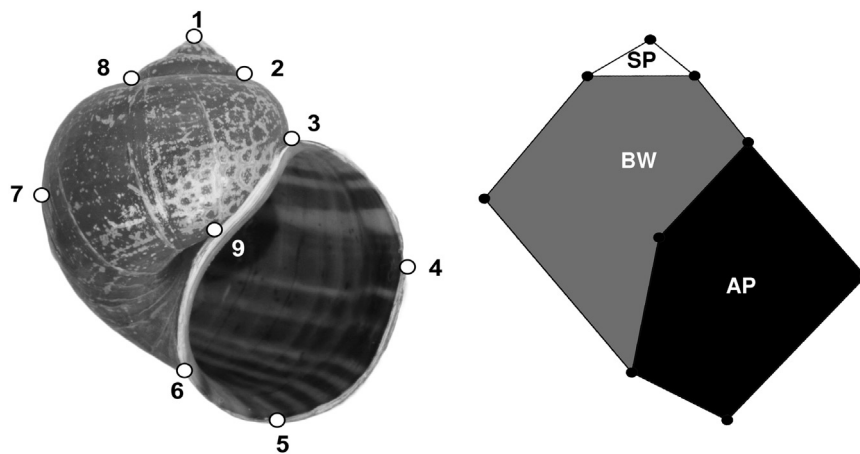


Fig. 1. Landmarks on the shell of *Pomacea canaliculata* and a scheme showing the main regions of the shell (SP: spire; BW: body whorl; AP: aperture).

et al., 2010). Temperature-induced changes in these biological functions may in turn differentially alter growth rates of different parts of the body and calcium carbonate deposition, and hence it is plausible that inter-population variation in shell characteristics among the diverse regions inhabited by this species could be explained, among other factors, by different thermal regimes.

The shell of *P. canaliculata* shows substantial variation in shape, thickness, color and banding pattern among populations and also between sexes (Cazzaniga, 1990; Estebenet, 1998; Estebenet and Martín, 2003; Estebenet et al., 2006; Tamburi and Martín, 2012). In its native range, the inter-population variability of shell shape is determined by both genetic and ecophenotypic causes (Estebenet and Martín, 2003; Estebenet et al., 2006). This great shell variability has contributed to taxonomic confusion in this genus (Cazzaniga, 2002), often compromising the development of rigorous and effective pest management strategies (Cowie et al., 2006). Although a hindrance in this aspect, the plasticity of the shell of apple snails may also be used to reconstruct paleo-environmental conditions (Goodfriend, 1992; Martín and De Francesco, 2006; Tamburi and Martín, 2012) or for bio-monitoring of water pollution levels (Márquez et al., 2011; Primost et al., 2015; Zdelar et al., 2017), but more information is needed about reaction norms to diverse environmental factors.

Relative shell weight (adjusted by shell length, shell volume or body weight) also shows great variation in *P. canaliculata*, differing among populations and between sexes, and is probably related to growth rates, water chemistry and to the investment of maternal calcium in the production of calcareous egg shells (Cazzaniga, 1990; Estebenet and Martín, 2003; Estebenet et al., 2006). These traits are in turn related to mechanical resistance to shell-crushing by predators and to reduction of the consequences of shell corrosion in carbonate corroding waters (Glass and Darby, 2009).

Most evidence of ecophenotypic variation in shell shape and relative weight comes from correlative studies of environmental conditions in the habitats where the snails live (e.g. Estebenet and Martín, 2003; Márquez et al., 2011; Moneva et al., 2012; Primost et al., 2015). Experiments involving controlled levels of the factors of interest are scarce for apple snails (e.g. Zdelar et al., 2017) and hitherto only the effect of food availability has been investigated in *P. canaliculata* (Tamburi and Martín, 2012, 2013).

In this study we analyzed the lifetime effects of different constant temperatures on shape and relative weight of *P. canaliculata* shells and whether there is sexual dimorphism in the reaction norms of these traits. Knowledge of the environmental factors producing ecophenotypic variation in the shells of this species will help to delineate interspecific limits, contribute to use of shells in paleo-environmental reconstruction and environmental monitoring and facilitate estimation of the effects of predators and turbulent water on mortality.

2. Materials and methods

2.1. Origin and maintenance of experimental snails

All the snails used in this work hatched from a group of ten egg masses collected during February 2012 in El Huáscar stream (36°55'3"S, 61°35'50"W, Buenos Aires Province, Argentina). Mean monthly air temperature in this region fluctuates between 7 and 21 °C and mean minimum and maximum temperatures are around 1 and 30 °C, respectively (Servicio Meteorológico Nacional, 2017). In the streams of this region it is common to record water temperatures around 0 °C and also higher than 35 °C (up to 36.2 °C; Seuffert et al., 2010).

The snails used in this work were reared at different constant temperatures from when they were one month old until their natural death (Seuffert and Martín, 2017). Subgroups of 12 individuals were randomly selected from the pool of hatchlings and placed in ten plastic aquaria of 20 L (30 × 35 × 20 cm); eight aquaria were maintained at four constant water temperatures with electric thermostats (20, 25, 30 and 35 °C; two aquaria for each temperature) and the other two aquaria were kept at room temperature, which was recorded hourly with a Hobo data-logger (accuracy = ± 0.67 °C). The snails were fed with fresh lettuce and once a week the aquaria were cleaned and the water was renewed. The shell length of each snail from the apex to the farthest point of the aperture was measured once a week with a Vernier caliper to the nearest 0.1 mm. The whole experiment lasted four years.

The sex of each snail was determined as early as possible during the experiment. Males were recognized by observation of the testis through the shell (Takeda, 1999) or by the humping of the operculum (Estebenet et al., 2006); snails without these male traits and bigger than the smallest male of each temperature treatment were considered to be females. Sexes were confirmed by examination of the soft parts after death. The shells were thoroughly cleaned from body remains immediately after the natural death of each snail. Digital images were obtained with a digital camera (DSLR, 55 mm lens), putting the shells in apertural view (Fig. 1) and keeping the plane between the most external border of the aperture and the columellar axis parallel to the supporting substrate, in order to minimize rolling and pitching. The shells were weighed with a digital scale to the nearest 0.001 g.

2.2. Morphological analyses

Following the geometric morphometrics methodology previously applied in this species (detailed by Tamburi and Martín, 2013), 9 landmarks (Fig. 1; Bookstein, 1997) were digitized using TPSdig2 software (Rohlf, 2017). Landmark 1 (LM 1) is the apex of the shell; LM 2 and 8 are left and right intersections of the last complete suture in the

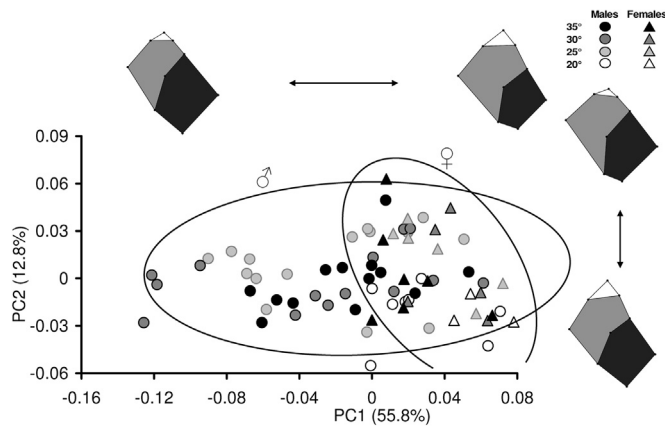


Fig. 2. Shell shape variation of *Pomacea canaliculata* reared at four different constant temperatures (20, 25, 30, 35 °C). The first two principal components show 68.6% of shape variation. Each sex is enclosed with 95% equal frequency ellipses. A scale factor of ± 0.1 was used to generate the snail wireframe graphs of shape variation along both axes.

shell outline; LM 3 is the upper suture between the body whorl and the aperture; LM 4 is the most external point of the aperture on the left margin and LM 7 is the most external point of the body whorl on the right margin of the shell; LM 5 is the extreme point of the aperture border opposite to the apex; LM 6 is the intersection of the aperture border and the outline of the body whorl; LM 9 is the intersection of the umbilicus border and the aperture border. MorphoJ Software (Version 1.05a; Klingenberg, 2011) was used to perform the Procrustes superimposition of images to account for differences in position, orientation and scale and, afterwards, to study in detail the aligned images (shape) and centroid size (size). The centroid size was calculated as the square root of the total quadratic distance between each landmark and the centroid of all landmarks in a particular specimen (Bookstein, 1991), constituting an integrated estimation of size based on all landmarks and not only on a single lineal measure, as it is shell length.

Possible differences in shell shapes of the snails reared under different constant temperatures were investigated by analyzing 50 males and 21 females (8, 15, 14 and 13 males and 3, 6, 5 and 7 females for the 20, 25, 30 and 35 °C treatments, respectively). The principal component analysis of the variance-covariance matrix of the Procrustes coordinates (Rohlf, 1993) was performed in order to investigate the most important variations in shape within this set of snails. Wireframe graphs, connecting selected landmarks with straight lines, were drawn to visualize the main differences in shape along each of the first two principal components. The spire, body whorl and aperture were coloured white, grey and black, respectively, to highlight the main regions of the shell mentioned along the text (Fig. 1) although the shell was analyzed as a unique module.

In order to determine if changes in shell shape are related to differences in size (allometry), regression analyses between shape variables and centroid size were performed for each sex. The allometric trajectories of males and females were compared through MANCOVA with centroid size as independent variable, by means of TPSregr software (following the methodology described by Viscosi and Cardini, 2011). Centroid size was log-transformed because of the wide range of shell lengths (20.7–70.4 mm). Multivariate tests of Pillai's trace were used because of unequal and small sample sizes in some treatments (Zar, 1984). When these results were significant, the subsequent analyses were performed using the residual values of the pooled within groups regression between shape and size, defined as allometry corrected shape (ACS), to explore shape changes separately from allometry (Tamburi and Martín, 2013). Allometric trajectories of the snails reared at different temperatures were compared within each sex to validate a global correction for allometry. A second set of multivariate regressions

was performed separately for each sex, with temperature as independent variable and shape or ACS as dependent variable, looking for a monotonic relationship between shape and temperature.

The shells of the snails reared at room temperature were used to study the effect of fluctuating temperature (FT) on shell shape. This treatment experienced temperatures between 9.4 and 31.9 °C with a mean of 23.2 °C (Seuffert and Martín, 2017). A discriminant analysis (DA) was performed to compare the shell morphology of snails that were reared under fluctuating temperature with those reared at a constant temperature of 25 °C. This temperature was selected for the DA because the mean of the FT treatment is closest to 25 °C and because only 6.5% of the records of FT were above 25 °C.

A measure of the snails' relative shell thickness was estimated following the methodology of Tamburi and Martín (2012). Relative shell weight (RSW, g cm^{-3}) was calculated as the shell dry weight divided by the internal volume of the shell; the latter was estimated as the difference between the weight of the shell filled with distilled water with a micropipette (considering a density of 1 g cm^{-3}) and that of the empty shell. Differences in RSW of snails reared at different temperatures were analyzed separately for females and males with ANCOVAs, using the centroid size as a covariate; the homogeneity of the slopes of the relationship of RSW with the covariate and the significance of the common slope were tested by F tests. The RSW of snails reared under fluctuating temperature was compared with those from 25 °C, for females and males separately, using t-tests.

3. Results

The first two principal components of the snails' shape reared under constant temperatures (Fig. 2) explained 68.6% of the morphological shell variation. Inter-sexual variation is appreciable in the first principal component with females exhibiting a narrower range of variation than males and some degree of overlap between both sexes. The allometric trajectories (Fig. 3) differ markedly between males and females (Pillai's trace: $\Lambda = 0.496$, $F_{14;97} = 6.83$, $p < 0.001$) and, consequently, we performed all subsequent analyses separately for each sex. Shell size explained 39.4% of the morphological variation in males (Pillai's trace: $\Lambda = 0.823$, $F_{14;35} = 11.67$, $p < 0.001$) while in females it explains only 5.04% (Pillai's trace: $\Lambda = 0.870$, $F_{14;6} = 2.88$, $p = 0.1004$).

The allometric trajectories in the different treatments showed the same pattern of change for both males (Pillai's trace: $\Lambda = 0.969$, $F_{42;93} = 1.06$, $p = 0.4054$) and females (Pillai's trace: $\Lambda = 2.818$, $F_{42;6} = 2.22$, $p = 0.1601$). In males, the common slope of these allometric trajectories was significantly different from zero (Pillai's trace: $\Lambda = 2.395$, $F_{56;140} = 3.73$, $p < 0.001$), so an allometric correction of shape was performed in subsequent analyses; in females the common slope

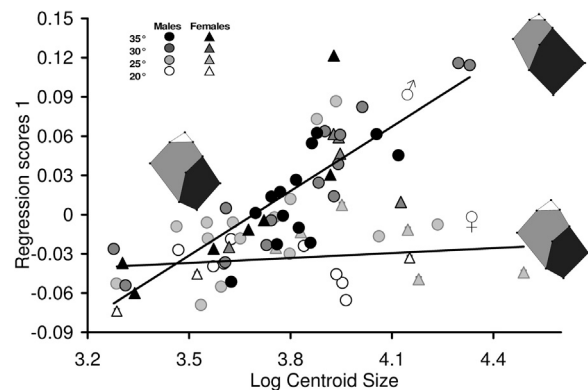


Fig. 3. Multivariate regression between size (log centroid size) and shape to detect allometry in females and males of *P. canaliculata*. A scale factor of ± 1 was used to generate the snails' wireframe graphs of shape variation along size axis; the snails' shell length ranged from 20.7 m to 70.4 mm.

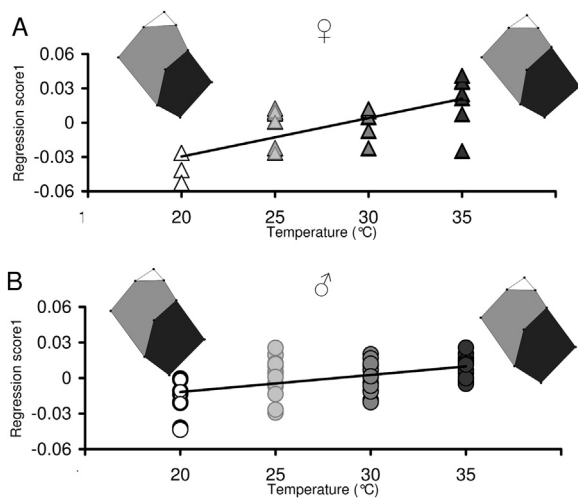


Fig. 4. Multivariate regression between temperature (20, 25, 30, 35 °C) and shape to detect changes in shape associated to treatments in females (A) and males (B). A scale factor of ± 10 was used to generate the snail wireframe graphs of shape variation along temperature axis.

was not significant (Pillai's trace: $\Lambda = 3.088$, $F_{56;24} = 1.45$, $p = 0.1592$), thus no allometric correction was performed.

Variation in shell shape attributable to different temperatures differed between sexes. The multivariate regressions between the different constant temperatures and shell shape showed a significant monotonic trend in females (Fig. 4A, $p = 0.0361$) but not in males (Fig. 4B, $p = 0.2097$). A 10.85% of shape variation was explained by temperature in the case of females whereas only a non-significant 2.82% was explained in males. In females, the location of the upper suture between the body whorl and the aperture (LM 3) change with temperature, resulting in narrower shells in the snails reared at lower temperatures, and more expanded apertures, relatively bigger than the body whorl, at higher temperatures (Fig. 4A).

In the analysis of females reared under fluctuating (FT) versus a constant temperature of 25 °C, the allometric trajectories showed the same slope for both treatments (Pillai's trace: $\Lambda = 0.627$, $F_{14;4} = 0.48$, $p = 0.8625$). The common slope of these treatments did not differ significantly from zero (Pillai's trace: $\Lambda = 0.651$, $F_{14;5} = 0.67$, $p = 0.7481$) and thus no allometric correction was performed. The discriminant analysis correctly classified only 42.9% of the specimens (Permutation test using T-square statistic: $p = 0.9298$).

The allometric trajectories of males under fluctuating (FT) and constant temperature of 25 °C showed the same slope (Pillai's trace: $\Lambda = 1.961$, $F_{42;18} = 0.81$, $p = 0.7223$) and the common slope did not differ significantly from zero (Pillai's trace: $\Lambda = 0.651$, $F_{14;5} = 0.67$, $p = 0.7481$). However, an allometry correction was performed anyway, since in the previous analysis of males with the four constant temperatures the common allometric trajectory was significantly different from zero. The discriminant analysis correctly classified only the 57.1% of the specimens (Permutation test using T-square statistic: $p = 0.0730$).

Relative shell weight (RSW) showed significantly higher variance in females than in males (variance ratio test: $F_{15;44} = 2.76$; $p = 0.004$), so the subsequent analyses were done for each sex separately (Fig. 5). ANCOVAs showed that centroid size explained a significant part of RSW variation in males but not in females (Table 1). In males, the adjusted means of RSW were significantly different among treatments, showing a significant decreasing trend with temperature ($F_{1;46} = 47.90$; $p < 0.0001$; $R^2 = 0.511$; Fig. 5). In females, the mean RSW (without covariate correction) showed no significant effect of temperature.

The RSW of females reared under fluctuating temperature showed no difference with females from the treatment of 25 °C (t -test for unequal variances: $t_{5,02} = 0.109$; $p = 0.917$; Fig. 5). Similarly, in the case

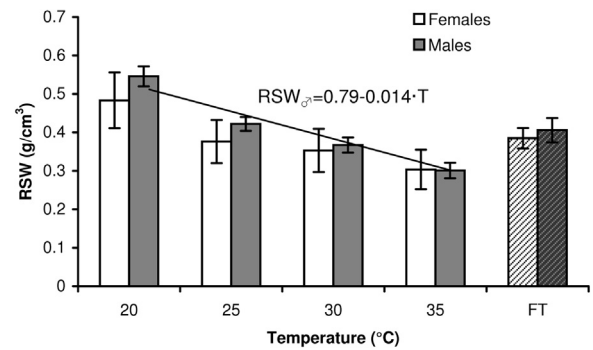


Fig. 5. Relative shell weight (RSW = shell weight/shell internal volume) in *P. canaliculata* males and females growing at different constant (plain pattern) or fluctuating temperatures (striped pattern). In the case of females bars are means \pm SE. For males the centroid size-adjusted means (\pm SE) and their regression line and equation to temperature are shown.

Table 1

Summary of one-way ANCOVAs of relative shell weight (RSW) of *Pomacea canaliculata* males and females reared under different constant temperatures. Centroid size was used as covariate; for females non-adjusted means were compared by ANOVA.

	Slope homogeneity	Common slope significance	Adjusted means homogeneity	ANOVA
Males	$F_{3,40} = 0.692$ $p = 0.5620$	$F_{1,43} = 8.09$ $p = 0.0068$	$F_{3,43} = 19.35$ $p < 0.001$	– –
Females	$F_{3,15} = 2.20$ $p = 0.1454$	$F_{1,14} = 2.43$ $p = 0.1412$	– –	$F_{3,15} = 1.39$ $p = 0.2835$

of males no difference was observed between RSW of snails reared under FT and 25 °C ($t_{19} = -0.482$; $p = 0.635$; Fig. 5).

4. Discussion

The main source of morphological variation of *Pomacea canaliculata* found in this work was allometry, which was detected in males but not in females. This intersexual divergence in allometric trajectories generates much of the morphological variation evidenced. Besides, significant morphological variation was induced in the shell of females when reared under different constant temperatures. Snails reared under regimes of constant or fluctuating temperature showed no difference in shell shape. Relative shell weight showed a negative relationship with water temperature.

The first axis of the principal component analysis showed that intersexual differences in *Pomacea canaliculata* constitutes an important source of shell shape variation, even in this set of snails reared under different temperatures throughout their entire lives. Similar results were obtained in *P. canaliculata* from the same region as the snails used in this work, when reared until maturity under different levels of food availability (Tamburi and Martín, 2013). Males exhibit a wider aperture than females and a humping in the operculum that is more evident when males are bigger (Cazzaniga, 1990; Estebenet, 1998; Estebenet and Martín, 2003; Tamburi and Martín, 2012, 2013). Our finding of significant allometry only for males indicates that intersexual differences in shell shape appear gradually during growth. Thereby, the isometric growth in females preserves a shape similar to that of juveniles while males depart from this initial shape. In previous work, Tamburi and Martín (2013) reported static allometry in both sexes but, as in this study, with a substantially smaller amount of morphological variation explained in females than in males.

The shell shape of female *P. canaliculata* showed a monotonic relationship with temperature, as was also the case between female shell shape and food availability (Tamburi and Martín, 2013). In both cases,

females reared at higher levels of each treatment (*i.e.* higher temperature and greater food availability) displayed a more expanded aperture, relatively bigger than the body whorl. The common point shared by these studies is that both an increase in food availability or in temperature generated an increase in growth rates. The morphological variation attributable to growth rate remains practically unchanged from maturity (Tamburi and Martín, 2013) to death (this work), suggesting that it could be useful in studies of paleo-environmental reconstruction based on the plastic shell of *P. canaliculata* (Martín and De Francesco, 2006).

The shell shape variation of *P. canaliculata* reared at different temperatures differed between sexes. In *Pomacea bridgesii*, temperature also generated differences in shell shape, with cooler waters producing shells with relatively smaller and rounder apertures (Zdelar et al., 2017), like the females in this study. Sexual shape dimorphism is not evident in *P. bridgesii* (Coelho et al., 2012), hence it is likely that this species shows a basal relationship between shape and temperature similar to that recorded in this study for *P. canaliculata* females. The visualization of this pattern is probably obscured in *P. canaliculata* males due to their allometric growth.

The analysis of the effect of constant (25 °C) against fluctuating temperature (FT) on shape showed no significant differences in males nor in females. Although more specific studies would be necessary, it is interesting to highlight that fluctuating temperatures do not seem to add morphological variation in this range of temperatures. In the same way, no difference was found in the relative shell weight (RSW) of snails reared under FT and 25 °C. On the whole, temperature fluctuation seems to have little influence compared with constant temperature. This is a desirable trait for the use of shell shape in paleo-environmental reconstruction.

Shells are relatively heavier and thicker in *P. canaliculata* males than females, probably because of the females' allocation of calcium reserves to calcareous egg shells and perivitelline reserves for the embryo (Estebenet and Martín, 2003). Snails growing with restricted food availability develop relatively heavier and thicker shells (Tamburi and Martín, 2012), probably because of their lower growth rates. Here we found an inverse relationship between relative shell weight and temperature, probably also attributable to the different growth rates generated by temperature. In fast growing apple snails, the area of most active deposition of CaCO₃ moves continuously forward, thus reducing the time that it stays at a given point of the shell and therefore resulting in a thinner and lighter shell relative to volume (Estebenet and Martín, 2003). Physicochemical effects cannot explain the pattern found since the solubility of calcium carbonate in water decreases with temperature (Graus, 1974). Nevertheless, this pattern was statistically significant only in the case of males. The smaller amount of data for females combined with the higher variance in their RSW probably blurred this difference. The greater variation in females' RSW could be a consequence of the differences in egg production, and hence in CaCO₃ allocation, among females at each temperature.

An increase in global temperature due to climatic change will probably influence considerably the distribution and performance of freshwater snails (Tomanek, 2008). For this reason, is of great importance understanding the gradient of responses of different traits to increasing temperatures, especially those exhibiting great plasticity, such as shell shape and thickness. The results of this work suggest that high temperatures could result in a faster growth and, in consequence, thinner and lighter shells. A similar result has been reported for the Florida apple snail, *Pomacea paludosa*, reared under different levels of Ca⁺⁺, with the snails at lower levels exhibiting thinner shells, implying a higher corrosion and lower crushing resistance (Glass and Darby, 2009). Therefore, invasion into warmer zones or an increase in temperature due to global warming would imply a greater vulnerability to predators, to corrosion and to harmful collisions, especially in flowing waters. These negative effects of high temperatures on snail vulnerability could interact with reduced fecundities and egg hatchabilities

above 25 °C (Seuffert and Martín, 2017), thus contributing to reduce the invasive potential of *P. canaliculata* in tropical areas where shell crushing predators are abundant.

Acknowledgments

This work was funded with grants from CONICET (“Consejo Nacional de Investigaciones Científicas y Técnicas”, PIP 112 200901 00473), UNS (“Universidad Nacional del Sur”, PGI 24/B185) and ANPCyT (“Agencia Nacional de Promoción Científica y Tecnológica, PICT 2012-1956). NET, MES and PRM are researchers in CONICET. We wish to thank to three anonymous reviewers for their helpful comments.

References

- Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Approach. Oxford University Press, New York.
- Bookstein, F.L., 1991. Morphometric Tools for Landmark Data. Cambridge University Press, New York.
- Bookstein, F.L., 1997. Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Med. Image Anal.* 1, pp. 225–243.
- Cazzaniga, N.J., 1990. Sexual dimorphism in *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Veliger* 33 (4), 390–394.
- Cazzaniga, N.J., 2002. Old species and new concepts in the taxonomy of *Pomacea* (Gastropoda: Ampullariidae). *Biocell* 26 (1), 71–81.
- Coelho, A.R.A., Calado, G.J.P., Dinis, M.T., 2012. Freshwater snail *Pomacea bridgesii* (Gastropoda: Ampullariidae), life history traits and aquaculture potential. *AACL Bioflux* 5 (3), 168–181.
- Costil, K., Bailey, S.E.R., 1998. Influence of water temperature on the activity of *Planorbis cornutus* (Pulmonata, Planorbidae). *Malacologia* 39, 141–150.
- Cowie, R.H., 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: Barker, G.M. (Ed.), *Molluscs as Crop Pests*. CABI Publishing, Wallingford, pp. 145–192.
- Cowie, R.H., Hayes, K.A., Thiengo, S.C., 2006. What are apple snails? Confused taxonomy and some preliminary resolution. In: Joshi, R.C., Sebastian, L.S. (Eds.), *Global Advances in Ecology and Management of Golden Apple Snails*. Philippine Rice Research Institute, Nueva Ecija, pp. 3–23.
- Estebenet, A.L., 1998. Allometric growth and insight on sexual dimorphism in *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Malacologia* 39, 207–213.
- Estebenet, A.L., Martín, P.R., 2002. *Pomacea canaliculata* (Gastropoda: Ampullariidae): life-history traits and their plasticity. *Biocell* 26, 83–89.
- Estebenet, A.L., Cazzaniga, N.J., 1992. Growth and demography of *Pomacea canaliculata* (Gastropoda: Ampullariidae) under laboratory conditions. *Malacol. Rev.* 25, 1–12.
- Estebenet, A.L., Martín, P.R., 2003. Shell interpopulation variation and its origin in *Pomacea canaliculata* (Gastropoda: Ampullariidae) from southern Pampas, Argentina. *J. Molluscan Stud.* 69, 301–310.
- Estebenet, A.L., Martín, P.R., Burela, S., 2006. Conchological variation in *Pomacea canaliculata* and other South American Ampullariidae (Caenogastropoda, Architaenioglossa). *Biocell* 30, 329–335.
- Gilioli, G., Schrader, G., Carlsson, N., van Donk, E., van Leeuwen, C.H.A., Martín, P.R., Pasquali, S., Vilà, M.h., Vos, S., 2017. Environmental risk assessment for invasive alien species: a case study of apple snails affecting ecosystem services in Europe. *Environ. Impact Assess.* 65, 1–11.
- Glass, N.H., Darby, P.C., 2009. The effect of calcium and pH on Florida apple snail, *Pomacea paludosa* (Gastropoda: Ampullariidae), shell growth and crush weight. *Aquat. Ecol.* 43 (4), 1085–1093.
- Goodfriend, G.A., 1992. The use of land snail shells in paleoenvironmental reconstruction. *Quat. Sci. Rev.* 11, 665–685.
- Graus, R.R., 1974. Latitudinal trends in the shell characteristics of marine gastropods. *Lethaia* 7, 303–314.
- Hayes, K.A., Cowie, R.H., Thiengo, S.C., Strong, E.E., 2012. Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). *Zool. J. Linn. Soc.* 166, 723–753.
- Hoefnagel, K.N., Verberk, W.C.E.P., 2017. Long-term and acute effects of temperature and oxygen on metabolism, food intake, growth and heat tolerance in a freshwater gastropod. *J. Therm. Biol.* 68, 27–38.
- Horgan, F.G., Stuart, A.M., Kudavidanage, E.P., 2014. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecol.* 54, 90–100.
- Ito, K., 2002. Environmental factors influencing overwintering success of the golden apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae), in the northernmost population of Japan. *Appl. Entomol. Zool.* 37, 655–661. <http://dx.doi.org/10.1303/aez.2002.655>.
- Joshi, R.C., Cowie, R.H., Sebastian, L.S., 2017. Biology and Management of Invasive Apple Snails. Philippine Rice Research Institute (PhilRice), Nueva Ecija.
- Klingenberg, C.P., 2011. MORPHOJ: an integrated software package for genetic morphometrics. *Mol. Ecol.* 11, 353–357.
- Márquez, F., González-José, R., Bigatti, G., 2011. Combined methods to detect pollution effects on shell shape and structure in Neogastropods. *Ecol. Indic.* 11, 248–254.
- Martín, P.R., De Francesco, C.G., 2006. Fossil record of *Pomacea* (Caenogastropoda:

- Ampullariidae) in Argentina and its paleoenvironmental implications. *Biocell* 30, 337–343.
- Martín, P.R., Estebenet, A.L., Cazzaniga, N.J., 2001. Factors affecting the distribution of *Pomacea canaliculata* (Gastropoda: Ampullariidae) along its southernmost natural limit. *Malacologia* 43, 13–23.
- Matsukura, K., Wada, T., 2007. Environmental factors affecting the increase in cold hardness in the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Appl. Entomol. Zool.* 42, 533–539.
- Moneva, C.S.O., Torres, M.A.J., Wada, T., Joshi, R., Demayo, C.G., 2012. Relative warp and correlation analysis based on distances of the morphological shell shape patterns of *Pomacea canaliculata* Lamarck from Japan and Philippines. *Int. J. Bioflux Soc.* 4, 12–21.
- Primost, M., Bigatti, G., Márquez, F., 2015. Shell shape as indicator of pollution in marine gastropods affected by imposex. *Mar. Freshw. Res.* 67 (12), 1948–1954. <http://dx.doi.org/10.1071/MF15233>.
- Rohlf, F.J., 1993. Relative warp analysis and an example of its application to mosquito wing. In: Marcus, L.F., Bello, E., García Valdecasas, A. (Eds.), *Contributions to Morphometrics*. Museo Nacional de Ciencias Naturales, Madrid, pp. 131–159.
- Rohlf, F.J., 2017. TPSDig2, version 2.30. Department of Ecology and Evolution, State University of New York at Stony Brook. <http://life.bio.sunysb.edu/morph/>. Accessed 2018 May 4.
- Servicio Meteorológico Nacional, 2017. <<http://www.smn.gov.ar/>>, (Accessed 15 April 2017).
- Seuffert, M.E., Burela, S., Martín, P.R., 2010. Influence of water temperature on the activity of the freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) at its southernmost limit (Southern Pampas, Argentina). *J. Therm. Biol.* 35, 77–84.
- Seuffert, M.E., Martín, P.R., 2013. Juvenile growth and survival of the apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) reared at different constant temperatures. *SpringerPlus* 2, 312.
- Seuffert, M.E., Martín, P.R., 2017. Thermal limits for the establishment and growth of populations of the invasive apple snail *Pomacea canaliculata*. *Biol. Invasions* 19, 1169–1180.
- Takeda, N., 1999. Histological studies on the maturation of the reproductive system in the apple snail, *Pomacea canaliculata*. *J. Anal. Biosci.* 22, 425–432.
- Tamburi, N.E., Martín, P.R., 2012. Effect of food availability on morphometric and somatic indices of the apple snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae). *Malacologia* 55, 33–41.
- Tamburi, N.E., Martín, P.R., 2013. Allometric and trophic effects on shell morphology of *Pomacea canaliculata* (Caenogastropoda, Ampullariidae) from a geometric morphometrics viewpoint. *Molluscan Res.* 33 (4), 223–229.
- Tomanek, L., 2008. The importance of physiological limits in determining biogeographical range shifts due to global climate change: the heat shock response. *Physiol. Biochem. Zool.* 81 (6), 709–717.
- Viscosi, V., Cardini, A., 2011. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. *PLoS One* 6 (10), e25630. <http://dx.doi.org/10.1371/journal.pone.0025630>.
- Zar, J.H., 1984. *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, New Jersey.
- Zdelar, M., Mullin, F., Cheung, C., Yousif, M., Baltaretu, B., Stone, J.R., 2017. Pollution-, temperature- and predator-induced responses in phenotypically plastic gastropod shell traits. *Molluscan Res.* <http://dx.doi.org/10.1080/13235818.2017.1358587>.