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Allometric and trophic effects on shell morphology of *Pomacea canaliculata* (Caenogastropoda, Ampullariidae) from a geometric morphometrics viewpoint

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Pomacea canaliculata, an invasive apple snail native to South America, is a serious pest of aquatic crops in several parts of the world. The origin of inter-population variation in shell shape is thought to be both genetic and environmental but the reaction norms to specific environmental factors are still poorly understood. Our aims were to analyze the existence of direct and indirect (allometric) effects of food availability (FA) on the shape of young adults of *P. canaliculata*. Full sibling hatchlings were reared under different levels of FA. Nine landmarks and 10 semi-landmarks were determined on photographs of mature shells and analyzed using geometric morphometrics. In both sexes significant allometry was found: a decrease in the spire height in both sexes, and an increase of the aperture size in males and of the last whorl in females. When this allometric component was removed a relationship between size-corrected shape and FA was found only in females, which were more globose and had a larger aperture when grown under high FA. This effect may be explained by the faster growth of the reproductive organs and the thinner shells of the best fed females.

Keywords: invader; apple snail; shape; landmark; food availability; maturity

Introduction

Pomacea canaliculata (Lamarck, 1822) is a freshwater snail with a great capacity to invade new environments (Cowie 2002). Native of southern South America, this apple snail and other congener species have been introduced into Southeastern Asia, North America and the Pacific islands (Cowie 2002; Rawlings *et al.* 2007; Hayes *et al.* 2008; Lv *et al.* 2012) where they caused severe impacts on wetland vegetation and aquatic crops such as rice and taro. The great shell variability makes their identification difficult, as well as complicating the study of several aspects of their biology (Estebenet *et al.* 2006). Their confused taxonomy has contributed to a delay in the development of effective pest management strategies (Cowie *et al.* 2006).

Morphometric studies of *P. canaliculata* in its native range showed the existence of sexual, genetic and ecophenotypic components of shell variability (Estebenet and Martín 2003; Estebenet *et al.* 2006). Estebenet (1998) also studied its ontogenetic allometry (i.e. changes in shell shape associated with size increase during individual development) and determined slight allometric patterns for different portions of the shell. However, the shape variation among adults of different sizes (phenotypic static allometry; Gould 1971; Cheverud 1982) has not been studied previously.

As yet, food availability (FA) is the only environmental factor that has been shown to affect shell shape on recently mature *P. canaliculata* (Tamburi and Martín 2012), but it also affects the size at maturity (especially in males; Tamburi and Martín 2009a). The shell shape variation revealed using linear dimensions and morphometric indexes (Estebenet and Martín 2003; Estebenet *et al.* 2006; Tamburi and Martín 2012) is difficult to interpret when the growth of different parts of the shell is allometric because some differences may be due to the difference in size. Some geometric morphometric techniques use landmarks (anatomical loci with correspondence within and among specimens) for morphological studies (Kendall 1977). An advantage of these techniques is that they maintain the geometric relationships between landmarks throughout the analysis (Rohlf and Slice 1990) and they also allow the relationship between size and shape to be disentangled (Klingenberg 2011). Geometric morphometrics based on shell landmarks have been used in several snails to discriminate among species, to study inter- and intra-specific variation, to detect sexual dimorphism or the effect of pollutants, etc. (Conde-Padín *et al.* 2007; Márquez *et al.* 2011; Minton and Wang 2011; Avaca *et al.* in press). Such techniques were recently used in *P. canaliculata* to study inter- and intra-population variation at the micro-geographic scale in the Philippines (Tabugo *et al.* 2010;

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Torres *et al.* 2011) and Japan (Moneva *et al.* 2012). The main aim of this study was to analyze the existence of phenotypic static allometry and the effect of FA on the shape of young adults of *P. canaliculata* using geometric morphometrics.

Materials and methods

The experimental design has been described in detail in previous studies (Tamburi and Martín 2009a, 2012). The snails used in the study were full-siblings to minimize genetic variation (hatchlings from a single egg mass laid by a laboratory couple originally from the Curamalal Grande stream, Argentina, 37° 14.731' S, 62° 07.795' W). Seventy-two three-week-old snails were randomly assigned to seven different levels of relative FA%. The snails were reared in individual 3 l aquaria with CaCO₃ saturated tap water at 25 ± 3°C, under a 14 h:10 h (light:dark) photoperiod and fed with fresh lettuce only. The daily amount of drained fresh lettuce that can be ingested by a snail of a given size (FA100%, g) was estimated using an empirical equation with shell length (SL) as the predictive variable (i.e. FA100% = 0.0033 · SL^{1.9322}; Tamburi and Martín 2009b) and the other levels of relative FA% (87%, 73%, 60%, 47%, 33% and 20%) were calculated accordingly. The weekly ration was calculated according to the mean SL of all the snails assigned to each FA% level in a given week.

The aquaria were cleaned once a week and the water was changed. The SL of each snail was measured and the sex was determined by observation of the testis through the shell (Takeda 1999) or of the humping of the operculum (Estebeñet *et al.* 2006) in males. The number of females varied from two to six for each FA level due to the random assignment of hatchlings to treatments, but the number of males was five in all cases.

In order to determine the moment in which each snail attained maturity, the experimental snails were paired each week for 24 h (after the water change) with a randomly assigned mature snail of the opposite sex. Four weeks after the first egg mass laid by an experimental female, or by the mate of an experimental male, the experimental snails were sacrificed by immersion in water at 80°C and digital images of the shell in apertural view were obtained.

A total of 19 landmarks, 9 true landmarks (LM, Figure 1) and 10 sliding semi-landmarks (SLM) (Bookstein 1997) were digitalized using TPSdig2 software (Rohlf 2010). Landmark 1 (LM 1) is the apex of the shell; LM 2 and 18 are left and right intersections of the last complete suture in the shell outline; LM 3 is the upper suture between the last whorl and the aperture; LM 6 is the most external point of the aperture on the left margin and LM 15 is the most external point of the last whorl on the right margin of the shell; LM 9 is the extreme point of the aperture border opposite the apex; LM 12 is the intersection of

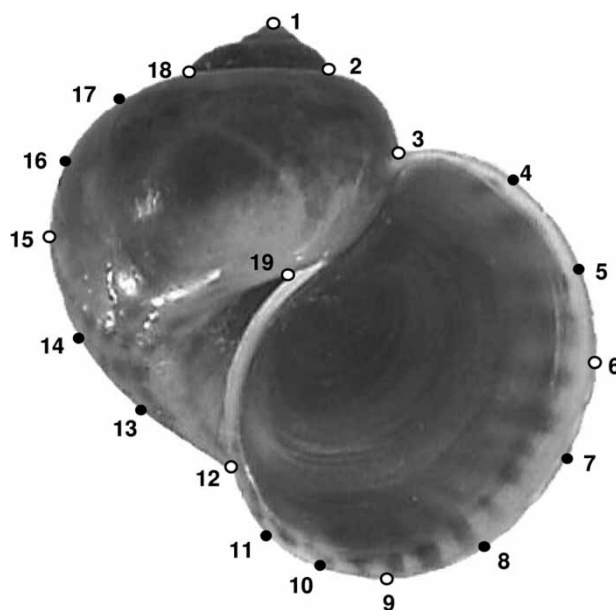


Figure 1. LM (white dots) and sliding SLM (black dots) on the shell of *P. canaliculata*.

the aperture border and the outline of the last whorl; LM 19 is the umbilicus of the shell. Sliding SLM were generated by drawing curves along the shell outline between selected pairs of adjacent landmarks and then using the TPSdig2 resampling tool to reduce the number of points until two equidistant SLM were obtained. Six SLM (4, 5, 7, 8, 10 and 11) were used to represent the left aperture profile and four SLM (13, 14, 16 and 17) to represent the right profile of the last whorl.

The software TPSrelw (Rohlf 2010) was used to relax the sliding SLM with the criterion of minimizing the bending energy matrix. After the Procrustes superimposition to account for differences in position, orientation and scale, the aligned specimens (shape) and centroid size (size) were saved for detailed study with MorphoJ Software (Version 1.05a; Klingenberg 2011). The centroid size is the square root of the total quadratic distance between each landmark point and the centroid of all landmarks in a particular specimen (Bookstein 1991); it was calculated with TPSrelw and introduced in MorphoJ Software as a covariate of size.

The relative warps ($\alpha = 0$) were calculated as the principal component analysis of Procrustes shape coordinates (Rohlf 1993). The consensus configuration and the deformation grids of the first relative warp were drawn in order to study the most important variations in shape among the specimens. Regressions between shape and centroid size were performed for each sex to study possible allometric effects. The residuals of these regressions were used in a new regression analysis for each sex to look for relationships between shape and FA that were independent of allometry.

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 adult snails (collected at the Curamalal Grande stream) registered with the collection numbers MACN-In 39436 and MACN-In 39437, respectively.

Results

The scatter plot of individual shell landmarks on the consensus configuration showed no important deviation from isotropy (Figure 2). The first two relative warps of the whole sample of snails showed 95% equal frequency ellipses (ellipse with 95% probability of containing a specimen) for males and females that are not overlapping in this morpho-space (Figure 2). Intersexual shape differences are noticeable in the first relative warp, with females showing a more globose shell; in males the aperture was clearly larger than the last whorl, whereas in females it was slightly smaller.

Thereafter males and females were analyzed separately with their own consensus shape on account of this sexual dimorphism. The regression analysis showed a significant relationship between shape and centroid size in females and males (Figure 3). The shells of males that matured larger in size showed a relatively less elevated

spire, smaller last whorl and larger aperture. The females that matured larger in size also showed a relatively less elevated spire and a slightly larger last whorl. The allometry was stronger in males than in females: the centroid size accounts for 16.69% of shape variation in males ($p = 0.0001$) and for only 7.33% in the case of females ($p = 0.0366$). In order to isolate the effect of FA on shell shape from the allometric effect we worked with the residuals of the regression between shape and centroid size (corrected shape). A significant relationship between size-corrected shape and FA was found in females (Figure 4(a); $p = 0.0126$). The FA level explained 8.79% of shape variation, the female shells being more globose and with larger apertures when grown at high FA levels (Figure 4(a)). In the case of males, there was no relationship between shape and FA after removing the allometric effect (Figure 4(b); $p = 0.9704$; $R^2:0.86\%$); in accordance with this any variation in shape observable in the photographs or shell outlines in Figure 4(b) is non-interpretable variation.

Discussion

Sexual dimorphism was the main component of morphological variation in our data set composed of snails at the same stage of life (recently matured) that were grown

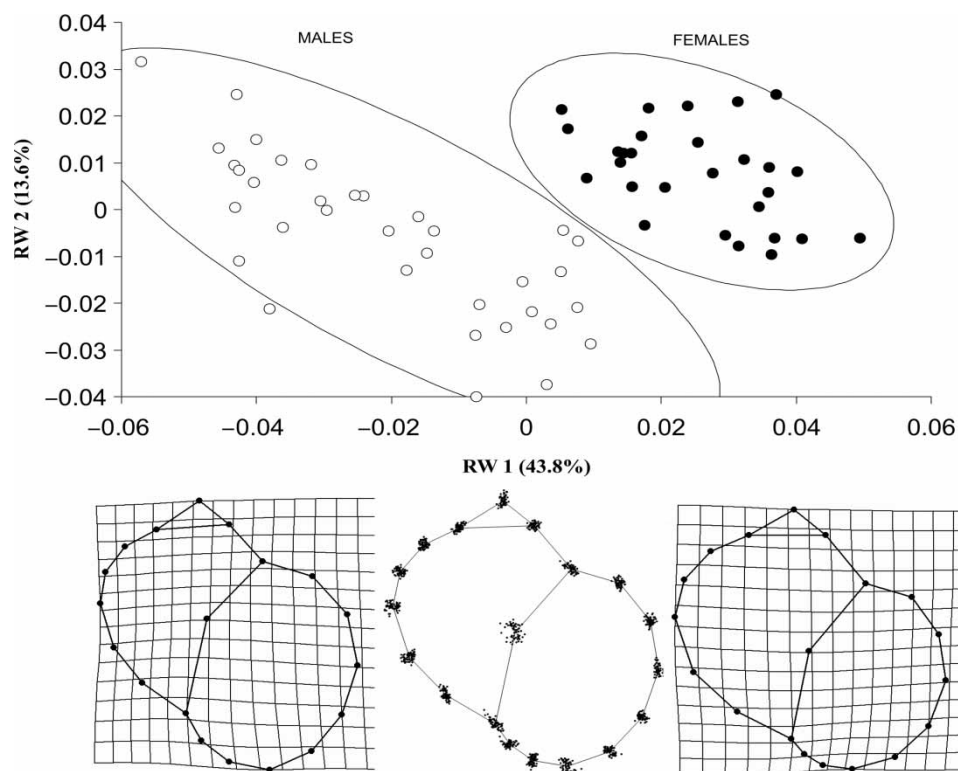


Figure 2. Principal component analysis of shape changes in *P. canaliculata* reared under a FA gradient. A total of 57.4% of shape variation is represented in the first two principal component axes. The figure includes the 95% equal frequency ellipses for males and females. The shell outline in the centre is the consensus shape with the specimen's dispersion of each landmark. The shell outlines in the deformation grids belong to the extreme specimens along the first component axes.

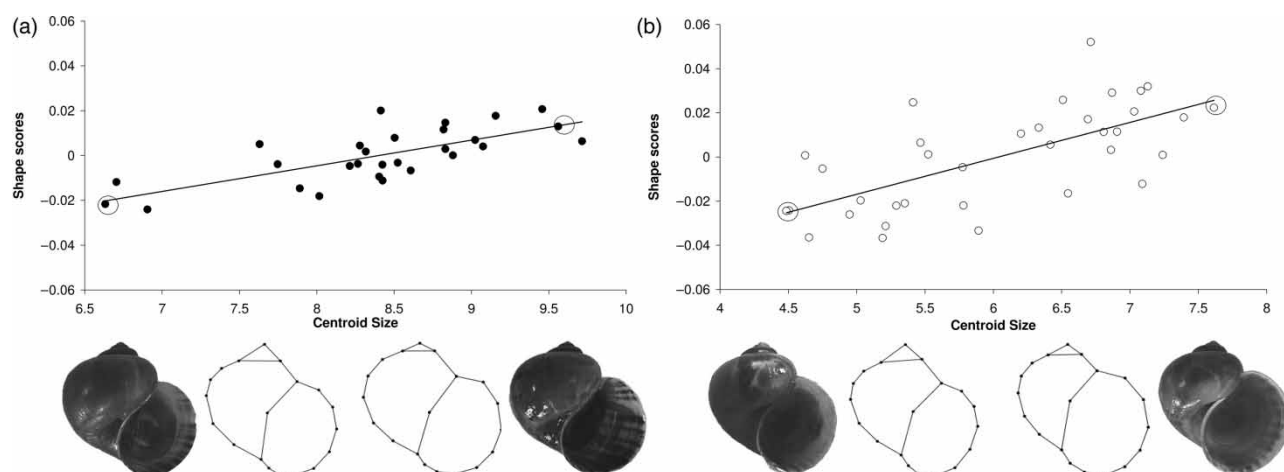


Figure 3. Regression between shape and centroid size in *P. canaliculata* reared under a FA gradient. (a) Females and (b) males. Left and right snail outlines are the allometric deviations from female consensus shape associated with low and high values of centroid size, respectively. Left and right snail photographs correspond to the left and right specimens circled in the scatter plot.

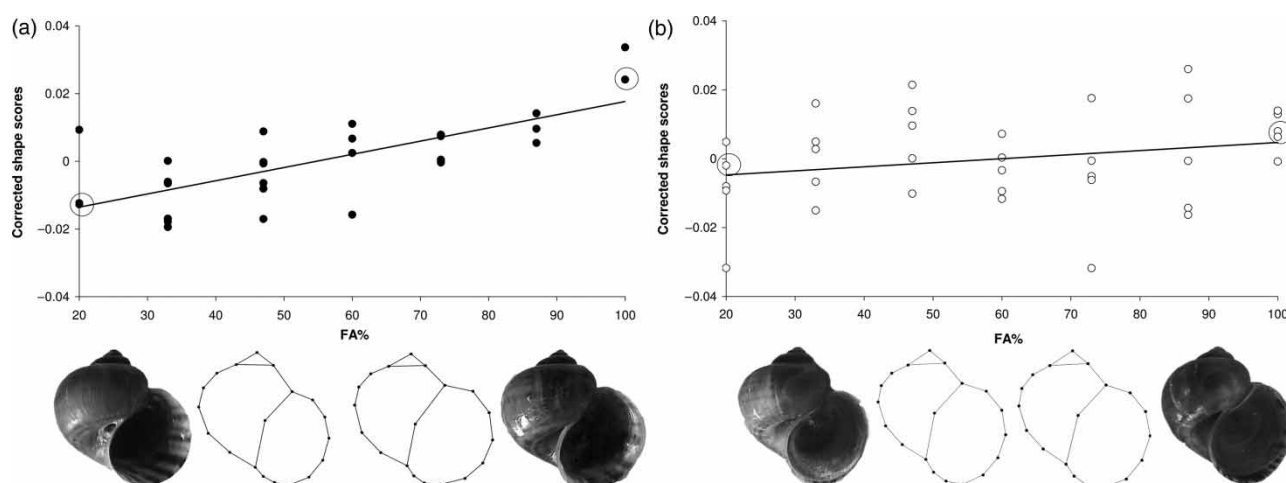


Figure 4. Regression between corrected shape and FA in *P. canaliculata* reared under a FA gradient. (a) Females and (b) males. Corrected shape values are the residual scores of the regression between shape and size previously calculated in Figure 3. Left and right snail outlines are the non-allometric shape changes associated with low and high FA levels, respectively. Left and right snail photographs correspond to the left and right specimens circled in the scatter plot.

in different trophic environments (FA levels). Moreover, our snails were full siblings and therefore they represented a narrow range of genetic variation. Tabugo *et al.* (2010) using geometric morphometrics also found that intersexual shape differences were in general greater than inter-population ones (probably both genetic and environmental in origin) for three lotic populations of *P. canaliculata* from the Philippines. However, intersexual differences in shape were less than inter-population ones when populations from Japan and the Philippines were studied using the same techniques (Moneva *et al.* 2012). These results indicate that the sex of snails must be taken

into account when studying morphological variation, especially at small spatial scales.

Sexual dimorphism in the genus *Pomacea* (Perry, 1810) was first detected in 1990 in *P. canaliculata* (Cazaniga 1990) and since then it has been studied in this species on different occasions (Estebeñet 1998; Estebeñet and Martín 2003; Estebeñet *et al.* 2006; Tamburi and Martín 2012). However, no studies are available for other species of the genus and only qualitative reports exist for a few species of apple snails (Demian and Ibrahim 1972). Geometric morphometrics is a very sensitive technique that has been used recently to reveal

cryptic sexual dimorphism in *Viviparus subpurpureus* (Say, 1829) (Minton and Wang 2011), a genus with a somewhat similar shell-shape to apple snails. Perhaps, the application of this technique will help to show that sexual dimorphism in shell shape is widespread among apple snails.

Both sexes of *P. canaliculata* showed static allometry as indicated by the significant association between shell shape and shell size. Probably part of the inter-population variation in shell shape detected in natural populations from the native range may be the result of the differences in size. For instance, the shell shape of both males and females from lotic and lentic habitats in the south of the Pampas differed significantly when studied using morphometric ratios (linear dimensions of the shell relative to SL; Estebenet *et al.* 2006). The maximum sizes attained by both sexes were also higher in lentic environments and hence the differences between the two habitat types may be related to size differences and not to a direct effect of the water current. On the other hand, Torres *et al.* (2011) did not find any relationship between shape and centroid size when working with field collected males and females from different rice fields in a small area of the Philippines. The differences between the results of studies undertaken in native and invaded areas may be due to environmental, genetic and methodological factors, but at the moment there is not enough information to perform any kind of meta-analysis.

The different effect of FA on the size-corrected shape in each sex is probably related to the strongly dimorphic effect of this factor on the age and size at which maturity is reached (Estoy *et al.* 2002a; Tamburi and Martín 2009a). The males used in the present study matured at a wide range of sizes (19–30 mm; coefficient of variation, CV = 27%) and in a narrow age range (12–24 weeks; CV = 10%) whereas females matured in a relatively narrower size range (32–42 mm; CV = 9%), and a wide age range (14–50 weeks; CV = 41%) (Tamburi and Martín 2009a). Static allometry in males is probably related to their wide range of size at maturity coupled with a common pattern of ontogenetic allometry (Estebenet 1998) but no direct effect resulting from FA was detected. In females, FA showed a significant effect on shell shape independently of the size variation between treatments; the combined effect of ontogenetic allometry and size was probably weak due to the small range of size variation in this sex. Under conditions of restricted FA, the age at maturity increases more than threefold (14–50 weeks) relative to that of better fed females and hence the underlying mechanism had more time to accumulate differences in shape between the FA levels.

A feasible mechanism that might explain the effect of FA in females relates to the growth of the albumen gland–capsule gland (AG–CG) complex, the most bulky component of the female reproductive system (Catalán

et al. 2006). This massive complex bulges into the mantle cavity (Andrews 1965) and modifies the water flow and compresses the pallial organs, which probably results in a modification in the shape of the aperture and consequently of the shell it generates. The development of the AG–CG complex is very plastic and can be significantly delayed by food deprivation (Estoy *et al.* 2002b), which in turn retards the onset of oviposition (Tamburi and Martín 2009a). In the most deprived females, the expansion of the aperture under the influence of the development of the AG–CG complex was probably delayed in comparison to well-fed females. Shell thickness decreases as FA increases in both sexes but this pattern is more noticeable in females (Estebenet and Martín 2003; Tamburi and Martín 2012) and hence the shell aperture of the best fed females was probably more susceptible to deformation.

In a previous study (Tamburi and Martín 2012), we found that the ratio aperture width/SL increases with FA, without any differences between males and females. In the present study, working with the same shells, we were able to detect that the common trend was apparently due to different causes in each sex: an allometric increase of aperture size related to the direct effect of FA on male size and the same allometric effect combined with a direct effect of FA on aperture size in females.

Allometric patterns in the shells of *P. canaliculata* and congeners seem to be slight (Estebenet 1998) but the size range among and within populations is often very wide (Martín and Estebenet 2002; Estebenet *et al.* 2006) and this may explain part of the shell shape variability in these snails. The landmark-based techniques used here allowed the detection of these allometric effects and their discrimination from direct environmental effects.

Several species of *Pomacea* have been introduced into different continents and their identity and origin has only recently been elucidated with the aid of molecular genetics (Rawling *et al.* 2007; Hayes *et al.* 2008; Lv *et al.* 2012). The development of strategies for their management as pests and invaders has been delayed due to the taxonomic confusion in this genus (Cowie *et al.* 2006). The high variability in shell shape, size and colour has greatly contributed to this confusion (Estebenet *et al.* 2006). The sensitivity of the morphometric techniques used here at intraspecific levels in other taxa (Cardin 2000; Ibañez *et al.* 2007) indicates that it will probably be useful for the characterization and identification of different species of *Pomacea* without using genetic-based techniques.

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