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ORIGINAL PAPER

Comparing applesnails with oranges: the need to standardize measuring techniques when studying *Pomacea*

Abigail K. Youens · Romi L. Burks

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Abstract Although invaders come in all shapes and sizes, several mollusks have recently achieved notoriety as both economically and ecologically costly invaders. Applesnails of the genus *Pomacea* get their name from reaching the size of an apple. Native to South America, the species *P. insularum* has recently established reproducing, and potentially invasive, populations in Texas, Alabama, Georgia, and Florida. In contrast to the widely invasive golden applesnail (*P. canaliculata*), few studies of the channeled species *P. insularum* exist. In studying similar invasive applesnail species, scientists use several methods of measurement. We have explored the relationships among shell height, operculum width, and weight among juvenile and adult *P. insularum* and tested their inter-measurer reliability. We also investigated the use of shell height, shell length, and operculum width measurements in *P. canaliculata* studies and observed whether or not those studies defined their measurements. We found that operculum width served as a significantly more reliable measure among researchers. Furthermore, operculum width better predicted weight than shell height. The majority of articles that measured *P. canaliculata* did not define their measurements, which may cause problems when comparing studies between native and exotic populations or when comparing the two species. We recommend that future studies of *P. insularum* use operculum width to measure snails and explore a possible sex dimorphism in the operculum width of adult *P. insularum.*

A. K. Youens · R. L. Burks (✉)
Southwestern University, 1001 E. University Avenue, Georgetown, TX 78626, USA
e-mail: burksr@southwestern.edu

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Introduction

In 1958, ecologist Charles Elton recognized that we live in a "very explosive world" where many exotic species successfully invade (Elton 1958). The magnitude of introductions continues to increase with globalization, changing land use and climate change (Pimentel et al. 2005). Levine and Antonio (2003) predicted that the number of invasive species in the United States would increase logarithmically as trade barriers open up around the world. Although invaders come in all shapes and sizes, several mollusks, including zebra mussels (*Dreissena polymorpha*), Asian clams *(Corbicula fluminea)* and the Asian freshwater mussel (*Limnoperla fortunei*) have achieved notoriety as both economically and ecologically costly invaders (Carlton 1999; Darrigran 2002; Drake and Bossenbroeck 2004). However, few mollusks have the size and appetite of applesnails of the genus *Pomacea*, which get their name from growing to the size of an apple (Joshi and Sebastian 2006). Large size and a taste for aquatic plants suggest high potential for ecological damage (Lach et al. 2000), which has been realized with the influx of *Pomacea canaliculata* (“golden” or “channeled” applesnails) into agricultural systems (Dancel and Joshi 2000; Joshi 2005).

Scientists have recently identified another exotic invasive applesnail (i.e., *P. insularum*) by genetic analysis (Cowie et al. 2006). This applesnail species recently established reproducing populations in Texas, Alabama, Georgia and Florida (Howells et al. 2006). No peer-reviewed literature exists for *P. insularum*, and extant studies occur at an early stage. Size represents a potentially important life history characteristic in *P. insularum.* Specifically, size and population density of females may affect clutch size (Tanaka et al. 1999) and size also serves as a key factor in assessing the macrophyte damage caused by herbivores, indicates the age-structure of a population and likely determines how many snails have reached reproductive maturity (Cazzaniga 1990).

We noticed that literature studies of *P. canaliculata* used different measuring techniques and wondered if these measurements would prove equivalent or comparable. In order to avoid the effects of comparing measures that lack meaningful relationships, like comparing apples to oranges, we set out to determine which measurements allow the fastest, most valid, and most reliable measurements for study of *P. insularum.* In the present study, we explored the extent of measurement use (primarily operculum width, shell height, and shell length) within past studies on *P. canaliculata.* In addition, we wanted to explore relationships between body size measurements (weight, operculum width and shell height) in *P. insularum* as a life history trait and as a way to discover which measurements best predicted weight. We expected that both operculum width and shell height would be predictive of weight. However, based on our own experience, we thought operculum width would exhibit less variation between measurers than shell height and serve as a more reliable measure for future field studies.

Methods

Lab measurements

In order to examine how morphological measures of applesnails compare in different life stages, we first collected 100 adult apple snails (*P. insularum)* from Armand Bayou and surrounding areas in Houston, Texas, in May 2006 and measured them on-site after collection. Juvenile snails came from our lab population, which hatched from egg clutches collected from Armand Bayou in May 2005. We used juveniles grown in the laboratory because of their scarcity and time-consuming collection in the field. We measured 144 juvenile snails every 4 days for 24 days. We measured the operculum width, or the widest distance across the operculum (in mm), with digital calipers touching the outside of the shell on either side of the aperture (Fig. 1a). Guedes et al. (1981) referred to it as “greater opercular diameter” and Estebenet and Martín (2003) referred to it as “apertural length.” Second, we measured shell height (in mm), or the tallest measurement of the shell perpendicular to the bottom edge of the aperture (Fig. 1b). Third, we patted snails dry with a paper towel and weighed them on a digital scale (in g; accurate to the g for adults and to 0.01 g for juveniles). We conducted regressions between all three combinations of body size relationships for juveniles, adults, and both life stages combined (SPSS 13.0). We chose the equation (linear or logarithmic) that represented the best predictive relationship between each pair of measurements and the R^2 values for each relationship.

In order to analyze the inter-measurer reliability of operculum width and shell height, we had six researchers who worked in the lab measure the same

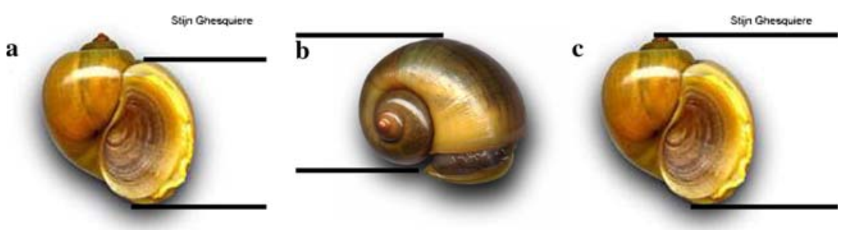

Fig. 1 Operculum width (**a**), shell height (**b**), and shell length (**c**) measurements for *P. insularum* in the present study. Pictures taken from http://www.applesnail.net

10 juvenile and 10 adult *P. insularum.* We gave the researchers a quick tutorial on measuring operculum width and shell height with digital calipers. We calculated the coefficient of variation (CV) between measurers for each snail and then we conducted *t*-tests between the averages of the CVs for operculum width and shell height. We conducted *t*-tests separately for adults and juveniles (SPSS 13.0). Lower CVs indicated a more reliable measurement.

Literature analysis

In order to explore the use of different measurements in *P. canaliculata* research as a guide for future *P. insularum* studies, we performed an extensive literature search of *P. canaliculata*-related articles to analyze their measurement methods. The articles came from Biological Abstracts and Science Direct search engines at Southwestern University and from the reference list of a recent book chapter on applesnails (Howells et al. 2006). From the Biological Abstracts search engine, we selected any article retrieved with the search criteria "apple snail" and "*Pomacea canaliculata*" in all text and dated no later than 1999, the approximate date that *P. insularum* established populations in Texas and researchers started to study it as an exotic invasive species. In the Science Direct search engine, any articles returned with the search criteria "apple snail" and "*Pomacea canaliculata*" in the title, abstract or keywords qualified. From the Howells et al. (2006) book chapter, we selected any peer-reviewed article that included the words *Pomacea canaliculata* (or reference to snails without the name of another species) and appeared in 1999 or later. For each article, we determined the name of the measurement used and whether or not the authors defined the measurement. We included articles that did not measure snail body size in our results, but did not obtain further articles from their reference lists. For all of the articles that did measure body size, we collected articles from their reference lists by the same criteria that we used for the book chapter. We only included articles with full-text available in English in the present study. Our analysis included percentages of articles that measured operculum width, shell height, and shell length (the distance across the snail from the whorl to the operculum, Fig. 1c), and the percentage of articles that defined their measurements.

Results

Lab measurements

We found strong, significant and linear regression relationships between all combinations of measurements. Some choices of regression equations made a marginal difference as logarithmic regressions better explained the relationships between weight and operculum width and weight and shell height for juveniles and for juveniles and adults combined (Table 1; Fig. 2). Differences in R^2 values between linear and logarithmic equation ranged from 0.013 to 0.022 for juveniles and from 0.028 to 0.045 for both stages combined. Weight and operculum width for

Table 1 Regression equations for all body size relationships

Pomacea insularum population	Panel	Measurements	Regression model	Equation
Juveniles	a	Shell height versus Weight	Logarithmic	$y = 4.756 \ln(x) + 10.997$
	b	Operculum width versus Weight	Logarithmic	$y = 5.372 \ln(x) + 11.896$
	c	Shell height versus Operculum width	Linear	$y = 0.834x + 1.284$
Adults	d	Shell height versus Weight	Linear	$y = 0.156x + 27.731$
	e	Operculum width versus Weight	Linear	$y = 0.137x + 33.075$
	f	Shell height versus Operculum width	Linear	$y = 0.908x + 0.926$
Juveniles and Adults	g	Shell height versus Weight	Logarithmic	$y = 7.27 \ln(x) + 9.258$
	h	Operculum width versus Weight	Logarithmic	$y = 7.798 \ln(x) + 0.978$
	i	Shell height versus Operculum width	Linear	$y = 0.929x + 0.201$

The small letters refer to the panel in Fig. 2

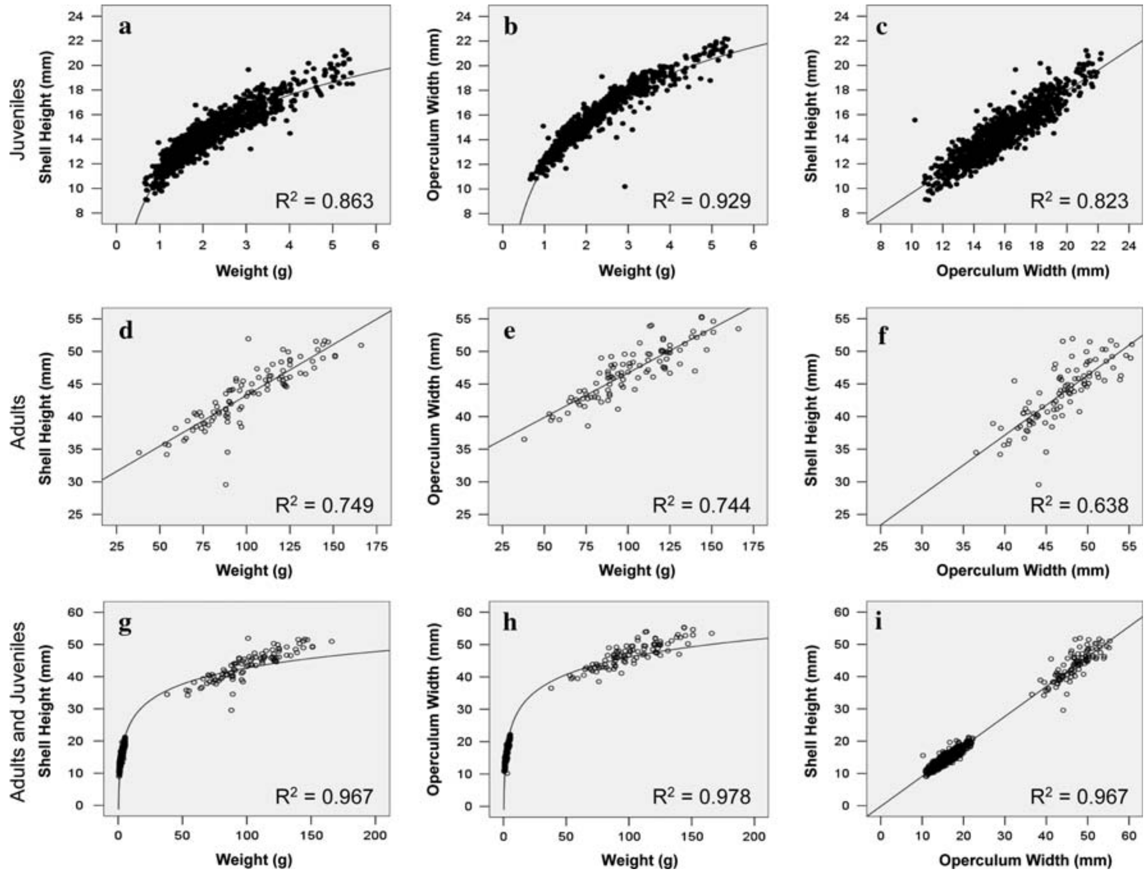

Fig. 2 Scatter plots of all body size relationships. Filled circles represent juvenile *P. insularum* (N = 989) and open circles represent adult *P. insularum* (N = 100). Regression equations occur in Table 1. For all relationships, $P < 0.001$

juveniles and adults combined exhibited the strongest relationship ($R^2 = 0.978$; $P < 0.001$). Adult operculum width and shell height resulted in the least predictive relationship ($R^2 = 0.638$; $P < 0.001$).

Operculum width exhibited a significantly higher inter-measurer reliability than shell height for both juvenile ($t_{29} = 9.912$; $P < 0.001$) and adult ($t_{29} = 12.981$; $P < 0.001$) life stages. The average CV between six measurers for *P. insularum* ended up 2.6 times higher for juvenile shell height than for operculum width (0.065 and 0.025, respectively) and 1.4 times higher for adult shell height than for operculum width (0.056 and 0.041, respectively).

Literature analysis

We found 48 articles that met our search criteria (for a list of specific articles and results, please contact the corresponding author). Of these articles, 11% measured operculum width, 48% measured shell height, and 28% measured shell length. Twenty-three percent of the articles that measured snails defined their measurements. Fifteen percent of articles did not measure snails. Of studies that measured native *P. canaliculata*, the majority measured shell length (83%). In contrast, the majority of studies of exotic *P. canaliculata* measured shell height (67%).

Discussion

Both of the body measurements investigated with lab measurements—operculum width and shell height—explained much of the variation in weight. Of all relationships between measurements, operculum width versus shell height in adult *P. insularum* had the weakest relationship ($R^2 = 0.638$), although this still provides a reasonable estimation by ecological standards. Juvenile and adult weight versus operculum width provided the best predictive measure ($R^2 = 0.978$). Furthermore, measuring operculum width resulted in significantly higher inter-measurer reliability than measuring shell height. *P. canaliculata* studies most commonly measure shell height (48%), but the majority of articles that measured snails did not define their measurements (77%). Two articles in particular (Estebenet and Martín 2003; Teo 2004) provided figures which greatly aided interpretation of their results. A noticeable difference existed between the measurements used by studies of exotic *P. canaliculata* (shell height) and those used by studies of native *P. canaliculata* (shell length). All measures are predictive of one another, but differences among measures in studies of *P. canaliculata* appear problematic nonetheless. Differences in measurements could impede the direct comparison of age structure and growth measurements between populations in experiments on *Pomacea.*

Our finding that operculum width exhibited higher inter-measurer reliability than shell height makes it a more promising measurement for snail body size. Operculum width is easy to define and it is easier to find the widest diameter across the operculum, a two-dimensional surface, than to find the tallest height of the snail, a three dimensional animal. None of these measurements harm the snails and ecologists can perform them easily in the field. Guedes et al. (1981) calculated regressions between measurements of snail size to devise a field measure for biomass. They measured the operculum by our method, which they called greater opercular diameter, and recorded the dimensions perpendicular to our measurement, which they termed lesser opercular diameter. Guedes et al. (1981) found that the product of greater and lesser opercular diameter correlated highly with the biomass ($R^2 = 0.9332$). Because one can view an applesnail shell as a convoluted cone, Guedes et al. (1981) argue that the operculum is analogous to the base and represents a valid dimension for measurement. We did not measure lesser opercular diameter in our study. However, our result that maximum operculum width may be a more effective measure of size than shell height adds to the potential problem created by differential measurements among researchers. For *P. insularum*, the possibility still exists to establish a standardized measurement among all ecologists to avoid having to compare apples to oranges. The reliability of measuring operculum width found in the present study and the validity of the measurement of the operculum posited by Guedes et al. (1981) for *P. canaliculata* make a strong argument for using operculum width as a standard measurement for *P. insularum.*

Our study separated *P. insularum* into juvenile and adult size classes, but did not take into account the sex of the snails. *P. canaliculata* display a sex dimorphism that may be similar in *P. insularum* (Cazzaniga 1990). Male *P. canaliculata* tend to have wider apertures than females of the species. This raises some concern that the measurements may not have the same relationships in male and female snails, and could be the cause of our lower R^2 among adult snails compared to juveniles or both life stages. It remains unclear whether the maximum width across the operculum varies or if only the operculum shell piece inside the aperture manifests these differences. Yusa et al. (2000) mentioned that all snails over the size of 20 mm had fully developed sex organs, so they could sex snails of this size by measuring their shells. Operculum width and shell height had a much stronger relationship ($R^2 = 0.967$) when we incorporated the juvenile life stage. Similar to our findings, Yusa et al. (2000) conducted a regression between operculum width and shell height for juvenile and adult *P. canaliculata* as a way to find snail size from only operculum width and found a strong predictive relationship ($R^2 = 0.968$). This suggests that these two measures of *P. insularum* have a similar relationship to the same measures in *P. canaliculata.*

Conclusions

P. insularum may pose a similar threat of invasion to *P. canaliculata*, an established exotic invasive species. In order to compare studies between species and between native and exotic populations, aquatic ecologists need to have valid, reliable, and consistent methods. Measuring operculum width appears to have these advantages when measuring body size of *P. insularum.* In order to refine methods further, future studies should look for a sex dimorphism in operculum width in adult *P. insularum.*

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