INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)

ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 20, No. 1, p. 24-32, 2022

RESEARCH PAPER | OPEN ACCESS

Morphological adaptation of *P. canaliculata* shell to the different ecosystems in Lanao Del Norte, Mindanao, Philippines

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Article published on January 10, 2022

Key words: Golden apple snail, Morphological adaptation, Shell chirality, Shell color, Shell length, Shell shape

Abstract

Different physiological, morphological, and behavioral adaptations of *Pomacea canaliculata* aided them in their survival to different adverse environmental conditions. Furthermore, the said adaptations can be very vital in the control and management strategies that can be employed in the areas where their population posed a threat to food security. The study employed an explorative-investigative study design for the gathering of data. Eight hundred seventy-three Golden Apple Snails from different freshwater ecosystems, namely stream, irrigational canal, and rice field were collected, cleaned, and examined. To elucidate the different adaptations of the GAS to the various ecosystems, their shell characteristics were observed, recorded, and examined. Consequently, this study found out that those shells from snails sampled in streams had bigger length, width, width of the aperture, a higher number of bands, and whorls when compared to those shells from irrigational canals and rice fields. Moreover, there was a negative correlation between pH and dissolved oxygen to the height, width, and width of the aperture. There was also a significant correlation between the temperature and width, weight, and the number of bands. It was concluded that to control and manage the population of the GAS the area should have less palatable food sources and less anthropogenic activities so that environmental parameters like high pH, lower temperature, and higher dissolved oxygen can be achieved.

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Introduction

The physiological, morphological, and behavioral adaptation of *Pomacea canaliculata* to various environmental conditions made them thrive and be one of the major pests in freshwater wetlands particularly affecting ricefields and other economically important agricultural areas. They had been known for their adaptive plasticity (Estebenet & Martin, 2002) and highly generalist and voracious macrophytophagous feeding nature (Morrison, *et al*., 2016).

P. canaliculata, or commonly known as golden apple snail, is one of the freshwater snails that underwent series of adaptations to thrive in any given freshwater environment. Hence, the golden apple snail can tolerate harsh environmental conditions such as low levels of salinity, lower temperature (Seuffert, *et al.,* 2013), low pH levels, metal and pollutant contamination, parasite infestations, period of drought (Silverwood, 2011), and low food availability (Tamburi & Martin, 2016). However, its survival in these adverse environmental conditions is highly dependent on the important physiological, morphological, and behavioral adaptations (Chukwuka, *et al.,* 2014) of these snails to their environment. Furthermore, according to Relyea (2002), as cited by Madjos, *et al.,* in 2015 that *P. canaliculata* can respond to the changes in the environment by producing alternative phenotypes as an adaptive strategy.

Consequently, there had been different functional parts of the snails that played an important factor in their survival especially in maintaining their homeostasis. One of those parts is the shell. Salient features of the snails' shells that ensured the snails' survival against adverse environmental conditions, predator cues, and various anthropogenic-related activities were: shell periostracum, shell chirality, shell color, shell shape, shell size and weight, and operculum shape and weight.

However, these characteristics could also be a potential target for control measures. A better understanding of the important characteristics of the species that helped them survive various environmental stresses should be understood by people implementing management and control programs. Henceforth, this paper was conducted to provide basic information on the adaptation of the snails based on the characteristics of their shells, so that proper management and control measures can be crafted to make them better suited in the area. The paper looked into three different ecosystems which have important economic value where the presence and manifestation of golden apple snails were seen to be of great concern.

Materials and methods

Sampling sites

Snails were collected from the different freshwater ecosystems in the Province of Lanao del Norte, Mindanao, Philippines. Site A was an agricultural area in Brgy. Tenazas, Lala, Lanao del Norte. Rice was the major product in the area and a very dense population of golden apple snail was observed, some can be found clustering around a shoot of the rice plant, and some were buried in the muddy rice field. Juvenile snails can be seen at the corners of each ricefield while eggs can be observed in the rice plants as well as on the plants found in the area. The second site was a distant dam connected to a stream located 10 kilometers from the first site near human settlement. Driftwoods, decaying plants, and trunks, solid waste, grasses, animal manure, and animals can be seen on the stream. A dense population of GAS can be observed on the sides of the stream and few individuals were observed floating on the water. Lastly, Site C was in an agricultural irrigation canal in the Municipality of Maigo, 45.6 kilometers east from Site A. Site C bed was cemented however, silt was observed with lesser vegetational cover. All sites had other species of snails; however, the collection was limited only to golden apple snail.

Snail collection

An explorative-investigative study design was employed in this study. A total of 873 samples were taken using hand-picking and through the use of a sieve. Out of the total collected samples, only 180 samples were utilized for this study comprising of 30 males and 30 females per area.

The snails were boiled and cleaned and the meat was removed by pin. Sex identification was made through shell shape (Mahilum & Demayo, 2014) and actual body parts examination. The presence of the penial complex would signify male and the absence thereof would be of female.

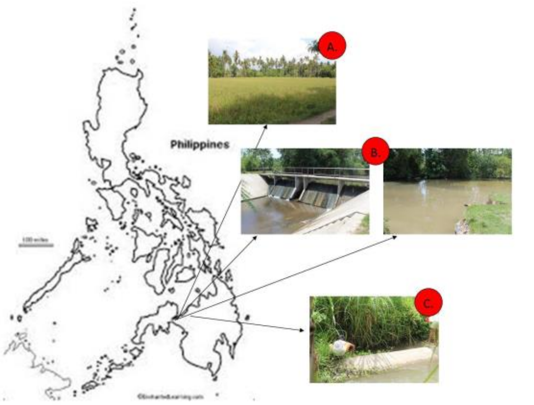


Fig. 1. Map of the Philippines. A) Rice field at Brgy. Tenazas, Lala, Lanao del Norte; B) Dam and Stream at Brgy. El Salvador, Lala, Lanao del Norte; and C) Irrigation Canal at Brgy. Balagatasa, Maigo, Lanao del Norte.

Sample Preservation

Representative samples of the species of freshwater gastropods were preserved in 70% ethyl alcohol and were kept for future referencing purposes. Shells were then air-dried and were subjected to different measurements.

Measurements of snail morphological traits

The different conchological characteristics were determined in this study:

a) Shell length

Shell length is the maximum measurement along the central axis of the snail. The measurement was done using a Vernier caliper (Fig. 2.a).

b) Shell width

The width of the shell was done by measuring the right angles to the central axis. Vernier caliper was used in the measurement (Fig. 2.a).

c) Shell weight

The dried weight of the shells was taken using a digital scale.

d) Width of aperture

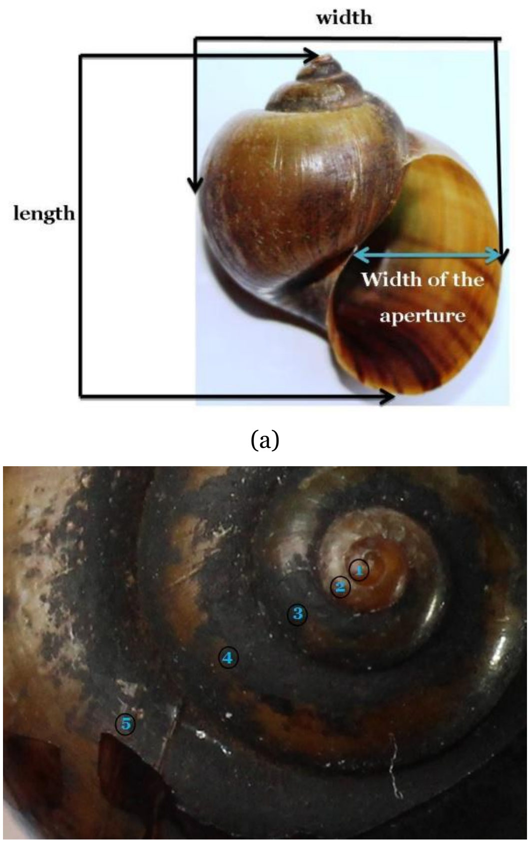
The width of the aperture was also measured using a vernier caliper (Fig. 2.a).

e) Shell chirality

Shell chirality was the orientation of the aperture opening. To determine the chirality, the shells were held with the spire pointing upwards and the aperture was facing the observer. Dextral shells are determined if the opening was facing right and sinistral if the opening was facing left.

f) Shell shape

Shell shape according to Gould (1966) as mentioned by the paper of Kemp, *et al.* (1984) was governed by a genetically allometric relationship. In this study, the snail shape was determined by the proportion of the height and the width. Oblong was determined if the height is much bigger than the width, globose – if the height and the width of the shell are proportional and depressed if the width is much bigger than the height.



(a)

(b)

Fig. 2. Determination of: a) shell length, shell width, and width of the aperture; and b) number of whorls.

g) Number of bands

Counting of the actual number of bands was done and was recorded.

h) Number of whorls

The whorl of the snails' shell is the single complete 360° revolution or turn in a spiral growth of the shell. The counting was made first by drawing a straight line and following the course of the whorl at a 90° angle (Fig. 2.b).

i) Shell texture

Visual observation of the texture of the shell was determined using smooth and rough consistency.

Physico-chemical Analysis

Physico-chemical parameters were evaluated in each sampling station to correlate the conchological characteristics. Standard protocols were observed in performing all the physicochemical analyses.

A pH meter (Tester 30, waterproof pH, and Temperature Tester Doubles Junction) was used in determining the pH, dissolved oxygen, and temperature of the different sampling stations. The device probe was dipped in the water surface for one minute and the data was taken and recorded in triplicates. Mean environmental parameters were shown in Table 1.

Table 1. Mean environmental conditions during the sampling of the samples.

Ecosystem	pH Level	Temperature	Dissolved Oxygen
Ricefield	7.6	31.7 °C	6.1 mg/dL
Dam and Stream	6.7	33.7 °C	2.7 mg/dL
Irrigation Canal	7.2	30.7°C	3.7 mg/dL

Data Analysis

Statistical tools like One-way ANOVA and MANOVA were used in this study. Pearson's correlation was also used to establish the correlation between the traits and the environmental factors. The data were recorded to excel and was analyzed and run in SPSS.

Results

Conchological traits

Table 2 summarized the mean scores from the data obtained from the study. From the data, it was evident that the female shells were larger than the male. Female shells also had more bands and whorls than their male counterparts. All shells displayed dextral orientation and regular spiral patterns. Moreover, all (100%) of the shell's shapes were globose and had a smooth texture.

Table 2. Mean scores of shell's characteristics in the three sites.

Location	Length (cm)	Width (cm)	Weight (grams)	Width of aperture (cm)	# of bands	# of whorl
Ricefield						
male	2.64	2.1	1.28	1.51	11	4.5
female	3.11	2.59	1.69	1.93	12.58	4.75
Stream/dam						
male	3.98	3.32	2.92	1.79	11.82	4.77
female	4.25	3.76	3.71	1.87	15.5	5
Irrigational canal						
male	2.77	2.29	1.39	1.23	12.5	4.4
female	2.87	2.42	1.47	1.28	9.34	4.31

Shell length, width, and weight

The snails found in the stream and dam were observed to be significantly higher in terms of the shell length, width, and weight when compared to the snails sampled from the irrigational area and ricefield regardless of the location. Analysis of variance showed significant differences in shell's length, width, and weight in the different environments with a p-value less than 0.05. Furthermore, it can be observed using Post-hoc analysis that streams and dams are significantly different from rice fields and irrigation canals in terms of shell's length, width, and weight (Fig. 3).

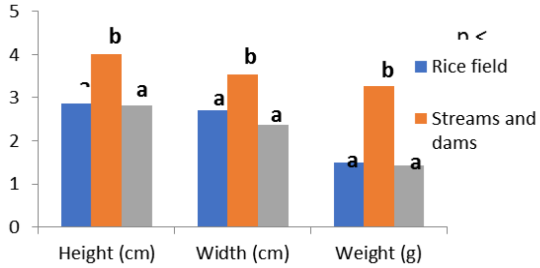


Fig. 3. Differences in shell's length, width, and weight in different environments.

Width of aperture, number of bands, and number of whorls

The same trend can be observed in terms of the width of the aperture, the number of bands, and the number of whorls in different environments. Results showed significant differences in terms of width aperture, the number of the band, and the number of whorls in the rice field, streams and dams, and irrigation canal ($p < 0.05$). Furthermore, stream and dam are significantly different from rice field and irrigation canal ($p < 0.05$) but no variation can be seen between rice field and irrigation canal (Fig. 4; Fig. 5; Fig. 6).

Correlation on the environmental factors with the shell's characteristics

A significant negative correlation was observed in pH and length of the shells (-0.703) and pH and width of the aperture of the shell (-0.784). A highly significant inverse correlation was also obtained between pH and width of the shell which means that when the pH increases the shell's length, shell's width and shell's width of aperture decreases. On the other hand, no significant correlation was observed in pH, shell's weight, and the number of bands. Temperature is directly correlated with the shell's width, weight, and width of the aperture ($p<0.05$) but not in the shell's length and the number of bands which showed no significant correlation. Additionally, a significant inverse correlation was seen in temperature and pH. Dissolved oxygen is one of the determinant factors of the health of the environment. In this study, table 3 further showed that dissolved oxygen is negatively associated with the shell's length, width, and width of the aperture. Apart from that, DO is not correlated with the shell's weight and the number of bonds.

Table 3. Pearson's correlation of shell's characteristics and environmental factor.

Variable	Variable[1]								
	Length	Width	Weight	WOA	NOB	NOW	pH	Temp	DO
Length	1								
Width	.361**	1							
Weight	.562**	.271**	1						
WOA	.652**	.278**	.490**	1					
NOB	.224**	.074^{ns}	.277**	.160*	1				
NOW	.340**	.163*	.297**	.234**	.014^{ns}	1			
pH	-.703*	-.804**	-.617^{ns}	-.784*	-.383^{ns}	.^{c}	1		
Temp	.434^{ns}	.713*	.755*	.672*	.408^{ns}	.^{c}	-.697*	1	
DO	-.706*	-.706*	-.453^{ns}	-.688*	-.299^{ns}	.^{c}	.943**	-.426^{ns}	1

WOA = Width of aperture; NOB = Number of bands; NOW = Number of whorl; Temp = Temperature; DO = Dissolved oxygen

^{ns} not significant; * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level. c. Cannot be computed because at least one of the variables is constant.

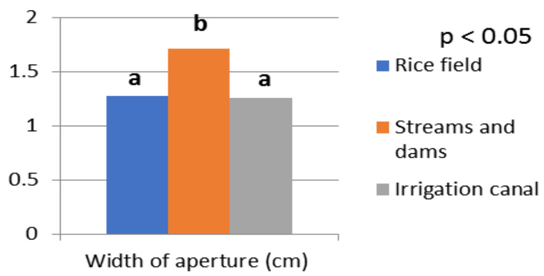


Fig. 4. Difference of width of the aperture as observed in the samples from the rice field, streams and dams, and irrigation canal.

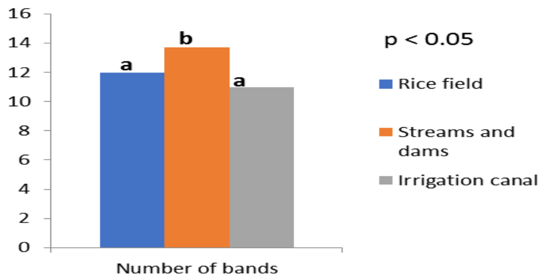


Fig. 5. Difference of the number of bands as observed in the samples from the rice field, streams and dams, and irrigation canal.

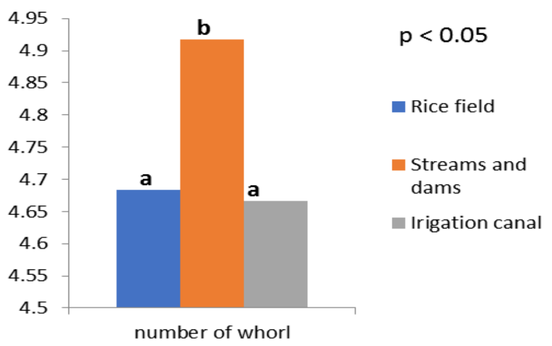


Fig. 6. Difference of the number of whorls as observed in the samples from the rice field, streams and dams, and irrigation canal.

Discussion

The physiological, morphological, and behavioral adaptation of *Pomacea canaliculata* to various environmental conditions made them thrive and be one of the major pests in freshwater particularly affecting rice fields and other economically important agricultural areas. Conchological studies, specifically, conducted for the species are just among the many factors that checked and elucidated how these species survived and adapted to different environments. This study looked into several shell characteristics that made them adaptable to the different ecosystems and found variations in the shells of the golden apple snail. The 45.6 kilometers distance of Site A to Site C did not produce a difference in the adaptations of the GAS, as shown in the different characteristics of their shells, this can be associated that they were of similar land use and had similar environmental disturbances. Whereas, Site B which is only 10 kilometers from Site A had been observed with different adaptations because of the different land use and anthropogenic activities observed since it was located near a settlement area. These findings were supported by the findings of Majos and Anies (2016) that noticed significant variations in snails that were geographically isolated and were exposed to various agricultural practices.

This study found out that the sampled male and female snails also had variations in terms of the size of the shell, the number of bands, and the whorls. The openings of the aperture were all oriented dextrally and all had regular spiral patterns. All of the shell's shapes were globose and had a smooth texture. It was observed from this study, that the shells from female snails were larger than the male snails, and is similar to the laboratory observation done by Estebenet and Martin in 2003 and 2002. This was also one of the adaptations of the female GAS that can attribute to favorable maternal functions.

Morphological adaptations of the snails were evident in the shells' length, width, and weight. There were observed significantly higher measurements of the shell length, width, and weight in snails' shells from Site B compared to those that were sampled from Site A and C. The difference in these traits can be attributed to several factors like ontogenetic, sexual, genetic, and ecophenotypic components of the snails (Estebenet, *et al.,* 2006); vast food sources (Estebenet and Martin, 2003) that were palatable to the snails ((Yam, *et al.,* 2016), anthropogenic disturbances, and environmental factors (Fink and Von Elert, 2006); water current (Minton, *et al.,* 2008); and the substrate of the bottom of the ecosystem where the snails were inhabiting as stated by Estebenet, *et al.* (2006) as proposed by Cazzaniga in 1987. These adaptions developed by the snails made them withstand different environmental factors and made them survive in the different sites.

Similarly, the width of aperture, number of bands, and number of whorls also showed significant differences only between Site B and Sites A and C. The similarity between Site A and C can be because of the similar land use and the environmental condition that these snails were exposed to including, but not limited, to pollution and other climate change-related disturbances. The number of bands in the shells of the snails corresponds to the secretory activity of the mantle edge (Comfort, 1950) and neurosecretory system of the mollusk (Boettiger, *et al.,* 2009; Ermentrout, *et al.,* 1986). In this study, it was evident that those snails in Site B had a higher number of disturbances when compared to Sites A and C.

The colors of the shells of the snails were observed to be closely related even if they were exposed to the different ecosystems, this can be attributed to the fact that all three sampling sites shared the same temperature level. In an article by Sokolova, *et al.* (2000) they cited that the color of the shells can be related to environmental gradients like climate, insolation, wave exposure, and salinity. In this case, all of the sites where the snails were sampled had a common climatic pattern, thus, this is a common factor that influenced its shell color.

When the different shell characteristics of *Pomacea canaliculata* were subjected to correlation analysis with the observed environmental parameters in the area, it was found out that these conditions also played a significant factor in terms of its adaptation. It was observed that the higher pH could limit the length, width, and width of the aperture. This can be related to the studies of Samsi and Karim (2019), Glass and Darby (2009), Marshall, *et al.* (2008) and Orr, *et al.* (2005) where the pH influenced the conditions of the shells of the GAS and other calcifying organisms. Accordingly, the shells of those snails in treatment with high pH produced small shell length and manifested signs of shell erosion.

Consequently, the higher level of dissolved oxygen was also negatively correlated to the length, width, and width of the aperture of the shell. The result was contrary to the result of Gupta, *et al.* (2017) that there was a linear growth rate in both the tank with elevated and lowered DO use in their study. Moreover, this study also observed the positive correlation of temperature to the width, weight, and number of bands of *P. canaliculata*.

This finding is related to the findings of Estebenet, *et al.,* 2002 that *P. canaliculata* were influenced in all aspects by temperature. Snails' growth rate was highest in elevated temperature (Kalinda, *et al.,* 2017; Tamburi, *et al.,* 2018; Memon, *et al.,* 2011) and could explain the increase in width, weight, and the number of bands. Furthermore, the result of this study is contrary to the findings of Galan, *et al.* (2015) that temperature together with pH and DO did not manifest correlation to the characteristics of the shell being tested in their study.

Conclusion and recommendations

As the environment played a vital part in the survival of the snails, the physiological, morphological, and behavioral adaptations of *Pomacea canaliculata* to various environmental conditions ensure their survival in an environment. The plasticity nature of the species also helped them adapt easily to the environment where they thrive. As is observed in this study, there were variations in the shells based on the observed characteristics. However, there was similarity in the shells that were relevant in Site A and C and this can be pointed to the closely related land use in the areas. Moreover, the proximity between Site A and B did not influence the similarity of the shells, hence, it was concluded that since they had different use and functions, the adaptations of the snails was different.

The noted difference can be because of the different anthropogenic activities, pollutants, and other environmental factors in the sites. The pH, temperature, and dissolved oxygen also played important roles in the adaptation of the snails. The pH and dissolved oxygen of the area had a negative correlation to the length, width, and width of the aperture of the snails. On the other hand, the temperature had a positive correlation to the width, weight, and number of bands.

Given this premise, to manage and control the population of the golden apple snails and minimize the damages they caused to agriculture, it is recommended that the areas should cater environment with less palatable food sources and should have adverse environmental conditions like higher pH, lower temperature, and higher dissolved oxygen. Further studies should also be conducted to test and check for the similarity of this study to different areas.

Acknowledgments

The researchers would like to thank the Department of Science and Technology Accelerated Science and Technology Human Resource Development Program (DOST ASTHRDP) for the scholarship grant that enabled the researchers to produce the paper.

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