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COMPARATIVE LIFE CYCLE STUDIES OF *Pomacea maculata* AND *Pomacea canaliculata* ON RICE (*Oryza sativa*)

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Apple snails, *P. canaliculata* and *P. maculata* are the two most successful invaders of many macrophytes especially rice in many countries of the world particularly Southeast Asia. Many studies have been conducted on ecology and management of *P. canaliculata* but little is known about the life cycle of *P. maculata* especially on rice. Therefore, comparative life cycle studies of *P. maculata* and *P. canaliculata* were conducted on rice. Results suggested that *P. canaliculata* showed significantly higher growth than *P. maculata* and matured during the 27th week of development, whereas the latter matured during the 32nd week. At maturity, no difference was recorded between two species for shell length and width. However, individuals of both species started consuming rice during the 4th week of the development. Shell length and width of both species were highly correlated with each other during the entire growth. Egg diameter was significantly higher in *P. canaliculata* than *P. maculata*, however, no significant difference was observed in the number of eggs, hatching period and rate for the two species. The results obtained can be utilized for the rearing technique of *Pomacea* spp. on rice to maintain cultures of different stages to study their unique features contributing to their successful invasion, and suggest appropriate management strategies to reduce their damage and spread.
Keywords: *Pomacea canaliculata*, *Pomacea maculata*, apple snails, life cycle, rearing technique

INTRODUCTION

Biological invasions are a great threat to human interests, often affecting ecosystem functionality, biodiversity and human health (Aragon *et al.*, 2010). Increased international trade has caused the rapid movement of many organisms, especially aquatic organisms including molluscs, from one location to another (McNeely, 2001). Apple snails, *Pomacea* spp., natives of South America were introduced in many countries, primarily for food or aquarium trade, are among the most invasive freshwater molluscs (Cowie *et al.*, 2006; Rawlings *et al.*, 2007; Hayes *et al.*, 2008; EFSA, 2012). They have caused substantial economic losses to rice in many countries of the world especially in Southeast Asia (Nghiem *et al.*, 2013). Besides rice, they also caused substantial damage to taro, water spinach, water chestnut and lotus (Carlsson, 2006). Due to the ever confounding identification of *Pomacea canaliculata* and *Pomacea maculata* (*P. insularum* and *P. gigas* synominized with it), initially it was assumed that only *P. canaliculata* was introduced to Southeast Asia (Cowie *et al.*, 2006; Hayes *et al.*, 2012). However, later investigations and genetic studies also confirmed the introduction of *P. maculata* in the region (Rawlings *et al.*, 2007; Hayes *et al.*, 2008; Hayes *et al.*, 2012).

The invasiveness of *Pomacea* spp. is based on their unique morphological and physiological characteristics such as high reproductive potential with rapid growth, polyphagous feeding habits, amphibious respiration and aestivating or hibernating potential (Cowie, 2002; Estebenet and Martin, 2002). All of these characteristics help them to survive under adverse environmental conditions and reproduce aggressively. In their native habitats, they caused only minor economic losses (Cazzaniga, 2006), but heavy economic losses are incurred in their introduced range, especially Southeast Asia, China, Japan and Hawaii (Horgan *et al.*, 2014; Nghiem *et al.*, 2013). The losses caused by the introduced apple snails to rice in three countries of Southeast Asia, viz. Philippines, Thailand and Vietnam, are estimated up to US$ 2.138 billion per annum (Nghiem *et al.*, 2013). They are also vectors of parasitic *Angiostrongylus* nematodes and cause infection of rice workers through cuts on their feet from the sharp edges of shells killed by pesticides (Horgan *et al.*, 2014; Nghiem *et al.*, 2013). Moreover, they also deteriorate many aquatic ecosystems to turn them into turbid and phyto-plankton dominated ones (Carlsson, 2006). Because of these impacts of *Pomacea* spp., one species, *P. canaliculata*, is included among "100 of the World's Worst Invasive Alien Species" (Lowe *et al.*, 2000).

Although many studies have been conducted recently on the biology of *P. canaliculata* (e.g. Wu *et al.*, 2011; Zhao *et al.*, 2012; Seuffert and Martín, 2013; Yoshida *et al.*, 2013), no significant studies have been conducted on the life cycle and biology of *P. maculata*. Moreover, no life cycle study of *P.*

canaliculata has been undertaken on rice, the most important crop invaded by the *Pomacea* spp. Therefore, this study was undertaken to study the comparative growth pattern of the two invasive *Pomacea* snails, *P. maculata* and *P. canaliculata*, by feeding them on rice. The relative age of the two *Pomacea* spp. when they started consuming rice was also determined. The results obtained could be helpful to estimate losses to rice seedlings at different developmental stages of the snails so that timely management strategies could be adopted against them to prevent any economic damage.

MATERIALS AND METHODS

Snails for rearing were collected from rice fields of Tanjong Piandang (N05°04.057' E100°23.116), Permatang Kuang (N05°31.414' E100°24.709) and Tanjong Karang (N03°27.308' E101°09.366) in Peninsular Malaysia. The two species were initially distinguished based on their shell morphology i.e., shell colour and later by the size of their eggs as suggested by Cowie *et al.* (2006); Marwato and Nur (2012) and Hayes *et al.* (2012). All snails were kept in separate aquaria (30×18×18 cm) at Glasshouse, Ladang 2, University Putra Malaysia and fed ad libitum with Yellow Burr Head (*Limnocharis flava*). Five freshly laid egg masses of each snail species were removed and kept in separate Petri dishes for hatching. The number of eggs per egg mass, hatching success and hatching period of each egg mass were recorded. After hatching, 15 hatchlings of each species with five replicates were kept in separate aquaria along with green algae (*Scenedesmus* sp.). Five snails from each replicate were used for measuring Shell Length (*SL*) and Shell Width (*SW*) (Fig. 1) on a weekly basis with the help of Dino-Lite digital microscope (AnMo Electronics Corp. Taiwan). When *SL* reached 1 cm and snails became easy to handle, measurements were taken using digital callipers (Mitutoyo, USA). A water level of 10 cm was maintained in the aquaria throughout the study, and the water was changed twice a week to avoid accumulation of fecal material and bad odours. Three-week old fresh paddy plants were provided daily to snails after the second week after hatching along with green algae and continued afterward. Observations continued until the snails reached maturity as confirmed by observation of mating in snails (Estoy *et al.*, 2002). The experiment was conducted at a naturally fluctuating temperature that normally ranged between 25 and 35°C with a photoperiod of 12:12.

The experiment was conducted using a completely randomized design (CRD). Analysis of Variance (ANOVA) was performed on *SL* and *SW* of *P. maculata* and *P. canaliculata* and significant differences were assessed at the 0.05% probability level using least square difference (LSD). Student's t-tests were used to analyze the egg data of the two snail species. In addition, regression analysis was applied to assess the relationship between growth parameters within individual *Pomacea* spp. and comparative growth pattern of the two species. All analyzes were performed using SAS version 9.2 (SAS Institute Inc. 2009).

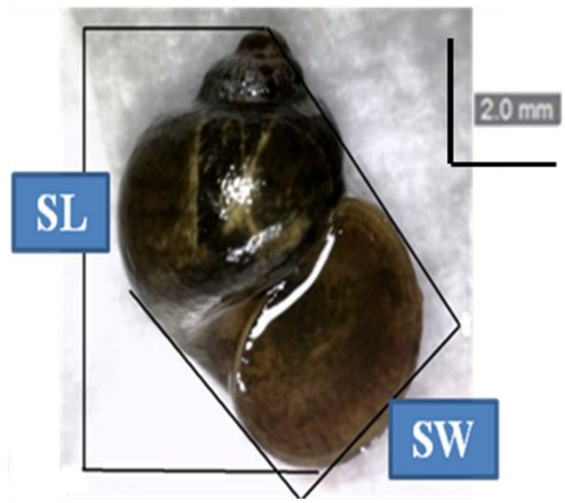

Figure 1. Measurements of Shell Length (*SL*) and Shell Width (*SW*) of *Pomacea* spp.

RESULTS AND DISCUSSION

Both species grew consistently with no mortality over the course of the experiment, with *P. canaliculata* growing more rapidly than *P. maculata*. *Pomacea canaliculata* became reproductively matured (first copulation observed) during the 27th week after hatching, while *P. maculata* grew more slowly, becoming reproductively matured in the 32nd week. However, neither *SL* nor *SW* at the time of maturity differed significantly between the two species (Table 1).

The more rapid growth of *P. canaliculata* compared to *P. maculata* may be because of the initial larger size of their hatchlings, which was also reported by Hayes *et al.* (2012). Moreover, under tropical conditions, growth in *P.*

Table 1. Growth parameters of *P. canaliculata* and *P. maculata* at the time of maturity.

	P. canaliculata (27th week)	*P. maculata* (32nd week)	*t*-value	*P*-value
	Mean ± SE (range)	Mean ± SE (range)		
SL (mm)	20.56 ± 1.09 (19.05-24.86)	20.25 ± 1.04 (18.29-23.82)	0.20	0.8452
SW (mm)	15.24 ± 1.02 (13.11-19.13)	14.69 ± 0.85 (12.59-17.62)	0.41	0.6897

SL = Shell Length, *SW* = Shell Width

canaliculata is continuous and snails became reproductively matured in seven months, whereas in fluctuating temperatures it took almost two years to reach maturity (Martin *et al.*, 2001; Cowie, 2002). Our results regarding the maturity of *P. canaliculata* and *P. maculata* are similar to above studies. However, study of Teo (2004) in Sabah, Malaysia, observed mating among field and indoor reared *P. canaliculata* in 82 days after hatching with a shell length of 38.2 mm. Reasons for the slow growth of snails in our study may be because of the type of food provided as we reared snails by feeding on fresh three-week old rice seedlings whereas studies by Teo (2004) were conducted on the weeds / wild plants. Another study on the effect of different food levels of fresh lettuce on the age of males and females of *P. canaliculata* at the first copulation confirmed the maturity of males and females in 17.96 and 8.141 weeks, respectively (Tamburi and Martin, 2009). Previous studies also confirmed the significant effect of different kinds of food on the growth and development of *Pomacea* spp. (Qiu and Kwong, 2009). Another factor that may have contributed to differences in growth and development of apple snails (*Pomacea* spp.) was the density of snails as growth is slower in denser populations (Yoshida *et al.*, 2013). In this study, a snail population of 15 snails per aquarium tank was maintained, whereas Teo (2004) used a snail density of 10 snails and the same may also have contributed towards faster growth in the study. Previous studies also demonstrated that a minimum length of 25 mm is required for females to attain sexual maturity and reproduce (Estebenet and Martin, 2002). Accordingly mating and oviposition of females is very much dependent on the growth rate of the females. Those studies also suggested that in addition to size, the maturity of snails also depends on the minimum development time of the growth. Accordingly, results of this study are in accordance with the above findings, but minimum development size of the snails was identified as a key factor for their maturity in comparison to minimum development time.

Fig. 2 shows the growth pattern of *P. canaliculata* and *P. maculata* in respect to increasing in *SL* and *SW*, respectively. For both species, *SL* and *SW* were highly correlated through the study (correlation coefficients >0.99). This finding is in accordance with the results obtained by Sharfstein and Steinman (2001) when studying the growth and survival of Florida apple snail, *Pomacea paludosa* Say.

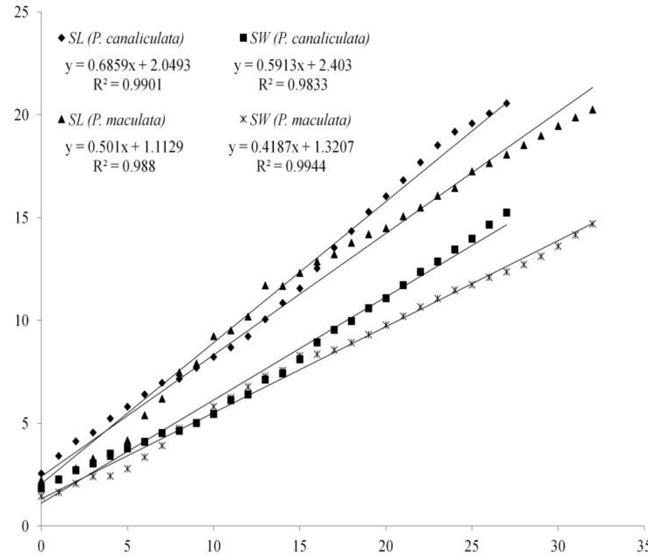

Figure 2. Comparative growth pattern in shell length of *P. canaliculata and P. maculata* at maturity.

Both *P. canaliculata* and *P. maculata* started consuming three-week old rice seedlings during the 4th week after hatching. At that stage, *SL* and *SW* of *P. canaliculata* were significantly higher than that of *P. maculata* (Table 2). Previous studies showed that *P. canaliculata* smaller than 16 mm were incapable of consuming rice seedlings and mostly fed on the detritus materials (Hirai, 1988), but 90% loss to rice seedlings by 10 mm sized snails have also been reported (Teo, 2003). Accordingly, findings of these studies did not validate results of this study. However, it was also observed that snails of even 2.5 mm size could consume lettuce and other macrophytes including rice seedlings in the absence of

Table 2. Growth parameters of *P. canaliculata* and *P. maculata* at the time of rice consuming.

	P. canaliculata (Mean ± SE)	*P. maculata* (Mean ± SE)	*t*-value	*P*-value
SL (mm)	5.24 ± 0.58	3.42 ± 0.29	3.09	< 0.05
SW (mm)	3.52 ± 0.97	2.44 ± 0.19	2.45	< 0.05

SL = Shell Length, *SW* = Shell Width

Table 3. Comparison of egg data for *P. canaliculata* and *P. maculata.*

	P. canaliculata	*P. maculata*		
	Mean ± SE (range)	Mean ± SE (range)	*t*-value	*P*-value
Total eggs	84 ± 2.46 (77-92)	123.8 ± 29.44 (56-231)	1.33	0.22
Egg diameter (mm)	2.85 ± 0.02 (2.62-2.95)	1.99 ± 0.02 (1.79-2.13)	29.29	< 0.0001
Hatching %	51.6 ± 6.42 (36-75)	62.4 ± 12.8 (49-81)	1.26	0.24
Hatching period (days)	13.94 ± 1.15 (10.31-16.45)	15.55 ± 1.32 (11.77-19.89)	0.92	0.38

any food (Estebenet and Martín, 2002), confirmed results of the study.

The only significant difference between the eggs of the two species was recorded in egg diameter, whereas no difference was observed between the two species regarding number of eggs per egg mass, hatching success and hatching period (Table 3). Barnes *et al.* (2008) and Hayes *et al.* (2012) also confirmed that *P. canaliculata* produce fewer eggs of larger size, whereas *P. maculata* produce many eggs of smaller size. Studies also reported highly variable pattern in number of eggs per clutch of the two species with a relatively higher number of eggs in *P. maculata* (Barnes *et al.*, 2008; Hayes *et al.*, 2012; Kyle *et al.*, 2013). Studies also showed a great variation in hatching success and period among *Pomacea* spp. mainly influenced by the temperature and other environmental stresses (Teo, 2004; Barnes *et al.*, 2008; Liu *et al.*, 2011).

***Conclusion*:** Comparative life cycle studies of *P. canaliculata* and *P. maculata* showed continuous growth in both species with *P. canaliculata* growing relatively faster and matured earlier than *P. maculata*. It is suggested that relatively faster growth and development of *Pomacea* spp. in rice fields as compared to the glasshouse studies is not because of the feeding on rice plants but may be due to feeding on many other weeds and simpler autotrophs plants that may provide more nutrients for their growth and development. Availability and quality of water also plays a key role in the life cycle of both apple snails. Therefore, further studies should be conducted to evaluate the effect of different macrophytes and autotrophs on the apple snail's growth along with the availability of water as it is the key factor for the snail's life cycle. Thus, life cycle studies may also contribute in the development of rearing technique that would enable researchers to cater to the understanding of many important biological aspects of these invasive apple snails and may help in their proper management in the fields.

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