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Effectiveness of an automated digital infrared CCTV multi-camera system in investigating mating and oviposition behavior of apple snails, *Pomacea* spp.

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Abstract
Pomacea canaliculata and *Pomacea maculata* attack rice growing areas with significant yield losses. Their success rate is mainly due to their high reproductive potential. This study aimed to investigate the reproductive potential of both *Pomacea* spp. using a digital infrared CCTV video camera system to ensure data accuracy by recording uninterrupted behavioral activities. Results of the study confirmed efficiency of digital infrared CCTV camera system used in recording and acquiring behavioral data with further potential for wide scale usage in future studies. Moreover, mating and oviposition in both species mostly occurred during night hours. *Pomacea maculata* showed higher mating frequency, oviposition duration and eggs per clutch, whereas egg diameter was higher in *P. canaliculata*. No difference was observed in other mating and oviposition parameters. Accordingly, higher reproductive potential of *P. maculata* as compared *P. canaliculata* could be one of the reasons for its large scale distribution in Peninsular Malaysia.

Keywords: *Pomacea*, invasive, mating, oviposition, Infrared digital camera system, apple snail.

1. Introduction
Exotic apple snails, *Pomacea canaliculata* and *Pomacea maculata* are the two most successful invaders of rice and other aquatic habitats in Southeast Asia and USA with the former being more widely distributed [1, 2, 3]. Their large scale negative impacts and invasive potential on the environment, human health and field crops necessitated the solid efforts to restrict their further spread [4, 5]. The invasiveness of these apple snails are based on their voracious feeding, long life span, high fecundity and high tolerance to environmental stresses [3, 6]. It is recognized that the invasiveness of exotic species, especially mollusks in new areas is very much dependent on their higher fecundity potential [7, 8]. This is clearly demonstrated in *Pomacea* spp. as the eggs are laid above the water surface to avoid the risk of siltation and aquatic predators. In addition, female *Pomacea* spp. has the capability to store sperm for longer duration to withstand the lower population densities at any later stage [9, 10, 11, 12]. Considering the importance of high reproductive potential for the invasiveness of the apple snails, many studies have been conducted on the reproductive potential of *P. canaliculata* [9, 12, 13, 14, 15, 16], but specific studies are still lacking on the reproductive potential of *P. maculata* and if done, are very scarce [6, 17]. Studies also lacks on the comparative mating and oviposition behavior of the two invasive species under the same experimental conditions to estimate the invasiveness of individual species except [15]. Moreover, all the research conducted to date on the mating behavior of invasive *Pomacea* spp. was mostly based on direct observations which lacks continuous monitoring and may create ambiguity in the data obtained. However, digital video cameras have been widely used for many behavioral studies of other invertebrates, especially insects [18], fish [19] and even in other mollusks [20, 21]. All such camera systems get variable success to achieve research objectives but the cost of the system remained a major constraint for their practical implication.

Therefore, the objectives of this study were to study the relative characteristics of mating and oviposition of *P. maculata* and *P. canaliculata* to ascertain the basis of their invasiveness regarding high reproductive potential by using low cost digital CCTV infrared video camera system. The CCTV system was installed for the continuous recording of all the behavioral activities of the snails regarding their mating to avoid any bias. The results obtained from this study are beneficial to understand the basic reproductive behavior of the two invasive apple snail species and the same could be exploited not only to reduce the damage caused by these snails but also to restrict their further spread to new localities.

2. Materials and Methods
2.1 Study site
This study was conducted in a Glasshouse, at Field 2, Faculty of Agriculture, University Putra Malaysia in September, 2013.

2.2 Recording system
A 24 hour digital recording system was established by modifying the commonly used CCTV recording system for the continuous monitoring of mating and oviposition behavior of the snails. Ten infrared IB68 Bullet Cameras (Skysonic, Taiwan) with variable focus were installed on two two-tier ladders with five cameras per ladder, each camera fixed over an aquarium tank of 30 x 18 x 18 cm size. All the cameras were then connected to an iCatch (Taiwan) 16 Channel Digital Video Recorder (DVR) with Two Terra Bytes (TB) Hard Disc memory for the continuous recording and storage of the data. The memory of the DVR was enough to store the recordings of the entire experiment. An ACER 17" monitor was also connected to the DVR for the display of the recordings so that data can be extracted for mating and oviposition behavior of the two species (Figure 1).

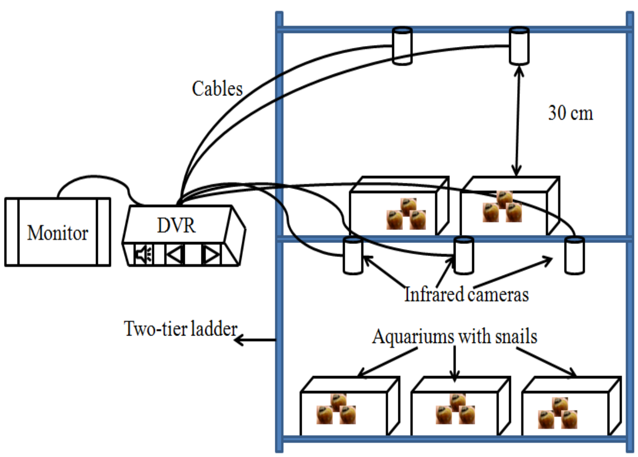

Fig 1: Experimental setup of CCTV camera system used in the experiment

2.3 Snail specimens
Sexually matured 3 cm snails of *P. maculata* and *P. canaliculata* were used. All the snails were obtained from the laboratory reared culture of the two species obtained from field collections in Bukit Kechik, Kelantan, Malaysia (N05º 50.942' E102º 29.353'). Identification of snails was done according to their shell morphology [22, 23], and final species confirmation was provided by Prof. Dr. R.H. Cowie. All the snails were obtained from the laboratory reared culture of the two species. Identification of males and females was done by observing the testicles through the translucent shell [24] along with convex operculum for males and concave operculum for females [25], and the latter confirmed by their mating [26]. One female with two males were kept in individual aquarium tanks to monitor their mating and oviposition behavior. Fresh 21 days old stems of *Limnocharis flava* were provided as food for the snails on a daily basis due to their less hindrance in the recording system. Five cm water level was maintained in the aquariums and changed twice a week to avoid foul odor.

2.4 Data collection and statistical analyses
The data for the experiment was collected daily for thirty days. A natural photoperiod of 12:12 was observed during the entire experimental duration. The data were extracted by watching recordings of all the cameras for the entire duration of the experiment at a speed of 64x. Mating in the snails was confirmed when the penis sheath of the male remained inserted into the mantle cavity of the female above the right nuchal lobe [10,27] and duration of mating was recorded accordingly until the complete removal of the penis sheath from female's mantle cavity. The oviposition duration was calculated from the laying of first to last egg. Egg diameter was calculated using Dino-Lite digital microscope (AnMo Electronics Corp. Taiwan), whereas the total number of eggs per clutch, hatching percentage and eggs per minute were calculated by direct observation.
The experiment was conducted in a completely randomized design with five replicates for each species. The data obtained for different parameters was analyzed using the Student t-test at 0.05 probability level and Pearson's correlation was used to estimate the relationship between different parameters of mating and oviposition. However, data for mating and oviposition frequency along with the timing of mating and oviposition was analyzed using non-parametric Wilcoxon-Mann-Whitney Test as data was not normally distributed (Shapiro-Wilk normality tests). All the analysis was done using SAS 9.3 computer software (SAS Institute Inc. 2009).

3. Results and Discussion
The highlight of the digital infrared CCTV video camera system used in this study was its effectiveness in producing clear and continuous recording of the mating and oviposition behavior of the two snail species, thus eliminating any bias in acquiring of the data (Figure 2). Previous studies of Albrecht *et al*. [28] on evaluation of the effect of food availability, different temperatures and day periods on the mating behavior of *P. canaliculata* observed different mating behaviors only three times a day during morning, afternoon and evening. Therefore, various behavioral activities that occurred in between the specific observations may have been missed and this could affect the accuracy of data. Previous studies also made observation on the mating behavior of apple snails twice a day during morning and evening and may also have overlooked many mating occasions in a day [10, 29]. Studies by Tamburi and Martín [26] on the effects of food levels on the reproductive output of *P. canaliculata* only recorded the number of egg masses laid, but did not include the exact timing and length of oviposition. Although the above mentioned studies may have accomplished their research objective, but the lack of accuracy as well as incompleteness of the above studies can be eliminated using the digital infrared CCTV system as applied in this study. In this study, the detailed data on the actual starting time of mating and oviposition in individuals of both species as well as the total duration of mating and oviposition was also recorded. The system used in the study has additional advantages of easy arrangement of the cameras, according to specific objectives of any study due to their variable focus lenses. Moreover, the system used is also time saving as recordings can be monitored at a speed of 64x. Therefore, the system applied in this study has the potential to be widely utilized in other behavioral studies of apple snails to increase the efficiency of data collection.

Fig 2: Snaps from CCTV recording showing mating and oviposition in *Pomacea* spp.

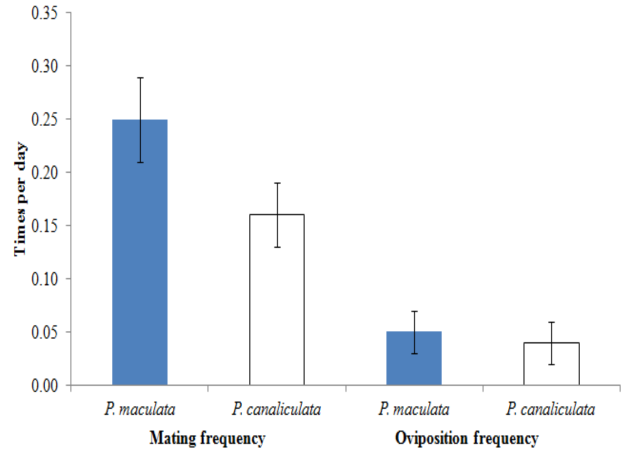

Fig 3: Comparison in mating and oviposition frequency of *P. maculata* and *P. canaliculata*

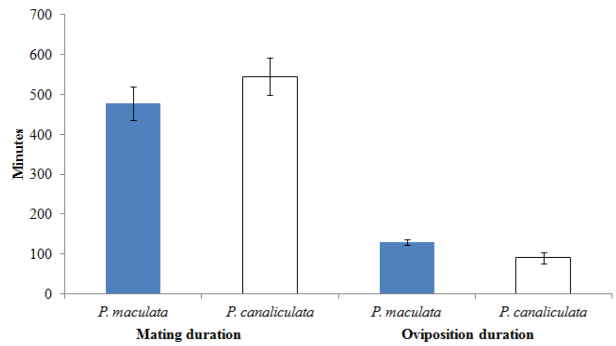

Fig 4: Comparison in mating and oviposition duration of *P. maculata* and *P. canaliculata*

The results of the comparative analysis of different mating and oviposition parameters of the two species are given in Figure 3 and 4. It was observed in the study that snails remain submerged in water during the entire duration of their mating. Significantly higher mating frequency (WMW, $P < 0.05$) was recorded for *P. maculata* (0.25 ± 0.04 times / day) as compared to *P. canaliculata* (0.16 ± 0.03 times / day), however, no significant difference was recorded for the mating duration of the two species ($t=1.04$, $P > 0.05$). The mean duration of mating in *P. maculata* and *P. canaliculata* was recorded as 478.5 ± 42.3 minutes and 545.5 ± 46.4 minutes respectively. Results also showed that mating in *P. canaliculata* was initiated only during night hours, whereas *P. maculata* individuals copulate equally during day and night. Often, a lengthy copulation, as observed in *P. canaliculata;* is required to transfer sufficient amount of sperms for the fertilization of thousands of eggs produced by female snails and often, a minimum 20% of copulation time is further required for proper genital contact between the male and female snails [12]. In addition, bigger male *Pomacea* spp. (over 3 cm) produces a comparatively higher amount of sperms and hence required a longer time to transfer all sperms to females [30, 31]. It has also been reported that the mating duration in apple snails could be influenced by the oviposition process in females whereby females who mated during the previous evening could lay eggs during the night; hence interrupting an ongoing mating process [27]. Previous studies conducted on *Pomacea* spp. also showed that mating is mostly observed either during the night or early morning hours and continued for a period of 8 to 20 hours. Studies also confirmed repeated copulation in the same individuals up to three times per week [9, 31].

In both the observed apple snail species, oviposition was recorded only during the night hours. Similar sighting has been confirmed to occur in *Pomacea* spp. based on previous studies and the probable reasoning is to lower the risk of predation and desiccation [9]. Generally for oviposition, females spent 20 minutes to 2 hours outside water to search the appropriate place for the oviposition and most of the oviposition was observed near the top of aquariums, indicating possible avoidance of eggs submersion. However, females immediately moved into the water after oviposition to regain the energy and freshness they exhausted during the oviposition. Results also showed no significant difference in oviposition frequency (WMW, $P > 0.05$) of *P. maculata* and *P. canaliculata* as the two species oviposit at 0.05 ± 0.02 times per day and 0.04 ± 0.02 times per day, respectively. Significantly higher egg laying duration ($t=2.91$, $P < 0.05$) was recorded for *P. maculata* (130.5 ± 7.1 minutes) as compared to *P. canaliculata* (90.1 ± 13.4 minutes). Meanwhile, no significant relationship between mating and oviposition of the two species was found. Previous studies had confirmed that oviposition in *Pomacea* spp. usually occur after 24 hours of copulation and continue up to 2 weeks, but females also have the ability to store the sperms up to 140 days and frequency of oviposition or egg production is not dependant on the frequency of mating [9,12]. It is also established that female *Pomacea* spp. can lay eggs up to 1.4 times per week [9]. Often, several other factors such as food deprivation [26] and temperature variation [32] also have a significant effect on oviposition, thus often influencing the egg mass size and weight and further regulating the seasonal onset of copulatory and spawning activities in relation to temperature increase.

Results of the study also confirmed comparatively higher fecundity in *P. maculata* as compared to *P. canaliculata* (Table 1). In the study maximum 441 and 214 eggs per clutch were recorded for *P. maculata* and *P. canaliculata,* respectively with a mean of 243.9 ± 22.8 and 124.8 ± 19.1. Accordingly, higher number of eggs per minute was also recorded for *P. maculata,* however, the egg diameter was significantly higher in *P. canaliculata* ($P < 0.05$). In a comparative study between *P. canaliculata* and *P. maculata*, higher fecundity in *P. maculata* than *P. canaliculata* was also reported [6]. Hayes *et al*. [22] also reported a comparatively higher number of eggs per clutch in *P. maculata* (average 1500) as compared to *P. canaliculata* (average 260). Another study on comparative features of eggs of *P. maculata* and *P. canaliculata* at their native habitats of Uruguay and USA suggested that width, mass and eggs were significantly higher in *P. maculata* clutches as compared to *P. canaliculata* [15]. The studies also confirmed the larger and fewer eggs in *P. canaliculata* as compared to *P. maculata* [22] and the results of this study also confirmed the same as fewer eggs of larger diameter were recorded in *P. canaliculata.* Previous studies also reported that a single *Pomacea* female can lay 4751 eggs per clutch, however; a high variability in clutch sizes was also observed [6]. High variation in the total number of eggs per clutch may be due to females of different sizes [10] under the influence of different environmental conditions because high population densities with low food availability results in snails of smaller sizes. Accordingly, theses snails may produce small egg masses with fewer eggs [32]. Moreover, the significant effect of diets and age of females is also observed on the clutch sizes and the number of eggs as older females produced less egg masses with fewer eggs [26, 29]. It was also observed that the high mating frequency was not positively correlated with high frequency of oviposition or egg production, however, fecundity is positively correlated with the size of females of *Pomacea* spp. [26, 35]. Moreover, in the study mean hatching of 70% and 73% was recorded for *P. maculata* and *P. canaliculata* respectively, and the same is in accordance with the previous studies of Barnes *et al*. [6] who recorded 70% and 30% hatching rate for *P. maculata* under field and laboratory conditions, respectively. High hatching success ranging from 87% to 100% is also reported among the individuals of *P. canaliculata* [36, 37]. The differences in hatching success in various studies may be attributed to temperature as studies showed the significant effect of temperature on the hatching success of *Pomacea* eggs where higher hatchability was recorded at constant higher temperatures [6, 17]. Studies also suggested huge variation in the hatching period of *Pomacea* spp. eggs ranging from 8 to 20.7 days and it mostly depends on the temperature and other environmental factors [36, 37]. Accordingly, above findings substantiate the results of the study as mean hatching of 13.2±0.4 and 12.7±0.8 were recorded for *P. maculata* and *P. canaliculata* respectively.

Table 1: Comparative analysis of egg mass data of *P. maculata* and *P. canaliculata*

	P. maculata	***P. canaliculata***	**P-value**
Total eggs	243.9±22.8	124.8±19.1	< 0.001
Egg diameter	1.99±0.02	2.97±0.02	< 0.001
Eggs / minute	1.9±0.2	1.4±0.1	< 0.05
Hatching %	69.9±3.2	73.1±4.1	> 0.05
Hatching Period (days)	13.2±0.4	12.7±0.8	> 0.05

No significant relationship between mating and oviposition of the two species was found as egg laying in *Pomacea* spp. often did not follow the mating and females can store the sperms up to 140 days [9, 12, 27, 35]. Moreover, duration of oviposition and total number of eggs per clutch were only highly correlated in *P. canaliculata,* however, no previous studies have included this relationship in their experiments.

Table 2: Correlation matrix (r) between mating and oviposition of *P. maculata* and *P. canaliculata*

P. maculata		**p-value**	***P. canaliculata***		**P-value**
	Egg laying			Egg laying	
Mating	0.1515	> 0.05	Mating	-0.0814	> 0.05

Table 3: Correlation matrix (r) between duration of oviposition and total number of eggs in *P. maculata* and *P. canaliculata*

P. maculata		**p-value**	***P. canaliculata***		**P-value**
	Total Eggs			Total Eggs	
Duration	0.4014	> 0.05	Duration	0.9251	< 0.05

4. Conclusion
The studies confirmed the higher mating frequency along with the higher oviposition duration in *P. maculata* as compared to *P. canaliculata.* Higher number of eggs per clutch along with eggs per minute was also recorded in *P. maculata.* Mostly mating and oviposition activities in two species were recorded during the night time, whereas no significant difference was observed for the duration of mating, oviposition frequency and hatching success between the two species. No correlation between mating and oviposition was observed for two species and only *P. canaliculata* showed highly significant correlation between duration of oviposition and total number of eggs per clutch. Higher reproductive potential of *P. maculata* may help it to over number *P. canaliculata* in invading countries of the Southeast Asia. The study also found the digital CCTV infrared video camera system effective in monitoring and acquiring the data on the behavior of the *Pomacea* spp. and possibly be exploited for more studies in future with accuracy and cost effectiveness. The results of the study could be helpful in understanding the basic patterns of mating and oviposition of the two most invasive *Pomacea* spp. Moreover, control measures should be so managed to disturb their mating and oviposition because it is the base of their invasiveness and further spread.

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