The effects of different water temperatures on survival and growth rate of juvenile invasive apple snail, *Pomacea canaliculata* (Lamarck, 1822) under controlled environment

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Abstract. Invasive apple snail, *Pomacea canaliculata* is one of the major mollusc pests affecting rice production in Malaysia. This pest not only gives fatal effects to rice but also decreases the water quality in the ecosystem due to the use of pesticides to control it. With the unpredictable changes on global climatic in Malaysia, it is important to have knowledge on the effect of water temperature on growth and survival rate of *P. canaliculata* in water bodies, in order to avoid loss in paddy field and its adverse effects on aquatic ecosystems. Water temperature is one of the most important environmental factors influencing the survival, growth rate, reproduction and behaviour of freshwater snails. The aim of this study is to evaluate the growth and survival rate of juvenile *P. canaliculata* at different temperature regimes. Growth and survival of juvenile *P. canaliculata* were evaluated at different constant water temperatures (15, 20, 25, 30 and 35°C). Our findings show, at 30°C the growth rate of *P. canaliculata* is higher compared with the other temperatures, however had low survival rate. Meanwhile, at room temperature (21 - 24°C), a higher survival rate (100%) was recorded. The results showed that different water temperatures affect the growth and survival rate of *P. canaliculata* and it is an important indicator for predicting and understanding the changes of water temperature when controlling this pest.

1. Introduction

Based on [1], golden apple snails (*Pomacea canaliculata*) are classified belonging to kingdom animalia, phylum of mollusca and class of gastropoda. Common names of golden apple snails are, channelled apple snail and golden apple snail. In Malaysia, *P. canaliculata* is called as siput gondang emas [2]. *P. canaliculata* are freshwater snails that are originally from South America [3]. *P. canaliculata* was introduced into Asia through Taiwan. First introduced to Taiwan in 1980, Japan in 1981, Philippines in 1982 and Thailand in 1986 for food commercial purposes because at that time, they expected the species to have high-protein food source [4]. It has been illegally brought into Malaysia for the same purpose. This species is now invading the environment and rice fields of Asian countries. Invasion by the *P. canaliculata* causes many losses to farmers. The economic impact of invasive species is a major concern throughout the world [5]. The distribution of invasive apple snails, *P. canaliculata* was spread widely at various rice fields in the five states of Peninsular Malaysia [6] especially in Kedah and Perlis. Its feeding
habit towards the young stems and leaves of paddy [4] and could consume 7-24 rice seedlings per day [7] thus, resulting in extreme damage to growing paddy. This pest not only gives fatal effects to rice but also decreases the water quality in the ecosystem due to the use of pesticides to control it.

The behaviour and physiological responses are influenced by surrounding factors. Therefore, understanding the behaviour of invasive species is crucial in developing measure control and management methods. Study on temperature is critical for predicting the dispersion and effects of invasive species, increasing in temperature of wetlands due to global warming [8]. Temperature is a favourable factor for population and establishment of invasive *P. canaliculata* to its effects on survival and growth rate, reproduction, lung ventilation frequency, feeding rate, and behaviour [9]. [9] also stated the percentage of the snail survival at low temperatures (15 and 20°C) was higher as compared to high temperatures (30 and 35°C). On the other hand, the behaviour of *P. canaliculata* was not active in water which is lower than 15°C [10].

A previous study showed the negative effects of invasive *P. canaliculata* in environments at higher temperatures have more closely match to their thermal optima. There is no doubt that the temperature is one of the major influences on *P. canaliculata* in terms of shell morphology. The main cause of morphological differences was the allometry. Temperature can alter growth of different parts of the body where it changes the calcium carbonate deposition thus, will produce variation in shell shapes by different thermal regimes. The growth of *P. canaliculata* can result faster due to optimal thermal regimes. According to [9], the *P. canaliculata* had a higher growth rate at 30°C compared to 15, 20, 25 and 35°C.

Behavioural periodicity of golden apple snail, *P. canaliculata* was assessed by [11] at different temperatures which were 15, 20, 25 and 30°C, and they found that at 15°C, the *P. canaliculata* showed less movement activity while, at high temperature, it spent more time moving behaviour such as bottom-crawling and side-crawling. This finding is similar to [12], where the *P. canaliculata* is active after being exposed to warm temperature and immobile when exposed in cool temperature at 13.5°C.

2. Material and methods

2.1. Location of sampling site

Thirty egg-masses of *P. canaliculata* were collected at paddy field Tanjung Karang, Selangor on 22nd March 2019. Collected egg-masses were placed in a box from the collection sites where they were counted and kept at room temperature (21-24°C) until they hatched.

2.2. Rearing methodology of Pomacea canaliculata

Hatched juvenile *P. canaliculata* were placed in a glass aquarium (1m length, 1m width and 0.45m height) with soil (5cm thick) and rainwater (approximate 70L) located at greenhouse Universiti Teknologi MARA Puncak Alam, Selangor. One week old of paddy seedlings, duckweeds (*Lemna minor*) and aquatic plants (*Hydrilla*) were transplanted inside the aquarium to imitate the actual environment in the paddy field. Juvenile *P. canaliculata* was fed with lettuce, *ad libitum* and cultured under this condition for 3-4 weeks until they were used in the experiments.

2.3. Preparation of different constant temperatures

Three waterbaths (SASTEC®) were used and each of them were set at different temperatures (25, 30 and 35°C). Meanwhile, temperatures 15 and 20°C were placed in two separate rooms with air conditioners.

2.4. Experiment preparation

A group of 300 juvenile *P. canaliculata* were collected randomly (5.00±1.00 mm shell length) and measured by using a Vernier caliper. The weight of the juvenile *P. canaliculata* was measured by
analytical balance. 300 of juvenile *P. canaliculata* were used for each treatment with 5 replications including control. Ten juveniles of *P. canaliculata* were placed in each 30 plastic aquaria (15cm length, 5cm width, 10cm height) and covered with lids to prevent them from escaping. Juveniles *P. canaliculata* were immersed at different temperature rates (15, 20, 25, 30 and 35°C) with room temperature (21-24°C) as a control method. Treatment control was placed in the room equipped with a data logger (Watchdog®) to record the temperature every hour. The aquaria were cleaned during the three-day interval, the water and soil were changed to avoid water fouling. The juvenile *P. canaliculata* were fed with lettuce every day according to their consumption. No artificial aeration was used during the experiment since the snails can easily breathe air inside the aquarium. Completely randomized block design (CRBD) was used in this experiment.

2.5 Data collection

The weight and shell length of juvenile *P. canaliculata* were measured every week for each temperature within 10 weeks [9]. The juvenile’s shell length (SL) were measured with Vernier caliper from apex to apertural lip [3]. While, the weight was measured by using analytical balance. The survival rates of juvenile *P. canaliculata* were observed every 24 hours over 10 weeks. The percentage of survival were counted using the equation by [9]:

\[
\text{Survival} = \left( \frac{\text{number of live snails}}{\text{total number of snails}} \right) \times 100
\] (1)

For determination either the juvenile *P. canaliculata* live or dead, the head/foot region of an unattached is prod with the toothpick. If the juvenile *P. canaliculata* fails to respond to this stimulation, more forcefully stimulate with the toothpick. Failure of the second, the juvenile *P. canaliculata* will be proclaimed dead. Dead juvenile *P. canaliculata* will be removed from the experiment.

The mean data of growth and survival of juvenile *P. canaliculata* in different temperatures was analysed using the One-Way Analysis of Variance (ANOVA) method at significant different, 0.05.

3. Results

3.1. Growth rate

The growth of juvenile *P. canaliculata* at five different temperatures within ten weeks is shown in figure 1. There was a significant difference between groups as revealed by one-way ANOVA (*p* < 0.000). A Bonferroni test showed that temperature 30°C were recorded being significantly further than temperature 15, 20, 21-24, 25 and 35°C (*p* = 0.000). Meanwhile, there was no significant difference between 21-24°C and 25°C (*p* = 1.000) or between 15 and 35°C (*p* = 1.000) within ten weeks. The highest growth rate of juvenile *P. canaliculata* was at temperature 30°C after ten weeks. The maximum mean shell length of juvenile *P. canaliculata* was 22.16 mm at 30 °C. Meanwhile, the lowest growth rate of juvenile *P. canaliculata* was at temperature 20 °C and the mean shell length was 6.94 mm.
Figure 1. Mean shell length (mm) pattern for the five cohorts reared at different constant temperatures (15, 20, 25, 30 and 35°C) including control (21-24°C) weekly within ten weeks.
3.2. Survival rate
The survival of juvenile *P. canaliculata* at six different temperatures including control (21 - 24°C) within ten weeks is shown in figure 2. The mean survival of juvenile *P. canaliculata* was different among the temperatures (Kruskal-Wallis test: $X^2 = 191.276$, $p = 0.000$) and was negatively correlated with temperature (Spearman’s $\rho = -0.262$; $p = 0.000$). The highest survival among the temperature treatment was at room temperature, 21-24°C (100%) followed by 20°C (72%), 25°C (24%), 30°C (12%), 15° (0%) and 35°C (0%). None of the juvenile *P. canaliculata* survived until week ten at temperature 15 and 35°C respectively.

![Figure 2. The percentage survival of juvenile *P. canaliculata* at six different temperatures and control (21 - 24°C) within 10 weeks.](image)

4. Discussion
This study showed the juvenile *P. canaliculata* had a higher growth rate at 30°C compared to 15, 20, 25 and 35°C. This finding has a similar result with [9] where at a temperature of 30°C, the mean of shell length is larger than the other temperatures. At temperatures 15 and 20°C the growth was normally slower compare to temperature 25, 30 and 35°C. The size of *P. canaliculata* was small at temperatures 15 and 20°C probably due to less mobility to reach food and decrease the food ingestion as stated by [13] observation during the experiment showed that the juvenile of *P. canaliculata* almost did not do any activity like, floating, feeding and crawling. This behaviour was also observed by [11] where they found the activity of *P. canaliculata* more likely to stay motionless which means, their activity and behaviour just dropped to zero at low temperature (15°C). It showed that at temperature 15°C, the mean shell length size of the juvenile *P. canaliculata* was smaller than other temperatures. While, at higher temperature the juvenile of *P. canaliculata* was more active such as crawling and feeding food. This statement has similar findings with [13] where they revealed that at temperature 30°C the *P. canaliculata* remain constant active on feeding. From this observation, the effect of the temperature that fit its thermal regimes can influence the growth of the juvenile of *P. canaliculata* by making them more active in food feeding. Also, can affect its growth which can result in an increase of shell length size.

At temperature 15 and 35°C, none of the juvenile *P. canaliculata* survived until week ten but this result contradicts from [9], whereas their study revealed that juvenile *P. canaliculata* at temperature 15 and 35°C survived until week ten and only suffered minor mortality at temperature 35°C. This is due to the different temperature and condition of the region where the juvenile *P. canaliculata* can tolerate low temperature during winter and resist to high temperatures during summer in Argentina. Meanwhile, Malaysia has tropical weather where it is quite humid and temperature ranges from 20 - 30°C and do not exhibit extremes of hot and cold temperatures. When the juveniles of *P. canaliculata* are exposed to 15°C and 35°C, they were not able to withstand the constant temperature they were in. [14] revealed the snails collected from summer cannot survived at 0°C in five days which shows same result from this paper where the snail in Malaysia will be dead when they exposed to cold water (<15°C). Water
temperature below 15°C and above 35°C is lethal for *P. canaliculata* at juvenile stage in Malaysia. Meanwhile, at temperature 21 - 24°C this species can tolerate well which indicates juvenile *P. canaliculata* prefer more to live in high humidity conditions with no mortality such as in Malaysia. Survival rate correlated negatively with temperature, with highest mortality at temperature 15 and 35°C.

5. Conclusion
This finding reveals the degree of survival and effect of the temperature on juvenile *P. canaliculata* that are able to prevent this species from invading further regions in Malaysia. This pest not only can harm paddy production but also the ecosystem. Temperature is a key factor for victorious formation of invasive apple snail to its effects on growth and survival. Unpredictable changes on global climatic and its effect on degree of invasive *Pomacea canaliculata* in water body in Malaysia is important in order to avoid loss in paddy field. Data on the influence of pH and temperature on *Pomacea canaliculata* is crucial for predicting and understanding changes and ready a better countermeasure on the potential problem. The result of this study is valuable to the industry in the assurance of how much it can measure invasion of *Pomacea canaliculata* in freshwater bodies and wetland agriculture in Malaysia as well as in contributing in new knowledge for the next generation.

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