COMBINED EFFECT OF TEMPERATURE AND LIGHT ON THE EGG HATCHABILITY IN *POMACEAE CANALICULATA* (GASTROPODA: AMPULIRIIDAE) FROM MALAYSIA

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Abstract

The Golden Apple Snail (GAS), *Pomacea canaliculata* is an invasive species that has become a serious pest organism in rice fields in Southeast Asia since 1980s. This study was conducted to assess the hatchability of eggs under a combination effect of temperature and light. The clusters of eggs were exposed to different temperature (28ºC, 30ºC, 32ºC, 34ºC and 36ºC) and under the presence and the absence of light. The finding showed that the cluster of egg hatched faster with an increase of temperature (2-way ANOVA; $P < 0.05$). However, the egg hatchability was not impacted by the presence or the absence of light. The combined effect between temperature and light on egg hatchability was also insignificant (2-way ANOVA; $P > 0.05$). Our findings indicate that temperature alone produces the main impact on the egg hatchability. Therefore, this knowledge provides an initial understanding to predict the population dynamic and geographical distribution of *Pomacea* from Malaysia particularly in the effort to hamper their ecological invasion.

Keywords: temperature, light, egg hatchability, *Pomacea canaliculata*, invasive species

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Introduction

Temperature regulates physiology of living organisms at various life stages. At adult stage, temperature alters the breeding patterns, sexual performances and aging processes (Walsh et al., 2019). In juvenile, temperature influences sexual maturation, frequency of ecdysis and the growth rate. Temperature also affects the behaviour, feeding mechanism, breathing processes and interaction of the juveniles with their environment. In the early life stage, temperature controls the viability of eggs and embryos from oviparous to viviparous organisms (Bellini et al, 2019). Consequently, temperature plays a vital role in regulating the life history of living organisms.

The role of light in relation to the physiology of organisms is also substantial. Most of animal skin and body contain receptor cells for light sensitivity. Such photoreceptor proteins occur as rhodopsin in mammals, chromatophore in aquatic invertebrates and phytochrome in plants. Studies on how living organisms react to photoperiodism showed that light affects diel vertical migration in most plankton (Kim et al., 2019), circadian rhythm in insects (Tomioka and Matsumoto, 2019), seasonal breeding in birds (Verhulst and Nilsson, 2008) and flowering of plants (Pearce et al., 2017). Some of camouflage animals protect themselves by manipulating light detecting pigments, melanophore, to reflect and to scatter light from being detected by their predators (Cheney et al., 2017).

The golden apple snail (GAS) or scientifically known as *Pomacea* is a freshwater, herbivorous snail. This species is originated from South America. They were introduced intentionally as an aquarium trade (Ng et al., 2017) and alternative protein source (Naylor, 1996) in Asian countries in 1980s. However, the intention as an alternative food is a failure among local people due to its unpleasant taste. In Thailand and China, health regulation restricted the introduction of GAS where the snail has been discovered as the host of many nematode parasites, commonly infested human and domestic animals (DeBoer, 2019). Consequently, the snail was discarded into local aquatic systems without proper cautions and regulations.

In Asian countries, the availability of warm water temperature throughout years provides stimulating environment for the growth of GAS (Hayes et al., 2015). High diversity and density of macrophytes in these regions additionally provide a wide range of food to this herbivorous snail. The GAS population has been observed to feed on native weedy plants (duckweed, water spinach, and water hyacinth) as well as commercial crops such as paddy, taro, lotus, water chestnut and water cabbage (Cowie, 2002). This snail also has the property of aestivation; the ability to survive under long dry conditions and possess amphibious respiration; ability to strive with and without water (Seuffert and Martin, 2010). Consequently, the population of GAS proliferates rapidly, invading almost all vegetation areas and becomes a threat to natural biodiversity and agriculture systems in Southeast and East Asia.

In Malaysia, the history of GAS introduction was reported in the late 1980’s as high-protein food and export item (Cowie, 2002). The first discovery in the rice fields was in Kampung Pengkalan Semeling, Kedah in 1991. It was reported that population of *P. canaliculata* was found in Kedah and Perak while its congeneric species, *P. maculata* was common in Sabah and Sarawak (Mat Hassan & Abdul Kadir, 2003). Just within 10 years after the first discovery, the snails become the key pest of rice in Malaysia (Yahaya et al., 2010). The snail favours on the rice seedlings which are less than 40 days. During 1991 there was no substantial damage to the rice seedlings but the damage became worsen in 2009 and 2010 as the estimated yield loss increased from RM43.6 million to RM 82 million respectively (Yahaya et al., 2017).

In this study, we aim to investigate the biology of *P. canaliculata* at the stage of egg after has been laid by the females. In general, hatching success greatly determines the sustainability of the population. Female snails need to copulate with the male before eggs are laid out. Eggs are fertilized internally and developed externally in calcareous shells. Eggs are laid out in clusters with bright pink colour on the stem of plants or on any surface above the water level. Normally, eggs will hatch into new hatchlings within 8 to12 days (Teo, 2014).

Factors that regulate the egg hatchability have been exploited by researchers in order to control the spread of invasion at early life stage. Horn et al. (2008) found that an immersion of eggs in water has reduced the hatching success by more than 75%. Hatchability of eggs was affected upon exposure to chemicals such as extract of *Barringtonia racemose* (Musman et al., 2013) and apple wax (Der-Chung et al., 2005). Biological controls by using fire ants that eat on snail eggs are successful to prevent egg hatching (Yusa, 2001). However, using ducks in biological control did not eliminate the eggs but caused the snail to lay eggs on sites that are unsuitable for hatchlings establishment (Liang et al, 2013). Therefore, the ongoing spread emphasis the urgent need for more research focusing on population dynamics, dispersal mechanisms and effects of *Pomacea* snails in different habitats. This study attempts to measure the pattern of egg hatchability under the influence of environmental parameters such as temperature and light.

Methods

Field Work

The samples of fresh eggs with bright pink colour were collected randomly around paddy fields heavily infested by GAS at Chenderong Balai, Perak, Malaysia. The collection was conducted consistently during afternoon. The eggs were found in clutches tightly attached at the weedy stem and wood sticks above the water surface. The freshness of eggs was determined with the presence of mucus covering the egg clutches. The ambient temperature during the egg collection was around 32ºC. Samples of egg clutches were transported back to Animal Laboratory of Universiti Teknologi MARA Perak Branch, Tapah campus in a moist and wet plastic container for further analysis.

Laboratory Work

The experiment was designed to assess thermal impact on the egg hatching performance. Five different temperatures were selected (28, 30, 32, 34 and 36ºC) with triplicate samples respectively. In each temperature, the egg samples were laid on petri dishes on top of moist cotton layer. Two set of petri dishes were prepared whereby one set was incubated under 24 hours of darkness and other set was exposed to 24 hours continuous light condition.

Under light condition, the petri dish contained egg samples were kept inside of a growth chamber to maintain the desired temperature. The light intensity was set up at 6400 lux. Under dark condition, all egg samples were kept in an incubator at the selected temperatures. The time required for egg hatchability was recorded in terms of the number of days taken for all eggs to hatch into new hatchlings.

Data Analysis

All data obtained from experimentation were analysed using SPSS version 20.0 through parametric test, two-way ANOVA. Post hoc test was conducted using Tukey HSD test. The confidence limit was set up at $p < 0.05$. All data have met normality assumptions and were proved to be homoscedastic when investigated with Levene's test.

Results

The rate of egg hatchability was insignificantly affected by the combined effect between light and temperature (2-way ANOVA: $P>0.05$; Table I). There was a significant main effect for temperature ($P<0.05$; Table I)) whereby the effect size of temperature (Partial $\eta^2 = 0.830$; Table I) was significantly larger than the effect of light (Partial $\eta^2 = 0.097$; Table I). The eggs hatched faster at 34ºC but did not differ significantly with days taken to hatch at 32ºC. The longest day taken for eggs to hatch was found at 28ºC. From our observation, none of the eggs hatched at 36ºC.

Meanwhile, there is no significant difference in the day of egg hatchability either in the presence or in the absence of light (2-way ANOVA: $P>0.05$; Table I). Our findings also observed that the eggs did not hatch and turned into dark colour under exposure to 36ºC either in the presence or the absence of light.

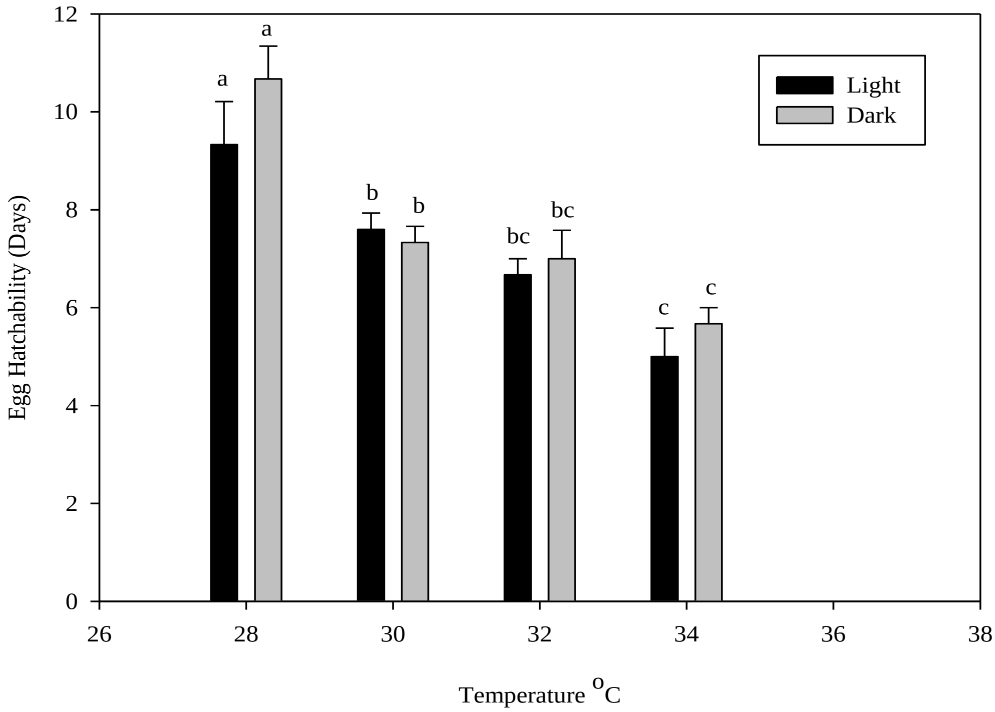

Figure 1. Egg hatchability (days) as shown by egg clutches of *P. canaliculata* exposed under different temperatures (°C) and in the presence or the absence of light.

Table I. Results of 2-way ANOVA on the egg hatchability of *P. canaliculata* exposed under different temperature and in the presence or the absence of light.

Source	df	SS	MS	F-value	P-value	Partial η^2
Light	1	1.50	1.50	1.71	0.209	0.097
Temperature	3	68.17	22.72	25.97	0.001*	0.830
Light x Temperature	3	2.17	0.72	0.82	0.499	0.134
Error	16	14.00	0.87			

df= degree of freedom; SS= sum of squares; MS= mean squares
** significant at p < 0.05*

Discussion

The study on egg hatchability can reveal the population dynamic of the golden apple snail, *P. canaliculata* from Malaysia. Our findings show there is no combination effect of temperature and light on the egg hatchability between 28 to 34°C and with or without the presence of light. A similar finding was obtained when there was no combination effect between temperature and light on the egg hatchability of another type of apple snail, *Marisa cornuarietis* within temperature of 22 to 25°C and 12L:12D photoperiodism (Aufderheide et al, 2006). Nonetheless, the egg hatchability of *Pomaceae patula* was significantly affected by the light and temperature (Meyer-Willerer and Santos-Soto, 2006). In this study, the lower temperature and lesser light significantly decreased the hatching efficiency than at higher temperature and the presence of more light. Possibly, the egg hatchability is species specific and further study on the egg morphology and physiology is required to explain the combination effect of light and temperature.

Temperature alone significantly produces the main effect on the egg hatchability of *P. canaliculata*. The eggs hatch faster with an increase of temperature from 28 to– 34°C. Another study on *P.*

canaliculata also revealed an increase in egg hatchability from 20 to 30ºC (Seuffert and Martin, 2017). This trend is typical when increasing temperature certainly accelerates the embryonic development inside eggs across different species such as *Marisa cornuarietis* (Aufderheide et. al, 2006), carp fish, *Cyprinus carpio* (Aanand & Rajeswari, 2018), poultry eggs (Boleli et l., 2016) and amphibian eggs (Wijethunga et al., 2016). Nonetheless, none of the *P. canaliculata* eggs hatched with an increase of temperature at 36ºC. A similar study by Seuffert and Martin (2017) on the same species has found that the adverse effect of temperature has started earlier at the temperature of 35ºC. It is expected that high temperature could lead to an adverse effect by destroying the enzyme configuration and protein structure required for embryonic development in many poikilotherms (Thépot & Jerry, 2015). As a consequence, embryo is not well-developed and no egg hatchability is observed.

Our study also revealed that eggs of *P. canaliculata* hatched within 5 to-6 days at 34ºC while at 28ºC the eggs hatched longer within 9 to10 days. Other studies have demonstrated that eggs of *P. canaliculata* hatched within 13 days at 21ºC (Zhou et al., 2003) and 22 to-24 days at 16ºC (Pizani et al., 2005). Clearly, the duration for egg hatchability depends on temperature where an increase of temperature stimulates for early egg hatching and vice versa.

While other studies had proven that light can influence the physical activities of *Pomacea* (Wagiman et al., 2016), our study revealed that the presence or the absence of light did not impact the egg hatchability. The egg hatchability also has not been affected under different exposure of photoperiodism 12D:12L, 14D:10L and 10D:10L (Trexler, 2011). The study by Lixia (2015), evidently showed that the duration for egg hatchability in *P. canaliculata* increased from 13.5 days to 16.6 days when exposed to 12D:12L and 0D:24L respectively. However, this study did not report statistical analysis to validate the significant difference between their photoperiodism treatments. Therefore, the presence or the absence of light is considered as insignificant factor for embryonic development. In fact, the presence of light is more relevant to other animals' physiological process such as sexual maturation (Sandhyarani & Vidyarani, 2015), mating behaviour and egg laying (Ter Maat et. al., 2012).

Conclusion

This study shows that the egg hatchability is not impacted with the combination of light and temperature. However, temperature alone gives a significant impact as the eggs hatch faster with an increase of temperature. Temperature of 36ºC is totally fatal to egg hatchability. Light seems has no impact to egg hatchability of *P. canaliculata*. The high tolerancy of *P. canaliculata* eggs towards daytime length offers the opportunity of this species to become an invasive species not only in tropical countries but also in temperate regions with different daytime length throughout years. Ultimately, temperature is the main controlling factor in order to find a solution in hampering the population invasion.

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