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Evaluation of Growth Performance of *Tachypleus gigas* (Muller, 1785) under Two Different Culture System

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ABSTRACT

Information on the growth of *Tachypleus gigas* is not well established as compared to its temperate counterpart *Limulus polyphemus*. Lack of documented study on *T. gigas* has further encouraged research on the growth and molting frequency under different culture methods. This report compares the size and weight increments and molting frequency of *T. gigas* larvae cultured using conventional (80-90% water change/day) and non-conventional (recirculating aqua culture system) methods. Size increment was measured based on prosomal width and weight increments of the larvae. Molting frequency was determined for the larvae from 6 to 11-month old. The larvae culture using these two methods molted 3 times during the culture period. Result of t-test showed that there was no significant differences ($p>0.05$) in the molting frequency of the larvae between the two methods. The final prosomal width for *T. gigas* larvae cultured using conventional method was 23.50 mm which is significantly ($p<0.05$) smaller as compared to 27.99 mm using non-conventional method. Similarly the final weight of the 11-month-old larvae cultured using conventional method was 0.61 g which is comparatively lower than those cultured under recirculating aqua culture system, 0.92 g. Water parameters (pH, salinity, temperature, dissolved oxygen and ammonia) for both systems were monitored and it was found that the conventional and non-conventional method of culture does not differ in salinity, temperature and pH except for dissolved oxygen (DO) and ammonia.

Key words: Growth performance, *Tachypleus gigas*, water quality

INTRODUCTION

Horseshoe crabs are extremely valuable to the medical research community. Currently, one major biomedical product is Limulus Amoebocyte Lysate (LAL), a cloting agent in horseshoe crab (*Limulus polyphemus*) blood that is used to detect human pathogens in medical equipment (Karl, 2004). The western countries have led the biomedical research and product development since the early 1900s. This crustacean is also used for vision studies because of similarity of its complex eye structure to the human eye. Until today, researches on horseshoe crabs continue. So far, products derived from horseshoe crabs have good potential for commercialization. There are only four existing species of horseshoe crabs today. They are *Limulus polyphemus*,

Tachypleus gigas, *T. tridentatus* and *Carcinoscorpius rotundicauda* (Brusca and Brusca, 1990). In spite of their commercial and medical importance, horseshoe crab populations are threatened by the loss of habitats. Human impact is be the major cause for this problem. The coastal changes and development in response to rapid population growth led to deterioration of water quality and habitat. For example, even though horseshoe crabs are able to tolerate the changes in environment, high concentration of pollutants (i.e., heavy metals) can cause detrimental effect on the growth of this invertebrate. Their populations declined have caused international interact in the conservation of this species.

In the US, *L. polyphemus* captured for blood extraction (LAL production) has approximately ten percent greater mortality than those in the wild (Karl, 2004). A more serious and immediate threat may be the recent, dramatic increase in horseshoe crabs harvests for bait for the eel and whelk fisheries. In Malaysia, horseshoe crabs are left to die in the sun when trapped in fisherman's fishing nets (Hunaini, 2007). Horseshoe crabs are also harvested and processed as a feed additive for poultry and livestock and as a compound fertilizers (Shuster, 2003). Horseshoe crabs take a long time to grow and it takes about 9-12 years to reach maturity. Horseshoe crabs are characterized by high fecundity but with high eggs and larval mortalities (Loveland *et al*., 1996). There are very few researches on the Asian Horseshoe crabs except by Sekiguchi *et al*. (1988) in particularly the embryonic development. During its formative years, the horseshoe crab molts, to accommodate its growing body. The new carapace then hardens. Juvenile horseshoe crabs may molt several times in the first and second years, then only once annually. However, in the laboratory where environmental temperatures remain constant and are often elevated above natural cyclic temperatures, juvenile horseshoe crabs were found to molt three to five times in the first year and then later once or even twice a year (Stephen and Berkson, 2005). In culture condition, molting problem was observed when the larvae when the larvae move out of the shell, legs, or telson, get stucked and this resulted in the mortality (Stephen and Berkson, 2005). Furthermore, during this molting process, these soft-shelled horseshoe crabs are highly susceptible to predation. Uncontrolled water quality such as ammonia toxicity, pH extremes, gas supersaturation and high turbidity have negative impact on their growth and survival.

Horseshoe crabs survive better in the natural environment. Few researchers have successfully used both natural seawater and commercially synthetic marine salt formulations to maintain horseshoe crabs for long period of time (Stephen and Berkson, 2005). The present study use seawater from Port Dickson. In order to reduce maintenance cost particularly in the cost for seawater, this study compared two methods (conventional and recirculating aquaculture system) for the culture of horseshoe crabs larvae in laboratory condition. Therefore this study to compare the effect of two culture methods on the prosomal width and weight increment and molting frequency of *T. gigas* larvae from the age of 6 to 11 month.

MATERIALS AND METHODS

Materials: The specimen and materials used in this study were 6 month-old *T. gigas* larvae. The larvae were produced from trough artificial fertilization using *T. gigas* brood stock captured from the wild. Other materials used were Recirculating Aquaculture System (RAS), rearing trays, micrometer and analytic balance, water testing kits, *Artemia nauplii* and seawater.

Methods

Samples and rearing trays: In this study, approximately 300 individuals of sixth month-old *T. gigas* larvae were used. Three trays for conventional rearing methods and another three for RAS. Each of these tray contained 50 *T. gigas* larvae. Seawater was used as the rearing media. Larval rearing was carried out in laboratory condition. The average prosomal width and weight measurements were taken at the initial stage of this study and once a month thereafter until larvae reach 11 month-old.

Recirculating aquaculture system, RAS: Generally, this system involved the water recirculation from the rearing trays into the mechanical and biological filters and the sterilized through UV light before being return to the rearing tray. In RAS, the mechanical and biological filter both functioned to filter to solid waste and remove ammonia from the system. Polyethylene (PE) tray were arranged to resemble drawers which can be pulled out for monitoring and maintenance purposes.

Feeding: *Artemia nauplii* was used as live feed for the horseshoe crab larvae. In this study, about 2.5 to 3.5 g of *Artemia* eggs were hatch everyday. Seawater with 18-20 ppt was used as a media for the culture. *Artemia* cysts were incubated for 24 h and the resulted nauplii were fed to horseshoe crabs larvae. Larvae was feed to station twice a day.

Water testing kit: There were five main water parameters measured in this study. The kits or apparatus used were; the refractometer (salinity), pH meter (pH), DO meter (dissolved oxygen and temperature) and ammonia kit (ammonia-nitrogen). Measurements were taken twice a week.

Size and weight increment: The larval growth was accomplished by molting their exoskeleton. Size and weight were measured after each molt. This measurement was carried out using a digital caliper (prosomal width measurement) and an analytical balance (body weight measurement). Length and weight measurements were carried once a month throughout out the culture period. Larvae were monitored daily to observe mortality and molting.

Molting: In order to grow, the larvae of *T. gigas* molt many times during their lifetime. The molting process depends on the relative growth. After molting, the size of *T. gigas* larvae becomes larger than before. Prosomal width and weight on horseshoe crab larvae were measured after each molting.

Statistical analysis: Data for prosomal width and weight increments by using t-test to compare the two rearing methods used.

RESULTS AND DISCUSSION

In this study, only prosomal width and weight were measured since the width considered as being the most reliable indication of the size increment and yields the smallest error in measurement (Sekiguchi *et al*., 1988). In this study, it was found the *T. gigas* larvae molt 3 times during the 6 month-old culture period. Three stages of body size increments for *T. gigas* larvae were observed for both RAS and conventional method. The result were as shown in Table 1. The weight and prosomal width of *T. gigas* larvae for both systems were

Table 1: Means data collected and result statistical analysis t-test for weight (g) and prosomal width (mm)

	Age (month)							
	System	6	7	8	9	10	11	T-test
Weight (g)	RAS	0.16±0.31	0.23±0.13	0.39±0.01	0.57±0.09	0.91±0.10	0.92±0.04	S
	C	0.61±0.01	0.28±0.17	0.40±0.01	0.52±0.12	0.57±0.33	0.61±0.19	
Prosomal width (mm)	RAS	15.86±0.06	17.46±3.07	22.83±3.45	23.42±0.87	28.08±0.59	27.99±0.83	S
	C	15.96±0.31	18.57±3.56	22.46±2.88	22.60±1.37	23.12±4.06	23.50±2.71	

Mean±SD of body sizes (mm) of *T. gigas* larvae reared in RAS and conventional method. RAS: Recirculating aquaculture system, C: Conventional method. S: Significant different ($p<0.05$)

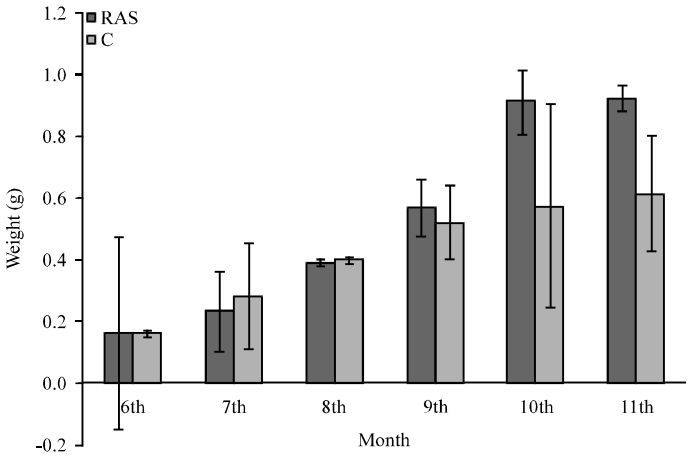

Fig. 1: Effect of RAS on total weight increments compared to conventional method

significantly different ($p<0.05$). This indicated that the different rearing methods namely the RAS and conventional method influence the increments in the prosomal width and weight of the larvae.

Figure 1 showed the final prosomal width achieved for *T. gigas* larvae cultured in RAS and conventional method was 27.99 and 23.50 mm, respectively. The final weight increment achieved for *T. gigas* larvae cultured in RAS was 0.92 and 0.61 g for conventional method (Fig. 2). The first molt occurred when larvae reached 187 days old, second at 250 days old and third at 278 days old for both RAS and conventional method. Therefore, RAS resulted in significantly better width and weight increments as compared to the conventional method. However, there was no different in molting frequency of *T. gigas* larvae cultured using these two methods using.

Estimation of molting frequency of *T. gigas*: Similar to other arthropods, horseshoe crabs must molt in order to grow. When an individual is ready to molt, a new soft and folded exoskeleton begins to form beneath the old one. The old carapace splits along the forward edge, allowing the animal crawl out. After molting, the size of *T. gigas* larvae is larger than before. In this study, it was found that the *T. gigas* larvae molted 3 times during the 6 month's cultured period. T-test results in Table 2 showed that the molting frequency for different molting stages for *T. gigas* larvae cultured for 6 month using RAS and conventional method was not significantly different ($p>0.05$). The animal will be stress in higher or lower solenoids so it will delays the deposition of calcium on the exoskeleton (Soundarapandian and Raja, 2008). Both RAS

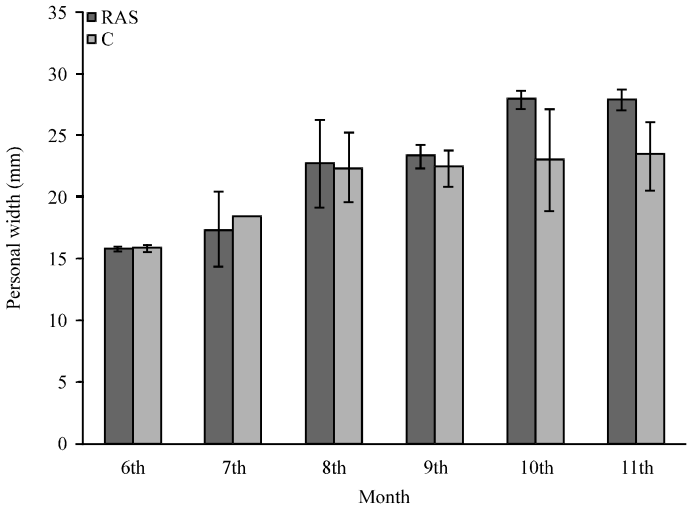

Fig. 2: Effect of RAS on prosomal width increments compared to conventional met

Table 2: T-test for the molting frequency at different molting stages for *T. gigas* larvae

Molting stages	Rearing system	Mean±SD	t-test
Stage 1	RAS1,RAS2,RAS3	48.00±1.73	NS
	C1,C2,C3	45.33±1.53	
Stage 2	RAS1,RAS2,RAS3	29.33±6.03	NS
	C1,C2,C3	15.33±1.16	
Stage 3	RAS1,RAS2,RAS3	6.00±1.00	NS
	C1,C2,C3	5.67±2.31	

Mean±SD (standard deviations) of molting frequency of *T. gigas* larvae reared in RAS and conventional method. RAS: Recirculating aquaculture system, C: Conventional method. NS: No. of significant different ($p>0.05$)

Table 3: Range of molting frequency of different molting stages for *T.gigas* larvae

	Range of molting frequency	
Molting stages	RAS	C
1st	92-98%	88-94%
2nd	46-70%	28-32%
3rd	10-14%	6-14%

RAS: Recirculating aquaculture system, C: Conventional method

and conventional culture method have no effect on the molting frequency at different molting stages for *T. gigas* reared from 6 to 11 moth-old. The range of molting frequency of different molting stages for RAS and conventional method is shown in Table 3.

Generally, the molting frequency of horseshoe crab larvae was observed to decrease against their age. The decreased in the molting frequency is basically due to the longer time needed for the larvae to grow until the exoskeleton could not longer contain them and then the molting occurs. The bigger larvae grow, the longer is the time taken for it to molt. During the molting process, some *T. gigas* larvae were unable to move out from their older shell, thus resulted in the mortalities of the larvae. Great amount of energy during the molting process may have caused this mortality. Overfeeding can be detrimental to the larvae. *Artemia* given as food will compete with the larvae

for oxygen especially in the conventional method. Poor water quality is also a factor to be considered contributing to detrimental effect on the larvae. Particularly, the DO and ammonia in the culture water quite different between the two systems.

Advantage of using RAS as compared to conventional method: This study showed the advantages and disadvantages of using RAS and conventional method for the culture of *T. gigas* larvae. In RAS, optimal water quality is being maintained and this provides a suitable condition for the culture of the horseshoe crab larvae. Although the initial cost of setting the system is higher but in the long term, it is more advantages and cost saving as compared to the conventional method, whereby the frequent water change will incur high cost. For the optimum growth and increasing biofilter efficiency and temperature are important in RAS (Rahman *et al*., 2012). During cultured period, it was observed that the *T. gigas* larvae cultured using the conventional method gets infected with fungus as compared to RAS (Fig. 3, 4). Ultraviolet installed in RAS sterilized and eliminated pathogens ultheaviolet, therefore reducing reduced disease problem for the *T. gigas* larvae.

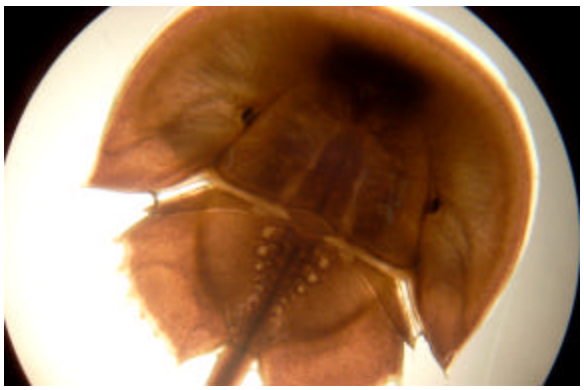

Fig. 3: *T. gigas* larvae reared in Recirculating Aquaculture System (RAS) appeared free from infection

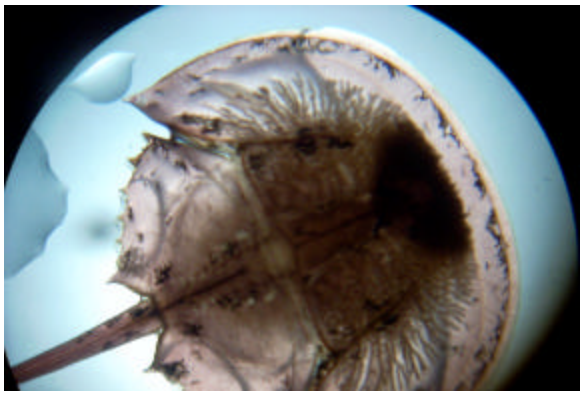

Fig. 4: *T. gigas* larvae reared in the conventional method showing fungal infection on shell

CONCLUSION

Based on the finding of this study, it can be concluded that culture method using RAS produced significantly better growth of *T. gigas* larvae as compared to the conventional method *T. gigas* larvae were generally easier to maintain under RAS in the laboratory as compared to the conventional method. RAS provides a solution for water quality problems such as dissolved oxygen DO and ammonia. Overall, RAS has more advantages, especially in reducing water wastage, human recourses and space requirements.

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