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EFFECT OF DIFFERENTIAL FEED ON THE MOLTING SUCCESS AND SURVIVAL OF HORSESHOE CRAB TRILOBITE (*Tachypleus gigas*)

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ABSTRACT

Mortality during early life stages of horseshoe crabs especially during the molting of third and fourth instar stage is significantly higher. This is perhaps due to the change in feeding behavior from filter feeding (first and second instar) to active feeding (third instar onwards). Hence, we offered differential microalgae feed (*Isochrysis*, Spirulina and *Isochrysis* + Spirulina) to induce molting and improve survival at third instar stages of Malaysian horseshoe crab (*Tachypleus gigas*). Third instar *T. gigas* were fed with 3 different concentration of microalgae [*Isochrysis* (7.5×10^6 cells/L and 0.75×10^6 n cells/L)], [(Spirulina (1 ml/L and 0. ml/L)], [(*Isochrysis* + Spirulina: (3.75×10^6 cells of *Isochrysis* + 0.5 ml spirulina/ L and 0.375×10^6 cells of *Isochrysis* + 0.25 ml spirulina/L)] every 3 days for 30 days interval and morphometric measures were documented. Results revealed that combination of microalgae feed induce molting and survival rate regardless of concentration employed. Significant difference in molting were observed between control and treated groups regardless of feed concentration ($p<0.05$). There was no mortality of crabs observed in *Isochrysis* feed tanks while 25% and 20% mortality of crabs (especially appendage lost) were recorded in spirulina and iso+spirulina feed tanks respectively. Molting was observed in all treatment tanks. We suggest that *Isochrysis* with the concentration of 7.5×10^6 cells/L can be used to induce faster molting in *T. gigas* instar stage.

Key words: *Tachypleus gigas*, horseshoe crab, molting, differential feed, microalgae

INTRODUCTION

The population of horseshoe crabs in particular Malaysian horseshoe crab *Tachypleus gigas* is facing serious problems such as human exploitation of natural stock, continuous exposure to pollution and loss of breeding and nursery grounds which eventually lead to the population decline in recent decades (Chatterji, 1994; Shin *et al.*, 2009; Kamaruzzaman *et al.,* 2011; Akbar John *et al.,* 2012). On the other hand, captive breeding and rearing techniques are the key towards successful restocking of their juvenile form are the most effective ways for enhancing their population in the wild in the short-term, in addition to the control of overharvest and long-term measures of habitat conservation (Li, 2008). To achieve higher success rate of the restocking, information on various growth parameters of these juveniles needs to be studied in the laboratory (Shin *et al.,* 2009). Research on diet and food preferences of horseshoe crabs in the wild has provided basic information on their nutritional requirements. However, most of these studies were on adult animals while studies on nutritional recruitment by their juvenile stages are still limited (Hu *et al.,* 2013; Hu *et al.,* 2014).

Selecting the optimal diet for culturing the newly hatched juvenile horseshoe crabs is one of the primary objectives for laboratory investigations. Due to high mortality rate of *T. gigas* during molting from third instar to fourth instar can be significantly reduced by offering better feed type as this is the transition stage for *T. gigas* (Akbar John *et al.,* 2012). During this molting period, the filter feeding behavior of instar is dramatically changing into active feeding type. Thus, offering better feed during this transition period would be crucial for better growth and survival of *T. gigas* in captive

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rearing practice. The present study was aimed to study molting success and survival of Malaysian horseshoe crab *T. gigas* by administering 2 different types of microalgae as their feed.

MATERIALS AND METHODS

A total of 20 healthy third instar stage *T. gigas* were transferred in to 3 L aquarium tanks containing 2 L of filtered sea water. Samples were acclimated in aquarium tank for 2 days without feeding. Experimental condition included salinity 32 ± 1 ppt, dissolved oxygen: 6.0–8.0 mg/L; Water temperature 26 ± 2°C; water ammonia concentration below 0.05 mg/L and photoperiod (12 D: 12 L). Feeding experiment consisted of 3 treatments (instars fed with *Isochrysis* sp., spirulina and combination of both) with 2 different concentrations (Table 1). The control group was un-fed throughout the experimental period and it includes 20 healthy third instar stage *T. gigas* in 3 L aquarium tanks in triplicate. *Isochrysis* 1800^{TM} (Size 5–7µ) Microalgae feed were procured from Reed mariculture Inc. USA while spirulina feed was from Uni Pharma FZC Malaysia Sdn Bhd. The experiment was carried out for 30 days with constant changing of filtered sea water once every 3 days. The crabs were fed with differential feeds every 3 days and the water was exchanged with new filtered sea water with salinity of 32 ± 1 ppt. Parameters such as total length, prosomal width, Inter-ocular width, opisthosomal and telson length were measured once every 3 days. All experimental feeding setup were run in replicate. Unlike previous study on *T. tridentatus* feeding in captive (Hu *et al.,* 2013), no sand bed was prepared in the tanks to avoid cross contamination and ensure minimal error during experiments. The data on mouth size of each instar stage were also recorded.

DATA ANALYSIS

Survival rate (SR) was calculated using SR (%) = survival individuals number/total individuals number x 100. Pearson correlation matrix analysis was used to check the interrelationship of different parameters. Paired sample *t* test was used to compare the molting between control and treated group. All data were expressed in Mean ± SD. Statistical analysis was performed using SPSS V21.

RESULTS AND DISCUSSION

In the present study, third instar *T. gigas* were fed with 2 different feeds (individually and combined) in 2 different concentrations to observe the molting success and survival rate. Molting occurred in control and treated tanks and significant molting difference was observed in experimental tanks compared to control. No significant difference was also observed in molting success of crabs fed with different concentration of feeds. However, laboratory observations showed that fast molting and active movement of juvenile crabs occurred in tank 1 (*Isochrysis* fed tank) and in tank 2 iso+spirulina (Refer Table 1 for concentration level of microalgae). This behavior in turn proves the selective feeding behavior of juvenile horseshoe crabs. Similar observation was made in juvenile *T. tridentatus* collected from wild where they predominantly feed on insect larvae (*Chironomus* sp.) (Zhou & Morton, 2004). Similarly, feeding behavior of mangrove horseshoe crab *C. rotundicauda* showed selective feeding behavior over bivalve species compared to other benthic invertebrates (Akbar John *et al.,* 2012). Interestingly, Hu *et al* (2013) has predicted frozen food could have an influence on feeding preference of juvenile horseshoe crabs in the laboratory culture where by he observed *Artemia salina* and *Mysis relicta* are more preferred by juvenile *T. tridentatus* compared to *Chironomus plumosus*. Hence, we conclude that juvenile of *T. gigas* has selective feeding behavior in captive condition even though differential feeds were supplied as dietary source.

Significant difference in molting were observed between control and treated groups regardless of concentration of feed fed with ($p<0.05$). No significant difference in the number of molting was

Table 1. Experimental feed concentration used to induce molting in *Tachypleus gigas* instar

Feed	Concentrations 1 (Tank 1)	Concentrations 2 (Tank 2)
Isochrysis	7.5×10^6 cells/ L	0.75×10^6 cells/ L
Spirulina	1 mL of powdered spirulina/L	0.1 mL of powdered spirulina/L
Isochrysis + spirulina	(3.75×10^6 cells of *Isochrysis* + 0.5 mL of powdered spirulina)/L	(0.375×10^6 cells of *Isochrysis* + 0.25 ml of powdered spirulina)/L
Control group	unfed	unfed

observed between the different concentrations employed. There was no mortality of crabs observed in *Isochrysis* fed tanks while 20% and 25% mortality of *T. gigas* (appendage lost) were recorded in spirulina and iso+spirulina treated tanks regardless of concentration of the feed. In terms of molting, significant variation were observed between *Isochrysis* fed tanks and spirulina fed tanks ($P<0.05$) while the molting pattern was the same between *Isochrysis* feed and combination of *Isochrysis* and spirlina treatment (Table 2). Significant increase in the molting was observed from fertilized egg stage to fourth instar ($P<0.001$) in control animals (Table 3). Pearson correlation matrix analysis

Table 2. Morphometric parameters of *Tachypleus gigas* instar fed with differential feed

Parameters	Fed with *Isochrysis*		
	Tank 1	Tank 2	Control
TL	15498.45 ± 2987	14191.84 ± 3209	12711.55 ± 3198
CW	12149.28 ± 2874	10212.21 ± 2911	9439.74 ± 1498
IOW	9149.21 ± 3813	5713.27 ± 2615	5949.67 ± 2581
OPL	6824.84 ± 1387	5728.77 ± 1894	4982.11 ± 1590
TailL	3871.43 ± 1811	3321.94 ± 1986	2691.18 ± 1102
	Fed with Spirulina		
	Tank 1	Tank 2	Control
TL	13280.58 ± 3768	12924.34 ± 4986	12711.55 ± 3198
CW	10982.47 ± 2711	92152.51 ± 4178	9439.74 ± 1498
IOW	6218.89 ± 1896	5891.66 ± 2813	5949.67 ± 2581
OPL	4718.25 ± 1781	4669.27 ± 1231	4982.11 ± 1590
Tail L	3024.39 ± 984	3114.39 ± 1642	2691.18 ± 1102
	Fed with *Isochrysis* + Spirulina		
	Tank 1	Tank 2	Control
TL	15291.28 ± 3561	13812.88 ± 3112	12711.55 ± 3198
CW	11482.31 ± 2890	11842.57 ± 2879	9439.74 ± 1498
IOW	7214.64 ± 2711	6894.19 ± 1219	5949.67 ± 2581
OPL	6412.56 ± 2122	5812.15 ± 1257	4982.11 ± 1590
TailL	2994.19 ± 987	3015.13 ± 1128	2691.18 ± 1102

Note: Tank 1 and Tank 2: differential concentration of feed (Refer Table 1); Data represented in Mean ± SD (µm). TL, IOW, CW, OPL and TailL represent Total length, Inter ocular width, Carapase width, opisthosomal length, Tail length respectively. All measurements are in micro meter.

Table 3. Molting rate and morphometric measures of control group animals during experimental period. Data based on the Mean±SD (µm)of 40 individual horseshoe crab developing stages (captive spawned)

Developing stages	Mean size of egg	Prosomal width	Optisthosomal length	Inter Ocular width	Total length	Tail length
Fertilized eggs	3459.32±587					
Developed eggs with perivitelline fluid	4687.18±1218					
First Instar (trilobite)		5966.4±535	2747.1±290	4016.89±261	6567.45±214	(Mouth size) 493.59±147 As there is no tail
Second Instar		90125.43±2116	3547.38±593	5295.78±1698	10587.47±2695	1605.28±265
Third Instar		9179.11±3132	4011.1±798	5581.67±2451	11298.19±3112	2019.14±472
Fourth Instar		9439.91±1498	4982.19±1590	5949.55±2581	12711.53±3198	2691.44±1102

Table 4. Pearson correlation matrix analysis between Morphometric parameters in *Tachypleus gigas*

Parameters	TL	CW	IOW	OPL	Tail L
TL	1				
CW	**	1			
IOW	**	*	1		
OPL	**	**	NS	1	
TailL	**	**	**	NS	1

Note: TL, IOW, CW, OPL and TailL represent Total length, Inter ocular width, Carapace width, opisthosomal length, Tail length respectively. ** and * represent significant difference at 99 and 95% confident interval.

showed significant positive correlation between all the morphometric parameters under study ($P<0.001$) (Table 4).

Many researchers have observed feeding preference is not the primary factor to determine the type of prey ingested (Shaw *et al.,* 2003; Nunn *et al.,* 2007); while, the availability of prey, mobility and distribution and a predator's catching efficiency should also be considered as important factors for food selection (Moore & Moore, 1976). For example, insect larvae were preferred by juvenile *Limulus* in nursery beaches (Botton *et al.,* 1992). However, this study has successfully reduced mortality rate of juvenile crabs molted from third to fourth instar stage by 0–25% , while the natural mortality of crabs from third to fourth instar stages was observed to be 27% in *Limulus polyphemus* (Carmichael *et al.,* 2003). This could probably be attributed to the good nutritional quality, as well as enough quantity of feed availability under culture condition. Though, *T. gigas* from Malaysia is comparatively smaller than *T. tridentatus* from Japan, their nutritional requirement in relation to their body size and developing stages remain same (Chatterji, 1994; Akbar John *et al*., 2012). Thus growth stagnancy during juvenile development can be overcome by offering differential microalgae feed as proposed in this study. Nevertheless, the effect of abiotic factors and nursery ground conditions might also have notable effects on the growth and survival of juvenile horseshoe crabs.

CONCLUSION

It can be conceded that *Isochrysis* with the concentration of 7.5×10^6 cells/L can be used to induce faster growth and mounting in *T. gigas* instar stages.

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