

Food Intake, Gut Transit Time and Defecation Pattern of Asian Horseshoe Crab, *Tachypleus gigas*

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Nowadays, the numbers of wild horseshoe crab harvested for study are increasing. However, general information on *Tachypleus gigas* satiation level, gut transit time, defecation pattern and acclimation period in captivity during holding period are still scanty. The aims of this study are to identify *T. gigas* food intake, gut transit time and defecation pattern during the rearing period by introducing the crabs with gastropod (*Turritella* sp.), crustacean (*Squilla* sp.) and bivalve (*Meretrix meretrix*) in several feed ratios; 0.2%, 0.6%, 1.0%, 1.4%, 1.8% and 2.2% expressed of crabs' body weight. The defecation of *T. gigas* was monitored for every three hours after being fed to measure their gut transit time and defecation pattern. Male crabs did not significantly eat until two weeks in captivity, while the females started to eat in the early first week after being harvested from the wild. The satiation levels of the females (gastropod: $1.8\% \pm 0.04$; crustacean: $1.7\% \pm 0.08$; bivalve: $1.8\% \pm 0.06$) were significantly higher than the males' (gastropod: $1.7\% \pm 0.08$; crustacean: $1.4\% \pm 0.02$; bivalve: $1.6\% \pm 0.05$) for all feed types. Gut transit time of the males were significantly longer than the females'. Male crabs took shorter time (1 ± 0 hr) to achieve their satiation than female crabs (2 ± 0 hrs). This study provides useful information for *T. gigas* rearing activity and for designing a better rearing system.

Keywords: *Tachypleus gigas*; acclimation period; intensive feeding; fasting period

I. INTRODUCTION

Horseshoe crabs are known as living fossils because of their perpetual ancient morphological appearance that is maintained in the past 150 years (Sekiguchi and Sugita, 1980; Briggs *et al.*, 2005). Malaysian horseshoe crabs, *Tachypleus gigas* can be found in the Malaysian West coastal waters (Kassim *et al.*, 2008; Tan *et al.*, 2009; John *et al.*, 2010). The horseshoe crabs' ability to withstand various environmental conditions for the past 150 million years is related to their adaptability to alter feeding behaviour according to the surrounding conditions (John

et al., 2012). However, there is no information that states about the limitation of *T. gigas* feeding adaptability to the surrounding conditions. Smith *et al.* (2013) stated that alternative reproductive tactics (ARTs) of horseshoe crabs would also influence their feeding behaviour. Male horseshoe crabs will extremely increase their gut transit time in order to increase food assimilation during the amplexus position. However, their study is limited to *Limulus polyphemus*. Studies on the amebocyte lysate content in *T. gigas* blood (Naqvi *et al.*, 2004; Gerhart, 2007) have increase the usage of *T. gigas* in biotechnological study and subsequently increase the numbers of wild *T. gigas* harvested for research purposes. The harvested horseshoe

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crabs will be priory retained in captivity within two weeks (Botton, 1984b; Hurton and Berkson, 2005; Chabot *et al.*, 2007; Coates *et al.*, 2012; John, 2012; Smith *et al.*, 2013) to synchronise their endogenous with the captivity environment (Chabot *et al.*, 2008; Watson and Chabot, 2010) before the subsequent analysis. Nevertheless, there is no justification on the optimum acclimation period of the *T. gigas*.

Moreover, there is no reliable information on feed consumption of *T. gigas* along the acclimation period. Besides that, their level of satiation, feeding period and defecation pattern are still unknown. Most of the previous reports are derived from the Atlantic species, *L. polyphemus* (Smith *et al.*, 2013). This information is important in improving relevant rearing method during the holding phase. Inappropriate feeding practice, i.e. inadequate feed quantity and over-feeding during holding phase might subsequently lead to nutrition deficiency and water deterioration problems that would affect the health of the crabs and their performance. Lower water quality is less hospitable to horseshoe crabs (Carmichael *et al.*, 2004) and might affect *T. gigas* health during the retention period. Thus, identification of horseshoe crabs' defecation pattern is needed in order to evaluate the efficiency of the applied aquaculture system in solving water deterioration problems that are mostly influenced by animal excretion. The aims of this study are to provide the information of the satiation, feeding period and defecation pattern of *T. gigas* during the rearing period by feeding the crabs with several benthic organisms namely; gastropod (*Turritella* sp.), crustacean (*Squilla* sp.) and bivalve (*Meretrix meretrix*) in captivity.

II. MATERIALS AND METHODS

A. Samples collection

30 pairs of adult *T. gigas*, male and female, were collected from their digging pit after the highest tide. The collected samples were brought to the Institute of Oceanography and Maritime Studies (INOCM) Research Station hatchery, cleaned of epibionts by the sea water, weighted and sexed. The conditions of each sample were inspected regarding their carapace colour, presence of pitting on the carapace and the presence of the body appendages. Only crabs with complete appendages can meet the prior requirement of this study. The male and female crabs were reared separately and divided evenly into five tanks respectively. Then, the crabs were tagged with numbering bands at the base of their telson. Water parameters were monitored regularly.

B. Feed consumption during acclimation period

The crabs were introduced with blood cockle (*Anadara granosa*) during acclimation feeding session. The crabs' feeding activities were then monitored within three hours (Smith *et al.*, 2013) in order to identify their acceptance towards the introduced feed. The quantity of the introduced feeds in each tank was maintained five pieces within three hours to avoid food competition between crabs. The crabs' responds towards the introduced feed were also recorded. The leftover feeds were removed after three hours. The three-hour feeding period was chosen according to Barlow *et al.* (1986), Chabot *et al.* (2007) and Smith *et al.* (2013)

studies. The crabs were left for 24 hours before conducting the next feeding session. Monitoring activity had been conducted every 24 hours until all of the crabs in the captivity started to consume the introduced feeds within three hours feeding session period. *T. gigas* significance response toward feed was identified using Analysis of Variance (ANOVA), Duncan test. Animals' acceptance toward food could be considered as the acclimation sign (Bayne *et al.*, 1993; Ibarrola *et al.*, 2000; Navarro *et al.*, 2009).

C. Satiation level test

Satiation experiment was conducted after all of the crabs in the captivity had started to consume the introduced feeds (acclimated) within three hours. Quantities of the crabs per tank were equalled with the previous acclimation experiment. The *T. gigas* were left for 24 hours without feed before beginning the test. The crabs were introduced to different food items: gastropod (*Turritella* sp.), crustacean (*Squilla* sp.), and bivalve (*Meretrix meretrix*) respectively. Each feed items had been cut to 1.03 ± 0.04 gm, and then were weighted to several percentages; 0.2%, 0.6%, 1.0%, 1.4%, 1.8%, and 2.2% that expressed the horseshoe crabs body weight. These percentages were chosen based on the observations from the previous experiment (acclimation period). Initially, the *T. gigas* in each tank were introduced with 0.2% of gastropod (*Turritella* sp.). Observation on their feeding activity was made within three hours. Quantities of the consumed and leftover feeds in the tank were recorded. Then, the percentages of feeds were increased to 0.6%, 1.0%, 1.4%, 1.8%, and 2.2% every 24 hours interval time. The

horseshoe crabs were fasted for 24 hours before the next feeding session so as to get the exact ratio of daily feed consumption. The same procedure was then repeated by introducing the *T. gigas* with crustacean (*Squilla* sp.) and then bivalve (*Meretrix meretrix*). ANOVA Tukey post-hoc test was used to determine the significance increases in daily feed intake quantity and satiation of male and female on the same feed item. Satiation level of different feed types between male and female were compared by using Two-way ANOVA.

D. Gut transit time and feed consumption period

Gut transit time test was conducted and compared between the male and female *T. gigas*. This test was conducted separately with the satiation level test because some feeds took more than 24 hours to be excreted. Male and female crabs were fed with gastropod (*Turritella* sp.), crustacean (*Squilla* sp.) and bivalve (*Meretrix meretrix*) respectively. Each feeding types had been cut to 1.03 ± 0.04 gm and then were soaked in carmoisine solution for 30 minutes (inert digestion marker). The weight of each introduced feed items were prepared according to the result of the previous satiation level experiment (1.8% of horseshoe crabs' body mass). The horseshoe crabs' feed consumptions were recorded every hour until the horseshoe crabs had stopped their feeding activity for two hours. The horseshoe crabs feeding period were analysed by using ANOVA, Tukey post-hoc test to determine the significant of feed intake per hour. Then, the horseshoe crabs' defecation was monitored every three hours until reddish faeces had appeared.

Three hours interval period was chosen based on regular observation on the crabs' defecation during previous feeding experiment (Smith *et al.*, 2013). Analysis of the gut transit time of different feed types between the males and females were analysed by using Two-way ANOVA.

E. Defecation analysis

Faeces produced by the male and female *T. gigas* during gut transit time experiment were collected in every three hours. The faeces were siphoned through a fine mesh filter (200 μ m) and were filtered using filter paper. Samples were dried within four hours at 60°C in an oven and weighted (total weight – filter paper weight in g) (Smith *et al.*, 2013). Data were then analysed to determine the defecation pattern of *T. gigas* in terms of defecation frequency and amount over times. The results were presented by accumulating the data for every 12 hours. Data were analysed by using ANOVA, Tukey post-hoc test to determine the significant of intensive defecation period. Relation between defecation frequency and amount were analysed by using the Pearson Correlation (Sig.2-tailed) analysis.

III. RESULTS

A. *T. gigas* acceptance toward feeds during acclimation phase

Male *T. gigas* did not significantly eat until second week in captivity ($p > 0.05$) after being harvested from the wild (Figure 1). Interestingly, 51.43% $\pm$ 25.54 of female *T. gigas* started to eat during first week in captivity ($p < 0.05$). All the females started to eat significantly dur-

ing every daily feeding session during the third week ($p < 0.05$). However, the males were only significantly started to eat (48.57% $\pm$ 15.74) during third week in the captivity ($p < 0.05$). During fifth week, all males in captivity started to eat actively during every daily feeding session ($p < 0.05$). Females *T. gigas*' acclimation toward feed was faster than males.

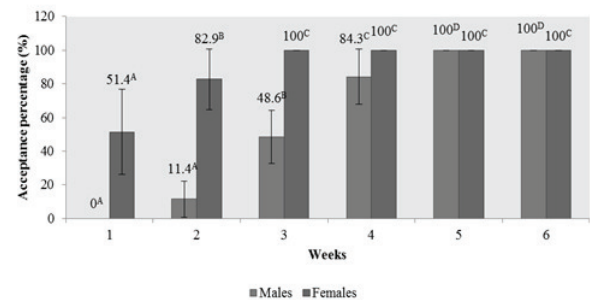


Figure 1. Horseshoe crabs acceptance toward introduced feeds during the acclimation phase. Different capital letters within same sex indicate the significant differences ($p < 0.05$)

B. Satiation level and gut transit time

Data showed that the male and female *T. gigas* started to consume the introduced feed during the first hour of feeding session ($p < 0.05$). They significantly achieved their satiation within three hours. There were no significant feeding activities for male and female could be observed after three hours ($p > 0.05$) (Table 1). Nevertheless, female *T. gigas* significantly took longer period (2 ± 0 hrs) to achieve satiation compared to the males (1 ± 0 hr) ($p < 0.05$). The satiation levels of the females (gastropod: 1.8% $\pm$ 0.04; crustacean: 1.7% $\pm$ 0.08; bivalve: 1.8% $\pm$ 0.06) were higher than the males (gastropod: 1.7% $\pm$ 0.08; crustacean: 1.4% $\pm$ 0.02; bivalve: 1.6% $\pm$

0.05)($F = 13.98$, $p < 0.05$)(Table 2 and Figure 2).

Interestingly, analysis showed that gut transit times of males (gastropod: 23 ± 4.6 hrs; crustacean: 17 ± 1.7 hrs; bivalve: 17 ± 1.7 hrs) were significantly longer than females (gastropod: 19 ± 1.7 hrs; crustacean: 10 ± 1.7 hrs; bivalve: 12 ± 0 hrs)($F = 3.72$, $p < 0.05$)(Table 2 and figure 3).

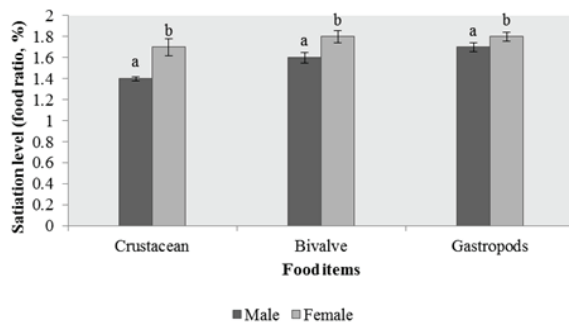


Figure 2. Male and female *T. gigas* satiation level. Different small letters between male and female in the same food item indicate the significant differences ($p < 0.05$)

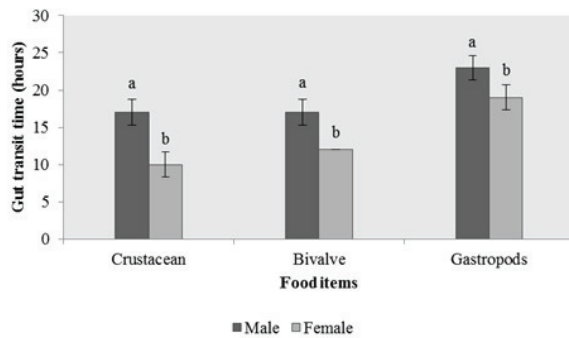


Figure 3. Male and female crabs' gut transit time. Different small letters between male and female in the same food item indicate the significant differences ($p < 0.05$)

C. Defecation pattern

Results showed that male *T. gigas* started to defecate during the second 12 hours, while female *T. gigas* started to defecate during the first 12 hours ($p < 0.05$). Males and females defecation amount were intense during the second 12 hours ($p < 0.05$). The most dramatic decrease in males' and females' defecation occurred after 36 hours after being fed. Defecation activity significantly stopped after the fifth 12 hours ($p < 0.05$) (Table 3 and Figure 4). The data shows a very strong significant correlation between defecation amount and frequency for males (Pearson Correlation = 0.969) and strong correlation for females (Pearson Correlation = 0.769). Defecation amount base on frequency of male and female *T. gigas* are correlated positively. Figure 4 shows that horseshoe crabs defecated intensively during the second 12 hours.

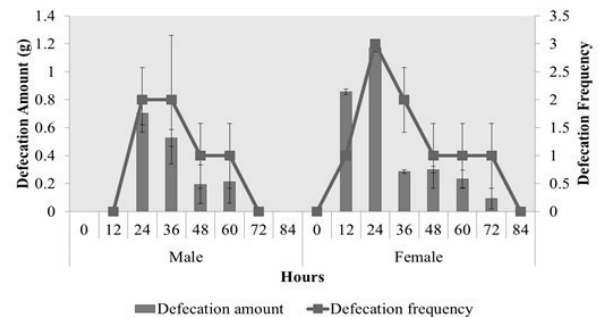


Figure 4. Defecation amount and frequency of horseshoe crabs

IV. DISCUSSIONS

During the amplexus position, male horseshoe crabs generally would hold the modified spines located at the edge of the females' opisthosoma (Botton *et al.*, 1996; Brockmann *et*

Table 1. Percentage of feed consumption per hour

Feeding period (hrs)	Males			Females		
	Gastropods	Crustacean	Bivalve	Gastropods	Crustacean	Bivalve
0	0 ^A	0 ^A	0 ^A	0 ^A	0 ^A	0 ^A
1	92.00 ± 8.54 ^B	100 ^B	100 ^B	96 ± 1.73 ^B	73.74 ± 17.58 ^B	72.51 ± 18.15 ^B
2	95.67 ± 5.13 ^B	100 ^B	100 ^B	99 ± 1.73 ^C	88.91 ± 12.74 ^{BC}	86.65 ± 13.38 ^{BC}
3	95.67 ± 5.13 ^B	100 ^B	100 ^B	100 ^C	100 ^C	100 ^C
4	100 ^B	100 ^B	100 ^B	100 ^C	100 ^C	100 ^C

Different capital letters in the same column indicate the significant differences (p<0.05)

Table 2. Male and female *T. gigas* satiation level and gut transit time

Feed types	Satiation level (food ratio, %)		Gut transit time (hours)	
	Male	Female	Male	Female
Crustacean	1.4 ± 0.02	1.7 ± 0.08	17 ± 1.7	10 ± 1.7
Bivalve	1.6 ± 0.05	1.8 ± 0.06	17 ± 1.7	12 ± 0
Gastropods	1.7 ± 0.04	1.8 ± 0.04	23 ± 1.6	19 ± 1.7

Different small letters in the same row under the same experiment indicate the significant differences (p<0.05)

al., 2000; Smith *et al.*, 2013) and would remain in this position until the end of spawning season (Brockmann, 2003). Study on the horseshoe crabs' feeding behaviour found that male crabs feeding movement are restricted during this position then intrinsically constrained their foraging activity. This would lead the horseshoe crabs to feed intensively before the spawning migration (Botton, 1984b; Kamaruzzaman *et al.*, 2011). Besides that, male crabs have adapted by slowing down their digestion in order to increase food assimilation. Suzuki *et al.* (1975) defined this behaviour as the adaptation of male crabs to deal with the restricted feeding ability. These adaptations might influence *T. gigas* gut fullness and subsequently influence their satiation and preference towards food. These behaviours delayed male *T. gigas* feeding activity until the second week in captivity after being harvested from the wild. Different behaviours have been observed on females, where they started to eat during the

first week in captivity. Furthermore, acclimation result of the present study shows that two weeks acclimation period that applied by Botton (1984b), Hurton *et al.* (2005), Chabot *et al.* (2007), Coates *et al.* (2012), John (2012) and Smith *et al.* (2013) studies were not suitable to be applied on *T. gigas*, since not all horseshoe crabs start to respond with the introduced feed during the second week in captivity. The *T. gigas* should be acclimated until all crabs start to respond towards introduced feeds. Bayne *et al.* (1993), Ibarrola *et al.* (2000) and Navarro *et al.* (2009) stated that animals' acceptance toward food could be considered as the acclimation sign to the new environment.

Analysis shows an interesting outcome where the ratios of food intake of female *T. gigas* (expressed of their body weight) were higher compared to males. The food intakes of female *T. gigas* (1.8% of body weight) in present study were close to Coates *et al.* (2012) study on the food

Table 3. Defecation pattern of male and female *T. gigas*

Period (hr)	Male		Female	
	Defecation frequency	Defecation amount (g)	Defecation frequency	Defecation amount (g)
0	0 ^a	0 ^a	0 ^a	0 ^a
12	0 ^a	0 ^a	1.00 ± 0 ^b	0.86 ± 0.02 ^b
24	2.00 ± 0.58 ^{bc}	0.71 ± 0.08 ^b	2.00 ± 0.58 ^d	1.17 ± 0.03 ^c
36	2.00 ± 1.15 ^c	0.53 ± 0.06 ^c	3.00 ± 0 ^c	0.29 ± 0.01 ^d
48	1.00 ± 0.58 ^{ab}	0.20 ± 0.14 ^d	1.00 ± 0.58 ^b	0.30 ± 0.02 ^d
60	1.00 ± 0.58 ^{ab}	0.21 ± 0.15 ^d	1.00 ± 0.58 ^b	0.23 ± 0.06 ^d
72	0 ^a	0 ^a	1.00 ± 0.58 ^b	0.09 ± 0.07 ^a
84	0 ^a	0 ^a	0 ^a	0 ^a

Different small letters in the same row indicate the significant differences (p<0.05)

intake of *L. polyphemus* (2% of body weight). John et al. (2012) stated that female *T. gigas* would spend more energy to bring the attached male through the gravid shore condition during spawning migration. This behaviour would lead the female *T. gigas* to increase their food intake. Statistically, the daily satiations of male *T. gigas* were lower compared to females. Different in satiation might be influenced by the gut transit time where, male *T. gigas* took 24 hours to significantly start the defecation, while the females only took 12 hours to significantly start the defecation. This factor might cause feed accumulation in the male *T. gigas* gut and decrease their satiation at every 24 hours feeding session. Feeding period of the *T. gigas* in this study is similar with several previous study that conducted by Barlow et al. (1986), Chabot et al. (2007) and Smith et al. (2013). They took less than three hours to achieve their satiation. However, male *T. gigas* took shorter feeding period to achieve the satiation compared to females. Shorter time taken to achieve satiation also could be classified as the adaptation of male crabs' feeding behaviour during the spawning season. Male crabs need to fill their gut with food promptly during incoming high tides, since they are engaged

solely in finding and competing for the mating partner along the spawning season (Brockmann, 1990) and have restricted feeding ability by remained passively in amplexus position (Botton et al., 1996). This can also be supported by Smith et al. (2013) study that the higher value of isotope $\delta^{15}\text{N}$ in the faeces of the amplexed males compared to the satellite males. Dealing with the restricted period during the incoming high tides has led the male crabs to divide the foraging, seeking partner and mating according to the priority. Thus, male *T. gigas* need to harness the limited time efficiently by shorting the time taken to achieve the satiation and use the left spawning period to mate. Generally, male *T. gigas* were having smaller gut size than females. Hence, male crabs would take shorter feeding period to achieve their satiation (Barboza et al., 2009).

Present study found that *T. gigas* defecation could be divided into two phases; intensive and extensive defecations. Intensive phase occurred at the early defecation period with higher defecation rate, while the extensive phase was during the lower faeces excretion volume before the defecation process had completely stopped. Obviously during intensive defecation phase, male

and female *T. gigas* respectively defecated more frequent and the volumes of the excreted faeces were higher. Our observation found that the peak defecation of male crabs started during the second 12 hours after being fed and slightly decreased during the third 12 hours. However, females started their defecation during the first 12 hours after being fed and decreased dramatically during the third 12 hours. Male crabs would slow down their digestion activities to increase food assimilation (Suzuki *et al.*, 1975). Although the times of initial defecation were different between sexes, there were similarities in the defecation pattern in which, their excretion activities were higher during the first and second 12 hours. Regular monitoring on water parameter during this phase was also vital to avoid the deterioration of the water quality in this study.

V. CONCLUSION

In conclusion, male crabs would not significantly eat during the first week after being caught from the wild. Aquaculturists are suggested to not feed the male *T. gigas* during the first week after been caught from the wild. Meanwhile, female *T. gigas* need to be fed during first week, since they started to significantly eat during first week in captivity. Three hours are the optimum periods for *T. gigas* feeding session and the excess feed should be taken out after

three hours. The defecation patterns between male and female crabs were similar where both sexes defecate intensively during the early 24 hours once the defecation starts. Crabs defecation must be monitored within the early 24 hours after being fed. For the applied aquaculture system, it should be efficient enough to maintain the water quality parameter namely; ammonia, nitrite, nitrate and etc. during this peak defecation phase. Further study is needed, since food types and conditions and also the environment factors intrinsically influencing horseshoe crabs feeding behaviour and defecation pattern. In the present study, all horseshoe crabs were fed with small and similarly sized feeds. Hence, the effect of food size toward defecation rate was not investigated. The data reported in the present study are limited to gastropod, crustacean and bivalve and should serve as baseline information for future studies.

VI. ACKNOWLEDGEMENT

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