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Feeding Mechanisms of Adult Tropical Horseshoe Crab, *Tachypleus gigas* toward Feeds' Conditions

Mohd Razali Md Razak[1] and Zaleha Kassim[1]*
[1]*Kulliyyah of Science, International Islamic University Malaysia,*
Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

Information on feeding behaviour of tropical horseshoe crabs, *Tachypleus gigas* (Müller, 1785) is still scanty compared to *Limulus polyphemus* (Linnaeus, 1758). Foods condition such as decayed, sheltered and etc., would substantially influence the edibility of food by the *T. gigas*. However, horseshoe crabs might have specific behaviour to manipulate the foraged food. The aim of this paper was to investigate the feeding mechanism of *T. gigas* toward different feed conditions. Experiments were conducted inside a glass tank. A total of 30 males and females respectively were introduced to five different natural potential feeds, namely, gastropods (*Turritella* sp.), crustacean (*Squilla* sp.), fish (*Lates calcarifer*), bivalve (*Meretrix meretrix*) and polychaete (*Nereis* sp.). We have manipulated the condition of introduced feed based on the several potential foods conditions in nature such as sheltered (protected with shell, hard outer skin or host tube), unsheltered and decayed before introduced the feeds to the crabs. In present study, female crabs took shorter response period towards surrounding feed compared to males. Overall, they prefer to consume freshly dead feeds and would totally reject the sheltered feeds, such as, shell, hard outer skin and tube host. Male crabs have particular feeding behaviours to manipulate the edibility of the decayed feeds. Detailed experiments on the feeding mechanism of *T. gigas* could provide more information on their foraging activity in the wild.

Keywords: *Tachypleus gigas*, feeding behaviour, feeding mechanism, selective feeder

I. INTRODUCTION

In estuarine ecology, horseshoe crabs are known as important predators that highly forage on benthic organisms (Carmichael *et al.*, 2004) and connecting the energy transfer within coastal food web (Botton *et al.*, 2003 and Bekson, 2009). Gut content analysis study found different types of animal species, sediment and macrophyte in the guts of adult horseshoe crabs (Botton *et al.*, 2003). However, study on *T. gigas* feeding mechanisms is still scanty compared with *L. polyphemus* that was intensively studied. Low commercial value from a few decades ago has led to the lack of extensive study (Adibah *et al.*, 2015) and reliable data (Tan *et al.*, 2009) on *T. gigas*. In addition, the study on *T. gigas* behaviour to the surrounding food at spawning area and during spawning migration is still unknown (Chatterji *et al.*, 1992).

Early study on horseshoe crabs feeding mechanisms was described by a number of authors that had been initially triggered by Lockwood (1870). Their studies identified the function of particular appendages regarding the feeding behaviour on the introduced feed (Shuster, 1982). However, the horseshoe crab feeding mechanisms study is restricted to *L. polyphemus*. There

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*corresponding author: drzack@iium.edu.my are several appendages descriptions in previous study that not similar compared with the Asian Horseshoe Crab, *T. gigas* morphology. *T. gigas* is suspected to engage in its own mechanisms in dealing with the surrounding feed since the feeding mechanisms is closely related with the feeding success.

Based on horseshoe crabs migration study, they approximately spend three hours in the spawning area; one hour before the highest tide and two hours after the highest tide (Barlow *et al.*, 1986; Chabot *et al.*, 2007). Carmichael *et al.* (2004) stated that horseshoe crabs may forage heavily from local sub-estuaries to allow assessment of their trophic position and diet composition before leaving into deep sea area. Hence, horseshoe crabs need to harness the limited time to find food and mating. However, food condition also could influence the edibility factor and affect the amount of available food. Thus, horseshoe crabs could have specific feeding adaptation to manipulate the condition of the forage food and meet the required nutrition within the limited time.

In addition, the reliable information on Asian Horseshoe Crabs behaviour to the surrounding food at the spawning area and during spawning migration is still scanty (Chatterji *et al.*, 1992). Experiment on *T. gigas* feeding mechanisms is needed to provide more information on their foraging activity in the wild. Experiment was conducted inside a transparent glass aquarium (Rudloe, 1979 and Botton, 1984a) to facilitate the monitoring on their feeding mechanisms. This data could be adopted in future feed suitability appraisals (Robert *et al.*, 2014) in the particular habitat. The aim of this paper is to investigate the feeding mechanism of *T. gigas* toward different feed conditions such as freshly dead, decayed and sheltered feed.

II. MATERIALS AND METHODS

A. Samples collection

A total of 30 pairs of adult *T. gigas* samples respectively were collected by hand from their spawning grounds in Pahang, Malaysia during the spawning season. Horseshoe crab samples were cleaned of epibionts using sea water then separated according to sex, measured, weighted and tagged at their telson. Males and females were kept separately in six large round shallow black tanks respectively (5 individual per tank) two weeks prior (acclimation) to the feeding test (Botton, 1984a and Hurton *et al.*, 2005). No sand was added to cover the bottom as to let them in the same condition as in the test tank. During the acclimation period, we offered the crabs with blood cockle, *Anadara granosa*. *T. gigas* acceptances to the introduced feed during acclimation period were monitored. Experiment was started after all crabs in the captivity showed respond towards blood cockle. Temperature and salinity of the treated seawater obtained from INOCEM Research Station, International Islamic University Malaysia were maintained between 26 – 28^{o}C and 29 – 31 ppt respectively. *T. gigas* was fasted 24hrs before the feeding test started (Smith *et al.*, 2013).

B. Manipulation of feeds condition

To achieve the objective of this experiment, conditions of the introduced feeds were manipulated to three conditions: sheltered (protected with shell, hard outer skin or host tube), unsheltered (removed shell, hard outer skin or host tube) and decayed. For the purpose of this study, 90 samples of gastropods (*Turritella* sp.), bivalve (*Meretrix meretrix*), crustacean (*Squilla* sp.), polychaete (*Nereis* sp.) and fish (*Lates calcarifer*) were respectively used. 30 samples from each food items were kept freshly inside the -20^{o}C refrigerator to maintain their freshness. Feeds shelter namely shell, hard outer skin and host tube were removed from the balance 60 samples of gastropods (*Turritella* sp.), bivalve (*Meretrix meretrix*), crustacean (*Squilla* sp.) and polychaete (*Nereis* sp.). A part of 30 samples from each feed items were kept in refrigerator to represent as fresh unsheltered feeds and the remained feed items including fish (*Lates calcarifer*) samples were divided respectively into 10 aquariums filled with sea water for 48 hours to represent as decayed feeds. Sea water in the aquariums was changed twice daily. The conditions of the feeds along the retention period inside the aquarium were monitored.

C. Horseshoe crabs behaviour and acceptance toward feed items analysis

We used the transparent aquarium (130×130×30 cm) in this test to facilitate the monitoring activity on horseshoe crabs feeding mechanisms (Botton, 1984a) (Figure 1). The bottom of the aquarium was attached with perspex strips to substitute the sand that relatedly assists the movement of crabs in nature. Five marine species namely gastropods (*Turritella* sp.), bivalve (*Meretrix meretrix*), crustacean (*Squilla* sp.), polychaete (*Nereis* sp.) and fish (*Lates calcarifer*) were placed in five different feeding trays. A crab had been released at the centre of the feeding test tank and the period for the crab to respond with the introduce feeds was immediately recorded. The feeding duration was recorded once the feeding activity started until the feed completely consumed by the crab. The feeding tests were then repeated for 30 times using 30 different individual male and female crabs respectively in each test. We rotated the position of the feeding trays clockwise once after each test. Seven appendages (hinge, chelicerae, pedipalps, pusher legs, gnathobases, genital operculum and telson) of the horseshoe crabs were observed to determine their feeding behaviour (Botton *et al.*, 2003) so as to estimate *T. gigas* acceptance, preference and rejection toward condition of feeds.

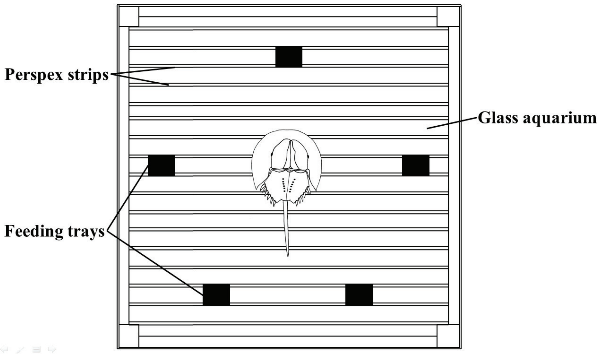

Figure 1. The arrangement of feeding trays inside the feeding test aquarium (130×130×30cm)

D. Statistical analysis

Mean comparison of two independent data for the parametric tests was performed using Independent-Samples T-Test. The analysis was carried out using SPSS package (SPSS 20.0 for windows, SPSS Inc., Chicago, IL, USA). Note that P values of less than 0.05 are considered as statistically significant.

III. RESULTS

Disparities in sizes were found to exist among the sexes, where the carapace lengths of males and females were around 16.5 ± 1.19cm and 22 ± 1.58cm and carapace width around 17.3 ± 1.07cm and 21.6 ± 1.34cm respectively. Males and females weight were around 374 ± 42.19g and 870 ± 171.76g respectively. Female crabs' carapace length, width and weight were 33.33%, 24.86% and 132.62% respectively more than males. The responses periods of male and female crabs to the introduced feeds were 13.14 ± 6.21 minutes and 3.42 ± 2.42 minutes, respectively (Table 1).

Statistical analysis shows that female crabs' response to the surrounding feed were significantly faster than males ($p<0.05$). Male crabs' response period toward feed was approximately 9.32 minutes slower than females. However, there were no significant differences between male and female crabs feeding duration ($p>0.05$); male: 51 ± 54 second, female: 54 ± 69 second. Feeding mechanisms test shows that both female and male crabs did not prefer the sheltered feed (shell, hard outer skin or host tube). Preferences of males and females toward fresh feeds were higher than the other feed conditions. Females did not prefer to consume decayed feeds. However, male crabs did not show rejection toward all food items, including the decayed polychaete and fish (Figure 2).

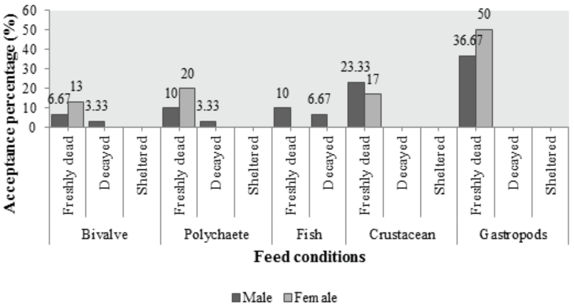

Figure 2. Percentage of *T. gigas*' acceptance toward different feed items and conditions

Feeding mechanisms of the male and female *T. gigas* toward different food items and conditions are presented in Tables 2 and 3 respectively. In specific, seven appendages were observed: hinge, chelicerae, pedipalps, pusher legs, gnathobases, genital operculum and telson to determine their feeding mechanisms (Figure 3, 4, 5 and 6). Observation shows that *T. gigas* would engage in different feeding mechanisms based on the condition of feeds. Besides that, males and females *T. gigas* have different feeding mechanisms that closely related to their preference toward feeds condition. Horseshoe crabs did not significantly move around while eating. Their feeding mechanisms closely depend on the condition of foraged feed. It is interesting to highlight that male and female crabs engaged in different feeding mechanisms. Male crabs have specific behaviours to manipulate the edibility of the decayed feeds. Differences in feeding mechanisms influence horseshoe crabs preference and rejection toward surrounding feed.

IV. DISCUSSION

Female crabs' response period toward surrounding foods was faster than the males because of the differences in energy consumption and food requirement of male and female

Table 1. Horseshoe crabs' measurements (size and weight), response time to the surrounding feeds and feeding duration

Sex	**Carapace size (cm)**		**Weight (g)**	**Response time (minutes)**	**Feeding duration (sec)**
	Length	**Width**			
Male	16.5 ± 1.19^{B}	17.3 ± 1.07^{B}	374 ± 42.19^{B}	13.14 ± 6.21^{B}	51 ± 54^{A}
Female	22 ± 1.58^{A}	21.6 ± 1.34^{A}	870 ± 171.76^{A}	3.42 ± 2.42^{A}	54 ± 69^{A}

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

Table 2. The behaviour of 30 males *T. gigas* toward different food items and conditions

Food items	Feed conditions	Appendage behaviours						
		Hinge	Chelicerae	Pedipalps	Pusher legs	Gnathobases	Genital operculum	Telson
Bivalve (*Meretrix meretrix*)	Freshly dead (opened shell)	Flexed to elevate the carapace upward to provide a feeding space at the ventral of the carapace.	Brought the small shredded flesh and held the flesh directly to the gnathobases.	Reached and brought the shell to the gnathobases. Flexed the joint between femur and patella to hold the shell under the gnathobases.	Flexed the joint between femur and patella to hold the shell under gnathobases.	Abducted and adducted to shred the flesh and push the shredded flesh into mouth.	Abducted the genital operculum to avoid the feed moves under the opisthosoma (Figure 3).	Flexed and pushed the bottom and elevate the carapace upward (Figure 5).
	Decayed						Flapped the genital operculum to remove the suspended decayed flesh from the ventral of the cephalothorax (Figure 4).	
Crustacean (*Squilla* sp.)	Freshly dead (removed hard outer skin)			Reached and brought the shell to the gnathobases.	Extended and pushed the bottom to elevate the carapace upward to provide a feeding space.		Abducted the genital operculum to avoid the feed moves under the opisthosoma.	Extended passively.
Gastropods (*Turritella* sp.)	Freshly dead (remove shell)							
Polychaete (*Nereis* sp.) & Fish (*Lates calcarifer*)	Freshly dead (removed host tube)							
	Decayed			Reached and shredded the flesh to remove the decayed part. Brought the feed directly to the gnathobases opening.			Flapped the genital operculum to remove the suspended decayed flesh from the ventral of the cephalothorax	

horseshoe crabs during spawning season. Female crabs would spend more energy during the migration inshore against the physiologically stressed gravid conditions. Besides that, prime in digging activity and locomotion during the amplexus position along the spawning seasons (John, 2012) led the female crabs to consume food more frequently in order to maintain their energy during migration and provide essential nutrition for gonad development compared to the males that commonly amplex passively on female opisthosoma along the spawning migration period. Thus, foods requirement might be higher compared to males. Male foraging ability is restricted during spawning season (Watson and Chabot, 2010). Nevertheless, Smith *et al.* (2013) found that fasting is not a nutritionally stress condition for male crabs. Male crabs might have specific feeding adaptation behaviours during the fasting period (Brockmann,

Table 3. The behaviour of females *T. gigas* toward different food items and conditions

Food items	Feed conditions	Appendages behaviours						
		Hinge	Chelicerae	Pedipalps	Pusher legs	Gnathobases	Genital operculum	Telson
Bivalve (*Meretrix meretrix*)	Freshly dead (opened shell)	Flexed to elevate the carapace upward to provide a feeding space at the ventral of the carapace.	Brought the small shredded flesh directly to the gnathobases.	Reached and brought the shell to the gnathobases. Flexed the joint between femur and patella to hold the shell under the gnathobases (Figure 6).	Flexed the joint between femur and patella to hold the shell under gnathobases (Figure 6).	Abducted and adducted to shred the flesh and push the shredded flesh into mouth.	Abducted the genital operculum to avoid the feed moves under the opisthosoma.	Flexed and pushed the bottom to elevate the carapace upward.
Crustacean (*Squilla* sp.)	Freshly dead (removed hard outer skin)			Reached and brought the feed to the gnathobases.	Extended and pushed the bottom to elevate the carapace upward.			Extended passively.
Gastropods (*Turritella* sp.)	Freshly dead (removed shell)							
Polychaete (*Nereis* sp.)	Freshly dead (removed host tube)							

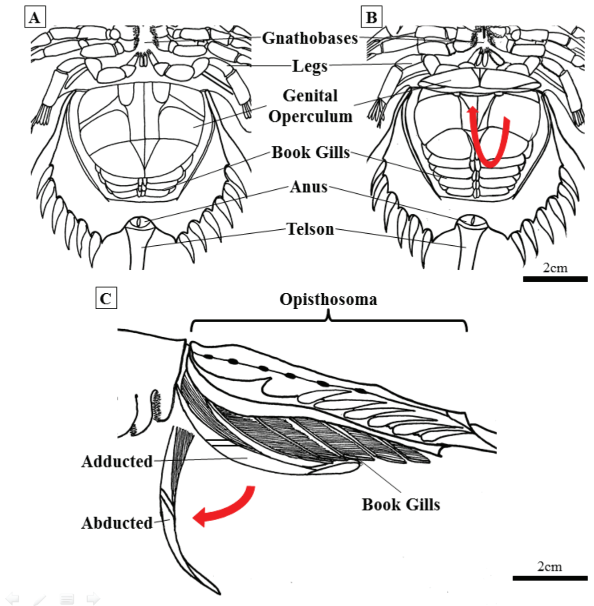

Figure 3. A: *T. gigas* adducted the genital operculum. B: *T. gigas* abducted the genital operculum downward during eating to avoid the feed moves under the opisthosoma. C: Movement and position of the abducted genital operculum (side view)

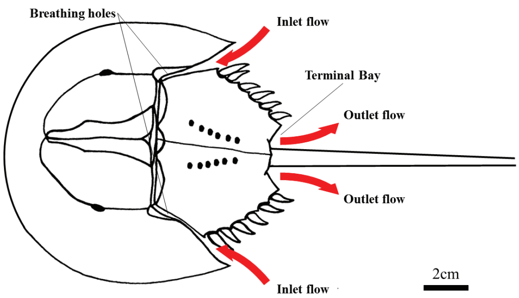

Figure 4. Water flowing direction under the opisthosoma to riddance the suspended decayed flesh

2003) by lowering their digestive enzymatic activity ultimately to increase the food assimilation (Suzuki *et al.*, 1975; Uys *et al.*, 1987 and John *et al.*, 2012).

During spawning season, the male crabs would engage solely to find the amplexus partner (Brockmann, 2003; Taborsky *et al.*, 2008; Brockmann and Smith, 2009 and Smith *et al.*, 2013) in highly competitive mating system (Brockmann, 1990). Thus, male crabs need to divide the restricted spawning period to finding spawning partner and foraging. These factors influence the horseshoe crabs satiation and response period

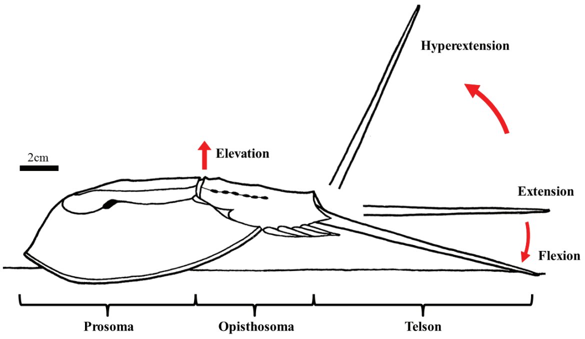

Figure 5. The movement of horseshoe crabs' telson. The telson was flexed to elevate the opisthosoma region

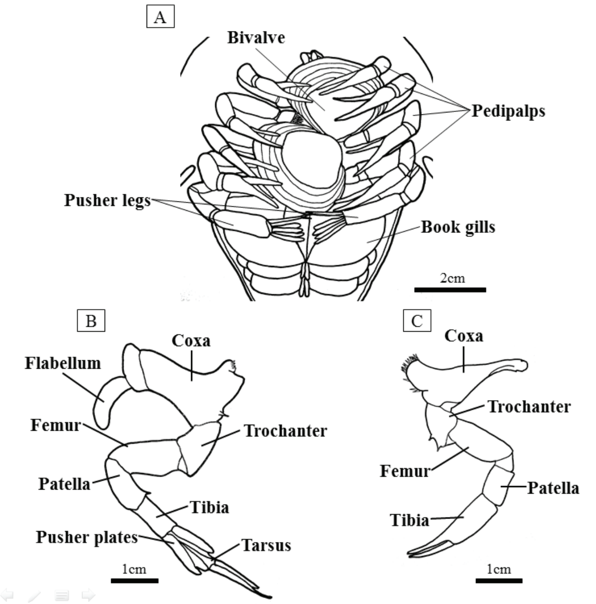

Figure 6. A: Horseshoe crabs flexed joint between femur and patella to hold bivalve shell under the gnathobases. B: Pusher leg. C: Pedipalp

toward feed in the experiment. Even though the size of the introduced feed were equalled and size of male crabs' gnathobases opening was smaller compared to females, we found that the feeding durations (sec) of male and female crabs were significantly equal. The result shows that the appendages movement of male horseshoe crabs during feeding were faster than females. In nature, their feeding durations would be closely influence by the size of the foraged feeds.

Both male and female crabs showed preference toward freshly dead feeds and would never consume the sheltered feeds. Interestingly, *T. gigas* has no ability to consume the sheltered food such as closed shells, hard outer skin and tube host. Despite the name, Asian horseshoe crabs' appendages are weaker than the real crab. Besides that, both of them are different in morphological appearance (Walls *et al.*, 2002). Strong appendages are related to self-defence mechanism and ambushed foraging technique that are substantially not applied by the *T. gigas* which has poor self-defence mechanism and forage as a scavenger. In this study, only female crabs shows rejection toward decayed feeds while male crabs have specific behaviour to manipulate the edibility of the decayed feeds. The rejection might be due to the degradation of food nutritional contents and toxin excretion by the microbial activity on the food.

This study found that male crabs have specific feeding mechanisms that used to manipulate the edibility of the decayed food. John *et al.*, (2010) study found that *T. gigas* could inhibit the microbial activity by the action of amebocyte lysate. However, the adaptation through feeding mechanism could be an additional prevention technique to avoid the introduction of toxin inside the body. Feeding mechanisms are related with the feeding efficiency during restricted spawning period. Botton *et al.*, (2003) stated that horseshoe crabs appendages (pedipalps, chelicerae and genital operculum) will work together to manipulate the foraged food to the gnathobases. Male crabs use pincers to separate the decayed parts from the flesh and bring the fresh part into the gnathobases. Genital operculum enrolled in two functions: to avoid the foraged food moves under the opisthosoma and to remove the suspended decayed flesh from the ventral of the cephalothorax. Recent study found that male crabs might consume any type of available food that could be found along the migration path in order to maintain the energy supply during amplexus and optimise the limited spawning period to mate. Previous study found that male horseshoe crabs tend to feed intensively during their migration toward inshore (Botton, 1984b and Kamaruzzaman *et al.*, 2011) and they would not be active in foraging activity during the amplexus (Brockmann, 2003 and Smith *et al.*, 2013).

V. CONCLUSIONS

Female *T. gigas*' responses to the surrounding food were faster than the males. However, males have ability to consume a large amount of feeds within a shorter time. Overall, they prefer to consume freshly dead feeds and would totally reject the sheltered feeds such as shell, hard outer skin and tube host. Male crabs have particular feeding behaviours to manipulate the edibility of the decayed feeds. Detailed experiments on the feeding mechanisms of horseshoe crabs can provide more information on their foraging activity in the wild.

VI. ACKNOWLEDGMENT

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