



## Relations of coral reef benthos composition and horseshoe crabs' gut content

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### Abstract

Intensive earlier studies on horseshoe crab feeding ecology are focused on the Atlantic species, *Limulus polyphemus* (Linnaeus, 1758). However, the distribution of species, food abundance, and availability of benthos in a particular environment might influence the horseshoe crab's preference for diet in a particular region. The aims of this research are to analyse the benthos composition in *Tachypleus gigas* spawning site and coral reefs located near the spawning ground in Cherok Paloh, Pahang, and compare it with the food composition inside the *T. gigas* digestive track. A total of 30 samples of male and female horseshoe crabs, *T. gigas* respectively, were harvested by using a fishing net during the incoming high tide. Their guts were dissected, and the contents were analysed according to Squires and Dawe criteria. Results showed that echinoderm was the main food composition in the gut of males (54.27%) and females (51.44%) during the open sea migration phase. Amuk-amuk coral reef (3°41'51"N 103°24'13"E), Raja Muda coral reef (3°38'01"N 103°28'23"E) and Air Leleh coral reef (3°35'38"N 103°29'00"E) are the nearest sea urchin habitats to the sampling area. Benthos analysis showed higher echinoderm composition in those coral reefs (31.58 – 42.11%). There is no echinoderm composition found in the spawning site sediment. Further study is needed to provide reliable data on the significance of coral reefs in the horseshoe crab's foraging ecology.

**Keywords:** *Tachypleus gigas*; Food composition; Foraging site; Echinoderm; Coral reef

### 1 Introduction

Prey selection is known as the horseshoe crab's feeding behaviour (Botton, 1984a; Chatterji et al., 1992). According to Botton et al. (2003), they tend to consume benthos, namely; bivalve, polychaete, crustacean, gastropod, and macrophytes. Macrobenthos in marine sediment play an important role in ecosystems, such as, energy transfer within the food web (Snelgrove, 1998). However, most of the previous studies intensively focused on *L. polyphemus* feeding behaviour instead of the Asian species, *T. gigas*. Serial studies conducted by Chatterji et al. (1992) along the Balramgari beach found that Asian horseshoe crabs, *T. gigas*, prefer to consume molluscs in the spawning site during the mating season. However, the preference towards food might be dependent on the availability and abundance of the feed in the particular environment (Razak et al., 2017). Besides that, Botton (1984b) and John et al. (2012) studies found that horseshoe crabs food selection is intrinsically influenced by the abundance of feed in a particular area.

Previously, many researchers stated that horseshoe crabs conduct foraging activity in the spawning site and would stay passively under the sand in the open sea area (Watson & Chabot, 2010). However, an isotope study conducted by Carmichael et al. (2004) stated that most of the food items inside the horseshoe crab's gut do not come from the spawning site. Razak et al. (2017) study found higher echinoderm composition in the gut of *T. gigas* from Cherok Paloh,

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Pahang. The composition is constituted by the Echinoidea class, sea urchins. Interestingly, the population of sea urchins is abundant in the coral reef area (Harborne et al., 2000). The aims of this study are to identify the benthos properties in *T. gigas* spawning sites in Cherok Paloh, Pahang, and coral reefs in front of the spawning ground and compare the results with food composition inside the *T. gigas* digestive tract.

## 2 Material and methods

### 2.1 Gut Content Analysis

A total of 30 male and female *T. gigas* were collected by the traditional method, hand picking during the incoming high tide at the spawning beach in Cherok Paloh, Pahang. The crabs were brought to the INOCEM Research Station, IIUM, Kuantan, Pahang. The crabs were killed priority by keeping them inside the  $-20^{\circ}\text{C}$  refrigerator before been dissected for the ethical purpose. The dissection was conducted according to Razak et al. (2017). Observations were initially made under the low power of a dissecting microscope. The identification of food composition in the gut was made according to Squires and Dawe (2003) criteria. The gut contents were classified according to polychaete, crustacean, gastropod, bivalve, echinoderm, macrophyte, and miscellaneous. Miscellaneous food items include foraminifera, insects, amphipods, isopods, annelid worms, larval, and juvenile stages of fish (John et al., 2012; Razak et al., 2017). Food items for each class were weighted accordingly.

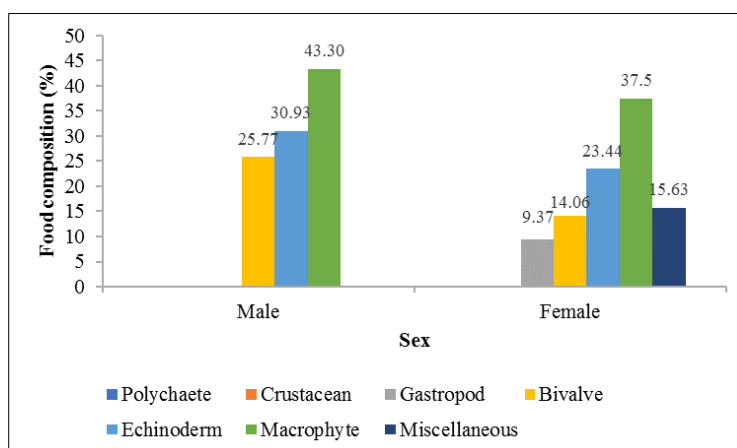
### 2.2 Benthos Analysis

Locations of the coral reefs were determined by the present of fisherman fishing trap buoyancy. The elevation of the coral reef around the fishing trap was detected by the echo sounder, 300c Garmin. SCUBA divers engaged in the following activities: 1) ensuring the location of the coral reef in the determined area and 2) collecting samples and taking picture of the animals. Coordinate and depth of the coral reefs area were recorded. Sediment samples were collected by using the core method. The samples were sieved through 0.5mm mesh size sieve then the remained macrobenthos were collected and preserved in buffered formalin containing Rose Bengal. Samples were analysed according to Squires and Dawe (2003) criteria.

## 3 Results and discussion

### 3.1 Food Composition

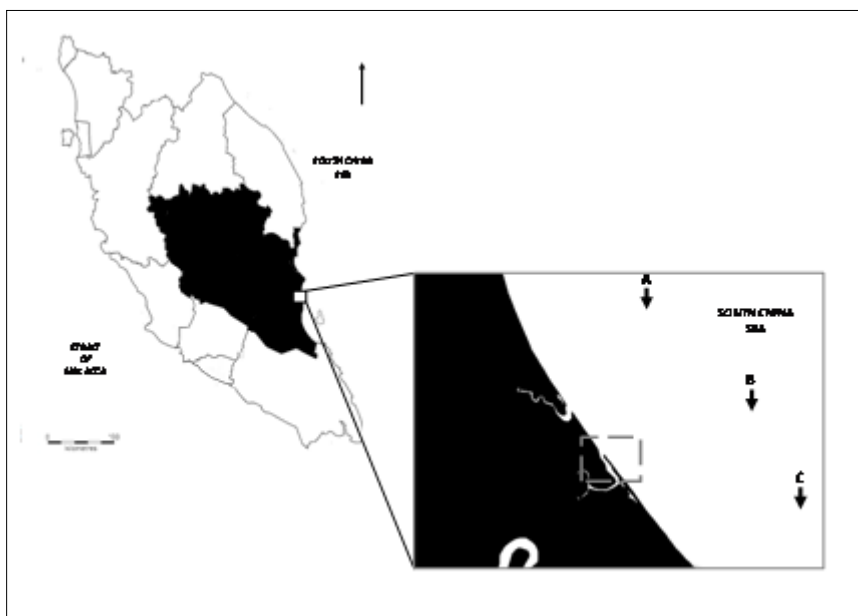
The food composition in males' gut was less variety compared to females. Only three food items; bivalve, echinoderm and macrophyte found in the gut of males while, five food items; gastropod, bivalve, echinoderm, macrophyte and miscellaneous in females. This present study found that echinoderm constituted as second major food composition in the gut of both males (30.93%) and females (23.44%) after macrophyte (male: 43.30 %, female: 37.5%) followed by bivalve that constituted 25.77% and 14.06% in gut of males and females respectively (Figure 1). Miscellaneous composition inside the females' guts also constituted by the foraminifera.



**Figure 1** The food composition inside the gut of male and female horseshoe crabs, *Tachypleus gigas*

### 3.2 Coral Reefs Location

There are three coral reef areas found in front of Cherok Paloh river mouth, Pahang; Amuk-amuk coral reef (3°41'51"N 103°24'13"E), Raja Muda coral reef (3°38'01"N 103°28'23"E) and Air Leleh coral reef (3°35'38"N 103°29'00"E) (Figure 2). Amuk-amuk and Raja Muda coral reefs are in front of Sepat and Cherok Paloh beach, Kuantan. Nevertheless, the location of Air Leleh Coral Reef is in front of Air Leleh beach, Pekan. Raja Muda is the deepest coral reef (12 m) compared to Amuk-amuk (9 m) and Air Leleh (10 m) (Table 1).



Dashed box: Sampling area, Cherok Paloh, Kuantan, Pahang. A: Amuk-amuk coral reef. B: Raja Muda coral reef. C: Air Leleh coral reef.

**Figure 2** Locations of the sampling area and coral reefs

**Table 1** Coordinate and depth of Amuk-amuk, Raja Muda and Air Leleh coral reefs

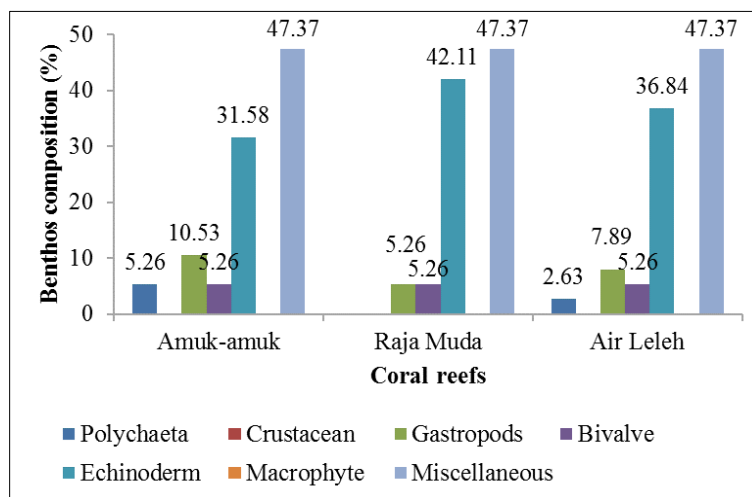
Coral Reef	Coordinate	Depth (m)
Amuk-amuk Coral Reef	3°41'51"N 103°24'13"E	9
Raja Muda Coral Reef	3°38'01"N 103°28'23"E	12
Air Leleh Coral Reef	3°35'38"N 103°29'00"E	10

### 3.3 Benthos Composition

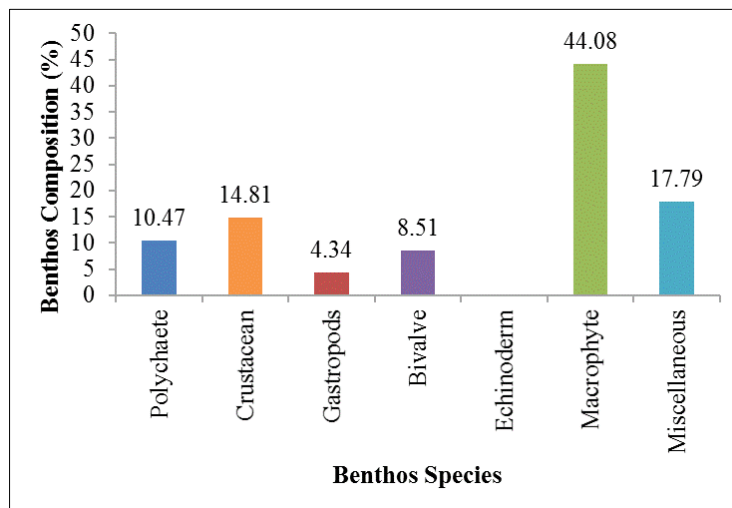
Echinoderm (Figure 3) was the second major benthos composition in Amuk-amuk (31.58%), Raja Muda (42.11%) and Air Leleh (36.84%) coral reefs sediment after the miscellaneous; Amuk-amuk (47.37%), Raja Muda (47.37%) and Air Leleh (47.37%). Compositions of gastropod (Amuk-amuk: 10.53%; Raja Muda: 5.26%; Air Leleh: 7.89%) in those coral reefs were higher compared to bivalve (Amuk-amuk: 5.26%; Raja Muda: 5.26%; Air Leleh: 5.26%) (Figure 4). Composition of polychaete was higher in Amuk-amuk (5.26%) compared to Air Leleh (2.63%) (Figure 4). No composition of polychaete found in Raja Muda coral reef sediment. Besides that, there were no compositions of crustacean and macrophyte found in all coral reefs. Macrophyte was the highest (44.08%) food composition in *T. gigas* spawning ground followed by crustacean (14.81%), polychaete (10.47%), bivalve (8.51%) and gastropods (4.34%). There was no composition of echinoderm found in the spawning ground sediment.



**Figure 3** A: Echinoderm (sea urchin). B: Gastropod.



**Figure 4** Benthos composition in Amuk-amuk, Raja Muda and Air Leleh coral reefs sediments



**Figure 5** Benthos compositions in the horseshoe crabs' spawning site

Information on food composition in the horseshoe crab's gut could be adopted in the process of defining the exact foraging grounds of horseshoe crabs in the wild. Carmichael et al. (2004) study found that most of the foods inside the gut of horseshoe crabs had not been foraged in the spawning ground. Razak et al. (2017) study stated that *T. gigas* arrive at the spawning site with full gut content, and the variety of food composition in the horseshoe crab's guts between spawning migration phases is prominent. Echinoderm was the second major food composition inside the gut of males and females. Most of the echinoderm that found in the horseshoe crab's gut constituted by the echinoidea class, sea urchin. According to Harborne et al. (2000), coral reef is the main habitat for the sea urchin. Amuk-amuk coral reef (3°41'51"N 103°24'13"E), Raja Muda coral reef (3°38'01"N 103°28'23"E) and Air Leleh coral reef (3°35'38"N 103°29'00"E) have been identified as the nearest sea urchin habitat to horseshoe crabs' spawning ground in Cherok Paloh, Pahang.

Interesting to state that, sea urchin contributed from 31.58% to 42.11% of benthos composition in those three coral reef areas. Carmichael et al. (2004) study on N and C stable isotopic in horseshoe crabs diet composition found that most of the food items inside the horseshoe crabs' gut are not come from the spawning ground. Unfortunately, there is no reliable previous study state about the composition of echinoderm inside the gut of *T. gigas*. This study showed zero echinoderm composition in the *T. gigas* spawning ground. Besides that, the macrobenthic diversity study in the horseshoe crab's nesting ground in Balok by John et al. (2013) also showed no significant composition of echinoderm in the spawning ground sediment. Macrophyte constituted as the major feed composition at the spawning site. The fragments of the macrophyte come from the continental area that has been washed away to the river opening site. Although the compositions of macrophyte were highest inside male and female *T. gigas* guts but, the study on *T. gigas* electivity index by Razak et al. (2017) showed that macrophytes were not the preferred food item during mating and post-mating phases.

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#### 4 Conclusion

Coral reef areas are important to marine organisms as they provide a plentiful food supply (Williams, 1982; Done, 1982; Fisher et al., 2015) to the marine organisms. This analysis showed that *T. gigas* would conduct their foraging activity at the open sea area before they arrive inshore to spawn. Coral reef might be the foraging ground of the *T. gigas*. However, further study is needed to provide reliable data on the significance of coral reefs in *T. gigas* foraging ecology.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

##### *Statement of ethical approval*

The ethical of horseshoe crabs usage in this study studies was monitored and approved by the IIUM Animal Care And Use Committee (I-ACUC), Kuantan, Malaysia.

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