

# Feeding Ecology and Dietary Preferences of *Tachypleus gigas* from East Malay Peninsula



Akbar John, Bryan Raveen Nelson, Hassan I. Sheikh, S. Hajisamae, and Jalal Khan

## 1 Introduction

Out of the four extant species for horseshoe crabs, namely *Limulus polyphemus*, *Tachypleus gigas*, *T. tridentatus*, and *Carcinoscorpius rotundicauda*, the latter three species are native to Malaysian coastal waters (John et al. 2018). Moreover, sympatric presence of *T. gigas* and *C. rotundicauda* populations in Balok, Pahang on the coast of East Peninsular Malaysia may indicate their habitat overlaps (Nelson et al. 2015; Nelson et al. 2016a). The populations are currently exploited for exotic protein (Nelson et al. 2016b, 2019, John et al. 2021). On a global scale, biomedical applications have

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A. John (✉)

Institute of Oceanography and Maritime Studies, Kulliyah of Science, International Islamic University Malaysia, Kuantan, Pahang, Malaysia

B. R. Nelson

Institute of Tropical Biodiversity and Sustainable Development, Universiti Malaysia, Kuantan, Pahang, Malaysia

e-mail: [bryan.nelson@umt.edu.my](mailto:bryan.nelson@umt.edu.my)

H. I. Sheikh

Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia

S. Hajisamae

Faculty of Science and Technology, Prince of Songkla University, Muang Pattani, Thailand

J. Khan

Department of Marine Science, Kulliyah of Science, International Islamic University Malaysia, Kuantan, Pahang, Malaysia

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increased the demand for horseshoe crab blue blood, especially *L. polyphemus* and *T. tridentatus*. Export of *T. gigas* from Malaysia and Thailand is anticipated apart from their local use (Zauki et al. 2019a, 2019b). Other threats to horseshoe crabs in Malaysia include impoverished food sources (Tan et al. 2011; John et al. 2012; John et al. 2013), water quality (John et al. 2011), and habitat degradation (John et al. 2012a; Fairuz-Fozi et al. 2018). Feeding ecology and behavior of wild and captive *L. polyphemus* (Able et al. 2019), *T. gigas* (Razak et al. 2017), *T. tridentatus* (Chiu and Morton 2004; Kwan et al. 2015a,b), and *C. rotundicauda* (John et al. 2012) were also examined. Electivity is used to indicate food availability while gastroscopic index indicates the *T. gigas*'s diets (Razak et al. 2017). However, only 20 samples were used in the study, and the results are preliminary and may be inconclusive. The present study was based on the approach described in Razak et al. (2017) but in a one-year timescale. This study enhances our understanding on *T. gigas* feeding ecology and food preference and also the way the population adapts during transitional weather at their spawning grounds.

## 2 Materials and Methods

### 2.1 Description of Study Area

Balok ( $3^{\circ}56.194' N$ ;  $103^{\circ}22.608' E$ ) and Tanjung Selangor (Pekan) ( $3^{\circ}36.181' N$ ;  $103^{\circ}23.946' E$ ) in Pahang state are important *T. gigas* spawning sites along the coast of East Malay Peninsula (John et al. 2018; Zauki et al. 2019a; 2019b) (Fig. 1). Adult horseshoe crabs migrate from the offshore to shallow continental shelf waters and spawn on intertidal sandy or sandy mud flats during every full and new moon period. Pahang has an average monthly rainfall between 250 and 300 mm and reaches



**Fig. 1** Location of the sampling area. Distance between two sampling zones (Balok and Pekan) is 67 km. These two stations were identified to be the major nesting and spawning grounds of horseshoe crabs in Pahang state, Malaysia

>3000 mm annually. The sediments at the sampling location were observed to be soft which may facilitate laying of eggs and their burial by the female horseshoe crab. Meteorological data from November to January reflected the wet monsoon (MMD 2011). Balok and Pekan coastal waters have active vessel movement connecting an upstream jetty and South China Sea to serve fisheries practices.

## 2.2 Sample Collection and Preparation

A total of 93 adult *T. gigas*, including 41 males and 52 females, were collected for gut content analysis by either handpicking or sampled using gill net placed across the nesting ground a day prior to the full and new moon days. The horseshoe crabs were euthanized injecting 50 ml of 70% ethanol at the opisthosoma-carapace hinge using a 16-gauge needle. At the laboratory, sterile scissors and forceps were used to separate gut contents from the gastrointestinal tract before transferring them into petri dishes containing 90% ethanol. After measuring the gastrointestinal length and weight, its gut contents were washed individually with 90% ethanol using dissecting and compound microscopes. The gut content was sorted under the stereoscopic microscope, identified to the lowest possible taxon and counted. Samples that were partially digested were considered as individuals of a representative group of animals and were counted accordingly. To photograph and measure the size of the ingested food stuffs, an epifluorescence microscope was used. In addition to horseshoe crabs, macrobenthos samples were also collected from the two nesting grounds in multiple random quadrats (1 m<sup>2</sup>) using hand scoop during low tide, and the samples were identified using standard taxonomic keys to the lowest possible taxon.

## 2.3 Data Analysis

The relative abundance of identifiable gut remnants in *T. gigas* was expressed as a percentage of total number of food items in the samples. Unidentified organic matters were considered as macrophytes based on microscopic observation following the method of Ferreira and Vasconcellos (Ferreira Soraia and João 2001). The intensity of feeding was studied by determining the gastrosomatic index (GSI), i.e., gut weight expressed as percentage of body weight. Electivity index (*E*) was calculated to understand *T. gigas* dietary composition and preferences (Chatterji et al. 1992). The *E* value ranged from +1 to -1, with a positive value indicates that a prey type is found in a higher proportion in the diet than in the prey community. A negative value indicates that a prey type is found in lower proportion in the diet than in the prey community. The *E* was calculated using the following formula:

$$E = \frac{r_i - P_i}{r_i + P_i}$$

Where,

$E$  = electivity index

“ $r_i$ ” = the relative abundance (%) of any food item in the gut, expressed as a percentage of total amount of food item.

“ $P_i$ ” = the relative abundance (%) of the same food item in the environment.

GSI = gastrosomatic index was calculated using the following formula:

$$GSI = \frac{\text{Weight of stomach contents}}{\text{Weight of the horseshoe crab}} \times 100$$

### 3 Results and Discussion

#### 3.1 Macrobenthos Composition at the Nesting Grounds

Gut content of *T. gigas* was predominated with bivalves, gastropods, polychaetes and crustaceans. Besides these major macrobenthos, there were other miscellaneous groups such as larval insects, amphipods, isopods, unknown larvae, fish larvae, foraminifera, and annelid worms. Among the macrobenthos, bivalves were encountered throughout the sampling periods in both sampling sites. In Balok, bivalve items accounted for 37.2% and 31.5% during the non-monsoon and monsoon seasons, respectively, while they constituted 28% and 21.5% during new moon days and on full moon days, respectively. In Pekan, bivalves accounted for 30.6% and 24.9% during the non-monsoon and monsoon seasons, respectively, while they accounted for 23.9% and 23.3% during new moon and full moon days, respectively. Gastropods were the second most dominant macrobenthic community followed by other organisms (i.e., insects, amphipods, isopods, larval and juvenile fishes, foraminifera, and annelidan worms) and polychaetes (Video 1). Crustaceans were the least dominant in both sampling locations. In general, the macrobenthic organisms were present in higher abundances during full moon compared with the new moon days. It was also apparent that the benthic percentage abundance was higher in Balok than that in Pekan. This might probably be due to the presence of nutrient-rich habitat together with better water quality along the Balok station compared with Pekan (Zauki et al. 2019a; 2019b). Some of the dominant macrobenthos in Balok include bivalves (*Anadara granosa*, *A. antiquata*, *Circe* sp., *Tellina virgata*, *Donax cuneatus*, *D. variabilis*, *Myadora striata* and *Codakia orbicularis*), gastropods (*Cerithidea cingulata*, *C. obtuse*, *Cerithium litteratum*, *Neritina* sp., *Littorina scabra*, *L. fasciata*, *L. undulata*, *Cliton oualaniensis*, and *Umbonium vestiarium*), polychaetes (*Ganoderma australe*, *Lentinus squarrosulus*, *Microporellus* sp., *Pycnoporus sanguineus*, *Rigidoporus* sp., *Lenzites elegans*, *Microporus* sp., and *Trametes* sp.) and crustaceans (juvenile penaeid shrimp, common crabs, and hermit crabs). Previous studies demonstrated that some polychaetes would feed on

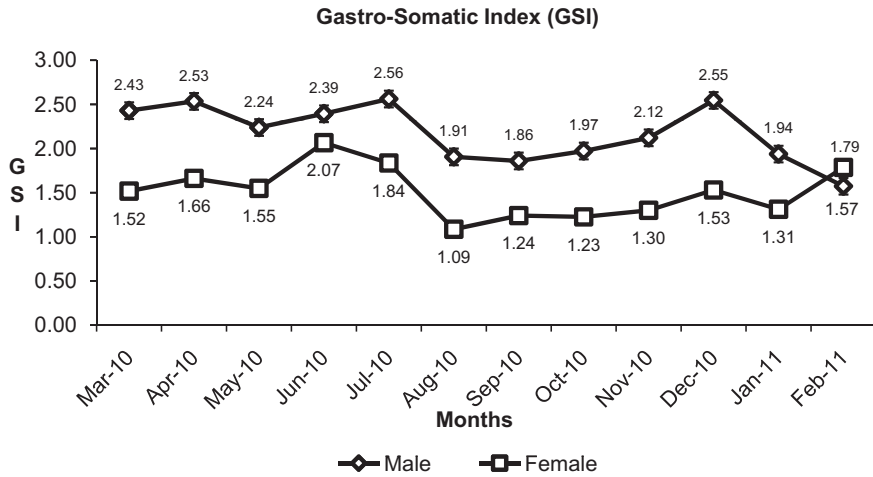
horseshoe crab eggs (John et al. 2013; Nelson et al. 2016b, Video 2) after habitat disturbance occurred at Balok (Nelson et al. 2019).

### 3.2 Feeding Composition of *T. Gigas*

Feeding composition of *T. gigas* from Balok and Pekan were pooled since similar food items were observed in their guts. Bivalve, gastropod, crustacean, and polychaete were the main food items for male *T. gigas*, while female horseshoe crabs primarily consumed bivalves during the non-monsoon and with the addition of polychaete during the monsoon (Table 1). Despite the fact that male *T. gigas* are smaller (311–383 g body weight, BW) compared with the females (725.7–1025 g BW), the males consumed in greater amount than the females, as observed between March 2010 and January 2011 (Fig. 2). This is probably due to the availability of foregut space in males, and their difference in metabolism and energy demands between sexes (Kyomo 1992). The maximum GSI value in the males was observed during July 2010 (2.56), followed by December 2010 (2.55) and April 2010 (2.53). The minimum GSI value in the males, however, was noted in February 2011 (1.57). The maximum value of GSI in the females was observed in June 2010 (2.07), followed by July 2010 (1.84) and February 2011 (1.79), while the minimum GSI in the

**Table 1** Relative composition of different food items in the gut of male (M) and female (F) *T. gigas* during the monsoon (MS) and non-monsoon (NMS) seasons. Data expressed as the number of individual prey items represented in percentage abundance in the gut

	Seasons		GSI	Relative composition of food items (%)				
				Bivalve	Gastropod	Crustacean	Polychaete	Others
M	NMS	Average	2.24	33.66	17.08	9.81	26.11	13.34
		Max	2.56	39.93	22.18	19.92	40.14	30.50
		Min	1.86	22.19	8.00	0.00	16.90	7.71
		SD	0.40	6.47	5.88	5.48	11.61	6.73
	MS	Average	2.04	25.80	11.91	4.73	30.45	27.11
		Max	2.55	33.39	19.01	10.09	42.12	34.45
		Min	1.57	19.68	6.69	0.00	18.11	20.67
		SD	0.40	6.47	5.88	5.48	11.61	6.73
F	NMS	Average	1.52	35.95	17.21	10.60	27.41	8.84
		Max	2.07	48.19	22.11	14.32	34.21	14.14
		Min	1.09	27.91	11.22	5.11	21.00	1.77
		SD	0.23	4.86	2.17	9.63	2.34	12.72
	MS	Average	1.48	26.68	11.61	11.13	31.01	19.58
		Max	1.79	31.20	14.12	22.21	33.18	31.92
		Min	1.30	20.10	8.89	0.00	27.71	6.34
		SD	0.23	4.86	2.17	9.63	2.34	12.72



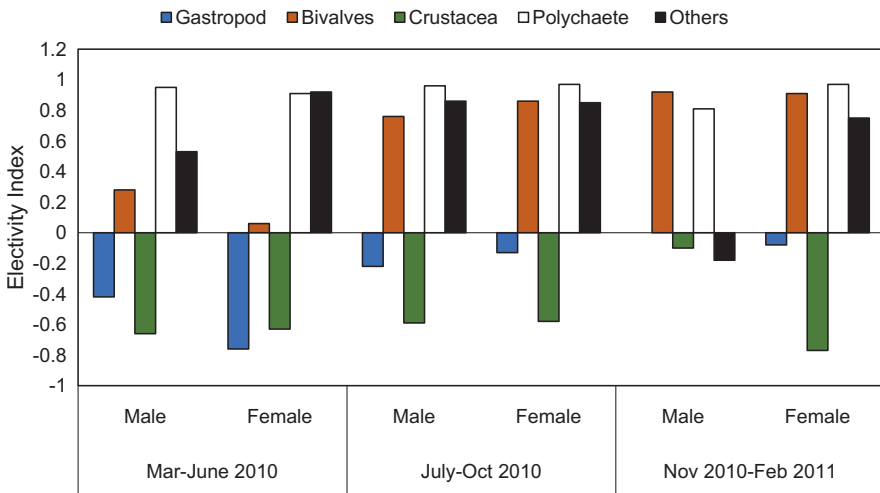
**Fig. 2** Gastro-somatic index shows the monthly variation in the feeding intensity of male and female *Tachypleus gigas*

females was noted in August 2010 (1.09) (Fig. 2). This observation was due to seasonal variation in feeding intensity of the crabs as recorded in the previous studies (Prasad and Neelakantan 1988; Pramanik and Mohanty 2016). Average feeding intensity values of the males during the monsoon and non-monsoon seasons were  $2.04 \pm 0.4$  and  $2.24 \pm 0.4$ , respectively (Table 1). Mean feeding intensity values of the females during the monsoon and non-monsoon seasons were  $1.48 \pm 0.23$  and  $1.52 \pm 0.23$ , respectively. Both male and female *T. gigas* endure exertive currents when migrating from deep into shallow waters. This also implies that they must feed to satiation before attempting the long-distance travel to the spawning grounds. Horseshoe crabs were previously reported to rely on bottom currents for long-distance locomotion (John et al. 2018). Their body weight will increase along with feeding activity regardless of their sex. For the males, a higher body weight may allow the increase of gravity to maintain an upright position when moving along inshore bottom currents. On the other hand, female *T. gigas* are larger in size to store more eggs in the bodies. Consuming more food increases chances of sinking and energy when traveling. Therefore, feeding less allows the females to remain buoyant when moving along the inshore bottom currents. Earlier research has indicated that intertidal sand flats with low energy currents are preferred by horseshoe crab as foraging grounds (Botton 1984; John et al. 2012; Botton et al. 2003). In addition, seabed perturbations cause flocculation and promote crustacean and polychaete thriving (Pinotti et al. 2014). Consequently, gastro-somatic index rose in February 2011 and reached 1.79, possibly due to additional crustacean and polychaete portions in female *T. gigas* diets. This finding indicated that both male and female *T. gigas* may migrate to and feed intensively in the shallow waters during the wet monsoon season (November to March) when the wave action is turning stronger.

### 3.3 Natural Food Selectivity

The electivity index ( $E$ ) calculated for the frequent food items during monsoon and non-monsoon seasons is presented in Fig. 3. Throughout the sampling period, the  $E$  was predominantly negative for gastropods and crustaceans, whereas positive values were found for bivalves, polychaetes, and miscellaneous food items (i.e., insects, amphipods, isopods, larval and juvenile stages of fishes, foraminifera, and annelid worms). During the non-monsoon season, *T. gigas* foraged on bivalves instead of polychaetes. This trend was reversed during the wet monsoon season. It was also apparent that during the peak mating season (June to August 2010), *T. gigas* may become less selective and prey on more food items. Seasonal variations in food composition showed that mollusks, especially bivalves, remained as the dominant food item. Unidentified organic matters in the gut of *T. gigas* were mostly macrophytes, apart from the presence of a considerable amount of sand particles in the foregut. Considering the uncertainty about the foraging time for *T. gigas*, we assumed that the negative values in  $E$  attained in this study would mean *T. gigas* has become selective and concentrates its effort on that resource. Comparisons in Table 1 indicated the gastropods and crustaceans are alternative but encompass major food portions for horseshoe crabs.

Recent studies on horseshoe crabs suggest that it consumes a variety of food items, including particulate organic matter (POM, such as algae), which may play a significant role in their diet (Gaines et al. 2002; Carmichael et al. 2004; Carmichael and Valiela 2005). We observed floc deposition with sedimentation, an attribute for organic matter enrichment in Balok (Nelson et al. 2016a; Zauki et al. 2019b). POM was reported to be major component in the diet of juvenile *L. polyphemus*,



**Fig. 3** The electivity index ( $E$ ) of different major food items consumed by *Tachypleus gigas* during March 2010 to February 2011

especially for the second to seventh instar, while the larger instars (8th–11th) relied less on POM and fed mainly on benthic organisms such as amphipods, isopods, and polychaete (Carmichael et al. 2009; Fan et al. 2017). POM was also found to be essential for *T. tridentatus*, *C. rotundicauda*, and *L. polyphemus*, especially in early stages. Seagrass and seagrass-derived organic matter were also crucial for juvenile *T. tridentatus* and *C. rotundicauda*; however, POM does not contribute as the major food item for the juveniles (Carmichael et al. 2009; Fan et al. 2017; Noor Jawahir et al. 2017). Our findings reported a dietary “calendar” for *T. gigas* where portions of food items changed with season due to the prey availability.

The relative percentage of food items in *T. gigas* guts varied monthly. This was correlated to the abundance of that particular food item in the environment. Such case in *T. gigas* was similar to selective feeding behavior as previously reported in many marine animals, despite their multiple feeding strategies, including filter feeding, deposit feeding, and hunting (Hughes 1980). To the best of our knowledge, studies concerning feeding preference and behavior of *T. gigas* are largely lacking, and therefore, we have to refer the relevant knowledge from their counterparts in Indian waters (Chatterji et al. 1992). The relationship between prey size and mouth size of horseshoe crabs were addressed in literature (Botton 1984; Botton and Haskin 1984; Botton et al. 2003; Botton and Shuster 2004). The highest values for gastrosomatic indices in our study confirmed that 87% of adult *T. gigas* diet comprises bivalves. Feeding behavior and food preference study on mangrove horseshoe crabs, *C. rotundicauda*, demonstrated that they prefer polychaetes more than bivalves during the non-monsoon season, while in monsoon season, their preferred gastropods (John et al. 2012). We hypothesize that the spawning season of gastropods and polychaetes was prior to the monsoon seasons (Hughes 1980; Pinotti et al. 2014), leading to their increase in abundance and subsequently contributing to horseshoe crab diets. Nevertheless, macrobenthos availability and their flourishing (Kwan et al. 2015a; b) may influence the shift in *T. gigas* feeding regimes.

Monthly variation in feeding intensity of *T. gigas* showed that male horseshoe crabs fed more intensely than the females. GSI analysis also showed that the adults fed more intensely during their mating season. No considerable variation was observed in the feeding intensity between the monsoon and non-monsoon seasons. An increased in food preference during mating season might be due to the increased availability of preferred food items in the nesting grounds. Kwan et al. (Kwan et al. 2015a, b) reported that a healthy environment with rich seagrass biomass can support the life of polychaetes, crustaceans, and bivalves. In such environments, there was no significant difference in the diet composition of the 6th–11th instar *T. tridentatus* in different sampling seasons.

Ever since the first attempt on horseshoe crab aquaculture (Kropach 1979) for biomedical bleeding practice, many attempts were carried out to successfully maintain horseshoe crabs in captivity (Tinker-Kulberg et al. 2020). Major issues for the present husbandry system for horseshoe crabs include the nutritional deficiencies that lead to panhypoproteinemia due to an administration of either single or limited number of mixed feed (Nolan and Smith 2009). However, in general, wild horseshoe crabs do not face this problem due to the availability and accessibility to



varying food sources. In such condition, gut content analysis data presented in this study could also be used to formulate better feed by understanding the feeding behavior and food preference of horseshoe crabs in the natural environment.

## 4 Conclusion

Adult *T. gigas* had different diets throughout the year as changes in seasonal weather and habitat condition influenced their foraging preferences. Our study showed the annual dietary “calendar” for *T. gigas*, which feed mainly on bivalves during the non-monsoon, while polychaetes were the major diet constituents during the monsoon seasons. Female fed on a mixed diet containing bivalves and polychaetes, while males fed mostly on mollusk (i.e., bivalves and gastropods). During the monsoon season when rough tides and heavy raining occur, *T. gigas* preferred polychaetes than bivalves, which was probably due to the easier access to polychaetes in the season. In general, adult horseshoe crabs were demonstrated to feed on different mollusks across the ranges. More research work can be carried out to determine the role of *T. gigas* in coastal food webs by addressing the dietary differences between adult and juvenile crabs. The obtained information in this study can also be used in long-term culture of *T. gigas*.

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**Conflict of Interest** Authors declare no conflict of interest.

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