Sains Malaysiana 41(7)(2012): 855–861

Feeding Ecology and Food Preferences of *Carcinoscorpius rotundicauda* Collected from the Pahang Nesting Grounds

(Ekologi Pemakanan dan Pemilihan Makanan *Carcinoscorpius rotundicauda* dari Kawasan Tapak Penetasan Negeri Pahang)

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ABSTRACT

The first time report on the feeding ecology and food preference of mangrove horseshoe crab Carcinoscorpius rotundicauda *(Latreille 1802) at their nesting grounds along the Pahang coast is given. Monthly sampling was carried out between March 2010 and February 2011 covering both monsoonal (March to October) and non-monsoonal (November to February) seasons. Major macrobenthic gut contents (bivalves, gastropods, crustaceans, polychaetes and miscellaneous food items including plant materials) were identified using microscopic examination. An electivity index (EI) was calculated for the frequent food items observed in the gut region of* C. rotundicauda *during monsoon and non-monsoon seasons. The EI was negative for crustaceans and positive for all the other food items including bivalves, gastropods, polychaetes and miscellaneous food items (which include insects, amphipods, Isopods, larval and juvenile stages of fishes, foraminifera and other Annelid worms). It is interesting to note that* C. rotundicauda *prefered less number of bivalves than polychaetes during non monsoon seasons but it was the reverse during monsoonal period. Male crabs intensely preyed on gastropods and female prefers polychaete worms during the peak mating/nesting season (June - August 2010). Seasonal variations in food composition showed that mollusks formed the main item especially gastropods. Unidentified organic matters in the gut content analysis of* C. rotundicauda *showed high preference towards plant materials. Gastro Somatic Index (GaSI) analysis showed that the feeding intensity of male crabs was higher during non-monsoon period while it was higher during monsoonal period in female crabs. In conclusion, the feeding ecology of mangrove horseshoe crabs were more similar to its closer and distant conspecifics. However, it was postulated that the higher preference of polychaete worms by the female* C. rotundicauda *during the peak mating season indicated its role in regulating the nesting behaviour.*

Keywords: Carcinoscorpius rotundicauda; *electivity index; gastro somatic index; horseshoe crab nesting*

ABSTRAK

Laporan pertama mengenai ekologi pemakanan dan makanan utama ketam ladam bakau Carcinoscorpius rotundicauda *(Latreille 1802) di tempat bertelur di sepanjang pantai Pahang telah diberikan. Persampelan bulanan telah dijalankan antara Mac 2010 dan Februari 2011 yang meliputi kedua-dua musim monsun (Mac hingga Oktober) dan bukan monsun (November hingga Februari). Kandungan usus makrobentik yang utama (bivalvia, gastropod, krustasia, polichaetes dan bahan makanan yang pelbagai termasuk bahan tumbuhan) telah dikenal pasti dengan menggunakan pemeriksaan mikroskopik. Indeks elektiviti (EI) telah dikira untuk bahan makanan yang kerap diperhatikan di bahagian usus* C. rotundicauda *semasa musim monsun dan bukan monsun. Keputusan memberikan EI yang negatif untuk krustasia dan positif untuk semua bahan makanan lain termasuk bivalvia, gastropod, polichaetes dan bahan makanan yang pelbagai (termasuk serangga, amfipods, Isopod, larva dan peringkat juvenil ikan, foraminifera dan cacing Annelid yang lain). Adalah menarik untuk diperhatikan bahawa* C. rotundicauda *lebih cenderung kepada bilangan bivalvia yang lebih sedikit daripada polichaetes semasa musim bukan monsun tetapi adalah sebaliknya ketika tempoh monsun. Ketam jantan giat menjadi pemangsa gastropod manakala ketam betina cenderung pada cacing polichaete semasa puncak musim mengawan/bersarang (Jun – Ogos 2010). Variasi yang bermusim dalam komposisi makanan menunjukkan bahawa moluska membentuk bahan utama terutamanya gastropod. Bahan organik yang tidak dikenali dalam analisis kandungan usus* C. rotundicauda *menunjukkan keutamaan yang tinggi terhadap bahan tumbuh-tumbuhan. Analisis Indeks Gastro Somatik (GaS) menunjukkan bahawa keamatan pemakanan ketam jantan adalah lebih tinggi semasa tempoh bukan monsun manakala bagi musim monsun, ketam betina adalah lebih tinggi. Kesimpulannya, ekologi pemakanan ketam ladam bakau adalah lebih serupa dengan konspisifikasi yang dekat dan jauh spesis tersebut. Walau bagaimanapun, adalah didalilkan bahawa keutamaan yang lebih tinggi terhadap cacing polichaete oleh* C. rotundicauda *betina semasa puncak musim mengawan menunjukkan peranannya dalam mengawal kelakuan bersarang spesis ini.*

Kata kunci: Carcinoscorpius rotundicauda; *indeks elektiviti; indeks gastro somatik; ketam ladam bakau*

Introduction

Out of four extant species of horseshoe crabs, *Tachypleus tridentatus, Tachypleus gigas* and *Carcinoscorpius rotundicauda* inhabit Malaysian coast while the distribution of *T.tridentatus* is restricted to East Malaysia (John et al. 2010; Kassim et al. 2008; Kamaruzzaman et al. 2011a & 2011b). Intriguing characteristic of horseshoe crabs is that they are morphologically similar in look and having virtually unchanged genetic makeup which helped them in withstanding various environmental stresses for the past 150 million years. This has attracted the attention of ecologists and conventional biologist who strongly believed that the adaptability of horseshoe crabs in terms of altering their feeding behaviour during the extreme environmental condition might have also played an important role in their evolution over time. During spawning time adult *C. rotundicauda* migrates from the offshore continental shelf to spawn on intertidal sandy mud beaches and mangrove area during full and new moon phase (Zaleha et al. 2010).

The digestive system of the four species of horseshoe crabs has many morphological similarities (except a few) in minute details, such as longitudinal ridges in the inner surface of the oesophagus, the proventriculus and the rectum (Yamasaki et al. 1988). These similarities may be due to evolutionary conservation, rather than convergence, and the basic feeding behaviour remains the same and unchanged in all extinct and extant horseshoe crabs. This leads to the final conclusion that the horseshoe crab's digestive system retains some primitive features like the well defined yellow connective tissue (Yamasaki et al. 1988). It was evident from several reviews of the natural history of horseshoe crabs (Sekiguchi 1988; Shuster 1982) that relatively very little is known of the merostomate's feeding biology, despite its familiarity to a large number of zoologists and palaeontologists, and its growing importance in biomedical research (Novitsky 1982, 1984). The first comprehensive study of feeding behaviour of *Limulus polyphemus* is of Botton (1984a, 1984b) which revealed a reasonable methodology to conduct the gut-content analysis in Indian horseshoe crab , *Tachypleus gigas* (Debnath 1985; Debnath & Choudhury 1987). Botton and Haskin (1984) provided a more intensive study of the feeding habits of *L. polyphemus* recovered from the continental shelf of Delaware Bay and the subsequent analysis of their stomach contents. Chattergi et al. (1992) had examined the food preferences of *Tachypleus gigas* along the Orissa Coast, India. However, no such study was conducted to prove or to support this statement by examining the food preferences of *C.rotundicauda* at their nesting grounds. Hence, the present study was aimed to investigate the feeding ecology and food preferences of mangrove horseshoe crabs at their nesting grounds.

Materials and Methods

Sample Collection and Laboratory Analysis

Balok (Lat3°56.194' N, Long103°22.608' E) and Tanjung Gosong (Pekan: Lat3°36.181' N, Long103°23.946' E) in Pahang, East coast of Malaysia were observed to be the nesting grounds of horseshoe crabs (Figure 1). A total of 42 horseshoe crabs (21 pairs) were handpicked alive from these two sampling locations after their nesting and

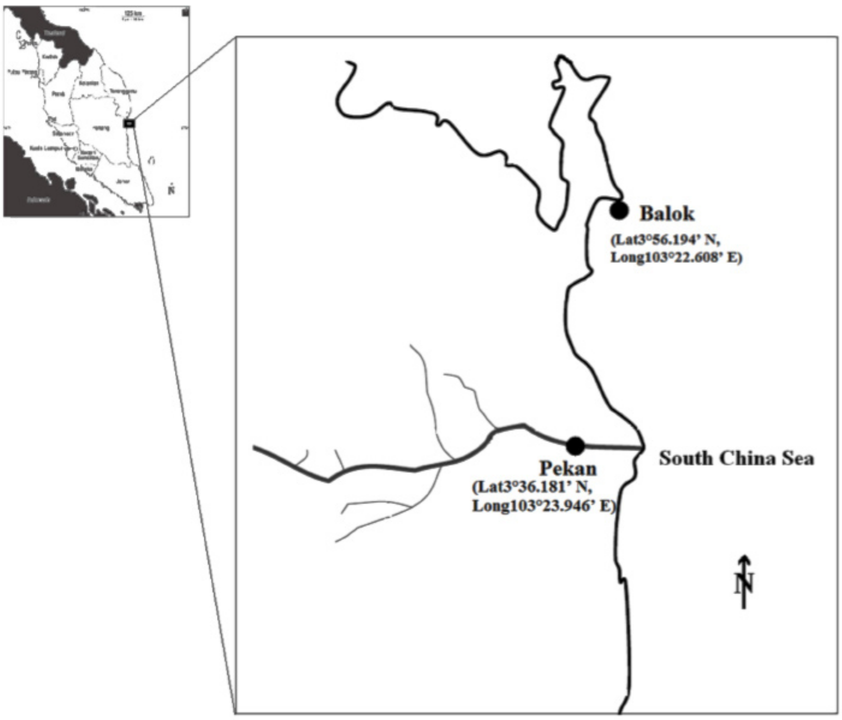

FIGURE 1. Location of the sampling area

immediately transported to the Institute of Oceanography and Maritime Studies (INOCEM) marine biology laboratory for further analysis. Horseshoe crab samples were primarily collected from Pekan station where they share the limited size of nesting ground with *T. gigas*. Field observation showed that the sediment nature of both the sampling locations were of loosely arranged sediments which might facilitate easy laying of eggs and egg burial by the female horseshoe crabs. Macrobenthic samples were also collected by hand operated van Veen grab from the nesting grounds and their percentage abundance in selected nesting grounds are given in Table 1. Samples were sexed, weighed and the Total length (TL) and Carapace width (CW) were recorded. The crabs were then ice killed and 30% of formalin was injected into their gut region. A sterile scissors and forceps were used to cut open the animal from postero-ventral region and the complete gut was removed and transferred to a clean petri dish containing 30% formalin. Wet weight of the gut was recorded and the gut contents were gently washed with formalin and transferred to another petridish. Squires and Dawe (2003) criteria were adopted for identification of semi digested and rigorously decayed food items in the gut content Gut contents were identified to a group level (bivalves, gastropods, crustaceans, polychaetes and others) using stereo microscope.

IDENTIFICATION OF MACROBENTHOS

The sediment samples were sieved through electrical shaker using 0.5 mm mesh size sieve as a final layer to collect the macrobenthic organisms. The retained macrobenthos were collected using smooth forceps and placed in appropriate vials containing 70% ethanol (Gurr 1965) in order to preserve the samples. The samples were identified to the group level as mentioned above using a stereo microscope.

TABLE 1. Monthly variation in the percentage abundance of different benthic organisms at Balok and Pekan during full and new moon days

Full moon percentage abundance		Non monsoon							Monsoon					Average in %	
		Mar-10	Apr-10	May-10	Jun-10	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11	Non monsoon	Monsoon
Bivalves	Balok	47.4	45.0	42.5	26.3	44.2	33.2	26.6	32.6	31.4	31.0	29.5	34.0	37.2	31.5
	Pekan	32.6	33.8	33.7	27.6	28.5	28.8	19.7	40.4	23.2	36.9	18.2	21.4	30.6	24.9
Gatropoda	Balok	32.0	33.3	19.8	19.4	22.8	32.6	19.2	25.4	26.8	34.1	30.5	28.9	25.6	30.0
	Pekan	28.8	12.5	21.8	32.4	26.7	30.9	15.0	17.2	21.5	18.5	22.4	28.2	23.2	22.7
Crustacea	Balok	4.6	7.9	4.7	8.1	3.3	6.3	6.3	11.8	8.0	2.6	8.3	5.7	6.6	6.2
	Pekan	4.3	12.8	19.0	9.7	9.5	14.0	5.8	5.4	5.3	2.4	8.3	6.1	10.1	5.5
Polychaeta	Balok	5.2	6.8	19.5	23.8	10.8	10.9	24.2	7.2	13.8	9.5	16.3	13.4	13.5	13.2
	Pekan	18.9	22.6	10.5	15.5	21.7	12.7	15.6	15.5	19.1	14.9	23.4	25.2	16.6	20.7
Others	Balok	10.8	7.0	13.6	22.3	19.0	17.1	23.8	22.9	19.9	22.8	15.4	18.0	17.0	19.0
	Pekan	15.3	18.2	15.1	14.8	13.6	13.7	43.9	21.5	30.9	27.4	27.6	19.1	19.5	26.2
New moon percentage abundance		Non monsoon							Monsoon						
		Mar-10	Apr-10	May-10	Jun-10	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11		
Bivalves	Balok	50.6	33.9	30.3	12.5	18.2	26.6	24.8	27.2	17.1	22.8	16.5	29.6	28.0	21.5
	Pekan	22.9	14.2	37.6	13.0	36.0	21.2	32.1	14.2	16.9	16.9	33.8	25.6	23.9	23.3
Gatropoda	Balok	15.1	36.4	16.0	25.6	28.4	26.0	27.7	17.3	24.5	19.4	22.3	21.8	24.1	22.0
	Pekan	22.8	8.4	23.6	11.6	19.6	47.3	22.0	22.1	21.3	26.3	14.9	13.7	22.2	19.1
Crustacea	Balok	7.4	6.1	3.4	16.3	9.9	12.5	7.7	6.8	5.7	12.6	10.8	10.1	8.8	9.8
	Pekan	2.6	6.7	3.2	9.2	3.6	3.5	3.7	8.8	5.5	12.7	11.0	8.2	5.2	9.4
Polychaeta	Balok	10.9	10.4	22.2	21.3	17.6	22.6	13.8	19.8	23.8	16.5	19.2	25.7	17.3	21.3
	Pekan	14.2	27.7	18.9	38.2	16.9	13.1	6.0	24.6	18.0	22.9	13.6	29.2	19.9	20.9
Others	Balok	16.0	13.2	28.2	24.3	25.9	12.3	26.0	29.0	28.9	28.6	31.2	12.8	21.8	25.4
	Pekan	37.5	43.0	16.6	28.0	23.9	14.9	36.2	30.4	38.3	21.2	26.6	23.3	28.8	27.3

DATA ANALYSIS

The intensity of feeding was studied by determining the Gastro-Somatic Index (GaSI) (gut weight expressed as percentage of body weight) as described by Chattergi et al. (1977). One way ANOVA test was performed to check the significant variation in the differential food preferences and sex linked food intake by *C. rotundicauda*. Percentage of decayed organic matter and sand particles was determined by eye estimation (Chattergi et al. 1992). Food preferences by mangrove horseshoe crabs in this study were compared with their conspecifics food preferences from the Indian and Mexican coastline (Botton 1884b; Chattergi et al. 1992).The electivity index (E1) was used as a measure of food selection (Chattergi et al. 1992). Values of this index range from +1 to -1, with positive values indicating that a prey type is found in higher proportion in the diet than in the prey community. Negative values indicate that a prey type is found in lower proportion in the diet than in the prey community. The electivity index (*E1*) was calculated using following formula:

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

where E is the electivity index, r_i is the relative amount of any food item in the gut (expressed as a percentage of total amount of food item) and P_i is the relative abundance (%) of the same food item in the environment.

Gastro Somatic Index (GaSI) value was determined using the following formula:

$$\text{Gastro Somatic Index (GaSI)} = \frac{\text{Weight of the stomach content}}{\text{Weight of the crab}} \times 100 \cdot$$

RESULTS AND DISCUSSION

Dietary study based upon analysis of stomach contents is now a standard practice in determining the ecology of selected animal group but surprisingly little literature exists upon the range of methods which may be employed. Studies have found that seasonal variation in the diet and/or dietary comparison between different sub-groups of the same species. To successfully culture horseshoe crab in hatchery and to aid conservation of natural population, one may need to understand the diet composition that yields the highest rates of growth and survival. Studies on gut content analysis and limited choice test suggest that protein sources, particularly bivalves, may be a main component of adult horseshoe crab diet (Botton 1984a; Botton et al. 2004; Chatterji et al. 1992; Debnath & Choudhury 1987). Gut content analysis however, are limited to detecting the most recent diet and may be biased towards the identification of species with hard skeletons or shells that remain in the gut (Alexander et al. 1996), However, in case of horseshoe crabs, male hooks the female in dorsal position and spends less energy during the migration towards the shore which might ultimately influence in reducing their digestive enzymatic activities (Suzuki et al. 1975; Uys et al. 1987). On the other hand, female crabs spent more energy during the migration in physiologically stressed gravid condition and hence they were allowed to graze on the available macrobenthos at the nesting grounds to determine their food preferences.

FOOD SELECTION

The electivity index calculated for the frequent food items of *C. rotundicauda* during monsoon (March-October) and non-monsoon (November-February) seasons is presented in Figure 2. The electivity index was negative for crustaceans and positive for bivalves, gastropods and polychaetes and miscellaneous food items (including insects, amphipods, isopods, larval and juvenile stages of fishes, foraminifera and Annelid worms) throughout the sampling period. Electivity Index (E1) analysis also showed that *C. rotundicauda* prefers more gastropods than bivalves (ANOVA $p < 0.05$) and hence gastropods formed most common food item encountered in its gut

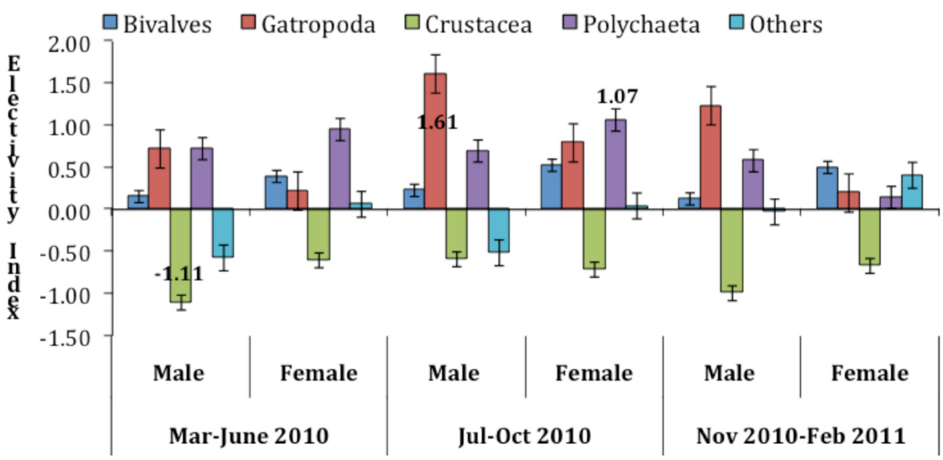

FIGURE 2. The electivity indices of different major food items consumed by *Carcinoscorpious rotundicauda* during March 2010- February 2011

region. Negative values of EI for crustacean samples clearly demonstrated that mangrove horseshoe crabs prefer considerably less number of crustaceans. Positive E1 value for grastropod might probably due to their higher abundance in the mangrove habitat compared to other macrobenthic community followed by polychaetes and bivalves (Lee 2008; Samidurai et al. 2011). It was apparent that during non monsoon seasons *C. rotundicauda* prefers lesser number of bivalves than polychaetes while during monsoonal period *C. rotundicauda* prefers higher number of bivalves than polychaetes which might probably due to their abundance in the mangrove sediment habitat (Mishra & Choudhury 1985). This observation might primarily due to their abundance. Polychaetes are more sensitive to the environmental stresses compared to bivalves and gastropods. Due to this reason, sensitive polychaetes could have been washed away during the monsoonal salinity stress while other macrobenthic community could withstand this condition. Though, horseshoe crabs are selective feeders, their adaptation to prefer wide variety of macrobenthic community (adaptive feeding) could have helped them in maintaining their population over the time (Botton 1984b). During the peak mating/nesting season (June - August 2010), male *C. rotundicauda* prey more on gastropods and female prefers polychaete worms. Unidentified organic matters in gut content analysis of *C. rotundicauda* showed high preference towards plant materials. As observed in *T. gigas*, dietary analysis revealed the presence of considerable amount of sand particles in *C. rotundicauda* gizzard stomach (Akbar John et al. 2011). Seasonal variations in food composition showed that mollusks form the main item especially gastropods as discussed above.

FEEDING INTENSITY

Monthly variation in sex related feeding intensity of *C. rotundicauda* showed that matured male horseshoe crabs (219.2-332.7 g body weight) fed more intensely than females (463.1- 890.8 g body weight). Maximum values of GaSI in male crabs were observed in April 2010 (3.00) followed by June-2010 (2.84) and March-2010 (2.65). Minimum GaSI in male was noted in January-2011 (1.5). Maximum values of GaSI in female crabs were observed in December 2010 (3.18) followed by June-2010 (1.5) and January-2011 (1.48). Minimum GaSI in female was noted in February-2011 (0.92) (Figure 3). The average intensity of feeding for matured male crabs during monsoon and non-monsoon seasons was 1.71±0.2 and 2.34±0.2, respectively. The mean intensity of feeding for matured female crabs during monsoon and non monsoon seasons were 1.71±1.01 and 1.32±1.01, respectively (Table 2). Overall, Gastro Somatic Index (GaSI) value showed that the intensity of feeding was not restricted to any particular month. However, the encountering of more food item in male crabs compared to the female ($p < 0.05$) might probably due to the low digestive enzymatic activity in male crabs during landward migration. Similar observation was also recorded in their closest conspecific (*T. gigas*) from Malaysian shoreline (Akbar John et al. 2011). There was an apparent fluctuation in the feeding intensity of female crabs throughout the sampling period. However, this does not affect their sizes collected during respective months.

The mechanism of feeding in horseshoe crab has been reported by many workers beginning with Lockwood (1970). The pieces of food, initially captured with the help of chelate walking legs, are grouped by chitinous gnathobase before their consumption (Sekiguchi & Shuster 2009). In the present study, plant materials were encountered in the gut in larger quantity. Similar observations have been reported previously; 90% of *L. polyphemus* consumed vascular plant and detritus (Botton 1984a). No significant difference in food consumption was noted between male and female horseshoe crabs ($p < 0.05$) which inturn

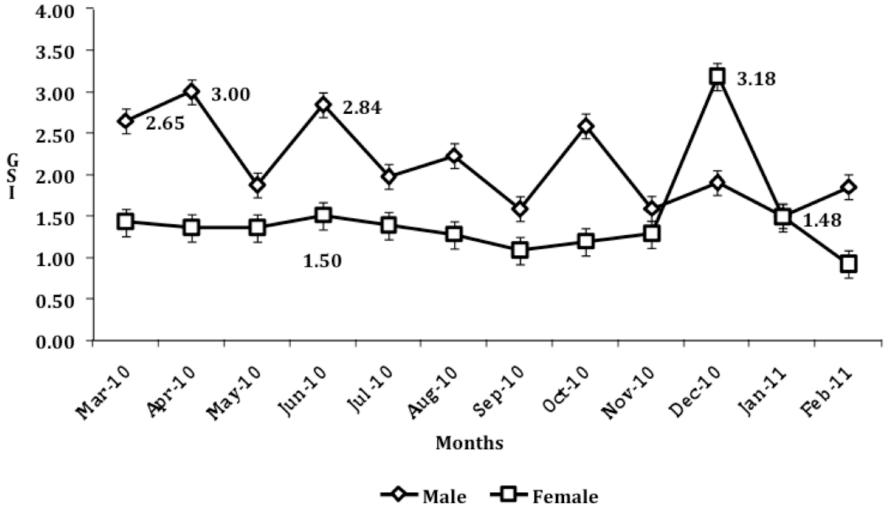

FIGURE 3. The values of Gastro Somatic Index (GSI) show the monthly variation in the intensity of feeding of male and female *Carcinoscorpius rotundicauda*

TABLE 2. Mean percentage composition of different food items in the gut of male and female *C. rotundicauda* during monsoon and non monsoon seasons

	Seasons		Food items encountered in *C. rotundicauda* gut					
			GaSI	Bivalve	Gastropod	Crustaceans	Polychaete	Others
Male	Non monsoon (N = 16)	Average	2.34	25.89	11.90	12.29	22.97	26.95
		Max	3.00	31.28	17.48	18.43	31.32	37.95
		Min	1.59	19.34	7.32	6.34	12.22	9.49
		SD	0.20	6.04	5.19	5.91	12.21	14.46
	Monsoon (N = 5)	Average	1.71	27.42	12.86	13.80	18.11	27.82
		Max	1.90	34.29	20.18	19.32	30.01	42.46
		Min	1.50	20.22	8.94	8.09	1.03	10.27
		SD	0.20	6.04	5.19	5.91	12.21	14.46
Female	Non monsoon (N = 16)	Average	1.32	22.37	17.08	12.47	26.98	21.10
		Max	1.50	27.09	25.42	22.32	38.09	32.16
		Min	1.08	19.24	11.42	1.49	12.93	4.07
		SD	1.01	7.39	5.09	4.12	13.84	18.68
	Monsoon (N = 5)	Average	1.71	20.44	21.65	11.37	23.01	23.54
		Max	3.18	30.11	28.54	16.48	32.34	51.42
		Min	0.92	12.42	17.42	6.39	2.42	12.94
		SD	1.01	7.39	5.09	4.12	13.84	18.68

proved their adaptable feeding behaviour. In contrast to its American conspecific, male *T.gigas* and *C. rotundicauda* tend to feed more intensely on differential food item during their migration towards the shore which could helped them in successful co-occurrence in Malaysian coast (Akbar John et al. 2011; Botton 1984a; Kamaruzzaman et al. 2011c). Similar observation was noted in horseshoe crab maintained in aquarium, whereby no significant difference in the electivity indices was observed (Botton 1984b). The increased food preferences during mating season might also be due to the increased availability of preferred food item in the nesting grounds.

CONCLUSION

Study on gut content analysis of mangrove horseshoe crabs (*Carcinoscorpius rotundicauda*) was in well accordance with previous report on the food preferences of its Indian and Mexican conspecifics. However, the selective food preference of mangrove horseshoe crabs showed that *C. rotundicauda* prefered lesser number of bivalves than polychaetes during non-monsoon period while it was the reverse during monsoon. Overall, the gut content analysis showed that the mangrove horseshoe crab prefered mollusks (especially gastropods) over other group of macrobenthos. Therefore, a study should be addressed to determine the influence of differential food item in improving the reproductive success and growth of horseshoe crabs. Due to the shrinking of horseshoe crab nesting grounds along the east coast of Peninsular Malaysia, a strong legislative step would help in maintaining their population along the coast.

ACKNOWLEDGEMENTS

This research was conducted with funding from the Ministry of Agriculture & Agro-based Industry, under the e-Science Fund and the IIUM Endowment Grant (EDW B 10-096-0435). The authors wish to express their gratitude to INOCEM Laboratory teams, Kulliyyah of Science for their invaluable assistance and hospitality throughout the sampling period.

REFERENCES

Akbar John, B., Kamaruzzaman, B.Y., Jalal, K.C.A. & Zaleha, K. 2011. Study on the Bionomics of Malaysian horseshoe crabs *Tachypleus gigas*. *International Symposium on Marine Ecosystems, Natural Products and Their Bioactive Metabolites*. 25 - 27 October, Bogor - Indonesia. p.137.

Alexander, S.A., Hobson, K.A., Gratto-Trevor, C.L. & Diamond, A.W. 1996. Conventional and isotopic determinations of shorebird diets at an inland stopover: the importance of invertebrates and *Potamogeton pectinatus* tubers. *Can. J. Zool*. 74: 1057-1068.

Botton, M.L. & Haskin, H.K. 1984. Distribution and feeding of the horseshoe crab, *Limulus polyphemus*, on the continental shelf of New Jersey. *Fish. Bull*. 82(2): 383-389.

Botton, M.L. 1984a. The importance of predation by horseshoe crabs, *Limulus polyphemus*, to an intertidal sand flat community. *J. Mar. Res*. 42(1): 139-162.

Botton, M.L. 1984b. Diet and food preference of the adult horseshoe crab, *Limulus polyphemus* in Delaware Bay, New Jersey, USA. *Mar. Biol*. 81: 199-207

Botton, M.L. & Shuster, C.N. 2004. Horseshoe Crabs in a Food Web: Who Eats Whom? In *The American Horseshoe Crab*, edited by C. N Shuster, R. B. Barlow & H. J. Brockmann, (eds.) Cambridge: Harvard Press.

Chatterji, A., Mishra, J. & Parulekar, A., 1992. Feeding behaviour and food selection in the horseshoe crab, *Tachypleus gigas*; (Müller). *Hydrobiol*. 246: 41-48.

Chatterji, A., Siddiqui, A.Q. & Khan, A.A. 1977. Food and feeding habits of Labeo gonius (Ham.) from the river Kali. *J. Bombay Nat Hist. Soc*. 75: 104-109.

Debnath, R. 1985. Studies on the distribution, abundance, breeding behaviour and feeding habit of Indian horseshoe crabs (Merostomata: Xiphosura) in the coastal water of Orissa and West Bengal, India. M. Phil. Diss. University of Calcutta, Calcutta (unpublished).

Debnath, R. & Choudhury, A. 1987. Studies on food preference and feeding habit of horseshoe crabs (Merostomata: Xiphosura) in the coastal waters of West Bengal, India *Internatial Symposium on Tropical Ecology Banaras Hindu Unit,* Varanasi, India

Gurr, E. 1965. *The Rational Use of Dyes in Biology*. Williams and Wilkins.

John, B.A., Jalal, K.C.A., Kamaruzzaman, Y.B. & Zaleha, K. 2010. Mechanism in the clot formation of horseshoe crab blood during bacterial endotoxin invasion. *J. App. Sci*. 10: 1930-1936.

Kamaruzzaman, B.Y., Akbar John, Jalal, K.C.A., Zaleha, K. 2011c.The *Living Fossil (Horseshoe crab)*. Kuala Lumpur: IIUM Publisher.

Kamaruzzaman, B.Y., Akbar John, B., Aqilah Megat, M.H. & Zaleha, K. 2011b. Bioaccumulation of heavy metals in Horseshoe crabs (*Tachypleus gigas*) from Pekan, Pahang, Malaysia. *Res. J. Environ. Toxicol*. 5 (3): 222-228.

Kamaruzzaman, B.Y., John, B.A., Zaleha, K. & Jalal, K.C.A. 2011a. Molecular phylogeny of horseshoe crab. *Asian J. Biotechnol*. 3: 302-309.

Kassim, Z., Shahuddin, H., Shaharom, F. & Chatterji, A. 2008. Abundance of three species of the horseshoe crab along the coast of Malaysia. *J. Bombay Nat. History Soc*. 105: 209-211.

Lee, S.Y. 2008. Mangrove macrobenthos: Assemblages, services and linkages. *J. Sea Res*. 59(1-2): 16-29.

Lockwood, S. 1970. The horseshoe crab. *American Narualist*. 4:257-274.

Mishra, A. & Choudhury, A. 1985. Polychaetes annalids from the mangrove swamps of Sundarbans, India. *Proceedings on the National Symposium on Biological Utilization and Conservation of Mangroves* pp. 448–452.

Novitsky, T.J. 1982. *Limulus* amoebocyte lysate: Live saving extract from horseshoe crab. *J. New England Aquarium* (A quasphere) 16: 26-31

Novitsky, T.J. 1984. Discovery to commercialization: The blood of the horseshoe crab. *Oceanus* 27(1): 13-18

Samidurai, K., Saravanakumar, A. & Kathiresan, K. 2011. Spatial and temporal distribution of macrobenthos in different mangrove ecosystems of Tamil Nadu Coast, India. *Environ. Monitor Assessment* pp. 1-18.

Sekiguchi, K. 1988. *Biology of Horseshoe Crabs*. Tokyo: Science House Co., Ltd.

Sekiguchi, K. & Shuster, C.N. 2009. Limits on the Global Disrribution of Horseshoe Crabs (Limulacea): Lessons Learned from Two Lifetimes of Observations: Asia and America. In *Biology and Conservation of Horseshoe Crab*s, edited by Tanacredi, J.T., Botton, M.L., Smith, D.R., Earle, S.A. Springer US pp. 5-24.

Shuster, C.N., Jr. 1982. A pictorial review of the natural history and ecology of the horseshoe crab, *Limulus polyphemus*, with reference to other Limulidae. In *Physiology and Biology of Horseshoe Crabs: Studies on Normal and Environmentally Stressed Animals*, edited by J. Bonaventura, C. Bonaventura, and S. Tesh New York: Alan R. Liss, Inc.

Squires, H. & Dawe, E. 2003. Stomach Contents of Snow Crab (*Chionoecetes opilio*, Decapoda, Brachyura) from the Northeast New found land Shelf. *J. Northw. Atl. Fish. Sci*. 32: 27-38.

Suzuki, K., Shiho, O., & Imahori, K. 1975. Fructose-diphophate Aldolase of Horseshoe Crab (*Tachypleus tridentatus*). *J. Biochem. 77*(2): 281-289.

Uys, W., Hecht, T. & Walters, M. 1987. Changes in digestive enzyme activities of *Clarias gariepinus* (Pisces: Clariidae) after feeding. *Aquaculture* 63(1-4): 243-250.

Yamasaki, T., Makioka, T., Saito, J. 1988. Morphology. In *Biology Of Horseshoe Crabs,* edited by K. Sekiguchi. Tokyo: Science House.

Zaleha, K., Kamaruzzaman, B.Y., John, B.A. & Ong, M.C. 2010. Cd, Cu and Pb Concentration Levels in Horseshoe Crab Nesting Grounds of Pahang Coast, Malaysia. J. *Biol. Sci*. 10: 790-794.

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Received: 18 August 2011
Accepted: 21 February 2012