Natural Diet of Blue Swimming Crab, *Portunus pelagicus* at Strait of Tebrau, Johor, Malaysia

(Diet Semula Jadi Ketam Renjong, Portunus pelagicus di Selat Tebrau, Johor, Malaysia)

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ABSTRACT

Knowledge of natural diet in Portunus pelagicus is essential to understand its nutritional requirements, its interactions with other organisms and its potential for culture. The natural diet of P. pelagicus was studied in Strait of Tebrau, Johor, Malaysia via gut content analysis and molecular analysis. A total of 30 identifiable fatty acids were detected in foregut content of P. pelagicus. Fatty acid $18:3\omega 3$ act as a mangrove detritus marker was found to be the most abundant in foregut content of both sexes of P. pelagicus and has higher value in female. PUFA was the main fatty acid found in foregut content of P. pelagicus and major contributed by fatty acid $18:3\omega 3$, $20:5\omega 3$ and $22:6\omega 3$. Marine animal's marker was found to be the dominant in foregut content of P. pelagicus and that indicated that marine animals were the main food source of P. pelagicus. In conclusion, this study showed that P. pelagicus is a primarily omnivores crab with preference of marine animal and with addition and/or incidental fed plant items.

Keywords: Dietary composition; fatty acid analysis; foregut contents; Portunus pelagicus

ABSTRAK

Pengetahuan mengenai makanan semula jadi ketam renjong, Portunus pelagicus adalah perlu dalam kajian keperluan nutrisinya, kaitannya dengan organisma lain dan potensi untuk menternaknya. Kajian makanan semula jadi P. pelagicus di Selat Tebrau, Johor, Malaysia dijalankan melalui kandungan analisis perut dan molekulnya. Sejumlah 30 asid lemak telah dikesan di dalam perut P. pelagicus. Asid lemak jenis $18:3\omega 3$ yang bertindak sebagai penanda sisa bakau telah dikesan sebagai yang terbanyak di dalam perut jantan dan betina P. pelagicus dan mempunyai nilai yang lebih tinggi dalam ketam betina. PUFA dikenal pasti sebagai asid lemak yang utama di dalam perut P. pelagicus dan penyumbang terbanyak asid lemak ialah $18:3\omega 3$, $20:5\omega 3$ dan $22:6\omega 3$. Penunjuk haiwan marin adalah yang paling banyak dikenal pasti di dalam perut P. pelagicus dan ini menunjukkan ia adalah makanan utama P. pelagicus. Kesimpulannya, kajian menunjukkan bahawa P. pelagicus adalah ketam omnivor dengan makanan utamanya adalah haiwan marin dan makanan tambahannya dengan/atau bahan-bahan daripada tumbuhan.

Kata kunci: Analisis asid lemak; kandungan perut; komposisi pemakanan; Portunus pelagicus

INTRODUCTION

Blue swimming crab, *Portunus pelagicus* is distributed throughout the coastal waters of tropical regions of the Indo-west Pacific region (Ikhwanuddin et al. 2012a). Locally known as 'ketam bunga' or 'ketam renjong', this species is an important income source for Malaysian fisherman community (Ikhwanuddin et al. 2012b). With wide geographical habitat, *P. pelagicus* consumes diverse variety of food sources accordingly (Chande & Mgaya 2004; Josileen 2011).

There are a few techniques that can be employed to study the natural diet of *P. pelagicus* such as gut content analysis and molecular analysis which is fatty acid composition. Some essential fatty acids cannot be synthesized by marine invertebrates (Meziane & Tsuchiya 2000). Thus, the fatty acid markers could determine the food sources consumed by the animals (Kharlamenko et al. 2001). Analysis of lipid markers can also be as a method for the study of food webs (Islam & Tsuchiya 2011).

The information on natural diet of *P. pelagicus* is important and will be useful for developing successful farming techniques for this species in the future. As the demand for *P. pelagicus* exceeds the production of crab fishery, it has indirectly developed the aquaculture industry recently (Ikhwanuddin et al. 2012a). The main objectives of this study were to determine the natural diet through foregut fullness, dietary composition and fatty acid composition of *P. pelagicus*.

MATERIALS AND METHODS

COLLECTION METHOD, DIETARY COMPOSITION AND FOREGUT FULLNESS

Crab samples were collected from Straits of Tebrau (1°22' N and 103°38' E), Johor, Malaysia, in December 2011. Carapace width (CW) and body weight (BW) of the crab samples were measured. Then, the crabs were

dissected to collect the foregut and used to determine fatty acid composition using fatty acid biomarker, dietary composition and foregut fullness.

The protocol used for determining the foregut fullness and dietary composition was modified from the method by Ikhwanuddin et al. (2009). Prior to dissection, each crab were thawed in water for about 30 min. Then, the crab foregut was removed for dissection and the fullness of its foregut was recorded using an index according to Ikhwanuddin et al. (2009). The foregut was divided into two parts which were one part used to determine dietary composition and another one part used to determine fatty acid composition. For each dietary analysis, each of the various dietary items was allocated to one of a number of broader dietary categories. The number of times that each dietary category was found in the foregut content of crabs was used to calculate its frequency of occurrence in the diets of crabs (de Lestang et al. 2000; Potter & de Lestang 2002).

FATTY ACID ANALYSIS

The fatty acid analyses were conducted following the Abdulkadir and Tsuchiya (2008). The freeze dried samples were ground using mortar and pestle and were stored in the freezer (-80°C). In order to carry out one-step method, extraction and etherification process are combined using a single tube. The powdered samples were weight and put in a 50 mL centrifuge tube. Later, each sample was mixed with 4 mL of hexane, 1 mL of internal standard solution, 2 mL of 14% Borontrifluoride (BF₃) in methanol and a magnetic stirring bar. The head space of tube was flushed with nitrogen gas and closed tightly with a Teflon-lined screw-cap, then heated on a hot plate at 100°C for 120 min under continuous stirring. After cooling to room temperature, 1 mL of hexane was added and followed by 2 mL of distilled water. The tube was shaken vigorously for 1 min and centrifuged for 3 min at 2500 rpm, which would result in two layers formed, where the upper part is the hexane layer containing the fatty acid methyl esters (FAMEs). The upper layer then was transferred into a clean sample vial using a Pasteur pipette to be injected into the gas chromatography (GC). The sample vial was labelled and stored in the freezer (-20°C). The samples was analysed using GC equipped with a Flame Ionization Detector (GC-FID) 6890N (PAgilent). The samples were analysed using DB-225Ms capillary column ($30m \times 0.25$ mm internal diameter, $0.25 \mu m$ film thickness), with temperature limits from 40 to 240°C. SupelcoTM 37 Component FAME Mix was used to determine the fatty acids. Helium gas was used as the carrier gas.

STATISTICAL ANALYSIS

The mean concentrations of total fatty acids were compared among the samples using one-way ANOVA to identify the significantly different mean values (p<0.05). The data were transformed using Kolmogorov-Smirnov to approximate normality. All statistics were performed using SPSS version 20 for Windows.

RESULTS

CRAB SAMPLES, DIETARY COMPOSITION AND FOREGUT FULLNESS

A 16 crabs samples sizes ranging from 10.87 to 13.37 mm in carapace width and only 10 samples have the foregut fullness index of 2, 3 and 4.

The specimens with these three foregut fullness index were used to examine the dietary composition. P. pelagicus specimens with foregut fullness index 1 (0-25% foregut fullness) were not examined for dietary composition as they were considered having empty foregut (Ikhwanuddin et al. 2009). The result showed that there were three different dietary categories (Table 1). The number of time that each dietary category was found in the foregut content of P. pelagicus was used to calculate its frequency of occurrence in the diets of P. pelagicus. The dietary categories found from 10 crabs' foregut content of P. pelagicus were decapods, eggs and unidentified material. The results showed that unidentified material was the most dominant dietary categories found in *P. pelagicus* with 90% of crab sampled. Then, followed by decapods with 20% of crab sampled. Figures 1 and 2 show the eggs and gill of crustaceans that have been found in foregut content of P. pelagicus.

Foregut fullness index 4 recorded the highest percentage at 87.50%. Figure 3 displays the overall recorded percentage (%) of foregut fullness index from 8 males and 8 females of *P. pelagicus*. The mean foregut

TABLE 1. Frequency of occurrence of different dietary categories and percentage of crab sampled from 10 crabs' foregut content of *P. pelagicus* from Johor coastal water

No.	Dietary categories	Frequency occurrence (Number of crab)		Percentage of crab sampled (%)	
		Male	Female	Male	Female
1	Decapods ¹	2	0	20	0
2	$Eggs^2$	0	1	0	10
3	Unidentified material	5	4	50	40

Note: 1 Decapods been identified based on the present of crustacean gills and parts of crab appendages (walking legs, swimming legs, carapace). 2 Eggs size = 305.0 μ m \pm 23.0 μ m

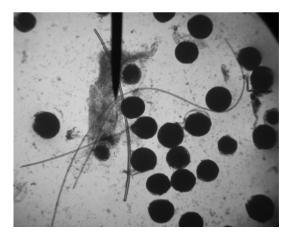


FIGURE 1. Eggs found in foregut content P. pelagicus



FIGURE 2. Gill found in foregut content of P. pelagicus

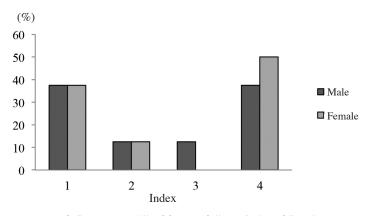


FIGURE 3. Percentage (%) of foregut fullness index of *P. pelagicus*

fullness index and mean carapace width for male of P. pelagicus were 2.50 ± 1.41 cm and 12.34 ± 0.96 cm and 2.63 ± 1.51 cm and 12.56 ± 0.75 cm for female of P. pelagicus. The results showed that the mean foregut fullness index and mean carapace width of female P. pelagicus are greater than those of male P. pelagicus.

FATTY ACID COMPOSITION

A total of 30 identifiable fatty acids were recorded in the foregut content of *P. pelagicus* (Table 2). Fatty acid 18:3ω3 was found to be the most dominant fatty acids in foregut content of both sexes of *P. pelagicus*. This fatty acid was detected higher in female of *P. pelagicus* with

219.4 g g⁻¹ dry weight compared with male with 195.4 g g-1 dry weight. The second dominant fatty acid was fatty acid 20:5ω3 and followed by fatty acid 16:0. The mean concentration of fatty acid was higher in female of P. pelagicus than in male in almost all fatty acids detected. Mean concentration (Table 3) of polyunsaturated fatty acid (PUFA) was the most abundant in foregut content of both sexes of P. pelagicus compared to saturated fatty acid (SAFA) and monounsaturated fatty acid (MUFA). PUFA was detected higher in female of P. pelagicus with 80.8 g g⁻¹ dry weight than in male with 71.0 g g⁻¹ dry weight. Fatty acid 18:3ω3 dominated the PUFA, which was 219.4 g g-1 dry weight in female and 195.4 g g-1 dry weight in male of P. pelagicus. Then, it was followed by MUFA, where the total mean concentration for both sexes of P. pelagicus was 83.8 g g-1 dry weight. Among the fatty acids in MUFA, fatty acid 18:1ω9 contributed the highest mean concentration with 74.5 g g⁻¹ dry weight in female and 48.6 g g⁻¹ dry weight in male of *P. pelagicus*. Figure 4 shows the concentration of fatty acid in SAFA, MUFA and PUFA in male and female of *P. pelagicus*. From the graph, it shows that female has the highest in mean concentration of all category of fatty acid. However, there is no significant different (p>0.05) between male and female of P. pelagicus in terms of SAFA, MUFA and PUFA. In both sexes of P. pelagicus, the most abundant food source that has been detected was marine animals (Figure 5). Longchain PUFA, fatty acid 20:5ω3, 22:6ω3 and ω3 which were marker for marine animals was detected highest in female of *P. pelagicus* than in male. Then, the second highest in mean concentration of fatty acid biomarker in P. pelagicus was mangrove detritus. Fatty acid 18:2ω6 and 18:3ω3 as marker for mangrove detritus was detected highest in female of P. pelagicus than in male. MUFA as marker for bacteria also was detected higher in female than in male of *P. pelagicus*. In term of fatty acid biomarkers, there was no significant different (p>0.05) between male and female of P. pelagicus in marine animals, mangrove detritus and also bacteria.

DISCUSSION

DIETARY COMPOSITION AND FOREGUT FULLNESS

Decapods, eggs and unidentified materials have been found in foregut content of *P. pelagicus*. Decapods were

TABLE 2. Fatty acid compositions (g g⁻¹ dry weight) in male and female of *P. pelagicus* from Johor coastal water. Value represents mean \pm SD, n= 8,- = not detected

Fatty acid	Male	Female
8:0	2.5 ± 1.8	2.3 ± 2.5
10:0	10.9 ± 8.7	13.7 ± 16.1
11:0	2.6 ± 0.8	3.9 ± 4.4
12:0	1.0 ± 0.4	4.9 ± 9.6
13:0	1.5 ± 0.8	2.1 ± 1.8
14:0	10.1 ± 7.1	15.7 ± 19.7
14:1	$3.4 \pm 0.0*$	$1.9 \pm 0.0*$
15:0	1.7 ± 0.9	2.7 ± 2.6
16:0	68.5 ± 21.3	92.6 ± 94.0
16:1	28.3 ± 11.4	48.0 ± 66.2
17:0	4.6 ± 2.0	5.1 ± 4.2
17:1	$1.4 \pm 0.0*$	$8.8 \pm 0.0*$
18:0	61.0 ± 12.7	79.4 ± 75.4
18:1w9	48.6 ± 11.4	74.5 ± 81.6
18:2w6	8.8 ± 5.2	6.9 ± 9.7
18:3w6	$2.9 \pm 0.0*$	$9.4 \pm 0.0*$
18:3w3	195.4 ± 74.6	219.4 ± 271.3
20:0	3.7 ± 2.2	6.5 ± 6.6
20:1	$4.6 \pm 0.0*$	$7.0 \pm 0.0*$
20:2	4.6 ± 2.7	$5.2 \pm 0.0 *$
20:3w6	-	$4.8 \pm 0.0 *$
20:3w3	44.0 ± 8.8	40.6 ± 16.0
21:0	-	$1.8 \pm 0.0*$
20:4w6	-	$1.2 \pm 0.0*$
20:5w3	82.8 ± 18.4	99.3 ± 76.9
22:0	± 0.0*	$4.3 \pm 0.0*$
22:2	± 0.0*	-
23:0	± 0.0*	$7.0 \pm 0.0*$
22:6w3	± 15.7	52.1 ± 34.5
24:1	± 0.0*	-

Note: * = Fatty acid only been detected in one sample of *P. pelagicus*

TABLE 3. Fatty acid compositions for fatty acid (g g^{-1} dry weight) in male and female of P .
pelagicus from Johor coastal water. Value represents mean \pm SD, n = 8, - = not detected

	Male	Female	
ΣSAFA	17.9 ± 26.5	25.8 ± 52.5	
Σ MUFA	31.3 ± 20.0	52.5 ± 69.9	
Σ PUFA	71.0 ± 72.9	80.8 ± 141.0	
ω3	371.9 ± 117.5	411.3 ± 398.6	
ω6	11.8 ± 5.2	24.5 ± 10.3	
$\omega 3 + \omega 6$	383.7 ± 122.7	435.8 ± 408.9	
18&20PUFA	338.6 ± 109.7	388.9 ± 374.4	
20:5ω3	82.8 ± 18.4	99.3 ± 76.9	
22:6ω3	49.7 ± 15.7	52.1 ± 34.5	
$18:2\omega 6 + 18:3\omega 3$	108.3 ± 109.9	129.3 ± 226.5	
Long-chain PUFA	183.9 ± 45.6	203.1 ± 127.3	

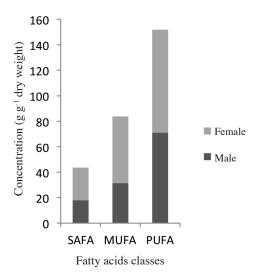


FIGURE 4. Concentration (g g⁻¹ dry weight) of fatty acid classes SAFA, MUFA and PUFA in male and female of *P. pelagicus* from Johor coastal water

consumed by male of *P. pelagicus* with percentage of crab sampled was 20% (Table 1). Ikhwanuddin et al. (2009) reported P. pelagicus consumed on a large amount of decapods with 25.86%. Decapods were determined based on the present of crustacean gill (Figure 2) and also part of appendages. The parts of appendages found in foregut content were walking legs, swimming legs and carapace. The present of this part of appendages may indicate this P. pelagicus is cannibalistic (Josileen 2011). Josileen (2011) also reported that P. pelagicus was consuming exuviae of other crabs during several occasions. Cannibalism occurs as a result of limited of the food availability in a particular area (Marshall et al. 2005). The mean size of eggs diameter found was 305.0 µm (Table 1). Ikhwanuddin et al. (2011) reported that, the mean size of eggs diameter of P. pelagicus was 383.6 µm and indicated that the eggs found in the present study were the eggs of P. pelagicus based on the similar size of eggs diameter. Unidentified material was found abundant in foregut content of P. pelagicus. P. pelagicus have been digested well the food

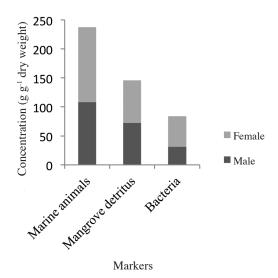


FIGURE 5. Concentration (g g $^{-1}$ dry weight) of markers marine animals, mangrove detritus and bacteria in male and female of coastal water *P. pelagicus* from Johor Value represents mean \pm SD

and it was difficult to be identified. Ikhwanuddin et al. (2009) also found that unidentified material as one of the most dominant dietary categories in the foregut content of *P. pelagicus*. De Lestang et al. (2000) found that less than 5% to the total volume of stomach contents of all inter-moult *P. pelagicus* in Peel–Harvey and Leschenault estuaries was contributed by unidentified material.

Foregut fullness percentage (%) that has been found in *P. pelagicus* was highest in foregut fullness Index 4 which means with full foregut (Figure 3). The results showed that, most of the foregut content of *P. pelagicus* was full. According to Kangas (2000), prior to and during molting, *P. pelagicus* cease feeding. Thus, the *P. pelagicus* in the present study were not at the stage of molting very soon or were molting. The foregut fullness Index 4 was recorded most from female of *P. pelagicus*. The mean foregut fullness index of female *P. pelagicus* also was greater than those of male *P. pelagicus*. However, the foregut fullness index did not affected by sexes of *P. pelagicus*.

FATTY ACID COMPOSITION

P. pelagicus has been collected at the coastal water of Gelang Patah, Johor and fatty acid 18:3ω3 was found to be the most abundant in foregut content of *P. pelagicus* (Table 2). In other study by Gunstone et al. (1994), it was stated that, fatty acid 18:3ω3 is an essential component of lipids in stems, leaves and roots. Fatty acid 20:5ω3 which was the second highest concentration detected in foregut content of P. pelagicus is one of the most important fatty acids and widely found in fish oils and algae (Christie 2011). Palmitic acid (16:0) also among the highest detected in P. pelagicus as this fatty acid was found in considerable amounts in the lipids of an animals (Christie 2011). PUFA was the main fatty acid found in foregut content of *P. pelagicus* with mean concentration of both sexes was 151.8 g g-1 dry weight (Figure 4). Fatty acid $18:3\omega 3$, $20:5\omega 3$ and $22:6\omega 3$ of PUFA were the major contributors to the highest concentration detected. PUFA of $\omega 3$ also was the most contributed to the highest concentration detected in foregut content of *P*. pelagicus. PUFA of ω3 and ω6 acids are essential dietary factors for all animals (Sargent et al. 1990). PUFA was not significantly differences between male and female. However, the study by Ayas and Özoğul (2011), showed that there was significant different in fatty acid 20:5ω3 and $22:6\omega 3$ in carapace meat of both sexes of *P. pelagicus*. In foregut content of P. pelagicus, MUFA was detected as the second highest in concentration (Figure 4). Oleic acid (18:1ω9) was identified as a primary MUFA in the foregut content of both sexes of P. pelagicus. In most animal fats, oleic acid has been the major component of fatty acid (Gunstone et al. 1994). Fatty acid 20:1 still able to be detected in *P. pelagicus* as this fatty acid play an important role in water transport and osmoregulation (Freas & Grollman 1980). In the present study, there was no significant differences of MUFA between male and female of P. pelagicus. Ayas and Özoğul (2011) found that, palmitoleic acid (16:1), oleic acid (18:1ω9) and cis-7-octadecenoic acid of MUFA in carapace meat of female P. pelagicus were significantly different with the male. SAFA was the less fatty acid been detected in foregut content of *P. pelagicus* (Figure 4). Fatty acids 16:0 and 18:0 of SAFA were the two most dominant fatty acids detected and this result was also the same in the study by Wu et al. (2010). Palmitic acid usually detected most in the marine animal source (Soundarapandian & Singh 2008) and also detected highest in *P. pelagicus*. Between male and female, there is no significant differences in terms of SAFA in foregut content.

In the study by Ayas and Özoğul (2011), there was also no significant differences in SAFA in carapace meat male and female *P. pelagicus*. Thus, the present study showed that, there was no significant differences between male and female of *P. pelagicus* in terms of SAFA, MUFA and PUFA. It can be inferred that the source of these fatty acid was the same in both habitat of male and female of *P. pelagicus*. The foregut content of *P. pelagicus* has been

analysed in the present study. Through the analysis of the foregut content, it only determined the food which has been ingested but not assimilated. Thus, the food that has been identified in the present study did not determine the natural diet of *P. pelagicus*.

The results of fatty acid concentration in the present study showed there were spread out over a large range of values. Marine animals were the most abundant of food source that have been found in foregut content of P. pelagicus (Figure 5). The highest concentration detected in this marker indicated that P. pelagicus consumed many marine animals. Long-chain PUFA (LC-PUFA), fatty acids $20.5\omega 3$, $22.6\omega 3$ (Graeve et al. 1997; Gunstone et al. 1994) and $\omega 3$ (Ackman 2002) can be used as the marine animals markers. Ackman (2002) stated that w3 fatty acids always present in fish flesh and are important to reduce hypertension and cholesterol absorption (Wu et al. 2010). Wu et al. (2010) reported that, fatty acid 20:5ω3 was dominant in P. pelagicus which was also found dominantly in edible tissue of brown crabs, Cancer pagurus (Barrento et al. 2010). The marine animals consumed by P. pelagicus consist of decapods, gastropods, bivalves, teleost and polychaetes (Ikhwanuddin et al. 2009). As P. pelagicus has been found consumed on a variety of marine animals, Chande and Mgaya (2004) suggested that, P. pelagicus population will not be affected if the availability of one prey group was reduced.

Mangrove detritus was the second highest concentration of fatty acid marker that has been detected in foregut content of *P. pelagicus* (Figure 5). The abundant of mangrove detritus as a result from the animal itself which unable to continue further desaturation of the fatty acids $18:1\omega7$, $18:1\omega9$, $18:2\omega6$ and $18:3\omega3$ (Bachok et al. 2003). The habitat of P. pelagicus which is near mangrove areas (FAO 2012) also influenced the abundance mangrove detritus detected in the foregut content. Study by Bachok et al. (2003) concluded that, the present of fatty acids $18:1\omega7$, $18:1\omega9$, $18:2\omega6$ and $18:3\omega3$ can indicated the sources of mangrove detritus. However, in the present study, only fatty acids 18:2ω6 and 18:3ω3 been detected in foregut content of P. pelagicus and were used as mangrove detritus marker. The primary source of mangrove detritus is leaf litter which fuels the ecosystem of mangrove (Tomascik et al. 1997). Once the mangrove detritus was eaten by P. pelagicus it can be detected by the mangrove marker as the mangrove marker signatures can persist on time scales of millions of years (Saint-Paul & Schneider 2010). Bacteria were the lowest concentration that has been detected in foregut content of *P. pelagicus* among the food sources (Figure 5). Fatty acid 18:1ω7 and odd-BrFAs are the main fatty acid in bacteria (Bachok et al. 2003). In the marine food webs, these fatty acids have been used as bacteria markers (Kharlamenko et al. 1995). However, in the present study, these fatty acids were not detected in foregut content of P. pelagicus. Bacteria have been detected in the foregut

content of P. pelagicus due to the presence of mangrove leaves. Bacteria may be qualitatively important, although they may be not quantitatively significant in the diet of invertebrates (Wright & Covich 2005). Alongi (1997) stated that, decomposition of mangrove litter and detrital material is a microbially mediated process. Thus, there are high bacterial densities and productivity as supported by the decomposing leaf material and fine particulate and dissolved organic matter in the mangrove soil (Tomascik et al. 1997). In all the markers that were used, the concentration in female of P. pelagicus was higher than in the male. The statistical analysis showed that there was no significant differences between male and female of P. pelagicus in terms of type of food source which were marine animals, mangrove detritus and bacteria. The food that was consumed by male and female did not give much difference in concentration of fatty acid detected. No difference in quantity of the food consumed by male and female also been reported by Josileen (2011).

CONCLUSION

In conclusion, 30 fatty acids were detected in the foregut content of *P. pelagicus*. PUFA was the most abundant in foregut content of *P. pelagicus* compared with SAFA and MUFA. The marine animal's marker was the highest in concentration detected in foregut content of *P. pelagicus*. The presence of decapods indicated that *P. pelagicus* is cannibalistic. This studies showed that *P. pelagicus* is a primarily omnivores crab with preference of marine animal and with addition and/or incidental fed plant items.

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