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Identification and Determination of the Fatty Acid Composition of *Portunus pelagicus* in Setiu Wetland Areas, Terengganu, Malaysia by GC-MS

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Abstract: A study was conducted to examine the fatty acid compositions at the early larval stages of blue swimming crab, *Portunus pelagicus* in wild at Setiu Wetland, Terengganu, Malaysia. The larvae were collected by towing across Setiu Wet Land Estuary using plankton net until adequate to do fatty acid analysis. The methods used in these experiments were one-step method. Fatty acid composition was analysed by gas chromatography-mass spectroscopy (GC-MS). The results showed that the fatty acids profiles were significant difference between Zoel stages ($p<0.05$). For Zoea 1 and Zoea 2, MUFA component was highest with concentration 5.45±0.52 mg g^{-1} dry weight and 9.8±0.23mg g^{-1} dry weight. While in Zoae 3 and Zoea 4, PUFA component showed the highest with concentration 14.89±0.37 mg g^{-1} dry weight and 10.54±1.81mg g^{-1} dry weight respectively. Fatty acids concentration of *P. pelagicus* larvae seemed to change as long as larvae metamorphosis from Zoea 1 to Zoea 4. Finally, measurement of fatty acid composition will provide valuable insights for better understanding of larval nutrition requirements of *P. pelagicus*.

Key words:*Portunus pelagicus* · Fatty Acids · Gas Chromatography-Mass Spectrometer · Setiu Wetland · Zoea

INTRODUCTION

Blue swimming crab, *Portunus pelagicus* is distributed throughout the coastal waters of tropical areas of the Indo-west Pacific region [1]. Locally known as 'ketam bunga' or 'ketam renjong', this species is an important income source for Malaysian fisherman community [2]. Recently, *P. pelagicus* has many attractive advantages as a model of Portunid crab species for fisheries, culture or scientific studies, [3-13]. This species has fast growth rate, high fecundity and relatively short larval duration. Furthermore, this species is an important source of animal protein and a favorite recipe in South East Asia and other parts of the world.

Fatty acids are important as nutritional substances and metabolites in living organisms. Many kinds of fatty acids play a vital role in the regulation of variety of physiological and biological functions. Biochemical tracer methods such as stable isotope or fatty acid (FA) analysis have the advantage of potentially providing less biased longer-term dietary information [14]. All marine animals contain *n*-3 polyunsaturated fatty acids (PUFA), which originate from both phytoplankton and symbiotic microorganisms, depending on solar energy [15] and from unique deep-sea barophilic bacteria [16, 17]. In particular, marine animals contain high levels of long chain *n*-3 PUFA, such as docosahexaenoic acid (DHA, 22:6*n*3) and Eicosapentaenoic acid (EPA, 20:5*n*3), in both their depot and tissue lipids [15, 18]. Similarly, *n*-6 PUFA, such as linoleic acid (LA, 18:2*n*6) and arachidonic acid (ARA, 20:4*n*6), are generally vital for terrestrial animals [19]. Either *n*-3 or *n*-6 PUFA usually dominates in marine or terrestrial animals, especially in their cell membrane phospholipids.

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Identifying the diets of animals is important for understanding their basic ecology, characterizing tropic interactions and predicting community level consequences of biotic and a-biotic change. In the marine environment, direct observation of feeding is often impractical or impossible, so ecologists have developed indirect methods for examining diet. Literature reviews showed that, there are no studies been done to investigate the FA composition of *P. pelagicus* larvae. From this study, the knowledge of FA in *P. pelagicus* larvae is useful to understand its nutritional requirements in each larval stage, its interactions with other organisms and will be useful for developing successful farming techniques for this species in the future. Thus, the objectives of this study are to investigate the FA composition of *P. pelagicus* larvae sampled from natural habitat of Setiu Wetland, Terengganu.

MATERIALS AND METHODS

Biological Sampling: In this study, the sampling was carried out at Setiu Wetland waters, Terengganu (102'46E; 5'39N) (Figure 1). The planktonic larvae were collected using serial filtration net with mesh size of 500µm, 200µm, 60µm and 20µm respectively. Detail of the biological sampling trips please refers to Table 1. Boat was used to tow across Setiu Wetland Estuary for 1 km. The 500µm mesh size was used to filter out the debris and unwanted elements. The collection of sample was according to depth; 5m and 15 m where was done twice providing two replicates for each depth per station. The net was wash thoroughly to make sure the maximum sample fill in at the end of the collector of the net. Towing was done until sample adequate at 200mg dry weight [20] to do FA analysis. Then, the sample was transferred into 250mL bottle with labelling. The larvae were separated according to Zoeal stages (Zoea 1 till Zoea 4). All the crabs larvae were distinguish individually by using pipette under compound microscope. Only *P. pelagicus* species was used and selected by observed their characteristic and morphology according to Arshad *et al*. [21]. The sample contains larvae were stored under deep freezer (-20°C) and were brought to laboratory to do FA analysis.

Sample and Data Analysis: After each larval Zoeal stage (Z1, Z2, Z3 and Z4) of samples were stored in deep-freeze for 48h, the samples were homogenized, freeze dried for 24h, then stored at - 40°C and finely ground before been analyzed. All chemicals used (hexane, chloroform, methanol and 14% BF_3 in methanol) were of analytical reagent grade for GC (Kanto Chemical Co. Inc. Japan). Non adecanoic acid (19:0) (Supelco Inc. USA) was used as an internal standard. The internal standard solution was prepared by dissolving100mg of 19:0 in 100 ml of hexane to obtain a final concentration of 1 mg/ml of 19:0. FA concentrations (C_{FA}, mg/g of dry sample) were calculated by comparing the peak area of FA in the sample with the peak area of internal standard as follows this equation:

$C_{FA} = A_S/A_{IS} \times C_{IS}/W_S$

$A_{S:}$ = Peak area of FA in the sample in chromatogram

$A_{IS:}$ = Peak area of internal standard in chromatogram

$C_{IS:}$ = Concentration of internal standard (mg)

$W_{S:}$ = Weight of sample (g)

Qualitatively (as a percentage), composition of individual FA was calculated by comparing the peak area of each FA with the total peak area of all FA in the sample. To ensure esterification was complete, the final extracts of samples were examined by Thin-Layer Chromatography (TLC) using Merck plates coated with kieselgel 60 silica (Darmstadt, Germany). The developing solvent was a mixture of hexane, diethylether and acetic acid (70:30:1). The TLC plate was immersed in phosphoric acid: 33% acetic acid:sulfuric acid:0.5% copper sulfate (5:5:0.5:90;v:v:v:v) and then dried and heated to visualize the spot of FA Methyl Ester (FAME).

The One-step Method Procedure for Fatty Acid Analysis: The one-step method procedures were principally carried out by combining the extraction and esterification processes using a single tube. Three replicates of each sample ±200mg were mixed with 4ml of hexane and 1ml of internal standard (Nonadecanoid acid) solution in a 50ml centrifuge tube. After adding 2ml of 14% BF_3 in methanol and a magnetic stirring bar, the head space of the tube was flushed with nitrogen gas and then closed tightly with a Teflon-lined screw-cap. The capped tube was heated on a hot plate at 100°C for 120 min under continuous stirring. After cooling to room temperature 27°C, 1ml of hexane was added followed by 2ml of distilled water. The tube was shaken vigorously for 1 min and centrifuged for 3 min at 2500 rpm (650 ×g). Of the two phase's which formed, the upper phase was hexane layer

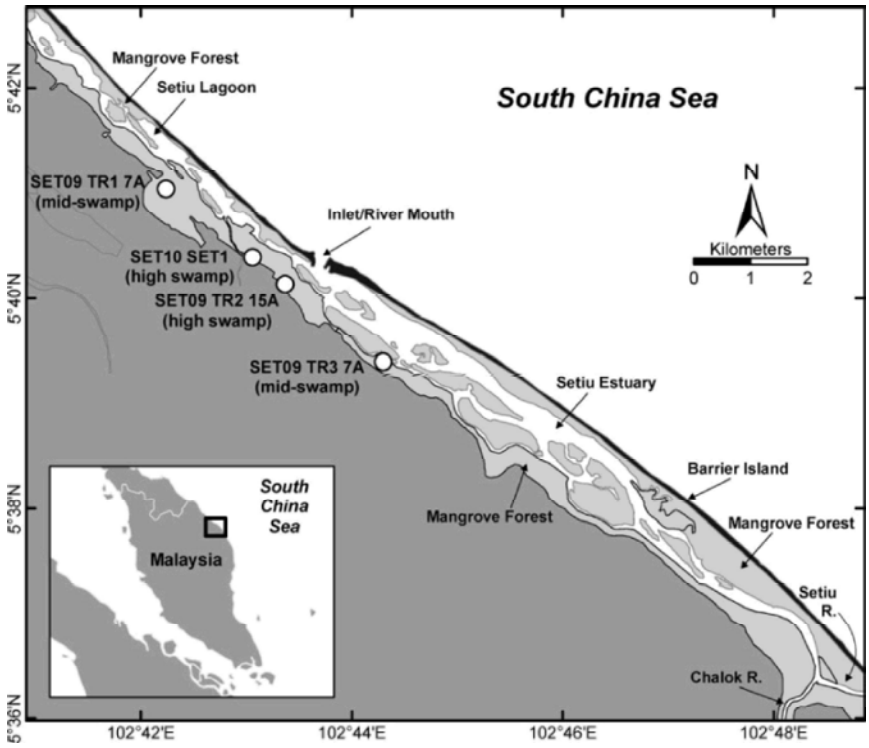

Fig. 1: Location of the biological sampling trips at Setiu Wetland Estuary, Terengganu, Malaysia.

Table 1: Details of the biological sampling trips at Setiu Wetland Estuary, Terengganu, Malaysia.

Sampling Trip	Date	Time	Towing Period	Location
1	15/8/2012	1015 h	20 min	Nearby seagrass area
2	3/10/2012	2300 h	30 min	Along muddy flat bottom area
3	4/12/2012	0915 h	25 min	Nearby mangrove creek
4	13/2/2013	1430 h	30 min	Nearby Seabass fish cage
5	9/4/2013	1730 h	35 min	Along estuary (many floating wastes)
6	7/6/2013	1100 h	30 min	Nearby fisherman jetty

containing the FAME's. Finally, ~1-2ml of the hexane layer was transferred using a Pasteur pipette into a clean sample vial to be injected into the GC for FAME analysis. In the experiment, the one-way statistical analyses were performed by using SPSS version 17.0. This comparison was displayed in graph form. All results will be presented as means ± SD The difference will be displayed as statistically significant when $p<0.05$.

RESULTS

Fatty Acid Characteristic of *P. Pelagicus* Larvae Stages

***Fatty Acid Composition*:** The stearic acid C18:0, SAFA component showed a broader range of fatty acid composition in Zoea 1. While in Zoea 2 the most dominant SAFA component was palmitic acidC16:0. In the other hand, Eicosapentaenoic acid (EPA) C20:5*n*3, showed the highest PUFA component in Zoea 3.In Zoea 4, Linoleic acid C18:2*n*6*c* showed the most dominant PUFA composition in these experiments (Table 2).

Concentration of Total Fatty Acids (TFA): Concentration of TFAin wild sampling in all larval stages were significantly ($P<0.05$) differ. The highest concentration of TFA were detected in Zoea 2, followed by Zoea 3, Zoea 4 and Zoea 1 with concentration 24.34±5.16 mg g^{-1} dry weight,18.31±1.21 mg g^{-1} dry weight, 14.3±2.49 mg g^{-1} dry weight and 13.57±1.21 mg g^{-1} dry weight, respectively (Table 2, Figure 2).

Fatty Acid Classes:

Saturated FA (SAFA), Monounsaturated FA (MUFA) and Polyunsaturated FA (PUFA): Stearic acid C18:0 was dominant FA component in Zoea 1. In Zoea 2, palmitic

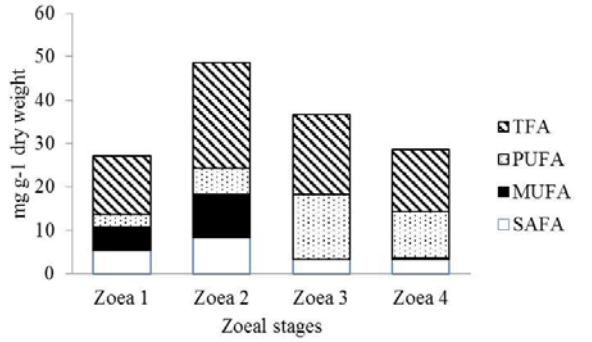

Fig. 2: Fatty acid concentration of P. pelagicus larvae at Setiu Wetland, Terengganu.

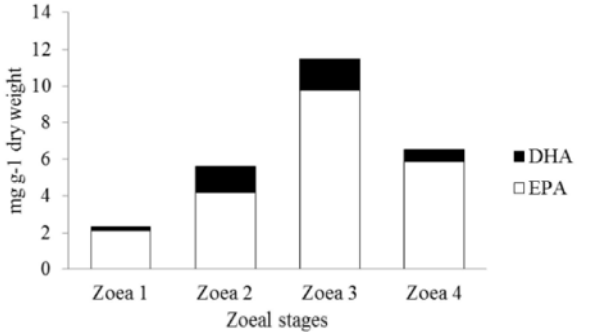

Fig. 3: EPA and DHA concentration of P. pelagicus larvae at Setiu Wetland, Terengganu.

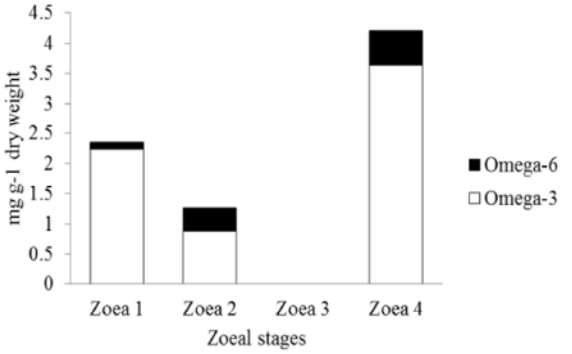

Fig. 4: Omega-3 and -6 concentrations of P. pelagicus larvae at Setiu Wetland, Terengganu.

acid C16:0 was highest FA composition. While in both Zoea 3 and Zoea 4, the most dominant FA component was heptadecanoic acid C17:0 (Table 2, Figure 2). The oleic acid C18:1*n*9*c* were most dominant FA component in both Zoea 1 and Zoea 2. While in Zoea 3, no MUFA component was found. In the other hand, only cis-10-Heptadecenoic acid C17:1 was found in Zoea 4 (Table 2, Figure 2). The most dominant PUFA component in Zoea 1, Zoea 2 and Zoea 3 was eicosapentaenoic acid

Table 2: Fatty acids characteristic (mg $^{-1}$ dry weight) in wild sampled of P. Pelagicus larvae at different larvae stages. Values are mean±S.D, n=3.

Wild Sampling P. Pelagicus at Different Larval Stages				
Fatty acid	Zoea 1	Zoea 2	Zoea 3	Zoea 4
C12:0	0.1±0.02	0.12±0.01	-	-
C13:0	0.06±0.01	0.11±0.01	-	0.12±0.03
C14:0	0.78±0.06	0.87±0.01	1.07±0.03	0.9±0.08
C14:1	-	0.04±0.00	-	-
C15:0	0.6±0.03	0.54±0.03	0.89±0.16	0.92±0.35
C16:0	0.16±0.00	5.08±4.32	-	0.48±0.05
C16:1	2.11±0.15	3.49±0.07	-	-
C17:0	0.43±0.21	1.09±0.05	1.46±0.65	0.96±0.11
C17:1	0.18±0.03	0.37±0.02	-	0.38±0.06
C18:0	2.8±0.21	0.22±0.01	-	-
C18:1n9c	2.55±0.17	4.18±0.09	-	-
C18:1n9t	-	0.08±0.01	-	-
C18:2n6t	0.14±0.03	-	-	-
C18:2n6c	-	0.37±0.01	3.44±0.07	4.02±0.49
C18:3n3	-	0.22±0.22	-	0.96±0.48
C18:3n6	0.06±0.01	-	0.26±0.01	-
C20:1	0.52±0.13	1.64±0.04	-	-
C20:2	0.1±0.02	0.09±0.01	-	-
C20:3n3	-	-	0.85±0.01	-
C20:3n6	0.06±0.01	-	-	-
C20:4n6	0.61±0.03	1.43±0.02	1.4±0.02	0.67±0.2
C20:5n3	1.66±0.05	2.44±0.18	7.06±0.17	3.75±0.5
C21:0	0.05±0.01	0.06±0.00	0.85±0.01	-
C22:0	0.21±0.01	0.37±0.03	0.79±0.01	-
C22:6n3	0.49±0.03	1.52±0.02	1.88±0.09	1.14±0.14
C23:0	0.11±0.01	-	-	-
C24:0	-	-	-	0.2±0.04

(EPA) C20:5*n*3. While, in Zoea 4 linoleic acid C18:2*n*6*c* showed the most dominant FA composition (Table 2, Figure 2).

The n-3/n-6 Ratio: Concentration of *n*-3 PUFAs was significant higher (p<0.05) to concentration of *n*-6 PUFAs (Table 1). The *n*-3 PUFAs predominantly over *n*-6 PUFAs in the most various *P. pelagicus* larvae stages of presence study (Table 2).

Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA): The highest EPA level was in Zoea 3 followed with Zoea 4, Zoea 2 and Zoea 1 with concentration 7.06±0.17mg g^{-1} dry weight, 3.75±0.5mg g^{-1} dry weight, 2.44±0.18mg g^{-1} dry weight and 1.66±0.05mg g^{-1} dry weight respectively. The most dominant DHA level was in Zoea 3 followed with Zoea 2, Zoea 4 and Zoea 1 with concentration 1.88±0.09mg g^{-1} dry weight, 1.52±0.02mg g^{-1} dry weight, 1.14±0.14mg g^{-1} dry weight and 0.49±0.03mg g^{-1} dry weight.

Omega-3 and Omega-6: The highest omega-3 concentration were in Zoea 3 followed by Zoea 4, Zoea 2 and Zoea 1 with concentration 9.79%, 5.85%, 4.18% and 2.16% respectively of TFA concentration (Table 2, Figure 4). The most dominant omega-6 concentration were in Zoea 3 followed by Zoea 2, Zoea 4 and Zoea 1 with concentration 1.66%, 1.43%, 0.67% and 0.18% respectively of TFA (Table 2, Figure 4).

DISCUSSION

Fatty Acid Composition Changes for Wild Larvae: FA 18:0 in Zoea 1 and 16:0 in Zoea 2 of SAFA were the two most dominant FA detected (Table 2) and this result was also the same in the study by Wu *et al*. [22]. Palmitic acid (16:0) was found in considerable amounts in the lipids of an animal [23]. It also usually detected most in the marine animal source [24] and detected highest in *P. pelagicus* larvae while roles for 16:0 and 18:0 have been suggested in supporting metabolic functioning rather than as a direct energy source [25]. In the other hand for Zoea 3, FA 20:5*n*3 was dominant. It is one of the most important FA and widely found in fish oils and algae [23]. For Zoea 4, the most dominant FA was Linoleic acid.

From this study, small amount of FA 18:3*n*3 was detected in Zoea 2 and Zoea 4 (Table 2). In other study by Gunstone *et al.* [26], it was stated that, FA 18:3*n*3 is an essential component of lipids in stems, leaves and roots. In this study, oleic acid (18:1*n*9) was detected as second dominant of MUFA group in Zoea 1. In most animal fats, oleic acid has been the major component of FA Gunstone *et al.* [26]. In the other hand, FA 20:1 was detected in Zoea 2 of *P. pelagicus* larvae as this fatty acid plays an important role in water transport and osmoregulation [27]. In Zoea 3, both EPA and DHA were dominant (Table 2). DHA and EPA are essential compounds required for cell membrane formation, osmoregulation, the synthesis of prostaglandins and they also appear to have an activating role in the immune system [28]. The results of FA concentration in the present study showed there were spread out over a large range of values. The highest concentration detected in this marker indicated that *P. pelagicus* larvae consumed many marine animals. Long-chain PUFA (LC-PUFA), fatty acids 20:5*n*3, 22:6*n*3 [29], Gunstone *et al.* [26] and *n*-3, [30] can be used as the marine animals markers. Ackman, [30] stated that *n*-3 fatty acids always present in fish flesh and are important to reduce hypertension and cholesterol absorption, [22]. Wu *et al.* [22] reported that, fatty acid 20:5*n*3 was dominant in *P. pelagicus* which was also found dominantly in edible tissue of brown crabs, *Cancer pagurus* [31].

The abundant of mangrove detritus as a result from the animal itself which unable to continue further desaturation of the fatty acids 18:1*n*7, 18:1*n*9, 18:2*n*6 and 18:3*n*3 [32]. The habitat of *P. pelagicus* larvae which is near mangrove areas FAO [33] also influenced the abundance mangrove detritus detected in the FA composition. Study by Bachok *et al.* [32] concluded that, the present of fatty acids 18:1*n*7, 18:1*n*9, 18:2*n*6 and 18:3*n*3 can indicate the sources of mangrove detritus. In the present study, only FA 18:1*n*9, 18:2*n*6 and 18:3*n*3 been detected in FA composition of *P. pelagicus* larvae and were used as mangrove detritus marker. The primary source of mangrove detritus is leaf litter which fuels the ecosystem of mangrove [34]. Once the mangrove detritus was eaten by *P. pelagicus* larvae it can be detected by the mangrove marker as the mangrove marker signatures can persist on time scales of millions of years [35]. FA 18:1*n*7 and odd-BrFAs are the main fatty acid in bacteria [32]. In the marine food webs, these FA have been used as bacteria markers [36]. However, in the present study, these FA were n ot detected in FA composition of *P. pelagicus* larvae. FA changes that occur during moulting cycles are an overriding physiological factor determining condition, especially in larval and juvenile crustaceans that have low energy reserves [37,38]. Our study indicates that *P. pelagicus* larvae proximate composition of TFA undergo changes in association with ecdysis.

The study by Muller *et al*. [39] show that, highest concentration of SAFA, MUFA and PUFA mostly in Zoea 2 and Zoea 3 showed that they used this fatty acid as energy to moult the next stage like metamorphosis. In the wild, DHA, EPA and AA are synthesized by primary producers and are concentrated as they move through the food web to higher level consumers [40].While crab larvae may consume microalgae, their nutritional value is relatively low compared with the rotifers or *Artemia* that the larvae consume. Study by Sargent [41] showed that in natural habitat, wild crab larvae feed on marine zooplankton, by feeding on phytoplankton, zooplankton mostly accumulate as a reserve of wax esters which are rich in highly unsaturated fatty acids (HUFA). This ensures that the full range of FA necessary for metabolic energy and cell membrane development of larvae.

In the other hand, the sustainable of structural and functional integrity of membrane cell were controlled by EPA according to Fox *et al.* [42] and Sargent [43]. In this study, Zoea 1 has low level of EPA. However, when larvae growth as increase the larval stage in Zoea 2 and Zoea 3, the level of EPA subsequently increased. But during Zoea 4 it was decreased. With this taken into account, when

larvae develop, the level was decreased and this may indicate the larvae utilized EPA as source for energy. This phenomenon same with has been reported for *J. edwardsii* larvae [25]. It shows that EPA level may decreased during development in some marine crustacean species.

The indicator for growth using *n*-3 to *n*-6 ratio in fish has been used for a long time ago [44] and also used as level indicator for FA metabolism and lipid nutrition for crustacean [45]. From this study the *n*-3 to *n*-6 ratio of *P. pelagicus* larvae was distinctly affected by *n*-3 to *n*-6 of the diet. An initially, in Zoea 1 showed high ratio of 11.94 suggested accumulation of significant level of *n*-3 fatty acid in oocyte yolk, but due to low *n*-3 levels in the rotifer fed to the early Zoea larvae with ratio 2.92 for Zoea 2 and 5.90 for Zoea 3. However, in Zoea 4 the *n*-3/ *n*-6 fatty acid ratio of larvae increase, reaching 8.73. These results indicate that diet, rather than nutritional superiority determines the balance between *n*-3 and *n*-6 fatty acid ratio in *P. pelagicus* larvae. Increase in TFA level was observed prior to metamorphosis at Zoea 4 stage, an accumulation that may be related to the energetically high cost of this challenging molt. Overall, the result from this experiment provides new insight into FA metabolism of *P. pelagicus* larvae during larval development, which important for identification of key FA requirement of larvae.

CONCLUSION

In conclusion, 27 fatty acids were detected in the FA composition of *P. pelagicus* larvae. PUFA was the most abundant in FA composition of *P. pelagicus* compared with SAFA and MUFA. The marine animal's marker was the highest in concentration detected in FA composition of *P. pelagicus*. 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.

The decreased level of EPA, DHA and AA suggested that HUFA requirement decreased when increased the larval stage. The TFA concentration of *P. Pelagicus* larvae observed to change as far as larval metamorphosis from Zoea 1 to Zoea 4. Larvae need some specific energy to undergo the next stages, like metamorphosis. That why, energy came from nutritional intake was very important for larval survival during larval development.

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REFERENCES

1. Ikhwanuddin, M., H.M. Juariah, A.B. Abol-Munafi and M.L. Shabdin, 2012a. Improved hatchery-rearing techniques for juvenile production of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758). Aquaculture Research, 43(9): 1251-1259.
2. Ikhwanuddin, M., M.N. Azra, A. Redzuari, Z.A. Aizam and A.B. Abol-Munafi, 2012b. Ingestion rates of *Brachionus* sp. and *Artemia* sp. nauplii by blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) larvae. Journal of Fisheries and Aquatic Science, 7(6): 402-411.
3. Sugumar, V. and P. Vasu, 2013. Effect of temperature on the biochemical constituents of the blue swimmer crab *Portunus pelagicus*. World Applied Sciences Journal, 28(3): 382-391.
4. Khoei, J.K. and A.A. Bastami, 2013. Mercury concentrations in tissues of blue swimming crab *Portunus pelagicus* and sediments from Khark Island. World Applied Sciences Journal, 21(9): 1391-1397.
5. Khoei, J.K., A.A. Bastami and M. Esmailian, 2012. The biochemical composition of the eggs blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) in the Persian Gulf coasts, Iran. Middle-East Journal of Scientific Research, 12(7): 915-920.
6. Bastami, A.A., M. Najafian and M. Hosseini, 2012. The distribution of the barnacle epizoites, *Chelonibita patula* (Ranzani) on blue swimmer crab, *Portunus pelagicus* (Linnaeus, 1758). World Applied Sciences Journal, 20(2): 236-240.
7. Hosseini, M., A.A. Bastami, J.K. Khoei, M. Esmailian, E.J. Songhori and M. Najafzadeh, 2012a. Concentrations of heavy metals in selected tissues of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) and sediments from Persian Gulf. World Applied Sciences Journal, 19(10): 1398-1405.

8. Hosseini, M., S.M.B. Nabavi, A.A. Bastami and Y. Parsa, 2012b. Mercury concentration in tissues of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) and sediments from Persian Gulf coasts, Iran. World Applied Sciences Journal, 18(3): 322-327.
9. Hosseini, M., A.A. Bastami, J.K. Khoei and S.A. Hosseini, 2012c. Seasonal and sexuality variation of mercury concentrations in selected tissues of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) and sediments from South Iran Persian Gulf. World Applied Sciences Journal, 18(3): 349-356.
10. Hosseini, M., A. Vazirizade, Y. Parsa and A. Mansori, 2012d. Sex ratio, size distribution and seasonal abundance of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) in Persian Gulf coasts, Iran. World Applied Sciences Journal, 17(7): 919-925.
11. Ikhwanuddin, M., A.D. Talpur, M.N. Azra, B. Mohd Azlie, Y.S. Hii and A.B. Abol-Munafi, 2012c. Effects of stocking density on the survival, growth and development rate of early stages blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) larvae. World Applied Sciences Journal, 18(3): 379-384.
12. Redzuari, A., M.N. Azra, A.B. Abol-Munafi, Z.A. Aizam, Y.S. Hii and M. Ikhwanuddin, 2012. Effects of feeding regimes on survival, development and growth of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) larvae. World Applied Sciences Journal, 18(4): 472-478.
13. Ikhwanuddin, M., T. Nor Adila, M.N. Azra, Y.S. Hii, A.D. Talpur and A.B. Abol-Munafi, 2011. Determination of live prey ingestion capability of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) larvae. World Journal of Fish and Marine Sciences, 3(6): 570-575.
14. Iverson, S.J., C. Field, W.D. Bowen and W. Blanchard, 2004.Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs, 74: 211-235.
15. Ackman, R.G., 1989. Fatty acids. In: Ackman RG (ed). Marine Biogenic Lipids, Fats and Oils, vol I. CRC Press Inc, Boca Raton, pp: 103-137.
16. De Long, E.F. and A.A. Yayanos, 1986. Biochemical function and ecological significance of novel bacterial lipids in deep-sea procaryotes. Applied and Environmental Microbiology, 51(4): 730-737.
17. Yano, Y., A. Nakayama, H. Saito and K. Ishihara, 1994. Production of docosahexaenoic acid by marine bacteria isolated from deep sea fish. Lipids, 29: 527-528.
18. Saito, H., Y. Seike, H. Ioka, K. Osako, M. Tanaka, A. Takashima, J. Keriko, S. Kose and J.C.R. Souza, 2005. High docosahexaenoic acid levels in both neutral and polar lipids of a highly migratory fish: *Thunnus tonggol* Bleeker. Lipids, 40: 941-953.
19. Ackman, R.G., 2000. Fatty acids in fish and shellfish. In: Chow CK (ed). Fatty acids in food and their health implications, 2nd edition. Marcel Dekker Press, New York, pp: 1-65.
20. Abdulkadir, S. and M. Tsuchiya, 2008. One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. Journal of Experimental Marine Biology and Ecology, 354(1): 1-8.
21. Arshad, A. M.S.K. Efrizal and C.R. Saad, 2006. Study on fecundity, embryology and larval development of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) under laboratory conditions. Research Journal of Fisheries and Hydrobiology, 1: 35-44.
22. Wu, X., B. Zhou, Y. Cheng, C. Zeng, C. Wang and L. Feng, 2010. Comparison of gender differences in biochemical composition and nutritional value of various edible parts of the blue swimmer crab. Journal of Food Composition and Analysis, 23(2): 154-159.
23. Christie, W.W., 2011. Fatty Acids: Straight-chain saturated. From the AOCS lipid library: http://lipidlibrary.aocs.org/Lipids/ fa_branc/index. html.
24. Soundarapandian, P. and R.K. Singh, 2008. Biochemical composition of the eggs of commercially important crab *Portunus pelagicus* (Linnaeus). International Journal of Zoological Research, 4(1): 53-58.
25. Smith, G.G., P.A. Thompson, A.J. Ritar and G.A. Dunstan, 2003. Effects of starvation and feeding on the fatty acid profiles of Stage I phyllosoma of the spiny lobster, *Jasus edwardsii*. Aquaculture Research, 34(5): 419-426.
26. Gunstone, F., J. Harwood and F. Padley, 1994. The Lipid Handbook. Chapman & Hall Electronic Publishing Division. pp: 709.
27. Freas, W. and S. Grollman, 1980. Ionic and osmotic influence on prostaglandin release from the gill tissue of marine bivalve, *Modiolus dimissus*. Journal of Experimental Biology, 84: 169-185.
28. Leger, P. and P. Sorgeloos, 1992. Optimized feeding regimes in shrimp hatcheries. In: Fast AW, Lester LJ (eds). Marines shrimp culture: principle and practice. Elsevier, pp: 225.

29. Graeve, M., G. Kattner and D. Piepenburg, 1997. Lipids in Arctic benthos: Does the fatty acid and alcohol composition reflect feeding and trophic interactions?. Polar Biology, 18: 53-61.
30. Ackman, R.G., 2002. Freshwater fish lipids - an overlooked source of beneficial long-chain n-3 fatty acids. European Journal of Lipid Science Technology, 104: 253-254.
31. Barrento, S., A. Marques, B. Teixeira, R. Mendes, N. Bandarra, P. Vaz-Pires and M.L. Nunes, 2010. Chemical composition, cholesterol, fatty acid and amino acid in two populations of brown crab *Cancer pagurus*: Ecological and human health implications. Journal of Food Composition and Analysis, 23(7): 716-725.
32. Bachok, Z., P.L. Mfilinge and M. Tsuchiya, 2003. The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan. Journal of Experimental Marine Biology and Ecology, 292(2): 187-197.
33. FAO., 2012. Portunus pelagicus: From Food and Aquaculture Organization of the United Nations: http://www.fao.org/ fishery/species/2629/en. (Accessed January 11, 2012).
34. Tomascik, T., A.J. Mah, A. Nontji and M.K. Moosa, 1997. The Ecology of the Indonesian Seas. Oxford: Oxford University Press. pp: 656.
35. Saint-Paul, U. and H. Schneider, 2010. Mangrove dynamics and management in North Brazil. New York: Springer. pp: 402.
36. Kharlamenko, V.I., N.V. Zhukova, S.V. Khotimchenko, V.I. Svetashev and G.M. Kamenev, 1995. Fatty acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). Marine of Ecology Progress Series, 120: 231-241.
37. Sasaki, G.C., J.M. Capuzzo and P. Biesiot, 1986. Nutritional and bioenergetic considerations in the development of the American lobster *Homarus americanus*. Canadian Journal of Fisheries and Aquatic Sciences, 43: 2311-2319.
38. Ouellet, P. and C.T. Taggart, 1992. Lipid condition and survival in shrimp (*Pandalus borealis*) larvae. Canadian Journal of Fisheries and Aquatic Sciences, 49: 368-378.
39. Muller-Feuga, A., R. Robert, C. Cahu, J. Robin and P. Divanach, 2003. Uses of microalgae in aquaculture. In: Stottrup, J. G. and L.A. McEvoy. (Ed.). Live feed in marine aquaculture. Blackwell Sciences. pp: 263-269.
40. Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. In: M. T. Arts, M. T. Brett and M. J. Kainz, (Ed.). Lipids in aquatic ecosystems. Dordrecht: Springer. pp: 309-326
41. Sargent, J.R., M.V. Bell, R.J. Hendersen and D.R. Tocher, 1990. Polyunsaturated fatty acids in marine and terrestrial food webs. In Animal Nutrition and Transport Processes: Nutrition in Wild and Domestic Animals. 11th Conference of the European Society for Comparative Physiology and Biochemistry, pp: 11-23.
42. Fox, C., J.H. Brown and M. Briggs, 1994. The nutritional of prawn and shrimp in aquaculture - a review of recent research In Muir, J.F. and R.J. Roberts, (Eds), recent advance in aquaculture. Blackwell Science, Oxford. pp: 131-206.
43. Sargent, A.R., 1995. Origins and functions of egg lipid nutritional implications.In Bromage, N. Roberts, L. (Eds), Broodstock management, egg and larval quality. Blackwood Science, Oxford, pp: 353-372.
44. Watanabe, T., 1982. Lipid nutrition in fish. Comparative Biochemistry and Physiology, 73B: 3-15.
45. Xu, X.L., W.J. Ji, J.D. Castell and R.K. O'Dor, 1994. Influence of dietary lipid source on fecundity, egg hatchability and fatty acid composition of Chinese prawn (*Penaeus chinensis*) broodstock. Aquaculture, 199: 359-370.