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Effects of various microalgae on fatty acid composition and survival rate of the blue swimming crab *Portunus pelagicus* larvae

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Present study is to examine effect of various microalgae on Fatty Acid (FA) of *Portunus pelagicus* larvae. Larvae were subjected to five different treatments; T1, T2, T3, T4 and T5. T1: rotifer and *Artemia* only; T2: rotifer, *Artemia* and *Chaetoceros* sp.; T3: rotifer, *Artemia* and *Chlorella* sp.; T4: rotifer, *Artemia* and *Isochrysis* sp. and T5: rotifer, *Artemia* and *Nannochloropsis* sp. Highest FA component in Zoea 3 was saturated FA group of T2 with concentration 26.06±26.23mg/g. For monounsaturated FA group, highest FA component in Zoea 1 was T5 with concentration 9.88±0.34mg/g. FA component in polyunsaturated FA group in Zoea 4, T4 with concentration 9.33±0.45mg/g was highest. For survival rate, highest survival rate obtained from T4 with 10.21%±0.45. In this study, essential FA requirement in each stage of *P. pelagicus* larvae can be reveal. Species of microalgae suitable for larvae can be characterized and selected for hatchery purpose.

[**Keywords:** *Portunus pelagicus*, Fatty Acids, *Isochrysis* sp., *Chlorella* sp., *Chaetoceros* sp., *Nannochlropsis* sp.]

Introduction

Blue swimming crab, *Portunus pelagicus* is distributed throughout the coastal waters of tropical regions 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, the crab fishery and culture operations are expected to continue to grow in the future[3]. However, problems associated with the use of live foods in aquaculture hatcheries have become a major bottleneck for expansion of the crab farming industry[4]. Improvement of current hatchery protocols is therefore necessary.

Although, both combination diet of *Brachionus* sp. and *Artemia* sp. was successfully used for *P. pelagicus* larvae[2,3] a different live food organism or algae gained popularity because the fact that a single or mixed organism or algae could not fulfil all larval-stage diet requirement. Studies showed that other than rotifer and *Artemia* nauplii, green plytoplankton and mixed diatom were also used to feed portunid crab larvae[5]. Microalgae play significant role as food for crustacean larvae stage and food for *Artemia*, rotifers and copepods which become food for post-larval, juvenile fish and crustacean. Microalgae are the best nutritional food for larvae during rearing period[6]. In peneid cultured, some microalgae have been used. *Chaetoceros calcitrans* gave better growth compared *Tetraselmis chui*i in *Penaeus monodon* culture[7].

Skletonema spp were fed by *Penaeus aztecus* and *Penaeus setiferus* larvae were grow faster[8]. *Tetraselmis chuii* and *C. ceratosporum* can be used as food for *Penaeus schmitti*[9]. Green microalgae and mixed diatom give successfully achievement in culturing *P. pelagicus* larvae[10]. We can conclude that the relationship between nutrition quality and larval development stage are important to produce high quality of postlarvae in any species. From this study, the knowledge of FA in *P. pelagicus* larvae is useful to understand its nutritional requirements in each stage, its interactions with other organisms and will be useful for developing successful farming techniques for this species in the future. As the demand for portunid crabs exceeds the production of crab fishery, it has indirectly developed the aquaculture industry recently[11].

Thus, the objectives of this study are to determine the fatty acid composition and survival rate of *P. pelagicus* larvae in hatchery trial using various microalgae.

Materials and Methods

Five tanks (1000L) for five treatments (T1, T2, T3, T4 and T5) were used in these

experiments. Larvae were reared from Zoea 1 until Zoea 4. Larvae in every treatment were sampled after reach Zoeal stage (Zoea 1 - Z1, Zoea 2 - Z2, Zoea 3 - Z3 and Zoea 4 - Z4) to run FA analysis. Larvae were sieved using 100µm plankton net and washed with distil water. Samples were brought to the laboratory and stored in deep-freezer under -80°C for 48 hours. The sample was freeze-dried for 48 hours before ready to do FA analysis. After each sample (Z1-Z4) completely dried, it was finely ground before analysis. All chemicals used (hexane, chloroform, methanol, and 14% BF3 in methanol) were of analytical reagent grade for GC (Kanto Chemical Co., Inc., Japan). Nonadecanoic acid (19:0) (Supelco Inc., USA) used as internal standard. Internal standard solution was prepared by dissolving 100mg of 19:0 in 100ml of hexane to obtain final concentration of 1mg/ml of 19:0. FA concentrations (CFA, 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:

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

Where A_S is the peak area of fatty acid in the sample in chromatogram, A_{IS} is peak area of internal standard in chromatogram, C_{IS} is concentration of internal standard (mg) and W_S is the weight of sample (g).

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

One-step method procedure consist extraction and esterification processes using a single tube. Three replicates of each sample (200-300mg) were mixed with 4ml of hexane and 1ml of internal standard (Nonadecanoid acid) solution in 50ml centrifuge tube. After adding 2ml of 14% BF3 in methanol and a magnetic stirring bar, the head space of 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 minutes under continuous stirring. After cooling to room temperature, 1ml of hexane was added followed by 2ml of distilled water. The tube was shaken vigorously for 1min and centrifuged for 3 minutes at 2500rpm (650×g). Two phases which formed, the upper phase was hexane layer containing the FAMEs. Finally, ~1–2ml of hexane layer was transferred using a Pasteur pipette into a clean sample vial to be injected into the GC for FAME analysis.

FAMEs were separated and quantified by gas chromatograph equipped with mass spectrometer (GCMS-QP2010 Ultra). Separation was performed with column ppms-5. Under pressure 50.0 kPa, with column flow 0.96ml/minute, linear velocity 35.5cm/second, and helium was used as carrier gas. 1µl of FAMEs samples in hexane were injected at 50°C and hold for 1min then oven temperature was raised to 300°C at a rate of 5°C/minute, and then finally held constant for 5 minutes.

Initial density larvae in each treatment tank were calculated. After larvae reach Z4 and initial stages of the megalope, density of larvae left were calculated as final amount. Survival rate (%) at particular treatment was met by dividing final stoking density with initial stocking density. Each treatment was replicated 3 times to get the mean value. Following formula was used to calculate the survival rate.

$$\text{Survival Rate} = \frac{\text{Final density}}{\text{Initial density}} \times 100$$

In this experiment, student t-test statistical analyses were performed by using SPSS. This comparison was displayed in graph form. All results will be presented as means±sd. Difference will be displayed as statistically significant when $P<0.05$.

Results

T5 showed a broader range of FA composition in Zoea 1, compared to other larvae feeding treatment (Table 1-5). The highest FA component showed in T2 was stearic acid C18:0 in Zoea 1. While in Zoea 2, Zoea 3 and Zoea 4, T2 showed broader range of FA composition compared to the other treated larvae with stearic acid C18:0 was the highest (Table 2). Concentration of TFA in larval feeding treatment in all larvae stages were not significantly ($P>0.05$) different. The highest TFA in Zoea 1 were detected in T2, followed by T5, T1, T3 and T4 with concentration of 34.43±4.50mg/g,27.66±7.76mg/g,19.02±6.74mg/g,12.62±1.18mg/g, and 7.52±2.76mg/g respectively, (Table 1-5). Concentration of TFA in Zoea 2, T2 was highest, followed by T1, T5, T4 and T3 with 15.8±0mg/g, 6.98±0.91mg/g,

Table 1—Fatty acids characteristic (mg/g) in *P. pelagicus* larvae (control, T1) at different larval stages. Values are mean±SD

	Larvae fed rotifer and *Artemia* only (T1)			
Fatty acid	Zoea 1	Zoea 2	Zoea 3	Zoea 4
C11:0	0.05±0.01	-	-	-
C12:0	0.07±0.00	-	-	-
C13:0	0.08±0.02	-	-	-
C14:0	0.78±0.20	-	0.54±0.03	-
C15:0	0.40±0.14	0.49±0.17	0.61±0.13	0.91±0.48
C16:0	1.70±2.73	-	0.77±0.04	-
C16:1	2.11±0.36	0.61±0.04	0.75±0.04	-
C17:0	0.69±0.21	0.49±0.14	0.78±0.19	0.58±0.29
C17:1	0.13±0.00	0.36±0.04	-	-
C18:0	3.61±0.47	-	5.64±0.3	-
C18:1*n*9*c*	1.76±1.51	-	-	-
C18:1*n*9*t*	2.32±0.09	-	-	-
C18:2*n*6*c*	0.18±0.13	3.27±0.35	3.79±0.19	2.27±0.65
C18:3*n*6	0.11±0.01	-	-	-
C20:1	0.88±0.42	-	0.53±0.02	-
C20:2	0.17±0.01	-	-	-
C20:3*n*3	-	-	-	2.08±0.32
C20:4*n*6	-	0.38±0.04	-	0.57±0.06
C20:5*n*3	2.23±0.19	0.88±0.09	-	1.38±0.49
C21:0	0.10±0.05	-	-	-
C22:0	0.24±0.01	0.49±0.04	-	0.41±0.03
C22:6*n*3	1.14±0.17	-	-	0.18±0.02
C23:0	0.25±0.01	-	-	-
C24:1	0.03±0.00	-	-	-
(*n*-3) / *n*-6) ratio	20.27	2.32	-	6.39
DHA / EPA ratio	0.51	-	-	1.56
SAFA / MUFA ratio	1.1	1.52	6.52	-
SAFA / PUFA ratio	2.08	0.32	2.2	0.29
MUFA / PUFA ratio	1.89	0.21	0.34	-

SAFA-Saturated fatty acid, MUFA-monounsaturated fatty acid, PUFA-polyunsaturated fatty acid, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid.

4.57±0.65mg/g, 4.31±0.71mg/g and 2.25±0.04mg/g, respectively (Table 1-5). Highest TFA concentration in Zoea 3 was detected in T2, followed by T1, T4, T5 and T3 with concentration 30.79±31.47mg/g, 13.41±0.92mg/g, 7.8±1.1mg/g, 6.11±0.9mg/g and 4.97±1.08mg/g, respectively (Table 1-5 and Figure 4). Concentration TFA of Zoea 4 was dominant in T2, followed by T4, T1, T3 and T5 with concentration 30.45±1.26mg/g, 13±0.8mg/g, 8.39±2.33mg/g, 6.74±0.55mg/g and 3.43±0.89mg/g. Survival rate of *P. pelagicus* larvae in both control and treatment was not significantly different (P>0.05) during days' culture. During day 3, the higher survival rate recorded in T3 with 79.96%±0.11. At same day, the lowest survival rate recorded in T1 with 72.34%±0.03. At megalopa stage, the highest survival rate from this experiment was obtained from T4 with 10.21%±0.45 followed with T3, T5, T2 and T1 with 8.93%±0.34, 8.35%±0.11, 6.96%±0.87 and 5.45%±0.33 respectively has been showed in Figure 9.

Discussion

The FA of newly hatching larvae *P. pelagicus* was shown in Fig. 1 to Fig. 4 to be influenced by the food eaten by broodstock through gametogenesis, same with other marine crustacean[12]. The broodstock used in these studies were sampled from the natural habitat. It may be consumed such animal like sea urchins, chitons and other crab considerably high in PUFA[13]. Another PUFA source, come from macrophytes, phytoplankton and benthic plankton are integrated, not altered become

Table 2—Fatty acids characteristic (mg/g) in *P. pelagicus* larvae (T2) at different larval stages. Values are mean±SD Larvae fed rotifer, *Artemia* and *Chaetoceros* sp (T2)

Fatty acid	Zoea 1	Zoea 2	Zoea 3	Zoea 4
C12:0	-	0.16±0.01	-	-
C14:0	0.72±0.7	1.24±0.04	0.72±0.7	0.75±0.65
C15:0	0.96±0.96	0.52±0.02	0.96±0.96	0.9±0.03
C16:0	7.31±7.24	0.24±0.02	7.31±7.24	7±0.04
C16:1	1.34±1.31	0.10±0.01	1.34±1.31	1.3±0.32
C17:0	3.49±3.84	0.64±0.03	3.49±3.84	3.4±0.01
C18:0	13.58±13.49	7.31±0.23	13.58±13.49	13.5±0.04
C20:1	-	0.26±0.15	-	0.25±0.06
C20:2	-	0.17±0.01	-	-
C20:4*n*6	1.38±1.62	1.03±0.03	1.38±1.62	1.35±0.09
C20:5*n*3	2.01±2.32	2.77±0.09	2.01±2.32	2±0.03
C22:0	-	0.39±0.01	-	-
C22:6*n*3	-	0.93±0.11	-	-
(*n*-3) / *n*-6) ratio	1.46	3.59	1.46	1.48
DHA / EPA ratio	-	0.34		
SAFA / MUFA ratio	2.28	28.43	19.45	16.48
SAFA / PUFA ratio	2.32	2.14	7.69	7.63
MUFA / PUFA ratio	1.02	0.08	0.4	0.44

SAFA-Saturated fatty acid, MUFA-monounsaturated fatty acid, PUFA-polyunsaturated fatty acid, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid.

neutral and polar lipids in crustacean, the balance change to EPA and DHA by elongation and desaturation process[14]. The TFA concentration of *P. pelagicus* larvae observed to change as far as larval metamorphosis from Zoea 1 to Zoea 4 (Fig. 4). In this case, larvae need some specific energy to undergo the next stages, like metamorphosis[15]. That why, energy came from nutritional intake was very important for larval survival during larval development. *Chaetoceros* sp. seemly can produce a natural antibiotic and can increase vitamin level in shrimp hatchery[16]. This probably due to high concentration of 16:0 and 18:0 FA component in *Chaetoceros* sp. at Z1, Z2, Z3 and Z4 observed in larvae fed rotifer, *Artemia* and *Chaetoceros* sp. (T2) compared to other treatment (Fig. 1). *Chaetoceros* sp. is after all contain high omega-3, especially EPA levels[17]. In this study, T4 showed high EPA and DHA (Fig. 5; Fig. 6) especially in Zoea 4. For *Isochrysis* sp., it was used to enrich *Artemia* nauplii[17]. High level of EPA and DHA level were found in *Isochrysis* sp. *Isochrysis* sp are rich in chloropyll, carotenoid (fucoxanthin, *β*-carotene, diadinoxantin, diatoxantin) and crisolaminarin (polysaccharide derived glucose glycosidic type *β*-1, 3)[18-19]. Another FA component of omega-6 HUFA was AA. It acts as essential nutrient for most crustacean species[20]. In this experiment, T5 showed level of AA highest at Zoea 1 stage (1.57±0.03mg/g), then dropped to (0.18±0.04mg/g) at Zoea 4 stage (Table 5). This decreased may result from the low level of AA in enrich rotifer and *Artemia* used to feed Zoea 2 and Zoea 3. As addition of appropriate amounts of AA to the diet has been reported to have a positive effect on survival in rearing larvae of other species, further research into exact AA requirement of *P. pelagicus* larvae should be conducted. Palmitic acid, stearic acid, AA, EPA and DHA were the dominant FA in *P. pelagicus* larvae. These FA were comparable to other live food and same with other reported crustacean like puerulus of *J. edwarsii*[21], *P. cygnus* phyllosoma[22] and juvenile Chinese mitten crab, *Eriocheir sinensis*[23]. High level of AA, EPA and DHA were found in *M. rosenbergii*[12] during larvae growth reflected with the decreased the level of SAFA and MUFA. EPA and DHA better than C18 PUFA, functioning as maintain the growth of crustacean like *P. japonicus*[24]. Inability of crustacean and marine fish to elongate shorter chain of PUFA to EPA and DHA at significant rate therefore must be taken through their feeding. High nutritional requirement make microalgae are known for their excellent nutrient properties[25].

Microalgae like *Chlorella* sp., *Isochrysis* sp., *Nannochloropsis* sp. and *Chaetoceros* sp. were used to increase PUFA level in larval diet[26].

Table 3—Fatty acids characteristic (mg/g) in *P. pelagicus* larvae (T3) at different larval stages. Values are mean±SD Larvae fed rotifer, *Artemia* and *Chlorella* sp. (T3)

Fatty acid	Zoea 1	Zoea 2	Zoea 3	Zoea 4
C12:0	-	-	-	0.15±0.01
C14:0	0.56±0.06	-	0.46±0.06	0.64±0.04
C15:0	0.5±0.1	-	0.62±0.18	0.46±0.01
C16:0	-	-	0.71±0.09	0.75±0.26
C16:1	1.55±0.12	1.03±0.02	0.62±0.07	-
C17:0	0.71±0.22	0.31±0.01	0.51±0.09	-
C17:1	0.47±0.04	-	0.45±0.1	-
C18:0	4.38±0.33	-	-	-
C18:2*n6c*	2.74±0.22	-	-	-
C20:1	-	-	-	1.13±0.10
C20:2	-	-	-	0.16±0.00
C20:3*n6*	-	-	-	0.07±0.01
C20:4*n6*	0.46±0.03	-	-	0.88±0.03
C20:5*n3*	1.25±0.06	0.91±0.01	1.41±0.15	1.49±0.03
C22:0	-	-	0.20±0.34	0.35±0.01
C22:6*n3*	-	-	-	0.71±0.06
(*n*-3) / *n*-6) ratio	2.72	-	-	2.32
DHA / EPA ratio	-	-	-	0.48
SAFA / MUFA ratio	3.04	0.3	2.33	2.09
SAFA / PUFA ratio	1.38	0.34	1.77	0.72
MUFA / PUFA ratio	0.45	1.13	0.76	0.34

SAFA-Saturated fatty acid, MUFA-monounsaturated fatty acid, PUFA-polyunsaturated fatty acid, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid.

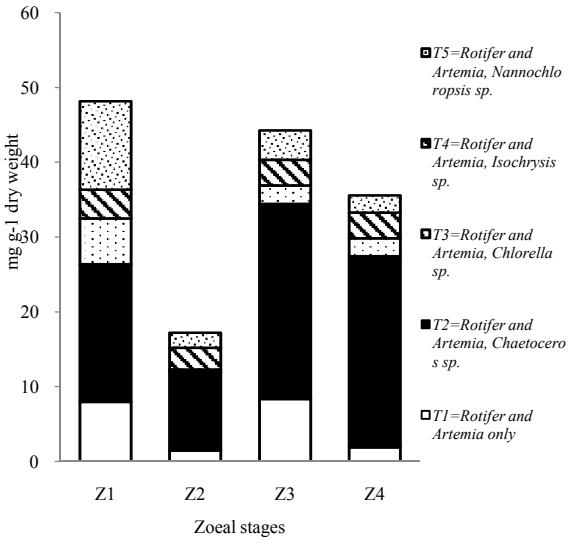

Fig. 1—Saturated fatty acid (SAFA) concentration in *Portunus pelagicus* larvae fed with different live food treatment at different larval stages from the hatchery trial.

Variability and complexity of live food is essential for animal's survival. Survival of the larvae strongly influenced by PUFAs like EPA and DHA[27,28]. From this study, the highest concentration of FA was PUFA component which detected in T2 during Zoea 1 (Figure 3). Most marine crustacean cannot produce HUFA from C18 FA[29]. EPA belonging to HUFA was contribute for larval survival[30] and DHA act as increased the intermolt process and make carapace width in mud crab *S. serrata* juvenile more broader[31]. The differences role of EPA and DHA are influence by varying their distribution in the tissues of organism such as DHA most available at neural tissue in brain and eyestalks, while EPA was distributed evenly at all the various type of tissue[32]. Supply microalgae have several beneficial effects on nutritional state of larvae.

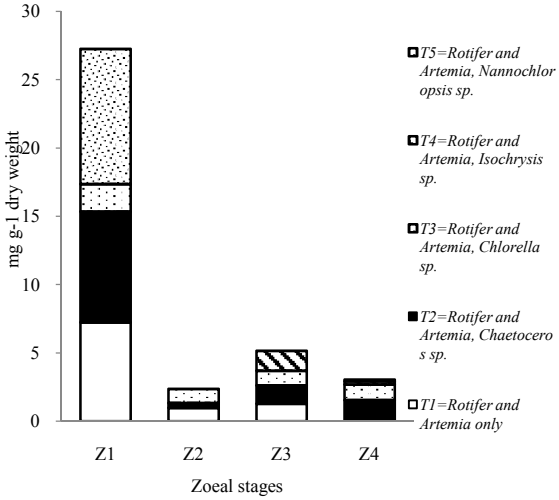

Fig. 2—Monounsaturated fatty acid (MUFA) concentration in *Portunus pelagicus* larvae fed with different live food treatment at different Fig. 2—larval stages from the hatchery trial.

Table 4—Fatty acids characteristic (mg/g) in *P. pelagicus* larvae (T4) at different larval stages. Values are mean±SD

Larvae fed rotifer, *Artemia* and *Isochrysis* sp. (T4)				
Fatty Acid	Zoea 1	Zoea 2	Zoea 3	Zoea 4
C13:0	1±0.15	0.46±0.05	-	-
C14:0	0.53±0.06	0.53±0.05	0.46±0.05	1.09±0.08
C15:0	1.44±0.47	0.4±0.1	0.71±0.06	0.75±0.20
C16:0	-	0.33±0.07	-	-
C16:1	-	-	0.84±0.07	-
C17:0	0.88±0.28	0.5±0.21	0.9±0.32	1.02±0.04
C17:1	-	-	0.63±0.05	0.24±0.03
C18:0	-	-	-	0.71±0.10
C18:2*n6c*	2.22±1.61	-	-	-
C18:3*n6*	-	-	-	0.21±0.01
C20:3*n6*	-	-	0.73±0.03	-
C20:4*n6*	0.49±0.07	0.55±0.06	0.89±0.07	1.10±0.07
C20:5*n3*	0.96±0.13	0.86±0.09	1.29±0.24	5.39±0.21
C21:0	-	-	0.39±0.08	-
C22:0	-	0.68±0.07	0.59±0.06	0.56±0.01
C22:6*n3*	-	-	-	1.92±0.06
C24:0	-	-	0.37±0.04	-
(*n*-3) / *n*-6) ratio	1.96	1.56	0.8	5.5
DHA / EPA ratio	-	-	-	0.36
SAFA / MUFA ratio		-	2.33	14.3
SAFA /PUFA ratio	1.04	2.06	1.18	0.37
MUFA / PUFA ratio		-	0.51	0.03

SAFA-Saturated fatty acid, MUFA-monounsaturated fatty acid, PUFA-polyunsaturated fatty acid, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid.

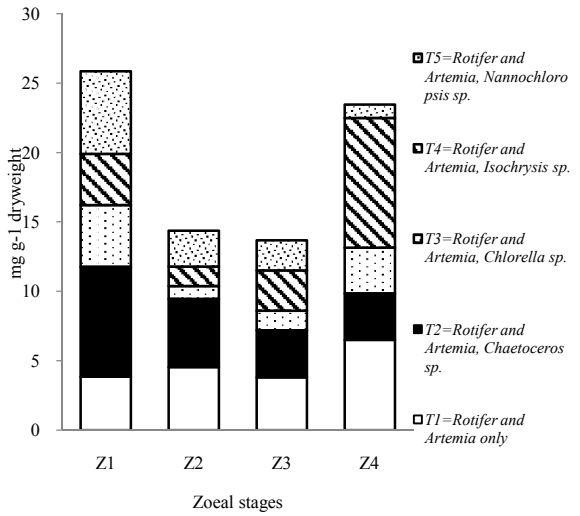

Fig. 3—Polyunsaturated fatty acid (PUFA) concentration in *Portunus pelagicus* larvae fed with different live food treatment at different larval stages from the hatchery trial.

Microalgae stimulate larval digestive enzyme production and colonization of digestive tract[33]. From this study after 5 day, highest survival rate of larvae was observed in T3. For day 8, T5 was significantly higher compared other treatment. This result similar with Ikhwanuddin[34] stated that, combination of instant frozen *Nannochlropsis* sp., *Artemia* and rotifer gives a better survival rate till 1st day juvenile crabs. However, in Zoea 3 and Zoea 4, T4 showed highest in survival rate of larvae. After 14 days, number of dead larvae in control tanks (T1) became conspicuously higher than that in other treatment tanks.

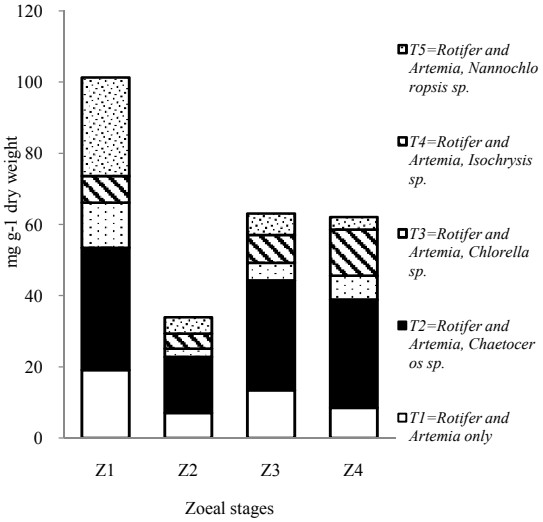

Fig. 4—Total fatty acid concentration in *Portunus pelagicus* larvae fed with different live food treatment at different larval stages from the hatchery trial.

Table 5—Fatty acids characteristic (mg/g) in *P. pelagicus* larvae (T5) at different larval stages. Values are mean±SD
Larvae fed rotifer, *Artemia* and *Nannochloropsis* sp. (T5)

Fatty acid	Zoea 1	Zoea 2	Zoea 3	Zoea 4
C12:0	0.12±0.02	-	-	-
C13:0	0.07±0.06	0.26±0.03	0.5±0.08	0.18±0.03
C14:0	0.66±0.58	0.41±0.06	0.51±0.1	0.22±0.1
C14:1	0.04±0.03	-	-	-
C15:0	0.39±0.34	0.42±0.19	0.52±0.15	0.15±0.03
C16:0	4.9±4.14	-	0.64±0.14	0.11±0.03
C16:1	2.39±2.07	-	0.98±0.18	0.12±0.01
C17:0	1.41±0.24	0.35±0.13	0.6±0.01	0.26±0.09
C17:1	0.32±0.03	-	-	-
C18:0	3.28±2.8	-	-	1.42±0.27
C18:1*n*9*c*	3.93±0.03	-	-	-
C18:1*n*9*t*	0.05±0.04	-	-	-
C18:2*n*6*c*	-	-	-	0.48±0.2
C18:3*n*3	-	0.59±0.07	-	-
C20:1	1.92±0.25	-	-	-
C20:4*n*6	1.57±0.03	0.72±0.09	0.69±0.12	0.18±0.04
C20:5*n*3	2.84±0.02	1.27±0.01	1.47±0.11	0.32±0.1
C21:0	0.07±0.01	-	0.54±0.08	-
C22:0	0.36±0.03	0.54±0.07	0.63±0.11	-
C22:6*n*3	1.55±0.04	-	-	-
(*n*-3) / *n*-6) ratio	2.80	2.58	2.13	1.78
DHA / EPA ratio	0.55	-	-	-
SAFA / MUFA ratio	1.2	-	-	19.42
SAFA / PUFA ratio	1.98	0.77	1.83	2.38
MUFA / PUFA ratio	1.68	0.77	1.83	2.38

SAFA-Saturated fatty acid, MUFA-monounsaturated fatty acid, PUFA-polyunsaturated fatty acid, DHA-docosahexaenoic acid, EPA-eicosapentaenoic acid.

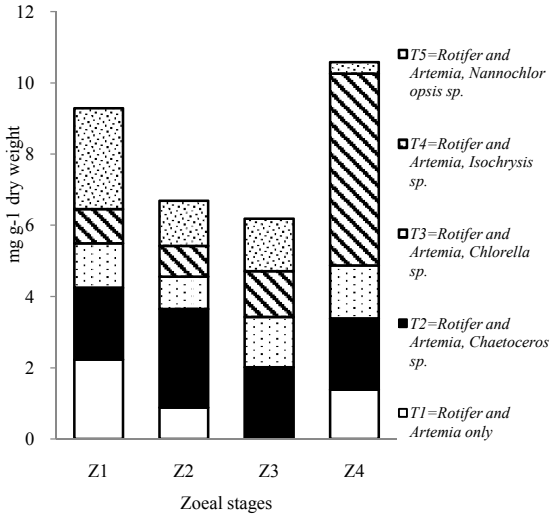

Fig. 5—Eicosapentaenoic fatty acid (EPA) concentration in *Portunus pelagicus* larvae fed with different live food treatment at different larval stages from the hatchery trial.

Moreover, water pollution was probable factor inducing larval mortality. Study from Tamaru[35], inorganic nitrogen (NH_4-N, NO_2-N, and NO_3-N) and phosphate (PO_4 -P) concentrations increased both in treatment and control tanks especially in control tanks. One of reasons for differences in concentrations of nitrogen and phosphate in rearing water between treatment tanks seemed to algal

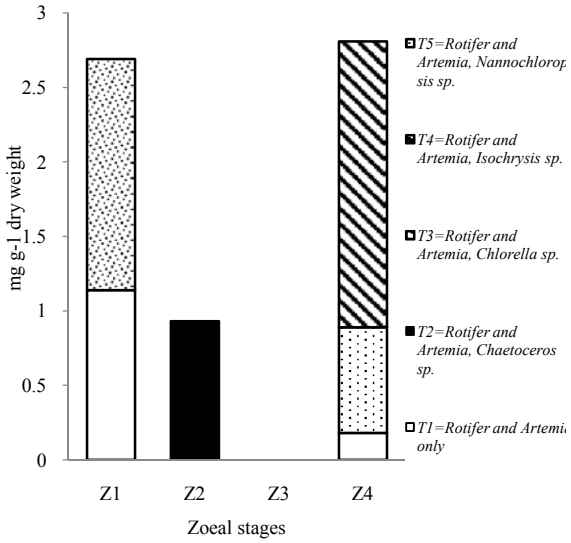

Fig. 6—Docosahexaenoic acid (DHA) concentration in *Portunus pelagicus* larvae fed with different live food treatment at different larval stages from the hatchery trial.

nutrient absorption. Microalgae absorbed dissoluble nitrogen and phosphate as nutrients. From this point of view, using microalgae was effective. It has been reported that microalgae added to culture water seemed to have 'beneficial' effect in larval fish culture in terms of survival by releasing oxygen into and removing certain metabolites like ammonia, from culture medium[36]. It was even suggested that microalgae also release antibiotic substance into culture medium[36]. Study from Tamaru[35] reported that nitrate nitrogen content in treatment tanks of mullet larvae was significantly higher than control tanks. They considered that reason was contamination from algal medium, because they used ammonium sulfate as a nutrient in medium. In this study, sodium nitrate was used instead of ammonium sulfate, so that toxicity of unionized ammonia was concluded as negligible. Moreover, all microalgae were used in this experiment reached stationary phase of growth. At stationary phase, almost all nutrients in medium were used for algal growth. Thus, contamination from algal medium would not raise nitrate nitrogen level in medium, but it was used for algal growth.

Conclusion

For conclusion, decreased level of EPA, DHA and AA suggested that HUFA requirement decreased when increased the larval stage. However, the precisely nutritional value of *n*-3/*n*-6 FA ratio and physiology involved still not fully understood. Fig. 7 and Fig. 8 showed the *n*-3/*n*-6 FA ratio in the present study. 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 for their survival, which important for identification of key FA requirement of larvae. Based on Fig. 9, there was no significant different (P>0.05) in survival rate between mean number of T1, T2, T3, T4 and T5. From the results of this experiment, it was shown that microalgae have have main two roles in the larval rearing water. The addition of microalgae into the rearing water was indispensable to enhance the FA component of rotifers and *Artemia* to a level suitable for the crab larvae. Moreover, the addition of the alga was effective to reduce the concentrations of the soluble nitrogen and phosphate.

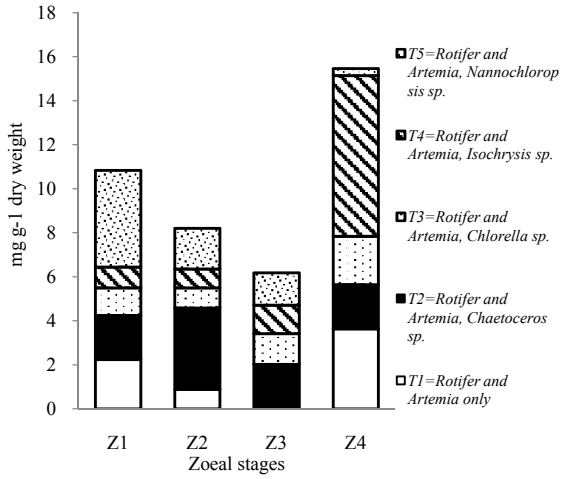

Fig. 7—Omega-3 (*n*-3) concentration in *Portunus pelagicus* larvae fed with different live food treatment at different larval stages from the hatchery trial.

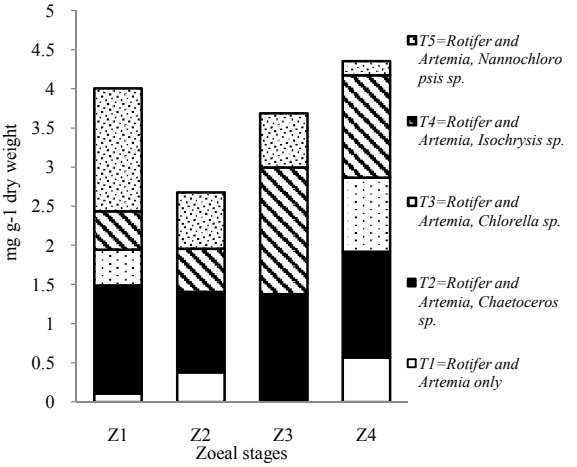

Fig. 8—Omega-6 (*n*-6) concentration in *Portunus pelagicus* larvae fed with different live food treatment at different larval stages from the hatchery trial.

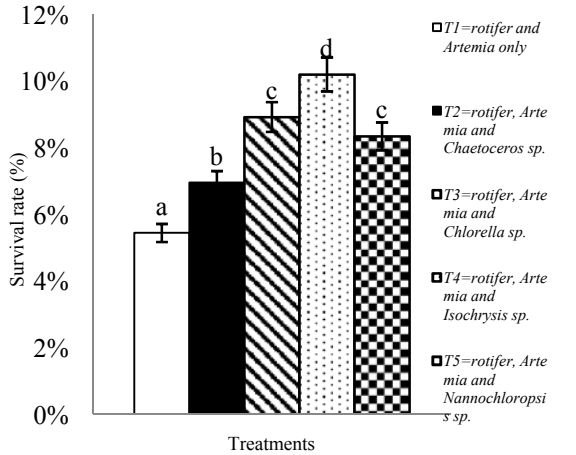

Fig. 9—Final survival rate (%) of *Portunus pelagicus* larvae (megalopa stage) fed with various microalgae and feeding schemes. Different letters in each treatment shows that there are no significantly different between treatments (P>0.05).

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