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Phospholipids Effect on Survival and Molting Synchronicity of Larvae Mud Crab *Scylla serrata*

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Effect of phospholipids on survival and molting synchronicity of mud crab larvae *Scylla serrata* were examined using *Artemia* enriched with five treatments of emulsion oil i.e. treatment with different level of soybean lecithin (SL) together with a level of DHA70G (referred to as DHA-SL0, 20 and 40) and treatment with SL and cuttle fish phospholipids (CPL) at 40 uL/L without DHA70G (referred to as WDHA-SL and WDHA-CPL). Survival rate, intermolt period, carapace width, and molting synchronicity were evaluated. Additionally, lipid classes and fatty acid composition of enriched *Artemia* were analyzed. Survival rate, intermolt period, and carapace width at the first crab (FC) stage of mud crab larvae fed DHA-SL0 to 40 were similar to that of WDHA-CPL but higher than that of WDHA-SL ($P < 0.05$). Moreover, mud crab larvae fed DHA-SL20, DHA-SL40, and WDHA-CPL had a significantly higher molting synchronicity index compared to that of larvae fed WDHA-SL and DHA-SL0. It can be concluded that combination of phospholipids and essential fatty acids exhibited an additive effect in improving molting synchronicity of mud crab larvae.

Key words: molting synchronicity mud crab, PC, phospholipids, *Scylla serrata*, survival

INTRODUCTION

The importance of phospholipids in marine crustacean nutrition has been demonstrated by some researches. It was postulated that crustaceans can synthesize phospholipids *de novo* (Sheih 1969) although the rate of synthesis was slow. Therefore it should be added in the diet to fulfill the requirement of rapidly growth in the early developmental stage of larvae (D'Abramo *et al*. 1981; Teshima *et al*. 1986a). It was reported that the supplementation of short-necked clam *Tapes philippinarum* phospholipids at the level of 1% to the diet containing 7% Pollack liver oil exhibited the highest weight gain of juvenile *Marsupenaeus japonicas* (Kanazawa *et al*. 1979a). The absence of phospholipids in the diet showed a negative effect for *Homarus americanus* and resulting molt death syndrome indicated by death during or suddenly after molting (Bowser & Rosemark 1981). Moreover, Teshima *et al*. (1986a) reported that prawn, *M. japonicus* larvae fed phospholipids deficient diet, almost larvae could not undergo to zoeal 2 stage and the inclusion of phospholipids in the level of 3.0% exhibited the survival and growth. Mokoginta and Suprayudi (1996) reported that the inclusion of phospholipids in the diet exhibited higher survival and growth of *Penaeus monodon* larvae and postlarvae.

It was reported that phosphatidylcholine (PC) is the active compound in the soybean phospholipids (SL) and the essentiality of phospholipids was affected by the phospholipids sources (D'Abramo *et al*. 1982). Coutteau *et al*. (1996) reported that supplementation of soybean phosphatidycholine (SPC) and de-oiled soybean lecithin (DSL) at 1.5 and 6.5% improved the growth response of *Litopenaeus vannamei* than that a-PC deficient diet. Kanazawa *et al*. (1985) demonstrated that among the phospholipids sources, SPC, and soybean phosphatidilinositol (SPI) and PC from bonito eggs are more effective in promoting the growth of larvae *L. vannamei* compared to the other phospholipids sources.

Several workers showed the importance of highly unsaturated fatty acids (HUFA) on the growth and survival of penaeid shrimps (Kanazawa *et al*. 1979b; Kayama *et al*. 1980; Merican & Shim 1996). Like other penaeid, we demonstrated that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were superior to that linoleic acid (LA) and lonolenic acid (LNA) in the term of survival rate, larger carapace width and intermolt period of mud crab *Scylla serrata* larvae (Suprayudi *et al*. 2004a). We also reported that during *Artemia* feeding the level of DHA and EPA should be adjusted to 0.1-0.5% and 0.7-0.9% to maintaining high survival and wider carapace width (Suprayudi *et al*. 2002b). It was also reported that the supplementation of PC increase the biological function of DHA and lipid retention in tissue of shrimp, that were linked to the growth and stress resistance (Harel *et al*. 1999). In stand point of cholesterol requirement it was found that mud crab larvae fed on live feed require 0.5% of cholesterol for supporting maximal growth and high survival rate (Suprayudi *et al*. 2012).

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A few studies have been conducted to evaluate the efficacy of phospholipids by using live food. However, it is not easy to obtain *Artemia* containing a fix ranges of phospholipids for nutritional studies (Rainuzo *et al*. 1994). However, Harel *et al*. (1999) showed that disire ranged of phosphoslid in live food could be obtained by enrich live food with diets containing phospholipids. They demonstrated that different of diet produce different level of polar lipid class and the range of nutrient in live food was independent to dietary phospholipids level, although higher polar lipid fraction were observed in *Artemia* enriched with mixture of phospholipids and DHA-sodium salt in contrast. Similar result also reported by Zhukova *et al*. (1998) where feeding *Artemia* with artificial diet induced changes in lipid fraction and fatty acid composition of Artemia.

On the other hand, besides the feeding regime and nutritional quality of live food, low survival rate of mud crab larvae in the seed production also affected by the molting synchronicity, especially from zoea 5 (Z5) to megalops. It was observed that megalops were grazed the Z5, eventually resulted in low survival rate at the first crab (FC) stage (Hamasaki *et al*. 2002). The present study was conducted to examine the effect of various dietary levels and sources of phospholipids in enriched *Artemia* on the growth and molting synchronicity of mud crab larvae.

MATERIALS AND METHODS

Culture Conditions. Selected healthy broodstock of *Scylla serrata* were obtained from Okinawa islands, Japan, and held in 5 kl fiberglass tank equipped flow trough water system. A berried female was transferred into 1,000 l aerated polycarbonate hatching tank with salinity maintained at 34‰ and temperature at 26 °C. One hour after the eggs hatched, actively swimming larvae were removed and used in the experiment (Suprayudi *et al*. 2002a).

Larvae were held in 1 l plastic beakers at a stocking density of 30 individuals per beaker. Water salinity of 33-34‰ was maintained during the rearing period of the zoeal stages. After larvae metamorphosed to the Megalops stage, the salinity was adjusted to 24‰. Water temperature was maintained at 30 °C using a controlled heater. All plactic beakers were gently aerated through Pasteur pipetts that were placed in the center of beakers. Every morning larvae were transferred into new beakers using a 5 ml pipette. During the transfer, larvae were counted and the developmental stages observed. Larvae were separated into different beakers depending on their larvae stages.

Larvae were fed on rotifers (40 ind/ml) once daily, from the first zoea (Z1) stage up to the second zoea (Z2) stage. From Z3 larvae fed *Artemia* at 1.5 naupplii/ml and at the megalops stage the density of *Artemia* were increased to 4 naupplii/ml.

Hatching *Artemia* Cyst and Enrichment Methods. The cyst (2 g/l) were incubated in filtered and UV exposed sea water at 28-29 °C under continuous strong aeration. After hatching, the nauplii were separated from the empty cyst and then transferred to 2 l plastic beakers at density of 180-200 nauplii/ml with continuous aeration. The beakers were kept a control room temperature (water temperature were adjusted 23 °C) and fed oil emulsion.

Oil emulsion were prepared by mixing 0.5 ml of oil and 0.1 g yolk egg in 100 ml water and mixed 10,000 rotation per minute for 2 minutes to get a good oil emulsion as described by Suprayudi *et al*. (2002b). A 40 ml of emulsion supplied into the enrichment medium and enrichment was carried out for 18 hours. Samples of *Artemia* for each treatment were frozen after being washed with freshwater.

Experimental Design. *Artemia* were enriched with soybean lecithin (containing > 35% of phosphatidilcholine (PC, Taiyo Yushi K.K., Japan) (SL) at 0, 20, and 40 µl (DHA-SL0, DHA-SL20, and DHA-SL40). In these treatment DHA70G were also added at 25 µl. Moreover to evaluate the essentiality of PL, two kinds of PL, i.e. SL and cuttlefish phospholipids (CPL) (containing 36 and 56% of DHA and PC, Taiyo Yushi K.K., Tokyo, Japan) were directly enriched at 40 µl, without the addition of DHA70G. Here after were referred to as WDHA-SL and WDHA-CPL. All treatments are summarized in Table 1.

Evaluating Parameters. Survival rate at each stage, intermolt period to reach each stage, carapace width at FC stage and molting synchronicity to megalopal stage were recorded for larvae in this experiment. Survival rate was calculated as the percentage value of larvae that successfully molted from the first zoeal stage. Carapace width was measured from the outer lateral spines and the differences between zoeal stage were distinguished by comparing the distance between eyes by using microscope compleatly with mikrometer (50x) (Suprayudi *et al*. 2002b, 2004a). In addition, crude lipid, fatty acid composition, and polar and non polar lipid classes of both rotifers and *Artemia* were determined.

Molting synchronicity index were calculated by the following method as described in Table 2. The calculation were based on the number of remain Z5 preyed by megalops and molting synchronicity index is then calculated as mean value of Z5 that survived to megalops.

Chemical Analysis. Total lipid was extracted from the samples of *Artemia* by homogenization in chloroform-methanol (2:1, v/v) (Folch *et al*. 1957). Lipids were saphonified by using of KOH (1 ml) in ethanol (15 ml) and heating for 40 min at 80 °C. The saphonified lipid then esterified by using 6.7% of BF_3 in methanol and heating for 20 min at 80 °C. Fatty acid methyl ester was diluted in

Table 1. Composition emulsion oil for enrichment of *Artemia*

Treatment Abbreviation	Oils (µl/l enrichment media)			
	OA*	DHA70G**	SL	CPL
DHA+SL0	75	25	0	0
DHA+SL20	55	25	20	0
DHA+SL40	35	25	40	0
WDHA-SL	60	0	40	0
WDHA-CPL	60	0	0	40

*Oleic acid ethyl ester (purity, < 95%); **Triglyceride type of docosahexaenoic acid (containing 70.7% DHA and 5.2% EPA).

hexane (10 mg/0.5 ml) and analyzed by using gas liquid chromatography (Shimadzu, GC-14B) equipped with a silica capillary column (30 m x 0.32 mm x 0.25 µm film thickness) (SUPELCO, Bellefonte, USA). Helium was used as the carrier gas and the pressure was adjusted to 100 kPa. Column, injection port, and detector temperatures were adjusted to 205, 225, and 250 °C respectively. Fatty acid methyl esters were identified by comparing the retention time against the standard ones.

Polar and nonpolar lipids were separated by using silica cartridge (Sep-Pak Waters Ass., USA). Both of polar and nonpolar lipids were injected into chromatorod (Rod S-III) and separated by using 98:2:0.1 v/v (1.2 dichloroetane:chloroform:acetic acid) and 65:35:4 v/v (chloroform:methanol:distilled water) as the mobile phase. Furthermore, polar and nonpolar lipids were fractionated by using Iatroscan MK-5 (Iatron Laboratories, Inc, Japan).

Statistical Analysis. Anova was utilized to analyze the effects of treatments on survival rate, intermolt period, carapace width, molting synchronicity, fatty acid composition, and PC. SNK multiple ranged tests were used to determine differences among means. All the statiscal analysis was performed using the SPSS 11.0 microcomputer software package.

RESULTS

Biological Parameters. There were no significant differences of phospholipids supplementation (DHA-SL0, 20, and 40) on means value of survival rate of mud crab larvae (Table 3) at the Z1-Z5 and MG-FC stages ($P < 0.05$). These mean values were similar to the WDHA-CPL treatment. However, all treatments had a higher survival rate compared to the WDHA-SL treatment. Similar patterns were also found on the carapace width (Figure 1) and intermolt periods (Table 4).

Supplementation of SL had a higher mean value of molting synchronicity index (Figure 2) in all larvae fed *Artemia* enriched with DHA70G (DHA-SL20 and 40). Larvae fed *Artemia* enriched with WDHA-CPL exhibited

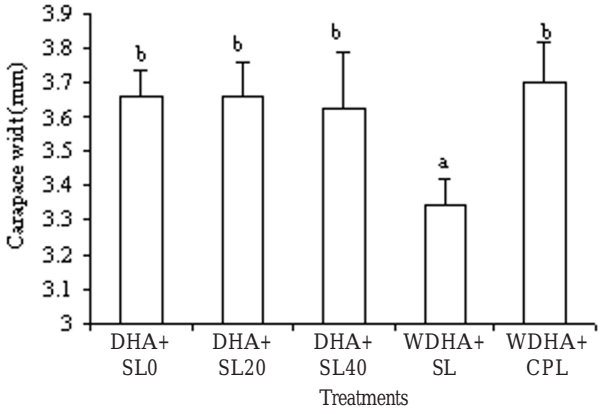

Figure 1. Carapace width (CW, mean SD, n = 3) of mud crab larvae at the first crab stag.

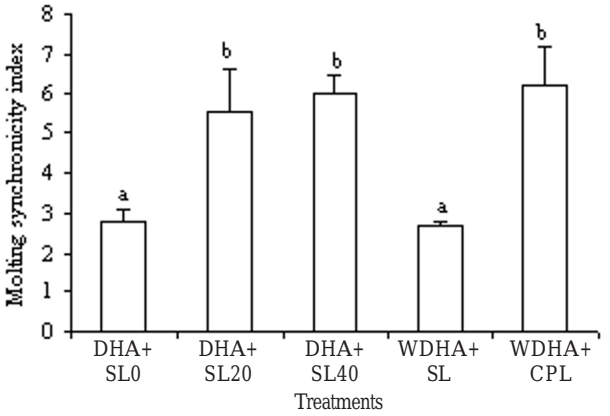

Figure 2. Molting synchronicity index (MSI, mean SD, n = 3) of mud crab larvaefrom Z5 to megalopa.

Table 2. Molting synchronicity index (MSI) of mud crab larvae according to various molting rate to Megalops

Molting rate (%)		Survival rate from zoea 5 (Z5) to Megalops (%)*								MSI***
zoea 5 (Z5)	Megalops	1**	2	3	4	5	6	7	8	
90	10	80	70	60	50	40	30	20	10	4.1
80	20	60	40	20	20	20	20	20	20	2.7
70	30	40	30	30	30	30	30	30	30	3.1
60	40	20	40	40	40	40	40	40	40	3.8
50	50	50	50	50	50	50	50	50	50	5.0
40	60	60	60	60	60	60	60	60	60	6.0
30	70	70	70	70	70	70	70	70	70	7.0
20	80	80	80	80	80	80	80	80	80	8.0
10	90	90	90	90	90	90	90	90	90	9.0
0	100	100	100	100	100	100	100	100	100	10.0

*Asumption, One megalop pray on one Z5 in a day; **Number day after moulting; ***Mean value of MG and survival rate from zoea 5 (Z5) to Megalops divided by 10.

Table 3. Survival rate (mean ± SD, n=3) of mud crab larvae

Stage	Fed enriched treatment of				
	DHA+SL0	DHA+SL20	DHA+SL40	WDHA-SL	WDHA-CPL
Z1-Z5	73.3 ± 3.3a	72.2 ± 1.9a	71.1 ± 3.8a	52.2 ± 3.8b	70.0 ± 3.3a
Z5-MG	88.0 ± 6.8	90.8 ± 4.4	92.1 ± 2.9	81.0 ± 5.2	90.5 ± 0.5
MG-FC	93.3 ± 7.6a	93.2 ± 2.8a	95.2 ± 4.8a	57.9 ± 3.9b	96.8 ± 10.5a

Value in the same row are significantly different ($P < 0.05$). Z: zoea, MG: megalops, FC: first crab.

Table 4. Intermolt period (mean ± SD, n=3) to reach each larval stage of mud crab larvae

Larval stage	Fed enriched treatment of				
	DHA+SL0	DHA+SL20	DHA+SL40	WDHA-SL	WDHA-CPL
Z1-Z5	16.9 ± 0.3	17.1 ± 0.1	17.1 ± 0.1	17.4 ± 0.2	17.1 ± 0.2
MG-FC	7.3 ± 0.09a	7.4 ± 0.09a	7.5 ± 0.20a	8.5 ± 0.2	7.3 ± 0.2

Value in the same row are significantly different (P < 0.05). Z: zoea, MG: megalops, FC: first crab.

Table 5. Crude lipid and fatty acid (Mean SD, n=3) of enriched *Artemia*

Crude lipid/ fatty acid	Fed enriched treatment of				
	DHA+SL0	DHA+SL20	DHA+SL40	WDHA-SL	WDHA-CPL
Lipid	19.59 ± 0.20[a]	21.00 ± 0.20[c]	20.62 ± 0.16[b]	19.37 ± 0.40[a]	20.99 ± 0.29[c]
Σn-3[1]	5.16 ± 0.24[b]	5.54 ± 0.26[b]	7.22 ± 0.15[c]	4.49 ± 0.10[a]	5.26 ± 0.15[b]
Σn-6[2]	1.29 ± 0.10[a]	1.57 ± 0.13[b]	2.28 ± 0.39[c]	1.48 ± 0.06[b]	1.32 ± 0.06[a]
Σn-3 HUFA[3]	1.13 ± 0.03[c]	1.18 ± 0.04[c]	1.19 ± 0.02[c]	0.39 ± 0.02[a]	1.04 ± 0.02[b]
EPA[4]	0.68 ± 0.03[b]	0.72 ± 0.03[b]	0.84 ± 0.10[b]	0.34 ± 0.01[a]	0.77 ± 0.05[b]
DHA[5]	0.31 ± 0.01[ab]	0.37 ± 0.04[c]	0.27 ± 0.06[ab]	0.00 ± 0.00	0.22 ± 0.03[a]

Value with superscript in the same row are significantly different (P < 0.05). 1. 16:3n-6, 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6; 2. 16:3n-3, 18:2n-3, 18:3n-3, 20:2n-3, 20:3n-3, 20:4n-3, 22:4n-3, 22:5n-3; 3. 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3. 4. Eicosapentaenoic acid. 5. Docosahexaenoic acid.

Table 6. Lipid classes (Mean SD, n=3) of enriched *Artemia*

Lipid classes	Fed enriched treatment of				
	DHA+SL0	DHA+SL20	DHA+SL40	WDHA-SL	WDHA-CPL
Crude lipid	19.59 ± 0.20[a]	21.00 ± 0.20[c]	20.62 ± 0.16[b]	19.37 ± 0.40[a]	20.99 ± 0.29[c]
Nonpolar					
SE[1]	0.01 ± 0.00[a]	0.09 ± 0.02[c]	0.07 ± 0.01[b,c]	0.06 ± 0.01[b]	0.06 ± 0.01[b]
TG[2]	10.47 ± 1.20	9.34 ± 0.95	8.61 ± 0.98	9.66 ± 1.32	9.78 ± 1.10
FFA[3]	0.84 ± 0.10[a]	1.24 ± 0.16[b]	1.08 ± 0.12[a,b]	0.85 ± 0.12[a]	0.95 ± 0.12[a,b]
FS[4]	0.55 ± 0.10[a]	0.79 ± 0.08[b]	0.80 ± 0.13[b]	0.42 ± 0.06[a]	0.73 ± 0.11[b]
MG+DG[5]	2.37 ± 0.12	2.02 ± 0.21	2.20 ± 0.21	2.24 ± 0.23	2.22 ± 0.17
Polar					
PE[5]	1.98 ± 0.21[a]	2.63 ± 0.35[b]	2.44 ± 0.16[b]	2.30 ± 0.11[b]	2.76 ± 0.21[b]
PC[6]	2.46 ± 0.11[a]	3.53 ± 0.32[b]	3.54 ± 0.24[b]	3.05 ± 0.24[b]	3.48 ± 0.35[b]
Others[7]	0.91 ± 0.10	1.35 ± 0.13	1.89 ± 0.21	0.82 ± 0.17	1.02 ± 0.22

Value with superscript in the same row are significantly different (P < 0.05). 1. Sterol esters, 2. Triglycerides, 3. Free fatty acids, 4. Free sterols, 5. Monoglycerides+diglycerides, 6. Phosphatidylethanolamine, 7. Phosphatidilcholine.

a higher mean value of molting synchronicity index compared to that of larvae fed *Artemia* enriched only (P < 0.05).

Chemical Parameters. Crude lipid in *Artemia* ranged from 19.37 to 21% of the percent dry basis, with value EPA and DHA ranging from 0.34 to 0.84% and from 0.0 to 0.37%, respectively (Table 5). The n-3HUFA level in *Artemia* ranged from 0.39 to 1.19%. The phospholipids enrichment markedly affected the inclusions of DHA in *Artemia* enriched with DHA70G. Except for "n-6 group of fatty acid, all value of other fatty acid that out lined in Table 5 was lower observed in *Artemia* enriched with WDHA-SL compared to WDHA-CPL or combination of SL and DHA70G (P < 0.05).

Nonpolar lipids were major constituent of the lipid fraction and dominated by triglycerides and followed by diglycerides and monoglycerides (Table 6). In addition the other components such as sterol esters, free sterols and free fatty acids in DHA-SPL20, DHA-SPL40, and WDHA-CPL were higher compared to that of DHA-SL0 and WDHA-SL (P < 0.05). The percentage of total polar lipids increased by the supplementation of phospholipids, with the level of PE and PC markedly increased according to the polar lipid fraction in enriched *Artemia* (P < 0.05).

DISCUSSION

Our result showed no differences on the survival rate, carapace width, and intermolt period of mud crab larvae fed by *Artemia* enriched with different level SL mixture with DHA. The level of PC at 2.46% (DHA-SL0) in *Artemia* suggested that is well within the ranged of the requirement level of mud crab for maintaining a normal growth. This may be a primary reason that elevating PC level from 2.5% (DHA-SL0) to 3.53% (DHA-SL20 and 40) in *Artemia* revealed a similar survival rate and carapace width of mud crab larvae. Teshima *et al*. (1986b) reported that *M. japonicus* required 3% of soybean lechitin for maintain high survival and growth rate. Good growth performance of *P. monodon* was achieved with the diet contains 2.0% soy lecithin (Piedad 1986). On the contrary, the supplementations of soy lecithin gave no significant effect on the growth and survival of fresh-water prawn *Macrobrachium rosenbergii* (Hilton *et al.* 1984).

The present study also demonstrated that phospholipids from cuttlefish (WDHA-CPL) was exhibited better growth performance in survival rate, intermolt period, carapace width and molting synchronicity index (96.8% , 24.3 days, 3.21 mm, and 1.4). It was reported that

the sources of phospholipids affecting the growth and survival of the shrimp. Coutteau *et al*. (1996) reported that the supplementation of soybean PC or de-oiled soybean lecithin at 1.5 or 6.5% in the diet exhibited the highest mean weight of postlarval *P. vannamei* compared to that of egg PC at 1.5%. Coutteau *et al*. (2000) stated that supplementation of 1.5% soybean PC significantly improved growth and reduced sensitivity to osmotic stress of post larvae *P. vannamei* compared to those fed with marine fish roe PC and the PC-deprived shrimp. Moreover, they also reported that marine fish roe PC yield better growth an survival than the PC-deprived shrimp. The effectiveness of phospholipids sources on the growth and survival of the shrimps are related to the concentration of essential fatty acid in the diet. *Artemia* enriched with WDHA-CPL and WDHA-SL is containing the same level of PC and PE (Table 6). However, WDHA-SL containing a lower level of EPA (0.34%) while DHA was no detected compared to WDHA-CPL (Table 5). Our previous study reported that the lower limit of EPA level in the *Artemia* was 0.6% for maintaining the survival and normal growth (Suprayudi *et al*. 2004b). Thus, the essentiality of phospholipids as growth promoters in mud crab larvae is related to the essential fatty acid. Koven *et al*. (1993) suggested that PL is a good source of essential fatty acids for *Sparus aurata* larvae that the digestive tract not completely developed.

Molting synchronicity to the megalopa stage influence the survival rate of mud crab larvae during mass seed production, since it was observed that megalopa graze remain Z5, and finally reduced the survival at the FC stage in mass seed production of mud crab (Hamasaki *et al*. 2002). The beneficial effect of interaction between PC and essential fatty acids on the improvement of molting synchronicity was observed in this study. The importance of phospholipids and its combination with essential fatty acids have been reported for *M. japonicas* (Kanazawa *et al*. 1985). They postulated that the elevating soybean lecithin level up to the range from 3.6 to 6.0% increased the survival rate, if diet contained 1.0% n-3HUFA. Moreover, Kontara *et al*. (1997) reported that supplying the combination of 1.5% soybean PC and 1% n-3 HUFA in the diet *M. japonicus* post larvae markedly improved the survival, growth, and resistance to osmotic stress. In this study elevating PC level from 2.46% up to 3.48 to 3.54% in combined with EPA (ranged 0.68 to 0.84%) and DHA (range from 0.22 to 0.37%) improved the molting synchronicity of mud crab larvae.

Many suggestions have been formulated to explain the role of dietary PL. These include their possible role in provision of choline, inositol or EFA, and emulsification and absorption of dietary lipid (Coutteau *et al*. 1997). Phospholipids probably contribute to the efficient transport of dietary lipid, thus improve the mobilization of neutral lipids such as fatty acid, triaglyceride and cholesterol from the gut to hepatopancreas in the from of high density lipoprotein and then transport to the cell target via hemolymph and also dietary PLs increase deposition in the tissue as well as an increase of the energy available for growth (Teshima & Kanazawa 1978, 1979, 1980, 1986a,b; Teshima *et al*. 1986b,c). Moreover Kontara *et al*. (1997) suggested that PL improves resistance to osmotic stress by its affect on lipid metabolism and improved incorporation efficiency of n-3HUFA. Taking all the above suggestions into the result of this study especially on molting synchronicity, we suggest that the elevating levels of phospholipids influence the acceleration of lipid transport (especially cholesterol and essential fatty acid) in the from lipoprotein via hemolymph and/or improve the utilization of EFA that were directly used during molting process.

Trough this study clearly brought out that the combination of phospholipids and EFA improved the molting synchronicity of mud crab. However, the additive effect of combination between phospholipids and EFA were directly influence the molting synchronicity by affecting hormonal regulation during molting is remain topic for continuing study.

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REFERENCES

Bowser PR, Rosemark P. 1981. Mortalities of culture lobsters, *Homarus americanus* associated with a molt death syndrome. *Aquacult* 23:11-18. http://dx.doi.org/10.1016/0044-8486(81)90003-X

Coutteau P, Camara MR, Sorgeloos P. 1996. The effect of different levels and sources of dietary phosphatidylcholineon the growth, survival, stress resistance, and fatty acid composition of postlarval *Litopenaeus vannamei*. *Aquacult* 147:261-273. http://dx.doi.org/10.1016/S0044-8486(96)01387-7

Coutteau P, Guerden I, Camara MR, Bergot P, Sorgeloos P. 1997. Review on the dietary effect of phospholipids in fish and crustacean larvae culture. *Aquacult* 155:149-164. http://dx.doi.org/10.1016/S0044-8486(97)00125-7

Coutteau P, Kontara EKM, Sorgeloos P. 2000. Comparison of phosphatidylcholine purified from soybean and marine fish roe in the diet of postlarval *Penaeus vannamei* Boone. *Aquacult* 181:331-345. http://dx.doi.org/10.1016/S0044-8486(99)00238-0

D'Abramo LR, Bordner CE, Conklin DE. 1982. Relationship between phosphatidylcholine and serum cholesterol in lobster, *Homarus* sp. *Mar Biol* 67:231-235. http://dx.doi.org/10.1007/BF00401289

D'Abramo LR, Bordner CE, Conklin DE, Baum NA. 1981. Essential of dietary phosphatidylcholine for the survival of juvenile lobster. *J Nutr* 111:425-431.

Folch J, Lee M, Stanley GHS. 1957. A simple method for the isolation and purification of totals lipids from animal tissue. *J Biol Chem* 226:477-509.

Hamasaki K, Suprayudi MA, Takeuchi T. 2002. Mass mortality during metamorphosis to megalops in the seed production of mud crab Scylla serrata (Crustacea, Decapoda, Portunidae). *Fish Sci* 68:1226-1232. http://dx.doi.org/10.1046/j.1444-2906.2002.00559.x

Harel M, Ozkizilcik S, Lund E, Behrens P, Place AR. 1999. Enhance absorption of docosahexaenoic acid (DHA, 22:6n-3) in *Artemia* nauplii using dietary combination of DHA-rich phospholipids and DHA-sodium salts. *Comp Biochem Physiol* 124:169-176. http://dx.doi.org/10.1016/S0305-0491(99)00106-6

Hilton JW, Harrison KE, Slinger SJ. 1984. A semi-purified test diet for *Macrobrachium rosenbergii*. and the of need for supplemental lecithin. *Aquacult* 37:209-215. http://dx.doi.org/10.1016/0044-8486(84)90153-4

Kanazawa A, Teshima S, Sakamoto M. 1985. Effect of dietary lipids, fatty acid and phospholipids on the growth and survival of prawn *Penaeus japonicus*. *Aquacult* 50:39-49. http://dx.doi.org/10.1016/0044-8486(85)90151-6

Kanazawa A, Teshima S, Tokiwa S, Endo M, Razek FAA. 1979a. Effect of short-necked clam phospholipids on the growth of prawn. *Bull Jap Soc Sci Fish* 45:961-965. http://dx.doi.org/10.2331/suisan.45.961

Kanazawa A, Teshima S, Tokiwa S, Kayama M, Hirata M. 1979b. Effect of linoleic and linolenic acids on growth of prawn. *Ocenol Acta* 2:41-47.

Kayama M, Hirata M, Kanazawa A, Tokiwa S, Saito M. 1980. Essential fatty acids in the diets of prawn III. Lipid metabolism and fatty acid composition. *Nippon Suisan Gakkaishi* 46:483-488. http://dx.doi.org/10.2331/suisan.46.483

Kontara EKM, Coutteau P, Sorgeloos P. 1997. Effect of dietary phospholipids on the requirement for and incorporation of n-3 highly unsaturated fatty acid in postlarval *Penaeus japonicus* Bate. *Aquacult* 158:305-320. http://dx.doi.org/10.1016/S0044-8486(97)00193-2

Koven WM, Kolkovki S, Tandler A, Kissil GW, Sklan D. 1993. The effect of dietary lecithin and lipase, as a function of age, on n-9 fatty acid incorporation in the tissue of lipid of Sparus aurata larvae. *Fish Physiol Biochem* 10:357-364. http://dx.doi.org/10.1007/BF00004502

Merican ZO, Shim KF. 1996. Qualitative requirement of essential fatty acid for juvenile *Peneaus monodon*. *Aquacult* 147:275-291. http://dx.doi.org/10.1016/S0044-8486(96)01379-8

Mokoginta I, Suprayudi MA. 1996. The effect of lechitin level in the diet on the growth and survival rate of tiger prawn *Penaeus monodon* larvae and postlarvae. *J Ilmu-ilmu Perairan dan Perikanan Indonesia* 4:55-64.

Piedad-Pascual F. 1986. Effect of supplemental lechitin and lipid sources on the growth and survival of *Penaeus monodon* juveniles. In: Maclean JL, Dizon LB, Hosillos LV (eds). *The First Asian Fisheries Forum*. Manila: Asian Fisheries Soc. p 615-618.

Rainuzo JR, Reitan KI, Jorgensen L, Olsen Y. 1994. Lipid composition in turbot larvae fed live culture by emulsion of different lipid classes. *Comp Biochem Physiol* 107A:699-710. http://dx.doi.org/10.1016/0300-9629(94)90372-7

Sheih HS. 1969. The biosynthesis of phospholipids in the lonster, *Homarus americanus*. *Comp Biochem Physiol* 30:679-684. http://dx.doi.org/10.1016/0010-406X(69)92146-X

Suprayudi MA, Takeuchi T, Hamasaki K, Hirokawa J. 2002a. Effect of *Artemia* feeding schedule and density on the survival and development of larval mud crab *Scylla serrata*. *Fish Sci* 68:1295-1303. http://dx.doi.org/10.1046/j.1444-2906.2002.00567.x

Suprayudi MA, Takeuchi T, Hamasaki K, Hirokawa J. 2002b. The effect of n-3HUFA content in rotifers on the development and survival of mud crab, *Scylla serrata*, larvae. *Suisanzoshoku* 50:205-212.

Suprayudi MA, Takeuchi T, Hamasaki K. 2004a. Effect Artemia enriched with EPA and DHA on the survival and the occurrence of molting failure to megalop of mud crab, *Scylla serrata* larvae. *Fish Sci* 70:650-658. http://dx.doi.org/10.1111/j.1444-2906.2004.00853.x

Suprayudi MA, Takeuchi T, Hamasaki K. 2004b. The essential fatty acids of larval mud crab *Scylla serrata*, (Crustacea:Decapoda): Implications of bioconversion of C18 unsaturated fatty acid to highly unsaturated fatty acid. *Aquacult* 231:403-416. http://dx.doi.org/10.1016/S0044-8486(03)00542-8

Suprayudi MA, Takeuchi T, Hamasaki K. 2012. Cholesterol effect on survival and development of larval mud crab *Scylla serrata*. *Hayati J Biosci* 19:1-5. http://dx.doi.org/10.4308/hjb.19.1.1

Takeuchi T, Toyota M, Watanabe T. 1992. Comparison of lipid and n-3 highly unsaturated fatty acid incorporation between Artemia enriched with various types of oil by direct method. *Nippon Suis Gak* 58:277-281. http://dx.doi.org/10.2331/suisan.58.277

Teshima S, Kanazawa A. 1986a. Effect of dietary phospholipids on lipid transport in the juvenile prawn. *Bull Jap Soc Sci Fish* 52:159-163. http://dx.doi.org/10.2331/suisan.52.159

Teshima S, Kanazawa A. 1986b. Role of dietary phospholipids in the transport of ^{14}C tripalmitin in the prawn. *Bull Jap Soc Sci Fish* 52:519-524. http://dx.doi.org/10.2331/suisan.52.519

Teshima S, Kanazawa A. 1979. Lipid transport mechanism in the prawn. *Bull Jap Soc Sci Fish* 45:1341-1346. http://dx.doi.org/10.2331/suisan.45.1341

Teshima S, Kanazawa A. 1978. Release and transport lipid in the prawn. *Bull Jap Soc Sci Fish* 44:1269-1274. http://dx.doi.org/10.2331/suisan.44.1269

Teshima S, Kanazawa A. 1980. Lipid constituent and serum lipoprotein in the prawn. *Bull Jap Soc Sci Fish* 46:57-62. http://dx.doi.org/10.2331/suisan.46.57

Teshima S, Kanazawa A, Kakuta Y. 1986a. Growth, survival and body lipid composition of the prawn larvae receiving several dietary phospholipids. *Mem Fac Fish Kagoshima Univ* 35:17-27.

Teshima S, Kanazawa A, Kakuta Y. 1986b. Effect of dietary phospholipids on the growth and body composition of the juvenile prawn. *Bull Jap Soc Sci Fish* 52:155-158. http://dx.doi.org/10.2331/suisan.52.159

Teshima S, Kanazawa A, Kakuta Y. 1986c. Role of dietary phospholipids in the transport 14C cholesterol in the prawn. *Bull Jap Soc Sci Fish* 52:719-723. http://dx.doi.org/10.2331/suisan.52.719

Zhukova NV, Imbs AB, Li LF. 1998. Diet-induced changed in lipid and fatty acid composition of *Artemia salina*. *Comp Biochem Physiol* 120b:499-506.