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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).