



Improving quality of broodstock and early larval stage of mud crab *Scylla serrata* through rearing and feeding methods

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Abstract. Production of mud crabs *Scylla serrata* larvae in hatchery are still facing high mortality in the early stage because of inadequate breeding technology. The aim of this study is to increase the survival rate and development of early larvae stage of mud crab which are generated from improving quality broodstock through different rearing treatment and feeding methods. The selected broodstocks which fed fresh fish 6% of wet body weight (BW) were reared in mangrove habitat. To obtain the mature ovaries of broodstocks, individual crabs were then treated with fresh food as much as 6% BW with different compositions as follows: 1) 3% fresh fish, 1.5% squid, and 1.5% shrimp; 2) 1.8% fresh fish, 3% squid, and 1.2% shrimp; and 3) 1.8% fresh fish, 1.2% squid, and 3% shrimp. Each treatment used 3 individual crabs as replication. Matured broodstock then incubated in aquarium until produced early stage of zoea larvae (Z1-Z2). The results showed that there were positive correlation between egg quality, survival rate and growth rate of early larval stage by treated broodstocks. Broodstocks fed with composition 1.8% fresh fish, 3% squid, and 1.2% shrimp of body weight reaching mature ovaries to undergo spawning and hatching larvae. Which are the ovaries mature time 14.5 ± 5.0 days, time of embryo incubation 11 ± 1.4 days and hatching rate $90.9 \pm 2.8\%$. In addition, early larvae stage from the broodstock which are supplemented with microencapsulation every 6 hours showed a better survival rate and growth rate than others.

Key Words: broodstock, rearing strategy, hatchery technique, maturation diet, growth of early larva.

Introduction. The mud crabs *Scylla* spp. has a high economic value as a fisheries product especially in the Asian region. The biggest crab consumer countries were China, USA, Japan, Korea, Thailand, Taiwan, Hong Kong, and Singapore where live crabs especially gravid females command premium prices (Agbayani 2001). Moreover, mud crabs represent a valuable component of small-scaled coastal fisheries in many countries in tropical and subtropical Asia (Nghia et al 2007a). Fishing activities of mud crabs increase in recent years as a result of market demand both locally and internationally (Ferdoushi et al 2010). Population of mud crabs decline drastically and it is characterized by less catches of mud crab and the smaller size of catches that have occurred in the last two decades, particularly in the Southeast Asian regions (Kosuge 2001; Le Vay 2001).

An attempt has been made to reduce over exploitation in the wild through cultivation. However, this effort is still facing many problems such as limited human resources with adequate cultivation techniques, differences in cultivation strategies due to variation in characteristics of local condition, limited broodstocks from the wild, with long maturation time and large variability of larval between batches (Shelley 2008; Pattiasina 2010).

Therefore, this research was conducted based on the mud crab hatchery techniques appropriate to local seawater sources, local resources, and hatchery management with regard to the islands region. In addition, the larval rearing methods of previous work were integrated between the broodstock feed quality and improvement

larval rearing environment. It is hope that cultivation of mud crabs that inhabit the mangrove ecosystem can be performed in their habitat by engaging local people to contribute rearing the adult or juvenile and it will in turn increase the wild population. Similarly, compliance with market demand for this commodity can be achieved and overfishing can be avoided. Also the ecological balance in nature will be preserved, as well as improve the local economy.

Material and Method. The research was developed from June to November 2012. Mud crab broodstocks were taken from different locations namely Dobo, Tual, West Seram and the Bay of Ambon. Broodstocks trial, spawning and larval rearing of mud crab were conducted in Mariculture Center, Waiheru, Ambon.

Broodstock rearing. Broodstocks of *Scylla serrata* iniatially were maintained in bamboo cage and plastic cage placed near the mangrove forest. Crabs were fed daily in the afternoon with fresh fish (*Decapterus* sp.) at 10% wet body weight. Observations were made at intervals of 4 days, and lasted until the crab has revealed ovarian tissue color changes to cream or pale yellow, as a sign that there has been a proliferation of eggs or early ovarian maturity. Crabs were then transferred to the semi-outdoor house and maintained in fiberglass tank (2.48 x 1.26 x 0.6 m) that had been disinfected with 10 ppm chlorine solution, rinsed with seawater and allowed 24 hours before used. Rearing tank was partitioned into 12 plots measuring 40 x 30 x 60 cm with 15 cm thick sandy substratum and filled with seawater as high as 25 cm to maintain one individual crab. Before being put into rearing tank, crabs were disinfected for 1 hour in ± 5 liters solution of KMnO₄ (37%). In addition to fiber tank, the broodstock crab is also maintained in the 16 L plastic container as a comparison. To ensure the quality of the water, flow-through seawater was applied for each individual plot which is also function as aeration. The all of containers were placed semi outdoor. As many as 9 inviduals undeveloped ovaries mud crab *S. serrata* with initial weights ranging from 500.7 \pm 103.4 g were used in this experiment.

To obtain the mature ovaries of broodstocks, individual crabs were then treated with fresh food as much as 6% of wet body weight (BW) with different compositions as follow: 1) 3% fresh fish, 1.5% squid, and 1.5% shrimp; 2) 1.8% fresh fish, 3% squid, and 1.2% shrimp; and 3) 1.8% fresh fish, 1.2% squid, and 3% shrimp. Each treatment used 3 individual crabs as replication. Observations of ovarian maturity stage was done every 4 days based on Pattiasina (2010) as presented in Table 1.

Table 1

Description ovarian tissue closure by which egg cells are observed externally through linkage under the carapace and the first abdominal segment and the scale value is used to determine the ovarian maturation stage (OMS) morphologically of the mud crab *Scylla serrata*

Ovarian tissue color	Scale	OMS	Description of egg cells in ovarian tissue
Transparent	1	I	Ovarian tissue has not been filled with egg cells
Milk white	1	I	Egg cells appear in the form of a point in the upper half of the ovarian tissue
Pale yellow	2	I+	Ovarian tissue begins to fill with eggs and started to appear color change
Dark yellow	3	II	Egg cells in the middle position of cross section of ovarian tissue
Dark yellow, orange, orange-reddish	4	II+	Egg cells already meet partial or half of cross section of ovarian tissue
Dark yellow, orange, orange-reddish	5	III	Egg cells have fulfilled in whole or almost $\frac{3}{4}$ of the ovarian tissue section and appear until the abdomen
Orange, orange-reddish	6	IV	Egg cells have filled the whole sections the ovarian tissue and appear until lower abdominal bulge

The berried crabs observed was then transferred as soon as possible after spawning to the 126 L aquaria that have previously been disinfected with a chlorine solution of 10 ppm. Container equipped with flow-through seawater and mild aeration. The incubator aquarium was siphoned daily to clean the dirt. To maintain water quality and to prevent contamination of the eggs, no food was given to the berried crabs. Observation of embryonic development was done every day under a microscope until larvae hatching occurs. When the hatching process was completed, larvae were selected based on their phototactic behaviour. Aeration in the incubator was turned off for several minutes and the active larvae swimming up to the surface were collected by gentle scooping.

Rearing of *S. serrata* larvae. As soon as the eggs hatch in the incubator aquaria, larvae were taken and then transferred into rearing containers with stocking density of 65 individuals/L. As many as 5 aquariums (12 L in volume) were filled with 9 L sterilised seawater and given mild aeration. The seawater turnover rate for each aquarium was 30%. Whenever aquaria were cleaned, larvae were transferred temporarily into 10 L plastic container. As much as 5 cylinder plastic containers of 10 L volume is filled with 9 L of seawater and floated with styrofoam sheets for placing larvae. Once cleaned, the larvae are returned to the aquaria.

Larval rearing system used a water-bath by placing all containers in the fiber tank measuring 2.48 x 1.26 x 0.60 m and filled with seawater as high as 30 cm. As many as 4 heaters and thermostats were used to stabilise temperature in fiber tank at 31-32°C. Layout of larval rearing system is presented in Figure 1. Overall larval rearing system takes place in an enclosed shaped house made of wood frame measuring 3.5 x 2 x 2 m. The space was entirely covered with plastic sheet with a fluorescent lamp above of the fiber tank and placed in a semi-outdoor location.

Larvae were fed with rotifer (*Branchionus plicatilis*) and *Artemia* sp. once a day in the morning. Rotifer (<30 µ) was given for newly hatching larvae at density 20 ind.mL⁻¹ while *Artemia* (<100 µ) was started at day 3 after hatching at density 10 ind.mL⁻¹. Larvae were also fed with the microencapsulated GAP (artificial plankton) at a dose of 0.01 mg.12 L⁻¹ aquaria every 3 and 6 hours.

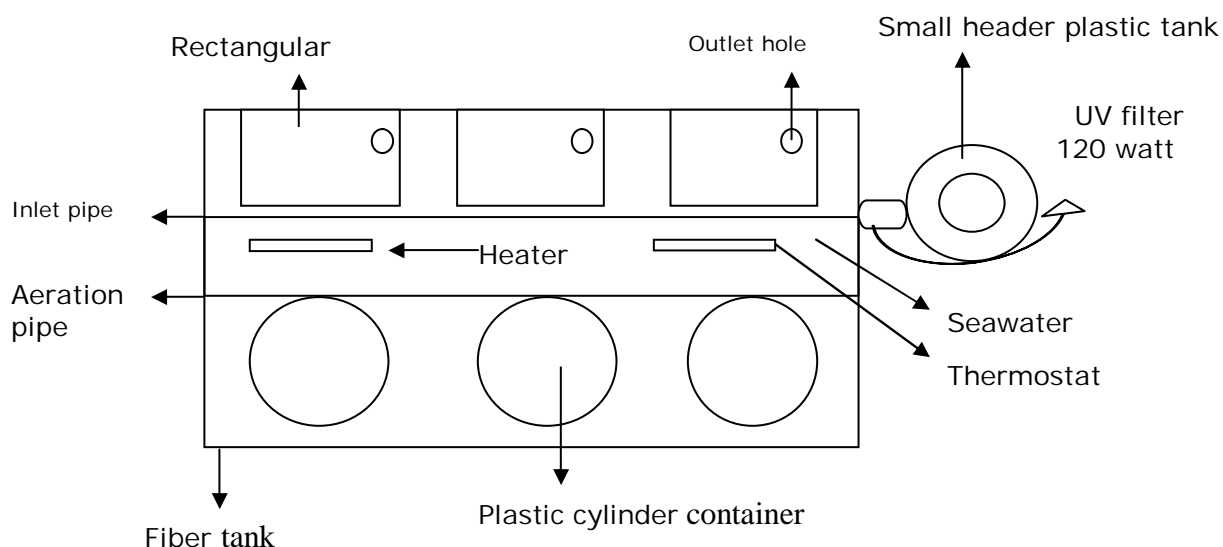


Figure 1. Layout of larval rearing system (original).

Data collection and analysis. Parameters of ovarian maturation spent time are determined by the time required to reach mature ovaries (ovarian maturation stage III) of the early ovarian maturation in mangrove site. The incubation time is the time required for embryo since starting spawning until larvae release. Hatching rate is the ratio between the numbers of larvae that successfully released with the number of eggs released multiplied by 100%. Total body length was measured under a microscope Nikon

eclipse 50i, by drawing a line from the tip of the carapace near the leading edge with an eye until at the end of the telson. Absolute length growth was analyzed using formula:

$$\Delta L = L_t - L_0.$$

Where: L_t - the average length (μm) of larvae at the end of the observation;
 L_0 - the average length (μm) of larvae at the beginning of the observation.

Data were analyzed descriptively, and presented in the tables and figures. While data of ovaries mature time were presented as the average \pm standard deviation of replicate measurements ($n = 3$). Statistical analysis of the data was carried out by an analysis of variance (ANOVA). The significance of differences was defined at $p < 0.05$.

Results and Discussion

Maturation and spawning strategies of brood *S. serrata*. A previous study conducted by Pattiasina et al (2012) reported that, brood *S. serrata* were kept in fiberglass tank with running water system, and they needed 2-3 weeks to obtained early mature ovary. Therefore, strategy to put immature *S. serrata* brood in mangrove area succed to shortening early ovarian maturation i.e. 4-7 days. By keeping immature brood *S. serrata* in the mangrove area means that those animals are living in their habitat in which they are still affected by tidal current and thus make them reach early ovarian maturation stage faster. In this experiment, containers are used to keep immature brood *S. serrata* in the mangrove area to undergo egg cells proliferation to reach the early ovarian maturation stage are made from bamboo (Figure 2a) and plastic basket (Figure 2b). This kind of containers is cheap and affordable by local farmers. In addition, both containers are applicable for narrow coastal area in small island. Eventhough both containers have those advantages, it seems that plastic cage is more effective because durable, cheaper, and it can be used more than once.



Figure 2. Confinement bamboo container (a) and plastic baskets (b) for the maintenance of *Scylla serrata* brood at mangrove area (original).

As soon as broodstocks show early ovarian maturation stage, they are put into the fibreglass tank which is partitioned in several plots (Figure 3a). This tank is durable and it can accommodate large number of broodstock. Apart from fibreglass tank, broodstocks are also kept in rectangular 16 L plastic containers (Figure 3b). In comparison, using rectangular 16 L plastic containers provide more benefits than fibreglass tank because they are relatively cheap and easy to clean as well as to replace sandy substratum. In addition, the *S. serrata* that are kept in a plastic container with through-flow seawater system result in more optimal conditions to accelerate the process of ovarian maturation.



Figure 3. The rearing container of fiberglass tank is partitioned (A) and rectangular plastic container (B) with through-flow seawater system (original).

In this experiment, broodstock has reached mature ovaries in preparation for spawning in a period ranging between 2 weeks and 1 month. Broodstocks fed with 3% fish, 1.5% squid, and shrimp 1.5% of BW reach ovarian maturation sooner (20.3 ± 9.0 days) than broodstock fed with fish 1.8%; squid 1.2% and shrimp 3% (37.3 ± 11.6 days). The feed composition with fresh fish (*Decapterus* sp.) as a source of protein can stimulate ovarian maturation of broodstock, due to higher protein content (82.0%) enough to fulfill the needs of increasing protein synthesis intensively during this process. The treatments show no differences ($P > 0.05$). It means that all feed treatments are eligible for the achievement of the ovarian maturation (Table 2).

Table 2

The ovaries mature time of the brood crab *Scylla serrata*

Fresh food composition (%)	Ovarian maturation time (days)
1) Fish 3%, squid 1.5%, shrimp 1.5%	20.3 ± 9.0^a
2) Fish 1.8%, squid 3%, shrimp 1.2%	14.7 ± 3.5^a
3) Fish 1.8%, squid 1.2%, shrimp 3%	37.3 ± 11.6^a

Same letters indicate no statistical differences ($P > 0.05$).

Nevertheless, only broodstocks were fed with 1.8% fish, 3% squid, and shrimp 1.2% of BW could be obtained mature ovaries to spawning and hatching larvae, which the mature ovaries reach in shortest time (14.5 ± 5.0 days), time of embryo incubation 11 ± 1.4 days and hatching rate $90.9 \pm 28\%$. It seems that the crabs fed with 3% squid of BW can stimulate the process of spawning and the success of hatching larvae compared with the same percent of fish and shrimp in each feed composition. Mixed fresh food composition with the squid 3% contains phospholipid, which is a specific nutrient needed for the reproduction of crustaceans. According to Castille et al (2004), food containing phospholipids is recommended to facilitate the utilization of cholesterol by crustaceans. There were 1.2% of shrimp in the composition of food, it is assumed as a significant source of cholesterol (Leera & Nwabugo 2012), so the fresh food mixture consisting of fish and squid can help the formation of lipoproteins that carry cholesterol in the hemolymph to the ovary. This may also explain the shortest time to achieve mature ovaries. In addition, Djunaidah (2004) stated that food composition of fresh fish, squid and shrimp contains high fatty acids DHA (docosa hexaenoic acid) also influence the content of DHA in the ovarian. Furthermore, Nghia et al (2007b) and Suprayudi et al (2012) reported that the *S. serrata* larvae require n-3 highly unsaturated fatty acids (HUFA) such as essential fatty acids (EFAs), especially eicosapentaenoic acid (EPA) and DHA to maintain their survival.

Rearing techniques strategy of early larvae stage. Most of larvae rearing facilities so far are expensive and not easy to be implemented for local farmer community. In this experiment, rearing facilities is designed as cheap as possible, easy to be implemented

and effectively maintain larvae at critical phase especially at early stage of zoea larvae (Z1-Z2). Larval rearing room is made of plastic sheets and sealed to maintain stable temperature and salinity (Figure 4a). Larval rearing container made of fiberglass is equipped with running seawater and aeration system as shown in Figure 4b. During the experiment, temperature and salinity of seawater in the aquarium which were embedded in the fiberglass container for keeping larvae are stable at 31-32°C and 33-34‰, respectively. This means that larvae rearing facilities in the experiment can stabilise temperature and salinity at optimal level for larvae. Thus, this rearing facility is not only can be used indoor but also outdoor as far as its location is protected and closed to the seawater.

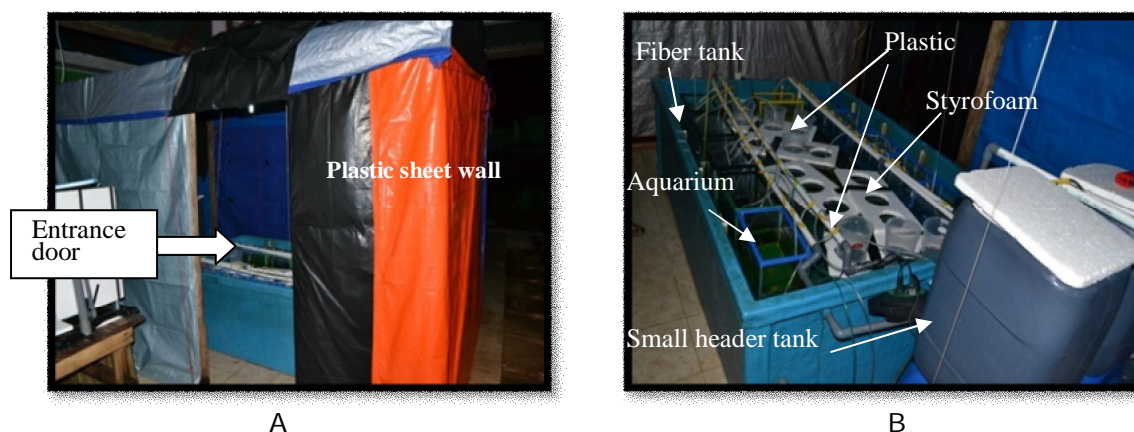


Figure 4. Models of larval rearing mud crab in a plastic wall room on the outside (A) and the inside (B) (original).

Larvae used in this experiment came from broodstock crabs fed with 1.8% fish, 3% squid and 1.2% shrimp of the body weight. These broodstock not only produced eggs sooner but also produced more active larvae. As stated above, larvae were fed with live food and microencapsulated GAP (artificial plankton) as a supplement. The result showed that larvae fed with artificial plankton at 6 h interval grow faster than those fed at 3 h interval (Figure 5).

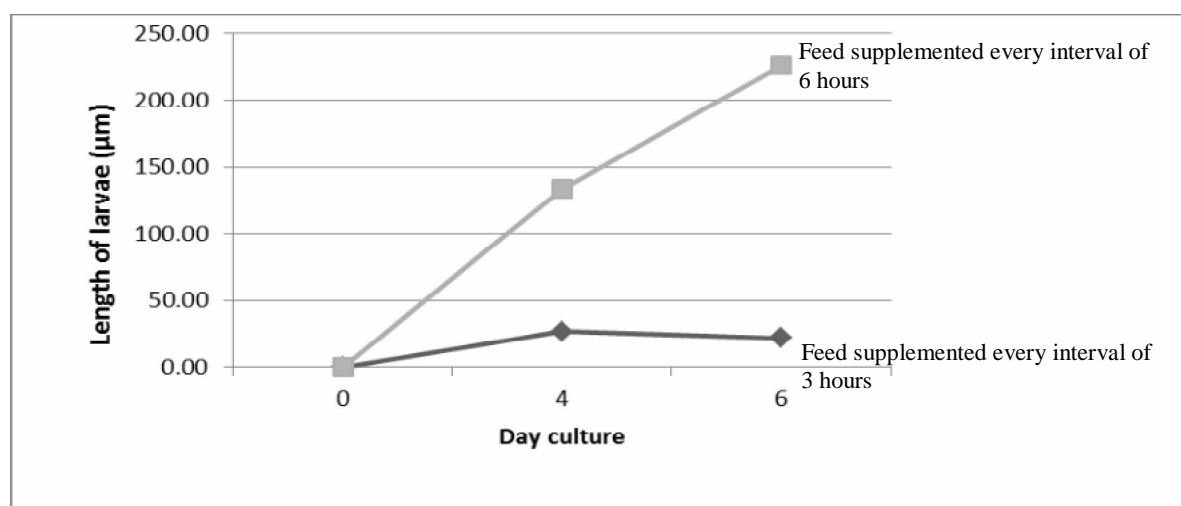


Figure 5. Growth of mud crab *Scylla serrata* larvae fed with artificial plankton at 3 hours and 6 hours interval ΔL .

Apart from live food, crude protein (43%) and crude fat (25%) in the microencapsulated artificial diets (GAP) supposedly are high enough to meet the needs of early stages of larvae to grow and to maintain their survival. The microencapsulation artificial diets are a form of artificial plankton suspension which is very useful to strengthening the immune system as well as to ensure growth and survival of larvae. Protein utilization by aquatic

organisms is intended to get amino acids that are essential because they can not be synthesized by the body (Pavasovic 2004). Thus, the need for protein by aquatic organisms is continuously required for the formation of new proteins either during the process of growth and reproduction or for the maintenance of body protein turnover (Wilson 2002). According to Holme (2008), the optimal level of protein required by larvae varies based on the species and the stage of larvae as well as the source of protein and it is strongly influenced by the digestion ability and amino acid composition. Furthermore, larvae of *S. serrata* as other penaeid larvae need a higher value of protein than juvenile and adult. The needs of protein for most crustaceans ranged from 30-60% (Djunaidah 2004; Holme 2008; Azra & Ikhwanuddin 2016). Apart from live food, the micro encapsulated food supplements given at the appropriate time can support the growth of *S. serrata* larvae in the early stages.

Conclusions. *S. serrata* broodstock maintained at the mangrove site effectively achieve the mature ovaries of early stages and shorten the time of ovarian maturation. The feed composition of 1.8% fish, 3% squid and 1.2% shrimp of the total dose of 6% of body weight brood crab shorten the time of spawning and hatching larvae. The microencapsulated food supplementation on early larvae stages at 6 h interval is better for growth than at 3 h interval. A practical feeding schedule of live food rotifer can be applied to early zoea larvae (Z1 to Z2) then *Artemia nauplii* for after the early larvae stage. Construction design larval rearing in semi outdoor made of plastic tarpaulin, with flow-through seawater and water bath system keep optimal temperature and salinity for sustaining survival of the larvae.

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