Reproduction Performances of Mud Crab (*Scylla olivacea*) Broodstocks with Different Feeds

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Abstract: *Broodstock maturation diets is important research in order to increase the reproduction performance of spawning females. This research was conducted to determine the appropriate feed for mud crab broodstock spawned and as information in support of efforts to provide quality stem in a controlled manner. The study was conducted at the Installation Research Marana Institute For Coastal Aquaculture (RICA). The test animals that used were 15 ind. mud crab broodstock having of size 200±15 g, carapace length 6±0,5cm, carapace width 12±0,5 cm with gonadal maturity level (TKG I) obtained from crab fishing locations. Research using completely randomized design which consists of 3 treatments and 5 replications. The treatments tested were: A (trash fish), B (squid) and C (golden apple snail). Variable measured were the rate of gonadal maturity, egg diameter, the degree of spawning, embryo incubation period, the number of larvae, hatching rate, larval amino acid and proximate analyze content and water quality variables. The results of proximate analyze showed that trash fish have the highest fat content (7.85 %) while the squid had a high protein content (73.72 %). Amino acids contained in squid is alanine, cystine, and leucine are thought to have an important role in the maturation of the gonads crab. The feed squid gives the best performing for mud crab S.olivacea broodstockin the achievement of gonadal maturity stage, fecundity and hatching rate.*

Keywords: *Reproduction performance; mud crab; broodstock; Scylla olivacea*

1. Introduction

Mud crabs belonging to genus Scylla have a high commercial value in international markets. Therefore, they are prospective to be cultured (Keenan, 1999; Trino & Rodriguez, 2002). In responding to economical benefits from mud crab trade, the Indonesia Government has supported the development of mud crab aquaculture. *S. olivacea* is one species of mud crabs commonly found in Indonesia region, particularly in South Sulawesi having the potential to be developed. However, the availability of seed is still a constrain in the increasing of mud crabs aquaculture production. Growt out or cultivation still depent on the natural seed, but the seed arrest excess (over fishing) being offset by conservation efforts led to a decline of mud crab population. Although the mud crab seeds have been already pro-

duced in hatchery, (Millamena & Quinitio, 2000; Djunaedi *et al*., 2002) butlow fecundity and survival rate are still considered to be improved (Keenan, 1999).

Some studies on the use of hormones and environmental manipulations to induce mud crab breeding have been reported (Primavera, 1985). Nevertheless, feed also influences mud crab maturity, breeding, egg quality and fecundity (Watanabe, 1988); in Indonesia, the use of local material for mud crab feed, particularly for *S. serrata* has been promoted by Hutabarat (1997). The natural diets produce better larval quality compared to the artificial diet, and the mixed diet is the better diet which resulted in better reproductive performance such as growth, survival, fecundity and maturation processes for Scylla broodstock (Azra and Ikhwanuddin, 2015) and there were only minor differences in reproductive performance between mixture fresh food and artificial diets (Djunaidah *et al.,* 2003).Trash fish, squid and golden snail are three raw materials that generally utilized for feed in fish aquaculture, as well as hatcheries. However, there is no sufficient information in feed improvement for *S. olivacea* broodstocks. Therefore, this study aimed to analyse reproduction performance of mud crab *S. olivacea* broodstocks feed with three different feed; which are trash fish, squid and golden snail.

2. Materials and Method

This study was conducted in a crab hatchery research installation in Maranak, Maros which is under the authority of Research Institute for Coastal Aquaculture (RICA), Maros. The study was conducted by acompletely randomized designwith three treatments which were trash fish, squid and golden snail as treatments A, B and C, respectively, and five replications. The crab feed were considered as the dependent factor, whereas ggonadal maturity stage, egg size (diameter), breeding percentage, period of embryonic incubation, amount of larvae, hatching rate, larval amino acid content and water quality were considered as independent factors.

A total of 15 individual of wild *S. olivacea* broodstocks originated were used in this study. The average (± standard deviation) of body weight, length and wide of samples were 200 ± 15 g, 6 ± 0.5 and 12 ± 0.5 cm, respectively;all crabs had gonadal maturity stage of I confirmed by gonadal maturity stages identification(Jhon and Sivadas 1978; Islam *et al.,* 2010; Rahmi *et.al.,* 2013) (see Table 1). Prophylaxis was then conducted by dipping all crabs in 25 ppm of formalin solution for 30 minutes to kill parasites that mightinfect those samples.

Samples were reared in 300 L conical re-circulating fiberglass tanks. Water depth was maintained in the range of 40 to 50 cm with salinity of 30 to 32 ppt. The tank bottom was covered with 5 cm of sand as substrate. During the study, those samples were feed based on their treatment where the feed doses were 15% of their body weight when the gonadal maturity stageof samples were I, then reduced to 5% of their body weight when the gonadal maturity stage was IV. Feed were applied once per day which was in the morning after removing uneaten feed from the initial feeding. The nutrients content of the feeds was proximate analyze in the Laboratory of Fish Nutrition of RICA, while amino acid contents of mud crab larvae was

Table 1. Gonadal maturity stageused in this study (Jhon and Sivadas 1978; Islam*et al.,* 2010; Rahmi *et.al.,* 2013)

Gonadal Maturity Stage	Morphological characteristics of ovarian
I (*Proliferation*)	Transparent ovarian (covered by thin peritoneum layer) and forms a structure of the *ribbon*-like.
II (*Pre-vitellogenesis*)	Off white to creamy white ovary, eggs are unclear, Approximately 2-3 mm thick and which occupy 1-2% of the cavity.
III (*Primary vitellogenesis*)	Ovary turns to pale or light yellow color, Eggs are visible, yet still covered by oil glands, 3-7 mm thick and occupy 10-20% of the cavity.
IV (*Secondary vitellogenesis*)	Yellow to orange ovary that are 7-12 mm thick and occupy 20-75% of the cavity. The layer can be separated. The oil glands is reduced.
V (*Tertiary vitellogenesis*)	Individual eggs are visible. Yellow orange to red orange ovary; 10-20 mm thick and occupy >75% of the cavity. Partly appears from cavity and obviously from abdomen

analyze in laboratorium of PT. Saraswanti Indo Genetech Bogor.

The gonadal maturity stages were identified every seven days through a visual observation after pressing the inferior part of ventral carapace. Breeding was observed based on the condition of abdomen of the crab where the eggs are attached. Water quality variables: temperature, salinity, pH and dissolved oxygen were measured daily through *in-situ* measurement water using a portable DO meter YSI MDS 650. Whereas, total ammoniacal nitrogen (NH3-N) was measured weekly where the samples were analysed in the Laboratory of Water Quality, RICA. The calculations of breeding ratio, eggs diameter, fecundity, hatching rate and embryonic period provided by equations below:

2.1 Breeding Percentage (%)

Amount of breeding broodstock in each treatment per total amount of broodstock in each treatmentmultiplied by 100 percent.

2.2 Egg Diameter

Diameter of the egg was measured under microscope attached with a micrometer ocular.

2.3 Fecundity

$$F = \frac{D \times V \times X}{Q}$$

Where:
F = fecundity
G = weight of gonad (g)
V = volume of diluted eggs sample (mL)
X = the number of egg contained in one mL of sample
Q = the weight of sampled eggs (g).

2.4 Hatching Rate:

The amount of zoea was calculated by collecting water containing zoea from hatching tanks with three replications. During the collection, the water in the tank was strongly aerated to ensure that the zoea was distributed homogeneity in the water column. In contrast, the un-hatching eggs were calculated by remove the aeration in the tank water then the tank was illuminated to attract the zoea accumulated at the water surface. The zoea then accumulated in the water surface, whereas the un-hatched eggs were deposited on tank bottom. The zoea and un-hatched eggs were separated by siphoning the zoea and then the un-hatching eggs. The un-hatched eggs were then calculated manually.

2.5 Embrionic period

Embrionic period was calculated based on the durance of broodstock incubation until the eggs were hatched. Larvae were harvest and then analyzed for amino acid content. The amino acid contents were analyses in The Laboratory of *Balai Besar Penelitian Teknologi Pasca Panen Pertanian* Bogor, West Java.Data of breeding percentage, egg diameter, fecundity, hatching rate and embryonic period were analyses by One-Way ANOVA to analyze the impact of dependent factor on the independent variables. The Turkeys test as post hoc analyses were conducted to find out the treatment resulted significant impact. Data of larval amino acid and water quality variables were analyzed descriptively The One-Way ANOVA was conducting using a software SPSS.

3. Results and Discussion

3.1 Gonadal Maturity Stage

Data of gonadal maturity stages for each mud crab broodstockat the specific period were qualitative data. The data were transformed in toquantitative data by ranking (Siahainenia *et al*., 2007); therefore statistical analysis for gonadal maturity stages could be conducted. The rank for each gonadal maturity stage was: 20, 40, 60 and 100 for gonadal maturity stages of I, II, III and IV, respectively. The gonadal maturity stage for each treatment during the study is provided by Table 2.

Morphological observation showed significant differences among the treatments. Mud crab broodstocks feed with squid reached its gonadal maturity earlier compared to that observed in broodstocks feed with trash fish and golden snail. In this study, the gonadal maturity stage of three of five broodstocksin treatment B reached stage IV within 21 days then all broodstock reached stage IV in the day 35. In contrast, only one of broodstockin treatment Areached stage IV within 21 day of rearing then all broodstock reached stage IV after 42 days of study. Broodstockin the treatment C required

Table 2. Gonadal maturity stages of mud crab broodstock observed during this study

Treatment	*Replication*	Gonadal maturity stages *Day of study*						
		0	7	14	21	28	35	42
Trash fish	1	I	I	II	III	III	IV	IV
	2	I	II	II	III	III	IV	IV
	3	I	II	II	III	IV	IV	IV
	4	I	II	III	IV	IV	IV	IV
	5	I	I	II	II	III	III	#
	average	20	32	44	60	76	92	80
Squid	1	I	II	II	IV	IV	IV	IV
	2	I	II	III	IV	IV	IV	IV
	3	I	II	II	III	III	IV	IV
	4	I	II	III	IV	IV	IV	IV
	5	I	II	III	III	IV	IV	IV
	average	20	40	52	84	92	100	100
Golden snail	1	I	I	II	II	III	#	#
	2	I	II	III	III	IV	IV	IV
	3	I	II	III	III	IV	IV	IV
	4	I	I	II	II	III	III	IV
	5	I	I	II	III	III	IV	IV
	average	20	28	48	52	76	72	80

Gonadal maturity stagerank scale: I =20; II=40; III=60; IV=100 (Siahaineniaetal., 2007); #: abortus

longer time to reach stage IV; this stage was reached in day 28 observed from two broodstocks then all broodstocks reached stage IV within period of 42 days.

The data of breeding percentage, fecundity, hatching rate, and embryonic period as well as the number of zoea observed in this study are presented in Table 4. The treatment did not significantly affect ($p>0.05$) breeding percentage and egg diameter. However, the performance of broodstocks feed with squid was higher compared that observed on broodstocks feed with trash fish and golden snail where all (100%) broodstocks were successfully breeding compared that observed on broodstocks feed with trash fish and golden snail (80%). There were three samples were died during this study which were 1 broodstockin treatment trash fish (day 42) and 2 broodstocks in treatment golden snail (day 35 and 42).

Table 3. The average (±SD) of spawning, egg diameter, fecundity, hatching rate, embryonic incubation period and the number of crab larvae from the broodstock.

Variable	*Treatment*		
	Trash fish	*Squid*	*golden snail*
Breeding percentage (%)	80±4.5[a]	100±0.0[a]	80±4.5[a]
Egg diameter (µm)	3.5±0.1[a]	3.5±0.1[a]	3.5±0.1[a]
Fecundity (million)	2.4±0.2[ab]	2.6±0.1[a]	2.2±0.2[b]
Hatching rate (%)	88.7±5.1[a]	92.3±2.5[a]	85.6±6.1[a]
Embryonic period (day)	14.4±0.6[a]	14.2±0.5[a]	14.8±0.5[a]
Number of zoea (million)	2.2±0.2[ab]	2.4±0.2[a]	1.9±0.3[b]

The value followed by the same letter on the same row were no significantly different (p>0,05)

3.2 Nutrient Content

The results of proximate analyzed showed that trash fish have the highest fat content (7.85 %) while the squid had a high protein content (73.72 %). The ash and crude fiber content of trash fish higher than squid and golden snail but the highest BETN reach in golden snail.

Table 4. Nutrient content of mud crab broodstock.

Nutrients *(% dry matter)**	*Typeof feed**		
	Trash fish	Squid	Golden snail
Ash	25.33	12.56	16.6
Protein	55.56	73.72	56.02
Lipid	7.85	0.95	5.14
Crude fiber	6.15	4.88	3.6
BETN	5.11	7.89	18.64

The value based on analyses result from the Laboratory of Fish Nutrition, RICA.

3.3 Amino Acid

Most of the highest amino acid contents was reach in trash fish but some of the amino acids contained in squid is alanine, cystine, and leucine (Table 5).

Table 5. Amino acid contents of mud crab larvae (% dry matter)

No.	*Type of amino acids*	*Type of feeds*		
		Trash fish	*Squid*	*Golden snail*
1.	*Aspartic acid*	5.08	3.28	4.26
2.	*Glutamic acid*	5.42	5.09	6.09
3.	*Serine*	2.34	0.21	1.81
4.	*Glysine*	2.98	2.01	2.63
5.	*Histidine**	1.15	0.13	1.01
6.	*Arginine*	3.45	2.64	2.57
7.	*Threonine**	1.58	1.42	1.45
8.	*Alanine*	2.05	2.16	2.22
9.	*Proline*	1.89	nd	1.75
10.	*Tyrosine*	2.15	1.37	1.68
11.	*Valine**	1.57	0.59	2.25
12.	*Methionine**	0.82	0.81	0.73
13.	*Cystine*	0.49	0.52	0.43
14.	*isoleucine*	0.22	1.57	nd
15.	*Leucine**	0.41	2.43	nd
16.	*Phenylalanine**	1.77	1.05	1.68
17.	*Lysine**	1.96	2.63	3.32

**essential amino acid*; nd : *not detected*

3.4 Water Quality

The successfulness of mud crab rearing and breeding is also influenced by water quality. Therefore, water quality during the rearing and breeding should be maintained. Data of water quality measured during this study are presented in Table 6.

Table 6. Average of water quality measured

Variable	Treatment (type of feeds)		
	Trash fish	Squid	Golden snail
Temperature (°C)	29-30	29-30	29-30
Dissolved Oxygen (ppm)	6.1-7.6	6.2-7.4	6.5-7.6
pH	7-8	7-8	7-8
NH_3-N(ppm)	3.0-3.4	3.1-3.7	2.9-3.1

The results of statistical analysis indicated that the type of feed influencing gonadal maturity stage of mud crab broodstocks ($p<0.05$), particularly after five days of rearing period. The significant gonadal maturity stage was observed in the 21 day of rearing when 60% of broodstocks feed with squid were already reached stage IV; whereas the gonadal maturity stages of broodstocks feed with trash fish and golden snail were vary between stage II and IV. The squid applied as feed in this study triggered gonadal maturity in mud crab broodstocks compared to trash fish and golden snail. Pattiasina *et al.* (2010) reported that the development of crab (S. *serrata*) gonad is influenced by source of feed and composition of feed nutritions.

There were no significant differences ($p>0.05$) in the means of egg diameters analyzed among treatments. Although the lipid content in squid is eight and five times lower than the lipid content in trash fish and golden snail, respectively (Table 1), the egg size produced from broodstock feed with squid were similar to that produced by broodstocks feed with the two other feeds. The finding in this study is different from the findings reported by Cerda *et al.* (1996) where broodstock feed with high lipid content generally produced bigger eggs. The contribution of lipid, particularly linoleic acid on vitellogenesis that a process to produce egg yolk, therefore the bigger size of egg yolk, the bigger egg diameter is produced. Thus, based on this study, the egg size produced by mud crab broodstocks feed with trash fish, squid and golden snail are comparably equal.

The mean fecundity of broodstocks feed with squid was significantly higher compared to that observed on broodstocks feed with golden snail; yet, although higher to that observed on broodstock feed with trash fish, no significant differences were observed between these feed; in addition, no significant differences also observed between broodstock feed with trash fish and golden snail (Table 3). The hatching rate and embryonic period between the treatments were no significantly different ($p>0.05$). However, there were significant differences ($p<0.05$) in the amount of zoea produced between the treatments. Higher mean amount of zoea was produced by broodstocks feed with squid compared that produced by broodstock feed with trash fish and golden snail; however, no differences in the mean amounts of zoea produced from broodstock feed with golden snail and trash fish.

These results indicated that the development of embryo of mud crab did not affected by these three feed. Thus, the use of squid resulted in high fecundity and eventu-

ally resulted in high amount of zoea.

Longer embryonic period increases the risk of fungal contamination, some studies conducted on tiger shrimp indicated that shrimp eggs were infected by *Haliphthoros philippinensis* and *Haliphtoros* spp, whereas *Lagenidium* infected eggs and larvae of *S. serrata*. The prevalence of *Lagenidium* sp. also reported on blue crab *Callinectes sapidus* (Sinderman, 1988). The severity of fungus on crustaceans has been reported by Zafran *et al*. (1993) where the fungus consumed the crustacean tissue then only remained the carapaces. In addition, *Lagenidium* sp. tolerant to wide ranges of salinity and pH, which are 20 – 40 ^{0}C and 4–11, respectively. In addition, Zafran *et al*. (1993) adviced to dip the eggs and larvae of mud crab in 10 ppm of formalin for 24 hours to combat *Lagenidium* sp.Bacteria, such as *Vibrio harveyi*, *V. carchariae, V. alginolyticus, V. parahaemolyticus*also contaminate mud crab eggs and larvae. In Indonesia, the prevalence of *Vibrio harveyi*was higher than that of those last two vibrios; in addition, the probability of vibrio contamination in mud crab is higher than in shrimp. Longer embryonic period (10 – 14 days) is suggested as the reason of high prevalency of vibrio on mud crab eggs and larvae; in addition, the eggs of mud crab developed in the external body of the mud crab then easier to exposure to the vibrio. This study, however, did not analyses the relationship between the feed and bacteria and fungi.

The proximate analyze result (Table 4) indicated that trash fish contains the highest lipid followed by golden snail and squid, respectively. The lipid content in squid utilized in this study is 11 times lower than-that in squid utilized by Hutabarat (1997) to promote local raw material for development of mud crab aquaculture in Indonesia. The influence of lipid contained in feed on hatching rate, where high lipid content in feed reduce hatching rate. Izquierdo *et al*. (2001) reported that lipid and the composition of fatty acids influence the successful of fish reproduction and larval survival rate. According to Mokoginta (1992) lipid contained in the yolk influences the earlier development stage of embryogenesis and eventually influence the development of embryo.

Yet another factor that influence reproduction performance was fed protein. Compared to trash fish and golden snail, squid had the highest protein conten (73.72%). This assumed be the main trigger for the better reproduction performance of the mud crab broodstock. This finding in the line with the some research of Ali *et al.* (2011), Azra and Ikhwanuddin (2015) and Djunaidah *et al.* (2003). Another finded of them were suggested to application of mix died. Based on the those study, for futher study on the mud crab *S. olivacea* broodstock maturation feed also combine natural feed (fresh squid) and artificial feed (dry pellet) in the hatchery seed production.

This study revealed that squid as feed for mud crab broodstocks resulted in better reproduction performance, particularly on fecundity and the amount of zoea or larvae. The species, feed and environmental condition as the main factors affecting fecundity, fertility and hatching rate of mud crab. In addition, the fecundity also influenced the size of mud crab (Mann *et al.,* 1997) where larger crab mostly has higher fecundity. The hatching rate is influenced by the quality

of the eggs and water quality; in addition, the availability of amino acids and fatty acids also influences the quality of mud crab eggs. Amino acids are the main components of protein. Amino acids are categorized in two different groups namely; essential fatty acids and non-essential fatty acids. Essential fatty acids cannot be synthesized by organism then this fat should be added to the feed of mud crab; in contrast non-essential fatty acids can be synthesized by mud crab (Sitompul, 2004). Essential amino acids required by fish larvae are arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, and valine. Histidine is required to maintain the balance of nitrogen in fish tissue. Arginine is essential for fish growth and biosynthetic of protein and urea. Methionine is limiting nutrient in some raw material for fish feed. The deficiency in methionine caused cataract in fish, whereas, in more specific to crustacean larvae, essential fatty acids contribute in growth and survival rate of larvae of *Penaeus japonicas* (Kanazawa *et al*., 1985).

Amino acids contained in the tissue of mud crab larvae for each treatment are presented in Table 5. Glutamate acid, alanine and leusine contribute on gonadal maturity in fish (Lochmann, 2004). The result of amino acids analyses indicated that amino acids content in larvae were similar to that of all treatments. However, larvae of mud crab broodstocks fed with trash fish have higher concentrations of amino acids than larvae of broodstocks fed with squid and golden snail. The results also indicated that larvae from broodstocks feed with trash fish is enriched with more various fatty acids; whereas proline was not detected from tissue of larvae originating from broodstocks feed with squid. In addition, two fatty acids, which are isoleucine and leucine, are not detected from the tissue of larvae produced from broodstocks feed with golden snail. High concentrations of most of essential fatty acids are found in tissue of larvae produced from broodstocks feed with trash fish, in exception for isoleucine and lysine that found to be high in larvae of broodstocks feed with squid and golden snail, respectively.

The concentrations of amino acids found in this study were generally higher than that reported by Ali *et al.* (2011); however, lower than that reported by Penaflorida (2004). It was suspected due to the difference of feed used in which two previous studies utilized artificial feed. Ali *et al.* (2011) and Penaflorida (2004) suggested that the profile of amino acids retained in the tissue of mud crabs is useful in feed formulation for larvae and broodstocks.

The alanine, leucine, serine, isoleucine, lysine and valine are the major six free catabolism fatty acids during embryogenesis in fish. The availability of these fatty acids is one of method in measuring the quality of fish eggs. The same method is also applicable to crustacean such as the high concentrationcontain of catabolism fatty acids. Free amino acids contribute as the source of energy for eggs with no oil globular. In addition, free amino acids are found in all tissues and plasma that contribute in protein synthesis-Organism required essential amino acids for physiological activities supporting metabolism. The exceed of amino acids, generally, are excreted through oxidative deamination in which the carbon bound is transformed in to acetyl or aceto-acetyl Co-A, piruvate or

one of molecule in intermediate reaction of tricarboxylate cycle, in which is then oxidized to produce energy. Protein is required for growth, and then when the protein is insufficient, the reserved protein is catabolized through deamination to produce energy (Luo *et al.,* 2006).

Water temperature is one of abiotic factor influencing activities, appetite, oxygen consumption and metabolism rate of crustacean. The optimum water temperature for mud crab wererangedfrom 26 to 32^{o}C. In addition to water temperature, pH influences process and rate of chemical reaction in water as well as biochemical reactions in mud crab tissue. According to Christensen *et al.* (2005), the optimum pH for mud crab ranges from 7.5 to 8.5.

Ammonia is a main product of nitrogen waste in waters originating from gill excretion (Neil *et al.,* 2005). Unionized ammonia (NH_3) is toxic to aquatic organism and is highly permeable that be able to infiltrate in to organism tissue (Eddy, 2005). Although the study was conducted in re-circulated tanks, ammonia resulted from mineralization from uneaten feed as well as mud crab excretion is potential to affect mud crabs (Fauzzia *et al.,* 2013). Concentrations of TAN (NH3-N) wasmeasured during this study ranged from 2.9 to 3.7 ppm. However, after transforming the concentration of TAN in to unionized ammonia mean concentrations of NH_3 was 0.02 ppm in which below the threshold concentrations of NH_3 (0.1 ppm).

4. Conclusion

Fresh squid as feed for mud crab *S. Olivacea* broodstocks provided better reproduction performance, particularly in fecundity and the amount of zoea or larvae produced from recirculated tank. The lipid content in squid is eight and five times lower than the lipid content in trash fish and golden snail, respectively. Squid had the highest protein content and this assumed be the main trigger for the better reproduction performance of the mud crab broodstock.

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