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## REVIEW

# A review of maturation diets for mud crab genus *Scylla* broodstock: Present research, problems and future perspective



Mohamad N. Azra <sup>a,1</sup>, Mhd Ikhwanuddin <sup>b,\*</sup>

<sup>a</sup> School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia

<sup>b</sup> Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia

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## KEYWORDS

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Reproduction

**Abstract** Study of broodstock maturation diets is important in order to increase the quality of berried females, which indirectly improve the larval quantity in the hatchery production of cultured species. This paper reviewed the studies on the maturation diets for mud crab broodstock, genus *Scylla* and compared independently to identify their effect on reproductive performance and larval quality. The broodstock is usually caught from the wild and held in the spawning or maturation tank for further use of hatchery seed production. Mud crab broodstock was fed either natural diet, artificial diet or mixed diet. Trash fishes were commonly used as a natural feed for mud crab broodstock; meanwhile artificial diets are from formulated fish meal and various kinds of feed. The results indicated that mud crab broodstock has a high dietary requirement for lipids, fatty acids and protein which are to be used during the maturation and breeding processes. However, the natural diet produce better larval quality compared to the artificial diet. The mixed diet is the better diet which resulted in better reproductive performances such as growth, survival, fecundity and maturation processes. This review also discusses the problems in the previous studies for the potential future research to develop very high quality and cost-effective formulated diet for the enhancement of

**Abbreviations:** FA, fatty acids; AA, amino acids

\* Corresponding author at: Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Mengabang Telipot, 21030 Kuala Terengganu, Terengganu, Malaysia. Tel.: +60 9 6683638; fax: +60 9 6683502.

E-mail addresses: [mohdnorazra\\_mdadib@yahoo.com](mailto:mohdnorazra_mdadib@yahoo.com) (M.N. Azra), [ikhwanuddin@umt.edu.my](mailto:ikhwanuddin@umt.edu.my) (M. Ikhwanuddin).

<sup>1</sup> Address: School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, Mengabang Telipot, 21030 Kuala Terengganu, Terengganu, Malaysia. Tel.: +60 1 29427795.

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broodstock and seed production technology. Information from this review can be useful in developing a better quality of crustacean broodstock's diet for commercial hatchery production.

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## 1. Introduction

The mud crab, genus *Scylla* is considered a new species in aquaculture with high expectation to continue to grow in the future. However, the hatchery technology of the mud crab's culture is in the developmental stage with a small number of breeding programs in a few countries. Due to rising market value of this product and its high demand, most crab farmers target the seed from the wild for stocking in the ponds (Noorbaiduri et al., 2014). The mud crab, especially the genus *Scylla* is commercially important because it is fished and cultured in various Asian countries such as Philippines, Indonesia, Vietnam, China, Taiwan, India, Sri Lanka and Malaysia. Currently, the mud crab aquaculture in Malaysia is associated with the mud crab fisheries and is likely to lead in changes to the enhancement of the ecological system, socio-economic and livelihood. Although some information is fundamental to the development of mud crab's seed production such as rearing techniques (Rabbani and Zeng, 2005) and culture systems (Quinitio et al., 2001), the status of broodstock nutrition should firstly be known before further breeding or restocking program. For mud crab hatchery culture, broodstock has never been an issue as generally female mud crabs

spawn readily and has high hatching rates. However, the development of hatchery seed productions currently relies on the wild caught broodstock. In addition, the consistent production of *Scylla* seed has never been successful anywhere in the world due to limited berried females produced in captivity. Furthermore, the wild ovigerous mud crab female especially the genus *Scylla* is difficult to obtain due to its migratory spawning behavior, especially in *Scylla serrata* which spawns in offshore water (Hill, 1994). They are also exploited by local individuals for immediate consumption (Ikhwanuddin et al., 2011). In order to obtain reliable ovigerous females, an appropriate diet should be analyzed to improve their reproductive performance without copulatory molting (changes of new exoskeleton for sexual mating) or further mating. In addition, the broodstock management technology is a critically important procedure that should be further improved, and dietary nutrition is an important issue for successful hatchery production.

Nutrition plays a critical role in the development of the ovary in mud crabs. Scientific studies on mud crab, *Scylla* broodstock nutrition have been conducted in the last three decades by several authors (Marichamy et al., 1986; Samarasinghe et al., 1991; Marichamy, 1996; Fortes, 1999a;

Saha et al., 2000; Trino et al., 2001; Christensen et al., 2004; Alava et al., 2007a; Manivannan et al., 2010). The mud crab broodstock reproductive performance and larval productions are majorly dependant on variation of maturation diets (Fortes, 1999b; Qiao et al., 2010). The natural diets are largely used for broodstock culture during the hatchery production. However, the use of natural feeds requires proper management to avoid deterioration of water quality which indirectly affects the culture environment. On the other hand, the formulated feed could provide sufficient nutrition to broodstock as well as to stimulate the ovarian maturation. The artificial diet offers many advantages compared to the natural diet or fresh feed which include, known nutrient content such as total lipid and protein, readily available supply throughout all the culture period and offering the opportunity to orally administer drugs either hormones or vitamins (Djunaidah et al., 2003). However, the preparation of the formulated diets will increase the production cost of mud crab hatchery (Petersen et al., 2013) and require more preparation time, which indirectly affects the continuance of the hatchery production.

This review can provide important information for a better understanding of the broodstock maturation diets and management of mud crab, *Scylla* species for further commercial seed and sustainable food productions.

## 2. Broodstock management

### 2.1. Sources of broodstock

Majority of broodstock comes from captive wild-caught, on-growing in earthen-ponds or domesticated improved broodstock (Shelley and Lovatelli, 2011). The mud crabs were usually collected from their natural habitat using trawl net, baited traps, hoop-and-bag traps, gill nets, hand-collected and hand-scoop or bought from the local fisherman. The broodstock's size used for breeding is important because it will affect the feeding ratio in the diet under the study. Commonly, for breeding purpose, the broodstock's eyestalk was ablated in order to increase their molting frequency and to accelerate the spawning and development of their gonads (Mann et al., 1999a). There are different procedures in the eyestalk ablation method such as constriction of eyestalk with catgut or removal using sterile surgical blades which reduced mortality to the minimum level (Allayie et al., 2011). There are two different management methods generally adopted between before and after spawning.

### 2.2. Before spawning

Usually, the broodstock may be dipped in antiseptic baths such as formalin and/or formaldehyde (Mann et al., 1999b; Millamena and Qunitio, 2000; Qunitio et al., 2011), potassium permanganate (Pattiasina et al., 2012), malachite green (Davis et al., 2004) or treflan (Shelley and Lovatelli, 2011) to eliminate microbial infection of the embryos and the eggs in broodstock.

The broodstocks were typically held in a maturation or spawning tank or pond for a period of a few months or more (1–3 months) and stocked with various stocking densities between 1 to 5 crab m<sup>-2</sup>, depending on the broodstock size.

Once the broodstocks were in captivity, they were provided with gravel (Alava et al., 2007a) or pebble substrate (Millamena and Qunitio, 2000) and topped with around 12–15 cm thick sand (Hamasaki, 2003; Millamena and Qunitio, 2000) for broodstocks to bury and extrude their eggs successfully. They were also supplied with sand-filtered seawater (Millamena and Qunitio, 2000) with good water quality (Ali et al., 2011) and adequate aeration (Qunitio et al., 2001; Millamena and Qunitio, 2000) to avoid stress to the brood and eggs. To prevent the attack by the other broodstock, each spawning tank was supplied with a wooden shelter (Alava et al., 2007a), half-cut vinyl chloride pipes (Hamasaki, 2003) or black nylon (Millamena and Qunitio, 2000). After an extensive care, they were fed with different types of diets including natural diet, artificial diet or mixed diet until they spawned.

### 2.3. After spawning

After the broodstocks spawned, they are reared in individual spawning tanks with sand-filtered seawater and flow through water system. During the incubation period, the broodstocks were not fed and one day before hatching, they were transferred in hatching tank with still water and gentle aeration (Hamasaki, 2003). After the broodstocks hatched, they were returned to experimental tanks and observed for re-maturation (Millamena and Qunitio, 2000). Usually, the broodstocks take a few weeks and they do not have to further mate for re-maturation.

## 3. Types of maturation diet

During the last 10 years, many attentions have been focused on the broodstock nutrition as well as the role and effect of various components of broodstock diet, such as lipid, cholesterol, protein especially on their maturation status (Anderson et al., 2004). Broodstock nutrition is without doubt one of the most outstanding reasons that affects the reproductive performance of most crustacean (Chung et al., 2011; Alava et al., 2007a). Broodstock nutrition affected directly the maturation of the ovary where the restriction of food can seriously inhibit the ovarian maturation in several *Scylla* species (Alava et al., 2007a; Djunaidah et al., 2003). In this review, three main diets were considered: natural diet (fresh feed), formulated feed (artificial diet), and mixed feed (combination of natural and artificial diet).

### 3.1. Natural feed

Generally, mud crabs were fed with the traditional food, which is natural feeds (or fresh feed) such as mussel meat, *Perna* sp., squid, *Loligo* sp., trash fish, *Leiognathus* sp. or *Oreochromis* sp., small bivalves, *Potamocorbula* sp., shrimp, *Fenner openaeus* sp. because these food can potentially reduce operational costs and can be recycled for daily use. However, frequent uses of the natural feed can also decline water quality in the cultural environment which indirectly influences the broodstock growth and maturation. The study by Millamena and Qunitio (2000) found that lack of essential dietary fatty acids in natural diets can reduce the reproductive performance of broodstock compared to the formulated diets or mixed diets.

### 3.2. Artificial feed alone (formulated feed)

The formulated feed data of mud crab broodstock are important for the development of nutritionally balanced and cost-effective commercial feeds. Millamena and Bangcaya (2001) suggested that future artificial diets are needed as a supplement to natural diet in order to promote more broodstock spawning with good quality eggs and larval production. Before the use of artificial diet alone, the broodstock was usually fed with natural diet for a few days and weaned till the end of the experiment. To develop suitable artificial feed for the mud crab broodstock, few studies refer to the modification of prawn broodstock diet (Millamena and Qunitio, 2000; Djunaidah et al., 2003). Majority of the artificial diets are from the fish, shrimp or squid meal with the addition of other ingredients such as fish or squid oil, wheat and binder (Djunaidah et al., 2003; Alava et al., 2007b).

### 3.3. Mixed feed (combination of natural and artificial feed)

The mixed diets are the combination of various natural diets and artificial feed for better effect of the reproduction and larval quality of cultured species. The study of mixed diet on the reproductive performance and larval quality of mud crab has been done by various authors (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001; Djunaidah et al., 2003; Rodriguez et al., 2003; Alava et al., 2007b). The proximate composition of crude protein, crude fat, crude fiber, ash and nitrogen free extract was calculated as percentage on a dry matter basis with mean percentage of natural diet and addition of formulated diet or artificial diet for each group.

## 4. Reproductive performance and larval quality

### 4.1. Natural feed

Table 1 shows the reproductive performance and larval quality of mud crab broodstock, genus *Scylla* fed with various natural diets. In general, the natural feed affected broodstock performances and larval quality of mud crab, genus *Scylla*. It's suggested that the natural diets can improve the broodstock's fecundity which indirectly increases the number of eggs produced from a single broodstock.

#### 4.1.1. Broodstock growth and survival

The study by Millamena and Qunitio (2000) showed that *S. serrata* broodstock fed with mussel meat, squid and trash fish produced better broodstock survival compared to the mixed diet or formulated diet. The broodstock growth also decreased when they fed with the natural diet as well as the total days taken from crab spawning to hatching (Alava et al., 2007b; Ali et al., 2011). Study by Millamena and Bangcaya (2001) indicated that broodstock fed with the natural fed showed lower survival compared to other diets.

#### 4.1.2. Broodstock spawned, time to matured and re-spawned

Studies on the effects of natural diet on mud crab broodstock also suggested that fresh natural food can be fed to the broodstock to accelerate the broodstock to mature, re-spawn and mating success (Alava et al., 2007b; Misieng, 2007; Ali et al.,

2011; Pattiasina et al., 2012). The study by Millamena and Qunitio (2000) showed that *S. serrata* broodstock fed with mussel meat, squid and trash fish produced more spawns compared to the other diets (mixed diet or formulated diet).

#### 4.1.3. Fecundity, egg production and fertilization

Both studies by Millamena and Bangcaya (2001) and Djunaidah et al. (2003) found that natural diet can enhance the fecundity of the broodstock. On the other hand, the studies by Millamena and Qunitio (2000) and Millamena and Bangcaya (2001) resulted the in the decrease of egg production and egg fertilization. The summary from the Table 1 of broodstock reproductive performance fed with natural food illustrates that fecundity, egg fertilization, hatching rate and survival of mud crab broodstock were affected by various kinds of natural diet (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001; Djunaidah et al., 2003).

#### 4.1.4. Larval quality

The natural feeds resulted in higher total zoea production according to Djunaidah et al. (2003) and Alava et al. (2007b). Djunaidah et al. (2003) also demonstrated that a combination of natural diet (shrimp, squid, trash fish and *Artemia* sp.) resulted in a higher survival and the strongest larvae production as determined by the starvation test with up to 120 h starvation compared to the artificial food which is as low as 60 h. In addition, Pattiasina et al. (2012) proved that broodstock fed with natural foods (fish and small bivalve) resulted in good larval quality with higher zoea survival compared to the artificial diet alone. On the other hand, both studies by Millamena and Qunitio (2000) and Millamena and Bangcaya (2001) showed that the natural foods could decrease the growth of larvae mud crab which was measured by the larval stage index (LSI).

### 4.2. Artificial feed alone

Table 2 shows different types of artificial diet which affect the reproductive performance and larval quality of mud crab broodstock. The use of artificial feed is suggested to increase the water quality and increase the reproductive performance of the mud crab broodstock (Ali et al., 2011; Pattiasina et al., 2012). In terms of the commercial hatchery production, the use of fish meal in the artificial diet could increase the total production cost which indirectly increases the price of the species cultured. However, a new approach has been done to overcome the usage of fish meal (see Section 6).

#### 4.2.1. Reproductive performance

Most of the formulated feeds consist of various protein types (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001), lipid classes (Djunaidah et al., 2003; Alava et al., 2007b) and essentials amino acids (Djunaidah et al., 2003; Ali et al., 2011) which are important to the ovarian maturation in mud crab broodstock. Artificial feed with higher protein percentage resulted in a better egg fertilization rate (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001) while on the other hand, broodstock fed with higher lipid had shown a shorter latency period and incubation time (Djunaidah et al., 2003). The preparations of the formulated

**Table 1** Reproductive performance and larval quality of mud crab broodstock, genus *Scylla* fed with various natural diets.

Species (country)	Eyestalk ablated – Study duration	Body weight – BW (g)	Types of mixture of natural feed	Summary			References
				Broodstock reproductive performance		Offspring quality performances	
				Lower/Worst	Higher/Better		
<i>Scylla serrata</i> (Philippines)	Yes – 3 months	300–400	Mussel meat, squid and trash fish	Fecundity, egg produced, egg fertilization rate and crab hatched	Crab spawned and broodstock survival	Lower total number of zoea and larval stage index	Millamena and Qunitio (2000)
<i>Scylla serrata</i> (Philippines)	Yes – 90 days	350–400	Brown mussel meat and fish by-catch	Egg fertilization rate and broodstock survival	Fecundity	Lower total zoea production and zoea growth index	Millamena and Bangcaya (2001)
<i>Scylla paramamosain</i> (Indonesia)	Yes – 2 months	200–300	Shrimp, squid, trash fish and <i>Artemia</i> sp.	Hatching rate, egg fertilization rate and egg hatching rate	Fecundity	Higher zoea production, phototaxis larvae and survival (> 120 h starvation test)	Djunaidah et al. (2003)
<i>Scylla serrata</i> (Philippines)	Yes – 112 days	625 ± 6.4	Fish and small bivalves	Longest incubation period*	Re-spawning (2 times) and shorter total days from ablation to spawned	Better zoea production	Alava et al. (2007b)
<i>Scylla olivacea</i> (Malaysia)	Yes – 84 days	n/a	Trash fish, cuttlefish and shelled prawn	n/a	Mating success and shortest period of ovarian development	n/a	Misieng (2007)
<i>Scylla serrata</i> (India)	No – 30 days	151 ± 5.3	Fresh fish meat	Weight gain (growth) and survival	Maturity stages	n/a	Ali et al. (2011)
<i>Scylla serrata</i> (Indonesia)	No – 34 days	500 ± 103	Fresh fish meat, squid and shrimp	Fecundity	Fast maturation	Higher zoea survival	Pattiasina et al. (2012)

\* Incubation time means the duration (day(s)) taken by broodstock from spawning to hatching.

feeds are usually from moist or dry feed forms. The study by Ali et al., (2011) indicated that crabs fed with dry form showed better performance compared to the semi-moist feed. They also found that the use of artificial diet resulted in a faster weight gain, the lowest feed gain ratio and a higher molting frequency compared to the broodstock fed with fresh natural diet. However, the formulated feed with a higher percentage of fish meal produced the worst result of total number of crab spawned, and fecundity (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001). In addition, the results from the studies by Alava et al. (2007b) and Pattiasina et al. (2012) found that artificial diets affected the maturation and survival of the mud crab broodstock with the longest duration to mature and the lowest broodstock survival till the end of the experiment.

#### 4.2.2. Larval quality

The study revealed that broodstock fed with a higher protein and lipid content of artificial feed caused the lowest larval quality (zoea production and starvation test) compared to the broodstock fed with natural feed (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001; Djunaidah et al., 2003; Alava et al., 2007b). The study on artificial diet showed that performance of broodstock can be increased by various artificial diets, but not the offspring quality of the mud crab. Thus, further analysis should be done to the mixed diet (combination of natural diet and artificial diet) in order to increase the larval quality of the mud crab.

#### 4.3. Mixed feed

Table 3 shows the reproductive performance and larval quality of mud crab broodstock fed with different mixed diets. The use of higher protein is obligatory in the formulation of the formulated diet. The higher protein level could enhance the reproduction of mud crab broodstock. In addition, the previous study showed that the protein has higher levels (>43%) in the diet composition of formulated feed compared to other diet composition (Fig. 1). In addition, a previous review on the mud crab, *S. serrata* showed that crabs grew well on diets containing 32–40% protein and 6–12% lipid, at dietary energies from 14.7 to 17.6 MJ/kg (Anderson et al., 2004). On the other hand, this review also discovered that the mixed diet results in a better larval quality especially in the number of zoea production (the number of larvae produced).

##### 4.3.1. Reproductive performance

The results from most studies showed that the mixed diet offered better reproductive performance of mud crab compared to other diets such as natural diet and artificial diet alone (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001; Djunaidah et al., 2003; Rodriguez et al., 2003; Alava et al., 2007b). The results from both studies by Millamena and Qunitio (2000) and Millamena and Bangcaya (2001) demonstrated that mixed diet achieved better number of crab spawned, crab hatched (the successful number of broodstock hatched) and the number of eggs produced. The studies by Millamena and Qunitio (2000), Millamena and Bangcaya (2001), Rodriguez et al. (2003) and Alava et al. (2007b) also found that mixed diet affects the survival of

mud crab broodstock. The results from the reproductive performance also indicated that broodstock fed with mixed diet resulted in the highest egg fertilization rate compared to the broodstock fed with natural diet (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001; Djunaidah et al., 2003). The result from Rodriguez et al. (2003) found that the combination of natural diet, brown mussels and artificial feed, cooked cracked corn showed an increase of final body weight and specific growth rate compared to natural diet alone. The mixed diet also shortened the latency period and duration from spawning to hatching of the mud crab broodstock (Djunaidah et al., 2003; Alava et al., 2007b). The result of mixed diet only affected the period from ablation to spawning of the mud crab broodstock (Alava et al., 2007b).

##### 4.3.2. Larval quality

The results of the previous study revealed that the mixed diet resulted in the highest zoea production of mud crab (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001; Djunaidah et al., 2003; Rodriguez et al., 2003; Alava et al., 2007b). All results from the study showed that the crab fed with mixed diet resulted in highest total zoea production except from the study by Rodriguez et al. (2003) because no data were available for larval quality performance. The LSI value also increased when the broodstock fed with the combination of natural and formulated diet (Millamena and Qunitio, 2000; Millamena and Bangcaya, 2001).

#### 5. Problems associated with mud crab broodstock

Poor nutrition on the broodstock also resulted in reduced fecundity, fertilization and larval starvation of Portunid crab (Oniam et al., 2012; Djunaidah et al., 2003). During embryonic development, the normal development is influenced by the availability of essential nutrients which are supplied via the broodstock diets (Izquierdo et al., 2001). These nutrients such as amino acids, vitamins and essential fatty acids are important during embryonic development which in turn affect the egg morphology, hatching rates as well as vitellogenin synthesis (Izquierdo et al., 2001). Moreover, the reliance of the embryo and newly hatched larvae on the egg yolk nutrition is essential in understanding the nutrient requirements of broodstock (Alava et al., 2007b). Other problems associated with the *Scylla* broodstock were also been done by the Lavilla-Pitogo et al. (2001) and they stated that shell disease, bacterial contamination of the hemolymph, parasitic infestation are the main factors to develop better broodstock for hatchery seed production. In addition, the preliminary study also showed that the fungus infections were also the main problem in the maintenance of the broodstock in the hatchery culture of the mud crab, genus *Scylla* (Ikhwanuddin, unpublished data). The lack of information on the changes in the ovary during reproductive cycle is one of the important factors that limits the expansion and development of the mud crab seed hatchery technology.

#### 6. Future research development

Improvements in mud crab broodstock maturation diets should involve the development of alternative natural feed

**Table 2** Reproductive performance and larval quality of mud crab broodstock, genus *Scylla* fed with selected artificial diets.

Species (country)	Feeding ratio (%* of body weight)	Types of artificial feed	Conclusion			References
			Broodstock reproductive performance		Offspring quality performances	
			Lower/Worst	Higher/Better		
<i>Scylla serrata</i> (Philippines)	2–3	Modified from prawn broodstock diet (Majority: fish meal, shrimp meal, squid meal and wheat)	Total number of spawning, fecundity and survival rate	Egg fertilization rate	Lower total number of zoea	<a href="#">Millamena and Qunitio (2000)</a>
<i>Scylla serrata</i> (Philippines)	2–3	Based on FA and AA* profile of matured crab ovaries (Majority: fish meal, shrimp meal, squid meal and wheat)	Total number of spawning with and without hatching, fecundity, broodstock survival rate	Egg fertilization rate	Lowest total number of zoea	<a href="#">Millamena and Bangcaya (2001)</a>
<i>Scylla paramamosain</i> (Indonesia)	3–5	Majority: squid meal, minced meat mussel and calf liver	Fecundity	Latency period** and incubation time***	Lowest zoea production and phototaxis larvae	<a href="#">Djunaidah et al. (2003)</a>
<i>Scylla serrata</i> (Philippines)	1–2	Based on different percentages of lipid – squid oil and soybean lecithin (Majority: fish and squid meal and bread flour)	Survival rate and re-spawn.	Lowest days from spawned to hatched	Lowest zoea production	<a href="#">Alava et al. (2007b)</a>
<i>Scylla serrata</i> (India)	10	Majority: fish meal, soya cake, wheat flour and squilla	n/a	Weight gain, feed gain ratio and molting frequency	n/a	<a href="#">Ali et al. (2011)</a>
<i>Scylla serrata</i> (Indonesia)	10	Based on protein and fat (Majority: –)	Days to matured and egg diameter	Not effected	Not effected	<a href="#">Pattiasina et al. (2012)</a>

\* %, percentage; FA, fatty acids; AA, amino acids.

\*\* Latency period means the duration (day(s)) of broodstock taken from eyestalk ablation to spawning.

\*\*\* Incubation time means the duration (day(s)) taken by broodstock from spawning to hatching.

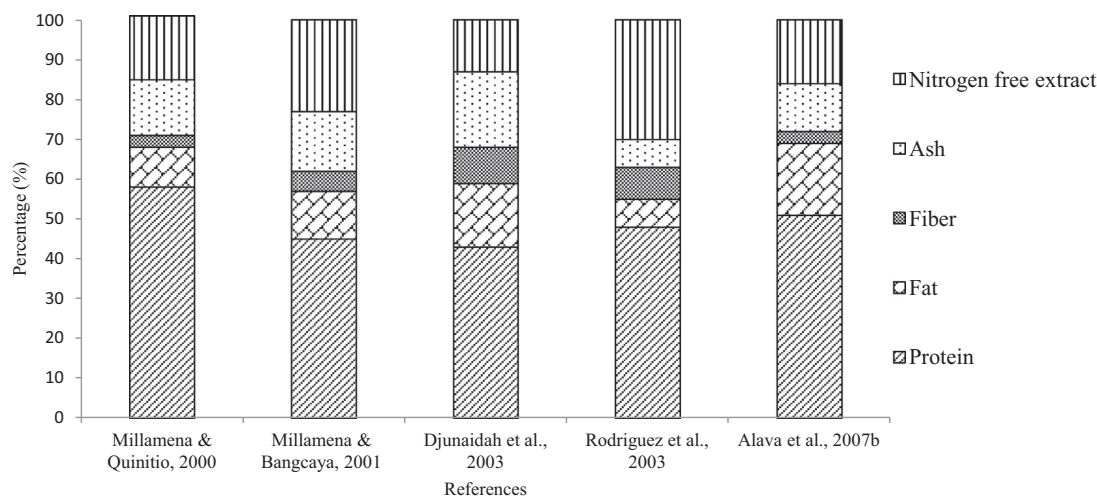
**Table 3** Reproductive performance and larval quality of mud crab broodstock, genus *Scylla* fed with different mixed diets.

Species (country)	Mixed diet and proximate composition						Conclusion			References
	Types of mixed diet	Mean percentage of dry matter basis (%)					Broodstock reproductive performance		Offspring quality performances	
		Protein	Fat	Fiber	Ash	N <sup>*</sup> -free extract	Lower	Higher/Better		
<i>Scylla serrata</i> (Philippines)	Squid, fish, mussel with formulated diet	58	10	3	14	16	None	Crab spawned, crab hatched, fecundity, egg fertilization rate and broodstock survival	Highest total zoea produced and highest mean larval stage index	Millamena and Qunitio (2000)
<i>Scylla serrata</i> (Philippines)	Mussel meat, fish with formulated feed	45	12	5	15	23	None	Crab spawned, crab hatched, fecundity and broodstock survival	Highest zoea produced and growth index	Millamena and Bangcaya (2001)
<i>Scylla paramamosain</i> (Indonesia)	Brine shrimp with formulated feed	43	16	9	19	13	None	Shortest latency period,** egg fertilization rate and egg hatching rate	Highest zoea production	Djunaidah et al. (2003)
<i>Scylla olivacea</i> (Filipina)	Brown mussel flesh and cooked cracked corn	48	7	8	7	30	None	Final body weight, weight gain, specific growth rate and broodstock survival	n/a	Rodriguez et al. (2003)
<i>Scylla serrata</i> (Philippines)	Squid, fish, mussel with artificial diet	58	18	3	12	16	Longest days from ablation to spawning	Shortest days from spawning to hatching and broodstock survival	Highest zoea produced	Alava et al. (2007b)

\* N, nitrogen.

\*\* Latency period means the duration (day(s)) taken from eyestalk ablation to spawning.





**Figure 1** Different mean percentages on dry matter basis in the diet composition of mixed feed for mud crab, genus *Scylla* broodstock.

and identify the important sources for gonad maturation which was one of the bottlenecks to the mud crabs' industry expansion. The hatchery production of mud crab is mainly focused on the better quality of seed production. Dietary lipid plays an important role as potential supplier of energy and essential fatty acids and fat soluble vitamins for the ovarian maturation. Thus, special diets are needed for future development of mud crab broodstock with more focus on various dietary compositions which will increase the quantity of berried females as well as the seed quality of mud crab production. Natural diet was also recommended in order to enhance the quality of the berried females such as polychaetes which were identified to have some steroid hormones for crustacean reproductive performance (Meunpol et al., 2007) which indirectly can reduce the production cost of formulated diet. Recently, the development of alternative sources of fish meal protein with gamma-irradiated soybean meal (Zhang et al., 2014) will also be suggested to replace more fish meal in the diet of mud crab broodstock. In addition, other than manipulation of diet requirement, other techniques such as using probiotic and antibiotic (Wu et al., 2014), alternative design of culture systems such as Re-circulating Marine Aquaculture Systems – RMAS (Ikhwanuddin, unpublished data) and genetic selection of broodstock (He et al., 2014) could increase the reproductive performance and larval quality of mud crab. Our future research will be focused on the development of high quality and cost-effective formulated diet such as development of alternative protein using single cell organism, enzymatic study, formulation of micro-bound diet with inclusion of natural ingredients and development of appropriate food type and feeding schedule to develop high quality of berried females either for restocking or breeding program.

## 7. Conclusion

The sufficient amount of diets such as protein (Millamena and Qunitio, 2000; How-Cheong et al., 1991), lipids (Alava et al., 2007b), cholesterol (Pattiasina et al., 2010), omega 3 and 6 (Millamena and Qunitio, 2000; Djunaidah et al., 2003),

carbohydrates (Pavasovic, 2004), vitamins and minerals (Trino et al., 2001) and other amino acids (Millamena and Bangcaya, 2001; Ali et al., 2011) should be provided in the broodstock diet to permit successful development of the mud crabs' ovaries and quality of eggs produced. The higher level of protein in the formulated diet of mud crab broodstock showed that protein must be included in the diet formulation for better reproductive performance. For lipid, it has been found that an artificial diet with a total lipid content of 10% composition (6–12%) can lead to improvement of larval production and quality (Shelley and Lovatelli, 2011). However, in-depth study should be done to identify the appropriate percentage of nutrition to enhance the broodstock maturation as well as larval performance. In conclusion, the combination of natural food and artificial feed can achieve more consistency in broodstock reproductive performance and enhance the quality of seed production in the hatchery (Table 3). Further study on the mud crab broodstock maturation diet should analyze the biochemical composition and enzyme profile of the diet in the wild first before using it the future hatchery seed production.

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