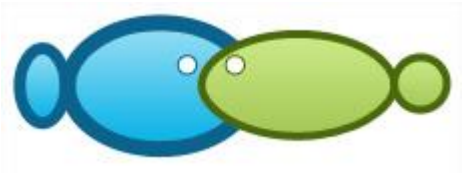

The mating success and hybridization of Mud crab, *Scylla* spp. in controlled tanks

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Abstract. Interspecific hybridization in mud crabs hardly occurs in uncontrolled conditions (in the wild). Therefore, the purpose of this study is to investigate the reproductive performance of female broodstock (fecundity, hatchability and crablet production) after mating with the same species and interspecific hybridization among *Scylla* spp. in controlled tanks. Four rounded fiberglass tanks, 1 m high and with a diameter of 2.1 m, were filled with 32 ppt saline filtered seawater. Then, 10 pairs (male/female) of mud crab broodstocks were stocked in each tank for mating and hybridization. The study involved four treatments: 1. *Scylla paramamosain* male paired with *S. tranquebarica* female; 2. *S. tranquebarica* male paired with *S. paramamosain* female; 3. *S. tranquebarica* male paired with the females of *S. paramamosain*, *S. olivacea*, and *S. tranquebarica*; 4. *S. paramamosain* male paired with females of *S. tranquebarica*, *S. olivacea*, and *S. paramamosain*. The number of precopulation and copulation incidences were recorded daily. Post copulated female crabs grew individually in different tanks until the gonads matured and the crabs spawned. The results of the research showed that the precopulation incidence obtained in treatment tanks 2, 3 and 4 were not significantly different ($P>0.05$), but they were significantly higher than the treatment in tank 1 ($P<0.05$). The interspecific hybridization between the female of *S. paramamosain* and the male of *S. tranquebarica* resulted in egg fecundities from 32200 to 1868000 eggs, and a hatching rate between 2 and 45%. It also resulted in crablet-D10 (after days 10) production between 32-123 individuals. Futhermore, *S. olivacea* female copulated with *S. tranquebarica* male had an egg fecundity of 758000 eggs, a hatching rate of 98%, and a crablet production of 620 individuals. The mating of *S. paramamosain* female with *S. paramamosain* male resulted in an egg fecundity of 1574000 eggs, hatching rate of 90% and crablet production of 1012 individuals. Futhermore, the mating of *S. tranquebarica* female with *S. tranquebarica* male resulted in an egg fecundity of 975500 eggs, a hatching rate of 89% and a crablet production of 628 individuals.
Key Words: crablet production, fecundity, hatching rate, hybridization.

Introduction. Mud crabs are brackish water commodities with high economic value in both the local and international markets (Keenan et al 1998; Ikhwanuddin et al 2010; Gunarto & Herlinah 2015). This has led to its intensive exploitation in some areas in Indonesia. However, as a means of preventing the over exploitation of these crabs, the Indonesian government, on behalf of The Ministry of Marine and Fisheries, issued "Permen KP" No. 1, 2015 (Ministry of Marine and Fisheries Republic of Indonesia, 2015) and the circular letter of The Ministry of Marine and Fisheries No. 18, 2015 (Ministry of Marine and Fisheries Republic of Indonesia, 2015) to regulate the fishing process. Some of the factors considered for regulation include the minimum catch size (200 g) and the prohibition of catching spawning females to ensure that their natural reproduction continues, and for the recovery of the wild population.

Mud crab, *Scylla tranquebarica,* is predominantly found in Indonesia in Aceh, West Sulawesi, South Sulawesi, Southeast Sulawesi, South and East Kalimantan, South coast of Central Java and West Papua. According to Gunarto & Sulaeman (2017), the culture of the mud crab *Scylla* spp. can be carried out in brackish water ponds. Generally, mud crabs, *Scylla* spp., can present rapid growth within the ponds (Syafaat & Gunarto 2018). The hybridization or cross breeding of mangrove crabs has been successfully carried out in controlled tanks by several researchers (Baiduri et al 2014; Fazhan et al 2017). Furthermore, Waiho et al (2015) reported that the successful mating of these crabs in

controlled tanks is influenced by their stocking density. Their mortality is more influenced by increasing stocking densities, as well as by the unequal ratio of males to females.

Hybridization among these animals aims quantitatively to produce crab offsprings with rapid growth and high survival rate, and qualitatively to produce good body shapes and sizes. According to Bondad-Reantaso (2007), the intraspecific hybridization is the mating between two individuals from the same species, while interspecific hybridization is the mating between two individuals from two different species. The process involved in the successful mating of female crabs has been studied by Fazhan et al (2017), by observing the spermatophore deposition in the seminal receptacle located in the gonophore, the round white compact sperm cell visible to the naked eye. Adolescent females were used in this research in order to obtain the first mating samples, having small rounded abdomens, an indication of entering adulthood.

Based on previous investigations carried out on the growth of *S. tranquebarica* from the crablet life stage, it was revealed that the mean weight of the first matured gonad is 205.2±0.3 g and the mean of carapace width is 116.7±0.7 mm (Gunarto et al 2019). However, *S. paramamosain* in Thailand has a carapace width of 112 mm when the first gonad is matured (Hamasaki et al 2011). Therefore, this study aimed at investigating the reproductive performance of female brood stock (fecundity, hatchability and crablet production) after mating with the same species and interspecific hybridization among *Scylla* spp. in controlled tanks.

Material and Method

Mating trial. The research was conducted in a mud crab hatchery located at the Barru station of the Research Institute for Coastal Aquaculture and Fisheries Extensions, Maros, South Sulawesi, Indonesia, from 10 March 2018 to 1 May 2018. Four rounded fiberglass tanks with a height of 1 m and a diameter of 2.1 m were used as the mating tanks in this experiment. Each tank was filled with 32 ppt salinity seawater filtered through a sand filter and sterilized with 100 mgL^{-1} chlorine, then neutralized using 100 mgL^{-1} natrium thiosulfate. The water volume in each tank was maintained at three tons with clean white sand placed in several plastic baskets coated with a green plastic net arranged at the bottom of the tanks, as burrowing places for the crabs. Adolescent female crabs of *S. paramamosain, S. tranquebarica* and *S. olivacea* with mean weight of 226.72±27.4 g, 230.73±25.47 g, and 208.42±35.51 g, respectively, and mean carapace width of 108.28±3.85 mm, 113.26±5.37 mm and 107.31±5.12 mm, respectively, were used. All the crabs presented immature abdominal morphology (small rounded abdomen) at the intermolt stage, as shown in Figure 1. The matured males of *S. paramamosain* had a mean weight of 214.83±16.34 g and a mean carapace width of 111.85±5.72 mm. The mature males of *S. tranquebarica* presented a mean weight of 373.73±64.75 g and a mean carapace width of 117.22±8.13 mm (Figure 2).

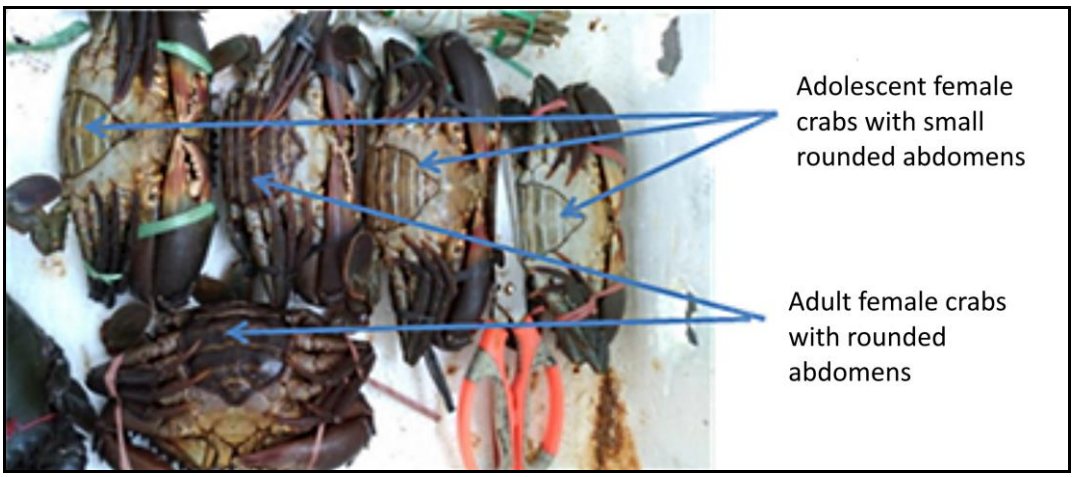

Figure 1. Adolescent female crabs and adult female crabs.

Figure 2. Adult male crab, *Scylla tranquebarica*, with tapered abdomen.

The mating trials were arranged in each tank, as follows:

1. Tank 1 - 10 males of *S. paramamosain* with 10 females of *S. tranquebarica*;
2. Tank 2 - 10 males of *S. tranquebarica* with 10 females of *S. paramamosain*;
3. Tank 3 - 10 males of *S. tranquebarica* with 3 females of *S. paramamosain*, 3 females of *S.olivacea* and 4 females of *S. tranquebarica*;
4. Tank 4 - 10 males of *S. paramamosain* with 3 females of *S. tranquebarica*, 3 females of *S. olivacea* and 4 females of *S. paramamosain*.

10 pieces of PVC pipe, 15 cm long and with a 8.8 cm diameter were used as shelters and placed on the bottom of the tanks. The crabs were fed squid and fish parts as much as 5% of the total weight, each day. The leftover feeds were removed every morning before supplying new food. 50% of the total volume of water in the tanks was replaced every two days. During the daily cleaning, the tanks and pipes were checked for crabs precopulation or mating pairs, which were recorded. According to Waiho et al (2015), the precopulation is a process in which the male takes up the female in a position known as 'precopulatory embrace'. Copulation is assumed to occur when the male overturns the newly molted female under itself and transfers the gametes to her through penetration with the gonopods.

Spawning and hatching. Each female crab was removed from the tank soon after mating. The carapace width and total body weight were recorded. The crabs were disinfected with 150 mgL^{-1} formalin solution 10% for two minutes, then stocked individually in a conical fiber glass tank (spawning tank) of 600 L, arranged in a backwash recirculation sand filtered system. The crabs were fed *ad libitum* with fish and squid daily, until they spawned. 80% of the water in the recirculation system was replaced every three days. The spawned female crabs were moved into the incubation tank after a 2-minute dip into the 150 mg L^{-1} formalin solution. The incubation tank is a one-ton rounded fiber glass tank filled with 700 L of sterilized seawater (salinity 32 ppt). There was no feeding until the larvae hatched. The fecundity was calculated based on the number of eggs at each weight unit (g) (Churchill 2003). The percentage of hatched larvae (hatching rate) was calculated by comparing the number of eggs and the resulted larvae. The hatched larvae number is estimated by the direct count of larvae for each volume unit (L) multiplied by the water volume in the hatching tank.

Larvae hatching rate = Total number of egg produced by a female mud crab/Total number of larvae produced by a female mud crab x 100%

The rearing of larvae was carried out in accordance with the method of Gunarto et al (2018). This involved feeding with rotifers until the zoea-3 stage, and then feeding with *Artemia* spp. nauplii and artificial feed in the subsequent stages until the end of the

megalopa stage, when they are sedentary at the bottom of the tank for a few days. Artificial feeds were only given to the crablet D-1 (day one) until harvesting at D-10 (day 10).

Statistical analysis. The precopulation incidences in each treatment were compared and discussed. The data about total weight and carapace width of copulated female crabs of various species were also compared and the significant differences were determined by one-way ANOVA using the IBM SPSS Statistics 24 package followed by Tukey's post hoc (multiple comparisons) test (α=0.05). In addition, the reproductive performance of female brood stock regarding egg fecundity, hatching rate, larvae production, and crablet production from different treatments were also compared and discussed.

Results and Discussion

Mud crab precopulations. During observation, there were 123 incidences of precopulation in all treatments. However, there was no pre-copulation event between the males of *S. paramamosain* and females of *S. tranquebarica* during the trials. The highest number of pre-copulations was found for the males of *S. tranquebarica* and females of *S. paramamosain*, with 44 events, which peaked at day 15 with 10 events (Figure 3).

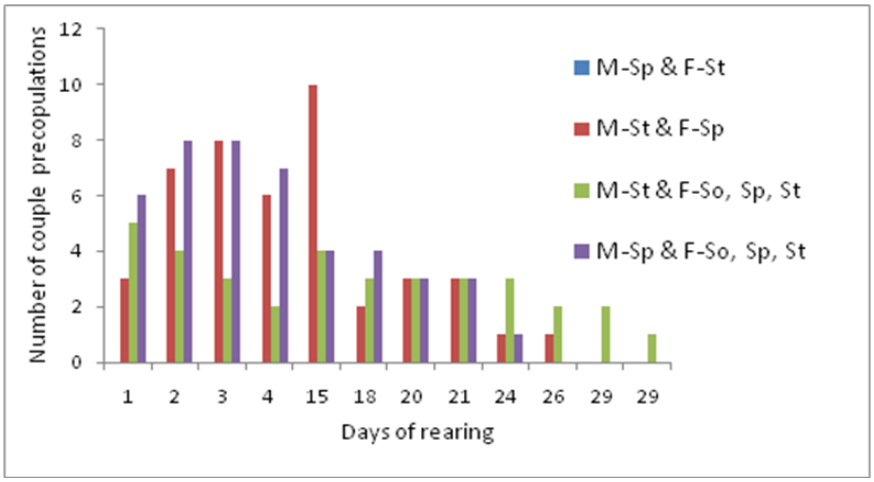

Figure 3. The number of pre-copulations of Mud crab, *Scylla* spp., during rearing in controlled tanks. M-sp - male of *Scylla paramamosain*; F-St - female of *Scylla tranquebarica*; M-St - male of *S. tranquebarica*; F-Sp - female of *S. paramamosain*; F-So, Sp, St - females of *Scylla olivacea*, *S. paramamosain*, and *S. tranquebarica*.

In the treatment where males of *S. tranquebarica* could mate with females from different species (*S. tranquebarica, S. paramamosain* and *S. olivacea*), pre-copulation started from day 1 with 5 incidences and then fluctuated between 1 and 4 incidences until the end of the experiment, with 35 events in total. Likewise, in the treatment involving the male of *S. paramamosain* and more females from different species (*S. tranquebarica, S. paramamosain* and *S. olivacea*), pre-copulation started immediately at day 1 and lasted until day 24, with the peak at day 2 and day 3, with 8 events each, having 44 total number of pre-copulation events. There was no significant difference ($P>0.05$) between the number of pre-copulation events in tanks 2, 3 and 4, but they were all significantly higher ($P<0.05$) compared with the treatment in tank 1.

Mud crab copulation. Overall, 12 copulation events were observed during the experiment. The highest number of interspecific copulation events was obtained in tank 2 (males of *S. tranquebarica* with females of *S. paramamosain*) having four occurrences out of the 10 pairs (40%) and the lowest was found between the males of *S. tranquebarica* and females of *S. olivacea*, with only two events (20%). However, the copulations between each pair of male of *S. tranquebarica* and female of *S. tranquebarica*

and between males of *S. tranquebarica* and females of *S. paramamosain* numbered three events (30%), as shown in Figure 4. There were no significant differences (P>0.05) between the female sizes (carapace width and body weight) and the success of copulation.

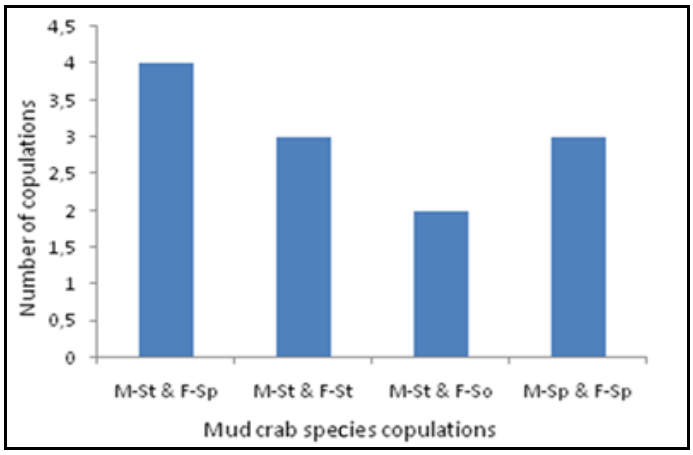

Figure 4. The number of crab copulations as intra and interspecific hibridization. M-st - male *Scylla tranquebarica*; F-Sp - female *Scylla paramamosain*; F-St - female *S. tranquebarica*; M-Sp - male *S. paramamosain*; F-So - female *Scylla olivacea*.

Size of the spawning female Mud crabs. From 12 individual successful matings, there were only six female crabs that subsequently successfully spawned with later egg hatching. The other six female crabs that mated successfully did not spawn, and some died later in the rearing tank. Figure 5 shows the total body weight, carapace width, and incubation period of the female crabs that spawned. The body weights and carapace widths of *S. paramamosain* females (soon after pre-mating molt) ranged from 181.5 to 318.8 g and from 99.5 to 137.7 mm, respectively. The body weight of the *S. tranquebarica* female was 210.0 g and the carapace width was 104.8 mm. The body weight of the first mating female of *S. olivacea* was 276.4 g and the width of the carapace was 129.5 mm. The mating duration was between 9 and 12 hours, starting at 5:00-6:00 PM and continuing until 4:00 AM, the next day. In addition, the period of egg maturation in female crab varied between species, from about 35 to 69 days in *S. paramamosain*, to 40 days in *S. tranquebarica*, and 79 days for *S. olivacea*. The incubation period of eggs was almost the same for all three species, ranging between 10 and 12 days, with the water temperatures between 27 and 32°C.

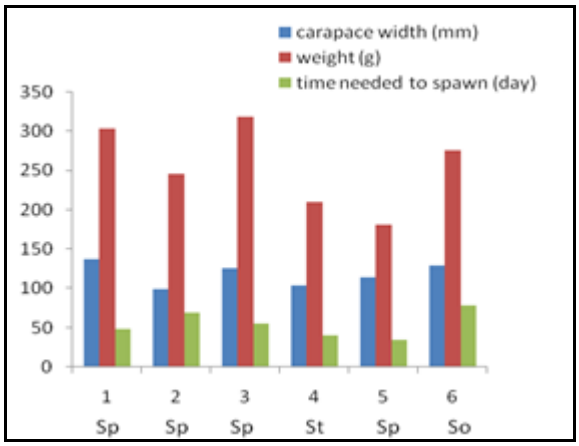

Figure 5. The size of female mud crab *Scylla* spp. after copulation (carapace width and weight) and total days needed from mating until the crab spawned. Sp - *Scylla paramamosain*; St - *Scylla tranquebarica*; So - *Scylla olivacea.*

Egg stage and further development. The relative egg fecundity, hatching rate and larvae production resulted from hybridization between females of *S. paramamosain* and males of *S. tranquebarica* ranged between 32200 and 1868000 eggs, 2-45.82%, and 2010 and 856000 larvae, respectively. The hybridization between females of *S. olivacea* and males of *S. tranquebarica* produced 758000 eggs, with a 98% hatching rate, and 738000 larvae. However, both pairs produced very low number of crablets, ranging between 23 and 123 individuals for the female *S. paramamosain* and male *S. tranquebarica* pairs and 620 individuals for female of *S. olivacea* and the male of *S. tranquebarica.*

The relative fecundity, hatching rate, larvae and crablet production from the mating of *S. tranquebarica* male and *S. tranquebarica* female is comparable to interspecies crossbreeding (above 975500 eggs, 89% hatching rate, 868200 larvae and 628 crablets). The mating of male *S. paramamosain* and female *S. paramamosain* produced 1574000 eggs, with a hatching rate of 90%, and a total crablet D-10 of 1012 individuals (Table 1).

According to Keenan et al (1998), there are frequent associations between species in mud crab populations dominated by *S. olivacea* and *S. tranquebarica*. In Don Sak Bandon Bay, Thailand, the population of crabs is dominated by *S. paramamosain*, but *S. olivacea* is also present (Hamasaki et al 2011). The species are also found in Indonesia. In the mouth of Cenranae river, Bone Regency, South Sulawesi, the predominant species of mud crab is *S. olivacea*, however, other species such as *S. tranquebarica* are found in fewer numbers. In the Malili River, East Luwu, South Sulawesi Province, Indonesia, *S. tranquebarica* predominates, but *S. olivacea* can also be found. Therefore, aside experimenting on intraspecific hybridization in one species, this study looked into the hybridization of female mud crabs from several species (*S. tranquebarica, S. paramamosain* and *S. olivacea*) paired with males from *S. tranquebarica* and *S. paramamosain*.

In this experiment, precopulation and copulation occurred between crabs from the same, but also from different species, except between females of *S. tranquebarica* and males of *S. paramamosain*. However, Fazhan et al (2013) reported that no natural crossbreeding was detected between males of *S. tranquebarica* and females of *S. olivacea* in the west coast of peninsular Malaysia. However, when males of *S. tranquebarica* were paired with females of *S. olivacea*, all the breeding steps (precopulation, copulation, eggs development, spawning and hatching) occurred, and eventually resulted in offspring. In addition, no breeding activity was observed between males of *S. paramamosain* and females of *S. tranquebarica*. However, Fazhan et al (2017) reported that the mating success of this pair was from 7 to 43%. This result is quite low if compared with the mating success of the pair of *S. tranquebarica* (male) and *S. olivacea* (female), of 58.3% (Fazhan et al 2013).

The highest number of crab precopulations (100%) and copulations (40%) were obtained in tank 2 (with females of *S. paramamosain* and males of *S. tranquebarica*). Conversely, there was no precopulation and copulation when the males of *S. paramamosain* were paired with females of *S. tranquebarica* in treatment 1. A mating success of only 20% was obtained in pairing the females of *S. olivacea* with males of *S. tranquebarica*. Also, there was only a 30% success in the mating between males of *S. paramamosain* and females *of S. paramamosain*, as well as between males of *S. tranquebarica* and females of *S. tranquebarica*. These differences might result from the different conditions of the female mud crabs, such as fattening, feed consumed and water quality, which all have the capacity to trigger molting. In this research, female molting naturally occurred before copulating with male crabs. However, limb autotomy could be carried out in the female crabs to stimulate molting (Baiduri et al 2014). This was not applied in this research, as freshly molted crabs were used. The *S. paramamosain* male crabs in this research had a mean carapace width of 111.85±5.72 mm and mean weight of 214.83±16.34 g, whereas the female *S. tranquebarica* crabs had a mean carapace width of 113.26±5.37 mm (after molting) and a mean weight of 230.73±25.47 g. The crabs were in the first genital maturity.

Table 1

Crablet production from interspecific hybridization and mating of mud crab, *Scylla* spp. in controlled tanks

No	Female crab size					Mating duration (hours)	Fecundity (egg number)	Larvae production (ind.)	Hatching rate (%)	Days required to reach megalopa stage (days)	Crablet production (ind.)
	male	Female	CL (mm)	CW (mm)	W (g)						
1	St	Sp	94.7	137.7	303.7	11	956600	15000	2	15	123
2	St	Sp	64.1	99.5	246.0	11	32200	2010	2	14	32
3	Sp	Sp	83.2	125.3	318.8	10	1.574000	1416000	90	15	1012
4	St	St	68.6	104.8	210.0	11	975500	868200	89	15	628
5	St	Sp	70.1	114.2	181.5	11	1.868000	856000	45.8	0	0
6	St	So	77.1	129.5	276.4	11	758000	738000	98	18	620
7	St	St	74.4	111.4	242.8	12	-	-	-	-	-
8	St	St	70.6	106.0	214.1	10	-	-	-	-	-
9	St	St	58.2	91.6	169.0	10	-	-	-	-	-
10	Sp	Sp	72.2	108.3	241.8	11	-	-	-	-	-
11	Sp	Sp	73.1	111.2	213.9	9	-	-	-	-	-
12	St	So	81.1	123.6	301.0	11	-	-	-	-	-

Note: ind. - individuals; CL - carapace length; CW - carapace width; W - weight after molting; Sp - *Scylla paramamosain*; St - *Scylla tranquebarica*; So - *Scylla olivacea.*

Also, the crabs undergo precopulation processes before mating and, according to Waiho et al (2015), the mating process requires about 82.0±10.8 hours in total, which includes precopulation, molting, copulation and postcopulation. The length of the precopulation process has an effect on the success of molting female crabs. During precopulation, the male crab climbs the female for a few days, which does not allow the female crab to eat, and if the precopulation process is too long (more than one week), it affects the vitality of the female crab, weakening it and, thereby, inducing failure in the molting process. Observing the duration of the precopulation process showed that the female crabs molted after 4 days from the ending of the precopulation. Usually the male crabs can only mate females that have successfully molted. The molting process makes it easier for male crabs to enter their spermatheca into the female thelicum, since its body is still soft (Ikhwanuddin et al 2011). Due to the exhaustion in the female molting process, the body parts of mud crabs such as the claws are often difficult to remove from the old carapace, further affecting the crab, causing even death in some cases. The fresh female crabs that died are often feed for male crabs.

The male crabs are often involved indirectly in the female molting process and the readiness for copulation is strongly influenced by the success of molting in female crabs, the health level of male crabs, as well as by some environmental parameters, such as water salinity and dissolved oxygen in the culture tanks. According to Fazhan et al (2017), the degree of successful mating between males of *S. tranquebarica* and females of *S. olivacea* and *S. tranquebarica* was around 50 to 70%. However, in this research, only a 30% success rate was recorded in the mating of *S. tranquebarica* males mated with *S. tranquabarica* females, and 20% for *S. tranquebarica* males with *S. olivacea* females. Hamasaki et al (2011) reported that the *S. paramamosain* female crabs in Don Sak have the first mature genitalia when they reach a 112 mm carapace width. However, *S. olivacea* male mud crabs have mature genitalia at a smaller carapace width, of about 87-103 mm (Islam & Kurokura 2012). In addition, Jirapunpirat (2008) reported that the *S. olivacea* female crabs show the first mature gonads at a carapace width of 9.55 cm in Thailand. Also, Islam & Yahya (2017) reported that the first sexual maturity of *S. paramamosain* appeared at about 5-7 months of age, for crabs reared in outdoor tanks from crablet size. The prolonged waiting period for spawning of the female crabs (also noted in this research) often lead to a high risk of diseases or failure in the molting process, which can later lead to death.

The females of *S. olivacea* were significantly bigger in size after molting ($P<0.05$), compared with the females of both *S. paramamosain* and *S. tranquebarica*, thereby resulting in higher hatching rates, higher larvae vitality, as well as higher crablet production. According to Gunarto & Herlinah (2015), the crablet production in the hatchery is affected by factors such as low larvae vitality, diseases, low food supply and quality at the larval stage, cannibalism in megalopa and crablet stages. In this study, low production of crablets might be due to the smaller size of the brood stock crabs, which produced low larval vitality. Moreover, *S. paramamosain* female crabs mated with different male species, such as *S. tranquebarica* could have led to problems in the development of embryo eggs, thereby causing low larvae production. The results showed that the cross breeding carried out among the mud crabs produced small numbers of crablets. However, the crossbreeding of *S. olivacea* resulted in the highest hatching rate (98%) and crablet production compared with *S. paramamosain*, with a hatching rate of only 2%. Sarower et al (2012) reported that *S. serrata* has a fecundity of 1000000±70750 eggs and a hatching rate of 5%, in Bangladesh. However, this research did not conduct any test on the quality of eggs, larvae, or crablets produced from both intra and interspecific hybridization. The fecundity, larvae hatching rate and crablet production were monitored.

The maximum values for egg fecundity in interspecific hybridization for females of both *S. paramamosain* and *S. olivacea* are lower than the maximum value of egg fecundity in the mating of *S. paramamosain* female mated with *S. paramamosain* male. According to Churchill (2003), the quality of the egg could be improved by increasing the EPA, DHA, as well as its total omega-3 levels. Other important parameters that determine egg quality include broodstock size, egg size, egg color, fatty acid content in

the egg, and fatty acid content in the larvae. Furthermore, other researchers reported that egg quality is also influenced by the age of broodstock (Brooks et al 1997), diet of the broodstock (Azra & Ikhwanuddin 2016), hormonal status of the broodstock (Campbell et al 1994), and bacterial colonization of the eggs (Hudson & Lester 1994). Therefore, a formalin solution of 150 mgL^{-1} was used in this research to eliminate bacterial involvement in the eggs before the female crab was introduced in hatching tank. Finally, the crablet production in both hybridization models is quite small due to low larvae vitality from females of *S. paramamosain* mated with males of *S. tranquebarica*. Other factors, such as infections with *Vibrio* spp. of the larvae, cannibalism in megalopa and crablet stages are also dominant factors capable of influencing the low numbers in the production of crablets.

Conclusions. The mud crab successful mating in controlled tanks occurred for *S. paramamosain* and for *S. tranquebarica*. Interspecific hybridization in controlled tanks occured between females of *S. paramamosain* and males of *S. tranquebarica*, as well as between females of *S. olivacea* and males of *S. tranquebarica*. In the interspecific hybridization, the highest hatching rate (98%) was obtained in the mating between the females of *S. olivacea* and males of *S. tranquebarica*, while the lowest was found between females of *S. paramamosain* and males of *S. tranquebarica* (2-45%). The highest hatching rate (90%) was obtained between females of *S. paramamosain* and males of *S. paramamosain*, while a hatching rate of 89% was obtained for females of *S. tranquebarica* and males of *S. tranquebarica*. Also, the production of crablets is higher in the mud crab mating with the same species when compared with the results obtained from interspecific hybridization.

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