Survival, growth, and molting frequency of mud crab *Scylla tranquebarica* juveniles at different shelter conditions

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Abstract. This study assessed the effect of different shelters on survival, specific growth rates and molting frequency in mud crab juvenile, *Scylla tranquebarica* for the enhancement in aquaculture production purposes. Mud crab juveniles with initial weight ranging from 0.3 to 0.5 g and initial carapace width ranging from 1.2 to 1.4 cm were chosen and placed in tanks of 135 liter volume capacity filled with 80 liter of water. There were 18 juveniles with triplicates for each treatment. Four treatments were introduced: treatment 1 (control - without shelter), treatment 2 (black net shelter), treatment 3 (green net shelter) and treatment 4 (sand substrate shelter). The study was done in triplicate and conducted for 50 days. Mud crab juveniles reared in treatment 4 showed significantly higher survival (59.26±2.62%) compared to other treatments. Besides, mud crab juveniles cultured in treatment 2 had the highest molting frequency. As a conclusion, the usage of black mesh net in the treatment 2 rearing increased the molting rate of mud crab juveniles, while higher survival rate was recorded in the treatment 4. As a recommendation, the effect of different substrate, shelters and material used can be done for further study. The finding from the present study can be applied in other mud crab or crustaceans. Full understanding on how mud crab juveniles reacted for a shelter is important for the beneficial in aquaculture especially mud crab farmers.
Key Words: *Scylla tranquebarica*, net, aggressive behavior, specific growth rates, molting frequency.

Introduction. Mud crabs (genus *Scylla*) are much in demand as a quality food due to their size, meat content and delicate taste (Azra & Ikhwanuddin 2016) and hence, command high prices in domestic and international markets. Mud crabs also have been cultured for many years in Asia, based primarily on the capture and fattening of juvenile crabs from the wild, particularly in Southeast region where production is increasing quickly (Petersen et al 2013; Dos Santos Tavares et al 2017). Increasing human population and market demand led to the constraint supply of seed stocks triggered by over exploitation (Ikhwanuddin et al 2015). The major difficulties in mud crab culture is the inconsistent production of crab seed due to unpredictability in reproductive performance including spawning, maturation and hatching rate (Ghazali et al 2017). Nevertheless, aggression and cannibalism, which are the main reasons of low survival in semi-intensive and intensive culture systems, still remain a challenge (Quinitio et al 2001; Williams & Primavera 2001; Allan & Fielder 2003; Mann & Paterson 2003; Paterson et al 2007).

In Malaysia, mud crab fisheries are confined to the estuaries and coastal areas, which support mangrove swamp development and they are also exploited by local individuals for immediate consumption (Ikhwanuddin et al 2011). First commercial mud crab culture operations in Malaysia was started in 1991 where mud crab farming is only concentrated within the mangrove forest area. Recently, due to the increasing price of mud crabs, it has encouraged many coastal communities or the local to initiate culture trials. Effort has been done to increase the Portunid culture production although the crab seeds supply is limited and breeding techniques have yet to be fully developed (Azra & Ikhwanuddin 2015).

The exploitation of wild crab seed has also lead to a tremendous effort in increasing the seed for the hatchery production through various culture techniques. For example, the usage of artificial reefs or shelters in the marine environment is a common practice worldwide, which has inspired technological research in seed production during the nursery culture phase (Mirera & Moksnes 2013). There are many artificial shelter designs, varying in materials and sizes according to their purpose; such as commercial and recreational fishing, habitat enhancement (Seaman & Sprague 1991), and habitat-damage mitigation (Dumbauld et al 1993). Artificial shelters are also used as a research tool to collect selected life stages of marine species such as spiny lobsters (Booth 1979). Generally, shelters are usually provided to reduce stress and prevent or minimize cannibalism (Millamena & Quinitio 2000; Millamena & Bangcaya 2001; Hamasaki et al 2002; Djunaidah et al 2003).

Most studies have limited success especially in the optimization of survival rate of the larvae which is in the large-scale culture (Djunaidah et al 2003; Hamasaki et al 2002). Major problems are caused by asynchronous molting, thus resulting in variation of size among mud crab juveniles. Hence, this may increase predation pressure on smaller individuals due to the higher proportion of larger crabs (Daly et al 2009). There are several factors affecting the survival in crab culture such as cannibalism, molting, salinity and temperature fluctuations, feed, shelter and stocking density (Ruscoe et al 2004; Holme et al 2007; Mann et al 2007; Rodriguez et al 2007; Mirera 2009; Quinitio & Estepa 2011).

Cannibalism is defined as the process of killing and consuming either all or part of an individual of the same species, which is a behavioral trait that is commonly observed in a wide variety of animals, including crustaceans (Elgar & Crespi 1992). Cannibalism of juvenile portunid crabs is known to be at its greatest when the cannibalizing crabs are large enough to kill other crabs in inter-moult with hard exoskeletons (Marshall et al 2005). Cannibalism is also recognized as a major problem due to the fact that cannibalism between larval and juvenile sibling has been documented as the major problem for mass mortalities in cultured crustaceans (Liao et al 2001).

Thus, this study will be beneficial for farmers involved in culturing mud crab as shelter plays an important role in reducing the cannibalism during the juvenile rearing process, especially in hatchery or intensive culture system. A clear understanding of how shelter and substrate is affecting crab survival, growth and development is important for increasing the number of adult crabs particularly in hatchery seed production. In this study, we aimed to evaluate the effects of different shelters on survival, growth rates and molting frequency, and observation on aggressive behavior and cannibalism in mud crab *Scylla tranquebarica* juveniles.

Material and Method

Sample of mud crab juveniles and treatment preparation. The mud crab juveniles were reared at Shrimp Hatchery, Universiti Malaysia Sabah and the juveniles are the same batch. Furthermore, idenfication of *S. tranquebarica* was done according to Keenan et al (1998).A total of 216 mud crab juveniles were chosen with the range of carapace width (CW) of 1.2-1.4 cm, and with the initial body weight (BW) of 0.3-0.5 g. There were 18 juveniles with triplicates for each treatment and there were 12 tanks that used in the experiment. Mud crab juveniles undergo acclimatization process for one week before the study was started. One week acclimation was conducted in order to reduce stress related with handling and laboratory environment (Fatihah et al 2014). This study was done at the Shrimp Hatchery, Universiti Malaysia Sabah. The study period was 50 days from 29th November 2016 to 17th January 2017. A total of 12 white fiber tanks with a volume of 135 liter each were used in the present study. Aeration tubes together with aeration stones were provided for each tank. The water volume for every treatment tank was 80 liter. Four treatments were introduced: treatment 1 (control, without shelter), treatment 2 (black net shelter), treatment 3 (green net shelter), and treatment 4 (sand substrate shelter). Triplicates were set up for each treatment and the replication was labeled as a, b and c. (i.e treatment 1a, 1b and 1c). All 12 tanks were arranged randomly in the present study to avoid any factors that might result in different reading during study as it could cause biased in the experiment.

Shelter preparation. Three types of shelter were prepared for the present study (two artificial crab shelters and sand shelter). The artificial crab shelters used in the study were made from netting materials which was black net mesh and square rigid mesh net in green colour, made from polymer high density polyethylene with mesh size 15 mm x 15 mm (Figure 1). The dimension of artificial shelter made from green colour rigid mesh net is 40 mm diameter and 26 cm height, while for black net mesh is 18 cm height and 40 cm width. Sand substrate used (Figure 1) was dried under the sun and soaked in the fresh water for 2 days. For the control treatment, there were three replicates of tanks, with no shelters provided in the tanks.

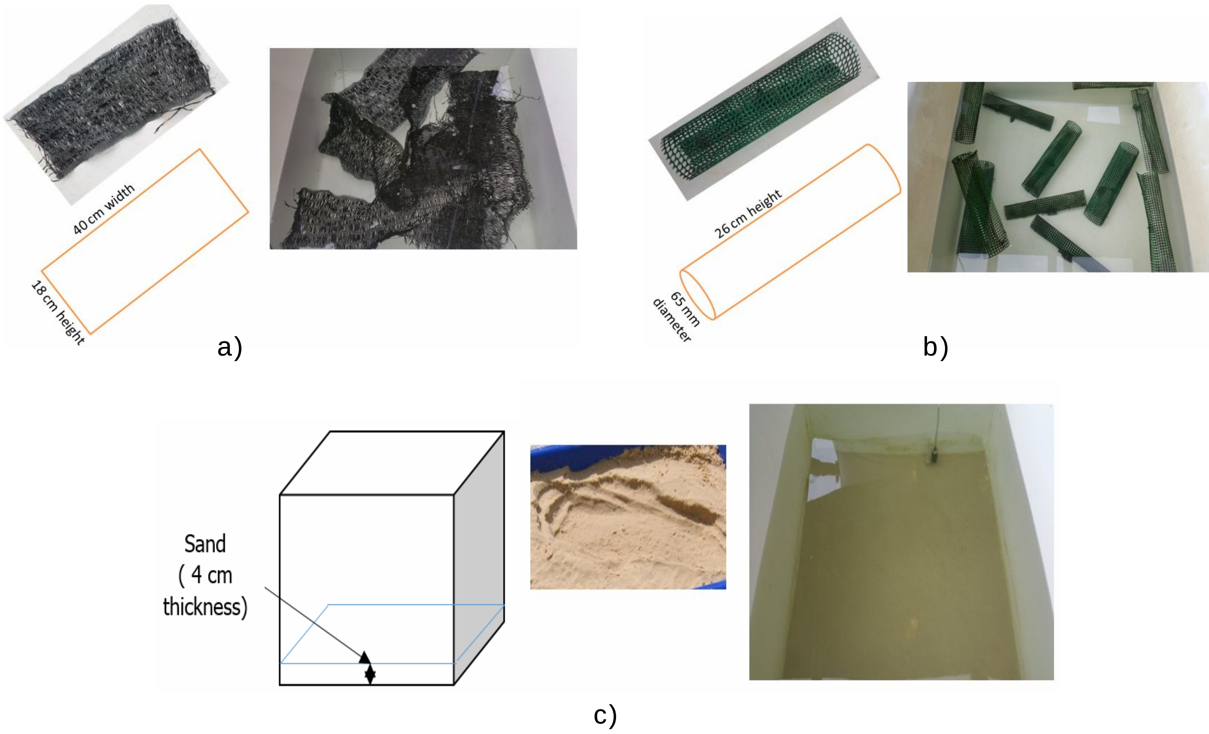

Figure 1. Three types of shelter used in the present study: a) black net, b) green net, and c) sand.

Juveniles and water management. The experiments were conducted for 57 days and all crab juveniles were fed with fresh fish (*Decapterus* sp.) and squid *ad libitum* twice daily (at 0800 h and 1600 h). The fillets fish and squid were cut dice shape. Approximately 50 % water changing was done for every day. Temperature, dissolved oxygen (DO), pH and salinity were monitored daily using an YSI 556 multiprobe meter. Water parameters were maintained at optimum level of temperature (27-29^{o}C), DO (5.5-6.8 mg L^{-1}), pH (8.2-8.9) and salinity (25-26 ppt).

Data collection. Data collection and observation were performed daily after feeding with initial calculation of BW and CW of all crab juveniles. After 50 days, the survival, specific growth rate (SGR), and molting frequency of mud crab juveniles were determined, along with the observation on molting behavior.

Survival of mud crab juveniles. In order to evaluate the survival rate of mud crab juveniles, the amount of surviving mud crab juveniles in each replication tanks were counted. The amount was compared with the initial number in this experiment. Survival rate was calculated using the formula below:

$$Survival\,rate = \frac{I_1}{I_2}\ X\ 100\%$$

The survival rates were examined based on Jobling (1995), where, SR was the survival rate, I_1 was the total number of crabs in end of experiment, and I_2 was the total number of crabs in beginning of experiment.

Total molting frequency. The evaluation on total molting frequency was done by calculating the total of molting that occurred based on the old exoskeleton in each replication which was recorded daily within 50 days of experiment.

Specific growth rate (SGR). For the regular collection of data, BW and CW of mud crab juveniles were measured within two weeks interval. The period of study was 50 days and therefore, there were five regular measurements for BW and CW including the first and last measurements. All data was recorded in the present study.

The specific growth rate was calculated at the end of the study (De Silva & Anderson 1995) based on the data from the first and last measurement of BW. The formula for specific growth rate used was as following:

$$SGR\ (\%\ per\ day) = \frac{W_2 - W_1}{t} \times 100\%$$

The formula was based on De Silva & Anderson (1995), where SGR was the specific growth rate, w_1 was the initial weight, w_2 was the weight at the end of experiment, and t was the duration of the experiment.

Data analysis*.* Data were analyzed by SPSS (Statistical Package for Social Science) version 20.0. One-Way Analysis of Variance (ANOVA) was used to determine the statistical differences among treatments. Besides, Tukey's multiple range test was then applied to detect significant differences between means ($p < 0.05$).

Results

Survival of mud crab juveniles*.* Table 1 shows the different treatments against the survival, molting frequency and SGR of mud crab juveniles for 50 days. The results of the present study showed significant differences for the survival rate between different treatments ($p < 0.05$). The control treatment had the lowest survival rate (20.37±2.62%) while treatment 4 had the highest survival rate (59.26±2.62%) for mud crab juveniles in 50 days of study. The survival rate for both treatments 3 and 4 using the artificial shelters (black net and green net) did not show any significant difference ($p > 0.05$), ranged 31.84-37.04%.

Table 1

Survival (%), molting frequency and SGR of juvenile mud crab, *S. tranquebarica* after 50 days

	Treatment 1	*Treatment 2*	*Treatment 3*	*Treatment 4*
Survival (%)	20.37±2.62	37.04±2.62	31.84±2.62	59.26±2.62
Molting frequency	38±1.25	57±1.41	44±2.05	36±0.47
SGR (%)	4.10±0.07	5.07±0.05	4.31±0.02	4.12±0.02

Total molting frequency*.* The molting frequency that occurred within 50 days was recorded and shown in Table 1. From the results, mud crab juveniles that were reared in treatment 2 had the highest molting frequency with 57±1.41 molting and it is significantly different ($p < 0.05$) from other treatments. Treatment 3 was significantly different with the others and recorded the second highest molting (44±2.05). Mud crab juveniles reared in treatment 4 had the lowest total frequency of molting with 36±0.47 molting within 50 days of experiments and showed significant different with the other treatments except the control ($p > 0.05$).

***Specific growth rate**.* Table 1 shows the SGR of mud crab juveniles after 50 days of experiment. The highest SGR was recorded in treatment 2 (5.07±0.05% per day) ($p < 0.05$). The second highest SGR (4.31±0.02% per day) was recorded in treatment 3 but not significantly different from treatment 1 ($p > 0.05$). Mud crab juveniles which were reared in treatment 4 had the lowest SGR with 4.12±0.02% per day and was significantly different ($p < 0.05$) to the juveniles that were reared in treatments 2 and 3, but there is no significantly difference ($p < 0.05$) to treatment 1.

The results of the present study indicated that the survival rate of the mud crab juveniles was strongly affected by the types of shelter (Pearson correlation: 0.862) and ranked as treatment 1 < treatment 3 < treatment 2 < treatment 4 (low to high survival rate). The sand shelter tends to increase the survival rate followed by those with artificial shelters. The control treatment without any shelter was recorded with the lowest survival rate. The specific growth rate of the juvenile mud crab in the present study was strongly correlated to the number of molting (Pearson correlation: 0.957). The higher the molting number, the specific growth rates increased.

Discussion

***Survival of mud crab juveniles**.* From the results, mud crab juveniles reared in treatment 4 showed the highest survival rate among all treatments, followed by treatment 2 and treatment 3. This may be due to the fact that sand as substrate provides refuge for mud crab juveniles. Black net and green net shelters provide complexity of habitat while there was no shelter provided in treatment 1.

Based on the results of the present study, the survival rate was the highest for mud crab juveniles reared with sand substrate because mud crab juvenile in treatment 4 tend to burrow themselves in the sand for hiding purpose. Therefore, it reduced the movement of mud crab juveniles. This is due to the provision of sand provided in treatment 4, hence easier for mud crab juveniles to rest and hide. When the movement of mud crab juvenile was reduced, the chance for mud crab juvenile to encounter each other was reduced. According to previous studies, it was known that habitat complexity influenced the decreasing rate of predator-prey encounter (Hill & Weissburg 2013; Hernandez Cordero & Seitz 2014). This result is inconsistent with other previous studies on portunid crabs, in which the survival of young juvenile stages was found higher in complex habitat relative to sand or mud habitats (Pile et al 1996; Moksnes et al 1998). This can be proved from the previous study, in which the survival in crustaceans is affected by the availability of substrate or refuge or shelter, hence increasing the survival (Moksnes et al 1998; Luppi et al 2001). The complexity of shelter in treatments 2, 3 and 4 provided hiding places for the mud crab juveniles from being attacked by other mud crab juveniles. Therefore, providing shelters and substrate into the rearing treatments caused prey to seek refuge from predators more easily. Other than that, availability of shelters also caused the predators to be less mobile or efficient in finding or catching prey if compared to rearing tanks that do not have any shelters, hence provide less habitat complexity (Crowder & Cooper 1982; Grabowski 2004).

***Specific growth rate of mud crab juveniles**.* Mud crab juveniles reared in treatment 2 had the highest result for growth in term of BW and CW, and treatment 4 had the lowest growth. The result of the Pearson correlation indicated that the SGR of the juveniles was closely correlated with the molting frequency. The molting frequency affects the growth of mud crab juveniles. Each molt tends to increase the size together with the weight of the mud crab. The highest molting frequency in the treatment 2 indicated the fastest growth of the juveniles. However, the high cannibalism that occurred in treatment 2 caused the lower stocking density if compared to treatment 4. Due to lower stocking density, there was less competition among each other for feeding. This result was found to be consistent with a previous study where growth of *S. serrata* increased as survival decreased (Genodepa 1999).

According to another previous study, feeding with squid was found to give higher BW gained for *S. serrata* (Susanto et al 2015). Besides, feeding with squid and mangrove habitats affected the growth, such as: an increase in the wide of fifth abdomen segment, the widest abdomen segment and body weight during egg development periods (Susanto et al 2015). Considering the highest survival rates, SGR as well as total molting frequency in treatments 2 and 4, culturing mud crab juveniles by using both black net and sand would improve survival, total molting frequency and growth at the same time.

Total molting frequency. In the present study, total molting frequency of mud crab juveniles was affected by the presence of shelter but not by the presence of substrate. Based on the present study, total molting frequency was greater in treatment 2, where shelter was provided. This may be due to the reason that treatment 2, in which black nets were provided as shelters provided mud crab juveniles a habitat that has more complexity compared to treatment 4 in which only sand was provided as the substrate. This result was proved by other studies on portunid crabs show that there was a higher molting frequency occurring with the presence of shelter in habitats relative to suboptimal substrata such as open sand, where the moult is delayed (Moksnes et al 1997, 2003).

According to Hamasaki (2003), the process of molting is necessary for growth, however, molting process itself is also known to be the cause of mud crab become more vulnerable to cannibalism or predation. In the present study, it was found that treatment 2 has higher in SGR. This may be caused by the higher total molting frequency that occurred in treatment 2 compared to other treatments. This result is consistent with previous studies, in which mud crab juveniles that moult frequently resulted in high growth rate (Ruscoe et al 2004; Catacutan 2002). Furthermore, it has been proved that the effect of shelter resulted in a weak positive effect on growth rates, where bamboo shelter was used for rearing *Scylla olivacea* (Fortes 1999).

Conclusions. Results from this experiment show that the survival, growth rates and molting of mud crab juveniles, *S. tranquebarica* were improved by the introduction of artificial shelter. The usage of black net as shelter tends to increase the molting frequency and the SGR, but lower the survival rate which is probably due to the material that had been used was too soft, which is insufficient to protect the crabs during molting. However, the presence of sand had improved the survival but not the growth performance of the mud crab juveniles. Growth performance and total molting frequency were improved by the usage of black mesh net as the shelter in the rearing treatment compared to the others. Survivals of mud crab juveniles were mostly improved by providing sand as substrate in the rearing treatment. Therefore, the growth and molting frequency of mud crab juveniles can be improved by using small pore size black net with more solid material.

As a recommendation, the effect of different substrate, shelters and material used can be done for further study. The finding from the present study can be applied in other mud crab or crustaceans. Yet, the design on the present study was only suitable on small scale production. Therefore, for bigger production, the modification on the shelter especially the size of shelter can be done to suit the culture area.

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