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GROWTH, SURVIVAL AND INTACTNESS OF GREEN MUD CRAB (*Scylla Paramamosain*) BROODSTOCK UNDER DIFFERENT CAPTIVE GROW OUT PROTOCOLS

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ABSTRACT

Development of broodstock of the green mud crab (*Scylla paramamosain*) was assessed under three different captive growout protocols *viz.* in the open fibre glass tanks (T1), in plastic boxes (T2) floating on fibre glass tanks and in plastic drawer/compartment (T3) for a period of 5 months under the Centre for Marine and Coastal Studies (CEMACS), Universitiy Sains Malaysia (USM). The male (M) and female (F) mud crab were cultured separately to maintain virginity. Suitability of both the sexes were evaluated considering the weight gain (size), survival and intactness of limbs during harvesting. Result of the present study revealed that, irrespectives of growout protocols, growth of mud crab happened following the sigmoid pattern. A noticeable intersexual weight attainment was observed with significantlyhigher ($p<0.05$) weight gain for the males. Meanwhile, growth was influenced by the culture protocols for both the sexes with significant ($p<0.05$) weight gain in outdoor tanks (M= 319.75 g, F= 246.17 g) followed by outdoor floating boxes (M= 250.50 g, F= 198.70 g) and indoor compartment (M= 246.40 g, F= 178.50 g). Survival and the proportion of intact crabs under indoor compartment and outdoor floating boxeswere significantly higher ($p<0.05$) than out door tank culture system. The result of the present study suggested that, outdoor growout protocol could be followed for faster broodstock development purposes to reduce the dependence on natural broodstock for hatchery operations.

Keywords: Captive condition, growth, survival, intactness, mud crab, brood stock.

INTRODUCTION

For successful aquaculture of any commercial species, a comprehensive understanding about the growth under different culture system is essential. Concepts

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on growth and surivalare considered as useful tools in aquaculture for projecting culture cycle and deciding the optimum harvesting time and size; thus ultimately minimizes the production cost and maximizes the farm output in terms of yield and revenues (Agbayani, 2001; Moksnes et al., 2014). On the other hand, growth, age and size are considered as necessary basic elements in determining the level of maturity for breeding purposes. In most crustaceans including mud crab, growth occurs through a moulting process (Shelley, 2008).

Mud crab is an aggressive and cannibalistic animal (Moksnes et al., 1998), which often lead a lowered survival and harvesting of limbless animals. For breeding purposes, an optimum sized and intact animal is the first desirable to achieve better reproductive performance and larvae quality (Quinitio and Parado-estepa, 2008; Thache, 2009). To understand the growth features of mud crab, several studies have been conducted (Le Vay et al., 2007) through tagging and recapture method on wild animals. Besides that, some of the information is also found on aquacultural growth of mud crab under pond culture (Ut et al., 2007), growth under drive-in cage culture (Shimpton and Hecht, 2007; Mirera, 2011). But all these results have been expressed from the aquaculture point of views. However, various aquaculture practices (viz. pond culture, cage culture, drive-in cage culture, pen culture) have been developed for mud crab to meet up the growing global demand. But specific documented information on broodstock development under captive condition is therefore scanty yet, except the findings of Quinitio et al. (2010) on *Scylla serrata* broodstockunder pond condition.

The green mud crab, *Scylla paramamosain*providing the lion share of mud crab aquaculture in the South-east Asian countries, including Malaysia.Scarcity of suitable broodstock often hinder the seed production operations (Islam et al., 2017). Development of captive brood stock is the emerging issue for successful seed production in hatchery condition (Shelley, 2008). But information on broodstock development under captive condition is therefore nil for this species. Considering the above, this experiment was aimed to find out the growth, survival and morphological features (intactness) of the green mud crab *S. paramamosain* under different indoor and outdoor grow out protocols for broodstock development.

MATERIALS AND METHODS

Experimental sites

The experiment was conducted at the Centre for Marine and Coastal Studies (CEMACS) under UniversitiSains Malaysia (USM), Penang, Malaysia during 2012 to 2015. Location of the site was at North-East part of Penang Island under 5° 28' 2.3664" N and 100° 12' 2.8728" E in Global Positioning System (GPS).

Experimental design

The experiment was designed with three grow out protocols for each sexes. The grow out treatments were: crabs reared in outdoor fibre glass tank bottom (T1), crabs grown individually in outdoor floating plastic boxes (T2) and crabs were individually grown under indoor plastic drawers/compartment (T3). Treatment T1 had three replicated tanks. Whilst, 40 boxes were set up in each tank and they were considered as replication for T2. On the other hand, three different layers of the compartment were considered as different replications for T3. Stocking density for T1 was 4 crabs/m^2, and for those of the box and compartment; it was single crab per box and compartment, separately.

Description and preparation of grow out protocols

A multi storied fabricated plastic compartments comprising 324 drawers were used for the T3 growout protocol (Plate 1: A). Each of the drawers had an area of 30cm × 25cm × 15cm. The drawers were arranged in such a way that one drawer was set upon another and formed 9 layers (row), 18 columns with two back to back stacks. All drawers were set up onto a base table holding a recirculation water tray in beneath with the volume of total volume of all drawers. On the other hand, soft shell crab shedding plastic boxes of 30cm × 25cm × 15cm each were used for the second treatment (T2; Plate 1: B). The boxes together with the floating plastic frame were set up in the fibre glass tanks with an area of 7 m^2 each (4.6 m × 1.52 m). For the T1 growout protocol, fibre glass tanks with the bottom surface area of 7m^2 (4.6 m × 1.52 m) were used (Plate 1: C). The tanks were prepared with inlet and outlet facilities, and provided a sand bed of 1m^2 (with a height of 12-15 cm). Seaweed (*Ulvalactuca*) was stocked in each tank at a rate of 150 g/m^2 (wet weight basis) before 1 month of crablet stocking to provide shelter and for water cleaning (Plate 1: D).

Stocking of crabs, feeding and management

For the first two months, crablets were reared in communal basis to grow to the juvenile stage. After that, all the juvenile crabs were harvested, sorted (male, female) and measured (individual total weight, carapace width). Then the intact crabs of both sexes were randomly selected for different treatments by ignoring the size to minimize the initial error among the treatments. Crabs were fed with chopped trash fish at the rate of 3 to 10% of body weight once a day at 9:30 am. For the boxes and compartments, single piece of trash fish was given to each chamber. Whereas, for the tank culture, total feed biomass was equally spread for the entire tank. Uneaten feeds and residuals were removed daily prior to feeding. Water salinity was maintained between 18 to 24 ppt.

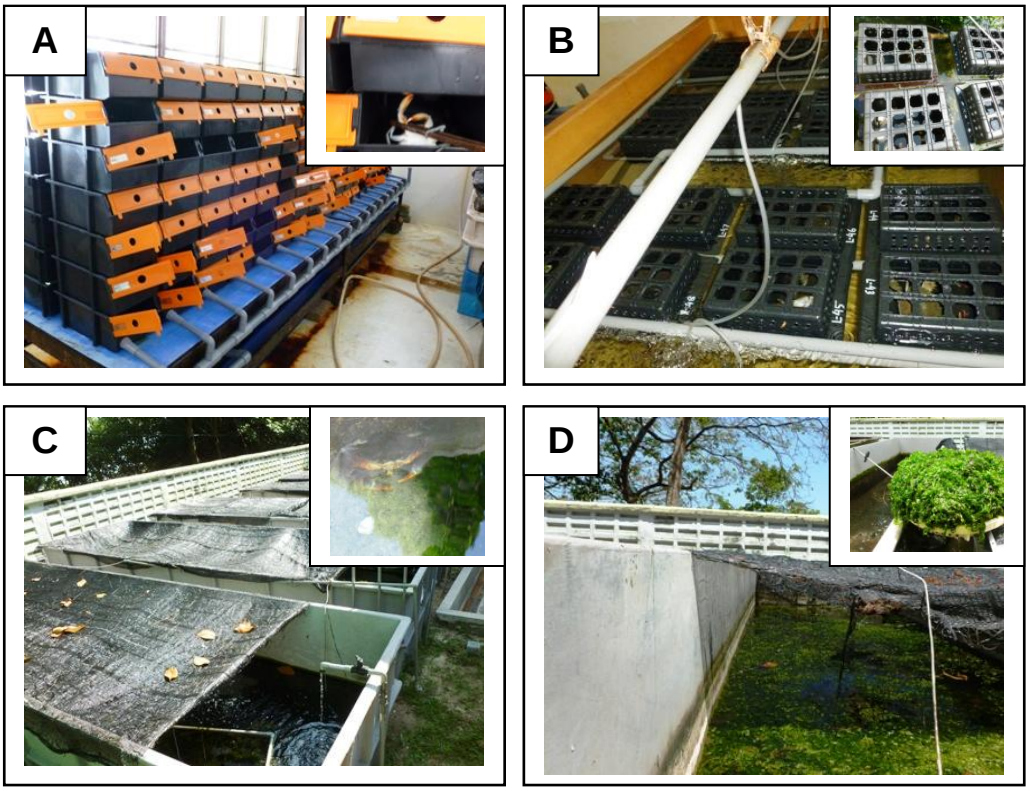

Figure 1. Photos on captive grow out protocols of mud crab,(A):grow out in plastic drawer/compartments, (B): grow out in plastic boxes, (C): grow out in open fibre glass tanks, (D):inner view of tank with sea weed (*Ulva*) as shelter

Monitoring and measurement of growth and water quality variables

The growth parameters of crab were monitored at monthly intervals. Five crabs of each sex were randomly taken from each grow out protocols and total weight (TW), and carapace width (CW) was measured following the standard method (Jantrarotai et al., 2006). Water quality variables were monitored on a monthly basis following standard methods (APHA, 1992). The water temperature ranged between 28.5-34.0 °C, salinity ranged between 18.0 to 24.0 ppt, water pH ranged between 7.7 to 8.9 and dissolved oxygen between 4.8 to 8.6 mg l^{-1} in all the treatments. All the water quality variables were within the acceptable range for grow out of crustaceans like mud crab (Cholik and Hanafi, 1992; Baliao et al., 1981; Baliao et al. 1999; Trino et al., 2001). The grow out experiment was continued up to five months (seven months from crablet). After that crabs were harvested, measured and data were processed.

Estimation and calculation of survival and SGR (specific growth rate)

Survival and SGR for each treatment replication for both the sexes (male and female

crabs) were calculated using the following formula:

$$\text{Survival (\%)} = \frac{\text{Initial number} - \text{final number}}{\text{Initial number}} \times 100 \quad \text{------ (1)}$$

$$\text{SGR (\%/day)} = 100 \times \frac{\{\text{Ln(Wf)} - \text{Ln(Wi)}\}}{\text{Time (total days of culture)}} \text{------ (2)}$$

Where, Wf= Final weight of crab, Wi= Initial weight of crab, Ln= Natural logarithm

Data analysis

Data was computed in MS Excel according to the treatment and sex. Data were analyzed through SPSS, version 22. ANOVA was performed to observe the differences between treatments and sexes. DMRT (Duncan's Multiple Range Test) was performed for ranking the differences. A confidence level of 95% ($p<0.05$) was considered as significant between the treatment for a specific variable.

RESULTS

Growth pattern of crabs

Irrespective of culture protocols, the increase in weight of both the sexes of mud crab seemed slower for the first two months, then it started to increase at a higher pace from the third month onwards (Figure 1&2). In the case of carapace width, initially the growth was faster and eventually it slowed down for the sixth and seventh month (Figure 3&4). The growth performance of both sexes of mud crab was faster in T1 (outdoor free culture) than that of T2 (outdoor box culture) and T3 (indoor compartment culture). Both weight and carapace width increment for both sexes showed a slow sigmoid pattern (s-shaped) against culture days in all the culture protocols (Figure1&4).Increment in weight (Figure1&2) and carapace width (Figure3 &4) started to vary under different culture protocols from first month when assigned into the treatment and continued up to sixth months for both the sexes.

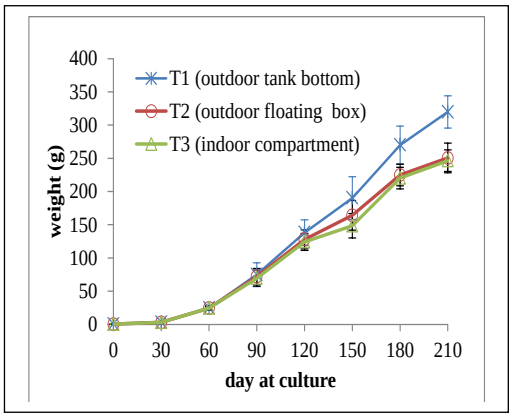

Figure 1. Growth pattern of male mud crab cultured under different captive growout protocols

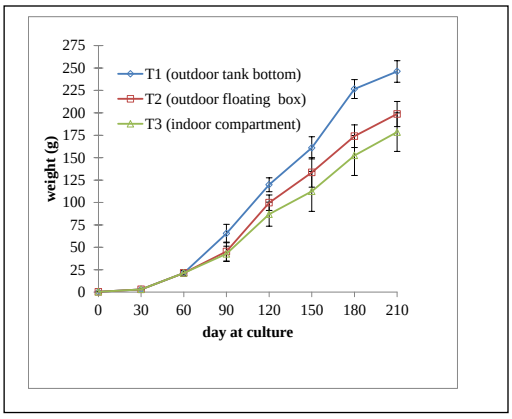

Figure 2. Growth pattern of female mud crab grown under different captive growout protocols

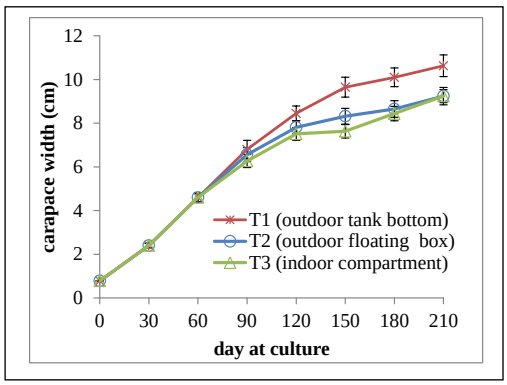

Figure 3. Growth trend of carapace width (CW) of male mud crab cultured under different captive growout protocols

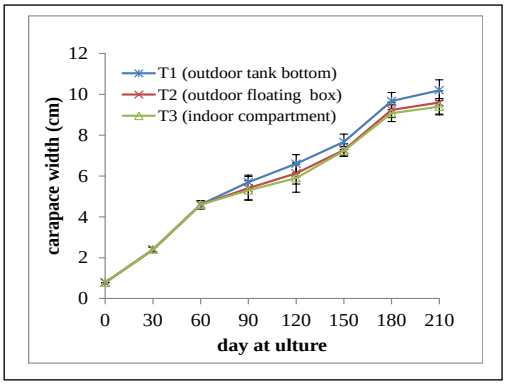

Figure 4. Trend of carapace width (CW) increment of female mud crab under different captive growout protocols

Growth, survival and intactness of female mud crab

Initial weight of female mud crab (21.32 g) and respective carapace width (2.80 cm) was similar in all the treatments. After five months of culture, female mud crab attained an average weight of 246.17 g, 198.70 g and 178.50 g in T1 (outdoor tank), T2 (outdoor box) and T3 (indoor compartment), respectively (Table 1). Weight gain was significantly higher ($p<0.05$) in T1 than T2 and T3. Similarly, SGR also differed statistically ($p<0.05$) among the treatments with the highest value (3.46%/day) in T1, but that was statistically similar (0>0.05) in T2 (3.25%/day) and T3 (3.14%/day).The lowest survival of female crab was 42.85% in T1 and it differed statistically ($p<0.05$) with other two treatments (65% in T2 and 60% in T3). During harvest, 16% and 2.5% of the female crabs were observed with broken appendages (limbless) in T1 and T2, respectively, whereas, none was observed in T3 (Table 1).

Weight gain, survival and intactness of male mud crab

As shown in table 2, final weight of male mud crab under outdoor free tank was 319.75 g and it was significantly higher ($p<0.05$) than outdoor boxes (250.50 g) and indoor compartment (246.4 g). Similarly, specific growth rate (SGR) of 3.63%/day was also statistically significant ($p<0.05$) followed by outdoor boxes (3.39%/day) and indoor compartments (3.37%/day).Survival rate of 67% under outdoor boxes and indoor compartment (64%) was statistically significant ($p<0.05$) than outdoor tanks (29%). Among the harvested male crabs 25.26%, 4.50% and 2.00% was limblost in outdoor free tank, outdoor boxes and indoor compartment system, respectively (Table 2).

Table 1. Growth, survival and intactness of female mud crab cultured under different captive grow out protocols

Parameters	Female grow out protocols		
	T1	T2	T3
Initial wt. (g)	21.32±3.18[a]	21.32±3.18[a]	21.32±3.18[a]
Initial CW (cm)	2.80±0.20[a]	2.80±0.20[a]	2.80±0.20[a]
Final wt. (g)	246.17±21.53[a]	198.70±13.98[b]	178.50±11.96[bc]
Final CW (cm)	10.20±0.51[a]	9.60±0.57[a]	9.40±0.40[a]
Survival rate (%)	42.85±7.15[b]	65.00±10.00[a]	60.00±15.00[a]
SGR (%/day)	3.46±0.09[a]	3.25±0.07[b]	3.14±0.07[b]
Limb lost (%)	16.03±5.74[a]	2.5±0.08[b]	0.00±0.00

Different superscripts in the same row indicates significant differences ($p<0.05$), shared superscript incate similarity, and a>b>c; CW: carapace width. Data are presented as Mean±SD.

Table 2. Growth, survival and intactness of male mud crab cultured under different grow out protocols in captive condition

Parameters	Male grow out protocols		
	T1	T2	T3
Initial wt. (g)	24.50±3.20[a]	24.50± 3.25 [a]	24.50±2.90 [a]
Initial CW (cm)	4.60±0.20 [a]	4.60±0.20 [a]	4.60±0.20 [a]
Final wt. (g)	319.75±24.20 [a]	250.50±22.40 [b]	246.40±16.20[cb]
Final CW (cm)	10.63±0.50 [a]	9.24±0.40 [b]	9.20±0.30[cb]
Survival rate (%)	29.00±3.57 [c]	67.00±12.40 [a]	64.00±10.50[ba]
SGR (%/day)	3.63±0.07 [a]	3.39±0.09 [b]	3.37±0.07[cb]
Limb lost (%)	25.26±3.18 [a]	4.50±1.00 [b]	2.00±1.00 [c]

Different superscripts in the same row indicate significant differences ($p<0.05$), shared superscript indicates similarity, and a>b>c; CW: carapace width. Data are presented as Mean±SD.

Comparison of bio-parameters between male and female crabs

Comparison of bio-parameters among male and female mud crab was done only for outdoor free tank culture system for both sexes. As presented in table 3, weight increment of male crab was 29.89% and SGR was 4.9% higher over the female, indicated faster growth of male. On the other hand, survival of male crab seemed 32.32% lower and appendages broken was 57.58% higher over the female mud crab (Table 3) designated the males as more aggressive and canabalistic than the female crabs.

Table 3. Comparison of bio-parameters (growth, survival and intactness) among different sexes of mud crab cultured under outdoor tank system

Parameters	Female	Male	% of increase or decrease over female
Initial wt. (g)	21.32±3.18[a]	24.50±3.20[a]	(+) 14.91
Initial CW (cm)	2.80±0.20[a]	4.60±0.20 [a]	(+) 64.28
Final wt. (g)	246.17±21.53[b]	319.75±24.20 [a]	(+) 29.89
Final CW (cm)	10.20±0.51[a]	10.63±0.50 [a]	(+) 4.22
Survival rate (%)	42.85±7.15[a]	29.00±3.57 [b]	(-) 32.32
SGR (%/day)	3.46±0.094[b]	3.63±0.07 [a]	(+) 4.91
Appendages broken (%)	16.03±5.74[b]	25.26±3.18 [a]	(+) 57.58

(+) indicates increase and (-) indicates decrease over the oposite sex; Different superscript in the same row indicates significant differences ($p<0.05$), shared superscripts indicate similarity, and a>b. CW: carapace width. Data are presented as Mean±SD.

DISCUSSION

Like other crustacean species, mud crab also grows through moultingand moulting might be affected by many factors (like, temperature, stress and scares from predator, lack of shedding/hiding places, inadequate nutritional feeding, hydrology) and any interruption in moulting might slower the growth (Kulmiye and Mavuti, 2004), thus longer time is needed to attain desirable size and even cause death to the victim. In this experiment, both male and female crab showed a sigmoid growth form (Figure 1-4). A discrete growth system of sharp changes in external size through moulting and slow growth in muscle content during the entire intermoult period might be the reason of such type of growth in crustaceans like mud crab (Shelley and Lovatelli, 2011). In crustaceans, moulting in juvenile stages is frequent and the moulting duration increases as the size grows bigger (Ehrhardt, 2008; Shelley and Lovatelli, 2011) and in many crustaceans, growth attainment rate per moult reduces with age, especially after pubertal moulting or maturity, resulted complex growth patterns (Ehrhardt, 2008). Meanwhile, a sigmoid growth pattern was modeled for *S. serrata* from mark-recapture methods, pond cultures and laboratory experiment (Moksnes et al.,2014). The growth pattern observed from this experiment on *S. paramamosain* is thus supported by the theme of the above mentioned authors.

In this experiment higher growth and SGR of both sexes of mud crab was achieved from the outdoor culture tank (T1) than outdoor box and indoor compartment (Table 1&2). The reasons behind this might be due to the provisions of semi-natural culture conditions in the outdoor tanks that might facilitate some diversified food choices like periphyton, aquatic insects and larvae of insectsthose grew onto the tanks and substrates (*Ulva*). In addition, it is also probably due to cannibalism on their siblings, which added extra nutrition to grow faster. Similarfaster growth in pond culture than

cages and indoor tanks was previously reported for *S. serrata* (Srinivasagam and Kathirvel, 1992). On the contrary, a higher growth of mud crab under drive-in cages set into mangrove than the pen culture was reported (David, 2009). Meanwhile, a 40% less growth of *S. serrata* in cage system than pond culture or natural growth was stated (Moksnes et al., 2014).

The survival of mud crab in this experiment showed wide variation between different culture protocols. Under outdoor boxes and indoor compartment, the survival seemed high (60-65%) and in tank system, it was 42.85%for female (Table 1) and 29% for male crabs (Table 2). Crabs restricted in a box and compartment might minimized the cannibalism in this study. Cannibalism in coupled with natural death (moulting death) lowered the survival in the tank culture system. Moulting death is the main fact of mortality in crustaceans (Cholik and Hanafi, 1992) and coupled with post moulting cannibalism reportedly reduce the survival and pointed as the main problem in mud crab aquaculture (David, 2009). In a study on *S. serrata,* the survial rate was 53.2% under drive-in cages and 31.25% under pen culture (David, 2009). Whereas, survival rate of *S. serrata* was 45-57% under the drive-in cage culture system (Mirera, 2011), and that for pond culture was 40.2-51.6% (Trino et al., 2001).

Specific growth rate (SGR) observed in this trial ranged between 3.46-3.14%/day for female crabs (Table 1) and 3.63-3.37%/day for males (Table 2) with highest values under outdoor tanks for both sexes. SGR value of 1.25g/day for the drive-in cage culture condition and 0.68 g/day for pen culture system for mixed sex culture of adult *S. serrata*was previously reported in Kenya (David, 2009). In another reporton *S. serrate* mentined the SGR of 10 g/month in tanks, 19 g/month in cages, and 29 g/month in ponds (Srinivasagam and Kathirvel, 1992).On the other hand, SGR value of 70 g/month (2.33 g/day) for *S. serrata* monoculture by starting with juveniles of 7 g size (Marichamy et al., 1986)seemed quite consistent with this study.

This study observed significantly higher ($p<0.05$) body weight and SGR values for the male than female for same culture protocols (Table 3). The SGR values from 1.8 - 1.9 g/day for *S. serrata* did not differ between sexes (Trino et al., 2001). While, a distinct difference in growth between sexes was noticed for *S. Oceania* and for *S. serrata* (Marichamy and Rajapachiam, 2001**)**. However, daily weight gain of 1-4 g was reported for mud crab that varies with species and sex, with males having a faster growth than females (Christensen et al., 2004); size of experimental animal (Ehrhardt, 2008); and location of experiment (Moksnes et al., 2014). Thus growth and SGR values obtained in this experiment for *S. paramamosain* are supported by the above mentioned authors.

Despite the higher growth of male over female, a lowered survival and higher appendages broken was noticed in male mud crab (Table 3) which indicated aggressiveness of the male. Male mud crab was reported as aggressive to secure territory (Shelley, 2008). All these are negative sides in point of production, market price, breeding and reproductive performance as cheliped lost is reported more

vulnerable to predation and cannibalism (Shelley, 2008). In addition, it caused lowered production because chelipod consisted about 40% of the total weight of male and 22% of the total weight of the female; of those has had a weight of 668 g (Heasman, 1980). Intact crabs of marketable size commands better prices than the injured or limb lost ones (Agbayani, 2001). Each of the appendages has had specific activities like feeding, escaping, defense or offense mechanism, mating and egg hatching for female. A suitable brood might be intact and has had a minimum weight of 450 g for *S. serrata* and 350 g for *S. Olivacea* and *S. tranqubarica* (Quinitio and Parado-Estepa, 2008). Intact brood provided better reproductive performance (Thache, 2009) and broods with injured appendages are not suitable due to low hatching rate and unsuitable larvae quality due to utilization of nutrients for regeneration of lost appendages (Zainoddin, 2001; Quinitio et al., 2010).

CONCLUSION

This experiment on growth of mud crab under the different protocols in captive condition has shown that growth in outdoor open tank is better than outdoor boxes or indoor compartments, but outdoor boxes and indoor compartment produced more intact animals. Both size and morphological features (intactness) of broodstockare regarded as pre-recusite for satisfactory reproductive performance and larvae quality. However, among the tested grow out protocols, outdoor culture system could be considered as superior option for quickest broodstockdevelopment of mud crab in captive condition.Extending the initial communal culture duration for a further one month and then arresting into boxes or compartment might be another option to achieve suitable sized and intact broodstockand that needs to be incorporated in future studies.

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