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Do Salinity, Limb Autotomy and Crab Sources Stimulate Shedding Efficiency of Mud Crab in Soft-Shell Production?

(Adakah Kemasinan, Autotomi Anggota dan Punca Ketam Merangsang Kecekapan Penanggalan Ketam Bakau dalam Penghasilan Cangkang Lembut?)

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ABSTRACT

The present study was conducted to optimize the water salinity, limb autotomy and growth performance of Juvenile mud crabs (JMC) (*Scylla olivacea*) cultured in control habitat (tanks, hatchery) compared to wild sources. In this regard, a study was performed in tanks with 5, 10, 15 ppt salinity level and in a pond having natural salinity level (control). After seven weeks of JMC culturing, it was observed that better growth attributes including survival rate, weight gain and shorter shedding period of JMC was attained at 15 ppt salinity in tanks compared to control. This optimized salinity level (15 ppt) was considerd for the second study where JMC legs were autotomized. In this study, better growth performance attributes were observed when both side walking legs were autotomized compared to one side walking legs autotomy and no autotomy. Considering the same salinity level (15 ppt), the third study was conducted to evaluate the growth performance of JMC cultured in hatchery (pilot scale) with their wild counterperts. Result showed that higher moulting rate and better growth performance (faster weight gain) of JMC were achieved in hatchery compared to wild cultured. However, JMC collected from hatchery and wild habitat acquired the same carapace width (CW) and body weight (BW) after twenty days rearing.

Keywords: Brackishwater; environmental stress; physical stress; hatchery; wild

ABSTRAK

Penyelidikan ini dijalankan untuk mengoptimumkan kemasinan air, autotomi anggota dan prestasi pertumbuhan juvenil ketam bakau (JMC) (*Scylla olivacea*) yang dikultur dalam habitat kawalan (tangki, tempat penetasan) berbanding punca liar. Dalam hal ini, kajian telah dilakukan di dalam tangki dengan paras kemasinan 5, 10, 15 ppt dan di dalam kolam yang mempunyai aras kemasinan semula jadi (kawalan). Selepas tujuh minggu pengkulturan JMC, diperhatikan bahawa sifat pertumbuhan yang lebih baik termasuk kadar kemandirian, pertambahan berat badan dan tempoh penanggalan JMC yang lebih pendek telah dicapai pada kemasinan 15 ppt dalam tangki berbanding kawalan. Tahap kemasinan yang dioptimumkan ini (15 ppt) telah dipertimbangkan untuk kajian kedua dengan kaki JMC diautomisasi. Dalam kajian ini, atribut prestasi pertumbuhan yang lebih baik diperhatikan apabila kedua-dua kaki berjalan sisi diautomisasi berbanding autotomi kaki berjalan sebelah dan tiada autotomi. Dengan mengambil kira tahap kemasinan yang sama (15 ppt), kajian ketiga telah dijalankan untuk menilai prestasi pertumbuhan JMC yang dikultur dalam tempat penetasan (skala perintis) dengan rakan liar mereka. Keputusan menunjukkan bahawa kadar penyalinan kulit yang lebih tinggi dan prestasi pertumbuhan yang lebih baik (pertambahan berat badan yang lebih cepat) JMC telah dicapai dalam tempat penetasan berbanding dengan kultur liar. Walau bagaimanapun, JMC yang diambil dari tempat penetasan dan habitat liar mempunyai lebar karapas (CW) dan berat badan (BW) yang sama selepas pemeliharaan dua puluh hari.

Kata kunci: Air payau; liar; tekanan alam sekitar; tekanan fizikal; tempat penetasan

Introduction

Mud crabs, which belong to the *Scylla* genus, are found across the Indo-Pacific area is known as green crab or mangrove crab (Ewel 2008; Gao et al. 2014; Keenan 1999). The majority of mud crabs are caught in brackish water farms, tide-fed shallow lagoons, estuaries, and mangrove swamps in Bangladesh (Shelley 2008). Aquaculture of mud crabs is therefore a long-standing tradition throughout the South and Southeast Asia (Rahman et al. 2020a). The farming method is primarily focused on capturing JMC from natural sources and fattening artificially to make as a value-added food source (Mirera 2011; Sujan et al. 2021). Apparently, gravid female mud crabs are grown and marketed by improving meat content and developing a gonad mantle within the body cavity (Muhd-Farouk et al. 2016). Meanwhile, soft-shell farming, a novel concept in mud crab aquaculture was begun at Shyamnagar, Bangladesh (Rahman et al. 2017).

Soft-shell crab production increased in Asia in the early 1990s as demand for soft-shell crabs increased in both local and foreign markets (Tobias-Quinitio et al. 2015). Soft-shell crabs can be made from any edible portunid crab species. However, crab species with significant commercial value and abundance in native geographical regions have received special attention. For instance, the Atlantic Blue Crab (*Callinectes sapidus*) is the most common soft-shell crab species in the United States (Chaves & Eggleston 2003); India's soft-shell producers primarily relied on blue swimming crab *Portunus pelagicus* (Maheswarudu et al. 2008). Many Asian countries favour mud crabs (*Scylla* spp.) for soft-shell crab production (Fujaya 2011; Shelley & Lovatelli 2011; Tobias-Quinitio et al. 2015). Soft-shell crabs can be eaten whole instead of without extracted flesh from hard exoskeleton, which makes mud crabs a high-valued food. On the other hand, soft-shell crabs are gaining popularity and are being viewed as a healthy food option due to their distinctive nutritional value and taste (Dana, Ghosh & Bandyopadhyay 2015). In particular, soft-shell crabs has more crude protein, moisture, ash, and phosphorus content than hard-shell crabs (He, Chen & Cai 2016).

The Asian sub-continent is the largest producer and exporter of soft-shell crabs (Hungria et al. 2017; Tobias-Quinitio et al. 2015). Soft-shell crab cultivation was introduced into Bangladesh in 2011 and has gained popularity as it has high value and has huge potential for artificial farm culture (Lahiri et al. 2021; Rahman et al. 2020b, 2018). Soft-shell crab farming is the most profitable among mangrove crab producers because the soft-shell mangrove crab can be consumed with the entire carapace and all limbs (Lwin 2018). Despite the demand and potential growth in the international market, many farms failed due to poor marketing chain links, seed shortages, and lack of proper technical knowledge (Lahiri et al. 2021; Rahman et al. 2020b).

The primary influences on mud crab moulting were environmental factors especially salinity (Liu et al. 2021; Zhang et al. 2021). The various tissues of the crab contain various insoluble and soluble proteins depending on the salinity of the environment (Venkatachari & Vasantha 1973). During crab moulting with different salinity levels, variations in uptaking water have also been observed (Neufeld & Cameron 1994). In addition to being a crucial component of reproduction, moulting is also dependent on the right salinity conditions (Harris 1982). Limb autotomy, a method of moult induction is often used in mud crab farms to produce a unique product called soft-shell (Waiho et al. 2021). A change in the crabs' hormonal condition is being caused by minor body damages (Dvoretsky & Dvoretsky 2012). Comparing crabs with autotomized and crabs with undamaged chelipeds, the autotomized crabs' moult interval is substantially shorter (de la Cruz-Huervana, Quinitio & Corre 2019). Therefore, the present study was aimed to optimize the salinity level for soft-shell aquaculture of *S. olivacea* and to understand the effect of limb autotomy on soft-shell farming practices. In addition to evaluate the stimulation of salinity and physical stress, this study investigates on the influence of crab sources, either from hatchery or wild, on farming of soft-shell mud crab, *S. olivacea*.

Materials and Methods

Study Site

The present study was conducted at Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI), Paikgacha, Khulna, Bangladesh (22°35'37" N; 89°18'54" E).

Pond and Tank Preparation

A rectangular pond (0.1 ha) was dried and re-excavated for making them suitable to start the experiment. Agricultural lime was broadcasted directly to the pond's bottom at a quantity of 250 kg.ha^{-1}. Pond edges were fenced by using bamboo splits and nylon nets (16 × 16 mesh.inch^{-1}) to avoid entry of other crabs in the experimental set up. Just after seven days, tidal water was pumped into the pond (about one-meter depth) from the adjacent river Kobadak. Triple Super Phosphate (TSP) amounting to 25 $kg.ha^{-1}$ and granular urea of 20 $kg.ha^{-1}$ were applied to complete the fertilization process before stocking. Indoor tanks (4.57 m × 1.52 m) were cleaned with stiff brush to remove debris from all surfaces. Henceforth, 30 ppt sea water was pumped into a tank and mixed with fresh water. Sea water salinity was adjusted with fresh water to make the desired salinity of 5 ppt, 10 ppt and 15 ppt to complete the soft-shell crab experiments. All tanks were equipped with aeration.

JUVENILE MUD CRABS

Juvenile mud crabs (*S. olivacea)* were selected to produce soft-shell crabs. This species is popularly known as orange mud crab and are abound in the Sundarbans region, Bangladesh. Many soft-shell farms are established around the Sundarbans mangrove forest and are operating their farms with this crab species for yielding soft-shell mud crab. Harvested wild mud crabs from the Sundarbans mangrove were purchased from the local markets for rearing them in ponds and tanks at Brackishwater station. In contrast, hatchery crabs were collected from the Brackishwater Station crab hatchery of Bangladesh Fisheries Research Institute.

EXPERIMENTAL DESIGN

EXPERIMENT 1 (E1): EFFECT OF DIFFERENT SALINITY LEVELS ON SOFT-SHELL SHEDDING OF *S. olivacea*

In this trial, the influence of salinity variation on the physiological parameters of *S. olivacea* was observed during moulting phase (Figure 1(a)). In this trial, three different salinities such as 5 (T1), 10 (T2) and 15 (T3) ppt were made in cemented tanks and in ponds with natural saline water as a control (T4) with three replications. The same-sized rafts (30 cm × 305 cm) and boxes (25 cm × 15 cm ×15 cm) were set in cemented tanks and in an earthen pond for the experiment. Each floated-raft was made up of PVC pipes and was able to hold 20 plastic boxes for each replication. In a single treatment, three rafts were attached all together for better management. A total 12 rafts were set at the surface of the water column for farming soft-shell crabs in all 240 boxes.

EXPERIMENT 2 (E2): EFFECT OF LIMB REMOVAL ON SOFT-SHELL SHEDDING PERFORMANCE OF *S. olivacea*

This trial was employed to observe the shedding response of a crab against the physical stress (Figure 1(b)). This experiment was designed with: without autotomizing (T1, control), one side autotomizing (T2) and both side autotomizing (T3) walking legs with three replications. As in E1, the same-sized rafts (30 cm × 305 cm) and boxes (25 cm × 15 cm × 15 cm) were set in cemented tanks and in an earthen pond. PVC pipes were used to make floated-rafts, which were able to hold 20 plastic boxes for each replication. Three rafts were attached all together in a single treatment for better management and calculation. Overall, 180 boxes were inserted into 9 rafts to conduct this study.

EXPERIMENT 3 (E3): COMPARISON OF SHEDDING PERFORMANCE BETWEEN HATCHERY-PRODUCED *S. olivacea* and WILD *S. olivacea*

This trial was conducted to understand the shedding performance between hatchery-produced crabs and wild crabs (Figure 1(c)). Brackishwater Station Crab Hatchery supplied the hatchery-produced mud crabs and wild mud crabs were procured from the Shibsa River Estuary of the Sundarbans. The first treatment (T1) was completed in a tank with hatchery-produced crabs. In the same tank, wild crabs were also stocked to conduct the trial as a second treatment (T2). Thereafter, 20 boxes (16 cm × 11 cm × 5 cm) were fixed into a single raft (17 cm × 221 cm) of PVC pipes. The total number of boxes were 120 which were inserted into the 6 rafts.

CRAB STOCKING, REARING AND MONITORING

Pre-moulted immature crabs were collected from the mangrove zones for stocking in the soft-shell experiments. Wild crabs were stocked in all treatments of three experiments (except T1 of E3). In contrast, hatchery-produced juvenile crabs were collected from the Brackishwater Station Crab Hatchery for T1 of E3 only, to compare moulting efficiency between hatchery -produced crabs and wild crabs.

Body weight (BW) and carapace width (CW) data were recorded before stocking in E1, E3, and before removing walking legs in E2. In E1, pre-moulted immature crabs (68.61±1.47 g) were stocked at the quantity of a single crab box^{-1} for T1, T2, and T3 in the three indoor cemented tanks. Likewise, the same stocking density was kept to stock crabs in the earthen pond. The floated raft on the pond was covered with a canopy of orchid net to protect crabs from the intense heat. Similarly, pre-moulted immature crabs (66.97±2.13 g) were stocked in the three indoor tanks for T1, T2 and T3 in E2. On the other hand, juvenile crabs (0.50±0.36 g) were stocked in a single indoor cemented tank for conducting T1 and T2 in E3.

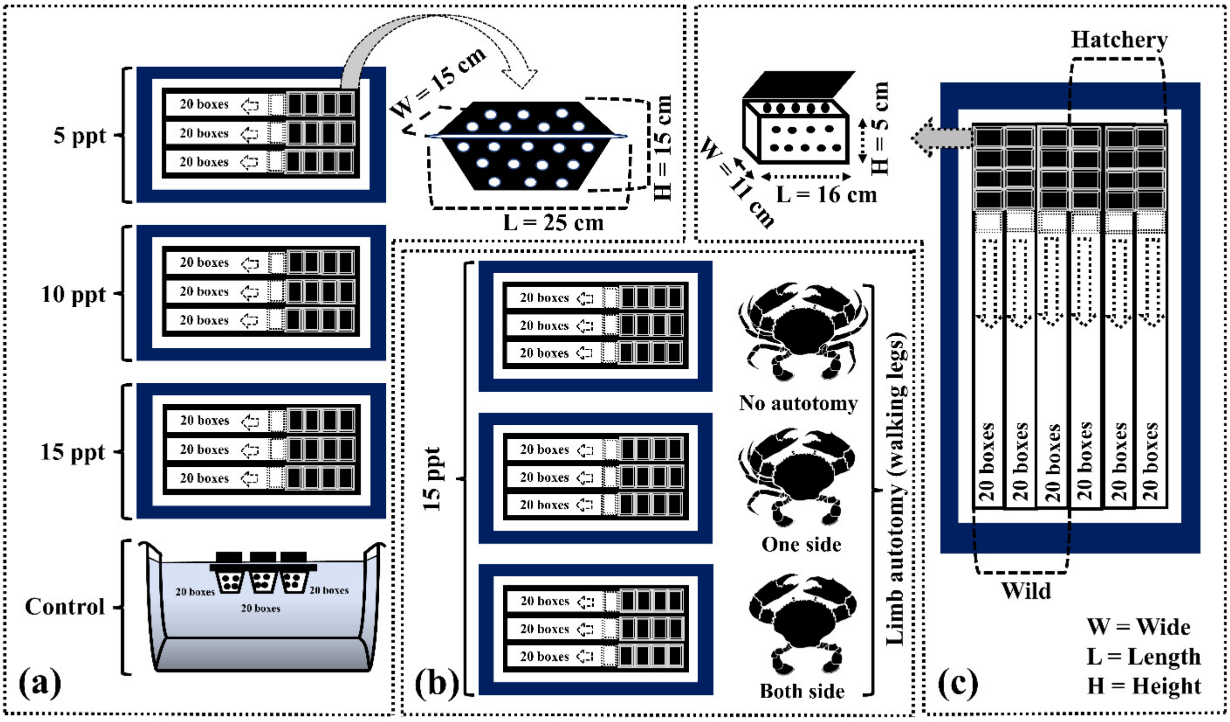

FIGURE 1. Diagram of soft-shell farming system of *S. olivacea*. (a) Experimental design of Experiment 1, (b) Experimental design of Experiment 2, and (c) Experimental design of Experiment 3

Low-valued chopped tilapia was fed at 5-10% of the crab body weight in every two days intervals for both E1 and E2. Besides, tiny pieces of trash fish (tilapia and dwarf prawns) were supplied as a daily meal (0.10 g) to the juvenile crabs of hatchery and wild in E3. The progress of crab mounting process was regularly monitored by hand and naked eye. Crabs were checked every day from each box in tanks and earthen pond by hand to examine the probability of moulting within next couple of hours. Just after shedding, the body weight (BW) and carapace width (CW) data were recorded. Generally, soft-shell crabs were picked by a scoop net from pond and tanks, and transferring them to the freshwater for remaining soft for next couple of hours. All crabs were harvested one by one from the boxes throughout the culture period (almost seven weeks) to complete E1 and E2. In E3, shedding performance between two sources (hatchery and wild) were observed within the first 20 days of culture period. During this period, CW-BW relationship were observed to compare the growth performance of mud crabs from both sources. A total of ninety-day culture was continued to collect the shedding frequency data by considering all lunar events for executing E3.

About 30% of water was siphoned and refilled by sea water with desired salinity in every week throughout the trial period. In every week, boxes were brought out to check the presence of unwanted vegetation or unexpected damage. Water quality parameters such as temperature, salinity, pH, dissolved oxygen concentration, alkalinity and ammonia was tested every week early in the morning at 07:00 am by following the methods of AOAC (1990) and APHA (1992).

The recorded water quality parameters viz. temperature, salinity, pH, dissolved oxygen concentration, alkalinity and total ammonia concentration of the experimental tanks and ponds during the culture period in this study are presented in Table 1. Temperature is one of the most fundamental environmental regulators that affects the growth, energy flow and biological effects of marine organism, mud crab. The temperature of the different experiments was observed between 25 °C and 31 °C. Water pH is also a vital indicator that can affect the aquatic life indirectly by altering other aspects of water chemistry. The recorded pH of this study ranged from 5.8 to 8.48. Dissolved oxygen is a critical parameter for

TABLE 1. Ranges in different water quality variables during the study period

Variables	Treatments							
	T1		T2		T3		T4	E3
	E1	E2	E1	E2	E1	E2	E1	
Temperature (^{0}C)	28↑	31↑	28↑	31.0↑	28↑	30.0↑	31↑	28.4↑
	25↓	26↓	25↓	26.0↓	25↓	26.5↓	27↓	27↓
Salinity (ppt)	5↑	15↑	10↑	15↑	15↑	15↑	9.0↑	15↑
	5↓	15↓	10↓	15↓	15↓	15↓	3.0↓	15↓
Water pH	7.8↑	8.4↑	8↑	8.3↑	8↑	8.48↑	8↑	8.37↑
	7↓	5.8↓	6.5↓	6.2↓	6↓	5.8↓	6.5↓	7.56↓
Dissolved oxygen (ppm)	7.2↑	8.6↑	8↑	8.8↑	6.5↑	8.6↑	7↑	8.8↑
	6↓	6.5↓	6↓	6↓	5↓	6.5↓	5.2↓	7↓
Alkalinity (ppm)	120↑	160↑	125↑	150↑	120↑	180↑	150↑	85↑
	90↓	110↓	100↓	115↓	80↓	110↓	80↓	76↓
Total ammonia (ppm)	1.5↑	1.0↑	1.0↑	1↑	1.5↑	1.0↑	0.5↑	0.5↑
	0↓	0↓	0↓	0↓	0↓	0↓	0↓	0.25↓

E1 = Experiment 1; E2 = Experiment 2; E3 = Experiment 3; ↑ = Max; ↓ = Min

all aquatic organisms as it is breathed by fish or shellfish and is needed by them to survive. The dissolved oxygen concentration recorded in these investigations varied between 5.0 ppm and 8.8 ppm. Moreover, the alkalinity and total ammonia levels of the experimental tanks and ponds ranged from 76 to 180 ppm and from 0 to 1.5 ppm, respectively.

DATA ANALYSIS

Microsoft Excel 2016, a computer program was used to compile, categorize and analyze the collected data from all experiments. Categorized data were transferred from Microsoft Excel 2016 to Statistical Product and Service Solutions (SPSS) ver. 26 for statistical tests of E1 and E2. Weight gain (in %), shedding period (days), survival rates (%) and production (kg.m^{-2}) of among treatments were compared by one-way ANOVA and Duncan's Multiple Range Test (Duncan 1955). Since E3 was designed with only two treatments, therefore, Microsoft Excel 2016 was used to run one tailed T-test to compare the shedding performance between the hatchery-produced crabs and wild crabs. For testing hypothesis, the statistical significance was set at the 5% probability level ($p<0.05$).

RESULTS AND DISCUSSIONS

WATER QUALITY

Soft-shell is the most profitable form for marketing of mud crab and this crustacean has a great aquaculture potential with high popularity in coastal areas (He 2015; Rahman et al. 2020b). Moulting is a process by which an organism sheds its old exoskeleton and replaces it with a new one (Chang & Mykles 2011). For mud crab, water quality is one of the major external factors that impacts moulting (Hartanti 2021). Above 5 ppm, the oxygen level was deemed to be suitable (Gaude & Anderson 2011). Moreover, a pleasant temperature was recorded as 25-35 °C (Hasnidar, Tamsil & Wamnebo 2021). Similarly, the pH ranged from 6.5 to 8.2 (Turano et al. 2007). In the mud crab culture, the optimal ammonia concentration was found to be below 1.0 ppm (Hasnidar, Tamsil & Wamnebo 2021). The parameters of the water quality viz. temperature, salinity, pH, dissolved oxygen concentration, alkalinity, and total ammonia, were recorded as 25-31 °C, 3-15 ppt, 5.8-8.48, 5.0-8.8 ppm, 76-180 ppm, and 0-1.5 ppm, respectively (Table 1). All recorded water parameters were validated with the findings of Shelley and Lovatelli (2011).

SALINITY INFLUENCE

The overall performance of soft-shell mud crab farming with salinity variations (E1) is presented in Table 2. Average gained weight of T3 was 55.92 ± 2.46 g which was significantly ($p<0.05$) higher than T1 (37.60 ± 0.28 g), T2 (45.84 ± 0.47 g) and T4 (40.17 ± 1.55 g), respectively (Table 2). The average shedding period of T3 was 30.56 ± 0.51 days after stocking which was also significantly ($p<0.05$) shorter than T1 (43.78 ± 2.01), T2 (38.89 ± 0.69) and T4 (41.78 ± 2.34), respectively. Moreover, the survival (93.33 ± 2.89 %) and production (2.24 ± 0.07 kg.m^{-2}) of T3 were significantly ($p<0.05$) higher than T1, T2 and T4, respectively (Table 2). The result indicated that 15 ppt salinity level gives better survival and production with higher weight gain and shorter shedding period.

Generally, estuarine salinity ranges from 0.10 to 20.0 ppt, with high salinity considered as 15-20 ppt in Bangladesh (Lara et al. 2009). Mud crabs with a salinity greater than 10 ppt fared well with the quickest time (0.54 days) to reach ecdysis (Triajie et al. 2020). In contrast, low salinity is likely to be more stressful for crabs than high salinity (Huang et al. 2019). Due to enforced osmotic stress, mud crab growth can also be significantly impeded in low salinity conditions (Rahi et al. 2020). Rather than high salinity levels, low salinity levels can result in decreased survival and growth rates of mud crab (Boonsanit & Pairohakul 2021). Crabs reared at 10 ppt and 20 ppt, showed significantly higher growth performance (Rahi et al. 2020). Similarly, the salinity level of 15 ppt showed high survival and production with increased weight gain and a shortened shedding period (Table 2).

TABLE 2. Growth performance (mean±SD) of soft-shell mud crab with salinity variations

Treatments (Salinity variation)	Weight gain (%)	Shedding period (days)	Survival (%)	Production (kg.m^{-2})
T1(5 ppt)	37.60 ± 0.28^{c}	43.78 ± 2.01^{a}	76.67 ± 2.89^{c}	1.60±0.06^{c}
T2 (10 ppt)	45.84 ± 0.47^{b}	38.89 ± 0.69^{b}	85.00 ± 5.00^{b}	1.84 ± 0.07^{b}
T3 (15 ppt)	55.92 ± 2.46^{a}	30.56 ± 0.51^{c}	93.33 ± 2.89^{a}	2.24 ± 0.07^{a}
T4 (Control)	40.17 ± 1.55^{c}	41.78 ± 2.34^{ab}	80.00 ± 5.00^{bc}	1.70 ± 0.11^{bc}

LIMB AUTOTOMY EFFECTS

The gross effects of limb autotomy on soft-shell mud crab culture are presented in Table 3. The average gained weight of T3 was 65.69 ± 1.23 g which was significantly ($p<0.05$) higher than T1 (48.77 ± 0.91 g) and T2 (56.6 2 ± 0.23 g). Likewise, the average shedding period of T3 was 20.00 ± 3.53 days which was also significantly ($p<0.05$) shorter than T1 (30.78 ± 6.01) and T2 (26.89 ± 1.50). Moreover, the survival (95.00 ± 2.89%) and production (2.30 ± 0.12 kg.m^{-2}) of T3 were significantly ($p<0.05$) higher than T1 and T2, respectively (Table 3). The result indicated that soft-shell mud crab

TABLE 3. Growth performance (mean±SD) of soft-shell mud crab with limb autotomy

Treatments (Walking legs autotomy)	Weight gain (%)	Shedding period (days)	Survival (%)	Production ($kg.m^{-2}$)
T1 (No autotomy)	48.77 ± 0.91^c	30.78 ± 6.01^a	83.33 ± 2.89^b	1.87 ± 0.06^b
T2 (One side)	56.62 ± 0.23^b	26.89 ± 1.50^{ab}	86.66 ± 2.89^b	2.01 ± 0.06^b
T3 (Both side)	65.69 ± 1.23^a	20.00 ± 3.53^b	95.00 ± 2.89^a	2.30 ± 0.12^a

farming with both limb trimming showed better survival rate, higher weight gain and shorter shedding period.

Appendage ablation causes quicker and more frequent moulting in mud crabs as found in some previous studies (He, Wu & Cheng 2016; Quinitio & Estepa 2011). In contrast, limb autotomy had no effect on survival of mud crab but the whole limb autotomy group took the lowest moult cycle time (Fujaya et al. 2020). In an indoor setting with a salinity range of 10 to 15 ppt, weight gain was greatly enhanced by both side limb autotomy (Rahman et al. 2020b). Soft-shell mud crab farming at 15 ppt salinity level with both limb trimming demonstrated better survival and production with higher weight gain and a shorter shedding period (Table 3). Studies showed that farnesoic acid O-methyl transferase (FAmeT) converts farnesoic acid into methyl farnesoate (MF) and FAmeT gene expression level in premoult was higher than in intermoult (Sunarti et al. 2016). Limb ablation increased MF and ecdylosteroid for growth and maturity of crustaceans (Laufer et al. 2002). Moulting performance of *S.olivacea* also has a positive correlation with hemolymph ecdysteroid concentration (Hasnidar, Tamsil & Wamnebo 2021).

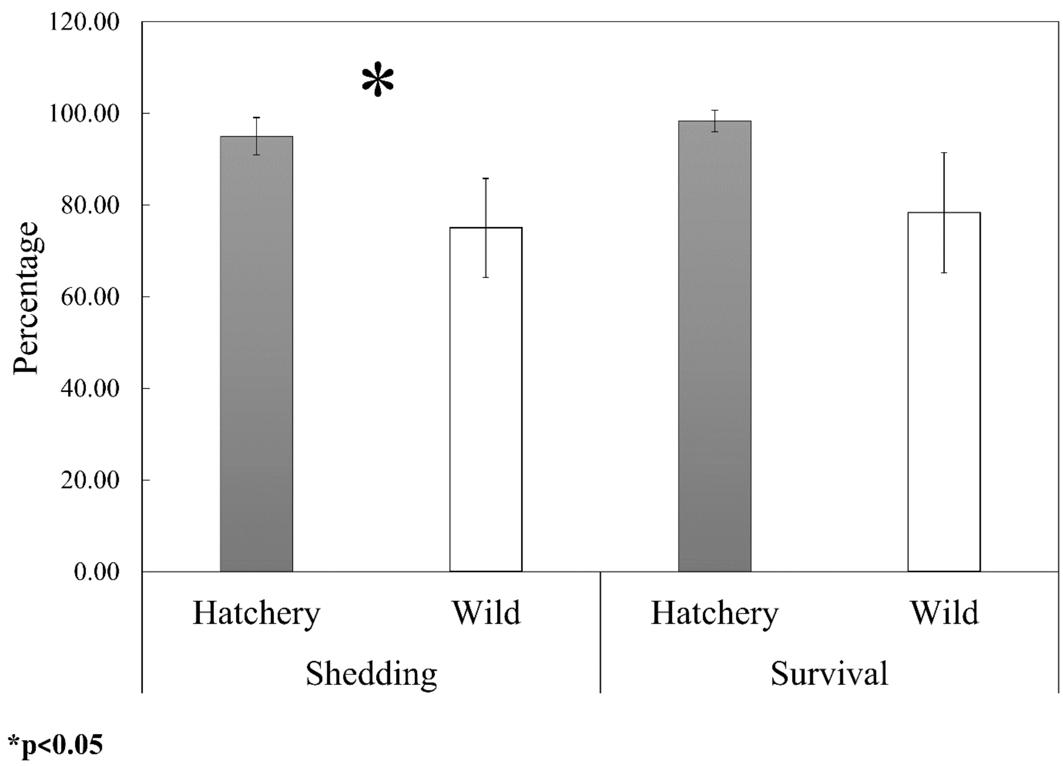

FIGURE 2. Comparing performances between mud crab juveniles of hatchery and wild

GROWTH PERFORMANCE OF MUD CRAB JUVENILES BETWEEN HATCHERY AND WILD

The survival and shedding rate of hatchery and wild juveniles of mud crab are presented in Figure 2. Survival and shedding of hatchery crabs were 98.33 ± 2.34% and 95.00 ± 4.08%, respectively. Whereas, the survival rate of wild crab was 78.33 ± 13.12% and shedding rate was 75.00 ± 10.80%. The result of this experiment focused those juvenile crabs from hatchery showed better performance than wild-sourced juveniles.

The hatchery-reared crablets had more protein and fat content than their wild counterparts (Sarower et al. 2021). The survival rate and production of normal

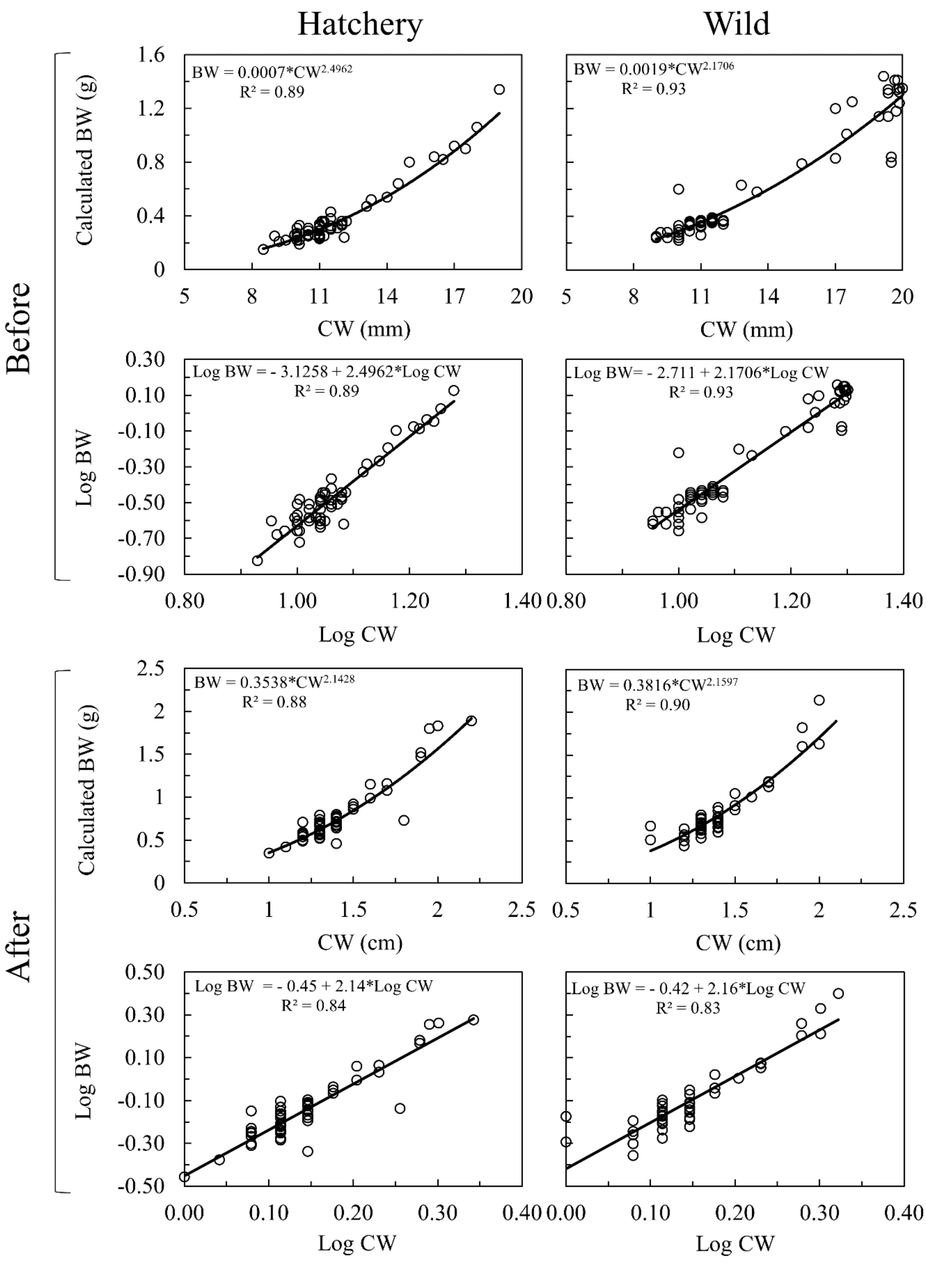

FIGURE 3. CW-BW relationship between hatchery and wild sourced mud crab

young crabs from wild were much lower than hatchery-produced crabs (Wu et al. 2018). In general, shedding performance was significantly higher in hatchery-produced crabs than wild (Figure 2). Moreover, survival rate was higher in hatchery-sourced crabs rather than in wild during the trial in the cemented tank. Besides, the same sized wild-sourced mud crabs were lighter in weight than hatchery-sourced mud crab before starting the experiment. But, after the experimental period, the relationship between carapace width and body weight were quite similar between crabs from hatchery and wild (Figure 3).

However, the relationship between carapace width (CW) and body weight (BW) of hatchery and wild-sourced *S. olivacea* for before and after the study is displayed in Figure 3. The logarithmic form of the equation ($BW = a*CW^b$) was considered to establish the CW-BW relationship. All values of carapace widths were plotted against the values of respective body weights to complete the scatter diagram for getting a curvilinear relationship (Figure 3). Parabolic curves were made by plotting the calculated value of the body weight against the carapace width of both hatchery and wild sourced *S. olivacea*. In contrast, the values of log total CW against their log calculated BW were plotted to get linear lines. Before the experimental study the estimated b values were 2.50 in hatchery and 2.17 in wild source.

On the other hand, after completing the study, the estimated b values were found as 2.14 and 2.16 for the hatchery and wild mud crab, respectively. The same Pearson correlation co-efficient (r) value (0.96) was observed for both hatchery and wild *S. olivacea* before the experiment. However, after the experimental study, the Pearson correlation co-efficient (r) values were found 0.93 in hatchery, and 0.92 in wild-sourced. It indicates highly significant relationships between CW and BW of this species. Both hatchery and wild crabs showed negative growth allometry before and after the experimental study. The result of this study also focused that the same-sized hatchery-sourced *S. olivacea* were heavier than the wild-sourced mud crab before starting the experiment. But, after completed the experiment, the relationship between carapace width and body weight were quite similar between both groups of crabs.

LUNAR CYCLE EFFECT ON MOULTING

Daily individual records of total moulting events (n=209) in indoor rearing of JMC during the whole 90-day moulting period considering lunar phases are presented in Figure 4. The maximum number of moulting occurrence (16) was found at the 11th day of the shedding period. However, moulting frequency values of 10 and 12 were observed on the 6th and 8th day, respectively. Thereafter, a gradual decrease of the events in the later days of rearing was observed due to the long-time taken for second moulting and regular death. The distribution of daily moulting frequency showed a trend characterized by peak values in between the new moon and full moon events (Figure 4).

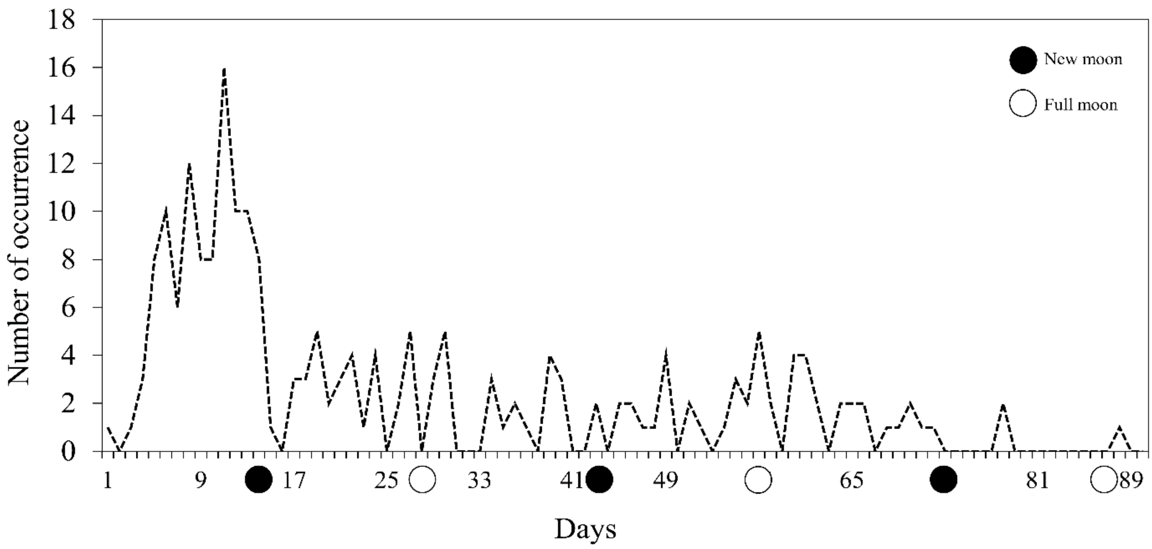

FIGURE 4. Moulting frequency polygon of juvenile mud crab *S. olivacea* during lunar phases

Ecdysteroids are hormones that help Arthropods and Crustaceans to govern their moulting events for regular growth (Hartanti 2021). The first quarter and third quarter of the lunar cycle are suitable times when mud crab usually contains the highest haemolymph ecdysteroid (Hasnidar & Tamsil 2019). Moulting usually starts at a great extent from the first quarter and continues to the full moon. Similarly, crabs also moult frequently from the third quarter to the new moon event. There were three new moon and three full moon events observed and the majority moulting picks were found just before the full moon and new moon events, as expected (Figure 4).

CONCLUSION

Salinity is a vital issue for farming mud crab. Here, soft-shell production was the highest at 15 ppt salinity level for indoor conditions. Both side limb autotomy worked well with the shortened duration of starting the moulting phase. In fact, shedding performance was significantly higher in hatchery-produced crabs than in wild crabs. Moreover, the majority moulting picks were found just before full moon and new moon events. These findings will guide the soft-shell farming in maintaining important parameters for getting better output.

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