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A NOVEL STUDY ON THE EFFECT OF RAPID BIOFLOC AS PELLET FEED ON THE SURVIVAL RATE AND WATER QUALITY OF MUD CRAB, *Scylla olivacea* CULTURE

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Abstract: In order to promote the optimum survival rate of mud crab, *Scylla* sp., in crablet culture, green technology approaches must be developed to increase the yield of mud crab production. The application of biofloc in *Scylla* sp. culture was proven successful in promoting a good crablet performance and sustaining a good water quality condition. Therefore, due to its importance, this study aimed to determine the survival rates of *Scylla olivacea* crablets cultured in tanks, fed with pelleted rapid biofloc aggregates. Three feeding trials were conducted consisting of commercial pellet (CP), rapid biofloc pellets (BP) and a mix of rapid biofloc pellets with commercial pellets (BP+CP) and were cultured up to 13 weeks of culture periods. The mud crab crablets were fed twice a day, while water exchanges were only conducted once per week. The survival rates were calculated at the end of the culture period while water quality parameters were regularly monitored. Overall, our findings showed that the survival rate in the BP+CP treatment was the highest with 30%, while 23.3% and 10% in CP and BP treatment, respectively. The water parameter was in optimum range in CP, BP and BP+ CP treatment tanks where pH ranged from 8.19 to 8.26, temperature between 26.68°C to 26.69°C and salinity between 32.17ppt to 33.16 ppt. Ammonium, NH_4^+ was below an optimum range with, 0.18mg/L in BP treatment and 0.21 mg/L in BP+CP as compared to CP. Meanwhile, nitrite, NO_2^- was identified below 1mg/L which ranged between 0.07 and 0.08 mg/L. Therefore, it can be concluded that combination of commercial and pelleted rapid biofloc aggregates have the potential capability to increase the survival rate of mud crab crablet culture while sustaining good water quality parameters and nutrient level.

Keywords: Sustainability, green technology, rapid biofloc, *Scylla* sp., survival, growth rates.

Introduction

The mud crab, *Scylla* sp. is a highly valuable and profitable aquaculture species due to its fast growth during adult stages and also has a good marketable acceptability (Sathiadhas & Najmudeen, 2004). The biofloc acts as alternative food for the mud crab larvae in an intensive mud crab culture system, while diet on *Artemia* allows the mud crab larvae to consume good nutritional level for survivability (Thirunavukkarasu *et al.,* 2014). Biofloc technology has been proven to successfully promote a maximum aquaculture production using microbial biotechnology and converted the toxic material in the nitrogen components into beneficial protein for the fish and crustacean consumption (Jamal *et al.,* 2020). Biofloc contains aggregation of heterotrophic bacteria, algae, zooplankton including protozoa, rotifers, diatoms, uneaten feed, dead organisms and a suspended particle that conglomerates together forming a microbial proteins diet to fish and crustacean (Manan *et al.,* 2017; Sharma *et al.,* 2018). This biofloc technology has been widely used in culturing numerous types of aquatic species such as tilapia, marine shrimp, freshwater prawn and also used to induce maturation in fish and shrimp (Emerenciano *et al.,* 2017; Kumar *et al.,* 2017).

In most cases, limited resources and low success rate on the optimization of survival rate of mud crab seedlings within large-scale culture (Djunaidah *et al.,* 2003). This is due to unsynchronised moulting periods that lead to variation of size among mud crab juveniles. Hence, it increases the cannibalism rate on the smaller size of its kin. The over-exploitation of wild crab stocks, destruction of mangrove swamps and poor quality of wild production to support the increasing demand are the main factors that have driven the development of hatchery technology for mud crabs (Lindner, 2005).

The supply of crab seed stocks from the wild is varied over time as recruitment to the fishery is seasonal (Walton *et al.,* 2006), as reflected in the variation of zoeal abundance in near shore waters (Sara *et al.,* 2006). The major threats towards mud crab and the seed supply includes algal blooms phenomenon, industrial and urban run-off waste discharge, and the over-exploitation of wild adult mud crabs (Ikhwanuddin *et al.,* 2011). Furthermore, supplies for mud crab seeds are also limited by the number of crabbers targeting only on certain species of mud crabs (Fazhan *et al.,* 2021). The requirement for a reliable supply of mud crab seed stock year-round to support farm expansion is essential and will enhance the future significance of mud crab hatcheries.

The declining survivability of mud crab larvae becomes the most significant problem. Thirunavukkarasu *et al.* (2014) dictated that the highest larval development of mud crab was only 6.9% in terms of survival rate in hatchery production. In addition, Schveitzer *et al.* (2013) reported that high amount of suspended solid in water will lead to health problems in cultivated species. Similarly, the impact of high concentration of total suspended solid (TSS) level in biofloc system on fish and shrimp is still unknown (Hargreaves, 2006). Linh *et al.* (2017) suggested that the supply of green water to the crab larvae helps promote plenty of natural food sources such as rotifer to the crab larvae and help supply beneficial nutrition diet for successful moulting process to reach the crablet phase. Gunarto *et al.* (2018) found out that only rotifer and Artemia nauplii were usually fed to the crab larvae until it reached crablet size. Syafaat *et al.* (2019); Gunarto *et al.* (2018) and Syafaat and Gunarto (2016) identified that the mixture between natural and artificial feed on the mud crab larvae is successful to promote the survival of the crabs to the crablet phase. Alternatively, the study on the feed supplementation using biofloc as enriched feed to the crablet phase is still scanty and no previous study has conducted on feeding trial using biofloc as a supplemented diet to the crablet phase. Thus, this study aimed to investigate the effect of pelleted rapid biofloc application on the survival rates and to the water quality assessment of mud crab crablet culture in biofloc system.

Materials and Methods

Experimental Set-up and Growth Performance Evaluation

This study was carried out at the marine hatchery, Institute of Tropical Aquaculture and Fisheries (AKUATROP), Universiti Malaysia Terengganu, Terengganu, Malaysia (5.4087° N, 103.0888° E) and mud crab of *Scylla olivacea* was used for the treatments. Three rectangular fiberglass tanks (6 ft x 3 ft x 3 ft) for control which consisted of commercial pellet feed (CP) for control, treatment tanks consist of biofloc pellets (BP) as T1 and rapid biofloc pellets with commercial pellets (BP+ CP) as T2 treatments were prepared with total capacity of 500 litre water . The feeding trials consist of commercial pellet (CP) from Gold Coin brand pellet feed, rapid biofloc pellets (BP) using biofloc pellets formulation and mix of rapid biofloc pellets and commercial pellets (BP+CP) respectively. Prior stocking of mud crab crablets, all treatment tanks were cleaned with chlorine, 30 ppm and filled with water overnight and then were rinse thoroughly to remove remaining chlorine. The tanks were left to dry before treated seawater with salinity 30 ppt was pumped into the tanks, adjusted to 1/3 of it's maximum capacity and were installed with aeration tubes.

Juveniles of *Scylla* sp. in crablet size, C4 at 1-2 cm, 0.04-0.09 gram were used and standardized for all samples used and then were placed individually in 1 litre cylindrical plastic containers with holes drilled for aeration. 1/3 of the containers were filled with beach sand and crablets were weighted before placed into the containers. For the stocking density, a total of 180 crablets were used, where 60 samples represented each treatment. The experiments were conducted up to 13 weeks.

Biofloc Pellet Preparation

For the rapid biofloc pellet (BP) preparation, samples of biofloc that were added with rapid biofloc bacterial inoculum were collected using imhoff cone (1 liter) and 5 gram of biofloc biomass for pellet preparation were collected everyday. For biofloc pellet preparation, method by Lee *et al.* (2017) with some modification was used. The collected biofloc was frozen at -80°C and continued with freeze drying using freeze dryer machine for 2 days. The dry biofloc was then ground into fine powder form. A fishmeal about 210 gram, biofloc powder about 380 gram, soybean meal about 220 gram, fish oil about 50 gram, vitamin about 5 gram, mineral mix about 5 gram, binder about 30 gram and wheat flour about 130 gram were mixed well using feed mixer. All the ingredients then were pelleted using the pellet machine. The biofloc pellets (BP) then were dried at 75°C for 12 hours. The BP were stored at -24°C freeze refigerator prior feeding the crablets. For the proximate analysis, the crude protein percentage was between 40% to 45% for the biofloc pellet

Water Exchange and Maintainance of Culture Tanks and Containers.

Water exchange was conducted about once a week where cultured seawater within the tanks was drained out completely for all treatments. Before seawater was pumped back, each container was scrubbed using a brush to remove any detritus or biofilm formed around the containers. The bottom of the tanks and aeration tubes were also scrubbed to remove any sand or algae formed and sprayed with seawater to completely remove all of the unwanted waste. After all cleaning procedures were conducted, new seawater was pumped into the tanks and aeration tubes were placed back.

Feeding Regimes

Upon 5% from body weight, the crablets were fed three times a day at 0800, 1600 and 2400 hrs following their feeding treatment BP, CP and mix of BP+CP, respectively. Before each feeding session, excess feed wastes were taken out by tilting the container sideways, allowing the feed wastes to be flushed out by the holes around the container.

Survival Rate (SR)

Once a week, the carapace width (CW), carapace length (CL) and body weight (BW) of the crablets were measured individually. The survival rate (SR) of mud crab was calculated as follows;

$$\text{Survival Rate (SR)} = \frac{\textit{Number of individuals alive the end of time period}}{\textit{Number of individuals alive the start of time period}} \text{ X } 100 \quad (1)$$

Monitoring of Water Quality Parameters

Pre-treated seawater following Standard Operation Procedure (SOP) were consistently used as stock water throughout the experimental study. The water quality parameters including pH, temperature (°C) dissolved oxygen, DO (mg L^{-1}) and salinity (ppt) were measured using YSI multiprobe model 556. In the lab, ammonium, NH_4^+ (mg L^{-1}) and nitrite, NO_2^- (mg L^{-1}) were evaluated following Phenate and Diazotization method (APHA, 2012), respectively.

Statistical Analysis

Statistical analyses were performed for each treatment tank using SPSS Software version 25.0 for the construction of graph and determination of mean and standard error. Two-way ANOVA was applied to determine the significant different between the means of water quality parameter and survival rate between control and treatment tanks.

Results and Discussion

In-situ Water Quality Parameters

pH was ranged between 8.19±0.13 in control and 8.26±0.01 in CP+BP of T2 treatment tank (Table 1). There was significant difference among treatments and control ($p = 0.002$, $p < 0.05$, $F = 19.471$). The highest pH recorded at T1 tank where crablets were fed solely with biofloc pellet (BP), while the lowest pH was recorded at control tank where crablets were fed with commercial pellet (CP). According to Fondriest Environmental (2013) the photosynthesis by algae can increase the pH level meanwhile the respiration and decomposition can lower the pH level in the water. This statement is supported by varied pH results achieved during the present study.

For the temperature, there was no significant difference identified between treatment and control tanks ($p = 0.495$, $p > 0.05$, $F = 0.791$). The temperature ranged between 26.69±0.51 in control (CP), 26.68±0.58 in (T1) consisting of BP and 26.68±0.54 in (T2) which consists of BP+CP (Table 1). The DO concentration ranged between 5.67±0.53 and 5.97±0.44 mg L^{-1}, whereas the salinity level ranged between 32.17±1.02 and 33.16±1.08 ppt. There were significant differences for DO and salinity with $p = 0.011$, $F = 10.629$ and $p = 0.363$, $F = 1.204$, respectively. For the whole study period, the highest salinity was observed in the control tank (33.16 ppt), while the lowest salinity level was in T2 (32.17 ppt). In all tanks, the salinity level was slightly low (± 25ppt) during exchange and refilling the water done once per week, and after water exchange it started to slightly increase during the whole culture period.

The highest ammonium, NH_4^+ was in the control tank with 0.29±0.22 mg L^{-1}, while the lowest was in T1 tank with 0.18±0.05 mg L^{-1} (Table 1). It was significantly different among the treatment and control tanks ($p = 0.002$, $p <$

Table 1: Water quality parameters under different feeding treatments for *Scylla* sp. culture over 13 week-experimental periods; Note that crablets were fed with Commercial Pellet, CP as control, only Biofloc Pellet, BP as treatment 1, T1 and mixed of Commercial Pellet and Biofloc Pellet, CP+BP as treatment 2, T2). There was significant difference between the treatment and control tanks ($p = 0.002$, $p < 0.05$, $F = 19.471$) for pH and ($p = 0.002$, $p < 0.05$, $F = 19.610$) for Ammonium, NH_4^+

Water Parameters	Control (CP)	T1 (BP)	T2 (CP+BP)
pH	8.19±0.13^a	8.26±0.01^b	8.22±0.12^a
Temperature (°C)	26.69±0.51	26.68±0.58	26.68±0.54
DO (mg L^{-1})	5.67±0.53	5.97±0.44	5.87±0.53
Salinity (ppt)	33.16±1.08	32.56±0.84	32.17±1.02
NH_4^+ (mg L^{-1})	0.29±0.22^a	0.18±0.05^b	0.21±0.09^b
NO_2^- (mg L^{-1})	0.08±0.07	0.07±0.02	0.08±0.03

0.05, F = 19.610). The fluctuated trends were observed in control and T2 tanks, as compared to consistent concentration of ammonia in T1 tank, which fall under optimum range. On the other hand, nitrite, NO_2^- was not significantly different among the treatments and control (p = 0.729, p > 0.05, F = 0.333). The similar trend was observed in all tanks (control, T1 and T2), where nitrite, NO_2^- concentrations fluctuated throughout the study periods.

Survival Rate of Mud Crab, Scylla. sp.

Figure 1 shows the survival rate of mud crab, *Scylla* sp. crablets cultured in three different diets which consist of commercial pellet (CP), biofloc pellet (BP) and mixed of both commercial and biofloc pellets (CP+BP). The highest survival rate at 30% was recorded in T2 where crablets were fed with mixed of commercial pellet and biofloc pellet (CP+BP). In captivity, normally survival of crablets crabs would usually be around 47.67% (Mia & Shah, 2010) and 40.3% to 46% (Syafaat *et al.,* 2019). The mortality was high due to unsuccessful moulting process (Greenaway, 1985) and might also be due to bacterial infection (Suprayudi *et al.,* 2004) that brought in high mortality even though raised in a single container. In control tank where the crablets were fed with only commercial pellet, the survival rate was 23.3% followed by the lowest survival rate (10%) in T1, where crablets were only fed with biofloc pellet. There was significant difference of survival rate among the treatments and control tanks (p = 0.00, p < 0.05, F = 108.29). The final weight gain achieved after the 13-week culture period was ranged between 50-60 gram /pc. The feed conversion ratio value (FCR) calculated was between 1.95 to 2.56, showing the good quality of pellet feed, BP.

It is crucial to provide nutritional feeds for *Scylla* sp. crablets with a good health environment to improve their growth and survival. Biofloc has great potential to deal with the optimum water quality in experimental tanks by optimizing the biomass of biofloc aggregates. In our trials, results showed that the biofloc could significantly maintain the water quality among the treatments and control tanks where (p = 0.002, p < 0.05, F = 19.610 for Ammonium, NH_4^+ level between control and treatments tanks (Table 1). The biofloc pellets in treatment tanks (BP and CP+BP) consist of composite nitrifying bacteria, inorganic matters and organic materials, harvested from fresh formulated biofloc (Kasan et al., 2019). The biofloc pellets are constantly produced as they contain high organic matter input, and those matters are floc together by biological polymer matrices

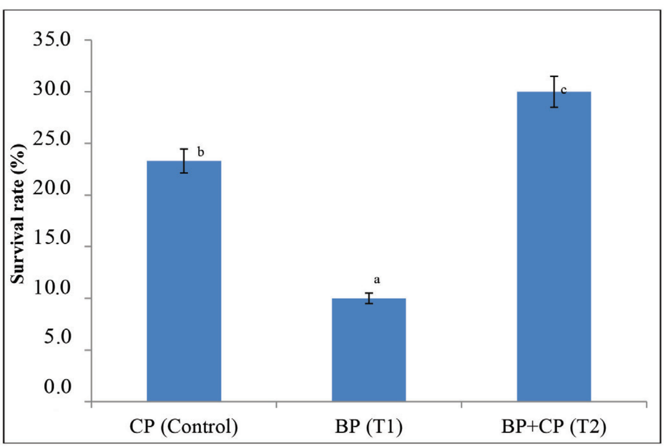

Figure 1: Survival rate (%) of mud crab, *Scylla olivacea* crablets fed with different biofloc pellet diets for 13-week culture periods; Note that crablets were fed with Commercial Pellet, CP as control has 23.3% survival rate, Biofloc Pellet, BP as treatment 1, T1 has 10% survival rate and mixed of Commercial Pellet and Biofloc Pellet, CP+BP as treatment 2, T2 has the highest survival rate of 30%

(Castro-Nieto *et al.,* 2012). Despite that biofloc can maintain good water quality, excess biofloc aggregates more than acceptable level and will cause poor water quality, changes in the biofloc composition and negative effects on the health of the cultured organisms, resulting in reduced development performance (Hargreaves, 2006).

The DO concentration identified fluctuated in the first 20-days of culture period, especially the lower DO level in the control tank. In the other treatment tanks (T1 and T2), the biofloc pellet seems to play its role in maintaining the DO level when supplied with constant aeration. This might be due to the microbial community consuming as much DO as possible to recycle and transform the excess nutrients and diverse metabolites into microbial biomass. Besides, there was also a possibility that the ammonia and nitrite oxidation occurred as supported by Manan *et al.* (2016). Dramatic changes in salinity will affect growth and survival of mud crab. *Scylla* sp. (Pedapoli & Ramudu, 2014). Mud crabs are highly tolerant to a wide range of salinity level, between 10 ppt to 34 ppt (Chen & Chia, 1996; Pedapoli & Ramudu, 2014). However, Jantrarotai *et al.* (2002) stated that the best survival occurred at 30 ppt, with salinity level ranged between 30 ppt – 35 ppt. In the early stage of mud crab larvae, the eyestalk had not yet developed which was the main organ function to produce water balance regulating hormone. An increasing trend of salinity (Table 1) indicates the osmolality mechanism boost up energy consumption on osmoregulation of mud crab larvae. High salinity level will alter the energy to regulate body fluid and eventually lowering the survival rate of larvae (Jantrarotai *et al.*, 2002; Long *et al.,* 2017).

Our findings also showed that heterotrophic bacteria (e.g., *Bacillus* sp.) successfully immobilized the ammonium, NH_4^+ concentration by conversion into nitrite, NO_2^-. Concentration of NH_4^+ and NO_2^- recorded during this experiment, remained within the optimum levels as recommended for decapod crustaceans, which was lower than 2 mg L^{-1} in the water (Philips *et al.,* 2002; Romano & Zeng, 2013). The presence of high concentration of NH_4^+ in the control tank indicated low nitrification processes of ammonium. It is related with high salinity; the larvae increased their ammonia or ammonium excretion, where there is possibility of the ammonium being released for hyper-regulation (Chen & Chia, 1996). However, the trend pattern of NH_4^+concentration in all tanks seems to be stable at the end of experiment. While NO_2^- concentration in both BP and CP+BP treatments seems to be relative stable, resulting from high rate of nitrification processes.

For the survival rate of crablets, results showed that the highest survival rate (30%) was when the crablets were fed with mixed commercial pellet and biofloc pellet (CP+BP) (Figure 1). Nghia *et al.,* (2007) and Baylon (2009) considered *Artemia* as the best first food for early *Scylla* sp. larvae and early crablet stage due to their small size, rapid reproduction and that it contained preferred nutrients. However, the early larval stages of *Scylla* sp. are passive predators, hence the fast-moving prey including *Artemia* nauplii are unable to be caught by them (Zeng & Li, 1999; Baylon, 2009; Quy, 2017). As a result, low survival rate due to the limitation in catchability of early zoea resulting in weak larvae and also to the early crablets stage that tend to promote dies (Quy *et al.,* 2017). Syafaat *et al.* (2019) also suggested feeding artemia nauplii with additional of artificial feed in the form of fresh feed formulation for better results of larvae zoea 5, megalopa to the crablets' rearing stages. Therefore, suspension of beneficial biofloc pellet provides better solution for enhancement of alternative feeds for mud crab production.

Conclusion

Our study demonstrated that the highest survival rate of mud crab, *Scylla* sp. is when they were fed with both commercial pellet and biofloc pellet (CP+BP), which successfully reached up to 30% of survival compared to when only CP and BP were used as feed. In addition, the water quality condition was also in the optimum level ranges which provided the most suitable environmental for survival of mud crab crablet culture condition.

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