ISSN 2962-9632

Performa Reproduksi Udang Pisang Domestikasi Non-Ablasi *Fenneropenaeus indicus*

Reproductive Performance of Non-Ablated Domesticated Banana Shrimp *Fenneropenaeus indicus*

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ABSTRACT

The aims of the study were to evaluate the reproductive performance of pond reared broodstock of *Fenneropenaeus indicus* without prior ablation for shrimp maturity. These technique being reported impair physiological process and finally offspring quality Pre-maturation was with (BFT) and without biofloc technology (non-BFT) system assessed during the trials. First trial, amount of 10 months old broodstock were acclimatized during four days before mating, while the second trial a total of 190 pairs shrimp at 11 months olds were culture separately (females and males) during four weeks and reared into biofloc system prior maturation performed. Molasse was used as carbon sources to maintain C/N ratio of 15:1. All maturation processed conducted using rectangular cement tanks at 10L and 14D condition. Shrimp fed on fresh diets consists of squid, oyster and live *Nereis sp* ca. 25% body weight (1:1:1). Daily water exchange rate of 100% in the morning and afternoon, respectively. It was found that the latency period from the trials (6-7 days) within the ranged of common intervals for *F. indicus*. However, daily spawners, nauplii production and postlarvae survival shows any significant differences ($p<0,05$) were 8.0% vs 11.4%; 33.850 vs 51.246 N spawner$^-$ and 30.7% vs 23.26%, respectively. Results finding shown that domesticated *F. indicus* can successfully produced healthy post larvae without prior broodstocks ablation. Study on BFT during pre-maturation of these species firstly report and there is the need for further research in terms of pre-maturation period, nutritional status of the broodstock, and compensatory growth in ponds.

Keywords : *Fenneropenaeus indicus*, non-ablated, reproductive performance, biofloc.

Introduction

Indonesia has a variety of shrimp species so that it is possible to diversify aquaculture commodities. These effort will further have an impact on national shrimp production and income generations as well. Diversification of a species is driven by many factors such as market and consumer needs, the desire to increase species resilience to climate changed, profit and competitive advantage (1). Amongst shrimps species available indigenously, *Fenneropenaeus. merguiensis* and *F. indicus* have a great potential for further development in Indonesia. In international trade, these two species are known as *banana shrimp* and morphologically almost similar but generally can be distinguished by the higher rostral crest in adult *F. merguiensis* (2). Both species contribute to global aquaculture production besides *Penaeus. vannamei, P. monodon, P. chinensis and P. japonicus* (3). Instead of high economic values species, *F. indicus* primarily has reported more tolerance to environment parameters such as temperature (4) and salinity(5)(6). Another technical advantages were broodstock availability in Indonesian waters, reproductive cycle is relatively short and even eggs can mature in rearing ponds. In 2006, production and global market contribution reached 96,000 tons for *P. merguiensis* and 41,000 tons for *P. Indicus.* Thus, production declined due to disease and farmers shifting to *L. vannamei* and *P. monodon* (3).

Two species of banana shrimp were initially domesticated in ponds since 2018 and 2020 for *F. merguiensis* and *F. indicus*, respectively. Seed production techniques basically adopted black tiger shrimp hatchery that being develop at the Main Centre for

Brackishwater Aquaculture (MCBA), Jepara. Shrimp broodstocks sourced on wild catches and gonadal maturity stimulated by eyestalk ablation. During that period, mass culture and post larvae production were successfully though some improvement needs to take into account.

Ablation technique (eyestalk cutting) is a common practice on maturation and reproduction system of penaeid shrimp (7)(8)(9). However, this method can changed the shrimps physiological process (10), resulting in damage to broodstock, eggs and larvae (11) and poor-quality offspring (12). The challenge for the future is to seek a viable alternative to eyestalk ablation (8)(13)(14) along with increasing consumer awareness on animal welfare and ethics (15). Adjustments to the pre-maturation phase, increasing stocking density and regulating sex ratios during maturation and mating process might be useful strategies (13). The application of gonadotrophic hormones as neurotransmitters were also reported by many authors (9,10,16,17).

Pre-maturation of shrimp broodstock through biofloc systems has been reported by many researchers and showed a positive response on spawning performance and larval survival of shrimp species (18), such as, *Farfantepenaeus duorarum* (19), *Litopenaeus stylirostris* (20)(21), *Farfantepenaeus brasiliensis* (22), and *L. vannamei* (23,24). The aim of this study was to evaluate the reproductive performances of non-ablated of *F. indicus* after pre-maturation with and without biofloc technology system.

Materials and Methods

Broodstock Production

The second generation of shrimp broodstock had been cultured in pond since February 5th, 2021. Initial stocking rate was 35 post larvae m^{-2} and reared under biosecurity conditions. All incoming water previously disinfected with calcium hypo-chloride (25-30 ppm) on treatment pond. Bird scaring and fencing along the dike of ponds were installed and prohibit or limit entrance of personnel except for engage staff only. All ponds used (include reservoir pond) were lined with high density polyethylene (HDPE). Generally, after three months olds the shrimp sex organ can be distinguished clearly, hence further sexing performed. Selected male and female population were culture into separate ponds to protect mating. Stocking density reduced until 2.5 shrimp m^{-2} and continuously to grown until attained suitable size for broodstock. Biweekly growth sampling was carried out for growth monitoring and health status was checked on monthly basis for the detection any pathogen by Polymerase Chain Reaction (PCR) test. Within the pathogen listed are White Spot Syndrome Virus (WSSV), Infectious Hypodermal Hematopoietic Necrosis Virus (IHHNV), Acute Hepatopancreatic Necrosis Disease), Infectious Myonecrosis Virus (IMNV), and Enterocytozoon Hepatopenaei (EHP).

After 10 months age, a number of male and female broodstock harvested by using lift nets to reduced stress. The selected broodstock based on suitable size, external organ and appendages are complete, no deformity sign, and swim actively performed. In order to minimize stress during selection, the shrimps were put into two fiber tanks (250 L capacity) and allow flow-through seawater system by using one inch submersible pump. A 100 watt mini blower was also installed to assure dissolve oxygen above 5 ppm. Transfer of selected broodstocks to the main hatchery unit by using to 50 L plastic drums and injected with pure oxygen.

Experimental Design

Gonadal maturation system during two consecutive trials have been performed without eyestalk ablation. Reproductive performance of the broodstock was observed through two series of experiments, namely: (A) broodstock maturation carried out after shrimp conditioned during four days without biofloc system (Non-BFT); and (B) Pre-maturation of the broodstock with biofloc system (BFT). The last trial conducted by using four units of cement tank (5.6x1.8x0.8 m) located indoor and translucent roof building. Both female and male shrimps were culture separately at stocking rate up to 10 shrimp m^{-2} and it was lasted for four weeks. Animals test fed commercial pellet 38% crude protein at a level of 3% biomass a day and divided into two meals a day. Floc formation was stimulated by adjusted carbon to nitrogen (C/N) ratio around 15:1 (25) by adding sugar cane molasses 2-3 times a week. On the third week culture period, amount of pellet diet reduced up to 75% and feed were substituted with fresh chopped squid and live polychaete, *Nereis* sp. (1:1) ca. 25% biomass per day. Renewal water media as compensation for evaporation and water exchanged is less than 10% week^{-}. Daily water quality monitoring includes salinity (Atago S/Mill-E), pH (Milwaukee pH meter), temperature and dissolved oxygen (YSI 55 OA) were recorded in the morning at 07.30-08.00 am. Floc volume was observed once a week using an imhoff cone. TAN, nitrite (NO_2^-), nitrate (NO_3^-) and TOM (total organic matter) were analyzed at Physics and Chemical Laboratory, MCBA Jepara. once a week.

Maturation and nauplii production

All broodstocks maturation process was carried out on concrete tanks 5x6x0.8m in dimension. Female and male ratio was 1:1 and numbers of shrimp during the first and second trial were 100 pairs and 170 pairs, respectively. Females and males weight of the broodstock selected were 37.35±4.90g and 25.15±2.66g (first trial) and 38.05±4.19g; 27.85±2.16g (second trial), respectively. Light condition in the maturation room was 10h light and 14h dark. Broodstock mainly fed on fresh food and consists of squid (morning), oysters (day time) and *Nereis* sp (late afternoon) up to 25% biomass per day. Total water exchanges was carried out in the morning and evening before feeding.

Gonadal development were observed in the afternoon and usually begun on the third day by using water resist-LED rechargeable headlight. Ready spawn broodstock transferred to fiber tanks (800 L active volume) into separate adjacent room. For spawning and hatching purpose, water media was filtered again using a serial cartridge filter and added 10 ppm EDTA. Spawning normally occurs at night and all the broodstock are returned it back into maturation tank at 04.00-05.00 in the morning. To optimized eggs hatching into nauplii stage, water temperature within the range of 30±1 ^{o}C and equipped with 100 watt automatic heater and lamp for illumination. Reproductive performance indicated by observing parameters such as: latency period, number of daily spawners, nauplii production and average nauplii production per spawner and post larvae survival. Nauplii were harvested by the next day based on their photo taxis response, then washed with pre-sterilized seawater. The number of nauplii was calculated by taking a triplicate samples using a 4 mL cap to determine the number of nauplii per mL.

Larviculture

Larviculture is the next important step for seed production in a shrimp hatchery. Provision of adequate and high quality seawater have important role during production cycles. Seawater pumped from the sea and went through a physical filtration (sand filter and pressure sand filter), sterilized with ca-hypochloride 25-30 ppm and then neutralized with thiosulfate solution. Addition of EDTA (*ethyl diamine tetraacetic acid*) as a chelating agent before used at a concentration of 10 ppm. Larvae tanks previously fill-up pre-treated seawater 1-2 days before nauplii stocked at a level of 70 cm water depth. Water temperature (30-32 ^{o}C) and and salinity (29-30 ppt) were adjusted and more preferred for the larvae. Nauplii were stocked at a density up to 100 nauplii L^{-} and shortly after metamorphosed into zoea stage, immediately fed *Skeletonema costatum* (20,000-30,000 cells mL^{-}). These live foods are maintained till late of mysis stage. Artificial feed was given in the form of a micro encapsulated diet (FRIPPAK) according to the larvae stages and it was ranged of 0.75 – 2 ppm and fed six times a day. *Artemia* nauplii was given at the earlier post larvae stage until harvesting at a concentration of 0.5- 2 nauplii mL^{-}. Water salinity is reduced around 2 ppt before metamorphosed into post larval and water depth increased to 100 cm. Daily larvae monitoring by observing feeding response, larva stages, and water condition in general. Water quality monitoring is carried out on daily (temperature, salinity, pH and oxygen solubility) and weekly (TAN, Nitrite-NO_2^-, Nitrate-NO_3^-, total bacteria and *Vibrio* concentration) basis. Survival was determined during harvest, *i.e.* calculating the percentage of seeds produced by the number of nauplii stocked. A number of Pl samples were taken during harvest for individual length and weight measurement.

Statistical analysis

IBM SPPS Statistics software was used (version 25.0) for statistical analysis. Spawning performances and larval survival were compared between BFT and non BFT treated broodstock by one-way analysis of variance. The level of statistical significance was $p<0.05$. The results are presented as mean ± SD.

Results and Discussion

Reproductive performance

In general, current experiment found that maturation, nauplii and seed production of domesticated *F. indicus* can be carried out and successfully without eyestalk ablation. Spawning performances of the broodstock from the two experiments is presented on Table 1. Shrimp weight is considered sufficient and suitable for maturation purpose. The reproduction study conducted by (26), used wild-caught broodstock and individual weight ranging from 20-91g (average 39.01±12.58g). Ablated of *F. indicus* broodstock with a mean weight of 40g was reported (27), even wild broodstock smaller than this size (11.5±3.1g) have been reported(28). Therefore, female weight of the broodstock used corresponded to age (10 and 11 months) was suitable for reproduction. Besides, most male-bearing sperm have been observed and it was another evidenced to pursuing maturation.

Table 1. Reproductive performance of the 2nd generation *F. indicus* with was (BFT) and non-BFT.

Parameters	Non-BFT	BFT
Shrimp weight (g)	37.35±4.90	38.05±4.19
Age (mo)*	10	11
Number of females (pcs)	100	170
Latency period (d)	7	6
Average daily spawner (pcs)	7.86	19.39
Percentage daily spawner (% d-)	8.0±2.9[a]	11.4±4.1[a]
Nauplii production (N spawner-)	51.246±8581[a]	33.832±9646[b]

*age was calculated after stocked in hatchery. Value (mean±SD) followed by different superscript within the same row denote significantly difference (p<0.05).

Latency period was observed on the sixth and seventh days and differ compared to ablated one that occur normally on forth day. (26), demonstrated that latency period of *F. indicus* from various wild catches in India waters reached 7-10 days. Hence, latency period observed within this experiment is considered normal primarily for domesticated broodstock. For *F. duorarum* species, the latency period had been reported and reaches 11 and 17 days for wild and domesticated broodstock, respectively (19). Daily spawner did not show any significant different ($p>0.05$) but conversely did on average nauplii production (Figure 1)..

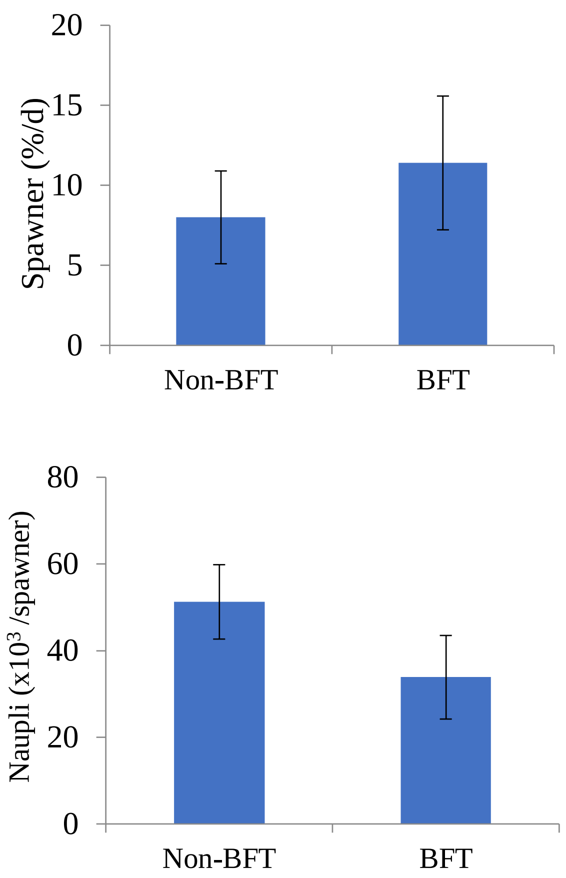

Figure 1. Daily spawner (% d-) and average nauplii production per spawner on pre-maturation experiment was with BFT and non-BFT.

Floc volume during pre-maturation ranged of 0.6-7 mL L^{-} (female's tanks) and 0.8 – 12 mL L^{-} (male's tanks). Shrimp survival at the end of pre-maturation was 98.5-100% indicated that shrimp broodstock can be cultured under biofloc conditions. Any mortality detected presumably due to cannibalism concurrent with the molting period. Molting mostly observed by the end of the pre-maturation period and it was counted of 22 and 13 for females and males, respectively.

The uses of BFT on *F. indicus* have been reported at various culture systems (29) (30) (31), and all those information proves that these species can utilize nutrients such as nitrogen from biofloc to support growth and immune parameters. Current results finding firstly confirmed regarding pre-maturation of *F. indicus* under biofloc technology system. Some improvement needs to take into account and bringing this technology more reliable primarily on non-ablated female shrimp. Non-ablated shrimp allow to prolong and efficient used of resources and more concerns on animal welfare in particular.

The combination of a biofloc system and fresh food during pre-maturation contributes to broodstock reproduction performance, such as number of daily spawner and seed survival rate. However, BFT treated broodstock shown lower nauplii production and significantly different ($p<0.05$) compared to non-BFT. Shrimps reared in biofloc performed healthier, more active and clean in appearances. However, there was an identified problems being reported for shrimp size above 39g were reduction in weight and condition factor (32). Current finding is expected to be a long run solution primarily on *F. indicus*. Study on *L. stylirostris* shown that shrimp broodstock reared with biofloc technology (BFT) compared to clear water (CW) exhibited a higher health status with a better spawning performance and larval survival (21). These finding were also demonstrated and confirmed on *P. vannamei* (24). This mechanism occurs through improving the nutritional status of shrimp in stimulating ovarian maturity and nutrient transfer to eggs (21). Unfortunately, there was no data available yet related to nutritional content within this experiment.

In trial-1 (Non-BFT) it was found that the number of daily spawners tends to decrease since the beginning of spawning period and start to increase after the second week (Figure 2). This may be explained and related to production process in ponds that relying on pelleted diets. Fresh food substitution such as squid and *Nereis* were relatively small quantity during the last few months and may not sufficient to support yolk development and pre –feeding larval (33).

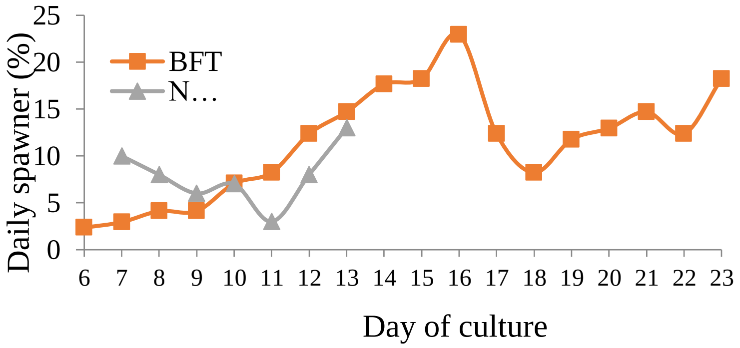

Figure 2. Latency periods and percentages daily spawner of *F. indicus* after pre-maturation with was BFT and non-BFT.

Lipid compounds are the main nutrients involved during the maturation process and are stored in oocytes to enriched subsequent larval development (34). Experiment on *F. merguiensis* during six weeks showed that feeding squid was more desirable for sperm quality produced (35). Polychaete worms such as *Nereis* sp. can improve the reproductive system of shrimp because of the arachidonic content (36,37). Squid is not only rich in cholesterol but also contains a lot of steroid hormones that play a role important on vitellogenesis process (33).

Average nauplii productions showed any significant difference (*p<0.05*) where BFT treated broodstock produced of 33,850 nauplii spawner^{-} and lower than non-BFT broodstock of 51,246 nauplii spawner^{-}. The difference on broodstock ages used were suspected affecting nauplii produced (38). Instead of broodstock size and age, sperm quality is an important feature for management of shrimp broodstock(39). Further, they explained that *L. vannamei* males at an age of 12 months have superior sperm quality than younger males. However, there were no studies yet being reported on domesticated of *F. indicus*. Study on pond-reared *P. merguiensis* was reported that peak spawning performance between 10 and 12 months old and suggested to bred within this period for seed production(40). Nutrition may another limiting factor for the current trial (BFT) that used 38% crude protein pellets during pre-maturation. Latest finding demonstrated that diet with 55/12 protein/lipid combination is best recommended for *P. indicus* gonad maturation (41).

Seed production

Instead of spawning performance, observations were made on larval development and seed production. Nauplii metamorphosed into zoea stage generally occur in the afternoon (17.00-20.00). Metamorphose larvae to the next stadia such as mysis and post larvae performed normally, which was taken about 3-4 days. Cannibalism begins to appear in the early post-larvae stage and affects the final survival (Figure 3). Meanwhile larvae prefer occupied at the tank bottom or stick to the wall. Studies over the last two years indicated that larval survival around 25% and its lower compared to *F. merguiensis* was 35% or more. (26), reported that low survival of this species due to lack of synchronized molting have been noticed during protozoea to mysis and mysis-3 to post larvae (PL) conversion.

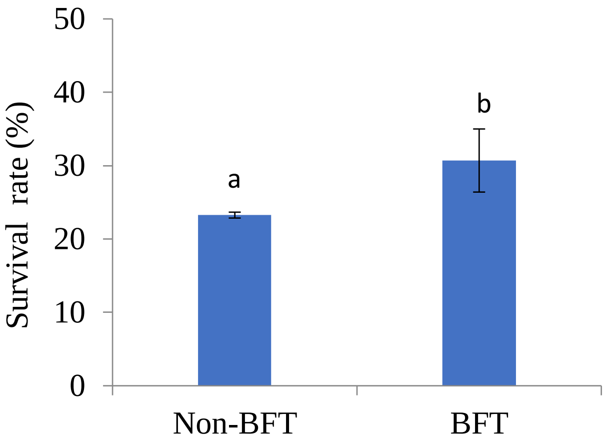

Figure 3. Survival rate (%) of *F. indicus* post larvae from broodstock was with BFT and non-BFT. Error bar followed by difference letter indicated significantly differences (*p<0.05*).

Efforts to increase larval survival have been studied such as the use of probiotic *Bacillus sp* into water media and *Artemia* nauplii (27) and adding the prebiotic inulin through *Artemia* enrichment (42)(43). However, their application into mass culture condition needs to address in the future. Final length and weight (Pl 10-11) from the two experimental series did not show any difference, and it was ranged of 8.0-8.15 mm and 1.9-2.2 mg, respectively. The post larvae produced showed more active, phototaxis, and relatively uniform in size.

Pre-maturation broodstock in BFT produced higher larvae survival (30.70%) and significantly different ($p<0.05$) compared to non-BFT (23.26%) as shown on Figure 3 above. Increasing the nutrients concentration in the hepatopancreas of broodstock reared on biofloc media will affect on ovarian development and nutrient transfer to eggs (19,21),(24). Nutrient sources not only derived from feed (fresh and dry pellets) given but also come from the floc formation as well. The combination of fresh food during pre-maturation with BFT may contribute to nutrient content of the broodstock. Unfortunately, fresh diet substitution within this experiment lasted on short period. Study on *L. vannamei* have been reported, and females that received fresh food for 20 days under biofloc rearing conditioned performed better eggs production, spawned more promptly, and presented higher levels of HUFA in eggs as compared to those that did not received fresh food (44). In male of *P. monodon*, spermatophore weight and total sperms count significantly higher than those pellet-fed groups after fed with polychaete for one month (45).

Water Quality

Water quality parameters at the pre-maturation, maturation and larvae rearing period are presented on Table 2. Measurements of N-compounds (TAN, NO_2-N, NO_3-N) and TOM during maturation period were not recorded since water exchanges were carried out on daily basis. Temperature and salinity are important water parameters and *F. indicus* can adapted well on both parameters. Larvae rearing of *F. indicus* in the tropics can tolerate the temperatures up to 35 ^{o}C (4) and wide salinity range of 5-40 ppt (46). However, best larval development at 20-25 ppt of salinity (47). Strains from certain locations can survive at salinities up to 58 ppt (6). Range of salinity shown any difference between the trials and it was purposely according to pond salinity condition.

Ammonia can be toxic to aquatic animals and cause damage to gills, skin and blood circulation (48). Ammonia-induced stress reduces growth rate, increases molting frequency and osmoregulatory capacity (49). As a major stressor, regular monitoring of ammonia concentrations is very important (50). Ammonia is represented by measuring the concentration of TAN (amount of ammonia-NH_3 and ammonium ions-NH_4^+) and is influenced by pH, temperature and salinity. The TAN value of the pre-maturation period (Experiment-2) tended to increase at the beginning and reached at a level of 4.34 ppm. This probably caused by nitrifying bacteria did not fully developed yet (51). Thus, through regular supply of carbon source (molasses) the TAN value attained zero ppm until the end of the pre-maturation period and followed by an increasing floc volume. During larvae rearing period, TAN values tended to increase till the end of rearing period along with the increase in *Artemia* concentration and the amount of feed given. Final conversion of TAN into ammonia (51) during larvae culture was 0.291 ppm and it was higher than recommended of <0.1 ppm (52).

Table 2. Water quality parameters monitored during pre-maturation, maturation and larvae rearing phases of *F. indicus.*

Water Parameters	Pre-maturation	Maturation	Larvae rearing
Non-BFT (without pre-maturiation)			
Temperature (°C)	-	27.8 - 28.8	28.90 - 31.20
Salinity (ppt)	-	28 - 30	20 - 31
pH	-	7 ,93 - 8.35	8.00 – 8.40
Dissolved oxygen (ppm)	-	4.94 - 5.70	5.16 – 5.91
TAN (ppm)	-	-	0.155-4,932
NO_2-N (ppm)	-	-	0.191-0.783
NO_3-N (ppm)	-	-	0.275-1.156
TOM (ppm)	-	-	82,290-131.540
BFT (with pre-maturation)			
Temperature (°C)	27.2- 29.1	27.2 – 28 ,9	31.1 – 32.7
Salinity (ppt)	26 – 33	28 – 30	28 – 30
pH	7.83 – 8.16	7.94 – 8.05	8.15 – 8.24
Dissolved oxygen (ppm)	5 ,03 – 5.85	4.64 – 5.42	4.23 – 4.52
TAN (ppm)	0.00 - 4.34	-	1.363-3,446
NO_2-N (ppm)	0.03 - 2.92	-	0.190-0.439
NO_3-N (ppm)	0.02 - 2.82	-	0.240-0.649
TOM (ppm)	102 - 301	-	87,350-168,650

General Discussion

The need for domesticated shrimp broodstock is allow taking complete control over the production plan and health-profile of successive filial generations(53). The use of captive reared broodstock may be the most ecological and economical approach to ensure a sustainable shrimp culture industry(53). The choice for *F. indicus* species were discussed previously and it has a great aquaculture potential in Indonesia due to technical and economic aspect of view. Further, the use of native species has many physiological advantages while avoiding many risks related to environmental issues and the introduction of exotic pathogens(26).

Eyestalk ablation commonly practice to reduces the level of gonadal inhibiting hormones (GIH), but this practice has been associated with high broodstock mortalities and poor-quality offspring (12),(17). Current experiments showed that maturation and spawning of *F. indicus* can be carried and successfully produced healthier post larvae without rely on ablation. However, there is the need such improvement mainly during pre-maturation to assure sufficient nutrient for maturation and successful spawning.

Pre-maturation into biofloc system firstly reported on *F. indicus* and it was considered environmentally friendly culture technique. The competitive value of biofloc technology is not only from an economic point of view but also from a biological perspective (18). Pre-maturation with biofloc system will reduced costs, energy and labor due to minimize use of seawater (pumping, sterilization) and improved biosecurity. Manipulating C:N ratio will encourages the uptake of inorganic nitrogen and converted into a microbial protein as an alternative nutrients source and other physiological functions for reared shrimp (25) (54). More studies on pre-maturation and reconditioning after spawning under biofloc culture condition are required. This protocol not only expected to improve the nutrient status of the broodstock, but it could have an impact on prolongs uses of resources.

Conclusions and Suggestions

Maturation, spawning and seed production of *F. indicus* without ablation successfully demonstrated. Higher nauplii production was observed for non-BFT treated broodstock (51,246 nauplii spawner^{-}) and significantly different (*p<0.05*) with BFT treated one (33,850 nauplii spawner^{-}), while post larvae survival was low (23.26% vs 30.70%). There was no significant in term of percentage daily spawner, but BFT-treated broodstock performed slightly increased and it was tended to increase from the beginning spawning period. Pre-maturation on domesticated shrimp was an important phase for nutrient improvement, and biofloc technology offer an alternative technique that can be applied.

Acknowledgments

The authors would like to sincere gratitude to all hatchery and grow-out teams for their dedication and hard work and make the experiment possible. Thanks are also given to all laboratory staff for their assistance to analyze on physic, chemical and biological parameters during the study. Last but not least, our express gratitude to Directorate General of Aquaculture through MCBA Jepara for financial support on this project.

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ISSN 2962-9632