Grow-out of transfection and non transfection black tiger shrimp broodstock, *Penaeus monodon* in concrete pond

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Abstract. Broodstock supply is part of the overall sustainability of tiger shrimp hatchery production. The purpose of this study aimed to evaluate the growth performance and survival rate of broodstock of tiger shrimp transfection and non transfection. This research was conducted at 1000 m² size of two ponds in Experimental Pond Installation, Research Institute for Coastal Aquaculture, in Punaga Village, Takalar Regency, South Sulawesi. The treatment was different kinds of broodstocks, which were: broodstock candidates of tiger shrimp transfection (A) and non transfection, (B). The 67.9 to 78.9 g/ind of broodstock candidates were stocked 0.2 ind.m⁻² and then reared for 90 days. During rearing period, these shrimp were fed using enrichment commercial pelleted feed with content 36-38% of protein in dosage of 10-4%/ body weight. Entering the last month of rearing period is given fresh feed were seaworms and squid (3% biomass weight). Feeding frequency was applied in the morning and in the evening. Measured variables were growth, daily growth rate, survival rate and water quality. The results showed that the average final weight of transfection and non-transfection black tiger shrimp broodstocks was 80.29 and 92.57 g/ind, respectively. Survival rate of tiger shrimp in this study ranged from 71.5 to 81.5%. The range of water quality during the study still supported the growth and survival rate of black tiger shrimp.

1. Introduction

Black tiger shrimp farming, *Penaeus monodon*, is one of the brackishwater aquaculture business activities that has long been developing in Indonesia and has produced significant foreign exchange. Nonetheless, since the 1990s, the farming has experienced many obstacles such as the case of shrimp death, both due to the less supportive water environment and the attack of diseases caused by pathogenic organisms such as viruses, bacteria, parasites and fungi [1]. Prevention of black tiger shrimp disease through the management of shrimp farming waste using reservoirs and biofilter, water recirculation systems and probiotic applications continues to be developed [2]–[7], but the results have not been stable and various cases of shrimp disease in ponds still occur frequently. Efforts to increase shrimp resistance to pathogen attacks are an alternative solution of disease problems in aquaculture. Transgenesis technology, especially genetic engineering to produce black tiger shrimps that are resistant to diseases, is one of the strategies that can be done in an effort to solve the disease problem that afflicts black tiger shrimp farming. Transgenic technology especially the transfer of antiviral genes in black tiger shrimp has been successfully carried out through transfection techniques. The
modification transgenic black tiger shrimp strains can improve the character traits such as increasing the rate of growth and survival of the shrimp against extreme environments and diseases [8].

Some previous studies on transfection black tiger shrimp have been reported by [9], about the characteristics of cDNA sequences coding for antiviral genes from black tiger shrimp. [10] regarding the expression analysis of the pmAV antiviral gene on black tiger shrimp, and the increasing resistance of black tiger shrimps to WSSV through the pmAV gene transfer [11]. The challenge test for black tiger shrimp, transgenic using Vibrio harveyi pathogenic bacteria [12]. Study on the performance of larvae of transgenic and non-transgenic black tiger shrimp after vitality test and morphological test [13]. Domestication of transgenic black tiger shrimp in fiber tank and ponds has been initiated by the Research Institute for Coastal Aquaculture. The black tiger shrimp morphologically weighed ≥ 100 g/ind for females and reached ≥70 g/ind for males for 13 months in the pond [14]. Research on the growth performance, size distribution and survival rate of transgenic black tiger shrimp of F0 and F1 generation [15]. Research about the reproductive performance of transgenic black tiger shrimp after artificial insemination using different spermatophore sources [16].

Domestication of the black tiger shrimp broodstock has been reported in several previous studies, including the development of the domestication technique of SPF broodstock in pond [17]–[23], and in a controlled tank [16], [23]–[30]. The use of shrimp broodstock which is farmed in ponds as an alternative to the use of wild broodstock can help the industry or hatchery in producing pathogenic free larvae and is one of the most important strategies for the success of shrimp farming. Some factors have a large impact on the quality and quantity of eggs such as broodstock age or size, origin, type of endocrine manipulation, genetic variation and broodstock feed [31]. To produce qualified larvae in the hatchery of black tiger shrimp, a good reproductive performance of a shrimp is obtained when the shrimp broodstock is fed with a mixture of fresh feed and formulation feed that has been mixed with various nutrients [32]. Therefore, a research to grow-out the candidates of transfection and non-transfection black tiger shrimp broodstock in a concrete pond is conducted. The purpose of this study is to evaluate the growth performance and survival rate of transfection and non-transfection black tiger shrimp broodstock in concrete pond.

2. Materials and methods
2.1. Location.
The study was carried out at the Experimental Pond Installation, Research Institute for Coastal Aquaculture in the Punaga Village, Takalar Regency, South Sulawesi, using 2 plots of 1.000 m² ponds. Pond construction was made of concrete with an exhaust system of central drain. Each plot was equipped with a 1 paddle wheel (1 HP).

2.2. Pond preparation and treatment experimental
The preparation of water uses a reservoir as many as 1 that has a function as a sedimentation of sea water. Pest eradication used chlorine 5-10 mg/L which was evenly distributed throughout the surface of the pond. The filling of water in the pond was carried out gradually until the depth reached 1-1.2 m. Growing the natural feedwas by applying urea fertilizer at 200 kg/ha and SP 36 at 100 kg/ha. The treatment applied in this experiment was the cultivation of transfection black tiger shrimp broodstock (A) and non-transfection black tiger shrimp broodstock as control (B). The stocking density used was 0.2 ind/m² or 200 shrimps/1,000 m² with initial weights ranging from 67.9-78.9 g/ind.

During the cultivation, the shrimps were given the commercial feed with protein content of 36-38% which has been enriched with various nutrient supplements. The ingredients for feed Enrichment used: 25 kg shrimp feed, fresh trash fish (14.2% dry) of 20 kg, 0.05 kg Vitamin C coated (0.2%), Carophyll pink (0.05 %) of 0.015 kg, Wheat flour (14%) of 3.5 kg and olive oil (0.5%) of 0.125 kg. Feed doses were given at 5% at the start of stocking and decreased by 3% in the last month of cultivation. Entering the last month of cultivation, it was given fresh food in the form of sea worms and squid [33], which was given 3% biomass weight. Frequency to give the commercial feed that has been enriched and fresh feed was done twice a day; morning and evening. The water was changed every 2 weeks as
much as 10-15%. In this study, RICA probiotics were also applied at a dose of 0.5-1 mg/L/week as a standard procedure to prevent the onset of disease [6]. Rearing periods of black tiger shrimps lasted for 90 days.

2.3. Observed variables
The variables observed were the weight growth of the black tiger shrimp including initial weight, final weight, absolute weight and daily weight growth rate. The weight was measured using an electric scale with an accuracy of 0.01 g. The number of samples of black tiger shrimp was 10-15 shrimps that were carried out every 2 weeks. The survival rate of the black tiger shrimp was observed at the end of the study by considering one by one all of the shrimp that lived at the end of the study. The observations of water quality variables (temperature and dissolved oxygen (DO meter), salinity (hand refractometer), pH (pH meter), were measured in situ that were carried out once a week, while parameters for ammonia (spectrophotometric techniques, indophenol methods), nitrite (spectrophotometric techniques, sulfanilamide method), nitrate (spectrophotometric technique, cadmium reduction method), alkalinity (titration method with sulfuric acid), phosphate (spectrophotometric technique, ascorbic acid method), TOM (permanganate technique, titration method), and TSS (gravimetric method) were observed every two weeks.

2.4. Analysis of data
The data on the growth, survival rate of the black tiger shrimp and water quality obtained was tabulated and analyzed descriptively.

3. Results and discussion
3.1. The growth of black tiger shrimp
The results of observations on the growth of prospective black tiger shrimps broodstock for 90 days of cultivation were varied and increased along with the increasing time of the cultivation for all of the treatments (Figure 1). The average final weight of the prospective transfection black tiger shrimp broodstock was 80.29 g/ind with a weight gain of 12.39 g/ind (18.25%), while the average final weight of the non-transfection black tiger shrimp broodstock (control) was 92.57 g/ind with a weight gain of 13.67 g/ind (17.36%). The final weight of the prospective black tiger shrimp broodstock obtained in this study was lower than some previous studies because of the differences in the size of the experimental animal used. The average final weight of the candidates of transfection black tiger shrimp broodstock, F₀ of 76.74 g/ind with the weight gain of 54.11 g/ind and prospective giant tiger shrimp broodstock transfection, F₁ of 73.27 g/ind with the weight gain of 44.69 g/ind [15]. The average final weight of the candidate of black tiger shrimp broodstock of 68 g/ind with the weight gain of black tiger shrimp broodstock of 33 g/ind (95%) after 220 days (about 7.3 months) [22]. The prospective female black tiger shrimp broodstock gained 98.5% and prospective male broodstock gained 98.13% for 120 days of cultivation in the pond [34]. The final weight of female black tiger shrimp ranging from 79.7-87.8 g/ind with the weight gain ranging from 35.8-46.4 (81.55-112.08%) and males ranging from 65.5-66.0 g/ind with the weight gain ranging from 32.3-33.3 (97.29-101.83%) in the recirculation tank for 165 days of cultivation [30].

Based on the figure 1, it could be seen that the growth pattern of black tiger shrimp for 90 days of cultivation showed a pattern that was relatively the same for both of transfection and non-transfection shrimps. This growth pattern showed linear growth with an average final weight ranging from 80.29 to 92.57 g/ind (Table 2). Meanwhile, the average final weight of the prospective black tiger shrimp broodstock transfection on the generation of F₀ and F₁ of 76.74 g/ind dan 73.27 g/ind, respectively [15]. The growth of penaeid shrimp in the nature was very fast in the first six to nine months after hatching and then reached a stagnant phase [35]. The female black tiger shrimps that were kept in a pond for 6 months had a weight between 48-50 g, while the male weighed about 35-38 g [19]. The first time of the gonad of female black tiger shrimp to be mature was at a weight of 70 g, but the egg quality was not really good and the number was small [36]. Cultivation time of 450 days, it could
obtain female broodstock ranging from 100-140 g/ind and male broodstock ranging from 80-100 g/ind which was cultivated in low salinity ponds of 8-12 ppt [18]. The size of a larger female shrimp was expected to produce more eggs per spawning. There was a relationship between the number of eggs per spawning and the number of spawning with the size of female black tiger shrimp [37]. The maturity level of shrimp was strongly influenced by the age and weight of the shrimp. Age and weight of male shrimps were positively correlated with spermatophore weight and sperm count. The age and weight of the shrimp broodstock were known to have a significant influence on sperm quality [38–40]. The were categorized as in the pre-maturation phase were weighted between 55-80 g [28]. Black tiger shrimp broodstock could be obtained from the cultivation in ponds or from catching in natural environment in mangrove areas or coastal areas. The minimum weight of female black tiger shrimp broodstock ranged from 80-150 g (7-12 shrimp/kg) and 50-120 g (8-20 shrimps/kg) for males. To produce a candidate broodstock, it took a long time of 8-10 months or 10-12 months from the spawning phase [25].

The growth rate of tiger shrimp obtained in this study was in the range of 0.13-0.15 g/day (Table 1). The daily growth rate obtained in this study was lower than the previous studies. The growth rate of black tiger shrimp from pond at 0.33 g/day [41]. Growth rate of prospective black tiger shrimp broodstock that was fast (using fast-growing DNA markers) reaching 0.50-0.52 g/day for females and 0.31-0.35 g/day for male broodstock [23]. Daily growth rate from prospective transfection black tiger shrimp broodstock of F0dan F1 of 0.42±0.04 g/day and 0.35±0.36 g/day respectively [15]. Growth rates of the black tiger shrimps ranging from 0.23 to 0.28 g/day which was cultivated at different densities for 63 days [42]. The daily weight growth rate of female black tiger shrimp ranging from 0.26 to 0.37 g/day and the daily weight growth rate of male black tiger shrimp ranging from 0.30 to 0.39 g/day [30]. The growth rate of the black tiger shrimps ranging from 0.19-0.35 g/day with an average of 0.26 g/day which was cultivated for 116-139 days [18]. The differences in the growth rate obtained in this study compared with the previous research were caused by the differences in the initial weights of the experimental animals used, the long time of cultivation, the cultivation container, the type and dosage of feed applied, stocking density used, environmental conditions and so on.

Figure 1. Growth pattern of transfection and non-transfection black tiger shrimp broodstock (Penaeus monodon) during 90 days rearing period
Table 1. Growth performance and survival rate of transfection and non-transfection black tiger shrimp broodstock during 90 days rearing period

| Variables                  | Black tiger shrimp broodstock |
|----------------------------|-------------------------------|
|                            | Transfection (A) | Non transfection (B) |
| Stocking density (shrimp/1000 m²) | 200 | 200 |
| Rearing period (day)       | 90 | 90 |
| Initial weight (g)         | 67.9 | 78.9 |
| Final weight (g)           | 80.29 | 92.57 |
| Absolute weight (g)        | 12.39 | 13.67 |
| DGR (g/day)                | 0.13 | 0.15 |
| Survival rate (%)          | 81.5 | 71.5 |
| Performa Index (PI)        | 10.60 | 10.73 |
| Produktivity (kg/ha)       | 130.87 | 132.39 |

3.2. The survival rate of the black tiger shrimp

The survival rate of the prospective transfection black tiger shrimp broodstock obtained was 81.50% higher than the prospective non-transfection black tiger shrimp broodstock obtained of 71.50% (Figure 2). The death found in all treatments was caused by environmental changes. Environmental factors affected the survival rate of the black tiger shrimps, such as the total organic matter content of pond water that was high during the cultivation (47.55-56.93 mg/L). The total organic matter content of pond water that was high triggered the proliferation of Vibrio spp also allowed viruses (especially WSSV) to attack weak shrimp due to various stressors [43]. The causes of stress on shrimp that would result in the onset of disease were the environmental conditions that were decreasing [44]. One of the causes of death of the black tiger shrimps in the pond was that generally they experienced unsuccessful molting in the form of imperfect carapace changes and the carapace of shrimp did not undergo hardening. The unsuccessful molting was thought to be closely related to water quality conditions during the cultivation of the black tiger shrimps [22]. The low survival rate of the black tiger shrimps was influenced by cannibalism factors and environmental factors, especially water quality, which contributed to the survival rate of the black tiger shrimp [30].

Figure 2. Survival rate of transfection and non-transfection black tiger shrimp broodstock (Penaeus monodon) during 90 days rearing period

The range of survival rate value of prospective black tiger shrimp broodstock obtained in this study was still better than the survival rate of prospective non-transgenic black tiger shrimp broodstock reported [45], who obtained a survival rate of non-transgenic black tiger shrimp ranging from 9.7-21.0%. Cultivated the black tiger shrimps from several families in the controlled tanks which obtained
survival rates of male shrimp ranging from 23.2-53.6% and female shrimp ranging from 20.8-45% for more than 14 months of cultivation [26]. The survival rate of the candidate black tiger shrimp broodstock growing fast by 10-30% in advanced cultivation from size of 20-30 g/ind to prospective broodstock size of >100 g/female and >70 g/male [46]. Survival rate of the black tiger shrimps from the pre-maturation phase ponds which were given different feed combinations ranging from 15.7-25.0% which were cultivated for 90 days [22]. Survival rate of transfection black tiger shrimp of 34-49% for 81 days of cultivation by feeding different protein content (30%, 40% and 50%) in a controlled tank [14]. Survival rates of prospective black tiger shrimp with a density of 1 shrimp/m² for 4 months ranging from 10.65-20.90% for females and 13.25-14.92% for males [34]. Survival rates of the black tiger shrimp ranged from 70.93-74.0% which was cultivated at different densities for 63 days of cultivation [42]. The survival rate of the black tiger shrimps from PL-15 to sizes >100 g/ind ranging from 25-40% [22]. Survival rate of the black tiger shrimps broodstock ranged from 64.0-88.7% which was cultivated for 108 days [47]. The survival rate of the transfection black tiger shrimp broodstock in the generation of F₀ and F₁ by 73.35% and 51.7% respectively which were cultivated for 128 days of cultivation in the pond [15]. Survival rate of the black tiger shrimps with different ratios of females and males in the recirculation tank, that was survival rate of male shrimp of 33.3-58.3% and females of 37.3-50.0% [30].

The high survival rate of prospective transfection black tiger shrimp broodstock compared to the non-transgenic shrimp was caused by the PmAV gene given that could form nonspecific defenses in shrimp. PmAV gene played an active role in responding WSSV virus infection which was useful in controlling diseases from a virus in a shrimp [10], [12]. The introduction of the TSV-CP gene, transgenic whiteleg shrimp showed a significant higher survival rate compared to normal (non-transgenic) shrimp [48].

3.3. Distribution of size and sex of black tiger shrimp broodstock

The distribution of transfection and non-transfection black tiger shrimp broodstock after being rearing for 90 days (Figure 3). The distribution of transfection shrimp broodstock is dominated by shrimp weighed <80 g/ind (58%), medium-sized shrimp weighed 81-90 g/ind (21%), and big-sized shrimp weighed >90 g/ind (21%). Meanwhile, non-transfection shrimp is dominated by shrimp weighed >90 g/ind (42.74%), shrimp weighed <80 g/ind (30.77%), and medium-sized shrimp weighed 81-90 g/ind (26.50%). Based on the distribution of sex, it can be seen that each of male and female broodstock get the same treatment (Figure 4). On transfection shrimp, the distribution of shrimp broodstock consists of 55.21% of male broodstock and 44.79% of female broodstock. Meanwhile, on non-transfection shrimp, the distribution of sex consists of 59.85% male broodstock and 40.14% of female broodstock.
During the cultivation period of the candidate of black tiger shrimp broodstock, it could be seen that female shrimps grew faster and were larger in size than the male shrimps. The female shrimps generally showed a faster growth rate than the male shrimps. The differences in growth of female and male shrimps was thought to be caused by the amount or rate of protein synthesis in females that were faster or there were certain genes that might be related to the production of hormones and enzymes possessed by females so that it was accelerating the growth. It was further stated that the RNA/DNA ratio of female black tiger shrimp was higher (4.96) compared to male (2.93) [12]. This was in line with the results of the research conducted [15], [49], [50], who stated that female shrimp grew faster than the male shrimp so that at the same age, female shrimps were larger than males. Some crustacean species exhibited that sexually dimorphic growth in female shrimp usually grew faster and reached a larger size than male shrimp in the same age [21], [51].

3.4. Water quality
Water quality played an important role as life and growth support of transfection and non-transfection black tiger shrimp in the ponds. The range of pond water quality variables during cultivation of the black tiger shrimp broodstock is presented in Table 2.

Table 2. Range of water quality variable’s values measured during 90 days rearing period

| Variables          | Treatment | Range value of water quality | Optimal range | References |
|--------------------|-----------|------------------------------|---------------|------------|
| Temperature (°C)   | A         | 27.9-30.70                   | 29.0-30.0     | [52]       |
|                    | B         | 27.7-30.90                   |               |            |
| Salinity (g/L)     | A         | 13.58-34.87                  | 10-35         | [53]       |
|                    | B         | 12.64-34.49                  |               |            |
| DO (mg/L)          | A         | 3.21-8.07                    | 4-7           | [54]       |
|                    | B         | 3.71-8.03                    |               |            |
| pH                 | A         | 8.00-8.86                    | 6.8-8.7       | [55]       |
|                    | B         | 8.01-8.78                    |               |            |
| Ammonia (mg/L)     | A         | 0.00627-0.0902               | 0.32-0.71     | [56]       |
|                    | B         | 0.1832-0.2632                |               |            |
| Nitrite (mg/L)     | A         | 0.0063-0.0304                | 0.01-0.05     | [57]       |
|                    | B         | 0.0036-0.0084                |               |            |
| Nitrate (mg/L)     | A         | 0.0215-0.0798                | 0.1 – 4.5     | [58]       |
|                    | B         | 0.0831-0.1438                |               |            |
| Alkalinity (mg/L)  | A         | 108.54-116.58                | 80-140        | [26]       |
|                    | B         | 104.52-108.54                |               |            |
| Phosphate (mg/L)   | A         | 0.0649-0.1647                | 1.0 ± 0.0     | [59]       |
|                    | B         | 0.0021-0.0419                |               |            |
| TOM (mg/L)         | A         | 47.55-50.06                  | <20           | [43]       |
|                    | B         | 48.8-56.93                   |               |            |
| TSS (mg/L)         | A         | 11-16                         | <40           | [53], [54] |
|                    | B         | 11-18                         |               |            |

Notes: A = transfection and B = non-transfection

The results of the temperature measurements in the two treatment pond were relatively the same, where the temperature ranged from 27.7-30.9 °C. The obtained water temperature was still relatively feasible to support the growth and survival rate of tiger shrimp in ponds. The value of the range of...
water quality in ripening containers of black tiger shrimp was the temperature of 27.0-28.8°C, pH of 7.8-8.2, dissolved oxygen of 5-6 mg/L, salinity of 28-33 ppt, ammonia of 0-0.5 mg/L and nitrate of 0-0.3 mg/L [37]. The results of monitoring the water quality during gonads maturation of the transgenic giant tiger shrimp covering temperatures of 28.2-29.8°C, pH of 7.8-7.9, dissolved oxygen of 4.5-5.5 mg/L and salinity of 34.2-34.8 ppt [16]. Temperatures that were common for tropical species that provide optimal growth were ranging from 29-30 °C [52]. The environmental parameters that supported the optimal growth of the black tiger shrimps were the temperature of 25-32 °C, dissolved oxygen of 4.8-8.9, salinity of 25-35 ppt, pH of 7.8-8.6 [47].

Salinity measurement results in both treatments ranged from 12.64-34.87 ppt. The salinity condition was still normal and could be tolerated by prospective black tiger shrimp broodstock. The optimal salinity for the growth of the black tiger shrimp was 10-35 ppt [53], [54]. The level of pond water salinity during cultivation of the black tiger shrimp ranged from 20-28 ppt [60]. Domestication of SPF black tiger shrimp broodstock in Thailand was carried out at the salinity of 8-12 ppt [18].

The results of the measurements of the dissolved oxygen in the cultivation plot of the prospective broodstock ranged from 3.21-8.07 mg/L. This value was feasible to support the growth and survival of the black tiger shrimp. The optimal water quality requirements for the black tiger shrimp were in a dissolved oxygen of 4-7 mg/L, temperature of 29-32 °C, salinity of 15-25 ppt and pH of 8.0–8.7 [61]. Dissolved oxygen limit for black tiger shrimp was 3-10 mg/L and optimum 4-7 mg/L [54].

pH was one of the important environmental parameters that determined the physiological processes of shrimp. The pH of the pond water was influenced by many factors, including the pH of the water source, the acidity of the subgrade, the input of shrimp farming and the biological activity that occurred in the pond [56]. The results of the pH measurements carried out on the transfection and non-transfectionshrimp ponds plot were ranged from 8.00-8.86. The pH value was still feasible and supported the life of the black tiger shrimp broodstock. The optimum range of pH 6.8 to 8.7 have to be maintained for maximum growth and production of the shrimp [55]. Water with a pH ranging from 7.5-9.0 was generally considered a suitable value for shrimp production [59].

Ammonia measurement results during the cultivation of black tiger shrimps ranged from 0.00627-0.2632 mg/L. This value could still support the growth and survival of the shrimps that were cultivated. The ammonia level in water should not exceed 1.2 mg/L [59]. The ammonia levels in shrimp broodstock culture ponds ranged from 0.02-0.10 mg/L [64]. Total value of ammonia nitrogen in the shrimp rearing media using probiotics and without probiotics was 0.32-0.71 mg/L and 2.1-2.7 mg/L, respectively [56].

The observation results of nitrate level obtained in the two culture ponds of the prospective black tiger shrimp broodstock ranged from 0.0036-0.0304 mg/L. The optimal range of nitrite for shrimp farming was 0.01-0.05 mg/L [54], [57]. The nitrate levels in the giant tiger shrimp broodstock ponds ranged from 0.05-0.10 mg/L [29]. The recommended limit of nitrite (NO₂-N) level for shrimp farming was <0.25 mg/L. However, the nitrate content obtained was about 0.00215-0.1438 mg/L [62]. Nitrate was the main form of nitrogen in natural water area and was indispensable for aquatic growth (algae), very soluble in water and stable. The nitrate content needed for the growth of algae in the waters was 0.2-0.9 mg/L and was optimal in the range of 0.1 – 4.5 mg/L [53].

Alkalinity had a function as a pH buffer, it was very important, especially to withstand the rise and fall and shock of pH. Shrimps have molting problem if alkalinity was below 75 mg/L and shell hardening after molting was inhibited at the low pH [22]. Alkalinity measurement results in both treatments ranged from 104.52-116.58 mg/L. That alkalinity condition was still feasible and supported the life of the black tiger shrimp broodstocks. The alkalinity range that needed to be maintained during the cultivation of black tiger shrimp was 80-140 mg/L. To increase the alkalinity of water during the cultivation of shrimp in the tank could be done by adding sodium bicarbonate regularly [26].

The results of the observation of phosphate level in the cultivation plots of the black tiger shrimp ranged from 0.0021-0.1647 mg/L, while the phosphate concentration was still feasible to support the growth and survival of the black tiger shrimps. The value limit of phosphate level (PO₄-P) suitable for shrimp farming activities was 0.05-0.5 mg/L [62]. The high levels of dissolved inorganic phosphate...
(1.0±0.0 mg/L) during the culture period could cause growth and shrimp harvest periods to decrease [59].

The range of Total Organic Matter (TOM) values obtained during the study ranged from 47.55-56.93 mg/L. The TOM value obtained during this cultivation was quite high and affected the growth and survival rate of the shrimp that were cultivated. The total organic matter should not exceed 20 mg/L. TOM level which exceeded 20 mg/L could trigger the increase of *Vibrio* spp. also allowed viruses (especially WSSV) to attack weak shrimp due to various stressors [43]. The level of the dissolved organic matter of a normal water area was a maximum of 15 mg/L, if the level of the dissolved organic matter was high, it could reduce the dissolved oxygen level in water so that it reduced the survival rate of the shrimp [52]. To suppress the level of TOM, probiotics were used during the cultivation period of prospective black tiger shrimp broodstock in the pond. The use of probiotics was able to improve the pond environment such as improving the value of redox potential of pond sediments, reducing ammonia concentration, total organic matter (TOM) and suppressing *Vibrio* spp population growth in pond water [3].

Total suspended solids (TSS) were solids that did not pass on 20 µm filter paper or did not dissolve in water and only floating. The range of TSS values obtained during the study was 11-18 mg/L. This value was still feasible and supporting the tiger shrimp life. TSS values during the cultivation of giant tiger shrimp broodstock in the range of 10-166 mg/L [45]. The total suspended solids at shrimp stocking were <30 mg/L, water in reservoir/reservoir plots were <20 mg/L, mid and late shrimp rearing were <40 mg/L and waste water were <30 mg/L [60].

4. Conclusions

The growth performance and survival rate of the transfection and non-transfection black tiger shrimp broodstock were relatively the same. The average final weight of the transfection and non-transfection black tiger shrimp broodstock was 80.29 and 92.57 g/ind, respectively. The survival rate of the black tiger shrimp broodstock produced in this study ranged from 71.5-81.5%. The range of water quality during the study still supported the growth and survival rate of the black tiger shrimp broodstock.

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Acknowledgments
The authors would like to thank you to all field technicians, and water quality laboratory analysts (Hamzah, Ilham, Wendy Santiajinata, Sarwono, Eko Apriliyanto, Krisno, Laode Muh Hafizh, Debora Ayu, Kurniah, St. Rohani, and St. Suleha) who have helped during the research.