Growth and survival of transfection and non-transfection tiger shrimp (*Penaeus monodon* Fabricius) broodstock candidate in pond cultivation

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**Abstract.** Tiger shrimp (*Penaeus monodon* Fab.) is one of important commercial species in Indonesia. Low quality of shrimp fry due to the lack of good quality broodstock availability is shrimp cultivation problem that need to be solved. The study was aimed to evaluate growth and survival rate of broodstock candidate of transfection and non-transfection tiger shrimp. The study was conducted using four ponds with 2000 m² in size in Takalar Regency. South Sulawesi. The treatment were A = transfection tiger shrimp broodstock candidate and B = non-transfection tiger shrimp broodstock candidate. Approximately 19.0-21.9 g/ind of shrimp were reared in 60 days in 0.25 individu/m² (500 individu/2000 m²) stocking density. Commercial pelleted feed which content 38% of protein was fed three times a day in 5-3% of body weight dosage. Measured variables were growth performance, size distribution and survival rate. The result showed that shrimp growth were no significantly different (P>0.05), while survival rate and size distribution were significantly different (P<0.05). The dominant size of transfection shrimp was 41-50 g/ind. Whereas, non-transfection shrimp was dominated by 31-40 g/ind in size (48.39%). Survival rate of tiger shrimp broodstock candidate in this study ranged from 30.80 to 64.10%.

1. **Introduction**

Tiger shrimp *Penaeus monodon* is one of the aquaculture commodities. It becomes the most-wanted commodity for Indonesian fishery exports until now. However, Since the issuance of Presidential Regulation No. 39 of 1980 concerning the prohibition of the use of trawl nets caused a decrease in catches. Especially for tiger shrimp commodities. Tiger shrimp culture has been decreasing since early 2000 until now due to disease attack and environmental degradation of coastal waters. Which has not been able to be solved until now? [1]. It is important to do some efforts to improve the tiger shrimp culture as a native species of Indonesia. The problem of decreasing quality of shrimp seeds needs to be overcome so that tiger shrimps do not get disease attacks during the culture process. In addition to the problem of the decreasing seed quality, the provision of broodstock candidates of shrimp from nature that has high quality is currently very rare. In fact, the shrimp broodstock highly determines the quality of shrimp.

In 2014, research on the culture of tiger shrimp broodstock in ponds focuses on improving the management of culture in ponds with a modular and thinning system. However, it has the problem of contamination by the *White Spot Syndrome Virus* (WSSV) in the ponds and such condition is still difficult to overcome. WSSV contamination in ponds is strongly influenced by biosecurity and maintenance management water quality, and feed. Thus, the activities in 2016 focused on the
management of culture in ponds using the selective breeding, both from the sea (F1) and the ponds (F2) that are diseases resistant through transgenesis. Based on the fact above, the provision of broodstock candidates of tiger shrimp selected in the pond becomes the right choice. The culture of shrimp broodstock candidates for the last 4 years in the area of ponds, separated from the community ponds, shows positive gonad growth and spawning. Therefore, aspects that need attention in improving the supply of tiger shrimp broodstock in ponds is the selection of broodstock candidates [2]. It is expected that this activity will be able to produce broodstock of size prematurity (30-60 g/ind).

The provision of broodstock candidates to meet the needs of hatcheries is very important to ensure the sustainability of shrimp seed production. So far, male and female shrimp broodstock still rely on catches in nature, which sometimes cannot be fulfilled since it is seasonal. It is expected that the quality seed production meet the needs of farmers, so the shrimp production from ponds increases. The limitation of good-quality shrimps produced by hatcheries becomes an obstacle faced by farmers so far. In shrimp hatchery, in the context of seed production, if very little eggs or larvae are obtained then the shrimp broodstock should be turned off (unproductive) [3]. Meanwhile, another obstacle often encountered in hatcheries is the supply of mature gonads, but there is only a small number of eggs produced and even the egg hatchability is very low [4]. Thus, due to those obstacles occurred in fulfilling the broodstock candidates of natural origin, broodstock candidates of tiger shrimp from a culture done in the ponds becomes one of the alternatives in the future.

The production of genetically modified tiger shrimp is obtained by transfection seed as an effort to improve the quality of tiger shrimp seed in the ponds, which is a collaboration of BRPBAPP Maros and Bogor Agricultural Institute through PmAV antivirus gene transfection technology [5], [6].

Based on that consideration, a series of studies on the culture of the broodstock candidates of tiger shrimp is carried out by utilizing the results of transfection and non-transfection in ponds that aims to determine the growth performance and survival rate of broodstock candidates of tiger shrimp from transfection and non-transfection. It is expected that the results of this study can be used as information material in the development of broodstock candidates of tiger shrimp farms in the ponds.

2. Materials and methods

2.1. Location.
This research was conducted at Experimental Pond Installation, Research Institute for Coastal Aquaculture and Fisheries Extension in Punaga Village, Takalar Regency, South Sulawesi, Indonesia.

2.2. Experimental design
This research was conducted at 4 ponds with size of 2,000 m² and equipped with a water drainage system using 8 and 10inch paralon pipes. This study compared the performance of growth and the survival rate of broodstock candidates of tiger shrimp from transfection (A) and non-transfection (B), and each treatment was 2 replications. Test animals used were broodstock candidates of tiger shrimp with initial weights ranging from 19.01-21.91 g/ind stocked with a density of 0.25 ind/m² (500 ind/2000 m²). To make the oxygen demand in the ponds remains optimal. Each plot pond installed 1 HP for 1 paddle wheel. During rearing period, shrimp were fed using commercial pelleted feed with content 36-38% of protein in dosage of 3-5% decreased as the increasing weight of shrimp biomass. The frequency of feeding the shrimps was carried out 3 times a day. This study applied liquid RICA probiotics that were first fermented at a dose of 0.5-1.0 mg/L/week as standard procedures to prevent disease [7]. Shrimp culture lasted for 60 days.

2.3. Observed variables
Variables observed during culture are the growth of weight of tiger shrimps using an electric scale that has a precision of 0.1 g as well as the length measured using a ruler with a precision of 1 cm which is done every 2 weeks. Absolute growth and distribution of size and survival rate broodstock candidates were observed at the end of the study [8]. Observations of water quality variables, such as
temperature, salinity, pH, and dissolved oxygen, were measured in situ once a week. Meanwhile, the parameters of ammonia, nitrite, nitrate, alkalinity, phosphate, TSS and BOT were observed every 2 weeks.

2.4. Analysis of data.
The data of growth and survival rate of shrimps was tabulated and analyzed by using T-Test in SPSS program and water quality data were analyzed descriptively.

3. Results and discussion

3.1. Growth of broodstock candidates of tiger shrimps
Based on the observation, growth of the weight of broodstock candidates of tiger shrimps for 60 days of culture vary and increase as the increasing time for both treatments (Figure 1). The average final weight of transfection broodstock candidates of tiger shrimp is 46.72 ± 3.61 g/shrimp with a weight gain of 24.82 g/shrimp (113.28%). Meanwhile, non-transfection broodstock candidates of tiger shrimp is 39.23 ± 1.51 g/shrimp with a weight gain of 20.23 g/shrimp (106.42%). The weight gain obtained in this study is higher than that in some previous studies, such as in a research by [9] which gained 95% of weight after 220 days (about 7.3 months), the weight growth of female broodstock candidates is 98.5% and male broodstock candidates is 98.13% after 120 days of culture in the ponds [10]. However, the results of this study are lower than that reported by [11]. It is stated that tiger shrimps kept in a tub for 8 months gain weight up to 200% when given 70% high protein pellet feed (50-60%) and 30% of fresh feed for 2 times. It is bigger compared to the shrimps fed only once a day with a slightly different feed composition, that is 60% commercial feed and 40% fresh feed. The broodstock candidates’ weight of F0 and F1 are respectively gained by 239% and 156% after 128 days of culture in the pond [12].

![Figure 1](image)

**Figure 1.** Growth of broodstock candidates of tiger shrimps for the each treatment after 60 days of culture

Based on the picture above, it can be seen that the growth pattern of tiger shrimps after 60 days of culture shows a relatively similar pattern of broodstock candidates’ growth both in transfection and non-transfection process. The growth pattern shows linear growth with an average final weight ranging from 39.23-46.72 g/shrimp. The growth pattern in female tiger shrimps kept in pond for 6 months weighs between 48-50 g. Meanwhile, males weigh around 35-38 g [13]. The female tiger
shrimps reach mature gonads at a weight of 70 g, but the quality of the eggs is not good and the numbers are less [14].

The daily growth rate of tiger shrimps obtained in this study is in the range of 0.34-0.42 g/day (Table 1). The daily growth rate obtained in the broodstock candidates of tiger shrimps from transfection is 0.42 ± 0.10 g/day and is not significantly different (p > 0.05) from the non-transfection one, which is 0.34 ± 0.11 g/day. Shrimp growth rate that has been achieved is quite good and included in the category of medium size based on the broodstock standards proposed by [15]. The results of this study are also not different from some previous studies, including a research by [16], who developed domestication of broodstock candidates of tiger shrimps in Jepara that obtained a daily growth rate up to the 6th generation of 0.322 g/day. The growth rate of tiger shrimp cultivated in ponds of 0.33 g/day [17]. The growth rate of fast-growing broodstock candidates of tiger shrimps (using fast-growing DNA markers) reaching 0.50-0.52 g/shrimp for female broodstock and 0.31-0.35 g/day for male broodstock [18]. The growth rate of broodstock candidates of F0 and F1 derivatives respectively of 0.42 g/day and 0.35 g/day after 128 days of culture in the pond [15].

Table 1. Weight gain, daily growth rate and survival rate of transfected and non-transfected tiger shrimp in each treatment for 60 days of rearing.

| Variables                      | Broodstock candidates of tiger shrimps |
|--------------------------------|----------------------------------------|
|                                | Transfection | Non-Transfection |
| Initial weight (g)             | 21.91 ±2.40  | 19.01 ±0.04      |
| Final weight (g)               | 46.72 ±3.61a | 39.23 ±1.51a     |
| Absolute weight (g)            | 24.82 ±6.00a | 20.23 ±1.55a     |
| Daily growth rate (g/day)      | 0.42 ±0.10a  | 0.34±0.11a       |
| Survival rate (%)              | 30.80 ±7.35a | 64.10 ±5.52b     |

Description: Values in the same row followed by the same letter indicate not significantly different (P > 0.05)

The survival rate of transfection broodstock candidates of tiger shrimps obtained in this study is 30.80 ± 7.35% and the non-transfection one is 64.10 ± 5.52% (Table 1). The death found in all treatments is due to extreme water quality factors, especially salinity that could reach 42 ppt in the initial culture phase. Transgenic seeds are the result of physical and physiological gene manipulation where shrimp must adapt to the new environment.

The range of survival rates of broodstock candidates of tiger shrimps obtained in this study (30.80-64.10%) is still better than the survival rates of broodstock candidates of tiger shrimps reported which is 9.7-21% after 120 days of culture [19]. The survival rates for black tiger shrimp broodstock in the ponds is about 29.0 to 37.5% [20]. Grow-out of tiger shrimps from several families in a controlled tub obtain male survival rate of 23.2-53.6%, and the females’ survival rate is ranging from 20.8-45% after more than 14 months of culture [11]. The survival rate of fast-growing broodstock candidates of tiger shrimps is 10-30% in further culture from the size of 20-30 g/shrimp to the size of the broodstock candidates (>100 g/female shrimp and >70 g/male shrimp) [21]. The survival rate of black tiger shrimp from the ponds in the prematuration phase given different feed combinations ranges from 15.7-25% after 90 days of culture [9]. The survival rate of tiger shrimp range 34-49% during 81 days of maintenance by feeding different protein contents (30%, 40%, and 50%) in a controlled tank [22]. The survival rates of broodstock candidates of tiger shrimps with a density of 1 shrimp/m2 for 4 months is 10.65–20.90% for females and 13.25-14.92% for males [10]. The survival rate of broodstock candidates of tiger shrimp obtained in this study is lower than that of [12], finding that the survival rate of transfected broodstock candidates of tiger shrimp is 51.7-73.35%. after 128 days of culture in the pond.
3.2. *Distribution of size of broodstock candidates of tiger shrimps*

The distribution of size of broodstock candidates of tiger shrimp after culture in the ponds for 60 days shows a significant difference (Table 2). The distribution of size of transfection broodstock candidates of tiger shrimp is dominated by size of 41-50 g/shrimp (34.09%), followed by shrimp sized 31-40 g/shrimp (26.30%), shrimps sized 51-60 g/shrimp (15.91%), shrimp sized ≤ 30 g/shrimp (12.01%), and shrimp sized above 60 g/shrimp as many as 11.69 g/shrimp. Whereas, the non-transfection broodstock candidates of tiger shrimp is dominated by the size of 31-40 g/shrimp (48.39%), followed by shrimp sized 41-50 g/shrimp (28.55%), shrimp sized 30 g/shrimp (18.72%), and shrimp sized above 51-60 g/shrimp (4.37%). This shows that the size of broodstock candidates of tiger shrimp from transfection is greater than that from non-transfection.

Table 2. Distribution of tiger shrimp broodstock candidate based on body weight

| Distribution of tiger shrimp broodstock based on weight (g) | Percentage of shrimp size (%) |
|----------------------------------------------------------|-------------------------------|
|                                                          | Transfection | Non Transfection |
| ≤30                                                      | 12.01         | 18.72          |
| 31-40                                                    | 26.30         | 48.36          |
| 41-50                                                    | 34.09         | 28.55          |
| 51-60                                                    | 15.91         | 4.37           |
| 61-70                                                    | 8.12          | 0              |
| 71-80                                                    | 1.95          | 0              |
| ≥81                                                      | 1.62          | 0              |

Based on sex distribution, there is no significant difference (Figure 2). In the broodstock candidates of tiger shrimp from transsection, the sex distribution consists of 55.84% males and 44.16% females, while the non-transsection consists of 51.64% males and 48.36% females. During the culture period, it is seen that female shrimps grow faster and are larger (41.90-46.62 g/shrimp) than male shrimps (36.87-38.88 g/shrimp). Some crustacean species exhibit dimorphic sexual growth in female shrimp, which usually grow faster and reach a larger size than male shrimp of the same age [23], [24].

Figure 2. Distribution of tiger shrimp broodstock candidate based on sex
3.3. Water quality

One of the factors that play a role in determining the success of aquaculture production is the management of water quality. Shrimps are aquatic animals of which life, health, and growth depend on water quality as their medium of life. The range of pond’s water quality variable during the culture of broodstock candidates of tiger shrimp is presented in Table 3. The results of temperature measurement during the treatments in two ponds are relatively the same, where the temperature in the transfection shrimps ranges from 27.04 to 31.45°C and the temperature in the non-transfection shrimps range from 27.44 to 31.97°C. The water temperature is still relatively feasible to support the growth and survival of tiger shrimp in ponds. Temperature that is common for tropical species to obtain optimal growth ranges from 29-30°C [25]. Water temperature affects the shrimp feeding response. where at high temperatures of 32°C and lower than 25°C [26], shrimp appetite drops to 30-50%. The optimal temperature for shrimps’ growth is 26-30°C [27].

Salinity measurement results in both treatments range from 30.55 to 45.53 ppt with an average of 42.54 ppt. The salinity condition is quite high, so it affects the growth and survival of tiger shrimps. Optimal salinity for the growth of tiger shrimps is 10-35 ppt [28]. That at high salinity, the transformation of energy is mostly used for the osmoregulation process rather than for the formation of meat. Thus, shrimp’s growth is slow. The slow growth of broodstock candidates of tiger shrimp probably be caused by the condition of water quality which is quite extreme, especially the salinity during culture, that ranges between 35-52 ppt. Salinity is closely related to the osmotic process and regulation of shrimps’ ions to their environmental fluids. Thus, energy in high salinity conditions will be used for osmotic processes, and not for growth [18].

**Table 3.** Range of water quality variable’s values measured during 60 days rearing period

| Variable          | Treatment | Minimum | Maximum | Average | Standard deviation |
|-------------------|-----------|---------|---------|---------|--------------------|
| Temperature (°C)  | A         | 27.04   | 31.45   | 29.49   | 1.26               |
|                   | B         | 27.44   | 31.97   | 29.56   | 1.35               |
| Salinity (ppt)    | A         | 30.55   | 44.95   | 42.18   | 3.69               |
|                   | B         | 30.61   | 45.53   | 42.88   | 3.88               |
| DO (mg/L)         | A         | 3.03    | 5.74    | 4.40    | 0.82               |
|                   | B         | 2.95    | 6.01    | 4.11    | 0.83               |
| pH                | A         | 7.39    | 9.40    | 8.15    | 0.52               |
|                   | B         | 7.50    | 8.98    | 8.15    | 0.48               |
| Alkalinity (mg/L) | A         | 140.25  | 176.88  | 140.25  | 20.04              |
|                   | B         | 136.18  | 148.74  | 136.18  | 8.71               |
| NH$_3$-N (mg/L)   | A         | 0.0623  | 0.2582  | 0.1408  | 0.0832             |
|                   | B         | 0.0660  | 0.3152  | 0.1596  | 0.0971             |
| NO$_2$-N (mg/L)   | A         | <0.0010 | 0.0210  | 0.0096  | 0.0077             |
|                   | B         | <0.0010 | 0.0069  | 0.0034  | 0.0037             |
| NO$_3$-N (mg/L)   | A         | 0.0053  | 1.2929  | 0.1147  | 0.0901             |
|                   | B         | 0.0066  | 0.1486  | 0.0599  | 0.0564             |
| PO$_4$ (mg/L)     | A         | <0.0021 | 0.4403  | 0.1339  | 0.1400             |
|                   | B         | <0.0021 | 0.0530  | 0.0085  | 0.0179             |
| TOM (mg/L)        | A         | 26.27   | 52.55   | 39.69   | 7.3969             |
|                   | B         | 13.13   | 45.67   | 35.34   | 10.4712            |
| TSS (mg/L)        | A         | 7.0     | 24.0    | 13.67   | 6.1033             |
|                   | B         | 5.0     | 45.0    | 19.88   | 14.6232            |

The results of measurement of dissolved oxygen of transfection shrimps range from 3.03 to 5.74 mg/L with an average of 4.40 ± 0.82 mg/L and non-transfection shrimps range from 2.95 to 6.01 mg/L.
with an average average 4.11 ± 0.83 mg/L. This value is feasible to support the growth and survival of tiger shrimps. That a minimum dissolved oxygen value is 3.9 mg/L and a maximum value is 4.2 mg/L during tiger shrimp culture in ponds [29]. A minimum dissolved oxygen value is 4.5 mg/L and a maximum value is 5.5 mg/L for 140 days of tiger shrimp culture in ponds, that support the growth of tiger shrimps up to 40.2 g/shrimp and survival rate of 85% [30].

The range of water pH values obtained during the study in transfection shrimps is 7.39 - 9.40 with an average of 8.15 and non-transfection shrimps is 7.50 - 8.98 with an average of 8.15. The results of the observations indicate that pH of water for shrimp culture media can still be tolerated by tiger shrimps. pH is one of the vital characteristics of the environment that affects the survival and growth of farmed shrimp, and has an effect on metabolism and physiological process of shrimps. The optimum pH ranging from 6.8 to 7.8 can increase growth and maximum production [31]. pH value is 7.5-8.8 during the tiger shrimp culture in ponds [29]. pH of pond’s water is an indication of fertility or potential productivity. Water with pH ranging from 7.5 to 9.0 is commonly considered suitable for shrimp production [32].

The results of observation on pond’s water alkalinity for transfection shrimps is 140.25-176.88 mg/L, with an average of 140.25 ± 20.04 mg/L while in non-transfection shrimps is 136.18-148.74 mg/L, with an average of 136.18 ± 8.71 mg/L. This value is still optimal enough to support the growth and survival of tiger shrimps. The alkalinity value of pond’s water is used as a pH stabilizer and normal growth of phytoplankton. Alkalinity value of pond’s water for tiger shrimps should be >100 mg/L or in the range of 120-160 mg/L [33]. Standard value in the total alkalinity of pond’s water is ≥ 80 mg/L. If the pond’s water alkalinity is below the standard, improvement can be made by applying lime [32].

The results of ammonia measurement during tiger shrimp culture range from 0.0623 to 0.258 mg/L (0.1408 ± 0.0832) on transfection shrimps and 0.0660-0.3152 mg/L (0.1596 ± 0.0971) on non-transfection shrimps. This value can still support the growth and survival of farmed shrimps. The ammonia content in water should not more than 1.2 mg/L [34]. The total value of ammonia nitrogen in shrimp culture media using probiotics is 0.32-0.71 mg/L [35]. The concentration of ammonia allowed for tiger shrimp culture is less than 0.1 mg/L [26].

The results of observations of nitrite content obtained in transfection shrimps range from <0.0010-0.021010 (0.0096 ± 0.0077) mg/L and in non-transfection shrimps range from <0.0010-0.0069 (0.0034 ± 0.0037) mg/L. The optimal range of nitrite for shrimp culture is 0.01 - 0.05 mg/L [36], the recommended nitrite (NO₂⁻) content limit for shrimp culture is <0.25 mg/L [37]. Nitrite content obtained in the culture of broodstock candidates of tiger shrimp is about 0.0010-0.6095 (0.0514 ± 0.1422) mg/L [12].

Nitrate content is around 0.0053-1.2929 (0.1147 ± 0.0901) mg/L in transfection shrimps and 0.0066-0.1486 (0.0599 ± 0.0564) mg/L in non-transfection shrimps. This value is sufficient to support natural food that grows in shrimp culture media. The nitrate content needed for algae’s growth in the water is 0.2-0.9 mg/L and is optimal in the range of 0.1-4.5 mg/L [38]. The optimal nitrate concentrations for shrimp is about 0.4-0.8 mg/L [39].

The results of observation show that phosphate content in transfection shrimps is about <0.0021-0.4403 mg/L with an average of 0.1339 ± 0.1400 mg/L. Meanwhile, phosphate content in non-transfection shrimps is <0.0021-0.05530 mg/L with an average of 0.0085 ± 0.0179 mg/L. Phosphate concentration during the study is classified as fertility level that is able to support the growth and survival of tiger shrimp. The limit value of phosphate content (PO₄-P) suitable for shrimp culture is 0.05-0.5 mg/L [37].

The range of total organic matter (TOM) values obtained during the study is 26.27 - 52.55 mg/L in transfection shrimps and 13.13-45.67 mg/L in non-transfection shrimps. TOM value obtained during this shrimp culture is quite high and affects the growth and survival of the farmed shrimps. The TOM should not exceed 20 mg/L. TOM content that exceeds 20 mg/L besides can trigger the proliferation of Vibrio spp. also enables the virus (especially WSSV) to attack shrimps that are weak due to various stressors [40].
The results of observations show that TSS content in transfection shrimps is 7.0-24 mg/L, with an average of 13.67 ± 6.1033 mg/L. Meanwhile, in TSS content in non-transfection shrimps is 5.0-45.0 mg/L with an average of 19.88 ± 14.6232 mg/L. TSS concentration during the study is relatively low and able to support the growth and survival of tiger shrimp. The optimal TSS value in intensive shrimp ponds is <40 mg/L. Thus, the TSS concentration obtained is still classified as abundant for shrimp growth and survival in ponds [41].

4. Conclusions
The performance of the growth of transfection broodstock candidates of tiger shrimp is not different from non-transfection broodstock candidates of tiger shrimp. The survival rate of transfection broodstock candidates of tiger shrimp is lower than that of non-transfection broodstock candidates of tiger shrimp. The distribution of size of transfection broodstock candidates of tiger shrimp is dominated by the size of 41-50 g/shrimp (34.09%) while the distribution of size of non-transfection broodstock candidates of tiger shrimp is dominated by the size of 31-40 g/shrimp (48.39%).

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