

Evaluation of growth performance of three strains Tilapia in brackish water pond in West Java, Indonesia

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Abstract: There is a lot of abandoned brackish water shrimp ponds in Indonesia, which can be utilize for tilapia fish farming in the context of increasing national tilapia production. This experiment aimed to evaluate the growth performance of three strains of Nile tilapia that is red tilapia (*Oreochromis* sp), Srikandi hybrid tilapia (*O. aureus x niloticus*) and blue tilapia (*O. aureus*) in brackish water pond. Fish fingerlings were produced by natural spawning of each strains in the freshwater earthen pond. The broodstock were mated at male to female ratio of 10:30. After 14 days of mating, larvae were harvested and reared in happa measuring 2x2 m² with a stocking density of 250 fish m⁻² for 90 days to reach the fingerling size. Rearing of fingerling was carried out in the 3x5 m² net installed in the coastal pond with water salinity of 20-30gL⁻¹ for 120 days. The fingerlings were stocked at density of 10 fish m⁻². Feeding was applied at a rate of 5-10% of biomass twice daily. The observed parameters included of growth rate, survival rate and feed conversion ratio. The results showed that Srikandi strains tilapia revealed the highest daily growth of 2.02 g day⁻¹, 3.34% higher than that of red tilapia and 21.45% higher than blue tilapia. The feed conversion ratio (FCR) of Srikandi tilapia was 1.69, lower than those of red tilapia and blue tilapia *i.e.* 1.72 and 1.92. Based on the growth rate, feed conversion ratio, and survival rate, Srikandi strain tilapia had the best performance among others and was recommended as the most suitable candidate for fish culture in the high salinity ponds in Indonesia.

Keywords: Growth, Tilapia, brackish water, salinity

Introduction

Tilapia has been widely cultivated in the world and become a source of animal protein. Therefore, it is then well known as 'aquatic chicken'. The global production of tilapia is steadily increase from 472,500 tonnes in 2010 to 1,030,000 tonnes in 2018, or with an increment of 217.99 % for eight years (FAO, 2020). In general, tilapia is a euryhaline fish that can live on a wide salinity range from freshwater to the sea. Some species of tilapia have wide tolerance to salinity and have the potential to be cultivated extensively in ponds with medium salinity (20 gL⁻¹) to high salinity ≥ 35 gL⁻¹ (El-Sayed, 2006).

Tilapia retain various characteristics which make them desirable species to be cultured in brackishwater farms. Some species of tilapia which have a wide level of salinity tolerance include *O. mossambicus*, *O. aureus*, *O. hornorum* and *Tilapia zilli*. Mozambique tilapia (*O. mossambicus*) is known to have a salinity tolerance of up to 120 gL⁻¹, grows and reproduces up to 49 gL⁻¹ salinity. Its seed lives and grows well until salinity of 69 gL⁻¹ (Whitefield and Blaber, 1979). Blue tilapia (*O. aureus*) is still able to grow well in the salinity ranged of 36-44 gL⁻¹ while its reproduction occurs at 19 gL⁻¹ salinity. With appropriate gradual acclimatization blue tilapia can grow at salinity of 54 gL⁻¹ (Popma and Masser, 1999). In addition, there were other tilapia strains that have rapid growth in brackish waters, namely red tilapia, red tilapia hybrid, and red Florida tilapia (Watanabe *et al.*, 1988; Pillay, 1991; Suresh & Lin, 1992). Red tilapia hybrid reveal growth better in brackishwater and seawater or at salinity up to 36 gL⁻¹ than in freshwater (Romana-Eguia and Eguia, 1999; El-Sayed, 2006).

In order to increase national fish production in Indonesia, it is necessary to develop the cultivation area of tilapia fish towards brackish water area, even to the seawater. The Ministry of Marine Affairs and Fisheries of

the Republic of Indonesia recorded the potential coastal land for pond fish cultivation reached 2.9 million hectares, while the utilization rate was only 24.15% of the available land in 2016 (Pusdatin, 2018).

Farming the regular tilapia were still constrained by the low growth and high mortality during their grow out in the brackish water ponds (Robisalmi et al., 2020). The mortalities were often caused by salinity fluctuations, especially during seasonal changes from the rain to dry season. Developing tilapia strain with higher salinity tolerance and ability to grow at high salinity up to 30 gL⁻¹ will overcome this issue.

Although Nile tilapia does not perform well at salinity higher than 15 gL⁻¹ it can tolerate a direct transfer from freshwater to 15-18 gL⁻¹ without mortality (Schofield et al., 2011; De Souza et al., 2019). If gradually acclimatized, Nile tilapia can tolerate salinity up to 36-40 gL⁻¹. The salt-tolerance ability of Nile tilapia also depends on water temperature as it can tolerate a higher salinity at a warmer temperature. In one study, no mortality was observed in 40 gL⁻¹ water at 29.4°C but all fish died at the salinity of ≥20 gL⁻¹ at 13.9°C (Schofield et al., 2011)

Research Institute for Fish Breeding (RIFB) of the Ministry for Marine Affairs and Fisheries of The Republic of Indonesia has released such superior tilapia strain which was then known as Srikandi strain in 2012. This salinity-tolerant tilapia strain was formed through hybridization between Nirwana strain of Nile tilapia (*O. niloticus*) and blue tilapia (*O. aureus*). It was considered as superior tilapia strain with good growth performance in salinity level up to 30 gL⁻¹ (Robisalmi et al., 2020) A study to evaluate the performance of this hybrid tilapia compared to other tilapia strains which have been known to have high tolerance to high salinity has not been conducted. This experiment aimed to evaluate the growth performance of three strains of Nile tilapia that is red tilapia, Srikandi hybrid tilapia and blue tilapia at brackish water pond.

Materials and Methods

Fish populations used in this study were three strains of tilapia, namely, red tilapia *Oreochromis* sp, Srikandi hybrid tilapia (*O. aureus x niloticus*) and blue tilapia *O. aureus*. These strains were treated as treatments in this study. Therefore, there were three treatments with three replicates.

Fish fingerlings were produced by natural spawning of each strains in the freshwater earthen pond. The mature broodstocks were mated in the spawning ponds at male to female ratio of 10:30. After 14 days of mating, larvae were harvested and reared in hapa measuring 2x2x1 m (length x width x height) with a stocking density of 250 fish m⁻². The fry were reared for 90 days. The fry were fed twice daily with feeding ratio of 10-15 % of fish biomass day⁻¹. The feed contained crude protein level of 28-30%.

At the end of fry rearing, the fingerlings were transferred to brackish water ponds for growing-out. Prior to stocking, tilapia fingerlings were adapted to higher salinity water by adding sea water to increase the salinity of the holding tank water. On the first day, three populations of fish fry were adapted to salinity of 10 gL⁻¹ and on the second day, the salinity was increased to 15 gL⁻¹ y. On the third day, the populations were adapted to salinity of 20 gL⁻¹, the desired salinity in the growing out ponds. The salinity of the adaptation tanks were regulated by sea water supply and measured using a salinometer (ATAGO S-10E, Japan) everyday.

The tilapia fingerlings were reared in the 3x5 m² nets installed in brackish water pond with water salinity of 20-30 gL⁻¹ for 120 days. The fingerlings were stocked at density of 10 fish/m². The fish were fed with commercial feed containing 30-32% of crude protein. Feeding was applied twice daily at a rate of 5-10% of biomass.

Sampling activities were carried out every month. The observed parameters included of growth rate, survival rate and feed conversion ratio. Data were analyzed using the ANOVA test with the SPSS 22 program.

There were some formulas involved to calculate the parameters values, as follows:

$$\text{Weight gain} = W_t - W_0$$

$$\text{Specific growth rate (SGR)} = \frac{(\ln W_t - \ln W_0)}{t} \times 100$$

$$\text{Daily growth rate (DGR)} = \frac{(W_t - W_0)}{t}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake}}{W_t - W_0}$$

$$\text{Survival rate (SR)} = \frac{\text{Number fish of the end}}{\text{Number fish of initial}} \times 100$$

Where W_0 and W_t were the initial and final fish weight, respectively, and t is the number of days in the rearing period.

Measurement of water quality is carried out periodically every month as supporting data which includes temperature, pH, dissolved oxygen, turbidity and salinity.

Results and Discussion

Three strains of tilapia were able to grow in high salinity brackish waters. The results showed that the Srikandi tilapia showed a higher growth value than the red and blue tilapia fish populations (Table 1). The highest absolute weight and biomass growth values were shown by Srikandi tilapia at 240.93 ± 22.95 g and 22194.67 ± 1904.41 , while the lowest value was in the blue tilapia population with growth in absolute weight and biomass of 214.54 ± 26.98 g and 16161.98 ± 1975.66 . The absolute weight and biomass of Srikandi and red tilapia were not significantly different ($P > 0.05$), but both were significantly different from blue tilapia ($P < 0.05$). These results were consistent with the finding of Siddiqui and Al Harbi (1995) who reported that hybrid tilapia fish had the highest weight of 327 g compared to *O. niloticus* 293 g, red tilapia 264 g, *O. aureus* 234 g and *O. mossambicus* 168 g after the rearing period of 392 days. Tayamen *et al.* (2004) also reported the similar finding that the growth of tilapia resulting from crossing *O. spilurus* x *O. aureus* kept in brackish water ponds with a salinity of 7-30 gL^{-1} for 120 days revealed a weight gain of 101.34 ± 41.65 g.

Table 1. The performances of weight and biomass of three populations of tilapia fingerlings grown in brackish water pond for 120 days

Parameters	Populations		
	Red Tilapia	Srikandi	Blue Tilapia
Initial Individual Weight (g)	4.26±0.25	4.38±0.12	4.30±0.23
Final Individual weight (g)	232.85±21.15a	246.73±21.65ab	218.83±26.75b
Weight gain (g)	228.59±20.90ab	240.93±22.95a	214.54±26.98b
Initial Biomass (g)	869.20±41.48	874.90±20.33	859.50±36.98
Final Biomass (g)	22938.97±832.73a	23069.57±1921.86a	17021.48±1838.98b
Biomass gain (g)	22069.77±799.64a	22194.67±1904.41a	16161.98±1975.66b

Values with the same superscript in each row were not significantly different from each other ($p>0.05$)

Ekmath *et al.* (1998) reported that tilapia results from crossing *O. niloticus* x *O. aureus* showed better growth than their parents and the cross between these two strains was a potential candidate for cultivation (Hulata *et al.*, 1993). Suresh and Lin (1992) reported that salinity tolerance differences among tilapia species and between species of *Oreochromis* genus. In this genus, *O. spilurus*, *O. mossambicus* and *O. aureus* showed higher salinity tolerance than Nile tilapia (*O. niloticus*). The Florida red tilapia hybrid, which was considered to be one of the high performing tilapia breeds cultured under high salinity ($15\text{--}30\text{ gL}^{-1}$) in tropical conditions, was derived from crossbreeding a mutant red *O. mossambicus* with *O. aureus*, *O. niloticus* and *O. urolepis hornorum*. However, this breed suffered from lack of cold tolerance (Watanabe *et al.*, 2006) and red tilapia strains generally show slower growth than Nile tilapia.

The body weight based growth pattern of these three tilapia strains steadily increased during grow-out period (Figure 1). During the first two months, the pattern of growth of three strains was relatively similar. The difference in growth among three strains of tilapia was seen since the third month and was more significant at the fourth or the last month of grow-out period. This difference in growth occurred due to the salinity of pond water. Salinity of brackish water ponds in the first two months of rearing period ranged from $15\text{--}20\text{ gL}^{-1}$, while in the third and fourth months increased to $20\text{--}30\text{ gL}^{-1}$. Setyawan *et al.* (2015) reported that tilapia which was reared for three months at a salinity of $30\text{--}33\text{ gL}^{-1}$ in marine floating cages showed a growth pattern that steadily increased until it reached a peak when fish growth began to stagnate and formed a sigmoid shape.

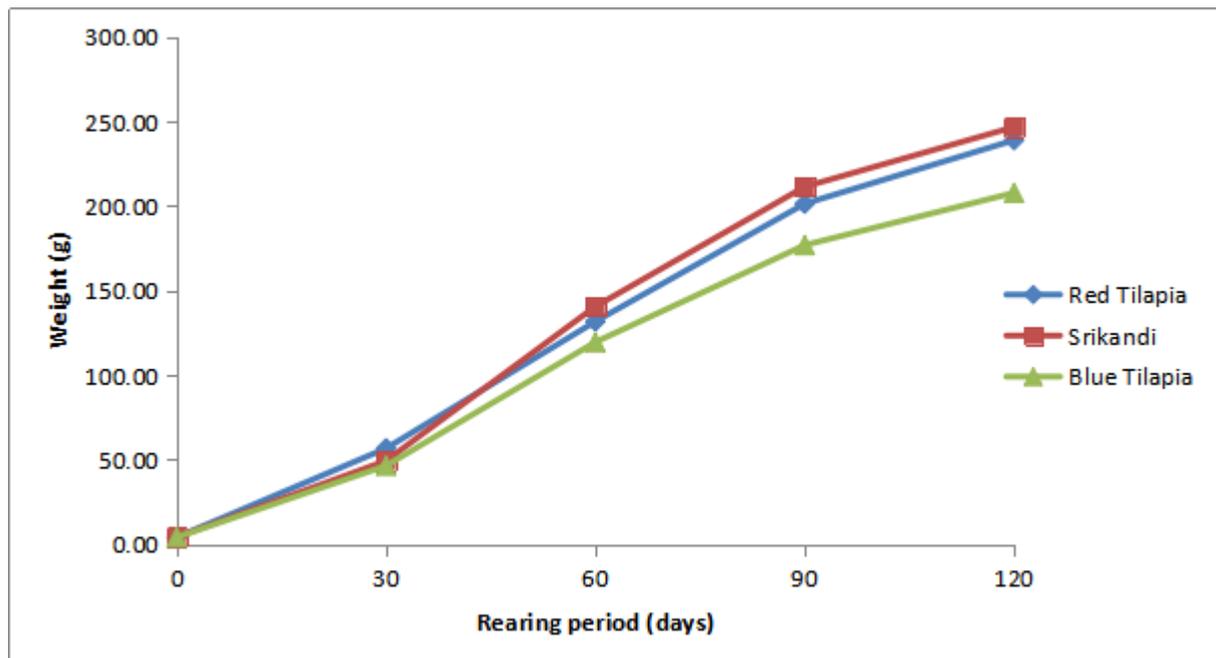


Figure 1. Body weight based growth pattern of three populations of tilapia fingerlings grown for 120 days in brackish water pond

At the end of grow-out phase, Srikandi tilapia revealed a body weight that 3.26% higher than it of red tilapia and 18.71% higher than it of blue tilapia. The high growth of Srikandi tilapia was due to the fact that Srikandi tilapia was the result of crossing of *O. niloticus* and *O. aureus*, where both parents were fish from breeding programs through family selection. Ninh *et al.* (2014) stated that a selection program on third generation tilapia in salinity of $15\text{--}20\text{ gL}^{-1}$ showed a significant increment performance in weight characters with an increase in the value of genetic variation from 0.30 to 1.62. Hadie *et al.* (2012) reported that Srikandi tilapia had a competitive advantage because it had the better growth performance at a salinity of $10\text{--}30\text{ gL}^{-1}$ compared to other tilapia strains. However, Chourasia *et al.* (2018) stated that *O. mossambicus*, which has a high salinity tolerance,

showed expression patterns and response similar to marine species, and differed from the salinity sensitive *O. niloticus*. Mozambique tilapia reared under tidally-changing salinities grew faster than fish reared in either fresh water or sea water and showed patterns of gene expression of elements of the GH/IGF axis that differed from those observed in fish held in constant fresh water or sea water conditions, such as the increase and decrease of GHR mRNA levels in muscle and liver with each phase of the tidal cycle (Moorman *et al.* 2016).

The fluctuation of salinity caused the ability of fish to adapt to the environment by carrying out different osmoregulation processes, depend on the individual conditions of fish. When the salinity rose, it was directly increased the osmotic pressure of the water proportionally. It was suspected that the osmotic pressure in water with a salinity of 20-25 gL⁻¹ was closer to the osmotic pressure of fish blood. Therefore, the ionic content of the water approached the ionic concentration of fish blood. As the consequences, the energy requirement for osmoregulation would be smaller, while energy for growth would available in larger quantities. On the contrary, the conditions of higher salinity would cause interference with osmoregulation process which caused stunted growth. According to Suresh & Lin (1992) there were several species of tilapia reared in hyper salinity conditions that showed better or the same growth performance as tilapia species reared in fresh water. D'Cotta *et al.* (2006) stated that there were two major stages that were involved in salinity adaptation, namely time to salinity tolerance and a slower process of salinity adaptation. Changes in salinity in waters would have direct implications to survival, growth, metabolism and distribution of fish because they affected the osmotic pressure and ion concentration of body fluids. Watanabe *et al.* (1985) reported that the larger the fish size, the osmoregulation ability and the tolerance to high salinity were higher than the smaller fish. Küçük *et al.* (2013) observed that *Oreochromis niloticus* acclimated to 8 gL⁻¹ salinity and transferred to four different salinities (12, 16, 20, and 24 gL⁻¹) showed no difference in their growth among the first three treatments; however, a decrease in growth rate was observed at 24 gL⁻¹ salinity.

In contrast to the weight gain, the specific growth rate values of the three tilapia populations had almost the same values, ranged from 3.21-3.37% weight day⁻¹ which results were not significantly different (P>0.05) (Figure 2). These results indicated that the three strains of tilapia had the potential to be developed in the brackish environment and can grow well at a salinity of 30 gL⁻¹. These results were consistent to Essa and Haroun (1998) who reported the growth rate in tilapia crossing of *O. niloticus* x *O. aureus* was 4.88% weight day⁻¹, while *O. niloticus* was 3.98% weight day⁻¹, *O. aureus* was 3.82% weight day⁻¹ and red tilapia was 3.73% weight day⁻¹. As seen from the daily growth value, in this study the population of Srikandi tilapia showed the highest daily growth value of 2.02 g day⁻¹. This value was 3.34% higher than red tilapia and 21.45% higher than blue tilapia. The daily growth value of Srikandi tilapia was not significantly different from that of red tilapia (P<0.05), but significantly different from blue tilapia (P>0.05).

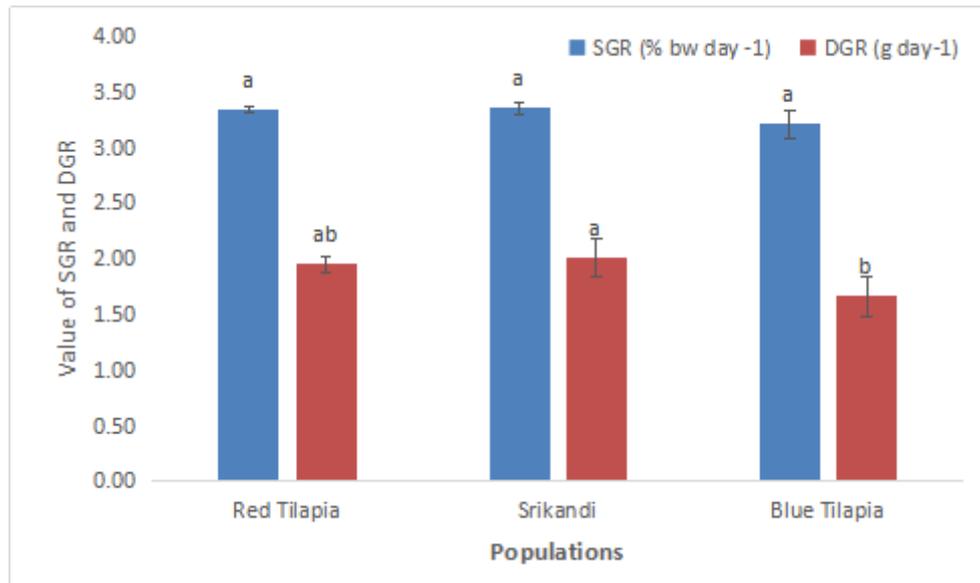


Figure 2. Specific growth rate and daily growth rate of three populations of tilapia fingerlings grown for 120 days in brackish water pond (Values with the same label letter in each category were not significantly different from each other ($p>0.05$)).

The value of the feed conversion ratio of the three tilapia populations reared in ponds ranged from 1.69–1.92 (Figure 3). The lowest feed conversion value was found in Srikandi tilapia followed by red tilapia and the highest FCR was in blue tilapia. There were no significantly different ($P>0.05$) between FCR of Srikandi and red tilapia, but their FCR value were significantly different from blue tilapia ($P<0.05$). The FCR value must also be taken into account when determining the best tilapia strain for farming in brackish water. The lower FCR in Srikandi tilapia could reflect the improved efficiency in metabolizing the commercial feeds and/or more effective utilization of available natural food. Based on these results it was known that this hybrid tilapia had the ability to adapt to higher salinity, which implicated to better digestibility of feed as an energy source to growth faster.

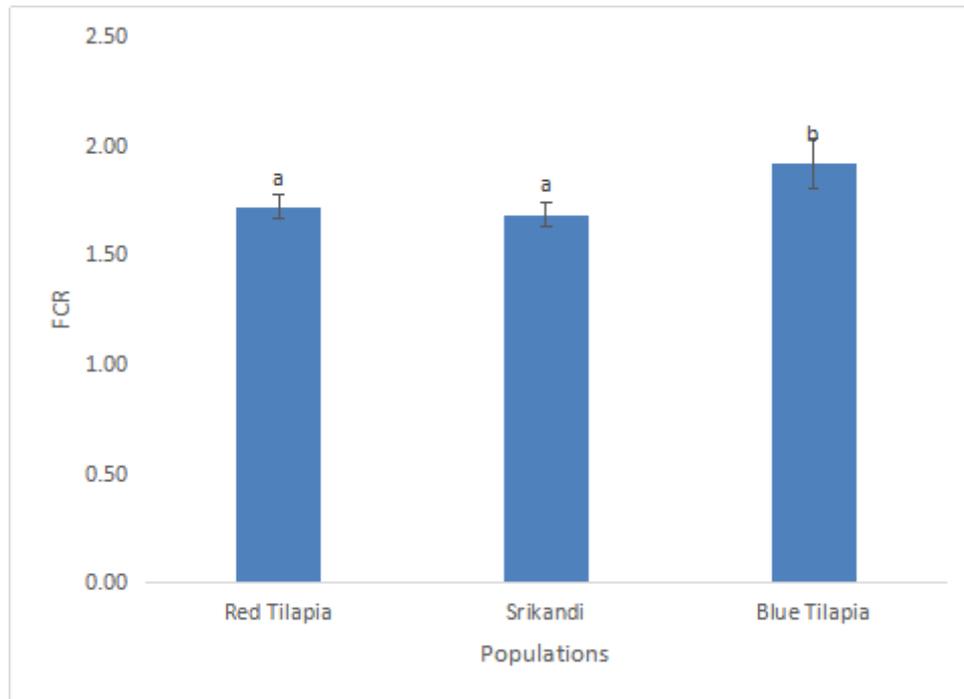


Figure 3. Feed conversion ratio of three populations of tilapia fingerlings grown for 120 days in brackish water pond (Values with the same label letter were not significantly different from each other ($p>0.05$)).

According to Liao & Chang (1983), high salinity will interfere with growth because the consumption rate decreased, this also affected the feed conversion ratio which causes inefficient. Watanabe *et al.* (1990) and Balcazar *et al.* (2004) found that red tilapia reared in sea water cages had a feed conversion ranging from 1.71-2.02, which was comparable to blue tilapia strain which was reared in ponds with a salinity of 25 gL^{-1} in the nursery phase had FCR ranged from 1.23-2.04 (Robisalmi *et al.* 2012). Moreover, Srikandi tilapia that was raised in pond with salinity up to 40 gL^{-1} had a FCR value ranged from 1.48-1.56 (Robisalmi *et al.* 2019). Wu *et al.*, (2021) found that in GIFT tilapia the optimal protein requirements at 8‰ salinity were higher than those at 0‰ salinity and the protein requirement at 8‰ and 0‰ were 37.70% and 33.70%, respectively. GIFT juveniles exhibited improved glycogen synthesis at 8‰ salinity and fat deposition at 0‰ salinity

The survival rates (SR) of three tilapia populations during 120 days rearing period were classified as high with the highest value was found in red tilapia of 96.00%, followed by Srikandi tilapia of 93.50% and blue tilapia 83.50% (Figure 4). There was no significant difference between SR value of red tilapia and Srikandi tilapia ($P>0.05$), while their values were significantly difference from blue tilapia ($P<0.05$). These results indicated that these three fish populations had a high tolerance for salinity. Siddiqui & Al Harbi (1995) reported the survival rates of several strains of tilapia during grow-out phase which reached 80% in hybrid tilapia, 74% in *O. niloticus*, 72% in *O. aureus*, 61% in *O. mossambicus* and 70% in red tilapia. In addition, McGeachin *et al.* (1987) stated that blue tilapia reared in sea water (salinity 36 gL^{-1}) showed good growth with low mortality. Nugon (2003) reported that juvenile blue tilapia and Florida red tilapia showed good survival of 80% at a salinity of 20 gL^{-1} while the survival rate of blue tilapia at a salinity of 35 gL^{-1} was slightly lower of 54%. Carmelo (2002) reported that *O. spilurus* grown in an open sea water cages with salinity of 37-38 gL^{-1} reached the average individual harvested weight of 483 g after reared for 150 days with a FCR of 1.6 and survival rate of 97.7%. According to El-Sayed (2006), the salinity levels that can be tolerated by tilapia in osmoregulation and for growth range from 20–35 gL^{-1} . Meanwhile, Ninh *et al.* (2014) reported that tilapia reared in ponds with salinity of 15–20 gL^{-1} had a survival rate ranging from 75.3–91.9%.

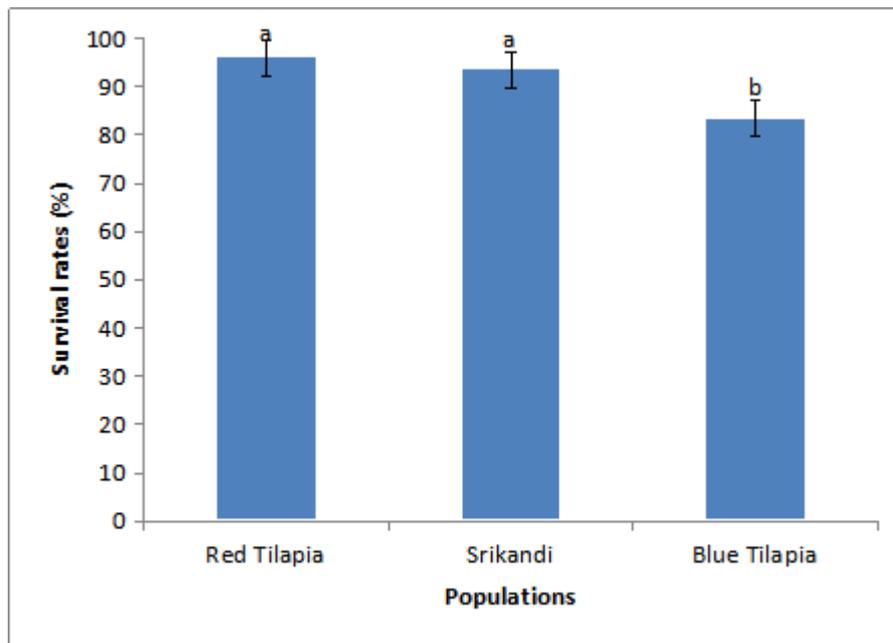


Figure . 4. Survival rate of three populations of tilapia fingerlings grown for 120 days in brackish water pond (Values with the same label letter are not significantly different from each other ($p>0.05$)).

According El-Leithy *et al.* (2019) the presences of the survival fish at higher salinity concentrations $30\text{--}34\text{ gL}^{-1}$ increase the chance for salinity resistance selection in the Nile tilapia. Higher expression of immune-related genes in the kidney with higher salinity concentration (20 gL^{-1}) associated with high mortality may indicate that the fish grown in higher salinity concentration were predisposed to infectious agents present in water. Malik *et al.* (2018) studied the effect of different salinity concentrations (0, 5, 10, 15, 20, and 25 gL^{-1}) on the breeding and survival of the Nile tilapia; their results showed that the highest fertility was at 15 gL^{-1} salinity with highest survival (92%), and a significant decrease in these parameters occurred at 20 gL^{-1} .

At salinity above 25 gL^{-1} , there was a significant increase in $\text{Na}^+\text{K}^+\text{-ATPase}$ activity (Guner *et al.*, 2005), accompanied by the secretion of NaCl by the gills and various forms of salt ions by the kidneys. The process of ammonia secretion through the gills and the osmoregulation process in order to maintain the osmotic pressure of the fish will absorb a certain amount of energy and convert ATP to ADP (Randal and Tsui, 2006). This becomes a selection for salinity resistance in the entire test population. At high salinity there will be media hyperosmotic pressure, as a result the fish will continue to try to respond to stress with an osmoregulation mechanism that requires a large amount of energy. Fish that had low osmoregulation capacity will not be able to respond to high hyperosmotic pressure in marine waters so that the mortality rate was much higher than in freshwater rearing.

As saltwater fish live in a hyperosmotic environment compared to the body fluids, it faces the continuous challenges of dehydration and salt gain. To compensate for water loss, marine fish must drink seawater continuously and the water is absorbed across the intestinal wall by solute-linked transport. The absorbed monovalent ions (mainly Na^+ and Cl^-) were later excreted via mitochondria-rich cells (chloride cells) at the gill. Most of the ingested divalent ions remain in the gut and were excreted with the feces; small quantities of these ions (mostly Mg^{2+} and SO_4^{2-}) were absorbed into the blood and excreted by the kidneys. Marine fish usually possess smaller and fewer glomeruli than the freshwater fish such that the glomerular filtration rate (GFR) and urine (isoosmotic) production were lower than the freshwater fish which has higher GFR and producing copious volumes of hypoosmotic urine (Evans, 2011; Laverty and Skadhauge, 2012). Fish were able to adapt salinity

stress by osmoregulation. Different pathways were included in this adaptation; they were involved mainly in osmotic homeostasis maintenance in fishes as reviewed by Kültz (2015).

Water quality has an important role to support the life and growth of fish and other aquatic biota. The results of observations on several air quality variables including temperature, pH, dissolved oxygen, turbidity and salinity in brackish water ponds were presented in Table 2. The health and subsequent growth of fish were directly related to the quality of water in which the fish were raised. There were several factors affecting growth offish including internal (nervous, endocrinological, and neu- roendocrinological) and external (temperature, salinity, photoperiod, ammonia, pH, and oxygen) factors (Bœuf and Payan 2001)

Table 2. The performances of water quality of the brackish water pond for grow-out of three populations of tilapia fingerlings for 120 days.

No	Parameter	Range
1	Temperature (oC)	29 - 31,5
2	pH	7,6 - 8,5
3	DO (mgL-1)	1,7 - 4,3
4	Turbidity (NTU)	124–148
5	Salinity (gL-1)	20-30

This research activity was carried out in standard conditions seen from the water quality parameters suitable for fish culture, except for the parameters of dissolved oxygen content. The optimum water quality parameters for cultured fish were dissolved oxygen content more than 5 mgL⁻¹, temperature 25-32°C, salinity 0-5 gL⁻¹, pH 6.5 - 9.0, nitrite less than 0.5 mgL⁻¹ and ammonia less than 1.0 mgL⁻¹ (Boyd, 1990). The low dissolved oxygen (DO) content had an impact on the decline in fish growth performance. In this study, the DO range was below 5 mgL⁻¹, however this range was still considered acceptable for tilapia. Tilapia can grow in waters with relatively low oxygen content below 3 mgL⁻¹, but if it was less than 1 mgL⁻¹, it will experience impaired metabolic growth and were susceptible to disease (Popma and Masser, 1999).

The higher salinity ranges in the ponds could result in stress, growth disturbance and death of tilapia, while This increase in salinity has implications for the activity of fish to move, because it provides high osmotic pressure so that the fish must often carry out the osmoregulation process to balance the condition of the fluid concentration in the body with the media. Meanwhile, the salinity that can be tolerated by tilapia to grow ranges from 20-35 gL⁻¹ (El-Sayed, 2006). Due to the fact that salinity in coastal ponds sometimes increases considerably when there is no fresh water supply and or rain, it is necessary to make observation on the performance of Srikandi strain tilapia in the salinity higher than 35 gL⁻¹.

Conclusion

Compared to two other strains, red tilapia and blue tilapia, Srikandi strain tilapia shown the best performance with daily growth rate of 2.02 g day⁻¹, feed conversion ratio of 1.69, and moderate survival rate of 93.5%, and was recommended as the most suitable candidate for fish culture in the high salinity ponds in Indonesia. This research focused more on phenotypic aspects and in the further research it was necessary to deepen to explore the physiological or molecular level of biological aspects.

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