Response of tilapia (Oreochromis niloticus) behaviour to salinity differences: a laboratory scale study

A A Fuadi¹,², I R J Hasly³, L I Azkia³ and M Irham⁴

¹Geospatial Information System Laboratory, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia.
²Department of Fisheries, Politeknik Indonesia Venezuela, Aceh Besar Indonesia.
³Marine Fisheries Technology - Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Indonesia.
⁴Centre for Environmental and Natural Resources Research (PPLH-SDA), Universitas Syiah Kuala, Banda Aceh, Indonesia.
*Corresponding Author: irham@unsyiah.ac.id

Abstract. Tilapia is one of the important commodities of freshwater aquaculture in Indonesia which has good immunity and adaptability. One of the adaptations that can be made by Tilapia is physiological adaptation to a high salinity range. Information on the resistance of Red Tilapia to different salinity on a laboratory scale provides basic information to test the use of Red Tilapia bait as an alternative bait in the Tuna longline fishery. The purpose of this study was to determine the behaviour and mortality rate of Red Tilapia (Oreochromis niloticus) to differences in salinity. This research was conducted at the Laboratory of fish behavior, Bogor Agricultural University in May 2018 with three stages. The first stage is the acclimatization of the test fish in the maintenance aquarium which is carried out in one week. The second stage is to calculate the salinity in water by diluting the desired salinity. The third stage tested the differences in salinity levels on the behaviour of the fish in the experimental aquarium. Comparative descriptive statistical analysis was used by comparing Tilapia between various salinity treatments. The random design used in this study was a randomized block design, namely between salinity and the length of time the fish remained in the aquarium. The behaviour of Tilapia during changes in salinity was light stress at salinity 10 and 20 ppt. However, when it rises to 30 ppt the fish are already experiencing severe stress, which is indicated by erratic swimming directions and the number of opercula that are getting slower.

1. Introduction

Tuna longlines are an effective fishing tool for catching tuna and other large pelagic fish in deep sea waters. Tuna longlines are passive but effective in catching tuna because their construction is able to reach the swimming depths of Tuna [1]. The catch of fish in the Tuna longline is largely determined by the fish's interest in eating the bait. Bait functions to attract fish to be caught [2]. The characteristics of a good bait are effective in attracting fish, easy to obtain, inexpensive, easy to store and durable [3].

The use of bait by the tuna longline fleet can be in the form of frozen bait or live bait. Several types of bait that are often used in longline fisheries by fishermen in Benoa are milkfish (Chanos chanos), Lemuru (Sardinella longiceps), Layang (Decapterus spp.) and squid (Loligo sp.) [1]. However, the availability of these feeds encountered problems. This is because fishermen usually only have a choice of natural bait, namely low-cost fish whose availability is highly seasonal and difficult to obtain [3, 4].
Besides that, the price used as bait is relatively expensive [4]. In addition, if the bait from fishing is used continuously, it is feared that it will disturb the balance of these fish resources in nature.

The availability of bait that is not continuous will affect the fishing operation. Another alternative is the use of artificial bait that only relies on color and does not have a specific odor which causes the catch to be less than optimal. For this reason, it is necessary to have an alternative substitute for bait in the longline fishery to overcome the limited bait. One of live bait that can be used as bait alternative is utilizing the abundance of freshwater fish resources which is affordable prices for fishermen [4].

Tilapia is one of the important commodities of freshwater aquaculture in Indonesia which has good immunity and adaptability. One of the adaptations that can be made by tilapia is physiological adaptation to a high salinity range because Tilapia is classified as a euryhaline fish and has the potential to adapt to sea water salinity (± 35 ppt) [5]. In a study [6], Red Tilapia had the best resistance between dead milkfish bait and dead tilapia bait. Red Tilapia is able to adapt to the environment such as lack of dissolved oxygen, high salinity and so on while in the sea. Tilapia has a red color that can attract Tuna fish to get closer. The use of Red Tilapia bait can also reach catches at depths of 40-60 and 155-175 m. Therefore, live Red Tilapia bait is more suitable for use as bait for long line tuna operating on the surface.

Consequently, it is necessary to study that Red Tilapia bait can be used as an additional alternative as live bait in longline fisheries by utilizing some of its resistance properties in sea water. The purpose of this study was to determine the mortality rate of red tilapia (Oreochromis niloticus) against differences in salinity and to determine the behavior of Tilapia on differences in salinity. The information on the resistance of Red Tilapia to different salinity on a laboratory scale provides basic information to test the use of Red Tilapia bait as an alternative bait in the Tuna longline fishery.

2. Method

This research was conducted at the Laboratory of Fish Behavior, Bogor Agricultural University in May 2018 with three stages. The first stage is the acclimatization of the test fish in the maintenance aquarium which is carried out in one week. The second stage is to calculate the salinity in water by diluting the desired salinity. The third stage tested the differences in salinity levels on the behavior of the fish in the experimental aquarium.

This study used five sized glass aquariums filled with 20 liters of fresh water. One aquarium for maintenance and the other four for the treatment aquarium. Each aquarium was treated with different salinity, namely 10 ppt, 20 ppt, 30 ppt and 40 ppt. Previously, the aquarium had a salinity of 0 ppt, then it was treated by making salinity by adding salt to each aquarium to obtain the desired salinity. The measurement of salinity using a refractometer.

The materials used in the study were as follows: (1) Live Tilapia obtained from cultivation at the Bogor Agricultural Institute. Fish are selected in various sizes with a total length (11-13.5 cm). The number of fish used was 4 fish, (2) Fresh water as a living medium for Tilapia, (3) Salt is used to make different salinity.

2.1. Preparation and preservation of Tilapia

The study was conducted on July, 2020. Sampel fish were collected from Bira Cot River at Aceh Besar district (05° 29.895' N and 095° 27.939' E). A total of twenty fish samples were monthly collected from Bira Cot River. The collected specimens were preserved in formalin (10%). Standard length and total length (mm) and body weight (g) was measured.

2.2. Data observation and data recording

Observations were made after the acclimatization process. Experiments were carried out using several aquariums, each with different salinity levels. This selection is used to determine how long the fish hold on to salinity levels. The test fish were left in normal conditions as control and then transferred to an aquarium that had different salinity (10, 20, 30 and 40 ppt).
Data collection was obtained every three hours. The assumption used for Tilapia is that in 1 aquarium, the type of fish is considered the same as measured by the length of the fish. So that the treatment used for each salinity uses fish obtained from the same aquarium. To determine the fish tolerance to salinity, the test is carried out every hour.

2.3. Data analysis
Comparative descriptive statistical analysis was used by comparing Tilapia between various salinity treatments. The random design used in this study was a randomized block design, namely between salinity and the length of time the fish remained in the aquarium. The mean number of operculum compared to normal time, then 10 ppt, 20, 30 and 40 ppt.

The experimental design used was a completely randomized design. To determine the effect of treatment on Tilapia, statistical analysis was used. Previously, a statistical test was carried out, first a normality and homogeneity test were carried out so that the data was spread normally and homogeneously. Normality test using Kolmogorov-smirnov and shapiro-wilk. Data were tested with SPSS 16.00 software. Variance analysis was used to test the effect of treatment (type of salinity) on Tilapia whether there was an effect or not. The test after analysis of variance is needed to determine whether there is a difference between the mean value of Tilapia between types of salinity 10, 20, 30 and 40, namely by performing the Duncan test with a test level of 5%.

The test hypothesis for decision making is:
Ho = salinity has no effect on fish behavior.
H1 = salinity affects fish behavior.

A follow-up test was carried out by observing the change in salinity from 20 to 30 ppt. The aim is to determine the trend of fish resistance to salinity. Based on the results of research [4] that the salinity tolerance of Tilapia at 21 ppt. So that in the next stage, the salinity of 22, 24 and 26 ppt is chosen. The choice is because at 30 ppt the fish no longer survive the first hour interval. The hypothesis for decision making is as follows:
Ho = 20-30 ppt increase in salinity has no effect on tilapia.
H1 = 20-30 ppt increase in salinity effect on tilapia

3. Results and Discussion

3.1. Behavioral response of Tilapia (Oreochromis niloticus)
The response of fish behavior to each salinity is seen from the movement of the fish. When the salinity is 10 ppt, the fish are still moving and tend to be active. When the salinity is increased to 20 ppt, the fish tend to be passive, which is indicated by the fish always swimming in the corner of the aquarium. However, fish still react when caught by hand. The average number of operculum openings was 87 per minute. This is in line with [7] and [4] that the fish movement began to slow down after the addition of salinity above 15 ppt.

Fish experienced death in the first hour is at 40 ppt salinity. What happens is that the fish always swim in the corner of the aquarium and then a few minutes later the fish move very actively with an erratic swimming direction before finally experiencing death due to not being able to adapt to environmental changes with a salinity of 40 ppt (Figure 1).

At the end of the second hour, mortality in fish occurred at a salinity of 30 ppt. Just like the previous mortality at a salinity of 40 ppt, previously the fish experienced erratic swimming movements. According to [8] the description of the behavior of tilapia under stress is that the fish swim to the surface to take oxygen along with the fast movement of the operculum, the movement becomes passive and the fish's reflex decreases.

Preliminary research results with salinity 10, 20, 30 and 40. Fish tend to tolerate 10 and 20 ppt salinity. To determine the tendency of fish to salinity with the length of time the fish took, further testing was carried out by dividing the range from 22, 24 and 26. The selection was based on previous research where observations were made of various salinity from 10 ppt, 20 ppt, 30 ppt and 40 ppt. The length of time for fish in the aquarium is one hour as the assumption from previous studies that
the fish can survive at an extreme salinity of 40 ppt for 1 hour. The results obtained are the fish still survive up to 24 ppt salinity. The fish were not able to tolerate the environment when the salinity was 26 ppt. This is in line with research [4] that fish is able to adapt to a salinity of 21 ppt.

![Figure 1. Fish mortality at a salinity of 40 ppt](image1)

Fish are water animals that are subject to various types of stressors because their hemoestatic mechanisms depend on the prevailing conditions in their surrounding environment. Common stressors faced are related to fishing, transportation, handling. Examples of additional stressors for fish include fluctuations in water salinity, pH, alkanes, dissolved solids, dissolved oxygen [9].

One way to determine the fish's tolerance to several salinity is by knowing its behavior and the fish's operculum opening. Fish response as a result of environmental changes that cause fish to try to adapt to the maximum and can survive to live.

3.2. Operculum opening

The operculum opening in fish was initially normal at around 48 per minute. Then when the addition of 10 ppt changed an average of 87 per minute. Meanwhile, 20 ppt the average number of operculum openings was 86 per minute. Fish experienced mild stress at salinity above 10 ppt. Then experience severe stress at salinity above 30 ppt. This is indicated by the number of changing operculum openings increasing at a salinity of 30 ppt by 20 per minute but before the death of the fish, the number of operculum openings reached 123 per minute then decreased to 60 per minute (Figure 2).

![Figure 2. Fish mortality at 30 ppt salinity](image2)

The graph on nine repetitions where one repetition is assumed to be one hour, will produce a graph of the number of operculum openings at various salinities (Figure 3) where at salinity 10 and 20 ppt the fish still tolerate fluctuating graphs. Whereas at salinity of 30 and 40 ppt the fish could not tolerate...
which was marked by death in fish, which was previously marked by a high number of operculum openings then decreased sharply.

Figure 3. Number of operculum openings with varying salinity

Several studies have revealed the potential morphological effects of salinity as the sole cause of stress by conducting experiments specifically evaluating the tolerance of hybrid tilapia (*Oreochromis mossambicus* x *O. urolepishornorum*) to hypersaline water and it was found that the main morphological indicator of hypersaline stress and the most sensitive of several test end points, are ultrastructural changes in gills [10].

Gills are the target of a stress response because of the relative fragility of the gills compared to other surface tissues and the gills are constantly exposed to the outer environment of the fish and what happens is that the structure of the gills is able to withstand and compensate for the chemical and physical attacks that are always faced [9].

3.3. Effect of Tilapia treatment

The statistical analysis used to determine the effect of treatment on the number of operculum openings of Tilapia was the Kruskal-Wallis non-parametric test. The test was carried out because the resulting data did not spread normally and was not homogeneous so it was analyzed through non-parametric analysis.

Based on the results of statistical analysis using the Kruskal Wallis Test, it was found that there was a statistically significant difference in salinity between groups 10, 20, 30 and 40 ppt, with a group average ranking of 10 ppt of 23.83; 20 ppt for 27.17; 30 ppt for 11.89 and 40 ppt for 11.11. For the value of H (Chi-square) in this test result is significant with $H = 17.645$ and $p = 0.001$ which is less than 0.05 (accept $H_1$). Further tests were carried out to determine the interaction between treatments. The test used was the Mann Whitney test between 10 vs 20; 10 vs 30; 10 vs 40; 20 vs 30; 20 vs 40 and 30 vs 40.

Table 1. Further Mann-Whitney test 10 vs 20; 10 vs 30; 10 vs 40; 20 vs 30; 20 vs 40 and 30 vs 40 ppt

| Asymp. Sig. (2-tailed) | The number of operculum opening |
|------------------------|-------------------------------|
| 10 vs 20               | .185                          |
| 10 vs 30               | .004                          |
| 10 vs 40               | .004                          |
| 20 vs 30               | .004                          |
| 20 vs 40               | .004                          |
| 30 vs 40               | .004                          |

A follow-up test in the form of the Mann Whitney test between salinity groups 10 and 20, found that there was no significant difference with $p$ value $> 0.005$. But, salinity group 10 with 30; 10 vs 40; 20 vs 30; 20 vs 40 and 30 vs 40 ppt, it was found that it was significantly different with $p$ value $< 0.005$. 
(Table 1). Tilapia can generally tolerate 20-35 ppt salinity. Tilapia salinity tolerance is also influenced by the sex and size of the fish. Adult fish can tolerate an immediate shift to 27 ppt with a 100% mortality rate at 37. Salinity tolerance depends on the fish species, strain and size, adaptation time and methods and environmental factors. [11, 12] stated that Oreochromis mossambicus, O. aureus and T. zillii were the most tolerant tilapia species. O. mossambicus can tolerate up to 120 water salinity, but they can grow normally and reproduce at 49 water salinity, and their seeds live and grow quite well at 69. Blue tilapia (O. aureus) and tilapia (O. niloticus) less tolerant of salinity.

Behavior as part of the fish response when the salinity is more than 15 ppt that occurs begins to indicate a stress response with passive movements then tends to swim actively and the direction of swimming is irregular. This is part of the stress response of fish due to high salinity pressure so that the fish try to adapt but the duration of their survival is shorter until they finally experience death.

The survival of some fish at higher salinity concentrations (30-34 ppt) increase the chance for selection for salinity resistance in the Nile Tilapia [13]. In the present study, the survival rate of Tilapia different based on salinity levels. [14] had similar observation on Tilapia and observed that survival rate of fish significantly varied with different salinity levels. Same with our and previous studies. O. niloticus is more moderate in its tolerance to saline conditions and dies at salinities >20,000 ppm [15]. [16] had a result in their research indicate that O. niloticus can survive and grow well at salinities up to 4000 ppm. The survival rate of 100% between 0 and 7‰ shows the fish were able to withstand a wide salinity range depends on the ability of the body fluids to function at least for short time in an abnormal range of internal osmotic and ionic concentrations [17, 18]. [19] reports on juveniles of Florida Red Tilapia can survive at 35 ppt salinity.

Increased size of the fish suggested that the fish were able to regulate osmotic pressure of the body fluid [20]. The more the osmo-regulatory adaptation, the greater the difference between the compositions and pressures of the internal fluid of the organism and its external environment. The salinity regimes of 0-7‰ were well tolerated by the fish [21]. [22] In their experiments, a direct response to salinity tolerance was outlined, indicating that Red Tilapias have greater capacity for osmotic regulation as intestinal and gill adaptations, drinking rate regulation and chloride cell proliferation an adaptive mechanism in hypersaline-tolerant fish species [23].

4. Conclusion
Red Tilapia has a salinity tolerance of up to 24 ppt. The behavior of tilapia during changes in salinity was light stress at salinity 10 and 20 ppt. However, when it rises to 30 ppt the fish are already experiencing severe stress, which is indicated by erratic swimming direction (up, down, to the right, to the left) and the number of operculums that are getting slower.

Acknowledgements
We thanked Universitas Syiah Kuala through the facility support at the Geospatial Laboratory of Marine and Fisheries Faculty of Universitas Syiah Kuala.

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