The motility and motion duration of jatimbulan tilapia
(Oreochromis niloticus) spermatozoa in different salinity

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Abstract. Tilapia hatchery is still conducted in freshwater and seeds are death simultaneously when cultivated in high salinity due to the acclimatization process. An alternative method to implement hatchery at high salinity is required. This study aims to determine the salinity of activation medium that provides the best Jatimbulan Tilapia sperm motility and motion duration at high salinity. The study applies completely randomized design (CRD), which consists of 5 treatments (0 ppt, 4 ppt, 9 ppt, 14 ppt and 19 ppt) and 4 repetitions. The parameters consists of sperm motility, motion duration, fresh sperm data (volume, color, odor, pH, consistency, and the concentration of sperm) and sperm abnormalities. The results exhibited that salinity significantly (p < 0.05). Influeneed the sperm motility and motion duration. Motility reaches its best at 0 ppt and 4 ppt (93.4 % and 87.8 %). For motion duration, best condition was in 0 ppt and 4 ppt treatments, totaling 2128 seconds and 1961.5 seconds. Meanwhile, sperm did not move when treated in waters with 9 ppt, 14 ppt and 19 ppt salinities.

1. Introduction
Tilapia is a species of fish with a high economic value [1] and euryhaline, which means that they are able to live and reproduce at a salinity above 30 ppt. The Freshwater Aquaculture Development Center (BPBAT) in Umbulan, East Java [2] has developed a superior tilapia seed through individual selection and has named as Jatimbulan tilapia (Jatimbulan stands for Jawa Timur (East Java) Umbulan). Similar to the case of milkfish (Chanos chanos), fish farmers have began to consider tilapia as a main commodity. Tilapia breeding has been cultivated in freshwater so when farmers cultivate seeds at high salinity, the seeds often die because of the abrupt change to salinity [3]. An alternative method that can be done to cope with death in high salinity is by hatching Jatimbulan tilapia in high salinity.

Research has been conducted as an efforts to hatch Jatimbulan tilapia at high salinity. Jatimbulan tilapia can tolerate 0 to 15 ppt salinity, but 10 ppt salinity is more recommended for to obtain optimum results. However, the results were only based on experiments performed in the laboratory with artificial fertilization. Hence further research on natural fertilization is required to prove the success of Jatimbulan tilapia hatchery at high salinity. The inhibiting factor in the natural fertilization of Jatimbulan tilapia is the suitable salinity for the motility and motion duration of the sperm remains unknown.
Fertilization in tilapia occurs externally [4]. According to Morita et al. [5] sperm motility is regulated by osmolality and calcium ion \([Ca^{2+}]\). When the sperm comes in contact with a hypotonic environment, it turns hypertonic to the environment. This causes the \(Ca^{2+}\) ion to increase and protein phosphorylation to take place so that the sperm becomes motile. According to Takai and Morisawa [6] argues that isotonic solution makes the sperm become nonmotile. When tilapia sperm is in contact with a hypertonic environment, its sleeve shrinks and its intracellular ion \([Ca^{2+}]\) is not increased so the sperm becomes nonmotile and dies [5]. The increase in salinity causes a decrease in the sperm motion duration of carp fish. If sperm motion duration and motility decreases, the ability of sperm to penetrate the micropyle is weak. Therefore, further research is required on the motility and motion duration level of Jatimbulan tilapia sperm at different salinities.

2. Methodology
The research was conducted using sperm derived from a Jatimbulan strain tilapia male parent, sperm coloring in form of eosin 2 %, \(NaCl\) 3 %, physiological \(NaCl\), saltwater, and freshwater. This research employed experimental methods with a completely randomized design (CRD), which consists of five salinity treatments, i.e: 0 (control), 4, 9, 14 and 19 ppt with four repetitions for each treatment.

Sperm retrieval was performed with the stripping technique, which provides subtle pressure on the belly of the fish starting from the linea lateral to the genital orifice. Sperm was collected in a container for different salinity treatments as stipulated in this study. Sperm was stored in a physiological \(NaCl\) with a 1:9 ratio of sperm to diluent. The sperm was stored in a refrigerator [7].

The main parameters of this study were sperm motility and motion duration. Observation of sperm motility was conducted through a light microscope with 400x magnification, while a hand tally counter was used to calculate the progressive motile sperm. Observation of motion duration was done through a 100x-magnification microscope, while a stopwatch was used to calculate its duration. Support parameter observations in this research includes the quality of fresh sperm (volume, color, odor, pH, and consistency) which was observed at baseline and sperm abnormalities that are observed at the end of the study. Support parameter observations include the examination of fresh sperm quality and sperm abnormalities. Macroscopic examinations covers volume, color, odor, pH, and consistency. Microscopic examination include sperm concentration [8]. Abnormality observations are carried out by examining the preparation of sperm smear.

Data of sperm motility and motion duration were analyzed using ANOVA. The mean were also compered using the Duncan's Multiple Range Test with the preset level of significant at \(p < 0.05\).

3. Results and discussion
Fresh sperm examination was conducted in macroscopic and microscopic manners. The results of the examination of Jatimbulan tilapia fresh sperm is illustrated in table 1. Table 1 exhibits the condition of fresh sperm from a Jatimbulan tilapia parent aged seven months weighing 502 grams and has undergone two spawnings. It is generally seen that the sperm volume was higher than the literature. The color, odor, and pH indicated the same condition with several results in the literature. Meanwhile, the concentration of sperm was higher than the literature.

The motility rate of the fish was observed by calculating the percentage of progressive motile sperm. Observations of motility rate are displayed in table 2. ANOVA test results indicated that salinity significantly \((p < 0.05)\) affected the sperm motility. The Duncan's multiple range test indicates that the highest average of motility is present in 0 ppt treatment (controls) that significantly differs \((p < 0.05)\) from 4 ppt, 9 ppt, 14 ppt, and 19 ppt treatments.
Table 1. Jatimbulan tilapia fresh sperm data.

| Macroscopic                       | Reference       |
|-----------------------------------|-----------------|
| Parameter                         | Research result |
| Volume                            | 2.3 ml          |
| Color                             | White milk      |
| Odor                              | Fishy           |
| Ph                                | 7               |
| Consistency                       | Viscous / Pekat |

Table 2. Jatimbulan tilapia sperm motility rate.

| Treatment | Motility Average (%) ± SD |
|-----------|---------------------------|
| 0 ppt     | 93.4 ± 1.5^c              |
| 4 ppt     | 87.8 ± 2.6^b              |
| 9 ppt     | 0.0 ± 0.0^d               |
| 14 ppt    | 0.0 ± 0.0^a               |
| 19 ppt    | 0.0 ± 0.0^a               |

Note: Different superscript in one column indicates significant difference (p < 0.05)

The average of motility in the Jatimbulan sperm motion duration was measured using a stopwatch and the sperm was observed in a single field of view. Table 2 below illustrates the motion duration.

Table 3. Motion duration of Jatimbulan tilapia sperm.

| Treatment | Average of motion duration (second) ± SD |
|-----------|-----------------------------------------|
| 0 ppt     | 2128 ± 50.2^c                           |
| 4 ppt     | 1961.5 ± 129.7^b                        |
| 9 ppt     | 0.0 ± 0.0^a                            |
| 14 ppt    | 0.0 ± 0.0^a                            |
| 19 ppt    | 0.0 ± 0.0^a                            |

Note: Different superscript in one column indicates significant difference (p < 0.05)

ANOVA test results indicated that salinity had a significant impact (p < 0.05) on the motion duration. The Duncan’s Multiple Range Test results show that the highest average of motion duration is in 0 ppt (control) treatment and was significantly different (p < 0.05) to 4 ppt, 9 ppt, 14 ppt, and 19 ppt treatments. The second highest average of motion duration was found in 4 ppt treatment which was significantly different (p < 0.05) from the lowest average low of motion duration found ini 9 ppt, 14 ppt, and 19 ppt treatments.

Observation of sperm abnormalities was conducted by observing the smear and calculating the percentage of sperm abnormality. The observation of sperm abnormality is exhibited in Table 4.
Abnormal sperm is found was 14 ppt treatment (tail was broken off, tailless head, curled tail) and 19 ppt treatment (curled tail and broken tail). Normal and abnormal sperm can be viewed in figure 6 and figure 7.

Table 4. Jatimbulan tilapia sperm abnormality.

| Treatment | Abnormality (%) |
|-----------|-----------------|
| 0 ppt     | 0               |
| 4 ppt     | 0               |
| 9 ppt     | 0               |
| 14 ppt    | 34              |
| 19 ppt    | 61              |

4. Discussion
The fresh sperm volume of 2.3 ml signifies that Jatimbulan tilapia sperm volume is higher than that of common tilapia. According to Herdiana [9] suggested that red tilapia sperm volume is just 1 ml. The sperm concentration of 9.00 x 10^8 cell/ml is still above the lowest range when compared to that of other tilapia. According to Musa [12], the highest concentration of tilapia sperm was 7.75 x 10^9 cell/ml and the lowest one was 6.83 x 10^8 cell/ml.

The characteristics of Jatimbulan tilapia sperm are milky white, quite thick, and fishy-smelling. This was similar to the physical characteristics of GIFT tilapia sperm: thick and milky white [13]. According to Mar’ati [11], one character of good sperm is the fishy odor (typical sperm odor). The result also indicates that the sperm has a pH of 7 (neutral). According to Condro et al. [7], good sperm has a pH of 7.

The highest level of sperm motility was found at 0 ppt (control) amounting to 93.4%, which was significantly different from that of 4 ppt treatment (brackish water), which amounted to 87.8%. Both were still in the normal range and were even far above the bottom threshold. According to Mar’ati [11] contends that normal motile sperm has motility of 70 % to 90 %.

Figure 1. Jatimbulan tilapia normal sperm (1000x magnification).
Note: na: normal (at 0 ppt); nb: normal (at 0 ppt); nc: normal (at 0 ppt).
Fish sperm turns motile when it is in contact with a hypotonic environment. This causes intracellular ion [Ca$^{2+}$] to increase and the midpiece part of the sperm containing the mitochondria (sleeve) to swell, so that the mitochondria is able to form ATP to become motile [5]. The motility rate at 9 ppt treatment reaches 0% because the sperm does not move at all when treated with 9 ppt water. Takai and Morizawa [6] suggests that isotonic solution causes the sperm to be nonmotile.

The sperm motility rate in 14 ppt and 19 ppt treatments that reaches 0% is supported by Morita et al. [5] who suggested that when fish spermatozoa comes in contact with a hypertonic environment, it causes its sleeve containing mitochondria to contract and its intracellular ion [Ca$^{2+}$] not to increase. This makes ATP to become unformed, the flagellar protein phosphorylation not happen, and eventually the sperm becomes nonmotile. According to Boryshpolets et al. [14] added that hypertonic solution can cause sperm cells to shrink and cause damage to the plasma membrane so that the sperm becomes nonmotile. The motility rate at 0 ppt treatment was significantly different ($p < 0.05$) from that at 4 ppt treatment which was consistent with the statement of Guthrie et al. [15]: the increase in salinity causes a decrease in the percentage of sperm motility. According to Janani et al. [16], the higher the osmolality of the activation media, the lower the amount of ATP will be. Moreover, the lower the amount of ATP, the lower the motility and the shorter the motion duration will be.

The longest sperm motion duration was found at 0 ppt treatment which endured 2128 seconds (35 minutes 46 seconds). Yet the motion duration found at 4 ppt treatment which lasted 1961.5 seconds (32 minutes 69 seconds) was still higher than that of other Oreochromis fish which are only able to move for 1800 seconds or about 30 minutes [5].

The sperm does not move when activated with 9, 14 and 19 ppt treatment waters. This is why 0 ppt and 4 ppt treatments were significantly different ($p < 0.05$) from 9 ppt, 14 ppt and 19 ppt treatments. At the interim, 0 ppt and 4 ppt treatments cause sperm to be motile. According to Morita et al. [5] asserted that freshwater fish sperm turns motile when it is in contact with a hypotonic environment. The sperm did not move when activated with 9 ppt treatment. According to Hannover [17], isotonic solution has a concentration of 9 ppt. According to Ridwan [18], argued that isotonic solution causes sperm to turn nonmotile. The sperm motion duration in 14 ppt and 19 ppt treatments which only lasted 0 seconds is in accordance with the opinion of Morita et al. [5] which argues that when fish sperm comes in contact with a hypertonic environment, it turns nonmotile.

The treatments of 0, 4 and 9 ppt show an abnormality percentage of 0%. This is consistent with the statement Sousa et al. [19] that the sperm plasma membrane is intact when in hypotonic condition (<180 mOsmol/kg). According to Gao et al. [20], isotonic solution has the same osmotic pressure with
that of sperm, thereby maintaining between the balance of osmotic pressure inside sperm cells and the one outside environment and making the cell membrane unbroken and intact. The treatments of 14 and 19 ppt show abnormality percentages of 34% and 61% respectively. According to Gao et al. [20], hypertonic solution may cause abnormality in the form of curled tail sperm. Hypertonic solution will make cytoplasmic vacuoles open and tail membranes become more permeable; hence the curled tail. The 19 ppt treatment caused sperm tail to break off. According to Sousa et al. [19] explains that the damage to the sperm tail can be caused by the difference in osmotic pressure between the sperm and the external environment. This ruptures the cell membrane. Tailless sperm may exist because hypertonic solution causes the cell membranes to contract so that they lose membrane integrity [20].

5. Conclusion
The higher salinity of the water and the activation medium lead to lower motility and shorter motion duration. The best motility rate and motion duration was found at 0 ppt and 4 ppt of salinity treatments. This study suggests that 0 and 4 ppt salinities can be performed as a benchmark to generate good fertilization because the motility rate and motion duration of Jatimbulan tilapia sperm are still in the normal range. Accordingly, Jatimbulan tilapia hatchery at high salinity can use 4 ppt salinity media with a natural fertilization method.

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