Different of aeration rate on hatching rate, abnormality, yolk sac absorption, and absolute length of newly hatched larvae of mahseer (Tor soro)

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Abstract. Mahseer, Tor soro is a local freshwater fish species that has an economical value in Asia including Indonesia. Environmental factors such as aeration, temperature, pH, and salinity affect the high of abnormality and unstable hatching rate. The purpose of this experiment is to determine of aeration rate on hatching rate, abnormality, yolk sac absorption, and absolute length of newly hatched larvae. The treatment of aeration rate are as followed: A) 0 mL/minute; B) 500 mL/minute; C) 1000 mL/minute; D) 1500 mL/minute; E) 2000 mL/minute. The result shows that the aeration rate affects the abnormality, yolk sac absorption, and absolute length of newly hatched larvae (p<0.05). The abnormality at 0 mL/minute was the highest (23.23 ± 0.26%) and the lowest (2.84 ± 0.36%) at 500 mL/minute. The yolk sac absorption 0.1268 ± 0.0056 mm³/day at 1500 mL/minute was the highest compared to the others (p<0.05). The absolute length of newly hatched larvae at 1500 mL/minute (4.6435 ± 0.2518 mm) was the highest compared to the others (p<0.05). The dissolved oxygen value was increased by increasing the aeration rate. The optimal aeration rate for egg incubation should be adjusted to 1000 mL/minute to reduce the abnormality and optimize for larval grow.

Keywords: abnormality; aeration rate; hatching rate; Tor soro; yolk sac absorption

1. Introduction
Mahseer is a local Indonesian freshwater fish consisted of four species, namely Tor soro, Tor tambra, Tor tambroides, and Tor douronensis. These fish are used as a special dish by the Batak tribe in North Sumatra. Batak culture presents fish as a requirement for traditional ceremonies such as marriage and childbirth [1]. Mahseer (Tor soro) is mainly distributed in Sumatera, Kalimantan, and Java Islands. The existence of Tor soro is also threatened with extinction due to overfishing, water pollution, and deforestation [2].

One way to reduce the threat of fish extinction is to cultivate it by creating an optimal environment and conditions for its growth. Aeration is used to increase dissolved oxygen which can reduce gas saturation. The decrease in the level of biological oxygen demand in waters is caused by the respiration of aquatic organisms, both plants and animals, such as fish, benthic animals, bacteria, and zooplankton [3]. The
existence of aeration can provide additional oxygen and circulation in the water. However, if the circulation is given too high, it can cause death in newly hatched larvae [4]. Research about *Clarias batrachus* has proven that water flow and aeration can affect embryo development [5]. The embryonic division phase can stop if the water has no aeration. Besides, the speed of aeration has been shown to affect the hatching rate, hatching time, and the survival rate of *Colossoma macropomum* [6] and *Clarias* sp. larvae [4].

*Tor soro* has been successfully cultivated such as broodstock management (natural, semi-natural, and artificial spawning), and larvae rearing technique. However, the seed production of this species is still unstable and high abnormality produced that caused by environmental conditions. Therefore, the research focused on the aeration rate is needed to increase seed production and reduce abnormality. The purpose of this experiment is to determine the hatching rate, abnormality, yolk sac absorption, and absolute length of newly hatched larvae which are influenced by aeration rate level.

2. **Materials and methods**

The experiment was conducted from July to August 2020 at Research Station for Freshwater Aquaculture Environmental Technology and Toxicology, Cibalagung, Bogor, West Java, Indonesia. Tools that used in this experiment are 15 glasses of aquariums with a size of 70 x 25 x 30 cm, stereo microscope, water checker, aerator, fiber tub, aeration infusion or air regulator, and camera.

The first step of this experiment was to select the male and the female of mature ones, then the female selected was stripped out of the eggs and also the stripped of the male to get the sperm. Finally, egg and sperm were mixed in the basin and added intravenous fluids, then it shook gently until homogenize. Thereafter, 200 eggs are introduced into each aquarium. The room temperature was adjusted to 23 °C using an air conditioner. Water quality parameters including temperature, dissolved oxygen, and pH were measured daily using a water checker. The treatment of this experiment is different aeration rates i.e. A) 0; B) 500; C) 1000; D) 1500; and E) 2000 mL/minutes. Parameters observed of this experiment were:

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\text{Hatching rate} \ [7]: \ HR \% = \frac{\text{Number of hatched eggs}}{\text{Total eggs}} \times 100 \quad (1)
\]

\[
\text{Abnormality} \ [8]: \ \text{Abnormality} \% = \frac{\text{Number of abnormal larvae}}{\text{Total of larvae}} \times 100 \quad (2)
\]

\[
\text{Yolk Sac Absorption Rate} \ [9]: \ \text{Yolk Sac Absorption Rate} \ (\text{mm}^3/\text{day}) = \frac{V_o - V_t}{t} \quad (3)
\]

\[
\text{Absolute Length} \ [7]: \ \text{Absolute Length} \ (\text{mm}) = \text{final length} - \text{initial length} \quad (4)
\]

All data such as hatching rate, abnormality, yolk sac absorption, and absolute length of newly hatched larvae were analyzed using one-way ANOVA. If there is a significant difference then continued to the Duncan test. All data were analyzed using IBM SPSS Statistic version 20 software. Water quality parameters such as temperature, dissolved oxygen, and pH were analyzed using descriptive.

3. **Results**

The result of the hatching rate of *Tor soro* on different aeration rates is presented in figure 1. The hatching rate of *Tor soro* eggs at different aeration rates indicated that the highest (90.63\% ± 2.67\%) was found in the treatment with an aeration rate of 500 mL/minute. Then followed by the treatment of 2000, 1500, 1000, and 0 mL/minute, which the percentage of each treatment are 84.50\% ± 1.19\%, 82.51\% ± 7.01\%, 82.05\% ± 5.91\%,
and 81.24 ± 4.71%, respectively (figure 1). The results of statistical tests showed that there are no differences (p>0.05) between the hatching rates of each treatment.

![Figure 1](image1.png)

**Figure 1.** Hatching rate of *Tor soro* at different aeration rates. The bars followed by the same letters show no significantly different (p>0.05).

Based on the result of the abnormality of *Tor soro* at different aeration rates is presented in figure 2.

![Figure 2](image2.png)

**Figure 2.** Abnormality of *Tor soro* at different aeration rates. The Bars followed by the same letters show no significantly difference (p>0.05).
The percentage of abnormality of *Tor soro* larvae (figure 2) shows that the highest value (23.23 ± 0.26%) was found in the 0 mL/minute treatment, followed by the 2000, 1500, 1000, and 500 mL/minute treatments with each values are 6.39 ± 2.40%, 6.16 ± 1.50%, 4.63 ± 2.24%, and 2.84 ± 0.36%, respectively. This result proves that the higher the aeration speed, the higher the abnormality. The statistical results showed that the abnormality was influenced by aeration rate (p<0.05). The Duncan test results showed that the aeration rate of 500 mL/minute was different from 0, 1500, and 2000 mL/minute (p<0.05). The aeration speed of 0 mL/min also showed different abnormalities (p <0.05) with aeration rates of 500, 1000, 1500, and 2000 mL/minute.

The result of the yolk sac absorption of *Tor soro* at different aeration rates is presented in figure 3. The yolk sac absorption rate (figure 3) shows that the highest value is 0.1268 ± 0.0056 mm³/day at an aeration rate of 1500 mL/minute, followed by aeration rate of 1000, 500, 2000, and 0 mL/minute, which each values are 0.1155 ± 0.0077, 0.0884 ± 0.0063, 0.0874 ± 0.0047, and 0.0770 ± 0.0074 mm³/day, respectively. Based on the ANOVA statistical test, it showed that the aeration rate affected the absorption rate of the yolk sac (p<0.05). The Duncan test results showed that the aeration rates of 1000 and 1500 were different from the aeration rates of 0, 500, and 2000 mL/minute.

![Figure 3. Yolk sac absorption of *Tor soro* at different aeration rates. The bars followed by the same letters show no significantly different (p>0.05).](image)

Based on the result of the absolute length of newly hatched larvae of *Tor soro* at different aeration rates is presented in figure 4. The absolute length of *Tor soro* larvae (figure 4) shows that the highest value is found in the treatment of aeration rate of 1500 mL/minute, which is 4.6435 ± 0.2518 mm, followed by aeration rates of 1000, 0, 2000, and 500 mL/minute with absolute length values respectively 4.2773 ± 0.2659, 4.0113 ± 0.2630, 3.5457 ± 0.1356, and 3.0837 ± 0.1215 mm. Based on the ANOVA statistical test, it was found that the absolute length of the larvae was influenced by the aeration rate (p<0.05). The Duncan test results showed that the aeration rate of 500 mL/minute was significantly different (p<0.05) from 0, 1000, 1500, and 2000 mL/minute. The aeration rate of 2000 mL/minute was significantly different from 0, 500, 1000, and 1500 mL/minute. The aeration rate of 0 mL/minute was significantly different from 500, 1500, and 2000 mL/minute.
Figure 4. The absolute length of *Tor soro* at different aeration rates. Bars followed by the same letters show no significantly difference (p>0.05).

Water quality parameters such as temperature, dissolved oxygen (DO), and pH at different aeration rates during the experiment are shown in table 1.

**Table 1.** Water quality such as temperature, DO, and pH during the experiment.

| Treatment       | Temperature (°C) | DO (mg/L) | pH     |
|-----------------|------------------|-----------|--------|
| 0 mL/minute     | 22.7–23.3        | 3.21–3.91 | 6.43–7.37 |
| 500 mL/minute   | 22.8–23.3        | 6.12–6.64 | 6.47–7.16 |
| 1000 mL/minute  | 22.8–23.4        | 6.58–7.31 | 6.62–6.85 |
| 1500 mL/minute  | 22.9–23.4        | 7.07–7.37 | 6.36–7.09 |
| 2000 mL/minute  | 22.9–23.4        | 7.45–7.9  | 6.57–7.19 |

Based on table 1 showed that the water temperature from the beginning up to the end of the experiment was relatively constant with a ranged from 22–23 °C and the pH values at all treatments were also relative constants with a range of 6 to 7. The dissolved oxygen (DO) was increased with increasing aeration rate. This indicated that the aeration rate affects dissolved oxygen concentration.

4. Discussion

The early stage of fish is a critical period, thus, to improve seed production in aquaculture, some limiting factors should be considered. The limiting factors in the early stage of fish are related to environments such as light intensity, photoperiod, temperature, salinity, pH, dissolved oxygen (DO), turbulence, and aeration rate have influenced embryogenesis, hatching time, hatching rate, growth, and survival in the early stage of larvae [10-12]. However, the present experiment shows that the aeration rate does not affect on hatching rate (figure 1).

Aeration rate is correlated with dissolved oxygen and turbulence or water current has been studied by Ellis *et al.* [10]. The present experiment shows that the highest number abnormality of newly hatched larvae
was found without aeration (control) while increased the aeration rate had also increased abnormality (figure 2). This condition caused by dissolved oxygen and also water current (turbulence) where the higher aeration rate had resulted in high on dissolved oxygen (table 1) and might also increase the water current. Dissolved oxygen is one of the limiting factors for the aquatic organism. Fish embryos developed under hypoxia lost their normal synchronization, and abnormalities in spinal and vascular development are commonly observed on Zebrafish. Moreover, the results of both laboratory and field studies showed a higher percentage of malformation in fish developed under hypoxic conditions, possibly through altering their normal apoptosis. Both in vitro and in vivo studies demonstrated that expression levels of certain genes directly or indirectly related to cell cycle, cell proliferation, and apoptosis, which underpin some of the fundamental processes related to development, are affected by hypoxia [13]. Therefore, the present experiment shows low dissolved oxygen at control treatment caused high abnormality and also the higher of dissolved oxygen at the aeration rate of 2000 mL/minute, suggesting that the appropriate aeration rate for *Tor soro* is 1000 mL/minute.

Yolk sac absorption rate has been affected by aeration [11, 12]. The present experiment shows that the yolk sac absorption rate increased by increasing aeration rate but then decreased at a higher aeration rate (figure 3). This indicated that the aeration rate may be related to energy spend on larvae development and growth. Inadequate of oxygen (control) and even over the dissolved oxygen (at 2000 mL/minute) the absorption is slow compare to aeration rate of 500, 1000, and 1500 mL/minutes. This fact that low and higher aeration rate inhibits the yolk sac absorption. Under this condition not only dissolved oxygen affected by aeration rate on the yolk sac absorption but the highest aeration rate has resulted in increasing the water current. The same result has been reported the absorption of oil globule in *Epinephelus coiodes* larvae show decreased with increasing aeration rate due to serve energy for water current and high dissolved oxygen concentration [11]. Oxygen is critical for multicellular existence. Its reduction to water by the mitochondrial electron transport chain helps supply the metabolic demands of life. The incompletely reduce or increase, reactive oxygen by product of this reaction can be quite toxic, thus, the oxygen should homeostasis [14].

The growth of absolute length at the present experiment show increase with increasing aeration rate but the highest aeration rate is decreased (figure 4). This can be caused by oxygen demands and yolk sac absorption. Inappropriate DO has inhibited the growth and also in immune response in Atlantic salmon [15]. The present experiment revealed that the suitable aeration rate for absolute of newly hatched *Tor soro* larvae is 1000 mL/minute.

Water quality parameters such as temperature and pH are within the optimal range. There exceptions for dissolved oxygen (DO) for newly hatched larvae of *Tor soro* with a range of 3–6 and >7.45 mg/L did not support absolute length. Commonly the larvae freshwater fish have tolerated to DO with ranging from 3–7 [16].

5. Conclusion
Based on the result of this experiment can be concluded that the aeration rate affects the abnormality, yolk sac absorption, and absolute length of newly hatched larvae. The optimal aeration rate for abnormality, yolk sac absorption, and absolute length of newly hatched larvae is found at 1000 mL/minute (with dissolved oxygen is ranging from 6-7 mg/L). Dissolved oxygen (DO) increased following by an increase in aeration rate. To maximize larva production and reduce the abnormality, the aeration rate of 1000 mL/minute should be applied.

References
[1] Siregar B, Barus T A and Ilyas S 2013 Hubungan antara kualitas air dengan kebiasaan makanan ikan batak (*Tor soro*) di perairan sungai Asahan Sumatera Utara *Jurnal Biosains Unimed* 1 (2): 1–11 [in Indonesian]
[2] Haryono, Agus H T, Jojo S, Asih S and Gema W 2010 Aquaculture Technique for Mahseer (Bogor: Puslit Oseanografi-LIPI) [in Indonesian]

[3] Meade J W 1989 Aquaculture Management (New York: Van Nostrand Reinhold)

[4] Nurmansyah R 2019 Effect of aeration rate on hatching and survival rate of Mutiara catfish (Clarias sp.) [Undergraduate Thesis] Bogor (ID): Bogor Agricultural University [in Indonesian]

[5] Rahman S M, Habib M A, Hossain Q Z, Siddiqui M N, Rahman M M and Ahsan M N 2013 Embryonic development of Clarias batrachus under the influence of aeration and water flow Ecoprint: An International Journal of Ecology 18 25–31 DOI: 10.3126/eco.v18i0.9395

[6] Hadi M S 2016 Effect of aeration flow on hatching and survival rates of pomfret (Colossoma macropomum, Cuvier) larvae [Undergraduate Thesis] Semarang (ID): Universitas Gadjah Mada [in Indonesian]

[7] Effendie M I 1997 Biology of Fishery (Yogyakarta: Yayasan Pustaka Nusatama) [in Indonesian]

[8] Wirawan I 2005 Effect of copper sulfate (CuSO₄) exposure on hatching rate, change on gill histopathology and abnormality of Zebra (Brachydanio rerio) larvae [Undergraduate Thesis] Surabaya (ID): Universitas Airlangga [in Indonesian]

[9] Ardimas Y A Y 2012 Effect of media temperature range on growth and survival of climbing perch (Anabas testudineus Bloch) larvae cultivation [Undergraduate Thesis] Semarang (ID): Universitas Gadjah Mada [in Indonesian]

[10] Ellis E P, Wade O W, Ellis S C, Ginoza J and Moriwake A 1997 Effects of turbulence, salinity, and light intensity on hatching rate and survival of larval Nassau grouper, Epinephelus striatus Journal of Applied Aquaculture 7 (3): 33–43

[11] Toledo J D, Caberoy N B, Quinitio G F, Choreasca C H and Nakagawa H 2002 Effects of salinity, aeration and light intensity on oil globule absorption, feeding incidence, growth and survival of early-stage grouper Epinephelus coioides larvae Fisheries Science 68 478–483

[12] Sugama K, Trijoko, Ismi S and Maha S K 2004 Environmental Factors Affecting Embryonic Development and Hatching of Humpback Grouper (Cromileptes altivelis) Larva: Advance in Grouper Aquaculture, ed M A Rimmer et al (Australia: ACIAR) p 134

[13] Wu R 2009 Effect of hypoxia on fish reproduction and development Fish Physiology 27 79–141

[14] Maltepe E and Saugstad O D 2009 Oxygen in health and disease: regulation of oxygen homeostasis-clinical implications Pediatric Research 65 261–8

[15] Burt K, Hamoutene D, Perez-Casanova J, Gamperl A K and Volkoff H 2013 The effect of intermittent hypoxia on growth, appetite and some aspects of the immune response of Atlantic salmon (Salmo salar) Aquaculture Research 45 (1): 124–137

[16] Boyd C E and Tukker C S 1998 Pond Aquaculture Water Quality Management (Boston: Kluwer Academic Publisher) p 700