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Determining Oxygen Consumption of Indonesian Mahseer (*Tor soro)* Fingerlings at Different Size and Stocking Density

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Abstract: Understanding oxygen consumption in fish is one of the essential factors in the survival performance of cultured fish. As this information is established, the appropriate size and optimum stocking density of cultured fish reared in an aquaculture system can be determined for successful larval rearing of mahseer in a hatchery. This study aimed to obtain information on the oxygen consumption rate and critical oxygen level of Indonesian mahseer fingerlings at different sizes and stocking densities. This study was conducted in the Laboratory of Fish Reproduction and Genetics, which belongs to the Research Institute for Freshwater Aquaculture and Fisheries Extension, Bogor. A factorial design with two factors (size and stocking density) and three replications were employed to run the experiments. Oxygen consumption rate was measured using a closed respirometer system (volume: 1.4 L) Parameters observed during the study are oxygen consumption rate and critical oxygen level. The results showed that the highest oxygen consumption rate was found at the size of 6-8 cm at 9.52 mg O_2/g/minute with a stocking density of 7 fish/1.4 L. Oxygen consumption rate of mahseer was increased with the increase of size and stocking density. In this experiment, the critical oxygen level of mahseer fingerlings was observed at 1.44 mg/L. The obtained critical oxygen consumption could be used as a reference to determine the amount of dissolved oxygen that could support the survival rate of mahseer. In rearing practice, it would be important information used for designing early warning and mitigating culture systems to avoid deterioration of dissolved oxygen levels in the water. Therefore, this study can be recommended for improvement mahseer rearing practice for better hatchery production with providing an appropriate oxygenation system for fingerling growth.

Keywords: aquaculture, mahseer, oxygen consumption rate, stocking density.

确定不同规格和放养密度的印度尼西亚马西尔托索罗鱼种的耗氧量

摘要：了解鱼类的耗氧量是影响养殖鱼类生存性能的重要因素之一。随着这些信息的建立，可以确定在水产养殖系统中养殖的养殖鱼的适当大小和最佳放养密度，以便在孵化场成功培育马西尔幼体。本研究旨在获取不同规格和放养密度的印度尼西亚马西尔鱼种的耗氧率和临界氧水平信息。这项研究是在茂物淡水水产养殖和渔业推广研究所所属的鱼类繁殖和遗

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传学实验室进行的。采用具有两个因素（大小和放养密度）和三个重复的因子设计来运行实验。使用封闭式呼吸计系统（体积：1.4升）测量耗氧率研究期间观察到的参数是耗氧率和临界氧水平。结果表明，放养密度为7条/1.4升时，6-8厘米的耗氧率最高，为9.52毫克O_2/克/分钟。和放养密度。在本实验中，观察到马赛尔鱼种的临界氧含量为1.44毫克/升。获得的临界耗氧量可以作为参考来确定可以支持马西尔存活率的溶解氧量。在饲养实践中，用于设计预警和缓解养殖系统以避免水中溶解氧水平恶化的重要信息。因此，可以推荐这项研究来改进马西尔的饲养实践，从而通过为鱼种生长提供适当的氧合系统来提高孵化场的产量。

关键词：水产养殖，马西尔，耗氧率，放养密度。

1. Introduction

Indonesian Mahseer *Tor soro* is a native freshwater fish with highly prospective for aquaculture commodities [1, 2]. In Indonesia, mahseer's farming activities in floating net cages were started in the 1990s [3] by collecting seeds from wild stock [4]. Collecting seeds of mahseer from hatcheries was not possible at that moment because its breeding technology was still not developed yet.

Studies on mahseer's breeding in Indonesia have started since the year 2000, which includes broodstock management [5, 6], artificial spawning [7], and larval rearing [8, 9].

An unsolved problem that becomes the drawbacks of mahseer's domestication is their optimum environmental condition [10, 11], especially oxygen consumption rate. Normally, mahseer lives in highly oxygenated waters [12, 13]. Dissolved oxygen concentration in aquaculture greatly affects fish survival [14, 15].

Information on fish oxygen consumption rate is critical to find optimum stocking density concerning dissolved oxygen availability in the water [16]. Low oxygenated water leads to hypoxic conditions and enforces specific adaptation mechanisms for aquatic biotas, such as lowering their metabolic rate.

Appropriate stocking density allows cultured species to optimize oxygen utilization for their optimum growth [17]. Apart from that, stocking density estimation based on oxygen consumption rate is crucial to avoid overcrowding that exceeds the carrying capacity of the rearing tank.

In addition, the oxygen consumption rate of fish is also affected by its size [18]. Therefore, studies on the effect of these factors are essential to support mahseer's production.

This study aimed to obtain information on mahseer's oxygen consumption rate critical oxygen level at different sizes and stocking densities for optimizing Indonesian mahseer's breeding program in the future.

2. Material and Methods

2.1. Data Collection and Experimental Design

The study was conducted in the Laboratory of Fish Reproduction and Genetics of the Research Institute for Freshwater Aquaculture and Fisheries Extension (BRPBATPP), Bogor, West Java, Indonesia for 60 days. The study was done from January to February 2020 to 144 Indonesian mahseer fingerlings in total.

Fish were acclimatized for seven days in 48 aquariums sized 40×40×40 cm filled with 20 L of water, before the experiment. The size and stocking density of acclimatized fish in each tank were set according to the experimental design.

Two factors factorial design (3×4) was employed for the experiment involving three range of sizes, i.e. 1-2 cm, 3-5 cm, and 6-8 cm, and four levels of stocking densities, i.e. 1 fish/tube, 3 fish per/tube, 5 fish/tube, and 7 fish/tube, see Table 1., while the water temperature is controlled at 27º-28ºC.

The experiment was run in a respirometer control tube (1.4 L) which was connected to a dissolved oxygen meter (Trans instruments HD 3030) following [18] modified protocol. The tube was filled with water until it is full, that no space left which might allow contact between free oxygen in the air and water. Fingerlings were put in the tube according to the experimental design (Table 1). The tube was sealed, and the dissolved oxygen meter attached to the tube was turned on. The fingerlings were observed every five minutes. The dissolved oxygen level was recorded, and the critical oxygen level (COL) was determined when fish started to collapse due to hypoxia.

Table 1 Treatment of the oxygen consumption rate (OCR) and critical oxygen level (COL) experiment

Size	Stocking Density (fish/tube)			Total
	Replication 1	Replication 2	Replication 3	
1-2	1	1	1	3
	3	3	3	9
	5	5	5	15
	7	7	7	21
3-5	1	1	1	3

Continuatioin of table 1

	3	3	3	9
	5	5	5	15
	7	7	7	21
6-8	1	1	1	3
	3	3	3	9
	5	5	5	15
	7	7	7	21
	Total			144

2.2. Data Analysis

Oxygen consumption rate (OCR) was computed based on equation as follows:

$$\text{OCR} = \frac{(\text{DO}_0 - \text{DO}_1) \times \text{V}}{\text{T}}$$

where:

OCR - Oxygen consumption rate O2 (mg O2/minute)

DO_0 - Dissolved oxygen concentration at the beginning of the experiment (mg/L)

DO_1 - Dissolved oxygen concentration at the end of the experiment (mg/L)

V - Water volume in respirometer tank

T - Duration when measuring the oxygen consumption (minute)

This formula was applied because it was relying on fish size and robustness against variability in body weight. Factorial MANOVA with a 95% level of significance was employed to analyze the data taking Pillai's trace as the multivariate test and post-hoc were done using the Games-Howell test. Data analysis was done using SPSS 16.0.

3. Results

Results showed that the oxygen consumption rate of mahseer fingerlings increased along with the increasing size and stocking density (Fig. 1-3). Groups of smaller fish with lower stocking density tend to survive longer even in lower oxygen concentrations compared to the larger fish with higher stocking densities.

The highest oxygen consumption rate occurs at fingerlings with sizes 6-8 cm with the stocking density of 7 fish/1.4 L (9.52±0.17mg O_2/g/minute).

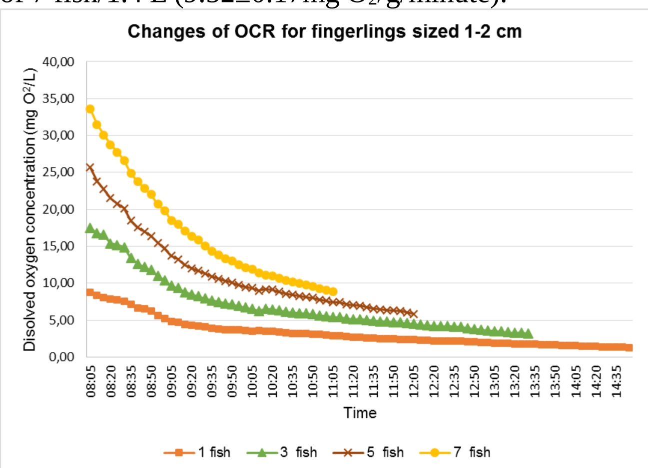

Fig. 1 Oxygen consumption rate changes for fingerlings sized 1-2 cm at four stocking densities.

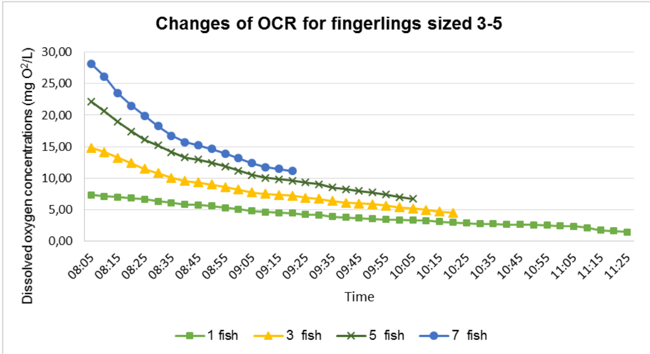

Fig. 2 Oxygen consumption rate changes for fingerlings sized 3-5 cm at four stocking densities

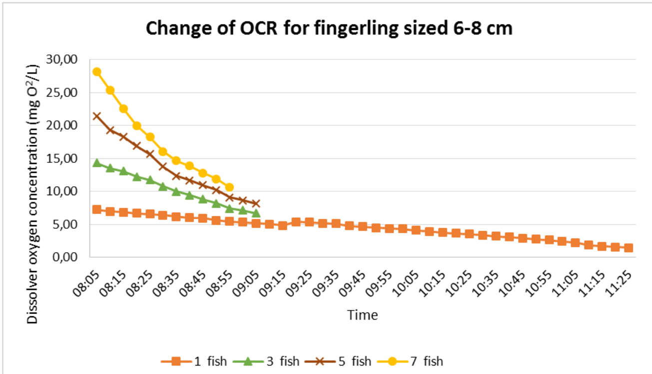

Fig. 3 Oxygen consumption rate changes for fingerlings sized 6-8 cm at four stocking densities.

On the other hand, the lowest rate of oxygen consumption was experienced by 1-2 cm fish size with a stocking density of 1 fish for each tube (1.78 ±0.15mg O_2/g/minute) (Table 2).

Table 2 Oxygen consumption rate and critical oxygen level of mahseer *Tor soro* fingerlings at different sizes and stocking density

Size (cm)	Stocking density (fish/1.4 L)	Oxygen consumption rate (mg O_2/g/minute)	Critical oxygen level (mg/L)
1-2	1	1.78±0.15	1.30±0.05
	3	2.07±0.07	1.32±0.12
	5	2.77±0.28	1.34±0.05
	7	3.15±0.03	1.44±0.06
3-5	1	2.66±0.29	1.41±0.06
	3	3.94±0.46	1.49±0.10
	5	4.97±0.45	1.52±0.13
	7	6.26±0.38	1.51±0.02
6-8	1	2.63±0.05	1.42±0.08
	3	8.41±0.12	1.52±0.08
	5	8.96±0.14	1.42±0.04
	7	9.52±0.17	1.53±0.04

Results of Pillai's MANOVA test suggested that the three factors (size, density, and interaction between size and density) significantly affect mahseer's both OCR and COL together, with the strength of association (partial eta squared) of 0.645, 0.533, and 0.571 respectively. Meanwhile, the test of the between-subject effect resulted in all the three factors also significantly affecting the OCR and COL individually, with the strength of association can be seen in Table 3.

Table 3 Test of between-subject effect for ORC and COL

Source of Variation	Mean Square OCR	Mean Square COL
Size	73.824 (0.989)*	0.69 (0.517)*
Density	26.542 (0.98)*	0.20 (0.316)*
Size × Density	6.196 (0.958)*	0.05 (0.184)*

*Effect significance at 95% confidence interval

Values between brackets show partial association (partial eta squared) of each factor to OCR and COL. The above results indicated that even though all factors were significantly contributed to the OCR and COL value, the effect of all three factors are stronger to the oxygen consumption rate (all association value are greater than 0.95) compared to the COL. Their association to the COL values was moderate and low. These results implied that OCR is more sensitive to changes in fish size and stocking density.

Games-Howell post-hoc test resulted in each size having to mean differences to be significant between groups implying that each group has a different effect on the OCR. However, results from analysis of fish size effect to COL show that the mean difference of group 2 (size 3-5 cm) does not differ significantly from group 4 (size 6-8), which means that fish from the two groups statistically have similar COL. Detail results can be seen in Table 4.

Table 4 Games-Howell post-hoc test of fish size effect to OCR and COL

Size (I)	Size (J)	Mean Difference (I-J) for OCR	Mean Difference (I-J) for COL
1-2	3-5	-2.0092*	-.1350*
	6-8	-4.9325*	-.1267*
3-5	1-2	2.0092*	.1350*
	6-8	-2.9233*	0.0083
6-8	1-2	4.9325*	.1267*
	3-5	2.9233*	-0.0083

*Mean difference is significant at 95% confidence interval

Stocking density also showed a significant mean difference between groups (Table 5) which suggests that each stocking density group also has a different effect on the OCR.

Table 5 Games-Howell Post-hoc test of stocking density to OCR and COL

Stocking Density (I)	Stocking Density (J)	Mean Difference (I-J) (OCR)	Mean Difference (I-J) (COL)
1	3	-2.4511*	-0.0633
	5	-3.2133*	-0.0456
	7	-3.9567*	-.1133*
3	1	2.4511*	0.0633
	5	-.7622*	0.0178
	7	-1.5056*	-0.05
5	1	3.2133*	0.0456
	3	.7622*	-0.0178
	7	-.7433*	-0.0678
7	1	3.9567*	.1133*
	3	1.5056*	0.05
	5	.7433*	0.0678

*Mean difference is significant at 95% confidence interval

4. Discussion

The success of fish fingerling rearing in an indoor aquaculture system depends on water quality conditions, appropriate feeding, and hatchery rearing practices. Among water qualities, oxygen consumption is mainly required for supporting metabolic activities in fish for excellent growth [19]. It is also an important physiological parameter which commonly influenced by environmental parameters changes, physical movement, sex, and bodyweight of fish [20]. In terms of hatchery rearing practice, different stocking density is an essential part impacting the availability of dissolved oxygen in fish rearing tanks [21, 22].

In the present study, the larger size of fish required a higher oxygen consumption rate. Similarly, the increase of stocking density significantly affects the decrease of oxygen consumption rate. Although the mean difference of critical oxygen level was only significant between-group 1 (1 fish/tank) and group 4 (7 fish/tank) (Table 5), this condition indicates that the increase of oxygen consumption rate in larger mahseer fingerlings with higher stocking density occurred because larger fish body mass needs more oxygen for metabolism and respiration process [9, 23, 24]. Furthermore, larger fish also have a more mature respirational system that consumes oxygen more effectively compared to smaller ones. Competition for dissolve oxygen intake is also intense in groups with more crowded fish kept in the tube.

The decrease of oxygen concentration in the tank indicates respirational and metabolic disruption that occurred in the experiment that mainly affects the survival of fish [25]. As there is no supplying oxygenation in the system, lack of oxygenized water affects stress levels in fish [26, 27] which increases ammonia concentration [15] in the tank. The excessive ammonia concentration in the tank can cause oxidative stress because gills cannot dispose of ammonia to the environment and it is retained in the blood system.

Therefore, this condition mainly affects hemoglobin loss in the blood which can bind to oxygen, and oxygen concentration in the blood becomes low.

Fish have different physiological responses to hypoxia [28, 29]. As fish started to move to the water surface in the experimental tank, it can indicate environmental pressures impacted fish mainly related to hypoxia condition. It was found that the critical oxygen level of mahseer fingerlings was 1.44 mg/L. Continued hypoxic conditions in rearing tanks mainly lead to fingerling mortalities [30]. In this experiment, it was observed that low oxygen intake resulted in gill bleedings and burst gall bladder in larger fish. In the meantime, smaller fish observe the loss of equilibrium when exposed to low oxygen concentration. These observations demonstrated that fish show different physiological responses to stressed oxygen levels based on their size. Understanding the oxygen consumption level of cultured mahseer in the hatchery will provide better hatchery practices in providing appropriate water quality for fingerling growth.

5. Conclusion

The oxygen consumption rate of Indonesian mahseer fingerlings was influenced by different sizes and stocking densities of fish reared in aquaculture production systems. Oxygen consumption rate increased with the increase in size and stocking density. It was found that the critical oxygen level of mahseer fingerlings was 1.44 mg/L. For the implementation of rearing practice, providing an appropriate dissolved oxygen system in mahseer hatcheries needs to consider the size and stocking density of fingerlings reared in aquaculture tanks. In addition, this research would be important information used for designing early warning and mitigating culture systems to avoid deterioration of dissolved oxygen levels in the water. Therefore, this study can be recommended for improvement mahseer rearing protocol for better hatchery production.

The use of a closed respirometry in this study to determine oxygen consumption may be relatively simple in construction and operational aspects, but it has not considered the increase and pressure of CO_2 and nitrogenous levels (ammonia and nitrite) in the chamber that possibly affect the resilience and physiological performance of the fish to hypoxia condition. Therefore, for better insights in investigating critical oxygen level on mahseer performance, further study would be better to be designed in intermittent-flow respirometry system to eliminate the impacts of CO_2 and nitrogen accumulation in the system, as well as its physiological effects, happen due to hypoxia condition.

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