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Correlation of Water Quality with OCR and SOD of Humpback Grouper in Net Cages in Concrete Ponds with Different Stocking Densities

Sefilia Adhi Nugroho[1], Gunanti Mahasri[2*], Abdul Manan[2]

[1]Master of Fishery Program, Faculty of Fisheries and Marine, Airlangga University, Surabaya, East Java, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine, Airlangga University, Surabaya, East Java, Indonesia

*Correspondence Author: gunanti.m@fpk.unair.ac.id

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ABSTRACT

The humpback grouper (*Cromileptes altivelis*) is one of the high-value commodities in Indonesia. Humpback grouper production from the cultivation sector has not been able to reach the expected production volume. The Floating Net Cage method is the latest technology in humpback grouper cultivation, but obstacles from weather and environmental factors are very large. The author is interested in conducting research on the stress levels of humpback grouper cultivated in the innovation of net cage cultivation media placed in concrete ponds with different stocking densities. The results showed that water quality parameters were no longer the main factor causing stress in fish raised in net cage cultivation media in concrete ponds. This was shown from the results of the SPSS model output with multiple linear correlation analysis with a significance value of OCR at 0.538 and SOD at 0.112. Stocking density treatment was more influential as a cause of stress in grouper fish at a significance value <0,05.

INTRODUCTION

Grouper is a leading marine aquaculture commodity in Indonesia due to its high selling price and significant market potential in both the national and international markets. Several species of grouper have been developed and cultivated in Indonesia, making them an important fish commodity due to their high prices in both domestic and international markets. Grouper production in 2024 increased from 2022 to 15,353 tons, but this figure only reached 24.34% of the target of 63,052 tons. (KKP, 2024)

According to the Technical Guidelines for Grouper Cultivation (KKP, 2017), the implementation of grouper cultivation using floating net cages (KJA) at sea currently faces many technical obstacles that are difficult to avoid, resulting in numerous losses and failures in grouper cultivation efforts. The main problem faced is that floating net cages at sea are highly dependent on weather and environmental conditions that occur in nature. Changing seasonal patterns, global warming, changes in currents and waves are factors that cannot be predicted and managed by humans. Increasing marine water pollution in coastal areas due to shipping waste or industrial and household waste from coastal settlements also plays a role in becoming a problem facing fish cultivation using the KJA system. Freshwater flows from rivers that carry various kinds of dissolved substances also pose a potential threat to the arrival of diseases and waste from land that gets caught in the cage nets. From a social perspective, cases of fish theft in KJA and damage to KJA nets due to the activities of local fishermen are also not uncommon.

Net cages placed in concrete ponds are expected to reduce the influence of natural conditions on the cultivation process. Concrete ponds refer to a combination of the words "tambak" and "concrete," which can be defined as a water area that is limited or created for cultivating fish, shrimp, or other aquatic animals made of concrete structures, both walls and the pond floor. The influence of currents, tides, and waves can be eliminated. Water quality can be better controlled and conditioned within the optimal range. Sedimentation or the accumulation of organic waste can be conditioned by routine and continuous water circulation. To increase the level of dissolved oxygen in the water, efforts can be made to use a water wheel.

Luh Mayda Ruspita Sari, et al. (2023) in their research stated that the oxygen consumption rate (OCR) is a physiological parameter that indicates the amount of oxygen consumed by fish at a certain time, related to DO concentration, and expressed in units of mgO2/g/hour. Indirectly, oxygen consumption can measure the metabolic rate in fish. The description of energy expenditure by living organisms in a physiological process is called metabolism. The more energy expended or used up for activity, the higher the OCR.

Superoxide dismutase (SOD) is a class of enzymes that limits the biological oxidant cluster enzyme system in the body, which can effectively respond to cellular oxidative stress, lipid metabolism, inflammation, and oxidation. (Zheng, M. et al. 2023). Superoxide dismutase (SOD) activity is an important indicator of fish physiological responses because it is related to fish growth, survival, and physiological immune responses to environmental changes. (Ismawati et al., 2020)

From the description above, the author is interested in conducting research on stress levels assessed from the levels of oxygen consumption (OCR) and superoxide dismutase (SOD) in mouse grouper fish cultivated in innovative containers and cultivation media in net cages placed in concrete ponds with different stocking density treatments.

LITERATURE REVIEW

The growth and development of grouper fish must be supported by good water quality. The optimal temperature for grouper cultivation is in the range of 28–

33°C, salinity 28–33 ppt, dissolved oxygen 4–8 ppm, pH 7.5–8.5, nitrite content 0–0.05 ppm, and ammonia <0.05 ppm. These parameters are in line with the ideal ecological conditions for grouper cultivation, namely a temperature of 24–31°C, salinity 30–33 ppt, dissolved oxygen >3.5 ppm, and pH 7.8–8.0. Optimal water quality conditions can trigger good physiological responses. (Manan & Eshmat, 2013; JAEI UNEJ, 2022; Octopus JIPK, 2024)

There are three types of physiological responses: primary, secondary, and tertiary. The primary stress response in fish is characterized by the production of hormones, namely catecholamines and corticosteroids. Secondary stress responses include impaired osmoregulation, increased lactate and blood glucose levels, and decreased glycogen stores. Tertiary stress responses include behavioral changes and decreased growth. (Prem Kumar et al. 2015)

Stress in fish can also be caused by oxygen consumption levels. Oxygen is a substance needed by fish for their functions, including energy production. It's called the oxygen consumption level (OCR) because the O2 level (OCR) reflects the amount of oxygen required by a given organism. Each organism has a different optimal O_2 level (OCR) (Devina, 2021). The O_2 level is calculated using the following formula according to Djauhari et al. (2019):

$$OCR = \frac{V\ x\ (D0_{t0} - D0_{tt})}{Wx\ t}$$

Description:
OCR : Oxygen Consumption Rate (mg O2/g/hour)
V : Water volume in the container (L)
DO_{t0} : Dissolved oxygen concentration at the start of the observation (mg/L)
DO_{tt} : Dissolved oxygen concentration at time t (mg/L)
W : Weight of the test fish (g)
t : Observation period (hours)

Oxidative stress in fish occurs when the production of reactive oxygen species (ROS) such as superoxide anion (O_2^-) exceeds the protection of antioxidant defense systems. Superoxide dismutase (SOD) helps break down potentially harmful oxygen molecules within cells, preventing tissue damage. Superoxide is produced as a byproduct of oxygen metabolism and, if unregulated, can damage cellular components. The enzyme superoxide dismutase (SOD) catalyzes the conversion of O_2^- to H_2O_2, which is then broken down into water by other enzymes such as catalase or glutathione peroxidase. SOD plays a role in the disproportionation of superoxide free radicals inside and outside the cell, thus protecting cell membranes and DNA from damage caused by oxygen free radicals (Zheng, M. et al. 2023).

METHOD

Tools and materials

The equipment needed in this study is a concrete pool with a size of 30mx14m, a net cage with a size of 3mx3mx1.5m in the form of a rectangular net bag, a mesh size of 25mm or 1 inch, a ½ PK water wheel 1 unit. Standard water quality equipment used such as thermometer, digital DO meter, digital pH meter, refractometer-salinometer, sechii disk, ammonia kit, and plankton net. The fish used are mouse grouper with an average length of ±14cm and an initial weight of ±28gr.

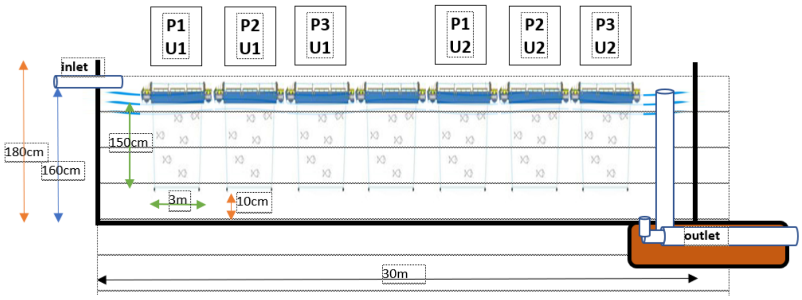

Figure 1. Illustration of net cage media in a concrete pond

Figure 2. Photo of a net cage field in a concrete pond

Research Method

The research method used in this study was an experimental method with a factorial completely randomized design (CRD) using three treatments and two replications for each treatment. Data collection applied an associative quantitative method with an experimental method. The data collected were numeric and continuous. Data collection techniques included observation and experimental measurements (Sugiyono, 2020).

The experiment involved three stocking density treatments with a ratio of 1:2:3 in each cage. The treatment was repeated twice for each stocking density value. The stocking density was determined based on the RSNI Grouper Breeding Reference 3 of 2024, with an average fish size of 13-14 cm and a weight of ±28 grams. The stocking density treatments applied were:

- P1: Stocking density below SNI standards = 8 fish/m3

- P2: Stocking density according to SNI standards = 16 fish/m3
- P3: Stocking density above SNI standards = 24 fish/m3

Analysis Method

Data analysis used quantitative analysis with multiple linear correlation techniques between water quality and stocking density treatment on oxygen consumption levels (O2D) and superoxide demand (SOD) using SPSS version 25 software. This analysis aims to determine how strong the relationship is between these variables and how the independent variables jointly affect the dependent variable. In this study, O2D and SOD are the dependent variables, and the measured water quality parameters, namely temperature, salinity, brightness, pH, and DO, are the independent variables (Kartiningrum, 2022).

RESULT AND DISCUSSION

Result

The results of the OCR and SOD measurements can be seen in Table 1 and Table 2 below:

Table 1. Average OCR Value

Treatment	OCR (mg O_2/L/jam)				
	M1	M2	M3	M4	Rata-rata
1	2,04±0.05	2,01±0,00	1,99±0,21	2,05±0,11	2,02±0,04
2	1,03±.001	1,01±0,00	1,01±0,00	1,09±0,01	1,04±0,01
3	0,69±0.02	0,67±0,01	0,66±0,04	0,69±0,01	0,68±0,02

Note: Values are the mean ± SD

Table 2. Average SOD Value

Treatment	SOD (U/mL)				
	M1	M2	M3	M4	Rata-rata
1	40,02±0,37	51,90±0,04	41,49±0,64	41,44±0,31	43,71±0,3
2	41,99±0,08	53,61±0,51	43,59±0,52	52,18±0,73	47,84±0,5
3	53,70±0,53	54,80±1,54	53,79±0,04	54,47±0,18	54,19±0,6

Note: Values are the mean ± SD

The results of the SPSS application calculations for the analysis of multiple linear correlations of water quality parameters against the OCR and SOD variables can be seen in Table 3 below:

Table 3. Correlation of Water Quality Parameters with OCR and SOD

Variabel Dependent	Mean	Sign. Anova	Temperature	Salinity	DO	pH	Brightness
TKO	1,25±0,58	0,538^{b}	0,338	0,432	0,140	0,378	0,469
SOD	48,58±6,05	0,112^{b}	0,201	0,126	0,004	0,008	0,326

Note: Values are the mean ± SD. Different alphabetic superscripts indicate values that are not significantly different between the dependent variable and the independent variable (water quality) ($p<0.05$, linear regression)

The results of the SPSS application calculations for the multiple linear correlation analysis of stocking density and time parameters on the OCR and SOD variables can be seen in Table 4 below :

Table 4. Correlation Test of Stocking Density and Time on OCR and SOD

Variabel Dependent	Mean	Sign. Multivariate test	Sign. Density	Sign. Time
OCR	1,25±0,58	0,000^{b}	0,000	0,318
SOD	48,58±6,05	0,000^{b}	0,000	0,000

Note: Values are the mean ± SD. Different alphabetic superscripts indicate significantly different values between the dependent variable and the stocking density and time variables (p<0.05, linear model-multivariate)

The results of water quality parameter measurements can be seen in Table 5.

Tabel 5. Average Water Quality Measurement Results

Water Quality Parameters	Average			Feasibility Value
	P1	P2	P3	
Temp	32°C	32°C	32,07°C	28-33°C*
DO	5,29 mg/L	5,32 mg/L	5,32 mg/L	>5*
pH	7,8	7,8	7,8	7,5 - 8,5*
Salinity	32 ppt	32 ppt	32 ppt	28 -33* ppt
Amonia	<0,05 ppm	<0,05 ppm	<0,05 ppm	<0,05* ppm

Description: * RSNI 3 9265:2024 Grouper Fish Breeding

Discussion

Table 1 shows the data results for observing the oxygen consumption level of each cage plot. From the table, it can be seen that in treatment 1 with a stocking density of 8 fish/m2, the OCR value tends to be greater than stocking density treatments 2 and 3. This indicates that at low stocking densities, mouse groupers are able to consume oxygen optimally due to low competition in the cage. In contrast to stocking density treatment 3, where the OCR lift tends to be low below 1, indicating high competition between mouse grouper individuals in consuming dissolved oxygen in the water. M.F Sahetapy (2013) in the results of previous research stated that the volume of water in a maintenance container greatly influences the level of oxygen consumption where the greater the volume of water in the maintenance container will increase the level of oxygen consumption of fish kept in the container. Shofihar Sinansari et al. (2020) in a previous study also stated that fish respond to increased stocking density by decreasing metabolic activity and increasing ventilation rate. However, the increase in ventilation rate is negatively correlated with oxygen consumption in one breath at higher stocking densities due to decreased fish activity, resulting in decreased oxygen consumption at higher stocking densities. This is consistent with the results of the OCR measurements in this study, where in treatment 3, the OCR value tended to be low, indicating a decrease in metabolic activity in grouper. The average OCR value in treatment 1 reached 2.02 with a standard deviation of ±0.04. Meanwhile, the OCR value in treatment 3 was 0.68 with a standard deviation of ±0.02.

Table 2 shows the results of observations and measurements of Superoxide Demand (SOD) values using the SOD Elisa Kit at the Central Research and Diagnostic Laboratory of the Satwa Sehat Malang Animal Clinic. The values obtained tended to be more fluctuating for treatments 1 and 2, while in treatment 3 the SOD value appeared to be more consistently high compared to treatments 1 and 2. Previous research by Sonny Lahati (2024) stated that the SOD enzyme was produced more when mouse grouper was applied with high stocking densities. High SOD activity indicates disturbed physiological activity in the fish. Low SOD activity is suspected to be due to the pressure of the cultivation environment not causing stress to the fish. In this study, the results of the high SOD value measurements in treatment 3 support the results of previous research that stated that the SOD enzyme was produced more when mouse grouper was applied with high stocking densities. The average SOD value in treatment 3 reached 54.19 with a standard deviation of ±0.6. Meanwhile, the SOD value in treatment 1 was 43.71 with a standard deviation of ±0.3..

In multiple linear correlation analysis, significance determination is made by examining the significance value (p-value) or Sig. value (2-tailed) generated from the analysis output. If the significance value is <0.05 (or 0.01 depending on the specified significance level), then there is a significant relationship between the independent and dependent variables simultaneously. Conversely, if the significance value is >0.05 (or 0.01), then there is no significant relationship. The significance of the model in Table 3 produces a value of 0.538b, indicating that the measured water quality parameters (temperature, salinity, DO, pH, brightness) do not significantly affect the Oxygen Consumption Rate (OCR) value. Similarly, for the SOD value, the significance of the model produces a value of 0.112b, which is in line with the significance results for OCR, which exceeds the standard significance value of 0.05. This indicates that the net cage media in concrete ponds assisted by water wheels is able to maintain constant water quality and the absence of fluctuations in water quality parameters is unable to affect the secondary stress response in grouper fish during the maintenance period.

Meanwhile, Table 4 above shows a significance value of .000, indicating that stocking density significantly impacted OCR values and SOD activity in humpback grouper during the rearing period. This finding is also supported by the following graph.

From the two graphs, the first indicator can be seen, that each graph line for each treatment does not intersect each other. This indicates that each treatment has a different influence value on the physiological response of the fish during the maintenance period.

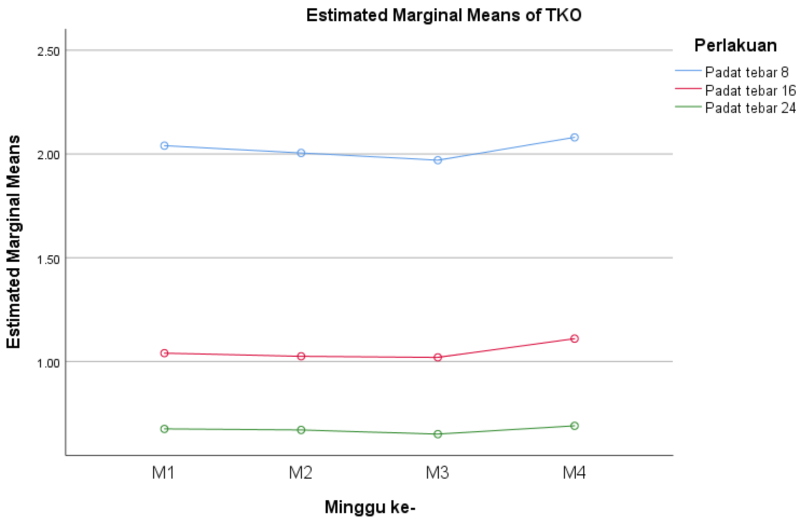

Figure 3. Correlation Graph of Scattered Density with TKO

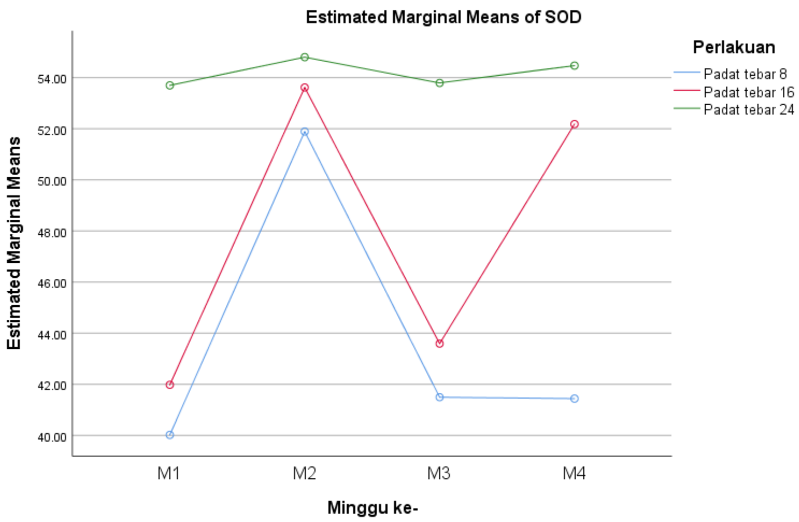

Figure 4. Correlation Graph of Stocking Density with SOD

The stocking density of 8 fish/m3 is classified as a low stocking density, showing a high TKO, which means the stress level in the fish tends to be low because there is no competition for oxygen in the cage. The results of this graph are also supported by the SOD graph at the same stocking density, showing a low SOD value, which means the fish are able to provide a good physiological response to their environment.

In contrast, the stocking density treatment of 24 fish/m3 showed the opposite results to the stocking density of 8 fish/m3, where the TKO graph line showed a low TKO value below 1, which means that competition for oxygen is quite high. This causes a significant physiological response with the SOD graph line at a high value which means the fish are less able to provide a good physiological response to their environment, and can trigger stress in the fish. The stocking density of 16 fish/m3 is the most ideal stocking density value with the graph in the middle. This shows that the fish are able to provide a good physiological response during the maintenance period.

The lowest OCR value was 0.62 at a stocking density of 24 fish/m3 in the third week of the rearing period. Meanwhile, the highest OCR value was 2.16 at a stocking density of 8 fish/m3 in the third week of the rearing period. The lowest SOD value was 39.1 at a stocking density of 8 fish/m3, while the highest SOD value was 55.89 at a stocking density of 24 fish/m3. The results of the measurements and analysis in this study are in accordance with the theory that the higher the stress level in fish, the lower the OCR value and the higher the SOD value.

From the average water quality values in table 5 above, it can be said that the use of the net cage method in concrete ponds with the help of a water wheel can produce optimal water quality parameter conditions for rearing mouse grouper fish.

CONCLUSION

The conclusion that can be drawn from this study is that the maintenance media of net cages in concrete ponds can minimize water quality fluctuations that commonly occur in floating net cages at sea. Therefore, the dynamics of water quality are no longer the main factor causing stress in mouse grouper. Stocking density treatment is a parameter that can cause differences in physiological responses in grouper during maintenance. The higher the stocking density value, the higher the level of stress that can occur in grouper as indicated by the TKO and SOD values. A stocking density of 16 fish/m3 is the most ideal stocking density value with the graph in the middle. It shows that the fish are able to provide a good physiological response during the maintenance period.

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