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Correlation Between Water Quality to Blood Glucose of Cantang Grouper (*E. fuscoguttatus* x *E. lanceolatus*) as an Indicator of Stress in Floating Net Cage

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Abstract. Cantang grouper is one of economical commodities in seawater and profitable in the resource fisheries sector. One of cultivation systems for Cantang Grouper is Floating Net Cage. Changes in water quality can cause stress in fish, can be seen in blood glucose value. This study aims to determine the correlation and water quality factors that influence to glucose level of kerapu cantang fish in floating net cage. This research was using a survey method with samples 10% of the population in 3 units of floating net cages with 4plots. The parameters are blood glucose of cantang grouper and water quality. Data analysis using SPSS ver.22 with linear regression test.The results showed water quality factors simultaneously has a strong correlation and influences to blood glucose of cantang grouper in floating net cage with linear equations $Y = 1170{,}248 + 1639{,}818(X_1) - 1010{,}567(X_2) + 17{,}093(X_3) - 39{,}998(X_4) - 127{,}117(X_5) + 23{,}691(X_6) - 15{,}418(X_7) + 0{,}257(X_8) + 225{,}965(X_9)$. Y = Glucose; X_1 = NO$_2$; X_2 = NO$_3$; X_3 = NH$_3$; X_4 =Dissolved Oxygen; X_5 = pH; X_6 = Temperature; X_7 = Salinity; X_8 = Transparancy; X_9 = Current. Therefore, current, pH, and dissolved oxygen are the influences to blood glucose of cantang grouper in floating net cage.

1. Introduction
One type of grouper that dominates the export world is the cantang grouper. It was recorded that in January 2019 grouper exports from Indonesia reached 277,006 tons. Furthermore, from August 2019 –

December 2019 it continued to increase every month. The export value of this grouper reached 2.20 million USD in December 2019, and became 1.74 million USD in January [1].

This cantang grouper is a hybrid type of grouper resulting from a cross between tiger grouper (*E. fuscoguttatus*) and male kertang grouper (*E. lanceolatus*). This hybridization is carried out in increasing grouper productivity, creating superior species diversification and also having aquaculture prospects that are very likely to increase fishery production in the future [2]. Cultivation of cantang grouper can be done with various systems, be it traditional, semi-intensive or intensive systems. One example of an intensive system that can be used for cantang grouper cultivation is the floating net cage (KJA). Grouper cultivation in floating net cages often fails because cultivators do not care about water quality conditions [3]. Good water quality can be used as a support for fish life that affects growth and physiological responses, but this can also be a stressor so that fish stress [4].

Water quality can include temperature, brightness, current velocity, salinity, dissolved oxygen, pH, ammonia, nitrite, and nitrate. When water quality conditions are poor or water quality fluctuations continue to change suddenly, it will affect the physiological responses of fish such as abnormal behavior. The existence of this response as an evaluation of the physiological response or stress response. Stress response in fish can be seen from changes in cortisol and blood glucose levels [5]. Blood glucose values are considered as one of the indicators used to identify fish responses when experiencing stress.

According to Nasichah *et al.* [6]. when fish experience stress there will be a primary response and a secondary response. Measurement of blood glucose levels is a simple method that can be used to determine when fish are under stress. The limits of optimal water quality for the survival of cantang grouper in KJA must be considered properly in order to support life and its physiological response. Based on this, it is necessary to conduct research related to water quality so that it can be analyzed and known more about these factors. In addition, it can also determine which water quality has a significant effect on triggering the stress response in hybrid cantang groupers kept in floating net cages (KJA).

2. Material and Method

2.1 Time and place
This reserach conducted at the Kerapu Lestari Bancar Pokdakan, Bancar District, Tuban, East Java. Chemical Analysis Laboratory of the Faculty of Fisheries and Marine Airlangga University, and Laboratory of the Environmental Service, East Java. The research was conducted for 3 months, on November 2020 – January 2021.

2.2 Tools and materials
The tools used in this study included nets, plastic buckets, styrofoam boxes, plastic tubs measuring 40 x as many as 13 pieces, aerator, aeration hose, aeration stone, wet cloth, 1 ml syringe, microtube, Glucose kit, tray, ruler. , scales, refractometer, pH meter, DO meter, ammonia kit, nitrate kit, nitrite kit, secchidisk, spectrophotometer, cuvette, erlenmeyer, pipette, analytical balance, and 1000 mL measuring cup. The materials required include cantang grouper 20-30 cm with a weight of ±1000 grams, glucose test strip, 10% EDTA, distilled water, 5% alcohol glycerin, tissue, Ammonium Chloride (NH_4Cl), Phenol solution (C_6H_5OH), Sodium nitroprusside ($C_5FeN_6Na_2O$), Alkaline citrate solution ($C_6H_5Na_3O_7$), Sodium hypochlorite (NaClO), NED Dihydrochloride, Sodium oxalate ($Na_2C_2O_4$), Ferrous Ammonium Sulfate ($Fe(NH_4)_2(SO_4)_2$), Sulfanilamide ($H_2NC_6H_4SO_2NH_2$) ($KMnO_4$), Potassium Nitrate (KNO_3), and Cloform ($CHCl_3$).

2.3 Research methods

The research method used in this research is a survey method. Cantang grouper samples were taken from 10% of the population in 3 units of floating net cages with 4 plots. Sampling was carried out every first week of month. The parameters are blood glucose of cantang grouper and water quality includes pH, temperature, dissolved oxygen, salinity, current, transparancy, ammonia, nitrate, and nitrite. Blood glucose measurements have been carried out by taking blood from the caudal vein [7] then measured with a glucose kit.Water quality measurements were measured directly in floating net cage water, then 200 ml of water samples were taken from each plot, and put into sample bottles. The sample bottle that already contains the sample water is put into a coolbox containing ice cubes [8]. After that the water samples will be taken to the laboratory for testing for ammonia, nitrate and nitrate using spetrophotometry. Data analysis using SPSS ver 22 with linear regression test.

3. Result and Discussion

3.1. Result

The results of multiple linear regression graphs between water quality factors and blood glucose of cantang grouper in floating net cages.
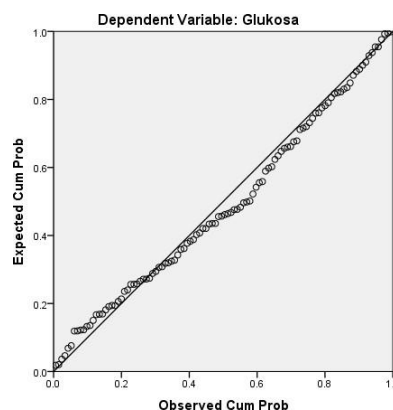

Figure 1. Regression graph of water quality on blood glucose of cantang grouper

Based on the results of SPSS output, the R value is 0.618. The value of R = 0.6 – 0.8 indicates a strong relationship [9] between all independent variables (water quality factors) simultaneously with the dependent variable (blood glucose levels of cantang grouper in floating net cages). The value of the coefficient of determination (R square) was obtained at 0.382. It can be interpreted that the contribution of water quality factors in influencing the glucose levels of cantang grouper in floating net cages is 38.2%, while the remaining 61.8% is influenced by other variables. Likewise, for the value of F obtained 6.725 with a value of sig. <0.05 indicates that water quality factors simultaneously affect blood glucose levels in cantang grouper in floating net cages.

Table 1. Regression results of water quality on blood glucose of cantang grouper.

		Coefficients[a]				
		Unstandardized Coefficients		Standardized Coefficients		
Model		B	Std. Error	Beta	t	Sig.
1	(Constant)	1170,248	1179,170		,992	,323
	NO_2	1639,818	1114,946	,475	1,471	,145
	NO_3	-1010,567	759,976	-,178	-1,330	,187
	NH_3	17,093	53,012	-,053	-,322	,748
	DO	-39,998	12,784	-,463	-3,051	,003
	pH	-127,117	34,266	-,404	-3,710	,000
	Temperature	23,691	17,419	,322	1,360	,177
	Salinity	-15,418	17,345	-,343	-,889	,376
	Transparancy	,257	,325	,132	,788	,432
	Current	225,965	75,068	-,476	-3,010	,003
a. Dependent Variable: Glukosa						

Based on the results of research data processing from multiple linear regression, the value of $Y = 1170{,}248 + 1639{,}818(X1) - 1010{,}567(X2) + 17{,}093(X3) - 39.998(X4) - 127{,}117(X5) + 23.691(X6) - 15{,}418(X7) + 0.257(X8) + 225{,}965(X9)$.

Information :

Y = Glucose   X5 = pH
X1 = NO_2 X6 = Temperature
X2 = NO_3 X7 = Salinity
X3 = NH_3 X8 = Transparancy
X4 = Dissolved Oxygen   X9 = Current

Based on nine independent variables that have been tested, the results of the regression analysis show that dissolved oxygen, pH, and velocity are water quality factors that significantly affect blood glucose levels of cantang grouper in KJA. Where the three variables have a significance value of <0.05 so it can be concluded that dissolved oxygen, pH, and current. significant effect on blood glucose. Based on the value of the variable regression coefficient, dissolved oxygen has a negative value to blood glucose levels, which means that dissolved oxygen increases, glucose decreases and vice versa. Meanwhile, based on the results of regression analysis, it shows that every time there is an increase in the pH value, it will decrease the value of glucose levels. This also applies the reverse is just as true because the pH value has a negative relationship so that it will affect the glucose level of the cantang grouper in the opposite direction. Meanwhile, when the current increases, the glucose level will increase and the other way, too.

3.2. Discussion

Based on the results of the analysis, water quality factors simultaneously have a strong correlation to blood glucose levels of cantang grouper in KJA. Water quality is one of the environmental factors that play a role in the survival of fish, this can be a supporting factor and can also turn into a stressor so that it is closely related to stress and death [4]. The pH value showed a significant effect on blood glucose of cantang grouper in floating net cages. According to Mota *et al.* [10] pH in the sea is rarely of low value because the sea itself has a high buffer capacity. Meanwhile, the pH value in the sea can change. The main cause of changes in pH is due to polluting activities that can cause an increase in theconcentration of carbon dioxide in the atmosphere [11].

Ammonia toxicity is strongly influenced by pH, where it can cause toxicity to the blood. The pH value is very influential because fish cannot adapt to inappropriate values or sudden changes so that fish cannot maintain acid-base regulation [12]. Changes in pH have physiological, ecological and toxicological effects that are significant and harmful to organisms so that they tend to increase fish glucose levels. In some fish species, exposure to low pH results in side effects of decreased growth and food intake. In addition, an inappropriate pH can increase cortisol and glucose levels, and cause stress that leads to immune activity [10], so the pH regression results obtained are negative, meaning that the lower the pH, the higher the grouper's blood glucose.

The dissolved oxygen value in the floating net cages was optimum, where 5 mg/L [13] for cantang grouper so that the glucose levels tended to be normal. However, it is possible to change the dissolved oxygen value. The regression results show a negative value. The negative meaning indicates the opposite direction, when oxygen is low it will cause high blood glucose. The consequences of global warming that occur can make the value of dissolved oxygen in the ocean zone very minimal [14]. If this happens, it will cause significant changes in the biogeochemical cycle accompanied by an increase in the toxicity of seawater. The lower the depth, the higher the temperature, and this high temperature can reduce the solubility of oxygen [14]. Particular the water temperature is important because it will affect the temperature of the fish's body, so that all of its behavior and physiology is influenced and limited by water temperature [15]. It is likely that dissolved oxygen also greatly affects other water quality values such as ammonia, nitrate and nitrite which can cause water to become toxic [16]. Dissolved oxygen is closely related to respiratory disorders, stress, and even death [17]. The values of nitrite, nitrate, and ammonia that cause toxicity in water can be carried actively into the gills and target blood, namely blood plasma which is oxidized by iron in hemoglobin so that blood does not have the ability to bind oxygen or is called methaemoglobin [18], and can cause stress in fish. However, based on the regression results, the values of nitrite, nitrate, and ammonia did not significantly affect the blood glucose of cantang grouper. This is probably because the bottom of the cage is far from the bottom of the water, as well as the current that changes the water circulation in the cage so that the nitrate, nitrite, and ammonia values are still within normal limits.

Current is a vertical or horizontal movement of either the surface mass or the mass of water in the sea. Currents usually move in a certain direction due to the wind and circulate water around the world, so it has a significant impact on organisms [19]. Based on the results of the analysis, the current can significantly affect the glucose levels of cantang grouper in KJA. This is probably because currents can affect other water quality factors, and can directly affect the physical condition of organisms. The current velocity itself can affect many other water quality factors such as high current velocity which can affect the distribution of existing salinity and will be related to dissolved oxygen values [20].

The salinity value obtained is still in the optimal level for cantang grouper, so that it is possible for the salinity value to not have much effect on blood glucose of cantang grouper. The elder of this

cantang grouper, the kertang grouper, has the advantage that it can tolerate changes in salinity, so this can also be one of the reasons for the insignificant salinity of the cantang grouper blood glucose [21].

In addition, the speed of the current can carry suspended substances in the water so that it affects the turbidity and brightness of the water. Current velocity can affect the distribution of waste which can affect the pH value. The value of nitrite, nitrate, and ammonia can also be influenced by the speed of the current, where this current will carry organic matter so that the value can be excessive [20]. The stronger water current velocity can be advantageous because higher water exchange can occur resulting in greater nutrient assimilation and better water quality. In extreme current conditions, currents can cause fish to be physiologically exhausted, and excessive energy expenditure resulting in stress and even death [22].

The low current speed will make the fish swim normally. The current velocity in the marine cage should be 20-50 cm/second [23]. The three KJAs have low current velocities so that the glucose levels of the cantang grouper are also normal. When there is a high current velocity, it will force fish to swim at a speed according to their environment to a certain extent, thus affecting their biological and physiological conditions [24]. This is related to the current velocity regression value obtained, which is positive. The positive meaning indicates that if the current increases, the blood glucose of the cantang grouper will also increase.

4. Conclusion
Based on the research results, it can be concluded that there is a strong influence and correlation between water quality factors simultaneously on blood glucose levels of cantang grouper in floating net cages. There are also water quality factors that have the most significant effect on blood glucose levels of cantang grouper in floating net cages, namely current velocity, pH, and dissolved oxygen.

5. References

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