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# Correlation of Density and Water Quality to Cortisol of Cantang Grouper Fish (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*) in Floating Net Cages

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## Abstract

Cantang grouper is a hybrid resulting from crosses between female tiger grouper (*E. fuscoguttatus*) and male kertang grouper (*E. lanceolatus*). Cultivation with floating net cages is one of the attempts made to cultivate cantang grouper fish. Problems that arise are the non-uniformity of the size of the fish when harvesting, this problem can lead to a decrease in selling prices and maintenance that tends to be longer. Metabolic disorders in living beings can be caused due to physiological stress and one of the causes can arise from water quality. Water quality is closely related to feeding and stocking density because the increase in feed and stocking density can affect the accumulation of feces to stress which causes slow growth. This study was conducted to find out the influence of density and water quality factors that play the most important role in the stress of cantang grouper fish based on indicators of cortisol levels. Cortisol testing is performed using the ELISA method with Bioassay Technology Laboratory E0014Fi kit type. Data are processed using multiple linear regression test analysis that serves to determine the relationship and influence of the free variable (independent) X on non-free variables (dependent) Y. Regression analysis is used to determine the relationship between factors that are causal relationships. After the regression test, then the Analysis of Variance (ANOVA) test is conducted, to find out the difference and influence of dependent parameters with significantly independent. There is a strong influence and correlation between water quality factors simultaneously to the cortisol levels of cantang grouper fish in floating nets, as can be seen from the R value of 0.546. The most influential water quality factor was nitrite with an effect of 19.7%. The next most significant water quality factor was ammonia with an effect of 37.1%. The indicated cortisol value can be known that fish are not in a state of stress due to increased $NO_2$ and $NH_3$. Density has no significant effect on cortisol because of its significant value above ($p>0.05$).

## INTRODUCTION

Cantang grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) is an export-scale marine fishery product. live grouper exports reached 277,006 tons, grouper export volume from August to December, the average reaches 205,572 tons per month. These fish exporter countries include Hong Kong, China, Malaysia, Singapore, the USA, Australia, and France (Alifatri *et al*., 2017). Cantang grouper is superior in growth, which is twice as fast as both parents (female tiger grouper (*E. fuscoguttatus*) and male kertang grouper (*E. lanceolatus*) as well as resistant to diseases and can be cultivated at salinity 15-33 ppt (Anita and Dewi, 2020). Culture using floating net cages is one of the efforts made to cultivate cantang grouper fish. The problem that arises is the non-uniformity of the size of the fish when harvesting. This problem can lead to a decrease in the sale price and longer maintenance. It is suspected that non-uniformity of body size may occur due to the presence of an obstructed metabolism of the body. Metabolic disorders in living beings can be caused due to the presence of physiological stress and this is one of the causes can arise from the quality of the water, so that it can cause negative effects on growth performance and disease resistance (Jentoft *et al*., 2005).

High demand makes farmers have to pay attention to aquaculture management, including water quality that can be caused by dense stocking of fish and feeding. According to Jentoft *et al*. (2005), metabolism disorders in living beings can be caused due to the presence of physiological stress and one of the causes can arise from the quality of the water, which can harm growth performance and disease resistance. According to Athirah *et al*. (2013), environmental factors or water quality are dominant determinants in cultivation so it greatly affects productivity. This is because water is a living medium that affects the growth and survival of fish.

Water quality is closely related to feeding and stocking density because increased feed and stocking density can affect the accumulation of feces in relation to stress which causes slow growth (Susanto *et al*., 2014). Custom stocking can affect the culture environment, especially dissolved oxygen, ammonia, nitrites, and nitrates. When the oxygen content in the waters is low, then nitrites and ammonia can increase, while nitrates are highly dependent on oxygen content in the oxidation process (Isnaini, 2011). Cultural environment will affect the primary and secondary responses of fish. The primary response changes in circumstances by the central nervous system (CNS) thus stress relieving hormones are cortisol and catecholamines. While the secondary response occurs as a result of the release of stress hormones that cause alterations in the blood and chemical tissues such as increased cortisol levels. This study was conducted to determine the influence of stocking density and water quality factors which play the most important role in the stress of cantang groupers based on cortisol level indicators.

## METHODOLOGY

### Place and Time

Research activities were carried out in the floating net cages owned by Fish Farming Group (Pokdakan) Grouper Lestari Bancar, Bancar Village, Tuban Regency, East Java, from October 2020 - February 2021. The ex-situ parameter consists of taking blood samples to check cantang grouper cortisol levels at the Microbiology Laboratory of the Faculty of Fisheries and Marine Affairs Universitas Airlangga and water quality measurements are carried out in the Laboratory Environment Agency, East Java.

### Research Materials

This study used cantang grouper as the main material obtained from the

Floating Net Cage Pokdakan Grouper Lestari Bancar, Bancar Village, Tuban Regency, East Java. Retrieval samples were carried out at station 1 which has a stocking density of 13 individual/$m^2$, station 2 with a stocking density of 18 individual/$m^2$, and station 3 with a stocking density of 20 heads/$m^2$. Fish samples were taken from as much as 10% of the pond population; in addition to blood and plasma of cantang grouper fish, cortisol kit, aquades, alcohol 70%, and EDTA 10% 0.1 ml are required. Water quality testing using water samples from streams of the North Coast of Java in the Tuban region, East Java.

## Research Design

This research uses the survey method, which is a study conducted based on data collection and general analysis of the facts available in the field (Hudjuala *et al*., 2017). The sampling method carried out refers to the time series method; this procedure is used because sampling is carried out with a time interval (Kurniawan *et al*., 2017). Cantang grouper sampling was carried out with three surveys; the time interval used was once a month in the period from October to December 2020. The collection was carried out randomly at several points, along with water samples.

## Work Procedure

The fish samples obtained were carried out through blood draws and measurements of length and weight. Sampling was carried out on three floating net cages units with different stocking solids. Each floating net cage unit had four plots containing fish, the size of each plot was 3 x 3 x 3 m. The sample in this study was a cantang grouper measuring 20-30 cm with a weight of ±1000 g. Grouper sampling was carried out at Grouper Lestari Bancar Pokdakan, Bancar Tuban Village, East Java. According to Umasugi and Burhanuddin (2015), sampling is taken as much as 10% of the total population, which is considered to represent the entirety of the fish population.

Water quality measurements include dissolved oxygen content and water temperature which are measured using a DO meter directly in the water of the floating net cage. Salinity and pH measurements are measured by sipping half of the measuring instrument into the floating net cage's water and waiting for the result. Ammonia ($NH_3$) measurements were carried out using a spectrophotometer with a fenat test method based on SNI 06-6989.30-2005. Measurement ($NO_2$) was carried out using a spectrophotometer with the method used by SNI 06-6989.9-2004. As for nitrate measurements ($NO_3$) these were using a spectrophotometer with an ultraviolet method. Cortisol levels of cantang grouper were measured using an ELISA reader. Cortisol measurements were tested through serum obtained from blood samples of cantang grouper fish, then testing was carried out using a cortisol ELISA kit. Blood was taken using a 1ml syringe with Ethylene anticoagulants diamine tetra acetic acid (EDTA) 0.1 ml through the caudal vein, as it is present in blood vessels located in the ventral part of the vertebrae bone (Hidayaturrahmah, 2015).

## Data Analysis

Data on the results of measuring water quality and blood glucose levels in fish cantang grouper in the collected Floating Net Cage Tuban, East Java were obtained. The closeness of relationships, influences, and factors that are significant between water quality and blood glucose levels of cantang grouper can be known by data analysis with multiple linear regression tests that serve to know the relationship and influence of the free (independent) variable X on non-free variable (dependent) Y. The data were processed using the SPSS 23 program application which was then explained in descriptive analysis. The variables X and Y were entered into the software to perform regression analysis.

## RESULTS AND DISCUSSION

Based on the results of the analysis, the water quality factor simultaneously has a fairly strong correlation to cortisol levels of cantang grouper in floating net cages. In the cultivation system of all water quality parameters, ammonia becomes the second limiting factor after oxygen. The average water quality, stocking density, and cortisol in cantang grouper measuring 20–30 cm with a weight of ±1000 g can be seen in Table 1.

Table 1. Average cortisol, water quality, and stocking density.

| Parameters | Station 1 | Station 2 | Station 3 | Reference |
|---|---|---|---|---|
| Cortisol( ng/mL) | 83.08 ± 11.69 | 91.72±13.96 | 97.16 ± 12.75 | < 400[a] |
| Stocking density (individual/m$^3$) | 13 | 18 | 20 | 20[b] |
| Dissolved Oxygen (mg/L) | 5.47 ± | 5.55 ± | 5.29 ± | ≥5[b] |
| Nitrite (mg/L) | 0.005 | 0.002 | 0.017 | ≤0.05[b] |
| Nitrate (mg/L) | 0.09 | 0.06 | 0.08 | <0.008[b] |
| Ammonia (mg/L) | 0.28 | 0.47 | 0.5 | <0.05[c] |
| Temperature (°C) | 31.5 | 31.25 | 31 | 26–32[b] |
| Salinity (ppt) | 37 | 38 | 38 | 27–35[d] |
| pH | 7.74 | 7.89 | 7.8 | 7.5–8.5[b] |
| Brightness (cm) | 93 | 82.5 | 105 | ≥500[e] |
| Current Speed (m/det) | 0.08 | 0.0625 | 0.013 | 0.2–0.5[f] |

Note: a. Apines-Amar *et al*., 2013; b. SNI 8036.2:2014; c. Wahyuningsih and Gitarama, 2020; d. Ngabito and Auliyah, 2018; e. Sulistiawati *et al*., 2020; f. Yulianto, 2013

Based on the regression results, It shows that the factors that have the most influence on cortisol are $NO_2$, $NH_3$, and current because each has a significance level value of < 0.05, while the stocking density has no effect because the significance level is> 0.05. It is suspected that stocking density has no effect on cortisol directly but can affect other water quality factors. Based on the results of the ANOVA test, an R square value of 0.546 showed a fairly strong relationship between all free variables and current simultaneously with bound variables. Specifically, the biggest factor that affects cortisol is $NH_3$, because it has an influence of 37.1% compared to $NO_2$ which is 19.7%.

Stocking density and water quality are independent factors that will affect the dependent factor, namely cortisol. Based on the results of the analysis, the water quality factor simultaneously has a fairly strong correlation to cortisol levels of cantang grouper at floating net cages. In the culture system of all water quality parameters, ammonia becomes the second limiting factor after oxygen. A low concentration of dissolved oxygen can be controlled by aeration; however, the high concentration of ammonia becomes a factor that is difficult to control (Wahyuningsih and Gitarama, 2020). This regression analysis is the initial stage used to determine the most influential factors between stocking density, temperature, salinity, brightness, pH, DO, current velocity, nitrites, nitrates, and ammonia against cortisol, so that specific regression results can be known as can be seen in Table 2.

Table 2. Output regression coefficients cortisol.

| Model | | Unstandardized Coefficients | | Standard Coefficients | T | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 3148.603 | 1847.163 | | 1.705 | .091 |
| | $NO_2$ | 1079.771 | 326.885 | .710 | 3.303 | .030 |
| | $NO_3$ | 857.426 | 710.261 | .444 | 1.207 | .230 |
| | $NH_3$ | 44.938 | 122.776 | .350 | .366 | .035 |
| | DO | 17.083 | 9.182 | .389 | 1.860 | .066 |
| | Temperature | -8.191 | 14.232 | -.137 | -.575 | .566 |
| | Current | -534.088 | 268.982 | -1.220 | -1.986 | .048 |
| | pH | -427.571 | 251.423 | -2.122 | -1.701 | .092 |
| | Salinity | 4.141 | 4.859 | .144 | .852 | .396 |
| | Brightness | 2.298 | 1.247 | 1.705 | 1.842 | .069 |
| | Stocking Density | .308 | 7.236 | .073 | .043 | .966 |

a. Dependent Variable: Cortisol

Based on the results of the regression, it is known that $NO_2$ and $NH_3$ currently have a significant effect on blood cortisol. The value of the variable regression coefficient, $NO_2$ has a positive value against blood cortisol levels, which means that $NO_2$ has increased, then cortisol has decreased, and vice versa. Based on the results of regression analysis, shows that every time there is an increase in the value of $NH_3$, it will increase the value of cortisol. This also applies the other way around because $NH_3$ values have a positive relationship so it will affect the cortisol values of fish in the opposite direction. A high concentration of ammonia is toxic, causing a decrease in oxygen supply in large numbers and changes in aquatic ecosystems. Fish have several mechanisms to tolerate excess ammonia and reduce toxicity ammonia including excretion and conversion (Cheng *et al*., 2015). But exposure to ammonia at an excessive level leads to disturbed ammonia excretion, resulting in increased ammonia absorption and even death (Sinha *et al*., 2012).

The cell walls of organisms are relatively impermeable to ammonium ions, but ammonia can easily diffuse across tissues where there is a gradient of concentration and acid-base balance. It is known that ammonia molecules move from water through the gill epithelium into the blood and also how ammonia molecules move from the blood to the tissues. This process occurs when ammonia levels in the environment increase, and cause disturbances to the excretion of ammonia or cause the uptake of ammonia from the environment, with the final result of ammonia becoming elevated in the body and causing an increase in the body's cortisol levels (Wahyuningsih and Gitarama, 2020). Meanwhile, if the current increases, the glucose level will decrease and vice versa. After knowing the factors that affect cortisol, an ANOVA test was carried out to determine the difference and its significance in influencing cortisol (Table 3).

Table 3. ANOVA test for cortisol value.

| Dependent Variable: Cortisol Value | | | | | | |
|---|---|---|---|---|---|---|
| Parameters | Type III Sum of Squares | df | Mean Square | F | Sig. | Influence Value |
| Corrected Model | 9098.395[a] | 10 | 909.839 | 11.675 | .000 | .546 |
| Intercept | 267937.900 | 1 | 267937.900 | 3438.16 | .000 | .973 |
| $NO_2$ | 1855.042 | 1 | 1855.042 | 23.804 | .000 | .197 |
| $NH_3$ | 4455.152 | 5 | 891.030 | 11.434 | .000 | .371 |
| Current | 120.257 | 2 | 60.129 | .772 | .465 | .016 |
| $NO_2$ * $NH_3$ | .000 | 0 | . | . | . | .000 |
| $NO_2$ * Current | .000 | 0 | . | . | . | .000 |
| $NH_3$ * Current | .000 | 0 | . | . | . | .000 |
| $NO_2$ * $NH_3$ * Current | .000 | 0 | . | . | . | .000 |
| Error | 7559,263 | 97 | 77.931 | | | |
| Total | 894881,000 | 108 | | | | |
| Corrected Total | 16657,657 | 107 | | | | |

a. R Squared = .546 (Adjusted R Squared = .499)

Based on the results of the ANOVA test output, an R square value of 0.546 was obtained. According to Harahap *et al.* (2013), the value of R ranges from 0.4 – 0.59 indicating a fairly strong relationship between all free variables ($NO_2$, $NH_3$, and current simultaneously with bound variables (cortisol). The R-value obtained can be interpreted to mean that there is an influence of 54.6% between the free variable and the bound variable. Similarly, for F values, it is 23.804 and 11.434 with sig values. < 0.05 indicates that water quality factors simultaneously affect blood cortisol levels in cantang grouper fish in floating net cages. Based on the ANOVA test, it can be seen that there is a significant difference between $NO_2$ and $NH_3$ against the cortisol of cantang grouper fish because it has a significance value of < 0.05. Current values seen in the table do not affect cortisol because the significant value is> 0.05. Specifically, the biggest factor that affects cortisol is $NH_3$, because it has an influence of 37.1% compared to $NO_2$ which is worth 19.7%. The growth of cantang grouper in the study was not affected due to stress. While the growth rate at station 1 is (3.72%), station 2 is (3.54%) station 3 is (3.68%).

The high content of nitrites and ammonia in the floating net cages environment can be caused due to the low current speed. According to Indrayana *et al.* (2014), the speed of the current itself can affect many other water quality factors such as the distribution of existing salinity and will be related to the value of dissolved oxygen and the chemical content of waters. The salinity value obtained is still at the optimal level for cantang grouper, so this is what allows the salinity value not to have too much effect on the cortisol of cantang grouper fish. In addition, the speed of the current can carry suspended substances in water, thus affecting the turbidity and brightness of the water. The speed of the current can affect the distribution of waste which can affect the pH value.

The value of nitrites, nitrates, 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 excess (Indrayana *et al.*, 2014). Under conditions of low currents, the circulation of waters will take place slowly so that the values of nitrites and ammonia can increase. According to Yulianto (2013), the current speed in floating net cages should be 20–50 cm/second. All three stations have low current speeds and do not affect cortisol values. The cortisol value of normal grouper fish was < 400 ng/mL (Apines-Amar *et al.*, 2013), while in this study all cortisol values were under normal conditions so that high nitrite and ammonia values could still be tolerated by fish because the cortisol levels of cantang grouper were relatively normal. The results of measuring the length, weight, and daily growth rate of fish in the floating net cage of The Sustainable Grouper Bancar Pokdakan, Bancar Tuban, East Java, can be seen in Table 4.

Table 4. Growth measurement results on floating net cages.

| Parameters | Station 1 | Station 2 | Station 3 | Optimum Value |
|---|---|---|---|---|
| SGR (%) | 3,72 | 3,54 | 3,68 | 3,48 (Sutarmat, 2012) |
| Average Length (cm) | 28,8 | 29 | 29 | |
| Average Weight (g) | 784,9 | 817,8 | 833,4 | |

The growth of cantang grouper in the study seems not to be affected due to stress, this can be seen from the daily growth rate which is still in line with research conducted by Sutarmat (2012). The study was conducted on cantang grouper fish raised at floating net cages for seven months. From the results of the study, the daily growth rate of cantang grouper was (3.48%). While the growth rate at station 1 is (3.72%), station 2 is (3.54%) while station 3 is (3.68%). This can be because the feed supply has been fulfilled for the formation of energy used in the body's metabolism and the absence of chronic stress occurs so that the energy used to maintain homeostasis and regenerate body cells can be diverted to the growth process. Other research conducted by Wen *et al*. (2012) also shows that differences in growth can be influenced by salinity, because it can affect various physiological processes in aquatic animals such as metabolism and osmoregulation.

Based on the results of the analysis, water quality factors simultaneously have a fairly strong correlation with cortisol levels of cantang grouper in floating net cages. In the cultivation system of all water quality parameters, ammonia becomes the second limiting factor after oxygen. Low dissolved oxygen concentrations can be controlled by aeration, but high ammonia concentrations are factors that are difficult to control (Wahyuningsih and Gitarama, 2020). High concentrations of ammonia are toxic, leading to a decrease in the supply of large amounts of oxygen and changes in the aquatic ecosystem. Fish have several mechanisms to tolerate excess ammonia and reduce ammonia toxicity including excretion and conversion (Cheng *et al*., 2015).

However, exposure to ammonia at excessive levels causes ammonia excretion to be disturbed, resulting in an increase in ammonia absorption and even death (Sinha *et al*., 2012). Exposure to ammonia has a sub-lethal effect whereby lower concentrations may not kill or adversely affect fish in a short period, but the same concentration can kill or damage aquatic life over a long period (Levit, 2010). The sublethal effect of ammonia can lead to growth inhibition, poor feed conversion, reduced resistance to diseases, decreased blood cell count, reduced oxygen levels in the blood, and structural damage to some types of organs (Wahyuningsih and Gitarama, 2020).

Wahyuningsih and Gitarama (2020) report that the most common and possible toxicity in culture is stunted growth rather than acute toxicity leading to death. In general, $NH_3$ concentrations in ponds should not exceed 0.05 mg/L. $NH_3$ concentrations of 0.02-0.07 mg/L have been shown to inhibit growth and cause tissue damage in some fish species. However, the threshold of ammonia toxicity depends largely on the type of species, size, fine solids, surface-active compounds, metals and nitrates (Levit, 2010). It is very likely that dissolved oxygen also greatly affects the value of other water quality such as nitrates and nitrites which can cause waters to become toxic (Ondara *et al*., 2017). Dissolved oxygen is closely related to impaired respiration, stress, and even death

(Zeitoun *et al.*, 2016). The values of nitrites, nitrates, and ammonia that cause toxicity to water can be actively carried into the gills and target the blood, namely blood plasma that is oxidized with iron in hemoglobin so that the blood cannot bind oxygen or called methemoglobin (Kroupova, 2005), and can cause stress in fish.

## CONCLUSION

There is a fairly strong influence of 54.6% between water quality factors simultaneously against blood cortisol levels of cantang grouper in floating net cage. Correlation of water quality factors shows an R Square value of 0.546 which means quite strong and the most significantly influential factor on levels the blood cortisol of the cantang grouper in the floating net cage is nitrites with an influence of 19.7% and ammonia with an influence of 37.1%. Density has no significant effect on cortisol because of its significance value above ($p>0.05$).

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