The health status of hybrid grouper *Epinephelus fuscoguttatus* (♀) × *E. lanceolatus* (♂) cultured in floating-net cage at Kelapa Dua Island, Seribu Islands, Indonesia

Status kesehatan ikan kerapu cantang *Epinephelus fuscoguttatus* (♀) × *E. lanceolatus* (♂) sp pada keramba jaring apung (KJA) di Pulau Kelapa Dua Kepulauan Seribu, Indonesia

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ABSTRACT

This study aimed to identify the fish health status, based on the total erythrocytes (cells/mm³), total leukocytes (cells/mm³), hemoglobin level (g/%), hematocrit, and histopathological profiles. This study was performed on February 2021 – January 2022. The study was located in a floating-net cage of Kelapa Dua Island, Seribu Islands, Jakarta, Indonesia. Sampling was performed by a purposive-sampling method, environmental parameter measurements containing temperature and salinity, clinical symptom observation in fish, total erythrocytes, leukocytes, hemoglobin, hematocrit, histopathological profiles of hybrid grouper liver and kidney, and the existence of viral nervous necrosis (VNN) virus. The observation results present the behavior changes in sick fish, namely whirling on the net cage surface, dead-sleeping or death-like condition below the net cage. Hybrid grouper has total erythrocytes of 2.16×10⁶ to 3.29×10⁶ cells/mm³, total leukocytes of 3.67×10⁴ to 6.31×10⁴ cells/mm³, hemoglobin level of 5.56 to 10.94 g/%, and hematocrit of 13.31 to 24.78 %. From the histopathological observation, necrosis, and vacuolysis occurred in the liver and tubular, leukocyte infiltrate, necrosis was occurred in the kidney and with the highest prevalence of VNN at 20–80 %. Therefore, hybrid grouper is sick due to an increased number of leukocytes at 5.13×10⁶ cells/mm³ above the normal range, followed by decreased hemoglobin and hematocrit levels at 5.56 to 10.94 g/% and 13.31 to 24.78 %, respectively, due to organ damager.

Keywords: hybrid grouper, hematology, histopathology, physical and chemical parameters

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi status kesehatan ikan meliputi total eritrosit (sel/mm³), total leukosit (sel/mm³), kadar hemoglobin (g/%) dan hematokrit (%), serta gambaran histopatologi. Penelitian ini dilaksanakan pada periode bulan Februari 2021 hingga Januari 2022. Lokasi penelitian pada keramba jaring apung (KJA) di Pulau Kelapa Dua Kepulauan Seribu. Metode pengambilan sampel ikan dilakukan secara purposive sampling, pengukuran parameter lingkungan perairan yaitu suhu dan salinitas, pengamatan gejala klinis ikan, perhitungan jumlah eritrosit, leukosit, kadar hemoglobin dan hematokrit serta gambaran histopatologi pada organ hati dan ginjal ikan kerapu cantang dan keberadaan virus VNN. Hasil pengamatan menunjukkan perubahan tingkah laku ikan sakit yaitu berputar ke atas permukaan KJA, terjadi sleeping dead atau ikan berada di dasar KJA seperti mati. Ikan kerapu cantang memiliki jumlah eritrosit 2,16×10⁶ sampai 3,29×10⁶ sel/mm³, leukosit 3,67×10⁴ sampai 6,31×10⁴ sel/mm³, hemoglobin 5,56 sampai 10,94 g/%, dan hematokrit 13,31 sampai 24,78 %, hasil histologi ikan mengalami perubahan sel pada hati yaitu hepatositis, vakuolisis, dan necrosis, serta ginjal mengalami perubahan infiltrasi leukosit, nicrosis, tubulus dan kehadiran virus VNN tertinggi berkisar 20 sampai 80%. Berdasarkan hasil penelitian tersebut maka dapat disimpulkan bahwa ikan kerapu cantang yang sakit karena jumlah leukosit meningkat 5,13×10⁶ sel/mm³ melebihi kisaran normal, kadar hemoglobin menurun 5,56 sampai 10,94% dan kadar hematokrit 13,31 sampai 24,78% rendah dan terjadi kerusakan jaringan pada organ dalam ikan.

Kata kunci: ikan kerapu cantang, hematology, histopathology, parameter fisika dan kimia
INTRODUCTION

Hybrid grouper is one of the highly economical species as a fishery commodity that has been developed in Indonesia. This fish was created from the artificial cross-breeding of two groupers: female tiger grouper *Epinephelus fuscoguttatus* and male giant grouper *E. lanceolatus* (Chaniago, 2020; Mahardika et al., 2020; Prihadi et al., 2020; Yang et al., 2022). Hybrid grouper has a speedy growth with high selling prices and demand in both domestic and foreign markets (Dahlia et al., 2019; Rochmad & Mukti, 2020). Nevertheless, grouper culture still faces a problem due to infectious disease attacks occurred during the production process, such as viral nervous necrosis (VNN) is a disease registered by the office international des epizooties (OIE).

VNN is a major problem in marine fishery production around the world. The infected fish can cause high mortality in aquaculture (Huang & Nitin, 2019; Leaño, 2019; Mahardika et al., 2020; Novriadi et al., 2014; Pradana et al., 2016; Shen et al., 2017; Nurlita et al., 2020; Kumalasari et al., 2022). Kelapa Dua Island is one of the potential hybrid culture locations with a floating-net cage system in Seribu Islands (Ghani et al., 2015). Culture activities with floating-net cage systems are never away from fish disease and environmental quality effects due to available water pollutants. To sustain fish culture activities, fish health management is necessary to conduct (Diniyah, 2019; Hermadi et al., 2021; Effendi et al., 2021; Tino et al., 2022).

If fish are exposed to chemical compounds or certain diseases, it will surely increase or decrease their hematological condition (Alipin & Sari, 2020). Fish health status can be identified through blood quality observation. Changes occurring in blood quality can be caused by disease or environmental conditions. Changes in hematocrit, hemoglobin, total erythrocytes, and total leukocytes are several indicators to determine the fish health condition (Hidayat, 2014; Anderson & Siwicki, 1995; Alipin & Sari, 2020). Based on the description above, this study aimed to identify the hybrid grouper health status based on the total erythrocytes (cells/mm³), total leukocytes (cells/mm³), hemoglobin (g%), hematocrit (%), and histopathological profiles of hybrid grouper liver and kidney.

MATERIALS AND METHODS

This study was performed on February 2021 - January 2022. Sampling was located in floating-net cages in Kelapa Dua Island, Seribu Island Administrative District, Jakarta, as shown in Figure 1.

Water quality

Water quality parameters, namely temperature and salinity, were measured around the floating-net cage area. Five live fish samples were transported with a plastic bag filled with water and oxygen at 30:70 ratio. Transportation was performed using taking grouper samples from floating net cages on Kelapa Dua Island to the fish health laboratory of the Aquaculture Department, IPB University with a closed transportation system, namely using sea transportation (ships) and land transportation (cars) as fish transportation.

Clinical symptoms

Clinical symptoms were observed on VNN-infected fish, including swimming behavior, sleeping-like movement, floating on the water surface, swim-bladder swelling, and appetite loss. Infected grouper displays a nervous disruption related to nervous and retina vacuolization (Novriadi et al., 2014).

Figure 1. Sampling location map.
Viral nervous necrosis (VNN) virus detection

RNA was extracted from the tissue with GENEzol™ (Geneaid, Taiwan), following the kit procedure. The cDNA was synthesized from 100 ng RNA using the RevertraAce qPCR cDNA synthesis kit with gDNA remover (Toyobo, Japan). PCR amplification was performed using the VNN primers to detect VNN and β-actin gene primers as internal sample control, VNN primer sequences 1: Forward: 5’-ACGCAAAGGTGAGAAGAAA-3’ reverse: 5’-TCCCAGATGCCCCA-3’ VNN2: Forward: 5’-AAGCAGACACGACACACCTT-3’ Reverse: 5’-TGTTGGAAAGGGAATCGTG-3’ (Rajan et al., 2016). Viral nervous necrosis was amplified using the nested-PCR method to avoid false-negative interpretation. Each reaction was followed by non-template control (NTC) to avoid false-positive interpretation. After undergoing the process with the nested PCR, the VNN DNA band will be formed at 313 bp. Primers for detecting VNN were specific coat-protein gene from GVNN (grouper VNN).

Histopathological profiles

Liver and kidney samples infected with VNN were cut their tissue at 5 μm thick, fixated with 10% buffer formalin, and stained with hematoxylin-eosin (HE). Histological samples were observed using binocular microscopes at 400x magnification (Cao et al., 2018).

Hematological profiles

Total erythrocytes

By following the Blaxhall and Daisley (1973) method, the formula for total erythrocyte calculation was:

\[ N = n \times 10^4 \]

Note:
\[ N = \text{Total erythrocytes (cells/mm}^3\text{)} \]
\[ n = \text{Total counted erythrocytes.} \]

An erythrocyte pipette absorbed the blood sample to reach the 0.5 limits with an aspirator. Then, Hayem’s solution was added until the 101 limits, before dropping onto the hemocytometer covered with a cover glass. The calculation was performed under the microscope at 400x magnification.

Total leukocytes

By following the Blaxhall and Daisley (1973) method, the formula for total leukocyte calculation was:

\[ N = n \times 50 \]

Note:
\[ N = \text{Total leukocytes (cells/mm}^3\text{)} \]
\[ n = \text{Total counted leukocytes} \]
A leukocyte pipette absorbed the blood sample to reach the 0.5 limits with an aspirator. Then, Turk’s solution was added until the 11 mark, before dropping onto the hemocytometer covered with a cover glass. Calculation was performed under the microscope at 400x magnification.

Hemoglobin (g/%) By Sahli method with Sahlinometer, according to Wedemeyer (1977); HCl 0.1 N was distributed to the diluter tube until reaching scale 2. Blood sample was absorbed by Hb-pippette until scale 20, then the diluter tube was included in a block comparator to compare blood solution color with standard solution. Blood solution height in the scale was counted as Hb value (g/ Hb).

Hematocrit (%)

Hematocrit was counted by ruler and presented in (%), according to Anderson and Siwicki (1993). The microhematocrit method was performed using the microcapillary tube filled with fish blood until reaching four or five of the tube part. Then, the tube was closed with a tube cover. Tube was distributed to the hematocrit centrifuge machine at 5000 rpm for five minutes. After five minutes, the machine was turned off and the tubes were removed, as the hematocrit value was determined by measuring the solution height with a ruler.

Data analysis

Data collected from erythrocytes, leukocytes, hemoglobin, and hematocrit were analyzed using an analysis of variance (ANOVA) and Tukey test. Meanwhile, VNN detection data were analyzed descriptively, following the prevalence calculation below:

\[ \text{Prevalence (100%) = } \frac{\text{Infected fish samples}}{\text{Total fish samples}} \times 100 \]

RESULTS AND DISCUSSION

Result

Temperature and salinity

The measurement results of temperature and salinity for a year in study location, namely on February 2021 – January 2022, are presented in Figure 2.
Clinical symptoms

The observational results of clinical symptoms based on behavior changes are shown in Figure 3. The clinical symptoms display behavior changes in grouper, such as whirling around the net surface, dead-sleeping or death-like on the net base, slow movement, and balance disruption.

VNN virus detection

The virus nervous necrosis (VNN) virus detection results can be descriptively shown as prevalence percentage, presented in Figure 4. The prevalence of VNN in grouper in February 2021 – January 2022 indicate different percentage during the observation, i.e., 60% in February, 80% in March and April, 0% in May and June, 20% in July, 60% in August, 20% in September, 0% in October, 40% in November, 0% in December, and 60% in January. The results indicate that the 80% incidence of VNN virus in March and in April can be caused by abnormal fish health conditions and environmental conditions in waters where temperature and salinity fluctuate due to rainfall. Whereas in July and September, the VNN virus was 20%, indicating an average fish immune system due to the size of the fish included in the consumption fish category and supported by proper aquatic environmental conditions, even though in August, the VNN virus was 60%.

Histopathological profiles

Fish histological condition in the liver and kidney during the rearing period displays changes Figure 5.

Hematological profiles

The observation results of hematological profiles in hybrid grouper on February 2021 – January 2022 are presented in Table 1. The blood profiles of hybrid grouper present the total erythrocytes at $2.16 \times 10^6$ to $3.29 \times 10^6$ cells/mm$^3$, total leukocytes at $3.67 \times 10^4$ to $6.31 \times 10^4$ cells/mm$^3$, hemoglobin at 5.56 to 10.94% g, and hematocrit at 13.32 to 20.71%. In detail, the total erythrocytes, total leukocytes, hemoglobin, and hematocrit based on the ANOVA and Tukey tests from floating-net cage in Kelapa Dua Island, Seribu Islands can be shown in the following Figure 6.

The highest data management results for erythrocyte parameters occurred in December and January, and the lowest occurred in March. This shows that the grouper’s erythrocytes decreased in March due to high leukocytes. The highest mean leukocytes occurred in March and August, and the lowest occurred in June. The highest number of leukocytes occurred in March, which was caused by pathogens (VNN virus and bacteria).

The highest percentage of hemoglobin is in October, and the lowest is in April. The hemoglobin level in the fish’s blood determines the resistance level in the fish’s body. These results indicate a normal hemoglobin condition. Meanwhile, the highest average hematocrit percentage was in September, and the lowest was in May. The decrease in hematocrit levels below 20% is caused by changes in behaviour, such as fish movements that are not balanced due to VNN virus infection.

![Figure 2. Temperature (°C) and salinity (ppt) levels.](image)

Table 1. Hematological profiles of hybrid grouper.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average *)</td>
</tr>
<tr>
<td>Erythrocytes (cells/mm$^3$)</td>
<td>$2.16 \times 10^6$ to $3.29 \times 10^6$</td>
</tr>
<tr>
<td>Leukocytes (cells/mm$^3$)</td>
<td>$3.67 \times 10^4$ to $6.31 \times 10^4$</td>
</tr>
<tr>
<td>Hemoglobin (g/%)</td>
<td>5.56–10.94</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>13.31–24.78</td>
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Note: *) Data were obtained on February 2021 – January 2022.
Figure 3. Behavior changes in VNN-infected grouper: (A) floating on the water surface, (B) abnormal swimming.

Figure 4. VNN prevalence.

Figure 5. Hybrid grouper histology in liver and kidney (H&E, 40×).
Note: A - Liver; B – Kidney; C – Norma liver (Ode et al., 2023); D - Normal Kidney (Apines-Amar et al., 2013); Ct - Tubular cells; Nc – Necrosis; Vc – Vacuolysis; IL - Leukocyte infiltration.

Figure 6. Hybrid grouper blood profiles; total erythrocytes, total leukocytes, hemoglobin, and hematocrit.
Discussion

Based on the clinical symptoms of hybrid grouper in floating-net cage system on Kelapa Dua Island, Seribu Islands, there are several behavior changes observed, namely whirling on the water surface and dead-sleeping by settling on the bottom net (Figure 2). These results were by Lestari and Sudaryatma (2014) and Novriadi et al. (2015), who reported that infected fish often swim on the water surface with slow movement or settled on the bottom net like dead fish. The water temperature in the floating-net cage was measured at 28-32°C. The temperature range required for grouper culture should between 27–30°C (Dedi et al., 2018; Syahputri et al., 2021).

Based on this statement, the water temperature in the floating-net cage is optimal and good for grouper growth, specifically in March, April, May, June, July, August, and October. Meanwhile, the temperature increased above 31–32°C, namely in January, February, September, November, and December. The water salinity in the floating-net cage was 31–34 ppt, which are common salinity range in Indonesian waters. Stated that the salinity level of Indonesian waters is commonly among 30–35 ppt. According to Sugama et al. (2012), seawater used for grouper culture must have a normal salinity range of 33–35 ppt.

Referring to these statements, the water salinity level in this study is quite optimal for hybrid grouper culture (Effendi et al., 2016; Hastari et al., 2017; Prasetyo et al., 2018). Erythrocytes are part of blood components, that are commonly counted for blood profile tests. Based on plots in Figure 5 and Table 1, total erythrocytes obtained the highest value in January at 3.29×10⁶ cells/mm³. While the lowest value was found in March at 2.16×10⁶ cells/mm³. Total erythrocytes obtained a significantly different value only in December, January, and March (P<0.05).

Different total erythrocyte values are affected by changes in fish behavior due to VNN attack and stress conditions. These results followed (Snieszko et al., 1974; Saparuddin, 2018; Lestari & Sudaryatma, 2014), as increased erythrocytes above the average level in normal fish means that the fish is in stress condition, while decreased erythrocytes indicates that the fish has been attacked by the disease. Normal erythrocytes for fish should be among 1.84×10⁶ to 3.35×10⁶ cells/mm³ (Johnny et al., 2003). Leukocytes are part of blood components that play a role in fish defense system, including pathogen attack, by phagocytizing microorganisms and producing antibodies (Robert, 2012).

The average total leukocytes of fish were found to have the highest leukocytes in March at 7.90×10⁴ cells/mm³, and the lowest leukocytes were found in November at 2.39×10⁴ cells/mm³ (Table 1). Based on the Tukey test and plots in Figure 5, total leukocytes were significantly different in March, April, January, and November (P<0.05). Thus, the leukocytes of hybrid grouper are above the normal values. These results are consistent with (Dopongtonung, 2008), as the normal leukocytes in fish are 2×10⁴ to 1.5×10⁵ cells/mm³.

Physiologically, the hemoglobin level in fish blood determines fish’s defense mechanism related to oxygen binding capability in the blood (Putra, 2015). In Table 1, hybrid grouper showed the highest hemoglobin value in October at 10.94 g%/ and the lowest value in April at 5.13 g/%. Hemoglobin level above 8 g/% occurred in September at 9.77 g/%, October at 10.94 g/%, and November at 9.48 g/% (Figure 5). This condition was suspected to occur a faster metabolic rate due to high hemoglobin level, that produced high amount of energy, resulting in fish stress.

This statement was made by Saparuddin (2018), whereas stress condition can be affected by physiological activity and hemoglobin levels in fish. According to Bastiawan et al. (2017), normal hemoglobin level in fish is 12–14 g/%. Hematocrit or packed-cell volume is a blood-formed percentage by erythrocytes (Saparuddin, 2018). This value indicates the percentage of erythrocytes in whole blood after the blood specimen has been centrifuged. Based on the Tukey test, the average hematocrit plot differed significantly in October, May, and April (P<0.05).

The highest hematocrit level was found in September at 24.78%, while the lowest level was found in May at 13.31%. Decreased hematocrit level below 20% was due to behavior changes, namely imbalanced movement, after VNN virus infection. This statement was supported by Royan et al. (2014) who reported that appetite loss occurred, when fish were exposed to environmental changes such as salinity, followed by a decreasing hematocrit level. A hematocrit value of 21% indicates a normal condition (Shabirah et al., 2019), while hematocrit value in teleost fish is among 20-30%, but several marine fish species average hematocrit level of around 42% (Yuliana et al., 2021).
The histological observation found specific cell or tissue changes in the target organs, namely liver and kidney in Figure 5, necrosis, vacuolysis were found around the liver tissue, while tubular, leukocyte infiltrates, necrosis was found around the kidney tissue, as reported by (Andini, 2018; Cao et al., 2018; Rahmawanti et al., 2021). At the same time, histological observation was performed to diagnose fish disease, due to extreme aquatic environment changes in temperature and salinity. In floating net cages (KJA), the presence of the VNN virus is 60% to 80% in June-April (Figure 6). These results indicate that the level of fish immunity will decrease, if the VNN infects continuously. These results were in accordance with (Ben-Asher et al., 2019; Novriadi et al., 2015; Cao et al., 2018; Yuwanita et al., 2018; Mahardika et al., 2020; Kurniawati et al., 2019; Nurlita et al., 2020), as VNN virus is one of the important diseases according to World Organisation for Animal Health (WOAH), which becomes an epidemic almost all over the world and can kill groupers up to 100% in a short time.

CONCLUSION

The health status evaluation of hybrid grouper in a floating net cage on Kelapa Dua Island, Seribu Islands, Jakarta concludes hybrid grouper are unhealthy with the increased total leukocytes at 5.13×10^6 cells/mm³, low hemoglobin level at 5.56-10.94 g/%, and low hematocrit level at 13.31 to 24.78%. From histopathological observation, necrosis was found in liver tissue, while congestion was found in kidney tissue with the prevalence of VNN is 60% to 80%.

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REFERENCES


technology using double net system for hybrid grouper (brown marbled grouper *Epinephelus fuscoguttatus* × giant grouper *Epinephelus lanceolatus*) and rabbitfish (*Siganus canaliculatus*). Earth Environmental Science 521.


Robert RJ. 2012. Fish Pathology, Fourth Edition. WILEY-BLACKWELL.


