Original article DOI: 10.19027/jai.22.2.106-114

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

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

Ruku Ratu Borut[1,2], Sukenda[1*], Sri Nuryati[1], Kukuh Nirmala[1], Irzal Effendi[1], Widanarni[1]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java 16680, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Pattimura, Ambon 97233, Maluku, Indonesia
*Corresponding author: sukenda@apps.ipb.ac.id

(Received January 11, 2023; Accepted January 30, 2023)

ABSTRACT

This study aimed to identify the fish health status, based on the total erythrocytes (cells/mm^3), total leukocytes (cells/mm^3), 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 2.16×10^6 to 3.29×10^6 cells/mm^3, total leukocytes of 3.67×10^4 to 6.31×10^4 cells/mm^3, 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 5.13×10^6 cells/mm^3 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/mm^3), total leukosit (sel/mm^3), 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 $2{,}16\times10^6$ sampai $3{,}29\times10^6$ sel/mm^3, leukosit $3{,}67\times10^4$ sampai $6{,}31\times10^4$ sel/mm^3, hemoglobin 5,56 sampai 10,94 g/%, dan hematokrit 13,31 sampai 24,78 %, hasil histologi ikan mengalami perubahan sel pada hati yaitu hepatosit, vakuolisasi, dan nekrosis, 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 $5{,}13\times10^6$ sel/mm^3 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, histopatologi, 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/mm^3), total leukocytes (cells/mm^3), 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'-AACTGACAACGACCACACCTT-3' Reverse: 5'-TGTGGAAAGGGAATCGTTG-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 = Total erythrocytes (cells/mm^3)

n = 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 400× magnification.

Total leukocytes

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

$$N = n \times 50$$

Note:

N = Total leukocytes (cells/mm^3)

n = 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 400× 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 nervosis 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 2.16×10^6 to 3.29×10^6 cells/mm^3, total leukocytes at 3.67×10^4 to 6.31×10^4 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.

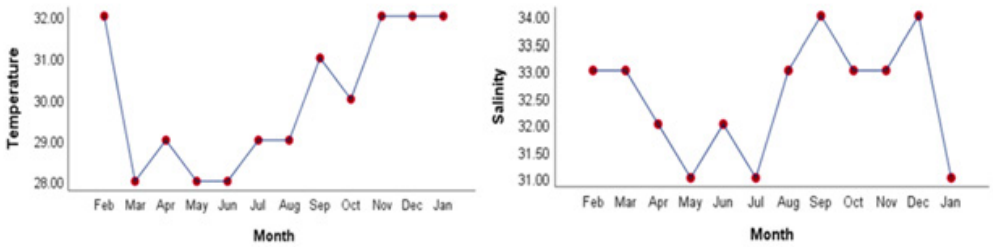

Figure 2. Temperature (°C) and salinity (ppt) levels.

Table 1. Hematological profiles of hybrid grouper.

Parameter	Values		
	Average *)	Normal Range	References
Erythrocytes (cells/mm^3)	2.16×10^6–3.29×10^6	1.84×10^6 – 3.35×10^6	(Johnny *et al.*, 2003)
Leukocytes (cells/mm^3)	3.67×10^4–6.31×10^4	2×10^4–15×10^4	(Dopongtonung, 2008)
Hemoglobin (g/%)	5.56–10.94	12–14	(Bastiawan *et al.*, 2017)
Hematocrit	13.31–24.78	21–40	(Shabirah *et al.*, 2019)

Note: *) Data were obtained on February 2021 – January 2022.

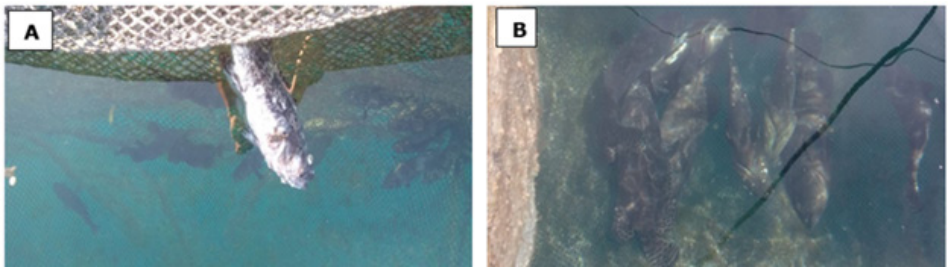

Figure 3. Behavior changes in VNN-infected grouper: (A) floating on the water surface, (B) abnormal swimming.

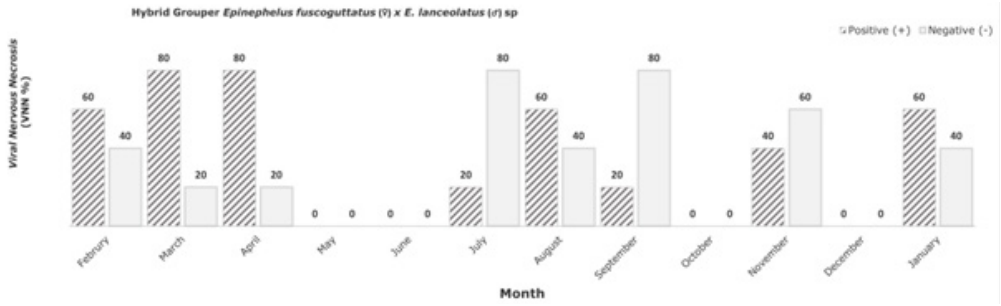

Figure 4. VNN prevalence.

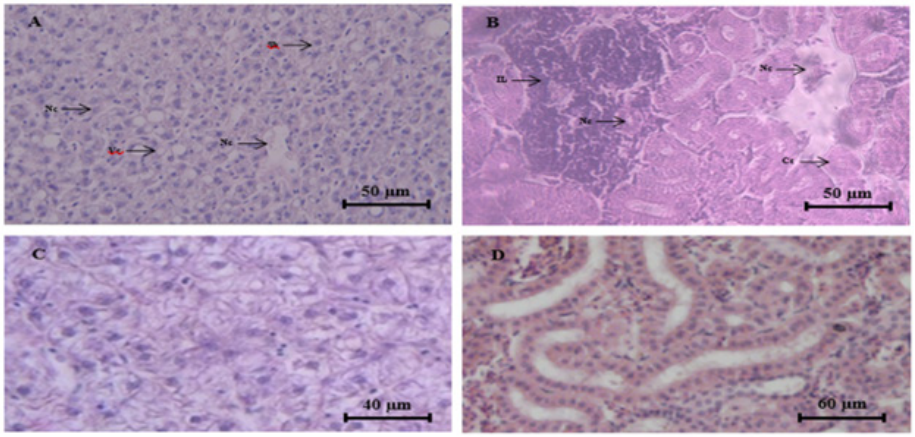

Figure 5. Hybrid grouper histology in liver and kidney (H&E, 40×).
Note: A - Liver; B – Kidney; C – Norma liver (Ode *et al*., 2023); D - Normal Kidney (Apines-Amar *et al*., 2013); Ct -Tubular cells; Nc – Necrosis; Vc – Vacuolysis; IL - Leukocyte infiltration.

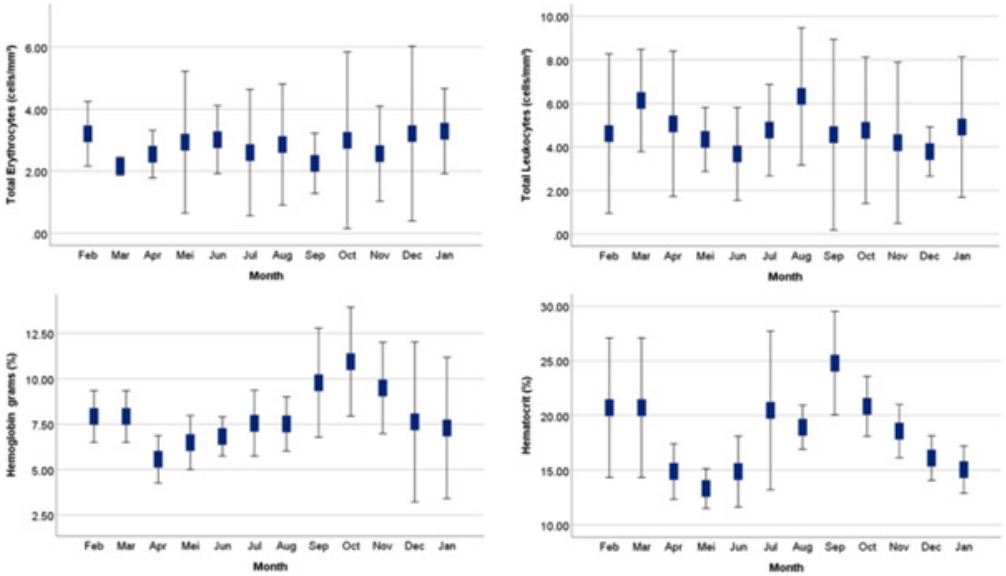

Figure 6. Hybrid grouper blood profiles; total erythrocytes, total leukocytes, hemoglobin, and hematocrit.

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 swam 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 3.29×10^{6} cells/mm^3. While the lowest value was found in March at 2.16×10^{6} cells/mm^3. 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 1.84×10^{6} to 3.35×10^{6} cells/mm^3 (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 2.39×10^{4} 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 2×10^{4} to 1.5×10^{5} cells/mm^3.

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 10.94g/% 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 occurend, 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 5.13×106 cells/mm^3, 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%.

ACKNOWLEDGMENT

The authors would like to thank National Postgraduate Educational Grant (BPPDN), Indonesian Ministry of Education, Culture, Research, and Higher Education, and the fish culturist group in Kelapa Dua Island, Seribu Islands, Jakarta, Indonesia.

REFERENCES

Alipin K, Sari TA. 2020. Hematological analysis as health indicator of (*Epinephelus* sp.) which culture in cages of east Pangandaran Beach, West Java. Metamorf Journal Biology Science 7: 285–292.

Anderson DP, Siwicki AK. 1995. Basic hematology and serology for fish health programs. Diseases Asian Aquaculture 6: 11–20.

Andini NS. 2018. Iridovirus diseases diagnosis in grouper fish (*Epinephelus* sp.) in Seribu Islands with polymerase chain reaction (PCR) method, immunohistochemistry and histopathology [Thesis]. Jogjakarta: Universitas Gadjah Mada.

Apines-Amar MJS, Amar EC, Faisan JP. 2013. Growth, plasma cortisol, liver and kidney histology, and resistance to vibriosis in brown marbled grouper, Epinephelus fuscoguttatus fed onion and ginger. AACL Bioflux 6: 530–538.

Bastiawan D, Taukhid T, Alifuddin M, Dermawati TS. 2017. Haemathologic and histhology changes of dumbo catfish (*Clarias gariepinus*) infected *Apbanomyces* sp. Jurnal Penelitian Perikanan Indonesia 1: 106–115.

Ben-Asher R, Ravid S, Ucko M, Smirnov M, Lahav O. 2019. Chlorine-based disinfection for controlling horizontal transmission of VNN in a seawater recirculating aquaculture system growing European seabass. Aquaculture 510: 329–336.

Blaxhall PC, Daisley KW. 1973. Routine haematological methods for use with fish blood. Journal Fish Biology 5: 771–781.

Cao S, Geng Y, Yu Z, Deng L, Gan W, Wang K, Ou Y, Chen D, Huang X, Zuo Z, He M, Lai W. 2018. Acinetobacter lwoffii, an emerging pathogen for fish in Schizothorax genus in China. Transboundary Emerging Diseases 65: 1816–1822.

Chaniago AA. 2020. Hybridization of tiger grouper (*Epinephelus fuscoguttatus*) x kertang grouper (*E. lanceolatus*). Makalah Ilmiah 1: 1–6.

Dahlia D, Suprapto H, Kusdarwati R. 2019. Isolation and identification bacteria on the seeds cantang grouper (*Epinephelus* sp.) from nursery pond at fisheries center brackish water aquaculture, Situbondo, East Java. Fish Health 6: 57–66.

Dedi D, Irawan H, Putra WKA. 2018. The influence of thyroxine hormone on megami Pellet feed against the growth of *Epinephelus fuscoguttatus-lanceolatus*. Intek Akuakultur 2: 33–48.

Diniyah CZ. 2019. Determining Potential Areas for Grouper Aquaculture in Seribu Islands, Jakarta Using Marxan as a Zoning Tool [Thesis]. Bogor: Institut Pertanian Bogor.

Dopongtonung A. 2008. Blood images of catfish (*Clarias* spp) From the Laladon-Bogor Area [Thesis]. Bogor: Institut Pertanian Bogor.

Effendi I, Diatin I, Budiardi T, Haderoseyani Y. 2021. Grouper Nursery Development through Group Capacity Building for Leading Commodity Strengthen of Seribu Islands Administrative District, Jakarta. Jurnal Ilmiah Pengabdian kepada Masyarakat 7: 148–161.

Effendi I, Suprayudi MA, Nurjaya IW, Surawidjaja EH, Supriyono E, Junior MZ, Sukenda. 2016. Oceanography and water quality condition in several waters of Thousand islands and its suitability for white shrimp *Litopenaeus vannamei* culture. Jurnal Ilmu dan Teknologi Kelautan Tropis 8: 403–417.

Ghani A, Hartoko A, Restiana WR. 2015. Analysis of land suitability from Pari Island waters in Seribu Island as a land fish farming grouper (*Epinephelus* sp.) at floating net cages by using GIS application. Journal Aquaculture Management Technology 4: 54–61.

Hastari IF, Kurnia R, Kamal MM. 2017. Suitability analysis of floating cage culture of grouper fish using GIS In Ringgung waters of Lampung. Jurnal Ilmu dan Teknologi Kelautan Tropis 9: 151–159.

Hermadi I, Fajar Nugraha A, Wahjuni S, Effendi I, Asfarian A. 2021. Design of marine environmental monitoring system to support the k1000 smart mariculture application using the MQTT protocol. Jurnal Ilmu Komputer Agri-Informatika 8: 20–30.

Hidayat AS. 2014. Isolation and dentification of vibrio sp bacteria from sunu group *Plectropomus Leopardus*. Teknosains 8: 209–216.

Huang K, Nitin N. 2019. Edible bacteriophage based antimicrobial coating on fish feed for enhanced treatment of bacterial infections in aquaculture industry. Aquaculture 502: 18–25.

Johnny F, Zafran, Roza D, Mahardika K. 2003. Hematology of several species of marine fish Cultivation. Jurnal Penelitian Perikanan lndonesia 9: 4.

Kumalasari H, Sudaryatma PE, Lestari AT, Nurlita W, Nugraha WA, Hasanah N. 2022. Identification of infectious spleen and kidney necrosis virus and viral nervous necrosis in seawater fish by multiplex polymerase chain reaction. Jurnal Sains Veteriner 40: 188–196.

Kurniawati MD, Sumaryam S, Hayati N. 2019. Application of conventional and Real Time-PCR Polymerase Chain Reaction (PCR) to detection of VNN virus (Viral Nervous Necrosis) in tiger group (*Epinephelus fuscoguttatus*). Techno-Fish 3: 19–30.

Leaño EM. 2019. Transboundary aquatic animal diseases: History and impacts in ASEAN aquaculture. Network Aquaculture Centres Asia-Pacific 72–79.

Lestari AT, Sudaryatma PE. 2014. Immunocytochemical Study on Blood and Organ Suspension of Tiger Grouper (*Epinephelus fuscoguttatus*) Infected with Field Isolate of Viral Nervous Necrosis. Journal Veterinary Science 32: 85–92.

Mahardika K, Mastuti I, Roza D, Syahidah D, Astuti WW, Ismi S. 2020. Monitoring the incidence of marine fish diseases in hatcheries and floating net cages in North Bali coastal water. Jurnal Riset Akuakultur 15: 89–102.

Novriadi R, Agustatik S, Dwi T, 2015. Identification of the presence of nervous necrosis virus, and iridovirus in marine fish farming in the working area of the Batam Marine aquaculture fishery center. Omni-Akuatika XIV: 54–62.

Novriadi R, Agustatik S, Wijayanti E, Kunci K. 2014. Study of the fish disease performance and environmental quality on batam island. Journal Overseer Fishery Aquaculture (Cd).

Nurlita W, Pandit IGS, Darmadi NM. 2020. Detection of the existence of viral nervous necrosis on fry cantang grouper at rain season. Sustainable Environmental Agricultural Science 4: 46–52.

Ode I, Wahjuningrum D, Yuhana M. 2023. The antibacterial activity of clove Syzygium aromaticum extract and its effects on the survival rate of hybrid grouper *Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂ infected with Vibrio alginolyticus. Jurnal Akuakultur Indonesia 22: 1–11.

Pradana D, Kusdarwati R, Sudarno S. 2016. Relation between water quality and endoparasite prevalency on nile tilapia (*Oreochromis Niloticus*) Intestine in Urban Farming Program Float Cage, Surabaya City. Journal Aquaculture Fish Health 5: 115.

Prasetyo ABT, Yuliadi LPS, Astuty S, Prihadi DJ. 2018. Correlation between substrate type and sedimentation rate with conditions of coral reef cover in Panggang Island Waters, Seribu Islands National Park. Journal Fisheries Maritime 9: 1–8.

Prihadi TH, Widiyati A, Hermawan M, Permana N, Radona D, Aubin J. 2020. Fish culture

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.

Putra WS. 2015. Collection of recipes & concoctions of medicinal plants for various health problems. Yogyakarta: Katahati. Rahmawanti A, Setyowati DN, Mukhlis A. 2021. Histopathological of brain, eye, liver, spleen organs of grouper suspected VNN in Penyambuan Village, North Lombok. Journal Biology Tropis 21: 140–148.

Rajan JJS, Praveena PE, Bhuvaneswari T, Jithendran KP. 2016. Design and evaluation of reverse transcription nested PCR primers for the detection of betanodavirus in finfish. VirusDisease. 27(2):123–129.doi:10.1007/s13337-016-0313-0.

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

Rochmad AN, Mukti AT. 2020. Enlargement technique for canntang hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) in floating net cages. Journal Biosains 22: 29–36.

Royan F, Rejeki S, Haditomo AC. 2014. The effect of different salinity on blood profile parameters of tilapia (*Oreochromis niloticus*). Journal Aquatic Management Technologhy 3: 109 – 117.

Saparuddin S. 2018. Effect of ethanol extract on increased hemoglobin concentration and hematocrit values of grouper and mouse Saintifik 4 :39–46.

Shabirah A, Mulyani Y, Lili W. 2019. Effect of types isolated lactic acid bacteria on hematocrit and differential leukocytes fingerling common carp (*Cyprinus carpio* L.) infected with *Aeromonas hydrophila* bacteria. World News Natural Sciences 24: 22–35.

Shen GM, Shi CY, Fan C, Jia D, Wang SQ, Xie GS, Li GY, Mo ZL, Huang J. 2017. Isolation, identification and pathogenicity of Vibrio harveyi, the causal agent of skin ulcer disease in juvenile hybrid groupers *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*. Journal Fish Diseases 40: 1351–1362.

Snieszko SF. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology 6 :197–208.

Sugama K, Rimmer MA, Ismi S, Koesharyani I, Suwirya K, Giri NA, Alava VR. 2012. Hatchery management of tiger grouper (*Epinephelus fuscoguttatus*): a best-practice manual. Australian.

Syaputri DR, Karim M, Yodfiatfinda. 2021. Risk Analysis of Bleach Grouper Cultivation (*Epinephelus fuscoguttatus x Epinephelus lanceolatus*) in The Waters of Lancang Island's. Thousand Island, DKI Jakarta. Jurnal Bioindustri 4: 69–83.

Tino W, Siregar VP, Gaol JL. 2022. The Application of Multicriteria Evaluation Model Using Fuzzy Ahp To Determine The Location Of Grouper Cultivation In Seribu Islands. Jurnal Ilmu Teknologi Kelautan Tropis 14: 363–378.

Wedemeyer, G.A and Yasutke. 1977. Clinical Methods for The Assessment on The Effect of Enviromental Stress on Fish Health. Technical Paper of The US Departement of The Interior Fish and the Wildlife Service 89: 1–17.

Yang X, Zhao X, Wang G, Dong X, Yang Q, Liu H, Zhang S, Tan B, Chi S. 2022. Improvement of hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂) by enzyme-digested poultry by-product: Growth performance, amino acid and peptide transport capacity, and intestinal morphology. Frontiers Nutrition 9: 1–17.

Yuliana A, Wulandari R, Zahra A. 2021. Provision of *Sargassum* sp. extract through commercial feed against hematocrit value and leukocyte differential in pomfret *Trachinotus blochi*. Jurnal Intek Akuakultur 5: 36–49.

Yuwanita R, Buwono NR, Eka Putra HF. 2018. Effect of dunaliella salina on polymorphonuclear leukocytes of cantang grouper (*Epinephelus fuscoguttatus x Epinephelus lanceolatus*) infected by viral nervous necrosis (VNN). Jurnal Ilmu Perikanan dan Kelautan 10 :124–130.