Water quality in floating net cages pond of humpback grouper (*Cromileptes altivelis*) infected by viral nervous necrosis

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Abstract. Humpback grouper (*Cromileptes altivelis*) is widely cultured in Indonesia due to its various advantages. Yet, the issue with humpback grouper pisciculture is the fact that it is prone to diseases, including bacteria, fungi, parasites, and viruses. The several disease-causing agents, viruses are quite dangerous agents. One of the viruses that can cause the failure of *C. altivelis* pond is Viral Nervous Necrosis (VNN), which causes stress due to poor water quality. The purpose of this study was to determine the condition of water quality in grouper floating net cages infected by VNN. The research method used in this research was the descriptive method with surveillance survey techniques, including measurement and analysis of water quality. Water quality observed include temperature, pH, Dissolved of Oxygen (DO), salinity, nitrate, and phosphate. Water quality sampling was carried out three times. Based on observations of the clinical symptoms of groupers that were indicated to have been infected by VNN, they showed physical signs of blackened skin and protruding eyes as well as swirling movements. The water quality of floating net cages was 26.7°C, 7.49, 8.53 mg L⁻¹, 32, 0.101 mg L⁻¹, and 0.002 mg L⁻¹, for temperature, pH, DO, salinity, nitrate level, and phosphate level, respectively. Based on the results of the study, the quality of water found in grouper aquaculture was still classified as normal. VNN infection in groupers was possible through a vertical pathway derived from the broodstock.

1. Introduction

Indonesia's sea territory shows significant fishery potential and business development, especially in the water conservancy. One of the commodities that have started to be developed due to its high economic value is humpback grouper (*Cromileptes altivelis*). Yet, the issue with humpback grouper pisciculture is it is prone to diseases, including bacteria, fungi, parasites, and viruses. Among numerous agents of diseases, a virus is a quite dangerous agent. One of the viruses attacking humpback groupers is Viral Nervous Necrosis, which can cause a major loss to fish farmers [1]. **Viral Nervous Necrosis (VNN)** is categorized as a virus that attacks fish larvae and juvenile fish. The symptoms shown are varied based on the fish age. VNN or Viral Encephalopathy and Retinopathy (VER) is an RNA virus that commonly attacks marine fish commodities [2]. The infection on the fish aged 20 days shows the decrease in appetite, while on 20-40 days old fish, it showed an abnormal behavioral change, such as staying on the bottom of the pond. Meanwhile, humpback grouper aged more than four months showed a symptom in which they swim float in the surface accompanied by the
enlargement of swimming bladders [3]. Moreover, VNN infection can trigger inflammation in fish tissues [4,5].

Important factors that need to be considered in supporting the success of aquaculture cultivation is a good environment and suitable for the grouper life so that high survival is obtained. The presence of bad water quality can aggravate the condition of the humpback grouper infected by VNN. Besides, the low survival value can also be caused by the humpback grouper that can be easily stressed [6]. The water used for Humpback Grouper (Cromileptes altivelis) pisciculture must be in the optimal quality so that the fish are not stressed [7]. According to Cao et al. [8], the triggers of diseases include the imbalance between environmental support and production quantity within one culture area (imbalance infection between the fish, pathogen, and its environment). The quality of water required for humpback grouper (C. altivelis) in the pisciculture pond includes the salinity, oxygen solubility, temperature, pH, nitrate, and phosphate. For grouper larvae, the appropriate temperature for VNN replication was 28°C, with a mortality rate reaching 100% [9]. Whereas if dissolved oxygen is out of balance, it will cause stress on the fish because the brain does not get enough oxygen supply. If the parameter of water quality as a habitat of humpback grouper is worsening, the pathogen organisms causing bacterial and virus diseases can enter and harm the fish. Fish that was harmed will die if it was not treated or prevented well [10]. Information about water quality status can affect the spread of VNN in grouper so that it can be used to evaluate and avoid outbreaks of VNN in aquaculture. Controlled water quality, it will improve the quality of life of groupers and produce fish with high quality and free from disease. Therefore, the purpose of this research was to observe the water quality in the floating net cages for humpback grouper infected with Viral Nervous Necrosis (VNN).

2. Methods

The method used in this research was descriptive. The water quality parameters measured in the floating net cages include temperature, pH, dissolved oxygen (DO), salinity, nitrate level, and phosphate level. The observation of clinical symptoms was conducted by observing the humpback grouper's body parts, which were taken as a sample, to be compared to the ones collected from a healthy environment. The water quality data analysis was conducted by comparing data obtained with the optimal value of the water quality parameter for the humpback grouper pisciculture. The water sample collection was conducted on one point of observation. It was conducted by taking water samples using three 600mL bottles with three repetitions to measure the nitrate and phosphate and analyzed in the laboratory. Other water quality parameters, such as temperature, pH, dissolved oxygen (DO), and salinity, were measured directly on site.

2.1. Temperature

According to Subarjanti [11], the measurement of water temperature was done by inserting the thermometer directly into the water for 2-5 minutes while avoiding direct sunlight. The thermometer scale reading was done as quickly as possible after lifting the thermometer from water.

2.2. pH

The pH measurement was done by using a pH meter [12] by inserting the pH meter into the water for 2 minutes. It was done by pressing the hold button until it showed a stable number on the pH meter display. The pH value was then recorded.

2.3. Dissolved Oxygen (DO)

According to Armita [13], measuring dissolved oxygen was initiated by calibrating the rod tip of the DO meter using distilled water. The DO meter rod was then dipped into the water sample. As the value was shown on the screen, it was recorded immediately.

2.4. Salinity
Salinity measurements was done using a refractometer. Then, a drop of water sample was placed on a refractometer. As soon as the value appeared, it was recorded. The salinity range was expressed in ppt units [14].

2.5. Nitrate Level
According to Boyd [15], the nitrate measurement procedure was conducted by filtering 12.5 mL of sample water and poured it into a porcelain cup. The water was then evaporated using a heater. 0.25 mL of disulphonic phenol and 5 mL of distilled water were then added into the cup and the mixture was stirred. Add NH₄OH with a ratio of 1:1 to form color and dilute the mixture with 12.5 mL of distilled water. The nitrate level was measured using a spectrophotometer (at a wavelength of 420 nm) with a standard solution for comparison.

2.6. Phosphate Level
According to Boyd [15], the procedure for measuring aquatic phosphates was done by making a 25 mL comparative standard solution in Erlenmeyer. 1 mL of ammonium molybdate - sulfuric acid was added to each standardized solution and homogenized. Five drops of SnCl₂ solution was then added and further homogenized. The measurement was done by comparing the color of the water sample with a standard solution using a spectrophotometer (at a wavelength of 690 nm).

3. Results and Discussion

3.1. Clinical Symptoms of Humpback Grouper Infected with VNN

Figure 1. A). infected fish with protruding eyes, B). the skin color of infected fish was darker, C). infected fish were floating on the surface of the water.

The environmental factors are very influential to the health of the humpback grouper. The main cause of the fish to be prone to disease is stress. Stress can happen due to the factor of poor water environment and the treatments in the pisciculture. During the first round of observation, infected humpback grouper experienced death, and had floated in the surface with a physical symptom of darkened skin and popping eyes. At the second observation, the humpback grouper was dead, had popping eyes, darkened skin, and darkened gills. The results of the third observation showed that the fish was still alive, yet had characteristics of abnormal movement (circling), darkened skin, and popping eyes. These symptoms aligned with the signs found by Asri et al. [10], who stated that in general, clinical symptoms of fish infected by VNN included abnormal movement, loss of appetite, body-color that seems to be darker, and the fish occasionally float due to the swimming bladder enlargement.

3.2. Measurement of Water Quality Parameters in Floating Net Cages
3.2.1. Temperature. The clinical symptoms occurred in humpback grouper can be caused by the temperature suitable to the VNN virus replication. The temperature is one of the factors that cause the growth of VNN virus in humpback grouper floating net cages. The temperature measurement during
three weeks of observation showed that the water temperature was around 27 – 28°C. Meanwhile, according to Asri et al. [10], the optimal temperature for VNN virus to grow in its host is at 24-32°C. For humpback grouper larvae, the suitable temperature for VNN to replicate is 28°C, with a death rate of 100%.

3.2.2. pH. The measurement of pH in the water is imperative because it is an indicator to acknowledge the concentration of hydrogen ions in the water. The condition of an acidic or base waters can be seen from the result of the pH measurement. Based on the pH measurement in the floating net cages in Sendangbiru, the pH was 7.8 – 8.1, which was still categorized as good. This is in line with Suhaimi et al. [16], who stated that the optimal pH for humpback grouper pisciculture in floating net cages is 6.5 – 8.5. The change of pH value influenced the respiration process in the pisciculture pond. If the pH value is too high, it can hamper the photosynthesis process due to the lowered content of CO₂, while if the pH value is too low, it can cause the fish to be limp or even death [17]. The water pH under or above the optimal will cause health disorder to the fish. The direct effect of low and too high pH value is the epithelial cells damage, both to the skin and gills, which will disturb the process of oxygen absorption primarily for fish.

3.2.3. Dissolved Oxygen (DO). The measurement of dissolved oxygen in the management of water quality is necessarily related to the fish's metabolism process, mainly in the respiration process, because the dissolved oxygen is related to the availability of oxygen in the waters. The result of DO measurement in the floating net cages in Sendangbiru was 8.45 – 8.62 mg/L, and it was still considered good. The result was aligned with Decree of the state minister of the environment [18], regarding seawater quality standard for biota that must be beyond 5mg/L. Furthermore, Tataningdatu et al. [19], stated that the balanced DO required for seawater creatures is more than 5mg/L. If the dissolved oxygen is not stable, it will cause stress for the fish because their brain cannot get the adequate amount of oxygen supply and cause death due to the lack of oxygen.

3.2.4. Salinity. Salinity is required by the fish to regulate their body liquid balance in accordance with the water environment. The liquid balance will stimulate faster growth [20]. The salinity in the floating net cages in Sendangbiru was 31-33 mg/L, which was still categorized as good for humpback grouper growth. Nitimulyo et al. [21], mentioned that the optimal salinity for humpback grouper growth was between 30-33 mg/L. The difference in seawater salinity can be caused by the stirring due to sea waves or water mass movement by gusts [22].

3.2.5. Nitrate Level. Nitrate is a form of main nitrogen compound in natural waters and is the primary nutrient for plants' growth. The level of nitrate in seawater is influenced by the input of domestic wastes to the waters. The source of nitrate content increase is generally urban, industrial, and agricultural wastes. The nitrate level of water from the floating net cages in Sendangbiru was 0.17 – 0.121 mg/L and it was still categorized as good for humpback grouper lives. the result was in line with Decree of the state minister of the environment [18], who suggested that the standard quality of seawater nitrate concentration appropriate for sea biota was 0.008 mg/L. The nitrate concentration in the surface layer is lower compared to the layer near the bottom because the nitrate in the surface layer is more utilized or consumed by phytoplankton. Besides, the slightly higher nitrate concentration near the bottom of the waters is also influenced by the sediments [23].

3.2.6. Phosphate Level. The presence of phosphate in natural waters is relatively small because the source of phosphate is fewer compared to the source of nitrate. Phosphate enters the water through numerous paths, including animal wastes, crop residues, and dead animals, as well as sediment grains that enter the sea [24]. The phosphate level in the floating net cages in Sendangbiru was 0.004 mg/L and it was still categorized as good or suitable for the humpback grouper. According to Anggraini et
al. [25], if the content of phosphate is lower than 0.051 mg/l, the water can be categorized as good. The maximum phosphate concentration suitable for sea biota is 0.015 mg/L [18].

Based on water quality measurement, it was suggested that the range of pH, DO, salinity, nitrate and phosphate in the floating cages could still be tolerated for the survival of groupers. Even so, we found VNN still infected several groupers. This was due to the temperature was in the range 27°C-28°C, that can indicate the presence of VNN virus activity. Nivriadi et al. [9], mentioned that for grouper larvae, the optimum temperature for VNN replication was 28°C with a mortality rate reaching 100%.

4. Conclusion

The observation of water samples from the floating net cages in Sendangbiru showed that water quality parameters was considered as a good condition. For each water quality parameter measured and compared, it was still categorized as good in accordance with the standard water quality for humpback grouper. Based on those results, VNN infection, which was evidenced by clinical symptoms, was not derived from horizontal factors. So, further studies was necessary to identify VNN infection.

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