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Analysis relationship of water quality to total hemocytes in intensive ponds for Vannamei Shrimp (*Litopenaeus vannamei*) cultivation CV TTB, Pasuruan City, East Java

Rahmahnia Febriyani[1,*], Muhammad Musa[2], Mohammad Mahmudi[3]

[1] *Master's degree program in Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, East Java, Indonesia.*
[2] *Department of Aquatic Resource Management, Faculty of Fisheries and Marine Science, University of Brawijaya*
[3] *Department of Aquatic Resource Management, Faculty of Fisheries and Marine Science, University of Brawijaya*

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ABSTRACT

Shrimp that is quite potential to be developed is the vannamei shrimp (*Litopenaeus vannamei*). This cultivation develops with intensive technology. This study aims to analyze the relationship between water quality and total haemocytes of shrimp in vaname shrimp ponds. This study uses a survey method with descriptive data analysis. The parameters measured include physical and chemical parameters, namely temperature, brightness, pH, dissolved oxygen, TSS (*Total Suspended Solid*), BOD (*Biochemycal Oxygen Demand*). while to see the health of shrimp seen by counting the total haemocyte (THC) of shrimp. The results showed that temperature measurements during the study, the average temperature value in the intensive vannamei shrimp pond CV.TTB Pasuruan City was 28°C within the normal range. Furthermore, for the measurement of pH obtained an average value of 7.9 mg/l. Furthermore, for DO content, the average value was 5.74 mg/l. Furthermore, for TSS content, the average value was 0.154 mg/l. Furthermore, the BOD content value obtained an average value of 32.26 mg/l., for the highest hemocyte value obtained from the sample in the 3rd sampling, which reached a value of 3.74 x 106 mg/l. Based on the results of the linear regression test, it was found that the TSS and BOD parameters were significantly correlated with total haemocytes in vannamei shrimp (Sig. <0.05), it can be concluded that the relationship between water quality parameters that affect total haemocytes is the BOD (*Biochemycal Oxygen Demand*) parameter and TSS (*Total Suspended Solid*).

Introduction

Water quality is a very important natural indicator for evaluating the feasibility of shrimp farming (Ma *et al*, 2013; Sahrijanna & Sahabuddin 2014). Since being introduced to Indonesia in 2001, vannamei shrimp (*Litopenaeus vannamei*) or white shrimp has become one of the leading commodities in the national aquaculture sector. Vannamei shrimp culture management in Indonesia is carried out with various cultivation patterns and systems. Starting from those still using traditional cultivation systems to super intensive ones with various technological applications (Suwoyo *et al*., 2015).

The application of intensive and super-intensive cultivation systems that are so popular with cultivators can have a polluting impact on the aquaculture ecosystem in the form of accumulation of aquaculture waste loads (Nakorn *et al*., 2017). The build-up of the waste load comes from aquaculture input activities such as wasted feed waste, fertilization, liming, shrimp moulting carapace and various other treatments during the cultivation period (Edhy, 2010). The addition of the waste load that continues to grow and the dynamic character of the pond ecosystem will affect the fluctuations in the dynamics of water physicochemical factors (Boyd, 1998). Physical-chemical factors or overall water quality in the culture environment are

* Corresponding author.
Email address: niafeb11@gmail.com

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important indicators for the comfort of aquatic organisms to live during the culture cycle (Carbajal-Hernàndez, 2013). Thus, indirectly the condition of physicochemical parameters with stable and ideal concentration levels will have a positive influence on the level of shrimp harvest productivity (Zafar *et al.,* 2015).

Strive to increase fishery production, especially shrimp, one of which is obtained using an intensive aquaculture system, namely increasing productivity through the application of stocking densities and high feed use. Vannamei shrimp is categorized as a superior commodity, but the obstacles faced in the cultivation are quite complex, one of which is the decline in water quality and sediment conditions in intensive pond systems. The high accumulation of organic matter in the ponds makes the self-purification process more difficult for microorganisms in the system and if this process is no longer effective, it can cause a drastic decrease in water quality and endanger the cultured shrimp.

One of the causes of a decrease in vannamei shrimp production is disease. Shrimp are very susceptible to disease, one of which is influenced by bad environmental conditions. Poor water quality can trigger stress in shrimp, thereby lowering the immune system (Oktaviana and Febriani, 2019). Hemocytes play an important role in the crustacean immune system. The composition of hemolymph can be measured and used as an assessment of crustacean health through the characteristics and activity of the defense system against infectious agents played by hemocytes. Hemocytes play a role in phagocytosis, encapsulation, degranulation and nodular aggregation against pathogens or foreign particles (Arifin *et al*., 2014).

The development of vannamei shrimp cultivation cannot be separated from disease attacks. Therefore, an alternative is needed to increase the production of vannamei shrimp by improving the health of vannamei shrimp.

Based on these descriptions. The purpose of this study was to analyze the relationship of water quality to shrimp health as seen from the hemocyte profile in intensive vannamei shrimp culture at CV TTB Pasuruan City, East Java.

Materials and Methods

Location and time of research

This research was conducted in intensive vannamei shrimp ponds CV. TTB, Bugulkidul District, Pasuruan City, East Java Province October 2021 - November 2021. This study uses a survey method with descriptive data analysis. This research was conducted in intensive pond aquaculture, round tarpaulin ponds with a total of 3 plots, plots with a pond area of 800 m^2, diameter 32 m, with a stocking density of 201 fish/m^2.

This study uses a survey method with descriptive data analysis. The survey method was carried out to obtain primary and secondary data in the field, while the descriptive method was used to describe the condition of intensive vannamei shrimp ponds in CV. TTB, Pasuruan City, East Java, by making direct observations through the object under study, namely water quality and vannamei shrimp as biomarkers. Sampling was carried out for two months with an interval of every two weeks to see the dynamics of water quality and shrimp growth.

Litopenaeus vannamei was obtained from intensive pond culture of vannamei shrimp, then the shrimp was taken hemolymph or blood in the shrimp to see the health of the shrimp as seen from the *total hemocyte* (THC). Physical and chemical water quality measurements as supporting data such as DO (*Dissolved Oxygen*), pH, temperature, TSS (*Total Suspended Solid*), BOD (*Biological Oxygen Demand*), which are measured according to SNI procedures. Water quality analysis is carried out in the Laboratory of the Implementation Unit Sumberpasir Freshwater Fishery Technical, Brawijaya University.

Data analysis used the descriptive method. Descriptive analysis was carried out on water quality data and *total hemocyte* data (THC). Analysis of the data to determine the relationship between water quality and *total hemocyte* (THC) and the relationship between them used a regression test (Steel, 1997). Furthermore, the data were tested for correlation (*relationship*) by looking at the value of r (*coefficient interval*) in the regression test. (Sugiyono, 2014). The correlation interval with the level of relationship between factors is as follows (Tabel 1).

Table 1. Correlation interval with the level of relationship between factors.

Number	Coefficient Interval	Relationship Level
1	0.00 – 0.199	Very low
2	0.20 – 0.399	Low
3	0.40 – 0.599	Medium
4	0,60 - 0,799	Strong
5	0.80 – 1.00	Very Strong

Table 2. Water quality in vannamei shrimp intensive tambien CV TTB Pasuruan.

Parameter	Research cities	S.1	S.2	S.3	Referensi
Temperature (C⁰)	Plot 1	28.5	27	28.2	28-32 (WWF Indonesia, 2014)
	Plot 2	28.5	28	28.3	
	Plot 3	28.4	27	28.1	
pH (mg/l)	Plot 1	8.1	8	7.9	7,5 - 8 (WWF Indonesia, 2014)
	Plot 2	8.2	8	7.8	
	Plot 3	8.2	8	7.8	
DO (mg/l)	Plot 1	7	5	5.1	>4 (WWF Indonesia, 2014)
	Plot 2	6.2	5	5.1	
	Plot 3	7	5	5.4	
TSS (mg/l)	Plot 1	0.202	0.302	0.094	50-300 (Avnimelech , 2009)
	Plot 2	0.268	0.278	0.177	
	Plot 3	0.272	0.259	0.192	
BOD(mg/l)	Plot 1	9.16	26	68.67	< 45 (KEPMEN KP No. 28 Thn 2004)
	Plot 2	15.19	18	58.12	
	Plot 3	14.98	19	61.72	

Table 3. Results of measurements of THC vannamei shrimp.

Research Cities	Sampling 1	Sampling 2	Sampling 3
Plot 1	2.52 x 10^6	2.04x10^6	3.40 x 10^6
Plot 2	2.42x 10^6	1.84x 10^6	3.51 x 10^6
Plot 3	2.36x 10^6	1.77x 10^6	3.74 x 10^6

Table 4. Regression Test Between Water Quality Parameters and THC.

Parameter	Sig.	Correlation	regression equation
Temperature (C⁰)	0.111	0.567	Y=-1987086 + 803439x
pH (mg/l)	0.795	0.101	Y= 5836843 - 404799x
DO(mg/l)	0.578	0.215	Y= 3.80115 - 204670x
TSS(mg/l)	0.009	0.801	Y= 4678972 – 9.042008x
BOD(mg/l)	0.003	0.866	Y= 1.738345,535+27498x

Results

Water quality measurement results

Table 2 shows the results of measuring water quality parameters. Based on the results of temperature measurements during the study, the average temperature value in the Vannamei shrimp intensive pond CV.TTB Pasuruan City was 28°C in the normal range. Furthermore, for measuring pH, an average value of 7.9 mg/l was obtained. The average value for DO content is 5.74 mg/l. The level of oxygen solubility in the water is a critical factor for shrimp life. Low dissolved oxygen content below 1.5 mg/l will be lethal to shrimp. In the waters, the average of TSS is 0.154 mg/l. Meanwhile, the BOD content is 32.26 mg/l. According to the Decree of the Minister of Marine Affairs and Fisheries No. 28 of 2004 concerning General Guidelines for Shrimp Cultivation in Ponds regarding the quality standard of shrimp pond effluent, it is stated that the permissible level of BOD in waste is < 45 mg/l, which is declared to have met the quality standard.

Total haemocyte count (THC)

Measurement of *Total Haemocyte Count (*THC) in vannamei shrimp samples during the study as shown in Table 3 was in the range of 1.88 x 106 – 3.55 x 106 ml. The range of THC values in this study was still within the normal range of THC values in vannamei shrimp.

Relationship between water quality and total haemocyte count (THC)

Table 4 shows the relationship between the measurement results of water quality parameters and *Total Haemocyte Count* (THC) in intensive vannamei shrimp ponds CV TTB Pasuruan.

Based on the results of the linear regression test, it was found that the TSS and BOD parameters were significantly correlated with total hemocytes in vannamei shrimp (Sig. <0.05). The results of the TSS regression equation show that if the TSS value increases by 1 unit, it will increase the total hemocytes with a correlation coefficient (r) of 0.801, which means that the correlation is strong, the higher the TSS value in ponds, the higher the total hemocytes in vannamei shrimp. The results of the BOD regression equation show that if the BOD value increases by 1 unit, it will increase the total hemocytes with a correlation coefficient (R) of 0.866, which means that the correlation is strong, the higher the TSS value in ponds, the higher the total hemocytes in vannamei shrimp.

Discussion

Good water quality parameters will affect productivity and the minimal distribution of disease infections in pond waters. Vannamei shrimp (*Litopenaeus vannamei*) is a type of shrimp that is very sensitive to changes in fluctuations environment, so that water as a living medium for shrimp culture must be meet the appropriate criteria both chemically, physically, and biologically (Sahrijanna and Sahabuddin, 2014).

The pH value in the research pond was 7.9 mg/l. This value is very suitable for use for intensive cultivation of vannamei shrimp. This reference is in accordance with the research hypothesis of Saraswathy *et al.* (2016), which states that for intensive ponds in India, cultivators are recommended to maintain a pH range of 7.5 - 8.5 mg/l with a morning-afternoon range of not more than 0.5 ppm. This is done to avoid stress on the shrimp, as well as the entry of pathogenic infections. The temperature during the cultivation period was recorded in the range of 28.72 - 29.22 ^{0}C. The temperature level in this figure is considered to be the desired temperature conditions for shrimp to grow, this statement is the same as the results of research by Wyban *et al.* (1995), which obtained the results of research that the optimum temperature for the growth of vannamei shrimp reared in controlled treatment obtained the optimum temperature range. which is good for shrimp growth is in the range of 27-30 $^{\circ}$C. The level of dissolved oxygen content in the research ponds obtained a concentration value of 5.74 mg/l. This shows that the oxygen demand in the pond is very sufficient, because for intensive vannamei shrimp ponds in Indonesia, the recommended oxygen level for shrimp farmers is > 4 mg/L (Supono, 2015). TSS content, an average value of 0.154 mg/l was obtained, the total suspended solids tend to increase during the cultivation period. Things that are thought to influence the formation of solids in shrimp ponds include high stocking densities, high amounts of feed given to shrimp every day during the rearing period. BOD content value obtained an average value of 32.26 mg/l According to Suriadarma (2011), that the highest BOD levels are in the sea rather than fresh waters because a lot of organic waste from upstream rivers, aquaculture areas and rice fields dumps it into the sea and accumulates in the sea.

The temperature values obtained are different but the difference is not too far. Differences in temperature values can be caused by differences in time and conditions of the research location. In addition, the difference in temperature is due to the increasing intensity of sunlight in the pond area, the heat exchange between water and air is also greater, resulting in an increase in temperature in the pond area. As a result, the growth and development of shrimp or aquatic biota will be hampered.

The pH concentration of the water will affect the shrimp's appetite. In addition, a pH that is below the tolerance range will disrupt the molting process so that the skin becomes soft and survival is low. In waters with a low pH, there will be an increase in the hydrogen sulfide (H2S) fraction and the toxicity of nitrite, as well as physiological disturbances in shrimp so that the shrimp becomes stressed, softens the skin (carapace), as well as decreases the survival rate and growth rate (Ip *et al.,* 2020).

DO values tend to be lower in the morning than in the afternoon and evening. This is because during the day there is photosynthetic activity from phytoplankton that produces oxygen. On the other hand, at night, phytoplankton do not photosynthesize and compete with shrimp for oxygen consumption (Kordi and Tancung, 2007). Dissolved oxygen below 3 mg/l can cause shrimp stress and death. To anticipate the lack of oxygen, the pond is equipped with a waterwheel or aerator.

The presence of suspended solids in high concentrations can indicate a decrease in the quality of the aquatic environment. This is because the high content of suspended solids can interfere with the penetration of sunlight which can further inhibit it. The high value of total suspended solids concentration can hinder the photosynthetic activity of marine plants, both micro and macro, resulting in reduced oxygen content in the water (Helfinalis *et al.*, 2012). According to Brant and Kauffman (2011), that high BOD levels are caused by high levels of pollution originating from organic waste.

The measurement of Total Haemocyte Count (THC) in vannamei shrimp samples was in the range of 1.88 x 10^{6} – 3.55 x 10^{6} ml. The increase of hemocytes is thought to be a response from the immune system in shrimp. This is because the body's defense system in shrimp plays a role in the body's defense mechanism by hemocytes, where they spread and increase in the total number of hemocytes are assumed to be a form of cellular immune response in the shrimp body (Van de Braak, 2002).

According to Yeh *et al.* (2009), the number of haemocytes of healthy shrimp weighing 11-12 g/head was 1.80 ± 9.28 x 107 cells/ml. Hemocytes are one of the defense systems in vannamei shrimp which are responsible for phagocytosis, nodulation, and encapsulation. To find out that hemocytes are cellular defenses of the body, it can be seen from their ability to a phagocytic activity which can increase in the event of infection. The presence of infection will stimulate the non-specific cellular defense system so that it is expected to be able to ward off disease attacks (Fontaine *et al.*, 1974). A high number of hemocytes indicates a good level of shrimp health (Oktaviana *et al.,* 2014).

Conclusion

It can be concluded that the relationship between water quality parameters that affect total haemocytes are BOD and TSS. The results of this study indicate that the highest total haemocytes were found in the vannamei shrimp sample obtained from the 3rd sampling in the CV TTB shrimp pond, Pasuruan City. This condition is closely related to the low levels of DO and high concentrations of BOD, TSS, in these locations.

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