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Phytoplankton Composition in White Shrimp (*Litopenaeus vannamei*) Pond Culture Infected White Feces Disease (WFD)

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KEYWORDS

Diversity index;
Dominance index;
Phytoplankton abundance;
Phytoplankton composition;
White shrimo.

Abstract The aim of this study is to find out the composition and condition of phytoplankton in the white shrimp (*L. vannamei*) culture inffected by the white feces disease so that one of the indicators of the onset of the white feces disease can be known. This research uses descriptive methods. The observed parameters are the identification of species diversity, abundance, diversity index and dominance index of phytoplankton. The sampling was conducted at three locations, Tuban area, Situbondo area, Lamongan area. The results of the study showed observations in Lamongan area, the total abundance of phytoplankton was 473 cells/L. Situpondo areas abundantity of phytoplankton total was 633 cels/L and Tuban areas abundance was 887 cells/L. In Lamongan area, the diversity index was 1,009. In Situbondo areas diversity Index was 1,013. In Tuban area, diversity indexes were 1,082. In Lamongan area, a dominance index was 0.477. In Situpond area, it was obtained a domination index of 0.544. The genus that dominates the three ponds are Cyclotella and Navicula. At all three locations have high variety of species and abundance of the genus members of the filum Cyanophyta and Bacillariofhyta are detrimental, the genuses that are found are indicators of contaminated waters. The dominance of Cyanophyta (blue-green algae) is also an indicator of the White Feces Disease.

Introduction

One of the most valuable aquaculture products in the world with a sizable export market is the white shrimp (*Litopenaeus vannamei*). In 2018 there were 4.9 million tonnes of white shrimp produced (FAO, 2020). increasing stocking density, intensive shrimp farming is encouraged by the increasing demand for shrimp, particularly in the export market. White shrimp (*Litopenaeus vannamei*) is one of the fishery commodities which is expected to be able to develop well, high demand and value of the world shrimp market (Budiardi, 2008). Aquaculture production has increased by an average of 29.9% per year, including brackish water cultivation which has also experienced a significant increase. One of the brackish water cultivation commodities that has quite a high selling value is vaname shrimp. Shrimp commodity production in 2014 reached 699,000 tons and will be increased to 755,000 tons in 2015, where around 70% of the production target is vaname shrimp (Directorate General of Fisheries and Maritime Affairs, 2015). To achieve this production target, many vaname shrimp are cultivated intensively. However, intensive cultivation of vannamei shrimp poses a higher risk of contracting disease. One disease that can easily threaten the sustainability of vaname

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shrimp cultivation is white feces disease. White feces disease causes a decline in vanname shrimp productivity. *L. vannamei* intensive ponds were invaded by this disease in Indonesia starting in 2014 (Anjaini *et al.,* 2018).

White feces disease causes production losses of up to 10-12% originating from decreased survival and lower crop yields. White feces disease is associated with high stocking density, poor water quality and pond bottom quality, dominance of one type of plankton, poor feed management and high levels of pollution in pond waters. The initial sign of an attack of white feces disease) is the appearance of white or sometimes yellow feces floating on the surface of pond waters (Mastan, 2015). According to Anjaini *et al.* (2018), the study's findings showed that white shrimp's gills, hepatopancreas, and intestines were infected with white feces disease tract all had protozoa (Gregarine).

Supito *et al.* (2016), said that white feces disease attacks often occur in shrimp culture which tends to be dark green water and sometimes also occur in ponds with brown water. Attacks of white feces disease also often occur when pond water changes drastically from green to brown. The change in water color from bright green to dark green or even blue indicates a shift in dominance by blue green algae. Changes in water color in white shrimp ponds are thought to indicate changes in the dominance of dissolved particles in the water. After stocking the PLs for 50–70 days, WFD incidences were noted. Blackish colouring of the gills, loose shells, atrophy (shrinkage) of the hepatopancreas, and intestines loaded with white strands of faeces rather than food are just a few of the clinical signs that can be seen. Hepatopancreatic microvilli in shrimps undergo metamorphosis, sloughing, and aggregation to generate vermiform entities, which at first glance resemble protozoan Gregarines. WFD results in a drop in shrimp output and an appetite decline that can stunt development and even result in mortality (Kurniawinata *et al.,* 2021).

White shrimp culture which applies a super intensive system using high stocking densities means that water quality parameters, namely physics, chemistry and biology in the shrimp rearing media, must be given great attention because they are one of the important factors that will determine the success of cultivation. The emergence of white feces disease in white shrimp culture can be caused by poor water quality in the rearing media.

Increasing phytoplankton is an issue that mostly impacts white shrimp production. In most cases, the nutrients in the ponds tend to increase accumulate during the culture period due to the accumulation of waste, both food waste and excretion of shrimp. Changes in the amount of phytoplankton in aquaculture ponds are generally correlated with changes in some of the nutrients in the water (Kunlapapuk *et al*., 2021)

Aquaculture pond environmental conditions have a big impact on shrimp culture. Particularly, there was a connection between the phytoplankton and the ponds' water quality variables. An increase in the amount of phytoplankton that was too high, for example, could have an impact on the lack of oxygen at night, (Dampin, 2011). The phytoplankton problem in the ponds was not well controlled, it would have a major negative impact on the productivity in the pond (Case *et al*., 2008). The presence of a dominant type of plankton is thought to be an indicator of the emergence of white feces disease. This is characterized by a drastic change in the color of the water. The presence of a dominant type

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of phytoplankton is thought to be the main indicator of color changes and a decrease in water quality. Plankton is an indicator of water quality which is very sensitive to changes in the environment, so it is necessary to identify phytoplankton in the vaname shrimp rearing media which is thought to be an indicator of the emergence of White Feces Disease.

The aim of this research is to determine the condition of phytoplankton in white shrimp ponds that are infected by white feces disease so that one of the indicators for the emergence of white feces disease can be identified. This research was carried out at the Fish Cultivation Laboratory, Fish Disease and Health Division and the Hydrobiology Laboratory, Aquatic Environment and Biotechnology Division, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Indonesia.

Materials and methods

Research Tools and Materials

Equipment used in the research included an Olympus CX21 microscope, film bottles, 15 L buckets, plankton net mesh size 25, sample bottles, the book The Plankton of South Vietnam by Shirota in 1966, the book The Marine and Freshwater Plankton by Davis in 1955, books The Freshwater Algae by Presscott in 1979, glass object, cover glass, Sedgwick Rafter, measuring cup, 1.5 L mineral water bottle, dropper pipette, beaker glass, washing bottle, cool box, tissue grinder, tray, refrigerator, vacuum pump. Materials used in the research included sample water, Lugol, aluminum foil, label paper, distilled water, filter paper, Whatman 41 paper and tissue.

Research Methods

The research method used in this research is a descriptive method. According to Hamdi and Baharuddin (2014), the descriptive method (descriptive research) is a research method aimed at describing phenomena that exist and are taking place at present or in the past. Descriptive research can not only be used to describe a situation, but can also describe the situation in stages of its development. Quantitative descriptive research emphasizes objective phenomena and is studied quantitatively. Maximizing the objectivity of quantitative descriptive research design is carried out using numbers, statistical processing, structure and controlled experiments. Quantitative descriptive research also does not rely on manipulation or changes to independent variables, but describes a condition as it is. With no control over the variables, descriptive research may be defined as a description of the current condition of affairs. Additionally, "descriptive studies may be characterised as simply the attempt to determine, describe or identify what is, while analytical research attempts to establish why it is that way or how it came to be" Descriptive research aims to shed light on current issues or problems through a process of data collection that allows them to describe the situation more (Manjunatha, 2019).

Sampling

The research was carried out in 2 stages. The first is sampling and the second is identifying and measuring samples in the laboratory. Sampling of phytoplankton in white shrimp ponds infected with white feces disease divided into three locations, which are Tasikharjo Village Tuban (11104506,076”E 6046’44,76”S), Peleyan Viilage of Situbondo (113058’35,503”E 7040’49,685”E) and Kranji Village of Lamongan Area (112022’25,487”E 6054’13,61”S). These three locations is one of the center of shrimp farming in east java. They have more than 10 ha of pond area, with intensive and traditional shrimp culture.

Plankton sampling methods in stagnant water such as reservoirs and ponds can be

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taken at a depth of 0.5 to 1 meter. Plankton sampling using Wisconsin plankton net (Rosada and Sunardi, 2021). Phytoplankton sampling was carried out quantitatively using a 15 L bucket which was then filtered with a plankton net mesh size 25 µm. Sampling was carried out in 3 parts of the pond, namely inlet, near feeding tray and outlet of the pond. The phytoplankton samples collected in the sample bottle are then dripped with 3-4 drops of Lugol and given a paper label as a marker.

Phytoplankton Identification

Identification of phytoplankton samples was carried out at the Hydrobiology Laboratory, Division of Environment and Aquatic Biotechnology, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang. Identification of plankton organisms was done using a 10x10 magnification binocular microscope. Observations and counts of organisms were done with the help of Sedgwick-rafter. Identification of plankton using the identification book Sachlan (1982). After the plankton species were identified, calculations were made to calculate species abundance, diversity index, and dominance index. Plankton identification only includes the genus.

The method for determining phytoplankton species diversity involves homogenising the sample water in the sample bottle and taking samples with a drop pipette. A cover is placed over the Sedgwick rafter after 1 cc of sample water has been dropped on it. seen under a microscope at a 100-fold magnification. matching the phytoplankton identification book with the taxonomic features.

Phytoplankton Abundance

The procedure to measure the abundace of phytoplankton, a water sample is taken using a film bottle. 3–4 drops of Lugol solution were added, and the paper was marked with a marker. Gently homogenising the water sample is done. A drop pipette was used to collect water samples, and up to 1 cc of the sample was then dropped onto a Sedgwick rafter. Sedgwick rafter was examined using a 100x magnification microscope. The following formula was used to determine phytoplankton abundance according to APHA (2005):

$$\mathrm{N} = n \times \frac{a}{A} \times \frac{v}{vc} \times \frac{1}{V}$$

Description:

N : Phytoplankton abundance (cells/L)
n : Number of phytoplankton counted (cells)
a : Sedgewick rafter area (mm^2)
v : Filtered water volume (ml)
A : Area of sedgewick rafter observed (mm^2)
vc : Volume of water in sedgewick rafter (ml)
V : Volume of filtered water (L)

Phytoplankton Diversity Index

The phytoplankton diversity index is calculated by the formula according to Odum (193) as follows:

$$\mathrm{H'} = -\sum \mathrm{Pi\ In\ Pi}$$

Description:

H' : Shannon-Wiener diversity index
Pi : Proportion of the number of individuals in 1 species divided by the total number of individuals

Table 1. Classification of Shannon-Wiener Phytoplankton Diversity Index Values

Index Value	Categories
3	High diversity, high distribution of individuals per species and high community stability
1-3	Medium diversity, medium distribution of individuals of each species and medium community stability

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<1	Low diversity, low distribution of individuals of each species and low community stability

Phytoplankton Dominance Index

The phytoplankton dominance index is calculated by the formula according to Odum (1993) as follows:

$$D = \sum [ni/N]^2$$

Description:
D : Simpson's dominance index
ni : Number of indovidu of the i-th genus
N : Total number of individuals of all genera

The dominance index category is if the D value is close to 0 then there is no dominating species and if D is close to 1 then there is a dominating species.

Data Analyst

Descriptive analysis is used to explain phytoplankton composition, abundance of phytoplankton, Domination index and diversity index during the culture period of white shrimp infected white feces disease, which are shown as tables or graphs.

Results and discussion

Diversity of Phytoplankton Types

Diversity is the total number of species in a region or in a certain area which shows the richness of species in a community as well as the balance of the number of individuals of each species (Yuliana *et al*., 2012). The results of the observations show that the pond areas that have the highest diversity of phytoplankton types from the highest to the lowest are in the Tuban pond area, then in the Situbondo pond area, and finally the Lamongan pond area. In ponds in the Lamongan area, 5 genus were found from 3 phylum, from the Bacillariophyta phylum there was 1 genus, namely Cyclotella, from the Chlorophyta phylum there were 3 genera, namely Chlorella, Xanthidium, Asterococcus, from the Cyanophyta phylum there was 1 genus, namely Chroococcus. The percentage of diversity of phytoplankton species in ponds in the Lamongan area can be seen in Figure 1A. In ponds in the Situbondo area, 6 genera were found from 3 phyla, from the phylum Chlorophyta there were 3 genera, namely Chlorella, Sphaerocystis, and Ulothrix, from the phylum Chrysophyta there was 1 genus, namely Navicula, From the Cyanophyta phylum there are 2 genera, namely Coelosphaerium and Oscillatoria. The percentage of diversity of phytoplankton species in ponds in the Situbondo area can be seen in Figure 1B. In ponds in the Tuban area, 7 genera were found from 3 phyla, from the Cyanophyta phylum there were 2 genera, namely Chroococcus and Coelosphaerium, from the Chrysophyta phylum there were 4 genera, namely Cyclotella, Nitzchia, Navicula, Coconeis, from the phylum Chlorophyta there is 1 genus, namely Scenedesmus. The percentage of diversity of phytoplankton species in ponds in the Tuban area can be seen in Figure 1C.

Plankton has a good role in the aquatic environment, however the very rapid growth of plankton and resulting in blooming can actually be detrimental to the pond environment and can cause a decrease in shrimp body resistance due to poor environmental quality. Environmental factors can cause shrimp antibody production to decrease so that the shrimp's immunity to disease attacks is reduced (Maimunah and Kilawati, 2015). Ponds infected with white feces disease often occur in pond water where the color of the water tends to be dark green or dark green and can occur in pond water that is brown. Attacks of White Feces Disease can occur when pond water conditions change

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drastically or suddenly from green to brown or vice versa. Based on the phenomenon of green pond water, it shows that pond water is dominated by plankton. The change in water color from bright green to dark green and even blue indicates a shift in dominance by blue green algae (Supito *et al.,* 2016).

Cyclotella is a type of diatom that is able to live in very extreme waters. The role of diatoms as producers in the food chain is that they produce organic matter and oxygen. Cyclotella can be used as a bioindicator in the aquatic environment (Harmoko and Krisnawati, 2018). The phylum Chlorophyta is often found in pond water environments. Xanthidium, Chlorella and Asterococcus are able to live well in environments that contain high levels of nutrients and utilize them to continue photosynthesis, reproduce and carry out other living activities. The ability of the Chlorophyta phylum plankton to utilize nutrients is expected to reduce the content of toxic compounds such as ammonium so that it can improve water quality in fisheries aquaculture (Aprilliyanti *et al.,* 2016).

Chlorella, Spaerocystis, and Ulothrix are algae from the Chlorophyta type. Plankton in the pond water environment is the main producer in an ecosystem which can be used as a natural food source and oxygen producer (Fauziyah and Laily, 2014). The Cyanophyta phylum which consists of Coelosphaerium and Oscillatoria has a role as an indicator in a polluted aquatic environment. Oscillatoria is known to have the ability to withstand changes in unfavorable environmental conditions. This is because Oscillatoria has cell envelopes that are layered and sheathed (Kamilah *et al.,* 2014). Navicula is a type of diatom that is often found in aquaculture waters and can be used as a natural food source for benthic and aquaculture organisms (Padang, 2012).

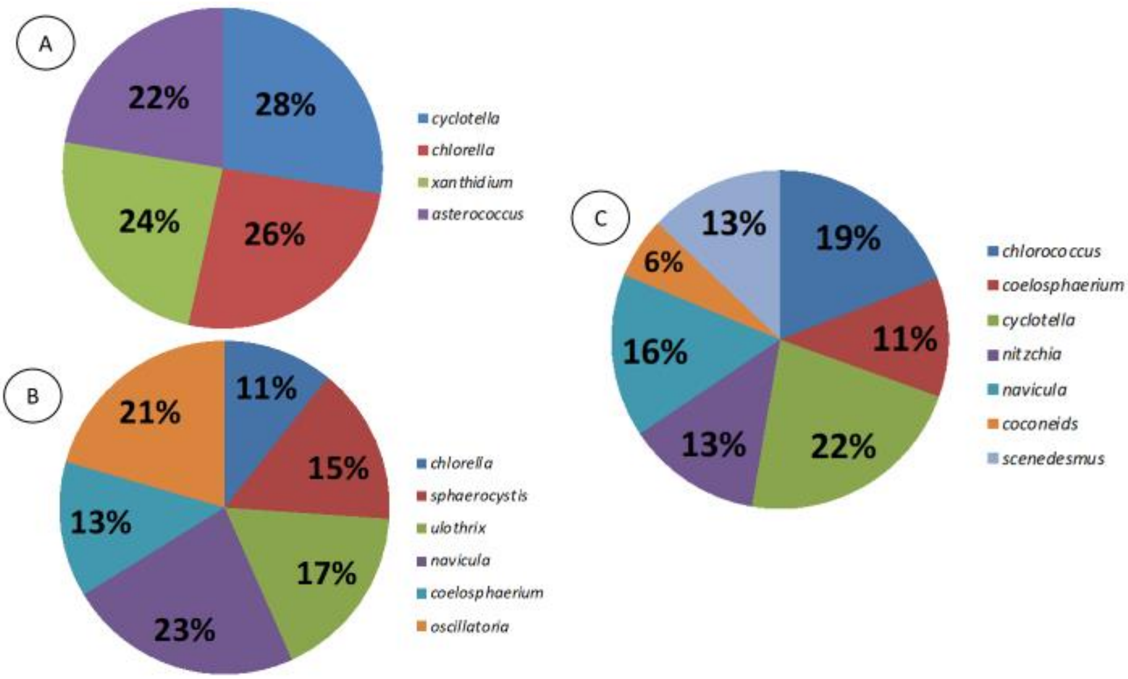

Figure 1. Percentage of Phytoplankton Diversity in A. Lamongan Pond; B. Situbondo Pond; and C. Tuban Ponds

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The diversity of Cyanophyceae in a body of water can be used as a bioindicator in determining water quality conditions. Cyanophyceae members include Chloorococcus and Coelosphaerium. In waters that are dominated by species from the Cyanophyceae class or blue-green algae, these waters can be indicated as being polluted (Lubis *et al*., 2016).

Phytoplankton from the phylum Chrysophyta have a role as the first producers which are a natural food source for zooplankton and cultivated organisms. The Chrysophyta phyla found include Cyclotella, Nitzchia, Navicula, Coconeids. However, Cyclotella plankton can also be used as an indicator of polluted aquatic environments (Heriyanto, 2011). Scenedesmus in quite large numbers in the aquatic environment indicates that the water conditions are polluted (Hariyadi, 2010).

As aquatic organisms, plankton have a range of tolerance to changes in various environmental factors such as temperature, water, pH, levels of dissolved oxygen (DO), nutrients, and light entering the waters. Changes in the values of various environmental factors will directly or indirectly affect plankton diversity (Barus, 2004).

Abundance, Diversity Index and Phytoplankton Dominance Index

The results of measuring the abundance, diversity index and phytoplankton dominance index in the three ponds can be seen in Table 1.

Table 2. Results of Phytoplankton Measurements

Pond Area	Parameter	Result
Lamongan Pond Area	Abundance (N) Sel/L	473
	Diversity Index (H^{I})	1,009
	Domination Indec (D)	0,477
Situbondo Pond Area	Abundance (N) Sel/L	633
	Diversity Index (H^{I})	0,544
	Domination Indec (D)	1,013
Tuban Pond Area	Abundance (N) Sel/L	887
	Diversity Index (H^{I})	0,526
	Domination Indec (D)	1,082

Phytoplankton Abundance

The Lamongan pond area showed a total abundance of phytoplankton of 473 cells/L, the Situbondo pond area showed a total abundance of phytoplankton of 633 cells/L and the Tuban pond area resulted in a total phytoplankton abundance of 887 cells/L. In ponds in the Lamongan area, the genus with the highest abundance was Cyclotella with 313 cells/L, while the lowest abundance genus was Asterococcus with 53 cells/L. The ponds in the Situbondo area with the highest abundance are the genus Navicula with 460 cells/L, while the lowest abundance genus is Chlorella with

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20 cells/L. The ponds in the Tuban area with the highest abundance are the genus Cyclotella with 633 cells/L, while the lowest abundance genus is Coconeids with 13 cells/L. L. The results of calculating phytoplankton abundance can be seen in Figure 2.

The abundance of phytoplankton can be influenced by the light used in the photosynthesis process. The rate of photosynthesis will increase if the light in the aquatic environment has an intensity that matches the needs of algae growth. Nutrients are also needed for the growth of phytoplankton. The existence of phytoplankton is closely related to the available nutrients, especially carbon, nitrogen, phosphorus and potassium and silica for the diatom group. Apart from that, grazing zooplankton also affects the abundance of phytoplankton. In a stable ecosystem, phytoplankton is available in abundant quantities compared to zooplankton, so that if grazing occurs by zooplankton, the balance of the ecosystem remains under control (Agustini and Madyowati, 2014).

The presence of plankton in a body of water can provide information about the condition of the water in a clean or polluted state, so that plankton as a biological parameter can be used as an indicator to evaluate the quality of a body of water or as a bioindicator. Plankton that are usually used as bioindicators are from the Cyanophyceae class and the Bacillariophyceae class (Kamilah *et al.,* 2014). Some algae that live in polluted aquatic communities are Navicula, Fragillaria, Synedra, Chlorella, Chlamydomonas, Oscillatoria, Phormidium, and Stigeoclonium. Navicula is a type of phytoplankton that is able to survive even in poor and polluted environmental conditions (Mayagitha *et al,* 2014). Phytoplankton types Bacillariophyta and Cyanophyta in the aquatic environment are capable of experiencing rapid and excessive growth which can result in Blooming Blue Green Algae (Wirabumi and Sudarsono, 2017).

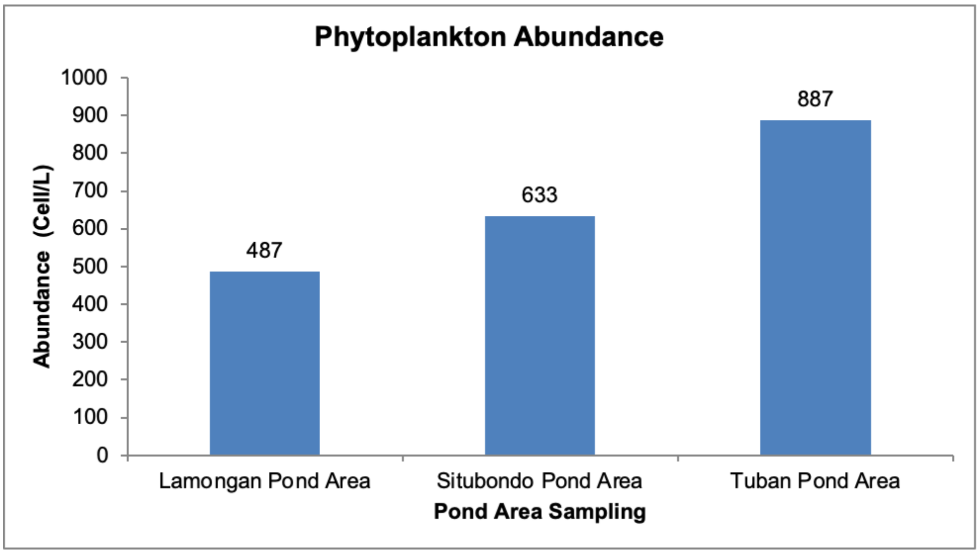

Figure 2. Abundance of Phytoplankton in vaname shrimp ponds

Phytoplankton Diversity Index

The diversity index is used to describe the structure of phytoplankton life mathematically and can be used to facilitate analysis of the types and numbers of organisms in waters. In ponds in the Lamongan area, the diversity index obtained was 1.009. In ponds in the Situbondo area, the diversity index obtained

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was 1.013. In ponds in the Tuban area, the diversity index obtained was 1.082.

The diversity index describes the richness of plankton types found in a body of water. If the diversity index value is less than 1, it is suspected that the biota community is in an unstable condition. A diversity index value between 1-3 means a medium biota community. A diversity index value of more than 3 indicates that the aquatic biota community is in a stable condition (Makmur e*t al.*, 2011). Classification of the degree of environmental pollution of aquaculture waters is based on the Shanon diversity index (H'), namely if H' > 2.0 (not polluted), 1.6 < H' > 2.0 (lightly polluted), 1.0 < H' > 1 .6 (moderately polluted) and H'< 1.0 (heavily polluted) (Rahayu *et al.,* 2015). In the three pond areas, the diversity index value shows the number 1, which means that the biota diversity in the vaname shrimp ponds is in moderate condition and based on the degree of environmental pollution, the value of the three vaname shrimp ponds is that the cultivation environment is in the moderately polluted category. According to Dewiyanti *et al*. (2014), moderate diversity indicates that the organisms are evenly distributed.

Phytoplankton Dominance Index

In ponds in the Lamongan area, a dominance index of 0.477 was obtained. In ponds in the Situbondo area, a dominance index of 0.544 was obtained. In ponds in the Tuban area, a dominance index of 0.526 was obtained. The dominant genera in the three ponds were Cyclotella and Navicula.

The abundance of Navicula species in aquatic environments is very high, this is because this species has the ability to adapt to various habitats, including less favorable habitats. Therefore, Navicula is often used as a bioindicator for environmental pollution in cultivation (Qiptiyah *et al*., 2008). Cyclotella belongs to the phylum Bacillariophyta. The Bacillariophyta class of phytoplankton dominates because of the phytoplankton's ability to adapt to environmental conditions and utilize nutrients optimally for growth. Cyclotella phytoplankton has a fast growth rate, high tolerance and is able to adapt to environmental changes and is able to utilize nutrients better, is cosmopolitan, resistant to extreme conditions, easy to adapt, and has a very high reproductive power (Dwirastina and Makri, 2015).

The presence of phytoplankton can be used as a bioindicator of changes in the quality of the aquatic environment caused by an imbalance in the aquatic ecosystem. Phytoplankton have certain tolerance limits to physicochemical factors so that they will form different phytoplankton community structures. This can be seen based on the presence of the types of phytoplankton that dominate these waters. A dominance index of less than 0.5 means that no species dominates. The greater the uniformity value and the smaller the dominance index value indicates greater species uniformity, meaning that there is no dominant species and no particular species is dominated (Mayagitha *et al*., 2014). White shrimp ponds in the Lamongan area show a value of less than 0.5, which means that there are no microalgae that dominate in shrimp ponds in Lamongan. However, vaname shrimp ponds in the Situbondo and Tuban areas show a dominance index value of more than 0.5, which means that there is biota dominance in these vaname shrimp ponds.

Conclusions and Suggestion

The results of observations in ponds in the Lamongan area showed a total abundance of phytoplankton of 473 cells/L, ponds in the Situbondo area showed a total abundance of

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phytoplankton of 633 cells/L and ponds in the Tuban area resulted in a total abundance of phytoplankton of 887 cells/L. In ponds in the Lamongan area, the diversity index obtained was 1.009. In ponds in the Situbondo area, the diversity index obtained was 1.013. In ponds in the Tuban area, the diversity index obtained was 1.082. In ponds in the Lamongan area, a dominance index of 0.477 was obtained. In ponds in the Situbondo area, a dominance index of 0.544 was obtained. In ponds in the Tuban area, a dominance index of 0.526 was obtained. The dominant genera in the three ponds were Cyclotella and Navicula. . At the three pond locations, species diversity and high abundance of members of the phyla Cyanophyta and Bacillariophyta were found to be detrimental, the genera found were indicators of polluted waters. The dominance of Cyanophyta (blue-green algae) is also an indicator of the emergence of White Feces Disease.

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