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Dynamic Ratio Correlation of N:P in relation to the Diatom Abundance in the Intensive System of the Vannamei (*Litopenaeus vannamei*) Shrimp Pond

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Abstract. Diatoms are one type of aquatic phytoplankton that has an important role. Diatoms, or *Bacillariophycyae,* are a phytoplankton that suits the needs of the shrimp cultivation in the pond because diatoms are an abundant primary producer and are needed as natural feed. Diatom needs nitrogen and phosphate in its life, while the nutrient itself in the water is not always in a stable condition. The research aimed to determine the dynamic ratio correlation of N:P in relation to the diatom abundance in intensive system vannamei (*Litopenaeus vannamei*) shrimp ponds in order to maintain aquatic stability or living media within intensive system vannamei shrimp cultivation. The main parameters observed were Ammonium, Nitric, Nitrate and Phosphate, as well as the abundance of diatom plankton. The supporting parameters measured were pH and clarity in the intensive vannamei shrimp pond in Banyuwangi. Based on results of the data analysis and the study about correlation of dynamic ration N:P toward abundance of Diatom, it can be concluded that the ratio value of N:P influences the composition of the level classification of the phytoplankton in aquatic cultivation. The differences in the level of ammonium, nitric, nitrate and phosphate provide different influences on the diatom abundance in the water. A high level of nitrate indicates an abundance of diatoms.

1. Introduction

At the present time, Vannamei shrimp (*Litopenaeus vannamei*) are an important fishery product. The production of giant tiger prawns in the agroindustry in Indonesia is decreasing, thus the development of Vannamei shrimp is a relevant cultivation alternative [4]. Water quality management is important for the success of shrimp cultivation. Water quality is focused on determining the availability of various organisms in a pond ecosystem, both for cultivating organisms and other biota as a compiler of the pond's overall ecosystem. Phytoplanktons are one factor that influences the water quality. Phytoplankton production in intensive cultivation is influenced by the availability of nutrients in the aquatic system, which mostly focuses on nutrients from Nitrogen (N) and Phosphate (P) [2].
Phytoplankton is expected to grow optimally in a water pond. In general, phytoplankton management is performed

by optimizing the organic compounds, through using fertilizer and changing the wate [2]. Nitrogen and phosphor are two influential parameters in water. There are many nutrients in the water, but only some of them able to be utilized by the phytoplankton [3].

Diatoms are one important species of phytoplankton in the water. The diatom *Bacillariophyceae* is a phytoplankton that is suitable to needs of shrimp cultivation in the pond [17]. Diatoms are also a potential bio-indicator compared to other organisms [15]. The growth supporting factors of plankton are complex and interact with one another between the physical and chemical factors in the water such as dissolved oxygen, temperature and the availability of nitrogen and phosphor [13].

The existence of diatoms in aquatic systems is influenced by various aspects. One of them is the availability of the N:P ratio, while the nutrients in the water are not stable. Regarding the condition above, it is necessary to apply further research of the correlation of the ratio of dynamic N:P in relation to the abundance of diatom in Intensive system Vannamei shrimp (*Litopenaeus vannamei*) ponds in order to maintain the water stability or living media in the intensive system in the Vannamei shrimp pond.

The problem formulated in this research was: how the correlation of the dynamic N:P ratio has a relationship on the diatom abundance in intensive systems of Vannamei shrimp *(Litopenaeus vannamei)*. The aim of this research was to discover the correlation of the dynamic N:P ratio toward the diatom abundance in intensive systems of Vannamei shrimp (*Litopenaeus vannamei*) in order to maintain the water stability or living media in intensive systems of shrimp cultivation.

The benefit of this research is that it seeks to provide information for the readers about the correlation of the dynamic N:P ratio toward diatom abundance in intensive systems of Vannamei shrimp (*Litopenaeus vannamei*). The information of this research and the results are useful to develop knowledge and its application for the community, in particular, for Vannamei shrimp farmers.

2. Methodology

2.1 Tools and materials

The tools used in this research were pH paper, secchi disks, plankton nets, bailers, a hand counter, hemocytometer, drop pipette, microscope, object glass, cover glass, test kit, and sample bottle. The materials used in the research included sample water from the intensive system of vaname shrimp (*Litopenaeus vannamei*) and lugol to deactivate the plankton movements.

2.2 Research methods

The method used in this research was a survey. The research preparation was applied by preparing the necessary tools and materials, i.e. the pH paper, secchi disk, plankton net, bailer, hand counter, hemocytometer, drop pipette, microscope, object glass, cover glass, test kit, sample bottle and lugol. The water sample, as the material used to observe the diatom abundance, was taken at 3 stations, which consisted of pond plots with 4 sample points for each corner of the intensive system of Vannamei shrimp (*Litopenaeus vannamei*) as a form of data clarification. The samples were taken using a plankton net, before calculating the density. The samples had to be directly brought to the laboratory in order to be observed and analyzed. The samples were observed at 100 and 400 times enlargement under a binocular microscope with the direct calculation done using the hemocytometer. The samples needed to be directly observed to maintain the quality of the phytoplankton. The samples were used to observe the ammonium, nitrite, nitrate and phosphate. The samples were taken in the morning at 5 AM, while the water samples to observe the plankton and water quality were taken in the afternoon at 4 PM.

2.3 Calculation and observation of the diatoms

The diatom observation consisted of identify and abundance in the pond. The diatom observation was applied to 3 plots of the pond, and the sample was taken at 4 points in the corners of each plot. The samples for the diatom observation were taken using a plankton net with a mesh size of 20 microns. The plankton net was able to filter the diatom phytoplankton class, as well as being able to enable the water to

come out through the micro holes of the plankton net.
The method used in the identification and observation of the phytoplankton was a direct calculation using a hemocytometer. It was applied by taking a 1 ml water sample from the bottle sample, and then covering it with a glass cover. The observation was done by identifying the phytoplankton and calculating its density using the hemocytometer. The sum of the phytoplankton was calculated using the method suitable for the size of the plankton. The samples had to be directly calculated in order to maintain the quality of observed phytoplankton, to make it easier to identify and conduct the density calculation when observing them under the microscope.

3. Results and discussion

The results of this research consisted of the dynamic N:P ratio and plankton abundance as the main data, and with the parameters of water quality like the level of acidity (pH) and water clarity as the supporting data. The observation was applied to 3 plots in the intensive system.

3.1 Grade of N:P ratio

The N:P ratio data was obtained from the data of the total nitrogen in the ammonium, nitrite and nitrate and this was compared to phosphate to get the dynamic N:P ratio for the 3 stations/plots. The data was taken from observations every 3 days. The results of the calibration of the dynamic N:P ratio can be seen in the following graphics below.
Based on the graphic above, the N:P ratio in plot 4 from day 24 to day 27 decreased, and then it increased from day 27 to day 33. On the next day, from day 33 to day 36, the N:P ratio decreased before starting to increase again at day 39. After this, the N:P ratio decreased until day 45. At day 45 and up to day 54, the N:P increased. From day 51 to day 57, it decreased, and then increased before day 60. The cause of the fluctuations in the grade of N:P ratio was due to the changing levels of ammonium, nitrate, nitrite and phosphate as mentioned below.
The calculation of the N:P ratio in plot 4 resulted in various fluctuating situation; at the shrimp age of day 24 to 30, it increased and then decreased from day 30 to day 39. At the shrimp age of day 30 to 42, the N:P ratio increased and then slightly decreased from day 45. From day 45 to day 54, the N:P ratio increased, decreasing again from day 54 to day 57 before increasing again until day 60. The cause of the fluctuating N:P ratio is due to the changing levels of ammonium, nitrate, nitrite and phosphate as mentioned below.

3.2 Plankton abundance

Plankton with a high abundance are a species that is able to support its life more efficient than other species in the same tropical level. This means that the species have important role for the plankton community in that particular aquatic system. The observation was applyied in 3 different stations/plots. The observation data was the result of every per-3-days calibration. This has been shown in the graph below.
Based on the graphic of the diatom journey above, it shows that on the initial day, the density of the diatoms remained low at a level of 0 cells/mL at day 24. From day 24 to day 39, the diatom density continually increased. The highest diatom density occurred on day 39 at 1,187,500 cells/mL. The diatom density decreased from day 39 to day 48, and then increased again until day 54. From day 57 to day 60, the diatom density decreased again.
Based on the graphic of the diatom density in plot 4, it showed initially on day 24, the diatom density remained at 0 cells/mL, which showed less density at the initial cultivation. Between day 24 to day 39, the density increased to 135,000 cells/mL. From day 39 to day 45, it decreased, and then from day 45 to day 54, diatom density continually increased. The highest level of diatom density in plot 4 was on day 54 at

682,500 cells/ml. The density then decreased until day 60.

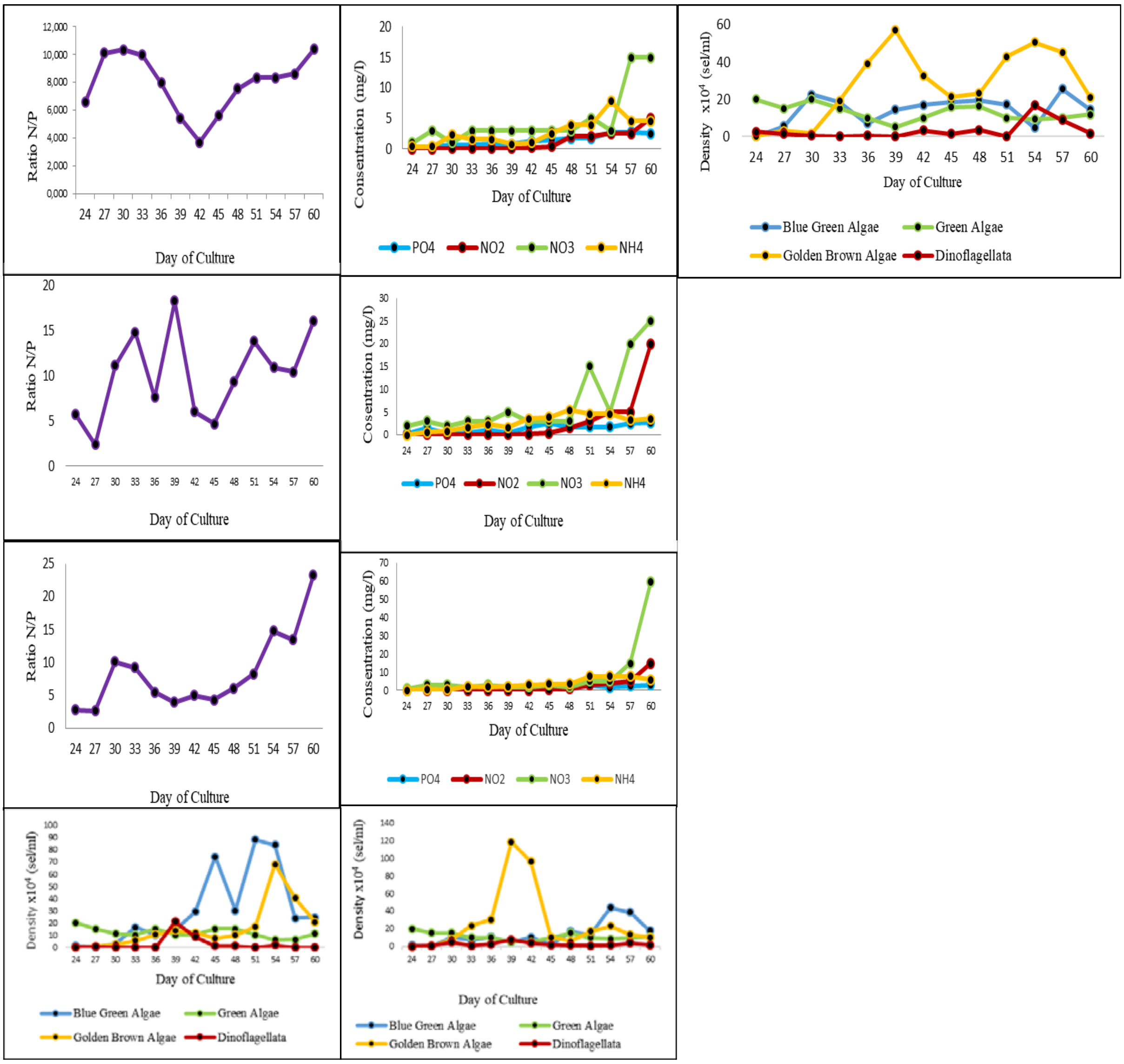

Figure 1) Ratio N: P at Plot 1 during the research; 2) Plot 1 Ammonium, Nitrite, Nitrate and Phosphate during the Research; 3) Ratio N: P Plot 4 during the research; 4) Plot Ammonium, Nitrite, Nitrate and Phosphate 4 during the research; 5) Ratio N: P Plot 7 during the research; 6) Ammonium, Nitrite, Nitrate and Phosphate during the research; 7) Phytoplankton Chart Plots 1 during the research; 8) Phytoplankton Chart 4 during the research and 9) Phytoplankton Chart 7 during the research.

Based on the graphic above, the density of the diatoms increased between day 24 and day 27, and then decreased until day 30. The density of the diatoms increased again from day 30 to day 39, and then decreased until day 45. The lowest level of diatom density in plot 7 was on day 24 with 2,500 cells/mL and the highest level occurred on day 39 at 570,000 cells/mL. From day 45 up to day 54, the diatom density increased and then started to decrease until day 60.

Diatoms, or Bacillariophyceae, are a suitable phytoplankton related to the needs of the shrimp cultivation in the pond [17]. This is because diatoms are an abundant primary producer and are needed as natural feed in both freshwater and the sea [1]. Diatoms are suitable feed for larva, shrimp, fish and mussels. This is because they contain complete unsaturated fatty acids, vitamins and amino acids, more than synthetic feed [16]. Diatoms are also bio-indicators related to water contamination because they have wall cells made from silica. Cell walls made from silica in general are strong and remain intact, thus the analysis of cells walls will indicate accumulated contamination particles in the aquatic system.

Nutrients are necessary parameter in relation to the life and growth of microalgae. They come in the form of a chemical compound needed in the metabolic process and cannot be produced by the organisms themselves; they are obtained from nature. The organisms need nutrients in order to develop and improve their body tissue, to manage the internal processes of the body and to provide energy for the body. The availability of nutrients is basically the availability of N and P. Nutrient sources of N and P include fertilizers and waste. The usage of N basis fertilizer (like Urea, $(NH_2)_2CO$) in an area of global cultivation has been increased by 100 times the N source in the water [8]. Nitrogen for plant or algae growth is mostly used in chlorophyll, which is important for photosynthesis and further growth [7]. Phosphor is very important for the viability of water organisms because its function in relation to the storage and transfer of energy in cells is useful in the genetic system [11].

The N:P ratio is one important factor in the pond and it is one of the success determinants in relation to activities of cultivation, in particular, in shrimp cultivation. It is in line with [6]'s findings that a high concentration of nutrients in the water will affect in the aquatic system's productivity. The composition of the nutrients in the N:P ratio is well-known as a red-field ratio. The N:P ratio is strongly affected by the plankton abundance. The N:P ratio can be calculated through a data combination regarding the ammonium, nitrite and nitrate as the component N and phosphate of P.

The ammonia resulted from shrimp feces, leftover feed and dead phytoplankton. Ammonia in water comes in two forms; not ionized (ammonia, NH_3) and ionized (ammonium, NH_4). Ionized ammonia is a harmful nutrient for water organisms because it is toxic, while non-ionized ammonia can be used as a nitrogen source by the phytoplankton [5].

Nitrite in water is often found in lesser amounts because it is unstable [6]. The nitrite concentration is less detectable in the water because of the nitrification process through the nitrosomonas bacteria [6]. Natural water contains nitrite of 0.001 mg/L, and it should not be more than 0.006 mg/L. Anything above 0.05 mg/L nitrite is toxic for sensitive organisms in the water [5]. This is in line with the conditions in plot 1, plot 4 and plot 7 on the last observation day or on day 60, where the extremely high nitrate concentration caused diatom density deprivation. Nitrate is a major form of nitrogen in natural water and a main nutrient for plant or algae growth. It is dissolved easily and is unstable [5]. [12] mentioned that for optimal growth, phytoplanktons need nitrate in the range of 0.9 – 3.5 mg/L.

An aquatic system with a low phosphate concentration (0.00 – 0.02 mg/L) will be dominated by the phytoplankton of the Bacillariophyceae class (diatom). In a medium phosphate concentration (0.02 – 0.005 mg/L), the water will be dominated by Clorophyceae. In a high phosphate concentration (>0.010 mg/L), the water will be dominated by Cyanophyceae. Orthophosphate is a form of phosphate that is able to be directly utilized by the phytoplankton for growth. Phosphate are usually available in lower amounts, and thus can be dividing factor for the growth of phytoplankton [14].

Based on the observation results of the phytoplankton during the research, there were various species of phytoplankton; Blue Green Algae, Green Algae, Golden Brown Algae and Dinoflagellata. This is in line with [20]'s statement indicating that the ratio of N:P in the water will influence the dominant composition of the plankton species. An N:P ratio of >20 means that the nature of the water will be dominated by the plankton diatom (Golden Brown Algae), while an N:P ratio of approximately 20 means that the nature of the water will be dominated by Green Algae. An N:P ratio of <10 means that the nature of the water is suitable for Blue Green Algae.

Based on the observation result over 60 days in 3 plots, it was determined that different levels of nitrogen and phosphor have different influences on the phytoplankton composition. An abundance of diatoms in the initial cultivation on day 24 remaining low in three plots at 0 – 2500 cells/mL. This possibly happened because of the low level of nutrients in the pond. This aligns with with the statement of [18], which states that along with the increase in cultivation time, the accumulation of leftover feed and shrimp feces will also increase. Both of them are organic compounds which will then be decomposed by bacteria into inorganic compounds (such as ammonia, nitrate, nitrite and orthophosphate). The water will therefore become more fertile. Fertility enhancement as a result of accumulated left-over feed and shrimp feces will affect the increase in diatom abundance, pushing it to the highest level of 1,187,500 cells/L.

Observations were done in plot 1 on day 27, 30, 42 and 51; in plot 4 on day 30, 33, 39, 42, 45, 48, 51 and 57 and in plot 7 at day 45, 48, 51, 54 and 57. It was discovered that the increase and decrease in the ratio of N:P was suitable for the increase and decrease of the diatom density level.

The N:P ratio was noted in plot 1 on day 33, 36, 45, 48, 54, 57 and 60; in plot 4 on day 27, 36, 54 and 60and in plot 7 on day 27, 30, 33, 39, 42 and 60. It was discovered that the increase and decrease in the N:P ratio is not in line with the increase and decrease in the diatom density level. This can be seen in the graphic on the ratio of N:P and in the graphic of the phytoplankton density mapped during the research in plots 1, 4 and 7. This has possibly happened because of the different composition levels of ammonium, nitrite, nitrate and phosphate. According to [12], an abundance of diatom can be influenced by nutrients like Nitrate and Phosphate.

Nitrate's fluctuating condition influences the increase in diatom density; this can be seen in plot 1 on day 27 and 33, in plot 4 on day 27, 33 and 51 and in plot 7 on day 24, 36 and 51, where increase nitrate caused an increase in diatom density. This aligns with the statement of [12] that nitrate (NO_3^-) is the main nutrient required for diatoms to grow and develop well. The high concentration of nitrate in the water will stimulate the growth of the diatom, because nitrate in certain concentrations provides a good condition for the diatoms to grow.

There were several conditions where there was a low level of nitrate but where the level of diatoms is increasing, like what happen in plot 1 on day 30, 51 and 54 and in plot 7 on day 33 and 39. This possibly occurred because in that condition, other classes of phytoplankton such as Blue Green Algae and Green Algae also need nutrients. However, the phytoplankton decreased as they can't absorb the nutrient very well. This means that the nutrients were more utilized by the diatoms.

As usual, phosphate was available at a few levels, therefore it can be used as dividing factor for phytoplankton growth [14]. In plot 1 on day 51 to day 60 and in plot 4 on day 57 out of 60, there was an increase in phosphate level. This was caused by an abundance of phytoplankton dominated by Blue Green Algae rather than diatoms. This aligns with a statement by [12] stating that aquatic systems with a low concentration of phosphate will be dominated by phytoplankton of the class Bacillariophyceae (diatom). In a high concentration of phosphate (>0.10 mg/L), the population will be dominated by Blue Green Algae.

Based on the plot 1, plot 4 and plot 7 data, the phytoplankton diatom density was more prevalent based on [9]'s statement that described Bacillariophyceae as a type of diatom that is more tolerant of environmental conditions such as temperature. It is able to adapt well to the environment and reproduces quickly and well. When there is an increase in nutrient levels, the diatoms are able to do mitotic division up to three times in 24 hours. Dinoflagella can only do so once every 24 hours in the same nutrient conditions.

In diatom cultivation activities, they are also able to adapt well by regulating their spores in accordance with the statement by [10], where they determined that the Bacillariophyceae class also has good adaptability. They can function in the context of silent spores that are usually smaller than other diatom cells. These silent spores can survive and grow in poor conditions as well as in the parameters of an environment that is still relatively stable for phytoplankton growth for the Bacillariophyceae class.

4. Conclusion

These results indicate that the relationship between the dynamic data the N:P ratio and the abundance of diatoms can be concluded as follows; the N:P ratio values affect the composition of the phytoplankton classes under cultivation. The differences in the values of ammonium, nitrite, nitrate and phosphate have different effects on the diatom abundance on the continent. Under high nitrate conditions, the value of abundance leans toward the diatom phytoplankton or golden brown algae.

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