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The dynamics of *Chlorella* spp. abundance and its relationship with water quality parameters in intensive shrimp ponds

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Abstract. *Soeprapto H, Ariadi H, Badrudin U. 2023. The dynamics of* Chlorella *spp. abundance and its relationship with water quality parameters in intensive shrimp ponds. Biodiversitas 24: 2919-2926. Chlorella* spp. is a plankton genus belonging to the phylum Chlorophyta and is commonly found in the shrimp farming ponds. This plankton might serve as natural feed especially for the post-larvae stage shrimps. This study aims to investigate the dynamics of *Chlorella* spp. abundance and its correlation with the N:P ratio in the intensively managed shrimp ponds. The study was conducted using a causal ex-post facto design with data collection through purposive sampling. The results showed that the plankton abundance in the observed ponds was moderate consisted of 4 class and 7 genera. The dominant genus was *Chroococcus* spp. and *Chlorella* spp. with abundance ranging from 2.50E+05 to 2.60E+05 cells/mL. Plankton abundance correlated with water quality conditions, including pH of 7.9, salinity of 19-20‰, temperature of 28.50-28.75°C, dissolved oxygen of 5.12-5.39 mg/L, nitrite of 0.035-0.072 mg/L, phosphate of 0.011-0.025 mg/L, organic matter of 95.77-102.32 mg/L, and alkalinity of 125-130 mg/L. There was also correlation between the increase in plankton biomass and nutrient solubility. The abundance of *Chlorella* spp. was negatively correlated with the N:P ratio of the water in the ponds. Based on dynamic model analysis, the most ideal N:P ratio is 1:20 from several simulations of 1:10, 1:20, 1:30, and 1:40. The dynamics of *Chlorella* spp. abundance followed an oscillatory pattern from the beginning of cultivation to harvest with the most ideal N:P ratio of 1:20. This understanding is important for making environmental engineering concepts in pond aquatic ecosystems.

Keywords: Biomassa, N:P, nutrient, plankton, shrimp

INTRODUCTION

Intensive shrimp farming is one of the aquacultural activities widely developed in coastal areas of Indonesia (Ariadi et al. 2019a). Shrimp farming in Indonesia has been developed intensively using various systems and technologies (Novriadi et al. 2022). There are several shrimp species and varieties widely cultivated in the country including *Penaeus monodon* (Fabricius, 1798), *Litopenaeus vannamei* (Boone, 1931), and *Penaeus merguiensis* (De Man, 1888) (Ariadi et al. 2021a). Indonesia's total shrimp production from pond culture in 2022 is 1,099,976 tons (Ariadi et al. 2022). The total production has increased by 15% from the 2021 period, which means that there is an increase in aquaculture production.

In the management of shrimp farming, one important aspect that needs to be considered is the presence of microorganisms that can serve as natural or live feed for the shrimp. The presence of live feed improves the efficiency of artificial feed and optimizes the conversion rate of shrimp culture (Ariadi and Wafi 2020). Live feed in shrimp farming can be used as an alternative feeding option when shrimps are still at post-larval stage (Fricke et al. 2023). Shrimps at post-larva require a higher level of nutritional needs than shrimps at adult stage (Wanna et al. 2023). This because the nutritional requirement for shrimp growth has a correlation with metabolic processes and the increasing amount of oxygen consumption (Xue et al. 2021). Shrimp tend to prefer live food types with high protein and mineral levels (Dien et al. 2018). In this regard, as live feed source, microorganisms provide adequate nutritional contents, which is very necessary in intensive shrimp culture.

Microorganisms that can be used as live feed in shrimp farming are plankton and detritus (Gonzalez et al. 2012). Plankton not only serves as natural feed in shrimp culture but also functions as a bioindicator to inform the quality of water environment in the shrimp ponds (Zhang et al. 2022). It strongly correlates with dynamic water quality throughout the shrimp cultivation cycle (Ariadi et al. 2021b). In many cases, water quality of shrimp pond fluctuates, which is affected by the plankton dynamics and water quality parameters that exist in aquatic ecosystems (Lavoie et al. 2022). Due to its importance, the dynamics of plankton in the shrimp cultivation system have attracted more in-depth investigations (Alam et al. 2021).

Previous studies showed that plankton dominance and fluctuation in water will have an impact on the stability of the pond ecosystem holistically. Plankton is active microorganisms whose presence can have a grazing effect on aquatic habitat ecosystems (Negrete-García 2022). Water fluctuations and extreme weather changes will impact the plankton community structure in shrimp pond waters (Ariadi et al. 2021c). Water treatment processes, such as liming, fertilizing, overfeeding and several other mitigating activities, can also contribute to changes in the dynamics of plankton in pond waters (Zhang et al. 2022). The plankton abundance and dominance also affect the community structure and composition of organisms that exist in the ponds water ecosystem (Ariadi and Mujtahidah 2022). The changes in plankton abundance can trigger dynamic fluctuations in the pond aquatic ecosystem conditions (Lu et al. 2022). Therefore, knowledge of plankton dynamics in shrimp farming activities is needed to support productive aquaculture cultivation activities (Tyrrell 2019).

One plankton genus that can be used as natural feed for shrimp is *Chlorella* spp. (Aprilliyanti et al. 2016). *Chlorella* spp. as a plankton type that is commonly found in pond ecosystems in tropical waters and plays an important role in aquaculture activities (Arumugham et al. 2023). *Chlorella* spp. is a cosmopolitan microorganism which easily blooms in various seasonal conditions (Yaobin et al. 2019). In some intensively managed ponds, *Chlorella* spp. often dominates and can be used as a biological agent to maintain the balance of the pond's water ecosystem (Ramanan et al. 2010). *Chlorella* spp. is highly desired to be used as live food when the shrimps are still at larva stage. This plankton is often used in controlled indoor culture systems to support the availability of live food for fry shrimp (Zhang et al. 2022). *Chlorella* spp. is also very sensitive to N:P ratio changes in the pond waters (Guo et al. 2022), thus ponds with a relatively high N:P ratio (>1:20) are suitable for the growth of this plankton.

The dynamic abundance of *Chlorella* spp. in the intensive shrimp cultivation ponds is very intriguing and needs further in-depth investigations. Therefore, this study aims to investigate the *Chlorella* spp. abundance and dynamic in intensive pond system and analyze their correlation with the N:P ratio. We expected that the results of this study might add knowledge regarding the relationship between plankton abundance and N:P ratio in pond water ecosystems. This understanding is important for making environmental engineering concepts in pond aquatic ecosystems.

MATERIALS AND METHODS

Study period and location

This study was conducted from December 2022 to February 2023 in intensive shrimp cultivation ponds located in Ulujami Village, Pekalongan District, Central Java, Indonesia. Two ponds were used as the observation site with extent of each pond was 200 m^2 and a stocking density of 125 shrimp/m^2. The observed ponds were given additional aeration using a paddle aerator of 8 HP.

Data collection procedure

The observed parameters included *Chlorella* spp. abundance, water quality parameters, and shrimp biomass productivity. The study used a causal ex-post facto design and purposive sampling method for data collection. The ex-post facto causal design method is a research method based on scientific data in the field without any engineering treatment. Samples were collected from three sides of the ponds including the edge, middle, and central, twice a week, while data were obtained based on parameters and sampling time.

Plankton abundance was observed using an Olympus CX 22 microscope and a NEUBEUR hemocytometer. Water quality parameters observed included pH, salinity, temperature, dissolved oxygen, nitrite, nitrate, ammonium, organic matter, phosphate, and alkalinity. Measurement of water quality parameters was carried out during the cultivation cycle.

Data analysis

Plankton abundance

Plankton abundance was calculated formula using APHA (1998) as follows:

$$N = Z \text{ x } \frac{x}{y} \text{ x } \frac{1}{v}$$

Where:

N : Individual abundance of plankton (ind/ltr)
Z : Individual number of plankton
X : Volume of filtered water sample (40 mL)
Y : Volume of 1 water drop (0.06 mL)
V : Volume of filtered water (100 L)

Water quality parameters

The sampled water was analyzed in the water quality laboratory at Pekalongan University Central Laboratory. The results of the water quality parameter analysis from the laboratory were then analyzed in the causal loop model indicators that have been made for assessment based on dynamic modeling system analysis. The causal loop model used refers to the multi-parameter linkages to observed research indicators. The assessment of the causal loop model is also based on existing theoretical concepts. In this study, the analysis of causal loop model used Stella software ver 9.02.

RESULTS AND DISCUSSION

Plankton diversity and abundance

The results of observation showed that the most frequently occurring genera in the ponds included *Chlorella* spp., *Chroococcus* spp., *Oscillatoria* spp., *Microcystis* spp., *Chlamydomonas* spp., *Nitzschia* spp., and *Coscinodiscus* spp. as shown in Table 1. The plankton genus abundance is strongly influenced by biological stability, the presence of a supportive water ecosystem (Utojo 2015), and the applied shrimp cultivation management treatment (Ariadi et al. 2022). The types of plankton found were green algae, blue green algae, and diatomae which tend to be abundant in intensive shrimp pond waters (Ariadi et al. 2021a).

The presence of abundant and diverse plankton indicates that an ecosystem has a good biological balance, meaning that the structure and composition of organism community are stable with high ecological integrity (Lyu et al. 2021). A stable plankton community structure and composition in the ponds will also reduce shrimp retention levels against stress (Shen et al. 2021). Shrimp will be easily stressed and infected by diseases if there are extreme fluctuations in environmental biological parameters (Sui et al. 2023).

A stability in plankton dominance and abundance indicates that aquatic ecosystems have minimal fluctuation of biological parameters (Wang et al. 2022). The stability of this aquatic ecosystem can also be caused by the shrimp cultivation standard applied, which is quite good and in accordance with the aquatic environment characteristics. Appropriate cultivation methods are needed in intensive shrimp farming activities to minimize crop failure (Huang et al. 2022). The abundance of plankton, which tends to be stable is caused by weather and seasonal factors, which are relatively stable throughout the cultivation period (Ibarbalz et al. 2019). Weather and season are the key factors that determine of temperature distribution in pond waters (Wan et al. 2021). Temperature in the plankton life cycle is a limiting factor whose existence will greatly determine of plankton succession (Ariadi and Mujtahidah 2022).

Among the plankton genera, *Chroococcus* spp. and *Chlorella* spp. had the highest abundance, as shown in Figure 1. *Chroococcus* spp. is a genus from the class of Chyanophyceae, while *Chlorella* spp. belongs to Chlorophyceae. The presence of *Chroococcus* spp. indicates that the ponds water is eutrophic (Manan et al. 2016), while *Chlorella* spp. is a cosmopolitan plankton genus (Ariadi et al. 2019b). Both species are considered not harmful as long as they are not blooming massively in water. If massive blooming of *Chroococcus* spp. occurs, the shrimp will experience gill damage and digestive disorders (Zhang et al. 2023).

In term of class, the plankton abundance in the ponds was quite diverse and balanced, ranging from Chlorophyceae, Chyanophyceae, Bacillariophyceae, and Coscinodiscophyceae. The plankton abundance results are in accordance with the data fluctuations as shown in Figure 1 and tended to be relevant with the real conditions in the pond waters. This stability will provide intervention in the food chain that occurs in water ecosystem (Sahoo et al. 2020). The plankton existence and dominance in water ecosystem are temporal and dynamic (Tulsankar et al. 2021). The plankton dynamic is commonly found in intensive shrimp pond waters due to the process of aquaculture inputs provided (Tien et al. 2019).

Abundance of *Chlorella* spp.

Chlorella spp. is one of the dominant plankton genera commonly found in intensive shrimp cultivation ponds (Wafi and Ariadi 2022b). In this study, the population of *Chlorella* spp. in the Pond 2 was higher compared to the Pond 1 as shown in Figure 2. The balance ratio of nitrite, nitrate, ammonium, and phosphate parameters will greatly determine the *Chlorella* spp. abundance in water (Angela et al. 2021). Besides, this plankton genus can be used as natural feed for shrimp (Ariadi et al. 2021a).

Table 1. The plankton genera found in the studied ponds during the observation period

Pond 1	
Class	Plankton genus
Chlorophyceae	*Chlorella* spp.
Chyanophyceae	*Chroococcus* spp., *Oscillatoria* spp., *Microcystis* spp.
Pond 2	
Class	Plankton genus
Chlorophyceae	*Chlorella* spp., *Chlamydomonas* spp.
Chyanophyceae	*Chroococcus* spp., *Oscillatoria* spp., *Microcystis* spp.
Bacillariophyceae	*Nitzschia* spp.
Coscinodiscophyceae	*Coscinodiscus* spp.

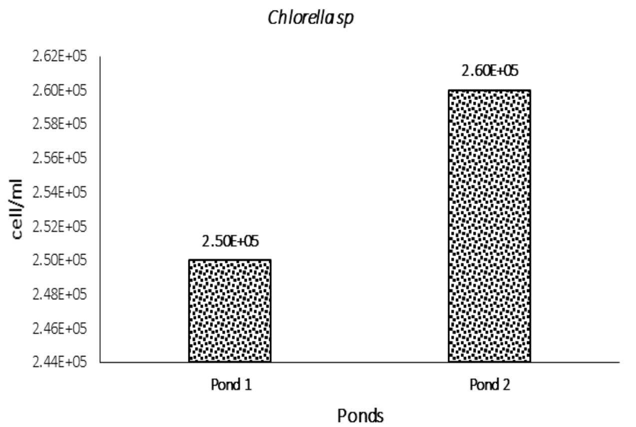

Figure 2. The abundance of *Chlorella* spp. in two ponds during the observation periods

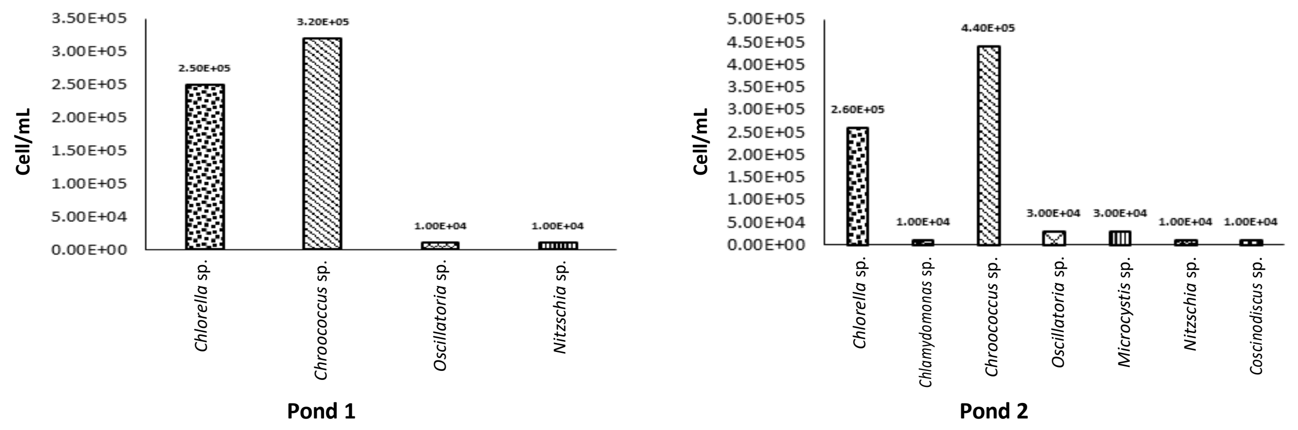

Figure 1. The abundance of each plankton genus in research ponds during the observation period

Chlorella spp. are commonly found in intensive shrimp pond waters due to the fertile and consistent character of pond waters (Lyu et al. 2021). The feeding intensive process and fertilization mechanism have another impact on the fertility level of pond waters (Maliwat et al. 2021). The amount of feed waste from shrimp farming activities is one of the main factors causing excessive fertility in pond waters (Li et al. 2019). Quantification of feed waste in pond ecosystems will accumulate in abundance and be aggregative. The aggregate increase in waste load is caused by the higher amount of feed given as a result of increasing by shrimp biomass reared (Maliwat et al. 2021).

Chlorella spp. are also tolerant to extreme weather changes (Shaari et al. 2011) and are not affected by dynamic ecosystem of the shrimp ponds. Aside from being used as a natural feed source, *Chlorella* spp. is an important microorganism that can indicate the balance of water quality (Santanumurti et al. 2022). This plankton acts as a competitor to *Vibrio* spp. for nutrients in the pond's ecosystem (Ariadi et al. 2022). Nutrients in pond waters have a limiting factor that is needed by aquatic organisms.

The process of competition to obtain nutrients by aquatic microorganisms occurs due to the processes of microorganism decomposition and respirations that take place at any time (Deng et al. 2021). The nutrient competition will also have an impact on the existence of *Chlorella* spp. in pond waters simultaneously (Iber and Kazan 2021). Nutrients will be reduced (in some case until zero nutrients) if the nutrient levels and the amount of aquaculture inputs in pond waters are not given at all.

Water quality

Table 2 shows that water quality parameters at the studied ponds were relatively good, and on average, were suitable for shrimp farming. Overall, the values were relatively ideal and comply with the recommended standards, except for the organic matter parameter. Water quality parameters are important indicators in intensive shrimp cultivation ecosystem (Ariadi 2020). The dynamics of water quality is caused by biotic and abiotic fluctuations in pond waters (de los Santos et al. 2021). The water quality parameters usually correlate with the dominant plankton, such as *Chlorella* spp. and *Oocystis* spp.

The excessive concentration of organic matter parameter might be due to the applied intensive treatment, which allows for the excess inputs and causes the accumulation of waste loads in the ponds (Ariadi 2019). The accumulation of organic matter in the ponds comes from feed waste, shrimp faeces, and other production waste (Ding et al. 2022). The high organic matter in the ponds also comes from a low siphon activity (Angela et al. 2021). The siphon frequency level will greatly determine several water chemical parameters, such as organic matter, total suspended solids, and dissolved oxygen (Wang et al. 2021).

The organic matter will be dangerous if the concentration is high and lasts for a long time (Liu et al. 2021). The presence of excessive organic matter will trigger an increase in *Vibrio* spp. dominance which is pathogenic (Ariadi and Mujtahidah 2022). High organic matter will also trigger shocks to water quality parameters due to the increasing of decomposition process by detritus in pond waters (Yang et al. 2019). The water quality dynamics will continue intensively during the shrimp cultivation period (Yaobin et al. 2019). The dissolved organic matter concentrations in pond ecosystems will have an impact on the physico-chemical processes that occur.

Dynamic model of the relationship between plankton abundance and water quality

Figure 3 presents the analysis results of dynamic model of multi-parameters correlation obtained from a literature study based on related theories. Multi-parameters analysis in a dynamic model system can be obtained from the correlation results between variables (Dykes and Sterman 2020). The variables created in this study were analyzed to ensure that they were all connected.

In the agribusiness sector, the correlation between variables can be obtained from parameters mutually correlated (Ariadi and Mujtahidah 2022). The variables analyzed in this study were nutrient loads and plankton biomass dynamic. Plankton in water is influenced by variables of nutrients, temperature, and the presence of the appropriate mineral elements as shown in Figure 3. The results of the dynamic modelling described in the causal loop model will be used as an tool analytical to find out the cause and effect of multivariable.

The correlation of *Chlorella* spp. with nutrients

Figure 4 shows that there is similar trend between on the dynamics of *Chlorella* spp. and nutrient, and between the N:P ratio and plankton biomass. Plankton in its natural habitat has a very close correlation with nutrient abundance in water (Guo et al. 2022). Furthermore, a trophic structure model can be formed from the correlation analysis and plankton growth dynamic in water ecosystem (Negrete-García 2022).

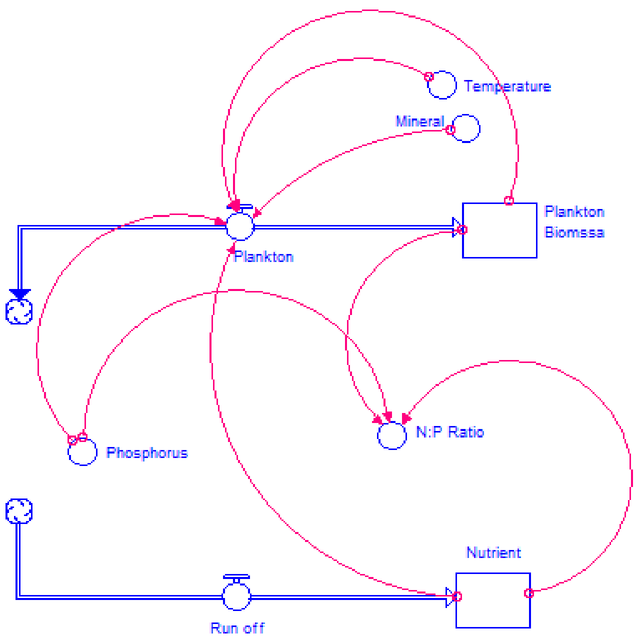

Figure 3. The causal loop model of the relationship between plankton and water quality parameters

Table 2. Water quality parameters in the research ponds during the observation period

Parameter	Pond		*Standards
	1	2	
pH	7.9	7.9	7.5-8.5
Salinity (‰)	19	20	0.5-35
Temperature (°C)	28.5	28.75	25-30
Dissolved Oxygen (mg/L)	5.39	5.12	>4.0
Nitrite (mg/L)	0.035	0.072	<0.100
Phosphate (mg/L)	0.011	0.025	<0.100
Organic Matters (mg/L)	95.77	102.32	<90
Alkalinity (mg/L)	125	130	>110

Note: *standards referred to Ariadi et al. (2019)

Based on Figure 4, the plankton biomass will continue to decrease as the N:P ratio reduces. The N:P ratio and water temperature dynamic determine the trophic status level (Recknagel et al. 2019). Additionally, the *Chlorella* spp. abundance during the farming cycle is described as oscillating, meaning that some factors drive its dynamic in the ponds water. Weather and hydrodynamic factors greatly determine the plankton abundance in the ponds (Accoroni et al. 2015).

Figure 4 also shows that nutrients in the ponds water will continue to increase, largely due to the accumulation of organic and inorganic waste loads (Nadapdap et al. 2020). According to a previous study, intensive shrimp cultivation is very likely to cause waste accumulation and water eutrophication. Improper handling of cultivation waste is also a serious problem for aquaculture activities (Aini and Parmi 2022).

The decrease of N:P ratio which affects plankton abundance is caused by nutrient consumption by microorganisms (Macias et al. 2019). The decrease of N:P ratio will also affect plankton dominance which becomes fluctuating (Tyrrell 2019). The fluctuation in plankton abundance is illustrated by the oscillatory pattern of *Chlorella* spp. abundance in which the impacts of increased nutrient element in pond waters will affect the dynamic pattern of plankton abundance as a whole. In intensive shrimp farming activities, the abundance of nutrients will continue to increase as the waste load in the pond increases (Yang et al. 2019).

The plankton found in shrimp ponds, such as the class of Cyanophyceae, Chloropyceae, and Dinophyceae tend to be fluctuated which is sensitive to nutrient elements in pond waters (Arumugham et al. 2023). The application of fertilizers containing N, P, and K elements is the main cause of nutrient levels in pond waters which tend to be high (Dien et al. 2018). The nutrient distributions in shrimp ponds also tend to fluctuate and are influenced by the dynamics of biotic and abiotic factors (Cardoso-Mohedano et al. 2018).

The effect of N:P ratio on the *Chlorella* spp. abundance

Based on the analysis using dynamic model, there is a negative correlation between the N:P ratio and the *Chlorella* spp. abundance. Different correlation patterns were obtained with N:P ratio estimates of A. 1:10, B. 1:20, C. 1:30, and D. 1:40 as shown in Figure 5. At a certain N:P ratio, plankton will easily experience blooming and growth decline (Reinl et al. 2020). The correlation between carbon, phosphorus, and nitrogen compounds in water ecosystem affects ecological balance and biological diversity (Gao et al. 2018).

As shown in Figure 5, the *Chlorella* spp. abundance remained stable at various N:P ratios in water, but the difference lies in the logarithmic growth phase. At an N:P ratio of A. 1:10, *Chlorella* spp. gradually bloomed, while at a ratio of C. 1:30 and D. 1:40, it experienced blooming phases faster than normal conditions (Figure 5). The presence of abundant nutrients in water tends to affect the fertility and abundance of organisms in water (Tuantet et al. 2019). Based on dynamic model analysis results, an N:P ratio of 1:20 is the most ideal for the growth cycle of *Chlorella* spp.

Chlorella spp. is a cosmopolitan plankton genus (Ariadi and Puspitasari 2021), which can grow optimally in a range of water temperature. The plankton abundance and biodiversity as well as stable primary productivity represent a healthy level of water environment (Tulsankar et al. 2021). Plankton also has the ability to adapt to abnormal conditions in its habitat. *Chlorella* spp. can also be used as natural feed to support cultivation activities (Maliwat et al. 2021), particularly in the growth phase of shrimps. It actively grows in several cultivation media with various conditions (Wafi and Ariadi 2022a). Furthermore, the presence of plankton itself can be used as a form of precise assessment for bioindicators of water environment (Aprilliyanti et al. 2016).

Overall, plankton diversity and abundance in the studied ponds were moderate with an even level of genus uniformity. Among all the identified genera, *Chroococcus* spp. and *Chlorella* spp. were the most dominant, presumably because both can survive under fluctuating environmental conditions (Utojo 2015). In particular, the water conditions in aquaculture ponds largely affect *Chroococcus* spp. and *Chlorella* spp. Water quality in the operational shrimp cultivation cycle is an important factor that determines the ponds ecosystem dynamic (Wafi and Ariadi 2022b). Dynamically, *Chlorella* spp. abundance and existence are closely correlated with the balance of the N:P ratio in the ponds.

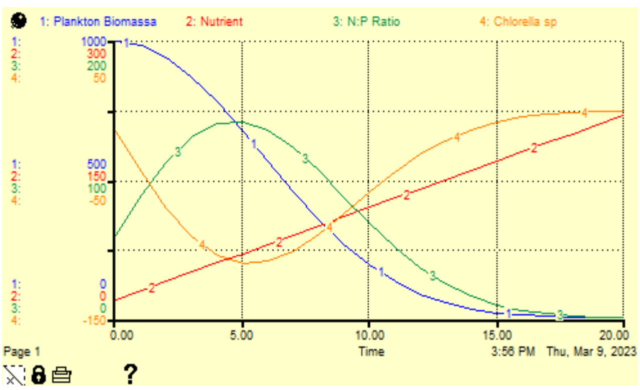

Figure 4. Model of the relationship between plankton abundance and nutrients in pond ecosystems

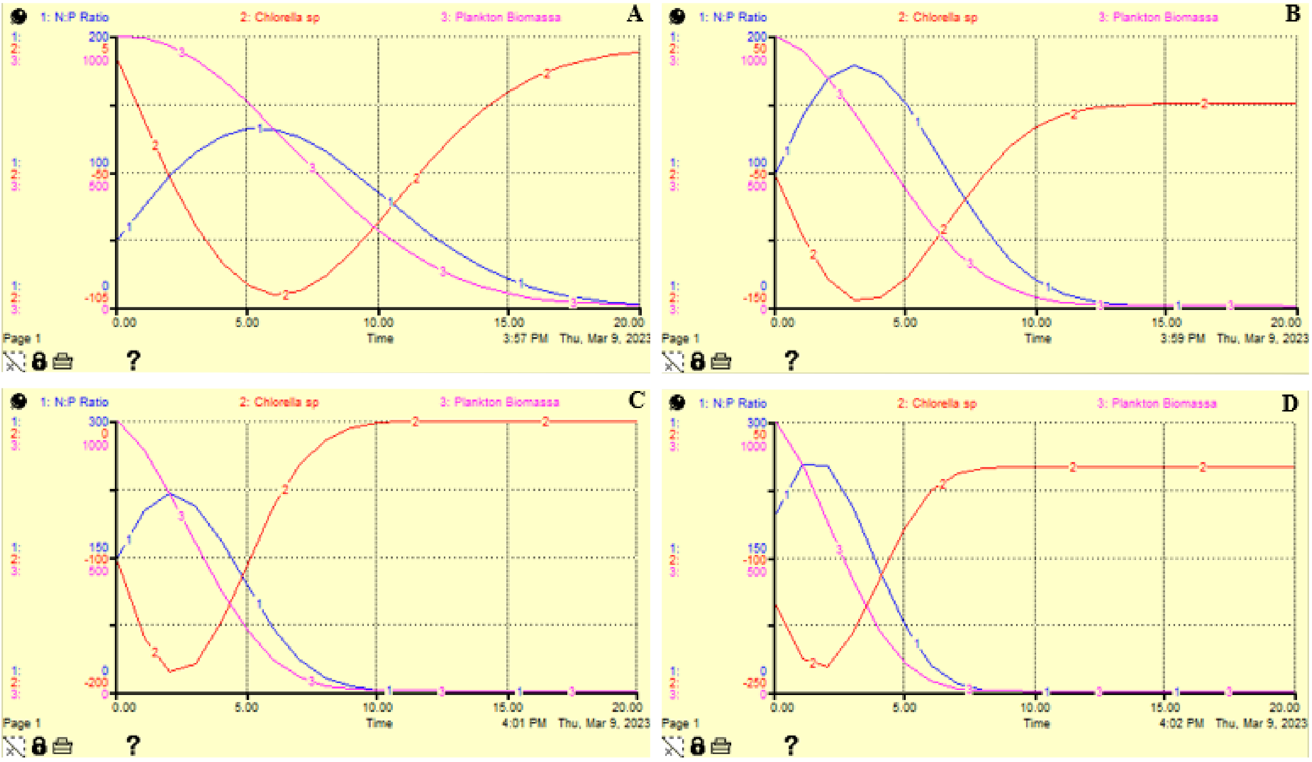

Figure 5. The correlation pattern between N:P ratio with the *Chlorella* spp. abundance from the estimated N:P ratio of A. 1:10, B. 1:20, C. 1:30, and D. 1:40

N:P Ratio is a key factor that determines of the plankton profil in pond waters (Liang et al. 2021). The process of grazing also greatly determines the nutrient consumption as well as the trend of the abundance of plankton dominance in pond waters (Aytan et al. 2018). *Chlorella* spp. is one of the plankton species that tends to be stable and dominant in pond water ecosystems (Alho et al. 2020). The plankton from chlorophyceae has the character of being able to live in overly fertile water conditions with an N:P ratio of 20:1 (Khanna et al. 2019).

In conclusion, based on dynamic model analysis, *Chlorella* spp. abundance was fluctuated from the beginning of cultivation to harvest with the most ideal N:P ratio of 1:20. In general, there was a negative correlation between *Chlorella* spp. abundance and N:P ratio in the ponds. In this study it was shown that *Chlorella* spp. is a type of plankton that is very sensitive to pond water quality conditions. The fluctuation of the N:P ratio is also very closely related to the dynamics of water quality which changes on a daily basis.

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