

Phytoplankton Community Stability as a Health Indicator of Whiteleg Shrimp in an Intensive Pond

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ABSTRACT

The goal of this study was to examine and assess the stability of phytoplankton communities in connection to the different health status type of shrimp pond (healthy and diseased) in an intensive culture. The research was conducted in two ponds located in Probolinggo region, which had different health status until the eight week of harvest period. Several water quality variables and phytoplankton community composition were observed. Non-metric dimensional scaling and analysis of similarity were used to compare the phytoplankton community structure and water quality parameters in both ponds. In addition, the stability of phytoplankton community in these ponds was measured using species turnover rate, rate change of composition community, and community stability indices. The results showed that phytoplankton community structure and water quality factors between a diseased and healthy pond were not statistically significant. On the other hand, the phytoplankton community in the healthy pond was more stable than that of the diseased pond. Furthermore, ammonia and phosphate were found as the dominant factors affecting the phytoplankton community in the studied ponds.

Keywords: aquaculture; community dynamics; *Litopenaeus vannamei*; microalgae; shrimp disease.

INTRODUCTION

The whiteleg shrimp (*Litopenaeus vannamei*) has been one of among the most widely cultivated crustacean species globally because of its great traits, which include speedy growth, a high rate of feed conversion, and an excellent potential for adaptation [Musa et al., 2020]. In order to achieve great yields, shrimp farming has lately been often conducted at high densities or intensive system [Emerenciano et al., 2022]. Nevertheless, this method generates a lot of waste food as well as associated substances that promotes the water nutrients and increases its susceptibility to algal blooming [Páez-Osuna et al., 1998]. This phenomenon compromises the viability of the microbial community structure and induced the outbreak of

many shrimp diseases [Kurniawinata et al., 2022]. Hence, intensification of shrimp farming has led to deterioration of water quality not only in the rearing pond but also its surrounding aquatic ecosystem, as well as the sustainability of this aquaculture business [Kurniawinata et al., 2022].

Dynamics of water quality condition of the aquaculture pond plays a great role in the successfulness of shrimp production. It takes a lot of data collection to identify the fluctuations in water quality that impacted aquaculture pond [Musa et al., 2023]. As a result, a number of studies have suggested using biological organisms to gauge the water quality of aquatic environments [McQuatters-Gollop et al., 2009; Parmar et al., 2016]. Phytoplankton is a water creature that is frequently used as a bioindicator [Mahmudi et al.,

2021]. These creatures have been considered as viable bioindicators for detecting long-term shifts in aquatic systems, specifically that related to algal blooms, climate change, and the management of water resources [Gökçe, 2016]. In this regard, this organism can swiftly adapt to environmental changes, resulting in a more rapid assessment of water quality [Parmar et al., 2016].

The development of a stable and favourable phytoplankton community would not only be beneficial to maintain good water quality but also to ensure the optimal growth and survival of shrimp [Ding et al., 2021]. The pollutants discharged into water and the deterioration of aquatic ecosystems can change the phytoplankton community composition [Qiao et al., 2020]. For instance, sewage flows can elevate nutrients, which might stimulate a bottom-up process [Abdel-Raouf et al., 2012] and cause the community structure of the aquatic ecosystem to be dominated by harmful algae [Mahmudi et al., 2020b]. In aquaculture ponds, this issue results in disease outbreaks of the cultured organism [Anderson et al., 2012]. When the quantity of phytoplankton abruptly fell within the natural process, it is possible that the disease outbreak was brought on by a disruption of the community's ideal state process [Tian et al., 2016]. Furthermore, vigorous fluctuations in the phytoplankton community frequently accompany mortality, irrespective of the diseases. This means that the phytoplankton community's stability and/or

shifting will probably be more significant than the community's composition and structure, as the former may have an indirect or direct impact on the development and even health of shrimp [Yang et al., 2020]. In order to enhance the accuracy of the phytoplankton community evaluation, it is recommended by previous research to employ a combination of community stability measurements and multiple temporal diversity indices to represent the dynamics of the community [Lyu et al., 2021].

Nonetheless, limited research has been done on the stability of phytoplankton communities, particularly on how shrimp dynamics affect their overall health/disease status. The purpose of this study was to analyse and evaluate the stability of phytoplankton communities in relation with the state of health (healthy or diseased) of shrimp pond under intensive culture system. This is significant in contributing the sustainability of aquatic environment as well as optimising the economic yield of shrimp farming industry.

METHODS AND MATERIALS

Study area

This study was conducted at two ponds located in coastal region of Probolinggo Regency (Figure 1). The first pond (diseased pond, DP) is owned by the Brackish and Marine Water Laboratory

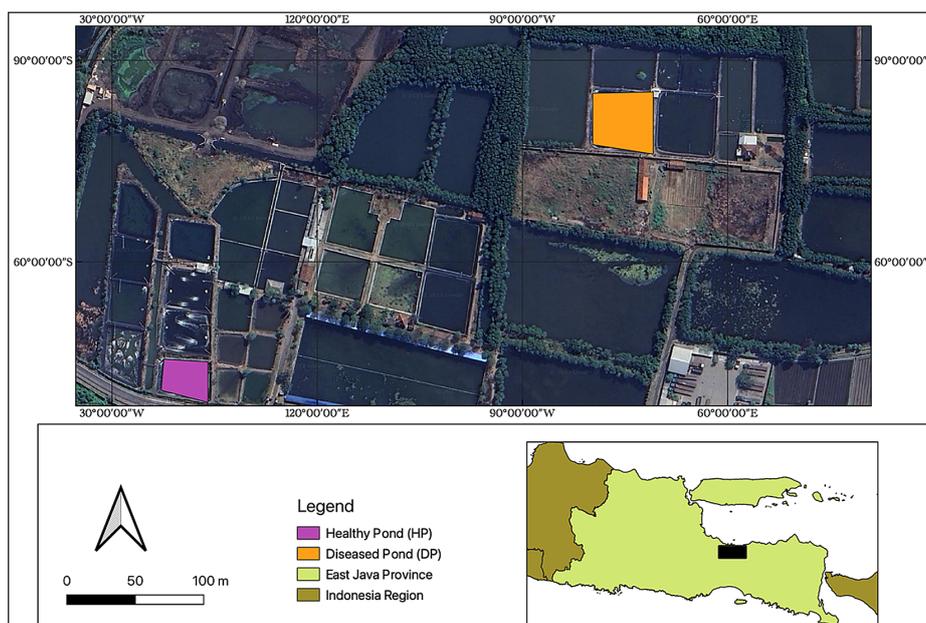


Figure 1. Study area at Probolinggo Regency consisted of healthy and diseased pond

at Brawijaya University, while the second pond (healthy pond, HP) is managed by marine and fisheries office of Probolinggo Regency. Due to disease outbreak that occurred in the first pond, early harvest had to be carried out at the eight week. The study took place from March to April of 2022.

Water quality parameters and phytoplankton observation techniques

By observing the water samples at the study sites, it was possible to measure several kinds of water quality parameter. To measure salinity (‰) and transparency (m), a secchi disc and refractometer were utilised. A Lutron PDO-520 DO meter was used to measure temperature (°C), dissolved oxygen (DO, mg/L), and pH. Additionally, test kits were used to assess the amounts of ammonia (mg/L), nitrate (mg/L), and nitrite (mg/L). Finally, total organic matter (TOM, mg/L) and orthophosphate (mg/L) underwent ex-situ colorimetric and titrimetric examination.

For the assessment of phytoplankton, 25 L of water samples were filtered using plankton nets with pore sizes of 25 . The samples were kept in a 30 mL vial bottle and preserved with 4% formalin. The samples were then tested in the laboratory. An Olympus CX-21LED light microscope was used to identify phytoplankton, and its 400x magnification was utilised to assess its morphological traits, which were then compared to Prescott's 1978 book on algal identification. Furthermore, to calculate phytoplankton density, the Sedgwick Rafter counting cell and the subsequent Lackey drop calculation were employed (APHA, 1989).

$$D = \frac{C \cdot A_t}{A_s \cdot S \cdot V} \quad (1)$$

where: D – phytoplankton density (cell/ml), C – counted organisms, A_t – area of cover slip (mm²), A_s – area of one strip (mm²), S – counted strips counted, V – water sample volume beneath the cover slip (ml).

Statistical data analysis

By utilizing Past software version 4.03, non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) were used to assess the general differences in phytoplankton community and water quality variables between diseased and healthy ponds. Furthermore, a number of metrics were employed, including

species turnover rate, rate change of community rate, and community stability, which are implemented in the R package “codyn,” to quantify and compare the periodical change as well as stability of the phytoplankton community in two ponds. The detailed foundation and algorithm of these methods can be found in Hallett et al. [2016]. Meanwhile, the relationship between phytoplankton community and water quality variables was analyzed using redundancy analysis (RDA) that was executed in the R “vegan” package.

RESULTS AND DISCUSSION

Comparison of phytoplankton community structure and water quality parameters of two ponds

In this study, a total of 21 genera were recognised, with the Chlorophyta and Chrysophyta divisions mostly dominating. Phytoplankton structure in both diseased and healthy pond mainly consisted of genera Achnanthes, Calanus, Chlorella, Cyclotella, Nitzschia.

The prevailing similarity of the two pond samples were analyzed using NMDS in terms of the Euclidean measurement of all environmental factors and the Bray-Curtis dissimilarity of community composition. The stress value for both ordinations was lower than 0.050, which considered as low stress. This indicates that the NMDS results were valid and acceptable. It is visible from Figure 2 that the ordination pattern of water quality between diseased and healthy pond is clearly distinct. In contrast, the pattern of phytoplankton ordination of these ponds is overlapped. Further analysis using ANOSIM (Table 1) showed that the phytoplankton community as well as water quality variables in diseased and healthy pond were not significantly different ($p > 0.05$). This is also supported by their determination coefficient (R-square) value which was small (R-square < 0.50).

Ordination of phytoplankton composition between diseased and healthy pond was overlapped (Figure 1) and not significantly differed (Table 1). The phytoplankton community of both ponds was dominated by genus Chlorella from Chlorophyta division. This is due to the use of Chlorella as the feed additive for whiteleg shrimp diet in studied pond. Chlorella is a frequently utilised microalgae in aquaculture, either as a direct feed or as an

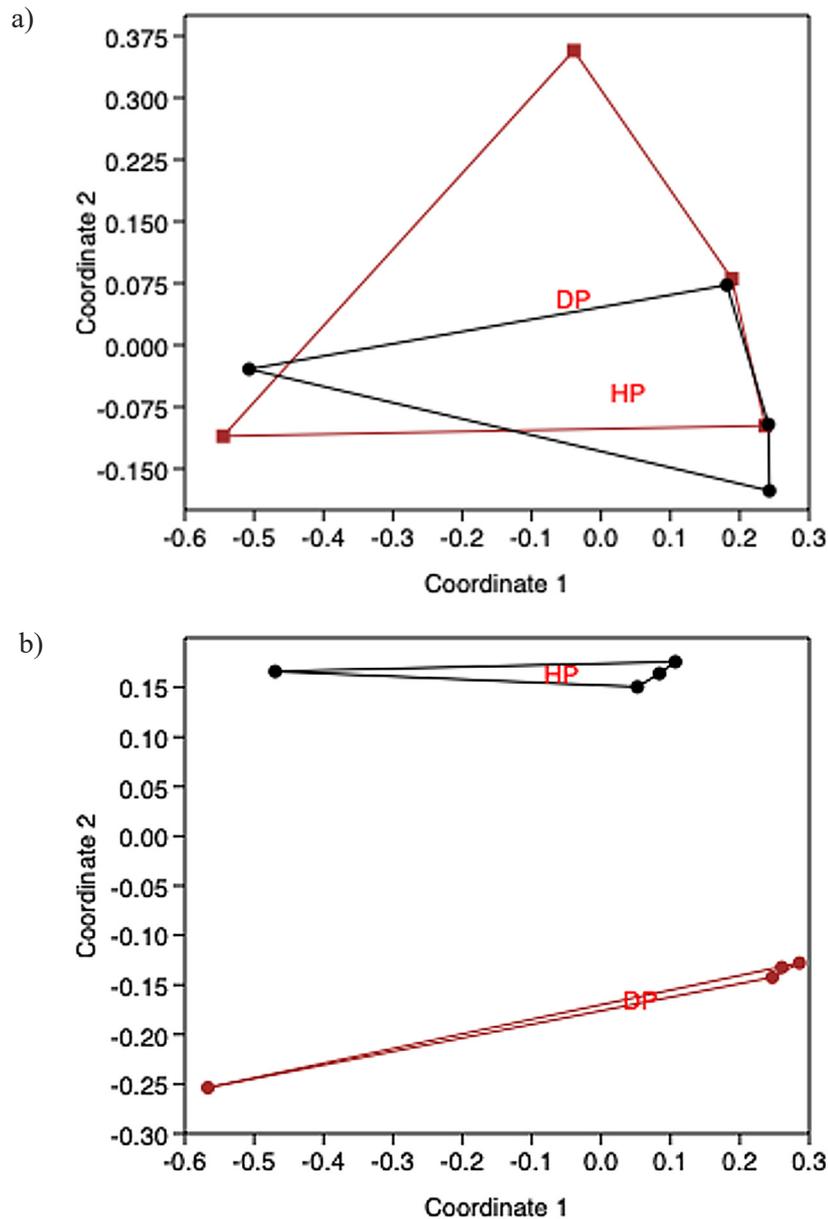


Figure 2. NMDS ordination results of (a) phytoplankton community composition; (b) water quality variables

addition for various species added to diet [Sukri et al., 2016]. The phytoplankton contains a high concentration of critical nutrients such as vitamins, coloring substances, amino acids, and other growth factors [Ajiboye et al., 2012]. Because of such nutritional benefits, the aquaculture sectors view its use as a sustainable and ecologically advantageous supply [Ahmad et al., 2020]. Chlorella has been effectively used as a food supplement in carp, shrimp, olive flounder, and Nile tilapia [Al-Musalam et al., 2014; Arsad et al., 2020; Maliwat et al., 2021; Safari et al., 2022]. Growth, survival rate, and immunological responses are all improved when the microalgae is added to diet [Maliwat et al., 2021; Eissa et al., 2023].

Investigation of the shifting and stability of phytoplankton communities in two ponds

In the diseased pond, the average turnover was 0.42 (i.e., 42% of the species examined in the preceding week did not show up in the subsequent week), compared to 0.11 in the healthy pond. The phytoplankton communities of both ponds displayed distinct variations in time (Figure 3). Species turnover in the diseased pond fell and subsequently rose until the end of the monitoring period. In comparison, a healthy pond experienced a contrast pattern. On the other hand, the composition of communities change is a multidimensional metric (Euclidean distance) that

Table 1. ANOSIM results on different test of phytoplankton community and water quality variables between diseased and healthy pond

Variables	R-square	p
Phytoplankton community	0.1458	0.2839
Water quality variables	0.1667	0.1455

measures how a community has changed over a period of time. The structure of the phytoplankton communities in the diseased pond exhibited a negative linear trend, as illustrated in Figure 4. In contrast, the trend for the healthy pond was upward. Figure 5 shows that the community stability

of the diseased pond was 1.04, which was lower than the healthy pond (1.30). This suggests that the community of phytoplankton in the pond that was in a healthy state was more stable.

The turnover rates in both diseased and healthy pond were fluctuated, with the overall tendency that this metric was lower in the latter pond. Meanwhile, the phytoplankton community rate change in healthy pond was decreased overtime. This pond was also proved to be more stable. The communities that have a greater variety of species should not only be more stable and exhibit a lower rate of turnover over a period of time, but also increased

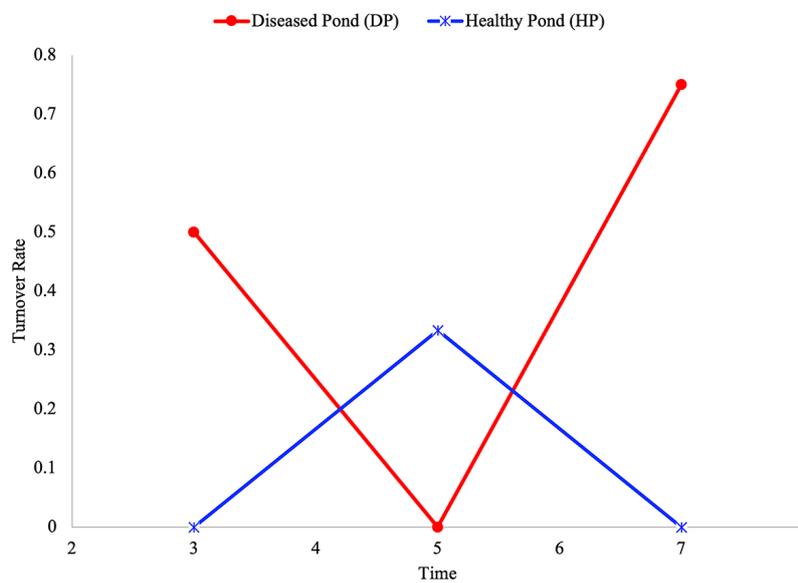


Figure 3. Species turnover rate of diseased and healthy pond

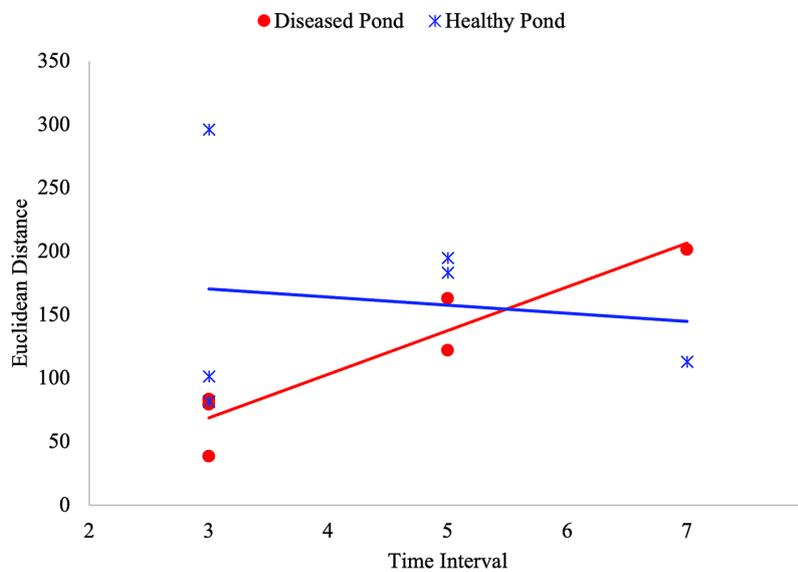


Figure 4. Rate change of phytoplankton composition of diseased and healthy pond

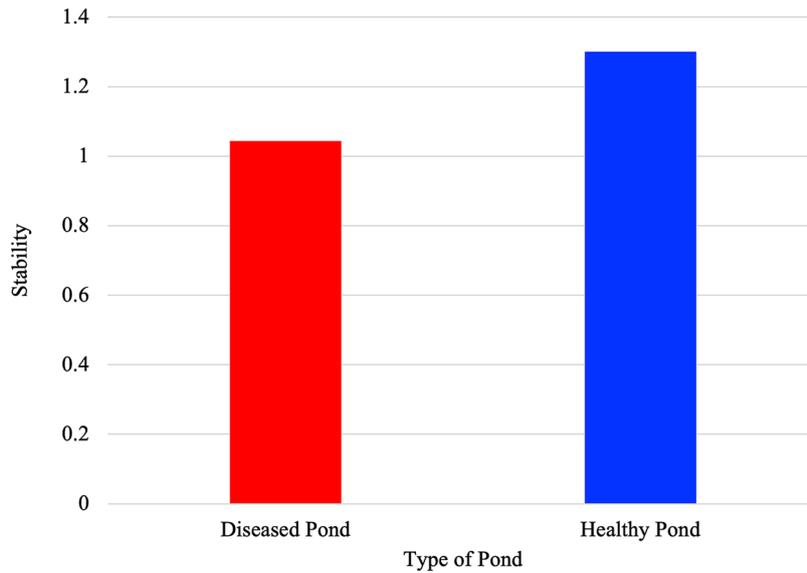


Figure 5. Phytoplankton community stability of diseased and healthy pond

biodiversity should encourage a more efficient use of resources [MacArthur, 1955; Tilman and Downing, 1994]. The connection among biodiversity and the rate of community turnover in plankton communities has been examined in earlier research [Shurin et al., 2007; Ptacnik et al., 2008]. These studies have demonstrated that species diversity decreases phytoplankton and zooplankton community time-based.

Previous studies revealed that phytoplankton diversity index in the whiteleg shrimp intensive ponds was low, even compared to that of traditional pond [Palupi et al., 2022]. It was a sign that

the shrimp pond water was contaminated [Musa et al., 2020]. The intensification of shrimp farming to achieve high production levels generates organic waste, which comes from feed wastes and significant portions of shrimp excrement [Musa et al., 2023]. Intensive aquaculture systems with high stocking densities have waste load issues that can impact the sustainability of the shrimp habitat and the surrounding aquatic ecosystem [Iber and Kasan, 2021]. These contaminants can lead to eutrophication, lower dissolved oxygen levels, and encourage the spread of several disease. Moreover, a decline in pond water quality

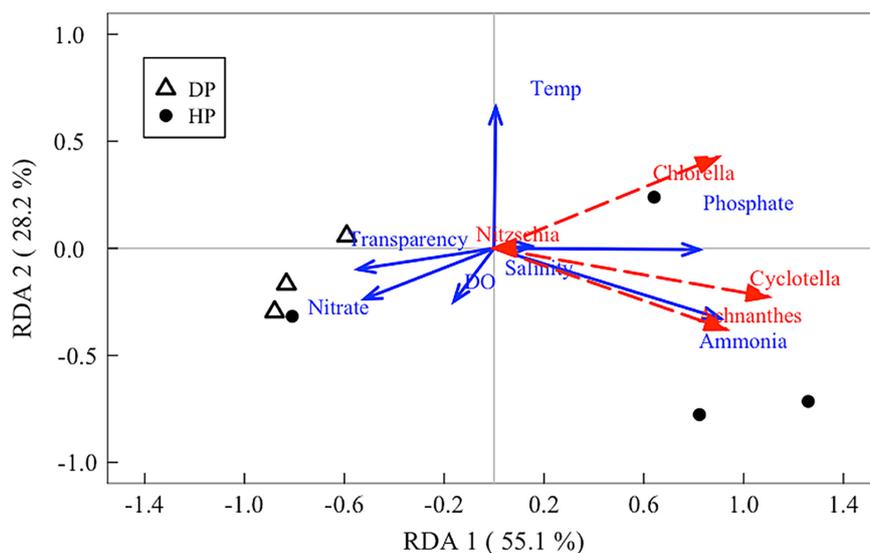


Figure 6. The biplot of RDA analysis for water quality variables and phytoplankton community in diseased and healthy pond

might cause a change in the phytoplankton community's composition and make shrimp more prone to infection [Anderson et al., 2012].

Factors affecting phytoplankton community in the two ponds

In order to investigate the fundamental causes of the phytoplankton community structure in the aquaculture waters of two ponds, RDA was used. The eigenvalues of axes 1 and 2 in Figure 6 biplot were 2.206 and 1.127, accordingly. When combined, these axes were able to account for 83.31% of the variation in the phytoplankton community throughout the sample period. Phosphate and ammonia had a strong association with axis 1, as seen by the vector length of environmental variables with the RDA axis. However, transparency and nitrate showed a considerable negative correlation with this axis. Meanwhile, temperature was strongly associated with axis 2. The findings suggested that these environmental variables were strongly correlated with the structure of the phytoplankton community in shrimp ponds. Furthermore, there was a noticeable clustering of the phytoplankton community in both the diseased and healthy ponds. Moreover, a considerable positive correlation was observed between phytoplankton belonging to the genera *Achnanthes*, *Cyclotella*, and *Nitzschia* with ammonia and phosphate.

Numerous studies on phytoplankton communities have demonstrated that phytoplankton-community characteristics such as species richness, community diversity, and community evenness can be driven by many abiotic factors and biological factors [Sun et al., 2023]. Studies have proven that phytoplankton community, species richness, community diversity, and species abundance are all closely related to nutrient concentrations [Soininen and Meier, 2014; Lusiana et al., 2020]. The excessive nutrients, such as nitrogen and phosphorus, in aquatic ecosystem have been proven to promote eutrophication and harmful algae blooms, which have become great threats to water quality and human health [Mahmudi et al., 2020; Akinawo, 2023]. In particular, phosphorus has been considered as the primary limiting nutrient for the development of phytoplankton in aquatic ecosystems [Bai et al., 2022]. Phosphorus levels increase the abundance of *Cyclotella* through regulating the rate and specificity of chitin, glucan, and lipid nanofiber synthesis. As the rate of phosphorus supply increased, so did the amounts of cell lipid and

glucan, which increased by 300 and 400%, respectively [Millie, 2011; Chiriboga and Rorrer, 2019].

Ammonia levels have consistently increased in recent years, owing primarily to increased shrimp aquaculture intensity. Aquaculture ponds often have a greater level of nitrogen ammonium than nitrate nitrogen, which appears to hinder diatom (such as *Achnanthes* and *Cyclotella*) formation and stimulate green algae (such as *Chlorella*) growth [Boyd, 2014]. High ammonia levels are toxic to shrimp, even though they are a rich source of nitrogen for phytoplankton, which raises the dissolved oxygen content in water and provides nutrition for the shrimp [Zhao et al., 2020]. The expression of chitinase, growth, moulting, phenoloxidase, and haemolymph antibacterial activity are all inhibited when shrimp pond ammonia levels above tolerance limits, which weakens the shrimp internal immune system [Cui et al., 2017].

CONCLUSIONS

This research revealed that phytoplankton community composition, as well as water quality variables of healthy and diseased ponds were not statistically significant. However, distinct cluster of observation from both ponds, in terms of water quality variables ordination results, were seen. This is due to the use of *Chlorella* as the additional feed for shrimp diet in both ponds. On the other hand, it has been shown that the phytoplankton community in a healthy pond is more stable compared to that in a diseased pond. This indicates that the contamination in diseased pond is more severe, which might drive by the level of ammonia and phosphate in the pond. These factors may affect the shrimp immune system as well as the composition of phytoplankton.

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