Research Article

Use of Phytoplankton to Assess Water Quality of Eco-Aquaculture System in Super-Intensive Whiteleg Shrimp (*Litopenaeus vannamei*) Pond

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Abstract | Rapid development of whiteleg shrimp farming industries has destroyed coastal environments due to land conversion, particularly mangroves. Recently, the concept of eco-green aquaculture by integrating mangroves and shrimp ponds has been discussed as an attempt to preserve mangrove forests as well as using them as wastewater treatment for aquaculture sewage. The aim of this study was to analyze the impact of integrated super-intensive shrimp aquaculture with mangroves in terms of water quality characteristics and phytoplankton as a bioindicator. The study was conducted at coastal area of Probolinggo region, Indonesia. Our research showed that the cultured ponds had high abundance of phytoplankton but low species diversity. Meanwhile, the water inlet and outlet of the shrimp farming which served at mangrove area had higher phytoplankton diversity. The composition of phytoplankton in this work was ideal for shrimp farming because it was dominated by the Chlorophyta and Chrysophyta divisions, which favour shrimp growth. On the other hand, water quality parameters across studied sites were quite similar, except for temperature and dissolved oxygen. Therefore, the study indicated that the existence of mangrove was supporting to increase the quality of water supply for the cultivation as well as the aquaculture water sewage in terms of phytoplankton bioindicator.

Keywords | Bioindicator; Coastal ecosystem; Integrated mangrove aquaculture; Aquaculture sustainability; Shrimp farming

Received | November 02, 2021; **Accepted** | November 26, 2021; **Published** | March 25, 2022
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Citation | Mahmudi M, Musa M, Arsad S, Lusiana ED, Bunga A, Wati NA (2022). Use of Phytoplankton to Assess Water Quality of Eco-Aquaculture System in Super-Intensive Whiteleg Shrimp (*Litopenaeus vannamei*) Pond. Adv. Anim. Vet. Sci. 10(5): 971-979.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.5.971.979
ISSN (Online) | 2307-8316

INTRODUCTION

Whiteleg shrimp (*Litopenaeus vannamei)* aquaculture has grown substantially in the previous decade, with a global output of approximately 4.16 million tonnes in 2016, accounting for 53 percent of total shrimp and prawn supply (FAO, 2018). The majority of this production is located in tropical areas of the world, primarily in Asian regions such as China, Thailand, Vietnam, and Indonesia (Ahmed et al., 2018). The main advantages of *L. vannamei* include high density tolerance, adaptability to diverse environmental pressures and speedier development even during the lesser culture periods (Suwoyo & Hendrajat, 2021). As a result of these characteristics, this organism has become the most popular shrimp species for large-scale production (Naylor et al., 2021).

The rapid development of shrimp farming industries has led to the destruction of coastal environments as a result of land conversion (Hamilton, 2013). Many mangrove forests in coastal areas have been transformed into shrimp ponds (Bournazel et al., 2015), and this change has negative impacts because mangroves have many environmental benefits, such as defence against tidal abrasion, extreme storms and sediment/nutrient trapping (Peng et al., 2009). As culture systems progress to become super intensive, the water quality in shrimp ponds tends to degrade due to increased shrimp mass and organic matter build-up from leftover feed, excrement and metabolism (Barraza-Guardado et al., 2013). The impairment of water quality caused by nutrient accumulation has been recognized as a possible cause of disease outbreaks in shrimp ponds (Alfiansah et al., 2018). Furthermore, in combination with municipal and agricultural effluents, shrimp farming wastewater discharges could contribute to algal blooms in the surrounding coastal environment. Hence, the shrimp farming industry is undergoing pressure to enhance its ecological sustainability (Briggs et al., 2004).

Recently, the concept of eco-green aquaculture by integrating mangroves and shrimp ponds has been increasingly discussed (Sampantamit et al., 2020). Such a strategy aims to preserve mangrove forests and allows them to function as wastewater treatment for aquaculture sewage (Ahmed et al., 2018). From a physico–chemical characteristics perspective, the system has been found to improve the water quality of extensive ponds' aquaculture wastewater (Peng et al., 2009); a similar study analyzed super-intensive ponds, but the results were not significant (Musa et al., 2020). Water physico–chemical characteristics, such as nutrients (nitrate and phosphate), dissolved oxygen (DO) and ammonia are routinely monitored either directly by water sampling and empirical studies or indirectly using remote sensing devices (Rahmanian et al., 2015). However, to identify water quality fluctuations, this approach requires a long series of data collection. Therefore, numerous studies have recommended that living organisms be used to measure the water quality of aquatic habitats (McQuatters-Gollop et al., 2009).

One aquatic organism commonly employed as a bioindicator is phytoplankton (Parmar et al., 2016). These organisms have been proposed as promising bioindicators for assessing long-term changes in aquatic habitats, particularly those linked to algal blooms, climate change and water resource management (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). Pollutants discharge into water, and the deterioration of aquatic ecosystems can change the phytoplankton community composition. For instance, sewage flows can elevate nutrients, which might stimulate a bottom-up process (Davis et al., 2010) and cause the aquatic ecosystem's community structure to be dominated by harmful algae (Berdalet et al., in press). In aquaculture ponds, this issue results in disease outbreaks of the cultured organism (Qiao et al., 2020).

Based on physico–chemical water quality monitoring and phytoplankton community structure, this study intended to analyze the impact of integrated super-intensive shrimp aquaculture on mangroves. Such an analysis is critical because this form of aquaculture has both significant economic interest and an increased risk of posing damage to the coastal environment. Coastal ecological degradation is predicted to be avoided by combining aquaculture with mangroves.

MATERIALS AND METHODS

Study Area

The research was carried out at Brawijaya University's Brackish and Marine Water Laboratory. This facility is located on the coast of Indonesia's Probolinggo region (Figure 1a), where it conducts super-intensive *L. vannamei* cultivation. The study took place between February and March 2021 with weekly sampling.

As shown in Figure 1b, we used six sampling locations. The river water near the entrance channel is utilized as the primary supply of water for the intensive pond (Site 1). Site 2 is a reservoir pond that serves as a water source for ponds as well as a water isolation system to interrupt the disease cycle. The intensive ponds are located at sites 3 and 4. The sewage disposal pond from these ponds is Site 5. The mangrove area is the last site (Site 6).

Water Quality and Phytoplankton Observation

We assessed several water quality characteristics, such as temperature (°C), transparency (m), pH, dissolved oxygen (DO, mg/L), salinity ($\%_o$), nitrate (mg/L), nitrite (mg/L), ammonia (mg/L), orthophosphate (mg/L) and total organic matter (TOM, mg/L) at the study sites by observed the water samples. A DO meter type Lutron PDO-520 was utilized to measure temperature, DO and pH, and a secchi disc and refractometer were utilized for transparency and salinity, respectively. Furthermore, nitrate, nitrite and ammonia were quantified by using test kits. Lastly, orthophosphate and TOM were analyzed ex-situ by utilizing colorimetric and titrimetric techniques.

Plankton nets with a pore size of 25 m were used to concentrate 25 L of water samples to identify phytoplankton. The samples were preserved with 4% formalin and stored in 30 mL vial bottle. After that, they analyzed in the laboratory. Phytoplankton identification process was carried out by using light microscope type Olympus CX-21LED with 400x magnification and the morphological characteristics was determined in accordance to Prescott's book algal identification (Prescott, 1978). In addition, we used the Sedgwick Rafter counting cell and the following Lackey drop formula to determine phytoplankton density (APHA, 1989).

$$D = \frac{C.A_t}{A_s.S.V}$$

where
D = phytoplankton desnity (cell/ml)
C = number of organisms counted;
A_t = area of cover slip (mm^2)
A_s = area of one strip (mm^2)
S = number of strips counted
V = volume of sample under the cover slip (ml).

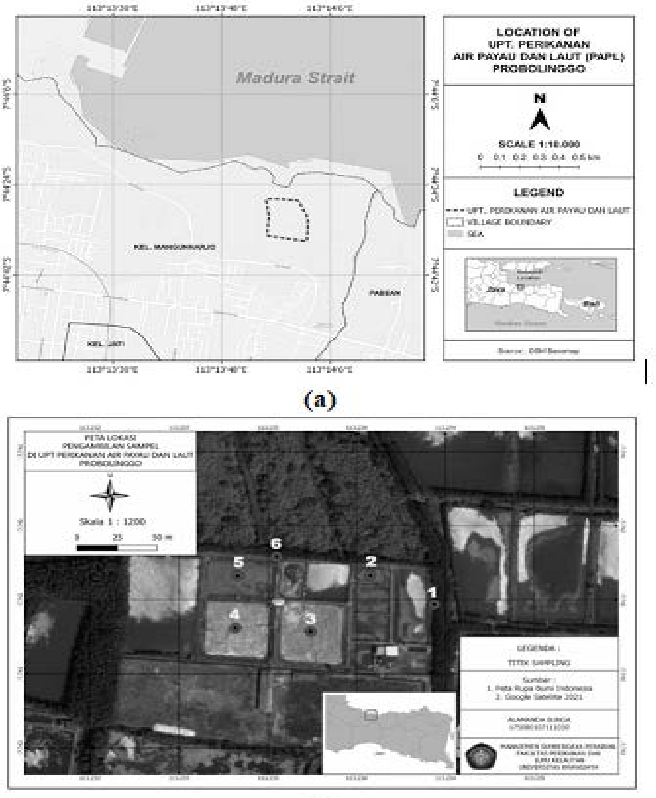

Figure 1: Research location (a) Brackish and Marine Water Laboratory of Brawijaya University, Probolinggo; (b) Sampling sites

Data Analysis

We employed three data analysis methods in this study. The water quality parameters were initially identified using box plots with an ANOVA/Tukey test. We then measured the diversity of phytoplankton composition using a Shannon–Wiener diversity index (Miao et al., 2019) and subsequently used canonical correspondence analysis (CCA) to investigate the connection between observed water quality indicators and phytoplankton abundance (Greenacre, 2010). The analysis was performed using both R and PAST software.

RESULTS

The results of temperature, nitrite and DO, as depicted in Figure 2, indicated that the sample waters differed between sites, as denoted by the unequal notation of the box-plot. Meanwhile, transparency, salinity, pH nitrate, ammonia, orthophosphate and TOM did not vary significantly between locations because these parameters shared similar letter notations. Site 5 had a higher temperature, whereas Sites 3 and 4 had the lowest values. In contrast, nitrite levels in these sites were far higher than in the other sites. A similar pattern occurred for the nitrate and ammonia measurement results, but the values of these indicators were not significantly different between sites. On the other hand, the highest and lowest DO levels were found at Sites 1 and 5, respectively. Furthermore, at all sampling sites, the transparency and salinity were 10-40 cm and 20–30 $^0/_{00}$, respectively. Relatively steady values across sites were shown for the orthophosphate and TOM measurement results.

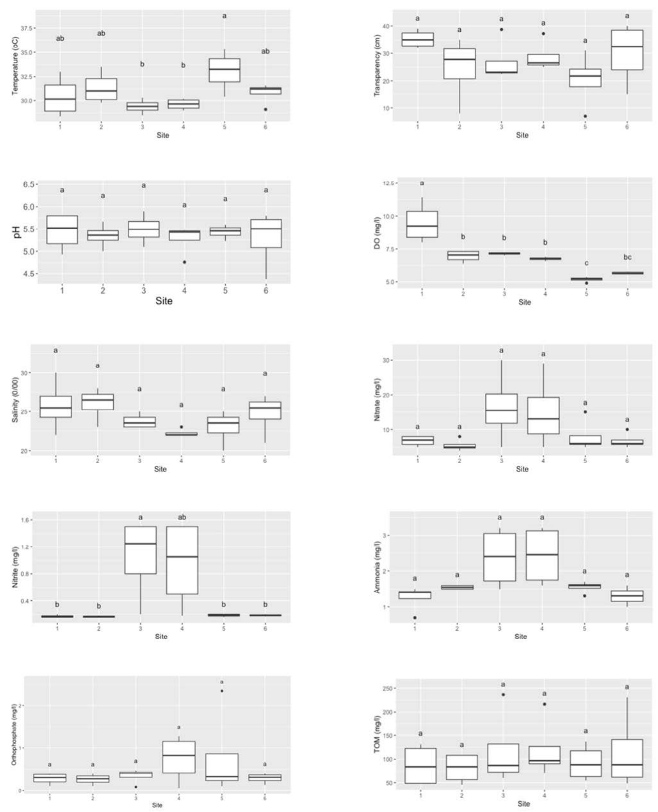

Figure 2: Physico-chemical water quality monitoring at studied sites

The composition of phytoplankton genera in the study site is shown in Table 1. A total of 21 genera were recognized, with the Chlorophyta and Chrysophyta divisions mostly dominating (Figure 3). *Peridinium* is a Dinophyta genus that was exclusively found at Site 4. Sites 1, 2 and 3 had the

Table 1: Phytoplankton community structure and diversity index (H')

Division	Genus	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Chlorophyta	*Chlorella*	-	-	+	+	+	+
	Oocystis	-	-	+	+	+	+
	Scenedesmus	-	-	-	-	+	-
	Cosmarium	+	-	+	+	-	-
	Ulothrix	-	+	-	-	-	-
	Chlamydomonas	-	-	+	-	-	-
Chrysophyta	*Nitzschia*	+	-	-	+	+	-
	Melosira	-	-	-	-	+	
	Amphora	-	+	+	-	+	+
	Cyclotella	+	+	+	-	+	+
	Navicula	+	+	+	-	+	-
	Gyrosigma	-	-	-	-	+	-
	Amphiprora	-	-	-	+	+	+
	Stephanodiscus	+	+	+	+	-	-
Cyanophyta	*Microcystis*	-	+	+	+	+	+
	Oscillatoria	-	-	-	-	+	-
	Anabaena	-	-	-	-	-	+
	Nostoc	+	-	-	-	-	-
	Chroococcus	-	-	+	+	-	-
	Spirulina	-	-	-	+	-	-
Dinophyta	*Peridinium*	-	-	-	+	-	-
Shannon-Wiener Diversity Index (H')		1.080	0.817	0.774	1.099	0.798	1.059

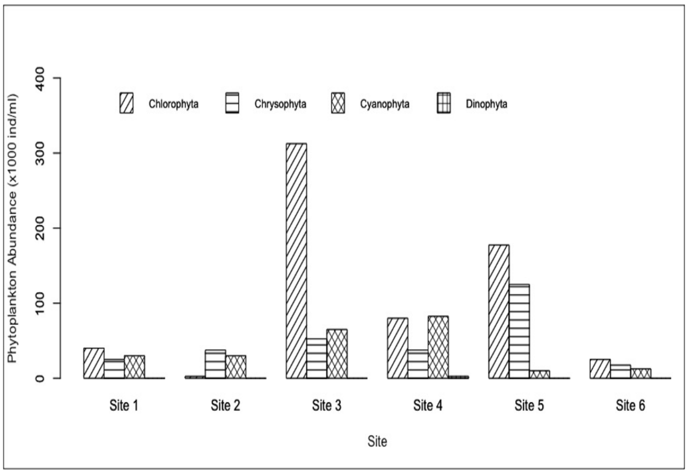

Figure 3: Phytoplankton abundance and division composition

lowest biomass and number of genera. On the other hand, sites 3 and 4, where the shrimp farming pond was located, as well as Site 5, exhibited a reasonably high quantity of phytoplankton. The diversity index (H') fluctuated across sites. A high diversity index was mainly found at Sites 1 and 6, whereas lower index values were observed at Sites 2, 3 and 5.

We performed a CCA to analyze the water quality factors that were associated with phytoplankton division presence. The relationship was obtained by taking the shortest projection of division point to the water quality parameter vector from the CCA triplot. According to Figure 4, Chlorophyta species were likely to occur in high concentrations of TOM, transparency, pH and nitrate, and low concentrations of temperature, DO, salinity and orthophosphate. The relationship between Dinophyta presence and water

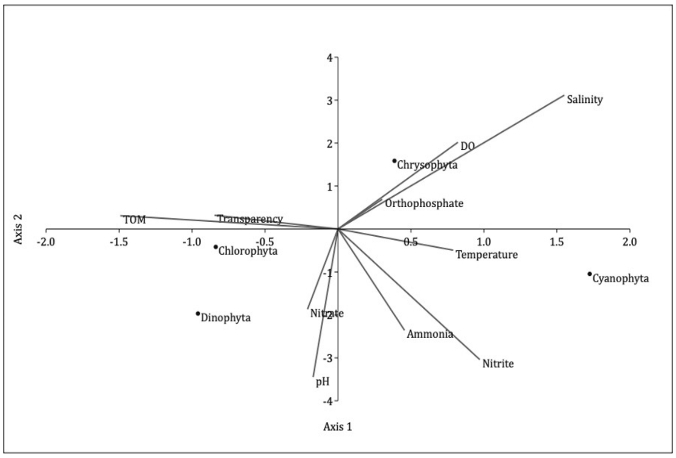

Figure 4: Triplot of Canonical Correspondence Analysis (CCA) for the relationship between phytoplankton abundance and physico-chemical factors of the studied sites

quality characteristics was found to be quite similar to that of Chlorophyta. Meanwhile, a contrasting pattern to Chlorophyta appearance was noted for the Chrysophyta division. On the other hand, the appearance of Cyanophyta corresponded with high temperature, ammonia, nitrite, orthophosphate and salinity; moderate nitrate and pH; low transparency and TOM.

DISCUSSION

Temperature is one of the important indicators for successful *L. vannamei* cultivation. This parameter can affect the growth, physiological performance and survival of this biota (He et al., 2018; Wang et al., 2019; Zhang et al., 2019). Previous research has shown that the optimum temperature for *L. vannamei* varied with body size—30 ˚C and 27 ˚C for small and large shrimp, respectively (Wyban et al., 1995). Similarly, transparency, as an essential characteristic of aquaculture ponds, is strongly related to photosynthetic activity (Abdel-Raouf et al., 2012). Despite there being no significant variation in transparency among sites, higher levels of transparency were found at Sites 1 and 6, indicating that the peculiarity of the mangrove root system can retain contaminants and silt (Kida & Fujitake, 2020).

The accumulation of aquaculture by-products in water might result in it becoming alkaline or acidic (Boyd, 2016). The pH levels in this study were lower than 6, which can be fatal to aquatic organisms (Velma et al., 2009). Meanwhile, the salinity was less than the recommended value for aquaculture (or 27 $^0/_{00}$) (Ministry of Environment, 2001). Low salinity, on the other hand, is unlikely to impair osmotic regulation to such a point that *L. vannamei* survival and growth levels would be affected (Gao et al., 2016). This biota has significant potential in coastal saline waters at salinities as low as 1 $^0/_{00}$ if the acclimatization technique is performed appropriately (Allen, 2004). Furthermore, in aquaculture, DO values of 4 to 5 mg/L or higher are considered optimum (Boyd, 2003). Low levels of DO (below 2.0 mg/L) are associated with reduced growth and a higher risk of organism death (Ferreira et al., 2011).

Commercial feed has been commonly utilized in intensive shrimp cultivation systems because farmers were compelled to feed their shrimp on a specified growth plan due to the reduction of the harvest period (Chaikaew et al., 2019). A high abundance of feed waste produces significant amounts of organic matter, which raises water's TOM concentration. As the amount of organic matter in the environment increases, so will the quantity of nutrients in the water (Lusiana et al., 2019a). Nutrients, such as nitrate and phosphate, are extensively used as indicators for eutrophication and have been linked to phytoplankton biomass (Lv et al., 2011). Phosphate is found in a variety of compounds, but only orthophosphate can be directly used by aquatic microorganisms (Wan et al., 2020). On the other hand, nitrite and ammonia, two of the three types of nitrogen investigated in this study, were found to surpass the quality threshold for fisheries (Ministry of Environment, 2001). High amounts of ammonia in water can harm the gills, reduce shrimp growth and moulting rate, as well as decrease the blood's ability to transport oxygen (Shaari et al., 2011). When any conversion of ammonia to nitrate is prevented, nitrite will be accumulated to significant levels, lowering the shrimp's immunity and making them prone to disease caused by the vibrio virus (Widanarni et al., 2020).

In contrast to the other sites, the Chlorophyta proportion at Site 2 was relatively low. Based on the CCA results, this finding might have been due to the high salinity level in this site contributing to the disappearance of Chlorophyta species. It has been reported that a high concentration of salinity can reduce the nutrient uptake rate of Chlorophyta organisms (Choi et al., 2010). On the other hand, specific factors that may enhance Chlorophyta assemblages in Sites 3, 4 and 5 were low temperature, DO, salinity as well as higher TOM levels. In this research, the common Chlorophyta genera in the studied sites were *Chlorella* and *Oocystis*. Various investigations have shown that *Chlorella* species can enhance nutrition, immunity, aquatic environmental remediation, stress relief, fish disease resistance and bacterial quorum sensing (Ahmad et al., 2020; Mtaki et al., 2021). Meanwhile, in subtropical aquaculture farms, *Oocystis* is a common-dominator genus with a steady population size and a high capacity to meet various environmental circumstances (Huang et al., 2012). The genus is also capable of reducing urea nitrogen levels in ponds (Liu et al., 2018). There were 8 genera from Chrysophyta or diatoms observed in this study, with most being *Cyclotell*a and *Navicula*. Diatoms are advantageous algae that play an essential role as a food supply for aquatic invertebrates (Boyd, 2016). Most shrimp aquaculture managers favour a high percentage of diatoms in a phytoplankton community because diatoms are advantageous algae that boost shrimp growth more than cyanophytes (Yusoff et al., 2002). Based on the CCA results, the assemblage of diatoms in this study was associated with high concentrations of DO, salinity and orthophosphate and low concentrations of TOM, transparency, pH and nitrate. This result contradicts previous research, which implied that high nitrate concentration enhances diatom growth (Cremen et al., 2007). From Figure 2 and Table 1, the diversity decreased as the DO level declined from Sites 2 to 3. Hence, DO level is a positive predictor for diatom species richness (Shaari et al., 2011).

Cyanophyta comprise 6 genera. The biomass of Cyanophyta was relatively high at Sites 3 and 4, which coincided with a high level of orthophosphate. Cremen et al. (2007)

also noted that high phosphate concentrations typically encourage the growth of Cyanophyta. Depressed diatom growth is most frequently followed by increased Cyanophyta growth (Yusoff et al., 2002). However, this study showed that since the beginning of the culture process, Cyanophyta were less dominating than diatoms, indicating that nitrate variation during the study did not hold diatom growth, whereas an insignificant increase of orthophosphate raised Cyanophyta growth. Finally, *Peridinium* was the only Dinophyta genus found at Site 4, with very little abundance. This organism is classified into the family Peridiniaceae—a bloom-forming microalgae (Gárate-Lizárraga & Muñetón-Gómez, 2008).

Both Cyanophyta and Dinophyta species are considered to be harmful algae (Baek et al., 2008; Mahmudi et al., 2020). Because of its ability to create toxins, such as microcystin, the presence of poisonous Cyanophyta in aquaculture systems is a reason for concern (Kimambo et al., 2019). Microcystin is the most common cyanotoxin found in freshwater ecosystems (Preece et al., 2017). *Microcystis* is an example of a Cyanophyta genus that can produce microcystin (Reichwaldt et al., 2013) and was the most common Cyanophyta genus found in this research, representing an alert for the sustainability of shrimp farming. Microcystin reaches the fish body through the gills, feed and food chain, causing liver tissue damage of aquatic organisms (Lehman et al., 2010). Microcystin can also assemble in fish tissues, posing a health risk to humans who consume them (Peng et al., 2010). Meanwhile, the blooming of Dinophyta, such as *Peridinium,* leads to water discoloration and reduced transparency, which affect the water system's primary production (Zohary et al., 2014; Rodríguez-Gómez et al., 2019). Algal blooming is generally caused by nutrient enrichment (Lusiana et al., 2019b) and water temperature increases up to 32 °C (Shaari et al., 2011). This phenomenon also causes a shift in the phytoplankton community and diminishes species richness/diversity (Wabnitz et al., 2018), as visible at Sites 3 and 5. In this study, we identified a serious threat to shrimp culture sustainability—nutrient enrichment—as a result of enhanced levels of orthophosphate that increased Cyanophyta biomass. Therefore, appropriate feed management during shrimp culture is required to control the accumulation of organic waste as well as nutrient enrichment (Lusiana et al., 2019a).

Sites 1 (water supply) and 6 (the mangrove area) were found to be the most diverse. In Site 6, the organic matter was absorbed by mangroves. Sedimentation in sewage ponds and organic matter build-up by mangrove plants diminish excessive organic matter content (Bao et al., 2013; Hossain & Nuruddin, 2016). The water supply for the studied sites' aquaculture systems was obtained from treated wastewater (Site 6), tidal and estuarine water. Due to dilution from sea-, estuary- and wastewater, these three sources result in good water quality for aquaculture practice.

CONCLUSION

The intensification of shrimp culture has resulted in a slew of environmental issues, as well as concerns about the activity's long-term sustainability. An integrated shrimp farming and mangrove system might be a solution to this issue. This research revealed that significant variation of temperature and DO level was found among sites. These affect the domination of Cholorophyta and Chrysophyta division in the studied ponds, which favour shrimp growth. Furthermore, the diversity index for the cultured ponds, which contain high amounts of organic waste and nutrients, was lower than the mangrove area site, which served as water inlet and outlet, demonstrating that mangroves can absorb organic matter and, therefore, improve water quality and increase phytoplankton structure diversity of the both water supply for the shrimp farming and aquaculture sewage.

ACKNOWLEDGEMENTS

This research was financially supported by Doctor Lektor Kepala Grant 2021 with contract number: 2105/UN10. F06/PP/2021, Faculty of Fisheries and Marine Science, Universitas Brawijaya.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

This research reports the investigation of the use of phytoplankton as a tool for water quality monitoring of eco-aquaculture system in super-intensive whiteleg shrimp pond. This is significant as an attempt to maintain the coastal ecosystem as well as the sustainability of shrimp farming.

AUTHORS CONTRIBUTION

Mohammad Mahmudi designed, coordinated, and supervised the research. Muhammad Musa and Sulastri Arsad supervised and revised the manuscript. Evellin Dewi Lusiana analyzed the data and wrote the manuscript. Alamanda Bunga and Nur Azlina Wati collected the data. All authors read and approved the final version of the manuscript.

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