

Cyanobacteria Community Dynamics and Trophic Status of Intensive Shrimp (*Litopenaeus vannamei*) Farming Pond in Situbondo, East Java Indonesia

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ABSTRACT

The objective of this study was to analyze the dynamics and community structure of Cyanobacteria, and trophic status of intensive shrimp culture (*Litopenaeus vannamei*) ponds in Situbondo based on Trophic Diatom Index (TDI). The ex post facto research was conducted in situ in the hamlet of Pond Mutiara Mas III Klatakan Situbondo, East Java Indonesia. The observation of Cyanobacteria and diatom community structure were conducted weekly during four cycles of shrimp farming ponds. Cycle of shrimp farming ponds is a cultivation period of the shrimps from seed to mature which ranges from 90-120 days. The dependent variables were the density of Cyanobacteria and diatom community, as well as chemical parameters, namely nitrite and orthophosphate. Trophic status was determined using TDI counted from diatom density. All of the data were then classified using cluster and biplot analysis program *PAST ver. 3.11* to describe the profile of ecosystem quality. The research results showed that four Genera of Cyanobacteria were identified as *Oscillatoria*, *Anabaena*, *Microcystis*, and *Merismopedia* during the farming cycles with *Oscillatoria* was the highest density. Based on the TDI values, the trophic status of the waters in the shrimp ponds was eutrophic until hyper-eutrophic. The water quality decreased along with the length of incubation time.

Keywords: *Cyanobacteria, intensive shrimp ponds, trophic status, TDI*

INTRODUCTION

Fishing commodity through direct fishing or aquaculture becomes one of the important industries which developing constantly throughout the years. World's fish supply development for consumption has exceeded the human population growth during last five decades, with the average of 3.2% in the period of 1961 – 2013 [1]. Crustacean group including white shrimp (*Litopenaeus vannamei*) is one of the important aquaculture products recently. In 2014, the production yields of Crustacean reached 6.9 million tons which was equivalent of US\$3.7 million [1, 2]. This achievement was supported by the development of aquaculture technology such as intensive farming. Intensive farming system is the farming system with the application of advanced technologies, high abundance of fry, and the artificial feed supplementation during the nursery [3].

Intensive farming system has an impact on the decline of water ecosystem services. This has been shown on shrimp farming ponds in Situbondo indicated that the application of intensive farming method did not guarantee the success of continuous shrimp harvest. The results of monitoring on water quality and plankton community structure indicated that the quality of ecosystem service has deteriorated [4]. The impact of this farming was the increase of organic wastes which can be derived from feed residues, shrimp feces, or dead shrimps and plankton bodies during the cultivation cycle [5, 6]. This will lead to decreasing the water quality such as eutrophication that can trigger harmful algal blooms (HABs) [7, 8]. A method that can be used to determine the trophic status of the waters is Trophic Diatom Index (TDI) determined from the composition and abundance of diatoms [9].

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Harmful Algal Blooms (HABs) comes from some algae groups such as Cyanophyceae, Bacillariophyceae, Dinophyceae, or Euglenophyceae [10]. These groups are able to naturally secrete toxic substances which can cause disturbance or even death of others aquatic biota [11]. The incidence of HABs in the shrimps' cultivation ponds might cause ecosystem shock and degrade the water quality of the habitat which can cause decreasing of shrimp production. This condition is also found in the intensive shrimp culture ponds in Situbondo. The intensive cultivation application system will cause the blooming of Cyanobacteria group [12].

Since this blooming incidence might cause a reduction of shrimp production, a conservational effort is required not only to maintain the stability of economic values but also to preserve the stability of ecosystem. This research was held to analyze the community structure of the Cyanobacteria group and the trophic status of shrimp (*L. vannamei*) farming pond in Situbondo based on the TDI during four cultivation cycles in order to further understand the impact of the intensive cultivation activities to the environment.

MATERIALS AND METHODS

Sample collection

Water samples were collected at five ponds of Mutiara Mas III Shrimp Pond, Klatakan Village, Kendit District of Situbondo Regency, East Java, Indonesia (Figure 1). Analysis of water quality and plankton diversity was performed weekly in Situbondo Laboratory of CJ. Feed Jombang during four cycles from March 2015 – June 2016. Cultivation cycle is the length of time required for the process of shrimp enlargement in the pond, which is about 90-120 days.

Water samples (~300 mL) were collected at a depth of 50 cm below the pond's surface. Then, water samples were sent to the laboratory in a dark condition with temperature below 4°C for further analysis. The analysis included chemical parameters (nitrite and dissolved phosphate) and community structure of plankton.

Chemical parameters and plankton analysis

Nitrite (NO_2^-) concentration was measured using Brucine method, while dissolved phosphate by Stannous Chloride method [15]. The absorbance values were measured using Scientific Genesys 20 Visible Spectrophotometer at 410 and 690 nm. Cyanobacteria and diatoms were identified using standard operating procedure of CJ Feed Jombang, combined with manual [13, 14]. The number of plankton cells was calculated using hae-

mocytometer counting chamber.

Data analysis

The data on the concentration of nitrite and dissolved phosphate during four cultivation cycles were analyzed descriptively on the average values of each parameter. The results of identification and component structure including composition and relative abundance were identified and analyzed. Total Diatom Index (TDI) was calculated based on diatom composition and density to determine trophic state of shrimp ponds (1). Lastly, all data were analyzed using cluster and biplot analysis with software of *PAST version 3.1.1* to determine the profile of intensive shrimp ponds in Situbondo [16].

$$TDI = (WMS \times 25) - 25 \quad (1)$$

$$WMS = (\sum a \cdot s \cdot v) / (\sum a \cdot v)$$

Note: WMS (weighted mean sensitivity); a= abundance or number of diatom taxa; s=sensitivity level for each taxon to pollutants (1-5); v= indicator value of each taxon (1-3) [9].

RESULTS AND DISCUSSION

Chemical parameters

Variation of nitrite and dissolved phosphate values during cultivation cycle ranging between 0.025 – 1.322 ppm and 0.013 – 0.148 ppm, respectively (Table 1). The rule of Indonesian Ministry of Marine Affairs and Fisheries No. 75 in 2016 about general guide for farming of giant black tiger prawn (*Penaeus monodon*) and king prawn (*L. vannamei*) states that water quality threshold of prawn farming for nitrite and dissolved phosphate are $\leq 1 \text{ mg.L}^{-1}$ and $0.1\text{-}5 \text{ mg.L}^{-1}$, respectively [17]. It can be interpreted that nitrite concentration during cultivation cycles met the standard, except at week 11 which was 1.322 mg.L^{-1} .

Nitrite in aquatic ecosystem is transformed into ammonium (NH_4^+) and nitrate (NO_3^-) which are done by *Nitrosomonas* and *Nitrobacter*, nitrogen fixation bacteria, as a part of nitrogen cycle [18]. It could be assumed that if the nitrite content in the waters is high then nitrate concentration in the waters is also high. High nitrate content in the waters will stimulate the development of phytoplankton biomass. Nitrate acts as an essential biochemical agent, such as the formation of chlorophyll and its role in the process of photosynthesis [19]. Furthermore, nitrate is an important component of nucleic acids such as DNA and RNA. In addition, the high nitrite concentration in the water can disrupt the growth

Table 1. The average value of chemical parameters during the cultivation cycle

Weeks	1	2	3	4	5	6	7	8	9	10	11	12
Nitrite (mg.L ⁻¹)	0.026	0.035	0.040	0.036	0.058	0.273	0.537	0.737	0.685	0.706	1.322	0.961
Dissolved Phosphate (mg.L ⁻¹)	0.013	0.013	0.021	0.043	0.124	0.148	0.114	0.102	0.093	0.055	0.044	0.065

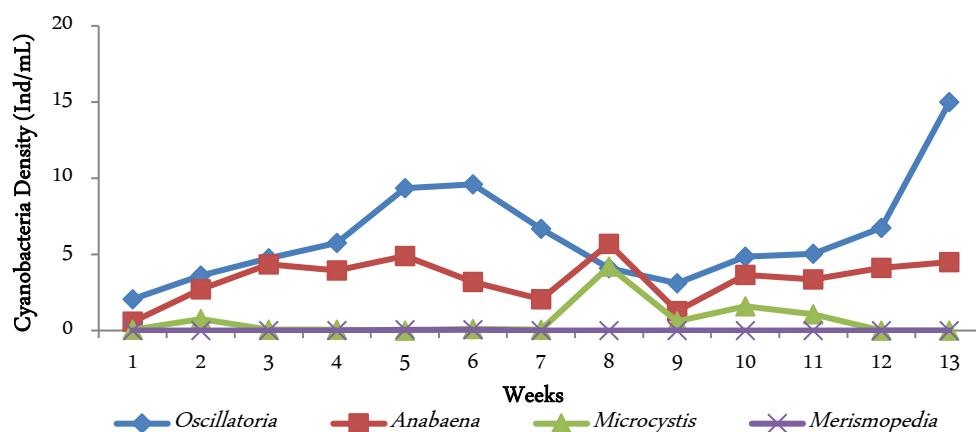


Figure 2. Composition and density of Cyanobacteria community during cultivation cycle

of shrimp because of its toxicity [20].

The values of dissolved phosphate were reported a different trend compared to nitrite concentration. The dissolved phosphate values gradually increased during cultivation cycle, and achieved the highest amount at week 6 by 0.148 mg.L⁻¹, then decreased until the end of the cultivation cycle. This fluctuation was caused by an accumulation of feeds during the cultivation cycle and decomposition process. Phosphate is a major limiting nutrient in the freshwater ecosystems primarily affecting the growth of phytoplankton [21]. Many species of phytoplankton from the Cyanobacteria group that dominate in the waters are usually initiated by the nutrient contents especially phosphate [7, 8, 22]. Phosphate is an ATP component that acts as a source of energy in intracellular transport for plankton, and nucleic acid components that organize the process of protein synthesis [18]. The utilization of dissolved phosphate in the waters can be evaluated from the increase of organism biomass in the aquatic ecosystem and is characterized by decreasing dissolved phosphate level until the end of the cultivation cycle.

Dynamics and community structure of Cyanobacteria and diatoms

Four genera of Cyanobacteria were identified during the cultivation cycle included *Oscillatoria*, *Anabaena*, *Microcystis*, and *Merismopedia*. The highest den-

sity of Cyanobacteria found during the cultivation cycle was genus of *Oscillatoria* (Figure 2).

Oscillatoria is commonly found in brackish waters and known as a tolerant organism towards organic pollutants at moderate-high level of nutrients [23]. It was observed that *Oscillatoria* density increased from the beginning to the week 6 then fluctuated until the end of the cultivation cycle. The density pattern of *Oscillatoria* is in agreement with the fluctuation pattern of dissolved phosphate (Table 1). *Oscillatoria* belongs to the diazotroph group of Cyanobacteria non-heterocyst [24]. The diazotroph genus has the ability to fix N₂ free from air, so that the organism can survive in the water with low nitrogen as long as phosphate is still available [25]. In addition, *Oscillatoria* can be used as a bio-indicator of shrimp pond quality, since the uncontrolled population of *Oscillatoria* might cause the quality of the waters getting worse indicated by the presence of other Cyanobacteria genera such as *Anabaena* and *Microcystis*.

In particular, *Anabaena* is commonly found in freshwater to brackish waters with low salinity [21]. Several species of Cyanobacteria genus are capable to produce toxin compounds such as microcystin (MCYs), anatoxin-a, anatoxin-a (S), and cilindrospermopsin (CYN), while *Anabaena circinalis* produces saxitoxin (STX) [11]. Not much different with *Anabaena*, the genus of *Microcystis* is also the most frequent blooming group of Cyanobacteria. One of the blooming cases has

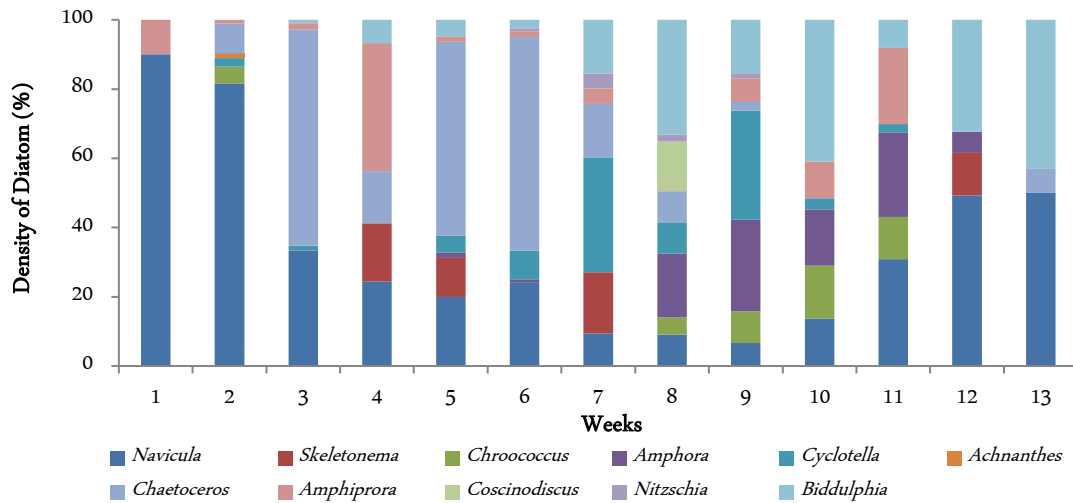


Figure 3. Composition and density of diatom community

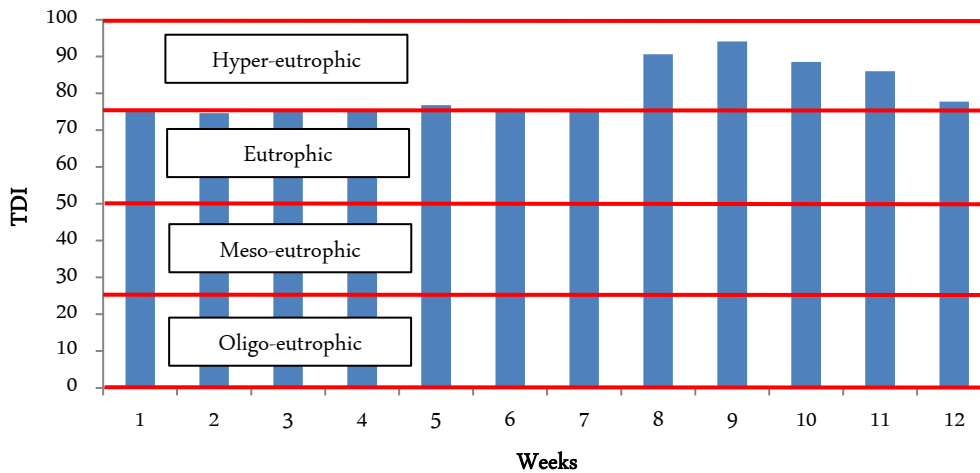


Figure 4. Biotic index (TDI values) of intensive shrimp farming ponds in Situbondo

occurred in the Sutami dam in Malang, East Java, Indonesia, and it was reported that the combination of nitrate 8 mg.L^{-1} and phosphate 0.4 mg.L^{-1} has been able to cause the highest abundance of *Microcystis* [26]. In addition, *Microcystis* is also capable to produce series of harmful compounds such as MCYs, anatoxin-a, and BMAA [11]. Various toxic compounds have targeted major organs in mammals that attack liver and nerve tissue, and caused death when exposed directly to aquatic organisms, as well as chronic effects when exposed to mammals [7, 11]. So, it is urgent to prevent the existence of these organisms.

Furthermore, the diatom composition found during the cultivation cycle consisted of 11 genera (Figure 3). Among these genera, there were sensitive and tolerant genera to organic pollutants [9, 23]. The result of this study found that various genera that sensitive to organic

pollutants were *Skeletonema*, *Chaetoceros*, *Achnanthes*, *Chroococcus*, *Coscinodiscus* and *Amphiprora*. The sensitive diatom genus found during the cultivation cycle has a higher diversity but lower density than the tolerant group. It was known that the density percentage values of sensitive diatom were quite high at the beginning of the cycle but decreased along with the length of cultivation period (Figure 3). *Skeletonema* and *Chaetoceros* were more sensitive genera to organic pollutants, which the optimum conditions of these organisms for growing were at the sea condition until low brackish, alkaline pH, and high level of cations. While genera of *Achnanthes*, *Chroococcus*, *Coscinodiscus* and *Amphiprora* were still able to survive in the presence of moderate organic matters.

The other genera that tolerant to organic pollutants were *Navicula*, *Amphora*, *Biddulphia*, *Cyclotella*, and

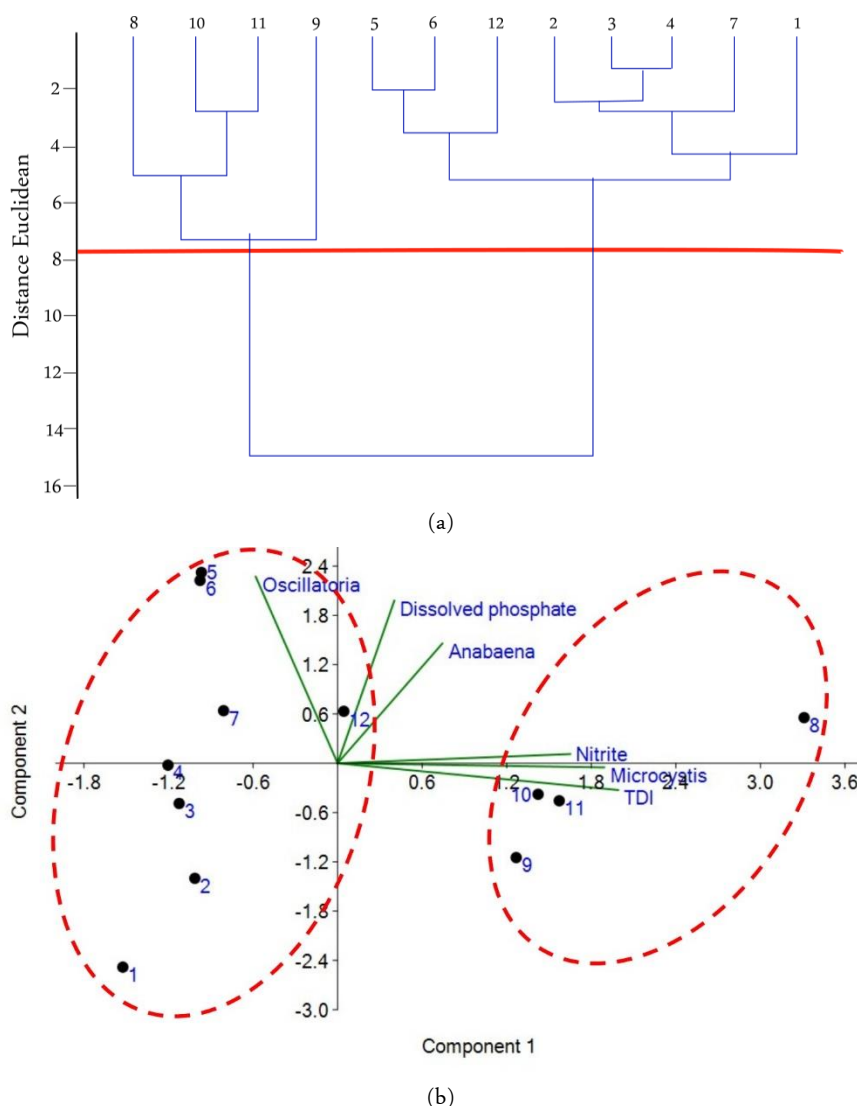


Figure 5. Characteristics of intensive shrimp farming pond Situbondo ecosystems based on PCA analysis: (a) cluster and (b) biplot PAST programme Ver. 3.1.1

Nitzschia. It was known by an increased percentage of density during the cultivation cycle, except for *Nitzschia* which only appeared at some observation time. While *Navicula* was known to have high density in the early and late weeks of the cultivation cycle. These genera were tolerant to the organic pollutants and could be used as a bio-indicator of water quality at a moderate-high level of nutrients [23].

Generally, the density of the Cyanobacteria group and tolerant diatom group increased during the cultivation cycle. It indicated that organic pollutants in the ecosystem were also increasing. The high density of *Oscillatoria* at mid-week of cultivation cycles can be used as a bio-indicator of pond water quality, because this group has diazotrophic capability. Thus, when the density of *Oscillatoria* is high, modification of shrimp en-

largement treatment is required in the ponds. Modifications can be performed by limitation of feeding in order to avoid the increasing of nutrients that can lead to increasing organic wastes.

Trophic status of intensive shrimp farming ponds

Total Diatom Index is a biotic index for water trophic status assessment determined by the composition and abundance of diatoms with a criterion of certain value [27]. TDI value criteria are categorized in several classes, among others, oligo-eutrophic with values 0 – 25; meso-eutrophic with values 25 – 50; eutrophic with a value of 50 – 75; and hyper-eutrophic with a value of 75 – 100. Based on the TDI analysis, it was known that the values of TDI during the cultivation cycle were quite varied and the longer cultivation time tended to increase

values about 74.66-94.15 and was categorized as eutrophic to hyper-eutrophic class (Figure 4).

Based on the values of TDI, eutrophication increased to its peak during the week 8 and 9 of cultivation cycle. It was due to the increased levels of organic pollutants derived from feed residues, feces matter, or the death of shrimps and plankton. One study of the Thai giant black tiger shrimp ponds (*P. monodon*) found that the main source of shrimp nutrients came from shrimp feeds so that it would stimulate phytoplankton growth in the shrimp farms that was indicated by the increasing of the concentration of chlorophyll-*a* [12]. This condition was in accordance with the observation that there was an increasing number of tolerant diatoms such as *Amphora*, *Biddulphia*, and *Cyclotella* (Figure 3).

Ecosystem characteristics of intensive shrimp farming ponds in Situbondo

Based on the cluster analysis, it showed that a longer time spent in the culture could be divided into two major groups (Figure 5a). Furthermore, based on the biplot analysis, each of these groups was marked by various parameters. The first major group (1st – 7th, 12th, and 13th week) was indicated by the high density of *Oscillatoria*, whereas in the second group (8-11th week) was indicated by the increasing density of *Anabaena* and *Microcystis* and also the increasing of dissolved phosphate, nitrite, and biotic TDI index (Figure 5b).

The increasing number of *Oscillatoria* at the beginning and end of the cultivation cycle could be used as early warning organism of shrimp pond quality in Situbondo. The longer cultivation time the worse water quality which was indicated by the increasing density of *Anabaena* and *Microcystis*, and also the increasing values of dissolved phosphate, nitrite, and TDI from 8th week of the cultivation cycle. This condition will require a comprehensive follow-up action due to the increasing density of *Oscillatoria* to avoid water degradation and threaten the sustainability of shrimp cultivation.

Water quality management need to be monitored periodically to overcome the bloom of microalgae, particularly of Cyanobacteria groups in the ponds. The addition of artificial feeding in the intensive shrimp farming system becomes a major factor of the increasing organic wastes in the ponds [12]. Artificial shrimp feeds might contain essential additives such as single cell proteins, growth promoting substances (steroid hormones) and some synthetic substances such as antibiotics and drugs for health and growth improvement of shrimps [29]. Hence, it is important to stop the use of artificial

feeding practices for several times to control the nutrients in the waters.

CONCLUSION

The Cyanobacteria genera were found during four cycles of intensive shrimp farming in Situbondo included *Oscillatoria*, *Anabaena*, *Microcystis*, and *Merismopedia*. Genus of *Oscillatoria* was the highest density during the cultivation cycle. Water quality in the shrimp ponds was categorized as eutrophic until hyper-eutrophic status.

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