Spatial and Temporal Distribution of Benthic Dinoflagellates on Artificial Substrate at Seribu Islands Waters

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Abstract

Damage to coral reef ecosystems and changes in water quality are capable of increasing the abundance of benthic dinoflagellates which could cause Ciguatera Fish Poisoning (CFP). Therefore, this research aimed to observe spatial and temporal abundance of those potential benthic dinoflagellates and analyze their relationship with environmental factors. Sampling was carried out on September 21-24, 2023 in Pramuka Island, Seribu Islands district, with different treatments, namely depth, location and time. Research conducted by deploy several series of artificial substrates into the waters, and the benthic dinoflagellates were collected from the artificial substrate placed in 1, 3, and 5 meter of water depths for 24, 48, and 72 hours at Odi, Mazu, and Villa Delima Piers. Cell densities were determined by enumeration using a Sedgewick Rafter Counting Chamber under a light microscope, and the relationship with environmental parameters was analyzed by using Principal Component Analysis (PCA). The results showed that 4 genera of benthic dinoflagellates which had the potential to cause CFP, were attached to the artificial substrate, namely Amphidinium, Coolia, Ostreopsis, and Prorocentrum. Prorocentrum cells were observed in highest number on artificial substrates at every treatment with different depths, locations, and times. Furthermore, the PCA analysis showed the abundance of Prorocentrum was influenced by the most typical environmental parameters at each depth, location, and time, namely temperature, nitrate, DO, and light intensity. The research provided valuable information on the benthic dinoflagellates both spatially and temporally, through international standard methods in order to prevent and anticipate negative impacts caused by CFP.

Keywords: *Benthic, HAB, PCA, Reefs, Substrate*

Introduction

Harmful Algal Blooms (HAB) is the phenomenon of microalgae population explosion in marine and freshwater waters capable of causing danger and loss. Based on occurrence, HAB is divided into microalgae with high biomass that can cause anoxia in water, and toxin-producing microalgae capable of contaminating seafood commonly consumed by humans. It is also a natural phenomenon that has occurred for a long time and is related to high anthropogenic activities in coastal areas. Anthropogenic activities such as industrialization, increasing coastal settlements, and agricultural activities in coastal ecosystems cause eutrophication that accommodates the growth of toxin-producing microalgae (GEOHAB, 2001; Davidson, 2012; Wells, 2015). Macroalgae communities attached to coastal structures and coral fragments due to human activities (tourism and sea transportation) become the preferred substrate for the toxin-producing microalgae (Steidinger and Baden, 1984). HAB can also cause economic, tourism, seafood, and ecosystem losses for coastal communities, because of damage to the biodiversity of coastal ecosystems (GEOHAB, 2001).

Based on GEOHAB (2001) and Elferink *et al*. (2020), dinoflagellates and diatoms are two groups of microalgae that have genera capable of producing toxins. Several genera of dinoflagellates can cause Ciguatera Fish Poisoning (CFP), a symptom of poisoning attributed to consuming seafood contaminated with benthic dinoflagellates. Moreover, benthic dinoflagellates from the genus *Gambierdiscus* produce ciguatoxin (CTX) causing CFP. Based on order, herbivorous predators consume CTX followed by other organisms in the aquatic ecosystem. CTX that accumulates in captured reef fish can cause symptoms of digestive tract and nervous system disorders in humans, such as diarrhea, vomiting, and hypotension, including

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muscle and joint pain (Friedman *et al*., 2008; Nasution *et al*., 2021). These toxins move from one organism to another through bioaccumulation and biomagnification processes in the food chain, with top predator fish being the location of the largest toxin accumulation (Seygita *et al*., 2015). CTX is also asscociated with dinoflagellates from the genera *Amphidinium, Coolia*, and *Prorocentrum* (Okamoto and Fleming, 2005; Solter and Beasley, 2013; Widiarti *et al*., 2022).

The occurrence of CFP in Indonesia has not been officially reported (Chan, 2015). However, the potential for occurrence has been shown by the discovery of benthic dinoflagellates that cause CFP in Indonesian water. According to Widiarti *et al*. (2007) and Fakhraini *et al*. (2018), toxic dinoflagellates with the potential to cause CFP were found on Pramuka Island, including *Amphidinium sp.* These include *Gambierdiscus toxicus, Ostreopsis lenticularis, Prorocentrum concavum, P. lima*, and *P. rhathymum* which are attached to the natural substrate of *Padina* sp. and *Sargassum* sp. Pramuka Island has the largest population in Seribu Islands, serving as a tourist destination (Handayani and Wahjuningsih, 2013). The productivity of human activities such as tourism, construction, and shipping cause damage to aquatic ecosystems, particularly coral reefs. Ardiansyah *et al*. (2012) stated that coral death index on Pramuka Island tends to increase from year to year. The damage to coral reef can serve a new substrate which are often occupied by benthic dinoflagellates. This phenomenon leads to the occurrence of new areas for macroalgae and seagrass, as well as various dead coral fractures (Widiarti and Pudjiarto, 2015).

The discovery of dinoflagellates that have the potential to cause CFP on Pramuka Island can lead to several losses for coastal communities and damage the biodiversity of ecosystems (GEOHAB, 2001; Widiarti *et al*., 2007). Despite this potential threat, there is no research related to the influence of depth, location, and time on the abundance of benthic dinoflagellates in Pramuka Island. This shows the need to investigate the abundance of benthic dinoflagellates with the use of an artificial substrate. Sampling of benthic dinoflagellates using artificial substrate can provide sufficient results comparable with another research. Generally, dinoflagellates sampling methods often use natural substrate that have varying surface areas, limiting the comparison of results with international standards (Razi *et al*., 2014; Fakhraini *et al*., 2018; Yong *et al*., 2018; Boisnoir *et al*., 2019; Tester *et al*., 2022). Artificial substrate has a measurable surface area, allowing for precise calculation of cell abundance and comparison, without obtaining natural substrate from their habitat (Tester *et al*., 2022).

Based on the description, this research aimed to identify and analyze the abundance of potential benthic dinoflagellates causing CFP in artificial substrate in Seribu Islands waters. Analysis was also conducted on the composition of benthic dinoflagellates on artificial substrate that differ spatially (location and depth) and temporally, as well as the relationship with environmental parameters. The results provided valuable information on the composition of benthic dinoflagellates both spatially and temporally, through international standard methods to prevent and anticipate negative impacts caused by CFP.

Materials and Methods

Sampling was carried out on 21–24 September 2023 in waters of Odi Pier, Mazu Pier, and Villa Delima Pier, at Pramuka Island in Seribu Islands district, as shown in Figure 1. Observations and sample enumeration were carried out at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. Artificial substrate consisted of three components, namely screen, float, and weights. The screen was made by cutting a 15 cm x 10 cm rectangular fiberglass mesh, which was connected to a fishing float and weighter, using a fishing line tied to the end of each component (Reguera *et al*., 2016).

Artificial substrate for depth treatment was placed in waters of Odi Pier at depth 1, 3, and 5 m with three replicates. This comprised a distance of 1 m between replicates in the natural substrate zone for 24 h (Tester *et al*., 2014). After 24 h, the thread connected to the screen was cut from an artificial substrate series using scissors. Subsequently, the screen and sample water were put in a ziplock plastic, and the remaining artificial substrate was lifted.

For location treatment, artificial substrate was placed in the water by inserting a weight at a depth of 1 m at three research stations for 24 h. Based on observation, Odi, Mazu, and Villa Delima Piers were dominated by macroalgae, coral transplant, and dead coral substrate, respectively. This treatment was carried out with three replicates, each separated by a 1 m distance so that the substrates among replicates were not too different. After predetermined time, the gauze on artificial substrate was cut from the main series using scissors and put into a ziplock plastic along with a water sample, and the remaining was lifted.

Another set of artificial substrates was placed

in water at a depth of 1 m with three replicates for time treatment of 24, 48, and 72 h. After the treatment was completed, each screen was placed directly into a ziplock, while the weights along with the fishing line and buoy were taken back.

Sample treatment

The screen and water sample in ziplock plastic were transferred to an airtight container and shaken for 1 minute to release dinoflagellates. Subsequently, the screen was separated from the water sample and the initial volume was measured using a measuring glass. Water sample was filtered using a 125 µm graduated sieve to filter out debris. Another filtration was carried out using a 25 µm graduated sieve. The sample retained on 25 µm sieve was sprayed with filtered sea water using a spray bottle, poured into a 100 ml bottle and then preserved using 96% ethanol.

Measurement of environmental parameters

Environmental parameters were taken at all stations in each treatment. Measurements were recorded during the installation and removal of artificial substrate at the sampling location. Salinity, DO, temperature, pH, and light intensity were measured in-situ using an ATAGO refractometer, Lutron D-5510 DO meter, Mquant Merck pH indicator strip paper, and LX-1010B LUX meter above water surface. Current speed and direction were measured using a floating droudge by placing it on the water surface and allowed to drift with surface current. Water samples were also collected using a 20 L container from each depth, and put into a bottle for analysis. The nitrate and phosphate content in the water sample was tested, by mixing water sample (0.50 ml for each test) with Sigma Aldrich nitrate and phosphate reagents (4.0 ml reagent-1 and 0.50 ml reagent-2 for nitrate, and 0.50 ml reagent-1 and 1 dose reagent-2 for phosphate). This step was followed by measuring the samples in the Merck Spectroquant Prove 300 UV/VIS spectrophotometer machine at the MERCK laboratory, Department of Biology, FMIPA Universitas Indonesia.

Sample observation

Water samples were pipetted and put into the Sedgewick Rafter Counting Chamber with a volume of 1 ml. The chamber was covered with a cover glass and observed using an Olympus CX23 light microscope at 100x magnification. Observation was performed on each sample with two replicates, and identification conducted by referring to the dinoflagellates identification books which were 'Marine benthic dinoflagellates: Unveiling their worldwide biodiversity' (Hoppenrath *et al*., 2014) and 'Marine phytoplankton of the Western Pacific' (Omura *et al*., 2012).

Cells abundance data processing

Data processing from the results of dinoflagellates tabulation were processed by calculating the surface area of the screen on artificial substrate. The screen was composed of cylindrical filaments which were structured to intersect each other forming the x and y axes, as shown in Figure 2.

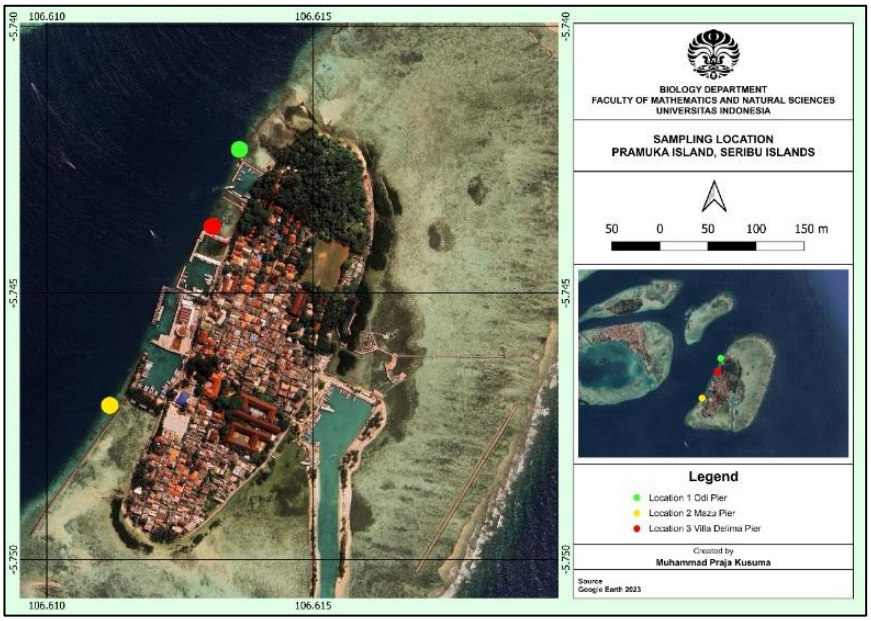

Figure 1. Location of artificial substrate placement and sampling on Pramuka Island, Seribu Islands

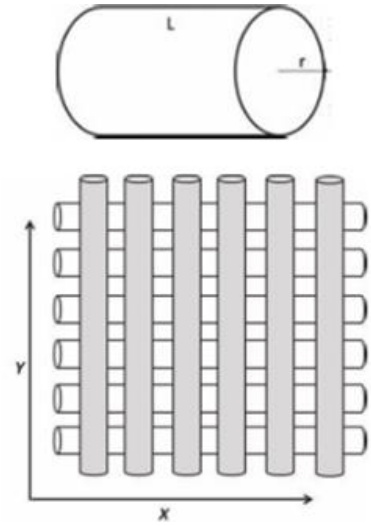

Figure 2. Screen area on artificial substrate and size of its constituent filaments (Reguera et al. 2016).

The screen on the artificial substrate consists of several filaments. Calculating the surface area of a single filament is the initial step that must be performed before determining the total surface area of the screen. The surface area of one filament and can be calculated by following formula of Reguera *et al*. 2016, which is:

$$A=2\pi rL+2\pi r^2$$

Note: A = Surface area of one filament; L = Filament length; r = Filament radius

Once the surface area of one filament has been calculated, the next step is to determine the total surface area of the screen, which can be calculated using the following formula of Reguera *et al.*, 2016, which is:

$$A\Sigma=AxNx+AyNy-NxNy16r^2$$

Note: Ax = Surface area of x filament; Ay = Surface area of y filament; Nx = Number of x filaments; Ny = Number of y filaments; r = Filament radius

The abundance of dinoflagellate cells was then calculated using the formula of Firdaus *et al.*, 2021, which is:

$$S = \frac{J}{H} x \frac{I}{G} x \frac{F}{N}$$

Note: S = Abundance of dinoflagellate cells (cells.cm^{-2}); J = Number of cells counted in the Sedgewick Rafter Counting Chamber; H = Volume of the sample observed in the Sedgewick Rafter Counting Chamber; I = Final volume of the sample after filtering and extraction (ml); G= Volume of the filtered sample (ml); F= Total volume of the sample collected from the field (ml); N= Surface area of the screen (cm^2)

Data analysis

The abundance of dinoflagellates can be associated with environmental parameters data at each treatment using Principal Component Analysis (PCA), which characterized environmental factors at each station (Razi *et al*., 2014; Fernando *et al*., 2019). PCA is a multivariate statistical test that analyzes the correlation of observation data with variables from several matrices. Furthermore, PCA can show patterns of similarity between observation data and variables in the form of mapping (Abdi and Williams, 2010). In this research, PCA was processed using Past 4.17 software, and variable characteristics were interpreted from the component values.

Results and Discussion

A total of three genera of dinoflagellates were found at depths of 1, 3, and 5m. These included *Prorocentrum* at each depth with total abundance of 55.0 cells.cm^{-2}, *Ostreopsis* at 1 and 5 m with total abundance of 18.0 cells.cm^{-2}, and *Amphidinium* at 3 m with total abundance of 6.0 cells.cm^{-2}. This was in accordance with research by Widiarti *et al*. (2007) and Fakhraini *et al*. (2018) who found the three genera in Pramuka island waters. Tester *et al*. (2014) and Boisnoir *et al*. (2019) also identified the presence of *Prorocentrum, Ostreopsis,* and *Amphidinium* attached to artificial substrate screens.

The benthic dinoflagellates were found more abundantly at 1 m depth with total abundance of 42.7 cells.cm^{-2}, whereas *Prorocentrum* cells were the most commonly found in that depth (30.7±11.0 cells.cm^{-2}), compare to the other genera. Lee *et al*. (2020) stated that *Prorocentrum* was very abundant and often found at various depths. *Amphidinium* could also be found in lower abundance in various microhabitats and depths compared to *Prorocentrum* and *Ostreopsis* (Fakhraini *et al*., 2018; Lee *et al*., 2020).

Based on the results, Mazu Pier became the station with the highest abundance of benthic dinoflagellates found (97.7 cells.cm^{-2}), while Villa Delima Pier had the lowest abundance with total of 24.3 cells.cm^{-2}, as shown in Table 2. The genera found were *Coolia*, *Ostreopsis*, and *Prorocentrum,* with abundance ranging from 12.0 to 128.7 cells.cm^{-2}. *Prorocentrum* was found at all stations in highest number compared to the other two genera, with a total cell abundance of 128.7 cells.cm^{-2}. Meanwhile, *Coolia* only observed at Odi Pier and Mazu Pier, with the lowest total abundance of 12 cells.cm^{-2}.

The highest abundance of cells found in Mazu Pier could be caused by several factors, including the diversity of its natural substrates. Several human activities commonly found at Mazu Pier, such as snorkeling or coral trampling, which have the potential to cause damage to the coral reef ecosystem (Ardiansyah, 2013). This damage can lead to the presence of dead coral fragments in the waters, creating new surfaces for macroalgae, such as *Padina*, which were one of the preferred substrates for benthic dinoflagellates (Widiarti *et al*., 2016). The discovery of dead coral fragments and the growth of macroalgae in large quantities at Mazu Pier, suggested the severe damage had already occurred to the coral reef ecosystems in the waters. Many coral transplant structures were also observed in Mazu Pier. The structures allowing potential formation of a new substrates for the attachment of benthic dinoflagellates (Subhan, 2014; Thoha *et al*., 2020).

The highest abundance of benthic dinoflagellates was found in the 72-hour treatment (129.2 cells.cm^{-2}), followed by 48 hours (54.1 cells.cm^{-2}) and 24 hours (20.8 cells.cm^{-2}), as shown in Table 3. *Prorocentrum* and *Ostreopsis* were found at every treatment (24 and 48 hours) with abundance values of 141,6 and 37,4 cells.cm^{-2}, respectively, while *Sinophysis* only observed at 72 hours treatment with abundance values of 25±33.1 cells.cm^{-2}.

Table 1. Abundance of Benthic Dinoflagellates (cells.cm^{-2}) at different depth

Genus	1m	3m	5m	Total
Prorocentrum	30.7±11.0	12.0±10.4	12.3±21.4	55.0
Ostreopsis	12.0±10.4	0.0±0.0	6.0±10.4	18.0
Amphidinium	0.0±0.0	6.0±10.4	0.0±0.0	6.0
TOTAL	42.7	18.0	18.3	

Table 2. Abundance of Benthic Dinoflagellates (cells.cm^{-2}) at different location

Genus	Odi Pier	Mazu Pier	Villa Delima Pier	Total
Prorocentrum	49.3±21.4	67.3±21.4	12.0±10.4	128.7
Ostreopsis	12.0±10.4	24.3±28.0	12.3±21.4	48.7
Coolia	6.0±10.4	6.0±10.4	0.0±0.0	12.0
TOTAL	67.3	97.7	24.3	

Table 3. Abundance of Benthic Dinoflagellates (cells.cm^{-2}) at different time

Genus	24 hours	48 hours	72 hours	Total
Prorocentrum	16.7±14.4	37.5±25.0	87.5±66.1	141,6
Ostreopsis	4,2±7.2	16,7±14.4	16,7±28.9	37,4
Synophysis	0±0.0	0±0.0	25±33.1	25,0
TOTAL	20,8	54,2	129,2	

Table 4. Environmental Parameters at Each Sampling Sites

Parameters	Unit	Odi Pier	Mazu Pier	Villa Delima Pier
Temperature	°C	29.95	29.35	30.25
Salinity	ppt (‰)	31	34	34
DO	$mg.L^{-1}$	7.2	7.2	8.55
pH	pH	7.5	7.5	7.25
Light Intensity	Lux	420.5	421.5	395.5
Nitrate	$mg.L^{-1}$	9.8	9.7	8.7
Phosphate	$mg.L^{-1}$	1.6	3.2	2.6
Water Current	$m.s^{-1}$	0.05	0.05	0.05

The higher abundance of *Prorocentrum* cells could be attributed to their high tolerance to diverse water conditions, and their wide distribution from tropical to temperate areas (GEOHAB, 2001; Zou *et al*., 2022). Fakhraini *et al*. (2018) also found that *Prorocentrum* was the most abundant dinoflagellates found in waters of Pramuka Island. Boisnoir *et al*. (2019) also observed that *Prorocentrum* and *Ostreopsis* cells had the highest abundance values, while *Sinophysis* showed the lowest.

The measurement of environmental parameters at each sampling sites in Pramuka Islands showed that waters temperature range from 29.35–30.25°C, salinity from 31–34‰, dissolved oxygen (DO) from 7.2–8.55 $mg.L^{-1}$, waters acidity from 7.25–7.5, light intensity from 395.5–421.5 Lux, nitrate from 8.7–9.8 $mg.L^{-1}$, phosphate from 1.6 –3.2 $mg.L^{-1}$, and water current velocity at 0.05 $m.s^{-1}$.

Relations between environmental parameters and cells abundance

Based on the results of PCA, the scatter plot with a variance of 67.943% showed that temperature, nitrate, and 1 m depth were grouped on the axis 1 negative, as presented in Figure 3. The 3 m depth was grouped with the environmental factor DO on the axis 2 positive, while the 5 m depth was pH on the axis 2 negative. The salinity and phosphate environmental factors were not grouped at any depth. The depth of 1 m was characterized by environmental factors such as nitrate levels and temperature, with the highest values of 9.8 $mg.L^{-1}$ and 29.95°C, respectively. Based on Accoronia *et al*. (2018), a higher temperature could increase the growth rate of *Prorocentrum*, showing elevated abundance at 1 m. This genus can survive at 5 - 30°C and grows optimally at a temperature of 25 - 30°C (Accoronia *et al*., 2018; Fakhraini *et al*., 2018). Yeremia *et al*. (2016) also found that *Prorocentrum* was most abundant at the station with the highest temperature compared to others. According to Abassi and Ki (2022) and Tasak *et al*. (2015), nitrate could increase the transcription process in dinoflagellates four times within 48 h, contributing to the growth rate of *Prorocentrum*. This genus was also found at the station with the highest nitrate levels in the research of Lumbantoruan (2009). *Amphidinium* was only found at 3 m which was characterized by high DO levels of 7.7 $mg.L^{-1}$, as shown by PCA results. The abundance of dinoflagellates was generally positively correlated with DO levels, serving as an indicator of dinoflagellates. Based on research by Razi *et al*. (2014), *Amphidinium* was only found at stations with higher DO than other stations.

The PCA results showed that axis 1 had a percentage variance value of 69.143% and axis 2 was 30.857%. The grouping on the axis 1 negative was formed by the variable dissolved oxygen (DO) at Villa Delima Pier, as shown in Figure 4. The grouping on the axis 2 negative was formed by the variable temperature of Odi Pier. Meanwhile, the grouping on the axis 2 positive was formed by the variables salinity and phosphate of Mazu Pier. The highest abundance of *Prorocentrum* was found in Mazu Pier, which was 67.3 $cells.cm^{-2}$, followed by *Ostreopsis* at 24.3 $cells.cm^{-2}$, and *Coolia* at 6.0 $cells.cm^{-2}$. The PCA results showed that the station was influenced by environmental factors in the form of salinity and phosphate. Salinity affects osmotic pressure in cells, as a higher value exceeding the tolerance range for dinoflagellates could trigger a decrease in cell activity and inhibit growth (Morton *et al*., 1992; Makmur *et al*., 2012).

Mazu Pier has a salinity of 34‰, which was considered optimal for dinoflagellates growth. Makmur *et al*. (2012) stated that dinoflagellates could grow when seawater salinity was in the normal range of 25-35‰. According to Morton *et al*. (1992), good salinity for *Prorocentrum* ranged from 20 to 43‰, while *Ostreopsis* and *Coolia* were between 24-43‰. The three genera had maximum growth at salinity <36‰, which was included in the optimal salinity range for dinoflagellates growth. Similarly, Morton *et al*. (1992) stated that the growth of the three genera is positively correlated with salinity. Phosphate is another environmental factor that characterizes Mazu Pier, serving as an essential nutrient for dinoflagellates growth. It is commonly introduced to water through industrial and domestic

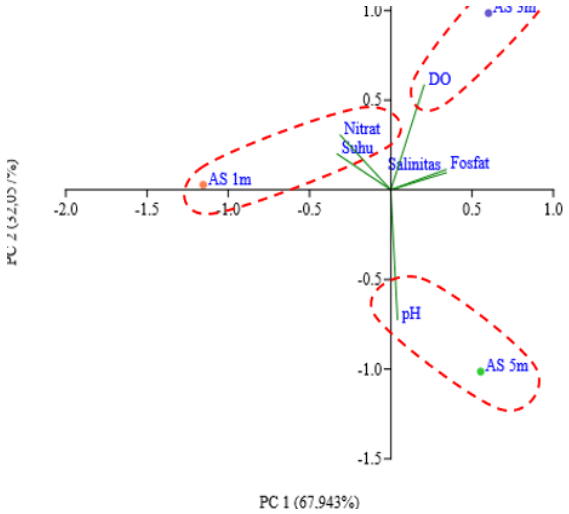

Figure 3. PCA Plot between Environmental Factors and Depth Treatment
Note: AS 1m = *Artificial Substrate* 1 m; AS 3m = *Artificial Substrate* 3 m; AS 5m = *Artificial Substrate* 5 m; PC 1 = Axis 1; PC 2 = Axis 2

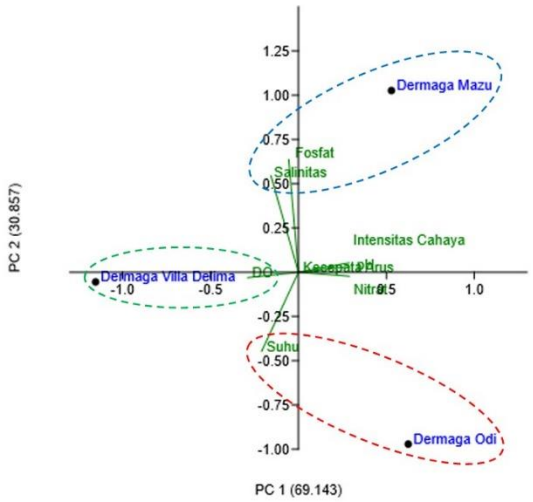

Figure 4. PCA Plot between Environmental Factors and Location Treatment
Note: Odi Pier = Location Difference Treatment 1; Mazu Pier = Location Difference Treatment 2; Villa Delima Pier = Location Difference Treatment 3; PC 1 = Axis 1; PC 2 = Axis 2

waste (Dwivayana *et al*., 2015). Suryadi *et al*. (2022) stated that phosphate concentration in waters could be influenced by sediment resuspension. According to Qin *et al*. (2021), phosphate is important for benthic dinoflagellates for metabolic purposes in cell growth and energy transmission. In this research, phosphate value at Mazu Pier was 3.2 mg.L^{-1}, which was known to be optimal for the growth of benthic dinoflagellates. This high value could be attributed to the closeness of the station to residential areas, where domestic waste contributed to the potential increase in phosphate content.

Herawati *et al*. (2023) stated that the phosphate content ranging from 0.27 to 5.51 mg.L^{-1} could be considered optimal for the growth of benthic dinoflagellates. Therefore, the value obtained in this research was in accordance with the optimal phosphate range for dinoflagellates growth (Qin *et al*. 2021). Results showed that an environment with high phosphate conditions would accelerate the growth rate of *Prorocentrum* cells. Similarly, Ibghi *et al*. (2022) showed that the abundance of *Prorocentrum, Ostreopsis,* and *Coolia* was positively correlated with phosphate. Herawati *et al*. (2023) stated that high salinity and phosphate in water could increase the abundance of dinoflagellates. Optimal salinity and phosphate content were the contributing factors for the discovery of the three genera in Mazu Pier waters.

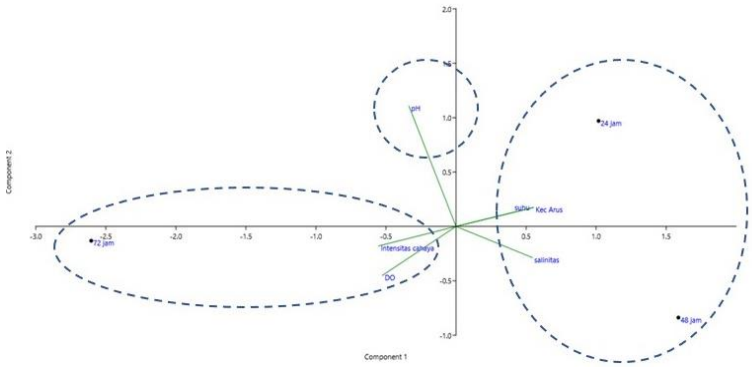

Figure 5. PCA Plot between Environmental Factors and Time Treatment
Note: 24 hours = 24 hours' Time Difference Treatment; 48 hours = 48 hours' Time Difference Treatment; 72 hours = 72 hours' Time Difference Treatment; PC 1 = Axis 1; PC 2 = Axis 2

The results of the PCA analysis produced two main components, principal component 1 (PC1) had a percentage variance value of 86.2%, and principal component 2 (PC2) of 13.8%. The grouping at the axis 1 positive was formed by environmental parameters of temperature, salinity, and current speed with treatment times of 24 and 48 h, as shown in Figure 5. Meanwhile, the grouping on the axis 1 negative was formed by DO and light intensity with a treatment time of 72 hours. The pH variable did not characterize any treatment time.

The highest abundance of *Prorocentrum* was found in the 72-h treatment at 87.5 cells.cm^{-2}, followed by *Sinophysis* at 25 cells.cm^{-2}. Based on the results of the PCA analysis, the 72-h treatment was characterized based on DO and light intensity. Sunlight is used by most autotrophic microalgae, including benthic dinoflagellates, for carbon fixation through photosynthesis. This allows microalgae to inhabit clear water columns to obtain intense light, with an optimal range of 1,000 to 10,000 lux (Fraga *et al*., 2012). In this research, light intensity measurements were carried out at noon during each treatment time (Maynardo *et al*., 2015). The high abundance of *Prorocentrum* in the 72-h treatment was supported by Heil *et al*. (2005) who reported an optimal growth of *Prorocentrum* at high levels of light intensity. Furthermore, research conducted by Brownlee et al. (2005) with a *Prorocentrum* culture also showed that DO concentration increased significantly in high-density cultures, then decreased until all oxygen had disappeared from the medium after 4 days, due to high oxygen consumption for respiration rates.

Conclusion

In conclusion, this research successfully identified the abundance of potential benthic dinoflagellates which could cause CFP, and analyzed the relationship between cell abundance and environmental factors. The results showed that benthic dinoflagellates which were found attached onto artificial substrate on Pramuka Island waters consists of *Amphidinium, Coolia, Ostreopsis,* and *Prorocentrum*, while *Prorocentrum* had the highest abundance of cells. The most common observed of the environmental parameters at each depth, location, and time treatment were temperature, nitrate, DO, and light intensity. These parameters affected the abundance of benthic dinoflagellates on artificial substrate.

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