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Study on The Potentially Harmful Benthic Dinoflagellates in Pari Island, Indonesia

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Abstract

Information on benthic dinoflagellates in Indonesia is rare since it often neglected in many microalgae researches. Thus, not much information is available about the ecology of benthic dinoflagellates in Indonesia, especially for three important genus, *Gambierdiscus*, *Prorocentrum* and *Ostreopsis*. Sampling were carried three times: in August, October, and December 2013. The sampling was conducted around Pari Island. Bethic Harmful Algal Blooms (B-HABs) were collected in each sampling site using a modified PVC rig with 15x10 cm screen (artificial substrate) which placed at the bottom of the water for 24 hours. Another form of a screen with leaf blade form, 30x5 cm was also deployed in each sampling site and left for 24 hours. In general, the different density of *Prorocentrum*, *Gambierdiscus*, and *Ostreopsis* were observed in various substrates in this research. Temporal variation of those three target genera was also found in this research, from the results so far, *Prorocentrum* was suggested as the most common benthic dinoflagellates in Pari Island. This genus was found in all substrates during two sampling periods in this study, except in sandy bottom substrate in October 2013. The highest density of *Prorocentrum* which observed in the screen placed in coral reefs area in October 2013, was 288 cells/100 cm^2. The lowest of *Prorocentrum* density was observed in coral reefs area in October 2013, was 0.53 cells/g of wet weight.

Keywords: Harmful Benthic Dinoflagellates, Pari Island, Indonesia.

Abstrak

Informasi mengenai dinoflagelata bentik di Indonesia masih sangat jarang dikaji. Oleh karena itu, tidak banyak informasi yang tersedia mengenai ekologi dinoflagelata bentik di Indonesia, khususnya pada tiga genus penting *Gambierdiscus*, *Prorocentrum* dan *Ostreopsis.* Pengambilan sampel dilakukan pada bulan Agustus, Oktober, dan Desember di sekitar Pulau Pari. *Benthic Harmful Algal Blooms (B-HABs)* dikoleksi pada setiap stasiun dengan menempatkan pipa PVC dengan screen sebesar 15x10 cm di dalamnya (substrat buatan) selama 24 jam. Secara umum, *Prorocentrum*, *Gambierdiscus*, dan *Ostreopsis* ditemukan pada berbaga isubstrat di setiap stasiun. *Prorocentrum* merupakan bentik dinoflagelata yang umum ditemukan di Pulau Pari. Genus tersebut ditemukan di setiap substrat selama dua kali pengambilan sampel, kecuali pada substrat berpasir sepanjang Oktober 2013. Kelimpahan tertinggi dari *Prorocentrum* ditemukan pada screen yang diletakkan pada substrat terumbu karang dengan kelimpahan sebesar 288 sel/100 cm^2. Sedangkan kelimpahan terendah ditemukan pada substrat natural yang berada pada area terumbu karang dengan kelimpahan sebesar 0.53 sel/g berat basah

Kata kunci : Harmful Benthic Dinoflagellates, Pulau Pari, Indonesia.

Introduction

Unlike their planktonic relative's benthic dinoflagellates are generally found attached to or otherwise associated with surfaces or substrates. These substrates include macrophytes, dead coral, rocks, bivalve shells, sediment, detritus, and benthic invertebrates (Delgado *et al*., 2006; Kibler and Litaker, 2012). Benthic dinoflagellates are microalgae that live attached to various kinds of substrates, such as rocks, sediments, dead coral reefs, seagrasses and macroalgae. Most genera of benthic dinoflagellates serve an important role in trophic level regulation in benthic community, but some others have known for its capability to produce a toxin or to induce Harmful Algae Blooms (HABs) phenomenon in the water column (Larsen & Mohammad-Noor, 2012; Almazan-Becerril *et al*., 2015).

Some benthic dinoflagellate genus such as *Gambierdiscus*, *Prorocentrum* and *Ostreopsis* have several species that produce maitotoxin, yessotoxin and palytoxin (Delgado *et al*., 2006; Tester *et al*., 2012; Litaker *et al*., 2012; Settlemier, 2012). Those toxins are harmful due to its high haemolytic activity and could cause deadly health problem for human (Tester *et al*., 2012; Litaker *et al*., 2012). In general, the target species of this study was known to cause Ciguatera Fish Poisoning (CFP) in human, and could also damaging the ecosystem by altering the food chain or degrading the environmental condition (Glibert *et al*. 2012)

Despite several attemps on studying the benthic dinoflagellates communities in Indonesia, such as study done by Skinner *et al*. (2011) in Bali, Anggraeni *et al*. (2013) in Pari Island, Razi *et* *al*. (2014) in Harapan Island, and Widiarti & Pudjiarto (2015) in Tidung Island, information on benthic dinoflagellates in Indonesia remains scarce since it often neglected in many microalgae researches and its appearance was often underreported. Thus, detailed information about the ecology of benthic dinoflagellates in Indonesia is still lacking. Another problem in the study of benthic dinoflagellates is lack of meaningful comparison between studies. This happens due to common sampling method which collecting natural substrates, such as macrophyte or macroalgae, to study the benthic dinoflagellates assemblages in a habitat. The difficulties in standardizing the amount or total surface area of natural substrates have become a concern in the GEOHAB working group. Thus, a need for standardized method arises to provide robust data for future research on worldwide benthic dinoflagellates distribution and abundance.

In this research, there were two main objectives: (1) to test new sampling method using standardized artificial substrate, with primary hypothesis that the density of benthic dinoflagellates in artificial substrate is correlated with cell density of adjacent natural substrates (H1); and (2) to study the distribution and abundance of three particular genus of dinoflagellates in Pari Island, which were *Gambierdiscus* spp., *Ostreopsis* spp., and *Prorocentrum* spp.

Methods

Samplings were carried three times, in August, October, and December 2013, which represent the end of dry, the intermediate, and the start of the wet season in Indonesia. Samples in this research were collected around Pari Island (Fig. 1), an island which is part of Thousand Islands (Kepulauan Seribu) located north from Jakarta. This island has some relatively pristine coastal ecosystems around smaller islands near Pari Island, which should have low anthropogenic influence. Research Centre for Oceanography has a research station, equipped with a small laboratory, which was used as the basecamp for this research. In this research, four permanent sampling sites have established, which consist of seagrass, seaweed, sandy bottom, and coral reefs area (Fig. 1). The depth of all selected sites was around 0.5 to 3 m, which make it possible to use only snorkelling equipment for sampling.

In each site, natural samples were taken at the same time with the retrieval of the artificial substrate. Sampling for both artificial and natural substrate was conducted on 28th of August, 22nd of October, and 18th of December 2013. There were a total of 94 samples from three sampling trips, in which 32 samples were collected each sampling, except in August sampling, due to bad weather condition.

A modified PVC rig was placed at the bottom of the water in each site. Each PVC rig consists of three 15x10 cm screen (artificial substrate). The shape, size, and material of the screens on this rig was standardized according to (Kibler & Litaker, 2012; Moreira & Tester, 2016). Additionally, one modified form of the screen (leaf blade form, 30x5 cm; green colour) was also deployed in each sampling site. The shape and colour of the modified leaf-blade screen were designed to mimic the shape and the movement of seagrass leaf in the water. As a note, all artificial substrates in this study were made of flexible fiberglass. After left in the water for 24 hours, the screen was then moved to the plastic sample jar (2 L) filled with ambient seawater. All sample were not preserved at this time.

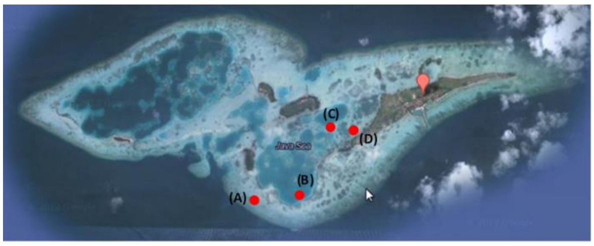

Figure 1. Aerial photograph of Pari Island (©Google Map Image). (A) seaweed, (B) sandy bottom, (C) coral reefs, (D) seagrass bed.

The natural sample that was collected consists of: (a) seaweed, (b) seagrass, (c) bottom sediment, and (d) coral rubble. Seaweed and seagrass samples were collected using snorkelling equipment. Small parts of seaweed's thallus or seagrass' leaf were cut by scissors or diving knife, then placed inside the plastic jar or plastic bag. Bottom sediment was collected using a small hoe, and only surface layer sediment was sampled in this research. Coral rubble was also collected with small hoe or hand-picked. All sample were not preserved at this time.

The water sample was also collected to find whether the targeted dinoflagellates were also present in the water column. The sample was taken using a modified 1 L Nansen bottle from 1 m depth. This water sample was not preserved at this time.

The method of benthic dinoflagellate extraction for all sample was done by following the protocol described in "YEOSU Project Information and Method: Use of an Artificial Substrate to Assess Field Abundance of Benthic HAB (BHAB) Dinoflagellate" (Kibler & Litaker, 2012), which later refined and described with more details in Moreira & Tester (2016).

The sample taken from the field was processed on the same day to avoid cell death or replication that might occur in the un-preserved sample. First, the plastic jar containing the sample was shaken vigorously (min. 30 times) to remove

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the dinoflagellate cells from seaweed, seagrass, sand, coral rubble, or screen. The sample then was poured through a stacked sieve (1000, 300, and 95 µm) placed on the top of 1 L graduated cylinder. After that, at least 50 ml of sieved sample was removed from the cylinder to be filtered using a circular nybolt mesh (mesh size 17 µm, diameter 47 mm). The volume of the filtered sample was different for each substrate type to avoid clogging of the filter.

The filtration was done using hand pump with pressure gauge, to avoid cell damages due to high pressure during the filtration process. The filter (nybolt mesh) then was transferred to 15 ml centrifuge tube filled with 10 ml of aqua bi-dest (double distilled pure water). This sample was preserved by adding 2 ml of Lugol's Iodine into the tube. In general, between 0.2 to 0.5 mL of Lugol's iodine solution is required per 100 mL water sample (Elder and Elbrachter, 2010). A higher concentration of Lugol's was added due to a possible higher concentration of living material or organic material in the filtered samples in this study. The wet weight of natural substrates (seagrass, seaweed, sand, and coral rubble) was measured using the electronic balance.

In this study, only *Gambierdiscus* spp., *Prorocentrum* spp., and *Ostreopsis* spp. cells were counted from the preserved sample. Identification and counting were done by removing a fraction of the sample using 1 ml stamp pipette to Sedgewick Rafter Counting Cell (SRCC). This fraction was then counted under 100 – 200X magnification using a Nikon Photophot DIC microscope. The number of cells counted in the sample was then converted to cells/100 cm^2 (artificial substrate), cells/gr wet weight (natural substrate), or cells/L (water sample). This conversion was conducted using formula as described in Kibler & Litaker (2012) and Moreira & Tester (2016).

The data was analysed using SPSS Statistic ver. 17 statistical software to: (1) determine the correlation value between the artificial substrate and adjacent natural substrate, and (2) determine its statistical significance. The data were further analysed with Bray-Curtis Cluster Analysis using BioDiversity Professional ver. 2 software (McAleece *et al*. 2007) to find the general pattern on habitat preference and effectiveness of using the artificial sample in this study.

Result and Discussion

From the analysis, *Prorocentrum* spp. was suggested as the most common benthic dinoflagellates in Pari Island (Fig. 2). This genus was observed in all type of habitat with relatively higher density compared to *Gambierdiscus* spp. and *Ostreopsis* spp. (Fig. 2). *Prorocentrum* spp. highest density was observed in the leaf-blade screen during December sampling, with a density of 729.6 cells/100 cm^2 (Fig. 2K). In contrast, the lowest density of benthic *Prorocentrum* spp. was observed in coral reefs in August and in the sandy bottom area in October, with density only 0.82 cells/g wet weight (Fig. 2). Planktonic form of *Prorocentrum* spp. also dominating the cell counts compared with the other two target species in this research. The highest abundance of planktonic *Prorocentrum* spp. was observed from the water sample in coral reefs area during August 2013 (Fig. 2D). Unfortunately, due to limitations of light microscopy method used to identify the dinoflagellate genus in this research, it was not possible to clarify whether the *Prorocentrum* spp. found in planktonic form is the same species with its benthic form or vice versa. As a note, only the planktonic species of *Prorocentrum* that are capable on forming the 'red tide' or ocean discoloration effect during its bloom, while the benthic species mostly associated with seafood poisoning due to the capability of many benthic *Prorocentrum* species to produce toxins (Faust *et al*., 1999). Even so, there is growing evidence that the benthic *Prorocentrum* is an important component in the benthic communities in many benthic ecosystems (Faust *et al*., 1999). Furthermore, the dominance of *Prorocentrum* in the benthic community is considered as a specific signature of benthic assemblages in tropical regions (Almazan-Becerril *et al*., 2015).

It was interesting to find a contradicting trend in the population of planktonic and benthic species of *Prorocentrum* in the coral reefs of this research. However, as described by Faust *et al*. (1999), some species of benthic and planktonic *Prorocentrum*, such as *P. concavum*, *P. emarginatum*, and *P. lima* were commonly found in the coral reef ecosystems of the subtropics and tropics. On the other hand, co-occurrence of *Prorocentrum* spp. with other benthic dinoflagellates, mainly *Gambierdiscus* toxicus, *Ostreopsis* spp., and *Coolia monotis*, was often observed in coral reefs ecosystem (Glibert *et al*., 2012). Thus, the occurrence of *Prorocentrum* in the coral reefs habitat in this study was not unusual phenomena. Unfortunately, due to lack of supporting data, we cannot confidently explain the reason behind the contradicting trend found in the population of *Prorocentrum* spp. of this study. Some specific environmental condition at the end of the dry season in August, such as temperature or nutrient concentration in the water, might cause such an unusual trend.

Unlike *Prorocentrum* spp., both *Gambierdiscus* spp. and *Ostreopsis* spp. were observed in lower cell density in most type of habitats and substrates (Fig. 2). However, a very high density of *Ostreopsis* spp. was observed in the leaf-blade screen sample from seaweed bed during October 2013 sampling (Fig. 2G). The cell density of *Ostreopsis* spp. at that time was

1,386.67 cells/100 cm^2 (Fig. 2G). It is important to note that this phenomenon was not observed in the screen sample (Fig. 2F), although both screen and leaf-blade screen were made from the same artificial substrates. Coincidentally, Skinner *et al*. (2011) reported a peak or bloom in Ostreopsis was occurred during October in the macroalgal bed in Bali that was dominated by *Sargassum*. In that study, the bloom of *Ostreopsis* was triggered by episodic nutrient load that enrich the water column. However, due to lack of nutrient data, we cannot confirm whether the high density of *Ostreopsis* during October in this study was related to increased nutrient concentration in the seaweed (macroalgae) bed of Pari Island.

Compared with *Ostreopsis* spp., *Gambierdiscus* spp*.* density was lower, and no high-density event observed in this study (Fig. 2). However, high density of planktonic *Gambierdiscus* spp*.* was recorded during December sampling in water column sample taken from seagrass area (Fig. 2L). Interestingly, study by Razi *et al*. (2014) in Harapan Island reported a high association of *Gambierdiscus* species with macroalgal, which indicated by its higher cell density in that habitat or substrate.

By looking at the comparison between the result from natural substrates (Fig. 2A, 2E, 2I) and artificial substrates (Fig. 2B, 2F, 2J, 2C, 2G, 2K), there almost no similarities found in the trend. In general, the density of the target genus in the natural substrate of seagrass and seaweed was higher compared to another type of substrate (Fig. 2A, 2E, 2I). However, a similar pattern was only observed in screen (artificial substrate) that was deployed in December 2013 (Fig. 2I). In contrast, the results showed by the usage of the leaf-blade screen (Fig. 2C, 2G, 2K) showed an almost completely different trend compared with the standardized screen and natural substrate. The cause for differences in result from the standardized screen and leaf-blade screen was not clear.

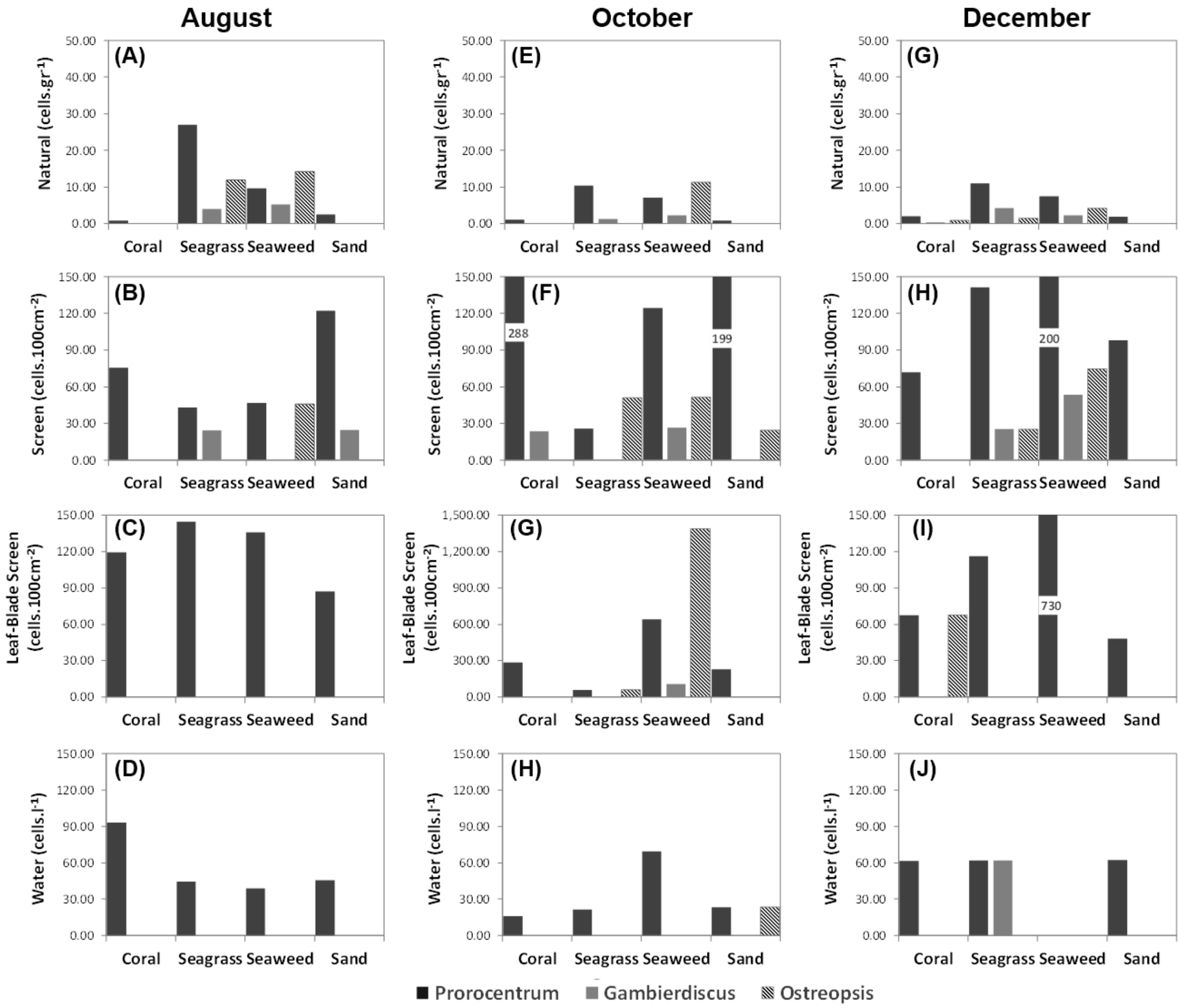

Figure 2. Result of benthic dinoflagellate sampling in August, October, and December 2013 from the natural substrate, the artificial substrate (screen), leaf-blade screen, and water column.

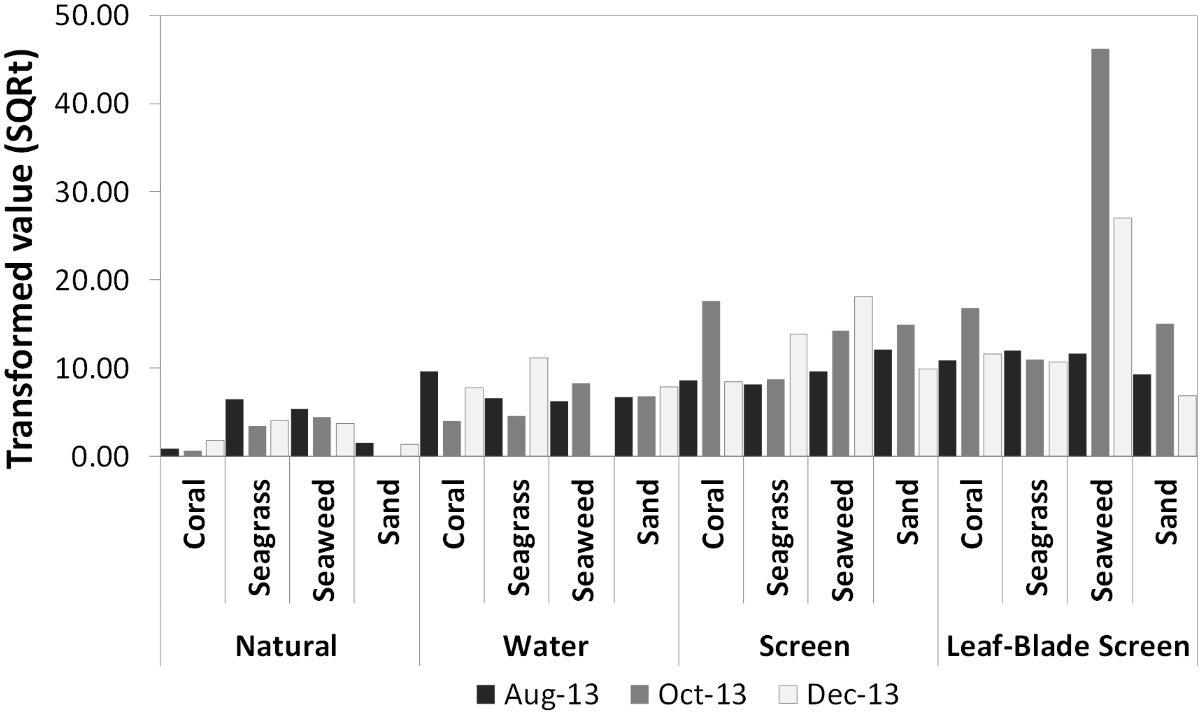

Figure 3. Transformed value (square root) of the cell count from all target dinoflagellate genus in the natural substrate, water column, screen, and leaf-blade screen.

By using square root transformation, all data were combined into one graphic to find a general trend in the data (Fig. 3). The results showed that benthic dinoflagellates in this study seem to correlate with seagrass and seaweed habitat. The total cell density of those two habitats was much higher compared to coral reef and sandy bottom (Fig. 3). *Prorocentrum* spp., *Gambierdiscus* spp., and *Ostreopsis* spp. were generally was found at a higher density in the seagrass and seaweed habitat of the Pari Island (Fig. 2). That trend was expected as the three target genus of this research were commonly found in high density at seagrass and seaweed habitats (Delgado *et al*., 2006). As a note, a study done by Aggraeni *et al*. (2013) on epiphytic dinoflagellates in seagrass (*Enhalus acoroides*) on the reef flat of Pari Island reporting that the density of benthic dinoflagellate was between 22 to 577 cells/cm^2, which generally higher than what was found in this study.

Bray-Curtis cluster analysis suggested that sampling using artificial substrate might not represent the natural composition of *Gambierdiscus* spp., *Prorocentrum* spp., and *Ostreopsis* spp. population in Pari Island (Fig. 4). The result showed that most samples from natural substrates are grouped into one cluster group, while samples from the screen, leaf-blade screen, and water column were separated into several cluster group (Fig. 4).

One important trend showed by the clustering analysis was that the result from the leaf-blade screen often resembles the community of target dinoflagellates in the water column (Fig. 4). Most of the samples from the leaf-blade screen (LS) was in the same cluster group with samples from the water column (W) (Fig. 4). That might indicate that our modified artificial substrate captured more planktonic species than the benthic species of *Gambierdiscus*, *Prorocentrum*, and *Ostreopsis*. Combination of both planktonic species and benthic species might be the reason why the density in leaf-blade screen (Fig. 2C, 2G, 2K) was much higher than the standardized one (Fig. 2B, 2F, 2J). However, it seems that the standardized screen was more effective to collect all three-target genus in this study, compared with the leaf-blade screen (Fig. 2).

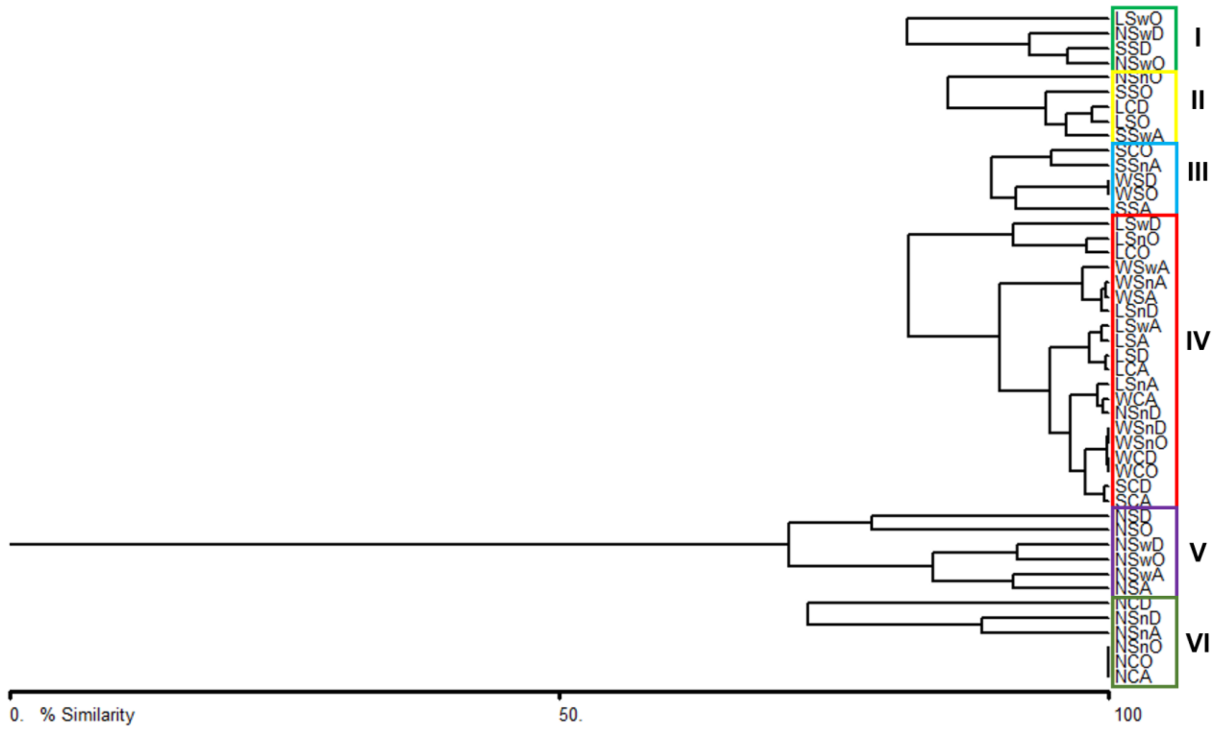

Figure 4. Bray-Curtis clustering analysis (simple average link) for each type of habitat and sample type in all sampling period. Six groups were formed based on 70% similarity in the genus composition of the target benthic dinoflagellate. Note: First Letter: N=natural substrate, L=leaf-blade screen, S=screen, W=water column; Second Letter: Sw=seaweed, S=seagrass, Sn=sand/bottom sediment, C=coral reef/rubble; Third Letter: A=August, O=October, D=December.

Table 1. Pearson correlation matrix showed weak ($r < 0.7$) and non-significant relationship ($p > 0.05$) between natural substrate and two types of artificial substrates.

	Natural	Screen	LB Screen
Natural	1.00		
Screen	-0.22	1.00	
LB Screen	0.20	0.38	1.00

From the result of simple Pearson correlation, it was known that there was no significant correlation between the artificial substrate and adjacent natural substrate in this study ($r < 0.7$; $p > 0.05$) (Table 1). The simple regression analysis also showed a weak negative relationship between the natural substrate and standardized screen (Fig. 5A). In contrast, the natural substrate seems to have a weak positive relationship between the natural substrate and leaf-blade screen (Fig. 5B). Those results indicating that the benthic dinoflagellate community in both artificial substrates (standardized screen and leaf-blade screen) were insufficient to represent the benthic dinoflagellate community in the natural substrates of the Pari Island.

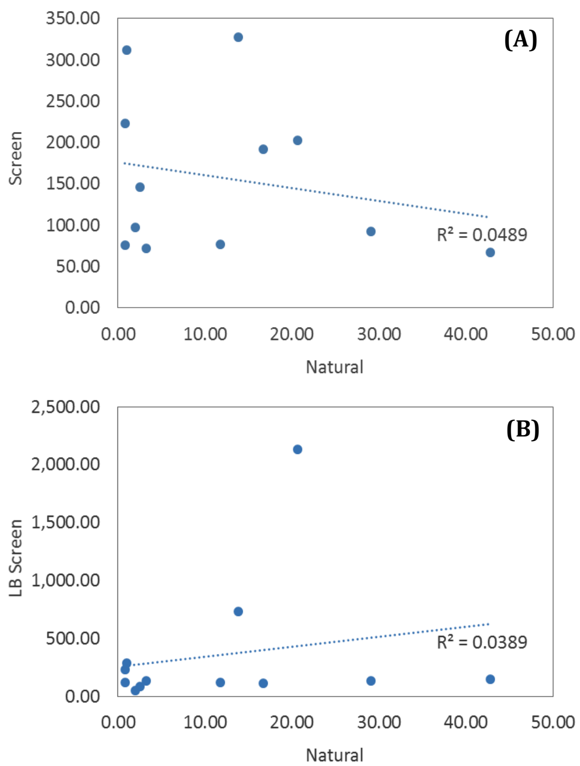

Figure 5. Regression graphic between natural substrates and: (A) standardized screen, (B) leaf-blade screen.

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Table 2. ANOVA table of regression between the natural substrate and standardized screen.

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4752.115	4752.115	0.514502	0.489612
Residual	10	92363.48	9236.348		
Total	11	97115.6	1.00		

Table 3. ANOVA table of regression between the natural substrate and leaf-blade screen

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	147837.6	147837.6	0.405012	0.538805
Residual	1	365020.1	365020.1		
Total	1	379803.9	1.00		

This result rejected our initial hypothesis that the density of benthic dinoflagellates in the artificial substrate is significantly correlated with the cell density of adjacent natural substrates. The results here were also different from what was reported by Tester *et al*. (2014). However, it was not clear why the density and composition of benthic dinoflagellates in the artificial substrates did not represent the condition in adjacent natural substrates or contradict to what was reported by other researchers. Also, it was not clear whether the shape and color of the artificial substrates, as showed by our leaf-blade screen, could affect the substrate preference of benthic dinoflagellates in Pari Island. However, we did have several thoughts on this:

(1) Important biological interactions, such as competition, predation, and allelopathy, might be missing in the benthic community of artificial substrate. Those factors might affect cell density and species composition. For example, a study in the coral reefs and seagrass bed in southern Belize suggested that low density of toxic benthic dinoflagellate in that ecosystems were caused by tannins produced by seagrass (*Thalassia testudinum*) that act as algaecides and preventing the growth of dinoflagellates in the ecosystem (Gomez & Pineda, 2019). On the other hand, the abundance of *Gambierdiscus* in the coral reefs area was heavily regulated by the grazing of small herbivorous fish (Loeffer *et al*., 2015). On the other hand, the target species of this study, *Gambierdiscus* spp., *Ostreopsis* spp., *Prorocentrum* spp., were known to have a high preference for macroalgae, and generally associated with green, brown, and red macroalgae (Delgado *et al*., 2006). Association between benthic microalgae species on a specific habitat, such as on *Amphiroa* sp. (Rhodophyta), were also suggested as a factor that drives the complexity of the benthic community on that habitat (Irola-Sansorest *et al*., 2018). However, a more detailed study on the species composition of all benthic organisms found in various type of substrates is required to prove this hypothesis. Analysis of the correlation or association with various biotic and abiotic factors are also required to explain some anomalies found in this study.

(2) Twenty-four hours deployment might insufficient for the benthic community in the artificial substrate to stabilize in Pari Island ecosystems. Thus the dominant species was the one which able to colonize the new substrate rapidly. However, it was known that *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum* could occupy a newly available substrate within 6-12 h and reached a population equilibrium within 24 h (Tester *et al*., 2014). Furthermore, Tester *et al*. (2014) also demonstrate that epiphytic diatoms and debris would replace the benthic dinoflagellates community at 48 h. It was unknown whether the same trend will occur if the artificial screen were deployed longer in various coastal ecosystems around Pari Island. As a note, a study by Caire *et al*. (1985) in Parsons *et al*. (2011) suggested that without a presence of suitable host species, *Gambierdiscus* takes time to colonize a new artificial substrate, which could take up to 4 months before it reached a stable population. Therefore, the study of the standardized artificial substrate (screen) using variation in deployment time might be valuable to determine the optimum time needed to obtain the best sampling result to describe the benthic dinoflagellate community in Pari Island.

(3) Despite a report by Tester *et al*. (2014) which stated that screen size did not affect the density of benthic dinoflagellate, we hypothesized that shape and color of the artificial substrate might affect the preference of some benthic dinoflagellates. As shown by our green colored leaf-blade screen, which able to capture the higher density of planktonic and benthic target species (*Prorocentrum* spp.) compared to the black colored standardized screen. The leaf-blade screen was designed to mimic the shape, color, and underwater movement of seagrass leaf, which was thought to attract more cells compared to the standardized screen. However, more detailed research needs to be conducted to test this hypothesis.

Conclusion

From this study, it was concluded that the result of sampling using artificial substrate might not represent the natural composition and density of target benthic dinoflagellates in Pari Island. The reason for that results was unknown, which signifying the requirement for further study. However, a general trend was found that *Gambierdiscus* spp., *Prorocentrum* spp., and *Ostreopsis* spp. were found in higher density around seagrass and seaweed habitat.

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