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The diversity of potentially toxic benthic dinoflagellates in Indonesian waters: Research study within 2010 - 2015

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ABSTRACT

Ciguatera Fish Poisoning (CFP) is a syndrome caused by ciguatoxin-producing benthic dinoflagellate, which are typically attached to macroalgae. The toxin is responsible for the human poisoning symptom observed after the consumption of contaminated reef fish. Research on the composition and abundance of benthic dinoflagellate on macroalgae had been conducted within the 2010 - 2015 at several coral reef areas in Indonesian waters, such as Weh Island - Aceh, Belitung Island, Lampung Bay, Seribu Islands, and Lombok Island. This study reviewed all the references concern on benthic dinoflagellate researches in Indonesia, with aims to raise awareness on the presence of potentially toxic benthic dinoflagellates in Indonesian waters, also to create an inventory of the species discovered. The study revealed four of the six genera which are potentially toxic, namely *Amphidinium*, *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum*. The *Prorocentrum* cells were identified in a larger number than any other genera in all the sampling locations. The relatively high number of benthic dinoflagellates were observed in Lampung Bay and Seribu Islands, which might be due to the high level of human activities. These findings are essential to compile a database on the CFP-causing species and to monitor the affected areas, specifically in highly populated locations or tourist sites.

1. Introduction

The CFP disease is a poisoning syndrome experienced by humans and other mammals after consuming marine fish associated with coral reefs (De Sylva, 1994; Lehane & Lewis, 2000). This condition is caused by benthic dinoflagellate, which produce toxic substances known as ciguatoxin. Fishes eating macroalgae, on which these dinoflagellate are attached, would become toxic. Subsequently, this toxin is bio-transformed through the food chain, with the largest predatory fish being the most toxic (Legrand, 1998). CFP is characterized by several gastrointestinal symptoms, including diarrhea, nausea, vomiting, and abdominal pains, as well as neurological symptoms, such as hot-cold inversion, joint and muscular aches, tingling sensation, numbness on lips and tongue, skin itching, and hypotension (De Sylva, 1994; Lehane & Lewis, 2000).

Ciguatoxin is mainly produced by *Gambierdiscus toxicus*. However, other species, such as *Ostreopsis ovata*, *O. siamensis*, *Prorocentrum lima*, *P. concavum*, *P. mexicanum (rhathymum)*, and *Amphidinium carterae* were only associated with *Gambierdiscus toxicus*, and can collectively produce CFP symptoms (Burkholder, 1998; Lehane and Lewis, 2000). Ciguatera is a symptom caused by the combination of several metabolites produced by numerous benthic dinoflagellates (Juranovic & Park, 1991). Besides maitotoxin, the Okadaic Acid (OA) which is produced by several species of benthic *Prorocentrum*, is also found in fish tissues during ciguatera poisoning outbreak (Gamboa et al., 1990; Thoha et al.,2019).

Indonesia is one of the several countries that have reported harmful algal bloom cases, though the majority were caused by planktonic species, such as *Margalefidinium* (*Cochlodinium*) and *Pyrodinium* (Thoha et al., 2019; Widiarti, 2004; Widiarti et all.,2000), there are no records of cases due to benthic species. However, the CFP-causing genera were discovered in several locations in the Indonesian waters, hence, the possibility of an outbreak is high. Benthic dinoflagellates, such as *Gambierdiscus*, *Prorocentrum*, and *Ostreopsis* were observed in Bali waters (Kuta, Sanur, and Nusa Dua) and Lombok (Gili Trawangan) (Skinner et al., 2011), west coast of South Sumatera and Bintan Island - Riau Islands (Thamrin, 2014), and the coastal waters of Padang - West Sumatera (Dwivayana et al., 2015; Eboni et al., 2015; Oktavian et al., 2018; Seygita et al., 2015). Therefore, research on the presence of benthic dinoflagellates in Indonesian waters is undoubtedly essential to collate a database on the CFP-causing species and to monitor the areas where they are located.

This study aims to raise awareness on the presence of potentially toxic benthic dinoflagellates and to create an inventory of the species discovered within 2010 and 2015 in various Indonesian waters (Razi et al., 2014; Widiarti, 2010; Widiarti & Adi, 2015; Widiarti & Pudjiarto, 2015b; Widiarti,

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Pudjiarto, & Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016). Also, the data obtained could be used for further research, specifically in phylogeny, to observe the distribution and variation of benthic dinoflagellate in Indonesian waters.

2. Materials and methods

Data used for this study were collected from research in several locations in the Indonesian waters within the 2010 - 2015 (Razi et al., 2014; Widiarti, 2010; Widiarti & Adi, 2015; Widiarti & Pudjiarto, 2015b; Widiarti, Pudjiarto, & Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016) which are Belitung Island waters in Bangka Belitung Province, Lombok Island in West Nusa Tenggara Province, Seribu Islands in Jakarta Province, Lampung Bay in Lampung Province, and Weh Island in Aceh Province (Table 1) (Fig. 1).

The samples were obtained from reef areas on small islands, which were already used for domestic, tourism, and fishery activities. The disturbance of reefs originated from those anthropogenic activities could damage coral reef areas and provide a new surface for various macroalgae growth, where the toxic dinoflagellates are more likely to attach (De Sylva, 1994; Hallegraeff, 2010; Lehane & Lewis, 2000). Belitung Island, Lombok Island, and Seribu Islands are small islands which already known as famous tourist sites, whereas Lampung Bay waters had already long used for aquaculture activities. Also, the Weh Island in Aceh is a well-known tourist site, though the disturbance of its coral reef areas was majorly due to the 2004 tsunami wave.

The macroalgae thallus of *Padina* and *Sargassum* were collected from the shallow reef flat at each location and placed in 250 ml wide-mouth plastic bottles filled with ambient seawater

Table 1. Sampling location and time within the 2010 - 2015 period

No.	Sampling location	Sampling time	Reference
1.	Belitung waters : Buyut Island, Keran Island, and Kelayang Cape	April 5 - 8th, 2010	(Widiarti, 2010)
2.	Seribu Islands waters : Harapan Island Tidung Island	 March 5 - 6th, 2013 September 19th, 2014	 (Razi et al., 2014; Widiarti and Pudjiarto, 2015a)
3.	North Lombok waters : Gili Meno and Gili Air	December 3rd, 2014	(Widiarti, Pudjiarto, and Pratama, 2016)
4.	Lampung Bay waters : Pahawang Besar Island Kelagian Kecil Island	 Augustus 5th, 2015 Augustus 6th, 2015	 (Widiarti and Adi, 2015)
5.	Weh Island - Aceh waters : Rubiah Island	December 12th, 2015	(Widiarti, Pudjiarto, Fathoniah, et al., 2016)

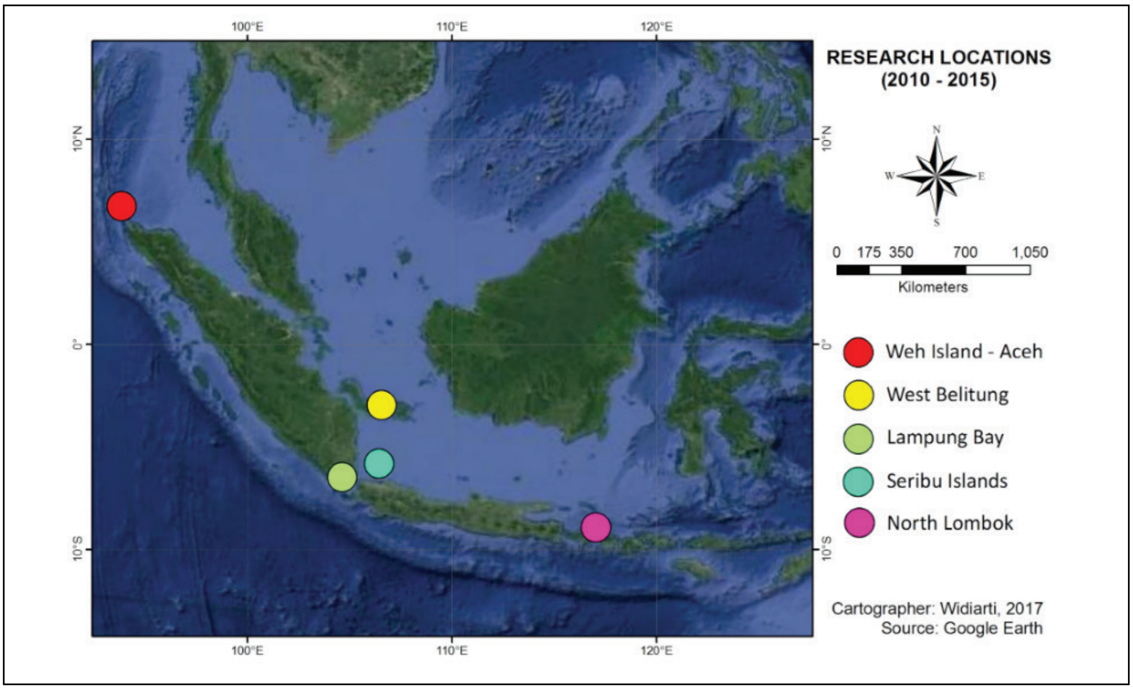


Fig. 1. Sampling locations in Indonesian waters within the 2010 - 2015 period.

(McCaffrey et al., 1990). Sampling process was conducted underwater (Jauzein et al., 2018; Tester et al., 2014), to prevent the sample from air and sunlight exposures. Subsequently, the contained macroalgae were vigorously shaken for 1 minute to detach the possible dinoflagellate (Tawong et al., 2015), and the seawater was filtered through a series of sieves (125 µm and 20 µm). The top sieve (125 µm) was used to filter detritus or sand (Heil et al., 1998), which was then retained in the 20 µm sieve and diluted in 100 ml of seawater as well as preserved with formaldehyde at 4% final concentration. In addition, the volume of macroalgae was measured by placing the thallus inside a measuring cup with a predetermined capacity, and observing the level of water displacement. The volume of water displaced is equal to the volume of macroalgae.

Data on the nutrient parameters were also collected. During sampling, ambient seawater was taken and placed inside a wide-mouth plastic bottle, covered with aluminum foil, and stored in a cooler box. Subsequently, the sample was analyzed further in the laboratory using spectrophotometer based on the colorimetric method (Sidabutar et al., 2016).

In the laboratory, material suspended were collected using a Pasteur pipette, placed in a Sedgewick rafter cell with two repetitions, and then observed under a Light Microscope with 10x10 magnifications for cell enumeration. The dinoflagellate cells were expressed as cells/L of macroalgae and the microphotographs were obtained by isolating the sample with a micropipette. Subsequently, the cells were dropped onto an object-glass under a microscope camera (Olympus IX73 Inverted Microscope). Data analysis include tabulation of discovered taxon to showed genus composition, and the cells abundance at each locations was presented by bar chart.

3. Results

There were six genera of benthic dinoflagellate that were discovered in this study (Razi et al., 2014; Widiarti, 2010; Widiarti & Adi, 2015; Widiarti & Pudjiarto, 2015b; Widiarti, Pudjiarto, & Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016) (Table 2). Some of these organisms, such as *Amphidinium*, *Gambierdiscus* (Fig. 2, Pl. 1.), *Ostreopsis* (Fig. 2,

Table 2. Benthic dinoflagellate species found in five sampling locations

No.	Species	West Belitung (2010)	Seribu Islands (2014 & 2015)	North Lombok (2014)	Lampung Bay (2015)	Weh Island - Aceh (2015)
1	*Amphidinium*	-	+	-	+	+
2	*Gambierdiscus*	+	+	+	+	-
3	*Ostreopsis*	+	+	+	+	+
4	*Prorocentrum*	+	+	+	+	+
5	Sinophysis	+	+	+	+	+
6	*Thecadinium*	-	-	-	-	+
	Reference	(Widiarti, 2010)	(Razi et al., 2014; Widiarti and Pudjiarto, 2015a)	(Widiarti, Pudjiarto, and Pratama, 2016)	(Widiarti and Adi, 2015)	(Widiarti, pudjiarto, fathoniah, et al., 2016)

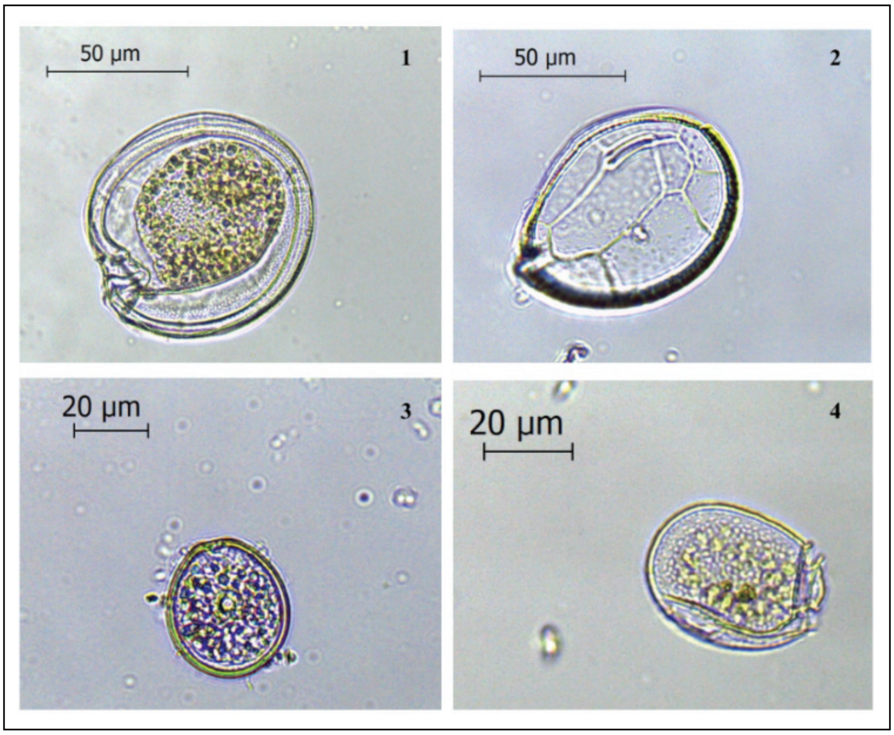


Figure 2. Potentially toxic benthic dinoflagellates found: 1. *Gambierdiscus*, 2. *Ostreopsis*, 3. *Prorocentrum*, 4. *Sinophysis*.

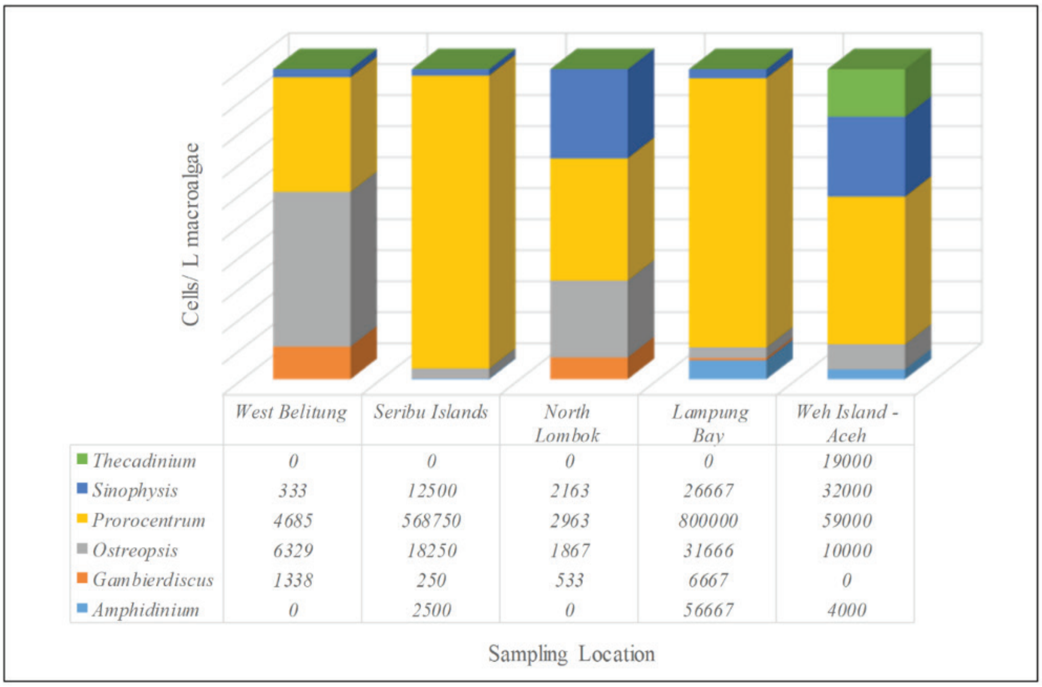
Pl. 2), and *Prorocentrum* (Fig. 2, Pl. 3) are potentially toxic and might induce CFP. Several species of *Gambierdiscus* are among the most toxic benthic dinoflagellates due to the production of ciguatoxin (Lehane & Lewis, 2000). *Thecadinium* and *Sinophysis* (Fig. 2, Pl. 4) are nontoxic genera, whilst the potential toxicity of *Sinophysis* is still unknown (García-Portela et al., 2017).

Prorocentrum were found in greater quantity than other genera (1,435,398 cells/L macroalgae) in every sample location, including Belitung, Seribu Islands, Lombok, Lampung Bay, and Weh Island waters (Razi et al., 2014; Widiarti, 2010; Widiarti and Adi, 2015; Widiarti & Pudjiarto, 2015b; Widiarti, Pudjiarto, & Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016) (Fig. 3). This genus was dominant in Lampung Bay waters and Seribu Islands with 800,000 cells/L and 568,750 cells/L macroalgae, respectively. Furthermore, *Amphidinium* (56,667 cells/L macroalgae), *Ostreopsis* (31,666 cells/L macroalgae), *Sinophysis* (26,667 cells/L macroalgae), and *Gambierdiscus* (6,667 cells/L macroalgae) were all observed to be abundant in Lampung Bay waters (Fig. 3). *Thecadinium*, which is a newly recorded species, was also discovered in Weh Island waters, with 19,000 cells/L macroalgae (Fig. 3).

Nutrient analysis showed that nitrate and phosphate concentrations ranged from 0.312 - 0.588 ppm and 0.139 - 0.180 ppm, respectively, in Lampung Bay (Widiarti & Adi, 2015), whereas nitrate concentration ranged from 0.009 - 0.17 mg/L and phosphate concentration ranged from 0.05 - 1.6 mg/L in Seribu Islands (Razi et al., 2014) (Table 3).

4. Discussion

The *Sargassum* and *Padina* were the only macroalgae collected in this research. Reports on the preference of ciguatera dinoflagellate on macroalgae substrate at Great Barrier Reef Australia showed that these cells were found abundantly on brown algae species (Heil et al., 1998). These benthic dinoflagellates are more likely to attach on a folious and filamentous macroalga with structural interstices, such as *Padina* and *Sargassum*. In 1998, research on substrate specificity for benthic dinoflagellate was conducted in West Penjaliran Island, Seribu Islands. This inquiry revealed that *Sargassum* and *Padina* were the most favorable surface for benthic dinoflagellate to attach (Widiarti, 2002).



Sources: Razi et al., 2014; Widiarti, 2010; Widiarti and Adi, 2015; Widiarti and Pudjiarto, 2015b; Widiarti, Pudjiarto, and Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016.

Fig. 3. The abundance of benthic dinoflagellate (cells/L macroalgae) in five sampling locations.

Table 3. Nutrient concentration in Lampung Bay and Seribu Islands

Location	Station	Nitrate (ppm)	Phosphate (ppm)	Location	Station	Nitrate (mg/L)	Phosphate (mg/L)
Pahawang Besar Island and Kelagian Kecil Island, Lampung Bay	**1.1**	**0.312**	**0.139**	**Harapan Island,** Seribu Islands	**North**	**0.017**	**0.05**
	1.2	**0.428**	**0.152**		**East**	**0.17**	**0.57**
	1.3	**0.465**	**0.163**		**South**	**0.08**	**0.12**
	2.1	**0.344**	**0.146**		**West**	**0.009**	**1.6**
	2.2	**0.389**	**0.155**				
	2.3	**0.588**	**0.180**				
Source	(**Widiarti and** Adi, 2015)			**Source**	(**Razi et** al., 2014)		

Prorocentrum cells were seen in greater abundance than other genera in every sampling location (Razi et al., 2014; Widiarti, 2010; Widiarti & Adi, 2015; Widiarti & Pudjiarto, 2015b; Widiarti, Pudjiarto, & Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016). *Prorocentrum* is known as dinoflagellate with high adaptation levels to strong water turbulence or low nutrient condition (Lee et al., 2020). Also, this genus is widely distributed and frequently found in planktonic and benthic habitats, in tropical and subtropical marine areas, and recently in temperate waters (Hoppenrath et al., 2013; Laza-Martinez et al., 2011). This genus may also be seen on almost every type of substrates, such as on macroalgae surfaces, floating detritus, benthic debris, corals, and sand (Faust, 1990, 1991; Hoppenrath et al., 2013, 2014).

The *Ostreopsis* cells were also observed in high number in every sampling location (Razi et al., 2014; Widiarti, 2010; Widiarti and Adi, 2015; Widiarti and Pudjiarto, 2015b; Widiarti, Pudjiarto, and Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016) (Fig. 3). Although this genus is more prevalent than *Gambierdiscus*, these organisms co-exist in several benthic substrates. Research on substrate preferences of harmful benthic dinoflagellates showed that *Gambierdiscus* and *Ostreopsis* were observed preferentially on turf algae, hard coral, and fleshy macroalgae (Lee et al., 2020). This similar preference suggested that *Ostreopsis* may serve as a good indicator for the presence of *Gambierdiscus* (Lee et al., 2020).

Gambierdiscus cells are absent in Weh Island waters and usually seen in lower abundance compared to *Ostreopsis* in every sampling location (Razi et al., 2014; Widiarti, 2010; Widiarti & Adi 2015; Widiarti and Pudjiarto, 2015b; Widiarti, Pudjiarto, & Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016) (Fig. 3). *Gambierdiscus* were either absent or present at very low concentrations in several microhabitats (Lee et al., 2020). However, macroalgae seemed to be an unlikely microhabitat for this genus.

Sinophysis is another benthic dinoflagellate, with high densities observed in every sampling location (Razi et al., 2014; Widiarti, 2010; Widiarti & Adi, 2015; Widiarti & Pudjiarto, 2015b; Widiarti, Pudjiarto, & Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016) (Fig. 3) and is associated with detritus in mangrove areas (Faust, 1993). Most of the sampling locations still preserve the mangrove ecosystem either where the mangrove was planted (such as in Harapan Island and Tidung Island - Seribu Islands), or it still exists in its natural state (such as in West Belitung, North Lombok, Pahawang Besar and Kelagian Kecil Islands - Lampung Bay, and Rubiah Island - Weh Island). This may explain why *Sinophysis* was also found in high numbers when compared to other genera. Moreover, this organism appears to be capable of attaching to various substrate types, such as detritus or sand (Hoppenrath et al., 2014), whereas in this research was observed on macroalgae. According to a Harapan - Seribu Islands report (Razi et al., 2014), *Sinophysis* were also found attached to seagrass and dead corals.

Thecadinium, a non-toxic genus not previously observed in other research, was seen in the sampling location (Widiarti, Pudjiarto, Fathoniah, et al., 2016) (Table 2) (Fig. 3) and was recently recorded in Weh Island waters. This organism is a dinoflagellate living in between sand particles (Hoppenrath et al., 2014). It is assumed that the cells were observed on macroalgae due to the process of water mixing, so that the cells from sand substrates were carried up into the water column and subsequently attached to the macroalgae. The massive 2004 tsunami wave also probably disrupted sands and sediments substrate in these areas, creating a change in the composition of the benthic assemblages in sediment.

Amphidinium cells were observed in low quantity when compared to other genera (Razi et al., 2014; Widiarti, 2010; Widiarti & Adi, 2015; Widiarti & Pudjiarto, 2015b; Widiarti, Pudjiarto, & Pratama, 2016; Widiarti, Pudjiarto, Fathoniah, et al., 2016) (Fig. 3). *Amphidium* is usually detected in low amounts and represents a minor component of benthic dinoflagellate assemblages (Lee et al., 2020).

Research on benthic dinoflagellate in the western part of the Indonesian waters, specifically Padang (West Sumatera) and Bintan Island (Riau Islands) was conducted between 2014 and 2015 (Dwivayana et al., 2015; Seygita et al., 2015; Thamrin, 2014). Also, three genera, which attach to macroalgae *Sargassum* were detected, namely *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum*. However, this research did not provide data on other benthic dinoflagellates, such as *Amphidinium*, *Sinophysis,* or *Thecadinium*. Although the two latter genera were not categorized as harmful due to the absence of toxins, they were nonetheless crucial in benthic communities, owing to their ecologically close association to other toxic benthic dinoflagellates (Irola-Sansores et al., 2018).

The relatively high number of benthic dinoflagellates observed in several locations, including the toxic ones, might be due to the high level of human activities (Furuya et al., 2010; Paerl, 1997). A majority of the sampling locations, such as in Lampung Bay and Seribu Islands had become resident, aquaculture, and tourist areas. Consequently, coral reef ecosystem degradation might occur due to tourist activities, including snorkeling, boat anchoring, and diving, which involve stepping on corals. This occurrence may also provide a new substrate area for macroalgae, which is ideal substrate for benthic dinoflagellate to attach (De Sylva, 1994; Hallegraeff, 2010; Lehane & Lewis, 2000).

Several Indonesian waters were recorded to have high levels of nutrients, with nitrate and phosphate ranging between 0.312 - 0.344 ppm and 0.139 - 0.146 ppm, respectively, in Lampung Bay. Meanwhile, Seribu Islands waters contained 0.009 - 0.17 mg/L nitrate and 0.05 - 1.6 mg/L phosphate (Razi et al., 2014; Widiarti & Adi, 2015). These nutrient levels were above the water quality standard from the Ministry of Forestry and Environment for marine biota, which was 0.002 mg/L and 0.015 mg/L for nitrate and phosphate (Ministry of Forestry and Environment, 2004). The high level of nutrients in these waters could result in eutrophication, thereby enhancing the proliferation of dinoflagellate. Blooming of dinoflagellate was often reported in Lampung Bay, such as *Pyrodinium bahamense*, which bloomed in 1998 and reached 2.4×10^9 cells/liter, limiting the water transparency to a depth of 2 meters (Widiarti, 2004). *Cochlodinium* also bloomed in 2013 and resulted in the sudden mass death of 360,000 caged fishes at Ringgung Beach (Irawan et al., 2017). In addition, mass fish death occurred in Jakarta Bay after *Prorocentrum micans*, also a dinoflagellate, bloomed in Ancol waters at 2004 (Thoha et al., 2007).

The benthic dinoflagellate community, toxic or nontoxic, is a part of the coral reef ecosystem. However, an increase in cell number or blooming due to the deterioration of the coral reef or water quality condition would negatively impact human health or economic activities in the surrounding area. Therefore, routine monitoring should be designed and conducted in locations where toxic benthic dinoflagellates are observed. This process can be performed by collecting macroalgae, such as *Sargassum* or *Padina,* and assessing them for the presence of attached toxic benthic dinoflagellates. Further monitoring for the presence of ciguatoxin in surrounding reef fish should also be conducted, specifically in species intensively fished by the locals and frequently consumed.

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