

Analysis of Benthic Dinoflagellate *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* Density in West Coast of Sumatera Island and Bintan Island Coast in Riau Archipelago, Indonesia

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ABSTRACT

Field experiment was conducted in Pulau Pesumpahan and Pulau Pisang Coast of West Coast of Sumatera Island and in Malang Rapat and Teluk Bakau Coast of Bintan Island coast, Riau Archipelago to examine the density of benthic dinoflagellate *Gambierdiscus*, *Ostreopsis* and *Prorocentrum*. The density was observed by field experiment using plastic screen. Four field stations (P. Pasumpahan and P. Pisang in the west of Sumatera Island waters, and Malang Rapat and T. Bakau in the east site of Sumatera island waters) were established. The results showed that the role of nutrients to increase number of dinoflagellates, including *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* is unclear. Increase of dinoflagellate including benthic dinoflagellate *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* may be indirectly as an effect of degradation of coral reefs, and increase of HAB including benthic dinoflagellate may be controlled by macroalgae and seagrasses.

Keywords: benthic dinoflagellate, density, gambierdiscus, ostreopsis, prorocentrum

INTRODUCTION

Many studies have been conducted on dinoflagellates of *Gambierdiscus* (Adachi and Fukuyo, 1979; Asuncion et al., 1994; Babinchak et al., 1996; Aligizaki and Nikolaidis, 2008; Parsons et al., 2012), *Ostreopsis* (i.e. Aligizaki and Nikolaidis, 2006; Adachi, 2008; Aligizaki et al., 2008; Abboud-Abi Saab et al., 2011, Parsons et al., 2012) and *Prorocentrum* (i.e. Barbier et al., 1999; Levasseur et al., 2003; Yoo, J., 2004; Aligizaki et al., 2009; Aligizaki and Nikolaidis, 2006). More recent studies focused on the ecology and toxicology of *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* dinoflagellates, with the most effort were put on the study of *Gambierdiscus* due to its role in ciguatera. In ciguatera endemic regions, the benthic dinoflagellate *Gambierdiscus toxicus* along with other dinoflagellates belonging to the genera *Ostreopsis*, *Prorocentrum* and *Amphidinium* are presumed to be the elaborators of ciguatoxins in fishes (Adachi and Fukuyo, 1979; Tindall et al., 1984; Holmes et al., 1990; Lewis and Holmes, 1993; 1994; Taylor et al., 1995).

Toxigenic microalgae of species from the genera *Gambierdiscus* and *Ostreopsis* present the biggest threats to human and environmental health (Parsons et al., 2012). Although many studies were conducted on three genera of *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* dinoflagellates, however, no information of those benthic dinoflagellates from both west coast and east coast (Riau Archipelago) Sumatera Island of Indonesia.

The objective of this study is to find out the different density of the benthic Dinoflagellate *Gambierdiscus*, *Prorocentrum* and *Ostreopsis* between west coast and east coast of Sumatera Island and to analyze condition of coral reef and occurrence of macro-algae and seagrasses to density of benthic dinoflagellate *Gambierdiscus*, *Prorocentrum* and *Ostreopsis*.

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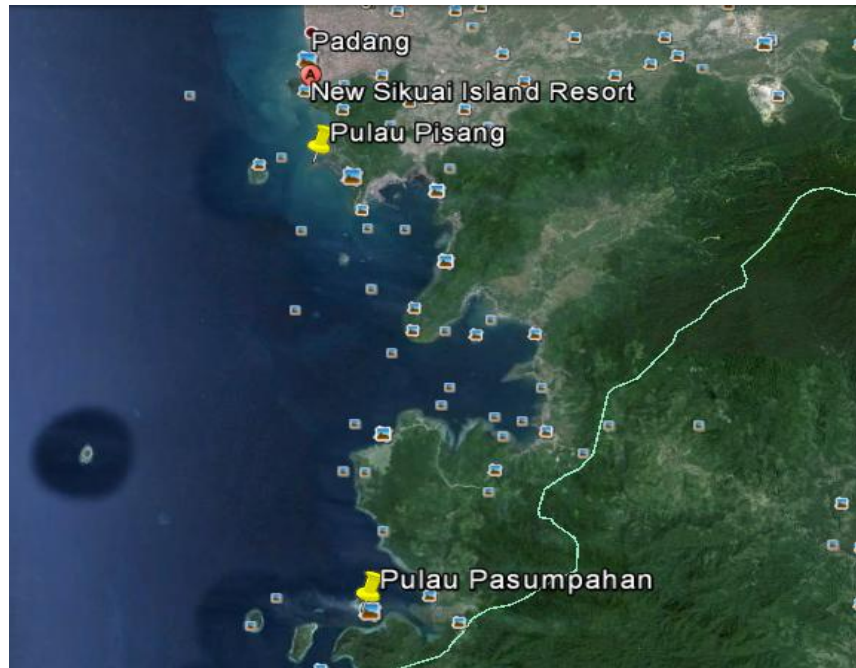
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MATERIALS AND METHODS

Experiment was conducted by snorkeling in Pulau Pasumpahan (P. Pesumpahan) coast ($1^{\circ}7'11,38''S$; $100^{\circ}22'2,78''E$) and in Pulau Pisang (P. Pisang) coast ($0^{\circ}59'24,35''S$; $100^{\circ}21'14,41''E$) of the west of Sumatera Island waters in August and between 21 to 22 September 2013 (Figure 1). Others experiments were conducted in Malang Rapat ($1^{\circ}6'44,78''N$; $104^{\circ}37'44,49''E$) and Teluk Bakau ($1^{\circ}3'1534''N$; $104^{\circ}39'7,15''E$) in Bintan Island waters side by snorkeling between 9 to 10 September and between 10 to 11 October 2013. For experiment, plastic screen were placed and recollected after keeping 24 hours both in Bintan Island side and in west of Sumatera Island side (P. Pesumpahan and P. Pisang)(YESOU Project Information and Methods, 2014).





Gambar 1. Sampling sides in P. Pasumpahan and P. Pisang coast in west coast Sumatera Island (A), and B, Study sites in Malang Rapat and T. Bakau coast in Bintan Island side (Google earth).

The suspension was then passed through a 350 micron and followed by 150 micron mesh sieve to remove large debris. The material that passed through were sieved again with a 20 micron mesh sieve. Material retained by the fine sieve was resuspended in sterile filtered seawater and examined by using Sedgwick Rafter under a compound microscope (Nikon compound microscope, Tokyo, Japan) for cell counting.

Environmental variables of the seawaters in this study were measured such as temperature, salinity, concentration of nitrate and phosphate. Data were analyzed with two-way ANOVA and when the results were significantly different, it was followed by Turkey-test (SPSS version 17).

RESULTS AND DISCUSSIONS

Four stations of these study sides have different environmental factors. Two stations in west coast of Sumatera Island, P. Pesumpahan and P. Pisang have macro-algae, sea-grasses and coral reefs ecosystem, but Station in P. Pesumpahan coast has much better coral reef ecosystem than Station in P. Pisang waters. Others two stations of sampling side in Bintan Island (Malang Rapat and T. Bakau coast) have macro-algae and sea-grasses. Malang Rapat and T. Bakau coast have also coral reefs ecosystem, with live coral cover and condition macroalgae and seagrass are much better in Malang Rapat (about 70 %) than T. Bakau coast (about 40 %). However, live coral cover in coastal area of P. Pisang was very low (about 2 %). Other environmental variables of four station show in Table 1.

Table 1. Environmental variables, temperature, salinity, nitrate and phosphate a measured in Pulau Pesumpahan, Pulau Pisang in west site of Sumatera Island, Teluk Bakau and Singgarang coast in Bintan Island sides.

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No.	Environmental variables	Stations			
		P. Pesumpahan	P. Pisang	Malang Rapat	T. Bakau
1.	Water Temperature (°C)	29.5	29	29	30
2.	Salinity (‰C)	35	34	35	35
3.	Nitrate (mg l ⁻¹)	0.1208	0.6354	0,3500	0.3610
4.	Phosphate (mg l ⁻¹)	0.0088	0.0376	0,1173	0.1155

Temperature among four stations were not so different, between 29 to 30°C. Water salinity was almost the same, there were between 34 to 35 ‰. Concentration of nitrate between 0.1208 in P. Pesumpahan to 0.6354 mgL⁻¹ in P. Pisang waters. whereas phosphate concentration was various among station, between 0.0088 mgL⁻¹ to 0.1173 mgL⁻¹.

The variety density of three genera of benthic dinoflagellate *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* were observed in P. Pesumpahan and P. Pisang coast in west of Sumatera Island waters and in Malang Rapat, and T. Bakau coast of Bintan Island waters, Riau Archipelago (east of Sumatera Island).

The results among density of *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* was the same trend between in P. Pasumpahan and P. Pisang, the highest number was *Ostreopsis*, and followed by *Prorocentrum* and *Gambierdiscus* in both P. Pasumpahan and P. Pisang waters (Figure 6). Density of *Ostreopsis* and *Prorocentrum* was much higher in P. Pasumpahan (1174 cells 100 cm⁻² and 1027 cells 100 cm⁻² respectively) than in P. Pisang (463 cells 100 cm⁻² and 347 cells 100 cm⁻² respectively). Difference density of *Ostreopsis* was more than two times higher in P. Pasumpahan than in P. Pisang coast. Whereas, difference *Prorocentrum* was also more than two times higher in P. Pasumpahan than in P. Pisang Island coast. However, density *Gambierdiscus* was the same in number between P. Pasumpahan (143 cells 100 cm⁻²) and P. Pisang (143 cells 100 cm⁻²).

Table 2 showed that density among three genera *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* were the same trend in number among 4 station (P. Pasumpahan, P. Pisang, Malang Rapat and T. Bakau coast). The highest density was consistently *Ostreopsis* among three genera of benthic dinoflagellae observed in each researched station. Comparison between density each genera between in the west (P. Pasumpahan and P. Pisang) and the east (Malang Rapat and T. Bakau) of Sumatera Island waters showed that the density all three genera higher in the east than the west of Sumatera Island waters. Difference density each of three genera dinoflagellate was much higher between in P. Pisang of in the west of Sumatera Island and both in Malang Rapat and T. Bakau in the east of Sumatera Island. But, the difference was not so different between in P. Pasumpahan and both in Malang Rapat and T. Bakau. The highest number was *Ostreopsis* (167 100 cells cm⁻²) in Malang Rapat in the east of Sumatera Island, compare to the highest number in the west of Sumatera Island was *Ostreopsis* (143 cells 100 cm⁻²) in P. Pasumpahan. Whereas the lowest number was observed *Gambierdiscus* (6 cells 100 cm⁻²) in P. Pisang on the west of Sumatera Island compare to the lowest number was *Gambierdiscus* (47 cells 100 cm⁻²) on T. Bakau in the east site of Sumatera Island.

Table 2. Mean density of *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* on plastic screen (12 hrs Experiment) in west coast of Sumatera Island and in Bintan Coast in Riau Archipelago.

No.	Station and Position	Genera and Sampling Time		
		<i>Gambierdiscus</i> (cells 100 cm ⁻²)	<i>Ostreopsis</i> (cells 100 cm ⁻²)	<i>Prorocentrum</i> (cells 100 cm ⁻²)
I	West coast of Sumatera Island		22-23 September 2013	
1	P. Pasumpahan	39	143	126



2	P. Pisang	6	42	14
II	Bintan Island in Riau Archipelago			
			9-10 September 2013	
1	Malang Rapat	141	335	304
2	Teluk Bakau	99	338	304

Cells counted of three dinoflagellate between the east and in the west of Sumatera Island was very different. Such as *Ostreopsis* in Malang Rapat and T. Bakau in Bintan Island waters were more two times higher than in P. Pasumpahan in the west of Sumatera Island waters. *Prorocentrum* density in Malang Rapat and T. Bakau was almost 2.5 times higher than in P. Pasumpahan in the west coast of Sumatera Island. Even, density of *Gambierdiscus* in Malang Rapat was almost 4 times higher than in P. Pasumpahan in the west coast of Sumatera Island. Whereas cells counted of all three genera *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* were the lowest and in T. Bakau coast.

Density among genera in the same station and between Station of the same genera (Figure 1). The three genera of dinoflagellates *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* were observed the same trend density in P. Pasumpahan Island coast in the west of Sumatera Island waters, in Malang Rapat and in T. Bakau of Bintan Island waters of Riau Archipelago (east of Sumatera Island waters), except in P. Pisang Island (Figure 2). Difference dinoflagellates among stations (P. Pasumpahan, P. Pisang, Malang Rapat and T. Bakau) was significant between *Ostreopsis* in P. Pasumpahan and *Gambierdiscus* in P. Pisang, *Prorocentrum* in P. Pasumpahan and *Gambierdiscus* in P. Pisang, *Gambierdiscus* in P. Pisang and *Ostreopsis* in Malang Rapat, *Gambierdiscus* in P. Pisang and *Prorocentrum* in Malang Rapat, and between *Prorocentrum* in P. Pisang and *Ostreopsis* in Malang Rapat (ANOVA, Turkey-test, $p < 0.05$). *Prorocentrum* was the only the same genera significantly different between different stations (P. Pisang and Malang Rapat and between P. Pisang and T. Bakau). Difference among 3 genera (*Gambierdiscus*, *Ostreopsis* and *Prorocentrum*) within station (within P. Pasumpahan, P. Pisang, Malang Rapat and within T. Bakau) were not significant (ANOVA, $p > 0.5$).

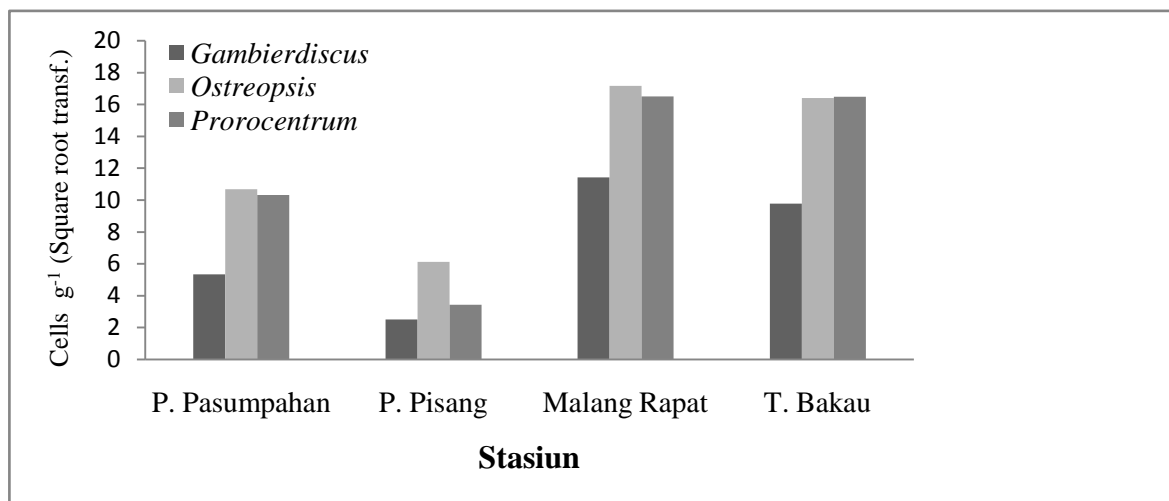


Figure 2. Mean density of *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* on plastic screen (Experiment) in P. Pasumpahan and P. Pisang coast of west Sumatera Island; and Malang Rapat and T. Bakau in east coast of Bintan Island, Riau Archipelago.

From experiment by using plastic screen during 12 hours was found that Density of benthic dinoflagellate *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* showed the same trend among three stations P. Pasumpahan, Malang Rapat and T. Bakau. The three genera of dinoflagellates *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* were observed the same trend density in P. Pasumpahan Island coast in the west of Sumatera Island waters, in Malang Rapat and in T. Bakau of Bintan Island waters of Riau Archipelago (east of Sumatera Island waters), except in P. Pisang Island. Difference dinoflagellates among stations (P. Pasumpahan, P. Pisang, Malang Rapat and T. Bakau) occurred significant between *Ostreopsis* in P. Pasumpahan and *Gambierdiscus* in P. Pisang, *Prorocentrum* in P. Pasumpahan and *Gambierdiscus* in P. Pisang, *Gambierdiscus* in P. Pisang and *Ostreopsis* in Malang Rapat, *Gambierdiscus* in P. Pisang and *Prorocentrum* in Malang Rapat, and between *Prorocentrum* in P. Pisang and *Ostreopsis* in Malang Rapat (ANOVA, Turkey-test, $p < 0.05$). *Prorocentrum* was the only the same genera significantly different between different stations (P. Pisang and Malang Rapat and between P. Pisang and T. Bakau). Difference among 3 genera (*Gambierdiscus*, *Ostreopsis* and *Prorocentrum*) within station (within P. Pasumpahan, P. Pisang, Malang Rapat and within T. Bakau) were not significant (ANOVA, $p > 0.5$).

DISCUSSIONS

Nutrient concentrations measured in September 2013, and no clear pattern between nutrients and three dinoflagellataes *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* among 4 stations. Phosphates and nitrates seemed to have no impact were associated with the trend results. Other studies have already mentioned that inorganic nitrogen, phosphorus and silicate concentrations appeared unassociated with benthic dinoflagellate distributions in tropical and Mediterranean areas (Grzebyk et al., 1994; Vila et al., 2001). Whereas in Hawaii, abundance of *Ostreopsis* spp. reported a positive correlation with concentration of nitrates, nitrites, phosphate and silicate (Parsons and Preskitt, 2007). Burkholder et al. (2008) concluded that there is an indirect link between development of *Ostreopsis* species (*O. ovata*, *O. siamensis* and *O. lenticularis*) and anthropogenic nutrient inputs via the stimulation of macroalgal habitat. However, the role of nutrients in the increase number of dinoflagellates, including *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* is still unclear.

Difference results were especially occurred significantly between genera of dinoflagellate in P. Pisang coast and in other three stations (P. Pasumpahan, Malang Rapat and T. Bakau). However, differences were observed between the same genera in different station only between *Prorocentrum* in P. Pisang and *Prorocentrum* in Malang Rapat coast, and between P. Pisang and T. Bakau coast, but *Prorocentrum* in P. Pasumpahan was not significantly different with *Prorocentrum* in both Malang Rapat and T. Bakau. Pulau Pisang and P. Pasumpahan are placed in the west of Sumatera Island waters and both Malang Rapat and T. Bakau are placed in the Bintan Island waters of Riau Archipelago (east of Sumatera Island waters). On the other hand, P. Pisang has coral reefs with macroalgae and seagrass as occurred in P. Pasumpahan, Malang Rapat and T. Bakau. But, coral reefs in P. Pisang is very bad condition live coral cover. Live coral cover in reef flat of P. Pisang is almost zero, and its macroalgae and seagrass are very rare.



Hence, it appears that there may not be much difference in the density of benthic dinoflagellate *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* between in the west coast of Sumatera Island waters (especially to the P. Pasumpahan coast) and in Bintan waters (Singgarang, Malang Rapat and T. Bakau coast) when their ecosystem condition are almost the same. Although, from the results of seagrass *Halophylla* and macroalgae *Padina* survey between west site of Sumatera Island waters (P. Pasumpahan and P. Pisang coast) and east site (Singgarang, Malang Rapat and T. Bakau coast) was significantly different. Attached *Prorocentrum* lima, *P. convacum*, *Ostreopsis* and *O. ovate* form epiphytic communities associated with coral reef, or rather with macro-algae attached to coral surfaces. These assemblages may vary in species composition and cell concentration between sites (Tindall and Morton, 1998).

Harmful algal blooms (HABs) have increased all around the world over the past several decades, with an increase in the diversity of the harmful species and the number of areas affected (Van Dolah, 2000; Cheng et al., 2005; Masoand Garcés, 2006), and these even may be associated with coral reef degradation (Bagnis et al., 1990, Lewis 2001). Degradation of coral reefs by physically or anthropogenic effect have caused increase of ciguatera, due to degraded reef has colonized by macroalgae as host by dinoflagellates (Kaly and Jones 1994). Hence, increase of dinoflagellate including benthic dinoflagellate *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* may be indirectly effect of degradation of coral reefs which is caused degraded reef will be dominated by macroalgae (Kaly and Jones, 1994), and increase of HAP including benthic dinoflagellate may be controlled by macroalgae and seagrasses.

CONCLUSION

They are many factors have reported in controlling increase of HAP, including temperature and nutrient. However, the role of nutrients in the increase number of dinoflagellates, including *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* is still unclear. Increase of dinoflagellate including benthic dinoflagellate *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* may be indirectly effect of degradation of coral reefs, and increase of HAP including benthic dinoflagellate may be controlled by macroalgae and seagrasses. *Acknowledgements.* I thank to Gires Usop who support me to this project. Financial support was by UNESCO.

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