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Research Article

Mass Mortality of Fish in Lampung Bay, Indonesia

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ABSTRACT

The surface waters in Lampung Bay appeared with dark reddish brown color from the beginning of October until November 2012 due to a bloom, an explosion in the phytoplankton population. This event resulted in the mass mortality of fishes that are cultured in floating nets there. Other smaller and less frequent blooms occurred in the bay the next year. This algal bloom was caused by the dinoflagellate species *Cochlodinium polykrikoides*. This was thought to be the first bloom of *Coclodinium* in these waters, or any in Indonesian waters. The highest abundance of *Cochlodinium* during the incident reached to 3.07 x 10^7 cells.l^{-1}. Mass fish mortality in the floating nets was due to oxygen depletion, mainly during the night time. The clogging of their gills by dense phytoplankton cells was assumed to be the cause. The population explosion of *Cochlodinium* was triggered by a high ratio of nitrate and phosphate concentration in the waters. The nutrient ratios of N/P in the water were high, indicating nitrate as a triggering factor and phosphate as a limiting factor.

Keywords: algal bloom, *Cochlodinium*, discoloration, fish mortalities, Lampung Bay

1. Introduction

Water discoloration occurred in Lampung Bay due to an explosion of the phytoplankton population. This kind of bloom incident is known as a red tide, nuisance bloom or harmful algal bloom (Smayda, 1990; Hallegraeff 1993; Andersen et al., 2002). The bloom resulted in damaging economic losses due to the mass mortality of fish, mainly grouper, which are cultured in floating nets there (Muawanah 2013). During the incident, a dark reddish brown discoloration of the surface waters could be seen covering a large part of the bay, especially in the location of the floating fish-culture nets. The algal blooms occurred in October 2012 and continued until June 2013, spreading partially on the surface water. The algal bloom was caused by high populations of several species (Hallegraeff et al., 1996; Andersen et al., 2008). Algal blooms can be detrimental to the health of consumers, cause mass mortality among fish and other biota in the area, damage the ecosystem, lower the aesthetic value of the waters that have an impact on tourism, and result in economic losses for the surrounding community (Smayda, 1990).

Generally, a red tide or harmful algal bloom (HAB) is an increase in the abundance of phytoplankton resulting in a perceived change of color in surface waters. The incidence of algal blooms is influenced by the nitrate and phosphate concentrations in the waters, where such organisms develop rapidly utilizing available nutrients (Gilbert et al., 2005; Andersen et al., 2008). Blooms may recur in the future and may even influence other microalgae if the triggering factor is always available in the waters. Blooms of toxic species can caused disasters endangering the health of consumers. Many aquaculture companies have suffered losses or even bankruptcy due to harmful algal blooms.

The existence of toxic phytoplankton species in Lampung Bay has now become a serious problem and will have a negative impact in the future if it is not addressed by the user community and the local government. The presence of red tide species in these waters can potentially contaminate other marine locations in various ways either through natural processes or human activities. This event was recorded as the first bloom of *Cochlodinium* in this bay, even as the first occurrence in Indonesian waters. Blooms of this species have occurred in some waters of the world where it is known as fish killer. This paper describes an aquatic disaster due to the unexpected occurrence of a phytoplankton bloom in Lampung Bay. It is also discuss the concentration of nutrients in the water, which is assumed to play an important role as a triggering factor. It is also partially describes the morphological features of the bloom species.

2. Materials and Methods

2.1. Site study

This bloom occurred in Lampung Bay which is located in the southern part of Sumatra (Figure 1). Most of cultured fish were grouper kept in the floating nets located along the coast in the western part of the bay. The floating nets mostly belong to local people and private companies. There is also a fisheries research station which is in charge in monitoring the water quality of the bay. During the bloom incident, observation was carried out from October- December 2012.

2.2. Sample collection

Samples were collected with a plankton net of 20 microns pore size, one meter in length and with a 25 cm diameter opening. It was equipped with a weight at the end so it could be lowered vertically to a depth of 5-10 meters. The net was hauled up slowly and steadily. After that phytoplankton samples were collected in sample bottles and preserved with Lugol solution by adding a few drops to the sample until it was light brown. Preserved samples were stored in a dark place to avoid damage. Samples were observed and analyzed qualitatively and quantitatively using a microscope in the laboratory. Enumeration of the phytoplankton was done using Sedgwik-Rafter Counting Cell (SRCC) and the results expressed in cells per liter (Michael 1995).

2.3. Nutrition analysis

Sea water samples for the analysis of phosphates and nitrates were collected using Nansen Bottles at a depth of one meter below the surface. Water samples were filtered through Millipore filter paper with pore size of 0.45 µm. Phosphate and nitrate analysis was conducted according to the method of Strickland and Parson (1972) using Shimadzu spectrophotometer wavelengths of 885, 543, 810 and 680 nm in units mg/l (ppm).

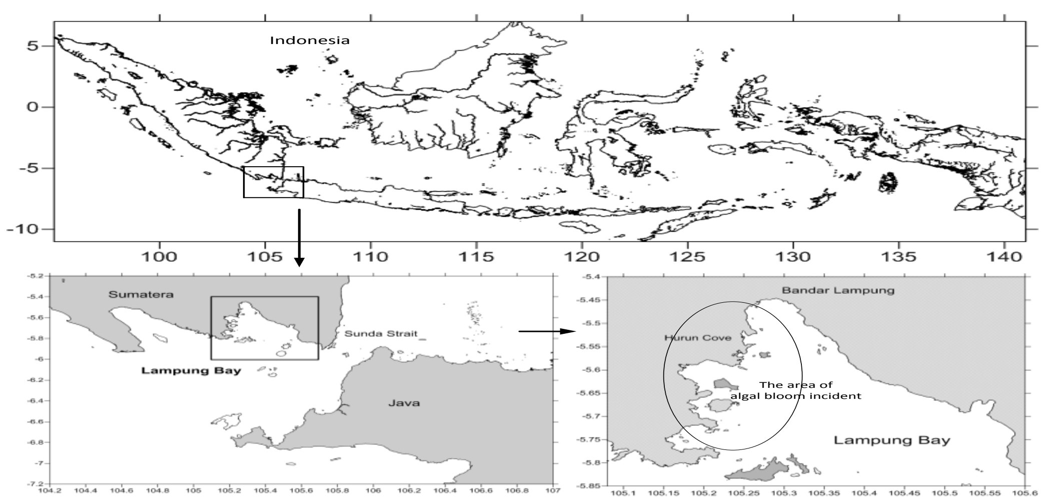

Figure 1. Map of the Indonesia, Lampung Bay and the location of algal bloom incident.

3. Results and Discussion

3.1 Episode of algal bloom

Outbreaks of rapid phytoplankton population growth have often occurred in Lampung Bay (Table 1) which has experienced algal blooms since the year 2000. The number of recorded bloom-causing species has increased significantly. Nine species are recorded to have caused problem blooms in Lampung Bay including *Pyrodinium, Noctiluca, Phaeocystis, Dinophysis, Trichodesmium, Ceratiun, Prorocentrum, Pseudonitzhia* and *Cochlodinium*. Since 2005, outbreaks of phytoplankton have been observed almost every year with different causative species. The species that bloomed most frequently in this bay from 2005-2010 was *Noctiluca*. This species is present in almost areas. Another species, *Pyrodinium sp,* was recorded in 2008, *Phaeocystis sp* in 2009, *Dynophysis sp* in 2010 and 2011, *Trichodesmium sp* in 2002-2012, *Ceratium sp* in 2011, *Pseudonitzchia sp* in 2009-2012 and the last species *Cochlodinium* occurred in 2012, 2013 and 2014 (Sidabutar 2006, Muawanah et al. 2013). Some of those species are classified as bloom makers but only a few as toxic species. Of all those species mentioned, the only one which is classified as very toxic and lethal is *Pyrodinium*. Previous research was conducted in 2005, 2006 and 2007 to study its occurrence (Sidabutar 2006). It was recorded that the first time this toxic species was found in abundance at Lampung Bay was at Hurun Cove .

The bloom incident of *Cochlodinium* in October 2012 was noted as the first occurrence ever known in this bay. This species has never been observed before in the samples collected there. The bloom covered a wide area of the bay, appearing as a dark reddish brown color on the surface water, like an oily suspension. It was assumed that the mass fish mortality occurred due to a lack of dissolved oxygen during night time when the fish had difficulty breathing because of the mucus substance covering the gills. The fish that died during the incident included cobia, pomfret, grouper and snapper. Fish mortality began in mid-October 2012 when the abundance of phytoplankton reached 10^7 cells/l, with the highest peak in mortality occurring in late October 2012. The death of fish occurred after the two-week bloom achieved its highest peak and fish deaths continued to rise until the end of month. A similar bloom of *Cochlodinium* happened in China, Japan and Korea (Kim CS et al. 2000, Kim D et al. 2002, Kim CJ et al. 2007, Yamatogi et al. 2005)

Table 1. The frequency of bloom occurrences in Lampung Bay (2002-2014).

Phytoplankton / Year	Periods of algal bloom events												
	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Pyrodinium	x	x		x					x				x
Noctiluca	x				x	x	x	x	x	x	x	x	x
Phaeocystis									x	x			
Dinophysis					x	x					x		
Trichodesmium				x			x		x	x	x		
Ceratiun										x			
Prorocentrum									x		x		
Pseudonitzhia									x		x		
Cochlodinium											x	x	x

Source : modified from Muawanah et al. (2013)

3.2. Shape and morphology of *Cochlodinium*

The bloom incident showed that the causative species is *Cochlodinium polykrikoides* (Muawanah et al. 2013). It was reported that *Cochlodinium* was known as the cause of blooms that often occur in Southeast Asian waters (Kudela et al. 2005). The distribution of this species is very broad ranging from the waters of the Atlantic, Europe and Asia, however, this species has never been found in Indonesian waters before. Prior to 1990s, the first bloom of *Cochlodinium* was recorded in Southeast Asia (Kudela et al. 2005). After that in the 1990s, fishing industries in South Korea suffered a loss of more than 100 million US dollars a year (Kim, 2010). Since that time, blooms of this species was recorded across the Asian region, on the coast of Europe and North America. Iwataki et al*.* (2007, 2008) identified this species into three sub-clades or ribotypes *Cochlodinum polykrikoides*: the Southeast Asian ribotype (which refers to the original clade Japan-Korea), the US-Malaysia ribotype and the Philippines ribotype. This species was also observed on the Mediterranean coast of Italy in the late 1990s (Rene et al. 2013) as well as the Black Sea in 2001 in Odessa and Tanjung Big Utrish where a large bloom occurred (http://phyto.bss.ibss.org.ua). In 2011 blooms of these species in North Tarragona were recorded (Rene et al. 2013).

Based on morphological characteristics, it is known that the species that bloomed in October 2012 belongs to the species of dinoflagellate named *Cochlodinium*. The characteristics of *Cochlodinium* is a rounded cell shape and they form chains of 2-8 cells or more (Steidinger and Tangen 1997). It has conical cells that are rounded at the peak forming the epitheca section. Cells have a spiral-shaped ring that surrounds the cell (spiral cingulum) with a cell size ranging from 20-30 µm (Figure 3). It has oval-shaped cells, forming a chain of 2, 4 and 8 cells and some are single and detached from the chain. There is a circular line pattern at the center of a cell. Each cell has a deep transverse furrow (cingulum) and spiral which commonly found on *Cochlodinium sp*. The size of cells ranges between 7-10 µm. This cell is small relative to *Cochlodinium polykrikoides* commonly found to bloom in the waters of Japan, China and Korea (Iwataki et al. 2007, 2008). Figure 2 shows the morphology of phytoplankton which bloomed in the waters of Lampung Bay.

Based on its morphology, this species showed similarity to the genus *Cochlodinium* although it was much smaller in size. It was necessary to study it more and, based on photographs, it has been clarified by Vera Trainer and Iwataki that the species name is *Cochlodinium polykrikoides*. According to Iwataki (pers. communication) there were two kinds of *Cochlodinium* identified in their samples, which were collected from the bay by a Japanese team in September 2014. They were *Cochlodinium polykrikoides* and *Cochlodinium fulvescens* (Figure 3). The existence of *Cochlodinium* in these waters has been proven through the presence of cysts (resting cysts) produced by the species in the sediment (Tang and Gobbler 2012). The results of cyst identification (Figure 3e) from the sediment proved that the species is *Cochlodinium polykrikoides*. Dinoflagellates can produce dormant cysts (diploid) and cell division that produces individuals (haploid) that are similar to their parent. This dormant cysts are produced when water conditions experienced extreme changes, in order to continue future generations.

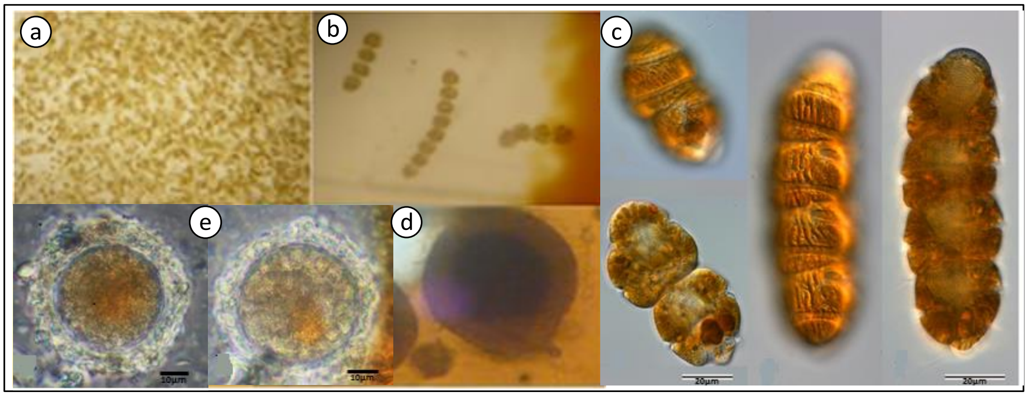

Figure 2. The shape morphology of bloom maker species in Lampung Bay waters.
(a) dense cells of *Cochlodinium*, (b-c) cells form a chain of 2, 4 and 8 cells,
(d) form of planozigote, (e) form of cysts in the sediment.
(a,b,d: photo by Muawanah, c : photo by Iwataki and e : photo by Estelle Masseret).

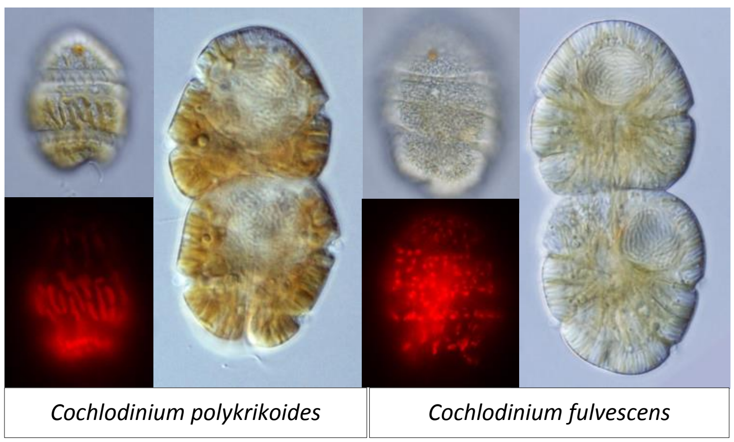

Figure 3. The difference in morphology of *C. polykrikoide*s and *C. fulvescens* (Iwataki et al. 2007).

3.3. Abundance of *Cochlodinium*

Figure 4 showing the graphic abundance of *Cochlodinium* when the bloom occurred in October 2012. The bloom achieved the highest peak on 29 October 2012 when abundance reached 10^7 cells per liter. During the bloom fish cultured in floating nets died. When the bloom occurred, the average density of *Cochlodinium polykrikoides* reached 3.07 x 10^7 cells per liter. The mechanism for the mass fish mortality due to the *Cochlodinium* bloom is still not known for certain, however, symptoms such as irritation of the fin, gill-damage and shortness of breath could be seen. In general, based on previous observations (Gobbler et al., 2008), mass mortality is caused by a lack of dissolved oxygen and clogging of the gills due to the high density of cells and mucus, showing that fish mass mortality was caused by the mucus from blooming *Cochlodinium* (polysaccharides). Other experts have said the fish deaths are caused by PSP or paralytic shellfish poisoning (Kim et al. 2000), but there is no other evidence that reinforces this opinion. Mass mortality can also be caused by the production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical (Kim et al., 2002) but this still needs further research (Gobler et at., 2012).

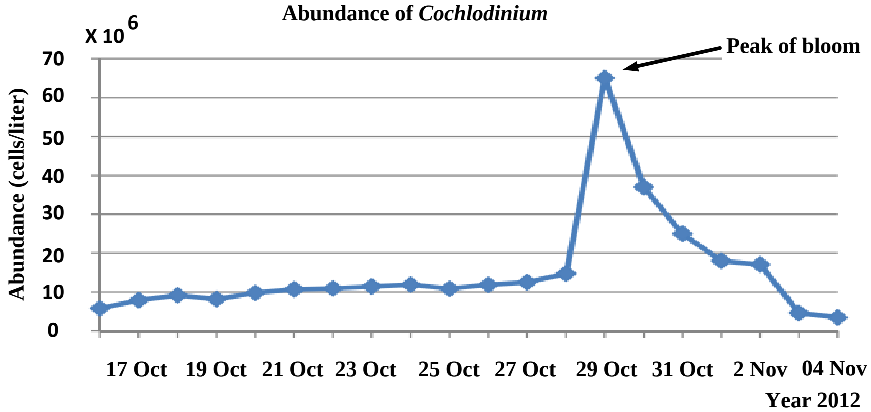

Figure 4. The abundance of Cochlodinium in Lampung Bay during the bloom in October 2012.

Table 2. The normal concentration of nutrients (mg/l) and after the incident in the bay.

Variables	Normal Concentration	Ranges (mg/l)	Averages	Increases
NO_2	0.012	0.030-0.020	0.080	0.7
NO_3	0.036	0.032-0.413	0.162	4.5
NH_4	0.103	0.085-1.241	0.670	6.5
PO_4	0.119	0.080-0.075	0.024	0.2

3.4. Concentration of nitrate and phosphate.

The results of measurement during the incident showed a high concentration of nitrates and phosphates in the water. This indicates that there had been a process of water enrichment or eutrophication, which is characterized by high concentrations of dissolved nutrients, especially dissolved nitrogen /DIN (Heisler et al. 2008, Anderson et al. , 2002; 2008). Nitrate levels were 4.5 times the average and likewise ammonia (NH_4) was 6.5 times higher than normal (Table 2). During the bloom incident, phosphate concentration increased 0.2 times of the usual conditions. On the contrary, nitrate concentration increased significantly. The ratio of N/P at that time was 35:5. When the ratio of N/P in the waters is ≤ 5, this indicates nitrate as a limiting factor (N-limiting), while if the ratio of N/P is between 5-10 it is referred to as intermediate, and a ratio of N/P ≥ 10 indicates phosphate as the limiting factor for the growth (Ian and Rissik, 2009). At the time the N/P ratio was high (N/P ≥ 10) the abundance of phytoplankton was increasing, whereas, when the N/P ratio was low (N/P ≤ 5), the abundance of phytoplankton decreased. It is clearly shown that that N/P ratio in these waters is much higher than the normal ratio there. Each type of phytoplankton has a specific response to the value of N/P ratio in the waters. The incident of blooming is strongly influenced by the ratio of nutrients, especially the nitrogen versus phosphorus ratio (Damar, 2012). The consequences of high nutrients will be an increase in total algal biomass but if the nutrient composition or N/P ratio changes, it can lead to changes in the community or species composition (Heisler et al., 2008 ; Gobler et al., 2012).

The height of the N/P ratio showed that nitrate was a triggering factor for the growth of *Cochlodinium* sp, while phosphate is a limiting factor. Figure 5 showed that the concentration of nitrate decreased during the bloom when it reached the peak of population growth. It appeared that nitrate decreased at the end of October 2012, when the population simultaneously decreased. The N/P ratio at the time *Cochlodinium* was most abundant at 10^4 cells/l was 28. This ratio is higher than the normal N/P ratio in marine waters, which is 10. The high value of the N/P ration showed that nitrate is a triggering element for the growth of *Cochlodinium*. The high concentration of nitrates may be caused by input from the mainland when there was heavy rain after a long drought in early October 2012. Other sources can be derived from the decomposition of organic matter in bottom water. Increased nutrients in the water are due to the impact of anthropogenic activities and especially the accumulation of forage fish for long-standing fish farming. The impact of the combination of those activities can trigger unusual growth of red tide species in these waters. The concentration of phosphate fluctuated after the population reached its peak, however the nitrate concentration dropped dramatically (Figure 6). This indicated that nitrate acts as a trigger for growth and phosphate as a limiting factor, though they are indispensable in small amounts.

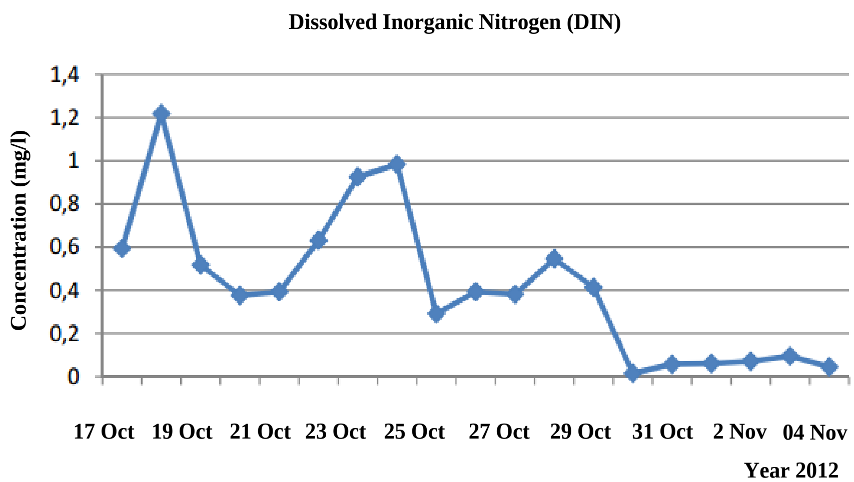

Figure 5. The fluctuations of nitrate concentration (DIN) in October 2012

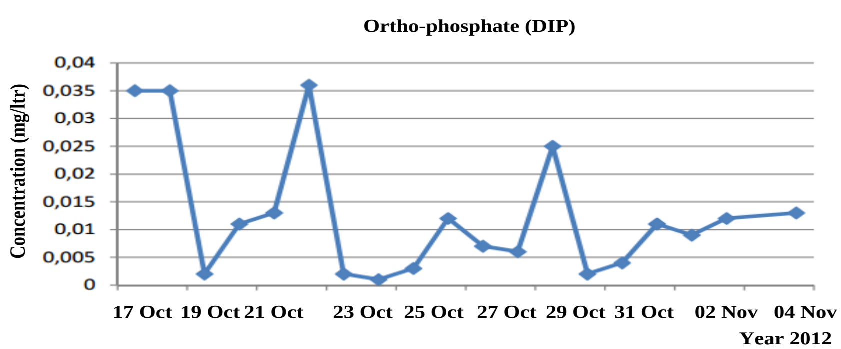

Figure 6. The fluctuations of phosphate concentration (DIP) in October 2012.

4. CONCLUSION

The micro-algal bloom and mass fish mortality in Lampung Bay during October to November 2012 was due to the occurrence of *C. polykrikoides*. The bloom of *Cochlodinium* was seemingly the first incidence ever reported in this bay and even in Indonesian waters. It is assumed that the fish deaths were due to oxygen depletion and the clogging of gills by the mucus produced by the algae. The bloom of *Cochlodinium* was triggered by the increasing ratio of nitrogen to phosphate, where nitrogen acted as a triggering factor and phosphate as a limiting factor.

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