Dinoflagellate cyst assemblages in surface sediment from Jakarta Bay

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Abstract. Dinoflagellate cysts play an essential role in the initiation, recurrence and geographical expansion of harmful algal blooms (HABs). The horizontal distribution and abundance of dinoflagellate cysts in marine sediments provide essential information in giving early warnings of the presence of toxic species and possible continuing recurrence of HABs in a given area. This research carried out in the waters of Jakarta Bay. Sediment samples collected from thirteen sampling stations using TFO gravity corer 2.1 cm diameter from sediment thickness 0-4 cm. The results showed that resting cysts belong to Gonyaulax, Alexandrium, Scrippsiella, Protoperidinium, Gymnodinium, Gyrodinium. Two of them, namely Gymnodinium sp and Alexandrium sp, known as toxic species. Cysts in the sediment thickness 0-4 cm mainly dominated by the group of Gonyaulax (Gonyaulacoid). Cyst abundance in the sediment depth-layer 0-2 cm is higher than in depth-layer 2-4 cm. The number of dino-cysts in sediment layer 0-2 cm ranged from 2.984 – 22.298 cysts cm\(^{-3}\) while in the layer 2-4 cm the amount varies from 1.063 – 8.132 cysts cm\(^{-3}\) of wet sediment. The result revealed that dino-cyst abundance is higher in the deposit collected from locations near to the coast. The assemblages of dinoflagellate resting cysts seemed to have a relation with some hydrological parameters.

1. Introduction
Nowadays, Jakarta Bay has been experiencing algal blooms events since 1991 [1]. The frequency and distribution of algal bloom phenomena have been increasing and had caused massive fish kills which lead to economic losses in local fisheries, decrease of water quality and also effected the areas of local tourism in the surrounding bay [2, 3, 4]. Algal bloom occurs due to population explosion, especially diatom, dinoflagellates and cyanobacteria groups [5]. Dinoflagellate classified that produces the most toxic species called harmful algal blooms, because they can infect fish and other biota and even poison humans as the consumers of marine products [5, 6, 7]. The dinoflagellate species are capable of producing resting cysts, prevalently through sexual reproduction [8]. Resting cysts have considerable ecological importance for cyst-producing species, as they ensure survival, favour dispersion, provide a source of genetic diversity, and promote bloom initiation and its recurrence [9, 10]. The resting cysts settle in sediments and remain viable for months, years, or even up to one century [11]. Consequently, cysts serve as a representative of plankton populations.

Cyst assemblage composition generally reflects temperature, salinity, productivity, and nutrient conditions [12], and several cyst assemblage signals can indicate eutrophication and pollution conditions [13, 14]. In recent years, reported, that cyst is a resting stage during the life cycle, and their formation is a protective mechanism against unfavourable conditions, such as nutrient depletion, grazing, competition, and high turbulence [15, 16, 17]. Therefore, resting cysts have a variety of potential functions in the overall ecology of the dinoflagellates such as seed population to initiate red
tides, a survival mechanism through environmental extremes, agents for species dispersal means for genetic recombination, direct sources of toxicity, and a factor in bloom termination [18, 19]

Cysts can remain in the sediments for decades and even centuries in deposits [20, 21], therefore providing a genetic repository [22] which can potentially lead to the emergence of harmful dinoflagellate cells that could form blooms once specific environmental factors optimum for their development arise. Dinoflagellate cysts are essential in initiating and terminating blooms [25, 24]. Some studies have implicated them as the source of toxins [25, 26, 27], whereas others have focused on their role in species distribution expansion. Cysts represent the potential diversity of a planktonic population and also its expressed diversity, in the form of vegetative phases inhabiting a particular site [28]. In some bloom-forming species, resting cysts are the key to bloom initiation as well as a tool for species maintenance when conditions become unfavourable [30, 31]. The resting cysts play an essential role in the initiation, recurrence and geographical expansion of harmful algal blooms (HABs). The horizontal distribution and abundance of resting cysts in marine sediments provide essential information in giving early warnings of the presence of toxic species and possible continuing recurrence of harmful algal bloom in this area.

This paper presents the study of cyst assemblages, abundance and distribution of dino-cyst species in recent sediments from Jakarta Bay. The survey carried out to provide baseline information on harmful algal bloom ecology for management purposes of the bay shortly.

2. Material and methods
2.1 Description of location
The survey took place in Jakarta Bay. Jakarta Bay is a bay in the waters of the Java Sea located north of the DKI Jakarta province, Indonesia. In this bay, 13 rivers flow into the city that divides the city of Jakarta. Jakarta Bay, which covers an area of about 514 km², is a shallow water area with an average depth of up to 15 meters. The sediment for the study collected at 13 sampling stations. Map of Jakarta Bay and the sampling station is showed in Figure 1.

2.2 Sediment Sampling
Surface sediment samples of about 1 cm in thickness each cut and kept in plastic bottles with a small amount of seawater above the sediment. The sample preparation for identification and quantitative analysis of the benthic cysts performed using the method described by Matsuoka et al [23]. The primary references used for identification purposes belongs to Matsuoka and Fukuyo [22, 24]. Both empty cysts and living cysts, they were identified and counted.
2.3 Sediment Analysis
The sediment was collected using TFO gravity corer, as shown in Figure 2. We collect samples from 4 cm thickness-deposits, then processed by the palynological technique [23]. Original pieces of sediments divided into two or three parts; one was oven-dried at 70°C for 24 h to determine the dry weight and to calculate the water content, and others treated using palynological or sieving procedures. In the palynological method, sediments were treated with HCl (10%) and HF (40%) solutions to remove calcium carbonates and silicate materials. Then the samples were repeatedly washed with distilled water to remove acid until the pH values were near 7.0. The chemically treated samples were sonicated for 30 s and then successively sieved through 125 and 20 um mesh-size metallic screens (two or three times) to remove coarse and fine materials. The sediments retained on the latter screen were transferred into a plastic tube and suspended in 10 ml distilled water.

For observation, we placed a 0.1–0.5 ml aliquot of the processed sample on a 1 ml counting chamber and diluted with distilled water. We used an inverted microscope (Olympus IX70) at magnifications of 100-900. For most examples, need identified a minimum of 200 dinoflagellate cysts and counted. Dinoflagellate cysts identified based on published descriptions. When species-level identification was not possible; however, the label made at the genus level. Cyst concentration described as cysts per gram of dry weight sediment (cysts/g).

2.4 Data analysis
Cysts were counted using a microscope on a Sedgwick-Rafter Counting (SRC) plate and identified using the specific guidelines for harmful algal bloom cysts by Matsuoka and Fukuyo [24] and other literature. The abundance of cysts calculated using the formula used by Lee and Matsuoka [6] as follows:

\[ N = n_i \times \frac{V_i}{V_o} \times \frac{1}{Bss} \times \frac{1}{D} \]

Where:
- \( N \): cyst abundance (cysts .cm\(^{-3} \))
- \( n_i \): the number of cysts observed
- \( V_i \): sample volume (100 ml)
- \( V_o \): observed sub-sample volume (1 ml)
- \( Bss \): the weight of sub-sample (gram)
- \( D \): ratio dry and wet weight

Figure 2. The TFO gravity corer and the sediment samples
Table 1. Weight of sub-sample (Bss) and dry and wet weight ratio (D).

| Sediment thickness | Sub-sample weight (Bss) | Dry and wet weight ratio (D) |
|--------------------|-------------------------|-----------------------------|
| 0 - 2 cm           | 57.422                  | 0.1109                      |
| 2 - 4 cm           | 65.466                  | 0.2874                      |

3. Results and discussion

3.1 Cyst assemblages

Cyst formation is a biological response from the dinoflagellate group in maintaining the survival of generations from unfavourable conditions, a strategy of spreading and sustaining generations. The cysts density as the indicator of the past and also an indicator of the aquatic environmental condition. In this study, the dino-cyst observed to provide baseline information on dinoflagellate ecology in the bay. The results showed that there are six types of dino-cysts were identified in the sediment samples from Jakarta Bay (Table 1). The six types of cysts, namely: Gonyaulax, Alexandrium, Scrippsiella, Protoperidinium, Gymnodinium, Gyrodinium. The highest abundance of dino-cysts belongs to Gonyaulax, both in the thickness layer 0-2 cm and 2-4 cm. Two types of cysts known as toxic species, such as Alexandrium and Gymnodinium (Figure 3). These two dinoflagellates are known as poisonous species because they produce toxins during their life, which can harm human health as a consumer of marine harvest. Previous research conducted in Jakarta Bay also found the dino-cyst of toxic species which also belongs to Gymnodinium [25]. In the sediment of the Jakarta Bay, it was observed many cysts belongs to Gonyaulax with various form, so it was tough to identify them due to the similarity form with the others dino-cyst. Most of the observed cysts are very similar to the cyst belongs to Spinifera (Figure 4).

The existence of dino-cysts in the sediment may prove the existence of dinoflagellates species occurring in the waters. Dinoflagellates generally have relatively low growth, abundance and low diversity when compared to other groups such as diatoms. However, the dinoflagellates group that can produce toxins are of more types. Dinoflagellates in a bloom-event can cause poisoning or infect toxins in consumption biotas such as crustaceans and shellfish. The cases of harmful algal bloom are generally dominated by the types of dinoflagellates that can produce harmful toxins [34, 37, 38].

Figure 3. Cysts of Gonyaulax sp (a), Alexandrium sp (b) and Gymnodinium sp (c)
3.2 Cysts abundance

The results showed that the total abundance of dinoflagellate cysts (dino-cyst) in the sediment (0-2 cm) collected from 13 stations is higher than the cyst in the sediment layer 2-4 cm. The total amount of cyst in sediment depth layer 0-2 is about 143.358 cyst /m$^3$, and the total amount cyst in sediment depth layer 2-4 cm is about 50.125 cysts/m$^3$. Graphic total of cyst abundance in both sediment depth layer collected from 13 stations in Jakarta Bay, shown in Figure 5. The high abundance of dino-cyst in the layer 0-2 cm, suggesting that there might be an unfavourable condition during a certain period, which leads the dinoflagellate to react with the situation by producing resting cyst. The population of dinoflagellate in the bay, during a certain period, may increase in number and frequency. Recently, the cyst is a resting stage during the life cycle, and their formation is a protective mechanism against unfavourable conditions, such as nutrient depletion, grazing, competition, and high turbulence. Several cyst assemblages signals can indicate eutrophication and pollution conditions [13, 14].

Table 2 showing the average abundance of species dinoflagellate in each sediment depth layer of 13 sampling stations in Jakarta Bay. There are six types of dino-cyst found in the sediment thickness of 0-4 cm. The cyst of Gonyaulax dominating the cyst assemblages with range abundance from 1.884-12.248 cysts.m$^{-3}$ (in the layer 0-2 cm), and in the layer 2-4 cm range from 52 - 4.199 cysts.m$^{-3}$. The cyst of Gonyaulax dominates the assemblage up to 50.71 per cent of the total abundance. The lowest abundance found belongs to the genus Gyrodinium with an average abundance of 132.85 cysts.cm$^{-3}$ (ranging from >0-785 cysts.cm$^{-3}$). Similarly, cyst in the sediment depth layer 2-4 cm, the highest abundance also belongs to species Gonyaulax with average number 1225.14 cysts.cm$^{-3}$, or around 31.75 per cent of the total cyst abundance. While the lowest abundance belongs to species Gyrodinium with average 202.38 cysts.cm$^{-3}$. The highest abundance of dino-cyst observed in the depth layer 0-2 cm, compared to the depth layer 0-2 cm. The abundance of dino-cyst in the depth layer 0-2 cm range from > 785 – 12.248 cysts.cm$^{-3}$, and in the depth layer 2-4 cm ranged from > 52 – 4.199 cyst. m$^{-3}$.

The average abundance of dino-cysts in the sediment depth layer 0-2 cm was 11.027 cysts.cm$^{-3}$, ranging from 2.984 – 22.298 cysts.cm$^{-3}$, while in the layer 2-4 cm the average abundance was 3.966 cysts.cm$^{-3}$, with values ranged from 1.063 – 8.132 cysts.cm$^{-3}$. The cyst abundance of each dinoflagellate species in both sediments layer, shown in Table 2. The comparison of dino-cyst abundance between depth layer 0-2 cm and 2-4 cm, as shown in Figure 5. In the sediment, was found six species of dinoflagellate cyst in the depth layer 0-4 cm. The highest abundance was Goniolacoid species (50%), and the lowest was Gyrodinium (1 %). The composition of dino-cyst in the sediment from Jakarta Bay shown Figure 6.
Figure 5. Graphic total cyst abundance in the sediment from 13 stations.

Table 2. The abundance of species dino-cysts in the depth layer of 13 sampling stations.

| No | Genus            | Sediment thickness 0-2 cm (Cysts.cm⁻³) | Sediment thickness 2-4 cm (Cysts.cm⁻³) |
|----|------------------|----------------------------------------|---------------------------------------|
| 1  | Alexandrium      | 628-4.083                              | 106-1.754                             |
| 2  | Gonyaulax        | 1.884-12.248                           | 319-4.199                             |
| 3  | Gymnodinium      | 0-1.884                                | 52-850                                |
| 4  | Gyrodinium       | 0-785                                  | 0-372                                 |
| 5  | Protoperidinium  | 471-2.041                              | 106-1.169                             |
| 6  | Scrippsiella     | 0-3.141                                | 0-1.169                               |
|    | **Range**        | >785-12.248                            | >52-4.199                             |

Figure 6 showing the graphic to compare the abundance of dino-cyst in the sediment collected from Jakarta Bay. We identified six types of dino-cyst which belong to phytoplankton, namely Alexandrium, Gonyaulax, Gymnodinium, Gyrodinium, Protoperidinium and Scrippsiella. The most abundance of dino-cyst belongs to Gonyaulacoid, reach a density up to 96,940 cysts/m³ in the sediment depth layer less than 4 cm. This type of dino-cyst is predominantly in the sediment, composed up to 50 per cent of the total density (Figure 7). The lowest abundance of dino-cyst in the sediment belongs to Gyrodinium. Marine dinoflagellates Gonyaulacoid are known to form resting cysts as part of their life history. During their life cycle dinoflagellates produce hypnozygotes during sexual reproduction for a resting phase of variable duration. Hypnozygotes of several species protected by an organic-walled cyst which fossilized in the sediment. More than 80 species of marine dinoflagellates are now known to produce these cysts [24][36].
3.3 Spatial distribution of cysts

Information on the distribution and density of cysts in sediment can provide important information about algal bloom episodes that have occurred in the waters. Cyst formation is a strategy to continue the generation of dinoflagellates when environmental conditions are unfavourable as well as a geographic distribution strategy [24].

The difference in cyst density in the depth layer of the sediment may indicate the frequency of dinoflagellate bloom occurring several years ago. Dinoflagellates that have bloomed in the waters can reproduce by dividing (fission) and forming cysts (encystment). The cysts that form accumulate in the sediment over a long period until conditions trigger the cysts to hatch (germination)[37].

Figure 8 showing the distribution of dino-cyst in Jakarta Bay. The abundance of dinoflagellate cysts appears to have a higher allocation in the coastal (inshore) than in the offshore. Of the 13 research stations, the number of dinoflagellate cysts higher at the sampling station which closed the beach area. The highest dino-cyst abundance recorded at St.10 (nearshore) with abundance 28.888 cyst/cm³, and the lowest at St.6 (offshore) with a bunch of 4.047 cyst/cm³. The quantity of cyst in the station near shore (St. 2, 5, 8, 10, 11) tend to be higher than the station in the offshore (St. 4, 7, 9, 12,
The distribution of cyst abundance is generally higher in the nearshore compared to the stations in the offshore. This suggesting that the occurrence of algal bloom or accumulation of dinoflagellate tends to occur in areas near the coast, mainly due to controlled by water movement. It might be any correlation with the physical factor of the bay which environmental factors that appear to have a relationship with the occurrence of cysts in sediment, such as pH, salinity, temperature, weight and depth. In contrast, the other parameters have an insignificant effect on the cyst assemblage (Table 3).

**Figure 8.** The comparison of cysts assemblages in coastal and offshore

| Variables       | Factor 1  | Factor 2 | Factor 3  |
|-----------------|-----------|----------|-----------|
| pH              | 0.908*    | 0.157    | -0.030    |
| Salinity        | 0.885*    | -0.104   | -0.084    |
| Temperature     | -0.799*   | 0.224    | 0.213     |
| Depth           | 0.781*    | -0.341   | -0.474    |
| Seston          | -0.686*   | 0.045    | 0.041     |
| NO$_3^-$        | -0.132    | 0.878*   | 0.049     |
| Current         | -0.351    | 0.746*   | -0.362    |
| DO              | 0.430     | 0.599*   | 0.212     |
| PO$_4^{3-}$     | -0.075    | -0.225   | 0.847*    |
| Transparancy    | 0.287     | -0.358   | -0.637*   |

**4. Conclusion**
There are six dinoflagellates in the assemblages of dino-cyst in the sediment, namely Gonyaulax, Alexandrium, Scrippsiella, Protoperidinium, Gymnodinium and Gyrodinium. The highest cyst density belongs to Gonyaulax, both in the deposit thick layer 0-2 cm and 2-4 cm, indicating that the frequency of this species, is higher than those of the other dinoflagellates, lately. There are two types dino-cysts found, already known as toxic species which produced toxin, namely Gymnodinium sp, and Alexandrium sp. The assemblages of dinoflagellate cysts in the sediment higher in the nearshore site comparing to the offshore area.
5. References

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