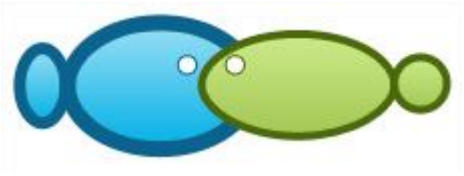

Cyst accumulation in the bottom sediments in Inner Ambon Bay and Kao Bay, Indonesia

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Abstract. Research to study occurrence, similarity and abundance of cyst of dinoflagellates which are accumulated in the bottom sediment was conducted in Inner Ambon Bay (IAB) and Kao Bay (KB) on June 2016. The cyst of dinoflagellates was collected from sediment at six stations in IAB and six stations in KB and then removed by using gradiant density method. Identification and counting of the cyst were carried out on the sedgewick rafter counting chamber by using light microscope. Dendrogram was performed by using cluster analysis. It was found that there were nine genera of cyst of dinoflagellates in both locations in which highest occurrence belonged to genus *Protoperidinium* while the lowest represented by genus *Ceratium* with the similarity values ranged from 25% to 100%. It also found that cyst of dinoflagellates in KB was more abundant than cyst in IAB. The result of cluster analysis showed that stations in both locations can be grouped into four groups.
Key Words: dinoflagellates, occurrence, similarity, abundance, Ambon Bay, Kao Bay.

Introduction. One of toxic dinoflagellate as harmful alga bloom is *Pyrodinium bahamense* var. *compressum* (Pbc) which can cause paralytic shellfish poisoning. This toxin accumulates easily in the tissue of shellfish such as oysters, green mussels, blood clams as they consume this dinoflagellate as their food (Setiapermana 1992). The accumulated toxin assaults neorosystem which caused major health security problems in human such as heart attack, paralysis and breathing difficulty (Setiapermana 1992). Meanwhile Praseno (1992) mentioned that fishes which consumed toxic dinoflagellate (Pbc) caused mass mortality of the fishes.

The first confirmation of Pbc bloom occurred in Kao Bay (Halmahera Island, North Moluccas Province) on March 1994 and in Ambon (Moluccas Province) on July 1994 (Sidabutar et al 1996). Red tide phenomena in Ambon Bay caused three children died and 33 persons were hospitalized as a consequence of consuming shellfish (Wiadyana et al 1996). They also stated that in 1994, harmful algae blooming in Ambon Bay was dominated by Pbc. In contrast, Wagey et al (2001) found *Alexandrium affine* was in the highest abundance in Inner Ambon Bay in 1997 which changed the water color in reddish-brown covering 1000 m^2 sea water. The bloom phenomena occurred again in Ambon Bay on July 2012, caused the death of thousands of cultured fish and seven people (two children and five adults) were hospitalized for a week after consuming shellfish. Over 2000 vegetative cells L^{-1} of Pbc were observed during that event and *Pyrodinium bahamense* (Pb) has become notorious for producing resting cysts which may accumulate in the bottom sediment (Likumahua 2013). It is considered that the accumulation of cysts, so-called cyst beds, has become a potential threat which may initiate blooms of their vegetative cells (Anderson 1989; Mizushima et al 2007). The abundance of the cyst of dinoflagellates was stimulated by the unsuitable of waters condition for the dinoflagellates blooming.

Harmful algae bloom phenomena have widespread progressively to other areas in Indonesia waters, such as in Piru and Elpaputih Bay (Banda Sea), Sorong, Biak, and also in Cendrawasih Bay (Northern Papua), Ujung Pandang surrounding waters (Sulawesi) and

Jakarta Bay (Wiadnyana et al 1996). Therefore, this research was conducted with the main objectives are to determine the occurrence, similarity and abundance of the toxic dinoflagellate in Inner Ambon Bay and Kao Bay through analysis of the cyst in the bottom of sediment.

Material and Method. The research was conducted on June 2016 at six fixed stations along inshore of Kao Bay (Northern part of Halmahera, North Moluccas Province) and Inner Ambon Bay (Moluccas Province) (Figure 1). Geografically, the position of sampling sites in Kao Bay is between 1°8'1.41"-1°8'49.77" N and 127°49'32.88"-127°54'14.29"E. While the position of sampling sites in Inner Ambon Bay is 06.66^{o} 39'29"-03.63^{o} 30'30"S and 128^{o}19'4.03"-128^{o}24'33"E.

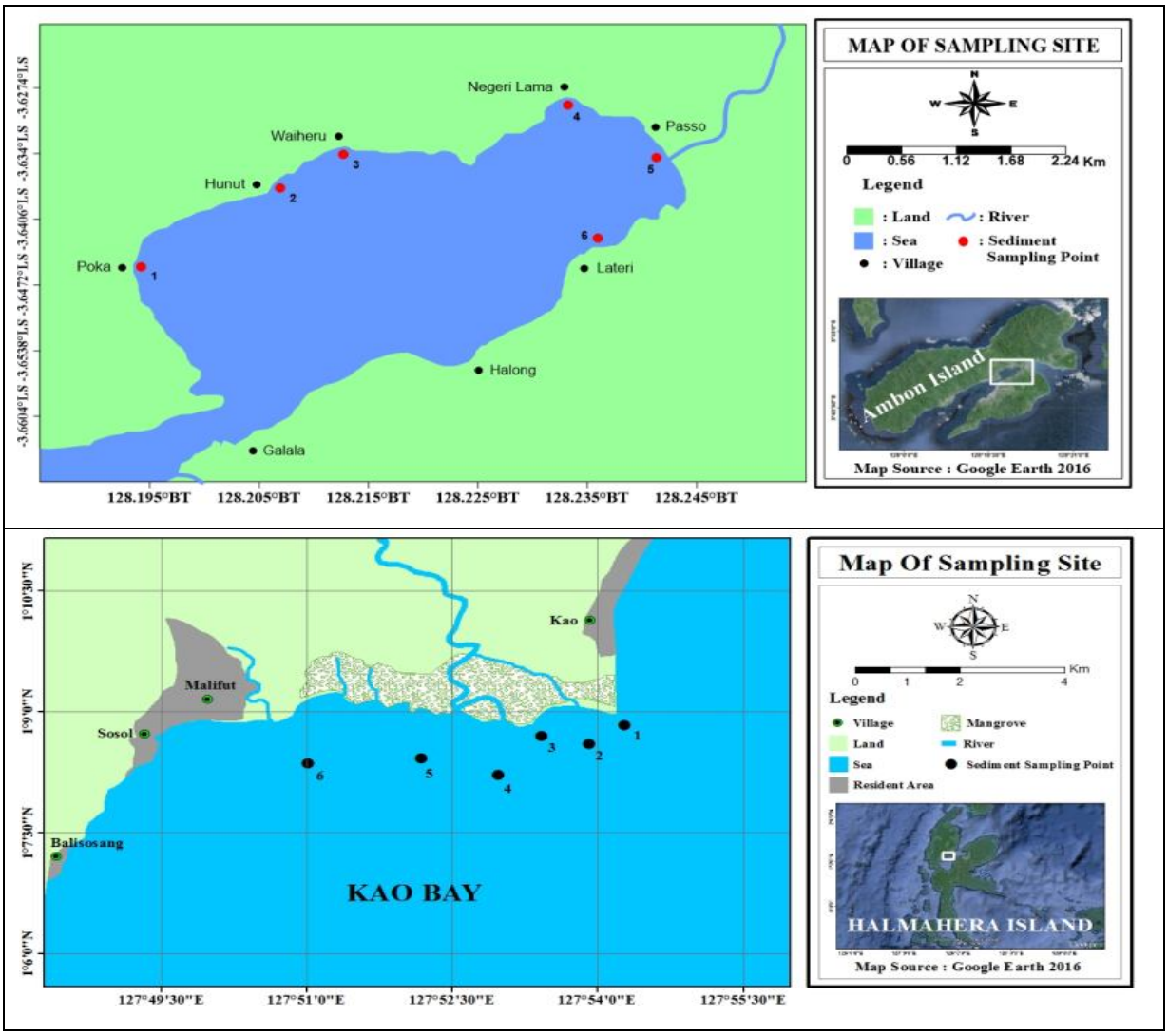

Figure 1. The map of sampling sites in Inner Ambon Bay and Kao Bay.

The sediment samples were collected from 12 stations along the coast of Kao Bay and Inner Ambon Bay by using Ekman Grab and then transferred to polyethylene bottles and stored in the dark at 4^{o}C prior to analysis. Sediment samples were taken from sediments along inshore because the greatest concentrations of dinoflagellata cysts are associated with sediments made up of small-diameter particles.

The cyst were separated from a gram of sediment using the gradient density method and diluted with a mL of 4% formaldehyde and then transferred to sedgewick rafter counting chamber. Identification and counting process were conducted under light microscope (Zeiss Primo Star and Zeiss AxioCam ERc5s) with magnified from 100-400x. Identification of cysts was carried out according to Al-Yamani & Saburova (2010).

Similarity indices (IS) of the cyst of dinoflagellata between stations in Inner Ambon Bay and Kao Bay were calculated according to Odum (1971) as followed:

$$IS = 2C / (A + B)$$

Where: A = total numbers of species at station A;
B = total numbers of species at station B;
C = total numbers of the similar species at stations A and B.

Criterion: IS = 75-100% : very similar;
IS = 50-75% : similar;
IS = 25-50% : dissimilar;
IS = < 25% : different.
Similarity indices were used to perform dendrogram by using cluster analysis with Primer 5.2 programme.

Results and Discussion

Occurrence and similarity. A total of nine genera of the cyst of dinoflagellates were found during the study in which seven genera occurred in Inner Ambon Bay (IAB) and eight genera encountered in Kao Bay (KB) (Table 1). The highest occurrence belonged to *Protoperidinium* which is found in all stations (12 stations), folllowed by *Pyrodinium* (10 stations) i.e. six stations in IAB and four stations in KB. On the contrarary, *Ceratium* had the lowest occurrence which was only found in one station i.e. station 2 at IAB. The rare occurrence of *Ceratium* in Inner Ambon Bay is also reported by Pello et al (2016) during the research on February 2015. The highest occurrence of *Protoperidinium* is not surprising because this genus is one of the most diverse and wide spread groups among marine phytoplankton (Faust 2002). Its species are distributed globally and they often dominate in coastal ecosystem. Meanwhile, distribution pattern of *Pyrodinium* especially *P. bahamense* mostly coincides with areas where mangrove forests are or have been well developed (Usup et al 2012). It is well known that both locations (IAB and KB) have mangrove forest in good condition.

Table 1
Cysts of dinoflagellates found at the 12 fixed stations in Inner Ambon Bay (IAB) and Kao Bay (KB)

Cysts Dinoflagellates	*Station*											
	IAB 1	IAB 2	IAB 3	IAB 4	IAB 5	IAB 6	KB 1	KB 2	KB 3	KB 4	KB 5	KB 6
Alexandrium	+			+	+	+	+	+	+	+		+
Dinophysis	+	+							+			
Ceratium		+										
Gonyaulax	+		+	+	+		+	+		+		+
Gymnodinium										+	+	
Prorocentrum			+	+	+			+				
Protoperidinium	+	+	+	+	+	+	+	+	+	+	+	+
Pyrodinium	+	+	+	+	+	+	+	+	+		+	
Scrippsiella							+	+	+			+

Similarity values of the cyst of dinoflagellates between stations in IAB and KB ranged from 25.0 to 100% or from dissimilar to very similar categories (Table 2). Table 2 enlightened that the similarity values between stations in IAB and KB contained of 66 pairs in which 21 pairs were categorized as very similar (IS = 75-100%), 36 pairs similar (IS = 50-75%) and nine pairs were dissimilar (IS = 25-50%).

The lowest similarity values (IS = 25%) occurred between IAB2 and KB4 as well as between IAB2 and KB6 while the highest occurred between IAB4 and IAB5 (IS = 100%). In general, station IAB2 contributes low similarity values because of genus

Ceratium which is only found in this station. The only similarity of the cyst of dinoflagellates between stations IAB2 and KB4 as well as between IAB2 and KB6 is caused by occurrence of genus *Protoperidium* which is found in all stations.

Table 2

Similarity values (%) of the cyst of dinoflagellates among stations in Inner Ambon Bay (IAB) and Kao Bay (KB)

	IAB1	*IAB2*	*IAB3*	*IAB4*	*IAB5*	*IAB6*	*KB1*	*KB2*	*KB3*	*KB4*	*KB5*
IAB2	66.7										
IAB3	66.7	50.0									
IAB4	80.0	44.4	88.9								
IAB5	80.0	44.4	88.9	100							
IAB6	75.0	57.1	57.1	75.0	75.0						
KB1	80.0	44.4	66.7	80.0	80.0	75.0					
KB2	72.7	44.4	80.0	90.9	90.9	66.7	90.9				
KB3	80.0	66.7	44.4	60.0	60.0	75.0	80.0	72.7			
KB4	66.7	25.0	50.0	66.7	66.7	57.1	66.7	60.0	44.4		
KB5	50.0	57.1	57.1	50.0	50.0	66.7	50.0	44.4	50.0	57.1	
KB6	66.7	25.0	50.0	66.7	66.7	57.1	88.9	80.0	66.7	66.7	28.6

Abundance and distribution of the cyst of dinoflagellates. The abundance of the cyst of dinoflagellates in the Inner Ambon Bay and Kao Bay is presented in Figure 2. In general, cyst of dinoflagellates in KB (mean 10.8 cells mL^{-1}) was more abundant than in IAB (mean 6.4 cells mL^{-1}). The highest abundance was found at station KB3 (47 cells mL^{-1}), whereas the lowest abundance was represented by two stations i.e. IAB6 and KB5 (8 cells mL^{-1}).

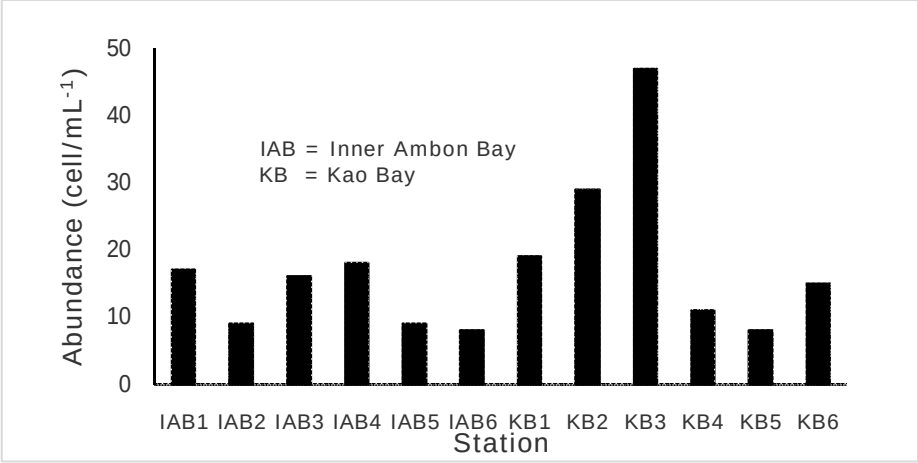

Figure 2. The abundance of the cyst in Inner Ambon Bay (IAB) and Kao Bay (KB).

Abundance of the cyst of Dinoflagellates (%) for each genera in every station is presented in Table 3. It can be seen in Table 3 that *Protoperidium* is the most abundant genera in all stations during the study, except for stations IAB2, KB2 and KB3 which are represented by genera of *Pyrodinium, Alexandrium* and *Scrippsiella*, respectively. Based on location, *Protoperidium* occupied 48.6% (mean 6.0 cells mL^{-1}) in IAB and 42.6% (mean 9.2 cells mL^{-1}) in KB of the total cyst of dinoflagellate found during the study. The others most abundance genera in both locations were *Alexandrium* and *Pyrodinium* which represented 15.5% (mean 2.7 cells mL^{-1}) and 12.1% (mean 2.1 cells mL^{-1}) of the total cyst of dinoflagellate, respectivelly. The abundance of *Alexandrium* in IAB during this study (11.7%) is higher than the abundance found by Pello et al (2016) on February 2015 (0.02%). On the contrarary, the abundance of *Pyrodinium* in IAB (3.2 cells mL^{-1}) is lower

than the abundance reported by Likumahua (2013) for the period of early July 2012 (5.99 cells mL^{-1}). The author also reported that during the bloom on 12 July 2012, the abundance of *Pyrodinium* reached >1000 cells mL^{-1} in red-brown water discoloration when sampling was conducted at center of the bloom.

Table 3

Abundance of the cyst of dinoflagellates (%) in Inner Ambon Bay (IAB) and Kao Bay (KB)

Dinoflagellata	*IAB1*	*IAB2*	*IAB3*	*IAB4*	*IAB5*	*IAB6*	*KB1*	*KB2*	*KB3*	*KB4*	*KB5*	*KB6*
Alexandrium	5.9	0.0	0.0	22.2	22.2	25.0	21.0	34.5	12.8	9.1	0.0	13.3
Ceratium	0.0	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dinophysis	5.9	11.1	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0
Gonyaulax	5.9	0.0	12.5	5.6	22.2	0.0	5.3	3.5	0.0	9.1	0.0	6.7
Gymnodinium	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.3	12.5	0.0
Prorocentrum	0.0	0.0	12.5	5.6	11.1	0.0	0.0	3.5	0.0	0.0	0.0	0.0
Protoperidium	52.9	33.3	43.7	50.0	33.4	62.5	57.9	31.0	29.8	54.5	62.5	66.7
Pyrodinium	29.4	44.5	31.3	16.6	11.1	12.5	5.3	3.5	4.3	0.0	25.0	0.0
Scrippsiella	0.0	0.0	0.0	0.0	0.0	0.0	10.5	24.0	51.0	0.0	0.0	13.3

Figure 3 shows the result of cluster analysis by using similarity index and its displayed that the cysts of dinoflagellates found on June 2016 in IAB and KB can be classified into four groups (A, B, C and D). The group was formed by the existing and the abundances of certain genera of the cyst found at both stations:

- group A, represented by station IAB2 is characterized by the existing of *Ceratium* which is found only in this station;
- group B, represented by stations IAB1, IAB3, IAB4 and IAB5, which is dominated by *Gonyaulax*;
- group C, represented by stations KB3, KB2, KB1 and KB6. This group is characterised by the existing of *Scrippsiella* and *Alexandrium*;
- group D, represented by stations KB4, IAB6, and KB5. This group is considered by the high numbers of *Protoperidinium*.

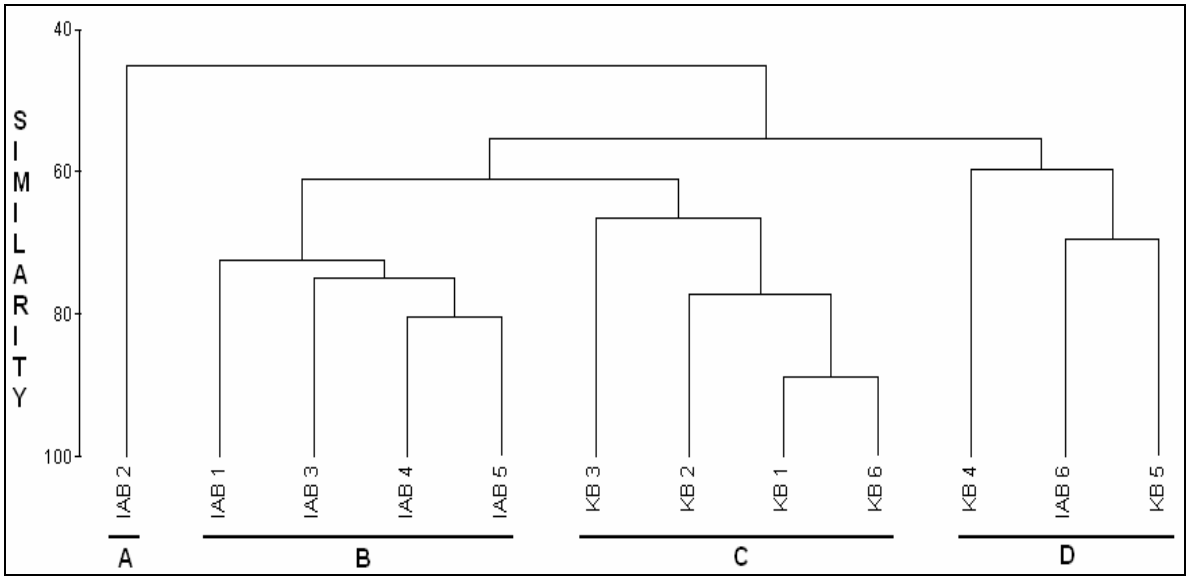

Figure 3 . Dendogram of stations grouping based on the similarity of cyst of dinoflagellata in Inner Ambon Bay (IAB) and Kao Bay (KB).

Conclusions. The cyst of dinoflagellates from the stations in Inner Ambon Bay and Kao Bay consists of nine genera, which are distributed almost in all stations. The exception are *Gymnodinium* and *Scrippsiella* which occurred only in Kao Bay and *Ceratium* which is found only in Inner Ambon Bay. The abundances of the cysts are highest in Kao Bay

especially at station 3, and the lowest abundance are in the Inner Ambon Bay (station 6) and in Kao Bay (station 5). Similarity among stations in the Inner Ambon Bay and Kao Bay can be categorised into very similar to dissimilar. Based on the similarity of the cyst, stations in this study can be grouped into four groups.

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