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Dinoflagellate cyst distribution in surface sediments of Ambon Bay (eastern Indonesia): Environmental conditions and harmful blooms

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ABSTRACT

The present study aimed to document dinocyst ecological preferences in Ambon Bay, Eastern Indonesia, and to investigate if the bay sediments serve as a seedbank for toxic bloom events. To this end, dinocyst and geochemical analyses of surface sediment samples were performed, along with physicochemical water column parameters. Twentythree dinocyst species were identified, and high dinocyst concentrations (up to ~12,000 cysts g⁻¹ dry sediment) were found in the inner bay. Environmental factors such as surface water temperature and salinity generally played an important role in dinocyst distribution. The concentration of *Polysphaeridium zoharyi* cysts showed a strong positive correlation with phosphorus. A statistically significant correlation was also found with the concentration of other autotrophic dinocysts in the sediments, and an inverse correlation was observed with the sediment C/N ratio. Cysts may serve as seedbanks for *Pyrodinium bahamense* blooms in the area.

1. Introduction

Dinoflagellates are a group of aquatic eukaryotic organisms that play an important role in the marine realm as primary (phototrophic/autotrophic ones) and secondary (heterotrophic ones) producers. Many dinoflagellate species are associated with harmful algal blooms (HABs), resulting in major negative socio-economic impacts and human health problems (e.g., review in Anderson et al., 2012; Hinder et al., 2012; Wells et al., 2020). Consequently, harmful representatives of this taxonomic group are subject to many studies focusing on their ecology, toxicology, and their complex life cycles (Bravo and Figueroa, 2014). Based on modern surface sediment studies, approximately 15% of the roughly 2000 marine dinoflagellate species are known to produce organic-walled resting cysts, hereafter referred to as dinocysts (Head, 1996; Matsuoka et al., 2013; Bravo and Figueroa, 2014), which are produced mostly after sexual reproduction. The cysts (benthic stages)

deposit and rest in the sediment until hatching occurs. Cysts may hence serve as a source of bloom initiation (seedbanks) in the water column when favorable conditions occur (Dale, 1983; Usup et al., 2012; Bravo and Figueroa, 2014). As dinocysts preserve well in sediments, they are one of the most important proxies used to study present and past dinoflagellate communities and environmental changes (deVernal and Marret, 2007; Uddandam et al., 2017). Successful reconstructions of past environments rely on the knowledge of modern dinocyst ecological preferences. Moreover, studies of modern dinocysts are useful to trace present and predict future (toxic) bloom events of a certain species in a particular area. Cysts provide insight in the ecology, biogeography, taxonomy and phylogeny of living species (Aydin and Uzar, 2014).

Environmental factors (e.g., water temperature, salinity, nutrients, and oxygen) are known to influence dinocyst distribution and community composition in marine environments (Zonneveld et al., 2013; Marret et al., 2019). In coastal areas, especially near river mouths,

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increased cyst abundance of some species (e.g., *Brigantidinium* spp., *Echinidinium* spp., *Lingulodinium machaerophorum*, *Polykrikos kofoidii/schwarzii*) and high total cyst concentrations are generally related to high nutrient input (Sangiorgi and Donders, 2004; Zonneveld et al., 2009; Zonneveld et al., 2013). In the inland bays of the Northwest Atlantic, a combination of elevated temperatures and enhanced nutrient load (typically linked to human-related eutrophication) positively correlated with the abundance of *Polysphaeridium zoharyi*, cyst of the toxic *Pyrodinium bahamense* (Price et al., 2017). This cyst was also reported to occupy tropical and subtropical coastal environments, characterized by high temperatures (e.g., Gulf of Mexico lagoons, Florida shelf; Limoges et al., 2013; Furio et al., 2012; Limoges et al., 2015; Price et al., 2016).

The toxic *Pyrodinium bahamense* var. *compressum*, has been recorded to thrive in Ambon Bay, Eastern Indonesia, and its density has been monitored since paralytic shellfish poisoning (PSP) events that occurred in July 1994 and 2012 (Wiadnyana et al., 1996; Likumahua, 2013). Other potential PSP species, *Gymnodinium catenatum* and *Alexandrium* spp., have been found frequently in phytoplankton samples obtained from the local monitoring program (Likumahua et al., 2020). In addition, recently, blooms of a non-toxic unidentified *Gonyaulax* species were recorded in the bay, resulting in water discolorations and low dissolved oxygen availability (data unpublished). Given the fact that various vegetative dinoflagellate cells have been found frequently and some of the species formed blooms and toxic outbreaks in the bay and some of them formed cysts, dinocyst assemblages in the upper sediments have become a highly relevant study topic. Mizushima et al. (2007) for the first time described dinocyst assemblages from one location in Ambon Bay (inner bay, red dot in Fig. 1) analyzing a sediment core collected in 1995. In their study, approximately 23 dinocyst species were determined in the upper 2 cm sediment, with a total density of 5631 cysts g⁻¹ dry sediment. Based on the ²¹⁰Pb-based age model, the sediment accumulation rate is ~0.39 cm/year (Mizushima et al., 2007), and the upper 2 cm sediment hence represents a time interval of ~4 years (1991–1995). Their results also showed that cysts of the toxic *Pyrodinium bahamense* var. *compressum* (*Polysphaeridium zoharyi*) were found in samples above 56–58 cm depth, suggesting the first appearance in ca. 1850, whereas the highest cyst densities were observed in more recent sediments.

This recent study is designed to expand our knowledge on dinocysts spatial distribution in the surface sediments of Ambon Bay. Here, we analyze dinocyst assemblages in 10 surface sediment samples throughout the inner and outer Ambon Bay (Fig. 1) to investigate whether the bay sediments serve as seedbanks for present and future harmful algal outbreaks. One of our surface sediments (sample 5, Fig. 1) is taken very close to the location of the published sediment core (Mizushima et al., 2007), as our surface sample represents a recent time interval compared to the uppermost sediment analyzed in the core. In addition, we wanted to provide information on dinocyst environmental preferences in shallow, coastal tropical environments by correlating surface water parameters (salinity, temperature, and nutrients such as phosphate and nitrate) and organic carbon (C_{org}), total nitrogen (N_{tot}) and phosphorous (P_{tot}) in surface sediments with dinocyst assemblages.

2. Materials and methods

2.1. Study area and sampling stations

Ambon Island (3°38'17"S and 128°07'02"E) is located in Maluku Province, Eastern Indonesia. The island is inhabited by roughly 450,000 people, of which the majority lives near the coastline. The island has a tropical climate with high rainfall during the wet season, associated with the southeast monsoon occurring from March to September. The dry season is characterized by relatively low precipitation and increased air temperature during the northwest monsoon between October and March. Monsoons are associated with upwelling and downwelling processes in the Banda Sea, which influence physicochemical processes in Ambon Bay (Wyrki, 1961; Boëly et al., 1990; Zijlstra et al., 1990). Relatively low surface water temperature and high surface salinity as consequence of upwelling in Banda Sea are detected in Ambon Bay between June and August (during the southeast monsoon) (Wenno and Anderson, 1984; Corvianawatie et al., 2014; Likumahua et al., 2019). The bay is a semi-enclosed estuary, which has a narrow sill dividing the bay into an inner and outer part (Figs. 1 & 2).

Plankton and water physicochemical parameters are monitored by the Centre for Deep Sea Research Institute (LIPI) since 2008 at stations 1, 2, 3, 4, 5, and 6 in the inner bay and at stations 8 and 10 in the outer bay. Stations 1 and 7 in this study were chosen as a massive bloom of

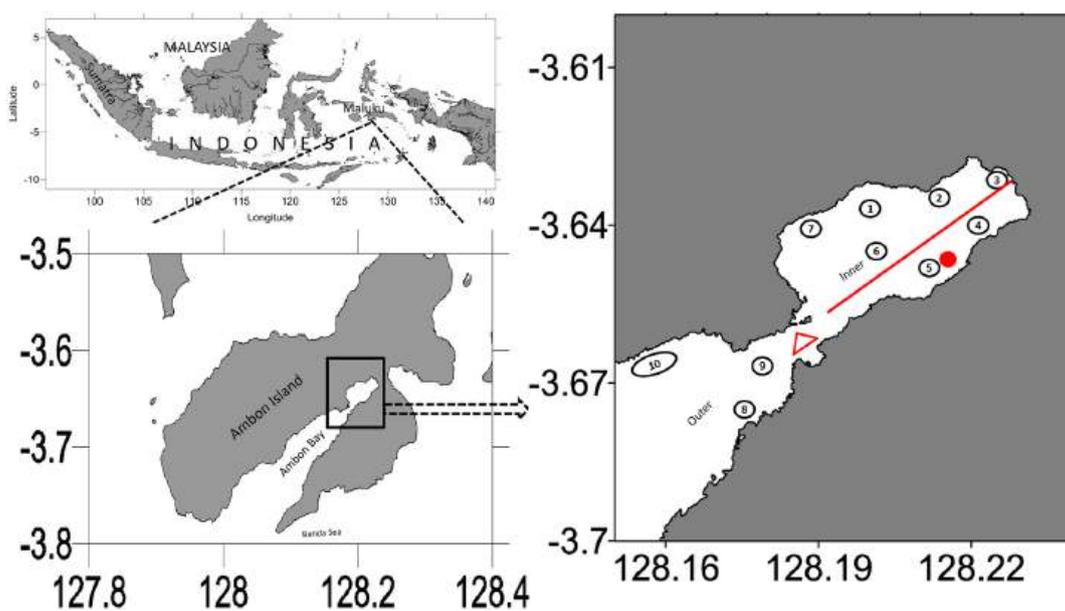


Fig. 1. Map of Ambon Bay with sampling stations (numbers 1–10). The red triangle shows the sill's location, the red dot is the approximate location of the sediment core collected by Mizushima et al. (2007), and the red line is the location of the cross section given in Fig. 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

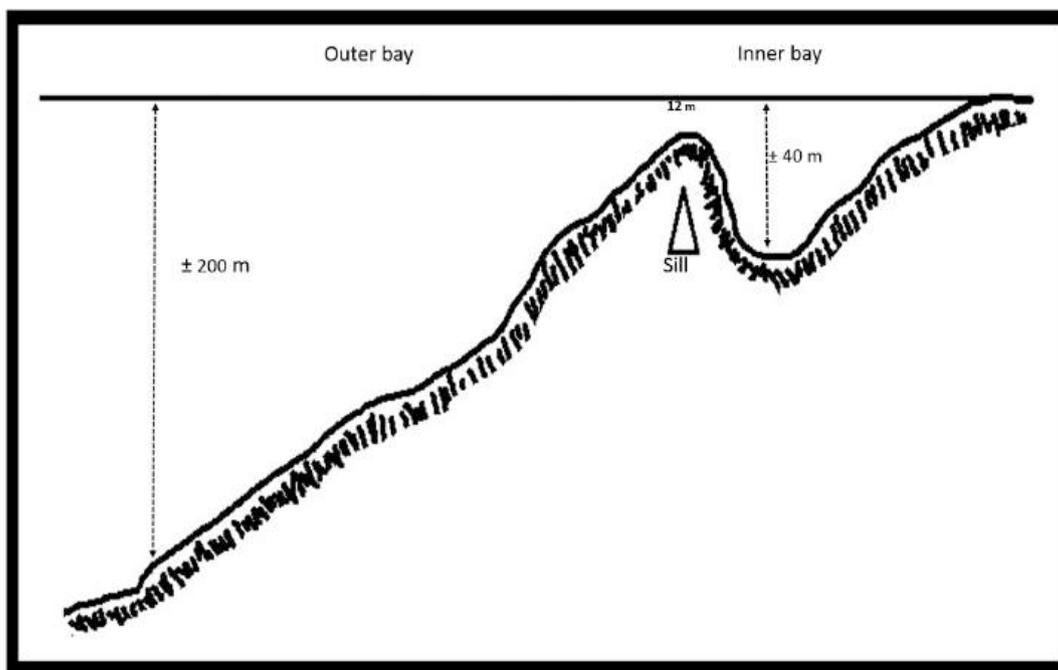


Fig. 2. Cross section of Ambon Bay.

Pyrodinium bahamense had occurred in that area during the dry season of 2013 (monitoring results, data unpublished). Two bloom events of this toxic species were also recorded at stations 4, 5, and 6 during the wet seasons of 1994 and 2012 (Wiadnyana et al., 1996; Likumahua, 2013). Furthermore, a massive *Gonyaulax* spp. bloom occurred recently in January 2019, during the dry season, which covered the area of all stations in the inner bay, except station 3 (data unpublished). Station 3 is located in the proximity of a highly populated area and receives a high level of untreated wastewater discharge. Substantial agricultural activity occurs in the area between stations 1 and 2. Here, two small rivers are streaming into the inner bay. Several floating fish cages (private aquaculture businesses) are located in the inner bay in the area close to stations 1, 4, and 5. Three stations in the outer bay were chosen as control (less disturbed) stations to observe dinocyst assemblages and abundance in areas where no toxic phytoplankton blooms had ever been recorded.

2.2. In situ water physicochemical parameters

Water physicochemical data used in the present study were obtained from LIPI monitoring program (2010–2014) supplemented with a high frequency field campaign carried out between 2018 and 2019. A

compact Alec CTD Model ASTD 687 was deployed to measure depth, temperature, and salinity. Data recorded by the CTD was used to calculate average temperature and salinity in the upper 2 m of the water column. Water samples for nutrient analysis were collected by deploying a 3.5 L Niskin bottle at approximately 0.30 m depth. Dissolved nutrients, nitrate (NO_3) and phosphate (PO_4) were measured using a spectrophotometer (UV-Vis Shimadzu 1700) following the method of Strickland and Parsons (1972) and APHA (1998). Seasonal and annual data for surface water physicochemical parameters were calculated and expressed as average values (Table 1) to be further used in statistical analyses.

2.3. Sediment sampling and analyses

Surface (0–2 cm) sediment samples were collected during the wet season of 2019 at 10 sampling stations, of which seven were located in the inner bay and three in the outer bay (Fig. 1). Sediment samples, likely representing the last ~5–10 years following the estimated sediment accumulation rates from Mizushima et al. (2007) were collected with a box core sampler at stations where water depths exceeded 18 m, and for the shallower stations with water depth < 15 m, sampling was done using scuba diving (Table 1). Polyvinyl chloride (PVC) cylinders

Table 1
Station numbers, depths and average surface water physicochemical parameters.

Station	Depth (m)	Temperature ($^{\circ}\text{C}$)			Salinity			Phosphate (μM)			Nitrate (μM)		
		dry	wet	annual	dry	wet	annual	dry	wet	annual	dry	wet	annual
Inner bay													
1	14	29.61	27.69	29.10	32.56	31.69	32.08	0.75	0.76	0.73	1.02	2.90	1.59
2	22	30.61	28.74	29.13	32.53	31.75	32.10	0.43	1.05	0.72	0.69	1.75	0.85
3	10	30.85	28.87	29.30	32.01	31.48	31.71	0.68	1.29	0.89	1.51	2.21	1.93
4	22	29.92	28.08	29.25	32.02	31.42	31.69	0.26	1.59	0.86	2.00	4.32	3.37
5	23	30.49	28.83	29.12	32.09	31.32	31.66	0.26	1.77	1.10	1.00	3.89	0.73
6	28	30.46	28.44	28.90	32.38	31.78	32.05	0.59	1.31	0.93	0.83	1.32	0.63
7	12	30.40	28.51	28.90	32.29	32.11	32.19	0.61	1.21	1.00	1.02	1.09	1.29
Outer bay													
8	20	28.87	27.81	28.28	33.58	32.95	33.23	1.37	1.49	1.54	1.97	3.21	2.64
9	31	28.96	27.86	28.35	33.67	32.97	33.28	0.86	0.71	0.84	0.88	1.16	1.17
10	17	28.94	27.70	28.25	33.69	32.75	33.17	1.26	0.30	0.72	1.54	0.98	1.19

(diameter 5 cm, length 15 cm) were used to collect surface sediments by the divers and from the boxcore. Samples were subsequently stored at -20°C until further analysis. The upper part 2 cm of the frozen samples was sliced and placed in small (6 cm \times 10 cm) plastic bags and stored in the freezer, and subsequently freeze dried before analyses.

Each sediment sample was subsampled for palynological (cyst species determination and counting) and geochemical analyses, both were performed at Utrecht University, The Netherlands. Approximately 5 to 7 g of freeze-dried sample was used for palynological analyses. Two *Lycopodium clavatum* tablets of 19,332 spores ($X = 9666$ per tablet, $S = \pm 2123$ and $V = \pm 2.2\%$) were added to each sample, which was subsequently treated with 10% hydrochloric acid (HCl) to remove carbonates and with 38% cold hydrofluoric acid (HF) to remove silicates. The treatment was repeated twice (e.g., Sangiorgi and Donders, 2004), and the residues were sieved through a 10- μm sieve. Samples were homogenized with a micropipette, and approximately 10–50 μL subsample was placed on a microscope slide, covered with a cover slip and sealed with nail polish. Dinocysts taxonomy follows Williams et al. (2017). Photographic materials in Rochon et al. (1999) and Zonneveld and Pospelova (2015) were also used for the identification of modern dinocysts. Cyst species determination and counting were performed under an Olympus LH 50A light microscope using 200 x and 400 x magnifications. Percentage (%) of each dinocyst species was calculated on the total dinocyst counts. The absolute abundance was expressed as the number of cysts per gram dry weight (cysts g^{-1} DW).

Geochemical parameters such as % organic carbon (C_{org}), total nitrogen (N_{tot}), and total phosphorous (P_{tot}) were analyzed from the remaining dried samples. Samples for the C_{org} (and N_{tot}) were first decalcified using 1 M HCl (Van Santvoort et al., 1996) and subsequently measured with a Fison-type NA 1500 NCS elemental analyzer. International reference material (internationally calibrated soil standard IVA2, certified value = 0.732 wt% C (Van Helmond et al., 2020)) and replicate standards showed a precision and accuracy <2%. The unit for C_{org} and N_{tot} were expressed in weight percentage (wt%). Aliquots of dried sediments were also digested with an $\text{HClO}_4\text{-HNO}_3\text{-HF}$ acid mixture and subsequently measured for P and other elements (expressed in part per million (ppm)) by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) with a Spectro Ciros Vision ICP-OES. The precision (relative standard deviation) was <5%.

2.4. Statistical analyses

Statistical analyses were performed using the Minitab 18 package. The relationship among dinocyst species, water physicochemical, and sediment geochemical parameters were evaluated using multivariate Principal Component Analysis (PCA) and Pearson correlation ($p < 0.05$) analyses. Data of all parameters were standardized to eliminate different physical units before the ordination in the PCA as described by Legendre and Birks (2012).

3. Results

3.1. Dinocyst percentages and concentrations in the upper sediment samples

Dinocysts were found in all sediment samples collected in Ambon Bay and comprised 23 cyst species. The heterotrophic *Brigantedinium* spp. (Fig. 3) was present in all samples with percentages ranging between 29.9% and 78.4% (average 54.6%) (Fig. 4A and Table S1). The cyst of the PSP toxin producer *Pyrodinium bahamense*, *Polysphaeridium zoharyi* (Fig. 3), was detected in almost all sediment samples and was found as the second most abundant dinocyst species. The percentage of this species ranged between 0% and 67% with an average of 30% of total cyst abundance. Other common species were *Lingulodinium machaerophorum*, *Operculodinium longispinigerum* and *Quinquecuspidata concreta* with the highest percentages of 8.1%, 9.9%, and 5.4%, respectively (Fig. 4A

and Table S1). Four species of *Spiniferites* were identified: *Spiniferites mirabilis*, *S. ramosus*, *S. delicatus* and *S. bentorii*, comprising the highest percentage of 9.7%. Heterotroph dinocyst species percentages were higher than autotrophs in all stations, with the highest percentage of 84% and 80% in stations 3 and 8, respectively (Fig. 4A and Table S1).

Generally, cyst abundances in the inner bay were higher than those in the outer bay. In the inner bay, total dinocyst abundances ranged between 3083 cysts g^{-1} DW and 11,700 cysts g^{-1} DW at stations 3 and 4, respectively (Fig. 4B and Table 2). Cyst concentrations at stations 1 and 5 were comparable with that at station 4. The concentration in the outer bay ranged between 425 cysts g^{-1} DW and 1433 cysts g^{-1} DW, of which the highest was at station 8. Similarly, high abundances of *P. zoharyi* were found at stations in the inner bay, of which the highest concentration was detected at station 4 (5975 cysts g^{-1} DW) (Fig. 4B and Table 2). Other than station 4, *P. zoharyi* abundances were also high at stations 1, 5, 6, and 7, while it was low at station 2 and absent at station 3 (Fig. 4B). In the outer bay, *P. zoharyi* abundances were detected in low concentrations, yet comparable among stations, ranging between 100 cysts g^{-1} DW and 175 cysts g^{-1} DW.

The dominant *Brigantedinium* spp. (Fig. 3) abundances ranged between 300 cysts g^{-1} DW and 5225 cysts g^{-1} DW with an average of 2249 cysts g^{-1} DW (Table 2 and Fig. 4C). Other abundant species such as *L. machaerophorum*, *Q. concreta*, cyst of *Protoperidinium nudum* and *Selenopemphix quanta* had average concentrations of 165 cysts g^{-1} DW, 74 cysts g^{-1} DW, 68 cysts g^{-1} DW, and 60 cysts g^{-1} DW, respectively (Fig. 4C). The four species of the genus *Spiniferites* comprised an average concentration of 148 cysts g^{-1} DW, and *S. bentorii* was the species with the lowest concentration (Fig. 4C).

3.2. Physicochemical properties of the water column

The average sea surface temperature (SST) during the wet season at the inner bay stations was one degree higher than that in the outer part. In the inner bay, the average temperature ranged between 27.69°C and 28.87°C , while it ranged between 27.70°C and 27.86°C in the outer bay (Table 1). During the dry season, the average SST in the inner bay was approximately 1.2°C higher than that in the outer bay: between 29.60°C and 30.49°C for the inner bay, and between 28.87°C and 28.96°C for the outer bay. The annual SST ranged between 28.25°C and 29.30°C (Table 1). Inversely, during the wet season, the average sea surface salinity (SSS) levels were higher in the outer bay than in the inner part. SSS levels ranged between 32.75 and 32.97 in the outer bay, whereas the level in the inner bay ranged between 31.32 and 32.11. Similarly, during the dry season, high SSSs were recorded in the outer bay ranging between 33.58 and 33.69, while the levels in the inner bay ranged between 32.01 and 32.56 (Table 1). Average phosphate concentrations were higher during the wet season (ranging between 0.30 μM and 1.77 μM) than during the dry season (ranging between 0.26 μM and 1.37 μM). The annual average of phosphate concentrations ranged between 0.72 μM and 1.54 μM (Table 1). Average nitrate concentrations were found to be higher during the wet season, ranging between 0.98 μM and 4.32 μM . During the dry season, the concentration ranged between 0.69 μM and 2.0 μM , while the annual averages were between 0.63 μM and 3.37 μM (Table 1).

3.3. Sediment geochemistry

Organic carbon (C_{org}) percentages were higher in the inner bay stations than in the outer stations, ranging between 2.24 (wt%) and 5.18 (wt%). The highest C_{org} percentage was recorded at station 3, and the lowest at station 8 (Fig. 5A). C_{org} between 1.28 (wt%) and 1.90 (wt%) were recorded in outer bay. Likewise, N_{tot} at the inner bay stations was higher than at the outer stations. Highest N_{tot} values of 0.35 (wt%) and 0.30 (wt%) were found at stations 5 and 3 in the inner bay with (Fig. 5B), while percentages in the outer bay were 0.10 (wt%) and 0.15 (wt%). Spatially, the distribution of P_{tot} in the sediment of Ambon Bay was

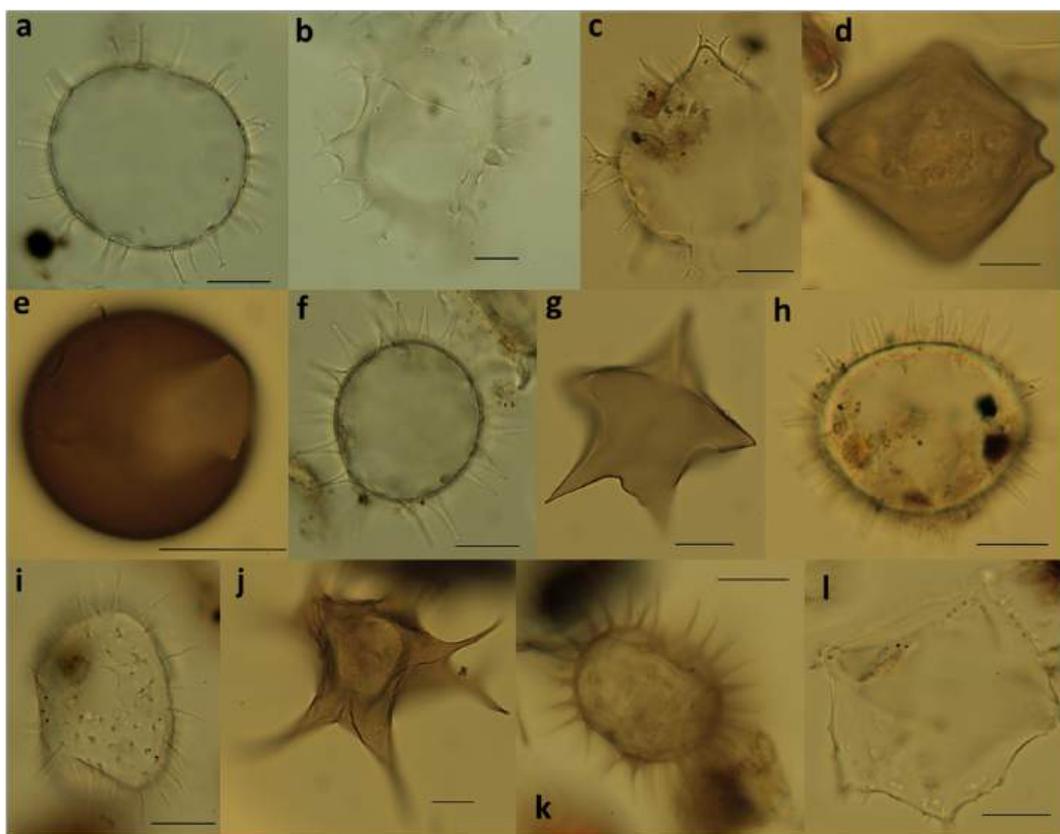


Fig. 3. Dinocyst light microscope photographs (scale bar = 20 μm). a) *Polysphaeridium zoharyi*, b) *Spiniferites mirabilis*, c) *S. bentorii*, d) *Selenopemphix nephroides*, e) *Brigantedinium* sp. f) *Dapsilidinium pastielsii*, g) *Lejeunecysta sabrina*, h) *Lingulodinium machaerophorum*, i) *Operculodinium longispinigerum*, j) *Stelladinium robustum*, k) cyst of *Protopteridinium nudum*, l) *Trinovantedinium applanatum*.

comparable between the inner and outer part. High P_{tot} concentrations were found at stations in the inner bay, ranging between 460.10 ppm and 638.10 ppm, at stations 3 and 5, respectively (Fig. 5C). In the outer bay, P_{tot} concentration was generally lower ranging between 394.33 ppm and 476.50 ppm at station 10 and at station 9, respectively.

We calculated the $C_{\text{org}}/N_{\text{tot}}$ ratio (C/N), which is often used to trace organic matter origin, whether predominantly marine (C/N ~ 7) or from soil and terrestrial (C/N 8 to >20) origin (e.g., Nasir et al., 2016). The ratio varied among stations in the inner bay stations, and the outer bay showed similar values. In the inner bay, $C_{\text{org}}/N_{\text{tot}}$ ratios ranged between 9.28 (station 5) and 17.00 (station 3), while in the outer bay, it ranged between 12.71 (station 8) and 13.76 (station 10) (Fig. 5D). The ratio of C and P ($C_{\text{org}}/P_{\text{tot}}$) was also calculated as it could indicate sediment anoxia and hence preferential release of P compared to C (Algeo and Ingall, 2007; Sulu-Gambari et al., 2018). The highest $C_{\text{org}}/P_{\text{tot}}$ ratio of 289.73 was recorded at station 3 in the inner bay (Fig. 5E). Ratios for other stations were generally two times lower than those with station 3. Generally, the inner bay stations showed higher ratios than those in the outer bay. In the outer bay, the ratio ranged between 81.25 and 102.61 (Fig. 5E).

3.4. Relationship between cysts and environmental drivers

The PCA plot analysis of all parameters analyzed showed distributions of predominant dinocysts and environmental factors (Fig. 6). The dinocyst *O. longispinigerum* plotted separately from other species, and it correlated positively with salinity and dissolved phosphate in the dry season (P_{dry}). Likewise, the dominant species, *Brigantedinium* spp., and in general, most of the heterotroph dinocysts positively correlated with $C_{\text{org}}/N_{\text{tot}}$ ratios (C/N in Fig. 6). Dinocyst species such as *L. machaerophorum*, *S. delicatus* and *Q. concreta* clustered with C_{org} , N_{tot} ,

$C_{\text{org}}/P_{\text{tot}}$ (C, N and C/P, respectively, in Fig. 6 and Table 3) in sediments. In addition, these species (except *S. delicatus*) positively correlated with water temperature (T_{annual} , T_{wet} , and T_{dry} in Fig. 6 and Table 3) and dissolved phosphate concentrations (P_{wet} in Fig. 6) during the wet season. *Polysphaeridium zoharyi* clustered with the % of autotrophic species, to which it contributed the most, and did not show any significant correlations with other parameters.

Pearson correlation analyses (Table 3) revealed that the autotroph dinocysts strongly correlated ($r = 0.95$; $p < 0.001$) with the concentration of *P. zoharyi*, while the heterotrophs and *Brigantedinium* spp. showed a strong relationship ($r = 0.97$; $p < 0.001$). A positive correlation was found between *L. machaerophorum* and *Q. concreta* ($r = 0.68$; $p < 0.05$). Dinocyst *O. longispinigerum* revealed strong positive correlations with environmental drivers such as seasonal and annual salinity ($r > 0.80$; $p < 0.01$) and dissolved phosphate concentrations during the dry season P_{dry} ($r = 0.89$; $p < 0.01$), while it showed inverse relationships with both seasonal and annual temperature ($r > -0.65$; $p < 0.05$) (Table 3). In contrast, *Q. concreta* was positively correlated to seasonal and annual average temperature ($r > 0.65$; $p < 0.05$). *L. machaerophorum* showed a positive significant correlation with total organic carbon (C in Table 3 and Fig. 6, $r = 0.66$, $p < 0.05$). *Q. concreta* also positively and significantly correlates with C_{org} ($r = 0.82$, $p < 0.05$) and $C_{\text{org}}/P_{\text{tot}}$ (C/P in Table 3 and Fig. 6, $r = 0.82$, $p < 0.05$).

4. Discussion

4.1. Dinocyst distribution

Dinocyst abundances in this study were higher (doubled) than those found in the upper 0–2 cm sediment collected in 1995 (Mizushima et al., 2007), implying that in recent years, dinocyst production in Ambon Bay

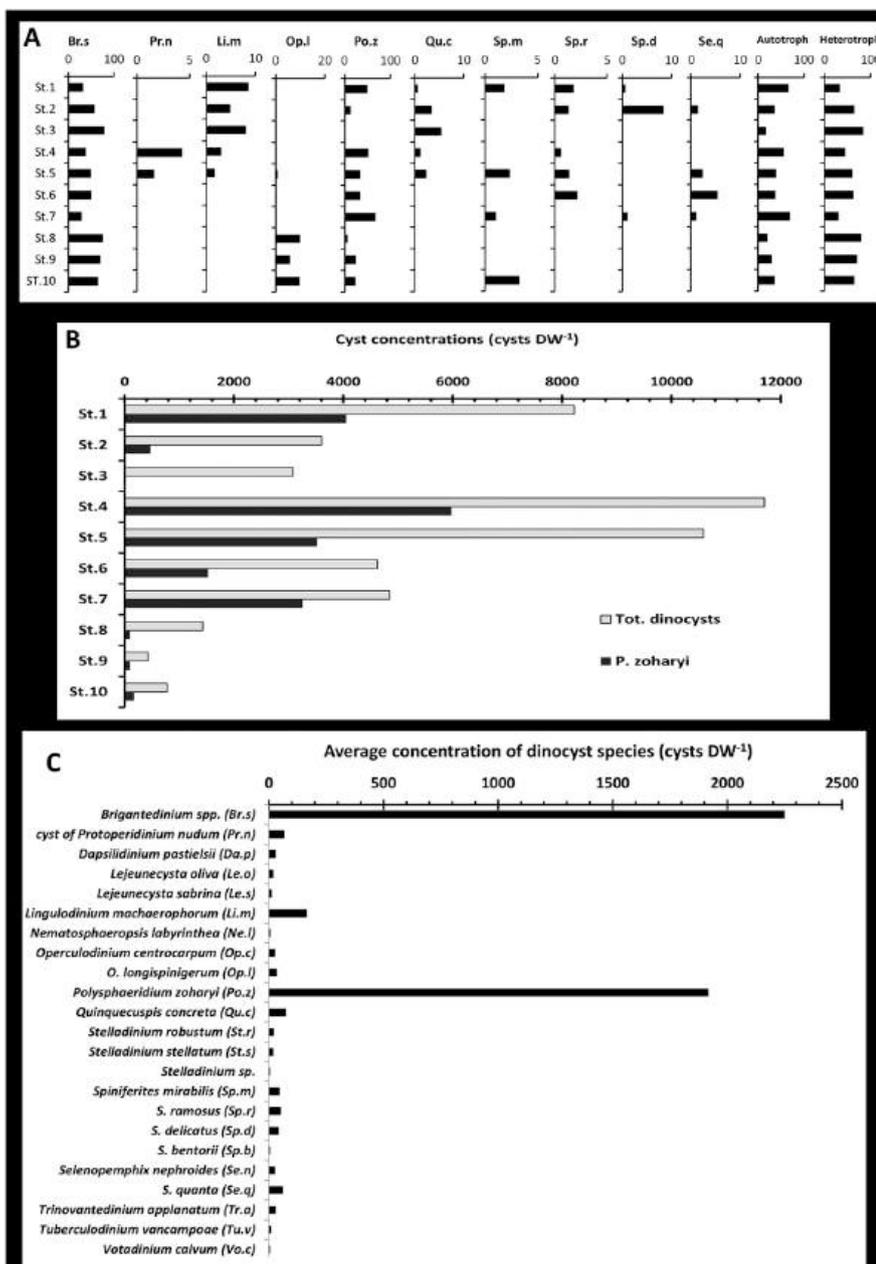


Fig. 4. Dinocysts in the upper 2 cm of sediment in Stations 1 to 10, Ambon Bay, Indonesia. A) Percentages of the most abundant dinocyst species. Abbreviations: *Brigantedinium* spp. (Br.s), cyst of *Protoperidinium nudum* (Pr.n), *Lingulodinium machaerophorum* (Li.m), *Operculodinium longispinigerum* (Op.l), *Polysphaeridium zoharyi* (Po.z), *Quinquecuspsis concreta* (Qu.c), *Spiniferites mirabilis* (Sp.m), *S. ramosus* (Sp.r), *S. delicatus* (Sp.d), *Selenopemphix quanta* (Se.q); B) Total dinocyst and *Polysphaeridium zoharyi* concentrations in sediments (cysts g⁻¹ dry weight) in each of the 10 Stations; C) Average concentrations (cysts g⁻¹ dry weight) of dinocyst species in the sediments of Ambon Bay. Stations 1 to 7 are located in the inner bay and 8 to 10 in the outer bay (see Figs. 1 and 2 for approximate water depth).

increased. *Brigantedinium* spp. was the most dominant dinocyst, found in all samples. The dominance of the heterotrophic *Brigantedinium* spp. in a dinocyst assemblage generally occurs in sediments where high nutrient discharges in densely inhabited coastal areas that fuels high primary productivity (Marret, 1994; Sangiorgi and Donders, 2004; Leroy et al., 2013; Zonneveld et al., 2013) as heterotrophic dinoflagellates thrive on organic matter (Gaines and Taylor, 1984; Jacobson and Anderson, 1986). *Brigantedinium* spp. is a cosmopolitan group, found in brackish to high saline environments (Price et al., 2018; Marret et al., 2019). It also dominates dinocyst assemblages in regions with high intensity of upwelling (e.g., Bringué et al., 2018). Thus, the combination of frequent nutrient discharges from land and regular upwelling during the wet season in Ambon Bay likely explain the dominance of this group in the surface samples.

Autotrophic cysts such as the four *Spiniferites* species did not show a clear distribution in Ambon Bay; however, they were more frequent in the inner bay. In contrast, *Operculodinium longispinigerum* was found only in the outer bay, where salinity is on average higher (Table 1). Most of

Spiniferites species are commonly distributed in temperate to tropical regions (Marret and Zonneveld, 2003; Zonneveld et al., 2013). Meanwhile, *O. longispinigerum* has been reported to be confined to tropical and equatorial regions, of which highest abundances were recorded in Java, western Indonesia (Zonneveld et al., 2013; Zonneveld and Pospelova, 2015). This species is known from oligotrophic open ocean regions. In Ambon Bay, *O. longispinigerum* is indeed inversely correlated with sediment geochemistry parameters (C_{org}, P_{tot} and N_{tot}), while it shows a strong and positive correlation with high water salinity. Our study reveals that *O. longispinigerum* can be present also in shallow areas, with more “open water” characteristics.

In the present study, the cyst of the PSP-producing species *Pyrodinium bahamense*, *P. zoharyi*, showed comparable (station 1, 5 and 7) and even higher (station 4) abundances than that found in the previous study in Ambon Bay (close to our station 5, Mizushima et al., 2007). The proliferations of the species in 2012 and 2013 and unrecorded blooms (in the last 6 years) were likely to contribute to high cyst accumulations, as high densities were found in the upper sediment at those stations (4,

Table 2
Dinocyst species concentrations in the upper 2 cm sediment in Ambon Bay, July 2019.

Dinocyst	St.1	St.2	St.3	St.4	St.5	St.6	St.7	St.8	St.9	ST.10
<i>Brigantidinium</i> spp. (Br.s)	2625	2050	2417	4500	5225	2350	1450	1075	300	500
cyst of <i>Protoperidinium nudum</i> (Pr.n)	0	0	0	500	175	0	0	0	0	0
<i>Dapsilidinium pastielsii</i> (Da.p)	175	0	0	75	0	50	0	0	0	0
<i>Lejeuncysta oliva</i> (Le.o)	0	0	0	0	50	150	0	0	0	0
<i>Lejeuncysta sabrina</i> (Le.s)	0	50	0	50	0	0	0	17	0	0
<i>Lingulodinium machaerophorum</i> (Li.m)	700	175	250	350	175	0	0	0	0	0
<i>Nematosphaeropsis labyrinthea</i> (Ne.l)	0	75	0	0	0	0	0	0	0	0
<i>Operculodinium centrocarpum</i> (Op.c)	50	150	0	0	25	50	0	0	0	0
<i>O. longispinigerum</i> (Op.l)	25	0	0	0	75	0	0	142	25	75
<i>Polysphaeridium zoharyi</i> (Po.z)	4050	475	0	5975	3513	1525	3250	100	100	175
<i>Quinquecuspidata concreta</i> (Qu.c)	50	125	167	150	250	0	0	0	0	0
<i>Stelladinium robustum</i> (St.r)	0	0	0	0	125	50	0	50	0	0
<i>Stelladinium stellatum</i> (St.s)	0	0	0	0	200	0	0	0	0	0
<i>Stelladinium</i> sp.	50	0	0	0	0	0	0	0	0	0
<i>Spiniferites mirabilis</i> (Sp.m)	150	0	0	0	250	0	50	0	0	25
<i>S. ramosus</i> (Sp.r)	150	50	0	75	150	100	0	0	0	0
<i>S. delicatus</i> (Sp.d)	50	300	0	25	0	0	50	0	0	0
<i>S. bentorii</i> (Sp.b)	50	0	0	0	0	0	0	0	0	0
<i>Selenopemphix nephroides</i> (Se.n)	0	50	0	0	125	100	0	0	0	0
<i>S. quanta</i> (Se.q)	0	50	0	0	250	250	50	0	0	0
<i>Trinovantedinium applanatum</i> (Tr.a)	0	0	250	0	0	0	0	50	0	0
<i>Tuberculodinium vancampoe</i> (Tu.v)	50	50	0	0	0	0	0	0	0	0
<i>Votadinium calvum</i> (Vo.c)	50	0	0	0	0	0	0	0	0	0
Total	8225	3600	3083	11,700	10,588	4625	4850	1433	425	775

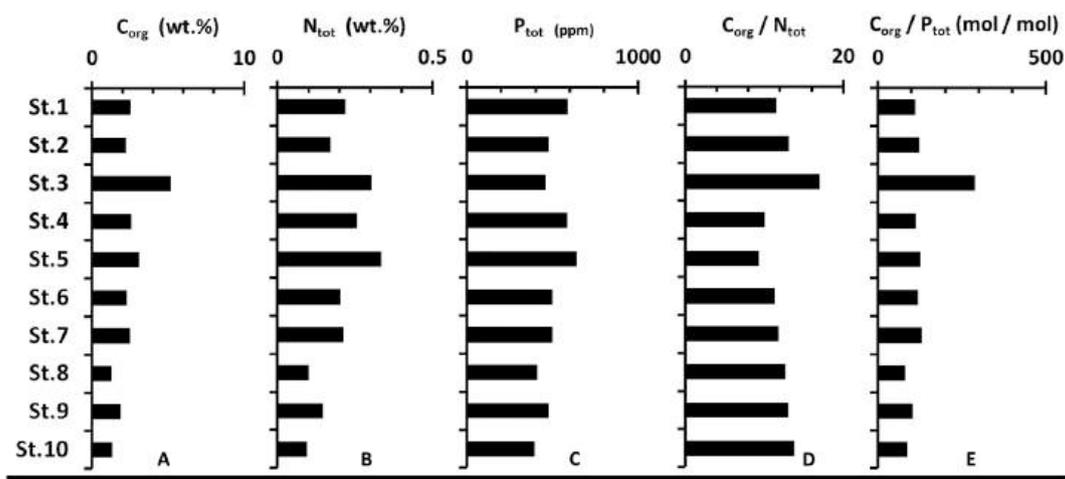


Fig. 5. Geochemical parameters in the upper 2 cm of sediment in Stations 1 to 10, Ambon Bay, Indonesia. A) Organic carbon (C_{org} , weight percentage); B) Total nitrogen (N_{tot} , weight percentage); C) Total phosphorous (P_{tot} , ppm); D) Organic carbon to total nitrogen ratio (C_{org}/N_{tot}); E) Organic carbon to phosphorous ratio (C_{org}/P_{tot} , mol/mol). Stations 1–7 are located in the inner bay and 8–10 in the outer bay (see Fig. 1).

5, 1 and 7) where blooms occurred. Another possible reason was that tides and internal currents or waves during upwelling might re-suspend cysts from the upper sediment, after which non-germinated cysts might settle again, thereby maintaining high cyst densities in the upper sediment. To date, no information regarding densities of re-suspended cysts (in particular *P. zoharyi*) in water columns is available for Ambon Bay. Hence, more studies using sediment traps would be needed to fill this gap. The *P. zoharyi* cyst concentrations were very low at stations located in the outer bay where no blooms were recorded. This implies that the inner bay is more prone to further PSP events than the outer bay, probably because of its relatively higher temperatures and (seasonally) low salinity, which are known to favor this euryhaline species.

High *P. zoharyi* abundances in surface sediments have shown to seed blooms in Philippines' and Mexican Pacific coasts (Yap-Dejeto et al., 2018; Yñiguez et al., 2018; Morquecho, 2019). Usup et al. (2012) concluded that the geographical distribution of *P. zoharyi* is wider than the observations of vegetative cells would suggest: Atlantic and Pacific regions, the Caribbean Sea, Baja California, and the Gulf of Persia. This

is interesting and shows the importance of mapping the presence of cysts of toxic species in the surface sediments to be used as a warning sign for potentially toxic blooms. In Asia, the cyst was recorded in Pleistocene sediments collected from the South China Sea and central Japan; in the 1920s in Manila Bay, the Philippines; in 1966 in Sabah Malaysia; and in the 1850s and 1860s in Ambon Bay and Hurun Bay, Indonesia (Mizushima et al., 2007; Siringan et al., 2008; Usup et al., 2012 and references therein). According to the sediment core investigation by Mizushima et al. (2007), the first appearance of *P. bahamense* cysts in Ambon Bay (~1850) predates the anthropogenic activities such as ballast water or transportation of shellfish seeds and could suggest that blooms are natural phenomenon.

4.2. Environmental properties

Average sea surface temperature during the wet season (T_{wet}) was slightly lower (27.79 ± 0.08) in the outer bay than in the inner bay (28.45 ± 0.43), coinciding with higher salinity levels (32.89 ± 0.12).

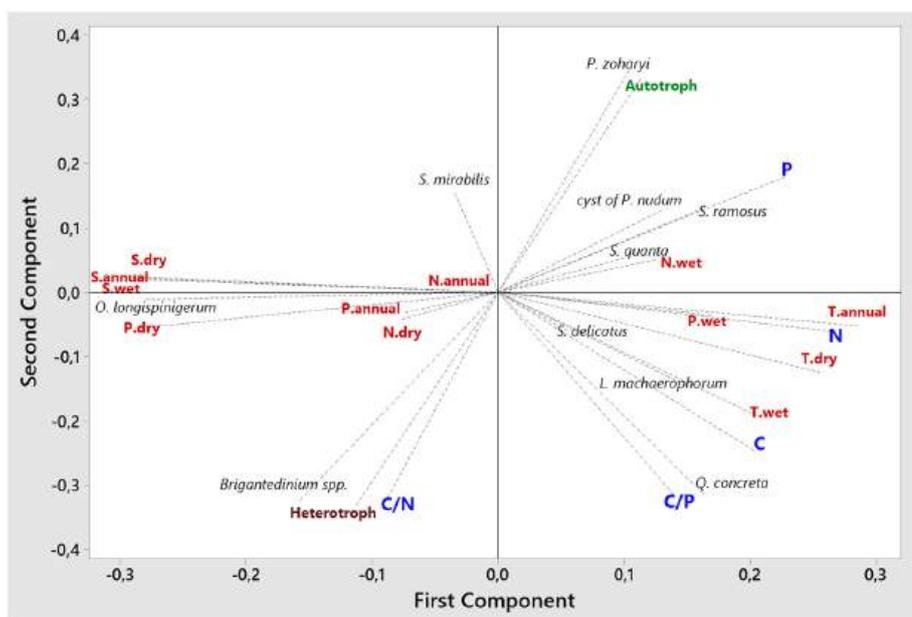


Fig. 6. PCA analysis between predominant dinocyst species, water physicochemical (red letters) and sediment geochemical parameters (blue letters). Abbreviations: S.dry, S.wet, S.annual) average salinity in the dry & wet season and annual; T.dry, T.wet, T.annual) average temperature in the dry & wet season and annual; P.dry, P.wet, P.annual) average dissolved phosphate in the dry & wet season and annual; N.dry, N.wet, N.annual) average dissolved nitrate in the dry & wet season and annual; C) organic carbon (C_{org}); N) total nitrogen (N_{tot}); C/N) ratio of C_{org} to N_{tot} (C_{org}/N_{tot}); P) total phosphorous (P_{tot}); C/P) ratio of C_{org} to P_{tot} (C_{org}/P_{tot}). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Pairwise Pearson Correlation between dinocysts and environmental parameters and among dinocysts (coefficient correlation 95%). Abbreviations: S.dry, S.wet, S.annual) average salinity in the dry & wet season and annual; T.dry, T.wet, T.annual) average temperature in the dry & wet season and annual; P.dry, P.wet, P.annual) average phosphate in the dry & wet season and annual; N.dry, N.wet, N.annual) average nitrate in the dry & wet season and annual; C) organic carbon (C_{org}); N) total nitrogen (N_{tot}); C/N) ratio of C_{org} to N_{tot} (C_{org}/N_{tot}); P) total phosphorous (P_{tot}); C/P) ratio of C_{org} to P_{tot} (C_{org}/P_{tot}).

Sample 1	Sample 2	Correlation	P-value
<i>L. machaerophorum</i>	C	0.66	0.040
<i>O. longispinigerum</i>	C	-0.64	0.047
<i>Q. concreta</i>	C	0.82	0.004
<i>O. longispinigerum</i>	N	-0.78	0.008
<i>Brigantedinium</i> spp.	P	-0.66	0.036
<i>O. longispinigerum</i>	P	-0.67	0.032
<i>Brigantedinium</i> spp.	C/N	0.69	0.027
<i>P. zoharyi</i>	C/N	-0.67	0.034
<i>Q. concreta</i>	C/P	0.82	0.004
<i>P. zoharyi</i>	<i>Brigantedinium</i> spp.	-0.94	0.000
Autotroph	<i>Brigantedinium</i> spp.	-0.98	0.000
Heterotroph	<i>Brigantedinium</i> spp.	0.97	0.000
Cyst of <i>P. nudum</i>	N.wet	0.74	0.015
<i>Q. concreta</i>	<i>L. machaerophorum</i>	0.68	0.030
<i>L. machaerophorum</i>	T.annual	0.68	0.031
<i>O. longispinigerum</i>	T.dry	-0.87	0.001
<i>O. longispinigerum</i>	T.wet	-0.66	0.038
<i>O. longispinigerum</i>	T.annual	-0.93	0.000
<i>O. longispinigerum</i>	S.dry	0.92	0.000
<i>O. longispinigerum</i>	S.wet	0.88	0.001
<i>O. longispinigerum</i>	S.annual	0.91	0.000
<i>O. longispinigerum</i>	P.dry	0.89	0.001
Autotroph	<i>P. zoharyi</i>	0.95	0.000
Heterotroph	<i>P. zoharyi</i>	-0.95	0.000
<i>Q. concreta</i>	T.dry	0.67	0.032
<i>Q. concreta</i>	T.wet	0.72	0.019
<i>Q. concreta</i>	T.annual	0.67	0.036

This suggest that upwelled waters in the Banda Sea reach Ambon Bay during the southeast monsoon (Likumahua et al., 2020). During this period, upwelled nutrient rich water from greater depths could be detected in the bay as a result of tidal forcing (Saputra and Lekalette, 2016). Ambon Island is a densely populated-coastal area that is likely to contribute to a continuous wastewater discharge through run-offs during the wet season, resulting in nutrient enrichment in the bay. Ikhsani

et al. (2017) reported a combination of sources of nutrient enrichment in Ambon Bay between May and August (during the southeast monsoon), as a result of upwelling and land derived inflow. During the dry season, water mixing triggered by wind flows may influence nutrient variability in the bay (Likumahua et al., 2019).

C_{org}/N_{tot} ratios in this study (9–17) were generally high for a marine environment (Emerson and Hedges, 1988; Meyers, 1994). This seems to indicate that Ambon Bay is likely to receive massive loads of soil and terrestrial organic matter (OM). The accumulation of carbon and nitrogen in coastal sediments is derived from complex mixtures of organic materials produced by marine algae, higher order organisms (autochthonous), soil organic matter, terrestrial vegetation, freshwater algae, and wastewater (allochthonous) (Schreiner et al., 2013; Cai et al., 2015; Li et al., 2016 and references therein). In addition, transformations of wetland to agriculture triggered the distribution of soil OM and nutrient inputs through river discharges, which eventually contributed to sedimentary organic carbon and nitrogen accumulation (Li et al., 2016). Thus, the recent increase in land utilization for local residencies in the hilly areas of Ambon Island (Pelasula, 2008) combined with intense local agriculture areas between station 1 and 2 (Fig. 1) are likely to contribute to high terrestrial OM inputs in Ambon Bay. The C_{org}/N_{tot} ratio is, however, very high in all samples, and this may also suggest that nitrogen is re-mineralized faster than C and that N from the sediments is released to the water column and fuel productivity. Nitrogen in the water column is indeed inversely correlated with C_{org}/N_{tot} , which could indicate that part of the N is released in the water column.

C_{org}/P_{tot} (mol/mol) ratios in sediments can be used as indicator of dissolved oxygen conditions in bottom water, as hypoxic and anoxic conditions would enhanced the regeneration of phosphorous (P) relative to carbon from organic matter and more efficient recycling of released P to the water column (C_{org}/P_{tot}) (Algeo and Ingall, 2007; Kraal et al., 2010; Sulu-Gambari et al., 2018). Generally, the C_{org}/P_{tot} ratios in our study indicated hypoxic to anoxic conditions in the inner bay (especially at station 3), while the outer bay was generally oxic. Liu et al. (2020) studied coastal areas close to an estuary and concluded that high input of anthropogenic phosphorous through rivers triggered nutrient enrichment and resulted in seasonal hypoxia, which subsequently promoted high P release from the sediments, promoting a positive feedback mechanism. The analysis of geochemical parameters in our study revealed that hypoxic conditions in the inner Ambon Bay, which is likely to receive high organic matter, nitrogen and phosphorous from land

through river streams during the wet season can be a source of extra nutrients to the water column, fueling primary productivity and (toxic) blooms. Regardless of these initial results, more studies on autochthonous OM and P sources as well as its recycling from sediments and potential links to toxic events (spatially and temporally) in Ambon Bay are needed.

4.3. Relationship between major dinocysts and environmental parameters

Based on the statistical analysis, the presence of *Brigantedinium* spp. in the surface sediments was strongly correlated with C_{org}/N_{tot} ratios and negatively correlated with P_{tot} (Table 3). This may mean that N and P are also released from sediments and acts to fuel productivity. This agrees with the general knowledge that the genus is generally found in waters with high primary productivity (from diatoms and rich in other organic matter) (Pospelova et al., 2010; Smayda and Trainer, 2010; Bringué et al., 2014) due to anthropogenically-mediated nutrient enrichment from river discharges (Sangiorgi and Donders, 2004; Zonneveld et al., 2009; Pospelova and Kim, 2010; Zonneveld et al., 2012; Price et al., 2018).

Lingulodinium machaerophorum, cyst of the red tide forming species *L. polyedrum* was found only in the samples 1 to 5 in the inner bay (Table 2), where temperature are slightly higher and salinity lower than in the outer bay (Table 1). The species positively correlated with the % of C_{org} in Ambon Bay (Fig. 6, Table 3). A similar result was also found in Izmir Bay (Eastern Aegean, Aydin et al., 2015). High relative abundances of the species were found to be associated with river water discharges, which were characterized by high productivity and low salinity as well as low oxygen in bottom waters (Sangiorgi and Donders, 2004; Zonneveld et al., 2009, 2013). The cyst of the species was found to dominate the dinocyst community in the polluted Gulf of Gemlik with high nutrient concentrations and hypoxic bottom water conditions (Balkis et al., 2016). Leroy et al. (2013) concluded that the distribution and bloom formation of the motile form of *L. machaerophorum* are not restricted to parameters such as salinity and high nutrient concentrations. Our results demonstrate that higher temperatures and lower salinity (and seasonal stratification) may represent the main controlling factors for the distribution of this species.

Many laboratory studies have revealed that temperature affected vegetative cell growth of the toxic species *Pyrodinium bahamense* (*P. zoharyi*) as well as cyst germination (Siringan et al., 2008; Morquecho et al., 2012; Usup et al., 2012; Morquecho et al., 2014). Sakamoto et al. (2009) concluded that the main factors triggering cyst formation are temperature (ranged between 22.5 °C and 32.5 °C), dissolved phosphate, and nitrate availability. In addition, anaerobic conditions govern cyst dormancy and quiescence. In an in vitro study, *P. bahamense* var. *compressum* temporary cyst or pellicle formation was found to be induced by low temperature, while viability was preserved at low temperature (Onda et al., 2014). Price et al. (2017) found that higher temperatures and eutrophication correlated with *P. zoharyi* presence. However, we found no correlation between *P. zoharyi* and water environmental parameters such as nutrient concentrations and temperature. Interestingly, although the relationship between P in sediments and % *P. zoharyi* is not significant, the PCA plot (Fig. 6) indicates that some relationship may exist. We re-ran the PCA with *P. zoharyi* concentrations (dinocysts $g^{-1}DW$) and found this to be significant ($r = 0.85$; $p < 0.001$) (data not shown). Its prevalent distribution in the inner bay and its highest concentrations (up to ~6000 cysts $g^{-1}DW$) there suggest that *P. zoharyi* prefers enclosed basin, with relatively higher temperature and seasonal lower salinity, shallow and (seasonally) stratified environment. Apparently, water column nutrients are not the drivers of *P. zoharyi* distribution.

Cyst production of *O. longispinigerum* in this study was positively correlated with salinity, and inverse relationships with temperature were found, implying that the species might prefer areas with relatively higher average salinity. Indeed it is mainly found in the outer bay area. A

contrasting result was found in the Gulf of Aqaba, where the relative abundance of this species was positively correlated with temperature and negatively correlated with salinity (Elshanawany and Zonneveld, 2016). These authors concluded that *O. longispinigerum* has a better adaptation to oligotrophic conditions as its concentrations are negatively related to nutrients such as phosphate and nitrate. This is also confirmed in our study since the species was negatively correlated with total nitrogen (N) and total phosphorous (P) in sediments (Fig. 6), while it showed no significant relationships with water nutrients, except with the dissolved phosphate level in the dry season (P_{dry} , Table 3). *Q. concreta* significantly correlates with C_{org} and C_{org}/P_{tot} in our study, implying that the species may be present or better preserved in areas where low dissolved oxygen conditions at the seafloor occur. This relationship was also recorded in the Mediterranean Sea, in which high *Q. concreta* concentrations were found in areas characterized by high productivity and lower bottom water oxygen levels (Elshanawany et al., 2010). Several studies have demonstrated that the preservation of heterotrophic dinocysts (among which *Q. concreta*) in low oxygen condition is favored (Zonneveld et al., 2007). In the South China Sea, the species showed a positive relationship with chlorophyll-a concentrations (Li et al., 2019). The complex relationship between dinocyst species and environmental drivers in a semi-enclosed estuary such as Ambon Bay, eastern Indonesia, shows the need for additional studies in other, ideally well-ventilated, bays in Indonesian waters.

5. Conclusions

Modern dinocyst assemblages were analyzed from 10 surface (0–2 cm) sediment samples distributed in the inner and outer Ambon Bay, Indonesia. This study revealed the occurrence of 23 cyst species and very high dinocysts concentrations (up to ~12,000 cysts $g^{-1}DW$ in the inner bay). One of our samples (station 5) was taken in the proximity of a sediment core collected in 1995 where dinocysts were also analyzed (Mizushima et al., 2007). We found total dinocyst concentration of ~10,000 cysts $g^{-1}DW$, twice that obtained from the upper 2 cm core sediment. Given the available sediment accumulation rate calculated for the area (0.39 cm/year, Mizushima et al., 2007) our samples cover the last ~5 years, while the upper 2 cm core sediments should be dated ~1990–1994. This implies that cyst productivity has doubled in the recent years. The dinocyst assemblages in the sediment samples are usually dominated by heterotrophic dinocysts (particularly *Brigantedinium* spp.). *Polysphaeridium zoharyi*, cyst of the toxin-producing dinoflagellate *Pyrodinium bahamense* var. *compressum* was everywhere in the bay, yet very high concentrations (up to 6000 cysts $g^{-1}DW$) were detected in the inner bay. This indicates that the sediments in the inner bay, where the blooms regularly occur, are likely acting as seedbanks to promote toxic outbreak each year upon cysts hatching. *Lingulodinium machaerophorum*, cysts of the red tide forming *Lingulodinium polyedrum*, was exclusively found in the inner part of the bay where mean annual salinity is lower and temperature slightly higher than in the outer part of the bay. *Operculodinium longispinigerum* has the opposite distribution, being more common in the outer, higher salinity part of the bay. Water column nutrients (nitrogen and phosphorous) do not seem to significantly correlated with any of the species, neither to drive their distribution. However, massive land run-offs and regular upwelling continuously provide high nutrients availability in the bay, which sustain the high productivity that the cysts data reconstruct. Significant correlations are instead found between some species and some of the sediment geochemical parameters (C_{org} , N_{tot} , C_{org}/N_{tot} , C_{org}/P_{tot}). However, all these parameters may be affected by the oxygen content of the bottom water and the sediment itself (e.g., Kraal et al., 2010; Nasir et al., 2016) and seem to indicated that dysoxic conditions occur in at least the inner bay. Further analysis in bays where bottom oxygen concentrations are higher would help the interpretation of our data.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2021.112269>.

Declaration of competing interest

The authors declare that they have no conflict of interest to disclose.

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