Dinoflagellate cyst composition, abundance, and assemblages in surface sediment of Paotere Port, Makassar, Eastern Indonesia: preliminary study for dinoflagellate cyst identification and collection

[1]Nita Rukminasari, [2]Akbar Tahir

[1] Department of Fisheries, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia; [2] Department of Marine Science, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia. Corresponding author: N. Rukminasari, nita.r@unhas.ac.id

Abstract. Paotere Port is a port in South Sulawesi, Indonesia which contributes significantly to the economic sector. The high levels of anthropogenic activities surrounding the port have resulted in increasing pollution, in particular organic matter pollution. This kind of pollutant affects dinoflagellate cysts communities. This study aimed to identify and collect dinoflagellate cysts, to determine dinoflagellate cyst abundance and assemblages and to examine the correlation between dinoflagellate cyst assemblages and diversity and environmental factors. Sediment samples were collected at Paotere Port, from 4 stations each having 3 substations as sampling replicates. We identified 31 species and 20 genera of dinoflagellate cysts, belonging to 5 families (Goniodomaceae, Ganyaulacaceae, Gymnodiniaceae, Protoperidiniaceae, and Peridiniaceae). Two of the species identified can potentially produce toxic compounds (*Alexandrium catenella* and *Cochlodinium polykrikoides*). There was a significant difference in dinoflagellate cyst assemblages between stations, however we found that dinoflagellate cyst abundance and diversity index values were low at all stations. *Pyrophacus horologium* was the most dominant species, contributing 23% of total dinoflagellate cyst abundance. Cyst abundance at Paotere Port is low compared to subtropical and temperate coastal regions.

Key Words: Dinoflagellate cyst, abundance and assemblages, surface sediment, Paotere Port, Eastern Indonesia.

Introduction. Harmful Algal Blooms (HABs) mostly occur due to anthropogenic nutrient enrichment of coastal waters (Hallegraeff 1993; Pospelova et al 2002; Smayda 1990). Dinoflagellates are key components of the phytoplankton community and some species contribute to the formation of HABs. During their life-cycle, dinoflagellates produce resting cysts which are preserved in the sediment. The dinoflagellate population is influenced by environmental factors such as temperature, salinity, nutrients, turbidity, and pollution (Granéli & Turner 2006) and they can also form cysts under unfavourable conditions.

In recent decades the study of dinoflagellate cysts has increased and has been mostly focused on using dinoflagellate cysts as biological indicators for the occurrence of eutrophication and changes in salinity and water temperature, and many studies have examined the distribution of dinoflagellate cyst assemblages in coastal sediments (Marret & Zonneveld 2003; Radi & de Vernal 2004; Rochon & Marret 2004). Cyst assemblages contain the cysts of some dinoflagellates that can cause harmful algal blooms, and therefore sediment analysis has also been conducted to document the historical occurrence of harmful dinoflagellates (Irwin et al 2003; Matsuoka et al 2006). The dinoflagellate cyst record is capable of providing important information on environmental parameters in productive estuarine systems that are characterized by high sedimentation rates (Pospelova & Kim 2010). The encystment of dinoflagellates is known to be affected by water temperature, nutrient availability, day length, and endogenous encystment rhythms (Granéli & Turner 2006), whereas cyst distribution in sediments has been related to environmental factors such as water temperature, salinity, turbulence, and the availability of nutrients (Marret et al 2004). This suggests that dinoflagellate cysts recorded from sediments can be utilized to reconstruct environmental conditions in a given study area.

The sedimentary cyst associations form natural archives that can be used to reconstruct upper water environmental conditions at times of deposition, and they have proven to be extremely useful as tracers of upper water productivity changes related to changes in the trophic state of upper waters (e.g. due to pollution-related to human activities such as urbanization and industrialization) (Dale & Fjellsa 1994; Pospelova et al 2002; Krepakevich & Pospelova 2010; Shin et al 2010; Zonneveld et al 2012; Satta et al 2013).

Coastal areas, including estuaries, are influenced by domestic and industrial wastewater discharge, urban sewage, and agricultural effluents caused by anthropogenic activities, as well as freshwater runoff from rivers; because of these influences, their biology and physical dynamics are often altered (Shin et al 2011). Pospelova et al (2002) reported on the relationship between dinoflagellate cyst abundance and human activity in the estuarine area of Massachusetts, USA, and suggested that more studies were needed to better understand such relationships as an indicator of eutrophication by dinoflagellate cysts which vary in different areas and at different eutrophication levels.

South Sulawesi province is one of the centres of economic activity in the eastern part of Indonesia, where South Sulawesi is considered a gateway to the area. One of the older ports within the port complex of Makassar City is the Paotere Port. Cargo and passenger ships frequently enter and leave the port. And this maritime traffic will tend to increase every year according to the current conditions and needs of the earth's population. The increasing volume and number of the vessels means that the discharge of wastewater containing oil (oily waste) is also likely to increase, causing pollution which, if it is not treated immediately, will damage the environment in the waters around the port (Irwan et al 2013).

The Paotere Port has long contributed greatly to the livelihoods of Makassar people, with activities such as fish auctions, transportation, and other services. Those activities have also caused the waters of the port to serve as the final disposal area for a wide range of pollutants causing declining levels of water quality (Irwan et al 2013). It is to be expected that the heavily anthropogenic activities at Paotere Port will also affect the dinoflagellate cysts community; however, there was a lack of studies conducted in this area. The purpose of our research was two-fold. Firstly, using cyst assemblages and the signals of nutrient enrichment, as previously described in the literature (Matsuoka 1999; Matsuoka et al 2003; Pospelova et al 2002, 2005), we aimed to identify dinoflagellate cysts from Paotere Port. Secondly, we attempted to determine whether the distribution of dinoflagellate cysts on small spatial scales correlates with available environmental and sedimentary data.

Material and Method

The study site. Dinoflagellate cysts sampling from surface sediment was conducted at Paotere Port (Figure 1). This area was selected due to high anthropogenic activity and high organic pollution from human activities surrounding Paotere Port (Figure 1).

Sediment sampling*.* Sediment samples were collected from Paotere Port (Figure 1) in July 2020. The sampling site consisted of 4 stations with 3 substations as sampling replicates at each station. The sediment samples were collected using a core sampler. The samples were stored in the dark at 10^oC until processed.

Sediment processing*.* Surface sediment samples were obtained from the top 1 cm of sediment from each core. Subsamples (2-3 cm^3) of sediment from each substation were suspended in filtered seawater (FSW) and sonicated for 15 minutes. The sonicated material was filtered through three-level sieves with mesh-sizes of 250 µm, 100 µm, and 20 µm. The residue (slurry) remaining on the 20 µm mesh size filter was washed with

FSW and collected in a 10 mL tube. Subsamples (5-7 mL) of the slurry were processed for cyst concentration as described by Amorim et al (2001) and Bravo et al (2006). The resulting samples were passed again through a 20 µm mesh size filter and collected in 5-15 mL of FSW.

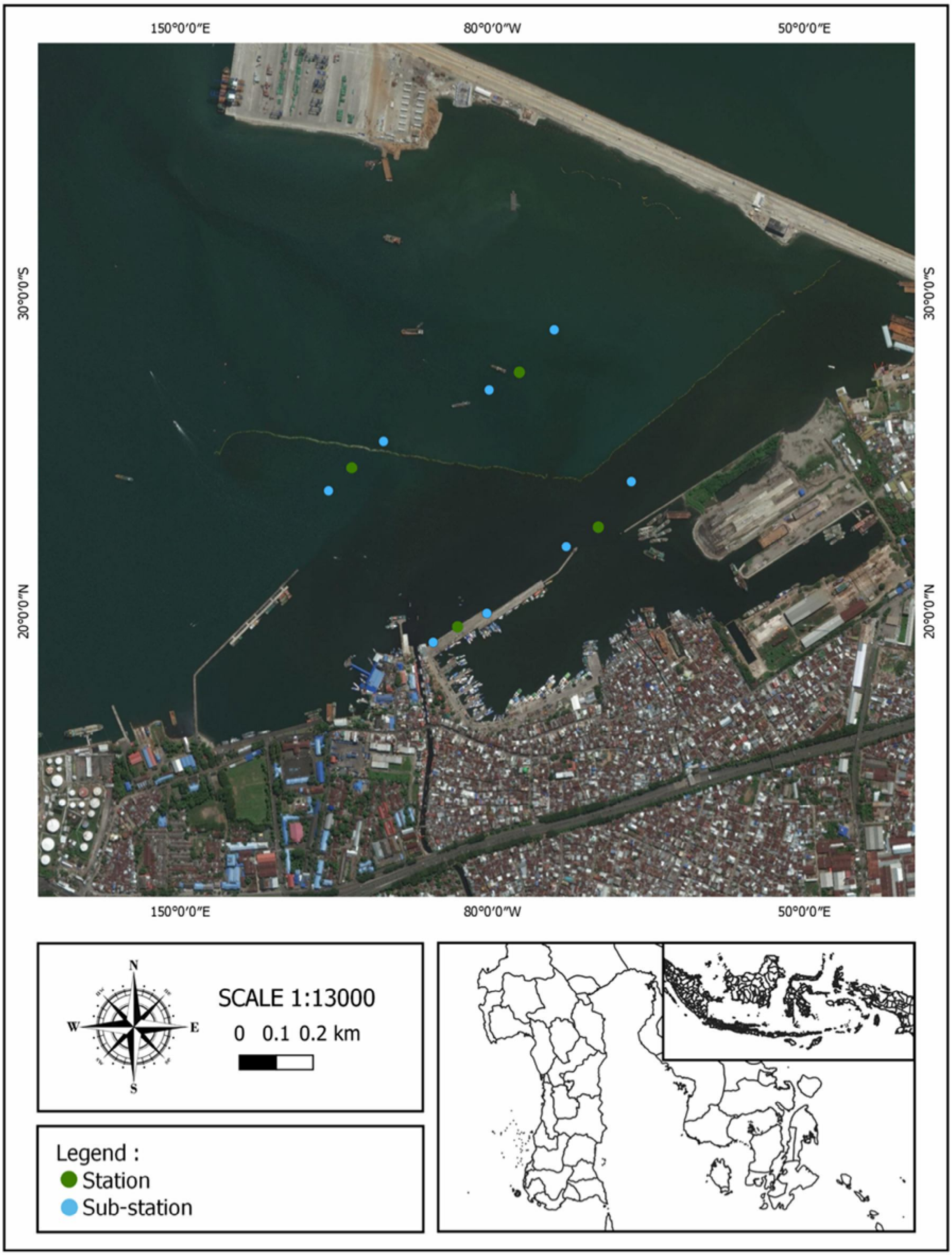

Figure 1. The study site at Paotere Port, Makassar City in South Sulawesi, Indonesia.

Cyst counts and identification. For dinoflagellate cyst analysis, the residue retained on the 20 µm mesh was transferred into a vial and suspended in 10 mL of filtered seawater. Aliquots of 0.5-1 mL of the processed samples were diluted to a total volume of 2.5 mL in a Petri dish (diameter 3.8 cm) and scanned under a light microscope at 100-400 times magnification. Dinoflagellate cysts were identified based on published descriptions (Nehring 1997; Godhe et al 2000; Orlova et al 2004; Joyce et al 2005; Bravo et al 2006; Pospelova & Kim 2010; Shin et al 2010, 2011; Aydin et al 2011; Alkawri 2016; Uddandam et al 2017). Cyst abundance was expressed as the number of cysts g^{-1} dry sediment weight. The water content was calculated according to the formula given by Matsuoka & Fukuyo (2000).

Water and sediment parameters. Water samples were collected with a 5-L Niskin sampler. Temperature, dissolved oxygen (DO), pH, and salinity were measured *in situ* using a multiparameter water quality checker. Nutrient concentrations of nitrate (NO_3–N), ammonia (NH_4–N), and phosphate (PO_4–P) were measured. Sediment samples were also collected using a sediment core sampler for sediment texture, total carbon, inorganic carbon, and total nitrogen analysis. The sediment texture was analysed by standard wet sieving and expressed as a percentage of sand, silt, and clay content. The total carbon (TC) and total nitrogen (TN) contents of 7-8 mg of dried, crushed, and homogenized sediment samples were determined with a CNS elemental analyser (CE instrument, NCS 2500). Total inorganic carbon (TIC) content was estimated with the help of a CO_2 Coulometer (CM 5014) following acidification of the samples.

Data analysis. For statistical analyses of the cyst dataset the Plymouth Routines In Multivariate Ecological Research (PRIMER) v5 software package was used. Univariate measures such as Shannon-Wiener's diversity index (H'), Margalef's species richness index (d), and Pielou's evenness index (J') were determined. Routines applied were MDS, ANOSIM, and SIMPER. Data was log (x+1) transformed to allowing the rare taxa to exert some influence on the similarity calculations. For similarity measures, the Bray-Curtis coefficient was used. Two-way ANOVA was performed on dinoflagellate cyst abundance, growth rate, Shannon-Wiener diversity index (H'), Margalef species richness index (d), and Pielou evenness index (J') to evaluate spatial variation using the GraphPad 7 software program.

Results

Composition and distribution of dinoflagellate cysts. A total of 31 species of dinoflagellate cysts representing 20 genera were identified from all stations and substations (Table 1, Figure 2). These dinoflagellate assemblages comprised 2 species of Goniodomaceae (6% of the total number of dinoflagellate cysts in all samples), 6 species of Gonyaulacaceae (19%), 5 species of Gymnodiniaceae (16%), 9 species of Peridiniaceae (29%), 7 species of Protoperidiniaceae (23%) and 2 species of unidentified family (6%). Cyst abundance at the study site ranged from 4 to 2147 cysts g^{-1} dry sediment weight. The most dominant taxon was *Pyrophacus horologium* which contributed 23% of total abundance.

The mean abundance of dinoflagellate cysts varied between stations. The highest mean abundance of dinoflagellate cysts was recorded at station III accounting for 484 cysts g^{-1} dry sediment weight, while the lowest mean abundance of dinoflagellate cysts was 55 cysts g^{-1} dry sediment weight at station I. Statistically there was a significant difference in dinoflagellate cyst abundance between stations I, II and IV; however, there was no significant difference in dinoflagellate cyst abundance between stations II and III (Figure 3a). The highest and the lowest species richness were found at stations II and III, with values of 1.5677 and 0.4517, respectively (Figure 3b). The species richness was significantly different between the stations. The highest and lowest Pileou's evenness index were found at a station I and II with values of 0.8777 and 0.7542, respectively; however, there was no significant difference in Pileou's evenness index between stations except for station II (Figure 3c). The Shannon-Wiener diversity index was calculated for all four stations. Station II had the highest diversity index; however, except for station III, the difference in diversity index between stations was not significant (Figure 3d).

Table 1
Species composition, abundance (cysts g^{-1} dry weight) and dominance of dinoflagellate cysts in surface sediments of Paotere Port, Makassar City, Eastern Indonesia

Resting cyst species	*Abundance (cyst g^{-1} dry sediment weight)*												*Dominance (%)*
	Station I			*Station II*			*Station III*			*Station IV*			
	1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	
Goniodomaceae													
Alexandrium catenella	0	0	116	0	0	0	0	76	0	0	0	0	2.13
Alexandrium cf. *tamiyavanachi*	0	0	0	13	0	0	0	0	0	0	23	0	0.40
Gonyaulacaceae													
Fragilidium subglobosum	0	0	0	0	0	34	0	0	0	0	0	0	0.37
Gonyaulax scrippsae	0	0	0	0	0	0	0	0	0	0	0	5	0.06
Gonyaulax verior	4	7	0	39	42	0	0	0	0	6	104	26	2.55
Pyrophacus horologium	0	0	755	0	0	0	900	277	215	0	0	0	23.85
Pyrophacus steinii	0	0	0	0	11	0	0	0	0	0	0	0	0.12
Spiniferites elongatus	0	0	0	0	0	22	0	0	0	0	0	0	0.25
Gymnodiniaceae													
Cochlodinium polykrikoides	0	0	886	0	0	0	79	0	0	0	0	0	10.72
Gymnodinium cf. *nolleri*	0	0	0	0	0	1198	0	0	0	0	0	0	13.31
Gymnodinium instriatum	0	0	0	13	0	0	0	0	0	0	0	0	0.14
Pheopolykrikos hartmanii	0	0	58	0	0	0	0	0	0	0	0	0	0.65
Polykrikos schwartzii	0	0	0	0	0	67	0	0	0	0	0	0	0.75
Peridiniaceae													
Bicarinellum bicarinelloides	0	0	0	0	21	0	0	0	0	0	0	0	0.24
Brigantedinium asymmetricum	0	0	0	0	11	0	0	0	0	0	0	0	0.12
Lejeunecysta oliva	0	0	0	0	11	0	0	0	0	0	0	0	0.12
Operculodinium centrocarpum	0	0	0	0	0	179	0	0	0	0	0	0	1.99
Scrippsiella cf. *lachrymosa*	4	7	0	78	85	0	0	0	0	19	46	0	2.65
Scrippsiella cf. *rotunda*	0	0	0	0	0	123	0	0	0	0	23	0	1.63
Scrippsiella crystallina	12	7	0	26	32	11	225	126	121	12	35	32	7.10
Scrippsiella trifida	0	0	261	13	42	22	252	113	40	0	0	5	8.33
Zygabikodinium lenticulatum	0	0	0	0	0	0	0	0	0	0	0	21	0.23
Protoperidiniaceae													
Archaeperidinium sp.	0	4	0	0	0	0	0	0	0	0	0	0	0.04
Pentapharsodinium tyrrhenicum	16	4	160	52	42	11	26	0	0	19	69	48	4.97
Protoperidinium avellana	0	0	0	0	21	0	0	0	0	0	0	0	0.24
Protoperidinium cf. *divergens*	0	0	0	26	0	0	0	0	0	0	0	11	0.41
Protoperidinium conicoides	0	0	944	0	0	0	0	0	0	0	0	0	10.48
Protoperidinium pentagonum	4	0	0	13	0	0	0	0	0	0	0	11	0.31
Protoperidinium subinerum	0	7	44	65	106	11	0	0	0	0	81	0	3.49
Unidentified diatom	0	0	0	13	0	0	0	0	0	0	0	0	0.14
Foraminifera	12	19	0	0	0	11	0	0	0	6	0	153	2.24

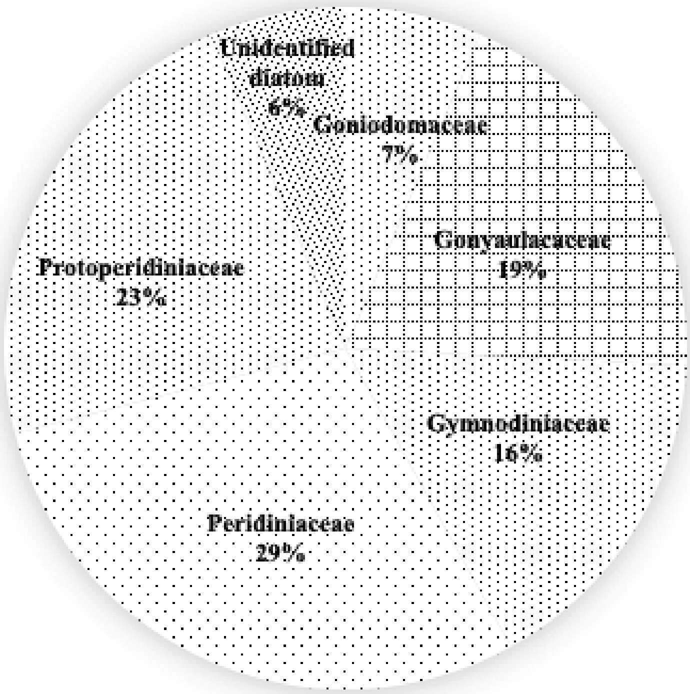

Figure 2. Percentage composition of cyst families (Goniodomaceae, Gonyaulacaceae, Gymnodiniaceae, Peridiniacea, and Protoperidiniaceae) in the surface sediment layer of Paotere Port, Makassar City, Eastern Indonesia.

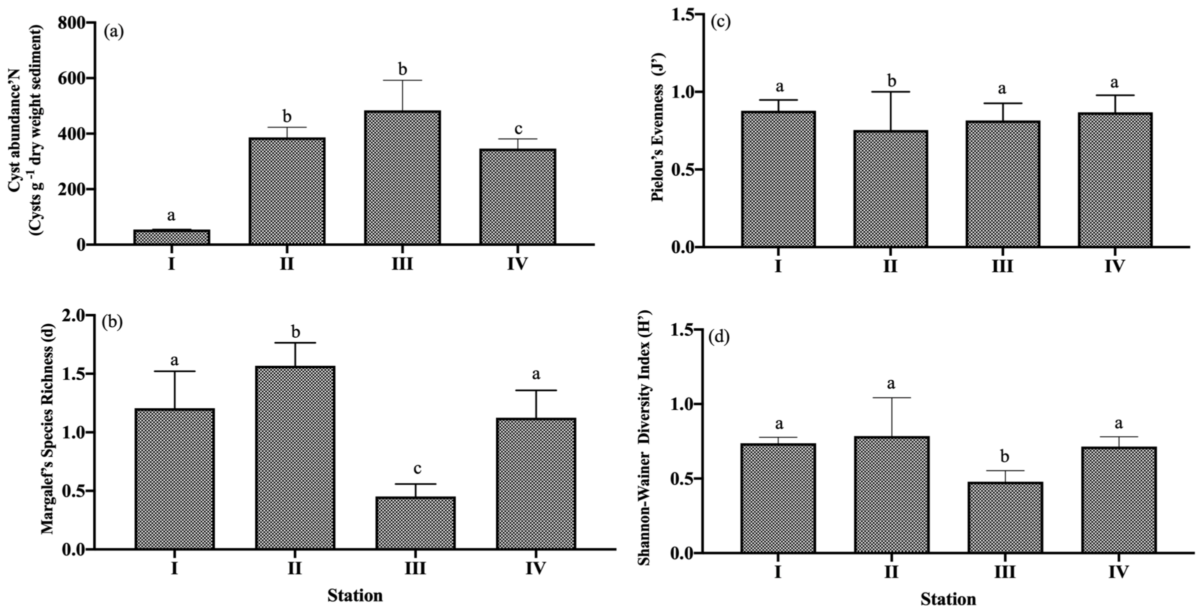

Figure 3. Comparison of the dinoflagellate cyst assemblages at four stations in Paotere Port, Makassar City, Eastern Indonesia (bars indicate mean values, whiskers indicate standard deviation): a. Cyst abundance in terms of the total number of cysts g $^{-1}$ DW of sediment for each station; b. Species richness; c. Pileou's evenness index; d. Shannon-Wiener diversity index.

Dinoflagellate cysts assemblages. When the log mean abundance of dinoflagellate cysts in each location was subjected to ordination, the plot depicting the relationships between the assemblages of dinoflagellate cysts in the different stations showed that the samples were initially widespread on the plot for all stations, with some substation points for different stations overlying each other (Figure 4).

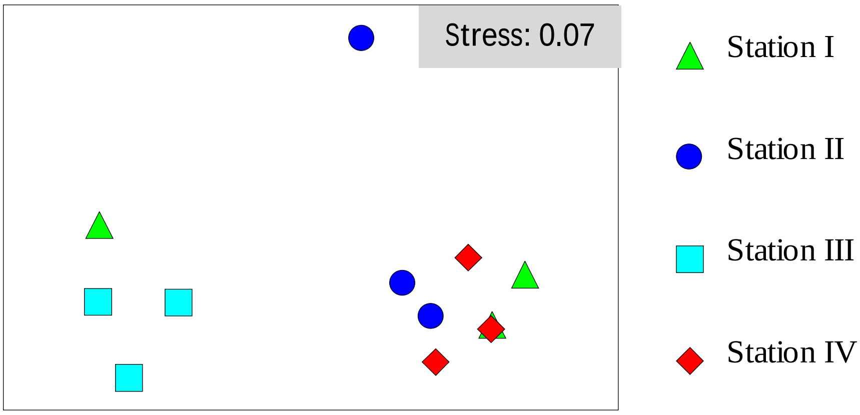

Figure 4. nMDS ordination plot of Log (X+1) transformation comparing the dinoflagellate cysts assemblages in term of total number of cysts g^{-1} DW of sediment for each station and substation.

The ANOSIM results showed that there was a significant difference in dinoflagellate cyst assemblages between stations ($p < 0.1$) (Table 2).

Table 2

Results from an ANOSIM Pairwise test and SIMPER analysis comparing dinoflagellate cyst assemblages between the four stations in Paotere Port, Makassar City, Eastern Indonesia

ANOSIM Pairwise Tests			*SIMPER Results*	
Station pair	*Global R*	*Significance level (%)*	*Dissimilarity (%)*	*Taxa most responsible for dissimilarity*
Station I vs Station II	-1.185	90	68.18	*Scrippsiella trifida* (7%), *Scrippsiella cf. lachrymosa* (6%), *Protoperidinium subinerum* (6%)
Station I vs Station III	0.037	20	72.75	*Pyrophacus horologium* (17%), *Scrippsiella trifida* (13%), *Scrippsiella crystallina* (11%)
Station I vs Station IV	-0.185	90	56.07	*Protoperidinium subinerum* (8%), *Foraminifera org living* (8%), *Scrippsiella cf. lachrymosa* (8%)
Station II vs Station III	0.778	10	74.89	*Pyrophacus horologium* (14%), *Protoperidinium subinerum* (9%), *Scrippsiella cf. lachrymosa* (7%)
Station II vs Station IV	-0.37	70	56.56	*Protoperidinium subinerum* (9%), *Scrippsiella trifida* (8%), *Gymnodinium cf. nolleri* (7%)
Station III vs Station IV	1	10	77.18	*Pyrophacus horologium* (19%), *Scrippsiella trifida* (13%), *Gonyaulax verior* (10%)

In general the most dominant species was *Pyrophacus horologium* which was contributed 23% of total dinoflagellate cyst abundance; however, the SIMPER results showed that the dominant dinoflagellate cyst taxon differed between stations. For stations I and IV, the dominant taxa was *Pentapharsodinium tyrrhenicum* (Protoperidiniaceae); meanwhile, for stations II and III, the dominant taxa were *Protoperidinium subinerum* and *Pyrophacus horologium*, respectively (Table 2). The percentage of dissimilarity between stations ranged from 56.07 to 77.18%. The highest similarity was found between station III and IV with the three taxa contributing most to the dissimilarity being *Pyrophacus horologium* (19%), *Scrippsiella trifida* (13%), and *Gonyaulax verior* (10%). The lowest dissimilarity was 56.07 between stations I and IV. The three taxa contributing most to this dissimilarity were *Protoperidinium subinerum* (8%), Foraminifera org living (8%), and *Scrippsiella* cf. *lachrymosa* (8%).

Water and sediment parameters. The highest temperature recorded at the stations was 31.57°C and the lowest was 30.62°C (Table 3). Salinity was generally highest at station IV and lowest at station I. The pH values ranged from 7.34 to 7.43 with the highest pH recorded at station III. The lowest DO concentration was recorded at station I (DO = 5.40 mg L^{-1}). The sediment texture was silty-sand at most of the stations/sub-stations sampled (Table 3). The Total $C_{organic}$ and TN content of sediment were highest at station I. The highest and lowest TIC values were found at stations II and III, with values of 15.58% and 7.38%, respectively. The highest $C_{organic}$:N ratio was found at station I (12.58); meanwhile station II had the lowest $C_{organic}$:N ratio (9.58).

Table 3

Water and sediment parameters at the sampling stations in Paotere Port

Station	*Water parameters*				*Sedimentary parameters*				
	Temp. (°C)	*Salinity (PSU)*	*DO (mg L^{-1})*	*pH*	*TOC (%)*	*TIC (%)*	*TN (%)*	*C:N ratio*	*Sediment type*
I	31.57	29.45	5.40	7.34	0.44	11.57	0.04	12.28	Sand
II	31.06	32.97	5.85	7.38	0.27	15.58	0.03	9.58	Sand
III	30.99	32.88	6.17	7.43	0.36	7.38	0.03	11.34	Sand
IV	30.62	33.02	6.30	7.38	0.31	12.32	0.03	11.40	Silt

Discussion. Our study found that the dinoflagellate cyst assemblages in Paotere Port were characterized by relatively low species diversity with Peridiniaceae taxa more dominant than Gonyaulacales, which is often the case in inner neritic environments (de Vernal & Giroux 1989; Grøsfjeld et al 2009). The assemblages of dinoflagellate cysts were dominated by Protoperidiniaceae. This indicates that the area had a high input of organic matter in the marginal-marine environment under restricted circulation conditions. Candel et al (2012) stated that assemblages dominated by Protoperidiniaceae along with foraminiferal linings and copepod eggs suggest the proximity of a terrestrial source with a high input of organic matter in the marginal-marine environment under restricted circulation conditions; meanwhile, Uddandam et al (2017) found that offshore assemblages were mainly comprised of heterotrophic protoperidinioid species. The recorded dominant species (*Pentapharsodinium tyrrhenicum, Protoperidinium subinerum* and *Pyrophacus horologium*) are consonant with low to moderate salinity and high nutrient content in surface waters due to river inputs.

It has been observed that differences in cyst abundance and assemblage composition such as those between the stations at Paotere Port are caused primarily by differences in the abundance of vegetative cells and their cyst production efficiencies, and/or by differences in hydrology and sedimentary regimes (Anderson & Lindquist 1985; Joyce et al 2005). Dinoflagellate cysts are believed to have hydrodynamic characteristics typical of fine silt-sized particles (Kawamura 2004) and can be transported by water currents. In the study area, the abundance of dinoflagellate cysts can be correlated with the texture of the sediment, i.e. sandy and silt sediment. A low cyst abundance was encountered in the sandy sediments, which are not suitable for cyst deposition (Montresor et al 1998). These results indicated that sediment grain size plays a major role in determining the cyst distribution in this area.

In general, tropical waters are characterized by dinoflagellate populations with low abundance and diversity (Rodrigues et al 2019). Pospelova et al (2002) suggested that low dinoflagellate cyst species richness and diversity are general indicators of polluted and highly eutrophic estuarine systems. Our study found that species richness and diversity indices were lower than in some previous studies from the other tropical waters (D'Silva et al 2013; Chen et al 2015, 2019; Uddandam et al 2017; Rodrigues et al 2019;). The species richness (0.4517 to 1.5687) indicates that Paotere Port has been subjected to a highly eutrophic regime due to the high level of anthropogenic activities surrounding the port. Our study found no correlation between total carbon and nitrogen in the sediment and the diversity index. This finding contrasts with previous studies (Pospelova & Kim 2010) which found that patterns of species assemblages and abundance seemed to be functions of water depth, sediment grain size, and nutrients (TOC, TIC, TN, and C:N ratios). Since we only conducted one sampling, there were no time series data to observe patterns or trends relating changes in environmental parameters with dinoflagellate cyst abundance and assemblages.

This study found two potentially harmful marine dinoflagellate cysts present in Paotere Port, *Alexandrium catenella* and *Cochlodinium polykrikoides*. Besides the potential for harmful algal blooms (HABs), these dinoflagellate cysts themselves can be very toxic, containing up to 10 times the toxin content of vegetative cells, thus constituting a possible source of poison to organisms long after the motile forms have disappeared from the water column (Zingone et al 2021). However, the number of these cysts was very low. They do not appear to pose a high risk of HAB occurrence in this area.

Conclusions. This study on dinoflagellate cyst distribution and assemblages in the Paotere Port, Makassar City, Eastern Indonesia recorded both low cyst abundance and diversity index values, with abundance influenced mainly by sediment texture (lower in sandy sediment compared to silty sediment). The 31 cyst-forming dinoflagellate species identified includes two potentially harmful taxa (*Alexandrium catenella* and *Conchlodinium polykrikoides*); these species were not previously reported in planktonic samples, and were recorded for the first time in this area. Cyst abundance at Paotere Port was low compared to subtropical and temperate coastal regions.

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Authors:
Nita Rukminasari, Department of Fisheries, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia, e-mail: nita.r@unhas.ac.id
Akbar Tahir, Department of Marine Science, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia, e-mail: akbar_tahirf@mar-sci.unhas.ac.id

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