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Species Assemblages and Distribution of Dinoflagellate Cysts from three Estuaries Sediment's of Makassar Strait, Eastern Indonesia

[1]*Nita Rukminasari and [2]Akbar Tahir

[1]Department of Fisheries, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia
[2]Department of Marine Science, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia

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Corresponding Author:
Nita Rukminasari
Department of Fisheries, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia
Email: nita.r@unhas.ac.id

Abstract: Makassar Strait encompasses high fisheries resources and high biodiversity and primary productivity. Algae blooms often occur in these areas, but there is a lack of studies that have been carried out on HABs in these locations specifically determining the collection and abundance of dinocyst. The study aimed to determine species assemblages of dinoflagellate cysts from three estuaries of Makassar Strait. Sampling was conducted at three locations, i.e., Jeneberang, Maros and Pangkep Estuaries during July 2020. Each location of sampling consisted of 4 stations and 3 substations as a sampling replication. Variables that were measured in this study were dinoflagellate cysts abundance species assemblages and distribution. Results showed that there was no clear group of dinoflagellate cysts assemblages between locations, however dinoflagellate cyst assemblages from Jeneberang Estuary was significantly grouped. We recorded 48 dinoflagellate cysts species that belonged to 5 families, namely Goniodomaceae, Gonyaulacaceae, Gymnodiniacea, Peridiniaceae and Protoperidiniaceae. The dominant species of dinoflagellate cysts from Jeneberang Estuary was *Scrippsiella crystallina*, meanwhile the dominant dinoflagellate cyst from Maros and Pangkep Estuary was *Gonyaulax verior* and *Pentapharsodinium tyrrhenicum*, respectively. *Scrippsiella trifida* was a dinoflagellate cysts species was contributed to the dissimilarity of cyst assemblages between locations. We also found potential five toxic dinoflagellate cyst species. In conclusion, the dinoflagellate cysts species composition, diversity, species richness and cyst abundance was not significant difference between stations of all locations. The abundance of dinoflagellate cysts was significantly correlated by sediment grain size. All sampling sites has a potentially occurred of HABs due to the five potential cysts toxic and harmful species discovered.

Keywords: Dinoflagellate Cysts, Species Assemblages and Dsistribution, Jeneberang, Maros and Pangkep Estuary and Makassar Strait

Introduction

In coastal and estuarine waters, phytoplankton is dominated by dinoflagellates and they are the most diverse organisms in the aquatic ecosystem. They could be as autotrophs, which produce their food and heterotrophs that utilize organic compounds for growth and reproduction (Tiselius and Kuylenstierna, 1996). Many dinoflagellates have two-phase of their life cycle, namely an asexual vegetative phase, which reproduction through the fusion of two cells and a sexual phase, which involving gamete fusion process (Mohamed and Al-Shehri, 2011). Sexual reproduction produces a motile cell, the zygote, which can be back to the vegetative stage or become a hypnozoite, or resting cyst. This vegetative state could be able as a plankton which swim in the water column and as a benthic that leave in the bottom sediments (Figueroa *et al*., 2007). Cysts can live in sediments for more than 5 years. When the favorable condition (mainly temperature) occurred, cysts that leave in the sediment as a feed bank could be creating plankton blooms in the water column (Lewis *et al*., 1999). Resting stage of many dinoflagellate species are very resistant to the changing of physical, chemical and biological factors (D'Silva *et al*., 2013).

One of an important role in the ecology of Harmful Algal Blooms (HABs) in particular for the dinoflagellate group was dinoflagellate resting cysts (Figueroa *et al*., 2007; Hallegraeff *et al*., 2004) and they are a fundamental attribute for dinoflagellate life cycles (Elbrächter, 2003). Hallegraeff *et al*., 2004; Nehring, 1993a) recorded that about 100 marine and freshwater dinoflagellates out of 2000 extant species have been shown to produce resting cysts. More than 20 of these dinoflagellates' cysts are known could cause HABs (Nehring, 1993b).

Temperature, salinity, nutrients and pollution are an environmental factors that influence the population of dinoflagellates and can also form cysts under unfavorable conditions. Dinoflagellate cysts in the sediment provide information on the dinoflagellates present in the water column (Anderson and Wall, 1978; Dale, 1976). The trophic form of dinoflagellate cyst (phototrophic and heterotrophic) is important information due to they have a different environmental and nutrition requirements (Harland *et al*., 2004).

Several biological and environmental factors: Primary production and physicochemical conditions (e.g., sea-surface temperature and salinity) could control a cysts distribution dynamics (Candel *et al*., 2012), including physical characteristics of sediment (Anderson *et al*., 2005; Anglès *et al*., 2010; Horner *et al*., 2011). Triki *et al*. (2017) found that fine sediment fractions are affected cyst abundances.

The information relates to the characterization of the distributional patterns of harmful species in both water column and sediments and identification of the microalgal community associated with these species are important (Glibert and Pitcher, 2001). Dinoflagellate cyst assemblages, distribution and environmental factors influencing their life are important to be studied for better utilization of dinoflagellate cysts as an indicator of environmental fluctuations (Eynaud *et al*., 2004; Holzwarth *et al*., 2007).

Makassar Strait has a high fisheries resources and also a high biodiversity and primary productivity. Increasing anthropogenic disturbance to those areas in particular increasing organic pollution from agricultural areas caused increasing nitrogen and phosphorous concentration. This condition caused eutrophication of waters. Eutrophication is one factor that could trigger the occurrence of algal bloom. Algal bloom in this area could negatively affect ecosystem health and large scale of fish death. The algal bloom often occurs in those areas, however, there is a lack of study that has been conducted about the distribution of dinoflagellate cysts in these areas. Based on this reason, it is important to conduct a study for determining the abundance, assemblages and distribution of dinoflagellate cyst at three estuarine of Makassar Strait, so that we could detect and map the occurrence of HABs at those areas.

Materials and Methods

Study Sites

In this study, we researched Makassar Strait, with several sites, namely Jeneberang Estuary (Gowa Regency), Maros Estuary (Maros Regency) and Pangkep Estuary (Pangkep Regency) (Fig. 1). Those areas were selected due to the presence of big rivers nearby respectively and extensive area of estuarine and also are very vulnerable to anthropogenic disturbance and with a high level of organic pollution and as a result, those area has a high potential for HABs occurrence.

Sediment Sampling

Sediment samples were collected from 3 locations in Makassar Strait (Fig. 1) in July 2020. Each location consisted of 4 stations and 3 substations as a replicate of sampling. The sediment sample was collected using Wilder hand corer and was taken 5 cm of surface bottom sediment. Before samples was processed, samples were stored in the dark at 10°C.

Cyst Analysis

Sediment Processing

Surface samples sediment were obtained by removing the top 1 cm of sediment from each core. For the profiles, sample sediment was weight. Weighing sediment of each section was suspended in Filtered Seawater (FSW) and sonicated using Granbo 008 digital ultrasonic cleaner for 15 min. The sonicated material was filtered through three levels of mesh-size sieved (250, 100 and 20 μm mesh-size) and collected on the 20 μm mesh size. The fine particle that passed 20 μm mesh size was washed with FSW and transferred into a 10 mL vial bottle.

Cyst Counts and Identification

For dinoflagellate cyst analysis, the top fraction (0-2 cm) which represents recent sediment sieved using the method of (Matsuoka and Fukuyo, 2000). Each sediment sample was sonicated for 15 min and sieved through 250, 100 and 20 μm mesh-sizes. Fine particle that has been passed on the 20 μm mesh was transferred to a petri dish and let sedimented for 15 min, upper layer of solution then transferred into 10 mL vial and observed under a light microscope with the magnification of 100-400 times. Dinoflagellate cysts were identified based on published descriptions (Alkawri, 2016; Aydin *et al*., 2011; Bravo *et al*., 2006; Godhe *et al*., 2000; Joyce *et al*., 2005; Kim *et al*., 2012; Mohamed and Al-Shehri, 2011; Narale and Anil, 2017; Nehring, 1997; Pospelova *et al*., 2004; Shin *et al*., 2011; Uddandam *et al*., 2017). The unit of cyst abundance was the number of cysts g^{-1} dry weight sediment. To measure the water content of each sample, a subsample

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was weighed wet and then dried in a 70°C oven for 24 h to obtain the dry weight. The water content and dinoflagellate cyst abundance were calculated following the formula: Cysts/g = N/W(1-R) (Matsuoka and Fukuyo, 2000). Where: N = Observed cyst number; *W* = wet sediment weight and R = water content rate.

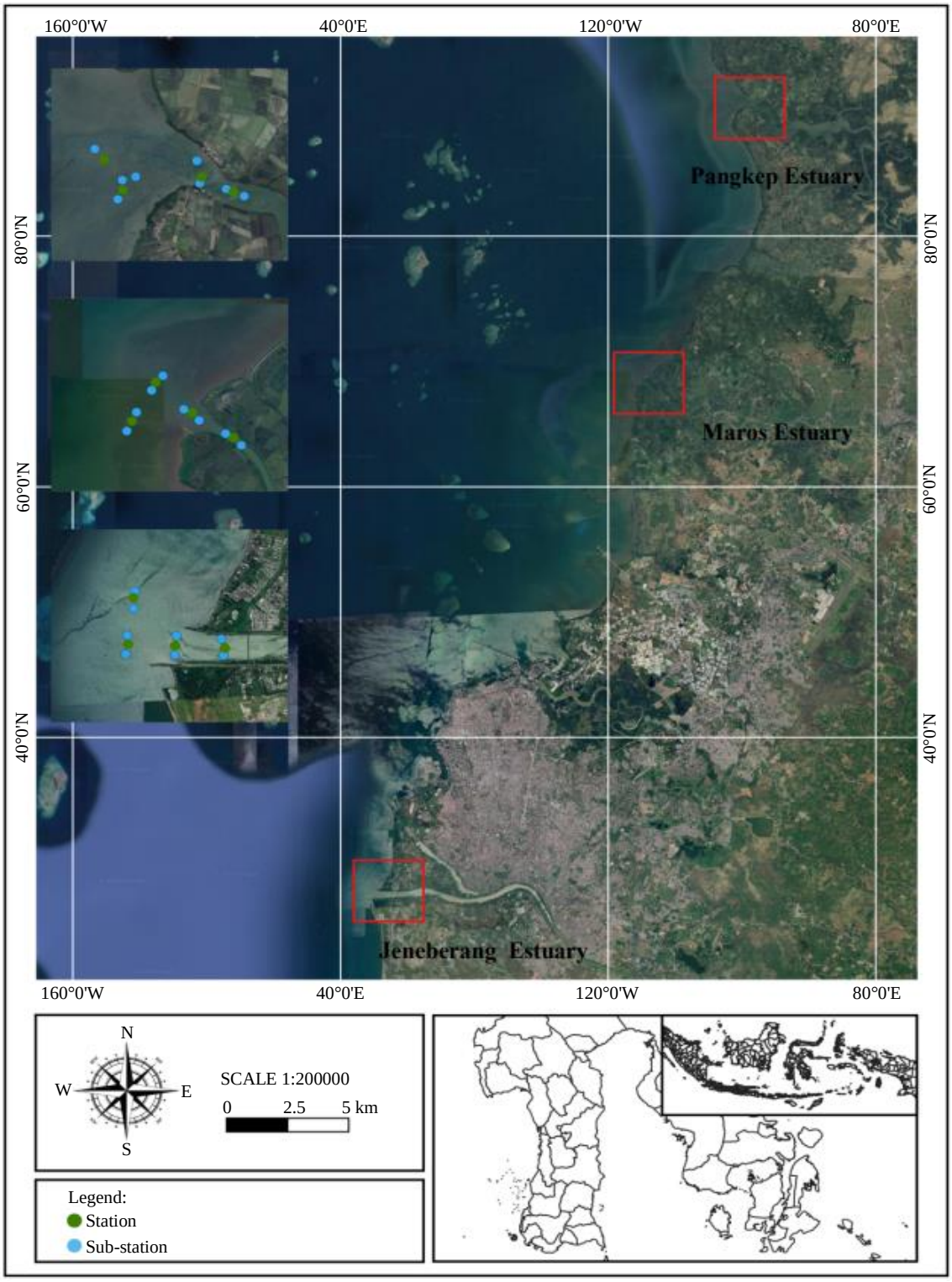

Fig. 1: Study sites map

Water and Sediment Parameters

Sediment samples were also collected using Eijkman Drag. Parameters from sediment that were analysis including sediment texture, total carbon, inorganic carbon and total nitrogen. Standard wet sieving was used for measuring sediment texture (percentage of sand, silt and clay content). The Total Carbon (TC) was analyzed using Loss of weight On Ignition (LOI %) according to (Meng *et al*., 2014). Total Nitrogen (TN) contents was analyzed using Kjeldahl method with detail analysis according to (Bremner, 1996). Total Inorganic Carbon (TIC) content was estimated with modified pressure-calcimeter method (Sherrod *et al*., 2002).

Data Analysis

PRIMER - version 5 (PRIMER–E Ltd. Plymouth, UK) was used for analysis Shannon–Wiener's diversity index (H'), Margalef's species richness (d) and Pielou's evenness (J')). nMDS, ANOSIM and SIMPER as a PRIMER routine were also applied. We used log (×+1) transformation of the abundant taxa. For measuring similarity Bray-Curtis coefficient was used. To examine a spatial variation of dinoflagellate cyst abundance, Shannon–Wiener's diversity index (*H*'), Margalef's species richness (*d*) and Pielou's evenness (*J*'), two-way ANOVA was performed using GraphPad 7 software program

Results

Dinoflagellate Cyst Taxa and Abundance

Overall, 48 dinoflagellate cyst species belonging to 25 genera were recorded in the sediment of three estuaries of Makassar Strait (Jeneberang Estuary, Maros Estuary and Pangkep Estuaries). The list of dinoflagellate cyst species found in surface sediment from three estuaries of Makassar Strait has been given in Supplement 1. The highest number of identified taxa per single sample was found in Maros Estuary station IV substation 2, 17 taxa, followed by 14 taxa in Pangkep Estuary station I substation 3, Maros Estuary station 3 substations 1 and Jeneberang Estuary station II substation 3 and the lowest, only 2 taxa in Maros Estuary station III substation 2. Total cyst abundance ranged from 35 cysts g^{-1} DW sediment (in Station 4 substation 3 of Pangkep Estuary) to 1135 cysts g^{-1} DW sediment (in station 1 substation 2 of Pangkep Estuary) (Fig. 2a) and did not show a significant spatial variation (two-way ANOVA). The number of species ranged from 2 to 17 within the investigated samples. Shannon diversity (H') ranges from 0.2959 to 1.159 (Fig. 2d). The highest species diversity was recorded in the Maros Estuary station IV substation 2. The lowest species diversity was found in Maros Estuary station III substation 2. Species richness ranged from 0.3102 (Jeneberang Estuary station I substation 3) to 2.9376 (Maros Estuary station IV substation 3) (Fig. 2b).

The highest and the lowest Pileou's evenness index (J') were found at Maros Estuary station III substation 2 and Pangkep Estuary station I substation 2 accounting for 0.9829 and 0.5034, respectively (Fig. 2c). However, there was no significant difference in the univariate measure of dinoflagellate cyst (cyst abundance, species diversity, richness and evenness) between locations and station samplings (Table 1).

Assemblages and Community Structure of Dinoflagellate Cyst

We were recorded of dinoflagellate cysts from all sediment samples (Table 1) belong to 5 families, namely Goniodomaceae, Gonyaulacaceae, Gymnodiniacea, Peridiniaceae and Protoperidiniaceae. Goniodomaceae consisted of five species: *Alexadrium cf. tamarense*, *Alexandrium catenella*, *Alexandrium cf. minutum*, *Alexandrium cf.tamiyavanachi*, *Alexandrium pseudogonyualax*. This family contributed 8% of the total species found (Fig. 3). The highest percentage of total species belonged to the family of Peridiniaceae, which consisted of 16 species (Fig. 3), namely: *Bicarinellum bicarinelloides*, *Brigatedinium asymmetricum*, *Dubridinium* spp., *Dubridinium* spp., Potter, *Echidinium granulatum*, *Lejeunecysta oliva*, *Operculodinium centrocarpum*, *Scrippsiella cf. lachrymose*, *Scrippsiella cf.rotunda*, *Scrippsiella crystallina*, *Scrippsiella trifida*, *Scrippsiella trochoides*, *Selenopemprix nephroides*, *Sxrystallin* sp, *Votadiniium spinosum*, *Zygabikodinium lenticulatum.* Gonyaulacaceae and Gymnodiniaceae were contributed the same percentage from the total dinoflagellate cyst species, accounting for 14%, which was contributing 7 species of dinoflagellate cysts (Fig. 3).

When the log mean abundance of dinoflagellate cyst in each location was subjected to ordination, the plot depicting the relationships between the assemblages of dinoflagellate cysts in the different station showed that the samples were initially widespread on the plot for all locations, with the point for Maros Estuary and Pangkep Estuary overlying each other (Fig. 4). The ordination plots for the dinoflagellate cyst communities at Jeneberang Estuary showed that the points for each station formed a relatively tight group that were discrete from each other (Fig. 4a). Furthermore, in three other locations, the points of the stations were overlapped each other (Fig. 4b and 4c). ANOSIM results showed that there was a significant difference in dinoflagellate cyst assemblages between stations for all locations (Supplement 2).

SIMPER result showed that each station for each location was dominated by different species from a different family. In general, family Peridiniaceae, Protoperidiniaceae and Ganyualacaceae was the dominant family at all stations for all locations (Supplement 2). Interestingly, *Pentapharsodinium tyrrhenicum* was dinoflagellate cyst which was dominant at station 2 for all locations, furthermore for Jeneberang Estuary and Pangkep Estuary in station III had the same species which was dominated, that was *Scrippsiella crystallina*.

The percentage of dissimilarity between stations for all locations ranged from 41.62 to 78.85. The highest dissimilarity percentage was found at the pair of the station I vs station II for Jeneberang Estuary with the taxa that the most contributed for dissimilarity was *Scrippsiella crystallina*. Furthermore, the pair of the station I vs station II for Maros Estuary had the lowest percentage of dissimilarity with the taxa most contributed for dissimilarity was *Scrippsiella trifida.*

Water and Sedimentary Parameters at three Estuaries of Northern Makassar Strait

A higher temperature (32.08°C) was recorded at station 1 of Jeneberang Estuary and lower temperature (30.40°C) was found at Maros Estuary station 3 (Table 2). The salinity value ranged from 25.41 PSU (Maros Estuary station I) to 32.95 PSU (Jeneberang Estuary station IV). The highest DO was recorded at Maros Estuary station IV accounting for 6.18 mgL^{-1}, Jeneberang Estuary station III had the lowest DO, which was 4.31 mgL^{-1}. pH was varied between station for each location, the ranged of pH value from 6.74 to 7.58. Locations of dinoflagellate cyst sampling in the study were mostly sandy sediment texture, where there were only two stations which have a silt sediment texture, namely Jeneberang Estuary station II and Pangkep Estuary station I. The ranged of TOC was from 0.09% (Jeneberang Estuary station IV) to 0.91% (Maros Estuary station III), meanwhile, we recorded TIC varied between stations and locations. The highest TIC was found at Jeneberang Estuary station IV accounting for 26.72% and Maros Estuary station I had the lowest TIC, which was 0.08%. We also found that undetected TIC for Maros Estuary station III and IV. TN content was similar value from all stations and locations, ranged from 0.01 to 0.05%. Furthermore, the highest and the lowest Corg: N ratio were found at Jeneberang Estuary station III and I, accounting for 32.77 and 6.64, respectively.

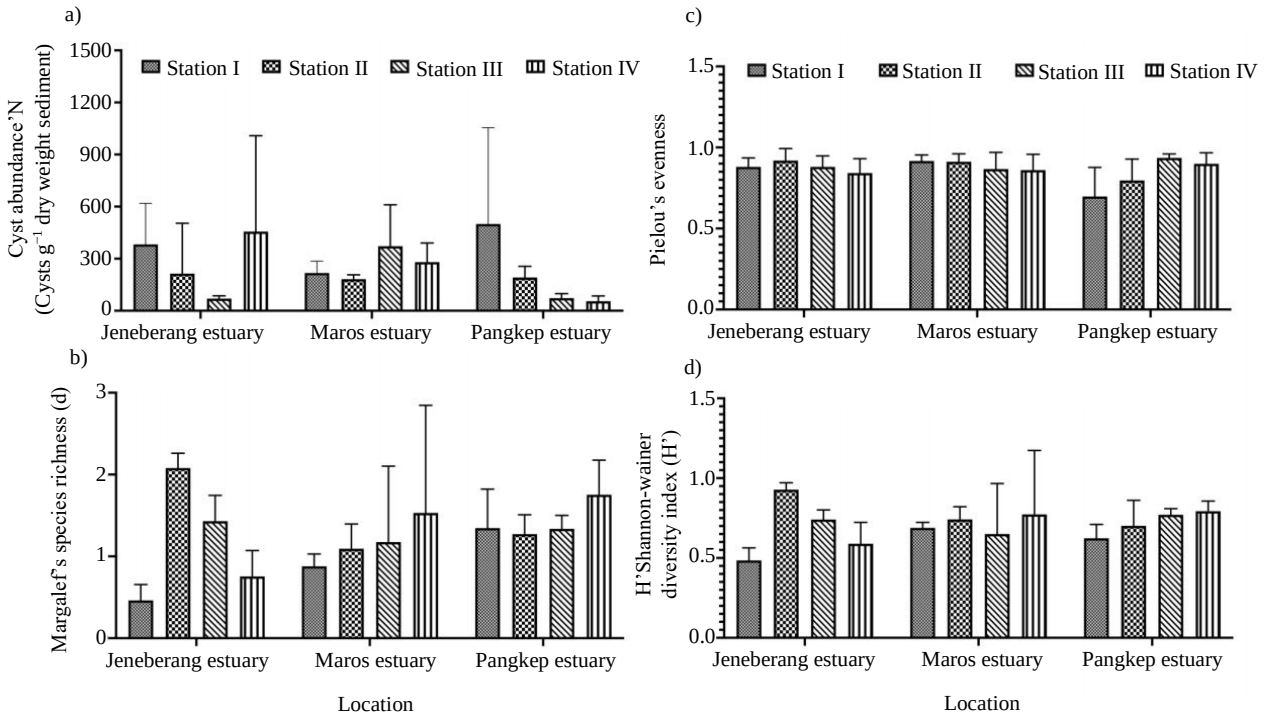

Fig. 2: Spatial variation in (a) dinoflagellate cyst abundance: N, (b) Margalef's species richness: d, (c) Pielou's evenness: J' and (d) Shannon- Wiener's diversity index: H' at three estuaries of MAakassar Strait

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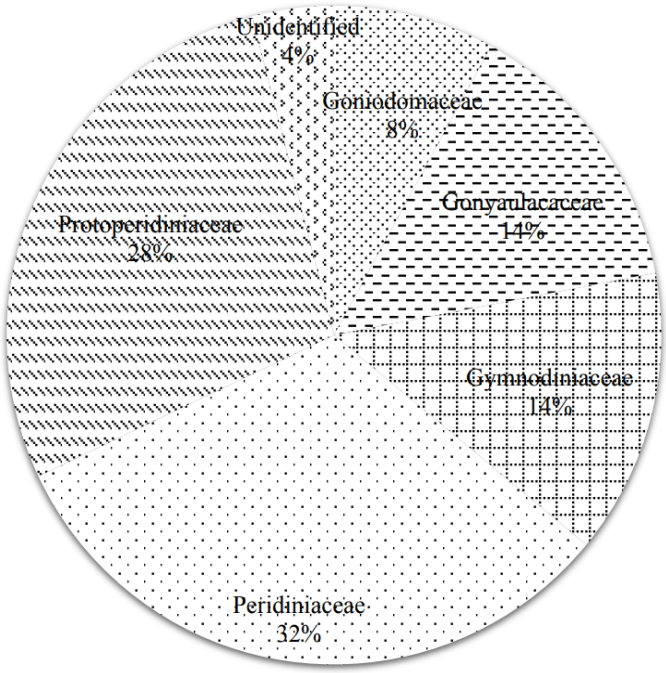

Fig. 3: Percentage composition cysts group: Goniodomaceae, Gonyaulacaceae, Gymnodiniaceae, Peridiniacea and Protoperidiniaceae in three estuaries of Makassar Strait

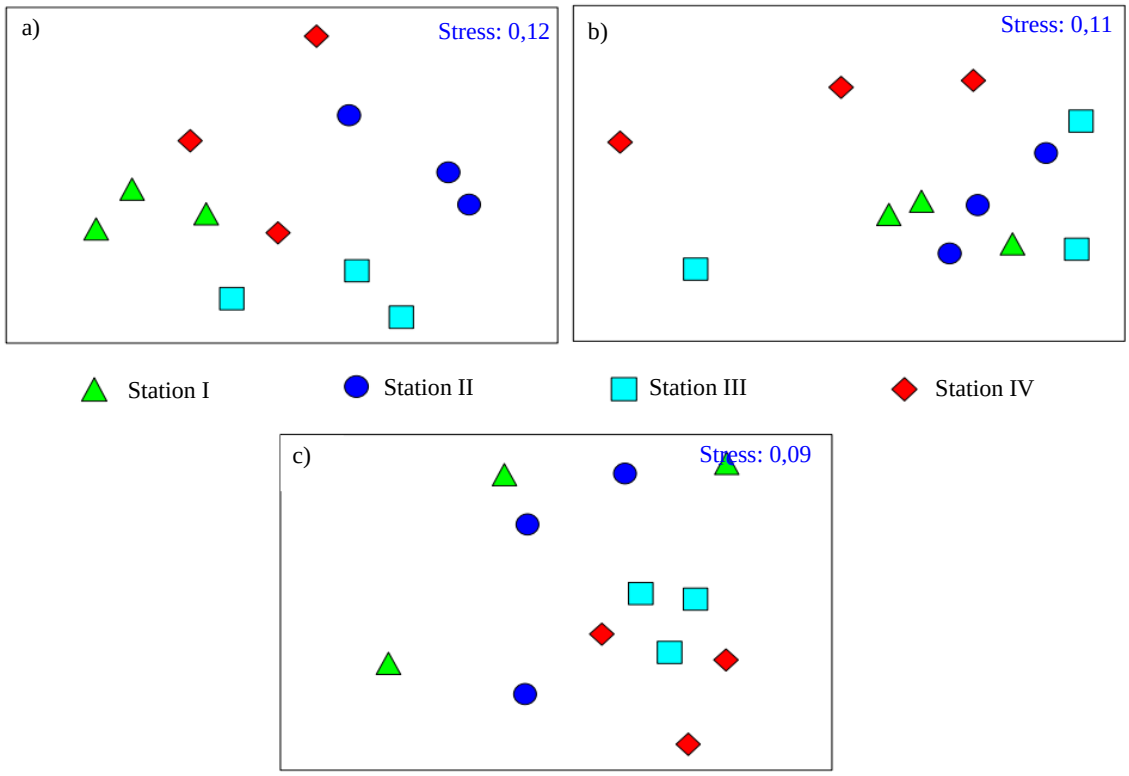

Fig. 4: nMDS ordination of Log (×+1) transformation comparing the dinoflagellate cysts assemblages in term of total number of cyst/g DW sediment between location for each station: (a) Jeneberang Estuary, (b) Maros Estuary and (c) Pangkep Estuary

Table 1: List of dinoflagellate cyst species with biological and paleontological name that found in surface sediment of three estuaries of Makassar Strait. Dinoflagellate cyst taxonomic name that used in this study are taken from (Alkawri, 2016; Aydin *et al*., 2011; Bravo *et al*., 2006; Godhe *et al*., 2000; Joyce *et al*., 2005; Kim *et al*., 2012; Mohamed and Al-Shehri, 2011; Narale and Anil, 2017; Nehring, 1997; Orlova *et al*., 2004; Pospelova *et al*., 2004; Shin *et al*., 2011; Uddandam *et al*., 2017)

Dinoflagellate biological name	HABs species	Dinoflagellate cyst (Paleaontological name)
Archaeperidinium sp.	Non-toxic	Cyst of Achaeperidinium sp.
Alexandrium catenella	Yes	Cyst of *Alexandrium catenella*
Alexandrium cf. minutum	Yes	Cyst of *Alexandrium cf.minutum*
Alexadrium cf. tamarense	Yes	Cyst of *Alexandrium cf.tamarense*
Alexandrium cf. tamiyavanachi	Non-toxic	Cyst of *Alexandrium cf.tamayavanachi*
Alexandrium pseudogonyualax	Non-toxic	Cyst of *Alexandrium pseudogonyualax*
Bicarinellum bicarinelloides	Non-toxic	Cyst of *Bicarinellum bicarinelloides*
Brigatedinium asymmetricum	Non-toxic	Cyst of *Brigatedinium asymmetricum*
Cochlodinium polykrikoides	Yes	Cyst of *Cochlodinium polykrikoides*
Diatom	Non-toxic	-
Diplopella symmetrica	Non-toxic	*Dubridinium spp*
Dubridinium spp Potter	Non-toxic	*Dubridinium spp*
Dubridinium spp	Non-toxic	*Dubridinium spp*
Echidinium granulatum	Non-toxic	Unknown
Foraminifera organic living	Non-toxic	-
Fragilidium subglobosum	Non-toxic	Unknown
Gonyaulax scrippsae	Non-toxic	*Spiniferites bulloideus*
Gonyaulax verior	Non-toxic	*Cyst of Gonyaulac verior*
Gymnodinium catenatum	Yes	*Cyst of Gymnodinium catenatum*
Gymnodinium cf. nolleri	Non-toxic	*Cyst of Gymnodinium cf.nolleri*
Gymnodinium instriatum	Non-toxic	*Cyst of Gymnodinium instriatum*
Lejeunecysta oliva	Non-toxic	*Unknown*
Operculodinium centrocarpum	Non-toxic	*Protoceratium reticulatum*
Pentapharsodinium tyrrhenicum	Non-toxic	*Unknown*
Pheopholykrikos hartmanii	Non-toxic	*Cyst of P. hartmannii*
Polykrikos kotoidii	Non-toxic	*Cyst of Polykrikos kotoidii*
Polykrikos schwartzii	Non-toxic	*Cyst of Polykrikos schwartzii*
Protoceratium reticulum	Non-toxic	*Perculodinium centrocarpum*
Protoperidinium avellana	Non-toxic	*Brigantedinium cariacoense*
Protoperidinium conicoides	Non-toxic	*Brigantedinium simplex*
Protoperidinium cf. americanum	Non-toxic	*Cyst of Protoperidinium americanum*
Protoperidinium cf. divergen	Non-toxic	*Echidinium aculeatum*
Protoperidinium claudicans	Non-toxic	*Votadinium spinosum*
Protoperidinium cf. excentricum	Non-toxic	*Echidinium delicatum*
Protoperidinium fukuyoi	Non-toxic	*Echidinium granulatum*
Protoperidinium oblongum	Non-toxic	*Votadinium calvum*
Protoperidinium pentagonum	Non-toxic	*Triovantedinium applantum*
Protoperidinium punctulatum	Non-toxic	*Echidinium sp*
Protoperidinium subinerum	Non-toxic	*Selenopemphrix nephroides*
Pyrophacus horologium	Non-toxic	*Unknown*
Pyrophacus steinii	Non-toxic	*Tuberculodinium vancampoae*
Scrippsiella cf. lachrymosa	Non-toxic	*Cyst of Scrippsiella cf.lachrymosa*
Scrippsiella cf. *rotunda*	Non-toxic	*Cyst of Scrippsiella cf.rotunda*
Scrippsiella crystallina	Non-toxic	*Cyst of Scrippsiella crystallina*
Scrippsiella trifida	Non-toxic	*Cyst of Scrippsiella trifida*
Scrippsiella trochoides	Yes	*Cyst of Scrippsiella trochoides*
Selenopemprix nephroides	Non-toxic	*Unknown*
Spiniferites elongatus	Non-toxic	*Unknown*
Sxrystallin	Non-toxic	*Unknown*
Votadiniium spinosum	Non-toxic	*Protoperidinium claudican*
Zygabikodinium lenticulatum	Non-toxic	*Unknown*

Table 2: Location of sampling station along with water and sedimentary parameters

	Water parameter				Sedimentary parameter				
Location/Station	Temp. (°C)	Salinity (PSU)	DO (mgl^{-1})	pH	TOC (%)	TIC (%)	TN (%)	C:N ratio	Sediment type
Jeneberang estuary									
I	32.08	31.42	6.13	7.29	0.24	25.35	0.04	6.64	Sand
II	31.01	29.21	5.76	7.34	0.31	21.22	0.03	10.69	Silt
III	31.00	31.39	4.31	7.30	0.24	12.33	0.01	32.77	Sand
IV	30.65	32.95	5.06	7.43	0.09	26.72	0.01	12.05	Sand
Maros estuary									
I	31.06	25.41	5.98	6.74	0.57	0.08	0.03	17.92	Sand
II	31.12	31.96	5.71	6.76	0.68	2.24	0.03	19.30	Sand
III	30.40	32.66	6.15	7.58	0.91	0.00	0.04	22.30	Sand
IV	30.28	32.91	6.18	7.48	0.67	0.00	0.04	17.15	Sand
Pangkep estuary									
I	31.55	29.10	6.07	7.36	0.84	0.93	0.05	17.63	Silt
II	31.41	29.91	5.95	7.22	0.36	2.71	0.04	10.51	Sand
III	30.85	31.83	5.72	7.07	0.63	7.60	0.03	18.46	Sand
IV	30.80	32.08	6.04	6.94	0.29	5.93	0.02	12.97	Sand

Discussion

Several biological parameters could be used as an effective indicator for environmental changing, namely: Phytoplankton composition, mainly diatoms and dinoflagellates cysts in particular (Rodrigues *et al*., 2019). Several diversity index, namely species composition, species diversity (Pospelova *et al*., 2002) and species-richness are also used to assess water quality including pollution and eutrophication (Rodrigues *et al*., 2019). Pospelova *et al*. (2004; 2002) used dinoflagellate cysts-based Fisher's α and species-richness (number of species) as indicators of environmental conditions in estuarine systems.

This study provided the first data about the abundance, composition and distribution of dinoflagellate cysts, including toxic species, in three estuaries of Makassar Strait. Species composition of dinoflagellate cysts from the sediment of three estuaries of Makassar Strait could be compared to in marine sediment of several previous study at Japan, Korea, Russia, India, Philippine, Aegean Sea, Sweden, Canada, Argentina, Saudi Arabia and China (Aydın *et al*., 2011; Baula *et al*., 2011; Candel *et al*., 2012; Godhe *et al*., 2000; Kim *et al*., 2012; Matsuoka and Fukuyo, 2000; Mertens *et al*., 2012; Mohamed and Al-Shehri, 2011; Pospelova *et al*., 2004; Shin *et al*., 2010; 2011) due to no early records of recent dinoflagellate cysts from Northern Makassar Strait. The comparison of dinoflagellate cyst assemblages in our study with nearby waters was not possible. The estuaries have different physical and environmental characteristics, therefore the response of dinoflagellate cysts to eutrophication in coastal waters may also depend on the type of estuary that is being investigated (Pospelova *et al*., 2002). A recent study by (Price *et al*., 2016) has shown that dinoflagellate cyst assemblages in estuaries vary by biogeographic province and that within a biogeographic province samples from the same estuary type generally had more similar assemblages compared to a different estuary type. In our study, the dinoflagellate cyst assemblages comprised mainly from cosmopolitan genera, such as *Alexandrium*, *Cochlodinium*, *Gymnodinium*, *Pentapharsoodinium*, *Protoperidinium*, *Gonyaulax* and *Scrippsiella* (Matsuoka and Fukuyo, 2000).

In general, tropical waters are characterized by low abundance and diverse dinoflagellate populations (Rodrigues *et al*., 2019). In our study sites, we found that there was a low diversity and species-richness. Furthermore, the diversity and species-richness values for dinoflagellate cysts in our study were lower than the values for temperate and tropical regions. Pospelova *et al*. (2004; 2002) suggested a decreasing in diversity and species-richness of dinoflagellate cyst taxa as a general indicator for toxic pollution and eutrophication estuarine system.

Our results showed that cyst species richness (0.31-2.94) and diversity (0.30-1.16) were significantly lower in this study than in others. Baula *et al*. (2011) found that cyst species diversity at Balinao, Pangasinan was low with range of diversity index from 1.9 to 2.7. A low dinoflagellate cyst species richness and diversity in our study indicated that the study sites were estuaries which a high nutrient loading. Pospelova *et al*. (2002) have been suggested that decreasing species diversity as an indicators of estuarine system which has a high nutrient loading. The Perinidiaceae (32%) and Protoperidiniaceae (28%) are abundant in all stations and locations. Gymnodiniaceae and Gonyaulacaceae species average 14% while Goniodoniaceae (8%) and unidentified (4%) were the represented group for a lesser contribution (Fig. 3). A high abundant of heterotrophic Protoperidiniaceae indicated that the waters in the study area was eutrophic. Previous study by (Hamel *et al*., 2002; Marret, 2003; Matsuoka, 1999; Matsuoka *et al*., 2003; Radi and de Vernal, 2004) found that Protoperidiniace was a good indicator for eutrophic waters. Kim *et al*. (2009) stated that Protoperidiniaceae as a heterotrophic dinoflagellate can be more abundant than autotrophic dinoflagellate in eutrophic coastal environment.

We found that sediment characteristics influenced cyst abundance, where sandy sediments tended to have a lower cyst concentration. The sediment characteristics in our study sites was mostly sandy sediment, consequently cysts abundance was low and ranged from 35 cysts g^{-1} DW sediment to 1135 cysts g^{-1} DW sediment. This finding in lined with previous study, which reported dinoflagellate cysts was higher abundance in muddy sediments than those of in sandy sediments (Anderson *et al*., 2005; Nehring, 1993b; Olli and Trunov, 2010). This may be due to behavior of dinoflagellate cyst to deposit their cyst in fine particles and that their abundance increases in sediments with a higher mud contents (De Vernal *et al*., 2013). Dinoflagellate cyst distributions in the sediments was influenced by the cyst production patterns in the overlying water column and specific encystment strategies of dinoflagellate species (Anderson *et al*., 2005; Dale, 1976; Kremp *et al*., 2009).

Our finding demonstrated trends and groupings of dinoflagellate cysts based on species composition and abundance. The pattern of species assemblages and abundant indicated a functions of water depth, sediment grain size and nutrients (TOC, TIC, TN and N:P Ratios). We found that there is a trend that sites with a lower TOC values have a higher values of species richness. This finding contradicts with previous study by (Pospelova and Kim, 2010) who found that there is a trend that sites with the higher TOC values have lower values of species richness. Marret (2003) have identified the most important environmental variables that can be related to the distribution patterns dinoflagellate cysts in the world, namely Sea Surface Temperature (SST), phosphate and nitrate concentrations. Marret *et al*. (2004) found that cysts are distributed according to stratification and grain size classes sediment. In Sunda Shelf, South China Sea, cyst distribution is related to water depth, total organic carbon and sediment grain size (Kawamura, 2004). In this study, Table 1 summarized the water and sediment parameters to dinoflagellate cyst species composition.

We also found dinoflagellate cysts species that potentially harmful in three estuaries of Makassar Strait sediments. The cysts belonged to the toxin-producing species of *Alexandrium catenella*, *A. minutum*, *Cochlodinium polykrikoides*, *Gymnodinium catenatum* and *Scrippsiella trochoidea* were found at several stations of sampling sites. The presence of harmful marine dinoflagellate cysts in marine sediments has been documented worldwide (Anderson *et al*., 2005; Fahnenstiel *et al*., 2009; Matsuoka *et al*., 2003; Pitcher and Joyce, 2009). In addition, the dinoflagellate cysts can be very toxic (up to 10 times more toxic than vegetative cells), it could be source of poison to organisms (Zingone *et al*., 2020).

Conclusion

Species composition, diversity, species richness and cyst abundance among the study sites was not a significant difference in. Sediment characteristics was strongly affecting the abundance of dinoflagellate cysts: Cyst abundances were low at all sampling sites due to sampling sites was mostly sandy sediment. Our study also found that there were five potentially toxic cysts and harmful species that was detected in all sampling sites, so the study areas was potential for occurring HABs.

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Author's Contributions

Nita Rukminasari: Designed a sampling method, conducted field sampling, data analysis and interpretation, writing a draft manuscript, submitting a manuscript.

Akbar Tahir: Designed a sampling method, data interpretation, final editing and proofreading of manuscript before submitting.

Ethics

This article does not contain any studies with animals performed by any of the authors.

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Supplement 1: Two-way ANOVA results to examine the spatial variation in dinoflagellate cyst abundance (N), Shannon-Wainer's diversity index (H'), Margalef's species richness (d), and Pielou's evenness (J') in three estuaries of Makassar Strait (Jeneberang Estuary, Maros Estuary and Pangkep Estuary).

Factor	df	SS	MS	fs	P value
N					
Location	2	37991	18995	0.1042	0.7815
Station	3	205387	68462	1.0690	0.4225
Location x Station	6	507530	84588	2.0880	0.2496
Cysts abundance x Location	4	729476	182369		
Cysts abundance x Station	6	384420	64070		
Cysts abundance	2	68015	34007		
Residual	12	486224	40519		
d					
Location	2	0.5071	0.2535	4.3010	0.1079
Station	3	1.7320	0.5775	2.8810	0.2301
Location x Station	6	4.0700	0.6784	1.6590	0.3200
Species richness x Location	4	0.2358	0.0590		
Species richness x Station	6	1.2030	0.2004		
Species richness	2	0.5805	0.2903		
Residual	12	4.9080	0.4090		
J'					
Location	2	0.0224	0.0112	1.1860	0.3904
Station	3	0.0186	0.0062	0.5266	0.5449
Location x Station	6	0.1016	0.0169	2.2460	0.2334
Evenness index x Location	4	0.0378	0.0094		
Evenness index x Station	6	0.0705	0.0118		
Evenness index	2	0.0003	0.0002		
Residual	12	0.0905	0.0075		
H'					
Location	2	0.0087	0.0044	0.5453	0.5768
Station	3	0.1705	0.0568	2.2310	0.2726
Location x Station	6	0.2438	0.0406	1.0140	0.4222
Shannon-Wainer index x Location	4	0.0321	0.0080		
Shannon-Wainer index x Station	6	0.1529	0.0255		
Shannon-Wainer index	2	0.0069	0.0035		
Residual	12	0.4807	0.0401		

Supplement 2: Results from an ANOSIM Pairwise test and SIMPER comparing dinoflagellate cyst assemblages between station for each location. Table displays Global R, Significance level (%), Dissimilarity (%) and the taxa most responsible for dissimilarity

	ANOSIM Pairwise Tests		SIMPER Results	
Pair	Global R	Significance level (%)	Dissimilarity (%)	Taxa most responsible for dissimilarity
Jeneberang Estuary	0.569	0.002		
Station I vs Station II	0.963	10	78.85	Scrippsiella trifida (11%), Pyrophacus horologium (11%), Pentapharsodinium tyrrhenicum (9%)
Station I vs Station III	0.815	10	68.28	Scrippsiella trifida (17%), Pyrophacus horologium (14%), Foraminifera org living (11%)
Station I vs Station IV	0.185	20	55.69	Scrippsiella trifida (16%), Pyrophacus horologium (15%), Alexandrium catenella (12%)
Station II vs Station III	0.556	10	64.08	Foraminifera org living (10%), Protoperidinium subinerum (9%), Scrippsiella trochoides (9%)
Station II vs Station IV	0.481	20	67.07	Pyrophacus horologium (10%), Scrippsiella crystallina (9%), Scrippsiella trifida (9%)
Station III vs Station IV	0.315	10	62.52	Pyrophacus horologium (12%), Foraminifera org living (11%), Scrippsiella trifida (11%)
Maros Estuary	0.105	0.21		
Station I vs Station II	-0.259	100	41.62	Scrippsiella trifida (13%), Alexandrium catenella (10%), Scrippsiella crystallina (9%)
Station I vs Station III	0.296	30	66.85	Scrippsiella crystallina (11%), Alexandrium catenella (10%), Scrippsiella trifida (9%)
Station I vs Station IV	0.296	10	69.34	Scrippsiella crystallina (9%), Protoperidinium subinerum (9%), Cochlodinium polykrikoides (8%)
Station II vs Station III	-0.037	50	64.33	Pentapharsodinium tyrrhenicum (10%), Scrippsiella trifida (9%), Scrippsiella crystallina (8%)
Station II vs Station IV	0.296	20	74.27	Scrippsiella trifida (9%), Pentapharsodinium tyrrhenicum (8%), Protoperidinium subinerum (8%)
Station III vs Station IV	-0.037	50	76.99	Alexandrium catenella (10%), Protoperidinium subinerum (8%), Scrippsiella trifida (7%)
Pangkep Estuary	0.253	0.042		
Station I vs Station II	-0.259	80	63.82	Pentapharsodinium tyrrhenicum (8%), Gymnodinium catenatum (7%), Foraminifera org living (7%)
Station I vs Station III	0.148	20	71.56	Zygabikodinium lenticulatum (7%), Pentapharsodinium tyrrhenicum (7%), Scrippsiella cf.lachrymosa (7%)
Station I vs Station IV	0.333	20	76.95	Scrippsiella cf.lachrymosa (7%), Pentapharsodinium tyrrhenicum (7%), Gonyaulax verior (6%)
Station II vs Station III	0.556	10	60.64	Scrippsiella trochoides (11%), Zygabikodinium lenticulatum (10%), Gymnodinium catenatum (7%)
Station II vs Station IV	0.556	10	67.58	Scrippsiella trochoides (9%), Scrippsiella cf.lachrymosa (8%), Zygabikodinium lenticulatum (7%)
Station III vs Station IV	0.148	20	45.57	Gonyaulax verior (15%), Foraminifera org living (11%), Scrippsiella crystallina (9%)