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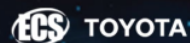
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# The abundance and species assemblages of dinoflagellate cysts at sediments in the three estuaries of the Southern part of South Sulawesi

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**Abstract.** Harmful algal blooms (HAB) are a phenomenon of drastically increasing phytoplankton populations, which can cause problems in aquatic ecosystems. Dinoflagellate cysts are a type of phytoplankton that are the main source of causing the HAB phenomenon; this occurs due to unfavorable environmental conditions. The Southern Estuary of South Sulawesi has high fishery resources and high primary productivity. The coastal areas around these waters are widely used for anthropogenic activities that trigger nutrient increases. There are no studies on HAB, especially the abundance and community structure of dinoflagellate cysts at this location. This study aims to identify the types, abundance, and species assemblages of dinoflagellate cysts in the three estuaries of southern South Sulawesi. Sampling was carried out at three locations, namely the Pappa River Estuary, the Kelara River Estuary, and the Calendu River Estuary, in January 2022. Each location consists of 4 stations, 3 sub-stations with 3 repetitions sampling for each substation. Results showed that there were 34 species of dinoflagellate cysts belonging to 6 families, namely Gonyaulacaceae, Gymnodiniaceae, Osteropsidaceae, Peridiniaceae, Polykrikaceae, and Protoperidiaceae. The highest abundance of dinoflagellate cysts was at the Calendu River estuary station III, accounting for 82 cysts g<sup>-1</sup>, while the lowest abundance of dinoflagellate cysts was found at Pappa River estuaries stations I and II, accounting for 8 cysts g<sup>-1</sup>. The level of diversity, uniformity, and richness of dinoflagellate cysts was not significantly different in the three study sites. The family Osteropsidaceae was the most common type of dinoflagellate cyst found in the three study sites. The Osteropsidaceae family is a type of dinoflagellate cyst that contains toxins, so it can be assumed that the three research sites have the potential for HAB.

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## 1. Introduction

Harmful algal blooms (HAB) are a phenomenon of drastically increasing phytoplankton populations, which can cause problems in aquatic ecosystems. Dinoflagellates are a unique group of eukaryotes that have various important ecological roles, especially as the core of the food chain [1]. Dinoflagellates can also form harmful and often toxic algae [2]. Dinoflagellates have a unique ability to survive the competition of natural selection. Under favorable conditions, dinoflagellates are able to reproduce in the water column in the form of swimming cells that actively divide in bulk. In poor environmental conditions, dinoflagellates stop dividing and then mate and produce a zygote which will continue at the encystment stage to produce resting cysts that can survive on the bottom of the water for years [3].

Dinoflagellate cysts are a type of phytoplankton that are the main source of causing the HAB phenomenon; this occurs due to unfavorable environmental conditions. Dinoflagellate cysts can survive



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in unfavorable environmental conditions. The dinoflagellate cyst stage is of great ecological importance as a “seed bank” for dinoflagellates [4].

Observation of cysts in waters is carried out to predict the possibility of rapid development of dinoflagellate species that can trigger the phenomenon of Harmful Algal Blooms (HABs). The continued discovery of new species suggests that there may be more dinoflagellate lineages to be identified [2].

The coastal areas around these waters are widely used for anthropogenic activities that trigger nutrient increases. There are no studies on HAB, especially the abundance and community structure of dinoflagellate cysts at this location. This study aims to identify the types, abundance, and species assemblages of dinoflagellate cysts in the three estuaries of the southern part of Makassar Strait, South Sulawesi.

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## 2. Materials and methods

### 2.1. Study site

The determination of the research location is based on the existence of community activities around the location with the assumption that community activities have the potential to affect the quality of the surrounding waters because they can produce anthropogenic disturbances with high levels of organic pollution. The study was conducted in January 2022. Each research location consisted of 4 stations and 3 sub-stations as sampling replication. The sampling locations selected were Pappa River Estuary (Takalar Regency), Kelara River Estuary (Jenepono Regency), and Calendu River Estuary (Bantaeng Regency). The three locations are Southern part of South Sulawesi (Figure 1).

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### 2.2. Sediment sampling

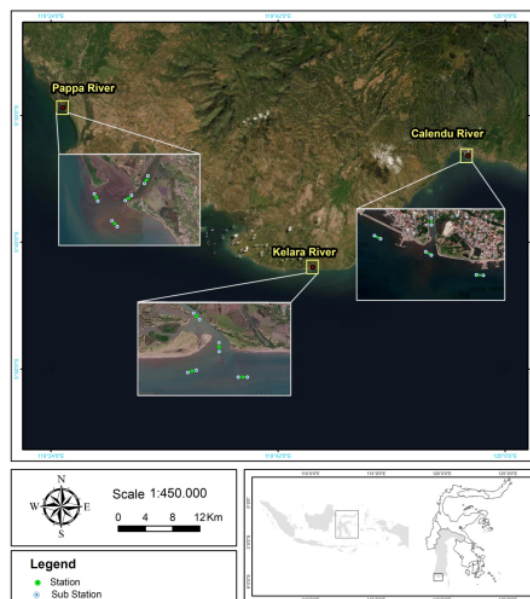
Sediment sampling was carried out using a corer sediment trap. The corer sediment is lowered vertically until it reaches the bottom sediment of the water. After reaching the bottom of the water, the corer sediment is inserted as deep as  $\pm 5$  cm for sediment collection. The sediment sample that has been taken will then be put into a box container. Before processing, the sediment sample is stored at 4°C.

### 2.3. Sediment processing

The separation between sediment and cyst uses the method [5] where the sample which has been weighed using an analytical balance is put into a beaker then added seawater until it reaches a size of 50 ml, then sonication is carried out, the process is carried out for 15 minutes using an ultrasonic sonicator. This process aims to release the cyst attached to the sediment particles. Furthermore, the sieving process is carried out using a sieve that is arranged in stages based on its size. In this study, three mesh sizes were used, i.e., 250  $\mu\text{m}$ , 125  $\mu\text{m}$ , and 20  $\mu\text{m}$ . The filtered sample was then deposited using a petri dish, then filtered again using a 20  $\mu\text{m}$  mesh size.

### 2.4. Cysts count and identification of dinoflagellate cysts using light microscope

Observation on the identification of dinoflagellate cysts was carried out by taking a sample to be observed using 1 ml dropper pipette, then transferred to haemocytometer container. Then, observed under a light microscope with a magnification 40-100 times. Each dinoflagellate cyst sample was identified at the species level. Dinoflagellate cysts were identified based on published descriptions [6-12]. The unit of cyst abundance was the number of cysts g<sup>-1</sup> dry weight sediment. To measure the water content of each sample, a subsample was weighed wet and then dried in a 70°C oven for 24 h to obtain the dry weight. Water content and dinoflagellate cyst abundance were calculated following the formula:  $\text{Cysts/g} = \text{N}/\text{W}(1-\text{R})$  [5]. Where: N = Observed cyst number; W = wet sediment weight and R = water content rate.



**Figure 1.** Research site map.

### 2.5. Identification dinoflagellate cysts using a scanning electron microscope

Dinoflagellate cysts samples were centrifuged for 5 minutes at 5000 rpm. Then a sample of 500  $\mu$ l was put into a microtube, and 1% osmium tetroxide was added with a ratio of 1:1. Then, the sample was left at room temperature for one hour and then filtered through a 0.22 mm filter membrane. The next step is the dehydration process in stages with the concentration of ethyl alcohol, including 30%, 50%, 70%, and 96%, and left for 10 minutes at each concentration. After that, the sample was allowed to dry overnight at room temperature. Then the sample is coated with gold powder. After that, the sample was identified using a scanning electron microscope.

### 18. 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 ( $J'$ ). To examine a spatial variation of dinoflagellate cyst abundance, Shannon-Wiener's diversity index ( $H'$ ), Margalef's species richness ( $d$ ), and Pielou's evenness ( $J'$ ).

### 3. Results and discussion

Dinoflagellate cyst species found based on observations in three river estuaries in the southern part of the Makassar Strait, South Sulawesi, were 34 species from 6 families (Table 1), namely Gonyaulacaceae, Gymnodiniaceae, Osteropsidaceae, Peridiniaceae, Polykrikaceae, and Protoperidiniaceae. In the Gonyaulacaceae family, 4 species were found, namely *Gonyaulax polygramma*, *Gonyaulax scrippsae*, *Gonyaulax spinifera*, and *Gonyaulax verior*. Two species were found in the Gymnodiniaceae family, namely *Cochlodinium polykrikoides* and *Gymnodinium instriatum*. Family Osteropsidaceae found five species, namely *Alexandrium affine*, *Alexandrium catenella*, *Alexandrium minutum*, *Alexandrium peruvianum*, and *Alexandrium tamarense*. Family Peridiniaceae found 9 species, namely *Kryptoperidinium foliaceum*, *Pentapharsodinium tyrrhenicum*, *Peridinium quinquecorne*, *Scripsiella crystalline*, *Scripsiella lachrymose*, *Scripsiella rotunda*, *Scripsiella trifida*, *Votadinium calvum*, and *Votadinium spinosum*. Family Polykrikaceae found 3 species, namely *Pheopolykrikos hartmannii*, *Polykrikos kofoidii*, and *Polykrikos schwartzii*. Family Protoperidiniaceae found 11 species, namely *Preperidinium meunieri*, *Protoperidinium achromaticum*, *Protoperidinium claudicans*, *Protoperidinium conycoides*, *Protoperidinium latissimum*,

*Protooperidinium leonis*, *Protooperidinium obtusum*, *Protooperidinium pentagonum*, *Protooperidinium punctulatum*, and *Protooperidinium punctulatum*. 25

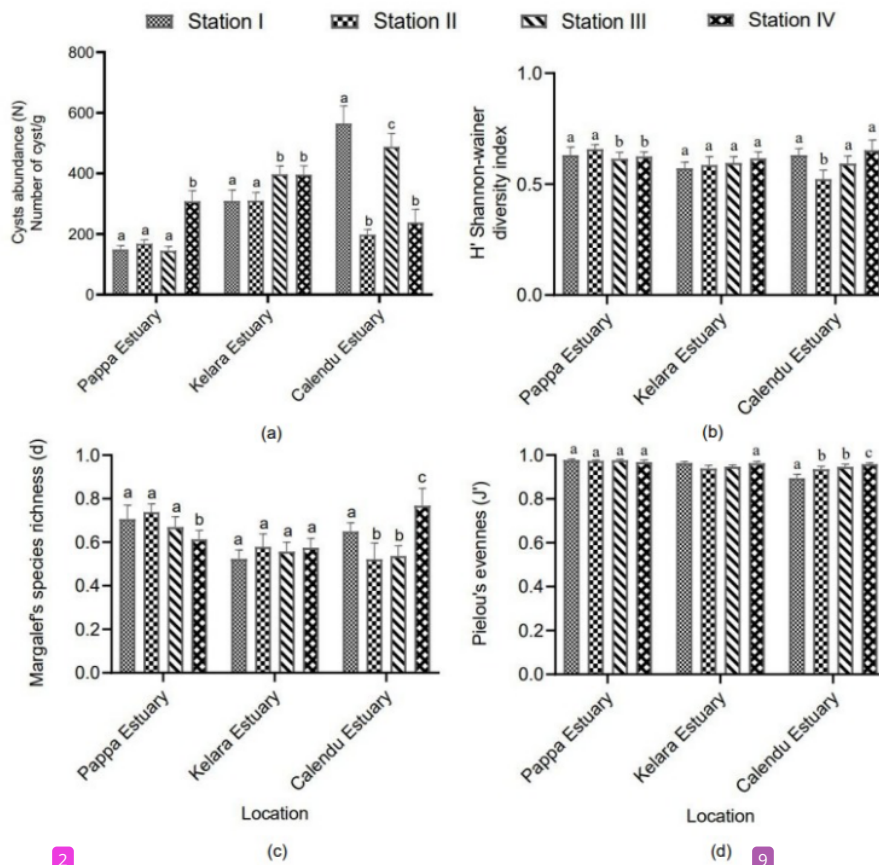
The results of the analysis of the abundance of dinoflagellate cysts in the Three Estuaries of the Southern Makassar Strait, South Sulawesi showed the highest abundance was at the Calendu River Estuary station I which had a value of 565 cyst g<sup>-1</sup> while the lowest abundance was at the Pappa River estuary station III which had a value of 145 cyst g<sup>-1</sup> (Figure 2a). The results of the analysis of the diversity index, uniformity, and richness of dinoflagellate cysts in the three Southern Estuaries of the Makassar Strait did not have significant differences (Figures 2b, 2c, and 2d).

The abundance of dinoflagellate cysts by family showed the highest abundance in the Peridiniaceae family with a percentage value of 44%, followed by the Osteropsidaceae family with a 40% value, the Gonyaulacaceae family with an 8% percentage value, the Protooperidiniaceae family with a 5% value, and the Polykrikaceae family with a 3 %, percentage value. and Family Gymnodiniaceae with a percentage value of 0% (Figure 3). The results of the morphology images of dinoflagellate cysts observed using a light microscope and scanning Electron Microscope based on Family can be seen in Figure 4.

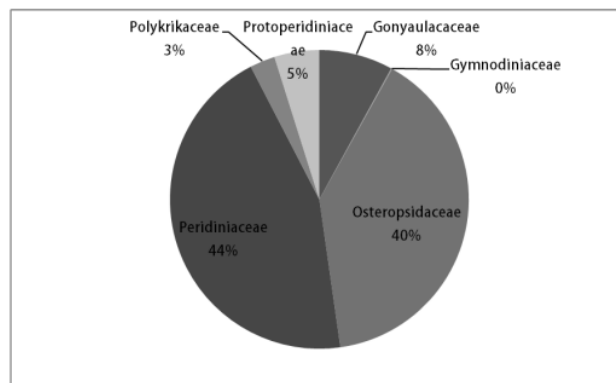
**Table 1.** Species composition of Dinoflagellate cysts in sediment of Three Estuaries Southern Part Makassar Strait, South Sulawesi.

Family	Genus	Species	River Estuary			
			Pappa	Kelara	Calendu	
Gonyaulacaceae	Gonyaulax	Gonyaulax polygramma	-	-	√	
		Gonyaulax scrippsae	-	-	√	
		Gonyaulax spinifera	√	√	√	
		Gonyaulax verior	-	√	-	
Gymnodiniaceae	Cochlodinium	Cochlodinium polykrikoides	√	√	√	
	Gymnodinium	Gymnodinium instriatum	-	-	√	
		Alexandrium affine	-	-	√	
Osteropsidaceae	Alexandrium	Alexandrium catenella	√	√	√	
		Alexandrium minutum	√	√	√	
		Alexandrium peruvianum	√	-	-	
		Alexandrium tamarensis	√	√	√	
		Kryptoperidinium	Kryptoperidinium foliaceum	-	-	√
Peridiniaceae	Pentapharsodinium	Pentapharsodinium tyrrhenicum	√	√	√	
		Peridinium	Peridinium quinquecomae	-	-	√
	Scrippsiella	Scrippsiella crystallina	√	√	√	
		Scrippsiella lachrymosa	√	√	√	
		Scrippsiella rotunda	-	-	√	
	Votadinium	Votadinium	Votadinium calvum	√	-	-
			Votadinium spinosum	-	√	√
Pheopolykrikos			Pheopolykrikos hartmannii	-	-	√
Polykrikos			Polykrikos kofoidii	-	-	√
	Polykrikos schwartzii	√	-	√		
Protooperidiniaceae	Protooperidinium	Preperidinium meunieri	-	-	√	
		Protooperidinium achromaticum	-	-	√	
		Protooperidinium claudicans	-	-	√	
		Protooperidinium conycooides	√	-	-	
		Protooperidinium latissimum	-	-	√	
		Protooperidinium leonis	-	√	√	
		Protooperidinium obtusum	-	-	√	
		Protooperidinium pentagonum	√	√	√	
Protooperidinium punctulatum	-	√	-			
Protooperidinium	Protooperidinium subinermis	-	-	√		
	Protooperidinium tyrrhenicum	-	-	√		

Description: (√) = there is; (-) = there is no any



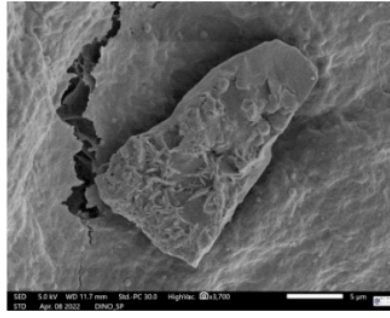
**Figure 2.** Spatial variation (a) dinoflagellate cyst abundance, (b) Shannon-Wiener's diversity index:  $H'$ , (c) Margalef's species richness:  $d$ , and (d) Pielou's evenness:  $J'$  at three Estuaries of Southern Part Makassar Strait.



**Figure 3.** Abundance of dinoflagellata cysts by family.

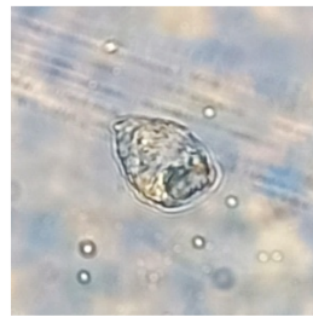
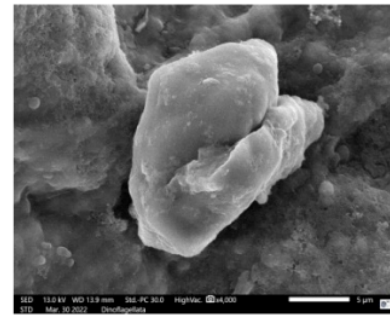
Family : Gonyaulacaceae

Genus : Gonyaulax



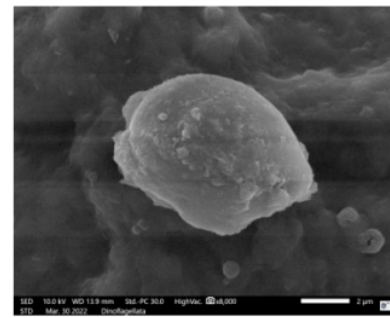
Family : Gymnodianiaceae

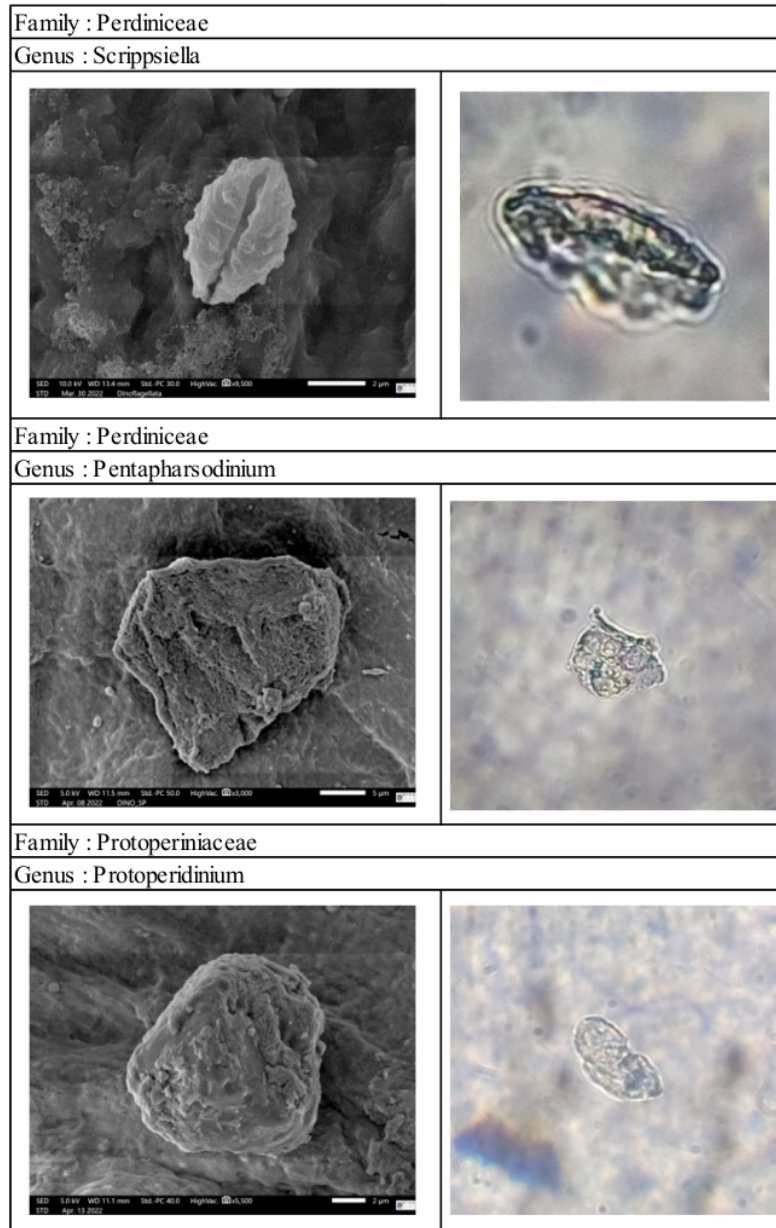
Genus : Gymnodinium



Family : Oosteropsidaceae

Genus : Alexandrium





**Figure 4.** Dinoflagellate cysts by family with light microscope and scanning electron microscope.

#### 24 Conclusion

The Results showed that there were 34 species of dinoflagellate cysts belonging to 6 families, namely Gonyaulacaceae, Gymnodiniaceae, Osteropsidaceae, Peridiniaceae, Polykrikaceae, and Protoperidiaceae. The highest abundance of dinoflagellate cysts was at the Calendu River estuary station I accounting for 565 cysts  $g^{-1}$ , while the lowest abundance of dinoflagellate cyst was found at Pappa River estuaries stations III, accounting for 145 cysts  $g^{-1}$ . The level of diversity, uniformity, and richness of dinoflagellate cysts was not significantly different in the three study sites. The family Osteropsidaceae was the most common type of dinoflagellate cyst found in the three study sites. The

Osteropsidaceae family is a type of dinoflagellate cyst that contains toxins, so it can be assumed that the three research sites have the potential for HAB.

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