Dinoflagel Muara Jawa Timur

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Diversity of dinoflagellate cysts isolated from estuarine sediments of the Bengawan Solo and Brantas rivers, Indonesia

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Abstract. *Andriyono S, Rukminasari N, Hidayani AA, Zakaria IJ, Alam MDJ, Kim HW. 2023. Diversity of dinoflagellate cysts isolated from estuarine sediments of the Bengawan Solo and Brantas rivers, Indonesia. Biodiversitas 24: 1083-1091.* Dinoflagellates are a major part of the phytoplankton and are commonly found in freshwater, brackish, and marine habitats and are major components that play an important role in marine ecosystems. Dinoflagellate cysts (dinocysts) are produced by dinoflagellates in an unfavourable environment and can be preserved well in sediments for long periods. More than 200 dinoflagellate species have been observed to produce resting cysts. That cyst is associated with the maintenance, discontinuation, and repetition of annual blooms. This study identified dinoflagellate cysts based on the morphological characteristics collected from the estuaries of the Brantas and Bengawan Solo rivers. Analysis of cyst diversity in the two regions shows that the Bengawan Solo River mouth has a higher Shannon-Wiener index compared to the Brantas river mouth. The type of dinoflagellate cyst that dominates at the mouth of the Bengawan Solo River is *Protoperidinium obtusum*, while that at the mouth of the Brantas River is *Polykrikos schwartzii*. Shade graph analysis shows a number of species to be found only in the estuary of the Bengawan Solo River, namely *Zygabikodinium lenticulatum, Polykrikos kofoidii, Protoperidinium pentagonum, Gymnodinium catenatum*, and *Votadinium* sp. Based on the results of this study, it is necessary to carry out DNA characteristic tests of isolated and cultured species to document genetic information. In addition, this information is very important in the management of coastal areas around the estuaries of the Bengawan Solo and Brantas rivers to prevent dinoflagellate population explosions that may have negative impacts on various sectors.

Keywords: Algae, algae bloom, aquatic, ecosystem, poisonous, toxic

INTRODUCTION

Changes in the phytoplankton community can occur when a number of harmful microalgae species grow and dominate the water column and proceed to cause environmental impacts. Such an event is known as a Harmful Algal Bloom (HAB) (Anderson et al. 2021). Following the concept of the Intergovernmental Oceanographic Commission (IOC-UNESCO), HABs are defined as a phenomenon of harmful algae growth in water which causes environmental problems (Anderson et al. 2021), losses to the fisheries sector, and even illness to humans (Trevino-Garrison et al. 2015). The harm caused by HAB events spans both spatial and temporal scales. Agricultural-scale losses include mass mortality of farmed fish, poisoning of marine food sources, itching of beachgoers, and others. HAB events are generally due to nutrient enrichment, high temperatures, low oxygen levels, and reduced light intensity as to enable dinoflagellates to overgrow. These incidents are widespread in estuary areas of rivers (Ajani et al. 2013). Most of the algae that cause HABs belong to the dinoflagellate group (Kudela and Gobler 2012).

Dinoflagellates are included in the microalgae group found in the sea and river waters (Senanayake et al. 2021). Dinoflagellates play an important role in these waters, namely as primary producers (Hoppenrath et al. 2014; Price and Bhattacharta 2017). As microalgae, dinoflagellates have a unique ability to specialize and survive in the competition of natural selection. Under productive conditions, dinoflagellates can reproduce rapidly in the water column in the form of actively dividing swimming cells. When environmental conditions are suboptimal, marked by nutrient enrichment (Faizal et al. 2012), warm temperatures (Coffey et al. 2019), low oxygen levels, and reduced light intensity, they stop dividing and mate to form cysts which hibernate and are able to survive in sediments for many years (Head et al. 2013). The hypnozygote is also produced by dinoflagellates through the fusion of gametes produced by vegetative dinoflagellate cells, securely protected within the thick walls of the cyst and highly resistant to adverse conditions (Bravo and Figueroa 2014). In this regard, a study of the biodiversity of dinoflagellate cysts in the estuaries of the Bengawan Solo and Brantas rivers in East Java was conducted using a morphological identification approach.

The water conditions of the Bengawan Solo River currently need serious attention. The river flow originating from Central Java emptying into East Java carries a number of materials including nutrients which can cause water enrichment. The case of enrichment in the waters of the Bengawan Solo River has been carried out by previous studies (Wijaya and Elfiansyah 2022). Enrichment at the mouth of the river plays an important role in the dynamics of plankton populations in water areas because phytoplankton will respond very quickly to changes in the concentration of the dissolved nutrients. It is reported that nutrient enrichment events can reduce the value of plankton biodiversity (Amorim and do Nascimento Moura 2021) and the productivity of an ecosystem (Isbell et al. 2013). The principal contributing factor is human intervention due to activities that have been carried out both on land and in aquatic (anthropogenic) ecosystems (Pawitan et al. 2007; Roosmini et al. 2018). This condition also has an impact on important ecosystems in the coastal area, namely coral reef ecosystems (D'Angelo and Wiedenmann 2014).

Meanwhile, the estuary of the Brantas river is also a major region in Java that crosses the provinces of Central Java to East Java. Conditions here are similar to those at the estuary of the Bengawan Solo River. The estuary waters of the Brantas river also receive a large amount of organic matter from human activities. Nutrient influx from the Brantas River has the potential to cause enrichment in the Madura Strait waters (Jänen et al. 2013). Increasing the proportion of nitrogen and phosphorus in the water results in a gradational change in marine conditions such that the highest concentrations of nutrients are accumulated in the estuary or coastal areas compared to river areas (Jennerjahn and Klöpper 2013). With the dynamics of the potential for enrichment and the variety of nutrient sources that enter the waters of the Bengawan Solo and Brantas rivers, research on the abundance and identification of dinoflagellate species is very important. Investigations of these key phytoplankton constituents are expected to yield valuable data toward the management of water areas that minimizes the occurrence of dangerous algae population bloom or HABs. Before the occurrence of HABs occurs in coastal areas, early detection of the presence of the cause needs to be done. Here the study and identification of the types of dinoflagellates in the form of dormant cysts in sediments were carried out in two estuary areas of the Bengawan Solo and Brantas rivers in East Java.

MATERIALS AND METHODS

Sample collection

Sediment at the bottom of either river mouth was collected with an Ekman Grab size 152x152x230 mm and put in plastic which was labeled according to the location of the sample. At each location of the river estuary, sampling was carried out at four stations with sampling being repeated three times. The coordinate points for sampling (Figure 1) at the mouths of the Bengawan Solo and Brantas rivers are presented in Table 1. These two locations were chosen in this study due to they are major rivers in Java and flow and terminated into East Java. In addition, many fisheries and aquaculture activities are scattered around the estuary of this river, so the existence of Bengawan Solo and Brantas Rivers plays an important role in this province. Sampling was carried out during the transition from the rainy season to the dry season so that the water conditions did not recede too much and were not affected by rainwater run-off from the two major rivers. Each river mouth was determined in four sampling locations with each location replicated 3 times for ensuring the data is quite valid.

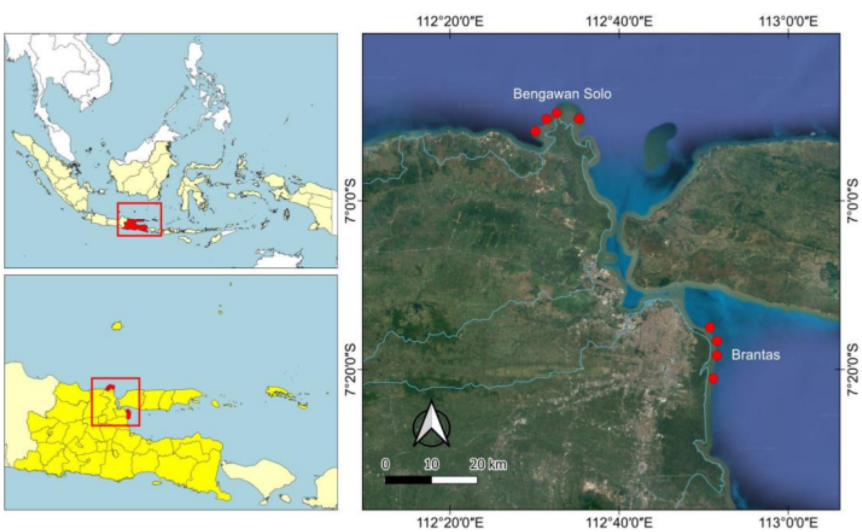

Figure 1. Sampling locations at the two river mouths of the Bengawan Solo and Brantas in East Java, Indonesia (https://earth.google.com/)

Table 1. Sediment sampling locations

River	Station coordinates
Bengawan Solo	06°51'44" S 112°30'06" E
	06°50'18" S 112°31'25" E
	06°49'36" S 112°32'36" E
	06°50'14" S 112°35'15" E
Brantas	07°14'59" S 112°50'45" E
	07°16'37" S 112°51'35" E
	07°18'19" S 112°51'34" E
	07°21'03" S 112°51'09" E

Sediment separation with dinoflagellate cysts

The separation between sediment and cysts uses the method (Matsuoka and Fukuyo 2000) where the sample is weighed using an analytical balance of 4-5 grams and then transferred to a 50 mL beaker glass container then introduced to sterile sea water until it reaches a size of 50 mL. Following the acid treatment procedure, a sonication process was carried out for 15 minutes using an ultrasonic agitator to remove sediment particles attached to cysts. Furthermore, the sieving process was carried out using a filter which is arranged in stages based on the size of the mesh openings. The sieving stage uses 3 filters with sizes of 250 µm, 125 µm and 20 µm mesh. The sample that has been filtered is then precipitated using a petri dish, then filtered again using a sieve with a mesh size of 20 µm. The filtered cysts were then put into a 10 mL sample bottle for morphological analysis.

Determination of the rate of water content

Determination of the percent of water content is carried out to establish the water contained in the sediment sample. Wet sediment samples were first weighed out at 4-5 grams using an analytical balance which was then put into the oven with a temperature of 150°C for 1 hour. After that, the dried sediment samples were weighed again. Previous research (Matsuoka and Fukuyo 2000) used the following formula wherein *Bb* is the wet sediment weight (g); *Bk* is the dry sediment weight (g), and *R* is the percent of water content (%):

$$R = \frac{Bb - Bk}{Bb} \times 100\%$$

Dinoflagellate cyst abundance calculation

The abundance of dinoflagellate cysts was observed under a light microscope using a haemacytometer with 8 fields of view on equipment. The abundance of dinoflagellate cysts was calculated based on the previous research formula of Matsuoka and Fukuyo (Matsuoka and Fukuyo 2000) wherein *R* is the rate of water content (%), *N* is the total number of cysts observed, and *W* is the weight of wet sediment (g):

$$Cysts\ per\ gram = \frac{N}{W(1-R)}$$

Identification of dinoflagellate cysts

Dinoflagellate cyst morphological analysis was carried out by taking organic sample residue using a dropper pipette of 1 mL and transferring it to a hemacytometer container for observation under a transmitted light microscope. Each dinoflagellate cyst sample was identified to the genus and species level based on the published description of the taxon by the researcher (Alkawri 2016; Mertens et al. 2020)

Data analysis

All data are presented in a table using Microsoft Excel software. Additionally, similarity analysis, shade plot analysis, and dinoflagellate abundance from two different sampling locations were analyzed using Primer-e software (Clarke and Gorley 2015). Biodiversity indices was measure including Margalef index, Pielou's Evenness Index and Shannon-Wiener Index. Margalef Index and Shannon-Wiener Index are indicate of species richness which measure by comparison total number of species and total number of individual in certain area (Fedor and Zvaríková 2019). While Pielou's Evenness Index indicate of species distribution in certain area or sampling site. The Evennes index value which is close to one means that the distribution of biodata in this area is almost perfectly distributed (Dewi et al. 2022).

RESULTS AND DISCUSSION

Dinoflagellate cyst diversity

The dinoflagellate cysts taxa present varied in the estuaries of the Berantas and Bengawan Solo rivers (Table 1). The Bengawan Solo River estuary yielded a higher diversity of four species of dinoflagellate cysts compared to that of the Brantas River estuary which only presented 12 species, which were collected from 4 sites and 3 replication each river. The type of *Protoperidinium obtusum* was found to dominate in the mouth of the Bengawan Solo River, while the type of *Scrippsiella lachrymose* dominated in the mouth of the Brantas River (Figure 2).

Biodiversity analysis of dinoflagellate cysts

Based on the dinoflagellate cyst abundance data, a biodiversity analysis was carried out to determine the dominating species and distribution patterns of cyst species in both river estuaries in East Java. Each sampling location was repeated four times to reduce the resulting data bias. Biodiversity analysis includes Margalef Index (d), Pielou's Evenness (J'), and Shannon-Wiener Index (H'). Referring to the Shannon-Wiener Index (H') value, it shows that the mouth of the Bengawan Solo River (2.1055) has higher diversity than the mouth of the Brantas River (2.0363). In this research, we have been collecting samples from mouth river as the main estuarine arean which trasition between fresh water and marine water ecosystem. This location also has several fisheries and aquaculture activities (Darmawan et al. 2019). Both biodiversity parameters (Margalef and Shannon-Wiener Index) show values between scores 1 and 3 which indicate a moderate level of diversity. Meanwhile, the biota is spread out almost entirely from the Pielou's Evennes Index which is close to a value of one (Siregar et al. 2014).

Analysis of similarity and abundance of dinoflagellate cyst types

The similarity analysis was carried out by comparing data on the abundance of dinoflagellate cysts found in both regions (Figure 3). The data was processed using Primer-e software and at the same time analyzing species abundance at each sediment collection station. Only the four sampling stations at the mouth of the Bengawan Solo River had the highest abundance and were different from other stations. Meanwhile, the sampling stations at the mouth of the Brantas River were in one branch which had a fairly high similarity between the four replicates. An abundance analysis (Figure 4) was carried out to ascertain species that have the potential to cause harmful algae blooms (HAB), which are known to be detrimental to fishing activities and even a danger to public health. The results of the ShaderGraph analysis showed that cysts of this type of dinoflagellate are only found in the mouth of the Brantas River, namely those of the *Protoperidinium compressum*. Meanwhile, at the mouth of the Bengawan Solo River, there are several species that are not found at the mouth of the Brantas River, namely *Zygabikodinium lenticulatum, Polykrikos kofoidii, Protoperidinium pentagonum, Gymnodinium catenatum*, and *Votadinium* sp.

Discussion

The diversity of dinoflagella cysts needs to be studied because certain types of dinoflagellates have the potential to become a plankton group that needs to be watched out for for reasons of health and public safety (Bravo and Figueroa 2014). This report is the first to identify dinoflagellate cysts in East Java, specifically at the mouths of large rivers in the Bengawan Solo and Brantas river estuaries. The existence of dinoflagellates in a number of areas has received intensive attention, including mapping the distribution and species present to avoid losses incurred when this type of dinoflagellate experiences a population explosion. Previous research on the presence of dinoflagellate blooms has been reported in a number of countries such as Australia (Roberts et al. 2019), Chile, and France (Luo et al. 2019). Meanwhile, studies of dinoflagellate cysts in Indonesia have been carried out in several areas such as Lampung Bay (Thoha et al. 2019), Jakarta and Flores (Matsuoka and Fukuyo 1999), Central Jawa (Matsuoka 1983, 1984), Makassar (Rukminasari and Tahir 2020), and Pankajene (Rachman et al. 2022). These incidents have proven harmful to activities related to aquatic resources such as fishing, which have had a huge impact, for instance in human health and wellbeing (Berdalet et al. 2016) and economic (Sanseverino et al. 2016). Losses due to the harmful algae blooms have been reported, which have caused the death of a number of fish in the waters and damaged the surrounding aquaculture industries (Jardine et al. 2020, Sakamoto et al. 2021). A number of regions also reported their impact on human health causing a numerous instances of illness (Grattan et al. 2016). Thus, it is essential for this research to be carried out to predict the potential for a dangerous algae population explosions.

In this study, two large river estuaries in East Java are shown to be important locations because of their proximity to many sites where fisheries are developing, especially realting to shrimp (*Litopenaeus vannamei*) and milkfish (*Chanos chanos*) ponds which are the mainstay of this region. The estuary of the Bengawan Solo River is in the Gresik area which is a high producer of milkfish (Rohman et al. 2021) in addition to intensive artisanal fishing activities carried out (Collier 2019). in addition to intensive artisanal fishing activities carried out (Collier 2019). Meanwhile, the Brantas River, which is also quite large, passes through the city of Surabaya which then branches toward North Surabaya and East Surabaya. The eastern Surabaya area is very close to Sidoarjo Regency, which is also one of the *Penaeus vannamei* white shrimp pond areas (Hukom et al. 2020).

Table 1. Types of dinoflagellate cysts isolated from sediments in the estuaries of the Bengawan Solo River and the mouth of the Brantas rivers

Species name	Average abundance of dinoflagellate cyst (cyst g^{-1} sediment)	
	Bengawan Solo	Brantas
Alexandrium catenella	35	48
Alexandrium minutum	66	93
Brigantedinium sp.	28	42
Cochlodinium polykrikoides	69	48
Gymnodinium catenatum	43	-
Gyrodinium sp.	32	50
Pentapharsodinium tyrrhenicum	150	106
Polykrikos kofoidii	39	-
Polykrikos schwartzii	89	170
Protoperidinium compressum	-	32
Protoperidinium leonis	50	-
Protoperidinium oblongum	251	112
Protoperidinium obtusum	126	139
Protoperidinium pentagonum	41	-
Scrippsiella crystallina	68	147
Scrippsiella lachrymosa	214	130
Votadinium sp.	27	-
Zygabikodinium lenticulatum	39	-

Table 2. Biodiversity analysis of dinoflagellate cysts at the mouths of the Bengawan Solo and Brantas rivers

River	Biodiversity Index		
	Margalef Index (d)	Pielou's Evenness (J')	Shannon-WienerIndex (H')
Bengawan Solo	2.7193	0.97933	2.1055
Brantas	2.4346	0.98888	2.0363

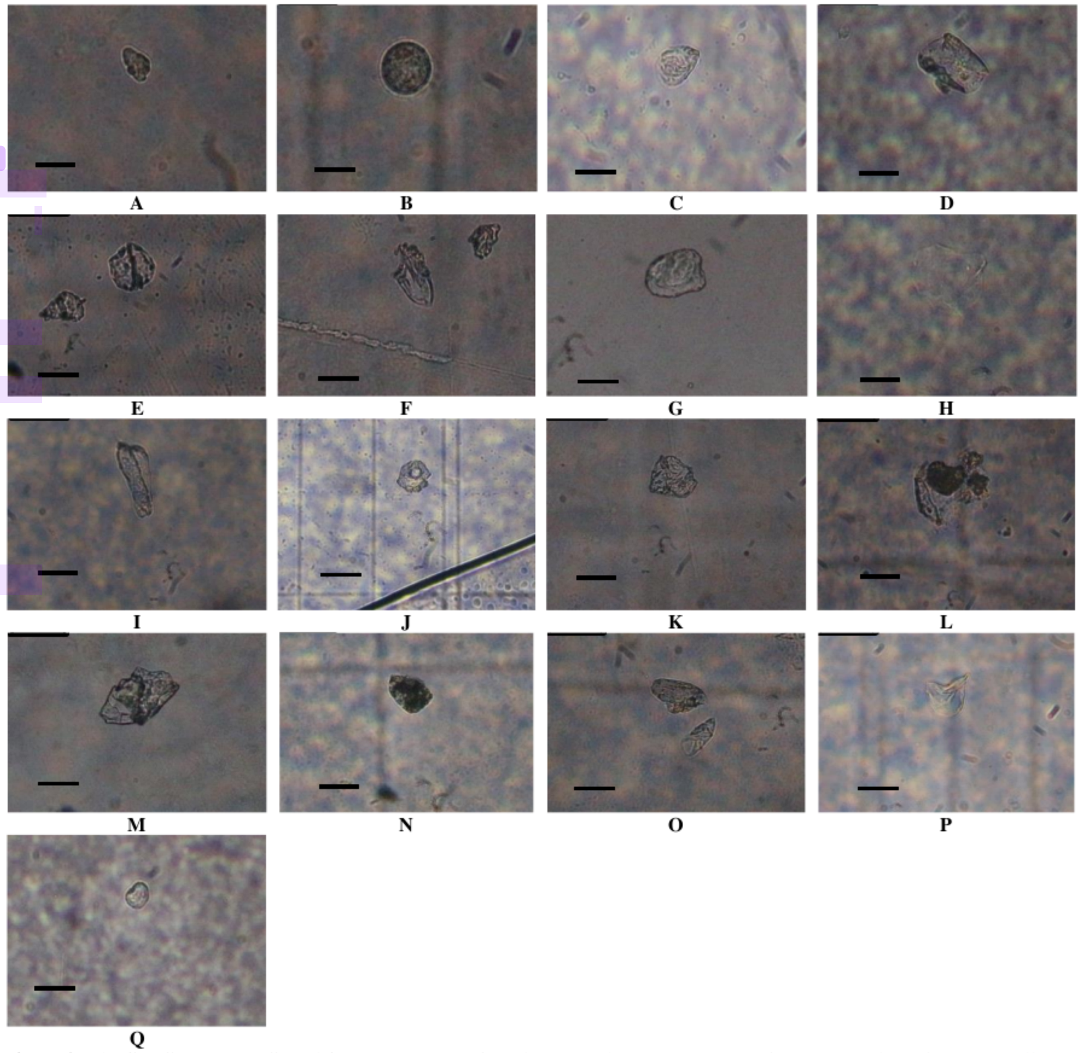

Figure 2. Dinoflagellate cyste collected from Bengawan Solo and Brantas river estuary. Bar scale 10 *μ* (A) *Alexandrium catenella*; (B) *Alexandrium minutum*; (C) *Brigantedinium sp*.; (D) *Cochlodinium polykrikoides*; (E) *Gymnodinium catenatum*; (F) *Gyrodinium sp;* (G) *Pentapharsodinium tyrrhenicum* ; (H) *Polykrikos kofoidii;* (I) *Polykrikos schwartzii* ; (J) *Protoperidinium leonis;* (K) *Protoperidinium oblongum*; (L) *Protoperidinium obtusum;* (M) *Protoperidinium pentagonum;* (N) *Scrippsiella crystallina*; (O) *Scrippsiella lachrymose*; (P) *Votadinium sp*; (Q) *Zygabikodinium lenticulatum*, (R) *Protoperidinium compressum*

There were two species found in all research locations, namely *Protoperidinium oblongum* and *Pentapharsodinium tyrrhenicum*. The ecological function of dinoflagellates in water has received the attention of a number of researchers (Furio et al. 2012; Naqqiuddin et al. 2014; Srivilai et al. 2012). Under unfavorable conditions, dinoflagellates will produce cysts that are able to survive and settle in aquatic sediments. Dinoflagellate cysts play an important role biologically and ecologically because cysts can help dinoflagellates survive in harsh environments, provide opportunities survive in unsuitable condition and facilitate their dispersal in aquatic habitats, and act as seeds in algae blooms (Bravo and Figueroa 2014). In addition, the dinoflagellates of some species can produce more toxins than the vegetative forms, seriously affecting other organisms through the food web and even human health (Kirkpatrick et al. 2004). Meanwhile for aquatic ecology, the impacts of algae blooms include beach pollution, lack of oxygen, and the death of marine species on a large scale (Park et al. 2013). *Alexandrium tamarense* has been identified as a toxic species (Oshima et al. 1992), and in this study we found two species in the genus *Alexandrium* (*A. catenella dan* and *A. minutum*). Therefore, accurate identification of dinoflagellates is an important first step in their early detection and function in ecological studies. Because dinoflagellates have limited or even non-existent taxonomic keys, molecular methods to identify dinoflagellates have become a priority as a continuation of this research. Acoording to the molecular approach, several studies have succeeded in identification of dinoflagellate by rDNA based on SSU, LSU and also ITS region gene (Branco et al. 2020; Mertens et al. 2015). In the current study, morphological identification was carried out by taking into account a number of general characteristics, such as the shape of the resulting cyst and its ornamentation, the structure and color of the walls, and the type of archeopyle (Matsuoka and Fukuyo 2000).

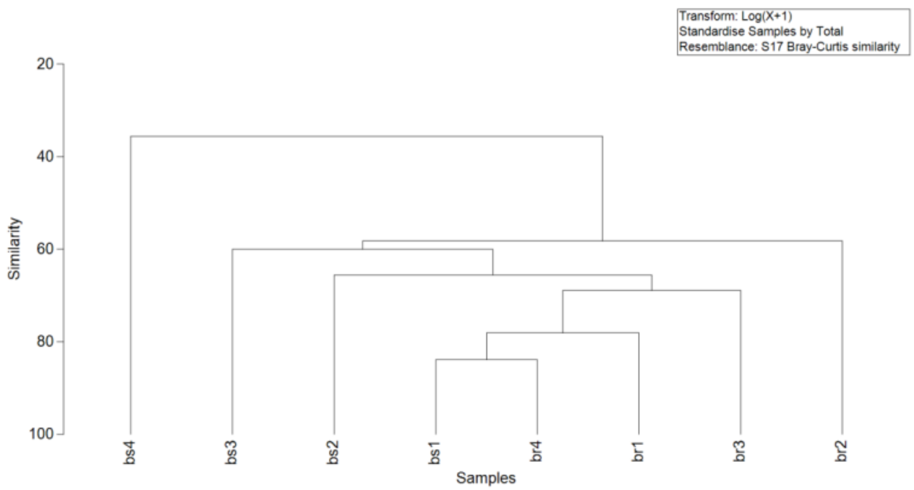

Figure 3. Analysis of similarity from eight sampling locations at the two river mouths of the Bengawan Solo and Brantas rivers in East Java

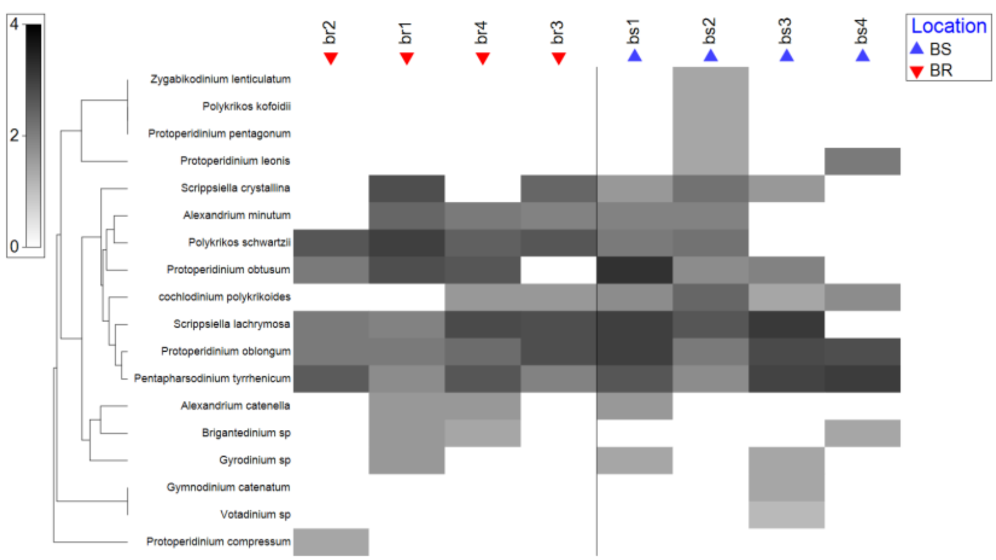

Figure 4. Shade plot of species distribution at each sampling station at the mouth of the Bengawan Solo River (bs) and mouth of the Brantas River (br). The color intensity indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher diversity index than the estuary of the Brantas River. The type of dinoflagellate cyst that predominates in the estuary of the Bengawan Solo River is *Protoperidium obtusum*. Index 0-4 is normalized data of species abundance using log10 which indicates species abundance in a certain area

The case of the dinoflagellate population explosion in Indonesia has not received serious attention even though several studies on dinoflagellate species have been identified in a number of areas. The main cause of the algae population explosion is nutrients in the waters which are abundant and support algae growth in a short timeframe (Piranti et al. 2021). Algae bloom events can occur in fresh water and marine waters. Cases that have been reported in a number of publications are the waters of the Jakarta Bay which caused mass fish die-offs (Nasution et al. 2021). Although, in East Java there have been no reports of algae blooms, and it is hoped that research on the potential for HABs in East Java waters can be mitigated from the start so that losses and impacts are expected to be minimized.

Biodiversity studies of dinoflagellate cysts in Java Island of Indonesia are still very limited (Matsuoka 1983, 1984; Matsuoka and Fukuyo 1999). Monitoring activities for the type *Pyrodinium bahamense* have been carried out to anticipate the presence of alagae blooms in Indonesia including, Lampung Bay, Jakarta Bay, Cirebon coastal waters and Ambon Bay (Rachman et al. 2021). Studies on the diversity index of dinofagellate cysts have been reported in Muara Jeneberang and Paotere Harbor with values ranging from 0.82-1.01 (Rukminasari and Tahir 2021). This diversity value is still relatively low when compared to the biodiversity of dinoflagella cysts in East Java with a Shannon-Wiener index ranging from 2.0363-2.1055. Another report in South Sulawesi, Maros estuary and Pangkep River estuary, which are included in the moderate category for the potential presence of HABs in this area (Rukminasari and Tahir 2021). Based on previous research, the abundance of dinoflagellate cysts can be an indication of the potential for HABs in a given region. There are four potential groups that have been classified, namely very low, low, medium, high and very high levels (McMinn 1991; Tian et al. 2018). Previous study show that abundance of dinoflagellate cysts in Lampung for *P. bahamense* is 1-100 cysts g^{-1} sediment (Rachman et al. 2021), while previous research found *M. polykrikoides* type cysts measured with abundances ranging from 100-1000 cysts g^{-1} sediment (Thoha et al. 2019). The abundance of *P. bahamense* in Cirebon waters was a serious case because it caused human death (Nurlina 2018), with a relatively high abundance of 100-1000 cysts g^{-1} sediment (Rachman et al. 2019). At the estuary of the Bengawan Solo River, the highest abundance was *Protoperidinium oblongum* (115 cysts g^{-1} sediment) and the lowest abundance was *Protoperidinium leonis* (21 cysts g^{-1} sediment). Meanwhile, *Alexandrium minutum* was found with the highest abundance (125 cysts g^{-1} sediment) in the mouth of the Brantas River, with an average of 73 cysts g^{-1} sediment having been found. Thus, the condition of the mouths of the Bengawan Solo and Brantas rivers still has a low potential for the occurrence of HABs, and an average abundance of 73 and 62 cysts g^{-1} respectively (McMinn 1991; Tian et al. 2018).

In conclusion, this report is the first study on the biodiversity of dinoflagellate cysts in the estuaries of major rivers in East Java. Research on dinoflagellate cysts revealed the diversity of dinoflagellates at the mouth of the Bengawan Solo and Brantas rivers. Analysis of cyst diversity in the two regions shows that the Bengawan Solo River mouth has a higher Shannon-Wiener index compared to that of the Brantas River. The type of dinoflagellate cyst that dominates at the mouth of the Bengawan Solo River is *Protoperidinium obtusum*, while at the mouth of the Brantas River it is *Polykrikos schwartzii*. ShadeGraph analysis showed a number of species found only in the estuary of the Bengawan Solo River, namely *Zygabikodinium lenticulatum, Polykrikos kofoidii, Protoperidinium pentagonum, Gymnodinium catenatum*, and *Votadinium* sp. Based on the results of this study, it is necessary to carry out DNA characteristic tests of isolated and cultured species to document genetic information. In addition, this information is very important in the management of coastal areas around the mouths of the Bengawan Solo and Brantas rivers to prevent dinoflagellate population bloom which are likely to have negative impacts on various sectors of fisheries industry and aquaculture activities.

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