Bukti Korespondensi Jurnal Internasional Bereputasi terakreditasi Q3 (Animal Science and Zoology)

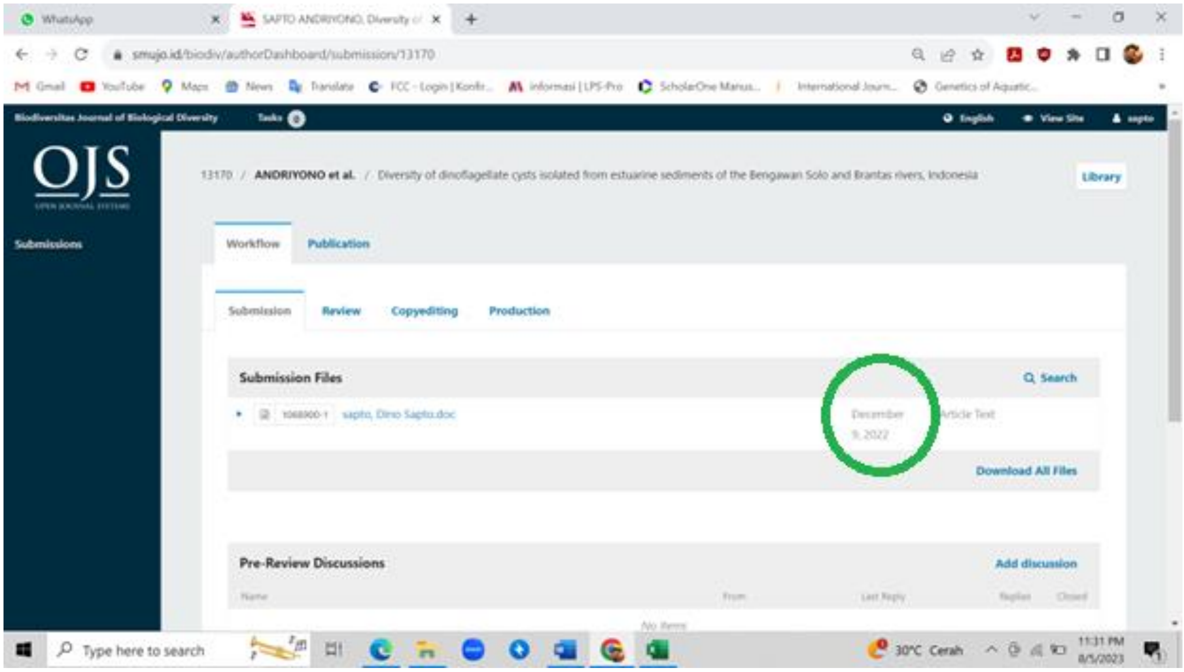
Judul : **Diversity Of Dinoflagellate Cysts Isolated From Estuarine Sediments Of Bengawan Solo And Brantas Rivers, Indonesia**

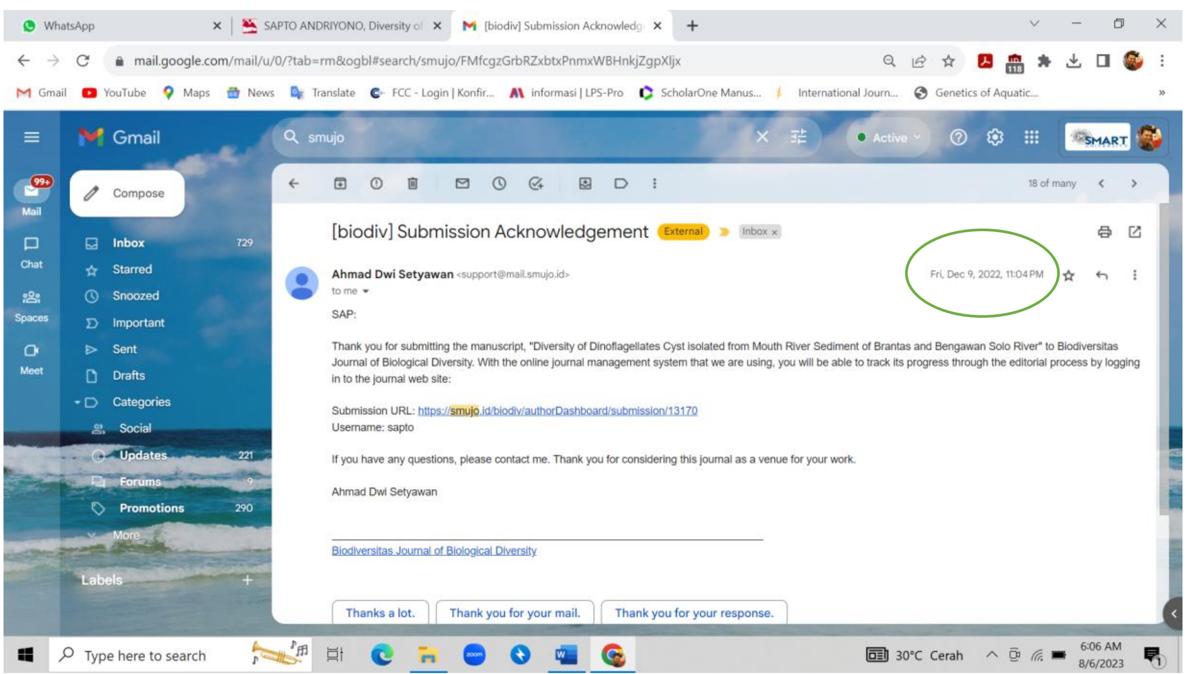
Jurnal : **BIODIVERSITAS**

Penulis : **SAPTO ANDRIYONO, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOBAIDUL ALAM, HYUN-WOO KIM**

No.	Perihal	Tanggal	Keterangan
1	Bukti Submit dan Artikel yang di submit	9 December 2022	Page : 2, Lampiran 1
2	Bukti review round 1, artikel revisi 1	12 December 2022	Page 3-4, Lampiran 2
3	Bukti review round 2, artikel dan revisinya	24 January 2023	Page 5, Lampiran 3-4
4	Bukti Review Round 3, artikel dan revisinya	02 February 2023	Page 6, Lampiran 5-6
5	Bukti Proof read	12 February 2023	Page 7
6	Bukti review round 4 Accepted	18 February 2023	Page 8
7	Link Artikel terpublikasi	18 February 2023	Page 8

1. Bukti Submit dan Artikel yang disubmit 9 Desember 2022 disystem OJS dan Notifikasi di Email





2. Review Roud 1.

Notifications

[biodiv] Editor Decision

2022-12-12 04:26 AM

Sapto Andriyono, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOBAIDUL ALAM, HYUN-WOO KIM KIM:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Diversity of Dinoflagellates Cyst isolated from Mouth River Sediment of Brantas and Bengawan Solo River".

Our decision is: Revisions Required

Reviewer A:

Dear Author,

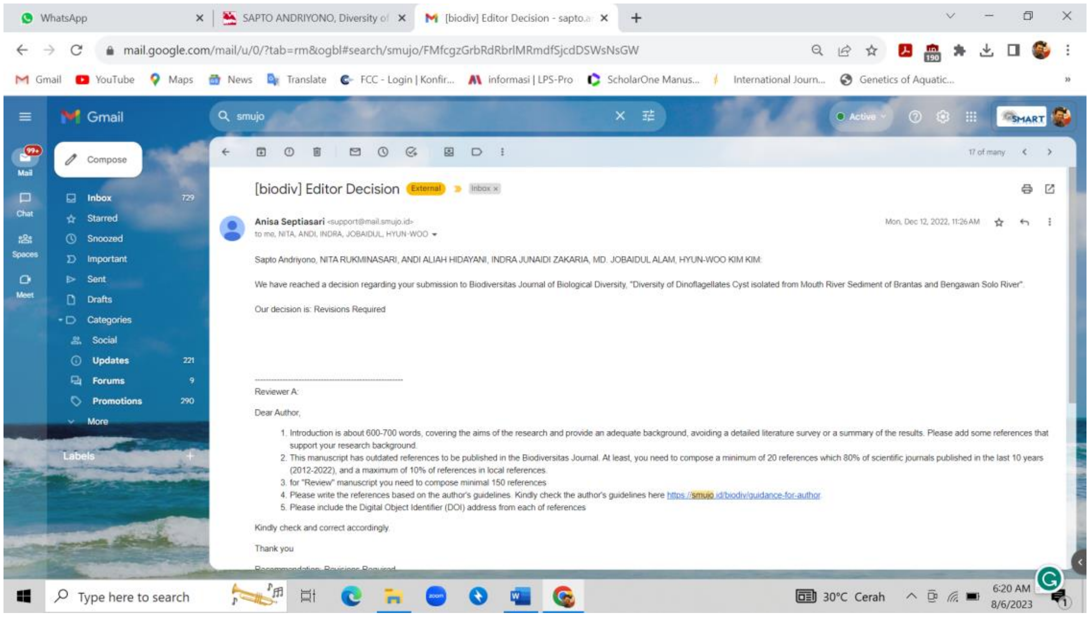
1. Introduction is about 600-700 words, covering the aims of the research and provide an adequate background, avoiding a detailed literature survey or a summary of the results. Please add some references that support your research background.
2. This manuscript has outdated references to be published in the Biodiversitas Journal. At least, you need to compose a minimum of 20 references which 80% of scientific journals published in the last 10 years (2012-2022), and a maximum of 10% of references in local references.
3. for "Review" manuscript you need to compose minimal 150 references
4. Please write the references based on the author's guidelines. Kindly check the author's guidelines here https://smujo.id/biodiv/guidance-for-author.
5. Please include the Digital Object Identifier (DOI) address from each of references

Kindly check and correct accordingly.

Thank you

Recommendation: Revisions Required

Notifikasi Rond 2 di Email


[biodiv] Editor Decision External Inbox

Anisa Septiasari <support@mail.smujo.id>
to me, NITA, ANDI, INDRA, JOBAIDUL, HYUN-WOO

Mon, Dec 12, 2022, 11:26 AM

Sapto Andriyono, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOBAIDUL ALAM, HYUN-WOO KIM KIM:

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2. This manuscript has outdated references to be published in the Biodiversitas Journal. At least, you need to compose a minimum of 20 references which 80% of scientific journals published in the last 10 years (2012-2022), and a maximum of 10% of references in local references.
3. for "Review" manuscript you need to compose minimal 150 references
4. Please write the references based on the author's guidelines. Kindly check the author's guidelines here https://smujo.id/biodiv/guidance-for-author
5. Please include the Digital Object Identifier (DOI) address from each of references

Kindly check and correct accordingly.

Thank you

3. Jawaban Review pertama round 2

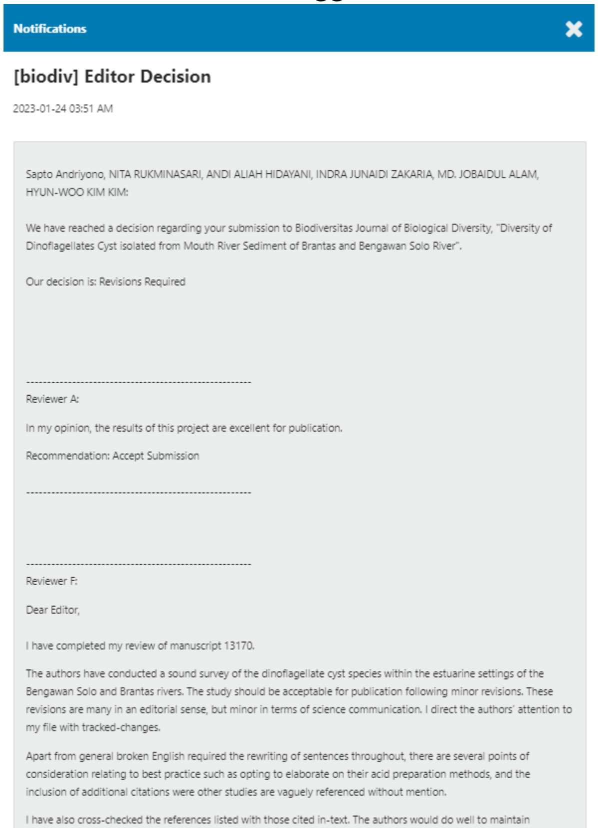
Dear Editor,

1. We have improved the Introduction part. This section is currently around 692 words (600-700 words), Besides, we also support some paragraphs about the adequate background.
2. He, we just 7 references out of 40 references of scientific journals published in the last 10 years (2012-2022),
3. We already follow the journal guidelines here https://smujo.id/biodiv/guidance-for-author for reference section.
4. We have input the Digital Object Identifier (DOI) address from each of the references

Best Regards

Sapto

3. Review Round 2 tanggal 24 Januari 2022



Notifications

[biodiv] Editor Decision

2023-01-24 03:51 AM

Sapto Andriyono, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOBAIDUL ALAM, HYUN-WOO KIM KIM:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Diversity of Dinoflagellates Cyst isolated from Mouth River Sediment of Brantas and Bengawan Solo River".

Our decision is: Revisions Required

Reviewer A:

In my opinion, the results of this project are excellent for publication.

Recommendation: Accept Submission

Reviewer F:

Dear Editor,

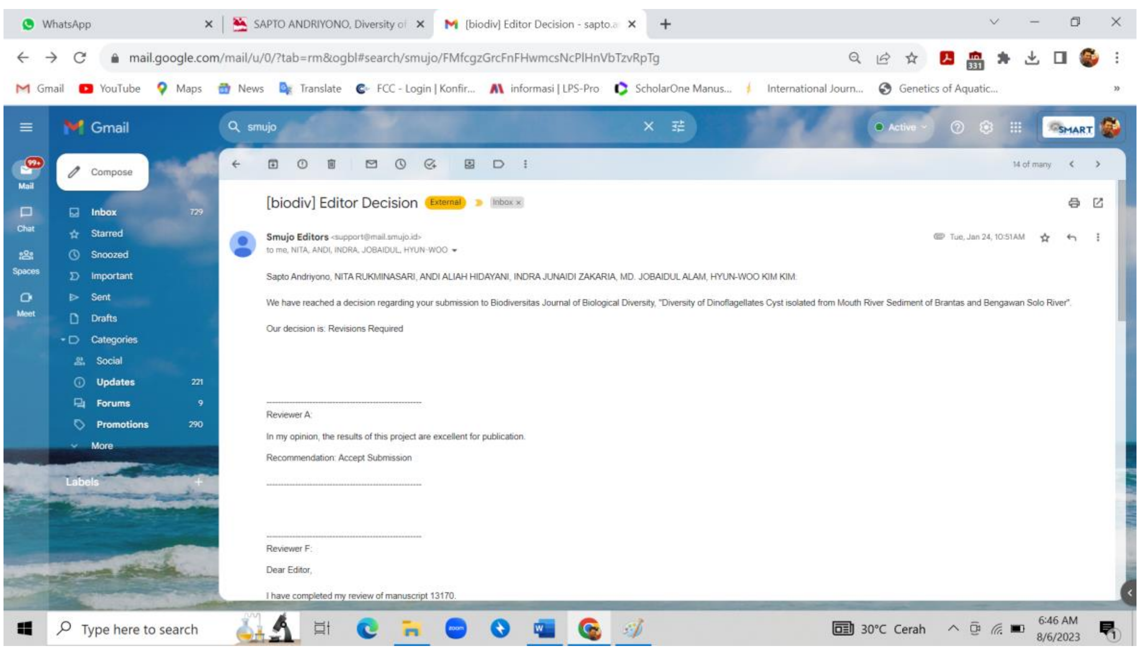
I have completed my review of manuscript 13170.

The authors have conducted a sound survey of the dinoflagellate cyst species within the estuarine settings of the Bengawan Solo and Brantas rivers. The study should be acceptable for publication following minor revisions. These revisions are many in an editorial sense, but minor in terms of science communication. I direct the authors' attention to my file with tracked-changes.

Apart from general broken English required the rewriting of sentences throughout, there are several points of consideration relating to best practice such as opting to elaborate on their acid preparation methods, and the inclusion of additional citations were other studies are vaguely referenced without mention.

I have also cross-checked the references listed with those cited in-text. The authors would do well to maintain

Email notifikasi



4. Review Round 3 tanggal 2 Februari 2023

Notifications

[biodiv] Editor Decision

2023-02-02 01:44 PM

Sapto Andriyono, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOBAIDUL ALAM, HYUN-WOO KIM KIM:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Diversity of Dinoflagellates Cyst isolated from Mouth River Sediment of Brantas and Bengawan Solo River".

Our decision is: Revisions Required

Reviewer A:

Dear Authors,
Thank you for submitting this manuscript that explores the diversity of dinoflagellate species as based on cyst detection along two rivers. This is an interesting and useful study with some human and animal health conequences. There are some revisions required in order to consider this manuscript for publication. I have included specific feedback on the word document version of the manuscript, please find attached. Make sure that any changes to the manuscript are shown using highlighted text or tracked changes. Additionally, please address the following key areas when making revisions:

1. Sample locations. Explain clearly how sample locations were selected and the time and season they were sampled. How many samples were taken per site? This is essential for repeatability.

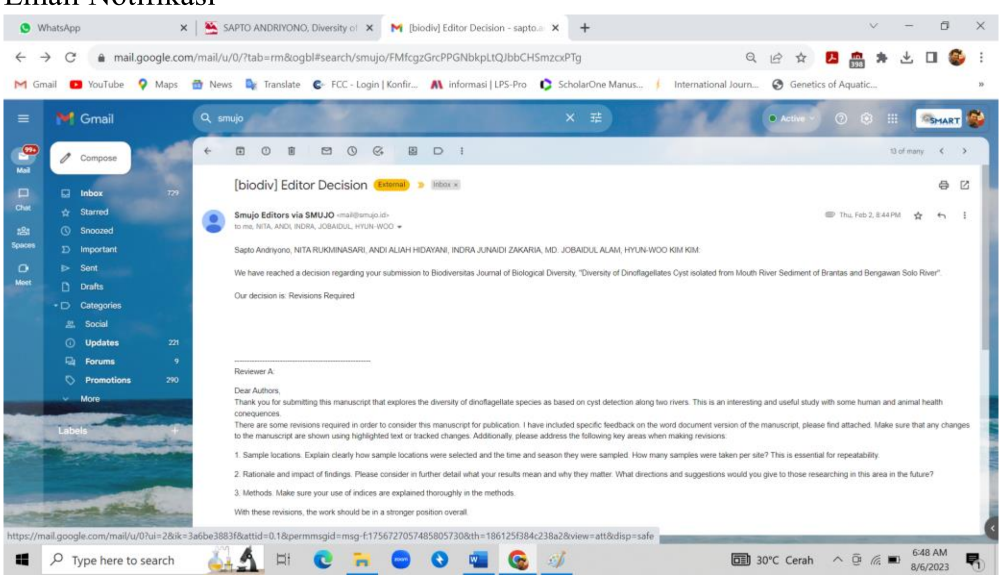
2. Rationale and impact of findings. Please consider in further detail what your results mean and why they matter. What directions and suggestions would you give to those researching in this area in the future?

3. Methods. Make sure your use of indices are explained thoroughly in the methods.

With these revisions, the work should be in a stronger position overall.

Recommendation: Revisions Required

Email Notifikasi



5. Proof Read tanggal 12 February 2023

Uncorrected Proof ✖

Participants

Fauzi Nugroho (faugro)

Smujo Editors (editors)

Sapto Andriyono (sapto)

Anisa Septiasari (aseptiasari)

DEWI NUR PRATIWI (dewinurpratiwi)

Messages

Note	From
Dear Author(s), Pls, find attached file for an uncorrected proof (Copyedited file). The revised manuscript (Corrected proof) is awaited. Do not worry about layout changes due to revision; our staff will fix it again. Note: Kindly TURN ON track changes when you make improvements. dewinurpratiwi, Dinoflagellate cysts isolated - Andriyono.doc	dewinurpratiwi 2023-02-11 11:47 PM
Dear Editor of Biodiversitas Journal Along with this email, we send the corrected file. Some revisions in number and affiliation. Thank you Sapto sapto, 13170-Article Text-1074642-1-18-20230211 (corrected1202).doc	sapto 2023-02-12 12:53 PM

Add Message

6. Accepted tanggal 18 Februari 2023

Notifications ✖

[biodiv] Editor Decision

2023-02-18 12:21 AM

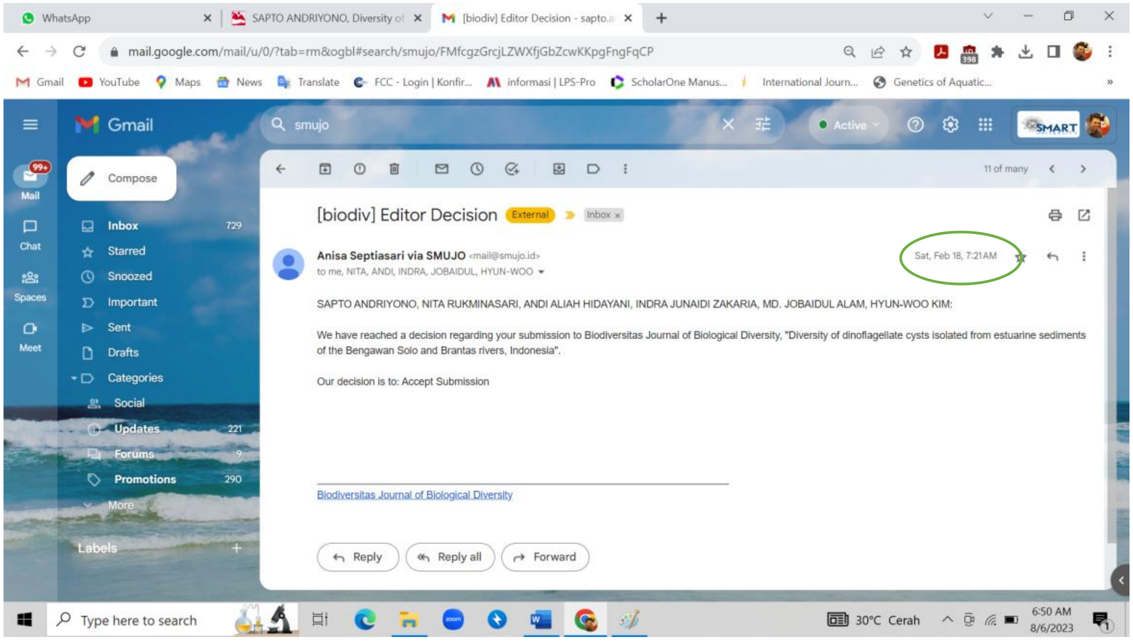
SAPTO ANDRIYONO, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOBAIDUL ALAM, HYUN-WOO KIM:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Diversity of dinoflagellate cysts isolated from estuarine sediments of the Bengawan Solo and Brantas rivers, Indonesia".

Our decision is to: Accept Submission

____

Biodiversitas Journal of Biological Diversity



7. Artikel terpublished tanggal melalui link https://smujo.id/biodiv/article/view/13170

COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

- ☑ The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
- ☑ The submission file is in OpenOffice, Microsoft Word (DOC, not DOCX), or RTF document file format.
- ☑ The text is single-spaced; uses a 10-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
- ☑ The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.
- ☑ Most of the references come from current scientific journals (c. 80% published in the last 10 years), except for taxonomic papers.
- ☑ Where available, DOIs for the references have been provided.
- ☑ When available, a certificate for proofreading is included.

SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:

- ☑ E-mail address
- ☑ Full postal address (incl street name and number (location), city, postal code, state/province, country)
- ☑ Phone and facsimile numbers (incl country phone code)

All necessary files have been uploaded, and contain:

- ☑ Keywords
- ☑ Running titles
- ☑ All figure captions
- ☑ All tables (incl title and note/description)

Further considerations

- ☑ Manuscript has been "spell & grammar-checked" Better, if it is revised by a professional science editor or a native English speaker
- ☑ References are in the correct format for this journal
- ☑ All references mentioned in the Reference list are cited in the text, and vice versa
- ☑ Colored figures are only used if the information in the text may be losing without those images
- ☑ Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate

Title:

Diversity of Dinoflagellates Cyst isolated from Mouth River Sediment of Brantas and Bengawan Solo River

Author(s) name:

SAPTO ANDRIYONO, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOBAIDUL ALAM, HYUN-WOO KIM

Address

(Fill in your institution's name and address, your personal cellular phone and email)

Department of Marine, Fisheries and Marine Faculty, Universitas Airlangga C Campus Jl. Mulyorejo Surabaya East Java, 60115, Indonesia Telp.+62-8232884599 email: sapto.andriyono@fpk.unair.ac.id

For possibility publication on the journal:

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- [x] Biodiversitas Journal of Biological Diversity
- [ ] Nusantara Bioscience
- [ ] Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia
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- [ ] Asian Journal of Tropical Biotechnology
- [ ] International Journal of Bonorowo Wetlands
- [ ] Cell Biology and Development
- [ ] Indo Pacific Journal of Ocean Life
- [ ] International Journal of Tropical Drylands

Novelty:

(state your claimed novelty of the findings versus current knowledge)

First report on dinoflagellate cyste from mouth river in East Jawa Province

Statements:

This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.

Author(s) has been read and agree to the Ethical Guidelines.

List of five potential reviewers

(Fill in names of five potential reviewers **that agree to review your manuscpt** and their **email** addresses. He/she should have Scopus ID and come from different institution with the authors; and from at least three different countries)

1. Munti Sarida, Ph.D ; munti.sarida@fp.unila.ac.id Scopus Author ID: 56278007100
2. Dr. Beginer Subhan; beginersubhan@apps.ipb.ac.id Scopus Author ID: 55991796500
3. Ni Kadek Dita Cahyani, Ph.D; nkdcahyani@gmail.com Scopus Author ID: 56028509600

Place and date:

Surabaya, 9 Desember 2022

Sincerely yours,

(fill in your name, no need scanned autograph)

SAPTO ANDRIYONO

Diversity of Dinoflagellates Cyst isolated from Mouth River Sediment of Brantas and Bengawan Solo River

SAPTO ANDRIYONO[1♥], NITA RUKMINASARI[3], ANDI ALIAH HIDAYANI[3], INDRA JUNAIDI ZAKARIA[4], MD. JOBAIDUL ALAM[4], HYUN-WOO KIM[5]

[1]Department of Marine, Fisheries and Marine Faculty, C Campus Jl. Mulyorejo Surabaya 60115. Universitas Airlangga, Surabaya, East Java, Indonesia
[2]Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Science, Andalas University, Padang, Indonesia
[4]Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka, Bangladesh
[5]Department of Marine Biology, School of Fisheries Sciences, Pukyong National University, Busan South Korea.
♥email: sapto.andriyono@fpk.unair.ac.id

Manuscript received: DD MM 2022 (Date of abstract/manuscript submission). Revision accepted: .... 2022.

Abstract. Dinoflagellates are a major part of the phytoplankton and are commonly found in freshwater, brackish, and marine habitats and are major components and play an important role in marine ecosystems. Dinoflagellate cysts (dinocysts) are produced by dinoflagellates in an unfavorable environment and can be stored well in organic matter for a long time. More than 200 dinoflagellate species have so far been observed to produce resting cysts and to be associated with the maintenance, discontinuation, and repetition of annual blooms. This study identified based on the morphological characteristics of dinoflagellate cysts from the estuary of the Bengawan Solo River and the estuary of Brantas river. 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 at the mouth of the Brantas river it is *Polykrikos schwartzii*. Shade graph analysis showed a number of species found only in the estuary of the Bengawan Solo River, namely *Zygabicodinium 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, dinoflagella, diversity, estuary, toxic

Running title: Andriyono et al. Diversity of Dinoflagellate Cyst

INTRODUCTION

Changes in the phytoplankton community in waters can occur when a number of harmful microalgae species grow and dominate the water column, and then cause environmental problems. This event is known as Harmful Algal Bloom (HAB). Following the concept of the Intergovernmental Oceanographic Commission (IOC-UNESCO), states that HAB is defined as a phenomenon of harmful algae growth in water which causes environmental problems (Hallegraeff 2003), losses to the fisheries sector and even causes death to humans (Hadisusanto and Sujarta 2010). 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 temperature, low oxygen levels and reduced light intensity, so that dinoflagellates can grow rapidly. This incident is very common in the estuary area of the river (Sudarmiati and Zaman 2007). Most of the algae that cause HABs belong to the dinoflagellate group (Hadisusanto and Sujarta 2010).

Dinoflagellates are included in the microalgae group found in sea and river waters (Senanayake et al. 2021). Dinoflagellates play an important role in waters, namely as primary producers (Hoppenrath et al. 2014, Price and Bhattacharya 2017). As microalgae, dinoflagellates have a unique ability to survive in the competition of natural selection. Under good water conditions, dinoflagellates can reproduce massively in the water column in the form of actively dividing swimming cells. When environmental conditions are bad nutrient enrichment (Faizal et al. 2012), high temperatures above 30ºC (Nitajohan 2008), 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 (Kurniawan 2008). The zygote is also produced by dinoflagellates by mating or fusion of gametes produced by vegetative dinoflagellate cells, securely protected within the thick walls of the cyst and highly resistant to adverse conditions (Alves-de-Souza et al. 2008, Bravo and Figueroa 2014, Dale 1983). In this regard, a study of the biodiversity of dinoflagella cysts in the estuaries of the Bengawan Solo and

Brantas Rivers in East Java was conducted using a morphological identification approach. Follow-up research from this activity confirms re-identification through a molecular approach to analyze diversity based on DNA characteristics possessed.

MATERIALS AND METHODS

Samples collection

Sediment at the bottom of the 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 locations with each location being repeated three times. The coordinate points for sampling (Figure 1) at the mouth of the Brantas River and the mouth of the Bengawan Solo River are presented in Table 1.

Figure 1. Sampling locations in two river mouths in East Java (https://earth.google.com/)

Table 1. Coordinate point of sediment sampling

No.	River location	Coordinate point
1.	Bengawan Solo	06º51’44” S 112º30’06” E 06º50’18” S 112º31’25” E 06º49’36” S 112º32’36” E 06º50’14” S 112º35’15” E
2.	Brantas	07º14’59” S 112º50’45” E 076º16’37” S 112º51’35” E 07º18’19” S 112º51’34” E 07º21’03” S 112º51’09” 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 added sterile sea water until it reaches a size of 50 ml, then the sonication process is carried out for 15 minutes using an ultrasonic sonicator to remove cysts attached to sediment particles. Furthermore, the sieving process is carried out using a filter which is arranged in stages based on the size of the pores. The sieving stage uses 3 filters with sizes of 250 µm, 125 µm and 20 µm. 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 cyst was then put into a 10 ml sample bottle for morphological analysis.

Determination of the rate of water content

Determination of the rate of water content is carried out to determine the water content contained in the sediment sample. Wet sediment samples were first weighed 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 dry sediment samples were weighed again. Previous research (Matsuoka and Fukuyo 2000) used the formulation and the calculation stage used the following formulation:

$$R = \frac{Bb - Bk}{Bb} x100\%$$

Information:
R = Rate of water content (%);
Bb = wet weight (g);
Bk = dry weight (g).

Dinoflagellate Cyst Abundance Calculation

The abundance of dinoflagellate cysts was observed under a light microscope using a haemacytometer with 8 fields of view. The abundance of dinoflagellate cysts was calculated based on the previous research formulation (Matsuoka and Fukuyo 2000)

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

Information :
N = Number of cysts observed (ind g-1);
W = Weight of wet sediment (g);
R = Rate of water content (%).

Identification of Dinoflagellate Cyst Morphology

Observation of dinoflagellate cysts was carried out by taking a sample to be observed using a dropper pipette of 1 ml, then transferred to a hemacytometer container, then observed under a light microscope. Each dinoflagellate cyst sample was identified to the genus and species level based on the published description of the dinoflagellate cyst by the researcher. (Alkawri 2016, Mertens et al. 2020)

Data Analysis

All data is presented in a table using Microsoft Excel software. Meanwhile, similarity analysis, Shade Graph analysis and dinoflagellate abundance from two different sampling locations were analyzed using Primer-e software (Clarke and Gorley 2015).

RESULTS AND DISCUSSION

Results

Identification of Dinoflagellate Cyst Morphology

Identification of the morphology of the dinoflagellate cysts varied quite a lot in the estuaries of the Berantas and Bengawan Solo rivers (Table 1). The Bengawan Solo estuary has a higher diversity of four species of dinoflagella cysts compared to the Brantas river estuary which only has 12 species. 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.

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

No.	Species name	Average abundance of dinoflagellate cyst (cyst g^{-1} sediment)	
		Brantas	**Bengawan Solo**
1	*Alexandrium catenella*	48	35
2	*Alexandrium minutum*	93	66
3	*Brigantedinium* sp.	42	28
4	*Cochlodinium polykrikoides*	48	69
5	*Gymnodinium catenatum*	-	43
6	*Gyrodinium* sp.	50	32
7	*Pentapharsodinium tyrrhenicum*	106	150
8	*Polykrikos kofoidii*	-	39
9	*Polykrikos schwartzii*	170	89
10	*Protoperidinium compressum*	32	-
11	*Protoperidinium leonis*	-	50
12	*Protoperidinium oblongum*	112	251
13	*Protoperidinium obtusum*	139	-

14	*Protoperidinium pentagonum*	-	41
15	*Scrippsiella crystallina*	147	68
16	*Scrippsiella lachrymosa*	130	214
17	*Votadinium* sp.	-	27
18	*Zygabikodinium lenticulatum*	-	39

Biodiversity Analysis of Dinoflagellate Cysts

Based on the data on the abundance of dinoflagellate cysts obtained, a biodiversity analysis was carried out to determine the dominating species and distribution patterns of cyst species in river estuaries in East Java. Each sampling location was repeated 4 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). This report is the first study on the biodiversity of dinoflagellate cysts in the estuaries of major rivers in East Java.

Table 2. Biodiversity analysis of dinoflagellate cysts at the mouth of the Bengawan Solo River and the mouth of the Brantas river

No.	Lokasi	Biodiversty Index		
		Margalef Index (d)	**Pielou's Evenness (J')**	**Shannon-Wiener Index (H')**
1	Bengawan Solo	2.7193	0.97933	2.1055
2	Brantas	2.4346	0.98888	2.0363

Analysis of Similarity and Abundance of Dinoflagellate Cyst Types

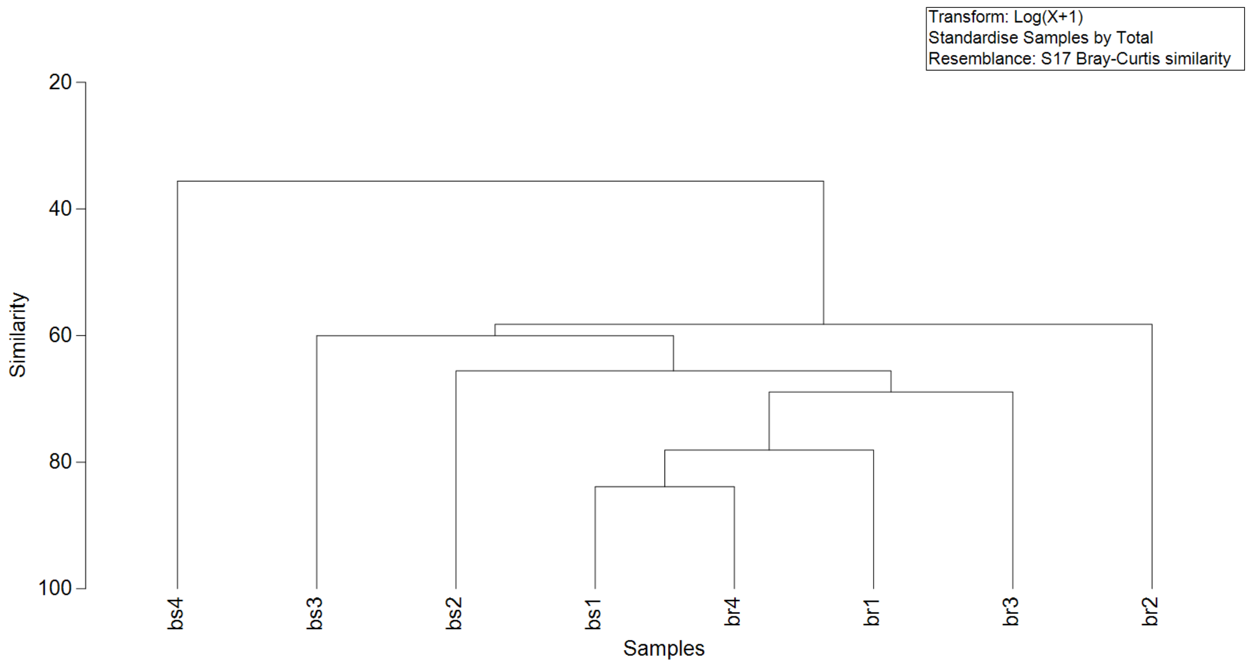

Figure 2. Analysis of similarity from 8 sampling locations in two different river mouths

The similarity analysis was carried out by comparing data on the abundance of dinoflagellate cysts found in both regions (Figure 2). The data was processed using Primer-e software and at the same time analyzing species abundance at each sediment collection location. Only the 4 sampling locations at the mouth of the Bengawan Solo River had the highest abundance and were different from other locations. Meanwhile, the sampling locations at the mouth of the Brantas river were in one clade which had a fairly high similarity between the four replicates. An abundance analysis (Figure 3) was carried out to find out certain species that have the potential to cause harmful algae blooms (HAB) which are feared to be detrimental to fishing activities and even endanger public health. The results of the Shadegraph showed that the type of dinoflagellate cyst was identified which was only found in the mouth of the Brantas river, namely the type of *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 *Zygabicodinium lenticulatum*, *Polykrikos kofoidii*, *Protoperidinium pentagonum*, *Gymnodinium catenatum*, and *Votadinium* sp.

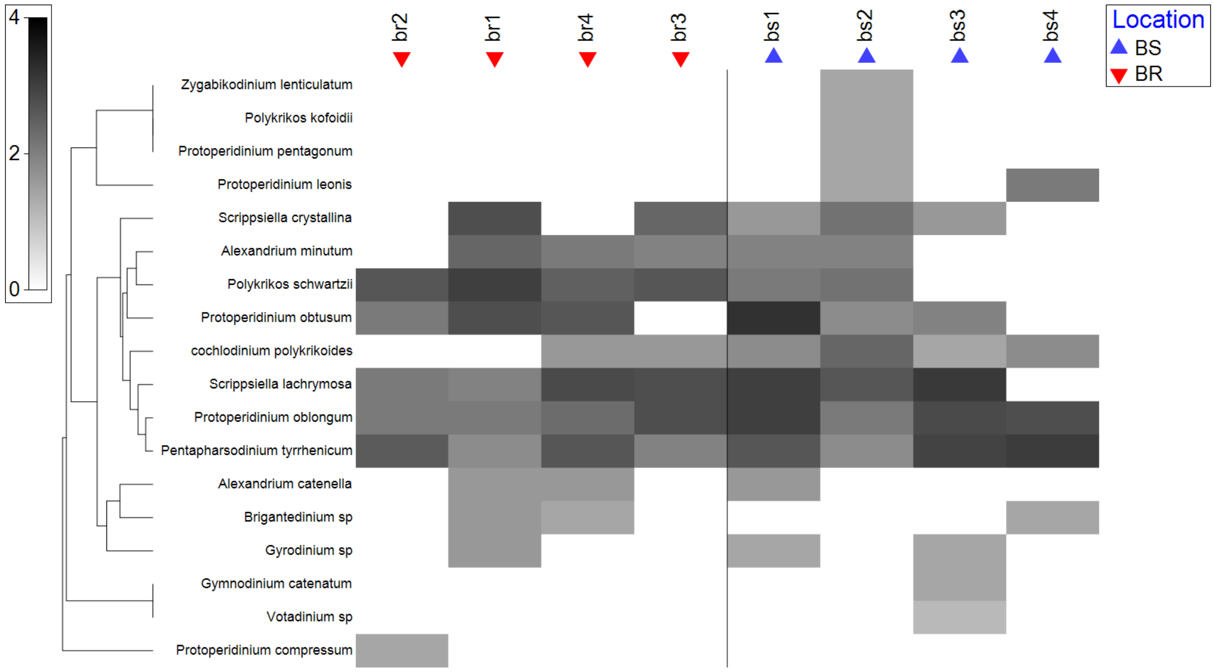

Figure 3. Graph of species distribution at each sampling location at the mouth of the Brantas river (br) and the mouth of the Bengawan Solo River (bs). The color intensity indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher diversity index than the estuary of the Brantas river. The type of dinoflagellate cyst that predominates in the estuary of the Bengawan Solo River is this type

Discussion

The diversity of dinoflagella cysts needs to be studied because this type of dinoflagella has the potential to become a plankton group that needs to be watched out for (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 River and Brantas river mouths. 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 dinoflagella explosions has been reported in a number of countries such as Australia (Riding et al. 2010), Chile (Alves-de-Souza et al. 2008), 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). This incident has harmed activities related to aquatic resources such as fishing activities which have had a huge impact. Losses due to the harmfull algae blooms have been reported which have caused the death of a number of fish in the waters and damaged the surrounding aquaculture activities (Jardine et al. 2020, Sakamoto et al. 2021). A number of regions also reported their impact on human health causing a number of disturbances (Grattan et al. 2016). Thus, this research is essential to be carried out to predict the potential for a dangerous algae population explosion.

In this study, two large river estuaries in East Java are important locations because around these locations many fishery activities are developing, especially shrimp (*Litopenaeus vanamae*) 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 quite 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 towards North Surabaya and East Surabaya. The eastern Surabaya area is very close to Sidoarjo Regency, which is also one of the Penaeus vanamae white shrimp pond areas (Hukom et al. 2020).

There were two species found in all research locations, namely the species *Protoperidinium oblongum* and *Pentapharsodinium tyrrhenicum*. The ecological function of dinoflagellates in water has received the attention of a number of researchers. 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 dinoflagellate species survive in harsh environments, provide opportunities 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 the species through its 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 (Bates and Trainer 2006, Park et al. 2013). The *Alexandrium tamarense* has been identified released of toxin (Oshima et al. 1992), and in this study we found two species in the genus *Alexandrium* (*A. catenella dan 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. 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 opening or archeopyle (Matsuoka and Fukuyo 2000).

The case of the dinoflagellate population explosion in Indonesia has not received serious attention even though a number of 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 time (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 deaths (Kurniawan 2008, Wouthuyzen et al. 2007). Although in East Java there have been no reports of algae blooms, 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 Indonesia are still very limited. 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 dinophagellate 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 particular region. There are four potential groups, namely very low, low, medium, high and very high levels has been classified (McMinn 1991, Tian et al. 2018). Pevious study show that abundance of dinoflagellate cysts in Lampung for the *P. bahamense* cyste is 1-100 cyste g^{-1} sediment (Rachman et al. 2021), while previous research found *M. polykrikoides* type cysts measured with abundances ranging from 100-1000 cyste 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 kiste g^{-1} sediment (Rachman et al. 2019). At the estuary of the Bengawan Solo River, the highest abundance was *Protoperidinium oblongum* (115 g-1 sediment kiste) and the lowest abundance was *Protoperidinium leonis* (21 g^{-1} sediment). Meanwhile, *Alexandrium minutum* was found with the highest abundance (125 kiste g^{-1} sediment) in the mouth of the Brantas river, with an average of 73 g^{-1} sediment cysts found. Thus, the condition of the mouth of the Brantas river and the mouth of the Bengawan Solo river still has a low potential for the occurrence of HABs and an average abundance of 73 and 62 g^{-1} kiste respectively (McMinn 1991, Tian et al. 2018).

CONCLUSIONS

Research on dinoflagellate cysts revealed the diversity of dinoflagellates at the mouth of the Bengawan Solo River and the mouth of the Brantas river. 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 dinoflagella 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. Shade graph analysis showed a number of species found only in the estuary of the Bengawan Solo River, namely *Zygabicodinium 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 dinogellate population explosions which may have negative impacts on various sectors.

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Artikel 9, LAMPIRAN 2

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Author(s) name:

SAPTO ANDRIYONO, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOBAIDUL ALAM, HYUN-WOO KIM

Address

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Department of Marine, Fisheries and Marine Faculty, Universitas Airlangga C Campus Jl. Mulyorejo Surabaya East Java, 60115, Indonesia Telp.+62-8232884599 email: sapto.andriyono@fpk.unair.ac.id

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SAPTO ANDRIYONO

Diversity of Dinoflagellates Cyst isolated from Mouth River Sediment of Brantas and Bengawan Solo River

SAPTO ANDRIYONO[1♥], NITA RUKMINASARI[3], ANDI ALIAH HIDAYANI[3], INDRA JUNAIDI ZAKARIA[4], MD. JOBAIDUL ALAM[4], HYUN-WOO KIM[5]

[1]Department of Marine, Fisheries and Marine Faculty, C Campus Jl. Mulyorejo Surabaya 60115. Universitas Airlangga, Surabaya, East Java, Indonesia
[2]Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Science, Andalas University, Padang, Indonesia
[4]Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka, Bangladesh
[5]Department of Marine Biology, School of Fisheries Sciences, Pukyong National University, Busan South Korea.
[♥]email: sapto.andriyono@fpk.unair.ac.id

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Abstract. Dinoflagellates are a major part of the phytoplankton and are commonly found in freshwater, brackish, and marine habitats and are major components and play an important role in marine ecosystems. Dinoflagellate cysts (dinocysts) are produced by dinoflagellates in an unfavorable environment and can be stored well in organic matter for a long time. More than 200 dinoflagellate species have so far been observed to produce resting cysts and to be associated with the maintenance, discontinuation, and repetition of annual blooms. This study identified based on the morphological characteristics of dinoflagellate cysts from the estuary of the Bengawan Solo River and the estuary of Brantas river. 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 at the mouth of the Brantas river it is *Polykrikos schwartzii*. Shade graph analysis showed a number of species found only in the estuary of the Bengawan Solo River, namely *Zygabicodinium 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, dinoflagellate, diversity, estuary, toxic

Running title: Andriyono et al. Diversity of Dinoflagellate Cyst

INTRODUCTION

Changes in the phytoplankton community in waters can occur when a number of harmful microalgae species grow and dominate the water column, and then cause environmental problems. This event is known as Harmful Algal Bloom (HAB). Following the concept of the Intergovernmental Oceanographic Commission (IOC-UNESCO), states that HABs is 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 causes 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 temperature, low oxygen levels, and reduced light intensity so that dinoflagellates can grow rapidly. This incident is very common in the estuary area of the river (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 waters, namely as primary producers (Hoppenrath et al. 2014, Price and Bhattacharya 2017). As microalgae, dinoflagellates have a unique ability to survive in the competition of natural selection. Under good water conditions, dinoflagellates can reproduce massively in the water column in the form of actively dividing swimming cells. When environmental conditions are bad 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 zygote is also produced by dinoflagellates by mating or 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 dinoflagella cysts in the estuaries of the Bengawan Solo and Brantas Rivers in East Java was conducted using a morphological identification approach.

The condition of the waters of the Bengawan Solo currently needs serious attention. The river flow originating from Central Java and empties 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. 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 in the waters. It is reported that nutrient enrichment events can reduce the value of biodiversity and the productivity of an ecosystem (Isbell et al. 2013). This condition is human intervention which is the main cause of the activities that have been carried out both on land and in aquatic (anthropogenic) ecosystems. 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 river in Java that crosses the provinces of Central Java to East Java. Conditions 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 enrichment 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 will change so that the highest concentrations can be 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. This is expected to be important data in the management of water areas that minimize the occurrence of dangerous algae population explosions or Harmfull Algae Blooms (HABs).

MATERIALS AND METHODS

Samples collection

Sediment at the bottom of the 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 locations with each location being repeated three times. The coordinate points for sampling (Figure 1) at the mouth of the Brantas River and the mouth of the Bengawan Solo River are presented in Table 1.

Figure 1. Sampling locations in two river mouths in East Java (https://earth.google.com/)

Table 1. Coordinate point of sediment sampling

No.	River location	Coordinate point
1.	Bengawan Solo	06º51’44” S 112º30’06” E 06º50’18” S 112º31’25” E 06º49’36” S 112º32’36” E 06º50’14” S 112º35’15” E
2.	Brantas	07º14’59” S 112º50’45” E 076º16’37” S 112º51’35” E 07º18’19” S 112º51’34” E 07º21’03” S 112º51’09” 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 added sterile sea water until it reaches a size of 50 ml, then the sonication process is carried out for 15 minutes using an ultrasonic sonicator to remove cysts attached to sediment particles. Furthermore, the sieving process is carried out using a filter which is arranged in stages based on the size of the pores. The sieving stage uses 3 filters with sizes of 250 μm, 125 μm and 20 μm. 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 cyst was then put into a 10 ml sample bottle for morphological analysis.

Determination of the rate of water content

Determination of the rate of water content is carried out to determine the water content contained in the sediment sample. Wet sediment samples were first weighed 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 dry sediment samples were weighed again. Previous research (Matsuoka and Fukuyo 2000) used the formulation and the calculation stage used the following formulation:

$$R = \frac{Bb - Bk}{Bb} x100\%$$

Information:
R = Rate of water content (%);
Bb = wet weight (g);
Bk = dry weight (g).

Dinoflagellate Cyst Abundance Calculation

The abundance of dinoflagellate cysts was observed under a light microscope using a haemacytometer with 8 fields of view. The abundance of dinoflagellate cysts was calculated based on the previous research formulation (Matsuoka and Fukuyo 2000)

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

Information :
N = Number of cysts observed (ind g-1);
W = Weight of wet sediment (g);
R = Rate of water content (%).

Identification of Dinoflagellate Cyst Morphology

Observation of dinoflagellate cysts was carried out by taking a sample to be observed using a dropper pipette of 1 ml, then transferred to a hemacytometer container, then observed under a light microscope. Each dinoflagellate cyst sample was identified to the genus and species level based on the published description of the dinoflagellate cyst by the researcher. (Alkawri 2016, Mertens et al. 2020)

Data Analysis

All data is presented in a table using Microsoft Excel software. Meanwhile, similarity analysis, Shade Graph analysis and dinoflagellate abundance from two different sampling locations were analyzed using Primer-e software (Clarke and Gorley 2015).

RESULTS AND DISCUSSION

Results

Identification of Dinoflagellate Cyst Morphology

Identification of the morphology of the dinoflagellate cysts varied quite a lot in the estuaries of the Berantas and Bengawan Solo rivers (Table 1). The Bengawan Solo estuary has a higher diversity of four species of dinoflagella cysts compared to the Brantas river estuary which only has 12 species. 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.

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

No.	**Species name**	**Average abundance of dinoflagellate cyst (cyst g^{-1} sediment)**	
		Brantas	**Bengawan Solo**
1	*Alexandrium catenella*	48	35
2	*Alexandrium minutum*	93	66
3	*Brigantedinium* sp.	42	28
4	*Cochlodinium polykrikoides*	48	69
5	*Gymnodinium catenatum*	-	43
6	*Gyrodinium* sp.	50	32
7	*Pentapharsodinium tyrrhenicum*	106	150
8	*Polykrikos kofoidii*	-	39
9	*Polykrikos schwartzii*	170	89
10	*Protoperidinium compressum*	32	-
11	*Protoperidinium leonis*	-	50
12	*Protoperidinium oblongum*	112	251
13	*Protoperidinium obtusum*	139	-
14	*Protoperidinium pentagonum*	-	41
15	*Scrippsiella crystallina*	147	68
16	*Scrippsiella lachrymosa*	130	214
17	*Votadinium* sp.	-	27
18	*Zygabikodinium lenticulatum*	-	39

Biodiversity Analysis of Dinoflagellate Cysts

Based on the data on the abundance of dinoflagellate cysts obtained, a biodiversity analysis was carried out to determine the dominating species and distribution patterns of cyst species in river estuaries in East Java. Each sampling location was repeated 4 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). This report is the first study on the biodiversity of dinoflagellate cysts in the estuaries of major rivers in East Java.

Table 2. Biodiversity analysis of dinoflagellate cysts at the mouth of the Bengawan Solo River and the mouth of the Brantas river

No.	**Lokasi**	**Biodiversty Index**		
		Margalef Index (d)	**Pielou's Evenness (J')**	**Shannon-Wiener Index (H')**
1	Bengawan Solo	2.7193	0.97933	2.1055
2	Brantas	2.4346	0.98888	2.0363

Analysis of Similarity and Abundance of Dinoflagellate Cyst Types

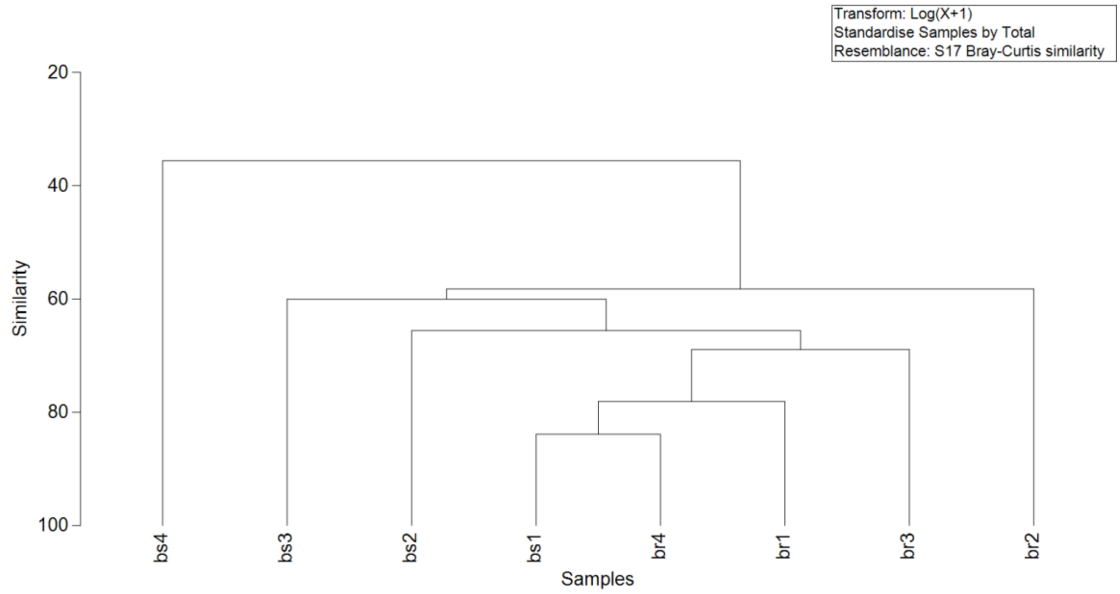

Figure 2. Analysis of similarity from 8 sampling locations in two different river mouths

The similarity analysis was carried out by comparing data on the abundance of dinoflagellate cysts found in both regions (Figure 2). The data was processed using Primer-e software and at the same time analyzing species abundance at each sediment collection location. Only the 4 sampling locations at the mouth of the Bengawan Solo River had the highest abundance and were different from other locations. Meanwhile, the sampling locations at the mouth of the Brantas river were in one clade which had a fairly high similarity between the four replicates. An abundance analysis (Figure 3) was carried out to find out certain species that have the potential to cause harmful algae blooms (HAB) which are feared to be detrimental to fishing activities and even endanger public health. The results of the Shadegraph showed that the type of dinoflagellate cyst was identified which was only found in the mouth of the Brantas river, namely the type of *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 *Zygabicodinium lenticulatum*, *Polykrikos kofoidii*, *Protoperidinium pentagonum*, *Gymnodinium catenatum*, and *Votadinium* sp.

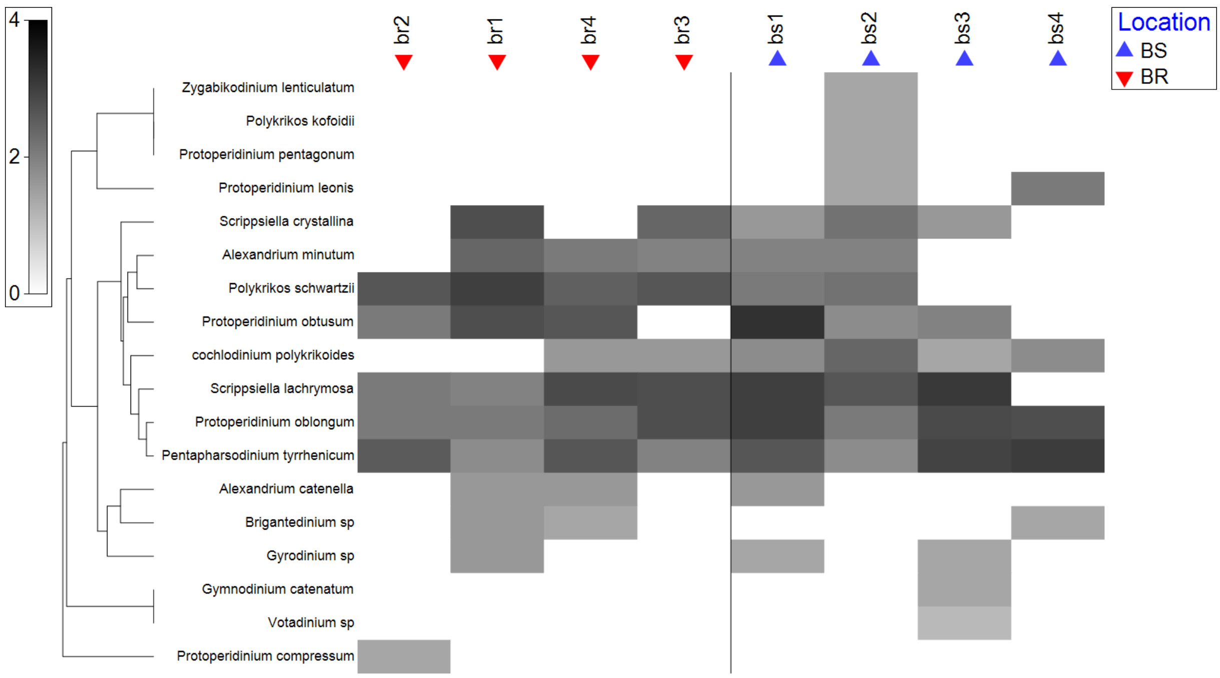

Figure 3. Graph of species distribution at each sampling location at the mouth of the Brantas river (br) and the mouth of the Bengawan Solo River (bs). The color intensity indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher diversity index than the estuary of the Brantas river. The type of dinoflagellate cyst that predominates in the estuary of the Bengawan Solo River is this type

Discussion

The diversity of dinoflagella cysts needs to be studied because this type of dinoflagella has the potential to become a plankton group that needs to be watched out for (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 River and Brantas river mouths. 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 dinoflagella explosions 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). This incident has harmed activities related to aquatic resources such as fishing activities which have had a huge impact. Losses due to the harmfull algae blooms have been reported which have caused the death of a number of fish in the waters and damaged the surrounding aquaculture activities (Jardine et al. 2020, Sakamoto et al. 2021). A number of regions also reported their impact on human health causing a number of disturbances (Grattan et al. 2016). Thus, this research is essential to be carried out to predict the potential for a dangerous algae population explosion.

In this study, two large river estuaries in East Java are important locations because around these locations many fishery activities are developing, especially shrimp (*Litopenaeus vanamae*) 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 quite 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 towards North Surabaya and East Surabaya. The eastern Surabaya area is very close to Sidoarjo Regency, which is also one of the Penaeus vanamae white shrimp pond areas (Hukom et al. 2020).

There were two species found in all research locations, namely the species *Protoperidinium oblongum* and *Pentapharsodinium tyrrhenicum*. The ecological function of dinoflagellates in water has received the attention of a number of researchers. 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 dinoflagellate species survive in harsh environments, provide opportunities 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 the species through its 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). The *Alexandrium tamarense* has been identified released of toxin (Oshima et al. 1992), and in this study we found two species in the genus *Alexandrium* (*A. catenella dan 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. 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 opening or archeopyle (Matsuoka and Fukuyo 2000).

The case of the dinoflagellate population explosion in Indonesia has not received serious attention even though a number of 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 time (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 deaths (Nasution et al. 2021). Although in East Java there have been no reports of algae blooms, 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 Indonesia are still very limited. 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 dinophagellate 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 particular region. There are four potential groups, namely very low, low, medium, high and very high levels has been classified (McMinn 1991, Tian et al. 2018). Pevious study show that abundance of dinoflagellate cysts in Lampung for the *P. bahamense* cyste is 1-100 cyste g^{-1} sediment (Rachman et al. 2021), while previous research found *M. polykrikoides* type cysts measured with abundances ranging from 100-1000 cyste 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 kiste g^{-1} sediment (Rachman et al. 2019). At the estuary of the Bengawan Solo River, the highest abundance was *Protoperidinium oblongum* (115 g-1 sediment kiste) and the lowest abundance was *Protoperidinium leonis* (21 g^{-1} sediment). Meanwhile, *Alexandrium minutum* was found with the highest abundance (125 kiste g^{-1} sediment) in the mouth of the Brantas river, with an average of 73 g^{-1} sediment cysts found. Thus, the condition of the mouth of the Brantas river and the mouth of the Bengawan Solo river still has a low potential for the occurrence of HABs and an average abundance of 73 and 62 g^{-1} kiste respectively (McMinn 1991, Tian et al. 2018).

CONCLUSIONS

Research on dinoflagellate cysts revealed the diversity of dinoflagellates at the mouth of the Bengawan Solo River and the mouth of the Brantas river. 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 dinoflagella 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. Shade graph analysis showed a number of species found only in the estuary of the Bengawan Solo River, namely *Zygabicodinium 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 dinogellate population explosions which may have negative impacts on various sectors.

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SUBMISSION CHECKLIST

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The first corresponding author must be accompanied with contact details: **Give mark (X)**

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All necessary files have been uploaded, and contain:

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• Manuscript has been “spell & grammar-checked” Better, if it is revised by a professional science editor or a native English speaker	v
• References are in the correct format for this journal	v
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• Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate	v

Artikel 9, LAMPIRAN 3

Diversity of d~~D~~inoflagellate~~s~~ c~~C~~ysts isolated from estuarine~~Mouth River~~ s~~S~~ediments of the ~~Brantas~~ Bengawan Solo and Brantas ~~Bengawan Solo~~ r~~R~~ivers

Abstract. Dinoflagellates are a major part of the phytoplankton and are commonly found in freshwater, brackish, and marine habitats and are major components which~~and~~ play an important role in marine ecosystems. Dinoflagellate cysts (dinocysts) are produced by dinoflagellates in an unfavorable environment, and can be ~~stored~~ preserved well in ~~organic~~ sediments~~matter~~ for ~~a~~ long periods of time. More than 200 dinoflagellate species have so far been observed to produce resting cysts and to be associated with the maintenance, discontinuation, and repetition of annual blooms. This study identified ___ based on the morphological characteristics of dinoflagellate cysts from the estuaries~~y~~ of the Brantas ~~Bengawan Solo River~~ and ~~the estuary of~~ Bengawan Solo rivers ~~Brantas river~~. 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 Ri~~ri~~ver ~~it~~ is Polykrikos schwartzii. Shade graph analysis shows~~ed~~ a number of species to be found only in the estuary of the Bengawan Solo River, namely Zygabicodinium 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, dinoflagellate, diversity, estuary, toxic

Running title: Andriyono et al. Diversity of Dinoflagellate Cyst

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INTRODUCTION

Changes in the phytoplankton community ~~in waters~~ can occur when a number of harmful microalgae species grow and dominate the water column~~,~~ and proceed to~~then~~ cause environmental ~~problems~~impacts. Such an ~~This~~ event is known as a Harmful Algal Bloom (HAB). Following the concept of the Intergovernmental Oceanographic Commission (IOC-UNESCO), ~~states that~~ HABs are~~is~~ 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 ~~causes~~ 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 ~~so that~~ dinoflagellates to~~can~~ grow rapidly. The~~i~~se incidents are~~is~~ very common in~~ the~~ estuary areas of~~f the~~ 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 Bhattacharya 2017). As microalgae, dinoflagellates have a unique ability to specialize and survive in the competition of natural selection. Under ~~good water~~productive conditions, dinoflagellates can reproduce rapid~~massive~~ly in the water column in the form of actively dividing swimming cells. When environmental conditions are suboptimal,~~bad~~ 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 ~~by mating~~ through the~~or~~ 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 r~~R~~ivers in East Java was conducted using a morphological identification approach.

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The water condition~~s of the waters~~ of the Bengawan Solo River currently need~~s~~ serious attention. The river flow originating from Central Java ~~and~~ emptying~~ies~~ 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. 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 ~~in the waters~~. It is reported that nutrient enrichment events can reduce the value of biodiversity and the productivity of an ecosystem (Isbell et al. 2013). The principal contributing factor~~is condition~~ is human intervention due to ~~which is the main cause of the~~ activities that have been carried out both on land and in aquatic (anthropogenic) ecosystems. This condition also has an impact on important ecosystems in the coastal area, namely coral reef ecosystems (D'Angelo and Wiedenmann 2014).

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Meanwhile, the estuary of the Brantas river is also a major ~~river~~ 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. T~~, t~~he estuary waters of the Brantas river also receive a large amount of organic matter from human activities. Nutrient influx~~enrichment~~ from the Brantas R~~r~~iver 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 ~~will~~ results in a gradational change in ~~-~~marine conditions such~~o~~ that the highest concentrations of nutrients are~~can be~~ 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 ~~This~~ are~~is~~ expected to yield valuable~~be important~~ data toward~~in~~ the management of water areas that minimizes the occurrence of dangerous algae population explosions or ~~Harmfull Algae Blooms (~~HABs~~)~~.

MATERIALS AND METHODS

Samples 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 sta~~loca~~tions with sampling~~each location~~ being repeated three times. The coordinate points for sampling (Figure 1) at the mouths of the Bengawan Solo and Brantas r~~R~~ivers ~~and the mouth of the Bengawan Solo River~~ are presented in Table 1.

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

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Table 1. ~~Coordinate~~S~~ point of s~~ediment sampling locations.

No.	River ~~location~~	Station cCoordinates ~~point~~
1.	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
2.	Brantas	07°14′59″ S 112°50′45″ E 076°16′37″ S 112°51′35″ E 07°18′19″ S 112°51′34″ E 07°21′03″ S 112°51′09″ E

Sediment s~~S~~eparation w~~W~~ith d~~D~~inoflagellate c~~C~~ysts

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~~added~~ to sterile sea water until it reaches a size of 50 ml.~~,~~ Following the acid treatment procedure,~~then~~ a~~the~~ ~~-~~sonication process wa~~i~~s carried out for 15 minutes using an ultrasonic agita~~sonica~~tor to remove sediment particles attached to cysts~~ attached to sediment particles~~. Furthermore, the sieving process wa~~i~~s carried out using a filter which is arranged in stages based on the size of the mesh openings~~pores~~. 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 w~~ere~~~~as~~ then put into a 10 ml sample bottle for morphological analysis.

Determination of the rate of water content

Determination of the rate of water content is carried out to ~~determine~~ establish the water ~~content~~ 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 dry sediment samples were weighed again. Previous research (Matsuoka and Fukuyo 2000) used the following formula~~tion and the calculation stage used the following formulation~~wherein Bb is the wet sediment weight (g); Bk is the dry sediment weight (g), and R is the~~=~~ r~~R~~ate of water content (%):~~;~~

~~Bb = wet weight (g)~~;

~~Bk = dry weight (g)~~.

~~;~~

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

Information:

R = Rate of water content (%);

Bb = wet weight (g);

Bk = dry weight (g).

Dinoflagellate cCyst aAbundance cCalculation

The abundance of dinoflagellate cysts was observed under a light microscope using a haemacytometer with eight8 fields of view. The abundance of dinoflagellate cysts was calculated based on the previous research formula oftion (Matsuoka and Fukuyo (2000) wherein R is the= rRate of water content (%), .N is the total number of cysts observed, and W is the weight of wet sediment (g):

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

Information :

N = Number of cysts observed (ind g-1);

W = Weight of wet sediment (g);

R = Rate of water content (%).

Identification of dDinoflagellate cCysts Morphology

Observation of Ddinoflagellate cyst morphological analysiss was carried out by taking organic sample residuea sample to be observed using a dropper pipette of 1 ml and, then transferringed it to a hemacytometer container for, then observationed under a transmitted light microscope. Each dinoflagellate cyst sample was identified to the genus and species level based on the published description of the dinoflagellate cysttaxon by the researcher. (Alkawri 2016, Mertens et al. 2020).

Data aAnalysis

All data is presented in a table using Microsoft Excel software. AdditionallyMeanwhile, similarity analysis, Shader-Graph analysis, and dinoflagellate abundance from two different sampling locations were analyzed using Primer-e software (Clarke and Gorley 2015).

RESULTS AND DISCUSSION

Results

Identification of Dinoflagellate cCyst diversityMorphology

TIdentification of the morphology of the dinoflagellate cysts taxa present varied significantly quite a lot in the estuaries of the Berantas and Bengawan Solo rivers (Table 1). The Bengawan Solo River estuary yieldedhas a higher diversity of four species of dinoflagellate cysts compared to that of the Brantas Rriver estuary which only presentedhas 12 species. The type of Protoperidinium obtusum was found to dominate

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in the mouth of the Bengawan Solo River, while the type of Scrippsiella lachrymose dominated in the mouth of the Brantas Rriver.

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

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No.	Species name	Average abundance of dinoflagellate cyst (cyst g^{-1} sediment)	
		Bengawan SoloBrantas	**BrantasBengawan Solo**
1	Alexandrium catenella	3548	4835
2	Alexandrium minutum	6693	9366
3	Brigantedinium sp.	2842	4228
4	Cochlodinium polykrikoides	6948	4869
5	Gymnodinium catenatum	43-	-43
6	Gyrodinium sp.	3250	5032
7	Pentapharsodinium tyrrhenicum	150106	106150
8	Polykrikos kofoidii	39-	-39
9	Polykrikos schwartzii	89170	17089
10	Protoperidinium compressum	-32	32-
11	Protoperidinium leonis	50-	-50
12	Protoperidinium oblongum	251112	112251
13	Protoperidinium obtusum	-139	139-
14	Protoperidinium pentagonum	41-	-41
15	Scrippsiella crystallina	68147	14768
16	Scrippsiella lachrymosa	214130	130214
17	Votadinium sp.	27-	-27
18	Zygabikodinium lenticulatum	39-	-39

Biodiversity aAnalysis of dDinoflagellate cCysts

Based on the dinoflagellate cyst abundance data on the abundance of dinoflagellate cysts obtained, 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 four4 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 Rriver (2.1055) has higher diversity than the mouth of the Brantas Rriver (2.0363). This report is the first study on the biodiversity of dinoflagellate cysts in the estuaries of major rivers in East Java.

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

No.	River~~Lokasi~~	Biodiversty Index		
		Margalef Index (d)	Pielou's Evenness (J')	Shannon-Wiener Index (H')
1	Bengawan Solo	2.7193	0.97933	2.1055
2	Brantas	2.4346	0.98888	2.0363

Analysis of s~~S~~imilarity and a~~A~~bundance of d~~D~~inoflagellate c~~C~~yst t~~T~~ypes

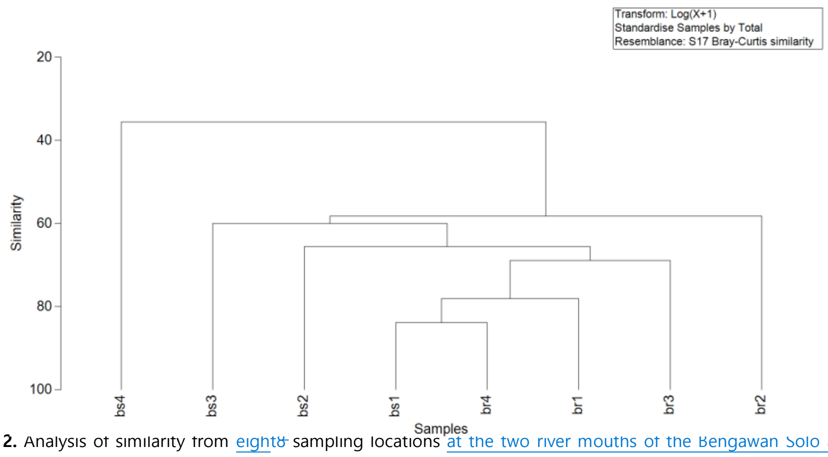

Figure 2. Analysis of similarity from eight~~8~~ sampling locations at the two river mouths of the Bengawan Solo and Brantas rivers in East Java.~~in two different river mouths~~

The similarity analysis was carried out by comparing data on the abundance of dinoflagellate cysts found in both regions (Figure 2). The data was processed using Primer-e software and at the same time analyzing species abundance at each sediment collection st~~loc~~ation. Only the four~~4~~ sampling st~~loc~~ations at the mouth of the Bengawan Solo River had the highest abundance and were different from other statio~~locatio~~ns. Meanwhile, the sampling st~~loc~~ations at the mouth of the Brantas R~~r~~iver were in one clade which had a fairly high similarity between the four replicates. An abundance analysis (Figure 3) was carried out to ascertain~~find out certain~~ species that have the potential to cause harmful algae blooms (HAB), which are known~~feared~~ to be detrimental to fishing activities and even a ~~en~~danger to public health. The results of the Shade~~r~~G~~g~~raph analysis showed that cysts of thi~~se~~ type of dinoflagellate ~~cyst was~~ are~~identified which was~~ only found in the mouth of the Brantas R~~r~~iver, namely th~~ose of thee type of~~ 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

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Rriver, namely Zygabicodinium lenticulatum, Polykrikos kofoidii, Protoperidinium pentagonum, Gymnodinium catenatum, and Votadinium sp.

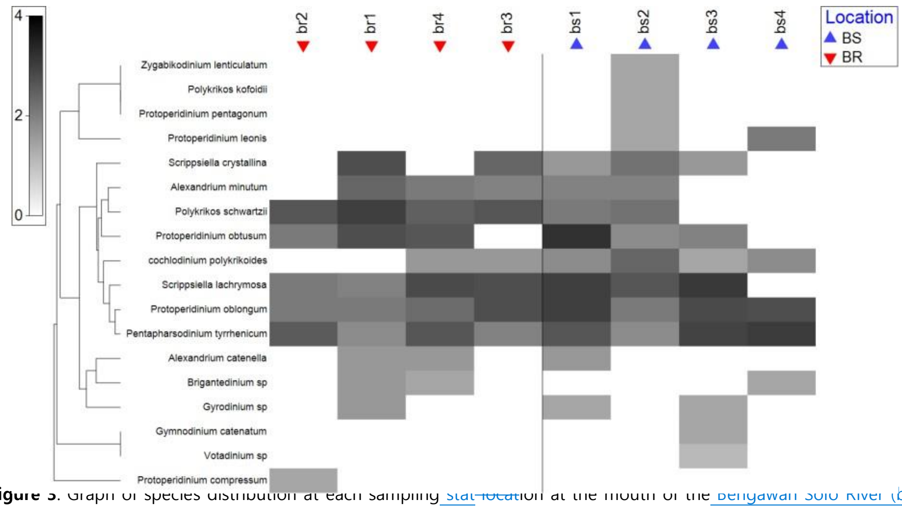

Figure 3. Graph of species distribution at each sampling stat locat ion at the mouth of the Bengawan Solo River (bs) Brantas river (br) and mouth of the Brantas River (br)the mouth of the Bengawan Solo River (bs). The color intensity indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher diversity index than the estuary of the Brantas Rriver. The type of dinoflagellate cyst that predominates in the estuary of the Bengawan Solo River is this type.

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Discussion

The diversity of dinoflagella cysts needs to be studied because certainthis type of dinoflagellates haves 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 River and Brantas river estuaries mouths. 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 bloomsexplosions 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). Theseis incidents haves proven harmful toed activities related to aquatic resources such as fishing, activities which have had a huge impact. Losses due to the harmfull algae blooms have been reported, which have caused the death of a number of fish in the waters and damaged the surrounding aquaculture industriesactivities (Jardine et al. 2020, Sakamoto et al. 2021). A number of regions also reported their impact on human health causing a numerous instancesber of illness disturbances (Grattan et al. 2016). Thus, it is essential for this research is essential 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 are shown to be important locations because around these locations of their proximity to many sites where fishery activities are developing, especially realting to shrimp (Litopenaeus vanamae) and milkfish (Chanos chanos) ponds which are the mainstay of this region. The estuary of the Bengawan Solo RRiver is in the Gresik area which is aa quite 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 towards North Surabaya and East Surabaya. The eastern Surabaya area is very close to Sidoarjo Regency, which is also one of the Penaeus vanamae white shrimp pond areas (Hukom et al. 2020).

There were two species found in all research locations, namely the species Protoperidinium oblongum and Pentapharsodinium tyrrhenicum. The ecological function of dinoflagellates in water has received the attention of a number of researchers. 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 dinoflagellate species survive in harsh environments, provide opportunities 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 the speciesother organisms through theits 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). The Alexandrium tamarense has been identified as a toxic species released of toxin (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. 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 of opening or archeopyle (Matsuoka and Fukuyo 2000).

The case of the dinoflagellate population explosion in Indonesia has not received serious attention even though a number of 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 BBay which caused mass fish die-offaths (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 Indonesia are still very limited. 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

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on the diversity index of dino~~fph~~agellate 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 R~~r~~iver 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 ~~particular~~ given region. There are four potential groups that have been classified, namely very low, low, medium, high and very high levels ~~has been classified~~ (McMinn 1991; ~~,~~ Tian et al. 2018). Pevious study show that abundance of dinoflagellate cysts in Lampung for ~~the~~ P. bahamense ~~cyste~~ is 1–~~-~~100 cyst~~se~~ g^{-1} sediment (Rachman et al. 2021), while previous research found M. polykrikoides type cysts measured with abundances ranging from 100–~~-~~1000 cyst~~se~~ 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 cy~~k~~ist~~se~~ 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 ~~kiste~~) and the lowest abundance was Protoperidinium leonis (21 cysts g^{-1} sediment). Meanwhile, Alexandrium minutum was found with the highest abundance (125 cysts~~kiste~~ g^{-1} sediment) in the mouth of the Brantas R~~r~~iver, with an average of 73 cysts g^{-1} sediment having been~~cysts~~ found. Thus, the condition of the mouths of the ~~Brantas~~ Bengawan Solo ~~river~~ and ~~the mouth of the~~ Brantas ~~Bengawan Solo~~ rivers still has a low potential for the occurrence of HABs, and an average abundance of 73 and 62 cysts g^{-1} ~~kiste~~ respectively (McMinn 1991; ~~,~~ Tian et al. 2018).

CONCLUSIONS

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 ~~River~~ and ~~the mouth of the~~ 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 R~~r~~iver ~~mouth~~. 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 R~~r~~iver it is Polykrikos schwartzii. ShadeG~~-g~~raph analysis showed a number of species found only in the estuary of the Bengawan Solo River, namely Zygabicodinium 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 explosions which are likely to~~may~~ have negative impacts on various sectors of industry.

ACKNOWLEDGEMENTS

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based on Research Grant No. 439/UN3.1.12/ PT/2022 on DNA Barcoding for fish from inland and marine water ecosystems. The authors have no conflicts of interest to declare.

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Artikel 9, LAMPIRAN 4

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

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Abstract. 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 unfavorable environment and can be preserved well in sediments for long periods of time. 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 dinoflagellates 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 Zygabicodinium 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, dinoflagellate, diversity, estuary, toxic

Running title: Andriyono et al. Diversity of Dinoflagellate Cyst

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). 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 grow rapidly. These incidents are very common 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 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 Bhattacharya 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 needs 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 biodiversity 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 explosions or HABs.

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.

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

Table 1. Sediment sampling locations.

No.	River	Station coordinates
1.	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
2.	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 rate 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 dry 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 rate 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 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 is 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).

RESULTS AND DISCUSSION

Results

Dinoflagellate cyst diversity

The dinoflagellate cysts taxa present varied significantly 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. 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).

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

No.	Species name	Average abundance of dinoflagellate cyst (cyst g^{-1} sediment)	
		Bengawan Solo	Brantas
1	Alexandrium catenella	35	48
2	Alexandrium minutum	66	93
3	Brigantedinium sp.	28	42
4	Cochlodinium polykrikoides	69	48
5	Gymnodinium catenatum	43	-
6	Gyrodinium sp.	32	50
7	Pentapharsodinium tyrrhenicum	150	106
8	Polykrikos kofoidii	39	-
9	Polykrikos schwartzii	89	170
10	Protoperidinium compressum	-	32
11	Protoperidinium leonis	50	-
12	Protoperidinium oblongum	251	112
13	Protoperidinium obtusum	-	139
14	Protoperidinium pentagonum	41	-
15	Scrippsiella crystallina	68	147
16	Scrippsiella lachrymosa	214	130
17	Votadinium sp.	27	-
18	Zygabikodinium lenticulatum	39	-

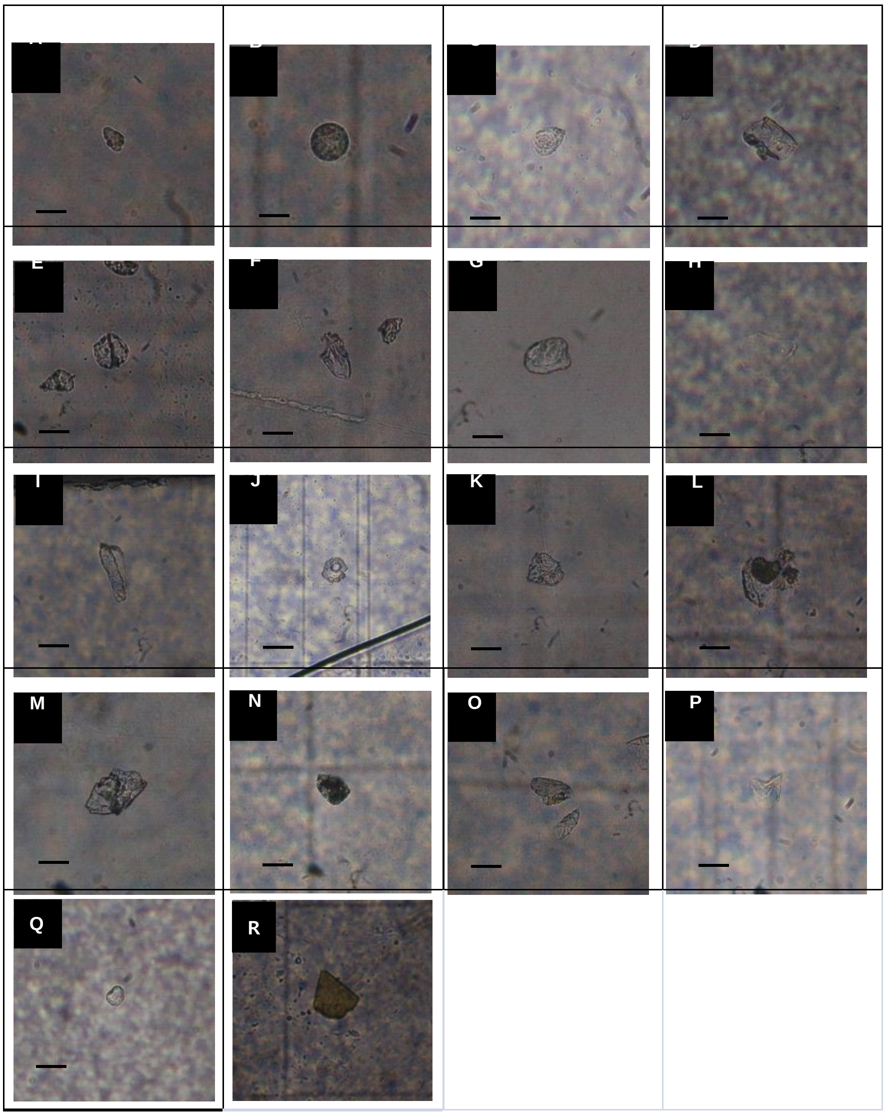

Figure 2. Dinoflagellate cyste collected from Bengawan Solo and Brantas river estuary. Bar scale 10 μ (A) Alexandrium catenella; (B) Alexandrium minutum; (C) Brigantedinium sp.; (D) Cochlodinium polykrikoides; (E) Gymnodinium catenatum; (F) Gyrodinium sp; (G) Pentapharsodinium tyrrhenicum ; (H) Polykrikos 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 .

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).

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

No.	River	Biodiversty Index		
		Margalef Index (d)	Pielou's Evenness (J')	Shannon-Wiener Index (H')
1	Bengawan Solo	2.7193	0.97933	2.1055
2	Brantas	2.4346	0.98888	2.0363

Analysis of similarity and abundance of dinoflagellate cyst types

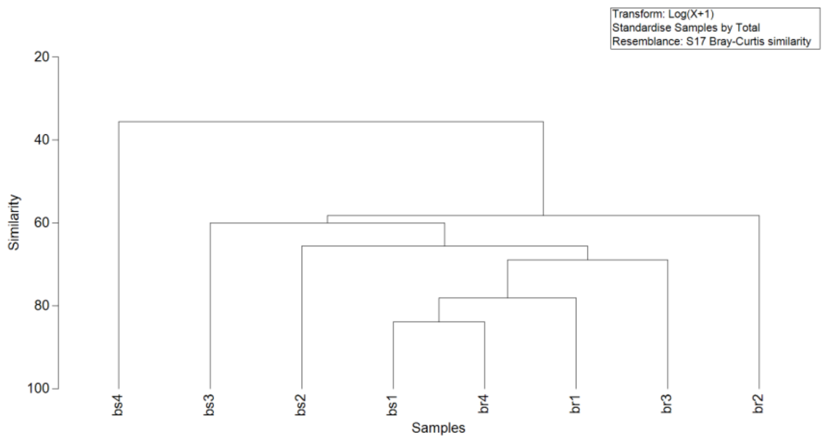

Figure 3. Analysis of similarity from eight sampling locations at the two river mouths of the Bengawan Solo and Brantas rivers in East Java.

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 Zygabicodinium lenticulatum, Polykrikos kofoidii, Protoperidinium pentagonum, Gymnodinium catenatum, and Votadinium sp.

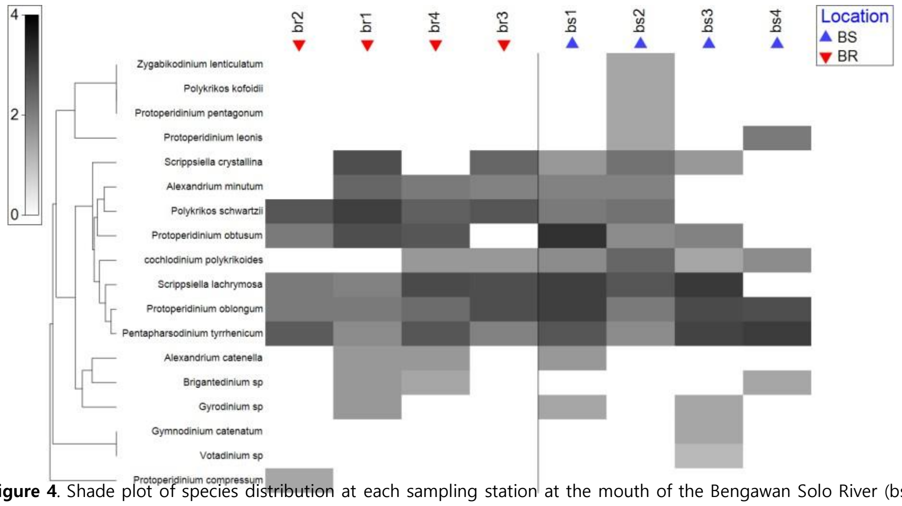

Figure 4. Shade plot of species distribution at each sampling station at the mouth of the Bengawan Solo River (bs) and mouth of the Brantas River (br). The color intensity indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher diversity index than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

Discussion

The diversity of dinoflagella cysts needs to be studied because certain type 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. Losses due to the harmfull 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 vanamae) 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 vanamae white shrimp pond areas (Hukom et al. 2020).

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 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. Berkenaan dengan pendekatan molekuler, beberapa penelitian telah berhasil mengidentifikasi berdasarkan rDNA pada SSU, LSU dan gen regio ITS (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).

The case of the dinoflagellate population explosion in Indonesia has not received serious attention even though a number of 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). Pevious 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).

CONCLUSIONS

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 Zygabicodinium 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 explosions which are likely to have negative impacts on various sectors of industry.

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

Abstract. 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 unfavorable environment and can be preserved well in sediments for long periods of time. 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 dinoflagellates 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 *Zygabicodinium 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, dinoflagellate, diversity, estuary, toxic

Running title: Andriyono et al. Diversity of Dinoflagellate Cyst

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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). 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 grow rapidly. These incidents are very common 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 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 Bhattacharya 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.

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The water conditions of the Bengawan Solo River currently needs 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 biodiversity 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 explosions or HABs.

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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.

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

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Table 1. Sediment sampling locations.

No.	River	Station coordinates
1.	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
2.	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 rate 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 dry 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 rate 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 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 is 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).

RESULTS AND DISCUSSION

Results

Dinoflagellate cyst diversity

The dinoflagellate cysts taxa present varied significantly 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. 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).

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

No.	Species name	Average abundance of dinoflagellate cyst (cyst g^{-1} sediment)	
		Bengawan Solo	Brantas
1	*Alexandrium catenella*	35	48

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2	*Alexandrium minutum*	66	93
3	*Brigantedinium* sp.	28	42
	Cochlodinium polykrikoides	69	48
5	*Gymnodinium catenatum*	43	-
6	*Gyrodinium* sp.	32	50
7	*Pentapharsodinium tyrrhenicum*	150	106
8	*Polykrikos kofoidii*	39	-
9	*Polykrikos schwartzii*	89	170
10	*Protoperidinium compressum*	-	32
11	*Protoperidinium leonis*	50	-
12	*Protoperidinium oblongum*	251	112
13	*Protoperidinium obtusum*	-	139
14	*Protoperidinium pentagonum*	41	-
15	*Scrippsiella crystallina*	68	147
16	*Scrippsiella lachrymosa*	214	130
17	*Votadinium* sp.	27	-
18	*Zygabikodinium lenticulatum*	39	-

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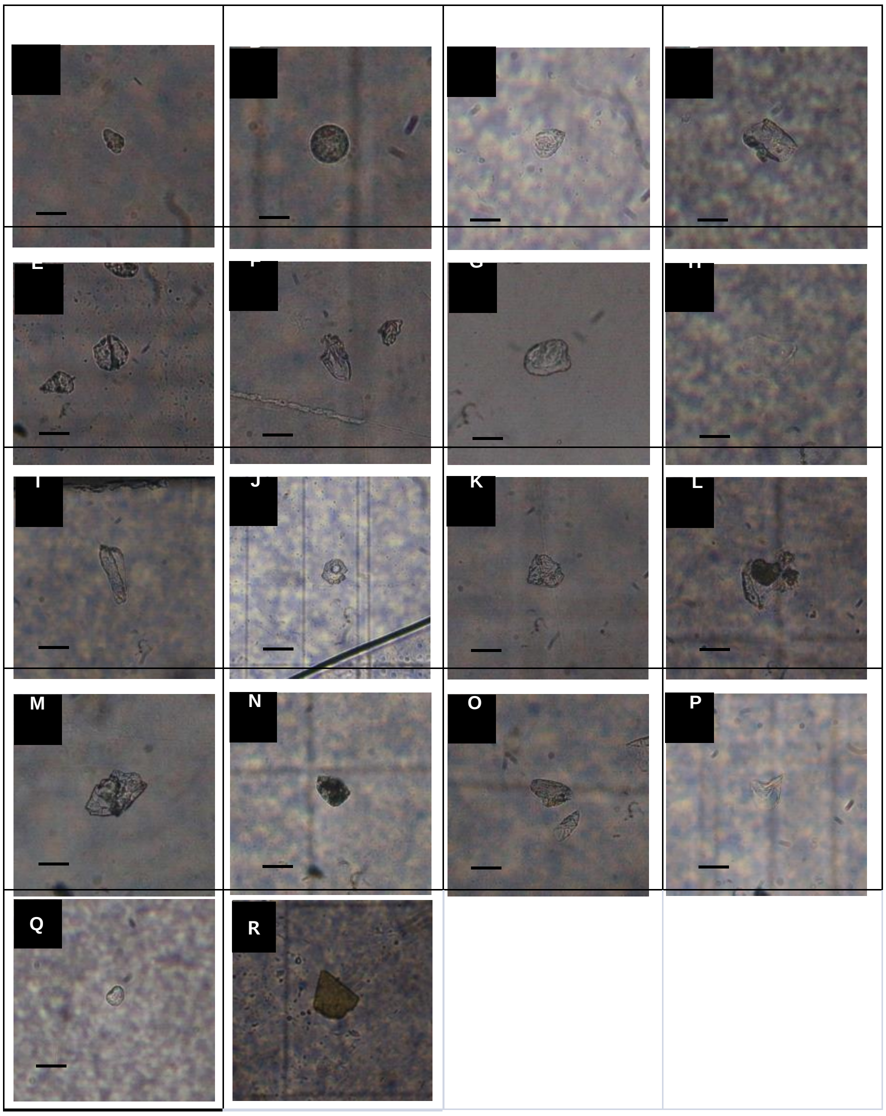

Figure 2. Dinoflagellate cyste collected from Bengawan Solo and Brantas river estuary. Bar scale 10 µ (A) *Alexandrium catenella*; (B) *Alexandrium minutum*; (C) *Brigantedinium sp*.; (D) *Cochlodinium polykrikoides*; (E) *Gymnodinium catenatum*; (F) *Gyrodinium sp;* (G) *Pentapharsodinium tyrrhenicum* ; (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* .

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).

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

No.	River	Biodiversty Index		
		Margalef Index (d)	**Pielou's Evenness (J')**	**Shannon-Wiener Index (H')**
1	Bengawan Solo	2.7193	0.97933	2.1055
2	Brantas	2.4346	0.98888	2.0363

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Analysis of similarity and abundance of dinoflagellate cyst types

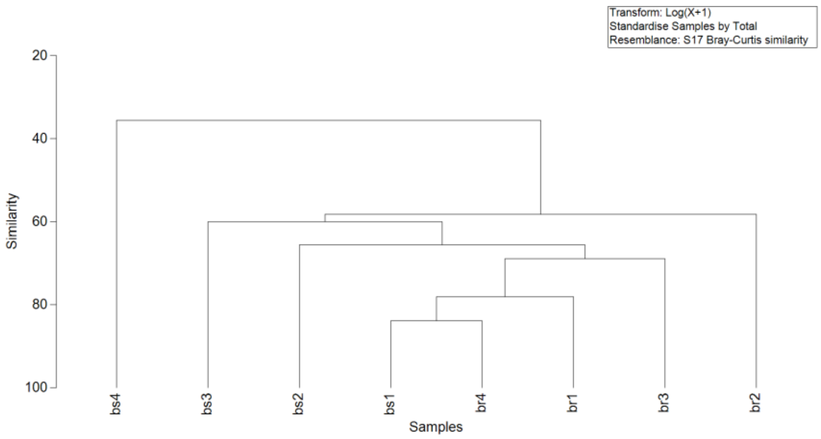

Figure 3. Analysis of similarity from eight sampling locations at the two river mouths of the Bengawan Solo and Brantas rivers in East Java.

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 *Zygabicodinium lenticulatum, Polykrikos kofoidii, Protoperidinium pentagonum, Gymnodinium catenatum*, and *Votadinium* sp.

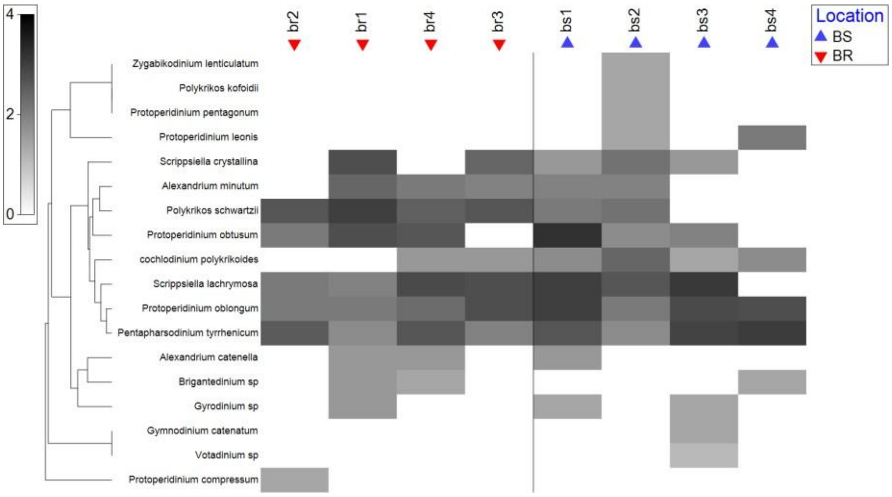

Figure 4. Shade plot of species distribution at each sampling station at the mouth of the Bengawan Solo River (bs) and mouth of the Brantas River (br). The color intensity indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher diversity index than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

Discussion

The diversity of dinoflagella cysts needs to be studied because certain type 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. Losses due to the harmfull 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 vanamae*) 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 vanamae* white shrimp pond areas (Hukom et al. 2020).

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 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

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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. Berkenaan dengan pendekatan molekuler, beberapa penelitian telah berhasil mengidentifikasi berdasarkan rDNA pada SSU, LSU dan gen regio ITS (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).

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The case of the dinoflagellate population explosion in Indonesia has not received serious attention even though a number of 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). Pevious 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).

CONCLUSIONS

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 *Zygabicodinium 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 explosions which are likely to have negative impacts on various sectors of industry.

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

Abstract. 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 unfavorable unfavourable environment and can be preserved well in sediments for long periods of timeperiods. 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 dinoflagellates 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 *Zygabicodinium 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, aquatic, algae bloom, ecosystem ~~dinoflagellate, diversity,~~ estuary, poisonous, toxic.

Running title: Andriyono et al. Diversity of Dinoflagellate Cyst

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 ~~grow rapidly~~overgrow. These incidents are ~~very common~~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) ~~(Hoppenrath et al. 2014, Price and Bhattacharya 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.

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The water conditions of the Bengawan Solo River currently ~~needs~~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 ~~explosions~~ 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 rivers in East Java (https://earth.google.com/).

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Table 1. Sediment sampling locations.

No.	River	Station coordinates
1.	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
2.	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 ~~rate of~~ 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 dr~~y~~ied 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 ~~rate~~ 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) ~~(Alkawri 2016, Mertens et al. 2020).~~

Data analysis

All data ~~is~~ 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).

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RESULTS AND DISCUSSION

Results

Dinoflagellate cyst diversity

The dinoflagellate cysts taxa present varied significantly 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 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).

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

N .	Species name	Average abundance of dinoflagellate cyst (cyst g^{-1} sediment)	
		Bengawan Solo	Brantas
~~1~~A	*Alexandrium catenella*	35	48
~~2~~B	*Alexandrium minutum*	66	93
~~3~~C	*Brigantedinium* sp.	28	42
~~4~~D	*Cochlodinium polykrikoides*	69	48
~~5~~E	*Gymnodinium catenatum*	43	-
~~6~~F	*Gyrodinium* sp.	32	50
~~7~~G	*Pentapharsodinium tyrrhenicum*	150	106
~~8~~H	*Polykrikos kofoidii*	39	-
~~9~~I	*Polykrikos schwartzii*	89	170
~~10~~J	*Protoperidinium compressum*	-	32
~~11~~K	*Protoperidinium leonis*	50	-
~~12~~L	*Protoperidinium oblongum*	251	112
~~13~~M	*Protoperidinium obtusum*	126	139
~~14~~N	*Protoperidinium pentagonum*	41	-
~~15~~O	*Scrippsiella crystallina*	68	147
~~16~~P	*Scrippsiella lachrymosa*	214	130
~~17~~Q	*Votadinium* sp.	27	-
~~18~~R	*Zygabikodinium lenticulatum*	39	-

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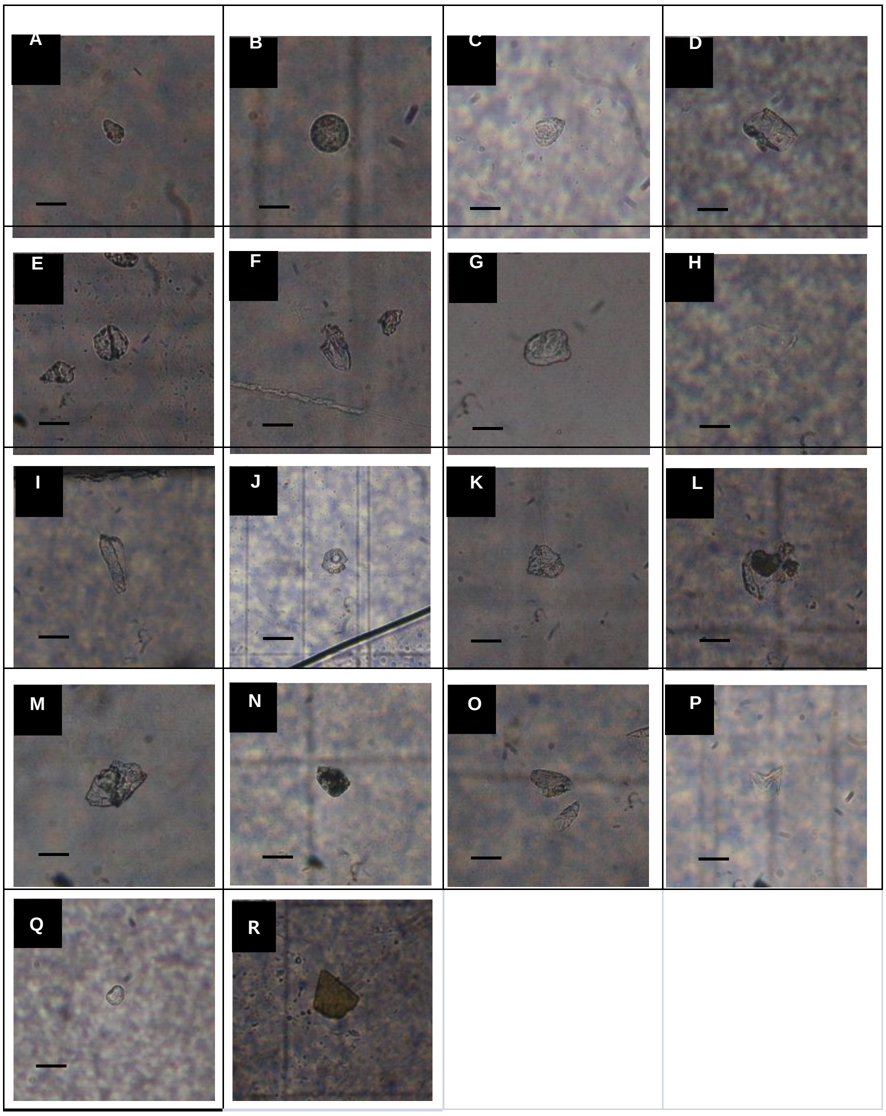

Figure 2. Dinoflagellate cyste collected from Bengawan Solo and Brantas river estuary. Bar scale 10 µ (A) *Alexandrium catenella*; (B) *Alexandrium minutum*; (C) *Brigantedinium sp*.; (D) *Cochlodinium polykrikoides*; (E) *Gymnodinium catenatum*; (F) *Gyrodinium sp;* (G) *Pentapharsodinium tyrrhenicum* ; (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* .

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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

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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).

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

No.	**River**	**Biodiversty Index**		
		Margalef Index (d)	**Pielou's Evenness (J')**	**Shannon-Wiener Index (H')**
1	Bengawan Solo	2.7193	0.97933	2.1055
2	Brantas	2.4346	0.98888	2.0363

Analysis of similarity and abundance of dinoflagellate cyst types

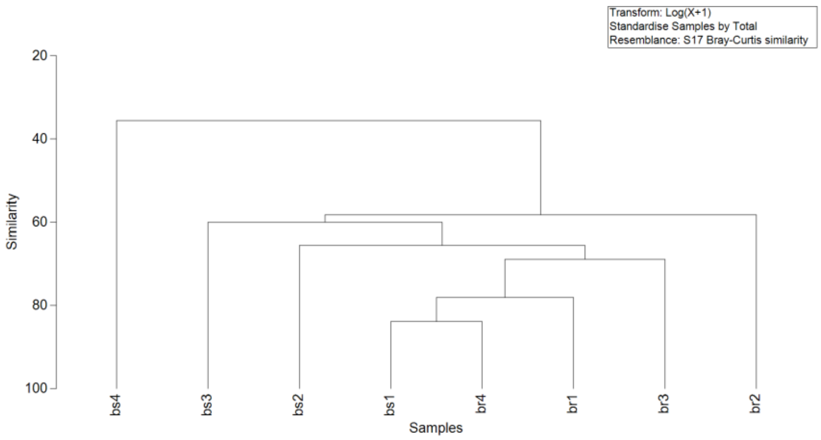

Figure 3. Analysis of similarity from eight sampling locations at the two river mouths of the Bengawan Solo and Brantas rivers in East Java.

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 *Zygabicodinium lenticulatum, Polykrikos kofoidii, Protoperidinium pentagonum, Gymnodinium catenatum*, and *Votadinium* sp.

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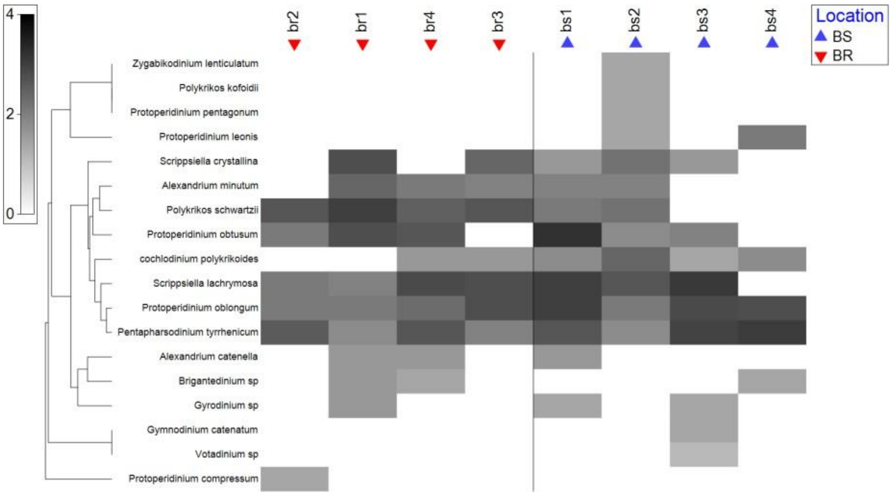

Figure 4. Shade plot of species distribution at each sampling station at the mouth of the Bengawan Solo River (bs) and mouth of the Brantas River (br). The color intensity indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher diversity index than 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.

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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 healt 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.

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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 vanamae*) 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 vanamae* white shrimp pond areas (Hukom et al. 2020).

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

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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. Berkenaan dengan pendekatan molekuler, beberapa penelitian telah berhasil mengidentifikasi berdasarkan rDNA pada SSU, LSU dan gen regio ITS~~. With regard~~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). ~~(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).

The case of the dinoflagellate population explosion in Indonesia has not received serious attention even though ~~a number of~~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). Pevious 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).

CONCLUSIONS

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 *Zygabicodinium 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 ~~explosions~~ bloom which are likely to have negative impacts on various sectors of fisheries industry and aquaculture activities.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude for the initiation of collaborative research. The authors also express our gratitude to Universitas Airlangga which provided partial funding for this research based on Research Grant No. 439/UN3.1.12/ PT/2022 on DNA Barcoding for fish from inland and marine water ecosystems. The authors have no conflicts of interest to declare.

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