

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

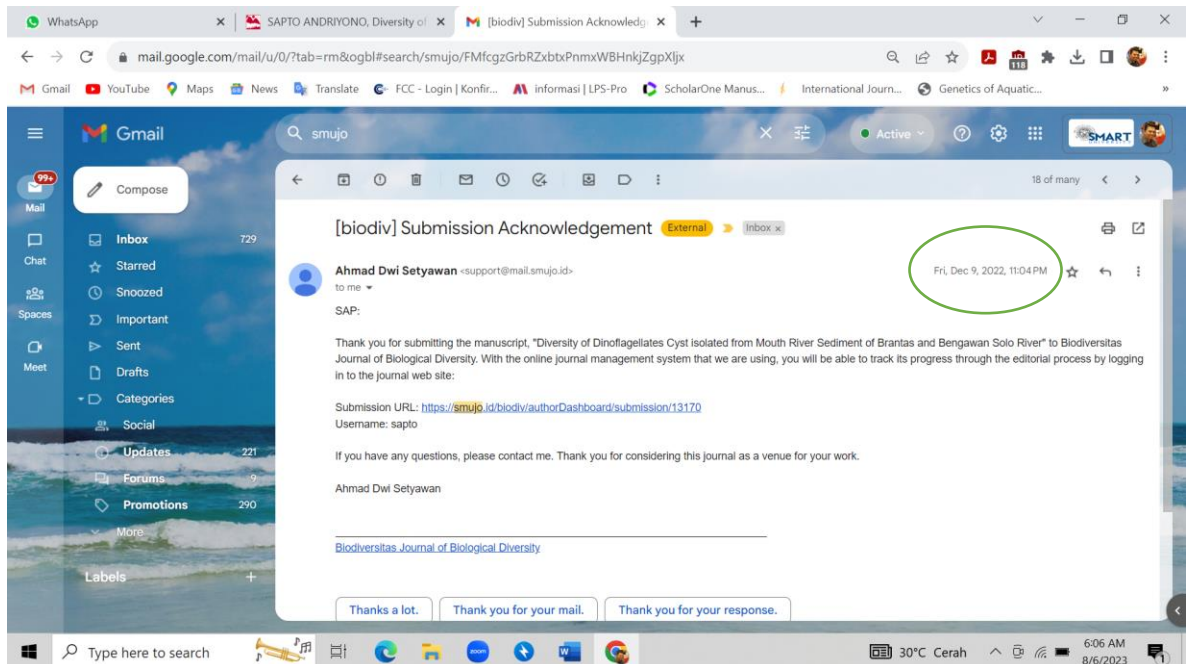
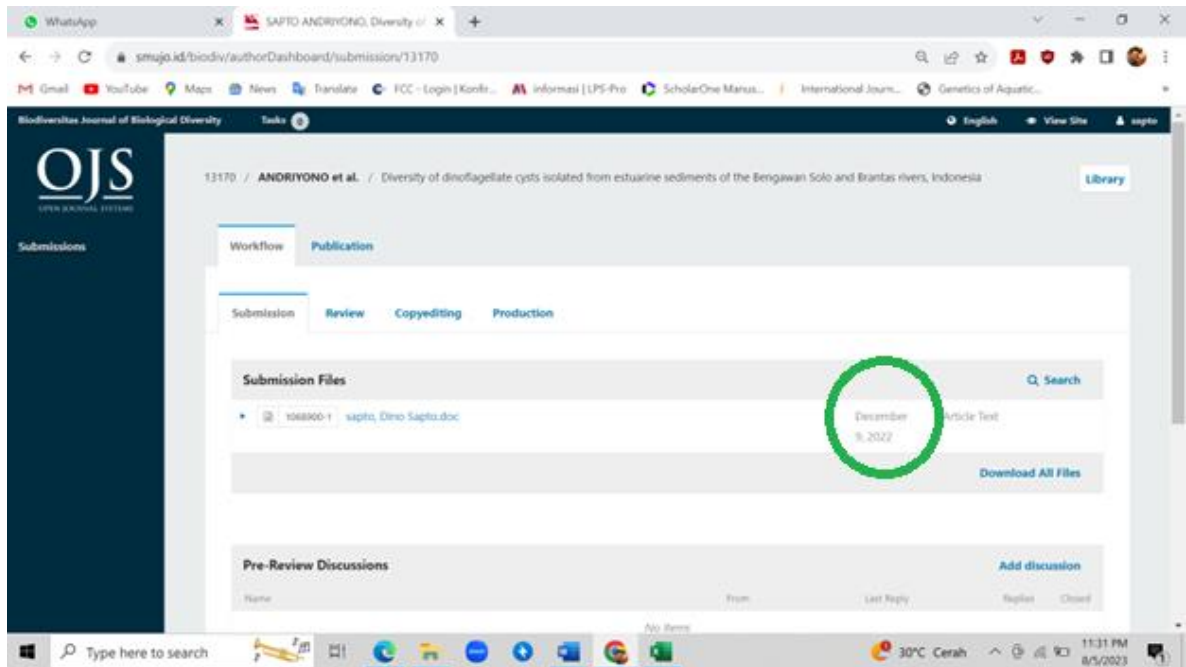
Judul : **Diversity Of Dinoflagellate Cysts Isolated From Estuarine Sediments Of Bengawan Solo And Brantas Rivers, Indonesia**

Jurnal : **BIODIVERSITAS**

Penulis : **SAPTO ANDRIYONO, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOB Aidul 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 JUNAI DI ZAKARIA, MD. JOB AIDUL 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 This manuscript has outdated references to be published in the Biodiversitas Journal.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

The screenshot shows a Gmail interface with a notification for an email from Anisa Septiasari. The email subject is "[biodiv] Editor Decision" and it is marked as "External" and "Inbox x". The email content is identical to the notification shown in the previous block, detailing the decision to require revisions for a manuscript submitted to Biodiversitas Journal. The email is dated Monday, Dec 12, 2022, at 11:26 AM. The Gmail interface includes a search bar with "smujo" entered, a left sidebar with navigation options like Compose, Mail, Chat, Spaces, Meet, and Categories, and a bottom taskbar with system icons and the date 8/6/2023.

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, INDRANA JUNAI DI 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 where 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

The screenshot shows a Gmail interface on a Windows desktop. The browser address bar displays the email URL: `mail.google.com/mail/u/0/?tab=rm&ogbl#search/smujo/FMfcgzGrcFnFHwmcNcPIHnVbTzvRpTg`. The email subject is "[biodiv] Editor Decision" and is marked as "External" and "Inbox". The sender is "Smujo Editors <support@mail.smujo.id>". The email content is identical to the notification above, dated "Tue, Jan 24, 10:51 AM". The Windows taskbar at the bottom shows the system tray with the date "8/6/2023" and time "6:46 AM".

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 JUNADI 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 consequences. 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

The screenshot shows a Gmail interface with the email content displayed in a preview window. The email is from 'Smujo Editors via SMUJO' and contains the same text as the notification above, including the subject '[biodiv] Editor Decision', the recipient list, the decision 'Revisions Required', and the reviewer's feedback points.

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 2023-02-11 11:47 PM
dewinurpratiwi, Dinoflagellate cysts isolated - Andriyono.doc	
Dear Editor of Biodiversitas Journal Along with this email, we send the corrected file. Some revisions in number and affiliation. Thank you Sapto	sapto 2023-02-12 12:53 PM
sapto, 13170-Article Text-1074642-1-18-20230211 (corrected1202).doc	

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6. Accepted tanggal 18 Februari 2023

Notifications 

[biodiv] Editor Decision

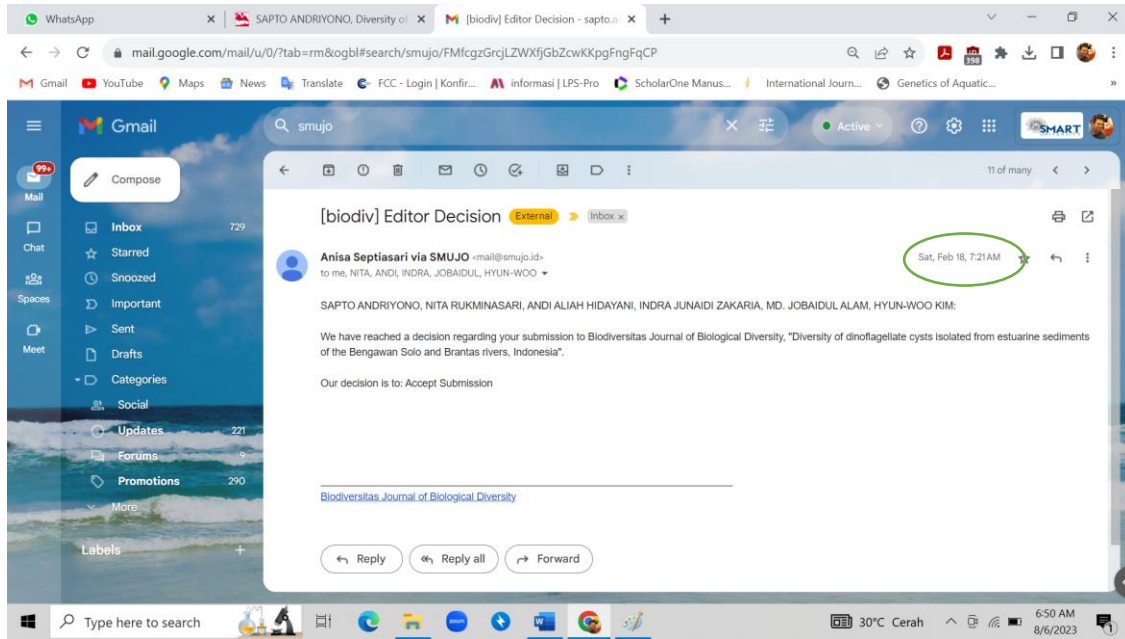
2023-02-18 12:21 AM

SAPTO ANDRIYONO, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAI DI ZAKARIA, MD. JOB AIDUL 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 terpublised 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 JUNAIIDI ZAKARIA, MD. JOB Aidul 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: spto.andriyono@fpk.unair.ac.id

For possibility publication on the journal:

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| <input checked="" type="checkbox"/> Biodiversitas Journal of Biological Diversity | <input type="checkbox"/> Nusantara Bioscience |
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| <input type="checkbox"/> Indo Pacific Journal of Ocean Life | <input type="checkbox"/> 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 manuscript** 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 ANDRIYONO¹✉, NITA RUKMINASARI³, ANDI ALIAH HIDAYANI³, INDRA JUNAIDI ZAKARIA⁴, MD. JOB Aidul Alam⁴, HYUN-WOO KIM⁵

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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 *Zygabacodinium 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 (Sudarmiyati 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 30°C (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

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

52 **MATERIALS AND METHODS**

53 **Samples collection**

54 Sediment at the bottom of the river mouth was collected with an Ekman Grab size 152x152x230 mm and put in plastic
 55 which was labeled according to the location of the sample. At each location of the river estuary, sampling was carried out
 56 at four locations with each location being repeated three times. The coordinate points for sampling (Figure 1) at the mouth
 57 of the Brantas River and the mouth of the Bengawan Solo River are presented in Table 1.



58

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

60
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Table 1. Coordinate point of sediment sampling

No.	River location	Coordinate 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
		07°16'37" S 112°51'35" E
		07°18'19" S 112°51'34" E
		07°21'03" S 112°51'09" E

62

63 **Sediment Separation With Dinoflagellate Cysts**

64 The separation between sediment and cysts uses the method (Matsuoka and Fukuyo, 2000) where the sample is
 65 weighed using an analytical balance of 4–5 grams and then transferred to a 50 ml beaker glass container then added sterile
 66 sea water until it reaches a size of 50 ml, then the sonication process is carried out for 15 minutes using an ultrasonic
 67 sonicator to remove cysts attached to sediment particles. Furthermore, the sieving process is carried out using a filter
 68 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
 69 and 20 µm. The sample that has been filtered is then precipitated using a petri dish, then filtered again using a sieve with a
 70 mesh size of 20 µm. The filtered cyst was then put into a 10 ml sample bottle for morphological analysis.

71

72 **Determination of the rate of water content**

73 Determination of the rate of water content is carried out to determine the water content contained in the sediment
 74 sample. Wet sediment samples were first weighed 4-5 grams using an analytical balance which was then put into the oven
 75 with a temperature of 150°C for 1 hour. After that the dry sediment samples were weighed again. Previous research
 76 (Matsuoka and Fukuyo 2000) used the formulation and the calculation stage used the following formulation:

77
$$R = \frac{Bb - Bk}{Bb} \times 100\%$$

78 Information:
 79 R = Rate of water content (%);
 80 Bb = wet weight (g);
 81 Bk = dry weight (g).
 82

83 Dinoflagellate Cyst Abundance Calculation

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

$$87 \text{ Cyst per gram} \frac{N}{W(1 - R)}$$

88 Information :
 89 N = Number of cysts observed (ind g-1);
 90 W = Weight of wet sediment (g);
 91 R = Rate of water content (%).
 92

93 Identification of Dinoflagellate Cyst Morphology

94 Observation of dinoflagellate cysts was carried out by taking a sample to be observed using a dropper pipette of 1 ml,
 95 then transferred to a hemacytometer container, then observed under a light microscope. Each dinoflagellate cyst sample
 96 was identified to the genus and species level based on the published description of the dinoflagellate cyst by the researcher.
 97 (Alkawri 2016, Mertens et al. 2020)
 98

99 Data Analysis

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

103 RESULTS AND DISCUSSION

104 Results

105 Identification of Dinoflagellate Cyst Morphology

106 Identification of the morphology of the dinoflagellate cysts varied quite a lot in the estuaries of the Berantas and
 107 Bengawan Solo rivers (Table 1). The Bengawan Solo estuary has a higher diversity of four species of dinoflagella cysts
 108 compared to the Brantas river estuary which only has 12 species. The type of *Protoperidinium obtusum* was found to
 109 dominate in the mouth of the Bengawan Solo River, while the type of *Scrippsiella lachrymose* dominated in the mouth of
 110 the Brantas river.
 111

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

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

14	<i>Protooperidinium pentagonum</i>	-	41
15	<i>Scrippsiella crystallina</i>	147	68
16	<i>Scrippsiella lachrymosa</i>	130	214
17	<i>Votadinium</i> sp.	-	27
18	<i>Zygabikodinium lenticulatum</i>	-	39

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116 Biodiversity Analysis of Dinoflagellate Cysts

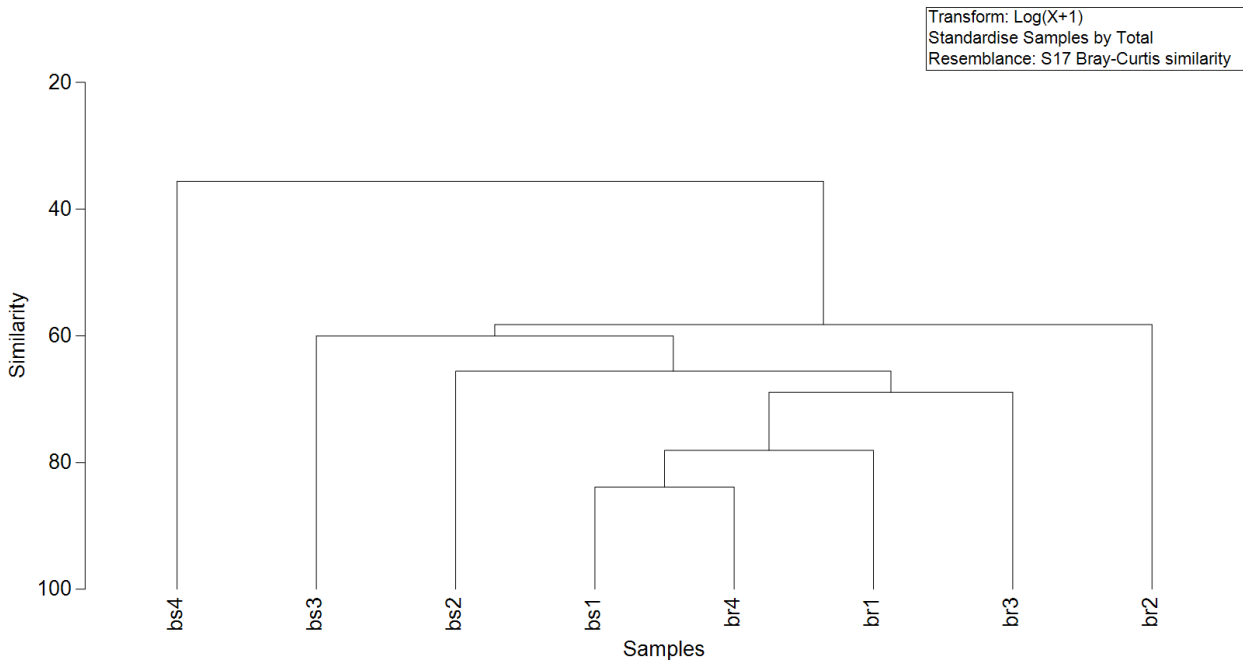
117 Based on the data on the abundance of dinoflagellate cysts obtained, a biodiversity analysis was carried out to
118 determine the dominating species and distribution patterns of cyst species in river estuaries in East Java. Each sampling
119 location was repeated 4 times to reduce the resulting data bias. Biodiversity analysis includes Margalef Index (d), Pielou's
120 Evenness (J'), and Shannon-Wiener Index (H'). Referring to the Shannon-Wiener Index (H') value, it shows that the mouth
121 of the Bengawan Solo river (2.1055) has higher diversity than the mouth of the Brantas river (2.0363). This report is the
122 first study on the biodiversity of dinoflagellate cysts in the estuaries of major rivers in East Java.
123

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

No.	Lokasi	Biodiversity 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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128 Analysis of Similarity and Abundance of Dinoflagellate Cyst Types



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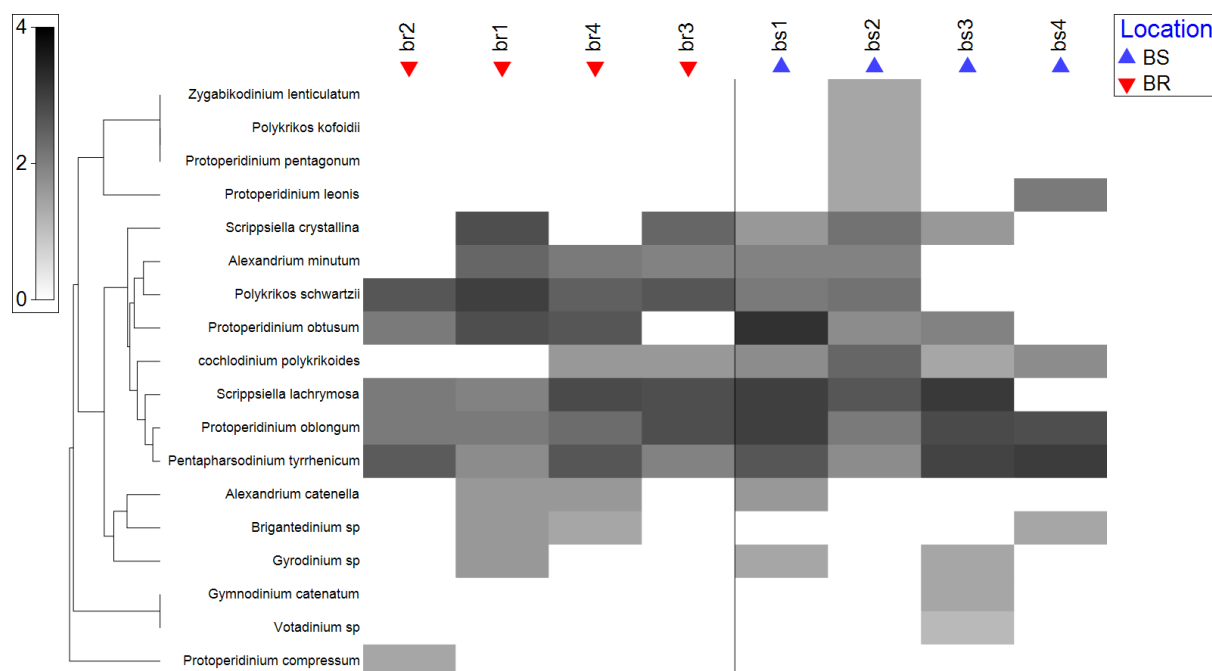
Figure 2. Analysis of similarity from 8 sampling locations in two different river mouths

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132 The similarity analysis was carried out by comparing data on the abundance of dinoflagellate cysts found in both
133 regions (Figure 2). The data was processed using Primer-e software and at the same time analyzing species abundance at
134 each sediment collection location. Only the 4 sampling locations at the mouth of the Bengawan Solo River had the highest
135 abundance and were different from other locations. Meanwhile, the sampling locations at the mouth of the Brantas river
136 were in one clade which had a fairly high similarity between the four replicates. An abundance analysis (Figure 3) was
137 carried out to find out certain species that have the potential to cause harmful algae blooms (HAB) which are feared to be
138 detrimental to fishing activities and even endanger public health. The results of the Shadegraph showed that the type of
139 dinoflagellate cyst was identified which was only found in the mouth of the Brantas river, namely the type of
140 *Protooperidinium compressum*. Meanwhile, at the mouth of the Bengawan Solo River, there are several species that are not

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found at the mouth of the Brantas river, namely *Zygabikodinium lenticulatum*, *Polykrikos kofoidii*, *Protoperidinium pentagonum*, *Gymnodinium catenatum*, and *Votadinium* sp.



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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

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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 harmful 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.

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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).

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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

181 more toxins than the vegetative forms, seriously affecting the species through its food web and even human health
182 (Kirkpatrick et al. 2004). Meanwhile for aquatic ecology, the impacts of algae blooms include beach pollution, lack of
183 oxygen, and the death of marine species on a large scale (Bates and Trainer 2006, Park et al. 2013). The *Alexandrium*
184 *tamarensis* has been identified released of toxin (Oshima et al. 1992), and in this study we found two species in the genus
185 *Alexandrium* (*A. catenella* dan *A. minutum*). Therefore, accurate identification of dinoflagellates is an important first step
186 in their early detection and function in ecological studies. Because dinoflagellates have limited or even non-existent
187 taxonomic keys, molecular methods to identify dinoflagellates have become a priority as a continuation of this research. In
188 the current study, morphological identification was carried out by taking into account a number of general characteristics,
189 such as the shape of the resulting cyst and its ornamentation, the structure and color of the walls, and the type of opening
190 or archeopyle (Matsuoka and Fukuyo 2000).

191 The case of the dinoflagellate population explosion in Indonesia has not received serious attention even though a
192 number of studies on dinoflagellate species have been identified in a number of areas. The main cause of the algae
193 population explosion is nutrients in the waters which are abundant and support algae growth in a short time (Piranti et al.
194 2021). Algae bloom events can occur in fresh water and marine waters. Cases that have been reported in a number of
195 publications are the waters of the Jakarta bay which caused mass fish deaths (Kurniawan 2008, Wouthuyzen et al. 2007).
196 Although in East Java there have been no reports of algae blooms, it is hoped that research on the potential for HABs in
197 East Java waters can be mitigated from the start so that losses and impacts are expected to be minimized.

198 Biodiversity studies of dinoflagellate cysts in Indonesia are still very limited. Monitoring activities for the type
199 *Pyrodinium bahamense* have been carried out to anticipate the presence of algae blooms in Indonesia including, Lampung
200 Bay, Jakarta Bay, Cirebon coastal waters and Ambon Bay (Rachman et al. 2021). Studies on the diversity index of
201 dinoflagellate cysts have been reported in Muara Jeneberang and Paotere Harbor with values ranging from 0.82-1.01
202 (Rukminasari and Tahir 2021). This diversity value is still relatively low when compared to the biodiversity of dinoflagella
203 cysts in East Java with a Shannon-Wiener index ranging from 2.0363-2.1055. Another report in South Sulawesi, Maros
204 estuary and Pangkep river estuary, which are included in the moderate category for the potential presence of HABs in this
205 area (Rukminasari and Tahir 2021). Based on previous research, the abundance of dinoflagellate cysts can be an indication
206 of the potential for HABs in particular region. There are four potential groups, namely very low, low, medium, high and
207 very high levels has been classified (McMinn 1991, Tian et al. 2018). Previous study show that abundance of dinoflagellate
208 cysts in Lampung for the *P. bahamense* cyste is 1-100 cyste g⁻¹ sediment (Rachman et al. 2021), while previous research
209 found *M. polykrikoides* type cysts measured with abundances ranging from 100-1000 cyste g⁻¹ sediment (Thoha et al.
210 2019). The abundance of *P. bahamense* in Cirebon waters was a serious case because it caused human death (Nurlina
211 2018), with a relatively high abundance of 100-1000 kiste g⁻¹ sediment (Rachman et al. 2019). At the estuary of the
212 Bengawan Solo River, the highest abundance was *Protoperidinium oblongum* (115 g⁻¹ sediment kiste) and the lowest
213 abundance was *Protoperidinium leonis* (21 g⁻¹ sediment). Meanwhile, *Alexandrium minutum* was found with the highest
214 abundance (125 kiste g⁻¹ sediment) in the mouth of the Brantas river, with an average of 73 g⁻¹ sediment cysts found. Thus,
215 the condition of the mouth of the Brantas river and the mouth of the Bengawan Solo river still has a low potential for the
216 occurrence of HABs and an average abundance of 73 and 62 g⁻¹ kiste respectively (McMinn 1991, Tian et al. 2018).

217 CONCLUSIONS

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219 Research on dinoflagellate cysts revealed the diversity of dinoflagellates at the mouth of the Bengawan Solo River and
220 the mouth of the Brantas river. Analysis of cyst diversity in the two regions shows that the Bengawan Solo River mouth
221 has a higher Shannon-Wiener index compared to the Brantas river mouth. The type of dinoflagella cyst that dominates
222 at the mouth of the Bengawan Solo River is *Protoperidinium obtusum*, while at the mouth of the Brantas river it is
223 *Polykrikos schwartzii*. Shade graph analysis showed a number of species found only in the estuary of the Bengawan Solo
224 River, namely *Zygabocodinium lenticulatum*, *Polykrikos kofoidii*, *Protoperidinium pentagonum*, *Gymnodinium catenatum*,
225 and *Votadinium* sp. Based on the results of this study, it is necessary to carry out DNA characteristic tests of isolated and
226 cultured species to document genetic information. In addition, this information is very important in the management of
227 coastal areas around the mouths of the Bengawan Solo and Brantas rivers to prevent dinogellate population explosions
228 which may have negative impacts on various sectors.

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SAPTO ANDRIYONO

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

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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 *Zygabacodinium 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

48 biodiversity of dinoflagella cysts in the estuaries of the Bengawan Solo and Brantas Rivers in East Java was conducted
49 using a morphological identification approach.

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51 The condition of the waters of the Bengawan Solo currently needs serious attention. The river flow originating from
52 Central Java and empties into East Java carries a number of materials including nutrients which can cause water
53 enrichment. The case of enrichment in the waters of the Bengawan Solo River has been carried out by previous studies.
54 Enrichment at the mouth of the river plays an important role in the dynamics of plankton populations in water areas
55 because phytoplankton will respond very quickly to changes in the concentration of the dissolved nutrients in the waters. It
56 is reported that nutrient enrichment events can reduce the value of biodiversity and the productivity of an ecosystem (Isbell
57 et al. 2013). This condition is human intervention which is the main cause of the activities that have been carried out both
58 on land and in aquatic (anthropogenic) ecosystems. This condition also has an impact on important ecosystems in the
59 coastal area, namely coral reef ecosystems (D'Angelo and Wiedenmann 2014).

60
61 Meanwhile, the estuary of the Brantas river is also a major river in Java that crosses the provinces of Central Java to
62 East Java. Conditions similar to those at the estuary of the Bengawan Solo River, the estuary waters of the Brantas river
63 also receive a large amount of organic matter from human activities. Nutrient enrichment from the Brantas river has the
64 potential to cause enrichment in the Madura Strait waters (Jänen et al. 2013). Increasing the proportion of nitrogen and
65 phosphorus in the water will change so that the highest concentrations can be accumulated in the estuary or coastal areas
66 compared to river areas (Jennerjahn and Klöpper 2013). With the dynamics of the potential for enrichment and the variety
67 of nutrient sources that enter the waters of the Bengawan Solo and Brantas rivers, research on the abundance and
68 identification of dinoflagellate species is very important. This is expected to be important data in the management of water
69 areas that minimize the occurrence of dangerous algae population explosions or Harmfull Algae Blooms (HABs).

70 MATERIALS AND METHODS

71 Samples collection

72 Sediment at the bottom of the river mouth was collected with an Ekman Grab size 152x152x230 mm and put in plastic
73 which was labeled according to the location of the sample. At each location of the river estuary, sampling was carried out
74 at four locations with each location being repeated three times. The coordinate points for sampling (Figure 1) at the mouth
75 of the Brantas River and the mouth of the Bengawan Solo River are presented in Table 1.



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77 **Figure 1.** Sampling locations in two river mouths in East Java (<https://earth.google.com/>)
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Table 1. Coordinate point of sediment sampling

No.	River location	Coordinate 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 07°16'37" S 112°51'35" E 07°18'19" S 112°51'34" E 07°21'03" S 112°51'09" E

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89 Sediment Separation With Dinoflagellate Cysts

90 The separation between sediment and cysts uses the method (Matsuoka and Fukuyo, 2000) where the sample is
91 weighed using an analytical balance of 4–5 grams and then transferred to a 50 ml beaker glass container then added sterile
92 sea water until it reaches a size of 50 ml, then the sonication process is carried out for 15 minutes using an ultrasonic
93 sonicator to remove cysts attached to sediment particles. Furthermore, the sieving process is carried out using a filter
94 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
95 and 20 µm. The sample that has been filtered is then precipitated using a petri dish, then filtered again using a sieve with a
96 mesh size of 20 µm. The filtered cyst was then put into a 10 ml sample bottle for morphological analysis.

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98 Determination of the rate of water content

99 Determination of the rate of water content is carried out to determine the water content contained in the sediment
100 sample. Wet sediment samples were first weighed 4-5 grams using an analytical balance which was then put into the oven
101 with a temperature of 150°C for 1 hour. After that the dry sediment samples were weighed again. Previous research
102 (Matsuoka and Fukuyo 2000) used the formulation and the calculation stage used the following formulation:

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

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Information:

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R = Rate of water content (%);

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Bb = wet weight (g);

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Bk = dry weight (g).

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Dinoflagellate Cyst Abundance Calculation

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

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

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Information :

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

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W = Weight of wet sediment (g);

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R = Rate of water content (%).

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Identification of Dinoflagellate Cyst Morphology

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

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Data Analysis

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

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RESULTS AND DISCUSSION

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Results

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Identification of Dinoflagellate Cyst Morphology

132 Identification of the morphology of the dinoflagellate cysts varied quite a lot in the estuaries of the Berantas and
 133 Bengawan Solo rivers (Table 1). The Bengawan Solo estuary has a higher diversity of four species of dinoflagella cysts
 134 compared to the Brantas river estuary which only has 12 species. The type of *Protooperidinium obtusum* was found to
 135 dominate in the mouth of the Bengawan Solo River, while the type of *Scrippsiella lachrymose* dominated in the mouth of
 136 the Brantas river.

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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 river

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

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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	Biodiversity 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

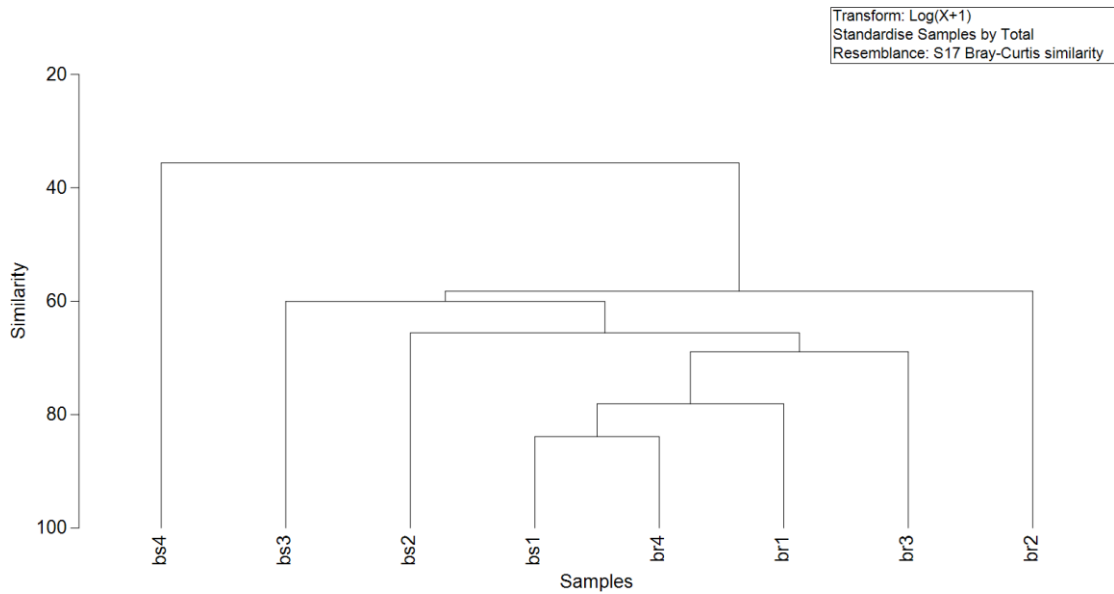
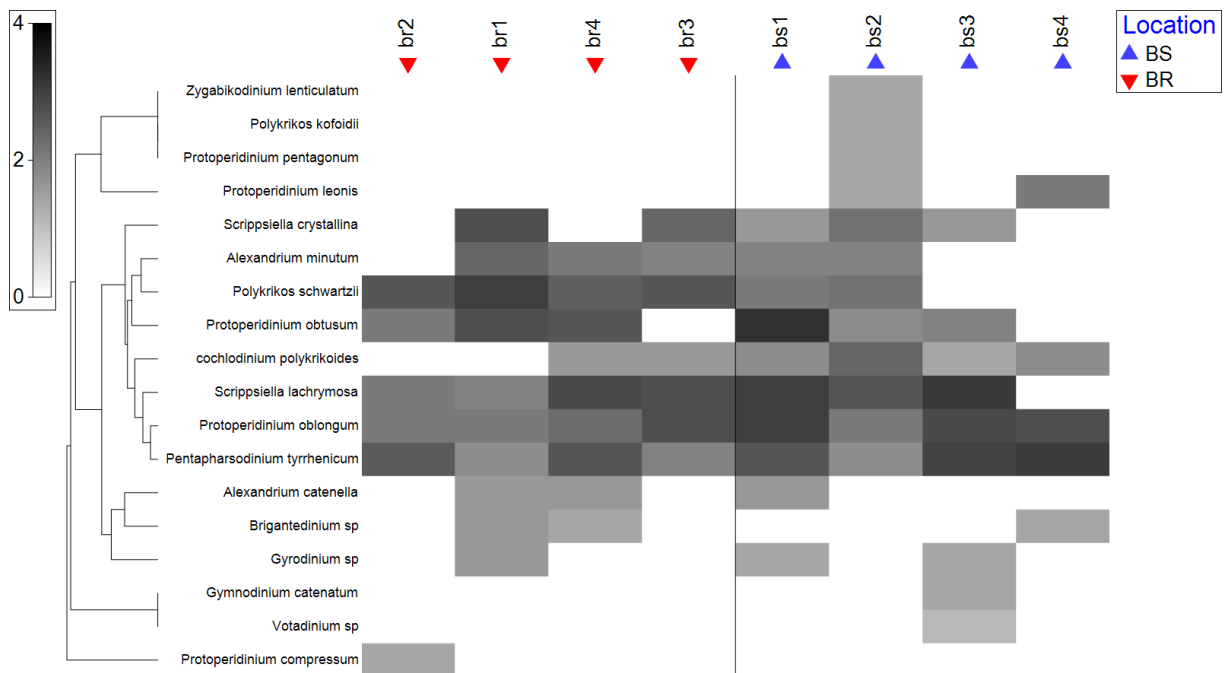


Figure 2. Analysis of similarity from 8 sampling locations in two different river mouths

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



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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

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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 harmful 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 *Pentaparsodinium 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 algae blooms in Indonesia including, Lampung Bay, Jakarta Bay, Cirebon coastal waters and Ambon Bay (Rachman et al. 2021). Studies on the diversity index of dinoflagellate 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). Previous 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

238 2018), with a relatively high abundance of 100-1000 kiste g⁻¹ sediment (Rachman et al. 2019). At the estuary of the
239 Bengawan Solo River, the highest abundance was *Protoperidinium oblongum* (115 g⁻¹ sediment kiste) and the lowest
240 abundance was *Protoperidinium leonis* (21 g⁻¹ sediment). Meanwhile, *Alexandrium minutum* was found with the highest
241 abundance (125 kiste g⁻¹ sediment) in the mouth of the Brantas river, with an average of 73 g⁻¹ sediment cysts found. Thus,
242 the condition of the mouth of the Brantas river and the mouth of the Bengawan Solo river still has a low potential for the
243 occurrence of HABs and an average abundance of 73 and 62 g⁻¹ kiste respectively (McMinn 1991, Tian et al. 2018).

244 CONCLUSIONS

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246 Research on dinoflagellate cysts revealed the diversity of dinoflagellates at the mouth of the Bengawan Solo River and
247 the mouth of the Brantas river. Analysis of cyst diversity in the two regions shows that the Bengawan Solo River mouth
248 has a higher Shannon-Wiener index compared to the Brantas river mouth. The type of dinoflagella cyst that dominates at
249 the mouth of the Bengawan Solo River is *Protoperidinium obtusum*, while at the mouth of the Brantas river it is
250 Polykrikos schwartzii. Shade graph analysis showed a number of species found only in the estuary of the Bengawan Solo
251 River, namely *Zygabocodinium lenticulatum*, *Polykrikos kofoidii*, *Protoperidinium pentagonum*, *Gymnodinium catenatum*,
252 and *Votadinium* sp. Based on the results of this study, it is necessary to carry out DNA characteristic tests of isolated and
253 cultured species to document genetic information. In addition, this information is very important in the management of
254 coastal areas around the mouths of the Bengawan Solo and Brantas rivers to prevent dinogellate population explosions
255 which may have negative impacts on various sectors.

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261

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SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:

Give mark (X)

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

All necessary files have been uploaded, and contain:

• Keywords	v
• Running titles	
• All figure captions	v
• All tables (incl title and note/description)	v

Further considerations

• 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
• All references mentioned in the Reference list are cited in the text, and vice versa	v
• Colored figures are only used if the information in the text may be losing without those images	v
• Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate	v

375

1 Diversity of ~~d~~Dinoflagellates ~~c~~Cysts isolated from
 2 ~~estuarineMouth River sSediments of the Brantas-Bengawan~~
 3 ~~Solo and Brantas Bengawan Solo rRivers~~

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14 **Abstract.** Dinoflagellates are a major part of the phytoplankton and are commonly found in freshwater, brackish,
 15 and marine habitats and are major components ~~whichand~~ play an important role in marine ecosystems. Dinoflagellate
 16 cysts (dinocysts) are produced by dinoflagellates in an unfavorable environment, and can be ~~stored-preserved~~ well in
 17 ~~organic-sedimentsmatter~~ for a long ~~periods of~~ time. More than ~~200~~ dinoflagellate species have so far been observed to
 18 produce resting cysts and to be associated with the maintenance, discontinuation, and repetition of annual blooms. This
 19 study identified _____ based on the morphological characteristics of dinoflagellate cysts from the estuar~~iesy~~ of the ~~Brantas~~
 20 ~~Bengawan Solo River and the estuary of Bengawan Solo rivers-Brantas river~~. Analysis of cyst diversity in the two regions
 21 shows that the Bengawan Solo River mouth has a higher Shannon-Wiener index compared to the Brantas river mouth.
 22 The type of dinoflagellate cyst that dominates at the mouth of the Bengawan Solo River is Protoperidinium obtusum,
 23 while ~~that~~ at the mouth of the Brantas ~~R~~river ~~it-is~~ Polykrikos schwartzii. Shade graph analysis show~~sed~~ a number of
 24 species ~~to be~~ found only in the estuary of the Bengawan Solo River, namely Zygaibicodinium lenticulatum, Polykrikos
 25 kofoidii, Protoperidinium pentagonum, Gymnodinium catenatum, and Votadinium sp. Based on the results of this study,
 26 it is necessary to carry out DNA characteristic tests of isolated and cultured species to document genetic information. In
 27 addition, this information is very important in the management of coastal areas around the estuaries of the Bengawan
 28 Solo and Brantas rivers to prevent dinoflagellate population explosions that may have negative impacts on various
 29 sectors.

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30 **Keywords:** algae, dinoflagellate, diversity, estuary, toxic

31 **Running title:** Andriyono et al. Diversity of Dinoflagellate Cyst

33 Changes in the phytoplankton community ~~in waters~~ can occur when a number of harmful microalgae
 34 species grow and dominate the water column, ~~and proceed to then~~ cause environmental ~~problems~~ impacts.
 35 ~~Such an~~ This event is known as ~~a~~ Harmful Algal Bloom (HAB). Following the concept of the Intergovernmental
 36 Oceanographic Commission (IOC-UNESCO), ~~states that~~ HABs ~~are~~ defined as a phenomenon of harmful
 37 algae growth in water which causes environmental problems (Anderson et al. 2021), losses to the fisheries
 38 sector, ~~and even causes~~ illness to humans (Trevino-Garrison et al. 2015). The harm caused by HAB events
 39 spans both spatial and temporal scales. Agricultural-scale losses include mass mortality of farmed fish,
 40 poisoning of marine food sources, itching of beachgoers, and others. HAB events are generally due to
 41 nutrient enrichment, high temperatures, low oxygen levels, and reduced light intensity ~~as to enable so that~~
 42 dinoflagellates ~~to can~~ grow rapidly. ~~The~~ ~~ise~~ incidents ~~are~~ very common in ~~the~~ estuary areas ~~off the rivers~~
 43 (Ajani et al. 2013). Most of the algae that cause HABs belong to the dinoflagellate group (Kudela and Gobler
 44 2012).

46 Dinoflagellates are included in the microalgae group found in ~~the~~ sea and river waters (Senanayake et al.
 47 2021). Dinoflagellates play an important role in ~~these~~ waters, namely as primary producers (Hoppenrath et
 48 al. 2014, Price and Bhattacharya 2017). As microalgae, dinoflagellates have a unique ability to ~~specialize and~~
 49 survive in the competition of natural selection. Under ~~good water~~ productive conditions, dinoflagellates can
 50 reproduce ~~rapidly~~ ~~massively~~ in the water column in the form of actively dividing swimming cells. When
 51 environmental conditions are ~~suboptimal, bad~~ ~~marked by~~ nutrient enrichment (Faizal et al. 2012), warm
 52 temperatures (Coffey et al. 2019), low oxygen levels, ~~and reduced light intensity~~, they stop dividing and mate
 53 to form cysts which hibernate and are able to survive in sediments for many years (Head et al. 2013). The
 54 ~~hypno~~zygote is also produced by dinoflagellates ~~by mating through their~~ fusion of gametes produced by
 55 vegetative dinoflagellate cells, securely protected within the thick walls of the cyst and highly resistant to
 56 adverse conditions (Bravo and Figueroa 2014). In this regard, a study of the ~~biodiversity of dinoflagellate~~
 57 cysts in the estuaries of the Bengawan Solo and Brantas ~~r~~Rivers in East Java was conducted using a
 58 morphological identification approach.

60 The ~~water~~ conditions ~~of the waters~~ of the Bengawan Solo ~~River~~ currently needs serious attention. The
 61 river flow originating from Central Java ~~and emptying~~ into East Java carries a number of materials including
 62 nutrients which can cause water enrichment. The case of enrichment in the waters of the Bengawan Solo
 63 River has been carried out by previous studies. Enrichment at the mouth of the river plays an important role
 64 in the dynamics of plankton populations in water areas because phytoplankton will respond very quickly to
 65 changes in the concentration of the dissolved nutrients ~~in the waters~~. It is reported that nutrient enrichment
 66 events can reduce the value of biodiversity and the productivity of an ecosystem (Isbell et al. 2013). The
 67 ~~principal contributing factor is condition~~ is human intervention ~~due to which is the main cause of the~~ activities
 68 that have been carried out both on land and in aquatic (anthropogenic) ecosystems. This condition also has
 69 an impact on important ecosystems in the coastal area, namely coral reef ecosystems (D'Angelo and
 70 Wiedenmann 2014).

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72 Meanwhile, the estuary of the Brantas river is also a major river-region in Java that crosses the provinces
 73 of Central Java to East Java. Conditions here are similar to those at the estuary of the Bengawan Solo River.
 74 The estuary waters of the Brantas river also receive a large amount of organic matter from human activities.
 75 Nutrient influxenrichment from the Brantas River has the potential to cause enrichment in the Madura Strait
 76 waters (Jänen et al. 2013). Increasing the proportion of nitrogen and phosphorus in the water will-results in
 77 a gradational change in -marine conditions suche that the highest concentrations of nutrients arecan be
 78 accumulated in the estuary or coastal areas compared to river areas (Jennerjahn and Klöpper 2013). With the
 79 dynamics of the potential for enrichment and the variety of nutrient sources that enter the waters of the
 80 Bengawan Solo and Brantas rivers, research on the abundance and identification of dinoflagellate species is
 81 very important. Investigations of these key phytoplankton constituents This areis expected to yield valuablebe
 82 important data towardin the management of water areas that minimizes the occurrence of dangerous algae
 83 population explosions or Harmfull-Algae-Blooms-(HABs).

84

MATERIALS AND METHODS

85 Samples collection

86 Sediment at the bottom of either river mouth was collected with an Ekman Grab size 152x152x230 mm
 87 and put in plastic which was labeled according to the location of the sample. At each location of the river
 88 estuary, sampling was carried out at four stalecations with samplingeach location being repeated three times.
 89 The coordinate points for sampling (Figure 1) at the mouths of the Bengawan Solo and Brantas rivers and
 90 the mouth of the Bengawan Solo River are presented in Table 1.

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92

93 **Figure 1.** Sampling locations at their two river mouths of the Bengawan Solo and Brantas irivers in East Java

94 (<https://earth.google.com/>)

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Table 1. Coordinates-point of sediment 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 07°16'37" S 112°51'35" E 07°18'19" S 112°51'34" E 07°21'03" S 112°51'09" E

105

106 Sediment sSeparation wWith dDinoflagellate cCysts

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

116

117 Determiration of the rate of water content

118 Determination of the rate of water content is carried out to determine-establish the water content
119 contained in the sediment sample. Wet sediment samples were first weighed out at 4–5 grams using an
120 analytical balance which was then put into the oven with a temperature of 150 °C for 1 hour. After that, the
121 dry sediment samples were weighed again. Previous research (Matsuoka and Fukuyo 2000) used the following
122 formula tion-and-the-calculation-stage-used-the-following-formulationwherein Bb is the wet sediment weight
123 (g); Bk is the dry sediment weight (g), and R is the =Rate of water content (%);:-

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125 Bb = wet weight (g);

126 Bk = dry weight (g);

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

Information:

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

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Dinoflagellate Cyst Abundance Calculation

The abundance of dinoflagellate cysts was observed under a light microscope using a haemocytometer with eight fields of view. The abundance of dinoflagellate cysts was calculated based on the previous research formula of Iton (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):

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

Information:

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

147

Identification of Dinoflagellate Cysts Morphology

Observation of Dinoflagellate cyst morphological analysis was carried out by taking organic sample residue a sample to be observed using a dropper pipette of 1 ml and then transferred it to a haemocytometer container for then 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 dinoflagellate cyst taxon by the researcher. (Alkawri 2016, Mertens et al. 2020).

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Data Analysis

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

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RESULTS AND DISCUSSION

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Results

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Identification of Dinoflagellate Cyst Diversity Morphology

Identification 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 yielded has a higher diversity of four species of dinoflagellate cysts compared to that of the Brantas River estuary which only presented 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)	
		Bengawan Solo	Brantas
1	Alexandrium catenella	3548	4835
2	Alexandrium minutum	6693	9366
3	Brigantidium sp.	2842	4228
4	Cochlodinium polykroides	6948	4869
5	Gymnodinium catenatum	43	43
6	Gyrodinium sp.	3250	5032
7	Pentapharsodinium tyrrhenicum	150406	106450
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 Analysis of Dinoflagellate Cysts

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 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). This report is the first study on the biodiversity of dinoflagellate cysts in the estuaries of major rivers in East Java.

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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	Biodiversity 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

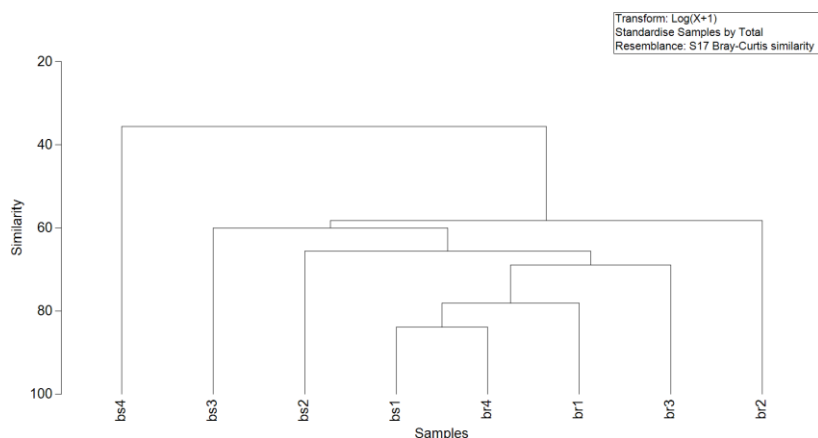


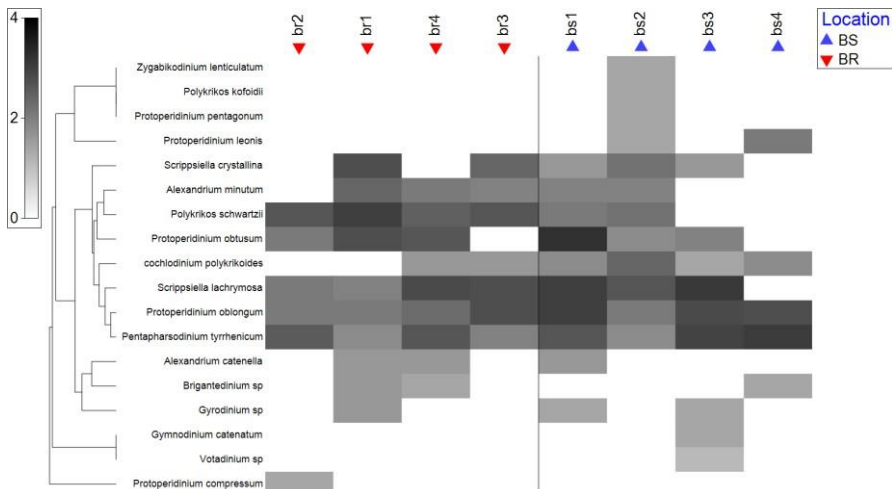
Figure 2. Analysis of similarity from eight sampling locations at the two river mouths of the Bengawan Solo and Brantas rivers in East Java in two different river mouths.

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 location. Only the four sampling locations at the mouth of the Bengawan Solo River had the highest abundance and were different from other station 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 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 endanger to public health. The results of the Shadegraph analysis showed that cysts of this type of dinoflagellate cyst was are identified which was only found in the mouth of the Brantas River, namely those of 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

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206 River, namely *Zygabikodinium lenticulatum*, *Polykrikos kofoidii*, *Protoperidinium pentagonum*, *Gymnodinium*
 207 *catenatum*, and *Votadinium* sp.

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 209



210
 211 **Figure 3.** Graph of species distribution at each sampling stat location at the mouth of the Bengawan Solo River (BS)
 212 Brantas river (br) and mouth of the Brantas River (br) the mouth of the Bengawan Solo River (bs). The color intensity
 213 indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher
 214 diversity index than the estuary of the Brantas River. The type of dinoflagellate cyst that predominates in the estuary
 215 of the Bengawan Solo River is this type.

216

217 **Discussion**

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

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233 ~~instances~~ber of ~~illness-disturbances~~ (Grattan et al. 2016). Thus, ~~it is essential for~~ this research ~~is essential~~ to
234 be carried out to predict the potential for a dangerous algae population explosions.

235 In this study, two large river estuaries in East Java ~~are~~ ~~are shown to be~~ important locations because ~~around~~
236 ~~these locations of their proximity to~~ many ~~sites where~~ fishery activities are developing, especially ~~relating to~~
237 shrimp (*Litopenaeus vanamae*) and milkfish (*Chanos chanos*) ponds which are the mainstay of this region.
238 The estuary of the Bengawan Solo ~~R~~river is in the Gresik area which is ~~aa~~quite high producer of milkfish
239 (Rohman et al. 2021) in addition to intensive artisanal fishing activities carried out (Collier 2019). in addition
240 to intensive artisanal fishing activities carried out (Collier 2019). Meanwhile, the Brantas River, which is also
241 quite large, passes through the city of Surabaya which then branches towards North Surabaya and East
242 Surabaya. The eastern Surabaya area is very close to Sidoarjo Regency, which is also one of the ~~Penaeus~~
243 ~~vanamae~~ white shrimp pond areas (Hukom et al. 2020).

244 There were two species found in all research locations, namely ~~the species~~ *Protoperidinium oblongum*
245 and *Pentapaharsodinium tyrrhenicum*. The ecological function of dinoflagellates in water has received the
246 attention of a number of researchers. ~~Under unfavorable conditions, dinoflagellates will produce cysts that~~
247 ~~are able to survive and settle in aquatic sediments. Dinoflagellate cysts play an important role biologically~~
248 ~~and ecologically because cysts can help dinoflagellate~~ ~~species~~ survive in harsh environments, provide
249 opportunities and facilitate their dispersal in aquatic habitats, and act as seeds in algae blooms (Bravo and
250 Figueroa 2014). In addition, the dinoflagellates of some species can produce more toxins than the vegetative
251 forms, seriously affecting ~~the species~~ ~~other organisms~~ through ~~theits~~ food web and even human health
252 (Kirkpatrick et al. 2004). Meanwhile for aquatic ecology, the impacts of algae blooms include beach pollution,
253 lack of oxygen, and the death of marine species on a large scale (Park et al. 2013). ~~The~~ *Alexandrium tamarense*
254 has been identified ~~as a toxic species~~ ~~released of toxin~~ (Oshima et al. 1992), and in this study we found two
255 species in the genus *Alexandrium* (*A. catenella* dan ~~and~~ *A. minutum*). Therefore, accurate identification of
256 dinoflagellates is an important first step in their early detection and function in ecological studies. Because
257 dinoflagellates have limited or even non-existent taxonomic keys, molecular methods to identify
258 dinoflagellates have become a priority as a continuation of this research. ~~In the current study, morphological~~
259 ~~identification was carried out by taking into account a number of general characteristics, such as the shape~~
260 ~~of the resulting cyst and its ornamentation, the structure and color of the walls, and the type~~ ~~of~~ ~~of opening~~
261 ~~or~~ archeopyle (Matsuoka and Fukuyo 2000).

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

270 Biodiversity studies of dinoflagellate cysts in Indonesia are still very limited. ~~Monitoring activities for the~~
271 ~~type~~ *Pyrodinium bahamense* have been carried out to anticipate the presence of ~~al~~algae blooms in Indonesia
272 including, Lampung Bay, Jakarta Bay, Cirebon coastal waters and Ambon Bay (Rachman et al. 2021). Studies

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273 on the diversity index of dinoflagellate cysts have been reported in Muara Jeneberang and Paotere Harbor
274 with values ranging from 0.82–1.01 (Rukminasari and Tahir 2021). This diversity value is still relatively low
275 when compared to the biodiversity of dinoflagella cysts in East Java with a Shannon-Wiener index ranging
276 from 2.0363–2.1055. Another report in South Sulawesi, Maros estuary and Pangkep River estuary, which are
277 included in the moderate category for the potential presence of HABs in this area (Rukminasari and Tahir
278 2021). Based on previous research, the abundance of dinoflagellate cysts can be an indication of the potential
279 for HABs in a particular given region. There are four potential groups that have been classified, namely very
280 low, low, medium, high and very high levels has been classified (McMinn 1991; Tian et al. 2018). Previous
281 study show that abundance of dinoflagellate cysts in Lampung for the P. bahamense cyste is 1–100 cysts
282 g⁻¹ sediment (Rachman et al. 2021), while previous research found M. polykrikoides type cysts measured with
283 abundances ranging from 100–1000 cysts g⁻¹ sediment (Thoha et al. 2019). The abundance of P. bahamense
284 in Cirebon waters was a serious case because it caused human death (Nurlina 2018), with a relatively high
285 abundance of 100–1000 cysts g⁻¹ sediment (Rachman et al. 2019). At the estuary of the Bengawan Solo
286 River, the highest abundance was Protoperidinium oblongum (115 cysts g⁻¹ sediment) and the lowest
287 abundance was Protoperidinium leonis (21 cysts g⁻¹ sediment). Meanwhile, Alexandrium minutum was found
288 with the highest abundance (125 cysts g⁻¹ sediment) in the mouth of the Brantas River, with an average
289 of 73 cysts g⁻¹ sediment having been found. Thus, the condition of the mouths of the Brantas Bengawan
290 Solo river and the mouth of the Brantas Bengawan Solo rivers still has a low potential for the occurrence of
291 HABs, and an average abundance of 73 and 62 cysts g⁻¹ respectively (McMinn 1991; Tian et al. 2018).

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292 CONCLUSIONS

293
294 This report is the first study on the biodiversity of dinoflagellate cysts in the estuaries of major rivers in
295 East Java. Research on dinoflagellate cysts revealed the diversity of dinoflagellates at the mouth of the
296 Bengawan Solo River and the mouth of the Brantas rivers. Analysis of cyst diversity in the two regions shows
297 that the Bengawan Solo River mouth has a higher Shannon-Wiener index compared to that of the Brantas
298 River mouth. The type of dinoflagellate cyst that dominates at the mouth of the Bengawan Solo River is
299 Protoperidinium obtusum, while at the mouth of the Brantas River it is Polykrikos schwartzii. Shade graph
300 analysis showed a number of species found only in the estuary of the Bengawan Solo River, namely
301 Zygaibacodinium lenticulatum, Polykrikos kofoidii, Protoperidinium pentagonum, Gymnodinium catenatum,
302 and Votadinium sp. Based on the results of this study, it is necessary to carry out DNA characteristic tests of
303 isolated and cultured species to document genetic information. In addition, this information is very important
304 in the management of coastal areas around the mouths of the Bengawan Solo and Brantas rivers to prevent
305 dinoflagellate population explosions which are likely to may have negative impacts on various sectors of
306 industry.

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311 water ecosystems. The authors have no conflicts of interest to declare.

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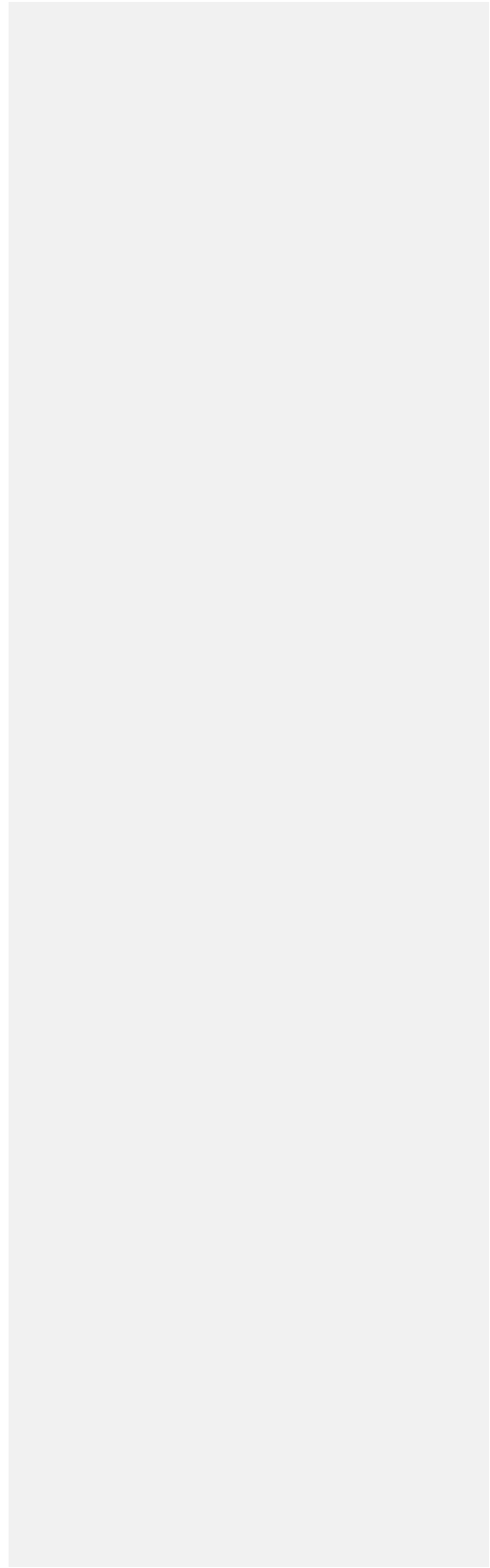
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Diversity of dinoflagellate cysts isolated from estuarine sediments of the Bengawan Solo and Brantas rivers

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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 *Zygabocodium 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

34 (IOC-UNESCO), HABs are defined as a phenomenon of harmful algae growth in water which causes
35 environmental problems (Anderson et al. 2021), losses to the fisheries sector, and even illness to humans
36 (Trevino-Garrison et al. 2015). The harm caused by HAB events spans both spatial and temporal scales.
37 Agricultural-scale losses include mass mortality of farmed fish, poisoning of marine food sources, itching of
38 beachgoers, and others. HAB events are generally due to nutrient enrichment, high temperatures, low oxygen
39 levels, and reduced light intensity as to enable dinoflagellates to grow rapidly. These incidents are very
40 common in estuary areas of rivers (Ajani et al. 2013). Most of the algae that cause HABs belong to the
41 dinoflagellate group (Kudela and Gobler 2012).

42
43 Dinoflagellates are included in the microalgae group found in sea and river waters (Senanayake et al.
44 2021). Dinoflagellates play an important role in these waters, namely as primary producers (Hoppenrath et
45 al. 2014, Price and Bhattacharya 2017). As microalgae, dinoflagellates have a unique ability to specialize and
46 survive in the competition of natural selection. Under productive conditions, dinoflagellates can reproduce
47 rapidly in the water column in the form of actively dividing swimming cells. When environmental conditions
48 are suboptimal, marked by nutrient enrichment (Faizal et al. 2012), warm temperatures (Coffey et al. 2019),
49 low oxygen levels, and reduced light intensity, they stop dividing and mate to form cysts which hibernate
50 and are able to survive in sediments for many years (Head et al. 2013). The hypnozygote is also produced
51 by dinoflagellates through the fusion of gametes produced by vegetative dinoflagellate cells, securely
52 protected within the thick walls of the cyst and highly resistant to adverse conditions (Bravo and Figueroa
53 2014). In this regard, a study of the biodiversity of dinoflagellate cysts in the estuaries of the Bengawan Solo
54 and Brantas rivers in East Java was conducted using a morphological identification approach.

55
56 The water conditions of the Bengawan Solo River currently needs serious attention. The river flow
57 originating from Central Java emptying into East Java carries a number of materials including nutrients which
58 can cause water enrichment. The case of enrichment in the waters of the Bengawan Solo River has been
59 carried out by previous studies (Wijaya and Elfiansyah 2022). Enrichment at the mouth of the river plays an
60 important role in the dynamics of plankton populations in water areas because phytoplankton will respond
61 very quickly to changes in the concentration of the dissolved nutrients. It is reported that nutrient enrichment
62 events can reduce the value of biodiversity and the productivity of an ecosystem (Isbell et al. 2013). The
63 principal contributing factor is human intervention due to activities that have been carried out both on land
64 and in aquatic (anthropogenic) ecosystems (Pawitan et al. 2007, Roosmini et al. 2018). This condition also
65 has an impact on important ecosystems in the coastal area, namely coral reef ecosystems (D'Angelo and
66 Wiedenmann 2014).

67
68 Meanwhile, the estuary of the Brantas river is also a major region in Java that crosses the provinces of
69 Central Java to East Java. Conditions here are similar to those at the estuary of the Bengawan Solo River. The
70 estuary waters of the Brantas river also receive a large amount of organic matter from human activities.
71 Nutrient influx from the Brantas River has the potential to cause enrichment in the Madura Strait waters
72 (Jänen et al. 2013). Increasing the proportion of nitrogen and phosphorus in the water results in a gradual
73 change in marine conditions such that the highest concentrations of nutrients are accumulated in the estuary

74 or coastal areas compared to river areas (Jennerjahn and Klöpper 2013). With the dynamics of the potential
75 for enrichment and the variety of nutrient sources that enter the waters of the Bengawan Solo and Brantas
76 rivers, research on the abundance and identification of dinoflagellate species is very important. Investigations
77 of these key phytoplankton constituents are expected to yield valuable data toward the management of
78 water areas that minimizes the occurrence of dangerous algae population explosions or HABS.

79 MATERIALS AND METHODS

80 Sample collection

81 Sediment at the bottom of either river mouth was collected with an Ekman Grab size 152x152x230 mm
82 and put in plastic which was labeled according to the location of the sample. At each location of the river
83 estuary, sampling was carried out at four stations with sampling being repeated three times. The coordinate
84 points for sampling (Figure 1) at the mouths of the Bengawan Solo and Brantas rivers are presented in Table
85 1.
86



87

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

90

91

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
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92

93 Sediment separation with dinoflagellate cysts

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

103

104 Determination of the rate of water content

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

110

$$111 R = \frac{Bb - Bk}{Bb} \times 100\%$$

112

113 Dinoflagellate cyst abundance calculation

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

118

$$119 \text{Cysts per gram} = \frac{N}{W(1 - R)}$$

120

121 Identification of dinoflagellate cysts

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

126

127 Data analysis

128 All data is presented in a table using Microsoft Excel software. Additionally, similarity analysis, shade plot
129 analysis, and dinoflagellate abundance from two different sampling locations were analyzed using Primer-e
130 software (Clarke and Gorley 2015).

131 RESULTS AND DISCUSSION

132 Results

133 Dinoflagellate cyst diversity

134 The dinoflagellate cysts taxa present varied significantly in the estuaries of the Berantas and Bengawan
135 Solo rivers (Table 1). The Bengawan Solo River estuary yielded a higher diversity of four species of
136 dinoflagellate cysts compared to that of the Brantas River estuary which only presented 12 species. The type
137 of *Protopteridinium obtusum* was found to dominate in the mouth of the Bengawan Solo River, while the
138 type of *Scrippsiella lachrymose* dominated in the mouth of the Brantas River (Figure 2).

139
140 **Table 1.** Types of dinoflagellate cysts isolated from sediments in the estuaries of the Bengawan Solo River
141 and the mouth of the Brantas River.

142

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

143

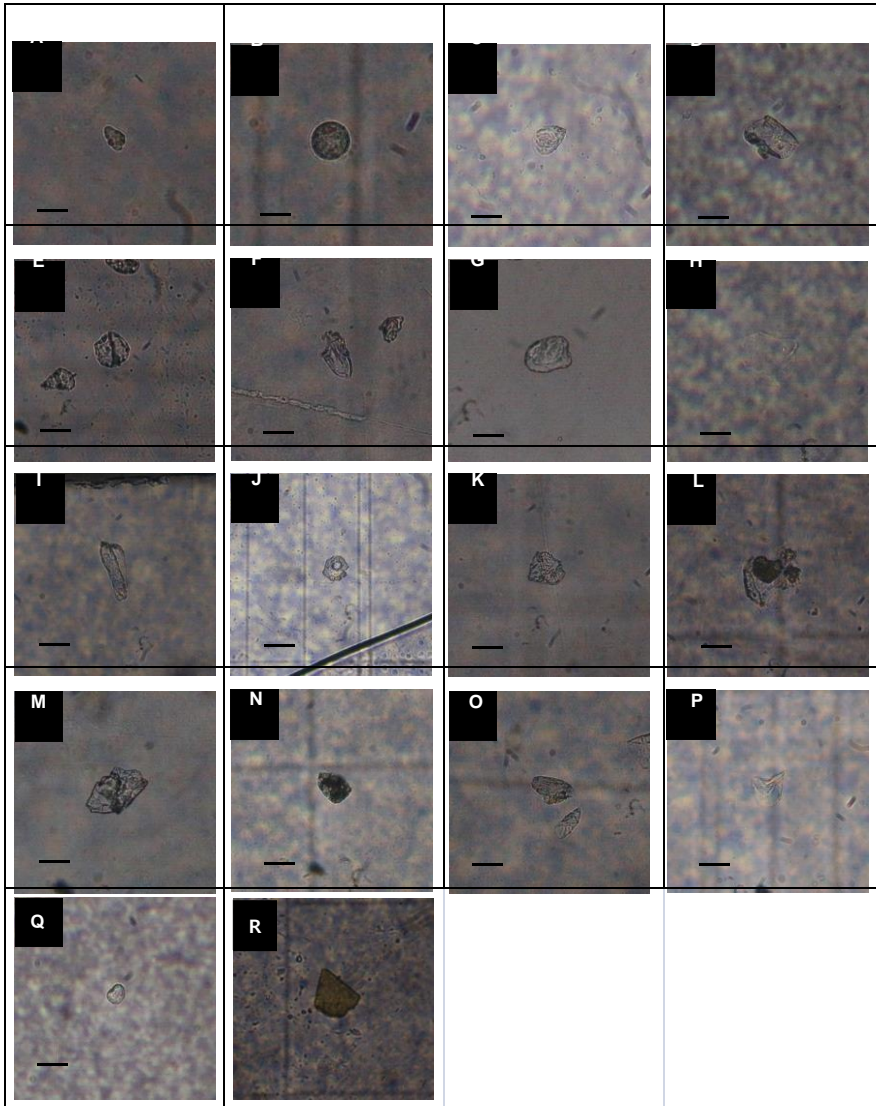


Figure 2. Dinoflagellate cyste collected from Bengawan Solo and Brantas river estuary. Bar scale 10 μ (A)

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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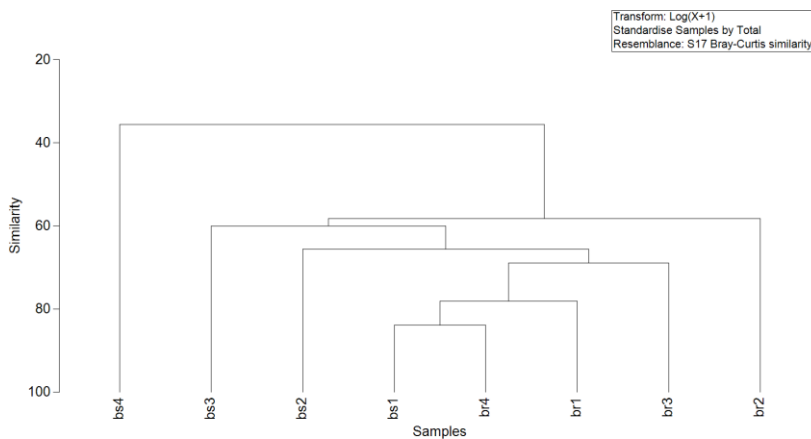
Biodiversity analysis of dinoflagellate cysts

153 Based on the dinoflagellate cyst abundance data, a biodiversity analysis was carried out to determine the
 154 dominating species and distribution patterns of cyst species in both river estuaries in East Java. Each sampling
 155 location was repeated four times to reduce the resulting data bias. Biodiversity analysis includes Margalef
 156 Index (d), Pielou's Evenness (J'), and Shannon-Wiener Index (H'). Referring to the Shannon-Wiener Index (H')
 157 value, it shows that the mouth of the Bengawan Solo River (2.1055) has higher diversity than the mouth of
 158 the Brantas River (2.0363).

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

No.	River	Biodiversity 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

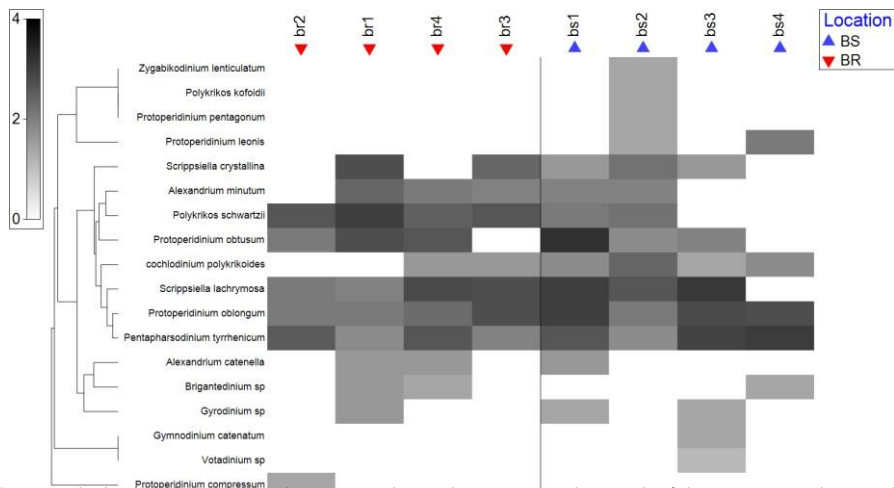
162
 163
 164 **Analysis of similarity and abundance of dinoflagellate cyst types**
 165



166
 167
 168 **Figure 3.** Analysis of similarity from eight sampling locations at the two river mouths of the Bengawan Solo and
 169 Brantas rivers in East Java.

171 The similarity analysis was carried out by comparing data on the abundance of dinoflagellate cysts found
 172 in both regions (Figure 3). The data was processed using Primer-e software and at the same time analyzing
 173 species abundance at each sediment collection station. Only the four sampling stations at the mouth of the
 174 Bengawan Solo River had the highest abundance and were different from other stations. Meanwhile, the
 175 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

176 the potential to cause harmful algae blooms (HAB), which are known to be detrimental to fishing activities
 177 and even a danger to public health. The results of the ShaderGraph analysis showed that cysts of this type
 178 of dinoflagellate are only found in the mouth of the Brantas River, namely those of the *Protoperidinium*
 179 *compressum*. Meanwhile, at the mouth of the Bengawan Solo River, there are several species that are not
 180 found at the mouth of the Brantas River, namely *Zygabikodinium lenticulatum*, *Polykrikos kofoidii*,
 181 *Protoperidinium pentagonum*, *Gymnodinium catenatum*, and *Votadinium sp.*
 182
 183



184 **Figure 4.** Shade plot of species distribution at each sampling station at the mouth of the Bengawan Solo River (bs)
 185 and mouth of the Brantas River (br). The color intensity indicates the higher the abundance of the species found. The
 186 estuary of the Bengawan Solo River has a higher diversity index than the estuary of the Brantas River. The type of
 187 dinoflagellate cyst that predominates in the estuary of the Bengawan Solo River is *Protoperidium obtusum*.
 188
 189

190 **Discussion**

191 The diversity of dinoflagella cysts needs to be studied because certain type of dinoflagellates have the
 192 potential to become a plankton group that needs to be watched out for for reasons of health and public
 193 safety (Bravo and Figueroa 2014). This report is the first to identify dinoflagellate cysts in East Java, specifically
 194 at the mouths of large rivers in the Bengawan Solo and Brantas river estuaries. The existence of dinoflagellates
 195 in a number of areas has received intensive attention, including mapping the distribution and species present
 196 to avoid losses incurred when this type of dinoflagellate experiences a population explosion. Previous
 197 research on the presence of dinoflagellate blooms has been reported in a number of countries such as
 198 Australia (Roberts et al. 2019), Chile, and France (Luo et al. 2019). Meanwhile, studies of dinoflagellate cysts
 199 in Indonesia have been carried out in several areas such as Lampung Bay (Thoha et al. 2019), Jakarta and
 200 Flores (Matsuoka and Fukuyo 1999), Central Jawa (Matsuoka 1983, 1984), Makassar (Rukminasari and Tahir
 201 2020), and Pankajene (Rachman et al. 2022). These incidents have proven harmful to activities related to
 202 aquatic resources such as fishing, which have had a huge impact. Losses due to the harmful algae blooms

203 have been reported, which have caused the death of a number of fish in the waters and damaged the
204 surrounding aquaculture industries (Jardine et al. 2020, Sakamoto et al. 2021). A number of regions also
205 reported their impact on human health causing a numerous instances of illness (Grattan et al. 2016). Thus, it
206 is essential for this research to be carried out to predict the potential for a dangerous algae population
207 explosions.

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

217 There were two species found in all research locations, namely *Protoperidinium oblongum* and
218 *Pentaparsodinium tyrrhenicum*. The ecological function of dinoflagellates in water has received the attention
219 of a number of researchers (Furio et al. 2012, Naqqiuddin et al. 2014, Srivilai et al. 2012). Under unfavorable
220 conditions, dinoflagellates will produce cysts that are able to survive and settle in aquatic sediments.
221 Dinoflagellate cysts play an important role biologically and ecologically because cysts can help dinoflagellates
222 survive in harsh environments, provide opportunities and facilitate their dispersal in aquatic habitats, and act
223 as seeds in algae blooms (Bravo and Figueroa 2014). In addition, the dinoflagellates of some species can
224 produce more toxins than the vegetative forms, seriously affecting other organisms through the food web
225 and even human health (Kirkpatrick et al. 2004). Meanwhile for aquatic ecology, the impacts of algae blooms
226 include beach pollution, lack of oxygen, and the death of marine species on a large scale (Park et al. 2013).
227 *Alexandrium tamarense* has been identified as a toxic species (Oshima et al. 1992), and in this study we
228 found two species in the genus *Alexandrium* (*A. catenella* dan and *A. minutum*). Therefore, accurate
229 identification of dinoflagellates is an important first step in their early detection and function in ecological
230 studies. Because dinoflagellates have limited or even non-existent taxonomic keys, molecular methods to
231 identify dinoflagellates have become a priority as a continuation of this research. Berkenaan dengan
232 pendekatan molekuler, beberapa penelitian telah berhasil mengidentifikasi berdasarkan rDNA pada SSU, LSU
233 dan gen regio ITS (Branco et al. 2020, Mertens et al. 2015). In the current study, morphological identification
234 was carried out by taking into account a number of general characteristics, such as the shape of the resulting
235 cyst and its ornamentation, the structure and color of the walls, and the type of archeopyle (Matsuoka and
236 Fukuyo 2000).

237 The case of the dinoflagellate population explosion in Indonesia has not received serious attention even
238 though a number of studies on dinoflagellate species have been identified in a number of areas. The main
239 cause of the algae population explosion is nutrients in the waters which are abundant and support algae
240 growth in a short timeframe (Piranti et al. 2021). Algae bloom events can occur in fresh water and marine
241 waters. Cases that have been reported in a number of publications are the waters of the Jakarta Bay which
242 caused mass fish die-offs (Nasution et al. 2021). Although, in East Java there have been no reports of algae

243 blooms, and it is hoped that research on the potential for HABs in East Java waters can be mitigated from
244 the start so that losses and impacts are expected to be minimized.

245 Biodiversity studies of dinoflagellate cysts in Java Island of Indonesia are still very limited (Matsuoka 1983,
246 1984, Matsuoka and Fukuyo 1999). Monitoring activities for the type *Pyrodinium bahamense* have been
247 carried out to anticipate the presence of algae blooms in Indonesia including, Lampung Bay, Jakarta Bay,
248 Cirebon coastal waters and Ambon Bay (Rachman et al. 2021). Studies on the diversity index of dinoflagellate
249 cysts have been reported in Muara Jeneberang and Paotere Harbor with values ranging from 0.82–1.01
250 (Rukminasari and Tahir 2021). This diversity value is still relatively low when compared to the biodiversity of
251 dinoflagella cysts in East Java with a Shannon-Wiener index ranging from 2.0363–2.1055. Another report in
252 South Sulawesi, Maros estuary and Pangkep River estuary, which are included in the moderate category for
253 the potential presence of HABs in this area (Rukminasari and Tahir 2021). Based on previous research, the
254 abundance of dinoflagellate cysts can be an indication of the potential for HABs in a given region. There are
255 four potential groups that have been classified, namely very low, low, medium, high and very high levels
256 (McMinn 1991, Tian et al. 2018). Previous study show that abundance of dinoflagellate cysts in Lampung for
257 *P. bahamense* is 1–100 cysts g^{-1} sediment (Rachman et al. 2021), while previous research found *M.*
258 *polykrikoides* type cysts measured with abundances ranging from 100–1000 cysts g^{-1} sediment (Thoha et al.
259 2019). The abundance of *P. bahamense* in Cirebon waters was a serious case because it caused human death
260 (Nurlina 2018), with a relatively high abundance of 100–1000 cysts g^{-1} sediment (Rachman et al. 2019). At
261 the estuary of the Bengawan Solo River, the highest abundance was *Protoperidinium oblongum* (115 cysts
262 g^{-1} sediment) and the lowest abundance was *Protoperidinium leonis* (21 cysts g^{-1} sediment). Meanwhile,
263 *Alexandrium minutum* was found with the highest abundance (125 cysts g^{-1} sediment) in the mouth of the
264 Brantas River, with an average of 73 cysts g^{-1} sediment having been found. Thus, the condition of the mouths
265 of the Bengawan Solo and Brantas rivers still has a low potential for the occurrence of HABs, and an average
266 abundance of 73 and 62 cysts g^{-1} respectively (McMinn 1991, Tian et al. 2018).

267 CONCLUSIONS

268
269 This report is the first study on the biodiversity of dinoflagellate cysts in the estuaries of major rivers in
270 East Java. Research on dinoflagellate cysts revealed the diversity of dinoflagellates at the mouth of the
271 Bengawan Solo and Brantas rivers. Analysis of cyst diversity in the two regions shows that the Bengawan
272 Solo River mouth has a higher Shannon-Wiener index compared to that of the Brantas River. The type of
273 dinoflagellate cyst that dominates at the mouth of the Bengawan Solo River is *Protoperidinium obtusum*,
274 while at the mouth of the Brantas River it is *Polykrikos schwartzii*. ShadeGraph analysis showed a number of
275 species found only in the estuary of the Bengawan Solo River, namely *Zygabiodinium lenticulatum*,
276 *Polykrikos kofoidii*, *Protoperidinium pentagonum*, *Gymnodinium catenatum*, and *Votadinium* sp. Based on
277 the results of this study, it is necessary to carry out DNA characteristic tests of isolated and cultured species
278 to document genetic information. In addition, this information is very important in the management of
279 coastal areas around the mouths of the Bengawan Solo and Brantas rivers to prevent dinoflagellate
280 population explosions which are likely to have negative impacts on various sectors of industry.

281

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286

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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 *Protooperidinium 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 *Zygabocodinium lenticulatum*, *Polykrikos kofoidii*, *Protooperidinium 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.

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

58 Meanwhile, the estuary of the Brantas river is also a major region in Java that crosses the provinces of Central Java to
 59 East Java. Conditions here are similar to those at the estuary of the Bengawan Solo River. The estuary waters of the Brantas
 60 river also receive a large amount of organic matter from human activities. Nutrient influx from the Brantas River has the
 61 potential to cause enrichment in the Madura Strait waters (Jänen et al. 2013). Increasing the proportion of nitrogen and
 62 phosphorus in the water results in a gradational change in marine conditions such that the highest concentrations of nutrients
 63 are accumulated in the estuary or coastal areas compared to river areas (Jennerjahn and Klöpffer 2013). With the dynamics
 64 of the potential for enrichment and the variety of nutrient sources that enter the waters of the Bengawan Solo and Brantas
 65 rivers, research on the abundance and identification of dinoflagellate species is very important. Investigations of these key
 66 phytoplankton constituents are expected to yield valuable data toward the management of water areas that minimizes the
 67 occurrence of dangerous algae population explosions or HABS.

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68 **MATERIALS AND METHODS**

69 **Sample collection**

70 Sediment at the bottom of either river mouth was collected with an Ekman Grab size 152x152x230 mm and put in plastic
 71 which was labeled according to the location of the sample. At each location of the river estuary, sampling was carried out at
 72 four stations with sampling being repeated three times. The coordinate points for sampling (Figure 1) at the mouths of the
 73 Bengawan Solo and Brantas rivers are presented in Table 1.
 74



75 **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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76 **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

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80 **Sediment separation with dinoflagellate cysts**

81 The separation between sediment and cysts uses the method (Matsuoka and Fukuyo 2000) where the sample is weighed
82 using an analytical balance of 4–5 grams and then transferred to a 50 ml beaker glass container then introduced to sterile sea
83 water until it reaches a size of 50 ml. Following the acid treatment procedure, a sonication process was carried out for 15
84 minutes using an ultrasonic agitator to remove sediment particles attached to cysts. Furthermore, the sieving process was
85 carried out using a filter which is arranged in stages based on the size of the mesh openings. The sieving stage uses 3 filters
86 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
87 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
88 morphological analysis.
89

90 **Determination of the rate of water content**

91 Determination of the rate of water content is carried out to establish the water contained in the sediment sample. Wet
92 sediment samples were first weighed out at 4–5 grams using an analytical balance which was then put into the oven with a
93 temperature of 150 °C for 1 hour. After that, the dry sediment samples were weighed again. Previous research (Matsuoka
94 and Fukuyo 2000) used the following formula wherein *Bb* is the wet sediment weight (g); *Bk* is the dry sediment weight (g),
95 and *R* is the rate of water content (%):
96

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97
$$R = \frac{Bb - Bk}{Bb} \times 100\%$$

98

99 **Dinoflagellate cyst abundance calculation**

100 The abundance of dinoflagellate cysts was observed under a light microscope using a haemocytometer with 8 fields of
101 view on equipment. The abundance of dinoflagellate cysts was calculated based on the previous research formula of
102 (Matsuoka and Fukuyo 2000) wherein *R* is the rate of water content (%), *N* is the total number of cysts observed, and *W* is
103 the weight of wet sediment (g):
104

104

105
$$\text{Cysts per gram} = \frac{N}{W(1 - R)}$$

106

107 **Identification of dinoflagellate cysts**

108 Dinoflagellate cyst morphological analysis was carried out by taking organic sample residue using a dropper pipette of
109 1 ml and transferring it to a hemacytometer container for observation under a transmitted light microscope. Each
110 dinoflagellate cyst sample was identified to the genus and species level based on the published description of the taxon by
111 the researcher (Alkawri 2016, Mertens et al. 2020).
112

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113 **Data analysis**

114 All data is presented in a table using Microsoft Excel software. Additionally, similarity analysis, shade plot analysis, and
115 dinoflagellate abundance from two different sampling locations were analyzed using Primer-e software (Clarke and Gorley
116 2015).

116

117

RESULTS AND DISCUSSION

118 **Results**

119 **Dinoflagellate cyst diversity**

120 The dinoflagellate cysts taxa present varied significantly in the estuaries of the Berantas and Bengawan Solo rivers (Table
121 1). The Bengawan Solo River estuary yielded a higher diversity of four species of dinoflagellate cysts compared to that of
122 the Brantas River estuary which only presented 12 species. The type of *Protoperidinium obtusum* was found to dominate in
123 the mouth of the Bengawan Solo River, while the type of *Scrippsiella lachrymose* dominated in the mouth of the Brantas
124 River (Figure 2).
125

125

126 **Table 1.** Types of dinoflagellate cysts isolated from sediments in the estuaries of the Bengawan Solo River and the mouth
127 of the Brantas River.
128

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No.	Species name	Average abundance of dinoflagellate cyst (cyst g ⁻¹ sediment)	
		Bengawan Solo	Brantas
1	<i>Alexandrium catenella</i>	35	48

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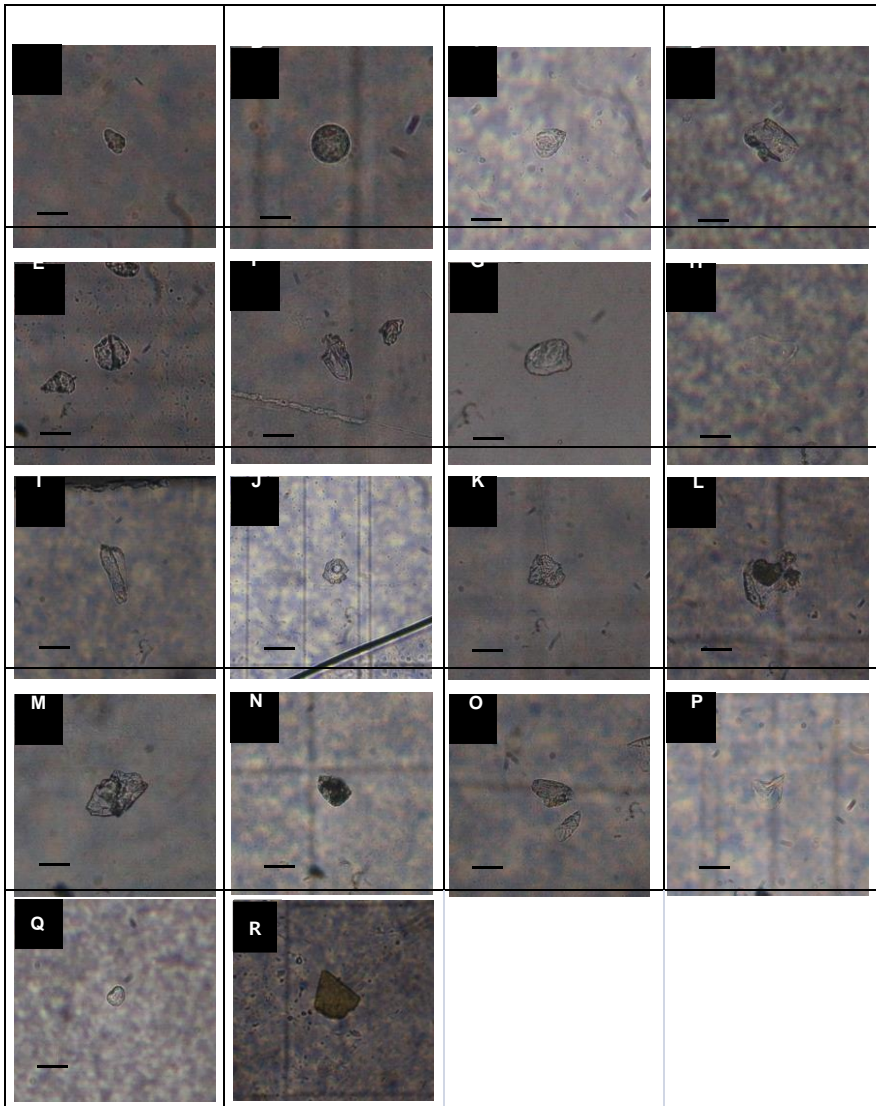
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2	<i>Alexandrium minutum</i>	66	93
3	<i>Brigantedinium</i> sp.	28	42
4	<i>Cochlodinium polykrioides</i>	69	48
5	<i>Gymnodinium catenatum</i>	43	-
6	<i>Gyrodinium</i> sp.	32	50
7	<i>Pentapharsodinium tyrrhenicum</i>	150	106
8	<i>Polykrikos kofoidii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

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

138 Based on the dinoflagellate cyst abundance data, a biodiversity analysis was carried out to determine the dominating
 139 species and distribution patterns of cyst species in both river estuaries in East Java. Each sampling location was repeated
 140 four times to reduce the resulting data bias. Biodiversity analysis includes Margalef Index (d), Pielou's Evenness (J'), and
 141 Shannon-Wiener Index (H'). Referring to the Shannon-Wiener Index (H') value, it shows that the mouth of the Bengawan
 142 Solo River (2.1055) has higher diversity than the mouth of the Brantas River (2.0363).

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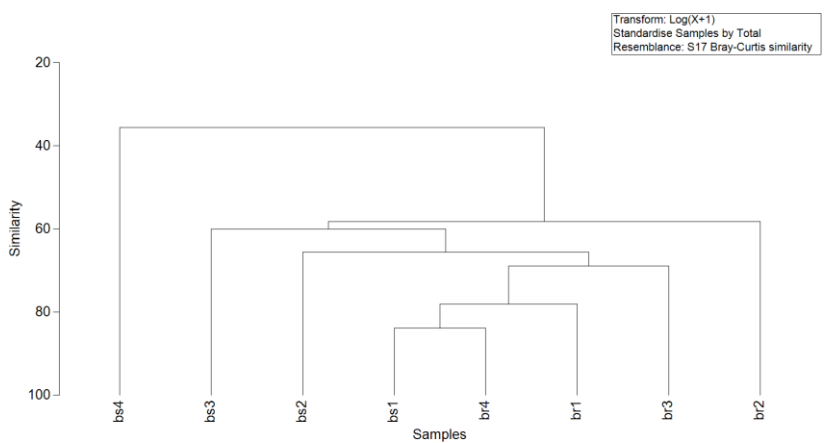
Table 2. Biodiversity analysis of dinoflagellate cysts at the mouths of the Bengawan Solo and Brantas rivers.

No.	River	Biodiversity 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



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

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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 *Protopteridinium 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 *Zygabocodinium lenticulatum*, *Polykrikos kofoidii*, *Protopteridinium pentagonum*, *Gymnodinium catenatum*, and *Votadinium* sp.

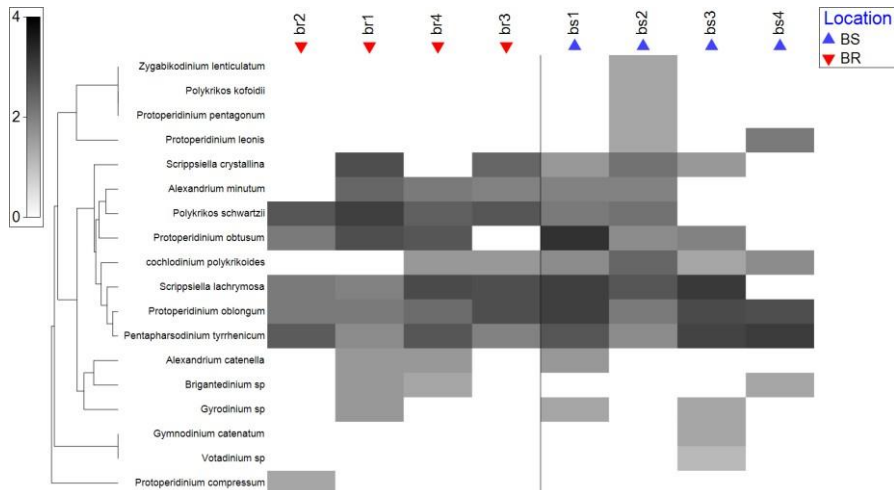


Figure 4. Shade plot of species distribution at each sampling station at the mouth of the Bengawan Solo River (bs) and mouth of the Brantas River (br). The color intensity indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher diversity index than the estuary of the Brantas River. The type of dinoflagellate cyst that predominates in the estuary of the Bengawan Solo River is *Protoperidinium 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 (Thoah et al. 2019), Jakarta and Flores (Matsuoka and Fukuyo 1999), Central Java (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 harmful algae blooms have been reported, which have caused the death of a number of fish in the waters and damaged the surrounding aquaculture industries (Jardine et al. 2020, Sakamoto et al. 2021). A number of regions also reported their impact on human health causing a numerous instances of illness (Grattan et al. 2016). Thus, it is essential for this research to be carried out to predict the potential for a dangerous algae population explosions.

In this study, two large river estuaries in East Java are shown to be important locations because of their proximity to many sites where fisheries are developing, especially realting to shrimp (*Litopenaeus 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 tyrrenicum*. 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 A. minutum*). Therefore, accurate identification of dinoflagellates is an important first step in their early

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207 detection and function in ecological studies. Because dinoflagellates have limited or even non-existent taxonomic keys,
208 molecular methods to identify dinoflagellates have become a priority as a continuation of this research. Berkenaan dengan
209 pendekatan molekuler, beberapa penelitian telah berhasil mengidentifikasi berdasarkan rDNA pada SSU, LSU dan gen regio
210 ITS (Branco et al. 2020, Mertens et al. 2015). In the current study, morphological identification was carried out by taking
211 into account a number of general characteristics, such as the shape of the resulting cyst and its ornamentation, the structure
212 and color of the walls, and the type of archeopyle (Matsuoka and Fukuyo 2000).

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

220 Biodiversity studies of dinoflagellate cysts in Java Island of Indonesia are still very limited (Matsuoka 1983, 1984,
221 Matsuoka and Fukuyo 1999). Monitoring activities for the type *Pyrodinium bahamense* have been carried out to anticipate
222 the presence of algae blooms in Indonesia including, Lampung Bay, Jakarta Bay, Cirebon coastal waters and Ambon Bay
223 (Rachman et al. 2021). Studies on the diversity index of dinoflagellate cysts have been reported in Muara Jeneberang and
224 Paotere Harbor with values ranging from 0.82–1.01 (Rukminasari and Tahir 2021). This diversity value is still relatively
225 low when compared to the biodiversity of dinoflagella cysts in East Java with a Shannon-Wiener index ranging from 2.0363–
226 2.1055. Another report in South Sulawesi, Maros estuary and Pangkep River estuary, which are included in the moderate
227 category for the potential presence of HABS in this area (Rukminasari and Tahir 2021). Based on previous research, the
228 abundance of dinoflagellate cysts can be an indication of the potential for HABS in a given region. There are four potential
229 groups that have been classified, namely very low, low, medium, high and very high levels (McMinn 1991, Tian et al. 2018).
230 Previous study show that abundance of dinoflagellate cysts in Lampung for *P. bahamense* is 1–100 cysts g⁻¹ sediment
231 (Rachman et al. 2021), while previous research found *M. polykrikoides* type cysts measured with abundances ranging from
232 100–1000 cysts g⁻¹ sediment (Thoah et al. 2019). The abundance of *P. bahamense* in Cirebon waters was a serious case
233 because it caused human death (Nurlina 2018), with a relatively high abundance of 100–1000 cysts g⁻¹ sediment (Rachman
234 et al. 2019). At the estuary of the Bengawan Solo River, the highest abundance was *Protoperidinium oblongum* (115 cysts
235 g⁻¹ sediment) and the lowest abundance was *Protoperidinium leonis* (21 cysts g⁻¹ sediment). Meanwhile, *Alexandrium*
236 *minutum* was found with the highest abundance (125 cysts g⁻¹ sediment) in the mouth of the Brantas River, with an average
237 of 73 cysts g⁻¹ sediment having been found. Thus, the condition of the mouths of the Bengawan Solo and Brantas rivers still
238 has a low potential for the occurrence of HABS, and an average abundance of 73 and 62 cysts g⁻¹ respectively (McMinn
239 1991, Tian et al. 2018).

240 CONCLUSIONS

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

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253 ACKNOWLEDGEMENTS

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256 439/UN3.1.12/PT/2022 on DNA Barcoding for fish from inland and marine water ecosystems. The authors have no conflicts
257 of interest to declare.
258

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

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

25 **Keywords:** algae, aquatic, algae bloom, ecosystem, dinoflagellate, diversity, estuary, poisonous, toxic.

26 **Running title:** Andriyono et al Diversity of Dinoflagellate Cyst

27 **INTRODUCTION**

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

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

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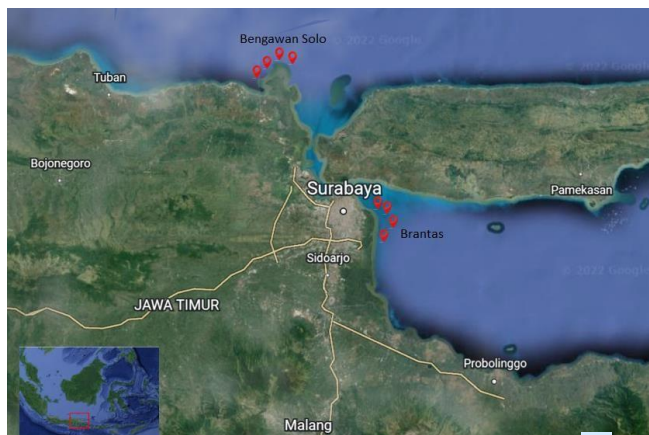
49 The water conditions of the Bengawan Solo River currently needsneed serious attention. The river flow originating from
50 Central Java emptying into East Java carries a number of materials including nutrients which can cause water enrichment.
51 The case of enrichment in the waters of the Bengawan Solo River has been carried out by previous studies (Wijaya and
52 Elfiansyah 2022). Enrichment at the mouth of the river plays an important role in the dynamics of plankton populations in
53 water areas because phytoplankton will respond very quickly to changes in the concentration of the dissolved nutrients. It is
54 reported that nutrient enrichment events can reduce the value of plankton biodiversity (Amorim and do Nascimento Moura
55 2021) and the productivity of an ecosystem (Isbell et al. 2013). The principal contributing factor is human intervention due
56 to activities that have been carried out both on land and in aquatic (anthropogenic) ecosystems (Pawitan et al. 2007, Roosmini
57 et al. 2018). This condition also has an impact on important ecosystems in the coastal area, namely coral reef ecosystems
58 (D'Angelo and Wiedenmann 2014).

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

72 MATERIALS AND METHODS

73 Sample collection

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



84 **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

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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.

103

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 ~~dried~~ sediment samples were weighed again. Previous research (Matsuoka and Fukuyo 2000) used the following formula wherein *Bb* is the wet sediment weight (g); *Bk* is the dry sediment weight (g), and *R* is the ~~rate-percent~~ of water content (%):

109

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

111

Dinoflagellate cyst abundance calculation

The abundance of dinoflagellate cysts was observed under a light microscope using a haemocytometer 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):

117

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

119

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)~~.

126

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 Zvariková 2019). While Pielou's Evenness Index ~~indicate of species distribution in certain area or sampling site. The Evenness 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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134 **RESULTS AND DISCUSSION**

135 **Results**

136 **Dinoflagellate cyst diversity**

137 The dinoflagellate cysts taxa present varied significantly in the estuaries of the Berantas and Bengawan Solo rivers
 138 (Table 1). The Bengawan Solo River estuary yielded a higher diversity of four species of dinoflagellate cysts compared to
 139 that of the Brantas River estuary which only presented 12 species, which collected from 4 sites and 3 replication each river.
 140 The type of *Protoperidinium obtusum* was found to dominate in the mouth of the Bengawan Solo River, while the type of
 141 *Scripsiella lachrymose* dominated in the mouth of the Brantas River (Figure 2).
 142

143 **Table 1.** Types of dinoflagellate cysts isolated from sediments in the estuaries of the Bengawan Solo River and the mouth
 144 of the Brantas River.
 145

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

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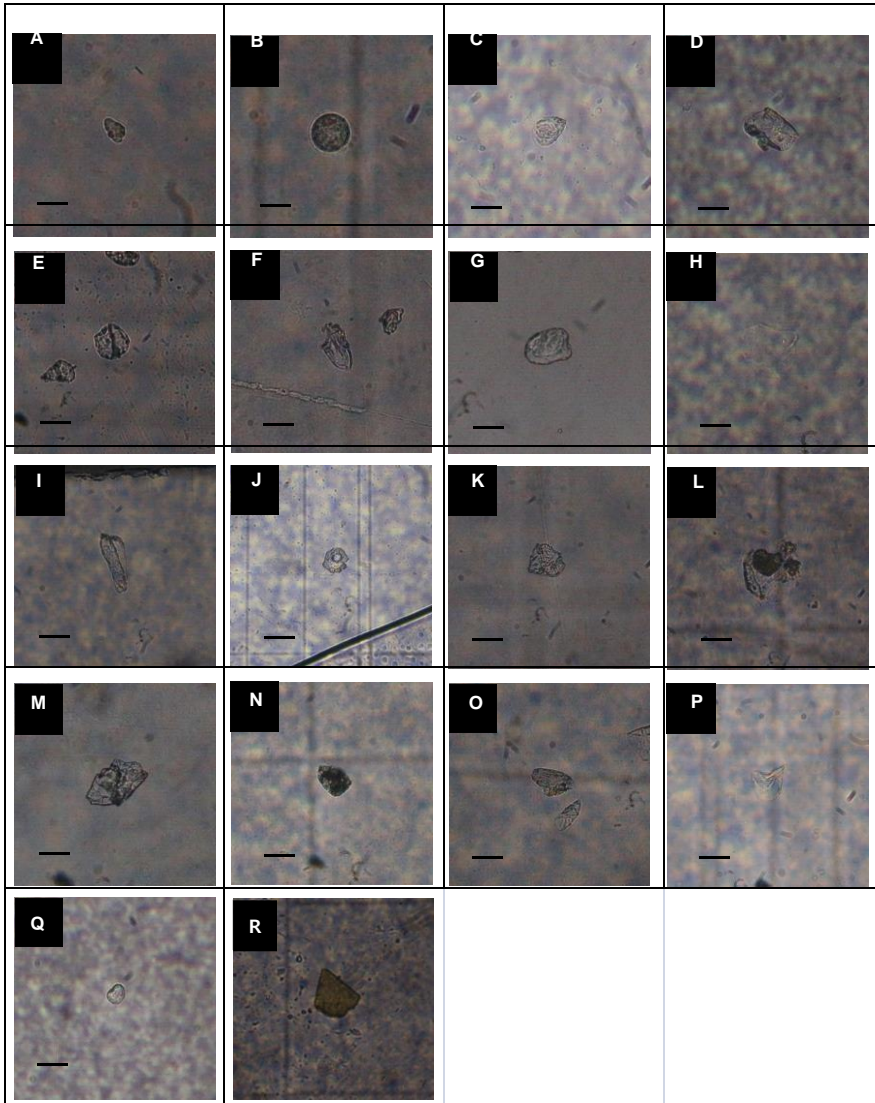
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147 **Figure 2.** Dinoflagellate cyste collected from Bengawan Solo and Brantas river estuary. Bar scale 10 μ (A) *Alexandrium catenella*;
 148 (B) *Alexandrium minutum*; (C) *Brigantedinium* sp.; (D) *Cochlodinium polykrikoides*; (E) *Gymnodinium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

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154 **Biodiversity analysis of dinoflagellate cysts**

155 Based on the dinoflagellate cyst abundance data, a biodiversity analysis was carried out to determine the dominating
 156 species and distribution patterns of cyst species in both river estuaries in East Java. Each sampling location was repeated
 157 four times to reduce the resulting data bias. Biodiversity analysis includes Margalef Index (d), Pielou's Evenness (J'), and
 158 Shannon-Wiener Index (H'). Referring to the Shannon-Wiener Index (H') value, it shows that the mouth of the Bengawan
 159 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 area which transition 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 Evenness 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	Biodiversity 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

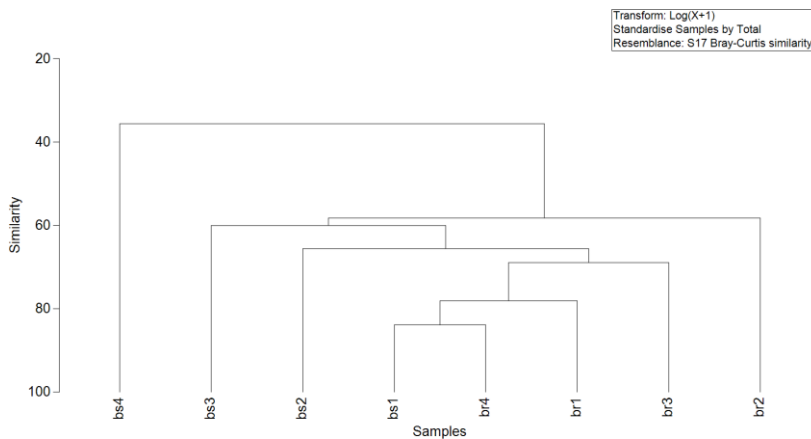


Figure 3. Analysis of similarity from eight sampling locations at the two river mouths of the Bengawan Solo and Brantas rivers in East Java.

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 *Protopteridinium 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 *Zygabocodinium lenticulatum*, *Polykrikos kofoidii*, *Protopteridinium pentagonum*, *Gymnodinium catenatum*, and *Votadinium* sp.

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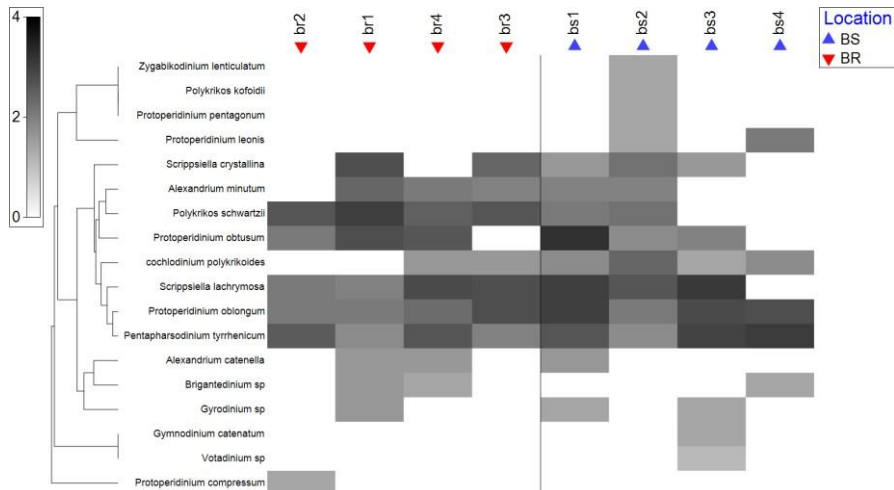


Figure 4. Shade plot of species distribution at each sampling station at the mouth of the Bengawan Solo River (bs) and mouth of the Brantas River (br). The color intensity indicates the higher the abundance of the species found. The estuary of the Bengawan Solo River has a higher diversity index than the estuary of the Brantas River. The type of dinoflagellate cyst that predominates in the estuary of the Bengawan Solo River is *Protoperidinium 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 health and wellbeing](#) (Berdalet et al. 2016) [and economic](#) (Sanseverino et al. 2016). Losses due to the [harmful](#) algae blooms have been reported, which have caused the death of a number of fish in the waters and damaged the surrounding aquaculture industries (Jardine et al. 2020, Sakamoto et al. 2021). A number of regions also reported their impact on human health causing a numerous instances of illness (Grattan et al. 2016). Thus, it is essential for this research to be carried out to predict the potential for a dangerous algae population explosions.

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

There were two species found in all research locations, namely *Protoperidinium oblongum* and *Pentapharsodinium tyrrenicum*. 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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229 al. 2013). *Alexandrium tamarense* has been identified as a toxic species (Oshima et al. 1992), and in this study we found two
230 species in the genus *Alexandrium* (*A. catenella* dan and *A. minutum*). Therefore, accurate identification of dinoflagellates is
231 an important first step in their early detection and function in ecological studies. Because dinoflagellates have limited or
232 even non-existent taxonomic keys, molecular methods to identify dinoflagellates have become a priority as a continuation
233 of this research. Berkenaan dengan pendekatan molekuler, beberapa penelitian telah berhasil mengidentifikasi berdasarkan
234 rDNA pada SSU, LSU dan gen regio ITS. With regard According to the molecular approach, several studies have succeeded
235 in identification of dinoflagellate by rDNA based on SSU, LSU and also ITS region gene (Branco et al. 2020; Mertens et
236 al. 2015). (Branco et al. 2020, Mertens et al. 2015). In the current study, morphological identification was carried out by
237 taking into account a number of general characteristics, such as the shape of the resulting cyst and its ornamentation, the
238 structure and color of the walls, and the type of archeopyle (Matsuoka and Fukuyo 2000).

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

246 Biodiversity studies of dinoflagellate cysts in Java Island of Indonesia are still very limited (Matsuoka 1983, 1984,
247 Matsuoka and Fukuyo 1999). Monitoring activities for the type *Pyrodinium bahamense* have been carried out to anticipate
248 the presence of algae blooms in Indonesia including, Lampung Bay, Jakarta Bay, Cirebon coastal waters and Ambon Bay
249 (Rachman et al. 2021). Studies on the diversity index of dinoflagellate cysts have been reported in Muara Jeneberang and
250 Paotere Harbor with values ranging from 0.82–1.01 (Rukminasari and Tahir 2021). This diversity value is still relatively
251 low when compared to the biodiversity of dinoflagella cysts in East Java with a Shannon-Wiener index ranging from 2.0363–
252 2.1055. Another report in South Sulawesi, Maros estuary and Pangkep River estuary, which are included in the moderate
253 category for the potential presence of HABs in this area (Rukminasari and Tahir 2021). Based on previous research, the
254 abundance of dinoflagellate cysts can be an indication of the potential for HABs in a given region. There are four potential
255 groups that have been classified, namely very low, low, medium, high and very high levels (McMinn 1991, Tian et al. 2018).
256 Previous study show that abundance of dinoflagellate cysts in Lampung for *P. bahamense* is 1–100 cysts g⁻¹ sediment
257 (Rachman et al. 2021), while previous research found *M. polykrikoides* type cysts measured with abundances ranging from
258 100–1000 cysts g⁻¹ sediment (Thoha et al. 2019). The abundance of *P. bahamense* in Cirebon waters was a serious case
259 because it caused human death (Nurlina 2018), with a relatively high abundance of 100–1000 cysts g⁻¹ sediment (Rachman
260 et al. 2019). At the estuary of the Bengawan Solo River, the highest abundance was *Protoperidinium oblongum* (115 cysts
261 g⁻¹ sediment) and the lowest abundance was *Protoperidinium leonis* (21 cysts g⁻¹ sediment). Meanwhile, *Alexandrium*
262 *minutum* was found with the highest abundance (125 cysts g⁻¹ sediment) in the mouth of the Brantas River, with an average
263 of 73 cysts g⁻¹ sediment having been found. Thus, the condition of the mouths of the Bengawan Solo and Brantas rivers still
264 has a low potential for the occurrence of HABs, and an average abundance of 73 and 62 cysts g⁻¹ respectively (McMinn
265 1991, Tian et al. 2018).

266 CONCLUSIONS

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

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282 439/UN3.1.12/ PT/2022 on DNA Barcoding for fish from inland and marine water ecosystems. The authors have no conflicts
283 of interest to declare.
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