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Pattern and germination rate of dinoflagellate cyst from three river estuaries (Jeneberang, Maros and Pangkep Estuary) of Makassar Strait

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Abstract. The research objective was to determine the rate and germination pattern of dinoflagellates from three estuaries (Jeneberang, Maros and Pangkep Estuary) of the Makassar Strait. The germination rate of dinoflagellate cysts from three estuaries of the Makassar Strait was studied at room temperature (28ºC) and at light: dark conditions 12:12 hours. Sediment samples were taken from three estuaries and treated for isolation of dinoflagellate cysts. The isolated dinoflagellate cysts were then incubated on 12 wells plates using f/2 medium for 28 days. The parameters measured in this study were the rate and pattern of germination measured every third day. The results showed that the germination rate of dinoflagellate cysts from Jeneberang Estaury was higher than other estuaries (Maros and Pangkep Estuary) for all stations. However, there was no significant difference in the germination rate between stations for all estuaries, except for the germination rate of Pangkep Estuary, there was a significant difference between station I and station IV. The germination pattern during the incubation period for all estuaries varied, however the abundance of dinoflagellate cysts from the Jeneberang estuary tended to increase with the incubation period. In conclusion, dinoflagellate cysts from Jeneberang Estuary had a higher germination rate and showed increased abundance during the incubation period.

1. Introduction

Dinoflagellate is one of the important organisms in aquatic ecosystems and plays an important role at the tropical level as a producer. Dinoflagellate has a life cycle where one of the stages of its life cycle is a non-motile stage (resting cyst) which remains in the bottom sediment or nears the bottom when conditions in the water column are not favorable for growth. [1] Cyst formation has contributed to the success of these organisms by allowing a switch between planktonic and benthic habitats in response to a seasonally variable environment[2–4]. Cysts are also very important for species dispersal and for the initiation and cessation of blooms. Therefore, it is important to examine the factors that influence the distribution, density and germination capacity of resting cysts to clarify the mechanisms of development by dinoflagellates [5].

There are two main processes associated with the formation of dinoflagellate cysts, namely encystment [6,7] and excystment [2,8]. Dinoflagellates spend most of their life cycle in the cyst stage, in response to adverse conditions [9]. This life cycle stage also plays an important role in the migration of species through sediment movement [10], and the seeding or maintenance of populations in the water column [11]. Excystment is influenced by various environmental factors including oxygen, light, temperature, salinity as well as internal regulation [8,12]. In addition, many factors can affect the growth and germination of cysts and more than one process needs to be considered in the study of dinoflagellate existence [13].

The growth rate is an important factor in determining the success of algal blooms; These physiological parameters can vary between strains [14,15]. Several studies have revealed significant intraspecific growth rate variability such as those for the *Karlodinium veneficum* strain [16]. [14] highlighted the same variation in growth rates of several *A. tamarense* (group I) strains isolated from the East coast of Scotland. [17] showed high intraspecific variability in oligo-nutrient requirements and growth rates among *A. tamarense* strains isolated from the same bloom that occurred in the Thau lagoon (France). [18] demonstrated high diversity in the growth rates of 19 Pseudo-nitzschia lines from the mid-Atlantic Coast of the United States.

The Makassar Strait has high fishery resources as well as biodiversity and high primary productivity. Increased anthropogenic disturbance to the area, especially the increase in organic pollution from agricultural areas, has led to an increase in nitrogen and phosphorus concentrations. This condition causes the eutrophication of the waters. Eutrophication is one of the factors that can trigger algae blooms. Uncontrolled growth of algae in these areas can negatively impact ecosystem health and fish mortality on a large scale. Algal blooming often occurs in these areas; however, minimal research has been done on the rate and pattern of germination / germination of dinoflagellate cysts from several river estuaries in the Makassar Strait. Based on this, it is necessary to conduct research to determine the pattern and growth rate of dinoflagellate cysts from the three estuaries of the Makassar Strait, so that it can be used as information on the abundance of dangerous dinoflagellates in the water column.

2. Materials and Methods

2.1. Sediment sampling

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

2.2. Isolation, sieving and incubation of dinoflagellate cysts

For dinoflagellate cyst analysis, the upper fraction (0–2 cm) of sediment was sieved using the method of [19]. Each sediment sample was sonicated for 15 minutes and sieved with mesh sizes of 250 µm, 100 µm, and 20 µm. The fine particles that have been passed on the net measuring 20 µm were transferred to a petri dish and allowed to stand for 15 minutes, the top layer solution was transferred to a 10 mL vial as a stock of dinoflagellate cysts for further experiments.

To determine the growth rate and pattern of dinoflagellate cysts, an experiment was carried out using 12 micro-plate well and f/2 growth media. Two ml of dinoflgellate cyst stock were added to the prepared f/2 medium. Previously, the initial abundance of dinoflagellate cysts from the stock was calculated. The dinoflagellate cysts were incubated in 12 micro-plate holes with f/2 medium for 28 days. The 12 micro plate well were then placed in a culture cabinet with a temperature of 28°C with a 12:12 hour light and dark period. Dinoflagellate cyst growth is checked periodically every 3 days for each isolated cyst by counting the increase in the number of cysts. The growth rate of the cyst is calculated using the formula:

$$\mu = \frac{\ln Nt - \ln No}{t}$$

Where No is the initial cyst concentration (sediment gr cyst $^{-1}$) and Nt is the cyst concentration after t day (sediment gr cyst $^{-1}$).

2.3. Data analysis

Three replicates for each site of origin of the dinoflagellate cyst were statistically analyzed by means of comparison, one-way ANOVA using the GrapPad version 7 computer program. Differences between treatments were tested by Tukey's test.

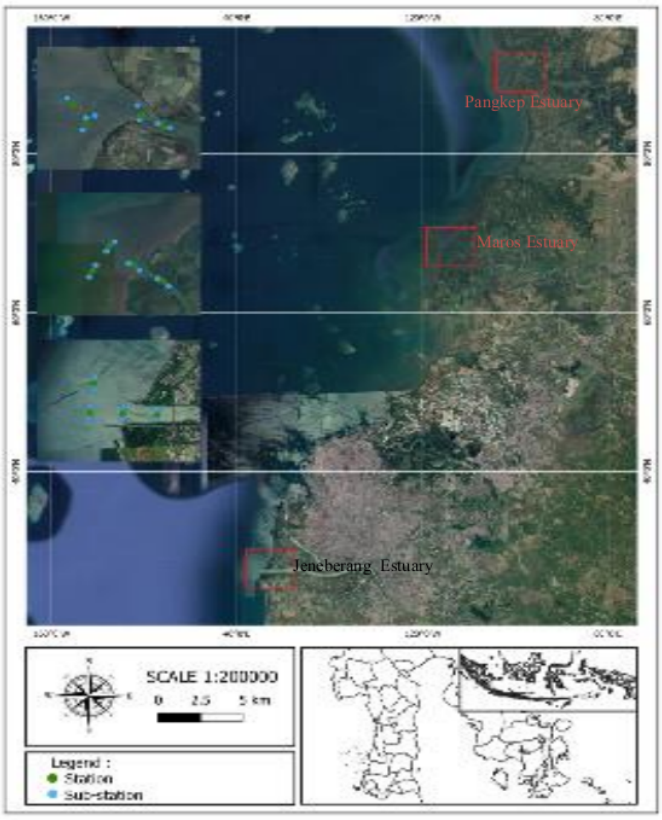

Figure 1. Study sites map

3. Results and Discussion

To determine the pattern and rate of dinoflagellate cyst growth from three estuaries, dinoflagellate cysts were incubated for 28 days. The growth patterns of dinoflagellate cysts from the three river estuaries showed a difference, wherein the dinoflagellate cysts from the Jeneberang River Estuary tended to continue to increase during the incubation period (Figure 2), while the growth patterns of dinoflagellate cysts from the Maros and Pangkep River Estuaries were relatively the same and fluctuated during the incubation period. The difference in the growth pattern of dinoflagellate cysts is thought to be due to differences in environmental conditions. These results are consistent with previous studies by [9] who found that the excysment of dinoflagellates in the Fal River Estuary is influenced by various conditions that facilitate the recovery of the dinoflagellate population in the water column.

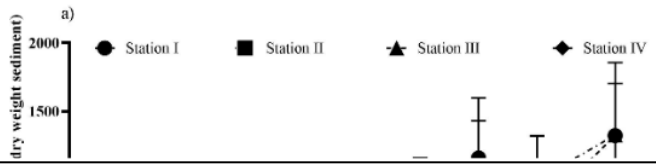

Figure 2. Growth pattern of dinoflagellate cysts from three estuaries of the Makassar Strait. a) Jeneberang Estuary, b) Maros Estuary and c) Pangkep Estuary.

Growth rate is an important factor in determining successful blooming; These physiological parameters can vary between strains [14,15]. The growth rate of dinoflagellate cysts from three river estuaries was determined by calculating the abundance of dinoflagellate cysts at the beginning and at the end of the experiment. The results showed that higher growth rates for all stations were found in dinoflagellate cysts from the Jeneberang River Estuary, with the highest growth rates found at station IV of 0.1613 cysts / day (Figure 3). Meanwhile, dinoflagellate cysts from the Maros River Estuary showed a low

growth rate for all stations and the lowest growth rate was found at station III, amounting to 0.05797 cysts / day. The growth rate range of dinoflagellate cysts in this study was lower than the range of growth rates in previous studies by [15] with a range of dinoflagellate cyst growth rates from species *Alexandrium pacificum* of 0.05 - 0.33. cyst/day. The low growth rate of dinoflagellate cysts in this study was suspected because in this study the dioflagellate cysts used were multi-species. This is in accordance with [15] that the growth rate of variation is high among dinoflagellate species. The results of statistical tests showed that the dinoflagellate cyst growth rate was significantly different between the three sampling locations, but the dinoflagellate cyst growth rate was not significantly different between stations in the three river estuaries, except between station I and station IV in the Pangkep River Estuary, the growth rate of dinoflagellate cysts was significantly different.

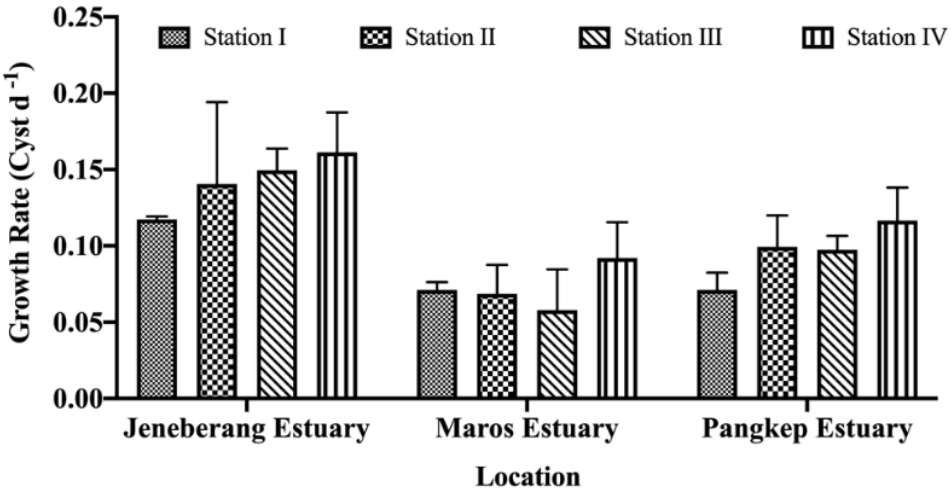

Figure 3. The growth rate of dinoflagellate cysts from the three estuaries of the Makassar Strait.

4. Conclusions

The growth patterns of dinoflagellate cysts from the three mouths of the Makassar Strait river varied and the growth patterns of dinoflagellate cysts from the Jeneberang River estuary tended to increase during the incubation period. The high variability of the dinoflagellate cyst growth rate from the three mouths of the Makassar Strait river was high due to the influence of intra-specific species of dinoflagellate cysts.

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