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Navicula sp Cell growth at different salinities

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Abstract
Navicula sp is one of the microalgae which plays an important role in fish and shrimp hatchery activities. *Navicula* sp. which can detect the surrounding environment and is able to adapt to various salinities. The purpose of this study was to determine the optimal salinity level for the growth of *Navicula* sp. The research was carried out on March 9 - 22 2022. The research location was carried out at the Natural Feed Laboratory of the Brackish Water Aquaculture Fishery Center (BPBAP) Ujung Batee Province of Aceh. This study used an experimental method with a completely randomized design (CRD), with 4 treatments and 3 replications. The treatment in this study is the difference in salinity: A (10 g/l), B (20 g/l), C (30 g/l), D (40 g/l). The results showed that treatment B with a salinity of 20 g/l had a significant effect (P <.0.05) on the other treatments. The results of the Duncan Significant Difference Test showed that the maintenance medium with a salinity of 20 g/l had the highest cell density (5.63x106 cells/ml), followed by the maintenance medium with a salinity of 30 g/l with a cell density (5.20x106 cells/ml).

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Introduction

Microalgae are unicellular microscopic photosynthetic eukaryotes that inhabit marine and fresh waters. Among microalgae, diatoms are responsible for approximately 20% of annual global carbon dioxide fixation through photosynthesis. This group of phytoplankton is about 20,000 different species distributed at depths of 5 m and up to several meters. Based on the cell's symmetry, diatoms can be pennate (bilateral symmetry) or cylindrical (radial symmetry). The uniqueness of diatoms is their pigment profile which is very different from that found in land plants and green algae. In fact, while diatoms have chlorophyll a and c, green algae and land plants have chlorophyll a and b (Villanova & Spetea, 2021) [42].

One of the microalgae species that can be cultivated for biomass utilization is *Navicula* sp. *Navicula* sp. has the highest fatty acid content, about 73 mg/gram on each weight per cell basis (2.1 cells/gram) and *Navicula* sp has the property of settling on the bottom of the waters and is easy to adapt. The properties that precipitate on *Navicula* sp. Can increase the production of higher biomass. (Andriani, 2021) [4]. The benthic diatom *Navicula* sp, incerta is the main component of phytoplankton and is also relatively easy to cultivate. It is used as live food in commercial mussel farming and a food source in aquaculture. In fact, a study explained the role of the plankton community in the success of vannamei shrimp farming with the biofloc system and the addition of *Navicula* sp. has had a significant effect on improving the performance of the shrimp culture (de Abreu *et al*., 2019) [11].

The culture technique of microalgae is the addition of nutrients that are given continuously to achieve maximum growth, because nutritional needs are always fulfilled at all times. Fertilizer added to plankton culture which functions to increase growth. Many factors influence this plankton culture, chemically such as seawater ions, pH and the base of the place (cement or soil). Besides that, phytoplankton is also influenced by temperature, salinity, sunlight and the ratio of Nitrogen Phosphate (N:P) (Soemarjati & Muqsith, 2014) [36].

The biochemical composition of microalgae can change with growth rate, environment (temperature, nutritional status, salinity, pH and light intensity). Temperature and light intensity are important factors controlling algal growth in natural environments and also the response of growth to temperature is important in regulating the dominance of plankton species. Therefore the growth characteristics are important in evaluating the potential for cultivating *Navicula* sp to be used for cultivation purposes. Growth rate, in terms of cell count or biomass; and biochemical composition should be optimized (Kang *et al*., 2011) [18].

Most of the diatoms are very sensitive to changes in the salt content of the water. The life of various types of phytoplankton including Skeletonema costatum depends on the salinity of the waters. The salinity factor is very important because it directly affects the osmotic pressure of the body. Productivity and adaptability of various types of algae are also closely related to the level of salinity of the environment (Rudiyanti, 2011) [31].

Vaname shrimp farming activities based on growth and survival parameters are affected by water quality conditions (Hernández *et al*., 2006) [16], one of which is salinity (Anita *et al*., 2018) [5]. Even though it has a wide range of salinity for growth, from 0 g/l to 40 g/l, vannamei shrimp will grow optimally at a salinity of 15 g/l to 25 g/l (Ponce-Palafox *et al*., 1997) [27]. As previously explained, the addition of *Navicula* sp. has provided an improvement in the performance of vannamei shrimp farming (de Abreu *et al*., 2019) [12]. On the other hand, salinity is also an environmental factor that influences the growth of microalgae (Armanda, 2013) [7]. Suitable salinity conditions for both vaname and *Navicula* sp shrimp will certainly increase the chances of success in cultivation. One strategy is to optimize salinity to determine the best salinity value to increase growth and cell content of *Navicula* sp.

Research Methods

This research activity uses several tools and materials used to assist data collection in the implementation of activities. The equipment and materials used during the research can be seen in table 1.

Table 1: Research Tools and Materials

No	Tool	Specification	Quantity	Utility
1	jar	Volume 5 liters	1 piece	Culture container
2	Aeration stone	Volume 2 cm	12 piece	Aeration
3	aeration pipe	Aeration hose	12 meter	Air duct
4	blowers	250 kwh	1 piece	Oxygen supply
5	Walne fertilizer	Liquid fertilizer	60 ml	Diatom culture
6	Sea water	Liquid	120 liter	Maintenance media water
7	Microscope	*Zoom in 100 times*	1 piece	Count cells
8	DO Meter	Digital 0.01	1 piece	Measure DO
9	pH Meter	Digital 0.01	1 piece	Measure pH
10	Refractometer	Digital 0.1	1 piece	Measure salinity

Experimental design

This study used an experimental method with an experimental design, namely Completely Randomized Design (CRD) with 4 treatments and 3 repetitions as follows:

$$Y = \mu + \tau + \varepsilon$$

Information

μ : The average (mean) value of expectations

τ : Effect of treatment factors for non-factorial studies or combination treatment factors for factorial studies (=α+β+αβ, if the study consists of two factors)

ε : Effect of error (experimental error)

Research Container Preparation

The container used in the *Navicula* sp culture is in the form of 5 ml volume jars of 12 pieces with a volume of 5 liters of water for 4 treatments and 3 replications. The container used is cleaned with clean water and rinsed with hot water to kill sticky bacteria after the previous use, then dried. Next, fill with media water according to the specified salinity, for treatments A (10 g/l), B (20 g/l), and C (30 g/l), dilution is carried out using the dilution formula (Akbar, 2021) as follows:

$$S = [(S1\ V1 + S2\ V2) : (V1 + V2)]$$

Information

S : Desired salinity (0/00) S1
: High salinity or sea water (0/00)
S2 : Low salinity or fresh water (0/00) V1
: High salinity water volume (liters)
V2 : Volume of low salinity water (liters)

For treatment D (40 g/l), dilution was not carried out because of the salinity content, because the salinity at the study site only reached 35 g/l, for this reason, additional salinity was carried out by drying it for 5 days, because to increase the salinity of 1 g/l it takes 1 drying day.

After the preparation of the container has been completed and the media water is in accordance with the desired salinity, fertilization is carried out using walne fertilizer (Na, H2PO4 .2H20 FeCl3 .6H2O, H3BO3, MnCl2 .4H2O) as much as 3 ml for 5 liters of water, then given Vitamin B, B 6, B 12 as much as 3 ml/container, then put *Navicula* sp. 10 x 106 cells for each container.

Monitoring

The steps for checking the growth of *Navicula* sp cells are as follows:

- Look at the water color of the *Navicula* sp media every day because on the fourth day the water color looks slightly brownish.
- On the fourth day, you can see that the color of the water

in the media is starting to brown. Check and count the samples under a microscope.
- To further check the development of *Navicula* sp. cells. every day.
- Counting *Navicula* sp cells using a 10x10 microscope.\
- Insert *Navicula* sp cells into the haemocytometer.
- Count the cells shown on the box and haemocytometer.

Data analysis

Data analysis was performed using analysis of variance or the F test (ANOVA). This test was conducted to determine the effect of the treatment (Independent Variable) on the different salinity measured or the F test. If the F test is significantly different or very significantly different, then proceed with the BNT test (Lessest Significant Difference), which is to find out the difference between each treatment. Research using Completely Randomized Design (CRD). To determine the effect of treatment on research variables, it was analyzed using ANOVA with the help of IBM SPSS Statistics 26.

Results and Discussion

Counting the number of cells *Navicula* sp. for 14 days of maintenance can be seen in Figure 1.

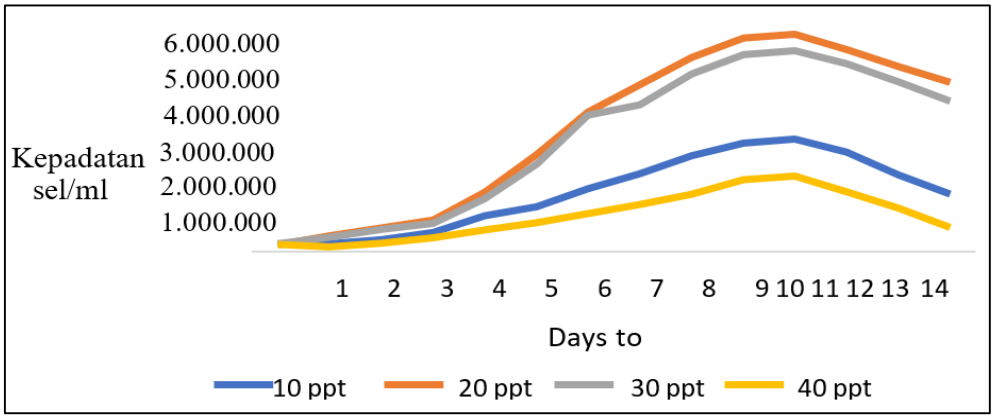

Fig 1: Number of *Navicula* sp cells during 14 days of rearing

Based on Figure 1, the cell density of *Navicula* sp. increased with the age of rearing, but at the beginning of rearing, from day 1 to day 4, the number of *Navicula* sp cells was still low, and the next day there was an exponential increase in the population and reached its peak on the 11th day. towards the 12th day began to decrease the number of cells until the 14th day. The difference in the increase in the number of *Navicula* sp cells based on the maintenance media at different salinities showed that the highest cell density was in the maintenance medium with a salinity of 20 g/l followed by the maintenance medium with a salinity of 30 g/l. this is justified by the opinion (Soemarjati, 2013) [34] that environmental factors play an important role in growth, one of which is the condition of salinity at 20-30 g/l which is still in the category of good conditions for growth. while the maintenance medium with a salinity of 10 g/l and 40 g/liter tends to have a lower cell density.

Based on the results of statistical tests, it can be seen that there were very significant differences between treatments ($p < 0.01$) (Table 2.2). The results of the Duncan Significant Difference Test showed that the maintenance medium with a salinity of 20 g/l had the highest cell density (5.63x106 cells/ml), followed by the maintenance medium with a salinity of 30 g/l with a cell density (5.20x106 cells/ml). according to research (Aziz, 1994) [7]. The two treatments did not show statistical differences, the difference was seen in the maintenance medium with a salinity of 10 g/l (2.93x106 cells/ml), and the lowest cell density was found in the maintenance medium with a salinity of 40 g/l (1.97x106 cells/ml).

Table 2: Population of *Navicula* sp at peak density (million cells/ml)

Repetition	Treatment (g/l)			
	10	**20**	**30**	**40**
1	2,70	5,40	5,00	1,90
2	2,90	5,70	5,20	2,30
3	3,20	5,80	5,40	1,70
Average	$2,93^{a}$	$5,63^{b}$	$5,20^{b}$	$1,97^{c}$

In general, the growth pattern of *Navicula* sp cells is the same as the characteristics of microalgae growth, where at the beginning of growth there is an adaptation process that causes a low number of cells, after the adaptation phase is complete, it will be followed by an exponential phase which is marked by a drastic increase in the number of microalgae cells, and reaches its peak at In the stationary phase, cell growth will decrease if the nutrients in the maintenance medium have decreased which is marked by the number of deaths (death phase) (Barsanti & Gualtieri, 2005) [8]. Most of the diatoms are very sensitive to changes in the salt content of the water. The productivity and adaptability of various algae are thought to be closely related to the level of salinity of the environment (Rudiyanti, 2011) [31].

Microalgae that live in marine waters such as *Navicula* sp generally have a wide tolerance for salinity (Khatoon *et al.*, 2010) [19] this can be seen from observations on rearing media with low salinity (10 g/l) to high salinity (40 g/l) still shows growth in *Navicula* sp. The results obtained during the study showed that the maintenance medium with a salinity of 20-30 g/l was a salinity suitable for the life of *Navicula* sp.

Salinity in a body of water is one of the limiting factors for the living things in it (Aziz, 1994) [7] appropriate osmotic conditions lead to optimal growth of both microalgae and living organisms in them. In aquaculture activities such as brackish water cultivation, the success of several commodities is largely determined by the salinity of the waters (Utami, 2016) [41]. Some crustaceans are able to adapt to wide salinity but differences in osmotic pressure in the waters and in the body will affect energy requirements, and will ultimately affect growth (Thabet *et al*., 2017) [40]. The results of observations of water quality for 14 days of *Navicula* sp maintenance can be seen in table 3.

Tabel 3: Kisaran Parameter Kualitas Air Selama Penelitian Tahap 1

Parameter	Salinity treatment (g/l)			
	10	20	30	40
Suhu ($_{o}$ C)	22.3-24	22.3-24	22.3-24	22.3-24
pH	7.7-8	7.7-8	7.7-8	7.7-8
DO (mg/l)	5-6	5-6	5-6	5-6

Parameters of water quality for the growth of Naviku sp cells. influenced by temperature, pH, DO, and salinity. Salinity becomes an influential factor in the cell growth of microalgae. Based on the results of the study, it was found that the temperature was 22.3-24 $_{o}$C, the range of these temperatures is still categorized according to the living environment of microalgae (Elicin *et al*., 2016) [13]. The pH obtained during the study was 7.7-8 in accordance with the pH of microalgae growth in general. pH or degree of acidity is a limiting factor for microalgae growth and has a limiting threshold for optimal growth. In general, the optimum pH range for microalgae culture ranges from 7-9, while the optimum pH for marine biota ranges from 7.8-8.5 (Andriani, 2021) [4]. Dissolved oxygen (DO) obtained during the trials ranged from 5-6 mg/l, in accordance with growth for microalgae cultures in general.

Dissolved oxygen is very important for respiration, growth, reproduction, metabolic processes by all living organisms of aquatic organisms. In addition, dissolved oxygen also plays a role in the decomposition of organic matter in waters (Sinaga *et al*., 2016) [33]. Salinity as a reference in this study is very influential on the growth process of *Navicula* sp. due to the nature of this microalgae easily change shape caused by differences in osmotic pressure. Good salinity in good tests is found in treatment B (20 g/l) with a density of (5.63x106 cells/ml), while the optimum salinity according to (H Suryanto Suwoyo & Mangampa, 2010) for microalgae growth can grow in the range of 15- 25g/l.

Conclusion

- *Navicula* sp cell densities in treatments of 10 g/l (2.93x106), 20 g/l (5.63x106 cells/ml), 30 g/l (5.20x106 cells/ml), and 40 g/l (1.97x106 cells/ ml).
- Optimum growth of *Navicula* sp as indicated by the highest cell density at a salinity of 20 g/l (5.63x106 cells/ml), followed by a salinity of 30 g/l (5.20x106 cells/ml).

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