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Growth, biomass, and carotenoids content analysis of *Navicula* sp. and *Chlorella* sp. in batch cultures with different salinities

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Abstract. *Haslianti, Sahidin, Asnani, Fristiohady A. 2023. Growth, biomass, and carotenoids content analysis of* Navicula *sp. and* Chlorella *sp. in batch cultures with different salinities. Biodiversitas 24: 4299-4306.* The physiological activity of microalgae is strongly influenced by culture conditions. Therefore, the rebound in this microscopic plant blooming phenomenon varies according to the aquatic environmental parameters, especially the salinity of its medium. This study aimed to analyze the cell density, biomass, and carotenoid content of two types of microalgal cultures isolated from the sea of Southeast Sulawesi. This research was conducted from October 2021 to October 2022. The independent variable in this study was salinity (30, 33, and 36‰), and the dependent variables were the parameters of cell density, biomass, and carotenoids content. This study consisted of 135 unit experiments using a completely randomized design (CRD). The same trend in cell density is observed in the two types of microalgae, where exponential growth occurred from day 2 to day 6 and decreased in growth rate on day 7. The biomass content of the two types observed was relatively the same, ranging from 0.0176-0.0181 g, while the carotenoids content ranged from 12.8294-25.5958 g/mL. The highest carotenoids level was found in the type of *Chlorella* sp. (25.5958 g/mL) while the lowest was in the type of *Navicula* sp. (12.8294 g/mL). The data indicate that the salinity parameter (>30≤36‰) greatly influences and thus spikes growth in cells due to their biomass and high carotenoid content. The environment parameter also contributes significantly to other physiological parameters (one-way ANOVA, $p<0.05$).

Keywords: Biomass, carotenoids, growth, microalgae, phytoplankton, salinity

INTRODUCTION

The extensive and rich marine natural resources of Southeast Sulawesi hold great potential for alternative raw materials, primarily coming from its diverse varieties of microalgae, which have the potential for exploring other beneficial utilities. The exploration of microalgae, widely known as Phytoplankton, found in the seawaters of Southeast Sulawesi, Indonesia. It has been reported by Indrayani et al. (2018) that at least ten types of microalgae were identified in a small area of Tanjung Tiram and Bokori Island waters. The immense potential of microalgae found rooted in diverse strains makes the seawaters of Southeast Sulawesi essential to be studied and explored spatially. The information that may be further explored can range from species according to their geography, growth promoter (Solimeno et al. 2015; Sathasivam et al. 2018; Zhuang et al. 2021) and contents for optimal utilization as prime raw material in the nutraceutical industry (Indrayani et al. 2018, 2019, 2021; Hynstova et al. 2018; Helena et al. 2020; Sathasivam et al. 2018; Zhuang et al. 2021).

Rapid microalgae growth can be achieved under a suitable climate within 24 hours to 3.5 hours during the exponential growth phase (Noer and Dessy 2012; Helena et al. 2020; Sathasivam et al. 2018; Zhuang et al. 2021). Generally, microalgae are known as phytoplankton, and most of them belong to the eukaryotic category. Microalgae such as Cyanophyta and Prochlorophyta are included in the prokaryotes while Glaucophyta, Rhodophyta, Heterokontophyta, Haptophyta, Cryptophyta, Dinophyta, Euglenophyta, Chlorarachniophyta and Chlorophyta are in eukaryotic microalgae groups (Barsanti and Gualteri 2006; Helena et al. 2020; Zhuang et al. 2021). The visual expression of microalgae cells varies, so these biotas can be distinguished based on the color of the cells by naked eye. The characteristic color of algal cells is primarily determined by the dominant type of pigment (photosynthesis) contained by the algae. For example, the *Chlorella* species have a lot of chlorophyll pigment so that their cells are green in color (Helena et al. 2020; Sathasivam et al. 2018; Zhuang et al. 2021; Andersen and Lewin 2018), and the Porpyridium species are reddish in color because they predominantly contain phycoerythrin pigment (Putri et al. 2020). Microalgae are unicellular microorganisms that can produce various types of carotenoids through photosynthesis. It also contributes significantly to the biological ecosystems of the oceans and freshwaters and has the potential to produce other bioactive compounds (Ambati et al. 2019; Sathasivam et al. 2018; Zhuang et al. 2021). Recent research related to the empirical study of the benefits of microalgae cells has been comprehensively discovered and disclosed through previous studies. Microalgal cells are rich in biochemical macromolecules which have functional role in meeting various human needs, including proteins, carbohydrates, lipids, pigments and other chemical molecules with high

commercial value. One of these macromolecules is a carotenoid. Carotenoids are fat-soluble pigments that give yellow, orange, or red colors to leaves, fruit, flowers, feathers, crustacean shells, meat, and fish skin (Del Campo et al. 2007; Negro and Garrido-Fernandez 2000). Naturally, carotenoid pigments are widely distributed in the biosphere; photosynthetic and non-photosynthetic organisms collectively produce and develop around 700 of their compounds (Lamers et al. 2008; Britton et al. 2008). The composition of carotenoids contained and the largest producer are from the microalgae group, where the types of carotenoids consist of astaxanthin, zeaxanthin, lutein, α-carotene, β-carotene, δ-carotene, myxoxanthin, fucoxanthin, violaxanthin, alloxantin, dinoxanthin, canthaxanthin, gyroxanthin, diadinoxanthin, cryptoxanthin, and peridinin (Borowitzka 2013; Solovchenko and Khozin 2017). Several environmental parameters, especially salinity, strongly influence the physiology of marine microalgae. Fluctuations in water salinity, or 'anomalies', directly affect microalgae biological activity (El-Baz et al. 2002; Indrayani et al. 2018, 2019, 2021; Pisal and Lele 2005). This observation of the salinity-induced activity phenomenon is the scientific basis for the different salinity treatments introduced to microalgae cultivation media obtained from Southeast Sulawesi waters. The different salinity treatments can presumably induce different expressions of cell growth and physiological conditions in microalgae.

Therefore, the research on "Growth, biomass, and carotenoids content analysis of the microalgae *Navicula* sp. and *Chlorella* sp. in batch cultures with different salinities" is essential to do, considering the limited availability of the latest related research, particularly in Southeast Sulawesi waters. Until this article was written, the current research was only conducted by Indrayani et al. (2021). Hence, this study aims to determine the effect of different salinities on cultivation media on the growth, biomass, and carotenoids content of *Chlorella* sp. and *Navicula* sp.

MATERIALS AND METHODS

Sources of algae strains

This research was conducted from October to December 2021. The samples used in this study were obtained from the seawater of Southeast Sulawesi. The microalgae strains were isolated using the agar plating technique (Andersen and Kawachi 2005) in medium f/2 (Guillard and Ryther 1962). The non-axenic strains are maintained and kept together with the microalgae culture collection at the Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia.

Culture condition

The *Navicula* sp. and *Chlorella* sp. were cultured in 300 mL Erlenmeyer containers with 150 mL Walne's nutrition at 30, 33, and 36 ppt salinity. Each treatment was conducted in three replications. Cultures were incubated at room temperature, and the culture growth was monitored by counting the number of cells every two days using a Neubauer hemocytometer (Moheimani et al. 2013). The harvesting process is carried out in two stages after reaching the 7th day of cultivation: first, to calculate biomass, and second, for carotenoid extraction.

The study aimed to observe the growth rate (by density), biomass, and carotenoids content of microalgae cultivated in different salinity media (30, 33, and 36‰) under room temperature (25°C) and 48-watt light exposure, from the initial phase of growth to the final phase (7 days). Observations of the cell density and growth rate were carried out every day using a microscope. Seawater as the medium culture had undergone a filtration process from *UPTD Balai Benih Udang* (The Regional Technical Implementation Unit of Shrimp Seed Center; unofficial translation) of Southeast Sulawesi Province. The seawater filtration is carried out to separate impurities using a filter bag (50 microns). The filtered seawater is then kept in a 1000 mL Erlenmeyer and wrapped in aluminum foil for the following sterilization process using an autoclave (Wiseclave WACS-1045). The seawater sterilization was done at 121°C for 15 minutes to ensure the absence of contamination by biotic pathogens (Indrayani et al. 2018, 2019, 2021). The seawater filtering and preparation were carried out at the Productivity and Aquatic Environment Laboratory, Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari, Indonesia.

Microalgae identification (DNA barcoding)

Identification species of microalgae obtained at the research location was carried out using the DNA Barcoding method. The analysis stages of DNA Barcoding were carried out through DNA extraction (Orozco-Castillo et al. 1994; Budiani et al. 2016), DNA amplification (PCR) (Ghosh and Love 2011), DNA electrophoresis, DNA sequencing, and bioinformatics analysis (Tamura and Nei 1993).

Data analysis

Microalgae cell density

The number of cultured cells was counted by a Neubauer hemocytometer (Moheimani et al. 2013). This step corresponds with Krebs (1989), who stated that aspects of the abundance of aquatic biota populations could be identified by measuring their density. Density is the size of the population in a unit of space expressed in the number of individuals from the population in a unit (Odum 1971). The density of microalgae cells obtained from the sea of Southeast Sulawesi was counted in 16 small squares by the hemocytometer, with the number of cells × 10^4 cells/mL. The counting chamber has a thickness of 0.1 mm. The suspended microalgae cells will fill the counting chamber so that the number of microalgae per unit volume can be known. Padang (2018) stated that the cell density could be calculated using the formula:

N x 10^4 cells/mL

For: N = The average number of cells per counting box

x 104 = The actual cell density in 1 mL of medium or water

cell/mL = Phytoplankton density units

After calculating the highest density in 1 medium box with 16 small boxes, the data are collected and calculated.

The results will be processed with Microsoft Excel to obtain graphs and further analysis.

Specific Growth Rate (SGR)

The specific growth rate of microalgae (*k*) is calculated by the formula by Hirata et al. (1981):

$$k = 3{,}22\,\frac{\log\left(\frac{Nt}{N0}\right)}{Tt - T0}$$

N_t is the microalgae density at time *t*, N_0 is the initial microalgae density, 3.22 is a constant, T_0 is the initial time, and T_t is the observation time.

Determination of dry weight (DW) and ash-free dry weight (AFDW) of biomass

The determination of DW and AFDW refers to standard methods for measuring microalgae growth (Moheimani et al. 2013). The DW was calculated according to the following equation:

$$\text{Dry weight (g/L)} = (\text{weight of filters plus algae}) - (\text{weight of filters})$$

The dried sample was then transferred to the furnace oven at 450°C for 5 hours. The organic dry weight or AFDW is calculated using the following formula:

$$\text{Ash-free dry weight (g/L)} = (\text{Dry weight}) - (\text{weight after ashing})$$

Determination of carotenoids content

The harvesting of carotenoid extraction steps from each microalgae sample from the same treatment was done by taking 150 mL of filtrate filtered with Whatman fiberglass paper. 2 mL of acetone was added to the sample and then centrifuged for 10 minutes at 3500 rpm. The sample will be separated into pellets and supernatants. The pellet was taken, added to another 2 mL of acetone, and centrifuged for 30 minutes at the same speed. The second result of the supernatant will be used. The supernatant was then transferred into a sterile test tube and diluted by adding 6 mL of acetone. The absorbance was measured using a spectrophotometer with wavelengths of 380, 450, 475, and 500 nm. The wavelength with the maximum absorbance will be used to calculate the total value of carotenoids (Rodriguez-Amaya and Kimura 2004; Sukarman et al. 2014).

$$\text{Total Carotenoids} = \frac{A_{max}}{K} \text{ X } \frac{V_P \text{ X } F_P \text{ X } 100}{\text{Mass sample (g)}}$$

For: A_{max} : Maximum Absorbance
F_p : Dilution Factor
V_p : Acetone volume
K : Constant (250)

Statistical analysis

The experimental design used in this study was a completely randomized design with 3 treatments and 3 repetitions, so the total experimental units were 9 units. The data obtained were analyzed statistically using a one-way ANOVA ($\alpha = 0.05$). The analysis will be proceed with the LSD test (Less Significant Difference) with a 95% confidence level for a significant or significantly different result from the ANOVA test to see how far the difference is between the salinity treatments (Steel and Torie 1981).

RESULTS AND DISCUSSION

Identification result (DNA Barcoding)

The analysis stages were carried out through DNA extraction, DNA amplification (PCR), DNA electrophoresis, DNA sequencing, and bioinformatics analysis shows that: (i) Sample Id : "KB" Identified as: *Navicula* sp., (diatom), (ii) Sample Id : "WW" Identified as: *Chlorella* sp., (green algae). It is certain that the samples we received was identified as *Navicula* sp., for sample KB, *Chlorella* sp., for sample WW (Table 1). The results of microalgae identification using the DNA Barcoding method are presented in Figure 1.

DNA sequence of the tufA gene obtained from samples

KB>TTGGCCNAAACACCGTGGAAACTATCTTTATTATCAAA
CAAGGTTGGGTGTTCCAAATATTGTTGTTTTCTTAAACAAAGAA
GATCAAGTTGATGATCCGGAATTATTAGAATTAGTTGAATTAGA
AGTTCGTGAATTACTTTCTGCGTATGACTTCCCTGGCGATGATA
TTCCAATCTGTCCAGGATCAGCATTACAAGCTGTTGAAGCAATT
ACAGCAAATCCAACTATTAAACGTGGTGATAACGAATGGGTTGA
TAAAATTTATGCATTAATGGATGCAGTTGATGAATATATTCCAA
CACCAGAACGTGACACTGAAAAAACATTCTTAATGGCCGTTGAA
GATGTATTCTCAATTACAGGGCGAGGTACTGTAGCAACAGGTCG
TATTGAACGTGGTGTTATAAAAGTAGGTGATAGCATTGAAATTG
TGGGAATCTCTGAAACTAAATCAACAACAGTTACTGGTATTGAA
ATGTTCCAGAAAACTTTAGAAGAAGGGTTTGCGGGTGACAACGT
TGGTATCTTATTACGTGGTGTAACACGTGAAGATATTGAGCGTG
GAATGGTTTTAGCAAAACCAGGTACAATTACTCCACATACAAAC
TTTGAATCAGAAGTTTACGTTTTAACAAAAGATGAAGGTGGTCG
TCACACTCCATTCTTTACAGGTTACCGTCCACAATTCTATGTTC
GAACAACTGATGTAACAGGTGCAATTACTCAATTTACAGCTGAT
GATGGATCAGTTGTTGAAATGGTAATGCCTGGTGATCGTATTAA
AATGACAGCTGAATTAATTTACCCTGTAGCAATCGAAGAAGGAA
TGCGATTCGCGATTCGAGAAGGCCCT

WW>AAGGAACCTTTATTTGTTTAGGCACAACAAGTAGGTG
TACCAAATATAGTTGTTTTTTTAAATAAAGAAGATCAAGTTGAT
GATGCTGAATTACTTGAATTAGTTGAATTAGAAATTCGTGAAAC
TTTAGATAAATATGAATTTCCAGGTGATGAAATTCCAATTGTTG
CAGGATCTGCTTTATTAGCGTTAGAAGGTTTAGCAGAGAATCCA
GAAGTTAAACCAGGTGAAAATAAATGGATTGACAAAATTTATAA
TTTAATGGACCAAGTCGATTCTTATATCCCAACCCCAGAGCGTG
CTACTGATAAACCATTTTTAATGGCTGTTGAAGATGTTTTTTCT
ATTACAGGGCGTGGAACTGTTGCTACAGGACGTGTTGAGCGTGG
AACTGTTAAAATTGGTGATTCTGTAGAATTAGTTGGTTTACGAG
AGACTAAAACATTAACAGTAACTGGTTTAGAAATGTTTCAAAAA
ACTTTAGACGAAAGTGTAGCTGGTGATAACGTTGGTGTTTTATT
ACGTGGTGTTCAAAAAACTGAAATTGAACGAGGTATGGTTTTAG
CAAAACCTGGTTCAATTACTCCACATACAAAATTTGAATCACAA
GTTTATGTATTAACTAAAGAAGAAGGTGGTCGTCATACACCATT
TTTTCCAGGTTATCGACCACAATTTTACGTTCGTACAACTGATG
TAACTGGTAAAATTGAATCTTTTCGCGCAGATGATGATAGTCCG
ACTCAAATGGTAATGCCAGGTGATCGTATTAAAATGATCGTGGA
ACTAATTCAACCAATTGCTATTGAAAAAGGTATGCGTTTCGCTA
TTCGGGAAGGCCCTCCATTT

Table 1. BLAST (Basic Local Alignment Search Tool) analysis result based on NCBI for species identification

Sample Id	Query Cover (%)	Per. Ident (%)	Species Identified
KB	98	89.02	*Navicula* sp.
WW	90	99.09	*Chlorella* sp.

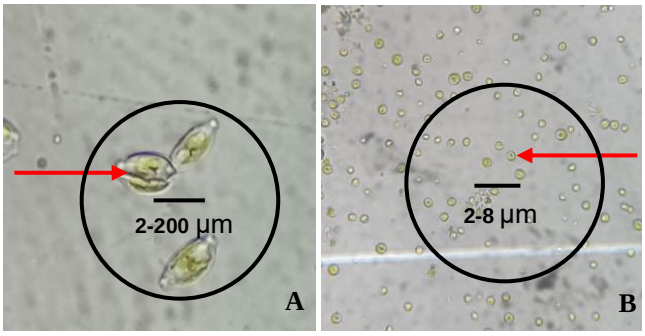

Figure 1. Identification result of microalgae based on DNA Barcoding, (A) *Navicula* sp., (B) *Chlorella* sp.

Microalgae density

The microalgae growth was observed by calculating the density of microalgae until the exponential phase ended on day 6 or before the phase of decreasing growth rate occurred (7th day). The histogram trend of microalgae growth is presented in Figures 2 and 3. The densities of both *Navicula* sp. and *Chlorella* sp. showed significant results or were significantly different by the salinity treatment (one-way ANOVA, $p<0.05$). Furthermore, in the analysis result of the LSD test, significant results were obtained between the salinity treatments on *Navicula* sp. (LSD, $p<0.05$). In contrast, a tendency towards an insignificant trend was observed in the *Chlorella* sp. data. For *Chlorella* sp., only treatment A was significantly different from treatment B, which occurred on day 6 (LSD, $p<0.05$), while the rest of the observations were not significantly different (LSD, $p>0.05$).

Specific Growth Rate (SGR)

The SGR of *Navicula* sp. showed a relatively similar trend with its cell density, where the highest growth phase was found on day 6 and was the peak of the exponential phase of *Navicula* sp. (Figure 4). Meanwhile, in *Chlorella* sp., the trend was inversely proportional to its cell density, where the second day was the peak of the exponential phase and tended to decrease in growth rate in the following days with a fluctuating trend until the end of the culture period (Figure 5). The SGR of *Navicula* sp. and *Chlorella* sp. showed significant or significantly different results on salinity. Therefore, the given salinity treatment affects both microalgae types' SGR (one-way ANOVA, $p<0.05$). Furthermore, the LSD test obtained results with the same trend as the cell density in each type. The results over salinity treatments were significant on *Navicula* sp. (LSD, $p<0.05$) but tended to be insignificant on *Chlorella* sp. (LSD, $p>0.05$), where only treatment A was significant over treatment B on the 6th day (LSD, $p<0.05$).

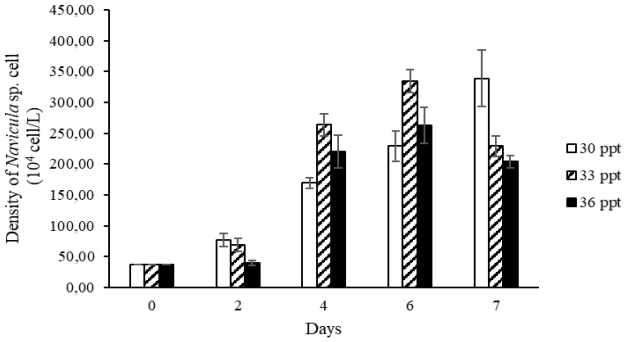

Figure 2. The average density of *Navicula* sp., cultivated at different salinities (A (30 ppt), B (33 ppt) and C (36 ppt))

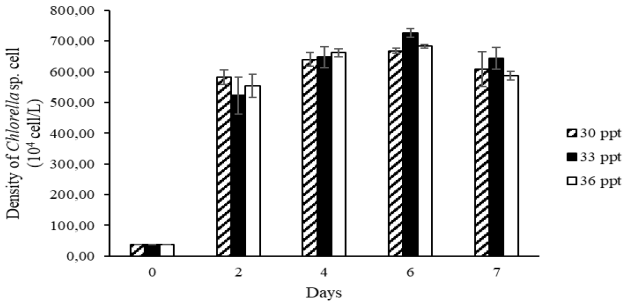

Figure 3. The average density of *Chlorella* sp. cultivated at different salinities (A (30 ppt), B (33 ppt) and C (36 ppt))

Microalgae biomass

The biomass of both microalgal types in each treatment showed moderately different values, both between treatments within one species or based on the treatment between types of microalgae. *Navicula* sp. and *Chlorella* sp. biomass showed significant or significantly different results for the salinity treatment (one-way ANOVA, $p<0.05$), while the further biomass test with LSD showed an inversely proportional trend or was not significantly different between salinity treatments (LSD, $p>0.05$). The average biomass of both microalgae in different salinity cultivations is presented in Figures 6 and 7.

Microalgal carotenoids content level

The carotenoid levels of both types of microalgae in each treatment are significantly distinct from each other. The high content of carotenoids shown in *Chlorella* sp., which produced 25.5958 g/mL of carotenoids, which is twice that of *Navicula* sp.'s 12.8294 g/mL, made *Navicula* sp. produce the lowest carotenoids level. Carotenoid levels in both types were observed to be affected by different media salinities (one-way ANOVA, $p<0.05$). LSD tests on the *Navicula* sp. data showed significantly different results between treatment A with B and C (LSD, $p<0.05$). Treatment B was not significantly different from C (LSD, $p>0.05$) but significantly different from A (LSD, $p<0.05$), as was treatment C, which was significant for treatment A (LSD, $p < 0.05$) but not significant for treatment B (LSD, $p>0.05$). In *Chlorella* sp., the results were not significantly different between the salinity treatments (LSD, $p>0.05$). The average levels of microalgae carotenoids cultivated at different salinities are presented in Figures 8 and 9.

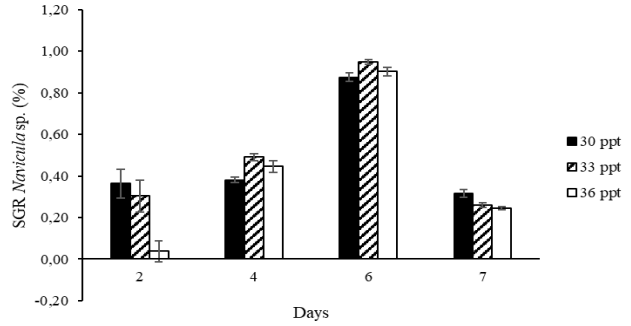

Figure 4. The SGR *Navicula* sp. cells cultivated at different salinities (A (30 ppt), B (33 ppt) and C (36 ppt))

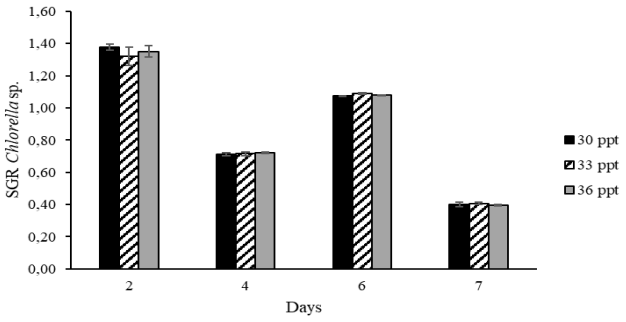

Figure 5. The SGR *Chlorella* sp. cells cultivated at different salinities (A (30 ppt), B (33 ppt) and C (36 ppt))

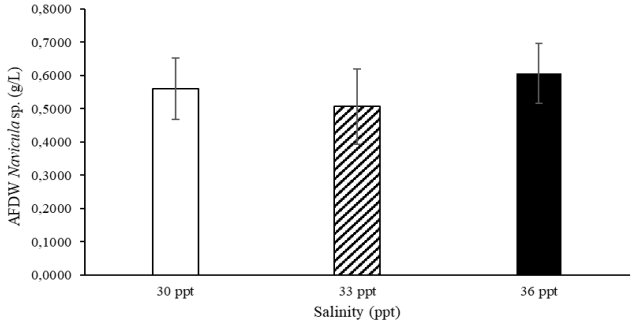

Figure 6. *Navicula* sp., biomass trend in different salinity (A (30 ppt), B (33 ppt) and C (36 ppt))

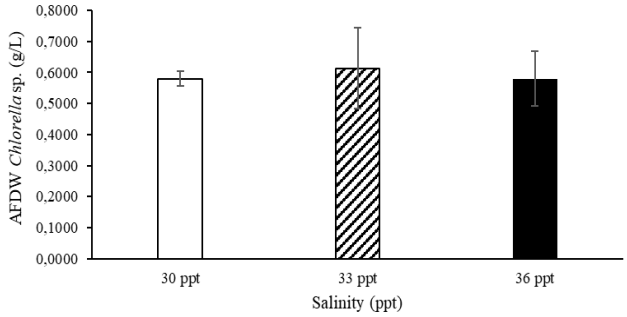

Figure 7. *Chlorella* sp. biomass trend in different salinity (A (30 ppt), B (33 ppt) and C (36 ppt))

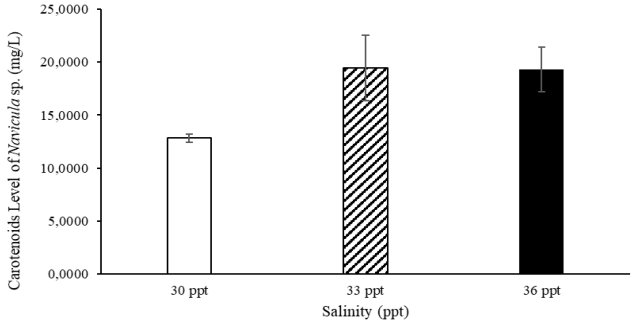

Figure 8. Levels of carotenoids of *Navicula* sp., according to different salinity (A (30 ppt), B (33 ppt) and C (36 ppt))

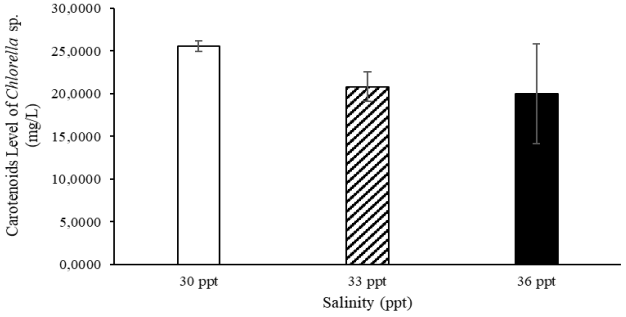

Figure 9. Levels of carotenoids produced by *Chlorella* sp., according to different salinity (A (30 ppt), B (33 ppt) and C (36 ppt))

Biomass productivity

The highest productivity of *Navicula* sp. biomass was obtained on the 6th day, while that of *Chlorella* sp. was obtained on the 2nd day. The growth of microalgae cells of the type *Chlorella* sp., therefore, is much faster than that of *Navicula* sp., which experienced a climax period of the exponential phase a few days later on the 6th day. The biomass productivity of *Navicula* sp. and *Chlorella* sp. was confirmed to be influenced by culture salinity (one-way ANOVA, $p<0.05$), but the LSD test showed that in *Navicula* sp., the biomass productivity tended to be insignificantly different between treatments during the culture period (0-7 days). Significantly different results were only obtained on day 2, namely treatment C over A and B (LSD, $p<0.05$), whereas B was not significantly different from A (LSD, $p>0.05$) but significantly different from C (LSD, $p < 0.05$). Treatment A was significant to the treatment C (LSD, $p<0.05$) and not significant for treatment B (LSD, $p>0.05$). Meanwhile, in *Chlorella* sp., the biomass productivity was not significantly different between the salinity treatments (LSD, $p>0.05$). Biomass productivity are presented in Figures 10 and 11.

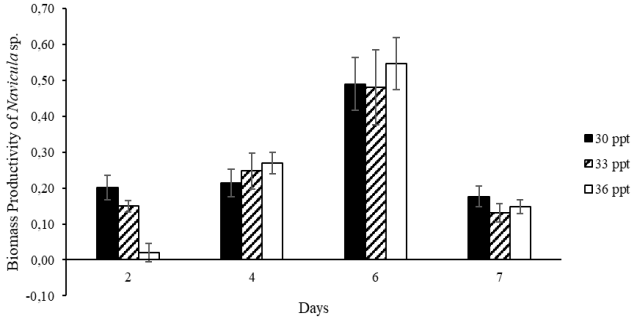

Figure 10. Biomass productivity of *Navicula* sp., (A (30 ppt), B (33 ppt) and C (36 ppt))

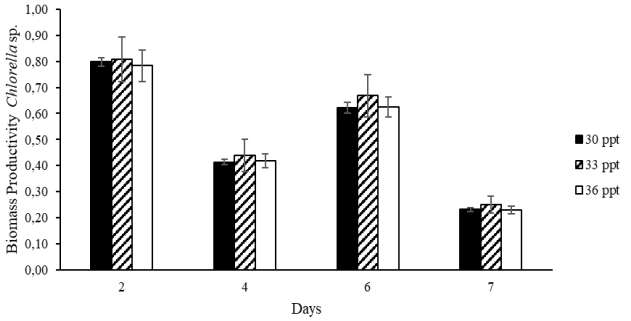

Figure 11. Biomass productivity of *Chlorella* sp., (A (30 ppt), B (33 ppt) and C (36 ppt))

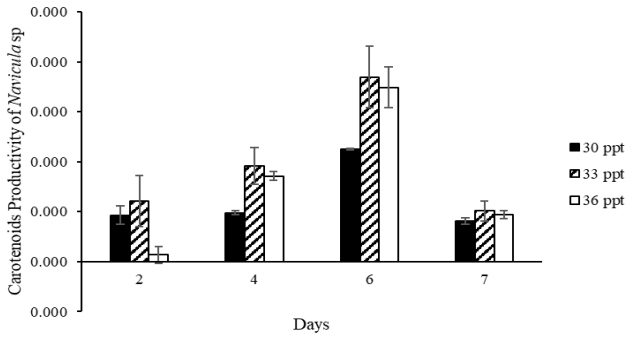

Figure 12. Productivity of *Navicula* sp. carotenoids by day (A (30 ppt), B (33 ppt) and C (36 ppt))

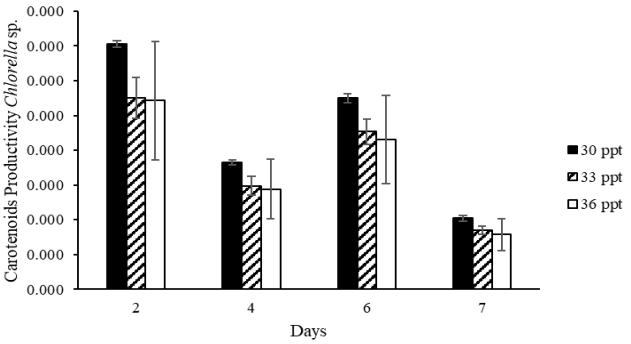

Figure 13. Productivity of carotenoids *Chlorella* sp., by day (A (30 ppt), B (33 ppt) and C (36 ppt))

Carotenoids productivity

The carotenoids production of *Navicula* sp. and *Chlorella* sp. is proportional to the productivity of their biomass, where the trends tend to be linearly increased in magnitude. The highest production of *Navicula* sp. carotenoids was obtained on the 6th day, while *Chlorella* sp. was on the 2nd. This result indicates the integral relationship between the two variables, which is directly related to the SGR of strains. The carotenoids productivity of both microalgae was confirmed to be influenced by different media salinities (one-way ANOVA, $p<0.05$).

The LSD test showed that the carotenoids productivity of *Navicula* sp. was not significantly different between treatments on day 7 (LSD, $p>0.05$) and significantly different on the other days (days 2 to 6), with details as follows: Day 2, treatment C was significant for A and B (LSD, $p<0.05$); treatment B was not significantly different from A (LSD, $p>0.05$) but significantly different from C (LSD, $p<0.05$); and treatment A was significant for treatment C (DSD, $p<0.05$) and not significant for B (LSD, $p>0.05$). On the other hand, on days 4 and 6, treatment A was significant to B and C (LSD, $p<0.05$), while B was not significantly different from C (LSD, $p>0.05$) but significantly different from A (LSD, $p<0.05$). Treatment C was significant for A (LSD, $p<0.05$) and not significant for B (LSD, $p>0.05$). Whereas for *Chlorella* sp., all the results were not significantly different between the salinity treatments (LSD, $p>0.05$). Carotenoids productivity are presented in Figures 12 and 13.

Discussions

Initial observation of the growth of microalgae cultivated on different salinities (30, 33, 36‰) was carried out by calculating the microalgae density until the climax of the exponential phase, namely on day 6. The microalgae cell density was calculated using a hemocytometer and observed with a light microscope with 40× magnification. The observations made over 7 days showed that the exponential phase occurred from day 2 to day 6, and on day 7, the growth decline phase began. The proportion of cell density from day 1 to day 3 shows the condition of cells that adapt or undergo metabolism before some divisions occur. On the 4th day, the cells begin to experience division by utilizing the nutrients provided in the culture container, so that cell growth slowly begins to increase until, on the 6th day, it reaches the end of the exponential phase. On the 7th day, the number of cells decreased. Therefore, it can be concluded that the average growth of microalgae in the culture media shows an exponential phase on day 6. A color change in the culture vessel can indicate increased cells in the culture.

In the experimental unit, the exponential phase occurred at the beginning of the treatment, namely on the first day of observation. After passing this phase, the cell growth rate began to decrease due to a lack of nutrients, light, or other chemical and physical factors. The cell growth in this phase

will continue into a stationary phase until the death phase. Nevertheless, some of the cells were still alive at the end of the observation due to the nutrient availability that was not completely depleted in the container. Balaira (2017) states that the exponential growth stage reaches its peak on days 6-8 in general. This phase is where microalgae proliferate until they decrease after approaching the growth decline phase, which is also influenced by the concentration of media salinity as a limiting factor for every microalgal physiological activity. In this study, the statement was confirmed that the end of the exponential phase that occurred on day 6 or day 7 of both types will change phase (into decreased growth). Furthermore, Lukitasari (2015) revealed that medium salinity significantly affects the growth rate, maintaining osmotic pressure between cells and water as a living environment for microalgae.

The results of the growth rate earlier discussed indicate the effect of medium salinity. Low salinity will cause microalgae cells to experience a hypotonic state, i.e., the condition of the solution in the environment has a lower concentration than inside the microalgae cells. Therefore, the cells will expand and swell due to the tendency of water to move across the osmotic pressure gradient, passing a partially permeable cell membrane. Meanwhile, high salinity will cause microalgae cells to experience a hypertonic state, where the solution conditions in the environment have a higher concentration than the inside cells, which causes the cells to shrivel and shrink. This trend also occurs in Aulia et al.'s (2021) study, where the growth of *Chlorella* sp. is in line with salinity, increment, and decrement. This trend shows a significant effect on the osmotic pressure in cells and osmoregulation mechanisms, which can directly affect the metabolic system, resulting in a decrease in the microalgae cell population (Sutomo 1991).

Salinity is essential for pigment formation, biomass production, and cell growth. It is also a limiting factor for the growth of microalgae. It is reported that the optimum salinity in the culture medium for *C. vulgaris* is around 30-35 ppt (Kasim et al. 2017). Other research also provided similar findings, such as Mahardani et al. (2017), who stated that the population density of *Dunaliella* sp. experienced the highest at salinity of 30 ppt (5.09×106 cells/mL) and 35 ppt (4.70×106 cells/mL) in 138 hours, whereas the population peak in 40 ppt achieved at 132 hours (4.15×10^6 cells/mL)—followed by the salinity of 45 ppt (3.98×106 cells/mL) at 156 hours. The average population density of *Dunaliella* sp. was the highest at 30 ppt, followed by a salinity of 35 ppt, 40 ppt, and 45 ppt, consecutively. The difference in cell density of *Dunaliella* sp. between these treatments is due to differences in salinity in the culture medium. Based on the research by Sukmawan et al. (2012), the salinity level in the culture medium affects the density of microalgae. Different salinity treatments gave a biomass trend that was mildly different or showed values that tended to be the same, ranging from 0.0176 to 0.0181 g/mL. This finding shows that the salinity treatments (30, 33, and 36‰) did not induce maximum stimulation for the somatic growth of the two types of microalgae that were cultured, but that does not mean that salinity does not have a strong correlation with microalgae biomass.

The salinity concentration in this study was picked from a range of optimal salinities to support the life of organisms in marine waters. Therefore, it will not harm nor cause significant fluctuations in microalgae biomass. Aulia et al. (2021) stated that salinity is one of the parameters that influences the accumulation of phytoplankton biomass in a culture. Salinity has a role in the survival of microalgae and can cause inhibition of metabolic activity that correlates to decreased photosynthesis and is related to biomass formation. The findings in this study are also in line with Gu et al. (2012) and Aulia et al. (2021), who stated that, in general, the formation of microalgae biomass experienced a declination in media with low (<30 ppt) or too high (± 45 ppt) salinity. This condition will relate to decreased photosynthetic efficiency due to stress caused by salinity. When microalgae grow on media that have too high or too low a salinity, the microalgae will require more energy to maintain osmotic pressure, causing a decrease in biomass or microalgae growth.

The carotenoid levels showed distinct differences between strains in this study, where carotenoid levels for both types ranged from 12.8294-25.5958 g/mL. The highest carotenoids were found in *Chlorella* sp. (25.5958 g/mL), and the lowest were in *Navicula* sp. (12.8294 g/mL). The finding in response to the change in salinity is in line with the research by Mahardani et al. (2017), who stated that the highest carotenoids content was produced by *Dunaliella* sp., particularly in the 35 ppt salinity treatment, which accumulated 1.0668 mg/mL carotenoids. Another treatment using 30 ppt salinity results in a carotenoid content of 1.0080 mg/mL. The carotenoid content in the 40 ppt treatment accumulated to 0.7980 µg/mL, while the sample using a 45 ppt salinity treatment resulted in 0.7056 µg/mL carotenoids. According to research by Pisal and Lele (2005), increasing salinity can increase the carotenoid content of *Dunaliella salina* microalgae from 2 pg/cell to 5.5 pg/cell.

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