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RESEARCH ARTICLE

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The effect of different light colors on the growth of *Nannochloropsis* sp.

Nurul Istiqomah[1,*], Alis Mukhlis[2], Zaenal Abidin[2]

[1]*Master Program in Fisheries and Aquatic Biotechnology, Universitas Airlangga, Indonesia*
[2]*Aquaculture Study Program, Universitas Mataram, Indonesia*

**Correspondence: nurul.istiqomah-2023@fpk.unair.ac.id*

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Abstract

The purpose of this study was to determine the effect of different light colours on population density, relative growth and self-replication time in *Nannochloropsis* sp. This study used a completely randomized design experiment involving 5 treatments and 4 replications. Treatment A uses red light, treatment B uses yellow light, treatment C uses green light, treatment D uses blue light, and treatment E uses white light. Density observations were made every 36 hours. The results of the study showed that differences in the colour of light had a significant effect on population density and relative growth of *Nannochloropsis* sp. but no significant effect on self-replication time.

Keywords: *Nannochloropsis*, growth, light

Introduction

Natural feed is a critical factor in supporting the growth and survival of cultivated organisms, particularly during the larval or seedling stages. The availability of natural feed that meets nutritional requirements can enhance seed quality and ensure the sustainability of seed production[1]. One of the microalgae species commonly developed as a natural feed source is *Nannochloropsis* sp[2]. This microalga is known for its high nutritional content, which includes 52.11% protein, 16% carbohydrates, 27.64% lipids, 0.85% vitamin C, and 0.89% chlorophyll-a[3,4].

As a photosynthetic microalga, light is a crucial factor in the growth of *Nannochloropsis* sp. Photosynthesis is a process by which inorganic compounds are converted into organic compounds with the aid of sunlight[5]. Sunlight consists of various electromagnetic spectra, each with different wavelengths, ranging from radio waves to gamma rays. The spectrum absorbed by plants for growth activities lies within the visible light range, which comprises seven colors: red, orange, yellow, green, blue, indigo, and violet[6]. Photosynthesis occurs due to the presence of chlorophyll, the green pigment in microalgae. In general, chlorophyll absorbs light with wavelengths between 600–700 nm, corresponding to the red spectrum, and between 400–500 nm, corresponding to the blue spectrum[7]. Therefore, this

study aims to investigate the ability of *Nannochloropsis* sp. to utilize different light color spectra for its growth.

Material and methods

Time and location

This study was conducted over 35 days, from May 5 to June 10, 2023, at the Independent Laboratory and the Environmental Aquaculture Laboratory, Aquaculture Study Program, University of Mataram.

Study design

The research employed an experimental approach by exposing *Nannochloropsis* sp. cultures to different light colors over a 10-day period. The experiment consisted of five treatments, each with four replicates. The treatments were as follows: Treatment A (*Nannochloropsis* sp. culture exposed to red light), treatment B (*Nannochloropsis* sp. culture exposed to yellow light), treatment C (exposed to green light), and treatment D (exposed to blue light), and treatment E (exposed to white light)[8] (**Figure 1**).

Figure 1. Layout of the experiment units

Research procedure

The culture containers were filled with sterilized seawater and pre-acclimated *Nannochloropsis* sp. stock cultures. Each container was filled with seawater and *Nannochloropsis* sp. stock according to the predetermined volumes, bringing the total volume of each container to 2 litres. Subsequently, each container was supplemented with 0.5 ml/L of KW21 fertilizer. After preparation, the containers were arranged based on the designated treatments and equipped with aeration. Observations of *Nannochloropsis* sp. density were conducted every 36 hours over a 9-day period, specifically at 36 hours, 72 hours, 108 hours, 144 hours, 180 hours, 216 hours, and 252 hours. Cell density was determined using a Neubauer improved haemocytometer and a binocular microscope with a 40x objective lens. Samples of *Nannochloropsis* sp. were collected using a micropipette (0.1 ml) and dispensed onto two chambers of the hemocytometer, then covered with a cover glass for counting[9].

Water quality parameters

Water quality measurements were conducted throughout the maintenance period, focusing on parameters such as temperature, dissolved oxygen (DO), pH, and salinity. Temperature was measured

using a thermometer and reported in degrees Celsius (°C). Dissolved oxygen was measured using a DO meter and expressed in ppm (mg/L). The pH was assessed with a pH meter, while salinity was measured using a refractometer and reported in ppt (gram/liter).

Research parameters

Growth and density of** Nannochloropsis **sp.

The growth and density of *Nannochloropsis* sp. were calculated using the following formula[10]:

$\frac{Cell\ count}{ml} = \frac{A+B+C+D+E}{5} \times 25 \times 10\ 4$

Where A, B, C, D, E is number of cells counted, 5 is number of squares observed in the counting chambers), and 25 is total number of squares in haemocytometer.

Relative growth of *Nannochloropsis* **sp.**

The relative growth rate (RGR) was calculated using the formula adapted from Mukhlis et al.[11]:

$RGR = (\frac{Ct-C0}{C0}) \times 100\%$

Where RGR is percentage of relative growth rate (%), C0 is initial cell population density (cells/mL), and Ct is maximum cell population density observed during the experimental period (cells/mL).

Doubling time of *Nannochloropsis* **sp.**

The doubling time (DT) was calculated using the formula adapted from Mukhlis et al.[11]:

$DT = \frac{\log 2 \times \Delta t}{\log Ct - \log C0}$

Where DT is double time (hours), C0 is initial cell population density (cells/ml), Ct is final cell population density (cells/ml), and Δt is duration of the observation period (hours).

Data analysis

The data collected from this study were statistically analyzed using Analysis of Variance (ANOVA) with a 5% significance level ($p<0.05$) in Microsoft Excel. If significant differences were observed, further analysis was conducted using the Least Significant Difference (LSD) test to identify specific treatment differences.

Result

Density of *Nannochloropsis* sp.

Figure 2 illustrates the growth trend of *Nannochloropsis* sp. over 216 hours. At the beginning of the culture period, the population density was low but began to increase in subsequent days. Treatments A, B, and E showed peak growth at 180 hours, with average densities of 25.198 x 103; 8.209x 103; and 12.385 x 103 cells/mL, respectively. In contrast, Treatments C and D reached their peak densities at 144 hours, with average densities of 6.385 x 103, and 9.688 x 103 cells/mL, respectively. These results indicate that the highest population growth was observed in Treatment A, which utilized red light. Analysis of variance revealed that the different light color treatments had a significant effect on the population density of *Nannochloropsis* sp. ($p <0.05$). Post-hoc analysis using the LSD test further confirmed that treatment A (red light) differed significantly from all other treatments.

RGR of *Nannochloropsis* sp.

Based on **Figure 3**, the relative growth of *Nannochloropsis* sp. indicates that the highest relative growth rate was observed in Treatment A (red light) at 1,700%. This was followed by treatment E, D, B, and C, with relative growth rates of 78.5%, 59.2%, 48.6%, and 35.6%, respectively. ANOVA ($p <0.05$) revealed that different light color treatments had a significant effect on the relative growth of

Nannochloropsis sp. Post-hoc analysis using the Least Significant Difference (LSD) test confirmed that treatment A (red light) significantly differed from all other treatments.

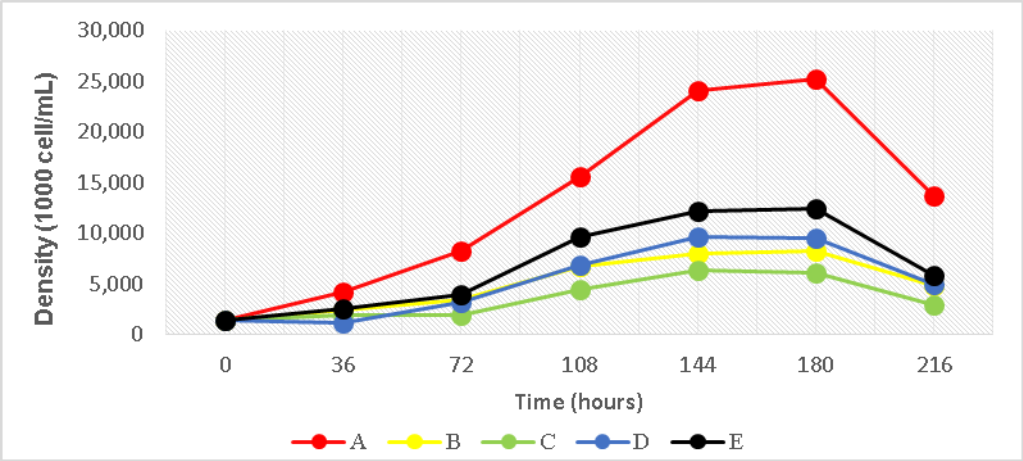

Figure 2. Population density of *Nannochloropsis* sp.

DT of *Nannochloropsis* sp.

Figure 4 shows the doubling time of Nannochloropsis sp.. The shortest doubling time was observed in treatment A (red light) at 43.90 hours. This was followed by treatment E, D, C, and B, with doubling times of 61.40 hours, 56.25 hours, 71.45 hours, and 75.32 hours, respectively. ANOVA ($p < 0.05$) indicated that different light color treatments did not have a significant effect on the doubling time of *Nannochloropsis* sp. Therefore, further post-hoc tests were not performed.

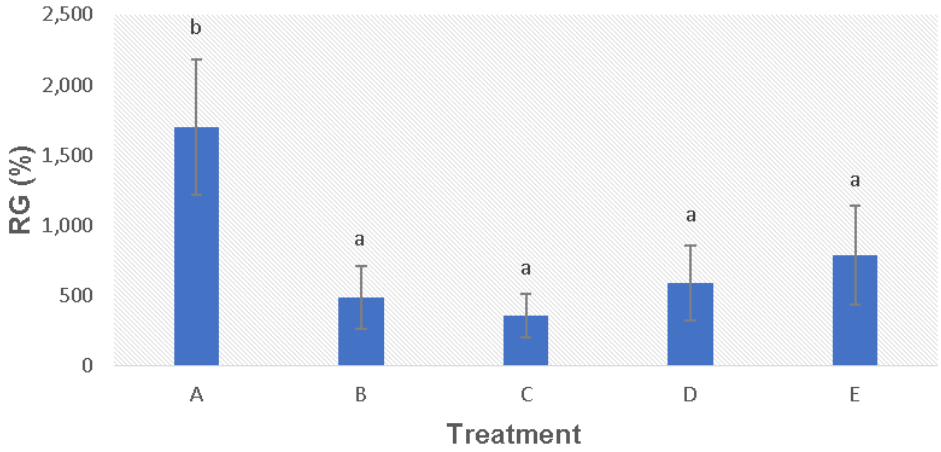

Figure 3. RG of *Nannochlorpsis* sp. (A: red, B: yellow, C: green, D: blue, and E: white).

Water quality

Result of water quality content is illustrated on **Table 1** consists temperature, pH, DO, and salinity with respective measurements and references.

Discussion

Based on **Figure 2**, the lag phase of *Nannochloropsis* sp. was observed between 0 and 72 hours. During this phase, the microalgae adapted to the light conditions and culture environment. According to

Prayitno (2016), the lag phase is characterized by the physiological adjustment of microalga cells to environmental conditions. During this time, cells prepare for division by synthesizing essential enzymes and metabolic compounds. However, cell division is limited, resulting in minimal population growth. The exponential phase occurred between 72 and 144 hours, marking a period of rapid cell population increase following the adaptation phase. As described by Prayitno[12], during the exponential phase, cells undergo rapid division, supported by the availability of enzymes and metabolites necessary for this process. This phase is also characterized by high rates of CO_2 absorption, rapid biomass production, and accelerated nutrient uptake from the culture medium, which gradually depletes available nutrients.

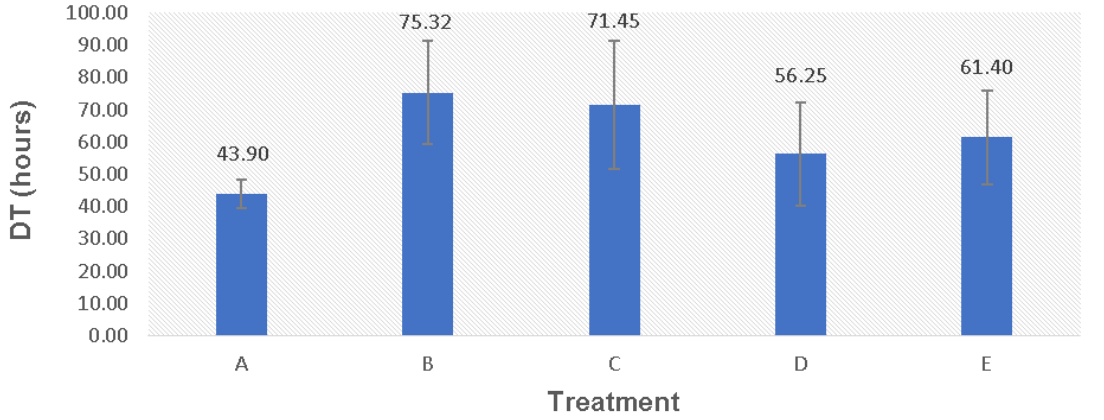

Figure 4. DT of *Nannochlorpsis* sp. (A: red, B: yellow, C: green, D: blue, and E: white).

After the exponential phase, *Nannochloropsis* sp. entered the stationary phase. In this study, the stationary phase was not clearly identified due to the 36-hour intervals between density observations. According to Istirokhatun et al.[13] , the transition between the stationary and decline phases is typically brief, requiring more frequent sampling, ideally more than once every 24 hours. Prayitno[12] further explains that the stationary phase is defined by a balance between the rates of cell growth and cell death. The final phase, the death phase, occurred between 144 and 216 hours for treatments C and D, and between 180 and 216 hours for treatments A, B, and E. This phase was marked by a significant decline in cell density. As noted by Prayitno[12], the death phase is characterized by the extensive mortality of cells and the near cessation of cell division due to the depletion of growth-supporting factors.

Table 1. Results of water quality measurements

Parameter	**Range**	**Reference**
Temperature (°C)	29.2-29.8	25-30
pH	7.7-8.0	8.0-9.5
Dissolved Oxygen/DO (mg/L)	5.1-5.3	4.0-7.5
Salinity (ppt)	36-38	25-35

The differences in the cell density of *Nannochloropsis* sp. are attributed to the varying light colours used in the treatments. Treatment A, utilizing red light, exhibited the highest cell density compared to other treatments. The increase in cell density is influenced by photosynthesis, a process that involves chlorophyll to absorb light. *Nannochloropsis* sp. contains chlorophyll-a is a green pigment essential in

photosynthesis for capturing light[14,15]. Furthermore, as noted by Saputra et al.[16], chlorophyll-a exhibits peak absorption at a wavelength of 660 nm, corresponding to red light. Therefore, the highest cell density observed in Treatment A is due to the ability of chlorophyll-a in *Nannochloropsis* sp. to effectively absorb red light.

Relative growth is determined by the percentage difference between the initial and peak cell densities, compared to the initial density. Treatment A demonstrated the highest relative growth percentage, attributed to the ability of chlorophyll-a in *Nannochloropsis* sp. to efficiently absorb red light, leading to enhanced growth under red light conditions. As described by Hanryani et al.[17], the red spectrum, with wavelengths ranging from 630 to 675 nm, is utilized to generate energy during photosynthesis. This energy is subsequently used to support cell growth.

The doubling time reflects the growth rate of *Nannochloropsis* sp., where a shorter doubling time indicates faster growth, while a longer doubling time corresponds to slower growth. The minimum doubling time is achieved when the specific growth rate reaches its maximum. This aligns with the statement by Afriza et al.[18], which asserts that a lower doubling time signifies a faster increase in population size, as cells require less time to divide, allowing the population to reach maximum density more quickly.

Water quality is a critical limiting factor for the growth and development of microalgae, which are aquatic organisms. The observed water quality parameters during the study are presented in Table 1. Phytoplankton density in aquatic environments is influenced by environmental factors, including temperature, pH, DO, and light. Temperature directly affects photosynthetic efficiency and plays a pivotal role in determining microalga growth. During the culture process, the temperature across treatments ranged from 29.2-29.8°C, which falls within the optimal range for *Nannochloropsis* sp. growth, as noted by Saputra et al.[16], who stated that the ideal temperature range for *Nannochloropsis* sp. is 25–30°C.

The culture medium's pH ranged from 7.7 to 8.0. According to Saputra et al.[16], *Nannochloropsis* sp. thrives within a pH range of 8.0–9.5. Therefore, the observed pH values are adequate to support the growth and survival of *Nannochloropsis* sp. The availability of dissolved oxygen is a critical determinant for the growth of Nannochloropsis sp., as it is essential for the synthesis of organic molecules during photosynthesis. In this study, dissolved oxygen levels ranged between 5.1 and 5.3 ppm, aligning with the optimal range of 4.0 to 7.5 ppm as identified by Damanik et al.[19].

Salinity levels across the treatments were measured between 36 and 38 ppt, which is considered suboptimal for the growth of *Nannochloropsis* sp. The optimal salinity range for *Nannochloropsis* sp. is 25–35 ppt. The higher salinity levels observed at 180 hours (7.5 days) near the end of the study are likely due to evaporation[20]. Damanik et al. [19] explained that changes in salinity from the beginning to the end of a study are often attributed to evaporation processes.

Conclusions

This study concludes that light color significantly affects the cell population of *Nannochloropsis* sp. Red light demonstrated a significant effect ($p < 0.05$) on the peak density and relative growth of *Nannochloropsis* sp. populations compared to yellow, green, blue, and white light. However, it did not have a significant impact ($p > 0.05$) on the doubling time.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest

References

1. Lestari U, Mukhlis A, Priyono J. Effect of nutrisil and KW21+SI fertilizer on *Chaetoceros calcitrans* growth. *Jurnal Perikanan UNRAM*. 2019;9(1):66-74. doi:10.29303/jp.v9i1.137.
2. Zanella L, Vianello F. Microalgae of the genus *Nannochloropsis*: Chemical composition and functional implications for human nutrition. *Journal of Functional Foods*. 2020;68:103919. doi:10.1016/j.jff.2020.103919
3. Erlania, Widjaja F, Adiwilaga E. Instant rotifer (*Brachionus rotundiformis*) storage at different temperatures, feeding with concentrate microalgae. *Jurnal Riset Akuakultur*. 2010;5(2):287-297.
4. Dangeubun J, Letsoin P, Syahailatua D. Growth of *Nannochloropsis* sp. in culture media enriched with shrub-like annual Clerodendrum minahassae leaf extract. *AACL Bioflux*. 2020;13(5):2807-2815.
5. Brown N, Newel IC, Stanley S, et al. Independent and parallel recruitment of preexisting mechanisms underlying C_4 photosynthesis. *Science*. 2011;331(6023):1436-1439. doi:10.1126/science.1201248.
6. Leister D. Enhancing the light reactions of photosynthesis: Strategies, controversies, and perspectives. *Moecular Plant*. 2023;16(1):4-22. doi:10.1016/j.molp.2022.08.005.
7. Santoso J, Suhardjono H, Wattimury A. Kajian nilai curs spektrum warna terhadap warna cahaya matahari dan cahaya buatan untuk pertumbuhan tanaman. In: *Seminar Nasional Magister Agroteknologi Fakultas Pertanian UPN "Veteran" Jawa Timur*. 2020:11-22. doi:10.11594/ nstp.2020.0602.
8. Vadiveloo A, Moheimani N, Cosgrove J, et al. Effect of different light spectra on the growth and productivity of acclimated *Nannochloropsis* sp. (Eustigmatophyceae). *Algal Research*. 2015;8:121-127. doi:10.1016/j.algal.2015.02.001.
9. Wahidin S, Idris A, Shaleh S. The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. *Bioresource Technology*. 2013;129:7-11. doi:10.1016/j.biortech.2012.11.032.
10. Rizky Y, Raya I, Dali S. Penentuan laju pertumbuhan sel fitoplankton *Chaetoceros calcitrans, Chlorella vulgaris, Dunaliella salina,* dan *Porphyridium cruentum*. *Research report*. 2012. Universitas Hasanuddin, Makassar.
11. Fery R, Nasution S, Siregar S. The effect of ammonium sulphate (ZA) fertilizer concentration on the growth of microalga population (*Nannochloropsis oculata*). *Asian Journal of Aquatic Science*. 2020;3(2):94-102. doi:10.31258/ajoas.3.2.94-102.
12. Prayitno J. Pola pertumbuhan dan pemanenan biomassa dalam fotobioreaktor mikroalga untuk penangkapan karbon. *Jurnal Teknologi Lingkungan*. 2016;17(1):45. doi:10.29122/jtl.v17i1.1464.
13. Istikhatun T, Aulia M, Utomo S. Potensi *Chlorella* sp. untuk menyisihkan COD dan nitrat dalam limbah cair tahu. *Jurnal Presipitasi Media Komunikasi dan Pengembangan Teknik Lingkungan*. 2017;14(2):88-96. doi:10.14710/presipitasi.v14i2.88-96.
14. Fithriani D, Ambarwaty D, Nurhayati. Identification of bioactive compounds from *Nannochloropsis* sp. *IOP Conference Series: Earth and Environmental Science***.** 2019;404(1):012064. doi:10.1088/1755-1315/404/1/012064.
15. Ryu A, Kang N, Jeon S, et al. Development and characterization of a *Nannochloropsis* mutant with

simultaneously enhanced growth and lipid production. *Biotechnology for Biofuels*. 2020;13:38. doi:10.1186/s13068-020-01681-4.
16. Saputra N, Tantu A, Amin M, et al. Pengaruh warna wadah berbeda terhadap pertumbuhan *Nannochloropsis* sp. *Journal of Aquaculture Environment*. 2020;3(1):1063. doi:10.35965/jae.v3i1.1063.
17. Hanryani P, Efriyeldi, Effendi I. The effect of different light colors on the biomass growth of *Spirulina platensis*. *Asian Journal of Aquatic Science*. 2020;2(2):132-137. doi:10.31258/ajoas.2.2.132-137.
18. Afriza Z, Diansyah G, Sunaryo A. Pengaruh pemberian pupuk urea (CH_4N_2O) dengan dosis berbeda terhadap kepadatan sel dan laju pertumbuhan *Porphyridium* sp. pada kultur fitoplankton skala laboratorium. *Maspari Journal*. 2015;7(2):33-40.
19. Damanik R, Komariyah S, Putriningtias A. Pengaruh penggunaan warna cahaya yang berbeda terhadap pertumbuhan *Nannochloropsis* sp. *Journal of Aquaculture Science*. 2020;5(2):99. doi:10.31093/joas.v5i2.111.
20. Erwandani, Sumahiradewi L, Astuti N, et al. pengaruh perbedaan warna cahaya lampu terhadap pertumbuhan *Nannocloropsis* spp.: The effect of different colors of light on the growth of *Nannocloropsis* sp. *Al-Qalbu urnalJ Pendidikan Sosial dan Sains*. 2024;2(2):89-95. doi:10.59896/qalbu.v2i2.114.