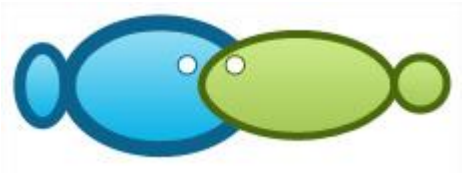

Effect of different light intensities on the growth and biomass productivity of a newly isolated thermophilic microalgae *Monoraphidium* sp. UNM-IND3 from Sulili hot spring, Pinrang Regency, Indonesia

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Abstract. Light is one of the important factors affecting the growth and productivity of microalgae biomass. This study aimed to analyze the effect of different light intensities on the growth and biomass productivity of microalga *Monoraphidium* sp. UNM-IND3. The *Monoraphidium* sp. UNM-IND3 was cultured in a 300 mL Erlenmeyer flask containing 150 mL of Guillard F/2 media under different light intensities (2000 lux, 4000 lux, 8000 lux), 12 hours light and dark cycles and temperature of 25±1°C (in triplicates). The results showed that the microalga could grow well under different light intensities tested. The highest specific growth rate was achieved at the light intensity of 8000 lux (1.04 day^{-1}) and the lowest obtained at the lowest light intensity of 2000 lux (0.71day^{-1}). The shortest doubling time was obtained in the light-intensity of 8000 lux (0.68 day) and the longest doubling time was obtained in the light-intensity of 2000 lux (0.97 day). There were no significant differences in the biomass yield and biomass productivity under different light intensities tested. The biomass yield ranged from 0.160 to 0.233 g L^{-1} and biomass productivity ranged from 0.152 to 0.214 g L^{-1} day^{-1}. This study indicates that microalgae *Monoraphidium* sp. UNM-IND3 prefers high light intensity for higher growth rate and biomass productivity and it has the potential for outdoor mass cultivation in open pond systems i.e raceway pond.
Key Words: extremophiles microalgae, microalgae biotechnology, *Monoraphidium* sp., raceway ponds.

Introduction. Microalgae are photosynthetic microorganisms that have many potential industrial applications i.e food, feed and pharmaceutical industries due to their ability to produce distinctive value-added products, and biologically active compounds including proteins, amino acids, enzymes, pigments, polyunsaturated fatty acids, polysaccharides, vitamins, antioxidants, and phytohormones (Chiaiese et al 2018; Ronga et al 2019). In addition, these microorganisms have several advantages such as rapid growth, minimal land and water requirements to produce high biomass, and the ability to adapt to various environmental conditions (Bhatnagar et al 2023). As a result, the United Nations' "Sustainable Development Goals" (SDGs) advocate microalgae biotechnology platforms for synthesizing industrially significant bioproducts (Kholani et al 2022).

For the commercialization of microalgae for various industrial applications, one of the important requirements is the ability of microalgae to be mass cultured in outdoor conditions, especially in open ponds system. Mass culture of microalgae using open pond systems such as raceway ponds is more advantageous than closed systems such as photobioreactors because it requires less energy, is cheaper to construct, and has lower operating costs (Gao et al 2024). The open raceway pond culture system is the most widely used culture system on an industrial scale (Costa et al 2019).

One of the environmental factors that affects outdoor cultures of microalgae is light intensity (Indrayani 2017). Light is the energy that drives the photosynthesis process, and the energy provided by light depends on the quality of light, light intensity, and photoperiod (Metsoviti et al 2020). Light determines the functional state, growth, and reproduction of microalgae and has a direct effect on microalgae metabolism (Zhang et al 2016; Erst et al 2018; Abiusi et al 2020). Therefore, it is very important to know how the growth and biomass production of a microalga respond to the light intensity received.

Monoraphidium sp. UNM-IND3 is a microalgae recently isolated from the Sulili hot spring located in Mamminasae village, Paleteang District, Pinrang Regency, South Sulawesi, Indonesia. To determine its potential for mass cultivation in open ponds outdoors, it is necessary to know the optimum light intensity for its growth and biomass production. Therefore, this study aims to analyze the effect of different light intensities on the growth and biomass production of *Monoraphidium* sp.UNM-IND3 microalgae. The results of this study are expected to be the basis for the development of the microalga as a source of raw materials for various industrial applications.

Material and Method

Microalga species. This study was conducted from February to May 2023. The microalga species used in this study is *Monoraphidium* sp. UNM-IND3, a newly isolated species from the Sulili hot spring, Pinrang Regency, South Sulawesi, Indonesia in June 2022 (Indrayani & Putra 2022) (Figure 1). The isolation method used a combined enrichment method and agar plating technique on f/2 agar media (Andersen & Kawachi 2005). This microalgae is maintained in the microalgae culture collection of the Agricultural Technology Laboratory, Faculty of Engineering, Universitas Negeri Makassar, Indonesia.

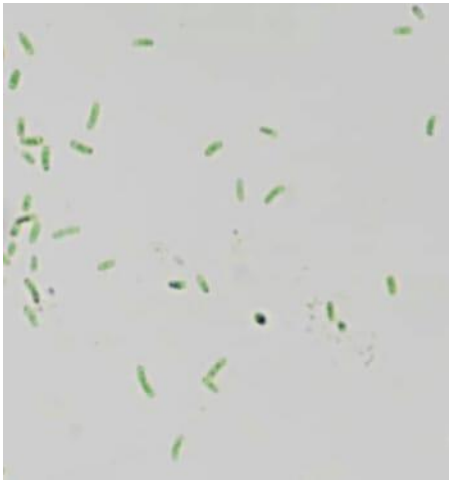

Figure 1. Microalgae *Monoraphidium* sp. UNM-IND03 under light microscope (400x magnification).

Culture conditions. Microalga was cultured using a 300 mL Erlenmeyer flask containing 150 mL of Guillard's f/2 media (Guillard 1975). The cultures were incubated in a culture room at 3 different light intensities namely 2000 lux, 4000 lux, and 8000 lux, temperature of 25±1°C, with a light-dark cycle of 12 hours (in triplicates). The culture was stirred manually at least three times a day (08.00 am, 12.00 pm, and 16.00 pm) and rotated every day.

Cell density. The density of culture cells was calculated daily using a haemocytometer. The growth curve was made by plotting time (days) against the number of culture cell densities.

Specific growth rate (SGR). The specific growth rate (SGR) (day^{-1}) was calculated based on the following formula (Moheimani et al 2013):

$$SGR = \frac{Ln(N_2/N_1)}{t_2 - t_1}$$

where: SGR is the specific growth rate, N_2 is the cell density at time t_2 and N_1 is the cell density at time t_1 in the exponential phase.

Doubling time (DT). Doubling time (DT) is the time required by cells or biomass to duplicate themselves. DT was calculated using the formula:

$$\mathbf{DT\ (day)} = \frac{\mathbf{0.693}}{\mathbf{SGR}}$$

where: DT = doubling time (day), Ln 2 = 0.693, SGR = specific growth rate (day^{-1})

Biomass yield. Biomass yield (g L^{-1}) was calculated based on the method of Moheimani et al (2013). Briefly, 5 mL of culture was filtered using pre-weighted Whatman filter paper (GF/25 mm) secured in a millipore filter apparatus and then vacuumed. The filter containing microalgae cells was then removed from the apparatus and dried in an oven at 75°C for 5 hours. After being in the oven, the filter was placed in a desiccator for approximately 30 minutes before being re-weighed. The biomass weight was calculated using the formula:

Biomass yield (g L^{-1}) = weight of filter containing alga – weight of filter

Biomass productivity. Based on the SGR and biomass yield, biomass productivity was calculated using the following formula (Indrayani et al 2021, 2022):

Biomass productivity (g L^{-1} d^{-1}) = SGR x biomass yield

Statistical analysis. Statistical analysis of the One-Way Analysis of Variance (ANOVA) test was used to determine whether there was a significant difference between treatments. Further testing was carried out using the Holm-Sidak method to determine which treatment was different. All statistical analysis was performed using Sigma-Plot 14 Systat Software Inc., USA.

Results

Growth of microalga Monoraphidium sp. UNM-IND3. The growth of microalga *Monoraphidium* sp. UNM-IND3 cultured at different light intensities (2000 lux, 4000 lux, and 8000 lux) during the culture period can be seen in Figure 2.

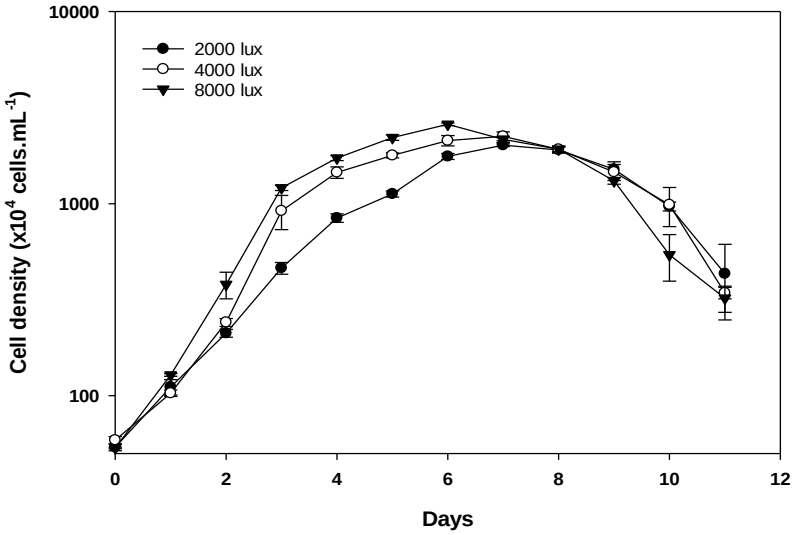

Figure 2. Growth curve of microalgae *Monoraphidium* sp. UNM-IND3 at different light intensities.

The results showed that microalga *Monoraphidium* sp. UNM-IND3 could grow well at all light intensities tested. The initial cell density in all treatments was about 55±2x10^4 cells mL^{-1}. One day after inoculation, cell density in all treatments increased exponentially until the 3rd day. Entering the 4th day, the increase in culture cell density began to slow down (early stationary phase) until reaching maximum density (late stationary phase) on the 6th day for the 2000 lux light intensity treatment and the 7th day for the 4000 lux and 8000 lux light intensity treatments. The culture entered the death phase on the 8th day, marked by a decrease in cell density (Figure 2).

The highest SGR was obtained from the light intensity of 8000 lux with an average of 1.04 day^{-1}, followed by the light intensity of 4000 lux with an average of 0.91 day^{-1}, and the treatment of 2000 lux with an average of 0.72 day^{-1}. The One-Way ANOVA test showed a significant difference between the average values of the SGR of microalga *Monoraphidium* sp. UNM-IND3 at different light intensities ($p = < 0.001$). The SGR at a light intensity of 2000 lux was significantly different from the SGR at a light intensity of 4000 lux ($p = 0.005$) and at a light intensity of 8000 lux ($p < 0.001$). Likewise, the SGR at a light intensity of 4000 lux was significantly different from the SGR at a light intensity of 8000 lux ($p = 0.019$) (Figure 3).

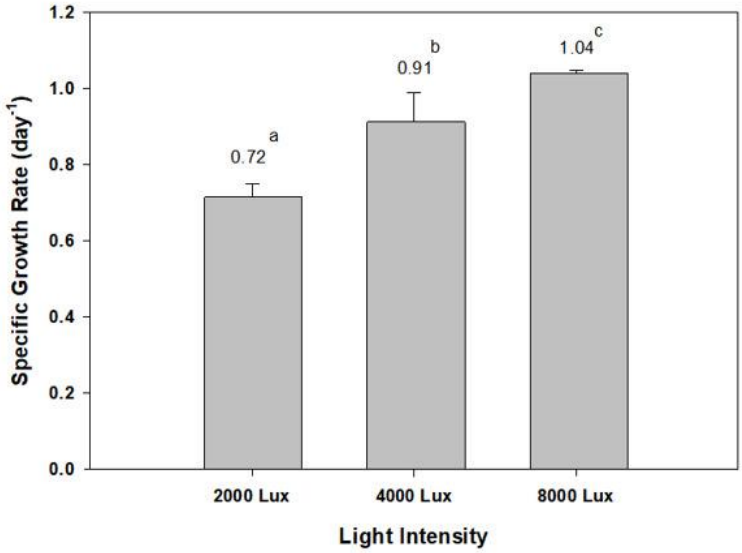

Figure 3. Specific growth rate of *Monoraphidium* sp. UNM-IND3 at different light intensities.

DT is the time required for cells or biomass to double. The shortest DT was obtained at the light intensity of 8000 lux (0.667 days) and the longest DT was obtained at the light intensity of 2500 lux (0.970 days). The statistical tests showed that there was a significant difference between the DT in each treatment (One Way Anova, $p < 0.001$). The DT at a light intensity of 2000 lux was significantly different from the DT at a light intensity of 4000 lux ($p = 0.003$) and 8000 lux ($p < 0.001$) as well as the DT at a light intensity of 4000 lux was significantly different from the DT at 8000 lux ($p = 0.038$) (Figure 4).

Biomass yield and biomass productivity of Monoraphidium sp. UNM-IND3. The results of the One-Way ANOVA analysis showed that there was no significant difference in the biomass yield of *Monoraphidium* sp. UNM-IND3 under different light intensities ($p = 0.516$). The average biomass value ranged from 0.160 to 0.233 g L^{-1} (Figure 5).

The statistical tests showed that differences in light intensity did not significantly affect the biomass productivity of microalga *Monoraphidium* sp. UNM-IND3 (One-Way Anova, $p = 0.486$). The average value of biomass productivity of *Monoraphidium* sp. UNM-IND3 ranged from 0.152 to 0.214 g $L^{-1}.day^{-1}$ (Figure 6).

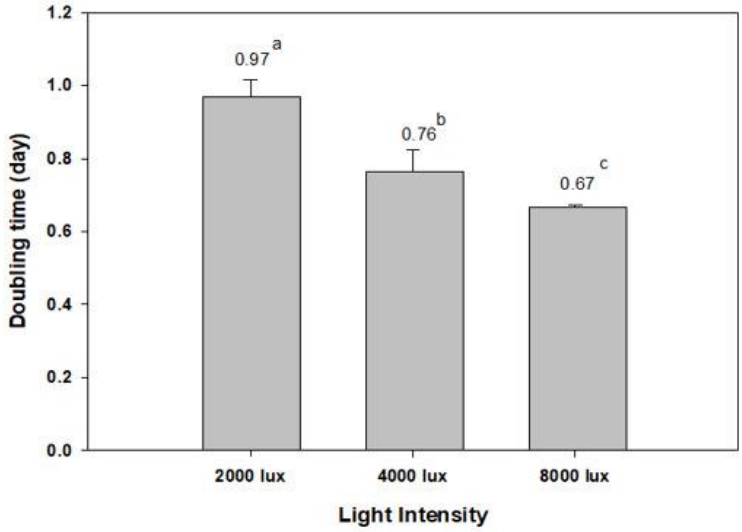

Figure 4. Doubling time of *Monoraphidium* sp. UNM-IND3 at different light intensities.

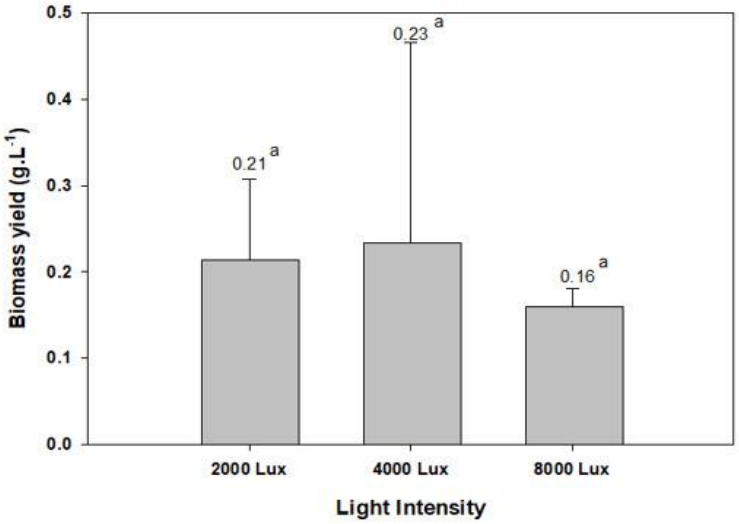

Figure 5. Biomass yield of microalga *Monoraphidium* sp. UNM-IND3 at different light intensities.

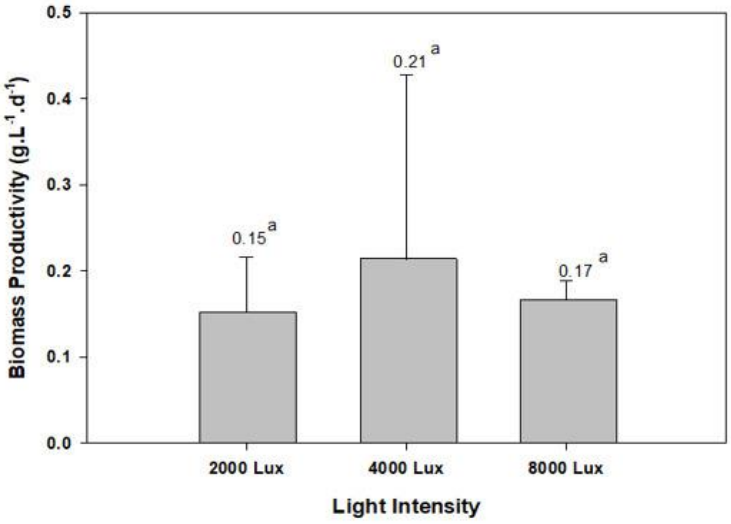

Figure 6. Biomass productivity microalga *Monoraphidium* sp. UNM-IND3 at different light intensities.

Discussion

Growth of microalga Monoraphidium sp. UNM-IND3. Light is one of the important factors affecting the growth and biomass productivity of microalgae (Li et al 2012). This study analyzed the growth response and biomass production of microalgae *Monoraphidium* sp. UNM-IND3 at different light intensities (2000, 4000, and 8000 lux). From this study, it is known that the microalga can grow well at all light intensities tested. One day after inoculation, all cultures showed immediate rapid growth until the 3rd day. This indicates that the culture is in the logarithmic or exponential growth phase. Rapid growth during the logarithmic phase is due to the abundance of nutrients, light, and CO_2 in the culture medium. It is also seen that the cultures in all treatments did not experience a lag phase or adaptation phase. This is because the inoculum was cultured using the same media and temperature conditions. In addition, the inoculum used is a freshly prepared inoculum that is in the exponential growth phase where the microalgae cells are actively dividing so that when transferred to a new medium with the same nutrient composition and culture condition, the culture will grow rapidly.

The exponential phase lasted until the 3rd day. Entering the 4th day, the cultures entered the early stationary phase marked by a slowdown in the increase in the number of cells until entering the final stationary phase on the 6th to 7th day. The slowdown in growth in the stationary phase is due to the decreasing availability of light and nutrients because they are utilized by the increasing number of cells. This is in line with the statement of Indrayani et al (2023), that microalgae growth is greatly influenced by the availability of nutrients. The relationship between nutrients and microalgae growth is directly proportional, if the nutrients in the culture medium begin to decrease, the increase in cell density will also slow down. Entering the 8th day, all cultures were in the death phase marked by a decrease in the density of culture cells due to running out of nutrients.

Although all cultures had the same growth pattern, the maximum cell density in each treatment was different. The highest light intensity of 8000 lux had the highest cell density and the lowest light intensity of 2000 lux had the lowest cell density. The reason for this is that in the final stationary phase, in addition to decreasing nutrients, the light intensity is also very limited and is no longer able to support the photosynthetic activity of increasingly dense microalgae cells. At this stage, cultures that have higher light intensity will still be able to supply energy for cell growth so that higher cell density is obtained compared to cultures at lower light intensity. As Wong et al (2016) stated, the growth and production of microalgae biomass are greatly influenced by culture cell density. High cell density culture will prevent light penetration which will ultimately reduce the intensity of photosynthesis (Gim et al 2016).

Based on the cell density data in the exponential phase, the SGR of microalgae can be calculated. The SGR is the speed of cell growth or microalgae biomass per unit time. In this study, it was found the growth rate of *Monoraphidium* sp.UNM-IND3 was positively correlated with the light intensity tested. The highest SGR of *Monoraphidium* sp. UNM-IND3 microalgae (1.04 day^{-1}) was obtained at the highest light intensity (8000 lux) and the lowest (0.72 day^{-1}) was obtained at the light intensity of 2000 lux. This result was higher than that of Vargas et al (2019) whose reported the highest SGR of *Monoraphidium contortum* of about 0.68±0.07 d^{-1} and Řezanka et al (2017) reported the growth rate of *Monoraphidium* sp. at value of 0.341 day^{-1}. This shows that *Monoraphidium* sp. UNM-IND3 prefer high light intensity for optimal growth. The preference for high light intensity is thought to be due to the original habitat of this microalgae being a shallow hot spring pool where the intensity of sunlight can penetrate to the bottom of the water. This is in agreement with statement of the Maltsev et al (2021), the light conditions for the growth of microalgae in natural habitas are determined by the geographical latitude of the area. For example, microalgal species that are found on open surfaces are adapted to grow in direct sunlight (Orlekowsky et al 2013; Solonenko et al 2020; Maltseva & Maltsev 2021) where the light intensity can reach up to 2000 µmol photons m^{-2} d^{-1} (Erickson et al 2015).

DT is the time required for a number of cells or microalgae cell mass to double the original number of cells. The higher the DT value, the more time the microalgae cells need for doubling. Conversely, the lower the DT value, the less time the microalgae cells need for doubling. In this study, we found that the lowest DT of *Monoraphidium* sp. UNM-IND3 was at a light-intensity treatment of 8000 lux (0.68 days) meaning that the cells need 0.68 days to grow to 2 times the original number of cells or biomass. A shorter generation time means faster population growth as the time required for cell division is shorter to reach maximum cell density. The fastest DT occurs in the exponential phase, which is the phase where cells divide rapidly. The doubling time of the *Monoraphidium* sp. UNM-IND3 in this study was faster than the DT of *Monoraphidium* contortum (1.03±0.1 days) reported by Vargas et al (2019).

The growth rate and generation time of microalgae are correlated with the cell size. According to Klin et al (2018), the growth of unicellular microalgae is inversely proportional to the size of its cells where large cells grow slower than small cells. Large cells use up metabolic energy faster so that growth becomes slower (Borowitzka 1992). In contrast, small microalgae cells grow faster because small cells have a larger surface area to volume ratio (SA:V ratio) so they are more effective in assimilating nutrients (Fogg 1975; Klin et al 2018). *Monoraphidium* sp. UNM-IND3 is a small green solitary microalga, longer than broad, spindle-shaped, straight or curved with cell length ranging from 5 to 9 µm, and cell width ranging from 2 to 3.5 µm (Lin et al 2019) and therefore its growth rate and doubling time are fast.

Biomass yield and biomass productivity of Monoraphidium sp. UNM-IND3. The biomass produced is a very important factor in determining the growth and productivity of microalgae. Light energy is an important factor in producing optimal biomass (Nzayisenga et al 2020). The appropriate light intensity for microalgae can increase biomass production and the increase in biomass and density of microalgae cells is directly proportional to the increase in light intensity (Maltsev et al 2021). In this study, the difference in light intensity tested did not significantly affect the production of microalgae biomass where the biomass ranged from 0.160 to 0.233 g L^{-1}. The biomass yield of microalga *Monoraphidium* sp. UNM-IND3 obtained in this study was lower compared to previous studies. For example, El-Sheekh et al (2024) reported the biomass yield of *Monoraphidium braunii* of 0.245 g L^{-1} under nitrogen deficiency culture as compared to the control of 0.582 g L^{-1} and 0.62 g L^{-1} under salt stress. Lin et al (2019) reported the biomass yield of *Monoraphidium* sp. HDMA-20 of 654±33 mg L^{-1}. Helamieh et al (2024) reported the biomass yield of *Monoraphidium braunii* under white light treatment reached 1.94±0.04 g L^{-1}, with red light reached a maximum of 1.62±0.04 g L^{-1} and with green light and blue light treatment led to a maximum biomass of 1.37±0.04 and 1.06±0.01 g L^{-1}, respectively.

Biomass productivity is influenced by the SGR and biomass yield. The higher the SGR and biomass, the more biomass productivity is produced, and vice versa. From this study, it was found that the biomass productivity of microalga *Monoraphidium* sp. UNM-IND3 ranged from 0.152 to 0.214 g L^{-1} day^{-1}. The biomass productivity of *Monoraphidium* sp. UNM-IND3 was higher than the biomass productivity of *Monoraphidium* sp. HDMA-11 isolated from Lake Ming, China (31.5 mg L^{-1} day^{-1}) (Lin et al 2018), *Monoraphidium* sp. HDMA-20 (36.3±1.8 mg L^{-1} day^{-1}) (Lin et al 2019), *Monoraphidium braunii* in nitrogen-depleted culture (0.03 g L^{-1} day^{-1}) as compared to the control (0.072 g L^{-1} day^{-1}) (El-Sheekh et al 2024) and *Monoraphidium* sp. (421 mg L^{-1} day^{-1}) (Khan et al 2024). Higher biomass productivity in this study is due to the higher growth rate of the *Monoraphidium* sp. UNM-IND3 compared to that of aforementioned *Monoraphidium* species or strains.

Conclusions. From this study, it can be concluded that different light intensities affect the specific growth rate and doubling time of *Monoraphidium* sp. UNM-IND3 microalgae where the highest specific growth rate and doubling time were obtained at a light intensity of 8000 lux with values of 1.04 day^{-1} and 0.68 day, respectively, and the lowest at a light intensity of 2000 lux with values of 0.72 day^{-1} and 0.97 day, respectively. Meanwhile, different light intensities did not significantly affect the biomass yield and

biomass productivity of *Monoraphidium* sp. UNM-IND3 where the average biomass yield value ranged from 0.160 to 0.233 g L^{-1} and the average biomass productivity ranged from 0.152 to 0.214 g L^{-1} day^{-1}. The ability to produce high biomass productivity under a wide range of light intensity will make this alga a potential candidate for outdoor mass cultivation in open pond systems such as race-way ponds.

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Conflict of interest. The authors declare that there is no conflict of interest.

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