

Cell density, growth response, and pigment accumulation of *Nannochloropsis* sp. culture under salinity concentration

¹Jurmin H. Sarri, ¹Al-Nasrif H. Kissae, ¹Melodina D. Hairol, ¹Khadiza H. Imlan, ¹Fatima Nhidzlah T. Ensano ¹Shardanika H. Kissae, ^{1,3}Rizal J. F. Robles, ²Enraida S. Imbuk, ¹Shada-Wati H. Kissae ²Nurshaifah M. Adjid, ²Fatmawati O. Albar

¹ Department of Aquaculture, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, 7500 Sanga-Sanga, Bongao, Tawi-Tawi, Philippines; ² Department of Aquaculture, College of Fisheries, Mindanao State University-Sulu, 7400 Patikul Site, Sulu, Philippines; ³ Institute of Tropical Aquaculture and Fisheries, University Malaysia Terengganu, 21030 Kuala Nerus, Malaysia.
Corresponding author: A. H. Kissae, al-nasrifkissae@msutawi-tawi.edu.ph

Abstract. Variations in salinity levels are a critical environmental factor influencing the physiology, growth, and productivity of microalgae. This study evaluated the effects of different salinity concentrations on the cell density, growth response, dry weight, and pigment accumulation of *Nannochloropsis* sp. Five experiments were prepared: control (0 g L⁻¹ NaCl), 5 g L⁻¹, 10 g L⁻¹, 15 g L⁻¹, and 20 g L⁻¹, each in triplicate, and cultures were maintained for 21 days. Results showed that a low salinity level (5 g L⁻¹) significantly enhanced growth, with the highest cell density (21.69±1.36 × 10⁶ cells mL⁻¹) and specific growth rate (SGR) (0.47±0.03 day⁻¹), outperforming the control and higher salinity experiments. In contrast, maximum dry weight was observed at 20 g L⁻¹, suggesting that elevated salinity induced cellular accumulation of storage compounds rather than rapid proliferation. Pigment analysis revealed that 5 g L⁻¹ salinity promoted the highest chlorophyll-a concentration (15.40±5.55 µg mL⁻¹), while all salinity treatments except the control increased total carotenoid accumulation. These findings indicate that low salinity favors growth and pigment production, whereas higher salinity enhances biomass accumulation, highlighting distinct physiological responses of *Nannochloropsis* sp. to salinity stress.

Key Words: cell density, growth response, pigment accumulation, *Nannochloropsis* sp., nutrient medium, salinity.

Introduction. Microalgae are microscopic photosynthetic organisms found in diverse aquatic environments, where they play an essential role in primary production, nutrient cycling, and oxygen generation (Coêlho et al 2019; Tan et al 2020; Hu et al 2023; Sarri et al 2024). Among them, *Nannochloropsis* sp. has gained particular attention because of its high lipid content, valuable pigments, and suitability as a feedstock for aquaculture and biofuel applications (Saadaoui et al 2021; Hashmi et al 2023). These microalgae are rich in proteins, lipids, vitamins, minerals, and omega-3 fatty acids, making them critical as live feed for zooplankton and, consequently, for fish and shellfish larvae (Chakraborty et al 2007). In addition to their nutritional value, the ability of *Nannochloropsis* to synthesize chlorophyll-a and carotenoids underscores their importance for both ecological and biotechnological purposes (Hosikian et al 2010). In addition, microalgal growth and biochemical composition are strongly influenced by external environmental factors, including nutrient availability, light, temperature, pH, and salinity (Gani et al 2019; Dolganyuk et al 2020).

Microalgae exhibit vibrant colors due to the presence of natural pigments, which are among their most important biochemical compounds (Silva et al 2020). Chlorophylls are the primary pigments, driving oxygenic photosynthesis and supplying the energy

needed for metabolic and reproductive processes (Da Silva & Lombardi 2020; Ahajan et al 2025). Carotenoids, on the other hand, act as accessory pigments with antioxidant properties and have growing applications in food, feed, and pharmaceutical industries (Jain et al 2022). Together, chlorophyll and carotenoids are essential for microalgal growth and survival, while also representing valuable bioactive compounds for human use (Brotosudarmo et al 2018; Ampofo & Abbey, 2022). Importantly, the synthesis and accumulation of these pigments can be strongly influenced by environmental conditions such as salinity, making them key indicators of physiological response in *Nannochloropsis* sp.

The microalga *Nannochloropsis* is recognized for its high fat content, making it very useful for producing biofuels, aquaculture feed, and nutritional supplements (Mazard et al 2016). *Nannochloropsis* sp. is a common microalgae species used in research and commercial applications because it grows quickly and can adapt to various environments (Premachandra et al 2023). Salinity, in particular, is a key determinant of microalgal physiology because it directly affects osmotic balance, photosynthesis, and metabolite accumulation. For *Nannochloropsis*, understanding the response to salinity stress is especially relevant since this genus is widely cultivated in coastal aquaculture systems where salinity can fluctuate. However, systematic evaluation of how different salinity concentrations influence growth, pigment accumulation, and biomass production in *Nannochloropsis* sp. remains limited. This study, therefore, investigates the effects of varying salinity levels on the cell density, growth rate, pigment accumulation, and biomass yield of *Nannochloropsis* sp., providing insights for optimizing its cultivation under controlled conditions.

Material and Method

The culture conditions of microalgae. Microalga *Nannochloropsis* sp. culture was conducted at the Regional Fisheries Research Center, Ministry of Agriculture, Fisheries, and Agrarian Reform, Pangasinan, Bongao, Tawi-Tawi, Philippines. 500 mL glass bottles were used for the culture. Cultures were maintained in 500 mL sterilized glass bottles containing BG-11 medium prepared with distilled water (Tables 1 and 2). Different concentrations of sodium chloride were added to the bottles to create various salinity levels, as shown in Table 3. The control sample did not contain any sodium chloride. The experiment was performed in triplicate. Each solution was autoclaved for 20 minutes at 121°C. After autoclaving, the samples were inoculated at an initial density of 1.83×10^5 cells mL⁻¹. Cultures were maintained under artificial laboratory lighting, with fluorescent lamps providing a continuous 24-hour photoperiod at an intensity of 200 μmol photons m⁻² s⁻¹. Ventilation was provided by an air motor, supplying ambient air (approximately 0.04% CO₂) filtered through 0.2 μm syringe filters to prevent contamination (Figure 1). An air conditioner maintained the temperature at 20±1°C.

Table 1

BG-11 nutrient medium

<i>Solution A</i>	<i>For 500 mL</i>
NaNO ₃	75.0 g
<i>Solution B</i>	<i>For 500 mL</i>
K ₂ HPO ₄	2.0 g
MgSO ₄ .7H ₂ O	3.75 g
CaCl ₂ .2H ₂ O	1.80 g
Citric acid	0.30 g
Ammonium ferric citrate green	0.30 g
EDTANa ₂	0.05 g
Na ₂ CO ₃	1.00 g

Source: Erbil et al 2021.

Table 2

Trace element composition

<i>Trace element solution</i>	<i>For 1000 mL</i>
H ₃ BO ₃	2.86 g
MnCl ₂ .4H ₂ O	1.81 g
ZnSO ₄ .7H ₂ O	0.22 g
Na ₂ MoO ₄ .2H ₂ O	0.39 g
CuSO ₄ .5H ₂ O	0.08 g
Co(NO ₃) ₂ .6H ₂ O	0.05 g

Table 3

Experimental samples and salinity concentration

<i>Experiment</i>	<i>Unit</i>	<i>Salinity Concentration</i>
Experiment A (Control)	g L ⁻¹	BG-11 without added NaCl
Experiment B	g L ⁻¹	5
Experiment C	g L ⁻¹	10
Experiment D	g L ⁻¹	15
Experiment E	g L ⁻¹	20



Figure 1. Experimental set-up. The glass bottles were enriched with different salinity concentrations (g L⁻¹) in a nutrient medium.

Growth response analysis. Each experimental sample of *Nannochloropsis* sp. microalga cultures was collected for cell counting and analysis every three days. A Neubauer hemocytometer was used to count cells daily under a light microscope, and contamination was visually checked daily. An analysis of the biomass of microalgae was conducted on a dry-weight basis. The dried weight of microalgae was determined by drying 5 mL of each experimental sample in an oven at 105°C for 2 hours. The specific growth rate (SGR) was calculated as follows.

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1}$$

Where:

N_2 = the cell number at the time (t_2).

N_1 = the beginning cell number at time t_1 .

Pigment analysis. Pigment analysis followed the method of Durmaz & Erbil (2020). Spectrophotometric analysis of *Nannochloropsis* sp. was determined by the chlorophyll-a and total carotenoid levels, respectively. Moreover, in experimental culture, a 5 mL test sample was centrifuged for 10 minutes at 3500 rpm, and then the supernatant was removed. Afterward, the tubes were then filled with 5 mL of methanol, which was then vortexed for 30 seconds to homogenize the sample. Finally, samples were remixed and centrifuged for 10 minutes at 3500 rpm. Analyses of supernatants were performed by spectrophotometry (DYNAMICA). Calculations of the values read at 666 nm for chlorophyll-a and 475 nm for total carotenoids in the spectrophotometer were made using the formulas given below.

$$\text{Chlorophyll-a } (\mu\text{g mL}^{-1}) = 13.9 A_{666} \text{ (Macias-Sánchez et al 2005)}$$

A_{666} wavelength 666 nm absorbance value

$$\text{Total carotenoids } (\mu\text{g mL}^{-1}) = 4.5 A_{475} \text{ (Zou \& Richmond 2000)}$$

A_{475} wavelength 475 nm absorbance value

Statistical analysis. The collected data on cell densities, growth responses, and pigment accumulation in *Nannochloropsis* sp. cultures were analyzed using IBM SPSS software version 20, with a significance level set at $p < 0.05$. Results were expressed as mean \pm standard error of the mean. Significant differences between groups were evaluated through One-way Analysis of Variance (ANOVA), while Levene's Test was applied to assess the homogeneity of variances. Duncan's post hoc Test was subsequently used to rank and compare the means (Hairol et al 2022; Sanuddin et al 2023).

Results

Cell density. The experimental sample of *Nannochloropsis* sp. was initiated at an initial density of 1.8×10^5 cells mL^{-1} and was maintained for 21 days of culture. Five experimental samples were investigated: experiment A (no salinity concentration), experiment B (5 g L^{-1} salinity concentration), experiment C (10 g L^{-1} concentration), experiment D (15 g L^{-1} concentration), and experiment E (20 g L^{-1} concentration). Figure 2 shows the cell number of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Before ANOVA, Levene's Test was performed to assess the homogeneity of variances, and the results indicated no significant differences among groups ($p > 0.05$), confirming that the assumption of equal variances was met. Results revealed that experiment B achieved the highest performance, reaching a maximum cell density of $21.69 \pm 1.36 \times 10^6$ cell mL^{-1} , which was significantly greater ($p < 0.05$) than experiment A ($16.98 \pm 0.44 \times 10^6$ cell mL^{-1}) and all higher-salinity treatments (experiments C and E, $7.85 \pm 0.44 \times 10^6$ to $6.19 \pm 0.22 \times 10^6$ cell mL^{-1}). In addition, the mean cell density of experiment B reached $8.82 \pm 1.80 \times 10^6$ cell mL^{-1} , and experiment A reached $8.08 \pm 2.42 \times 10^6$ cell mL^{-1} , were statistically higher ($p \leq 0.05$) than experiment C

($3.51 \pm 0.84 \times 10^6$ cell mL⁻¹), experiment D ($3.38 \pm 0.40 \times 10^6$ cell mL⁻¹), and experiment E ($2.80 \pm 0.22 \times 10^6$ cell mL⁻¹) (Figure 3). Overall, moderate salinity (5 g L⁻¹) supported the highest growth, while elevated salinity (10-20 g L⁻¹) strongly suppressed cell proliferation.

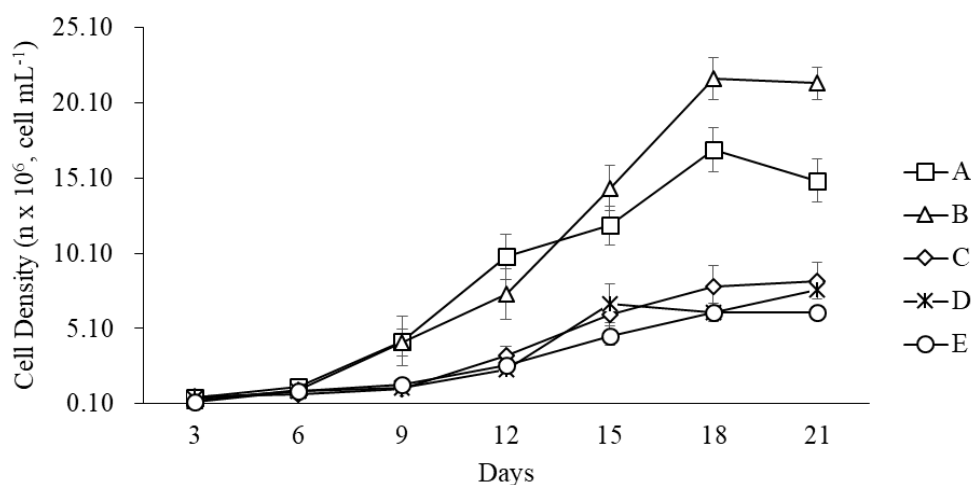


Figure 2. Cell density ($n \times 10^6$, cell mL⁻¹) of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Experiment A (no salinity concentration), experiment B (5 g L⁻¹ salinity concentration), experiment C (10 g L⁻¹ concentration), experiment D (15 g L⁻¹ concentration), and experiment E (20 g L⁻¹ concentration).

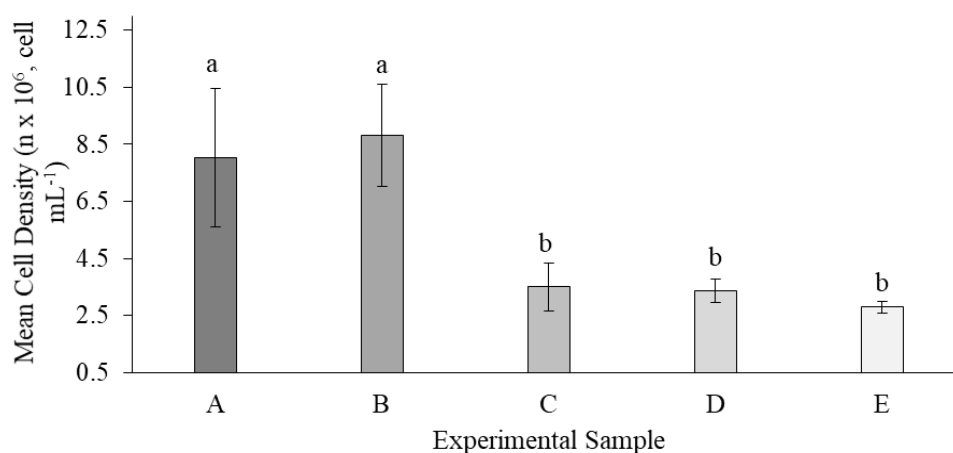


Figure 3. Mean cell density ($n \times 10^6$, cell mL⁻¹) of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Experiment A (no salinity concentration), experiment B (5 g L⁻¹ salinity concentration), experiment C (10 g L⁻¹ concentration), experiment D (15 g L⁻¹ concentration), and experiment E (20 g L⁻¹ concentration).

Specific growth rate and dry weight. The maximum SGR was obtained at nine days of culture for all experimental samples. SGR of experiments A, B, C, D, and E were 0.38 ± 0.05 day⁻¹, 0.47 ± 0.03 day⁻¹, 0.15 ± 0.07 day⁻¹, 0.18 ± 0.04 day⁻¹, and 0.15 ± 0.12 day⁻¹, respectively (Figure 4). Levene's Test confirmed homogeneity of variances ($p > 0.05$), allowing valid ANOVA comparisons. As a result of the study, experiment B was significantly different ($p \leq 0.05$) from the other experimental samples. Additionally, in terms of mean SGR, experiment B obtained an SGR of 0.23 ± 0.00 day⁻¹, and experiment A obtained an SGR of 0.22 ± 0.01 day⁻¹, which was significantly different ($p \leq 0.05$) than experiment C (0.18 ± 0.01 day⁻¹), experiment D (0.18 ± 0.00 day⁻¹), and experiment E (0.17 ± 0.00 day⁻¹) (Figure 5). Moreover, the dry weight was determined at the end of the culture period for the *Nannochloropsis* sp. culture. It has been revealed in the present

study that maximum dry weight was obtained from experiment E ($26.21 \pm 0.33 \text{ g L}^{-1}$), which was significantly greater ($p \leq 0.05$) than experiment D ($20.34 \pm 0.40 \text{ g L}^{-1}$), experiment C ($15.28 \pm 0.43 \text{ g L}^{-1}$), experiment B ($10.51 \pm 0.17 \text{ g L}^{-1}$), and experiment A ($4.42 \pm 0.11 \text{ g L}^{-1}$) (Figure 6). This finding appears contradictory to the SGR and cell density results, where higher salinity reduced growth performance. A likely explanation is that elevated salinity (20 g L^{-1}) imposed stress that limited cell division but promoted the accumulation of storage compounds, thereby increasing dry weight per unit volume. Thus, while moderate salinity (5 g L^{-1}) enhanced growth rates and proliferation, extreme salinity (20 g L^{-1}) suppressed cell division yet triggered stress-induced biomass accumulation, reconciling the observed discrepancy.

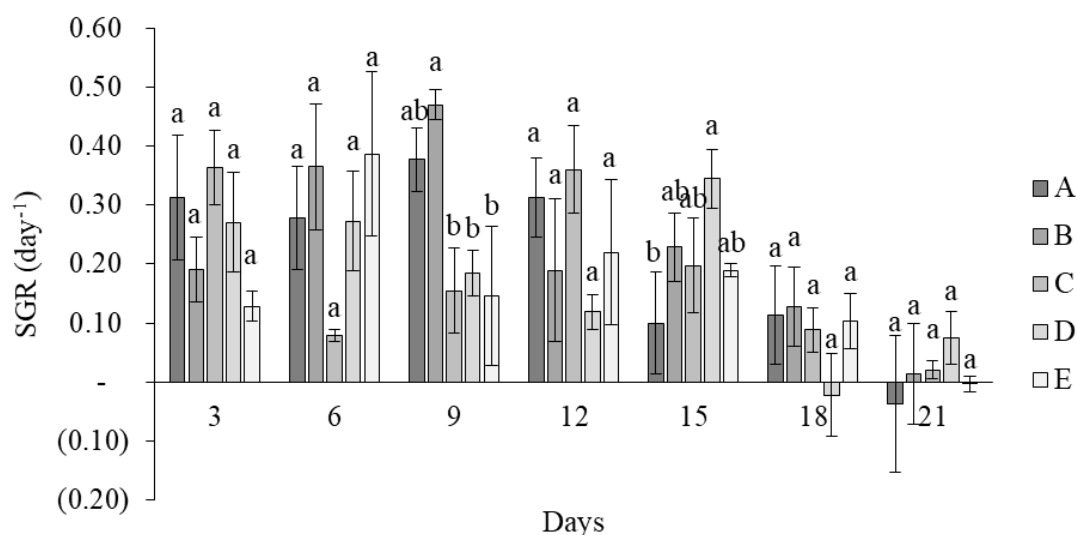


Figure 4. Specific growth rate (SGR, day^{-1}) of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Experiment A (no salinity concentration), experiment B (5 g L^{-1} salinity concentration), experiment C (10 g L^{-1} concentration), experiment D (15 g L^{-1} concentration), and experiment E (20 g L^{-1} concentration).

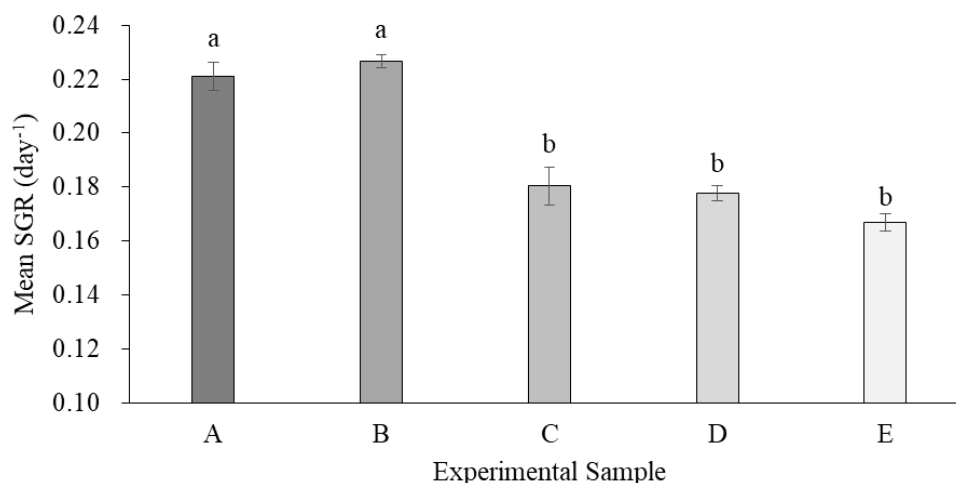


Figure 5. Mean specific growth rate (SGR, day^{-1}) of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Experiment A (no salinity concentration), experiment B (5 g L^{-1} salinity concentration), experiment C (10 g L^{-1} concentration), experiment D (15 g L^{-1} concentration), and experiment E (20 g L^{-1} concentration).

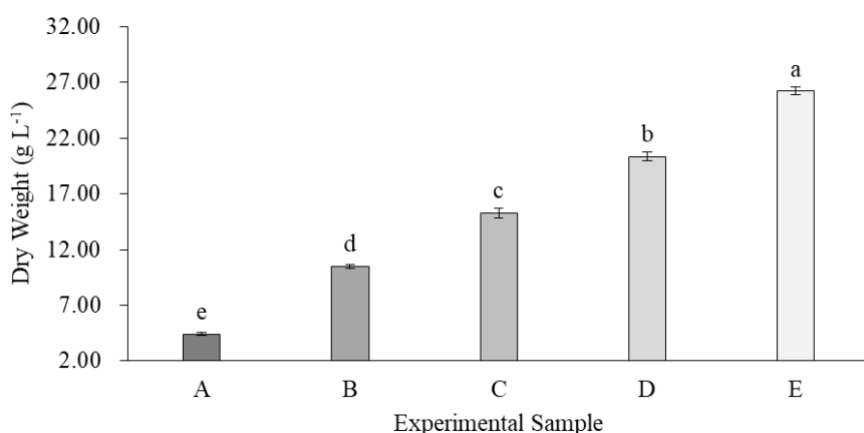


Figure 6. Dry weight (g L⁻¹) of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Experiment A (no salinity concentration), experiment B (5 g L⁻¹ salinity concentration), experiment C (10 g L⁻¹ concentration), experiment D (15 g L⁻¹ concentration), and experiment E (20 g L⁻¹ concentration).

Chlorophyll-a pigment accumulation. Figure 7 shows the chlorophyll-a pigment accumulation of *Nannochloropsis* sp. culture at various salinity concentrations in a nutrient medium. The measurement of chlorophyll-a pigment accumulation was done in triplicate. The chlorophyll-a concentrations of experiments A, B, C, D, and E were $9.46 \pm 2.14 \mu\text{ mL}^{-1}$, $15.40 \pm 5.55 \mu\text{ mL}^{-1}$, $9.68 \pm 2.27 \mu\text{ mL}^{-1}$, $9.61 \pm 2.07 \mu\text{ mL}^{-1}$, and $9.19 \pm 1.28 \mu\text{ mL}^{-1}$, respectively. The Levene's test confirmed variance homogeneity ($p > 0.05$), and ANOVA results indicated no significant differences ($p > 0.05$) among treatments. Thus, no treatment can be considered superior in terms of culture-level chlorophyll-a accumulation, and the higher mean observed in experiment B should be interpreted only as natural variability rather than a meaningful trend.

In terms of cellular chlorophyll-a content, experiments A, B, C, D, and E yielded $0.64 \pm 1.10 \text{ pg. cell}^{-1}$, $0.73 \pm 0.28 \text{ pg. cell}^{-1}$, $1.23 \pm 0.38 \text{ pg. cell}^{-1}$, $1.24 \pm 0.23 \text{ pg. cell}^{-1}$, $1.50 \pm 0.21 \text{ pg. cell}^{-1}$, respectively (Figure 8). Although no statistically significant differences were found ($p > 0.05$), the results suggest wide variability across treatments, with some indication of higher cellular chlorophyll-a values at elevated salinity. However, this pattern remains inconclusive without statistical support.

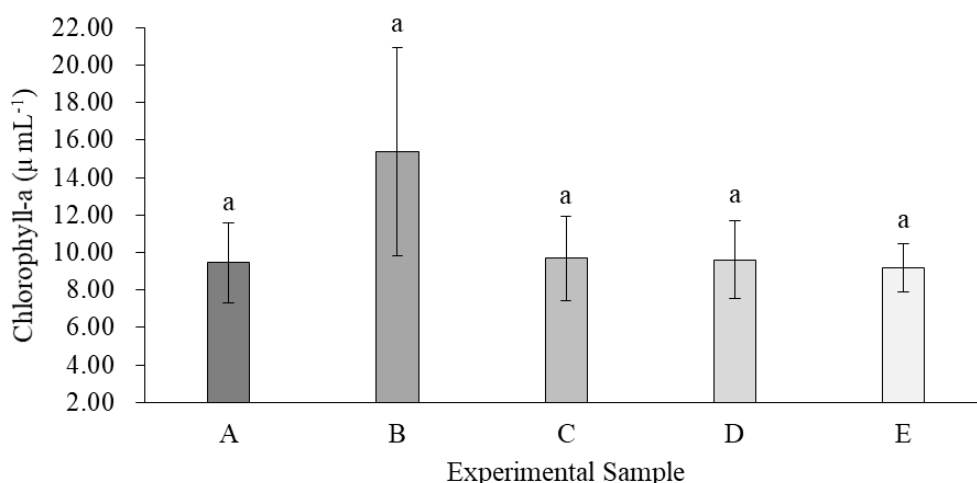


Figure 7. Chlorophyll-a pigment accumulation of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Experiment A (no salinity concentration), experiment B (5 g L⁻¹ salinity concentration), experiment C (10 g L⁻¹ concentration), experiment D (15 g L⁻¹ concentration), and experiment E (20 g L⁻¹ concentration).

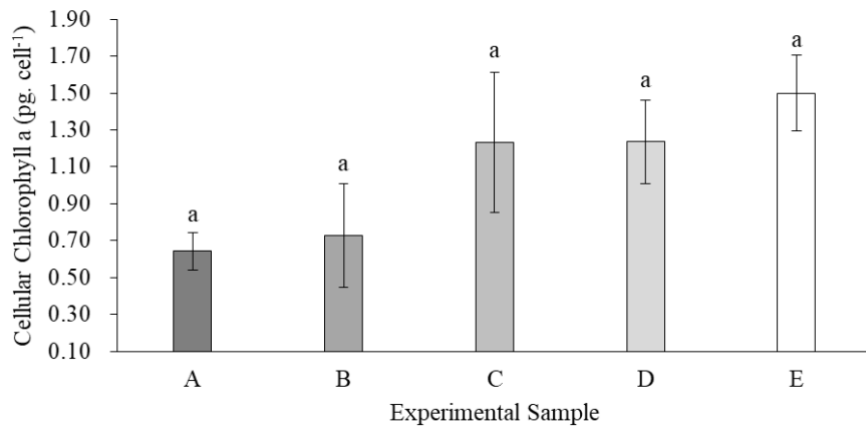


Figure 8. Cellular chlorophyll-a pigment accumulation of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Experiment A (no salinity concentration), experiment B (5 g L⁻¹ salinity concentration), experiment C (10 g L⁻¹ concentration), experiment D (15 g L⁻¹ concentration), and experiment E (20 g L⁻¹ concentration).

Total carotenoid pigment accumulation. Figure 9 shows the total carotenoid pigment accumulation of *Nannochloropsis* sp. culture at different salinity concentrations in a nutrient medium. The total carotenoid concentrations in experiments A, B, C, D, and E were 2.95±0.34 μ mL⁻¹, 3.62±0.82 μ mL⁻¹, 3.46±0.54 μ mL⁻¹, 3.67±0.35 μ mL⁻¹, and 4.71±0.84 μ mL⁻¹, respectively. The Levene's Test confirmed variance homogeneity ($p > 0.05$), and ANOVA indicated no significant differences ($p > 0.05$) among treatments. Nevertheless, experiment E showed a trend toward higher total carotenoid accumulation compared with the other treatments. In terms of cellular carotenoid content, experiments A, B, C, D, and E yielded 0.20±0.03 pg. cell⁻¹, 0.17±0.03 pg. cell⁻¹, 0.45±0.13 pg. cell⁻¹, 0.51±0.02 pg. cell⁻¹, 0.78±0.17 pg. cell⁻¹, respectively (Figure 10). Here, experiment E was significantly higher ($p < 0.05$) than the other experimental samples, indicating that increased salinity promoted carotenoid accumulation on a per-cell basis. This suggests that while overall culture carotenoid yield did not increase substantially, individual cells under high salinity invested more in carotenoid production, likely as part of a stress-adaptive mechanism.

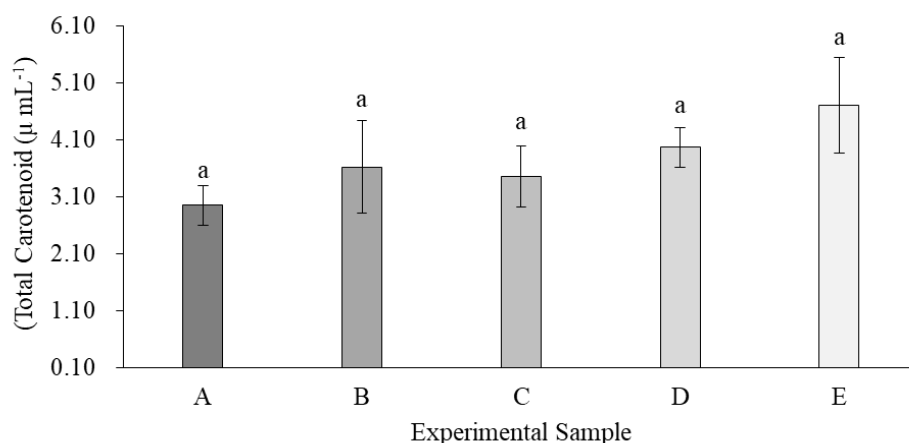


Figure 9. Total carotenoid pigment accumulation of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Experiment A (no salinity concentration), experiment B (5 g L⁻¹ salinity concentration), experiment C (10 g L⁻¹ concentration), experiment D (15 g L⁻¹ concentration), and experiment E (20 g L⁻¹ concentration).

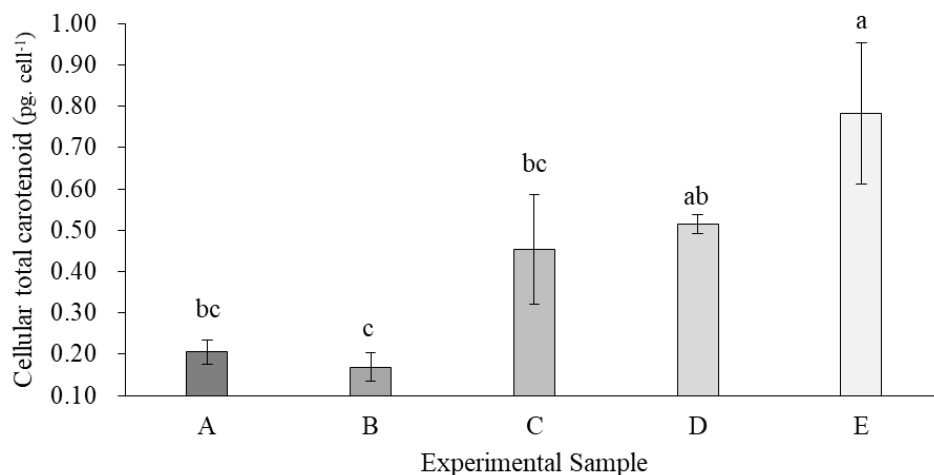


Figure 10. Cellular total carotenoid pigment accumulation of *Nannochloropsis* sp. culture at different concentrations of salinity in a nutrient medium. Experiment A (no salinity concentration), experiment B (5 g L⁻¹ salinity concentration), experiment C (10 g L⁻¹ concentration), experiment D (15 g L⁻¹ concentration), and experiment E (20 g L⁻¹ concentration).

Discussion. The goal of producing microalgae is to maintain a high cell density in continuous culture, as this is necessary for microalgae to reproduce efficiently. Continuous culture enables cells to proliferate more rapidly and effectively. A significant factor affecting the growth of microalgae cultures is salinity. This study investigated the effect of varying salinity concentrations on the culture of the microalga *Nannochloropsis* sp. As a result, a lower salinity of 5 g L⁻¹ obtained a higher cell density of $21.69 \pm 1.36 \times 10^6$ cell mL⁻¹ than the control experiment, with a mean cell density of $8.82 \pm 1.80 \times 10^6$ cell mL⁻¹. However, higher concentrations of salinity lower the cell density of *Nannochloropsis* sp. culture. Thus, the present study indicated that *Nannochloropsis* sp. produced high cell density at lower salinity compared to higher salinity and control experiments. This study, similar to that of Gu et al (2012), found that *Nannochloropsis oculata* exhibited better growth in low-salinity conditions and slower growth when cultivated in high-salinity conditions. In addition, *Nannochloropsis* sp. cultivated at a salinity level of 5 g L⁻¹ reached a maximum cell density of 12.00×10^6 cells mL⁻¹ (Fakhri et al 2017).

Additionally, microalga *Chlorella capsulate* cultivated at a salinity level of 25 g L⁻¹ reached a maximum cell density of 3.1×10^6 cells mL⁻¹ (Ebrahimi & Salarzadeh, 2016). Their study had a lower cell density than the current study, in which a salinity level of 5 g L⁻¹ in a nutrient medium achieved a cell density of 21.69×10^6 cells mL⁻¹ in *Nannochloropsis* sp. culture. Several studies have demonstrated that salt fluctuations induce an oxidative stress response in microalgae, alter the water's external water potential, disrupt its turgor pressure, and inhibit cell division and growth (Kirst, 1990; Kaur et al 2022; Vrana et al 2022). It has been reported that microalgae have adapted to salt stress by altering their photosynthesis and adjusting their osmotic balance (Mutale-joan et al 2021). Although higher salinity (20 g L⁻¹) reduced cell density and growth rate, it simultaneously resulted in the highest dry weight. This apparent contradiction can be explained by stress-induced accumulation of storage compounds (such as lipids and carbohydrates), which increase biomass even when cell proliferation is suppressed. Similar stress-driven biomass accumulation has been reported in other microalgae under salt stress (Mutale-joan et al 2021; Kaur et al 2022). Therefore, while lower salinity favored rapid cell division and higher cell numbers, extreme salinity promoted stress tolerance strategies that increased per-cell biomass, leading to greater dry weight despite reduced cell density. The present study confirmed that higher salinity concentrations in a nutrient medium lower the cell growth of *Nannochloropsis* sp. culture, but may simultaneously promote stress-induced biomass accumulation.

The SGR of *Nannochloropsis* sp. culture in a lower salinity concentration was higher at 0.47 day^{-1} for the first nine days of the culture period than in the higher salinity concentration and the control experiment; however, it decreased after 21 days of the culture period. Additionally, the lower salinity concentration also achieved a higher mean SGR of 0.23 day^{-1} than other experimental samples. Erbil et al (2021) produced microalga *Chlorella* sp. using the BG-11 medium in a tubular photobioreactor with no salinity concentration. They achieved a mean SGR of 0.078 day^{-1} , which is lower than the present study, where microalga *Nannochloropsis* sp. culture enriched BG-11 medium with lower salinity obtained a higher SGR. Generally, determining growth-limiting substances can be done by analyzing the SGR of cell cultures. Jaiswal et al (2020) reported that several factors can influence the growth of microalgae, including salinity. Due to their ability to overcome osmotic pressure, algal species can adapt to salinity (Zafar et al 2021). The present study found that in *Nannochloropsis* sp. culture, the SGR increased when lower salinity concentrations were present in the nutrient medium.

Furthermore, the yield of *Nannochloropsis* sp. cells cultured at different salinity levels was estimated by measuring the biomass of *Nannochloropsis* sp. after 21 days of culture. The present study revealed significant differences among the experimental samples, with the highest salinity concentration of 20 g L^{-1} producing a dry weight of $26.21 \pm 0.33 \text{ g L}^{-1}$, which was higher than the dry weight of the other experimental samples. Researchers from various studies have cultured the microalga *Nannochloropsis oculata* in a fiberglass-reinforced plastic panel photobioreactor enriched with F/2 medium, achieving a dry weight of 0.81 g L^{-1} (Durmaz & Erbil, 2020). An additional study found that continuous cultures enriched with f/2 medium achieved productivity levels of 2.02 and 3.03 g L^{-1} in helical tubular photobioreactors (Briassoulis et al 2010). Their results were comparable to those of the present study, which found that higher salinity levels in a BG-11 medium resulted in an increase in dry weight in *Nannochloropsis* sp. cultures. However, researchers have stated that obtaining high biomass in microalgae cultures depends on the salinity concentration of the nutrient medium (Liu & Yildiz, 2018; Sánchez-Bayo et al 2020; Ratomski & Hawrot-Paw, 2021; Kissae et al 2025). This creates an apparent contradiction: low salinity favored higher growth rates and cell densities, while high salinity reduced cell division but still produced greater dry weight. The most likely explanation is that under salinity stress, *Nannochloropsis* sp. diverts energy away from cell proliferation and instead accumulates storage compounds such as lipids and carbohydrates, increasing biomass per cell. This stress-induced accumulation of storage products has been widely reported in microalgae exposed to unfavorable growth conditions (Kaur et al 2022; Mutale-Joan et al 2021). Thus, the present study suggests that while lower salinity promotes rapid population growth, higher salinity enhances biomass accumulation per cell, reconciling the observed differences between cell density/SGR and dry weight.

The pigment accumulation in the present study showed that the chlorophyll-a accumulation of $15.40 \mu\text{g mL}^{-1}$ tended to increase with a lower salinity concentration (5 g L^{-1}) compared to a higher salinity concentration and the control experiment, although this difference was not statistically significant. Researchers reported that a Bold Basal medium with a 12 g L^{-1} salinity concentration achieved a chlorophyll-a accumulation of 4.5 mg g^{-1} in the microalga *Chlorella vulgaris* culture (Kebeish et al 2014). Additionally, Teh et al (2021) reported that an F/2 medium with a higher salinity concentration of 30 g L^{-1} obtained a chlorophyll-a accumulation of $14.62 \mu\text{g mL}^{-1}$ for microalga *Chlorella vulgaris* culture. These results support the general trend observed in the present study, even though differences between treatments in chlorophyll-a content were relatively small. The researchers reported that physical stress factors, such as salinity manipulation, influence the accumulation of the chlorophyll-a pigment in microalgae cultures (Esakkimuthu & Wang 2022). Moreover, a high total carotenoid pigment accumulation of $4.71 \mu\text{mol L}^{-1}$ was obtained in the highest salinity concentration of 20 g L^{-1} in *Nannochloropsis* sp. cultures. Ali et al (2021) reported that a salinity concentration of 10 g L^{-1} in BG-11 nutrient medium resulted in a total carotenoid content of $4.37 \mu\text{g/mL}$ in *Chlorella vulgaris* microalga culture. The present study found that a higher salinity concentration (20 g L^{-1}) in BG-11 nutrient medium resulted in higher total carotenoid

pigment accumulation, which is lower than the reported value. In a study of freshwater microalgae species, salt stress is a more effective alternative to light stress in promoting carotenoid production, as increased salinity levels result in increased biomass and pigment production, particularly carotenoids. However, as the salinity concentration increased, microalgae growth decreased (Asulabh et al 2012). The results of this study showed that higher cell densities are not associated with higher carotenoid pigment accumulation. Numerous studies have confirmed that microalgae accumulate carotenoids at higher levels under conditions of nutrient stress, such as salinity (Ravi et al 2012; Saha et al 2013).

Conclusions. Salinity is a significant factor influencing cell density, growth responses, biomass, and pigment accumulation in *Nannochloropsis* sp. cultures. The present study revealed that lower salinity (5 g L⁻¹) promoted higher cell density and SGR, while higher salinity (20 g L⁻¹) favored greater dry weight biomass accumulation. Pigment responses also varied, with chlorophyll-a tending to increase under lower salinity, whereas total carotenoid content was enhanced at higher salinity. These results demonstrate that different physiological parameters of *Nannochloropsis* sp. peak at different salinity levels, highlighting the importance of tailoring culture conditions to desired outcomes, whether maximizing cell density, biomass yield, or pigment production.

Acknowledgments. The authors would like to thank Mindanao State University, Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), the College of Fisheries (COF), and the Department of Science and Technology-Science Education Institute (DOST-SEI) for their ongoing support throughout the research. The authors would also like to express their heartfelt gratitude to Ma'am Arlyn A. Abdulla from the regional Fisheries Research Center, Ministry of Agriculture, Fisheries, and Agrarian Reform, Pangasinan, Bongao, Tawi-Tawi, Philippines, for allowing them to conduct this research in their facilities.

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

References

- Ahajan N. A., Robles R. J. F., Ancheta R. A., Imlan K. H., Talaid E. M., Sarri J. H., Mañalas A. A., 2025 Assessing the effects of organic fertilizer vermicompost on the growth and chlorophyll of sea lettuce *Ulva lactuca* under laboratory conditions. *AACL Bioflux* 18(5):2075-2087.
- Ali H. E. A., El-fayoumy E. A., Rasmy W. E., Soliman R. M., Abdullah M. A., 2021 Two-stage cultivation of *Chlorella vulgaris* using light and salt stress conditions for simultaneous production of lipid, carotenoids, and antioxidants. *Journal of Applied Phycology* 33(1):227-239.
- Ampofo J., Abbey L., 2022 Microalgae: Bioactive composition, health benefits, safety, and prospects as potential high-value ingredients for the functional food industry *Foods* 11(12):1744.
- Asulabh K. S., Supriya G., Ramachandra T. V., 2012 Effect of salinity concentrations on growth rate and lipid concentration in *Microcystis* sp., *Chlorococcum* sp., and *Chaetoceros* sp. In the National Conference on Conservation and Management of Wetland Ecosystems. School of Environmental Sciences Mahatma Gandhi University, Kottayam, Kerala.
- Briassoulis D., Panagakis P., Chionidis M., Tzenos D., Lalos A., Tsinos C., Berberidis, K., Jacobsen, A., 2010 An experimental helical-tubular photobioreactor for continuous production of *Nannochloropsis* sp. *Bioresource Technology* 101(17):6768-6777.
- Brotosudarmo T. H. P., Limantara L., Chandra, R. D., 2018 Chloroplast pigments: Structure, function, assembly and characterization. In *Plant Growth and Regulation-Alterations to Sustain Unfavorable Conditions*. IntechOpen. London, UK. pp. 43-80.

- Chakraborty R. D., Chakraborty K., Radhakrishnan E. V., 2007 Variation in fatty acid composition of *Artemia salina* nauplii enriched with microalgae and baker's yeast for use in larviculture. *Journal of agricultural and food chemistry* 55(10):4043-4051.
- Coêlho D. D. F., Tundisi L. L., Cerqueira K. S., Rodrigues J. R. D. S., Mazzola P. G., Tambourgi E. B., Souza, R. R. D., 2019 Microalgae: cultivation aspects and bioactive compounds. *Brazilian Archives of Biology and Technology* 62:e19180343.
- Da Silva J. C., Lombardi A. T., 2020 Chlorophylls in microalgae: occurrence, distribution, and biosynthesis. *Pigments from microalgae handbook* pp. 1-18.
- Dolganyuk V., Belova D., Babich O., Prosekov A., Ivanova S., Katserov, D., Patyokuv N., Sukhikh, S., 2020 Microalgae: a promising source of valuable bioproducts. *Biomolecules* 10(8):1153.
- Durmaz Y., Erbil, G. Ç., 2020 Comparison of industrial-scale tubular photobioreactor to FRP (fiberglass reinforced plastic) panel photobioreactor on outdoor culture of *Nannochloropsis oculata* in the marine hatchery. *Ege Journal of Fisheries & Aquatic Sciences (EgeJFAS)/Su Ürünleri Dergisi* 37(3):303-308.
- Ebrahimi E., Salarzadeh A., 2016 The effect of temperature and salinity on the growth of *Skeletonema costatum* and *Chlorella capsulata* *in vitro*. *International Journal of Life Sciences* 10(1):40-44.
- Erbil G. Ç., Durmaz Y., Elp M., 2021 Indoor growth performance of *Chlorella* sp. production at tubular photobioreactor. *Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi* 7(2):90-95.
- Esakkimuthu S., Wang S., 2022 Physical stress for enhanced biofuel production from microalgae. In *Handbook of Algal Biofuels*. Elsevier. pp. 451-475.
- Fakhri M., Arifin N. B., Yuniarti A., Hariati M. A. 2017 The Influence of Salinity on the Growth and Chlorophyll Content of *Nannochloropsis* sp. *BJ17. Nature Environment and Pollution Technology An International Quarterly Scientific Journal* 16(1):209-212
- Gani P., Sunar N. M., Matias-Peralta H. M., Apandi N., 2019 An overview of environmental factors effect on the growth of microalgae. *Journal of Applied Chemistry and Natural Resources* 1(2):1-5.
- Gu N., Lin Q., Li G., Tan Y., Huang L., Lin J., 2012. Effect of salinity on growth, biochemical composition, and lipid productivity of *Nannochloropsis oculata* CS 179. *Engineering in Life Sciences* 12(6):631-637.
- Hairol M. D., Nian C. T., Imlani A. H., Tikmasan J. A., Sarri J. H., 2022 Effects of Crab Shellmeal Inclusions to Fishmeal Replacement on the Survival, Growth, and Feed Utilization of Mangrove Crab *Scylla serrata* (Forsskal 1775). *Yuzuncu Yil University Journal of Agricultural Sciences* 32(4):714-726.
- Hashmi Z., Abbas S. H., Osama S. M., Muhammad A., Usman M. T., Jatoi A. S., Bozdar M. M., 2023 Microalgae technology in aquaculture applications: a comprehensive literature review. *AMPLITUDO: Journal of Science and Technology Innovation* 2(2):61-69.
- Hosikian A., Lim S., Halim R., Danquah M. K., 2010 Chlorophyll extraction from microalgae: A review on the process engineering aspects. *International journal of chemical engineering* 2010(1):391632.
- Hu J., Meng W., Su Y., Qian C., Fu, W., 2023 Emerging technologies for advancing microalgal photosynthesis and metabolism toward sustainable production. *Frontiers in Marine Science* 10:1260709.
- Jain P. K., Jain P., Pandey B., Sarangi P. K., Prakash A., Singh A. K., Srivastava R. K., 2022 Carotenoids and pigment generation using the microalgal production system. In *Micro-algae: Next-generation Feedstock for Biorefineries: Contemporary Technologies and Future Outlook*. Springer Nature Singapore, Singapore, pp. 129-143.
- Jaiswal K. K., Banerjee I., Singh D., Sajwan P., Chhetri, V., 2020 Ecological stress stimulus to improve microalgae biofuel generation: a review. *Octa Journal of Biosciences* 8(1):48-54.

- Kaur M., Saini K. C., Ojah H., Sahoo R., Gupta K., Kumar A., Bast F., 2022 Abiotic stress in algae: Response, signaling and transgenic approaches. *Journal of Applied Phycology* 34(4):1843-1869.
- Kebeish R., El-Ayouty Y., Hussein, A., 2014 Effect of salinity on biochemical traits and photosynthesis-related gene transcription in *Chlorella vulgaris*. *Egyptian Journal of Botany* 54(2):281-294.
- Kirst G.O., 1990 Salinity tolerance of eukaryotic marine algae. *Annual Review of Plant Physiology and Plant Molecular Biology* 41:21-53.
- Kissae A.-N. H., Sarri J. H., Amlani M. Q., Jumsali M. H., Jamil W. M., Imbuk E. S., Jalilul J. M., Jeva M. A., Hairol M. D., 2025 Incorporation of salinity and AMPEP concentration in nutrient medium on dry weight, cell density, growth response, and pigment accumulation of *Nannochloropsis* sp. culture. *AACL Bioflux* 18(3):1593-1604.
- Liu Y., Yildiz I., 2018 The effect of salinity concentration on algal biomass production and nutrient removal from municipal wastewater by *Dunaliella salina*. *International Journal of Energy Research* 42(9):2997-3006.
- Macias-Sánchez M. D., Mantell C., Rodríguez M., De La Ossa E. M., Lubián L.M., Montero, O., 2005 Supercritical fluid extraction of carotenoids and chlorophyll *a* from *Nannochloropsis gaditana*. *Journal of Food Engineering* 66(2):245-251.
- Mazard S., Penesyán A., Ostrowski M., Paulsen I. T., Egan, S., 2016 Tiny microbes with a big impact: the role of cyanobacteria and their metabolites in shaping our future. *Marine drugs* 14(5):97.
- Mutale-joan C., Rachidi F., Mohamed H. A., Mernissi N. E., Aasfar A., Barakate M., Mohammed D., Sbabou L., Arroussi, H. E., 2021 Microalgae-cyanobacteria based biostimulant effect on salinity tolerance mechanisms, nutrient uptake, and tomato plant growth under salt stress. *Journal of Applied Phycology* 33:3779-3795.
- Premachandra E., Balasooriya B., Premarathna M., Ekanayaka, I., 2023 *Nannochloropsis* sp.: culturing and potential for fish feed production. In *Proceedings of 21st Agricultural Research Symposium* 141:145.
- Ratomski P., Hawrot-Paw M., 2021 Influence of nutrient-stress conditions on *Chlorella vulgaris* biomass production and lipid content. *Catalysts* 11(5):573.
- Ravi S., Ambati R. R., Kamath S. B., Chandrappa D., Narayanan A., Chauhan V. S., Ravishankar G. A., 2012 Influence of different culture conditions on yield of biomass and value-added products in microalgae. *Dynamic Biochemistry, Process Biotechnology and Molecular Biology* 6(2):77-85.
- Saadaoui I., Rasheed R., Aguilar A., Cherif M., Al Jabri H., Sayadi S., Manning S. R., 2021 Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. *Journal of Animal Science and Biotechnology* 12(1):76.
- Saha S. K., Moane S., Murray P., 2013 Effect of macro-and micro-nutrient limitation on superoxide dismutase activities and carotenoid levels in microalga *Dunaliella salina* CCAP 19/18. *Bioresource Technology* 147:23-28.
- Sánchez-Bayo A., Morales V., Rodríguez R., Vicente G., Bautista L. F., 2020 Cultivation of microalgae and cyanobacteria: Effect of operating conditions on growth and biomass composition. *Molecules* 25(12):2834.
- Sanuddin N. B., Hairol M. D., Nian C. T., Robles R. J. F. Illud, H. A. Muyong J. S., Ebbah J. H., Sarri J. H., 2023 Impact of different nutrient enrichment concentrations on the growth of microalga *Nannochloropsis* sp. (Monodopsidaceae) culture. *Acta Natura & Scientia* 4(1):87-93.
- Sarri J. H., Ibno D. C. V., Hassan R. K., Hairol M. D., 2024 Investigation of the effect of AMPEP concentration in nutrient medium on the cell density, growth response, and pigment accumulation of *Nannochloropsis* sp. culture. *AACL Bioflux* 17(6):2886-2898.
- Silva S. C., Ferreira I. C., Dias M. M., Barreiro, M. F., 2020 Microalgae-derived pigments: A 10-year bibliometric review and industry and market trend analysis. *Molecules* 25(15):3406.

- Tan J. S., Lee S. Y., Chew K. W., Lam M. K., Lim J. W., Ho S. H., Show, P. L., 2020 A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids. *Bioengineered* 11(1):116-129.
- Teh K. Y., Loh S. H., Aziz A., Takahashi K., Effendy A. W. M. Cha, T. S., 2021 Lipid accumulation patterns and role of different fatty acid types towards mitigating salinity fluctuations in *Chlorella vulgaris*. *Scientific Reports* 11(1):438.
- Vrana I., Bakija Alempijević S., Novosel N., Ivošević DeNardis N., Žigon D., Ogrinc N., Gašparović, B., 2022 Hyposalinity induces significant polar lipid remodeling in the marine microalga *Dunaliella tertiolecta* (Chlorophyceae). *Journal of Applied Phycology* 34(3):1457-1470.
- Zafar A. M., Javed M. A., Hassan A. A., Mehmood K., Sahle-Demessie, E., 2021 Recent updates on ions and nutrients uptake by halotolerant freshwater and marine microalgae in conditions of high salinity. *Journal of Water Process Engineering* 44:102382.
- Zou N., Richmond A., 2000 Light-path length and population density in photoacclimation of *Nannochloropsis* sp. (Eustigmatophyceae). *Journal of Applied Phycology* 12(3):349-354.

Received: 02 July 2025. Accepted: 25 August 2025. Published online: 26 November 2025.

Authors:

Jurmin Hamad Sarri, Aquaculture Department, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, 7500 Sanga-Sanga, Bongao, Tawi-Tawi, Philippines, e-mail: jurminsarri@msutawi-tawi.edu.ph

Al-Nasrif Halikul Kissae, Aquaculture Department, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, 7500 Sanga-Sanga, Bongao, Tawi-Tawi, Philippines, email: al-nasrifkissae@msutawi-tawi.edu.ph

Melodina Dindin Hairol, Aquaculture Department, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, 7500 Sanga-Sanga, Bongao, Tawi-Tawi, Philippines, e-mail: melodinahairol@msutawi-tawi.edu.ph

Khadiza Hairul Imlan, Aquaculture Department, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, 7500 Sanga-Sanga, Bongao, Tawi-Tawi, Philippines, e-mail: imlankhadz@gmail.com

Fatima Nhidzlah Tan Ensano, Aquaculture Department, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, 7500 Sanga-Sanga, Bongao, Tawi-Tawi, Philippines, e-mail: fatimanhidzlahensano@msutawi-tawi.edu.ph

Shardanika Halikul Kissae, Aquaculture Department, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, 7500 Sanga-Sanga, Bongao, Tawi-Tawi, Philippines, e-mail: kissaesharda19@gmail.com

Rizal Jhunn Falcatan Robles, Aquaculture Department, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, 7500 Sanga-Sanga, Bongao, Tawi-Tawi, Philippines; Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Malaysia, e-mail: rizaljhunrobles@msutawi-tawi.edu.ph

Enraida Sabri Imbuk, Aquaculture Department, College of Fisheries, Mindanao State University-Sulu, 7400 Patikul Site, Sulu, Philippines, email: imbukenraida@gmail.com

Shada-Wati Halikul Kissae, Aquaculture Department, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, 7500 Sanga-Sanga, Bongao, Tawi-Tawi, Philippines, e-mail: shadawatikissae@msutawi-tawi.edu.ph

Nurshaifah Mohammad Adjid, Aquaculture Department, College of Fisheries, Mindanao State University-Sulu, 7400 Patikul Site, Sulu, Philippines, email: nursaifah.adjid@msusulu.edu.ph

Fatmawati Omar Albar, Aquaculture Department, College of Fisheries, Mindanao State University-Sulu, 7400 Patikul Site, Sulu, Philippines, email: fatmawati.albar@msusulu.edu.ph

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

How to cite this article:

Sarri J. H., Kissae A. N. H., Hairol M. H., Imlan K. H., Ensano F. N. T., Kissae S. H., Robles R. J. F., Imbuk E. S., Kissae S. W. H., Adjid N. M., Albar F. R., 2025 Cell density, growth response, and pigment accumulation of *Nannochloropsis* sp. culture under salinity concentration. *AAFL Bioflux* 18(6):2547-2560.