

Temporal dynamics of cell density and culture performance of live feed *Nannochloropsis oculata, Isochrysis galbana*, and *Chaetoceros* sp. cultured in guillard f/2 medium

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Received: March 2026 Accepted: June 2026

Abstract

This study was investigated to observe the growth kinetics of marine microalgae *Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros* sp. The culture was maintained in three different vessels: sizes 250 mL flask using Guillard f/2 medium and natural seawater. All three species amplified distinct growth phases, including lag, exponential, stationary, and declining phases. Maximal cell densities were achieved during the exponential phase, with *Nannochloropsis oculata* reaching the highest at 96.76 ± 41.87 x 10^5 cells/mL on day 8. *Isochrysis galbana* exhibited a steady growth trend, peaking at the highest cell density (120.48±76.91×10^5 cells/mL) among three species on day nine. In contrast, *Chaetoceros* sp. grows substantially more slowly, reaching a maximum density of 26.83±4.62×10^5 cells/ml, regardless of the culture vessel size. Surprisingly, *Nannochloropsis oculata* demonstrated the fastest growth rate (0.93 cells/day) on day three. These findings highlight how important it is to optimize culture conditions in order to maximize the development potential of specific microalgal species. It is required to conduct more research on how these strains are affected by nutrient addition and light intensity.

Keywords: Microalgae, Live feed, Growth kinetics, Cell density, Aquaculture

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[DOI: 10.22034/ANFS.1.1.21]

Introduction:

Under the group of algae that are accounted for producing atmospheric oxygen via photosynthesis, microalgae are unicellular microorganisms that thrive in both saltwater and freshwater environments. Marine microalgae are the floating microscopic unicellular plants found in seawater (Tahir *and Ransangan,* 2021; Hachicha *et al.*, 2022). They offer limitless opportunities for utilization in different sectors for the benefit of mankind. They are generally free-living and pelagic with size ranges from 2 to 20µm. The important classifications of microalgae are the diatoms, dinoflagellates, green algae, blue-green algae, and coccolithophores. Among them the species *Nannochloropsis oculata* belongs to the class of green algae, specifically within the order Eustigmatophyceae, *Isochrysis galbana* is a member of the class coccolithophores, which are a type of haptophyte, and *Chaetoceros* sp. which belongs to the class of diatoms (Bacillariophyceae) (Volkman *et al.,* 1993; Shi *et al.,* 2008; Brown 1991; Zhang *et al.,* 2023; Reitan *et al.,* 2021).

One of the major intents of the study was to focus on one of the major contributors in the shrimp industry in Bangladesh, Cox's Bazar. Cox's Bazar district has a 42125-hector area for shrimp production whose total production was estimated in FY 2021-22 as 14394.55 metric tons. The hatchery sector of Cox's Bazar is also contributing to the production of PL (Post Larvae) to meet the demand of the country's annual shrimp PL demand DoF (2022). Focusing on improving the value chain activities within the shrimp firms by integrating microalgae production, is one of the focuses of the Bangladesh Fisheries Research Institute (BFRI), which can lead to increased competitiveness and value creation in the industry. By enhancing microalgae production alongside shrimp farming through a government or private-based approach, Cox's Bazar can potentially strengthen its position in the global market, ensuring long-term growth and economic stability for the coastal community (Nagarajan *et al.,* 2024).

Microalgae supplementation in shrimp culture offers numerous benefits by improving nutritional quality, enhancing growth rates, and supporting environmental sustainability. As a valuable source of lipids, proteins, vitamins, and minerals, they serve as a contributor to the overall health and development of various growth stages of shrimp (Bhambri *et al.,* 2023; Ma *et al.,* 2024). Microalgae plays a vital role in aquaculture to meet the nutritional requirement of the larvae as well as for bioencapsulation (Perumal *et al.,* 2012; Abreu *et al.,* 2025). Additionally, microalgae also play a crucial role in maintaining a healthier ecosystem for the culture environment by efficiently removing ammonia, nitrite, and nitrate from aquatic environments, thus reducing nitrogen pollution for shrimp farming (Huang *et al.,* 2022; Mahmudi *et al.,* 2023). Microalgae are also crucial to aquatic ecosystems, contributing 40% of global photosynthesis and producing nearly half of Earth's atmospheric

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oxygen at any given time (Moreno-Garrido, 2008). Specific microalgae strains have also been identified to effectively ennoble the growth of shrimp and enhance their survival rates, ultimately increasing shrimp maximum yield in culture tanks (Duan *et al.,* 2022)

These organisms can develop photoautotrophically, using light and CO_2 as sources of energy and carbon, respectively, and converting them into biomass and O_2 (Khan *et al.,* 2018). This makes their growth straightforward and potentially economical. As a component of a bio-refinery idea, microalgae farming can produce a wide range of goods with uses in medicine, food, pharmaceuticals, nutraceuticals, and biofuels (Ibañez and Cifuentes, 2013).

Coutteau (1991) described three basic types of culture systems batch culture, semi-continuous culture, and continuous culture (also subdivided into turbidostatic and chemostat culture) based on the intention of uses and techniques used in culture (Dhert *et al.,* 1992; Tzovenis *et al.,* 1997; Sorgeloos *et al*., 2009). Lavens and Sorgeloos (1996) authored a comprehensive guideline on how and in which way the algal growth in batch culture experiences five different phases. The guideline describes the kinetics as different phases in the growth stage: lag, exponential, stationary, and declining. The initial stage named the lag phase is an initiation of slow growth which experiences the rapid growth of cell division in the exponential phase, slow cell division due to the lack of nutritional resources in stationary phases, and rapid declination of cell density due to death of microalgae.

Increasing energy demand has led to concerns about fossil fuel depletion as well as anthropogenic carbon dioxide (CO_2) emissions which have contributed to global climate change (Daneshvar *et al.,* 2022; Asma Sarwer *et al.,* 2022). Capturing CO_2 using microalgae addresses these environmental concerns and offers additional benefits, making it a potentially economically viable system (Onyeaka *et al.,* 2021). Consequently, microalgae have been suggested as excellent candidates for carbon sequestration and biofuel production. Among their advantages are high photosynthetic efficiency, high biomass production, and rapid growth compared to other crops used for energy purposes. The growth of microalgae requires sunlight, water, CO_2, and nutrients for photosynthesis (Stoppato, 2008). Thus, optimizing culture conditions and developing a multi-stakeholder engagement strategy for various sectors related to shrimp, crab, and other mollusk production and consumption can benefit significantly from a sustainable microalgae production scheme.

Materials and methods

Research location

This study was conducted at the “Live Feed Culture” laboratory of the Bangladesh Fisheries Research Institute, Cox's Bazar. The laboratory is well-equipped with modern scientific equipment and facilities for the culture and testing of microalgae. Essential

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equipment, including aerators, beakers, conical flasks, measuring cylinders, burettes, microscopes, autoclaves, LED light systems, and hemocytometers, were available. Strict hygienic conditions were maintained, and the laboratory was well-furnished.

Seed source and management

Marine microalgae species—*Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros* sp.—were collected and cultured in the "Live Feed Culture" laboratory at the Bangladesh Fisheries Research Institute, Cox's Bazar.

Water source and management

Fresh seawater was sourced from the sub-littoral zone along the coast of the Bay of Bengal. The water was pumped, filtered, and stored in tanks for 24 hours to allow siltation. Afterward, the seawater was pumped into treatment tanks, passed through cartridge filtration, and subjected to ultraviolet radiation for disinfection.

Environmental control

Continuous 24-hour aeration was provided via aeration tubes to ensure optimal oxygen levels and prevent the settling of algal cells. The temperature was maintained between 26-27°C, and light intensity was regulated at around 4500 lux using LED lights. This helped maintain the necessary conditions for microalgal culture.

Sterilization of equipment

All culture vessels, including test tubes and flasks, were sterilized in an autoclave at 121°C (29.4 psi) for 10 minutes (small flasks and test tubes) and 1 hour for larger 10-liter containers (Kawachi and Noël, 2005).

Culture of microalgae

Media preparation

To achieve high microalgal cell densities in the shortest possible time, a nutrient-rich culture medium is essential, as natural concentrations of nutrients in freshwater and seawater often do not support optimal algal growth. Numerous media recipes have been developed, tailored to specific microalgae species. For this study, we used Guillard's f/2 medium, a widely accepted and effective formulation for marine microalgae culture. This medium is rich in essential macro and micronutrients, including nitrogen, phosphorus, silica, and trace metals, which are necessary for robust algal growth. The f/2 medium is specifically designed to provide balanced nutrition for a wide range of marine species, ensuring high-density cultures for experimental purposes. This nutrient mixture was prepared and sterilized prior to inoculation to promote optimal conditions for the targeted microalgae species. By using this proven medium, we aimed to achieve rapid algal growth and efficient culture maintenance for the study's requirements (Table 1).

Inoculation and culture

In this study, three marine microalgae species, *Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros* sp., were cultured under controlled conditions.

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Table 1: Guillard's F/2 media used to culture marine microalgae (Guillard, 1975)

Major nutrient	Chemical formula	Concentration (g/L)
1.Nitrate	$NaNO_3$	75g/L
2.Phosphate	NaH_2PO_4	5g/L
3.Silicate	$Na_2SiO_3.9H_2O$	30g/L
4.Trace Metals		
	$FeCl_3.6H_2O$	3.5g/L
	Na_2EDTA	4.36/L
Dissolved in 900mL distilled water. Added 1mL of each of the following trace metal solutions.		
	$CuSO_4.5H_2O$	0.98g/100 mL
	$ZnSO_4.7H_2O$	2.20g/100 mL
	$CoCl_2.6H_2O$	1g/100 mL
	$MnCl_2.4H_2O$	18g/100 mL
	$Na_2MoO_4.2H_2O$	0.63g/100 mL
Make up the volume to 1 liter with distilled H_2O. Added 1mL per liter of seawater of the above solutions (1-4)		
5. Vitamins		
	Biotin	1mg
	B_{12}	1mg
	Thiamine	20mg

Dissolving vitamins in 1liter of distilled water. Store frozen. Adding 0.5mL vitamin solution for every 1-liter seawater

The culture environment was meticulously maintained to ensure optimal growth, with a consistent temperature range of 26-27°C. The pH of the culture medium was kept between 7.5 and 8.5, which is ideal for the growth of marine microalgae. The salinity level was maintained between 32-33 ppt (parts per thousand), replicating the natural seawater conditions these species thrive in. Light intensity was carefully controlled at 4500 lux to provide the necessary illumination for photosynthesis (Fig. 1). Guillard's f/2 culture medium was used for the cultivation, providing the essential nutrients for the algae's growth, including nitrogen, phosphorus, trace metals, and vitamins. The inoculation process involved transferring specific volumes of microalgae from a batch culture into fresh culture medium. For example, 10-15 ml of *Nannochloropsis oculata* culture was added to 150 ml of prepared medium in a 250 ml flask. This method ensured a controlled inoculation of each species in the appropriate growth vessel.

Estimating algal density

Algal density was estimated by counting the cells using the Palmer-Maloney slide or a hemacytometer, which is a specialized counting chamber. The chamber contains two identical grids, each divided into nine large squares, which helps in counting cells under a microscope. A small sample (100 µL) of the cultured algae suspension was removed and placed into a microcentrifuge tube. The suspension was then diluted by adding 80 µL of Trypan blue dye, a vital stain that distinguishes viable from non-viable cells. Living cells remain colorless, while dead cells absorb the dye and appear blue under the microscope.

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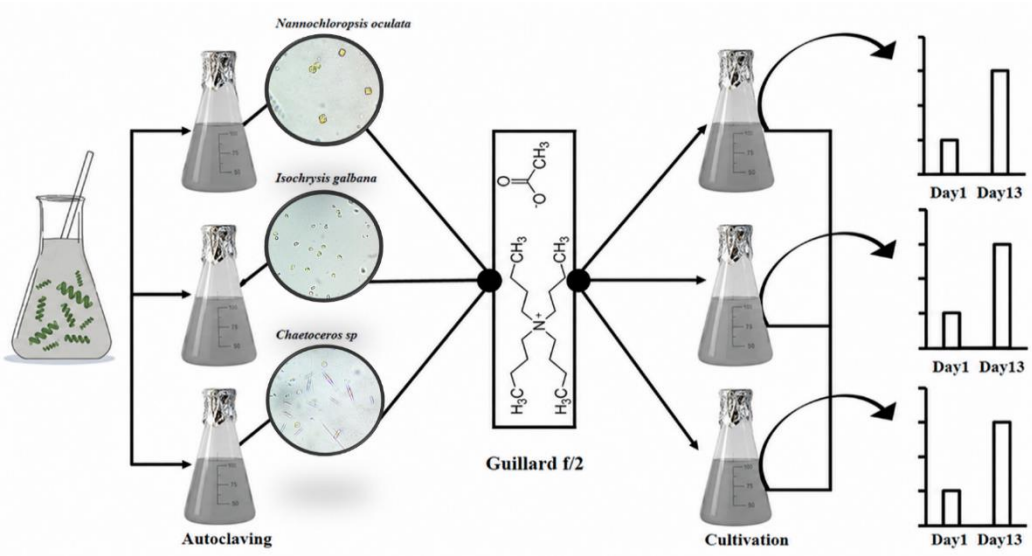


Figure 1: Methodological framework of the total culture.

This method allowed for accurate cell counting and differentiation, facilitating the calculation of algal cell density.

Method

Number of viable cells/mL= Average number of cells/square ×Dilution factor $\times 10^4$ (vlab.amrita.edu, 2011)

$$\text{Average number of cells/squares} = \frac{\text{Total number of cells in square}}{\text{Number of squares}}$$

$$\text{Dilution factor} = \frac{\text{Final volume}}{\text{Volume of cells}} = \frac{\text{Volume of cells+Volume of Trypan Blue}}{\text{Volume of cells}} = \frac{100+80}{100} = 0.8$$

Data recording and data analysis

During successive 13 days of observation, the number of cells of each culture vessel was counted and recorded by hemacytometer. Data were statistically analyzed and interpreted to determine the phases by Microsoft Excel 2013. Growth kinetics modeling was also done by plotting all the calculated data into Microsoft Excel 2013 and different phases in the growth of these microalgae were observed and presented in the graph.

Statistical analysis

One-way Analysis of Variance (ANOVA) was performed on three different microalage *Nannochloropsis oculata*, *Isochrysis galbana* and, *Chaetoceros sp* from the cell count data analysis using the Statistical Package for Social Science (SPSS) version 25 and Microsoft Excel to make the graphs.

Growth rate

The growth rate of the cultures was calculated from the expression; (Phatarpekar *et al.,* 2000):

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$K = (\ln N_1 - \ln N_0) / t$

Where, N_1=cell count at the time 't', N_0=initial cell counts at time '0', and t=time in days.

Growth kinetics

Growth kinetics of three different cultured microalgae species- *Nannochloropsis oculata*, *Isochrysis galbana* and, *Chaetoceros* sp. *sp*- were observed and represented graphically in Box and Whisker statistical chart using Microsoft Excel.

Results

The growth patterns of three microalgae species, *Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros* sp., were observed over a 13-day culture period. The cell density of *Nannochloropsis oculata* exhibited a gradual increase starting from the third day of culture, reaching its peak on the eighth day, with a cell density of $96.76 \pm 41.87 \times 10^5$ cells/mL (Fig. 2).

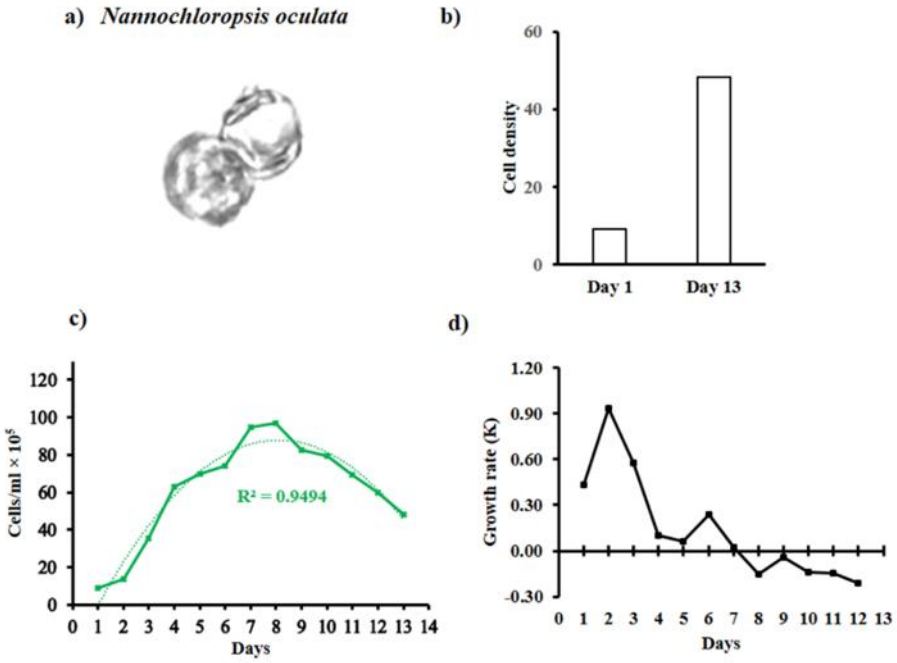


Figure 2: a) Identical sketch of *Nannochloropsis oculata*; b) Cell density of *Nannochloropsis oculata* on Day 1, illustrating the initial status, and Day 13, showing the final status after media application; c) Changes in cellular density (cells/mL); d) Growth rate (K) of *Nannochloropsis oculata*.

This was followed by a sharp decline on the ninth day, after which the cell density remained at lower levels throughout the remainder of the culture period. The decrease in cell density post-peak may be attributed to several factors, including nutrient depletion, accumulation of metabolic waste products, or a shift from exponential to stationary phase growth.

In contrast, *Isochrysis galbana* demonstrated more vigorous growth compared to the other species. Its cell density reached its highest point of $120.48 \pm 76.91 \times 10^5$ cells/ml on the ninth

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day, with a slight reduction in the subsequent days, indicating that the species had reached its optimal growth phase by this time (Fig. 3). This rapid initial growth and subsequent slight decrease may suggest a brief but intense exponential growth phase, after which the species entered a stationary phase.

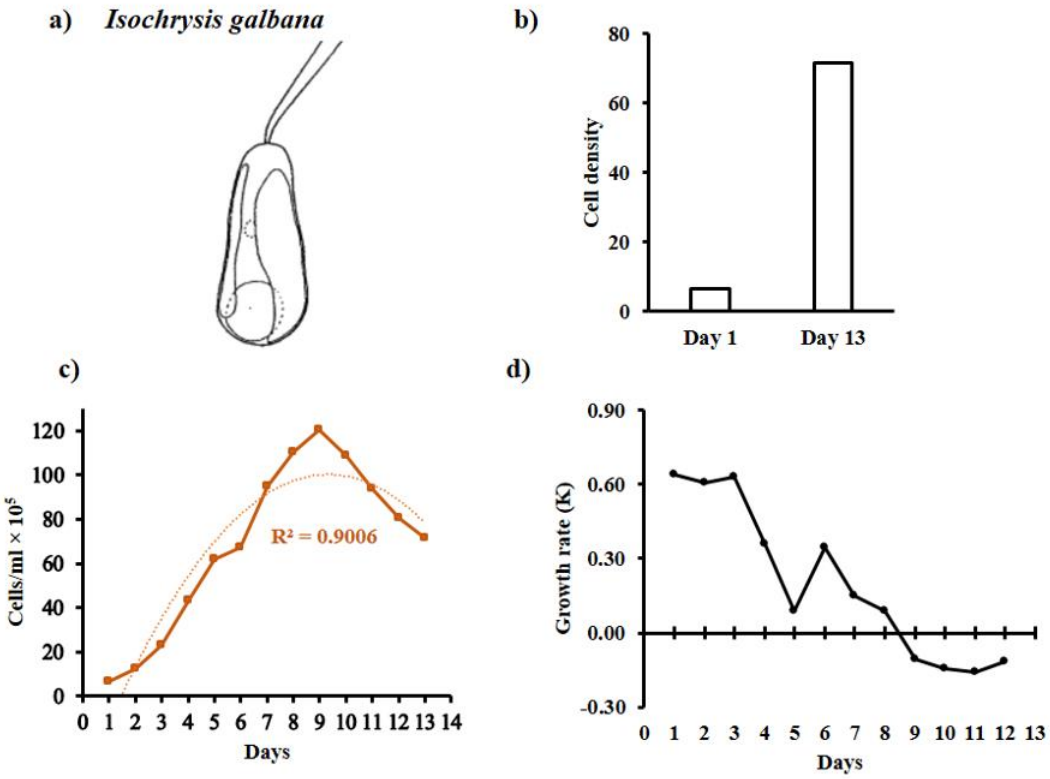


Figure 3: a) Identical sketch of *Isochrysis galbana*; b) Cell density of *Isochrysis galbana* on Day 1, illustrating the initial status, and Day 13, showing the final status after media application; c) Changes in cellular density (cells/mL); d) Growth rate (K) of *Isochrysis galbana*.

On the other hand, *Chaetoceros* sp. exhibited a slower and more modest growth trajectory. The cell density peaked at 26.83±4.62×10^5 cells/mL, a much lower value compared to the other two species. This species showed a more gradual increase in cell number, and its growth rate was consistently lower than that of *Nannochloropsis oculata* and *Isochrysis galbana*. Following its peak, the cell density rapidly declined until the thirteenth day, indicating a transition to the death phase after reaching its peak. This slower growth could be attributed to several factors, including its biological characteristics, nutrient requirements, or environmental conditions that may not have been optimal for this species.

During the first five days of culture, significant variations in cell density (p<0.05) were observed among the three microalgae species. This early-stage variation indicates that each species responded differently to the culture conditions, with distinct growth rates and adaptations. The difference in cell density during the initial five days was

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statistically significant, highlighting the unique growth patterns of each species. However, after the fifth day, the differences in cell density among the species became statistically insignificant, suggesting that by this time, the growth of all three species had either slowed down or stabilized in a similar range. This could indicate that, beyond the initial rapid growth phase, all species had entered a stationary phase where factors such as nutrient availability, light intensity, and space limitations began to equally affect their growth rates.

These findings underscore the importance of understanding the species-specific growth dynamics when culturing microalgae, as each species exhibits unique growth characteristics that influence their cell density and overall productivity in culture systems (Fig. 4).

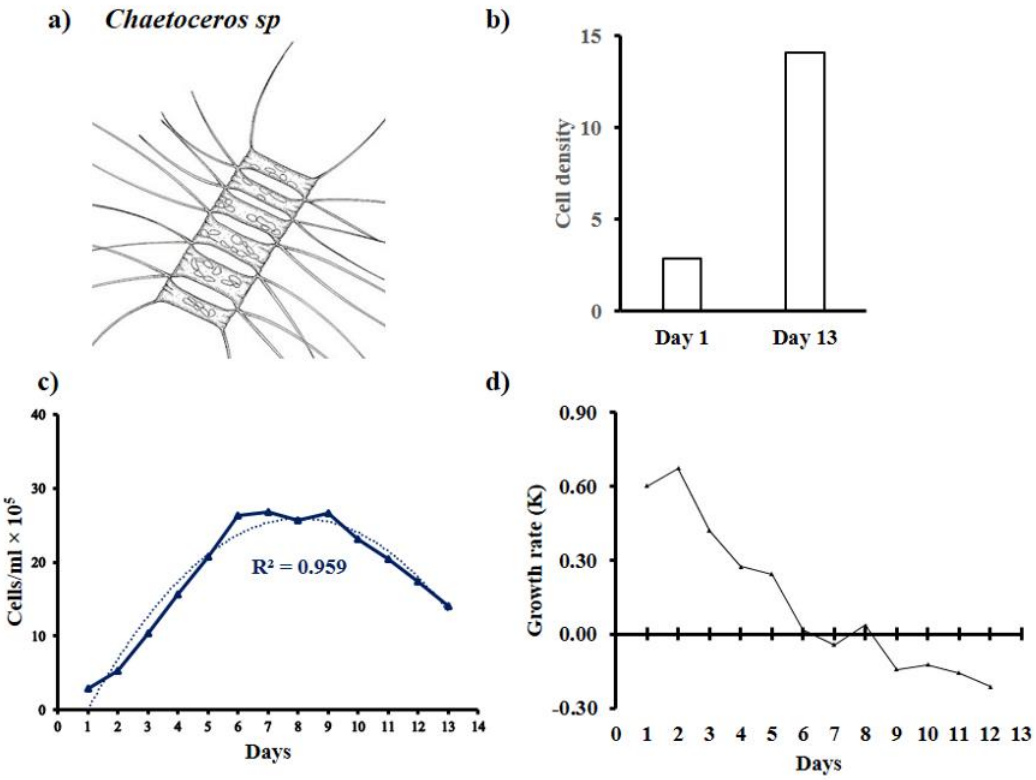


Figure 4: a) Identical sketch of *Chaetoceros sp*; b) Cell density of *Chaetoceros sp* on Day 1, illustrating the initial status, and Day 13, showing the final status after media application; c) Changes in cellular density (cells/mL); d) Growth rate (K) of *Chaetoceros* sp.

For the species factor, significant differences were observed between *Isochrysis Galbana* and *Chaetoceros* sp. (p<0.0001), as well as between *Nannochloropsis Oculata* and *Chaetoceros* sp. (p<0.0001). However, the comparison between *Nannochloropsis Oculata* and *Isochrysis Galbana* was not statistically significant (p=0.3747), indicating no substantial difference between these two species.

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Regarding the phase factor, the comparison between the Mid (D4-D7) and Initial (D1-D3) phases showed a significant difference in cell density (p=0.0000006). A similar significant difference was found between the Tertiary (D8-D13) and Initial (D1-D3) phases (p<0.0001). However, the difference between the Tertiary (D8-D13) and Mid (D4-D7) phases was not significant (p=0.2244).

For the Species: Phase interaction, the differences were more nuanced. Significant differences were observed between several species-phase combinations. For example, *Isochrysis Galbana* during the Mid (D4-D7) phase compared to *Chaetoceros* sp. during the Initial (D1-D3) phase exhibited a large difference in cell density (60.66, p<0.0001), and similarly, *Nannochloropsis Oculata* during the Mid (D4-D7) phase compared to *Chaetoceros* sp. during the Initial (D1-D3) phase showed a difference of 69.34 (p<0.0001). However, some combinations, like *Isochrysis Galbana* in the Tertiary (D8-D13) phase compared to *Isochrysis Galbana* in the Mid (D4-D7) phase, showed no significant difference (p=0.05).

Growth rate

The growth rates of three microalgae species cultured under controlled conditions are presented in Figure 5, showing distinct patterns over the 13-day culture period. For *Nannochloropsis oculata*, the growth rate fluctuated during the first three days of culture, with rates of 0.63±0.26 cells/d. This initial variation suggests an adaptation phase, where the algae are adjusting to the culture environment.

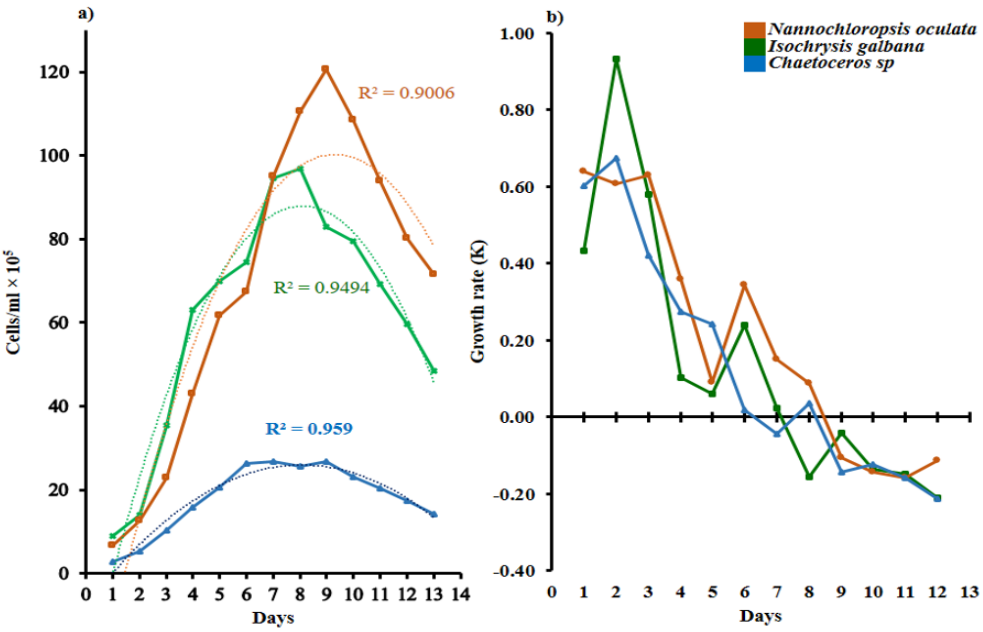


Figure 5: a) Growth curve of *Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros sp.* observed over 13 days of culture. b) Changes in cellular density (cells/mL) and growth rate (K) of *Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros* sp.

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Following these fluctuations, the growth rate declined significantly over the next few days, with values of 0.1, 0.06, 0.24, and 0.02 cells/d from days 4 to 8. These rates suggest that the algae entered a phase of slower growth, possibly due to nutrient depletion or environmental limitations. On the ninth day, the growth rate turned negative, reaching -0.21 cells/d and continued to decrease steadily until the thirteenth day. This decline indicates the transition to the stationary and death phases, typical of microalgal cultures once they have exhausted available nutrients or other growth factors. In the case of *Isochrysis galbana*, the growth rate remained relatively stable during the first three days, with rates of 0.63±0.02 cells/d, suggesting an optimal initial growth phase. However, a decline was observed on the fifth day, where the growth rate decreased to 0.36 cells/d. The growth rate then exhibited a series of fluctuations, decreasing rapidly on the sixth day, increasing on the seventh day, and then decreasing again on the eighth and ninth days. From the tenth day onwards, the growth rate turned negative, starting at -0.11 cells/d and continuing at this rate until the end of the culture period. This pattern suggests that *Isochrysis galbana* reached its peak growth early and then experienced a rapid decline, possibly due to the depletion of key nutrients or accumulation of metabolic waste products. *Chaetoceros* sp. exhibited a different growth profile, with initial growth rates of 0.57± 0.12 cells/d on days 1 to 3. These rates were relatively moderate compared to the other two species. From the fourth day onward, the growth rate stabilized at 0.28 cells/d, followed by a slight decline to 0.24 cells/d on the sixth day. After this point, the growth rate fluctuated between positive and negative values, with cells experiencing a gradual decline. Negative growth rates of -0.02, -0.04, 0.04, -0.14, -0.12, -0.16, and -0.21 cells/d were observed from days 7 to 13, indicating that the species entered a prolonged phase of stagnation and eventual senescence. This steady decline further reflects the species' limited adaptability to the culture conditions compared to *Nannochloropsis oculata* and *Isochrysis galbana*, which displayed more rapid initial growth but followed by quicker declines. In summary, the growth rates of the three microalgae species reveal species-specific patterns, with *Nannochloropsis oculata* and *Isochrysis galbana* experiencing more rapid growth followed by sharper declines, while *Chaetoceros* sp. showed a slower, steadier growth followed by a prolonged decrease. These differences highlight the unique physiological responses of each species to the culture environment.

Growth dynamics

Figure 6 illustrates the various growth phases of the cultured microalgae from the initial culture phase to the decline in cell abundance due to nutrient depletion. The graph identifies key phases such as the lag, exponential growth, stationary, and declining phases, each marked by specific trends in cell abundance.

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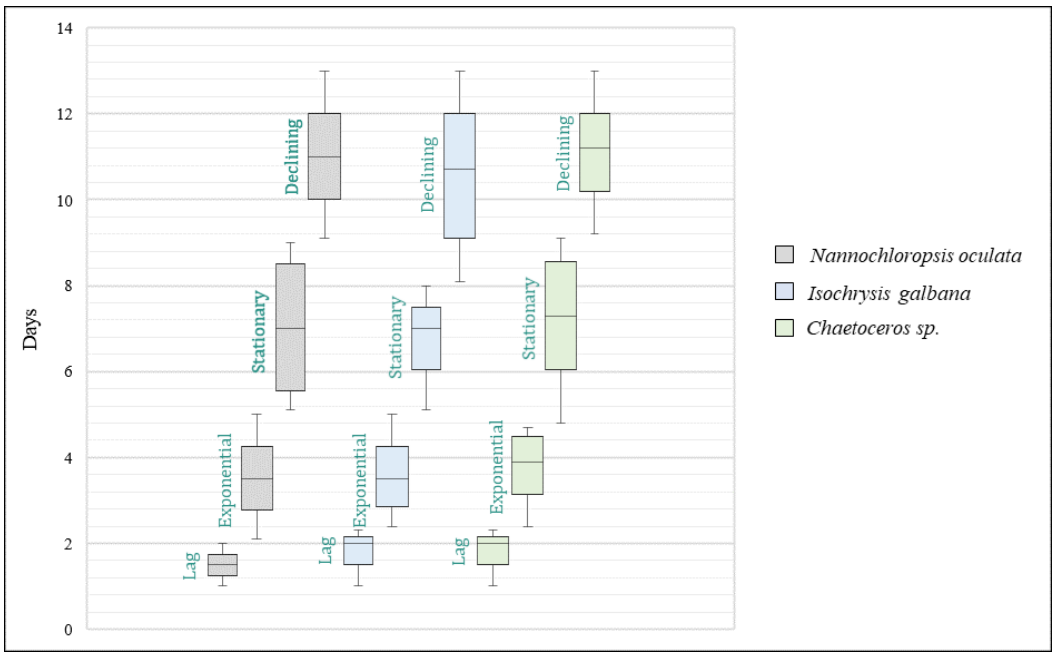


Figure 6: The growth phases of *Nannocloropsis oculata, Isocrysis galbana* and *Chaetoseros sp* within 13 days of culture observation.

Both *Nannochloropsis oculata* and *Isochrysis galbana* exhibited a lag phase during the first two days of culture, where the cells acclimatized to the culture environment. This is typical for many microalgae species as they adjust to new growth conditions, preparing for cell division. In contrast, *Chaetoceros* sp. experienced a slower initial growth, and its lag phase extended until the third day, indicating that it required a longer adaptation period compared to the other two species. Following the lag phase, the exponential growth phase began, marked by rapid cell division and a significant increase in cell density. *Nannochloropsis oculata* exhibited an exponential growth phase lasting three days, while *Isochrysis galbana* continued its rapid growth for five days, and *Chaetoceros* sp. for four days. This phase is typically characterized by a steep upward slope in cell abundance as cells divide rapidly and resources are used efficiently for growth. After the exponential growth phase, the algae transitioned into the stationary phase, where growth slowed as nutrients became limited, and cell division decelerated. For *Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros* sp., the stationary phase lasted for 4, 3, and 4 days, respectively. During this period, the algae were still alive but were no longer reproducing at the same rapid rate due to the depletion of essential resources required for growth. Eventually, the declining phase was observed, where rapid cell death occurred due to the exhaustion of nutrients and accumulation of waste products. This phase marks a sharp decrease in cell density. *Nannochloropsis oculata* entered the declining phase after the ninth day, *Isochrysis galbana* after the tenth day, and *Chaetoceros* sp. after the eleventh day. The decline in cell abundance

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during this phase was characterized by the gradual death of cells as they reached the limits of their growth potential under the given conditions. In summary, the growth dynamics of the three species displayed distinct patterns, with variation in the duration of the lag, exponential growth, stationary, and declining phases, reflecting their unique growth characteristics and nutrient requirements.

Discussion

In the current study, three marine microalgae species—*Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros* sp.—were cultured and monitored for 13 days under controlled laboratory conditions. Temperature, pH, and light intensity were kept consistent across all species, while the salinity of the seawater and cultured vessels varied, acknowledging that each microalga has a distinct ideal growth environment.

Isochrysis galbana and *Nannochloropsis oculata* exhibited optimal growth at temperatures between 26°C and 28°C with salinities of 33 ppt, aligning with their known preferences for warmer and saltier waters. Although Hoff *et al*., (2008) suggest an ideal salinity of 15–30 ppt for *Isochrysis galbana*, our data indicate that this species thrives in environments with slightly higher salinity. Additionally, Ukeles (1976) found that *Isochrysis galbana* does not expand its cells effectively at temperatures between 27°C and 35°C. However, in our experiment, *Isochrysis galbana* demonstrated robust growth at temperatures ranging from 26°C to 27°C, suggesting its adaptability to slightly different conditions than previously reported.

In contrast, *Chaetoceros* sp. showed relatively slower growth compared to the other two species. Hoff *et al.,* (2008) highlight that *Chaetoceros* sp. may require higher light intensities (8000–10,000 lux) to achieve optimal growth. The less favorable growth rate observed in our study may be attributed to the environmental conditions not fully meeting the species' light requirements.

Nannochloropsis oculata reached its peak cell density of $96.76\pm41.87\times10^5$ cells/mL on the eighth day, while *Isochrysis galbana* reached its peak of $120.48\pm76.91\times10^5$ cells/mL on the ninth day. *Chaetoceros* sp., on the other hand, reached its peak cell density of $26.83\pm4.62\times10^5$ cells/mL on the seventh day. These findings align with the general growth trends observed in other studies, such as Phatarpekar *et al.,* (2000), who noted that optimal cell density often occurs on the eighth and sixth days of culture for similar species. The peak densities observed across all species correspond to the exponential growth phase, a period known for rapid cell division.

Chaetoceros sp. exhibited slower growth than the other two species, likely due to environmental limitations, though all species were cultured in identical media. Despite these challenges, *Chaetoceros* sp. reached its peak cell density on day seven, indicating that it can still grow effectively under the given conditions.

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The stationary phase, which occurs when growth slows due to nutrient depletion, is often considered favorable for harvesting microalgae due to higher concentrations of valuable compounds such as lipids (Sacristán de-Alva *et al.,* 2022). However, this phase can also present challenges, such as cell death and potential bacterial proliferation, as noted by Maleki *et al.* (2020). The exponential phase, characterized by active cell division, is considered ideal for high biomass production and efficient harvesting, as suggested by Tourlouki *et al.* (2020). Our data show that the stationary phases of *Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros* sp. persisted for 4, 3, and 4 days, respectively. These findings enhance our understanding of the growth dynamics of these species and provide valuable insights for optimizing microalgae harvesting for commercial applications, such as food and biofuel production.

Conclusion

For large-scale commercial microalgae cultivation, understanding the development conditions and growth kinetics of key species is crucial. *Nannochloropsis oculata*, *Isochrysis galbana*, and *Chaetoceros* sp. are among the most widely cultured microalgae globally, valued for their nutritional and commercial potential. In the context of advancing this sector within our country, research focusing on the growth kinetics and optimal culture conditions is essential. By understanding the population dynamics of these species, as demonstrated in our study, we can identify the most favorable developmental phases for harvesting, which is a critical aspect of commercial microalgae production. The insights gained from this research can contribute significantly to refining culture techniques and optimizing harvesting strategies, thereby enhancing the sustainability and productivity of the microalgae industry in the future

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