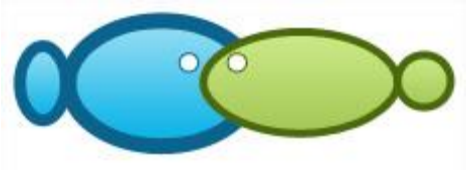

Investigating the optimum silicon to nitrogen ratio (Si/N) for diatom (*Chaetoceros* sp.) culture

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Abstract. Diatoms, including *Chaetoceros* sp., are nutrient-rich microalgae that serve as a beneficial food source for fish and shrimp larvae, providing essential nutrients necessary for the growth and development of aquatic organisms. A perfect balance of macro and micronutrients is required for *Chaetoceros* sp. growth and productivity. This study examined the ideal silicon to nitrogen ratio (Si/N) for *Chaetoceros* sp. culture. The experiment was carried out in a wet laboratory for eight days, employing a Completely Randomized Design (CRD) method, using the different Si/N ratios (1:1, 2:1, 1:2, and 1:4) as a single treatment, each with three replicates, resulting in a total of 12 experimental units. The results showed that the Si/N=1:1 ratio treatment had a greater average density of *Chaetoceros* sp. cells, peaking at 538,000 cells mL^{-1} by day 6, followed by treatment Si/N=2:1 with a maximum average density of 479,333 cells mL^{-1}. The lowest average cell density of 388,000 cells mL^{-1} was found in the Si/N=4:1 treatment. Both treatments, Si/N=1:1 and Si/N=2:1, maintained a consistently higher average cell density and exhibited better growth phases, indicating the optimal Si/N ratio for the growth of *Chaetoceros* sp. Poor water quality was noticeable during stationary and death growth phases, especially for treatments Si/N=1:1 and Si/N=2:1, indicated by the highest NH_3 values reaching 0.85 mg L^{-1} on the last day of the experiment. The highest average silica concentration was found in the treatment Si/N=2:1, which remained at an average concentration higher than 7.0 mg L^{-1} until the end of the culture period. Regarding proximate compositions, the study also revealed that the protein, lipid, and carbohydrate contents were higher in the S/N=1:1 treatment than in other treatments. The findings of this study are expected to improve the effectiveness and efficacy of fertilizer both in the laboratory and in mass production of *Chaetoceros* sp.
Key Words: diatom culture, nutrient ratio, silica, nitrogen.

Introduction. Diatoms have been recognized among the microalgae groups that play a crucial role in marine ecosystems and have various industrial applications. Because of their significance as primary producers, diatoms are essential to coastal and marine ecosystems, helping to maintain the food chain, carbon cycle, and nutrient cycles (Benoiston et al 2017; B-Béres et al 2023). Their contribution to organic matter generation and oxygen release through photosynthesis highlights their significance in preserving the health and equilibrium of aquatic ecosystems. Diatoms are beneficial as food for fish and shrimp larvae because they supply essential nutrients for the growth and development of aquatic organisms (Kumaran et al 2017; Brindley et al 2022; Boyd 2014). In addition to their nutritional value, diatoms contribute to water quality maintenance in aquaculture systems by absorbing excess nutrients and reducing the concentration of potentially harmful substances such as ammonia and nitrite (Kumaran et al 2017; Marella et al 2020). Diatoms also facilitate sediment formation at the bottom of aquaculture ponds, providing an additional food source for benthic organisms and fostering microorganism habitat. Given their significance, diatoms are integral components in the design and management of aquaculture systems, ensuring cultured organisms' optimal growth and health.

The fast proliferation of diatoms like *Chaetoceros* sp. in favorable environments and their nutrient-rich composition make them popular natural feeds for shrimp in fish hatcheries and aquaculture. The *Chaetoceros* genus has many species that are good food for fish or shrimp larvae. Aquatic creatures, such as clam and shrimp larvae, might benefit significantly from the consumption of *Chaetoceros* sp. due to their tiny cell size, fast growth rate, and capacity to adapt to different environmental conditions (Kumaran et al 2017; Krichnavaruk et al 2005). Species of *Chaetoceros*, the most common of which are *C. muelleri*, *C. gracilis*, *C. calcitrans*, *C. simplex*, and *C. affinis*, are most often seen in culture media. The availability of nutrients and environmental conditions are two major factors influencing the growth behavior, composition, and biomass production of *Chaetoceros* sp. (Sánchez-Saavedra & Voltolina 2006; López-Elías et al 2008).

Chaetoceros sp., like other diatoms, can absorb silicon and convert it into silica, a necessary component of their cell walls. As a result, silicon is a crucial element for *Chaetoceros*, contributing significantly to its growth and overall development. Previous research has shown that the silicon to nitrogen (Si/N) ratio significantly affects *Chaetoceros* sp. growth and productivity (Tantanasarit et al 2013; Gilpin et al 2004; Li et al 2017a). *Chaetoceros* sp. cell walls require silicon, whereas protein synthesis and cell growth need nitrogen. Moreover, the presence and uptake of silicon compared to nitrogen can substantially influence the cellular processes and metabolic makeup of diatoms. As a result, understanding the optimal Si/N ratio is critical for improving diatom growth in culture conditions. Further investigation into this relationship can yield innovative insights for enhancing the mass production and conservation efforts of *Chaetoceros* sp. This information may lead to advancements in industrial applications and ecological conservation efforts involving the diatom species. Despite its importance, there is a scarcity of research on the ideal conditions for the growth of *Chaeotoceros* sp.

Successful culturing of *Chaetoceros* sp. requires a precise balance of macro and micronutrients, especially silicon (Si) and nitrogen (N). Reaching the ideal Si/N ratio is critical for optimizing resource efficiency, minimizing waste, and assuring efficient nutrient usage in culture media. The present study investigated the optimal Si/N concentration ratio for *Chaetoceros* sp. culture. The results of this study are expected to specifically enhance the effectiveness of fertilizer efficacy both in the laboratory and mass-production of *Chaetoceros* sp. In a broad application, it potentially deepens our knowledge of the ecology of diatom production in both aquaculture and naturally aquatic environments.

Material and Method

Culture preparation. Diatom-*Chaetoceros* sp. used for this study was obtained from the Hatchery Installation Unit of the Research Institute for Brackishwater Aquaculture and Fisheries Extension (RICAFE) in Maros, South Sulawesi, Indonesia. The five-day-old culture of *Chaetoceros* sp. was used as an inoculum, cultured in 250 mL Erlenmeyer flasks to establish a pure stock before it was subsequently transferred to 2,000 mL jars for an intermediate production, reared for 3 days, for utilization in the next stage of the experimentation. Before the *Chaetoceros* sp. culture was commenced, sterilization procedures were applied to experimental equipment and seawater, employing filtration (filtered through pores of 10, 5, and 1 µm), autoclaving techniques, chlorine disinfection (about 24 h; the residual was removed with sodium thiosulfate), and UV light exposure. After the sterilization process, the quality of the sterilized seawater was analyzed to determine the initial concentration of major nutrients nitrate (NO_3), ammonia (NH_3), and Si (Table 1).

Chaetoceros sp. culture and experimental design. The *Chaetoceros* sp. culture process involved the preparation of culture media, including the formulation of fertilizers. Based on the initial seawater quality and fertilizer data in Table 1, the Si/N ratios for the experiment were calculated. The variations in Si/N ratio were determined employing the "silica to nitrogen calculator" available online at http://algaenviro.com.au/silica-calculator/ (Tannock 2024).

Table 1
The initial seawater quality and fertilizer data were used to determine the Si/N ratios

Variable	*Value (mg L^{-1})*
Initial ocean water quality	
NO_3	0.6874
NH_3	0.0452
Silica (SiO_2)	6.5
Nitrogen fertilizer	
Sodium nitrate ($NaNO_3$)	54.7059
Silica fertilizer	
Sodium silicate (Na_2SiO_3)	43.0189

In this experiment, several fertilizers were employed to help enrich the seawater medium for growing *Chaetoceros* sp. including potassium nitrate (KNO_3), sodium hydrogen phosphate (Na_2HPO_4), sodium ethylene diamine tetra acetic acid (Na-EDTA), Clewat-32 (substitutable for ferric chloride ($FeCl_3$)), and Na_2SiO_3. Following the guideline of "f Medium" (Guillar 1975), 75 g L^{-1} $NaNO_3$, was applied as a nitrogen source for the seawater medium. Therefore, considering the initial value of NO_3 in the seawater medium, the concentrations of NO_3=55.39 mg L^{-1}, NH_3=0.0452 mg L^{-1}, and SiO_2=6.5 mg L^{-1} were used as inputs into the "silica to nitrogen calculator" application. The Si/N calculation estimated that the total silica fertilizer (Na_2SiO_3) concentrations of 47.5 mg L^{-1}, 101.5 mg L^{-1}, 20.5 mg L^{-1}, and 7 mg L^{-1} were required to achieve a Si/N ratio of 1:1; 2:1; 1:2; 1:4, respectively. The f medium is equivalent to a Si/N=1:1 ratio so it was assigned as a control. Following the completion of culture media preparation, the pure stock of *Chaetoceros* sp. was put into 2,000 mL Erlenmeyer flasks for the experiment. The experiment was carried out for 8 days. During the culture period, the salinity of the culture medium was maintained at 25 to 28 ppt by diluting the seawater using deionized water. The cultures were continuously aerated with sterile, humidified air using an aquarium pump and illuminated by white fluorescent lamps at an intensity of 2,000 Lux for 24 hours (Sivasubramanian & Rao 2014). The room temperature of 24±1°C was maintained until the end of the experiment. This study used a Completely Randomized Design (CRD) method, using the different Si/N ratios as a single factor (treatment), each with three replicates, resulting in 12 experimental units. The Si/N ratio treatments in this study were established based on the Si/N ratios as the following:

- Treatment A (control)=Si/N ratio=1:1
- Treatment B=Si/N ratio=2:1
- Treatment C=Si/N ratio=1:2
- Treatment D=Si/N ratio=1:4

Analytical methods. The cell density growth pattern of the *Chaetoceros* sp. was assessed based on daily sampling taking 2 mL sample. The cell counting was conducted using a light microscope and hemocytometer for division rate determination. Meanwhile, water quality parameters such as pH and salinity were measured daily. Other water quality parameters including nitrogen ammonia-nitrogen (N-NH_3); nitrate-nitrogen (N-NO_3); nitrite-nitrogen (N-NO_2), SiO_2, and phosphate (PO_4) were only measured at the end of the culture period (Table 2).

After 7 days of culture in the stationary phase, a 2 L sample was taken from each of the four treatments and centrifuged at 6,000 rpm (revolutions per minute) speed for 6 min. The *Chaetoceros* sp. pellets were rinsed with ammonium formate solution (washing agent) to eliminate salts and then subjected to drying at 70°C until a constant weight was achieved (Salas-Leiva et al 2016). The ash-free dry weight was determined by exposing the sample to a temperature of 500°C for 6 hours. The proportions of proteins, lipids, carbohydrates, and organic matter were assessed for *Chaetoceros* sp. in the four treatments. The proximate analysis was conducted using colorimetric techniques, employing a Shimadsu absorption spectrophotometer (UV150-02). The analysis of proteins, lipids, and carbohydrates followed the procedure described by Anning et al (2000).

Table 2

Water quality parameters and analysis methods used in the study

Variable	*Analysis methods*
pH, Salinity (ppt)	pH meter, hand-refractometer (APHA 2017)
NH_3-N (mg L^{-1})	Spectrophotometric (APHA 2017)
NO_3-N (mg L^{-1})	Spectrophotometric (APHA 2017)
NO_2-N (mg L^{-1})	Spectrophotometric (APHA 2017)
PO_4-P (mg L^{-1})	Spectrophotometric (APHA 2017)
SiO_2 (mg L^{-1})	Atomic Absorption Spectrophotometer (AAS) (APHA 2017)

Statistical analysis. The R Studio software was used to perform a statistical analysis. Analyses of variance (one-way ANOVA) were performed to compare the *Chaetoceros* sp. average cell density between treatments. Data showing significant differences ($P<0.05$) were analyzed by paired comparisons using Tukey's honestly significant difference (HSD) post-hoc test.

Results

Effect of different Si/N ratio treatments on Chaetoceros sp. density. The cell density of *Chaetoceros* sp. in each treatment during the culture period is presented in Figure 1 and Table 3. The Si/N ratio of treatment A had a greater average density of *Chaetoceros* sp. cells since the first day of culture, compared to other treatments. Most treatments reached peak cell density by day 6, with a maximum density of 538,000 cells mL^{-1} observed in treatment A, followed by treatment B with an average density of 479,333 cells mL^{-1}. The treatment with the lowest average cell density was treatment B, with a maximum average cell density of 388,000 cells mL^{-1}.

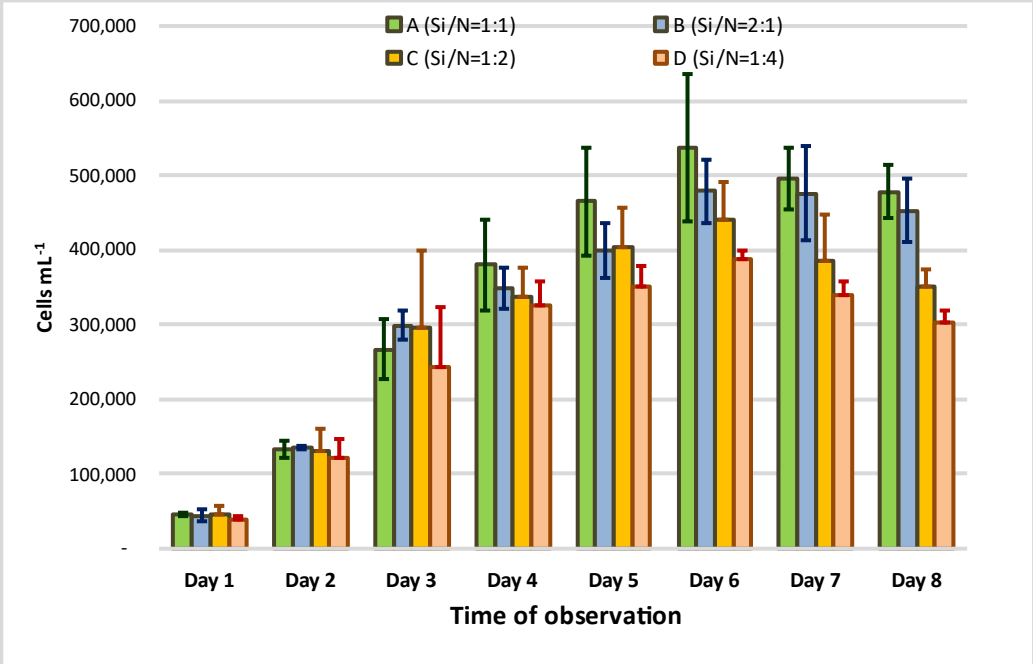

Figure 1. The mean cell density of *Chaetoceros* sp. for different Si/N ratios.

The ANOVA results for *Chaetoceros* sp. show that the P-values were 0.015 and 0.000 ($p<0.05$) on days 7 and 8, respectively. At a significance threshold of alpha=0.05, it can be inferred that there was a highly significant difference between treatments on days 7 and 8 (Table 3). Due to significant differences in the treatment, additional Tukey's HSD post-hoc tests were carried out to examine the impact of different treatments. Based on the

post hoc test, the average difference between treatments started on day 5, resulting in two subgroups. Subset 1 included treatments A, B, C, and D, with treatments C and D showing different average cell densities. The condition remained consistent from day 5 to day 6. On day 7, three subsets were created: subset 1 (A and B), subset 2 (C), and subset 3 (D). Only two subgroups were identified on the 8th day: Subset 1 containing elements A and B, and Subset 2 containing elements C and D. Throughout the investigation, treatment A and treatment B maintained a consistent average, indicating the optimal Si/N ratio for the growth of *Chaetoceros* sp.

Table 3

The results of ANOVA on the effect of different Si/N ratio treatments on the *Chaetoceros* sp. average cell density

Treatments	*Average density (cells mL^{-1})*							
	Day-1	*Day-2*	*Day-3*	*Day-4*	*Day-5*	*Day-6*	*Day-7*	*Day-8*
A(C)=1:1	38,667^{a}	120,667^{a}	244,000^{a}	325,333^{a}	352,000 ^{a}	388,000^{a}	340,667^{a}	302,667^{a}
B=2:1	44,000 ^{a}	131,333^{a}	266,667^{a}	337,333^{a}	400,000^{ab}	440,000^{ab}	385,333^{ab}	351,333^{a}
C=1:2	45,333^{a}	132,000^{a}	296,667^{a}	348,667^{a}	403,333^{ab}	479,333^{ab}	476,000^{bc}	453,333^{b}
D=1:4	45,333^{a}	135,333^{a}	299,333^{a}	380,000^{a}	465,333^{b}	538,000^{b}	496,000^{c}	478,667 ^{b}

Different superscript letters within the same column indicate significant differences ($p<0.05$)

Chaetoceros sp. growth phases. Figure 2 illustrates the growth phases of *Chaetoceros* sp. in different Si/N ratios. There was a continuous logarithmic increase in the number of cells from the first day up to day 6. This phase is known as exponential growth, marked by a rapid increase in cell numbers due to logarithmic cell division. From days 6 to 8, the *Chaetoceros* sp. cell density seemed to plateau, marking the stationary phase. After day 8, there was decreased cell production, characterized by a reduced amount of nutrients in the environment. During this phase, algae cells undergo cell death instead of cell division, leading to changes in the color of the culture water, including the presence of froth on the water surface, fading hue, and the accumulation of clumps of algal cells at the bottom of the container. Based on the growth curve, treatments A and B exhibited better growth phases than treatments C and D. This was evident from a longer duration of exponential phase (6 days) and greater average cell density. Treatment D exhibited the lowest growth phases, with the exponential phase terminating on day 4 and lower cell density.

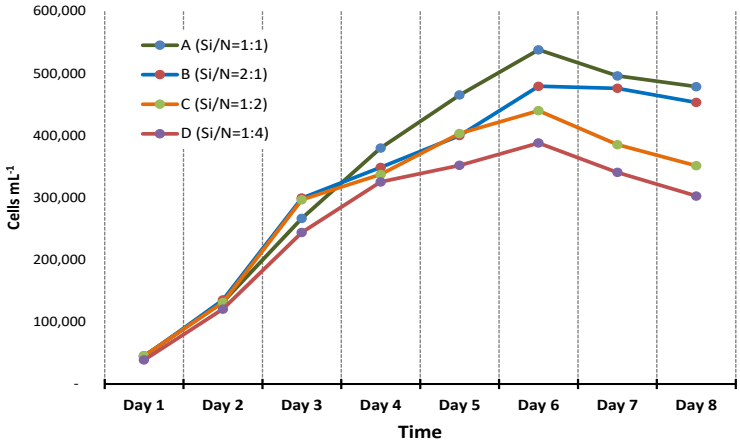

Figure 2. Average growth curve of *Chaetoceros* sp. in different Si/N ratios (mean cell density, n=3).

Water quality. Figure 3 shows water quality changes indicated by pH and salinity variables during the *Chaetoceros* sp. culture. Since light intensity and DO concentrations were controlled at optimum levels, only salinity and pH exhibited significant changes during the experiment. As described in Figure 8, although the pH values on the first day (day 1) for treatments A and B were higher compared to the other treatments, changes in water quality generally occurred on the last day (day 8) of the rearing period for all treatments,

when increased salinity and pH values were noticeable before the death phase of the cultured *Chaetoceros* sp.

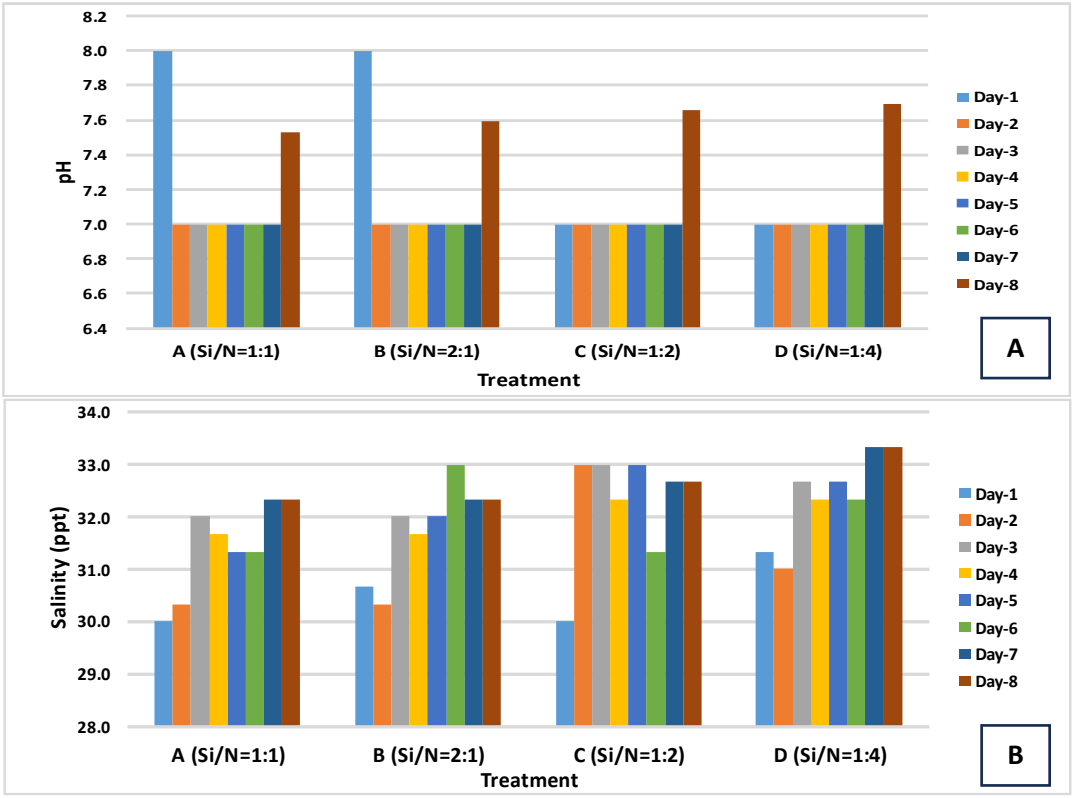

Figure 3. Daily changes in water quality (A=pH and B=salinity) at each treatment during the culture period of *Chaetoceros* sp.

Table 4 presents the water quality data comparing the initial and the last day of culture. Changes in water quality were noticeable, especially for the variables NH_3-N and NO_3-N in the treatments of C and D. The elevated NH_3 reached 0.85 on the last day of the experiment Si/N of 1:4. The highest average silica concentration was found in the treatment B which remained at an average concentration of 7.7 mg L^{-1} until the end of the culture period. Although the Si concentration in treatment A was lower than that of C and D, the Si/N ratio was much higher.

Table 4

Comparison of water quality data on the initial and the last day of *Chaetoceros* sp. culture

Measurement period	*Treatments*	*NH_3-N (mg L^{-1})*	*NO_2-N (mg L^{-1})*	*NO_3-N (mg L^{-1})*	*PO_4 (mg L^{-1})*	*Si (mg L^{-1})*	*Si/N**
Initial (seawater media)		0.037	0.004	0.155	0.037	0.275	
Final (culture media)	A (Si/N=1:1)	0.487	0.017	0.090	0.062	3.680	2.04
	B (Si/N=2:1)	0.384	0.014	0.201	0.030	7.700	4.97
	C (Si/N=1:2)	0.491	0.037	1.490	0.035	4.485	1.41
	D (Si/N=1:4)	0.847	0.270	2.089	0.032	4.596	0.92

Si/N* was based on the "silica online calculator" (http://algaenviro.com.au/silica-calculator/) employing NH_3-N, NO_3-N, and Si variables values.

Proximate compositions. Table 5 compares the composition of essential nutrients for both diatom species with different Si/N ratio treatments. However, Table 5 presents only the results of treatments A and B, while treatments C and D did not have enough samples due to the dominance of dead cell *Chaetoceros* sp. The moisture content of treatments A

and B was relatively small, ranging from 3.28% to 12.61%, because the diatom samples tested had gone through the drying process first. The results show that the ash content for treatment B was greater than that of A. The protein contents of treatments A and B were 18.45% and 16.34%, respectively. The lipid content of *Chaetoceros* sp. was found to be 1.72% and 1.42% in the Si/N ratio treatments A and B, respectively. As with the fat content, the highest carbohydrate content was also obtained in the treatment A (21.98%).

Table 5

The composition of proximate analysis results for *Chaetoceros* sp. at Si/N ratios of 1:1 and 2:1

Proximate composition	*A (Si/N=1:1)*	*B (Si/N=2:1)*
Ash content (%)	57.85	65.51
Lipid content (%)	1.72	1.47
Protein content (%)	18.45	16.34
Crude fiber content (%)	6.04	9.07
Carbohydrate content (%)	21.98	16.68

Discussion. The results have demonstrated that the ratios of silica to nitrogen substantially influence the growth and density of diatoms, such as *Chaetoceros* sp. This study indicated that the Si/N=1:1 ratio resulted in a higher average density of the *Chaetoceros* sp. than those with lower or higher ratios. For optimal diatom growth, it is usual to use a Si/N=1:1 ratio, which increases protein and chlorophyll synthesis, stimulates frustule creation, and achieves an optimum equilibrium between the Si and N elements. This optimal ratio promotes the growth and photosynthetic activity of *Chaetoceros* sp., resulting in higher density and improved general health of the culture (Tantanasarit et al 2013). In natural waters such as coastal, marine, river, or brackish water aquaculture ponds, the 1:1 ratio of Si/N ratio creates a beneficial environment that can outperform other diatom species, therefore boosting growth success (Hamm et al 2003; Wilken et al 2011). A decreased Si/N ratio could result in inadequate silica for frustule development, which would restrict the structural strength of diatoms and impede their growth (Wilken et al 2011). Reducing Si/N ratios can promote dinoflagellate growth over diatoms, causing a shift in the ecology with diverse negative impacts (Davidson et al 2012; Ren et al 2020; Zhou et al 2017; Neha et al 2021). Conversely, a greater Si/N ratio can cause excess silica, leading to uneven nutrient intake and probable nitrogen availability limits, ultimately severely affecting the growth and density of diatoms (Gilpin et al 2004). Thus, it is essential to maintain a 1:1 Si/N ratio to ensure the optimal balance for promoting the vigorous growth and competitive capabilities of *Chaetoceros* sp. In aquaculture practice, increasing the Si/N ratio from 1:1 to 2:1 in low-silica environments through fertilizer is expected to improve frustule production and nutrient uptake in *Chaetoceros* sp., leading to more significant growth and density compared to cultures with a 1:1 ratio. Therefore, optimizing the silica-to-nitrogen ratio according to the current nutritional conditions in the experiment improved the growth and competitive advantage of *Chaetoceros* sp.

Understanding population dynamics or growth phases of diatoms during culture is vitally important because it enables researchers to optimize and control diatom biomass production (Mohan et al 2021). It also helps study the factors that influence diatom growth and identify strategies to enhance their productivity for various applications such as bioenergy, food, and environmental remediation (Marella et al 2020; Li et al 2017b). Based on the results, the *Chaetoceros* sp. cells are better harvested before the stationary phase (day 5 or day 6). This phase is characterized by rapid and consistent cell division, which significantly increases biomass (Sánchez-Saavedra & Voltolina 2006). Harvesting at this stage ensures that the culture's maximum productivity is achieved. The growth phase of the diatom also influences the fatty acid production in these organisms (Lee et al 2014; Miller et al 2012). The Polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) derived from diatoms, determine cultured animal species' quality, survival, growth, and disease resistance (Santin et al 2021; Krishnan et al 2020). Hence, being rich in primary fatty acids, this diatom can be cultured in mass to satisfy the needs of

microalgae as live feeds or other potential applications. Pratiwi et al (2009) found that saturated fatty acids in *C. gracilis* tend to increase when entering the stationary growth phase in all the culture media. Harvesting at the correct stage is crucial for obtaining the highest yield and quality of the *Chaetoceros* sp. biomass. Furthermore, understanding diatoms' metabolic profile and growth phases is crucial for their physiological characterization (Hano & Tomaru 2019).

Water quality can significantly influence the growth and density of diatoms during culture. Although some argue that diatoms are highly adaptable organisms that can tolerate a wide range of water quality conditions, factors such as pH, temperature, salinity, nutrients, and the presence of contaminants can all influence the capacity of diatoms to grow. Therefore, understanding and maintaining the chemical and physical quality of the water in diatom culture is essential for successful cultivation. Since light intensity, temperature, and DO concentration were maintained at optimum levels, only salinity and pH exhibited significant changes during the experiment. Adenan et al (2013) found that *Chaetoceros* sp. exhibited variable responses to different salinity levels, with optimal growth occurring within specific ranges of 20 to 30 ppt. Water salinity influences microalgae's ability to maintain osmotic pressure between the protoplasm and its environment, by which high or low salinity can disrupt cell activity (Talebi et al 2013; Mo et al 2020). Based on the results, the average salinity of the seawater was initially 30 ppt and reached 35 ppt at the end of the study, which might be one of the primary reasons affecting *Chaetoceros* sp. growth and perhaps leading to die-off. *Chaetoceros* sp. can survive in pH levels ranging from 7 to 8.5, with optimal growth occurring between pH 7.9 and 8.5 (Hasani et al 2022; Iwasaki et al 2021). If the pH is not suitable, the growth of microalgae will not proceed normally. The changes in pH during microalgae culture might occur due to a decrease in the solubility of CO_2 and minerals in the culture medium (Dolganyuk et al 2020; Iwasaki et al 2021). Therefore, monitoring and maintaining proper water quality parameters is essential for ensuring the overall health and productivity of the diatom culture.

Diatoms require nutrients like phosphate, nitrogen, and silica for metabolism, physiological functions, and biochemical processes (Orefice et al 2019). The results indicated that adequate availability and the appropriate ratio of nutrients comprising N, P, and Si will encourage diatom growth and health. Nutrient deficiencies can particularly limit diatom growth and production. As pH rises, free ammonia concentrations above 0.5 mg N L^{-1} will significantly inhibit the growth and photosynthesis ability of cultured diatoms (Shoener et al 2019; Rossi et al 2020). Furthermore, Gilbert et al (2016) stated that although phytoplankton requires nitrogen in nitrate form for growth, excess concentrations can impair algal cell production. Similarly, excess or insufficient phosphorus nutrient concentration could also negatively affect cell proliferation. Diatoms also have an N/P ratio of 15:1 and generally prefer nitrate over ammonia as a nitrogen source (Geider & La Roche 2002). Additionally, diatoms require sufficient silicon for cell construction and DNA synthesis (Krichnavaruk et al 2005). The findings of this study revealed that, despite the maximum silica concentration in the treatment with a Si/N ratio of 2:1, Si utilization was more effective in the Si/N=1:1 treatment, indicated by a Si/N ratio of around 1. Si shortage was noticeable in the Si/N=1:4 treatment, which had less than 1 Si/N ratio. Empirical data suggests that the diatom growth rate is reduced when the nitrogen to silicon ratio exceeds 3:1. Additionally, if the silica concentration in seawater falls below 6.4 mg SiO_2 L^{-1}, silicate can be used to achieve the desired concentration (Boyd 2014).

As a natural food and indicator of the quality of the aquatic environment, diatoms, *Chaetoceros* sp. is not only expected to grow with a high cell density but must also contain enough several important nutritional components. The nutrient content of *Chaetoceros* sp. can be influenced by factors such as algal growth phase, cell density, environmental conditions, and growth media formulation (Kumaran et al 2017; Brindley et al 2022). Essential nutrients that must be available in the feed include protein, lipid, carbohydrates, vitamins, and minerals. Like other diatoms, *Chaetoceros* sp. obtains nutrients from the water medium containing nutrients by absorbing them directly through the cell membrane. Ash content, representing the mineral content of a substance, plays a vital role in maintaining cellular functions. The high ash content in diatoms is due to frustules in their

cell walls, containing a significant amount of silicate (Liu 2017). The results revealed that the protein, lipid, and carbohydrate contents were higher in the S/N=1:1 treatment. The higher ash content in the Si/N=2:1 ratio than in the Si/N=1:1 ratio was due to higher silica input. Proteins are essential for microalgae cell structure and function. They are critical in membrane and light-harvesting complex formation containing several photosynthetic enzymes (Barkia et al 2019). In addition, protein is necessary for cell and body structure. It provides energy and maintains water equilibrium. The protein content makes it a good nutrient source, especially in feed applications for aquaculture. Adequate silica addition can enhance the growth and productivity of *Chaetoceros* sp., thereby increasing protein production (Saxena et al 2022). However, the direct influence of silica addition on protein content may be insignificant, as protein is vital for cell growth. It may only sometimes be directly affected by silica availability (Sahebi et al 2015). Lipids in *Chaetoceros* sp. usually consist of mono and polyunsaturated fatty acids, which can provide good nutritional value, especially for organisms requiring essential fatty acids (Sahebi et al 2015). Growth conditions and cell metabolism can influence lipids in *Chaetoceros* sp. (Lin et al 2018). *Chaetoceros* sp. also contains carbohydrates, often consisting of polysaccharides such as cellulose, glucosamine, and others (Synytsya et al 2023). Carbohydrates in *Chaetoceros* sp. can be influenced by nutrient availability and growth conditions. According to Vitova et al (2014), appropriate silica addition can enhance algal growth and productivity through increasing carbohydrate accumulation as an energy reserve.

Conclusions. The findings have shown that the ratio of silica to nitrogen significantly impacts the growth and density of *Chaetoceros* sp. The *Chaetoceros* sp. reached a higher average density when the Si/N ratio was 1:1, compared to lower or higher ratios. The ideal Si/N ratio increased the cell density and photosynthetic function of *Chaetoceros* sp., leading to improved growth and enhanced overall health of the culture. The study emphasizes that Si/N=1:1 and Si/N=2:1 consistently maintained a higher average cell density and showed better growth phases, confirming the optimum Si/N for the growth of *Chaetoceros* sp. It was obvious that the water quality was poor during the stationary and death growth phases, particularly in the Si/N=1:2 and Si/N=1:4 treatments, demonstrating the need for additional silica. The treatment Si/N=2:1 had the greatest average silica concentration, consistently higher than the natural seawater concentration throughout the culture period. The study also revealed that the S/N=1:1 treatment had more significant protein, lipid, and carbohydrate contents than the other treatments. The findings of this study have the potential to enhance the efficiency and effectiveness of fertilizer in both laboratory and large-scale production of *Chaetoceros* sp. It can enrich our understanding of diatom production ecology in aquaculture and natural aquatic environments.

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