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Effects of Different Silicate Concentrations on Growth Performance of *Thalassiosira* sp. as Live Feed for Shrimp Larvae

Andri Hendriana[1], Imam Tri Wahyudi[1], Sheny Permatasari[1*]

[1] Technology and Management of Applied Aquaculture, School of Vocational Studies, IPB University, Indonesia

*Correspondence: E-mail: shenypermata@apps.ipb.ac.id

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ABSTRACT

Background: Diatoms require silicate for frustule formation and optimal cell division, making silicate availability a critical factor in improving microalgal production for aquaculture. Thalassiosira sp. is one of the most widely used live feeds in shrimp hatcheries because of its high nutritional value and suitable cell size for larval consumption.
Aims: This study evaluated the effects of different silicate concentrations on the growth performance of Thalassiosira sp. by simultaneously assessing cell density, specific growth rate (SGR), and division rate (DR).
Methods: A completely randomized design with four treatments was employed: control (without silicate), 15 ppm (SL15), 20 ppm (SL20), and 25 ppm (SL25), each with three replicates. Cell density was monitored daily for 14 days using a haemocytometer, while SGR and DR were calculated from the exponential growth model.
Results: The results showed that silicate supplementation significantly affected all growth parameters ($p < 0.05$). The highest cell density (4.60×10^6 cells mL^{-1}) was obtained in the SL25 treatment on day 12, whereas the highest SGR ($29.17 \pm 0.32\%$ day^{-1}) and DR (0.422 ± 0.005 divisions day^{-1}) were recorded in SL20. However, Duncan's Multiple Range Test indicated no significant differences between SL20 and SL25 for SGR and DR, suggesting comparable physiological responses at these silicate concentrations. These findings indicate that silicate concentrations between 20 and 25 ppm provide the optimal range for enhancing the growth performance of Thalassiosira sp. and may improve the efficiency of live-feed production for shrimp hatchery applications.
Conclusion: Silicate supplementation significantly improve the growth performance of *Thalassiosira* sp., as reflected by increased cell density, specific growth rate, and division rate.

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1. Introduction

The increasing demand for high-quality shrimp post-larvae has intensified the need for stable and nutritionally rich natural feed sources. Microalgae play a crucial role in hatchery systems, serving as both direct and indirect feed through the zooplankton trophic chain (FAO, 2020). One of the microalgae commonly utilized is *Thalassiosira* sp., a marine diatom known for its high nutritional value, including proteins, carbohydrates, lipids, and essential fatty acids that support shrimp larval growth (Nur *et al*., 2017; Costard *et al*., 2012; Vega *et al*., 2004). *Thalassiosira* sp. contains approximately 44.5% protein, 26.1% carbohydrates, and 11.8% lipids on a dry weight basis (Panjaitan *et al*., 2015; Jessica *et al*., 2024). In addition, its relatively small cell size, ranging from 4–32 µm, is suitable for the mouth opening of shrimp larvae (Devianti *et al*., 2022).

The availability of natural feed is essential to fulfill the nutritional requirements of shrimp larvae. Therefore, the culture of natural feed organisms must be conducted continuously and consistently (Wahyudi *et al*., 2022). As a live feed organism, *Thalassiosira* sp. offers several advantages, particularly its ease of cultivation (Jessica *et al*., 2024). In marine ecosystems, *Thalassiosira* sp. also functions as an important primary producer involved in the carbon cycle through the biological pump mechanism (Benoiston *et al*., 2017).

The successful cultivation of *Thalassiosira* sp. is highly dependent on nutrient availability, particularly silicate, which plays an essential role in the formation of the frustule or diatom cell wall. Silicate deficiency may disrupt cell division, cause frustule deformation, and significantly reduce biomass production (Xu *et al*., 2025). Conversely, silicate supplementation at optimal concentrations has been reported to enhance cell density and photosynthetic activity in microalgae (Cannavaro *et al*., 2024).

Several studies have demonstrated that silicate requirements and responses vary depending on the strain, culture medium, and environmental conditions (Erlangga *et al*., 2021; Afianti & Endrawati, 2024). However, studies simultaneously evaluate the effects of silicate concentration on quantitative growth parameters, such as population density, specific growth rate (µ), and division rate (k), in *Thalassiosira* sp. remain limited. These parameters are critically important for determining the efficiency of microalgal production as natural feed for shrimp larvae.

Although previous studies have demonstrated that silicate supplementation improves the growth of *Thalassiosira* sp., most investigations have primarily focused on single response variables, such as cell density, chlorophyll-*a* content, or biomass production. Consequently, the effects of silicate concentration on multiple quantitative growth indicators have not been comprehensively evaluated. Population density reflects biomass accumulation, specific growth rate describes the rate of biomass increase during cultivation, and division rate represents cellular reproductive activity. Simultaneous evaluation of these three parameters provides a more comprehensive understanding of growth dynamics and culture performance than any single indicator alone. Therefore, the present study fills this knowledge gap by assessing the combined responses of population density, specific growth rate, and division rate under different silicate concentrations to identify the optimal silicate dosage for efficient *Thalassiosira* sp. production in shrimp hatchery systems.

2. Methods

2.1 Time and Study Location

This study was conducted in May 2025 at the Natural Feed Laboratory, Aquaculture Production Laboratory, Vocational School of IPB University. The laboratory is located at Central Bogor District, Bogor City, West Java, Indonesia.

2.2 Equipment and Materials

The equipment used in this study included 1-L glass bottles, 15-L gallon containers, measuring cylinders, pipette bulbs, chlorine test kits, culture racks, neon box lamps, aerators, aeration hoses (microtubes), beakers, culture containers, microscopes, haemocytometers, droppers, and cover glasses.

The materials used included freshwater, sea salt, *Thalassiosira* sp. inoculum, silicate fertilizer, walne fertilizer, and vitamins.

2.3 Experimental Design

This study employed a Completely Randomized Design (CRD) consisting of four treatments with three replications for each treatment. The treatments were different silicate concentrations (Erlangga *et al*., 2021):

- K : Control (without silicate supplementation)
- SL15 : 15 ppm silicate
- SL20 : 20 ppm silicate
- SL25 : 25 ppm silicate

2.4 Water Sterilization

The water used for culture was sterilized prior to use to prevent contamination by unwanted microorganisms. A total of 10 L of water was placed into a sterile gallon container, followed by the addition of chlorine at a concentration of 1 mL/L. Continuous aeration was applied for 14 days to ensure complete removal of residual chlorine before the water was used as a culture medium. Residual chlorine is toxic to microalgae because it can damage cell membranes and inhibit photosynthetic activity. A prolonged aeration period effectively promotes chlorine volatilization and ensures that the culture medium is free of disinfectant residues prior to inoculation. The 14-day aeration period was adopted following previous studies on microalgal culture (Boyd *et al.,* 2013; Erlangga *et al.,* 2021). Water sterilization is a critical step in microalgal cultivation to maintain aseptic conditions and ensure stable growth.

2.5 Preparation and Cultivation of *Thalassiosira* sp.

Total of 12 glass bottles with a capacity of 1 L were sterilized using a 50ppm chlorine solution and subsequently rinsed until no chlorine odor remained. Each bottle was filled with 600 mL of sterile water. Aeration systems and neon box lamps were then installed as constant light sources to support microalgal photosynthesis. Light exposure and aeration are important factors in microalgal culture because they influence photosynthetic rates and nutrient distribution (Torzillo & Vonshak, 2013).

The culture medium was prepared to a final volume of 700 mL per bottle. A total of 420 mL of sterile water was supplemented with Walne fertilizer consisting of N:P (1.05 mL), trace metals (0.5 mL), and vitamins (0.42 mL). Silicate supplementation was applied according to the respective treatments as follows:

- K : without silicate
- SL15 : 6.3 mL
- SL20 : 8.4 mL
- SL25 : 10.5 mL

Subsequently, 280 mL of *Thalassiosira* sp. inoculum was added into each culture container. Continuous aeration at moderate intensity was provided to maintain nutrient homogeneity and oxygen availability. Silicate is known to play a vital role in frustule formation and significantly influences diatom growth rates (Hildebrand, 2008).

2.6 Observation Parameters

2.6.1 *Cell Density*

The cell density of *Thalassiosira* sp. was observed daily using a haemocytometer under a microscope. This method is commonly applied in microalgal enumeration due to its accuracy for laboratory-scale cultures. The calculation of the population growth of *Thalassiosira* sp. was performed using the formula by Muhklis *et al.,* (2017):

$$N = \frac{(n_1+n_2+n_3+n_4+n_5)}{5} \times 25 \times 10^4$$

where:

N = cell density (cells/mL)
$n_1 - n_5$ = number of cells counted in each square
5 = number of observed squares
25 = total number of haemocytometer squares
10^4 = haemocytometer volume conversion factor

2.6.2 *Specific Growth Rate*

The specific growth rate (μ) represents the population growth rate of microalgae. This parameter was calculated according to Sas *et al.,* (2023):

$$\mu = \frac{\ln N_t - \ln N_0}{t} \times 100$$

where:

μ = specific growth rate (% day^{-1})
N_t = cell density at time t
N_0 = initial cell density
t = culture period (days)

2.6.3 *Division Rate*

The division rate (k) indicates the number of cell divisions per day and was used to describe the reproductive dynamics of microalgae under specific culture conditions. The parameter was calculated using the following equation:

$$k = \frac{\log_2 N_t - \log_2 N_0}{t}$$

where:

k = division rate (divisions day^{-1})
N_t = cell density at time t
N_0 = initial cell density
t = culture period (days)

2.7 Data Analysis

The data were analyzed using analysis of variance (ANOVA) to determine the effects of the treatments on the observed growth parameters. When significant differences were detected, Duncan's Multiple Range Test (DMRT) was subsequently performed at a 95% confidence level ($\alpha = 0.05$). Statistical analyses were conducted using IBM SPSS Statistics.

3. Results and Discussions

3.1 Growth and Density of *Thalassiosira* sp.

Based on the results of the study, all treatments exhibited fluctuating growth patterns throughout the 14-day culture period. During the initial observation period, from day 1 to day 3, all treatments remained in the adaptation phase (lag phase), characterized by relatively low cell density. Subsequently, the cultures entered the exponential growth phase between day 4 and day 12, as indicated by a significant increase in cell abundance across all treatments. After reaching peak growth, all treatments experienced a decline in cell density toward the end of the cultivation period. This growth pattern is consistent with the general growth characteristics of microalgae, which consist of lag, exponential, stationary, and death phases (Riski *et al.*, 2021).

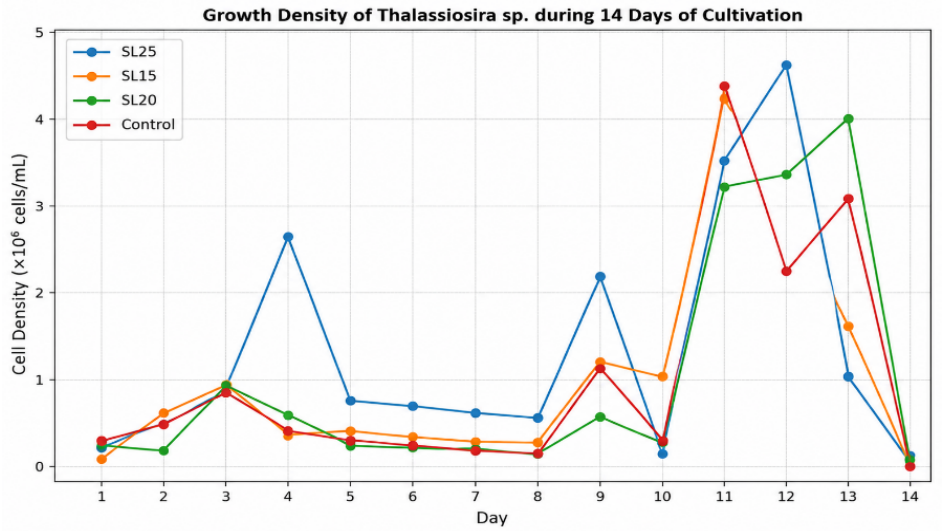

Figure 1. The density dynamics of *Thalassiosira* sp. during the 14-day observation period.

The SL25 treatment exhibited the highest cell density, reaching 4.60 × 10^6 cells mL^{-1} on day 12. These findings indicate that silicate supplementation significantly influenced the growth of *Thalassiosira* sp., although the growth response varied depending on the silicate concentration applied. The control treatment without silicate addition still demonstrated cell growth; however, the resulting density was consistently lower than that observed in treatments supplemented with silicate. This condition suggests that silicate plays a crucial role in supporting cell division and the formation of diatom cell structures (Cannavaro *et al*., 2024).

The present findings are consistent with those reported by Cannavaro *et al*., (2024), demonstrated that silicate supplementation enhanced both cell density and chlorophyll-a content in *Thalassiosira* sp. cultures. Similarly, Umiatun *et al*., (2017) observed a positive correlation between increased silicate concentration in aquatic environments and the abundance of benthic diatoms. The results also revealed that all treatments experienced a decline in cell density after reaching the peak growth phase. This decline was presumably associated with nutrient depletion in the culture medium, increased intercellular competition, and the accumulation of toxic metabolic by-products generated during the culture period. According to Aisyah *et al*., (2024), *Thalassiosira* sp. cultured under static conditions without additional nutrient supplementation tends to undergo rapid population decline during the late cultivation phase. Furthermore, Sanjaya & Danakusumah (2023) stated that an imbalance between silicate and other nutrients, such as nitrogen and phosphate, may destabilize culture growth even when silicate is available in sufficient quantities.

The deterioration of culture medium quality caused by the accumulation of organic matter and respiratory metabolites also contributed to the reduction in cellular physiological activity. Under such conditions, photosynthetic efficiency decreases, leading the culture to gradually enter the death phase

(Riski *et al*., 2021). In addition to nutrient availability, environmental factors including water quality, light intensity, salinity, temperature, osmotic pressure, pH, and nutrient composition also influence the growth performance of *Thalassiosira* sp. cultures during the cultivation period (Garcia *et al*., 2012; Nurfalaa *et al*., 2016; Marthia, 2020).

3.2 *Specific Growth Rate*

The ANOVA results demonstrated that silicate fertilizer application had a significant effect on the specific growth rate (μ) of *Thalassiosira* sp. ($p < 0.05$) (table 1). Based on Duncan's multiple range test, treatments SL20 and SL25 were grouped within the same subset (a), indicating no significant difference between these treatments; however, both differed significantly from SL15 and the control treatment. The SL20 treatment produced the highest average SGR value of 29.17 ± 0.32% day^{-1}, followed by SL25 at 28.90 ± 0.47% day^{-1}, SL15 at 23.17 ± 0.18% day^{-1}, while the control treatment exhibited the lowest SGR value at 18.15 ± 0.17% day^{-1}. The elevated SGR values observed in SL20 and SL25 indicate that silicate supplementation at these concentrations was able to optimally support cellular growth during the exponential phase of the culture.

Table 1. Effect of silicate on the specific growth rate values of *Thalassiosira* sp.

Treatments	Mean ± SD
SL20	29.17 ± 0.32^{a}
SL25	28.90 ± 0.47^{a}
SL15	23.17 ± 0.18^{b}
Control	18.15 ± 0.17^{c}

Numbers followed by the same letter(s) within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

The increase in SGR values following silicate supplementation suggests that silicate plays an essential role in frustule formation, cellular metabolism, and cell division processes in diatoms. Silicate is utilized in the synthesis of silica-based cell walls formed within the silica deposition vesicle (SDV). Adequate silicate availability enhances frustule formation efficiency, thereby accelerating cell division and promoting culture growth (Cannavaro *et al*., 2024). Erlangga *et al*. (2021) also stated that silicate is an essential nutrient for diatoms due to its direct involvement in frustule formation and cell division activity. Furthermore, increased silicate availability in the culture medium may accelerate biomass accumulation and improve microalgal culture productivity.

Although the SL20 treatment exhibited a slightly higher mean value than SL25, the numerical difference was relatively small compared with the variation among experimental replicates. Consequently, Duncan's Multiple Range Test classified both treatments within the same homogeneous subset. This finding suggests that silicate concentrations between 20 and 25 ppm produced comparable physiological responses, indicating that the growth of *Thalassiosira* sp. may have reached a near-optimal or saturation range where additional silicate no longer resulted in a statistically detectable improvement in growth performance. Nevertheless, excessively high silicate concentrations may potentially induce ionic imbalance and silicate precipitation within the culture medium, thereby reducing nutrient uptake efficiency (Erlangga *et al*., 2021).

Variations in growth patterns among treatments may also have been influenced by environmental factors such as temperature, light intensity, aeration, pH, and the availability of other nutrients in the culture medium. These factors play important roles in regulating metabolic activity and photosynthetic efficiency in microalgae, ultimately affecting the overall growth rate of the culture (Riski *et al*., 2021).

3.3 *Division Rate*

The ANOVA results demonstrated that silicate fertilizer application had a significant effect on the division rate (μ) of *Thalassiosira* sp. ($p < 0.05$) (table 2). Based on Duncan's multiple range test, treatments SL20 and SL25 were classified within the same subset (a), indicating no significant difference between these treatments; however, both treatments differed significantly from SL15 and the control. The SL20 treatment produced the highest division rate value of 0.422 ± 0.005 divisions day^{-1}, followed by SL25 at 0.418 ± 0.007 divisions day^{-1}, whereas the control treatment exhibited the lowest value at 0.263 ± 0.003 divisions day^{-1}. The elevated division rates observed in SL20 and SL25 indicate that silicate supplementation at these concentrations effectively enhanced cellular division activity during the exponential growth phase of the culture.

Table 2. Effect of silicate on the division rate values of *Thalassiosira* sp.

Treatments	Mean ± SD
SL20	0.422 ± 0.005[a]
SL25	0.418 ± 0.007[a]
SL15	0.336 ± 0.003[b]
Control	0.263 ± 0.003[c]

Numbers followed by the same letter(s) within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

Silicate plays an essential role in the formation of the frustule, the silica-based cell wall required for diatom cell division. Adequate silicate availability enables faster frustule synthesis, thereby enhancing cellular division activity. In addition, silicate has been associated with increased chlorophyll-a content and improved photosynthetic efficiency, which support cellular energy metabolism (Cannavaro *et al.,* 2024).

The present findings are consistent with Erlangga *et al.,* (2021), who stated that silicate is an essential nutrient in diatom cultures because of its direct involvement in cell wall formation and cellular reproduction. Similarly, Sanjaya & Danakusumah (2018) reported that silicate supplementation at optimal concentrations enhanced both growth and cellular division activity in *Thalassiosira* sp. cultures. Furthermore, Umiatun *et al.,* (2017) demonstrated that increasing silicate concentrations in aquatic environments significantly correlated with higher benthic diatom abundance.

Although the SL20 treatment produced the highest division rate value, Duncan's test indicated that it was not significantly different from SL25. This result suggests that silicate concentrations ranging from 20–25 ppm remain within the optimal range for supporting the cell division activity of *Thalassiosira* sp. In contrast, the control treatment produced the lowest division rate, indicating that silicate limitation may inhibit frustule formation, thereby slowing the cell division process.

3.4 The Role of Silicate in the Growth of *Thalassiosira* sp.

Silicate (SiO_2) is an essential nutrient for diatoms because it is directly involved in the formation of the frustule, the silica-based cell wall characteristic of diatoms. The frustule consists of two overlapping parts (thecae) that function in cellular protection and structural maintenance. Frustule formation occurs within a specialized organelle known as the silica deposition vesicle (SDV), where silicic acid undergoes polymerization into complex silica structures (Cannavaro *et al.,* 2024).

Adequate silicate availability plays a crucial role in supporting cell division and metabolic processes in *Thalassiosira* sp. Each daughter cell requires the formation of a new frustule prior to cell division. Therefore, silicate limitation may inhibit frustule synthesis, resulting in reduced or stagnant population growth. Furthermore, silicate deficiency may also reduce photosynthetic efficiency and cellular

enzymatic activity (Cannavaro *et al.,* 2024). According to Petrenko & Page (2025), frustule formation is regulated by complex interactions among organic molecules such as silaffins, silacidins, and long-chain polyamines, which act as templates during silica precipitation. Imbalances in silicate concentration may interfere with these processes, thereby affecting frustule morphology and the success of diatom cell division.

The relationship between silicate availability and diatom abundance was also reported by Umiatun *et al*., (2017), who demonstrated that increasing silicate concentrations in aquatic environments significantly correlated with higher benthic diatom abundance. These findings further emphasize the important role of silicate in regulating the growth and productivity of diatoms in both natural waters and laboratory cultures. Erlangga *et al*., (2021) stated that silicate is an essential macronutrient in diatom cultures, particularly for *Thalassiosira* sp., because it directly contributes to cell wall formation and reproductive processes. Insufficient silicate availability in the culture medium may disrupt frustule synthesis, thereby preventing optimal cell division. Consequently, population growth becomes slower and overall culture productivity declines.

In addition to its role in frustule formation, silicate is also associated with increased chlorophyll-a content, reflecting enhanced photosynthetic efficiency and cellular energy metabolism (Cannavaro *et al*., 2024). Therefore, optimal silicate availability is considered a key factor in improving the growth and productivity of *Thalassiosira* sp.

3.5 Environmental Factors Affecting Growth

The growth of *Thalassiosira* sp. is influenced by interactions between biological and environmental factors. Water quality serves as an important environmental determinant in microalgal growth. Temperature is a major factor influencing phytoplankton productivity because it affects photosynthetic rate and growth velocity. Temperature plays a significant role in regulating cellular metabolism and the division rate of *Thalassiosira* sp., with an optimal range of approximately 4–35°C (Boyd *et al*., 2013; Nurul *et al*., 2023; Jessica *et al*., 2024). Elevated temperatures may reduce dissolved oxygen concentrations, thereby affecting the metabolic activity of the culture (Riski *et al*., 2021). Conversely, low-temperature conditions may inhibit algal growth (Han *et al*., 2013).

In addition to temperature, pH is another limiting factor affecting the adaptive capacity of aquatic organisms. The optimal pH range for diatom growth is approximately 8–9.77 (Jessica *et al*., 2024; Kurniaji *et al*., 2025). pH values are influenced by photosynthetic activity, temperature, and the presence of ions within the culture medium (Aisyah *et al*., 2024). pH affects phytoplankton metabolism and growth through several mechanisms, including altering the balance of organic carbon, influencing nutrient availability, and affecting cellular physiology (Padang, 2014).

Salinity also affects cellular osmotic pressure and nutrient uptake efficiency. *Thalassiosira* sp. can grow optimally within a salinity range of 25–34 ppt (Panjaitan, 2015; Sas *et al*., 2023; Jessica *et al*., 2024). Extreme salinity fluctuations may additionally affect the metabolism of *Thalassiosira* sp., which can be expressed at the molecular level, including changes in gene expression (Stenger-Kovács *et al*., 2023).

Light intensity is another critical factor influencing photosynthesis and the formation of organic compounds. The optimal light intensity for supporting microalgal growth ranges from 5,000 to 10,000 lux (Arifin *et al*., 2025). Appropriate light intensity can enhance growth rate and increase microalgal cell density (Dewi, 2017). Light intensity plays a fundamental role in microalgal photosynthesis because it is one of the primary limiting factors of primary productivity. Therefore, light intensity must be adjusted according to the physiological requirements of the microalgae. Light levels outside the optimal range may disrupt cellular metabolism and inhibit microalgal cell division (Utami *et al*., 2012).

4. Conclusions

Silicate supplementation significantly improved the growth performance of *Thalassiosira* sp., as reflected by increased cell density, specific growth rate, and division rate. Although the highest cell density was achieved at 25 ppm silicate, no significant differences were observed between 20 and 25 ppm for specific growth rate and division rate. Therefore, silicate concentrations of 20–25 ppm are recommended as the optimal range for laboratory-scale cultivation of *Thalassiosira* sp. to support efficient live-feed production for shrimp hatcheries.

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6. Authors Note

The authors declare that there is no conflict of interest regarding to the publication of this article. Authors confirmed that the paper was free of plagiarism.

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