The effect of commercial nutrients to increase the population of *Skeletonema costatum* on laboratory and mass scales

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Abstract. *S. costatum* is one type of phytoplankton used in the maintenance of fish, shrimp, and crab larvae, but most are given in the maintenance of tiger shrimp larvae (*Penaeus monodon*) from the phase of nauplius phase after zoea. *S. costatum* has the advantages found in the autolysis enzyme itself so that it is easily digested by the larvae and does not pollute the cultivation media. Therefore knowledge about how is the effect of nutrients in *S. costatum* commercial life of food culture on laboratory scale up to mass scale. The method *S. costatum* is generally started by sterilizing tools and materials, preparation of culture media for laboratory scale (2 liters), semi-mass scale (80 liters) and mass scale (2 tons), fertilizer application, seed selection, the cost of counting *S. costatum* cells with peak population depends on the 36th hour with the highest number of cells in each culture scale items, namely laboratory scale of $602.547 \times 10^3$ cells/mL, semi-mass scale of $484.713 \times 10^3$ cells/mL and mass scale of $133.757 \times 10^3$ cells/mL then the last process is harvesting until it becomes flour. The obstacles encountered are environmental problems such as unstable weather and can be contaminated.

1. Introduction

Life feed or seed required for larval fish and shrimp because it can stimulate the movement of larvae to be more active due to feeding like plankton life will always move to stimulate the larvae to pursue in order to get that feed life [1]. *S. costatum* is one type of phytoplankton that is used in the maintenance of larval fish, shrimp, and crabs, but most are given on the activities of larval rearing tiger shrimp (*Penaeus monodon*) from phase to phase zoea nauplius [2, 3, 4, 5, 6].

Life feeds *S. costatum* has the advantage that there is enzyme autolysis itself so easily digested by the larvae and does not pollute the media culture [7], as well as sufficient nutrition complete on *S. costatum*. According to [8] stated that the content of nutritive *S. costatum* reaches 37% protein, 7% fat, and 21% carbohydrates. Cultivation of *S. costatum* life feeds can be done in three different cultures scale the laboratory scale in order to produce pure phytoplankton or monospesies [9], then in the semi mass scale that has a goal to increase in the number of cells of *S. costatum* so that it can be cultivated on a mass scale, the next is done on a mass scale sized tub 1-20 Ton and is the last link before harvesting [9]. Therefore it is necessary to do a study on the effect of nutrients in *S. costatum* commercial life of food culture on laboratory scale up to mass scale.
2. Material and methods

2.1. Material

The process of cultivating life feeds S. costatum performed on live food laboratory at the great hall of Brackish Water Aquaculture (BBPBAP) Jepara. The cultivation process feeds S. costatum life generally begins with the stage of sterilization equipment and materials, preparation culture media, fertilizer, seed selection, maintenance, and cell counting and the last stage is the process of harvesting.

2.2. Methods

2.2.1. Sterilization equipment

Sterilization is done by using soap and rinsed with fresh water and dry sterilization done for equipment glass by using an oven at 150 °C for 15 minutes. This sterilization device typically used to sterilize glass, metal tools, materials such as oils and powders [10]. Sterilization material that is water is done by using a mechanical filter and chlorinating with a dose of 60 ppm for 24 hours and the provision of Na-thiosulfate with a dose of 30 ppm.

2.2.2. Culture media

The culture medium used in the cultivation of feed S. costatum life can be divided into three types based on the scale of cultivation and the media volume: Laboratory Scale (2 Liter), semi mass scale (80 Liter) and mass scale (2 Ton). The salinity of water used in aquaculture feed S. costatum life amounted to 26 ppt.

2.2.3. Fertilizer

Fertilizer given in aquaculture Life feeds S. costatum differences between laboratory-scale with semi mass scale and mass. The composition of the fertilizer for the cultivation of a laboratory scale is as follows:

| Table 1. Nutrient laboratory scale. |
| --- | --- | --- |
| No. | Nutrient | Dose (ppm) |
| 1. | KNO₃ | 100 |
| 2. | NaH₂PO₄ | 10 |
| 3. | Na₂SiO₃ | 10 |
| 4. | Na₂EDTA | 5 |
| 5. | FeCl₃ | 1 |
| 6. | Vitamin B₁₂ | 0.001 |

As for the composition of the semi fertilizer and bulk mass scale is as follows:

| Table 2. Nutrient semi mass scale, |
| --- | --- | --- |
| No. | Nutrient | Dose (ppm) |
| 1. | UREA | 15 |
| 2. | TSP | 15 |
| 3. | Na₂SiO₃ | 10 |
| 4. | NPK | 15 |
| 5. | FeCl₃ | 1 |
| 6. | Vitamin B₁₂ | 0.001 |

2.2.4. Seed selection

Seeds used in aquaculture Life feeds S. costatum is derived from mass culture scale is then used as the initial inoculant both at laboratory scale and semi bulk.
2.2.5. Maintenance and counting cells
The process of cell conducted over four days and is followed by the calculation of the growth of cells every four hours. Counting of cell growth *S. costatum* done using Sedgewick rafter counting cell [11, 12, 13] and calculated using the formula [14] as follows:

\[
\frac{\sum Sel \times 10^3 \times P}{3.14 \times 10}
\]

Information:
- \(\sum Sel\) = The number of cells of 10 broad point of view
- \(10^3\) = The number of boxes on SRCC
- \(P\) = Factor Diluent
- \(3.14 \times 10\) = \(\pi\) (Phi) x 10 as broad view of circular

2.2.6. Harvesting
The latter process is the process of harvesting *S. costatum* done on a mass scale and bulk semi. Harvesting is done by filtering *S. costatum* with a bag filter plankton with a mesh size of 60 nm and then rinsed with freshwater and then dried for 3-4 days and then mashed.

3. Results and discussion
The results obtained during the production process life feeds *S. costatum* is a graph of cell growth of *S. costatum* at each scale aquaculture and water quality during the production process.

3.3.1. Cell growth of *S. costatum*
*S. costatum* cell growth was observed every 3 hours. Initial density culture characterized by a lag phase where the *S. costatum* is still experiencing physiological adaptation to the environment. Exponential phase occurs at 21 hours after the stocking of seedlings. It is in accordance with the opinion of [15] which states that this phase is characterized growth rate that reached a maximum with rapid cell culture and constant.

Density cell *S. costatum* on a laboratory scale exponentially reaching peak at the 36th hour after the initial seed with a maximum cell density at all three cultures that do that I culture as much as 585.350 \(\times 10^3\) cells/mL, culture II as many as 602.547 \(\times 10^3\) cells/mL and culture III as much as 570.382 \(\times 10^3\) cells/mL.

![Figure 1. Graph of cell growth *S. costatum* laboratory scale.](image-url)
After experiencing the peak phase \textit{S. costatum} going through a phase of decline or death of this happens at around 42 hours after the stocking of seedlings but not until it reaches the cell death overall and the density of the cells on the last observation that hour - 90 cell density of its cultures I as much as 35.350 \times 10^3 cells/mL, in cultured II as many as 42.675 \times 10^3 cells/mL and culture III as much as 32.484 \times 10^3 cells/mL.

[16] that \textit{S. costatum} have exponential phase/log phase with a sharp increase in the growth rate of 12 to 39 hours with the increase in population density 14 790 cells/mL to 428 050 cells/mL. After experiencing the peak phase of \textit{S. costatum} going through a phase of decline or death of this happens at around 42 hours after the stocking of seedlings but not to reach the overall cell death and cell density on the last observation 90 hours into his cell density in the culture I as much as 35.350 \times 10^3 cells/mL, in cultures of II as many as 42.675 \times 10^3 cells/mL and culture III as much as 32.484 \times 10^3 cells/mL. According to [17] This phase is characterized by a higher mortality rate than the rate of reproduction because the physical and chemical qualities cultured organisms are at a point where no longer have cleavage. The fall in the rate of growth of \textit{S. costatum} can be caused by three things, namely the reduction of micronutrients as a limiting factor because it has been used extensively during the exponential phase, the presence of toxic produced by the microalgae itself as a result of a metabolic poison itself microalgae and declining water quality due to cell who have experienced the death-cells would be a clot and cause transparency that would impede the process of photosynthesis [18].

\begin{figure}
\centering
\includegraphics[width=\textwidth]{cell_growth_graph.png}
\caption{Graph of cell growth \textit{S. costatum} semi mass scale}
\end{figure}

\textit{S. costatum} cell density on a semi mass scale achieve exponential peak at the 36th hour after the initial seed with a maximum cell density in the 2nd culture made that I culture as much as 462.101 \times 10^3 cells/mL, culture II as many as 484.713 \times 10^3 cells/mL.

After experiencing the peak phase of \textit{S. costatum} going through a phase of decline this happens at around 42 hours after the stocking of seedlings but not to reach the overall cell death and cell density on the last observation 90 hours into his cell density in the culture I as much as 81.528 \times 10^3 cells/mL and cultured II as many as 82.484 \times 10^3 cells/mL.

This phase is characterized by a higher mortality rate than the rate of reproduction because the physical and chemical qualities cultured organisms are at a point where no longer have cleavage [17]. The fall in the rate of growth of \textit{S. costatum} can be caused by three things, namely the reduction of micronutrients as a limiting factor because it has been used extensively during the exponential phase, the presence of toxic produced by the microalgae itself as a result of a metabolic poison itself.
microalgae and declining water quality due to cell who have experienced the death-cells would be a clot and cause turbid that would impede the process of photosynthesis [18].

Harvesting is done by filtering on each culture by using a sieve, water culture taken with a bailer and slowly poured into the filter. The harvesting practice is by the opinion [19] is by using a sieve. The results of *S. costatum* filter is rinsed with freshwater and allow it to dry and then removed and weighed using an analytical balance and covered with aluminum foil so getting wet weight of the culture I amounted to 3.12 grams and culture II 1.96 gram ago dried to dry and then weighed again and the dry weight is obtained in cultures of *S. costatum* I of 0.10 grams and 0.27 grams of culture II.

![Graph of cell growth *S. costatum* mass scale.](image)

**Figure 3.** Graph of cell growth *S. costatum* mass scale.

Cultivation of *S. costatum* at this scale mass *S. costatum* cell density can not be calculated until the entire growth phase, stationary phase and cause cell loss phase on *S. costatum* mass scale should be harvested when it reaches the exponential phase at the 27th hour by the number the density of cells in culture I of $126.751 \times 10^3$ cells/mL and the culture II of $133.757 \times 10^3$ cells/mL because the condition of the cells are still in good condition. This is by [20] that this phase is the best stage in the harvesting of fish feed or industrial purposes.

*S. costatum* growth of cells on a mass scale if cultivated throughout the entire phase of his life, as was done in the research [21] which states that the concentration of *S. costatum* cells were cultured on a 10 ton concrete tub with commercial fertilizers media reached peak growth cell on the third day with the cell number reached 750,000 × 103 cells/mL.

### 3.3.2. Water quality

Monitoring of water quality in aquaculture feeds *S. costatum* life do every day once the parameters measured include temperature, pH, and salinity. Water quality is another factor that significantly affects the cell growth of *S. costatum* for water as a medium of life. Water quality parameters that affect, among others, temperature, salinity, and pH. Environmental parameters play an important role in regulating the metabolic processes of aquatic organisms [22].

| Day | Temperature (°C) | pH   | Salinity (ppt) |
|-----|------------------|------|----------------|
| 1   | 26-28            | 8-8.2| 26             |
| 2   | 26-28            | 8-8.2| 26             |
| 3   | 26-28            | 8-8.2| 26             |

Table 3. Measurement of water quality *S. costatum*. 
Temperature affects an organism's life cycle stage and a factor limiting the spread of a species [22] and differences in the composition and abundance of *S. costatum* [23]. Temperature conditions on the cultivation of *S. costatum* were observed range in temperature 26-28°C both in laboratory cultures, semi-bulk and bulk. The results of temperature measurements are still in good condition, because *S. costatum* nature eurythermal diatoms that can grow on a wide temperature range is 3-30°C [24], while the optimum temperature of 25-27°C [25].

Another water quality parameters are salinity is one of the water quality parameters that affect the osmotic pressure between the organic cell protoplasm with the environment [26]. In general marine microalgae normal life at optimum salinity of 25-35 ppt [27]. Salinity value used in the cultivation of *S. costatum* is valued at 26 ppt salinity that is still within the range that can be tolerated by *S. costatum* between 15-34 ppt, optimal growth at 25-29 ppt salinity [19].

The next parameter of water quality is the pH or acidity. The degree of acidity (pH) is a factor that can affect the culture of microalgae. Microalgae require a pH of 7 to 8.5 for optimum growth [28]. [29] also states that one of the optimum conditions of growth of *S. costatum* is pH 7-8. This would correspond to the measurement results of pH value on the cultivation of *S. costatum*, with the value of 8 to 8.2.

*S. costatum* growth of cells on a mass scale if cultivated throughout the entire phase of his life, as was done in the research [21] which states that the concentration of *S. costatum* cells were cultured on a 10 ton concrete tub with commercial fertilizers media reached peak growth cell on the third day with the cell number reached 750,000 × 103 cells/mL.

Water quality is another factor that greatly affects the cell growth of *S. costatum* for water as a medium of life. Water quality parameters that affect, among others, temperature, salinity, and pH. Environmental parameters play an important role in regulating the metabolic processes of aquatic organisms [26]. *S. costatum* which is eurythermal the diatoms are capable of growing at a wide temperature range is 3-30°C [24], temperature affects an organism's life cycle stage and a factor limiting the spread of a species [22]. In defending the survival and reproduction of ecological changes in temperature cause differences in the composition and abundance of *S. costatum* [23]. The temperature can be tolerated by *S. costatum* ranges from 25-34°C, while the optimum temperature of 25-27°C [25].

Another water quality parameters are salinity is one of the water quality parameters that affect the osmotic pressure between the organic cell protoplasm with the environment [26]. In general, normal life marine microalgae at optimum salinity of 25-35 ppt [27], while salinity value range that can be tolerated by *S. costatum* between 15-34 ppt, optimal growth at 25-29 ppt salinity [19].

The next parameter of water quality is the pH or acidity. The degree of acidity is a picture of the amount of the hydrogen ion activity in general waters. pH value describes how much the level of acidity or alkalinity of water. Waters with pH = 7 is neutral, pH <7 is acidic, while a pH <7 is said to be alkaline water conditions [30]. The degree of acidity (pH) is a factor that can affect the culture of microalgae. Microalgae require a pH of 7 to 8.5 for optimum growth [28]. [29] also states that one of the optimum conditions of growth of *S. costatum* is pH 7-8. This would correspond to the measurement results of pH value on the cultivation of *S. costatum* with values ranging from 8.

4. Conclusion
The cultivation technique of *S. costatum* begins with the sterilization process, preparation of culture media for laboratory scale (2 L), semi-mass scale (80 L) and mass scale (2 metric ton), fertilizer application, seed selection / inoculant, then the cultivation process of *S. costatum* including the calculation of cell growth with a peak population at the 36th hour with the highest number of cells of each culture scale ie the laboratory scale of 602,547 × 10³ cells/mL, semi-mass scale of 484,713 × 10³ cells/ml and mass scale of 133,757 × 10³ cell/ml then the last process is harvesting to become flour.
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