

Culture of *Chaetoceros calcitrans* as Natural Feed for *Mytilopsis adamsi* Cultivation at National Research and Innovation Agency, North Lombok

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ABSTRACT

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This study was conducted to explore the culture technique of *Chaetoceros calcitrans* as a natural feed and its role in supporting the maintenance of the brown mussel (*Mytilopsis adamsi*). The microalga was chosen because it provides essential nutrients such as proteins, lipids, and carbohydrates that are important for the growth of aquatic organisms. The work took place over one month, from May 6 to June 4, 2025, at the National Research and Innovation Agency (BRIN), North Lombok. The culture followed a stepwise approach, starting with sterilization of equipment and media, the addition of KW21 fertilizer and silicate, inoculation with five-day-old seed cultures, daily maintenance, and cell density monitoring. The observations showed that *C. calcitrans* density rose steadily from the first day and peaked on day 5 at 4,020,000 cells/ml, before declining by day 7. This trend reflects the typical growth phases of microalgae, from lag and exponential to stationary and death phases. Feeding trials involved offering *C. calcitrans* to brown mussels twice daily at a dose of 500 ml each time. The results indicated consistent weight gain across all replicates, although growth levels varied among treatments. Overall, the study confirms that stepwise culture with appropriate nutrient supply and water quality management can produce *C. calcitrans* at optimal densities. Its application as a natural feed also provides measurable benefits for the growth of brown mussels in culture systems.

INTRODUCTION

Success in aquaculture is influenced by four main factors: water quality management, seed management, broodstock management, and feed management. Feed management is a crucial factor in aquaculture, as feed is rich in the nutrients needed by the organisms being cultivated. Efforts to meet the availability of feed for cultivated organisms include producing natural feed (Igo *et al.*, 2020). Natural feed has a balanced nutritional content, making it suitable for organisms in the larval stage. Furthermore, natural feed is easy to culture, has a

movement that can stimulate the organisms to prey on it, and has the ability to reproduce rapidly in a relatively short time, ensuring its availability at all times (Yusuf *et al.*, 2015).

One group of aquatic organism that serves as a natural food source for various types of larvae is microalgae. Microalgae are plant-like aquatic organisms that can photosynthesize and survive on inorganic nutrients and produce organic substances through the process (Diniariwisan & Rahmadani, 2023). The nutritional content of microalgae provides a source of micronutrients, vitamins, oils, and trace elements for aquatic communities. One type of phytoplankton that serves as a natural food source is *Chaetoceros calcitrans*, which has been successfully cultured and is commonly cultivated (Imelda *et al.*, 2018).

C. calcitrans is widely used as a type of natural feed due to its several advantages, namely being easily digestible and having a high nutritional content. According to Prasetyo *et al.* (2022), *Chaetoceros calcitrans* contain 35% protein and between 14 and 17% lipid. Mukti (2018) said that *Chaetoceros* genus has high potential as a producer of high-value chemical compounds such as omega fatty acids. Therefore, *C. calcitrans* is commonly used as a natural feed because it contains high amounts of carbohydrates, proteins, and lipids that support larval growth. Therefore, this activity was carried out to determine the natural feed culture technique and to determine the effect of providing *Chaetoceros calcitrans* as a natural feed in the maintenance of brown mussels (*Mytilopsis adamsi*) at the National Research and Innovation Agency, North Lombok.

METHODS

Time and Place

This activity was carried out for 30 days, from May 6, 2025, to June 4, 2025, at the National Research and Innovation Agency (BRIN), West Pemenang Village, Pemenang District, North Lombok Regency, West Nusa Tenggara (NTB). Data collected during this activity included primary and secondary data. Primary data is data from the original source obtained directly through several methods, namely observation, interviews, active participation, and documentation (Ardiansyah *et al.*, 2023). Meanwhile, secondary data was obtained from various literature such as books, journals, articles, and so on.

Culture Method

The laboratory-scale culture method for *C. calcitrans* at the National Research and Innovation Agency (BRIN) is carried out using a multilevel culture method. The multilevel culture technique is a method of cultivating natural food, either phytoplankton or microalgae, which is carried out in stages at several levels or culture scales. According to Alim (2018), multilevel culture is based on the principle that plankton culture is carried out by starting with a small volume and gradually increasing to a larger one, generally called plankton culture. Multilevel culture is a method designed to utilize space to increase productivity in producing natural food. This is in line with Encinas-Arzate *et al.* (2020), who stated that the culture process is influenced by regulating light intensity and aeration, as well as monitoring cell density using a hemocytometer to ensure the culture remains in the optimal growth phase.

Density Calculation

C. calcitrans cell counts were performed using a hemocytometer mounted on a microscope. Samples were taken from sterilized culture bottles, then 1–2 drops were placed in the previously cleaned and sterilized counting chamber of the hemocytometer. The sample was covered with a coverslip and observed under a microscope at 10×10 magnification. Counts were performed using four different viewing angles within the largest box. The viewing

angle used was the large box containing 16 squares with three large boundaries. Plankton counting in this activity used the left and upper L fields of view to count plankton that touched the boundaries of the field of view. If plankton touched the right and lower boundaries of the fields, they were not counted. This is in accordance with the statement by Tewal *et al.* (2021), who stated that density calculation with a hemocytometer involves observing the cells suspended in the counting chamber, or the small or large boxes of the hemocytometer. The formula for calculating density with a hemocytometer is as follows:

$$\text{Density} = \frac{\text{cell count (cell)}}{4 \text{ field of view}} \times 10^4 \text{ cell/ml}$$

Weight Growth Calculation

The formula for calculating weight growth of *Mytilopsis adamsi* is adopted from the absolute weight calculation formula based on (Purwati & Diniariwisan, 2025):

$$\Delta W = W_t - W_o$$

where:

ΔW = Absolute weight (g)

W_t = Average weight at the end of rearing (g)

W_o = Average weight at the start of rearing (g)

RESULTS

Chaetoceros caltricans Density

C. caltricans density measurements were conducted daily at the same time until harvest to determine the growth rate of the phytoplankton. Based on these observations, the density data for *C. caltricans* cultured in a volume of 5,000 ml is shown in the graph below:

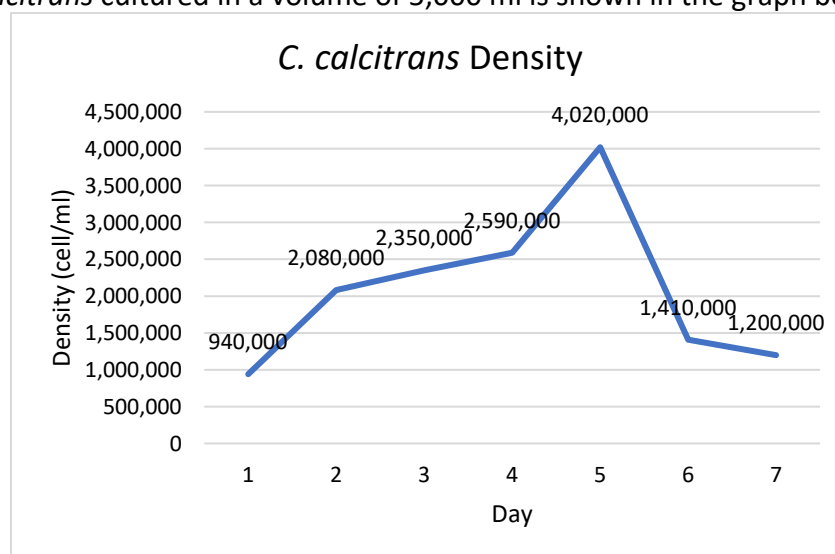
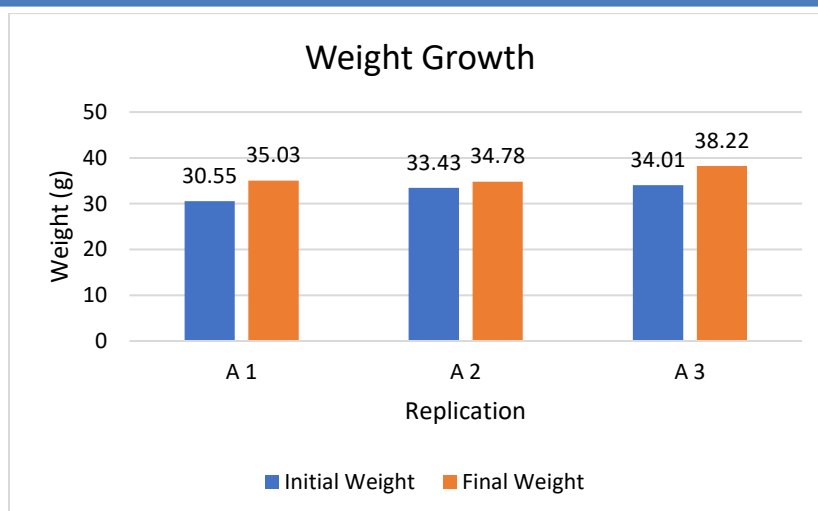


Figure 1. *C. caltricans* Density Chart

Brown Mussel Weight Growth

C. caltricans provided to brown mussels raised at the North Lombok Research and Innovation Agency (BRIN) in North Lombok significantly impacted their growth. The growth graph of brown mussels fed the *C. caltricans* natural feed is shown in Figure 2.

Figure 2. Weight Growth of Brown Mussels (*Mytilopsis adamsi*)

DISCUSSION

Equipment Preparation and Sterilization

Equipment and material preparation are necessary before conducting culture activities. Preparation of culture equipment begins with washing the previously used culture container. The culture container used is a 10 L jar. Sterilization of equipment begins by soaking the jar and aeration device in 15 ml of laundry bleach (ByClean) for 30 minutes. According to Widiastiti *et al.* (2023), an ideal disinfectant will quickly destroy bacteria, fungi, viruses, and protozoa without damaging the objects exposed to the liquid. Common disinfectants used in laboratories are alcohol and Bayclin (chlorine) solution. The purpose of this soaking is to eliminate any microorganisms remaining in the container from the previous culture activity. After soaking for 30 minutes, rinse with clean running water until the odor of ByClean is eliminated. This aligns with the opinion of Mufidah *et al.* (2017), who stated that the initial stage in laboratory-scale culture is the preparation of equipment and materials, which aims to kill unwanted microorganisms resulting from the previous culture.

Water Media Sterilization

The media water used in laboratory-scale *C. calcitrans* culture activities is seawater. Laboratory-scale culture media water sterilization at the National Research and Innovation Agency (BRIN) is carried out using clothes bleach (Byclean). Culture media water sterilization using Byclean is carried out in a 150 L culture container. Before sterilizing the media water, the container is first rinsed with clean water, then filled with seawater to the predetermined limit of 140 L, then 100 ml of Byclean is added with aeration for 24 hours. After 24 hours, 50 ml of sodium thiosulfate is added. According to Peneyra *et al.* (2020), Sodium thiosulfate works by neutralizing chlorine by converting it into chloride ions, this process is a redox reaction to eliminate the oxidative and toxic properties of chlorine, so that chlorine residues in the culture media or cultivation water do not harm the organisms being maintained. Sterilization is important in the preparation of natural food culture media. to ensure that the water used is free from contaminants (bacteria, fungi, viruses, and other organisms) that could interfere with culture growth. This is consistent with Febrinawati *et al.* (2020) statement that sterilization is carried out to kill microbes that can cause contamination during culture.

Fertilizer and Silicate Preparation

In natural food culture activities, the availability of fertilizer and silicate is a crucial factor that must be considered, both in type and dosage. Fertilizer plays a role in providing nutrients for optimal growth of microalgae (Diniariwisan & Muahiddah, 2024). In this study, KW21 fertilizer was used at a dosage of 1 ml/L, so 6 ml of fertilizer is required for 6 L of culture media. According to Lestari *et al.* (2023), the high nitrogen content of KW21 fertilizer is essential for supporting the growth of phytoplankton, including *C. calcitrans*. This is in accordance with the statement of Prafanda *et al.* (2020), who stated that the availability of nutrients in the media is a major component that determines the growth and quality of microalgae. In addition to nitrogen, *C. calcitrans* also requires silicate as a basic material for the formation of frustules or cell walls. Silicate deficiency can inhibit growth because the cell structure is not fully formed. In this culture, liquid silicate is added at a dose of 1 ml/L, which is given early before seeding so that it is immediately available to the diatom cells. According to Lestari *et al.* (2019), adding silicate at the right time and amount supports the resistance of diatom cells to environmental stress, increases biomass, and makes it a suitable natural food for crustaceans and bivalves. This is in accordance with the statement of Aguire *et al.* (2018), who stated that diatoms have a unique cell wall, called a frustule, which is composed of silica. This structure is formed through a process known as silicification, which is when cells absorb silicate from their environment to build this protective layer.

Distribution of *C. calcitrans* Seeds

After the culture medium has been sterilized and supplemented with nutrients, the next step is to spread the *Chaetoceros calcitrans*. The seeds used are from a previous culture, generally five days old or in the peak exponential phase. The number of seeds spread is adjusted to the media volume, which is approximately one-quarter to one-fifth of the total culture volume. For example, a 5 L culture requires 1 L of seeds. Before spreading, the seeds are first inspected to ensure they are healthy and free from contamination. Examination is carried out using a microscope, as recommended by Febrinawati *et al.* (2020), to detect the presence of foreign particles or microorganisms that should not be present. Furthermore, the visual condition of the culture is also observed; healthy seeds are golden brown, while a change in color to green indicates contamination.

After carrying out the seed inspection process, the next step is to take the seeds using a measuring cup that has been sterilized beforehand with alcohol/distilled water to prevent contamination. After that, the seeds in the measuring cup are immediately spread into the new culture medium that has been prepared beforehand. The seeds used are approximately 5 days old, which is when entering the exponential phase/peak phase. This is in accordance with the statement of Mufidah *et al.* (2017), that the seeds cultured from the peak exponential phase are seeds that are experiencing maximum growth and their density is also maximum, so that if these cells are cultured on a larger scale, the cells will develop more quickly. Kim *et al.* (2018), also said that the test results showed that seeds taken in the exponential phase, especially near the end of the phase, provide more stable and higher biomass growth compared to seeds from other phases, this occurs because in the exponential phase the cells are in the best physiological condition to actively divide.

Figure 1 shows that the peak density of *C. calcitrans* occurred on day 5 with a number of 4,020,000 cells/ml. On the day of culture until day 1 when the calculation was obtained, the density of *C. calcitrans* cells was 940,000 where this day was the lag phase. The lag phase is the period of adaptation of plankton to the new environment, growth in this phase is still slow because *C. calcitrans* cells are still adjusting to the conditions of the culture medium. On day

1 after the calculation until day 5 there was an increase in cell density from 940,000 cells/ml to 4,020,000 cells/ml which was the peak cell density. This indicates an exponential phase where *C. calcitrans* cells divide rapidly due to the availability of optimal nutrients and light. This phase is the phase where cell population growth reaches its highest rate. After reaching its peak on day 5, growth began to slow and cell density decreased to 1,410,000 cells/ml on day 6. Then on the 7th day, the cell density decreased further to 1,200,000 cells/ml. This indicates that the cultivation period has entered the death phase, which is a continuation of the stationary phase, where the rate of cell death exceeds the rate of cell division. As a result, the number of living cells in the culture medium begins to decrease significantly. In this plankton density growth cycle activity, a very rapid stationary phase occurs which is influenced by the absence of available nutrients in the culture medium, resulting in microalgae nutrient deficiencies and death, causing a short stationary phase. This is in line with Prasetyo *et al.* (2022), who stated that the time span between the stationary phase and the death phase is relatively short. Microalgae experience nutrient deficiencies, so that nutrient availability becomes increasingly limited, which causes a decrease in cell density and ultimately leads to microalgae death.

Feeding *C. calcitrans* for the Growth of *Mytilopsis adamsi*

Based on their feeding habits, *Mytilopsis adamsi* are filter-feeders that rely heavily on natural food sources available in the water. Phytoplankton is the primary source of nutrients needed to support the growth and survival of mussels. The advantages of natural food include its complete nutritional content and its presence floating in the water, making it easy for mussels to filter. Among the various types of phytoplankton, *C. calcitrans* is a good natural food for brown mussels because it is rich in essential fatty acids and protein, and is easily digested, supporting optimal growth.

One of the natural feeds provided for *Mytilopsis adamsi* cultivation at BRIN North Lombok is *C. calcitrans*, which is known to have a high nutritional content that supports optimal mussel growth. Feeding is carried out in a volume of 500 ml per feeding and at a frequency of 2 times a day, namely at 08:00 and 16:00 WITA, the aim is to ensure sufficient food availability to support the development of mussels that are in the growth stage. According to Silina (2023), mussel growth is highly dependent on the availability of food in their environment. When the number of phytoplankton is at a sufficient level, mussels can grow faster because their nutritional needs are met. This is in line with Fabra *et al.* (2025), which states that the filtration capacity of bivalves is highly dependent on environmental conditions and physiological aspects. Therefore, feeding should be regulated according to the species capacity and the existing situation. Feeding was carried out directly from a 10 L jar, poured into a measuring cup, and then added to the mussel rearing tank. Before the feeding process, the measuring cup was washed first to remove contamination, so that the *Mytilopsis adamsi* could utilize it optimally. According to Panjaitan *et al.* (2015), who stated that providing natural feed to cultivated biota during the growth phase must be done with adjusted timing to ensure the feed is available at the right developmental stage.

Based on the cultivation activities, the results of observations of the initial weight and final weight of *Mytilopsis adamsi* in replications with *C. calcitrans* feed, there were differences in the growth of mussel weight in each replication (figure 1). The results of providing natural feed with *C. calcitrans* species can affect the increase in weight growth of brown mussels. This is due to the nutritional content in *C. calcitrans* which supports the nutritional needs of mussels, including protein, carbohydrates, and fats which are essential for optimal growth. According to Prasetyo *et al.* (2022), there are several components contained in *C. calcitrans*

that are used for the development of cultivated biota, especially in the development of larval life, including fat 14-17%, protein 35%, carbohydrates around 17%.

CONCLUSION

This activity demonstrated that *C. calcitrans* culture can be carried out effectively using a multistep method, starting from media sterilization, the addition of KW21 fertilizer and silicate, and the use of seeds in the exponential phase. The culture results reached a peak density on the 5th day of 4,020,000 cells/ml. Providing *C. calcitrans* as natural feed has also been shown to support the growth of brown mussels (*Mytilopsis adamsi*), as seen by the increase in weight during cultivation.

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