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Natural Feed *Nitzschia* sp. Culture on Laboratory Scale

Lena Susianti[1], Rangga Idris Affandi[1]*

[1]Aquaculture Study Program, Faculty of Agriculture, University of Mataram
Pendidikan Street No. 37 Mataram, West Nusa Tenggara

***Correspondence**:
ranggaidrisaffandi@unram.ac.id
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ABSTRACT

Natural feed is fish feed that has the form of microscopic organisms found in waters. Commonly used natural feeds are phytoplankton, zooplankton, protozoa, microcrustaceans, microscopic invertebrates, and others. The need for natural feed is growing in line with the increasing fisheries cultivation efforts. Phytoplankton has a very important role in waters, its ecological function as a primary producer and the beginning of the food chain causes phytoplankton to often be used as a measure of the fertility of waters. One of the phytoplankton that is abundant in waters because of its ability to survive even in extreme conditions and has a fat content is *Nitzschia* sp., a member of the Bacillariophyceae class. *Nitzschia* sp. plays an important role as a primary producer and is widely used as natural feed for marine organism larvae such as crustaceans, bivalves, and fish. The purpose of this study was to determine the laboratory-scale *Nitzschia* sp. natural feed culture technique to support cultivation activities. This research was conducted from March to April 2024 at the Marine Aquaculture Center (BPBL) Lombok, West Sekotong, West Lombok Regency, West Nusa Tenggara. The research methods used are descriptive methods and field observations (surveys). The results that the laboratory scale *Nitzschia* sp. natural feed culture technique includes equipment sterilization activities, culture preparation, seed distribution, density calculation and harvesting. Factors that affect the growth of *Nitzschia* sp. are culture media that are free from contamination, initial stocking density, light intensity of 2000-5000 Lux, water quality (pH, salinity, and temperature).

INTRODUCTION

Natural feed is fish feed that has the form of microscopic organisms found in waters. The types of aquatic organisms used as natural feed vary greatly, depending on the type of fish and its age. Commonly used natural feeds are phytoplankton, zooplankton, protozoa, microcrustaceans, microscopic invertebrates, and others. The need for natural feed is growing

in line with the increasing fisheries cultivation efforts. Every cultivation activity requires the availability of seeds in sufficient quantities, on time and continuously. To meet these needs, natural feed culture installations must be available as part of the production components in cultivation activities (Andriani et al., 2022). In fisheries cultivation activities, many use natural feed, namely phytoplankton, to stimulate the growth of cultivated organisms.

Phytoplankton are the largest oxygen producers on earth and live near the surface of the water where there is enough light to support the photosynthesis process. Phytoplankton have a very important role in waters, their ecological function as primary producers and the beginning of the food chain causes phytoplankton to often be used as a measure of the fertility of waters. The more phytoplankton in a body of water, the more fertile and beneficial the body of water can be. Phytoplankton are also often called plant plankton because they contain chlorophyll (Putri et al., 2021).

One of the phytoplankton that is abundant in waters because of its ability to survive even in extreme conditions and has a fat content is *Nitzschia* sp., a member of the Bacillariophyceae class. *Nitzschia* sp. is a diatom that has characteristics such as long cells at each end, has thin cell walls and cell sizes ranging from 10-40 µm. *Nitzschia* sp. plays an important role as a primary producer and is widely used as natural food for marine organism larvae such as crustaceans, bivalves, and fish. This microalgae has a high growth rate, is easy to cultivate, and has a fairly high lipid content (Ilhami et al., 2015).

Therefore, to maintain the availability of natural food for marine organisms, it is necessary to carry out natural food culture that is good in quality and quantity continuously. Therefore, knowledge of good phytoplankton culture is needed so that it can meet the feed needs for cultivation activities. The purpose of this study was to determine the technique of natural feed culture of *Nitzschia* sp. on a laboratory scale to support cultivation activities.

METHODS

Time and Place

This research was conducted from March to April 2024 at the Marine Aquaculture Center (BPBL) Lombok, West Sekotong, West Lombok Regency, West Nusa Tenggara.

Research Method

The research methods used are descriptive methods and field observations (surveys). Descriptive research is research conducted to determine the value of independent variables, either one or more variables (independent) without making comparisons, or connecting with other variables. Based on this understanding, it can be concluded that descriptive research is conducted by seeking information related to existing symptoms, clearly explaining the objectives to be achieved, planning how to approach it, and collecting various types of data as material for making reports. Survey research is a research method that aims to obtain a general picture of the characteristics of the population described by the sample. Survey research can be conducted in various fields including economics, business, politics, government, sociology, education, and in science and technology fields. The data obtained during this research were analyzed descriptively, namely describing all activities carried out clearly and in detail which are supported by literature studies so that they can provide clear and complete information (Annisa & Affandi, 2024; Irawati & Affandi, 2024; Mas'ud & Affandi, 2024; Ningsih & Affandi, 2023; Pebrianti & Affandi, 2024).

Density Count

Observation of *Nitzschia* sp. culture is carried out every day by calculating the daily density on the scale used. The growth observation time is adjusted to the time at the beginning of the *Nitzschia* sp. culture. Samples are taken and put into a sample container, then taken to the observation place under a microscope. The calculation is done by taking 1 ml of sample in the sample container using a dropper pipette, then dropped on the hemocytometer and covered using a cover glass. The counting process is carried out using a microscope with 10x magnification, the number of cells is counted in 5 medium boxes located in the center of the box, namely in the upper left corner, upper right corner, lower right corner, lower left corner, and center. Cells in each box are counted with the help of a hand tally counter.

RESULTS

Pure culture of diatoms is a monospecies of plankton cultured in a controlled room carried out in the natural food laboratory of the Marine Aquaculture Center (BPBL) Lombok. The diatoms cultured are the *Nitzschia* sp. type. *Nitzschia* sp. culture on a laboratory scale is the beginning of culture activities in an effort to provide stock to maintain the sustainability of *Nitzschia* sp. maintenance and is the beginning of culture activities that will be continued in semi-mass and mass-scale culture activities.

Natural food culture of *Nitzschia* sp. Laboratory scale at BPBL Lombok was carried out in an AC room with a temperature between 21-23°C with aeration, with neon lighting with a light intensity of 2000-5000 Lux. Pure culture started with a scale of 10 mL, 100 mL, 250 mL, 500 mL, 1000 mL and 5 L culture with a jar container.

Tools Sterilization

Tools sterilization is an initial activity in the culture of *Nitzschia* sp. which aims to eradicate organisms attached to the culture tool and to avoid contamination from unwanted organisms. In general, sterilization of tools for natural feed culture in the BPBL Lombok laboratory is carried out in three stages, namely soaking with vixal, washing with soap and the boiling process. The container to be used is first wet sterilized by washing all equipment using cleaning soap after that it is rinsed with fresh water and dried for one day, then containers such as jars, aeration stones and aeration hoses are boiled using a pan filled with water that has been heated using a stove until boiling for 2 minutes at a temperature of 100°C.

Culture Media Sterilization

The culture preparation process at BPBL Lombok is carried out 1 day before the spread of seeds with the stages of the seawater sterilization process that will be used for the culture media. The seawater sterilization process is carried out in 2 ways, namely Erlenmeyer flasks with a volume of 250-1000 mL are sterilized using an autoclave for 20 minutes at a temperature of 121°C then stored at room temperature. While the 5 L jar container is sterilized using chemicals, using a chlorine solution with a dose of 1 mL, left for 24 hours with aeration so that all bacteria can be destroyed and the water in the container is completely sterile. After 24 hours, 0.2 g of sodium thiosulfate is added and left for 30 minutes which functions to neutralize water containing chlorine.

Fertilizer Application

The most important elements needed in diatom culture are N, P, and Si. The main nutrient that phytoplankton needs most for growth is nitrogen in the form of nitrate. Diatoms cannot survive with insufficient Si supply because silicate is not only needed in the formation of cell walls but also to have high resistance to environmental pressures such as extreme conditions. In addition, the addition of silicate fertilizer affects the cell density of microalgae.

Biomineralization is the ability of living organisms to synthesize solid inorganic materials using nutrient sources in their natural environment. An example is diatoms which are unicellular microalgae (Bacillariophyceae) that are able to produce cell walls by taking silicic acid from their aquatic habitat. Their cell walls contain amorphous silica and special biomolecules.

Seed Distribution

One of the seed sources used is from the Gondol area, Bali. The culture was carried out in an air-conditioned room with a temperature between 21-23°C, aerated, with 18 watt neon lighting. The culture started with a scale of 500 mL, namely 400 mL of sterilized seawater, added with 100 mL of *Nitzschia* sp. seeds, then added 0.5 mL of commercial fertilizer KW21 and 0.5 mL of diluted sodium silicate. Then the culture was raised using a 5 L jar. From a 500 mL culture, 4 L of sterilized seawater was added, then 5 mL of commercial fertilizer KW21 and 5 mL of diluted sodium silicate were added. *Nitzschia* sp. Culture laboratory scale is ready to be observed and its density is calculated from day 1 to day 6. Then the results of this laboratory scale will be used as seeds for semi-mass scale.

Density Count

Analysis of the calculation results on a 1 liter Erlenmeyer flask using a microscope and a haemacytometer, the results of the density calculation were carried out on the 1st day after spreading the seeds and the initial density level obtained was 14,450,000 cells/mL and increased to 17,300,000 cells/mL. And the results of the calculation of the final density level on the 6th day of the peak phase were 39,850,000 cells/mL.

Figure 1 shows that the growth graph of *Nitzschia* sp. is in accordance with the normal phytoplankton growth pattern. From the beginning of the culture to the 6th day of *Nitzschia* sp. did not decrease. The optimal density of phytoplankton is influenced by the culture time depending on the type of phytoplankton, the initial density of the inoculant and environmental conditions.

The growth of *Nitzschia* sp. did not experience a lag phase but experienced an increase in density, namely on the 1st to 6th day (exponential phase). The stationary phase occurred on the 7th day which experienced stability. Increasing growth will cause competition between cells in obtaining nutrients while the availability of nutrients decreases every day, therefore renewal must be carried out by continuing the culture in a container with a larger volume. Based on the graph, it can be concluded that the peak growth of *Nitzschia* sp. occurred on the 6th day with a density of 39,850,000 cells/mL.

Table 1. Nitzschia sp. Density Count on Laboratory Scale

Day	1	2	3	4	5	6
Top left	44	55	82	93	128	150
Top right	75	81	77	131	133	161
Bottom left	63	63	80	164	133	176
Bottom right	46	78	97	146	154	154
Center	61	69	100	140	156	156
Density	$1{,}445 \times 10^4$	$1{,}730 \times 10^4$	$2{,}180 \times 10^4$	$3{,}370 \times 10^4$	$3{,}520 \times 10^4$	$3{,}985 \times 10^4$

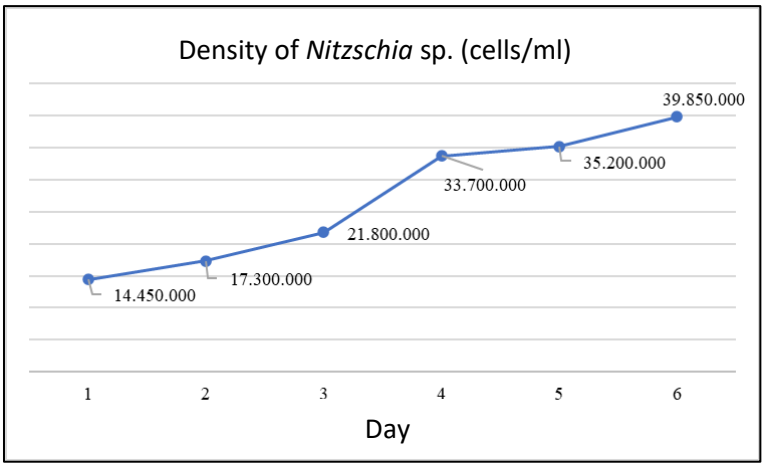

Figure 1. Growth of *Nitzschia* sp.

Harvesting

The laboratory-scale harvesting process at BPBL Lombok with a volume of 500-1000 mL is then renewed by continuing the culture in a container with a larger volume, namely a 5-10 L jar container.

Factors Affecting the Growth of *Nitzschia* sp.

Environmental factors are very important in the growth of microalgae. Factors that affect the growth of microalgae are as follows:

1. Light

Light plays an important role in the process of photosynthesis, namely as a source of light that is utilized by autotrophic organisms into chemical energy through chlorophyll activity. In addition, adequate lighting also plays a role in maintaining the temperature of the media so that it remains in the optimum range of microalgae growth. Carbon fixation is carried out by phytoplankton by regulating its photosynthetic pigments to light. The white light spectrum can increase the chlorophyll content of *Nitzschia* sp.

2. Temperature

Temperature is one of the abiotic factors whose presence greatly affects the growth of microalgae. Increasing the temperature in the optimum range will increase the metabolic rate and photosynthetic activity of microalgae. The optimum temperature for microalgae cultivation at BPBL Lombok ranges from 21-28°C, and can change depending on the composition of the media used and the type of microalgae cultivated. Changes in temperature cause differences in the composition and abundance of diatoms.

3. Acidity (pH)

The acidity (pH) is one of the important parameters that affects the growth of microalgae. Waters are greatly influenced by the concentration of CO_2 and acidic compounds. During photosynthesis during the day, microalgae use CO_2 from the water so that this causes the pH of the water to increase while at night photosynthesis does not take place but respiration continues, reducing the pH of the water. Phytoplankton at BPBL Lombok are able to grow optimally at a pH range of 8-8.6. This shows that the pH of the culture media is still safe for the growth of *Nitzschia* sp.

4. Salinity

Salinity is a description of the amount of salt in water. The distribution of salinity in seawater is influenced by various factors such as water circulation, evaporation, rainfall and river flow. Salinity is also one of the factors that affects cell growth because salinity affects osmotic pressure which will directly affect the metabolic process, respiration process, thus

inhibiting the growth of vegetative cells which will then gradually affect the density of the microalgae population. The optimum salinity for phytoplankton growth in BPBL Lombok is 33 ppt. High or low salinity can cause osmotic pressure inside the cell to be lower than outside the cell so that cell division activity is disrupted.

5. Nutrients

Nutrients in the maintenance media are the most important components in the growth of microalgae, reducing the percentage of nutrients can affect the physiological processes of microalgae and have an impact on growth. Microalgae growth is not only influenced by the availability of adequate essential macronutrients but also by the availability of sufficient micronutrients according to needs. Macronutrients and ions are generally not toxic to microalgae and can be used in high concentrations to support microalgae growth, but on the other hand micronutrients have toxic properties and inhibit microalgae growth if used in excessive concentrations.

DISCUSSION

The quality of the results of culture activities on a laboratory scale will determine the success of subsequent culture activities, therefore it must meet technical requirements such as being free from contamination, nutrition and environment as well as light and water quality. Pure culture activities of *Nitzschia* sp. on a laboratory scale are carried out in a sterile room and are not exposed to direct sunlight so that neon lights are used as a light source so that *Nitzschia* sp. can carry out photosynthesis (Suriadnyani & Aryani, 2017).

According to Putri & Salim (2024), which states that sterilization is an effort to kill unwanted microorganisms with the aim that activities do not experience culture failure due to contamination. Cleanliness of laboratory equipment such as Erlenmeyer flasks, droppers, test tubes, jars, aeration hoses and aeration stones is a very basic part of laboratory activities. According to Safitri (2022), the preparation of the media water used in the culture of *Nitzschia* sp. must be completely sterile, aiming to kill pests and diseases that cause contaminants.

According to Kamariah et al. (2023), excessive nitrogen content can inhibit the biosynthesis process of algae cells. KW21 is a Japanese-made liquid culture medium consisting of several elements and chemical compounds packaged in 1 liter bottles. In 1 liter of KW21 contains chemical elements and compounds, namely nitrogen 49 grams/liter, phosphate buffer 4 grams/liter, boric acid, manganese, cobalt, zinc, EDTA, amino acid complex, and vitamin mix (B1, B12, biotin and others) (Walid, 2018).

According to Sujarwani & Rusmana (2020), the ratio of water and fertilizer volume is 1: 1 and for the provision of plankton seeds as much as 10% of the water volume. According to Rahayu et al. (2022), stated that the diatom adaptation phase occurs on the 1st-2nd day after inoculation, on the 3rd to 6th day there is a very rapid increase in density (exponential phase), while on the 7th day there is a stationary phase in this phase *Nitzschia* sp. experienced stability and there was no increase in the number of microalgae populations. According to Anggraeni (2016), stated that the decrease in the development of the culture algae population in limited media is caused by several factors, namely the composition and nutrient content of the media which are decreasing.

According to Anggraeni (2016), *Nitzschia* sp. harvesting is carried out at the peak of its growth or when it is old, namely on the 5th to 6th day, because at that time the growth reaches maximum density. According to Kamariah et al. (2023) stated that light is an environmental factor that affects the pigment content of microalgae. According to Ernawati et al. (2023)

stated that temperature directly affects the efficiency of photosynthesis and is a determining factor in growth. According to Sukron (2018), changes in pH cause the life of biota in waters to be disrupted due to CO_2 imbalance. The optimal pH range for diatom growth is 7-8. According to Hujannah (2023), diatom microalgae can live at a salinity of 26-35 ppt. According to Wahyudi et al. (2022) stated that the nutrients needed by microalgae to grow and reproduce consist of micronutrients and macronutrients. The macronutrients needed include C, H, N, P, K, S, Mg and Ca and for micronutrients such as Fe, Cu, Mn, Zn, Co, Mo, Bo, Vn, and Si.

CONCLUSION

Based on this study, it can be concluded that the laboratory scale *Nitzschia* sp. natural feed culture technique includes equipment sterilization activities, culture preparation, seed distribution, density calculation and harvesting. Factors that affect the growth of *Nitzschia* sp. are culture media that are free from contamination, initial stocking density, light intensity of 2000-5000 Lux, water quality (pH, salinity, and temperature).

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