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Original research

Study of *Nannocloropsis oculata* and *Tetraselmis chuii* As Natural Feed for *Brachionus plicatilis* on Laboratory Scale at Center for Marine Aquaculture (BBPBL) Lampung

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Abstract
A very influential factor in marine fish production is the provision of efficient and appropriate feed and feed management for marine fish larvae. Natural feed has content that can fulfill the nutritional needs of marine fish larvae. One of the natural feeds that can be utilized in marine fish farming is rotifer (*Brachionus plicatilis*). This study aims to determine the laboratory scale culture technique of *Brachionus plicatilis*, determine the growth rate of *Brachionus plicatilis* with different phytoplankton feeding, and determine the optimal phytoplankton density for *Brachionus plicatilis*. This research used observation and preliminary test methods in data collection and processing. Data collection starts from the preparation of tools and materials, calculation of *Brachionus plicatilis* and natural feed to water quality. Preliminary tests were carried out on each type of natural feed with 3 different densities, namely, *Nannocloropsis oculata* with a density of 75,000 cells/ml; 100,00 cells/ml; 125,000 cells/ml and *Tetraselmis chuii* with a density of 1000 cells/ml; 2000 cells/ml; 3000 cells/ml. The best results from both types of feed are *Nannocloropsis oculata* with a density of 100,000 cells/ml and *Tetraselmis chuii* with a density of 3000 cells/ml. These two types of feed with optimal density were used to feed *Brachionus plicatilis* for 7 days. The results obtained from the calculation of the number of *Brachionus plicatilis* individuals are, *Brachionus plicatilis* with *Nannocloropsis oculata* feed produces 72 ind/ml with an exponential phase on the fourth day and *Brachionus plicatilis* with *Tetraselmis chuii* feed produces 100 ind/ml with an exponential phase on the fifth day, so the best natural feed for *Brachionus plicatilis* growth rate is *Nannocloropsis oculata.*

Keywords: Brachionus plicatilis, Culture technique, Growth rate, Nannochloropsis oculate, Tetraselmis chuii

Introduction

In the hatchery phase, fish need to adapt to their environment and do not yet have a good digestive system (Muchlisin et al, 2003). Natural feed can help the digestive process of fish larvae and provide good energy and nutrients for marine fish larvae (Dhert, 1996). The main requirement in improving the development of fish larvae is the availability of quality natural feed (Suriansyah, 2012). *Brachionus plicatilis* is the most commonly used natural feed in marine fish hatcheries. *Brachionus plicatilis* has advantages as a natural feed, namely having a body size that matches the mouth opening of fish larvae, is not difficult to digest by fish larvae, has a slow movement so that it is easily preyed upon by fish larvae, has a high nutritional content, and also has a very fast growth and development (Redjeki, 1999). The quality of *Brachionus plicatilis* is influenced by the type and amount of natural feed given (Melianawati et al, 2006).

Natural feed commonly used in *Brachionus plicatilis* cultivation are *Nannochloropsis oculata* and *Tetraselmis chuii* (Lubzenz, 1987). The use of natural feed is one of the efforts to improve nutrition and accelerate the development of *Brachionus plicatilis* (Fulks & Main, 1990). The nutritional content of the natural feed includes protein, fat, carbohydrates, minerals, and amino acids. This content can increase the density of *Brachionus plicatilis* in a shorter time (Nontji, 2002).

This study aims to compare two natural feeds that can accelerate the development and improve the nutrition of *Brachionus plicatilis*. The natural feeds available at Center for Marine Aquaculture (BBPBL) Lampung are *Nannochloropsis oculata* and *Tetraselmis chuii*.

Material and Methods

Tools and Materials

The tools used in this study are culture containers with a volume of 3 liters, erlenmeyer, glass jar, aerator, drip pipette, erlenmeyer, beaker, aerator, light microscope, hand counter, haemocytometer, Sedgwick rafter cell, cover glass, glass plate, measuring cup, preparation, measuring cup, filter bag, bucket, sample bottle, aeration hose, aeration stone, aluminum foil, tissue, stationery, and label paper. The materials used in this study are *Brachionus plicatilis* seedlings, *Nannochloropsis oculata*, *Tetraselmis chuii,* sterile sea water, fresh water, chlorine, iodine, 70% alcohol and detergent.

Methods

1. Tools sterilization

Sterilization of tools began with soaking tools such as jars (culture media), measuring cups, erlenmeyers, drop pipettes, aeration hoses, aeration stones into a fresh water solution that has been mixed

with 30 ppm chlorine for 24 hours. The soaking stage aims to remove the remaining sediment from the feed in the culture container. Then, the tools were washed using soap and rinsed with fresh water until clean. Next, the tools were sprayed with 70% alcohol for aseptic conditioning and then air dried.

2. Water sterilization

Sterilized water consisted of a mixture of seawater and fresh water in a ratio of 80%: 20% with a salinity of around 25 ppt as measured by a refractometer. This sterile water was sterilized by boiling at ±150°C for 15-20 minutes which aims to denature the enzymes of microorganisms in the media (water). After that, the water is filtered with a 20 µm filter cloth to prevent contaminants from being carried away by the water. Then, sterile water is put into a 3 liter erlenmeyer and covered using aluminum foil to prevent evaporation.

3. *Brachionus plicatilis* culture

Brachionus Plicatilis culture began with the calculation of the seeds to be used. *Brachionus plicatilis* seeds used in laboratory-scale culture were taken from the harvest of mass-scale *Brachionus plicatilis. Brachionus plicatilis* seeds were diluted in a ratio of 1: 1, then the density of *Brachionus plicatilis* was calculated using a Sedgwick rafter cell with the help of a hand counter under a microscope at 4x magnification to determine the initial density of seeds, after which it was calculated using the formula:

$$V1 = \frac{N2\ x\ V2}{N1}$$ (Ekawati, 2005)

Description:

V1: sample water volume (ml)
N1 : density of *Brachionus plicatilis* seedlings (ind/ml)
V2 : volume of desired culture media (ml)
N2 : desired density of *Brachionus plicatilis* seedlings (ind/ml)

After obtaining the initial density, *Brachionus plicatilis* seedlings were inoculated into culture containers with a media volume of 1.5 liters of sterile water which had previously been acclimated for approximately 3 hours to adjust to the new environment. The initial density of *Brachionus plicatilis* used was 30 ind/ml, then aeration was installed as an oxygen supply.

4. Feeding

The density of *Nannochloropsis oculata* and *Tetraselmis chuii* was first calculated using a haemocytometer with the help of a hand counter under a microscope with 10 times magnification. Haemocytometer is a tool made of glass that is divided into boxes in two places of the field of view. The amount of food density in each culture vessel is adjusted to the desired density, namely for *Nannochloropsis oculata* with a density of 100,000 cells/ml and *Tetraselmis chuii* with a density of 3000 cells/ml. Calculations using the formula :

$$\frac{\sum \text{cells}}{\text{ml}} = \frac{N\ x\ 25\ x\ 10000}{5}$$

(Mudjiman, 2008)

Description:

∑cells/ml : phytoplankton density
N: average number of cells (from 5 boxes)
5 : number of boxes counted
25: total number of boxes on the haemocytometer.

After obtaining the desired density of *Nannochloropsis oculata* and *Tetraselmis chuii*, the phytoplankton can be given to *Brachionus plicatilis* as food. Feeding is done every day with a period of 24 hours. The amount of food given to *Brachionus plicatilis* during the 7 days of observation can be seen in Table 1.

Table 1. Feed density of Brachionus plicatilis

Day	*Feed Quantity*	
	Nannochloropsis oculata	*Tetraselmis chuii*
	100.000 cell/mL	3000 cell/mL
1	1,42 x 10^6	4,4 x 10^5
2	1,64 x 10^6	8,3 x 10^5
3	3,5 x 10^6	1,03 x 10^6
4	2,11 x 10^6	3,63 x 10^6
5	2,73 x 10^6	2,13 x 10^6
6	2,51 x 10^6	1,97 x 10^6
7	1,78 x 10^6	1,88 x 10^6

5. *Brachionus plicatilis* population counts

Prepare the tools and materials to be used, namely microscope, Sedgwick rafter cell, hand counter, cover glass, drop pipette, sample, lugol, and 70% alcohol. Then, the Sedgwick rafter cell is cleaned first, the surface of the Sedgwick rafter cell is sprayed with 70% alcohol and then wiped with a tissue in a direction to make it sterile. Next, drop lugol at 5 points in the Sedgwick rafter cell which aims to keep *Brachionus plicatilis* from moving so that it is easier when doing calculations, then cover with a hexagonal cover glass so that there is room to drip the sample. Then, take a 1 ml sample of *Brachionus plicatilis* using a drop pipette. Next, drop the sample on the Sedgwick rafter cell and close the cover glass completely until there are no air bubbles. Observe the *Brachionus plicatilis* sample counted on the sedgwick rafter cell by observation under a microscope with a magnification of 4 times, observations were made 3 times repeated with the help of a hand counter. Number of individuals in Sedgwick rafter cell = actual number of individuals.

Results and Discussion

Late observations were made for 7 days to see the growth of *Brachionus plicatilis* with feed that had been adjusted at a certain density. The following graph of *Brachionus plicatilis* growth with the provision of different types of feed and the amount of feed density can be seen in figure 1.

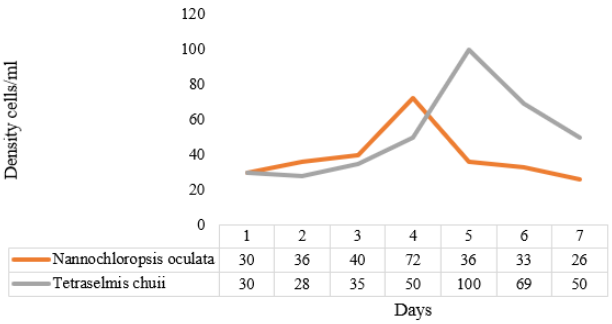

Figure 1. Growth rate of Brachionus plicatilis

Based on the observation of *Brachionus plicatilis* growth, it can be seen that there are several phases of growth in *Brachionus plicatilis*. On feeding *Nannochloropsis oculata* with a density of 100,000 cells/ml rotifers entered the adaptation phase on day 1 to day 3 consecutively with an initial density of 30 ind/ml, 36 ind/ml, and 40 ind/ml. Meanwhile, *Brachionus plicatilis* treated with *Tetraselmis chuii* with a density of 3000 cells/ml entered the adaptation phase on days 1 to 4 consecutively with densities of 30 ind/ml, 28 ind/ml, 35 ind/ml, 50 ind/ml. According to Widjaja (2004), *Brachionus plicatilis* at the beginning of the culture experienced a growth that is not too high allegedly because *Brachionus plicatilis* is still adjusting to the new environment, from a mass scale environment (outdoor) to a laboratory scale (indoor).

Water quality measurements were carried out at the beginning and at the end of the test which aims to determine changes in *Brachionus plicatilis* growth media, can be seen in table 2. Water quality.

Table 2. Water quality

Parameter	*Brachionus plicatilis*			
	Nannochloropsis oculata		*Tetraselmis chuii*	
	Start	End	Start	End
Temperature (°C)	22	26	22	26
Salinity (psu)	25	31	25	30
pH	7,5	7,7	7,6	7,7
Dissolved Oxygen	5,5	5,9	5,6	5,8

The measurement results showed that the water temperature of the rearing medium during observation ranged from 22-26 °C. This temperature range is still within the range that can be tolerated for the growth of *Brachionus plicatilis*. According to (Redjeki, 1999) the optimal temperature range for Brachionus plicatilis growth is 20°C-30°C.

Discussion

Brachionus plicatilis enters the exponential phase, where the cells have undergone good development, so they have rapid and constant growth. This is in accordance with the opinion of Isnansetyo and Kurniastuty (1995) in (Wina et al, 2013) that the exponential phase begins with cell division at a constant growth rate with optimum culture conditions, the growth rate in this phase reaches a maximum density. *Brachionus plicatilis* with *Nannochloropsis oculata* feed experienced an exponential phase or peak density on day 4 with a density of 72 ind/ml, while *Brachionus plicatilis* with *Tetraselmis chuii* feed experienced an exponential phase one day later than *Brachionus plicatilis* with *Nannochloropsis oculata* feed on day 5 with a density of 100 ind/ml. This is in accordance with the statement Heriantii & Sarnita (1982) that *Nannochloropsis oculata* helps accelerate the growth of *Brachionus plicatilis* so that the peak density is obtained faster, while *Tetraselmis chuii* helps in optimizing the growth of *Brachionus plicatilis* so that it has a higher density but is slightly slower to reach the peak or exponential phase. Furthermore, the nutrient content available in the culture media is sufficient to be utilized by *Brachionus plicatilis* for growth so that there is a rapid increase in the number of individuals. This is in accordance with the statement Priyambodo (2011) that in culturing *Brachionus plicatilis* the availability of food greatly determines the growth rate of the *Brachionus plicatilis* population.

If there is a lack or excess of nutrients in the media material can cause a decrease in the growth rate for *Brachionus plicatilis*. The growth of *Brachionus plicatilis* with *Nannochloropsis oculata* food decreased from day 5 to day 7 with the number of individuals in a row, namely 36 ind/ml, 33 ind/ml, and 26 ind/ml. While *Brachionus plicatilis* with *Tetraselmis chuii* food experienced a decline phase from day 6 to day 7 with the number of individuals respectively, namely 69 ind/ml and 50 ind/ml. This is thought to be due to the amount of density of *Brachionus plicatilis* population growth has reached the maximum growth limit, thus narrowing the space for movement and causing disruption of *Brachionus plicatilis* activity and competition between organisms in fighting for food to survive. Changes in water quality of the maintenance media can also be a factor in reducing the growth of *Brachionus plicatilis* (Nurlinda et al, 2019). The decline in growth after peak growth is caused by limited culture media, both nutrient content and volume of culture media (Riesya & Nurhidayati, 2013).

The difference in the growth rate of *Brachionus plicatilis* with two different types of feed can be caused by differences in the content of each feed. According to Payne & Rippingale (2000) in Sutomo (2007), *Nannochloropsis oculata* contains Eicosapentaenoic acid (EPA) of 44.26%, has Vitamin B12 content, and has a total omega 3 content of 42.7%.

The nutritional content of *Nannochloropsis oculata*, especially for EPA, Vitamin B12, and omega 3 is not possessed by other feed tested, namely *Tetraselmis chuii*. In the proximate content test, *Nannochloropsis oculata* has better nutrition than *Tetraselmis chuii*. *Nannochloropsis oculata* contains 57.02% protein compared to 26.4% content of *Tetraselmis chuii*, protein is used to form amino acids that play an important role in the process of growth and reproduction (Yudha et al, 2013).

Water quality

Temperature affects the process of substance exchange or metabolism of a living thing. When the temperature increases, the metabolism and respiration of aquatic organisms increases rapidly and results in an increase in oxygen consumption which has an impact on decreasing dissolved oxygen levels (Effendi, 2000).

Water salinity during the study was between 25-31 ‰ and is a normal salinity for the growth of *Brachionus plicatilis.* Good salinity levels to support the growth of *Brachionus plicatilis* range from 20-31 ‰ (Rusyani, 2007). According to Redjeki (1999) if there are fluctuations in salinity in a short period of time can cause rotifer stress to affect the swimming activity or movement of rotifers.

Conclusion

Nannochloropsis oculata is a better natural feed to accelerate the growth rate of *Brachionus plicatilis* than *Tetraselmis chuii* but to increase the number of *Brachionus plicatilis* faster with *Tetraselmis chuii* feed. The increase in growth of *Brachionus plicatilis* is faster with *Nannochloropsis oculata* feed, namely the exponential phase occurs on the fourth day with a total of 72 individuals / ml, while *Brachionus plicatilis* with *Tetraselmis chuii* feed enters the exponential phase later, namely on the fifth day but with a higher number of individuals, namely 100 individuals / ml. Based on the growth rate of *Brachionus plicatilis*, the best natural food for *Brachionus plicatilis* growth is *Nannochloropsis oculata*.

Author Contribution

Desvita Putri Ramadhani : Conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; wrote the paper. Gres Maretta and Lisana Husna Imaniar : Comments and suggestions regarding this analysis.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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