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Population-level of *Nannochloropsis* sp. as an enrichment diet for marine rotifer *Brachionus rotundiformis* in mass culture tanks

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ABSTRACT

Rotifers are a natural food that has the potential as a major source of food for marine fish larvae. This study aims to observe the population density of *Nannocloropsis* sp. which plays a role in rotifer productivity. Culture of *Nannocloropsis* sp. and rotifers carried out in a volume of 50,000 L tanks using water media and ZA fertilizer mixture at a dose of 100 ppm, urea 10 ppm, TSP 10 ppm, NaEDTA 5 ppm, and $FeCl_3$ 2 ppm. The density of *Nannochloropsis* sp. and rotifers was observed using a microscope, hemocytometer, and Sedgewick rafter cell. The monitoring of water quality (temperature, salinity, and light intensity) was carried out every day in the morning and afternoon. Data were analyzed using the Microsoft Excel and displayed descriptively. The highest increase in culture density of *Nannochloropsis* sp. occurred on the 6^{th} day of 20×10^6 cells/mL while rotifer culture occurred on the 5^{th} day of 98 individuals/mL. The water quality observation in *Nannochloropsis* sp and rotifer culture showed water temperatures ranging from 27-30 °C (*Nannochloropsis* sp.) and 29-33 °C (rotifers), salinity range between 30-35 ppt (*Nannochloropsis* sp.) and 32-33 ppt (rotifers), as well as the value of light intensity obtained results ranging from 098-305 lux (*Nannochloropsis* sp.) and 085-554 lux (rotifers). The result showed that *Nannochloropsis* sp. quality is a factor that can affect rotifers' productivity.

Key word: *Nannochloropsis sp., Rotifers, Density, Mass culture, Diet*

Introduction

Natural food has a major role in supporting the initial growth of fish larvae cultivated (Ferreira *et al*., 2018). Marine fish larvae in their development are very susceptible to environmental changes, so the first feeding is important for the success of this initial phase (Hamre *et al*., 2013). Abnormalities, digestive tract damage, decreased food efficiency, and feed activity is closely related to the lack of egg yolk reabsorption in fish larvae (Pittman *et al*., 2013). Rotifers are known as a natural feed that has important potential to support the growth of most marine fish larvae (Dhert *et al*., 2014), (Takeuchi and Haga, 2013). Rotifers have several advantages such as their small size and ability to manipulate the quality of

artificial nutrients (Kotani *et al*., 2017). Rotifer's nature as a filter feeder can be useful as enrichment of natural feed along with other active ingredients contained in microalgae (Rahman *et al*., 2018).

Utilization of microalgae as a diet can run optimally through the supply of high-quality microalgae biomass and a constant growth rate (Ferreira *et al*., 2018). Commercial rotifers enrichment media commonly used are *Chlorella vulgaris* which is enriched with docosahexaenoic acid (DHA), frozen *Nannochloropsis oculata*, and taurine enrichment media (Waqalevu *et al*., 2019). Moreover, the super fresh Chlorella-V12 product can also increase DHA levels enriched by *Chlorella vulgaris* (Hagiwara *et al*., 2014; Kim *et al*., 2014; Thépot *et al*., 2016; Kotani *et al*., 2017). Several previous studies have provided insights into the enrichment materials application to enable the optimization of natural food (Dhert *et al*., 2014; Radhakrishnan *et al*., 2017).

However, information regarding the enrichment of *Nannochloropsis* sp. for rotifers feed is still not well known. *Nannochloropsis* sp. is known to contain vitamin B12 and eicosapentaenoic acid (EPA) (5.91%) and total omega 3 HUFAs (42.7%) and protein (57.02%) (Ma *et al*., 2016) so that they can be used as enrichment ingredients for marine fish farming.

The production process of *Nannochloropsis* sp biomass in outdoor culture tanks is known to have advantages such as relatively low costs. In this study, the treatment of *Nannochloropsis* sp. used to determine the optimal population level that can be supplied to rotifers. The population rate of *Nannochloropsis* sp. and rotifers biomass productivity will be observed during the study.

Materials and Methods

The study area

This research was conducted at the Indonesian Center for Marine Aquaculture Research and Fisheries Counseling (BBRBLPP), Buleleng, Bali, Indonesia.

Microalgae culture of *Nannochloropsis* sp.

Nannochloropsis sp. containing vitamin B12, eicosapentaenoic acid (EPA) (30.5%), omega 3 HUFAs (42.7%), protein (52.11%), carbohydrates (16%), vitamin C (0.85%), and chlorophyll A (0.89%) cultured outdoors in a concrete volume of 50,000 L. Distribution of seedlings was carried out after the sterilization of culture media by brushing dirt or moss attached to the walls and sides of the tub, and rinsed with freshwater or seawater and left for 24 hours. After 24 hours, the tub was then filled with seawater as a medium for *Nannochloropsis* sp. as much as 18 m^3 of the total body volume of 24 m^3. Seawater was then sterilized using as much as 50 mL of chlorine. A few hours after being given chlorine, seawater was neutralized by using sodium thiosulfate at a dose of 25 mL. Subsequently given ZA fertilizer at a dose of 100 ppm, urea 10 ppm, TSP 10 ppm, NaEDTA 5 ppm, and $FeCl_3$ 2 ppm (Ferreira *et al*., 2018). The entire fertilizer composition was mixed and dissolved with ±2 L of freshwater, except TSP fertilizer must be dissolved separately because it was difficult to dissolve. Fertilizer mixing was done in a 3,000 L tank equipped with aeration to facilitate mixing and prevent sedimentation. Besides, the stirring process was assisted by ±5 cm diameter pipes. Spreading the seeds of microalgae *Nannochloropsis* sp. done after the fertilization process was complete. *Nannochloropsis* sp. was taken from other mass culture tanks that were ready to be harvested and stocked using ±30 cm diameter pipes and filtered with filter bags.

Daily population rate monitoring of *Nannochloropsis* sp.

The daily density of *Nannochloropsis* sp. calculated by carrying water samples on culture media as much as 1 mL and observed under a microscope by using a hemocytometer. The calculation was done using the following formula:

$$\textbf{Total Population}\left(\frac{\mathbf{S}}{\mathbf{L}}\right) = \frac{\mathbf{N}}{\mathbf{5}} \times \mathbf{25} \times \mathbf{10^4}$$

Note: N = Population density

Rotifers *Brachionus rotundiform* mass culture

Rotifers culture used a 5,000 L volume tank and had previously been cleaned of residual dirt and mildew. The tub was then filled with water taken from the *Nannochloropsis* sp. by supplying water using a pipe 10 cm in diameter and assisted by a pump engine (Sales *et al*., 2019). Rotifers culture tanks were equipped with aeration to supply oxygen. The spreading of the rotifers seeds was done after filling the culture media. Rotifers seeds were obtained from the harvest of culture stock in other tanks. Seedlings were stocked using a 1,500 mL volume measuring cup. Spreading was done at the aeration point so that rotifers were easier to spread. The density of the rotifer stocked could be calculated under

a microscope by taking a culture medium sample of 1 mL and dripping on a Sedgewick rafter cell. Furthermore, rotifer observation was carried out with the addition of iodine.

Preservation of rotifers culture

Culture preservation was carried out by monitoring water quality, aeration, enrichment feed requirements, and rotifer population rates. Water quality monitoring was carried out every day in the morning and afternoon. It also includes temperature (°C), salinity (ppt), and light intensity (lux) variables (Ferreira *et al*., 2018).

Rotifers harvesting

Rotifers harvesting was carried out on the 6th day after stocking. In addition, harvesting could be done by two methods, namely partial method (only 50% of the total volume from culture harvested) and total harvest. Harvesting was done using a 2.5 inch hose or through a pipe mounted at the bottom of the culture tub and accommodated using a bucket and plankton net measuring 50-60 µm as a filter. Harvesting was done in the morning at sunrise because at that time many rotifers gathered at the surface.

Daily monitoring of rotifer density

Rotifers daily density was monitored by taking 1 mL of a water sample from culture media. Observations using a microscope, Sedgewick rafter counting chamber, and hand counter. The initial culture density (D0) was 12 individuals/mL, density observations were carried out for 5 days. Rotifer density was calculated using the following formula:

$$\mathbf{N} = \frac{\mathbf{Total\ of\ Individual}}{\mathbf{mL}} \times \mathbf{Tub\ Volume}$$

Note: N = total number of individuals (Ind/L)

Data analysis

Data were calculated using Microsoft Excel, Microsoft and displayed in the graph, image, and table form.

Results

Population rate of *Nannochloropsis* sp.

The observation on *Nannochloropsis* sp density showed that the initial density of culture was 3.4×10^6 cells/mL. Density on the 1st day increased to 5.0×10^6 cells/mL and successively continued to increase until the third day of 18×10^6 cells/mL. This phase on 3rd day was called the exponential phase and was harvested 50% and upscaling. The microalgae density began to experience a significant decrease on the 4th day which was 12×10^6 cells/mL. The density rate increased significantly on the 5th day to 19×10^6 cells/mL. Likewise, the microalgae density on the 6th day was still approaching the 5th day and equal to 20×10^6 cells/mL. Optimal density *Nannochloropsis* sp. obtained on the 6th-day culture (exponential phase) so that it could be used for further enrichment of rotifers feed *Nannochloropsis* sp. could be seen in Figure 4.

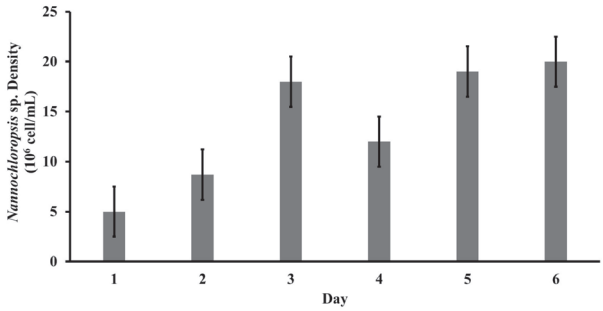

Fig. 1. Density of microalgae *Nannochloropsis* sp. which was mass-cultured for 6 days.

The result of observation on environmental factors from *Nannochloropsis* sp. shown in Table 1. Furthermore, the observation of water quality during the maintenance period showed that the average water temperature ranges between 27-30 °C, the range was still within the optimal limits for the *Nannochloropsis* sp growth. Salinity measurement in the culture tank was obtained in the range of 30-35 ppt and the value of light intensity obtained in the morning until noon was obtained in the range of 098-305 lux.

Rotifers *Brachionus rotundiformis* daily density rate

The observation on the rate of rotifers density enriched with *Nannochloropsis* sp. shown in Figure 5. The initial rotifers density stocked into a mass culture bath was 12 individuals/mL. It rate began on day one by 46 individuals/mL and increased significantly on the day two by 84 individuals/mL or increased 82% from the previous day. The decline occurred on the 3rd day by 51 individuals/mL and gradually increased again on days four and five, with 72 individuals/mL and 98 individuals/mL, respectively.

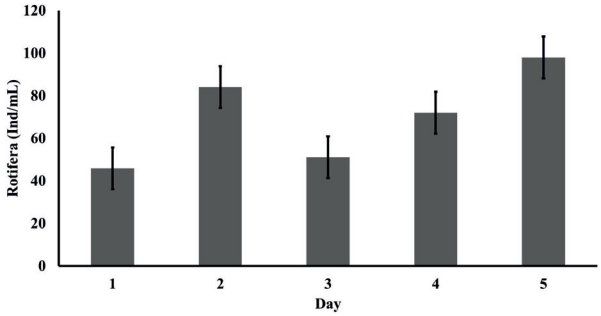

Fig. 2. Rotifers *Brachionus rotundiformis* density during the enrichment period with *Nannochloropsis* sp.

Discussion

Rotifers of the genus *Brachionus* can be used as the first natural feed for most marine fish larvae because they can manipulate their nutritional quality with enrichment methods (Kotani *et al*., 2013). Moreover, the composition of rotifer fatty acids also depends on the effectiveness and enrichment of material biomass (Kotani *et al*., 2017; Hamre, 2016).

The use of microalgae *Nannochloropsis* sp. as a mass enriched rotifers enrichment material, it showed an increase in rotifers growth, although further research is still needed to determine the nutritional content and effectiveness of marine fish larvae. Figure 4 showed the maximum density of *Nannochloropsis* sp. obtained in the exponential phase on the 3rd day on mass culture media that is equal to 18×10^6 cells/mL. The growth rate decreased on the 4th day, which was 12×10^6 cells/mL. Similar results were also reported by Chebil and Yamasaki (1998) that the maximum density in *Nannochloropsis* sp. was 19.9×10^6 cells/mL and 46% less than enriched media water which reached the final biomass at the stationary phase of 43.2×106 cells/mL. Decreased biomass from microalgae can be caused by a lack of micronutrients in culture media that inhibits the growth of phytoplankton and in this case, algae have absorbed almost all micro substances contained in culture media (Pedruzi *et al.*, 2020; Metsoviti *et al.*, 2019).

Meanwhile, the addition of micronutrients can help in supporting the *Nannochloropsis* sp biomass. Waqalevu *et al*. (2019) reported that the strengthening effect of DHA given through the addition of *C. vulgaris* can increase DHA composition in the treatment and the addition of micronutrients in microalgae mass culture has the potential to strengthen the nutrient content in rotifers. Furthermore, the protein and arachidonic acid (ARA) content were three times higher in *Nannochloropsis gaditana* where nutrition was sufficient (Ferreira *et al*., 2018). The ARA content is important to tolerate stress, pigmentation, growth and survival rates, and the formation of eicosanoids in marine fish larvae. Eicosapentaenoic acid (EPA) is one of the components of Omega-3 that functions in helping the formation of blood cells and the heart, stabilizing the circulatory system by expediting blood circulation. In general, EPA is beneficial for the growth of brain cells, organs of vision and bones, and preserves blood vessel and heart cells. EPA is needed to help the growth and development of nerve cells to be optimal. Lack of these substances will make the nerve cells lack energy for the development process so that it can disrupt the work and function of nerves drastically. Not only for nerves, but EPA also plays an important role in the organs of vision and bone (Rasmussen and Johnson, 2013). EPA can affect growth, the formation of blood vessels and heart cells, regulation of blood circulation (Gammone *et al*., 2018).

Docosahexaenoic acid (DHA) functions as a nerve-wrapping network that plays a role in launching nerve commands and delivering nerve stimulation to the brain. DHA is a derivative of omega-3

Table 1. Water quality parameters in the *Nannochloropsis* sp.

Time	Temperature (°C)	Salinity (ppt)	Light intensity (lux)
Morning	27-28	30-32	98×100 – 112×100
Afternoon	28-30	33-35	178×100 – 305×100

Table 2. Water quality parameters on rotifers mass culture media for five days.

Time	Temperature (°C)	Salinity (ppt)	Light intensity (lux)
Morning	29-30	32-33	085×100 – 172×100
Afternoon	31-33	32-33	160×100 – 554×100

which can help in making blood healthy and the mechanism of action of blood vessels and the heart. Changes in omega 3 fatty acids to EPA and DHA in the body of an organism can go through a process with the help of an enzyme that is the enzyme delta-6-desaturase can convert omega 3 or linolenic acid (C18: 3, n-3) into stearidonic acid, then with the help, the enzyme delta-5-desaturase is converted by the body into eicosapentaenoic acid (C20: 5, n-3) and with the help of the delta-4-desaturase enzyme is converted to docosapentaenoic acid (C22: 6, n-3) (Gammone *et al*., 2018).

Rotifer density rate after 24 hours since initial stocking was 46 individuals/mL and increased significantly on the second day by 84 individuals/mL or increased by 82%. These results are smaller when compared with the results of the study of Ferreira *et al*. (2018) which ranged between 196 and 232 individuals/mL after 24 hours of enrichment with *Nannochloropsis gaditana* which was cultured with sufficient nutrition.

Conclusion

The result showed that the nutritional quality of microalgae is an important factor that can affect the productivity of biomass from mass-cultivated rotifers. The highest density of rotifers occurred on the 5th day that was equal to 98 individuals/mL where on that day there was an increase in the *Nannochloropsis* sp density from the previous day which amounted to 19×10^6 cells/mL. Calculation of nutrient content and addition of nutrients to microalgae *Nannochloropsis* sp. be a topic that need to be considered for future studies to produce adequate and high-quality natural feed products to support the growth of marine fish larvae.

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