The Growth Rate of *Branchionus Plicatilis* O.F. Muller Population With The Addition of A Mixture of Yeast, Chicken Manure Fertilizer, Urea, And Triple Super Phosphate (CAKAP)

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Abstract. The study of the growth rate of *Branchionus plicatilis* O.F. Muller population with the addition of a mixture of yeast, chicken manure fertilizer, urea, and triple super phosphate (CAKAP) was conducted in June 2012. Four treatments namely MK, MP1, Mp2, and MP3 were used in this experiment. MP1 consisted of CAKAP medium, 0.3 g/2L of yeast; MP1 consisted of CAKAP medium, 0.3 g/2L of yeast, and 0.1 ml/2L of fish oil. MP2 and MP3 have the composition as MP1 but show different concentrations of fish oil which were 0.2 and 0.3 ml/2L each. Every treatment had six replicates. The result showed that the highest growth rate of *Branchionus plicatilis* O.F Muller was obtained from the first observation (day 2) of MP2 medium with the rate 2.605 individual x 2 x 10⁻³ per day while the lowest one was shown by MP3 medium which was 2.282 individual x 2 x 10⁻³ per day.

Keyword: *Branchionus plicatilis*, CAKAP medium, fish oil, growth rate, yeast bread

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1 Introduction

The potential for aquaculture in Indonesia is still the largest in Southeast Asia with a land area of more than 15.59 million hectares, consisting of 2.23 million hectares of freshwater cultivation potential, 1.22 million hectares of brackish water cultivation and 12.14 million hectares of marine cultivation potential. Although the current utilization of aquaculture potential is not optimal, aquaculture production has shown a significant increase from 4.78 million tons in 2009 to around 6.97 million tons in 2011. It should be noted that in 2011, aquaculture production contributed 56.33 percent of total fishery production. Nationwide, with a growth rate that is
much higher than that of capture fisheries, amounting to 21.83 percent. Aquaculture continues to increase, so that the future of fisheries will focus on developing aquaculture [2].

An important factor that supports hatchery business is quality natural food. One way to improve the quality of natural food is enrichment of the feed so that the production of cultivated larvae produces good quality [3].

Currently, natural food which is one of the natural resources of animal groups that is widely used for fish seeds is from the rotifers (*Brachionus plicatilis*) group. This natural feed can increase the amount of fish production that is cultivated by the community in order to improve the economic conditions of the fisheries and marine sectors because it can save expensive feed ingredients [8].

Inadequate nutrition for feed bodies used as larvae feed has been identified as one of the causes of failure in larval rearing. Fish larvae really need some content of EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid), while the EPA and DHA content in rotifers are usually insufficient to support larval growth. Considering that the source of EPA and DHA are fish oils, various types of oil on the market contain fatty acid compositions so that they Fish oil is one of the ingredients that can be used to enrich rotifers. Fish oil contains many types of fatty acids, both saturated and unsaturated fatty acids. The main content of fish oil is fatty acids which have high unsaturation. Marine fish oil is rich in linolenic fatty acids, EPA 20: 5n-3 and DHA. The EPA and DHA content of fish oil were 17.2% and 13.2% [13], [14].

Research on the growth rate of *Brachionus plicatilis* has been widely carried out, but it is not yet known how the effect of giving yeast and fish oil to CAKAP medium on the growth rate of the *Brachionus plicatilis* population. The potential for aquaculture in Indonesia is still the largest in Southeast Asia with a land area of more than 15.59 million hectares, consisting of 2.23 million hectares of freshwater cultivation potential, 1.22 million hectares of brackish water cultivation and 12.14 million hectares of marine cultivation potential. Although the current utilization of aquaculture potential is not optimal, aquaculture production has shown a significant increase from 4.78 million tons in 2009 to around 6.97 million tons in 2011. It should be noted that in 2011, aquaculture production contributed 56.33 percent of total fishery production. nationwide, with a growth rate that is much higher than that of capture fisheries, amounting to 21.83 percent. Aquaculture continues to increase, so that the future of fisheries will focus on developing aquaculture[2].

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Research on the growth rate of Brachionus plicatilis has been widely carried out, but it is not yet known how the effect of giving yeast and fish oil to CAKAP medium on the growth rate of the Brachionus plicatilis population.
2 Materials and Methods

Research Methodology

The method used in this study was an experimental method with non-factorial completely randomized design (CRD) analysis with 4 media treatments and 6 replications, as follows:

MK: The control medium consisted of CAKAP medium + 0.30 g of yeast
MP1: Media treatment, consisting of CAKAP medium + 0.30 g of yeast + 0.1 ml of fish oil
MP2: Media treatment, consisting of CAKAP medium + 0.30 g of yeast + 0.2 ml of fish oil
MP3: The treatment medium consisted of CAKAP medium + 0.30 g of yeast + 0.3 ml of fish oil

Note: CAKAP medium is the best medium that has been used in existing research. The concentration used was (200 mg / 2L chicken manure + 4 mg / 2L Urea fertilizer + 3 mg / 2L TSP fertilizer).

Media Material Preparation Brachionus plicatilis

The feed media used in this study was chicken manure that had been dried in the sun first. The dried chicken manure, Urea and TSP fertilizers, and yeast are mashed and sieved, then weighed according to the composition of the treatment (CAKAP medium + yeast + fish oil). In this case the yeast used is dry yeast fermipan and fish oil used is scotts emulsion, then chicken manure, urea and TSP are put into a strimin bag.

Media Acclimation

The water used for acclimation is obtained from the pool water of the North Sumatera University Library in Medan which has been filtered using a 15 micron filter edged plankton net. The pool water is put into a 25 liter aquarium. Then the media (according to its composition) is put into a strimin cloth and immersed in the aquarium and acclimated for 2 days.

Media Treatment

The water used for the treatment media was obtained from the pool water of the North Sumatera University Library in Medan which had been filtered using a 15 micron filter edged plankton net. The pool water was put into 30 3L glass jars, each side of 2 liters of pool water. Then each CAKAP medium was inserted into a strimin cloth and hung into each jar, then the jar was closed again with a strimin cloth with a diameter of 1 mm to prevent the entry of insects, then incubated for 7 days. By doing fertilization, it means that it will change the concentration of nutrients so that it will affect Zooplankton, in this case B. plicatilis.
After 7 days, *Brachionus plicatilis* seedlings were put from the acclimation media into each treatment medium as many as 25 individuals. Then the media jar was closed again with a strimmed cloth. Then the media jars were put into a closed cabinet shelf and given a 20 watt TL lamp (so that the room temperature was between 28-29 °C) with a distance from the surface of the treated media jars of about 20 cm.

In research that has been carried out, the physical properties of water media such as temperature and pH are checked 3 times in 16 days, namely on days 4, 9 and 13. For temperature is measured by a thermometer and pH is measured with a pH meter. Furthermore, the treatment media was aerated every day for 3 minutes using an aerator so that the dissolved O₂ content was not too low.

**Preparation Seeds *Brachionus plicatilis***

*Brachionus plicatilis* used in this study was obtained from the Universitas Sumatera Utara Library pool in Medan. *Brachionus plicatilis* was collected using a plankton net and put into a 10 liter bucket. Furthermore, enough *Brachionus plicatilis* seeds are inserted into the aquarium to be acclimated for 5 days. The aquarium is placed under a 20 Watt lamp with a distance of 20 cm (so that the room temperature is between 28-29°C) and aeration is carried out for 5 days.

**Treatment the Addition of The Leaven***

*Brachionus plicatilis* used in this study was obtained from the North Sumatra University Library pool in Medan. *Brachionus plicatilis* was collected using a plankton net and put into a 10 liter bucket. Furthermore, enough *Brachionus plicatilis* seeds are inserted into the aquarium to be acclimated for 5 days. The aquarium is placed under a 20 Watt lamp with a distance of 20 cm (so that the room temperature is between 28-29°C) and aeration is carried out for 5 days.

**Treatment of Addition of Yeast and Fish Oil***

The treatment of adding bread yeast and fish oil was carried out after adding *Brachionus plicatilis* into the jar and adding bread yeast and fish oil every day according to the composition of each treatment.

**Observation and Counting of Growth Rate *Brachionus plicatilis***

Based on existing research, observations and calculation of population growth rates are carried out every 2 days for 16 days or 8 observations (H1 = day 2, H2 = day 4, H3 = day 6, H4 = day-8, H5 = day 10, H6 = day 12, H7 = day 14, H8 = day 16). Each treatment medium was repeated 6 times. This is based on the lifespan of *Brachionus plicatilis*, which is 12-19 days.
Before taking *Brachionus plicatilis*, the media water is first stirred slowly using a glass stirring rod so that the *Brachionus plicatilis* contained in the media is evenly distributed, so that the individuals caught in the serology pipette can represent all the *Brachionus plicatilis* in the jar. Furthermore, the *Brachionus plicatilis* contained in the 20 ml serology pipette was counted with the naked eye, that is, by being exposed to the light. This method is in accordance with that carried out by the Serang Marine Aquaculture Research and Development Center. After counting the individual population of *Brachionus plicatilis*, they are put back into the jar (treatment medium). This observation was carried out until the 16th day of observation [6].

**Data Analysis**

From the results of observations / research, the calculation of the increase in the population of *Brachionus plicatilis* has been completed, then the population growth rate is searched for analysis using the following formula:

$$K = \frac{\ln N_t - \ln N_o}{t}$$

Notes:  
- $K$ = Population growth rate *Brachionus plicatilis* per day  
- $N_t$ = Population size of *B. plicatilis* after $t$ day  
- $N_o$ = Initial population size of *B. plicatilis*  
- $t$ = Observation time (per day)

The data obtained from the observations are arranged in tabular form. The quantitative data (dependent variable) obtained were tested for their significance on the effect of the treatment group (independent variable) with the help of a computer statistical program, namely the SPSS release 13 program. The test sequence begins with the normality test, the homogeneity test. If $p > 0.05$, it is followed by one-way analysis of variance test for data with repeated observations (more than 2 times) or more than 2 treatments and if it is significantly different, it is continued with the Post Hoc-Bonferroni analysis test at the 5% level. But if the normality and homogeneity test $p < 0.05$, continue with the transformation and if the transformation $p > 0.05$ continues the analysis of variance test, but if $p < 0.05$ continues the Kruskal-Wallis non-parametric test and if $p < 0.05$, the Mann-Whitney test is performed to see the difference. each treatment.

**3 Result and Discussion**

From the results of the research that has been carried out, it is found that the average number of *Brachionus plicatilis* individuals increases, as shown in Table 1 below:
Table 1. Average increase in the number of individuals population of *Brachionus plicatilis* (ind / 2 L).

| Time Observation (P) | Media and Treatment |
|----------------------|---------------------|
|                      | MK  | MP1 | MP2 | MP3 |
| P0 (day 0)           | 26  | 26  | 26  | 26  |
| P1 (day-2)           | 2.333 | 2.222 | 2.417 | 1.333 |
| P2 (day-4)           | 4.444 | 4.000 | 6.694 | 3.361 |
| P3 (day-6)           | 8.500 | 9.139 | 9.222 | 6.556 |
| P4 (day-8)           | 10.944 | 9.861 | 12.944 | 9.389 |
| P5 (day-10)          | 9.111 | 9.083 | 11.639 | 8.000 |
| P6 (day-12)          | 8.111 | 8.111 | 10.639 | 7.028 |
| P7 (day-14)          | 7.139 | 7.361 | 9.389 | 6.361 |
| P8 (day-16)          | 6.139 | 6.361 | 8.583 | 5.333 |
| **Total**            | 82.721 | 82.138 | 97.527 | 73.361 |
| **Average**          | 9.191 | 9.126 | 10.836 | 8.151 |

From Table 1, it can be seen that the increase in the number of individuals in the population of *Brachionus plicatilis* with treatment media and observation time varies widely, where the highest total number of individual increases is in MP2 media of 97.527 ind / 2L (average 10.836 ind / 2L) and the lowest was found in MP3 media of 73.361 ind / 2L (average 8.151 ind / 2L). The high average individual growth in MP2 media is due to the provision of 0.2 ml fish oil which is very good for supporting the life of *Brachionus plicatilis*, so that the increase in the number of individuals looks sustainable and there is still adequate nutrition in this medium.

HUFA (Highly Unsaturated Fatty Acids) n-3 essential fatty acids, especially EPA and DHA, are very important in determining the successful development and survival rates of larvae, especially in the early stages of larval development [12].

The lowest increase in the number of *Brachionus plicatilis* individuals was on MP3 media with the addition of 0.3 ml fish oil, which was 73.361 ind / 2L (average 8.151 ind / 2L). This situation is due to the provision of fish oil as much as 0.3 ml which is too high which causes fat accumulation so that it does not support the survival of *Brachionus plicatilis*. 
Essential fatty acids play a very important role in the formation of new cell components. The occurrence of growth is the result of tissue formation or cell multiplication of the organism concerned, but the excess and lack of ω-3 fatty acids cause cell membranes to malfunction, enzyme activity in cells is inhibited so that metabolism is disturbed and the growth rate is low [7].

In the treatment for all media, the highest average individual gain was obtained at P4 (day 8), that is, the highest was obtained in MP2 of 12,944 ind / 2L and the lowest was found in MP3 9,389 ind / 2L. The high individual increase in P4 was because the nutrient content in the media was the most optimal media condition in supporting the growth and addition of Brachionus plicatilis individuals, while the lowest was found at P8 (day 16) in all media treatments, this condition shows that at P8 it is suspected to occur. decreased availability of food ingredients needed by Brachionus plicatilis for growth and reproduction.

Good media conditions and the availability of adequate media nutrition in culture media can cause an increase in the number of individual populations of B. plicatilis quickly, but will also experience a rapid decline in the number of individual populations if the media and nutrition conditions are not. again sufficient and can support life [4].

The Rate of Population Growth Brachionus plicatilis

The results of data analysis showed that the population growth rate of Brachionus plicatilis is quite variable, as shown in Figure 1 below:

![Figure 1. Average Population Growth Rate of Brachionus plicatilis (ind x 2 x 10⁻³ x day⁻¹) on the Treatment Media.](image)

From Figure 1 it can be seen that after observation on day 2 there was a drastic reduction in the population growth rate of Brachionus plicatilis. This situation was due to the fact that at the time of observation on the second day there was a very high growth rate, which resulted in a
decrease in the availability of foodstuffs for *B. plicatilis* during the observation of the following days.

The production of *B. plicatilis* is very dependent on its feed supply, if there is a lot of feed available then the production of *B. plicatilis* will also be a lot. Increase availability of live food (rotifers) during larval rearing depends entirely on the availability of natural food, because in terms of its nutritional value, live food tends to be more nutritious when fed Chlorella [1], [9].

Based on the research that has been carried out on the population growth rate of *B. plicatilis* on the four media with the addition of yeast and fish oil during the study time, after statistical analysis seen from the normality and homogeneity test the results showed that P < 0.05 so that it must be transformed and obtained. P < 0.05 and continued to the non-parametric kruskal-wallis test to test 2 or more treatments. From the non-parametric Kruskal-wallis test, the results obtained P > 0.05, which indicates the value is not significantly different, more details can be seen in Table 2 below:

**Table 2.** The average difference test on the treatment media during the observation time from day 2 to day 16

| Media | Average Growth Rate from Day 2\textsuperscript{nd} to Day 16\textsuperscript{th} |
|-------|---------------------------------------------------------------------------------|
| MK    | 5.069                                                                            |
| MP1   | 5.043                                                                            |
| MP2   | 5.212                                                                            |
| MP3   | 4.961                                                                            |

Table 2 shows that the results of the comparison statistical analysis between MK media and the media (MP1; MP2; MP3) are not significantly different. This indicates that the addition of fish oil has no significant effect on the population growth rate of *B. plicatilis*. The addition of yeast has the most influence on the growth rate of *B. plicatilis* and not the addition of fish oil.

The addition of 0.30 g of yeast is the best media composition and can optimally support the life of *B. plicatilis* and its reproduction. Baker's yeast is one of the organic substrates that has the potential to increase the growth of *B. plicatilis*. Baker's yeast is a source of feed derived from yeast group fungi. Baker's yeast has a high carbohydrate and protein content which is very good for the growth rate of the *B. plicatilis* population [10], [11].

From the results of research that has been carried out on the population growth rate of *B. plicatilis* with the addition of yeast and fish oil to the CAKAP media, it can be concluded that the provision of yeast and fish oil has no significant effect on the growth rate of *B. plicatilis* on each observation day.
4 Conclusion

From the results of research that has been carried out on the population growth rate of *B. plicatilis* with the addition of yeast and fish oil to the CAKAP media, it can be concluded that the provision of yeast and fish oil has no significant effect on the growth rate of *B. plicatilis* on each observation day.

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