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Addition of water from the treatment pond of pangasius fillet waste (*Pangasius sp.*) with different concentrations in the cultivation medium due to the population growth of *Daphnia sp.*

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Abstract. Live feed is very important in fish farming, especially in hatchery. Wastewater from processing fillets (*Pangasius sp.*) has a high organic nutrient content and has the potential to be used as a *Daphnia sp.* culture medium. This research aims to study the effectiveness of the addition of the liquid waste of Pangasius fillets in the *Daphnia sp.* culture medium using a completely randomized design (CRD); 5 treatments with 4 replications were conducted. The treatments were conducted by adding the liquid waste of Pangasius fish fillets at a concentration of 5%, 10%, 15%, and 20%. The control treatment for the live feed cultivation was in the form of a dry chicken manure addition with a dose of 2.4 g/L in the medium and without treatment. The main observed parameter in the study was the population growth of *Daphnia sp.* The supporting parameters observed were in the form of phytoplankton and bacterial abundance and water quality. The results showed that the addition of the water from the Pangasius fish fillet waste, as much as 20%, had the best results related to the population growth of *Daphnia sp.* Meanwhile, the water quality was still within the normal range during the *Daphnia sp.* culture.

1. Introduction

The need for natural feed is important in the activity of freshwater fish cultivation, both for consumption and ornamental fish. *Daphnia sp.* are a type of natural food that is often used in freshwater fish farming activities because it has a high protein content. *Daphnia sp.* is an animal that is a non-selective filter feeder. Food from *Daphnia sp.*, among others, comes in the form of bacteria, yeast, single-celled algae, detritus and dissolved organic matter [1]. Besides that, *Daphnia sp.* is able to eat various sizes of particles dissolved in water [2]. Up until now, a common way to grow *Daphnia sp.* is by adding fertilizer to the culture medium. The use of organic fertilizers has several advantages, including its low cost and the contents of the several micro and macronutrients[3]. The fertilizer commonly used today is in the form of chicken manure [4].

The growth of *Daphnia sp.* depends on the nutrient source contained in the culture medium. The improvement of the natural feed can be done by adding organic fertilizers that aim to increase the availability of nutrients in the water. Wastewater from washing the fish fillets has the potential to be



used to grow *Daphnia* sp. due to its high organic matter content. From the previous study, it is known that the liquid waste of fish fillets contains 1.58% wet protein, 0.05% wet fat, and 0.35% wet ash [5]. Therefore, by applying the right fertilizer strategy, cultivation environment, improvement in feed management, productivity increments and sustainability in aquaculture, production will be positively affected [3].

2. Material and methods

2.1. Origin of organism

The *Daphnia* sp. used was from Balai Benih Ikan Jepun (BBIJ), which were then maintained and reproduced in the PSDKU UNAIR Fisheries Laboratory in Banyuwangi. The species was chosen specifically with a minimum size of 1 mm by filtering using a net scope measuring 1 mm.

2.2. Preparation of tools and media

The tools and media were electrified in advance before use. The tools were in the form of plastic containers, hoses, and aeration stones that were washed thoroughly using soap and then dried. The water, as the culture medium, was sterilized by using chlorine at a dose of 25 ppm and it was aerated for 24 hours and deposited for 3 days. To reduce the residue from the chlorine, Na Thiosulfate at a dose of 5 ppm was used.

2.3. Wastewater collection

The wastewater used as a mixture on the *Daphnia* sp. culture medium originated from a treatment pond for *Pangasius* fillet waste treatment equipped with an aeration system (Figure 1). Samples of wastewater were taken from 8 points using a sample bottle at a depth of 10 cm.



Figure 1. Aeration treatment pond for pangasius fillet waste treatment

Table 1 Shows the results of the organic C analysis on the various media used. The c-organic test was carried out using the Walkey Black method.

Table 1. Analysis of organic C in the various culture media

Treatment	C-Organic (%)
Dry Manure Chicken	2,60
Wastewater Concentration of 5 %	7,80
Wastewater Concentration of 10 %	11,50
Wastewater Concentration of 15 %	49,60
Wastewater Concentration of 20 %	66,30

2.4. Research design

Twenty-four plastic tubs were filled with 3 L of water of the *Daphnia* sp. culture medium with a constant aeration. The *Daphnia* sp. used was 20 pcs/L. The maintenance process was carried out for 12 days. *Daphnia* sp. was maintained for 12 hours in a bright condition and for 12 hours in a dark condition. Every day, the population sampling process and the checking of the water quality parameters was conducted in the form of pH, temperature and DO. The treatments given in this study included:

- A. Treatment 1: Positive control in the form of adding dry chicken manure 2.4 g/L
- B. Treatment 2: Negative control without additional dry manure chicken
- C. Treatment 3: Adding water from the treatment pond to the fillet fish waste - 5% of the total media
- D. Treatment 4: Adding water from the treatment pond to the fillet fish waste - 10% of the total media
- E. Treatment 5: Adding water from the treatment pond to the fillet fish waste - 15% of the total media
- F. Treatment 6: Adding water from the treatment pond to the fillet fish waste - 20% of the total media

The picture of the research design can be seen in **Figure 2**.

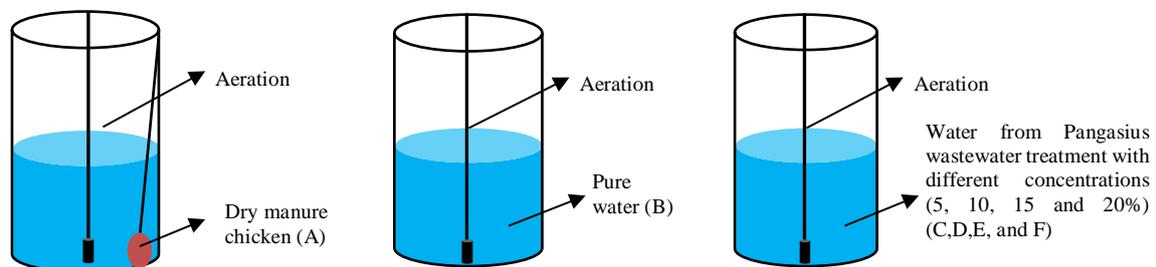


Figure 2. Illustrations of the research design.

2.5. Preparation of culture media

The culture media was first prepared before the maintenance process was carried out. The media used was added according to the treatment given. It was then aerated for 3 days before stocking the *Daphnia* sp. The goal was to homogenize the water and to grow natural food.

2.6. Sampling *Daphnia* sp.

The sampling was carried out every day for 12 days of maintenance done using a 5 mL glass beaker. The samples were taken from 5 different points. The sampling results were then averaged and multiplied by the overall volume of the water. Phytoplankton and bacterial density measurements were carried out at the beginning and end of the maintenance period. Water quality checking included temperature, pH, DO and TOM.

2.7. Observations

Calculations of the *Daphnia* sp. population were carried out every day using the sampling method. The calculation of the phytoplankton density was carried out using a hemocytometer. For calculating the bacterial abundance, the spread plate method was used. The observation of *Daphnia* sp. was carried out using a microscope (Olympus CX23).

2.8. Data analysis

The data obtained was then processed in the Excel 2010 program. The data analysis was performed using ANOVA (Analysis of Variance) with a 95% confidence interval. If it was significantly different, then it was continued with the Duncan Multiple Range Test (DMRT). The statistical analysis was performed using the IBM SPSS statistics version 16.0 software.

3. Result and discussion

3.1. Result

3.1.1. *Daphnia* population

The population of *Daphnia* sp. based on the calculation results during the 12-day maintenance period can be seen in **Figure 3**.

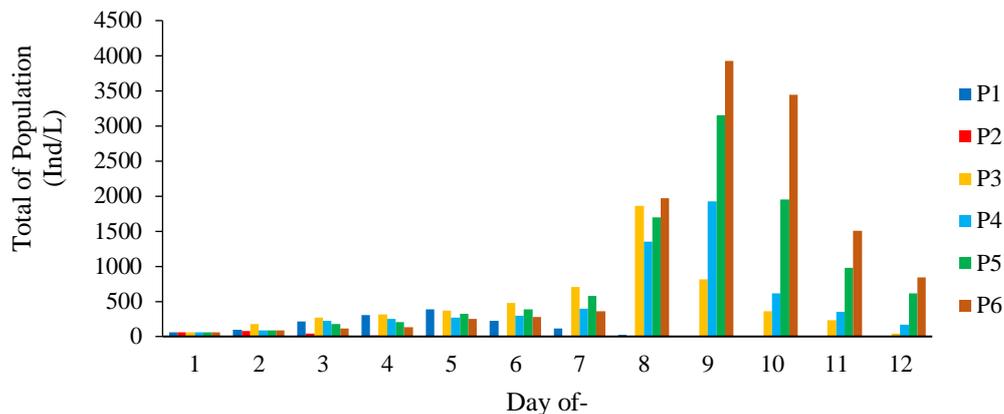


Figure 3. The growth of the *Daphnia* sp. population using different culture media

Based on Figure 3, the highest spike in population growth was found in treatment 6 (20% addition). The maximum population among all of the treatments occurred in the period from day of 7 to day 9. The lowest population was found in treatment 2 (pure water), where in day 5, the phase of death was experienced. The *Daphnia* sp. population density, with a variety of different media cultures, is shown in **Figure 4**.

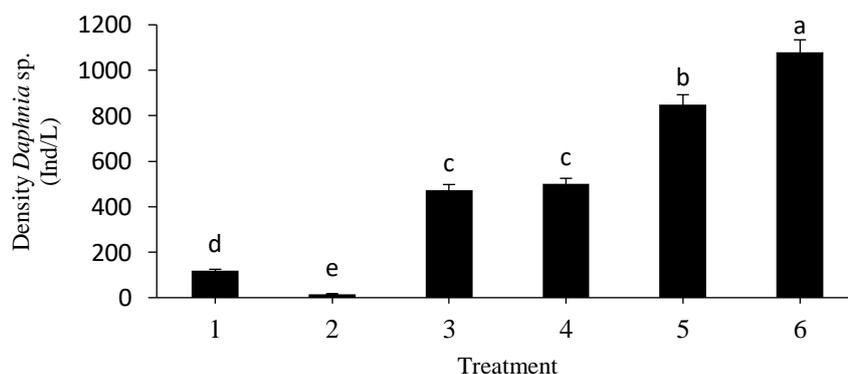


Figure 4. *Daphnia* sp. population density in all treatments

Based on Figure 4, it is known that the highest *Daphnia* sp. density was found in treatment 6 (addition of 20% wastewater) and the lowest was in treatment 2 (without treatment).

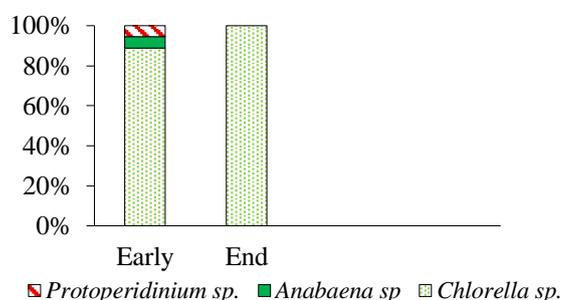
3.1.2. Phytoplankton and bacterial density

Increased phytoplankton density was found at the beginning and end of the maintenance except in treatment 2 (pure water). The plankton density can be seen in **Table 2**.

Table 2. Plankton density in different culture media

Sampling	Class	Phytoplankton density in the culture media of <i>Daphnia</i> sp. (Cell/ ml)					
		Dry chicken manure	Pure water	5%	10%	15%	20%
Initial	<i>Chlorophyceae</i>	25000	0	12500	12500	15000	35000
	<i>Cyanophyceae</i>	0	0	0	0	0	2500
	<i>Dinoflagellata</i>	0	0	0	0	0	2500
	<i>Bacillariophyceae</i>	0	0	0	0	0	0
	Total	25000	0	12500	12500	15000	40000
Final	<i>Chlorophyceae</i>	35000	7500	25000	110000	32500	75000
	<i>Cyanophyceae</i>	0	0	0	0	0	0
	<i>Dinoflagellata</i>	0	0	0	0	0	0
	<i>Bacillariophyceae</i>	0	0	0	0	0	0
	Total	35000	7500	25000	110000	32500	75000

Based on Table 2, the Chlorophyceae class was found at the beginning and end of the maintenance period using different culture media. The highest initial density was found in the treatment with the addition of 20% fillet wastewater (Treatment 6). The highest final density was found in the treatment with the addition of 10% fillet wastewater (Treatment 4). The dominant type of plankton found in all treatments, both at the beginning and at the end of the maintenance period, was *Chlorella*. The type of plankton found can be seen in **Figure 5**.

**Figure 5.** Percentages of the plankton type in the *Daphnia* sp. cultivation medium

The abundance of bacteria at the beginning and end of the maintenance in all *Daphnia* sp. culture media as seen in **Table 3**.

Table 3. The abundance of bacteria at the beginning and end of the maintenance in different culture media

Treatment	Initial (cfu/ mL)	Final (cfu/ mL)
1 (Dry manure chicken)	784×10^2	33×10^3
2 (Pure water)	358×10^2	18×10^2
3 (Wastewater 5%)	605×10^2	20×10^3
4 (Wastewater 10%)	771×10^2	28×10^3
5 (Wastewater 15%)	794×10^2	31×10^3
6 (Wastewater 20%)	967×10^2	47×10^3

Based on **Table 3**, the highest abundance of bacteria at the beginning of maintenance was in treatment 6. The highest abundance of bacteria at the end of the maintenance period was in treatment 5.

3.1.3. Water Quality

The water quality at the beginning and end of the different *Daphnia* sp. culture media can be seen in **Table 4**.

Table 4. Water quality range in the maintenance media for *Daphnia* sp.

Treatment	Time	Parameter of water quality					
		pH	Temp (°C)	DO (ppm)	TAN (ppm)	NH ₃ (ppm)	TOM (ppm)
1 (Dry Chicken manure)	Initial	6,9	25,4	5,1	0,012	0,001	69,52
	Final	8,4	27,4	4,2	1,532	0,211	22,75
2 (Pure Water)	Initial	7,0	24,6	4,9	0,001	0,000	7,58
	Final	7,9	27,1	5,5	0,008	0,001	14,98
3 (Wastewater 5%)	Initial	6,9	24,1	4,2	0,696	0,039	63,83
	Final	8,0	27,9	5,0	0,944	0,165	42,34
4 (Wastewater 10%)	Initial	7,3	25,1	4,3	0,814	0,046	66,99
	Final	8,0	27,1	5,1	1,695	0,253	55,24
5 (Wastewater 15%)	Initial	7,0	24,9	4,1	0,854	0,048	65,72
	Final	8,1	27,9	5,4	1,802	0,254	54,98
6 (Wastewater 20%)	Initial	7,1	24,9	4,0	0,964	0,054	67,72
	Final	8,4	28,1	5,1	2,348	0,293	23,38

Based on **Table 4**, the water quality parameters in various culture media were in the range of normal conditions for the maintenance of *Daphnia* sp. However, there was an increase in TAN level at the end of the maintenance period in all treatments.

3.2. Discussion

Daphnia sp. is a type of zooplankton that has non-selective filter feeder properties. *Daphnia* sp. growth depends on nutritional and environmental factors [6]. In Cladoceranscultivation, the type and amount of foods and the composition of nutrients in the feed must be considered. *Chlorella* sp. is a type of phytoplankton found to dominate all cultural media. The peak phase was found on days 8 and 9 and began to decline on day 10. *Daphnia* sp. is a type of zooplankton that can consume phytoplankton, and is well compared to other types of freshwater zooplankton [7]. The decline of the population in this study could have been caused by a decrease in the water quality in the maintenance media. This was indicated by the increase in the TAN content at the end of the maintenance period in all media.

Phytoplankton and bacteria are food sources for *Daphnia* sp. The phytoplankton's nutrient content is an important factor for Cladocerans' growth ([8], [9], [10]). According to [11], *Daphnia*, besides being a phytoplankton eater, is also able to utilize protozoa and bacteria as a source of nutrition. Based on the results of the identification, the researchers determined the results stating the type of *Chlorella* sp. found at the beginning and end of the observation. The administration of *Daphnia* sp. using *Chlorella vulgaris* provided the maximum/optimal results compared to using *Azollapinnata* and yeast [12]. Plankton abundance tended to increase at the end of the maintenance period. This could have been caused by the phytoplankton utilizing the inorganic nutrients found in the culture media. Bacterial abundance decreased at the end of the maintenance period. This was caused by the decrease in the organic matter in the culture medium because it had been used by *Daphnia* sp. In the *Daphnia* sp. culture, the amount of carbon (C) and phosphorus (P) must be of a constant quantity [13], [14].

Water quality, including temperature and DO, was in the normal range of conditions for *Daphnia* sp. life. The pH value at the end of the treatment increased in all treatments. The increasing pH at the end

of the maintenance period can be caused by the effects prompted by phytoplankton abundance in the water. The TAN content in all treatments increased at the end of the maintenance period. This can be caused by the decomposition of dead organisms in the culture medium. An increase in the number of phytoplankton in the pond will increase the pH value of the water, so as to increase the ammonia that had not been ionized in the water [15]. The TOM content decreased in all treatments except in treatment 2. The decrease in TOM content was caused by the *Daphnia* sp. eating the organic matter in the water, causing the content of the organic matter in the media to decrease.

4. Conclusion

The addition of water from the waste of *Pangasius* fillets at a concentration of 20% (Treatment 6) provided significantly different results out of all of the treatments. The peak population started on the 8th and 9th days and the decline started on the 10th day. This was due to a decrease in the content of the organic matter as evidenced by a decrease in the TOM content at the end of the maintenance period. The water quality was still in the normal range in all of the treatments.

5. References

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