Effect of Feeding *Daphnia* sp. Enriched with EM_4 Against the Seed Growth and Survival of Striped Catfish (*Pangasianodon hypophthalmus*)

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ABSTRACT

*Catfish is one of the leading freshwater fish commodities and has the potential to be developed and continuously boosted in productivity. This research aimed to determine the optimal dose of EM_4 on Daphnia sp. enrichment as a natural food striped catfish (*Pangasianodon hypophthalmus*) towards survival and growth. The study was conducted from November 2021 – January 2022 at the Fisheries Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. Researchers chose it by using experimental with a completely randomized design. It consisted of 4 treatments and three replicates. A treatment (control), B (10mL/L), C (20 mL/L) and D (30 mL/L). The fish used in this study were 240 striped catfish aged 15 days with a length of 2.5±0.55 cm obtained from fish seed sellers in Bekasi. Analysis of growth data used the normality test and then analyzed the data for variance (ANOVA) with a 95% confidence level, while data analyzed water quality descriptively. The results showed that feeding* Daphnia *sp. enriched with EM_4 at a concentration of 10mL/L resulted in a length growth of 2.96%, specific growth rate of 7.40%, and feeding efficiency value of 34.35%, and survival rate of 88.33% on striped catfish seeds.*

Keyword: - *Daphnia sp., EM4 Probiotic, Striped catfish, Survival and Growrth*

1. INTRODUCTION

Catfish is one of the leading freshwater fish commodities and has the potential to be developed and continuously boost its productivity. The demand for catfish comes from the domestic market and export needs, especially in the Middle East [16]. In 2020, catfish production will reach 124,412 tons, and in 2040 it is projected to be 909,493 tons. One of the steps we can take to increase the productivity of catfish is to produce superior and efficient seeds in aquaculture activities. One of the ways to improve the performance of fish seed production is to improve nutrition from natural feeds. Generally, the raw meal used is phytoplankton and zooplankton, which have several advantages, such as the size that fits the fish's mouth opening and the fish's interest that arises due to the movement of natural food that makes fish want to eat it [18]. This study used Stripped catfish seeds that were 15 days old. Usually, 15-day-old catfish are fed with Artemia nauplii, Tubifex and artificial feed in the form of crumble pellets because the size of the meal is by the mouth opening of the catfish [5]. The feed used in this study was *Daphnia* sp. this is done because of the abundant availability of *Daphnia* and easy cultivation, compared to tubifex worms which are seasonal and expensive feed.

Daphnia sp. in this study is expected to function as a substitute for feed that is often given. *Daphnia* sp. is one type of zooplankton that is commonly used as natural food. The nutritional value contained in *Daphnia* sp. in wet weight is 4% protein, 0.54% fat, and 0.67% carbohydrate [11]. Nutrient enrichment technology is carried out to

improve *Daphnia's* value by providing probiotics. Adding probiotics can improve feed efficiency, make feed easier to digest, allow enzymes to work more effectively, and promote fish growth. One of the probiotic brands used is EM_4. EM_4 consists of 90% lactobacillus sp., phosphate solvent, photosynthetic bacteria, *Streptomyces* sp, cellulose-decomposing fungi and yeast. Based on the above background, the identification of the problem from this research is how much influence Daphnia sp. enriched using EM_4 with different concentrations can increase the growth and survival of Stripped catfish (*Pangasianodon hypophthalmus*).

2. MATERIALS AND METHODS

The research process lasted 40 days, from November to December 2021, at the Fisheries Laboratory, Faculty of Fisheries and Marine Sciences, Padjadajran University. The tools used in the research are an aquarium, ruler, analytical scales, pH meter, DO meter, thermometer, heater, stationery and documentation tools, while the materials used in the research are striped catfish with a length of 2.5±0.55 cm, *Daphnia* sp. and EM_4.

Research chose the method used in this research by using experimental with a completely randomized design. It consisted of 4 treatments and three replicates. Parameters observed were length growth, specific growth rate, feeding efficiency, survival rate, and water quality which we kept every ten days. Analysis of length growth data, specific growth rate, feeding efficiency, and survival rate used the normality test and then analyzed the data for variance (ANOVA) with a 95% confidence level. At the same time, water quality was analyzed descriptively and compared with SNI.

Experimental A: control (feeding Daphnia without EM_4)
Experimental B: Enrichment of Daphnia with EM_4 at a dose of 10 mL/L
Experimental C: Enrichment of Daphnia with EM_4 dose of 20 mL/L
Experimental D: Enrichment of Daphnia with EM_4 dose of 30 mL/L

Preparation for enrichment is done by taking EM4 using a measuring cup with a size of 50 mL, then inserting EM4 according to the dose into a 3 L glass jar containing 1 L of water, then stirring slowly. Daphnia sp. was put into each aquarium with a stocking density of 500 ind/L, filled with 1 litre of water. Total Daphnia sp., stocked on the whole jar, is 3000 individuals. Daphnia sp., which has been stored, will be enriched for 4 hours, is done because that time is the most effective and efficient) [13].

The test feed used in this study was Daphnia sp. which has been enriched with various doses of EM4 10 mL/L, 20 mL/L and 30 mL/L, respectively. fish gave feed to the test fish fry. fish carried out the test feed for each treatment ad libitum. Feeding is given starting from 09.00-15.00 WIB. Feed was given back if fish used feed up in each research container. Fish seed maintenance was carried out for 40 days. Parameter observations were carried out every 10 days by scooping the seeds, then weighing and measuring growth (length and weight). Meanwhile, to maintain the water quality of the maintenance media in optimal conditions, water siphoning is carried out every day, and water changes are carried out every week. Water siphoning is carried out as much as 10% of the water in the media, and then the water is refilled as before.

Next, the measured data is analyzed using the normality test, homogeneity test, Kruskal-wallis test, survival rate, specific growth rate, feeding efficiency [7-24] With the following formula:

Normality test: The formula used for this normality test uses the Shapiro-Wilk test with the following formula:

1) Divider (d) W test

$$d = \sum_{i=1}^{n} (x_i - \bar{x})^2 = \sum_{i=1}^{n} x_i{}^2 - \frac{1}{n} (\sum_{i=1}^{n} x_i)^2$$

n = The amount of data to be tested

2) Limiting (k) test W

If n is even $k = \frac{n}{2}$

If n is odd $k = \frac{n-1}{2}$

3) The formula W calculates

$$W = \frac{1}{d}[\sum_{i=1}^{k} a_i(x_{[n-i+1]} - x_{[i]})^2$$

The value of d comes from the calculation of the first formula. The limit value (k) comes from the calculation of the second formula. The Shapiro-Wilk test has 2 hypotheses being tested, namely:

H_0: Sample comes from a normally distributed population

H_1: the sample comes from a population that is not normally distributed

The test criteria used in the Shapiro-Wilk test are if Wcount ≤ 0.05, then the data is said to be not normally distributed (H0 is rejected). Conversely, if the Account value is > 0.05, the information is said to be normally distributed (H0 is accepted).

Homogeneity test: Homogeneity test is used as a reference material to determine statistical test decisions. The basis or guidelines for decision making in the homogeneity test are as follows (Pratama and Permatasari 2021):

$$F = \frac{biggest\ variant}{smallest\ variant}$$

Information:

a. If the value is significant or Sig. <0.05, it is said that the variance of two or more groups of data population is not the same (not homogeneous)
b. If the value is significant or Sig. > 0.05, it is said that the variance of two or more data population groups is the same (homogeneous).

Kruskal-Wallis test: The Kruskal-Wallis test is a hypothesis test developed from the one-way ANOVA method for conditions where some requirements cannot be met for parametric analysis. By the general formula kruskal-wallis (Quraisy et. al, 2021):

$$H = \frac{12}{N(N+1)} \sum_{i=1}^{k} \frac{Ri^2}{ni} - 3(N+1)$$

Information:

H= the value of kruskal-wallis from the calculation results
Ri = the number of ranks from the i-th group/category
ni= the number of cases in the sample in the i-th group/category
k = number of groups/categories
N= the total number of observations (N = n1 + n2 + n3 + … +nk)

Lenght growt : The lenght growth is calculated using the formula

Pm = Pt – Po

Where:

Pm = absolute length

Pt = average length of individuals on day t (cm)

Po = average length of individuals at the start of the study (cm)

The Specific Growth Rate: The specific growth rate is calculated using the formula

$$SGR = \frac{Ln\ Wt - Ln\ W0}{t}\ x\ 100\%$$

Information:
SGR = Specific Growth Rate
Wt = Average weight of fish at the end of rearing (g)
W0 = Average weight of fish at the beginning of rearing (g)
t = Maintenance time (days)

Feeding efficiency: Feeding efficiency is calculated at the end of maintenance, namely by the formula of feed efficiency :

$$EP = ((WT + D)-W0)/Fx\ 100\%$$

Information:
EP = Feed efficiency (%)
Wt = Final fish weight (g)
Wo = Initial fish weight (g)
D = Weight of dead fish during the study (g)
F = amount of feed consumed (g)

Survival Rate: The survival rate (SR) can be calculated by the formula as follows:

$$SR = \frac{Nt}{N0}\ x\ 100\%$$

Information:
SR = Survival rate (%)
N0 = Number of fish at the beginning of maintenance (tails)
Nt = Number of fish at the end of the study (tails)

Water quality: Water quality measurements were carried out to determine the condition of the water as the environment where live fish were measured, namely temperature, dissolved oxygen (DO) and pH, measurements were carried out every 7 days.

3. RESULTS AND DISCUSSION

3.1 Normality test

A normality test is a test carried out to know the distribution of data in a group data or variable that is usually or not normally distributed [23]. Data of more than 30 numbers (n>30) can be assumed to be normally distributed. To determine whether the data obtained has a standard or abnormal distribution, data can do it by conducting a normality test. The test used is the Shapiro-Wilk. Test. After completing the normality test on the research data, the results show that the data is normally distributed, as in table 1 below.

Table 1. Normality test of research data after transformation

Data	W table	W count
Absolute length growth	0,859	0,17349
Specific Growth Rate	0,859	2,79665
Feeding Efficiency	0,859	0,12860
Survival Rate	0,859	0,08532

Information: (Source: Primary Data)

1. $W_{tavle} < W_{count}$ then H_0 is accepted
2. $W_{table} > W_{count}$ then H_0 is rejected

Table 1. above shows that the Shapiro-Wilk value after data transformation is carried out, namely the specific growth rate of 2.79665. These results show that the specific growth rate leads to W table < W count; data can conclude that the data can be normally distributed [17]. Then, the result will use the data from this transformation to conduct further statistical tests. However, for absolute length growth data, survival and feed efficiency, because the data is not standard, then the non-parametric Kruskal-Wallis test will be carried out.

3.2 Homogeneity test

A homogeneity test can be conducted to determine the underlying assumption in the analysis of variance (ANOVA) that the conflict of the population is the same. The similarity test between clashes for used to determine whether the data distribution is homogeneous by comparing the variations [23]. The homogeneity test used in this study is using the Levene test. After carrying out the Levene homogeneity test on the research data, the results can show in the following table.

Table 2. Test the homogeneity of research data

Data	L liven	F table
Absolute Length Growth	0,015	4,07
Specific Growth Rate	1,319	4,07
Feeding Efficiency	0,376	4,07
Survival Rate	4,260	4,07

Information: (Source: Primary Data)

1. $F_{table} > F_{count}$ then H_0 is accepted
2. $F_{table} < F_{count}$ then H_0 is rejected

Based on the results of the Levene test calculation in table 4 shows that the calculated F value of absolute length growth is 0.015, the specific growth rate is 1.319, and the feed efficiency is 0.376. These results indicate that F table > F arithmetic, which can conclude that the data obtained shows homogeneous results [26]. As for the survival data, the Levene test value is 4.260; from these results, we can conclude that the survival data is not homogeneous because the F table < F count.

3.3 Absolute length growth

Growth is a biological process that occurs continuously in the body of fish which is characterized by an increase in length or weight in a specific time. The result can measure absolute length growth by measuring body length based on a particular unit of time [4]. Overall, the average individual size of striped catfish continues to increase. Indicates that the feed given has been optimally utilized for growth.

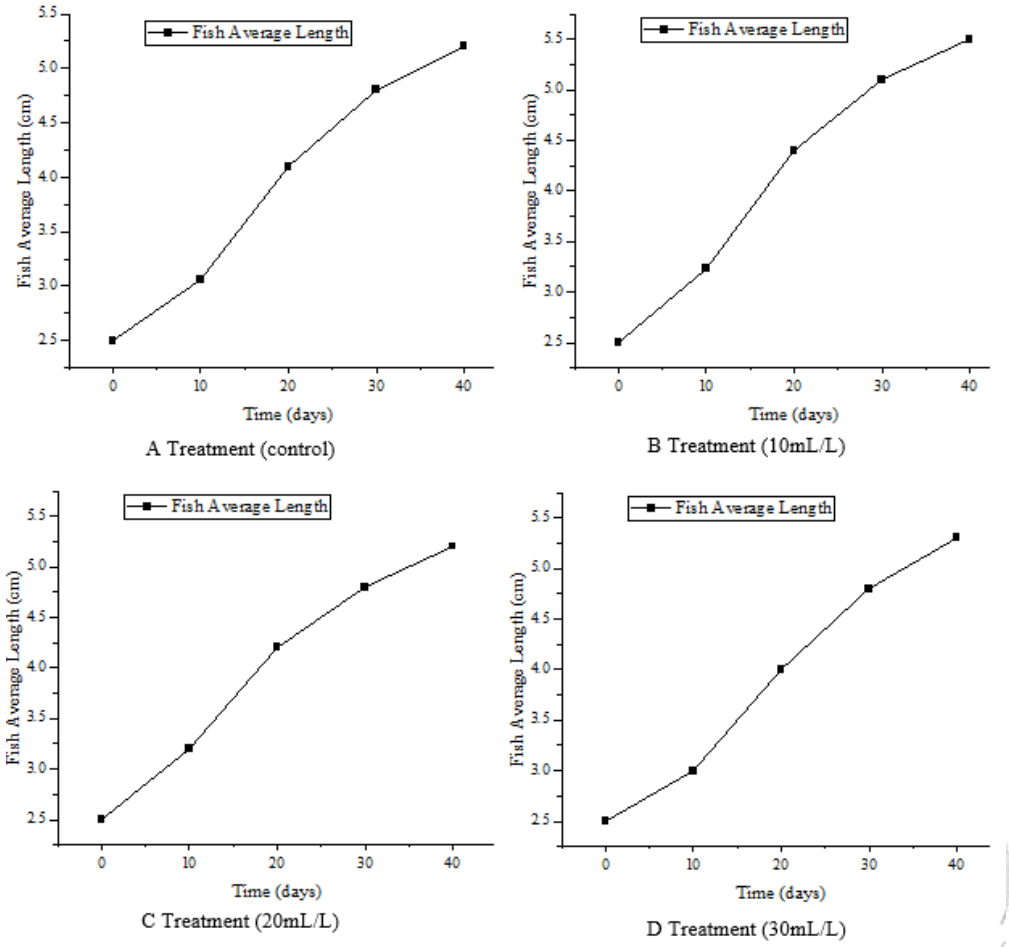

Fig -1: Graph of average length gain of test fish ame of the figure
(Source: Primary Data)

The results of observations made on the growth in length of fish for 40 days showed an increase in growth rates which varied quite a bit for each treatment, as seen from the pattern of increase in the average length, which ran at each time of observation of each treatment (Figure 1).

Table 3. Growth in absolute seed length of striped catfish

Treatment	Absolute length growth (%)
A (control)	2.67 ± 0.0577^{a}
B (10 mL/L)	2.96 ± 0.2517^{a}
C (20 mL/L)	2.73 ± 0.2517^{a}
D (30 mL/L)	2.80 ± 0.1000^{a}

Information: Values followed by the same lowercase letter are not significantly different based on the Multiple Comparison test at the 5% level. (Source: Primary Data)

The results of the Kruskal-Wallis test showed that *Daphnia* feeding had no significant effect on the growth in the absolute length of striped catfish fry (Table 3). In line with the research by [6], the administration of *Daphnia*,

which was enriched using probiotics, also did not have a significantly different effect on the growth and survival of bang fry. This can occur due to the low nutritional value of the protein contained in *Daphnia*, so it does not meet the needs of striped catfish seeds. In addition, the enzyme content in *Daphnia*, which has enriched with EM_4, is thought to accelerate the digestive process when the feed is in the intestines of fish fry. With the presence of enzymes, the process of breaking down nutrients into simple ingredients is easily absorbed by the seed's directives. Thus, the striped catfish fry becomes hungry quickly because the stomach emptying rate is faster, but they do not receive feed immediately.

3.4 Specific Growt Rate

The results of observations made on the specific growth rate for 40 days show an increase in the growth rate, which is quite varied for each treatment, as seen from the pattern of average weight gain that varies at each observation time of each treatment in Figure 2. The result shows that the administration of *Daphnia* sp., enriched using EM4, gave an excellent response to the growth of striped catfish fry.

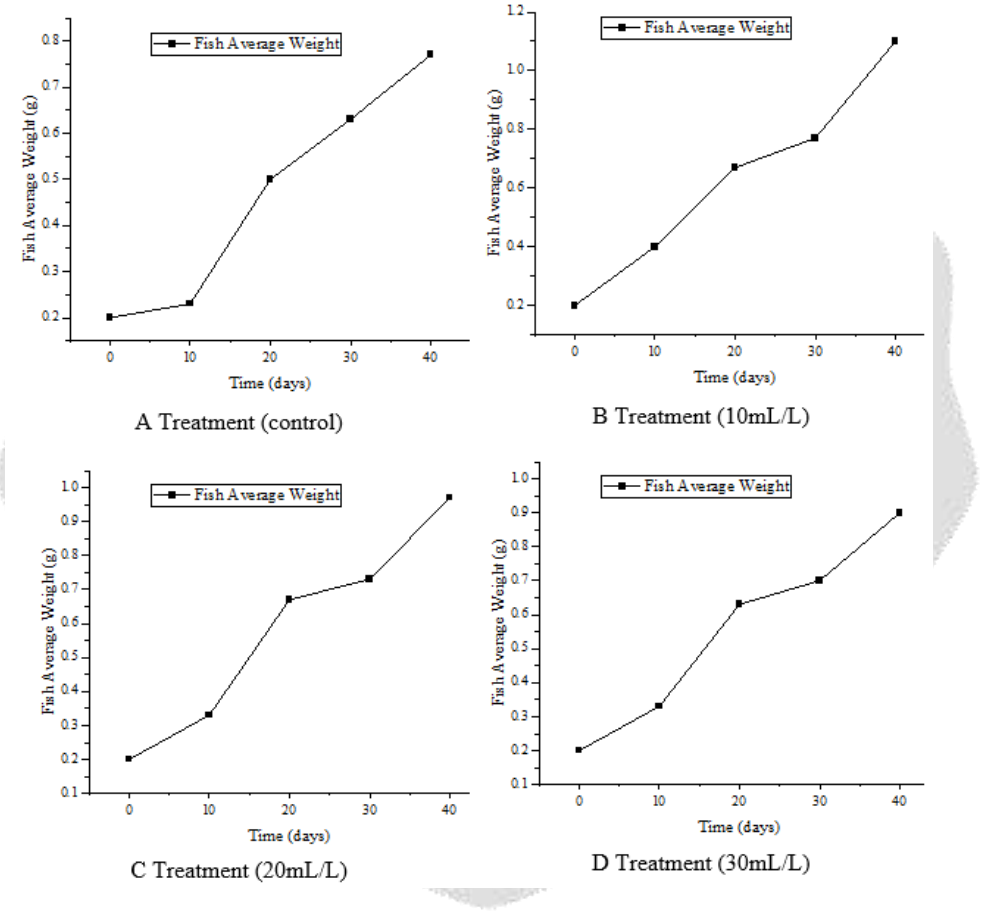

Fig -2: Graph of average weight gain of test fish ame of the figure
(Source: Primary Data)

Based on the analysis of Anova variance, the administration of *Daphnia* sp. which had been enriched with EM_4 as fresh feed for striped catfish did not have a significant effect on the specific growth rate of striped catfish fry (table 4).

Table 4. Specific growth rate of striped catfish fry

Treatment	Spcific Growth Rate(%)
A (Control)	7,30 ± 0.0040[a]
B (10 mL/L)	7,40 ± 0.0040[a]
C (20 mL/L)	7,37 ± 0.0040[a]
D (30 mL/L)	7,35 ± 0.0000[a]

Information: Values followed by the same lowercase letters are not significantly different based on Duncan's multiple-distance test at 95% level. (Source: Primary Data)

Table 4. shows that the specific growth rate of Stripped catfish fry did not have a significant effect, but from the existing treatments, we saw that the highest growth was found in treatment B (10 mL/L) at 7.40%, while the lowest growth rate was in treatment A. (Control) of 7.30%. The research conducted by [7] regarding the enrichment of *Daphnia* sp. using veteran at a dose of 10 mL/L showed the highest growth of pearl fish fry.

Striped catfish seed growth from feeding *Daphnia* sp. enriched with each dose showed different results in each treatment. Differences in fish growth with *Daphnia* sp. improved using EM4 with different amounts could be due to the additional nutritional content of the feed. Nutrients such as carbohydrates, fats and proteins are energy that can affect growth [12].

The growth of Stripped catfish fry from treatment B (10 mL/L) was higher than the other treatments. The research conducted by [13] showed that the highest growth of catfish fry was 1.885% by feeding *Daphnia* sp. enriched with a Viterna dose of 10 ml/l. The protein content in *Daphnia* sp. increased the EM4 amount of 10 mL/L at 2.50%, with a fat content of 0.40% and carbohydrate content of 96.11%. This value may need to be larger, but it can provide optimal results for the growth of Stripped catfish fry.

3.5 Feeding Efficiency

After the research, the average feed efficiency data was 32.16% - 34.35%. Based on the results of the Kruskal-Wallis test, *Daphnia* sp., with EM4 enriched as fresh feed for striped catfish, did not have a significant effect on the efficiency of feeding catfish fry (Table 5).

Table 5. Feeding Efficiency

Treatment	Feeding Efficiency (%)
A (Control)	32.16 ± 2.2578[a]
B (10 mL/L)	34.35 ± 1.7253[a]
C (20 mL/L)	34.25 ± 1.8726[a]
D (30 mL/L)	32.89 ± 2.5251[a]

Information: Values followed by the same lowercase letter are not significantly different based on the Multiple Comparison test at the 5% level. (Source: Primary Data)

One of the advantages of probiotics in increasing growth and feed efficiency in fish is the presence of probiotic bacteria in the digestive tract. Probiotics that enter the intestines of fish will help the digestive process of feed efficiently utilized by fish because the nutritional content of feed will be easily absorbed by the fish body [21]. In table 6, we can see that the highest feed efficiency value is found in treatment B (10mL/L) at 34.35%, followed by treatment C (20mL/L) at 34.25%, treatment D (30mL/L) at 32.89 % and the lowest feed efficiency value was in treatment A (control) of 32.16% seen in the efficiency value between treatments which showed no significant difference.

The results of feed efficiency conducted [1] by giving a 6ml dose of probiotics to catfish showed an effect of 43.93%, and research conducted [8] with a quantity of 1% probiotics resulted in a feed efficiency value of 31.55%

for catfish. The low weight of feed efficiency in treatment A (control) of 32.16% that thought to be due to inefficient absorption. The statement of [3] regarding the low absorption of feed because the control treatment of *Daphnia* did not receive additional nutrients from probiotics; could cause slower digestive activity than other treatments. Probiotics benefit the host by improving nutritional value and feed utilization [14].

3.6 Survival rate

After conducting the research, the data obtained that the average survival rate of striped catfish seeds was 68.33%-88.33%. The results of the nonparametric test show that there is no significant difference in Table 6.

Table 6. Kruskal-wallis test

	Score
H	2,744
df	3
χ^2	7,815

(Source: Primary Data)

Information:

1. $x^2_{table} > H_{count}$ is H_0 accepted
2. $x^2_{table} < H_{count}$ is H_0 rejected

From the results of table 7 above, the calculated H value is 2.744 with the value of 2table, which is 7.815 and 2table > H figured. So this indicates that the test results are in the H0 acceptance decision, which means there is no difference in the survival value of Stripped catfish fry in treatments A, B, C and D.

Table 6. Stripped catfish fry survival rate

Treatment	Survival Rate (%)
A (control)	81,67 ± 17,5594[a]
B (10_mL/L)	88,33 ± 12,5831[a]
C (20_mL/L)	86,67 ± 7,63763[a]
D (30_mL/L)	68,33 ± 34,0343[a]

Information: Values followed by the same lowercase letter are not significantly different based on the Multiple Comparison test at the 5% level. (Source: Primary Data)

In table 8. we can see that data found the lowest survival rate in treatment D, which was 68.33%, then followed by treatment A, which was 81.67%, treatment C was 86.67%, and the highest survival rate was in treatment B, which was 88, 33%. Their nutrition determines the survival rate of fish; one effort is made to improve life by giving suitable feed both from the meal and the nutritional content contained in the feed [10].

The use of EM4 probiotics is essential in fish farming activities. Still, the amount of EM4 we will use is considered because if it is excessive, it can increase mortality in fish [24]. Therefore, the dose of EM4 to increase survival must follow the amount. If the concentration is low, the survival value of fish will also be lower. This is in line with research conducted by [2], which showed that the lowest survival value was the administration of probiotics at a dose of 0.5 mL/L and control on tilapia culture media. Based on the results of the research that data carried out, the lowest survival value of Strapped catfish fry found in treatment D at 68.33%, and this can occur because the concentration of EM4 is too high so that EM4 is absorbed in *Daphnia* sp. more than fish needs. The value of good fish survival is in the range of 75%-80% [22].

3.6 Water Quality

Water quality is one of the essential role holders in aquaculture activities for the sustainability of fish—the following table of water quality during the maintenance of striped catfish. The results of water quality measurements generally indicate that the water quality during the study was still in an excellent range to support the maintenance of striped catfish seeds.

Table 7. Catfish resring water quality

Treatment	Parameter		
	Temperature (°C)	DO (mg/L)	pH
A (Kontrol)	27,4	6,47	7,1
B (EM_4 10 ml/l)	27,5	6,47	7,1
C (EM_4 20 ml/l)	27,5	6,38	7,2
D (EM_4 30 ml/l)	27,3	6.72	7,3
Standard	27 – 30*	>5*	6,5 – 8,5*

*Note: *SNI (2000)*

(Source: Primary Data)

4. CONCLUSIONS

Based on the results of research on the effect of Daphnia enrichment with EM4 at different concentrations, the results were not significantly different for striped catfish seeds in treatments A, B, C and D. Enrichment of Daphnia with EM4 at a concentration of 10mL/L could increase the highest growth performance, namely long growth absolute growth of 2.96%, specific growth of 7.40%, efficiency of feeding 34.35% and survival of 88.33%.

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