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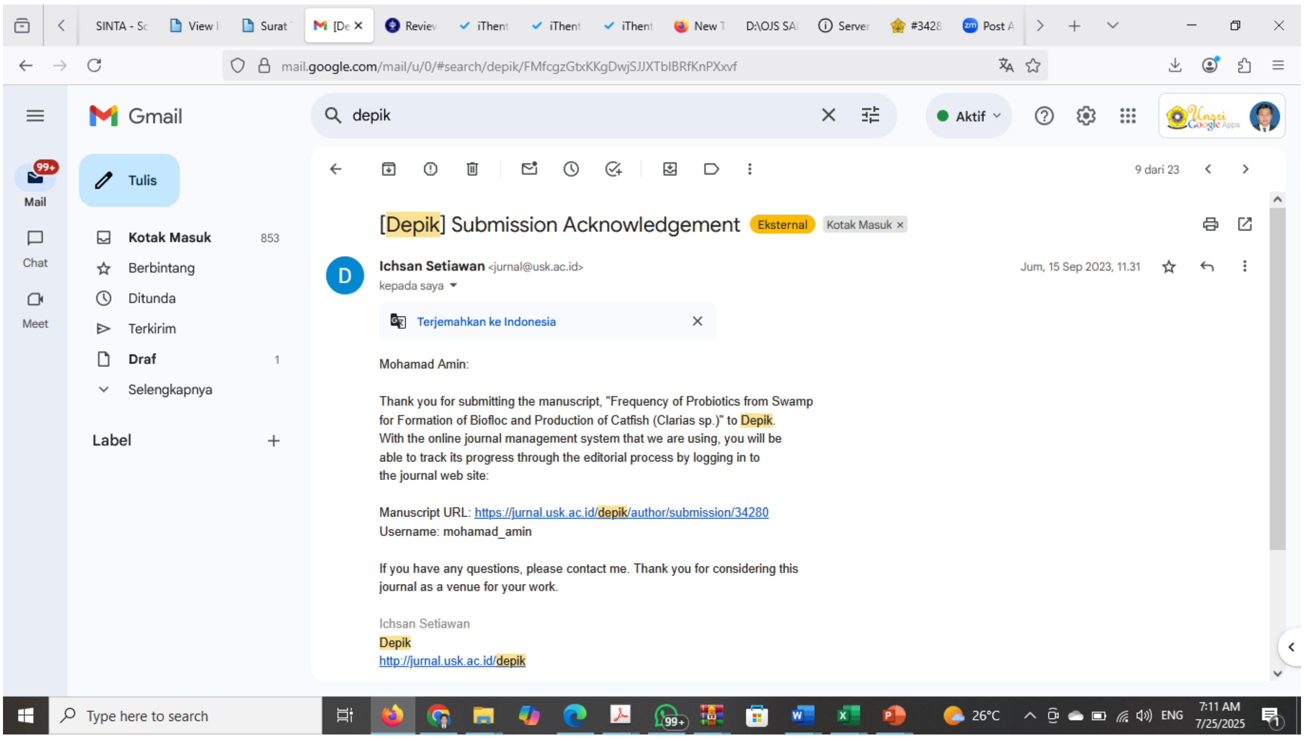

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Frequency of Probiotics from Swamp for Formation of Biofloc and Production of Catfish (*Clarias* sp.)

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ABSTRACT

Catfish (*Clarias* sp.) cultivated with a combination of the addition of probiotics in the biofloc technology are thought to increase production. The frequency of adding probiotics from swamps to cultured water media has never been studied to ensure flocks' availability in rearing media. This study aimed to determine the appropriate frequency of probiotics from swamps in the biofloc technology to improve the parameters of successful cultivation, especially increasing catfish production and biofloc formation. This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given was different in the frequency of giving probiotics from swamps: (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. The results showed that P2 was the best treatment with a floc volume of 68.33 mL L^{-1}, absolute length growth 8.18 cm, absolute weight growth 19.30 g, feed efficiency 135.24 %, survival 89.33%, biomass production 24639.50 g, temperature 28.85-29.59ºC, pH 7.27-7.42, dissolved oxygen (DO) 3.91-5.72 mg L^{-1}, ammonia 0.45-1.15 mg L^{-1} and total dissolved solid (TDS) 717.33-885.50 mg L^{-1}. Therefore, probiotics from swamps should be given to fish culture media with a frequency of 2 times during 42 days of rearing or once every 21 days.

Introduction

Catfish (*Clarias* sp.) is a freshwater fishery commodity widely cultivated due to high market demand. According to data from the Statistics from the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia (2022), catfish production in Indonesia in the enlargement cultivation sector in 2019 was 289 thousand tons and reached 384 thousand tons in 2020, while in 2021, it was 360 thousand tons. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and with an environmental perspective by minimizing waste disposal in the surrounding waters (Fauzi *et al*., 2022). According to Putra *et al.* (2017), technology biofloc by utilizing probiotics in the form of heterotrophic bacteria can produce natural feed from the flocs that are formed, thereby increasing the value of feed efficiency and improving water quality.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the media. Swamp microorganisms identified are *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti*et al*., 2018). Giving probiotics from swamps (*Bacillus* sp. and *Streptomyces* sp.) has been studied in feeding the agility fish (Tanbiyaskur *et al*., 2022) and the rearing medium with biofloc technology in catfish (Wijayanti *et al*., 2021) and snakehead fish (Wati, 2021; Anil, 2022). In this study, swamp probiotics can increase growth, feed efficiency, fish survival, and aquaculture water quality (Wijayanti *et al*., 2020).

Probiotics from swamps containing bacteria *Bacillus* sp. and *Streptomyces* sp. can be used as a starter in the biofloc system for floc formation. In research by Wijayanti *et al.* (2021), administration of probiotics from swamps with a frequency of 1 time for 42 days of rearing resulted in a floc volume of 40 mL L^{-1}

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(42nd day). Using these flocs for 60 days of rearing resulted in the final average weight and length growth from the initial average of 7.16 g and 9.50 cm to 36.95 g and 18.50 cm and FCR 0.97. Floc volume results (Wijayanti *et al*., 2021) are lower than Bakar *et al.* (2015) research results, whose floc volume reached 92.5 mL L^{-1}. According to Zaidy (2022), the maximum floc volume for catfish is 100 mL L^{-1}. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water, around 70-80% of the water volume. These flocks must continue to be available in the rearing medium in sufficient quantities as natural food for fish to increase fish growth and improve feed efficiency and water quality. According to Feroza *et al.* (2021), the flocs in the rearing media will decrease because they are consumed by fish every day, so it is necessary to have the availability of flocs in the rearing medium or not cause blooming microorganisms due to excess floc volume. Therefore, it is necessary to research the effect of the frequency of probiotics from swamps on the formation of biofloc and catfish production.

Materials and Methods

Location and time of research

This research was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University in December 2022–January 2023.

Research Materials

The materials used were catfish seeds (7 ± 0.5 cm in length) and bacteria *Bacillus* sp., *Streptomyces* sp., molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tool used is a tarpaulin pool with a round diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, imhoff cone, loop needle, erlenmeyer, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter and ammonia test kit.

Research Design

This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given is the difference in the frequency of giving probiotics from swamps, which are as follows:

P1 = 1 time during 42 days of rearing
P2 = 2 times during 42 days of rearing

Work Procedure

Swamp Origin Probiotic Culture

Pure culture obtained from the isolation of bacteria from swamps *Bacillus* sp. and *Streptomyces* sp. results of previous studies (Wijayanti *et al*., 2018) liquid culture was carried out. Bacterial liquid culture *Bacillus* sp. was done on the media Nutrient Broth (NB) liquid by taking one ose of bacteria for deep culture Erlenmeyer, which already contains NB 20 mL media. In contrast, *Streptomyces* sp. was done on the media Yeast Malt (YM) liquid by taking one ose of bacteria and culturing in liquid YM media 20 mL deep Erlenmeyer. The two bacteria were agitated with a magnetic hot plate stirrer for three days for *Bacillus* sp. and five days for *Streptomyces* sp. The density of bacteria obtained from liquid culture results was calculated using the cup counting or TPC method (Total Plate Count). Then, do the mixing of bacteria with the formulation. Liquid media for storage media formulations in 5% molasses and filled with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish were reared in 6 round ponds made of tarpaulin with a diameter of 2 m and a height of 1.2 m. Rearing begins with pond preparation, including cleaning, water filling, and pond incubation. The pond is cleaned by brushing the entire inside of the pond and drying it for one day to kill the pathogens. Then, fill with water as high as 0.7 m and incubate for three days (Ma'ruf, 2016). Then salt at a dose of 1 kg m^{-3} and camphor dolomite 50 g m^{-3} (Sucipto *et al*., 2018) were added to the rearing medium and incubated for one day (Wijayanti *et al*., 2021). Installation of aerators is carried out at 4 points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process uses catfish measuring 7 ± 0.5 cm with a stocking density of 500 m^{-3} (Ma'ruf, 2016) and maintained for 42 days. Fish stocking was carried out in the morning when water conditions were normal and acclimatized for seven days to reduce stress on the fish. During rearing, probiotics were added to the biofloc pond at a density of bacteria *Bacillus* sp. 10^5 CFU mL^{-1} and *Streptomyces* sp. 10^5 CFU mL^{-1} (Wijayanti *et al*., 2020) with a frequency according to treatment, namely (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. During rearing, the addition of a carbon source in the form of molasses was also carried out at a dose of 200 mL m^{-3} with a frequency of once every seven days (days 0, 7, 14, 21, 28, 35) (Putra *et al*., 2017). Fish are fed using commercial feed with a

39-41% protein content. The feeding frequency is three times daily, namely at 08.00 WIB, 12.00, and 16.00 WIB with the at satiation. If there are dead fish, the fish are weighed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental pond unit.

Research Parameters

Volume floc and Floc Composition

Floc volume was measured using an Imhoff cone by taking media water maintenance at 1 point of collection as much as 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on days 7, 14, 21, 28, 35 and 42. After measuring the floc's volume, the precipitated floc is taken to observe the composition of floc microorganisms. Floc composition was microscopically observed (40x magnification) on days 0, 1, 21, and 42.

Total Bacterial Colonies

Total bacterial colonies formed in the rearing medium were counted with the cup count method by taking water as a maintenance medium. After The sample water is diluted through dilution steps 10-5, 10-7, 10-9. Bacterial density calculations were done on days 0 (before adding probiotics from swamps), 1, 21, and 42. Growing bacterial colonies were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1) = Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth

The formula used to measure absolute weight growth, according to Hopkins (1992) is:

$$W = Wt - Wo$$

Information:

W	=	Growth in absolute weight of fish kept (g)
Wt	=	Average fish weight at the end of the study (g)
Wo	=	Average fish weight at the start of the study (g)

The formula used to measure absolute length growth, according to Hopkins (1992), is:

$$L = Lt - Lo$$

Information:

L	=	Absolute length growth of reared fish (cm)
Lt	=	Average length of fish at the end of the study (cm)
Lo	=	Average fish length at the start of the study (cm)

Feed Efficiency

Feed efficiency (FE) is calculated based on a formula based on Afrianto & Liviawaty (2005):

$$FE\ (\%) = \frac{(Wt+D)-Wo}{F}\ x\ 100$$

Information:

FE	=	Feed efficiency (%)
Wt	=	Fish biomass at the end of the study (g)
Wo	=	Fish biomass at the start of the study (g)
D	=	Biomass of fish that died during the study (g)
F	=	Amount of fish feed given during the study (g)

Survival Rate (SR)

The survival rate is calculated using a formula based on Aliyu-Paiko*et al.* (2010) as follows:

$$SR\ (\%) = \frac{Nt}{No}\ x\ 100$$

Information:

SR	=	Survival (%)
Nt	=	The final quantity of fish at the end of rearing (g)
No	=	The initial quantity of fish at initial rearing (g)

Biomass Production

The formula can calculate the level of biomass production according to Shang (1982):

$$P\ (g) = W\ x\ N$$

Information:

P	=	Production of biomass (g)
W	=	Average weight of fish at the end of rearing (g)
N	=	The final quantity of fish at the end of rearing (g)

Water quality

Water quality parameters included temperature and pH, measured every day at 08.00 WIB and 16.00 WIB. Dissolved oxygen level, TDS (*Total Dissolved solid),* and ammonia were measured every seven days (day 0, 7, 14, 21, 28, 35, 42) once in 42 days of the rearing period.

Data analysis

Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively.

Results and Discussion

Volume Floc

The results of the T-test analysis of flock volume during rearing are presented in Table 1.

Table 1. Data and results of the T-test analysis of flock volume during rearing

Days to-	Volume flock (mL L^{-1})	
	P1	P2
7	6.00±2.00	5.33±3.06
14	11.67±2.89	13.00±3.00
21	17.00±2.65	21.67±2.89
28	35.00±5.00[a]	53.33±5.77[b]
35	43.33±7.64[a]	66.67±11.55[b]
42	48.33±2.89[a]	68.33±10.41[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the results of the T-test analysis showed that floc volume on the Based on the results of the T-test analysis showed that the floc volume in treatment P1 was not significantly different from treatment P2 on days 7, 14, and 21 and was significantly different on days 28, 35, and 42. Floc volume is the amount of suspended solids over a certain period in an inverted conical container (Effendi, 2003). The highest floc volume was found in the P2 treatment given probiotics with a frequency of 2 times during 42 days of rearing, reaching 68.33 mL $L^{-1,}$ while P1 is 48.33 mL L^{-1}. The floc volume obtained in this study was higher than that in the study by Wijayanti *et al.* (2021), which is 40 mL L^{-1} (42nd day). This is presumably due to the influence of the time-frequency of giving probiotics to the rearing medium. According to De Schryver *et al.* (2008), the factors that influence the formation of bio floc are the administration of probiotics (starter) with floc-forming microbial composition, agitation intensity by aeration device, organic carbon source, and water quality. This is also reinforced by the results of Malaputra *et al.* (2016), namely the administration of commercial probiotics with a frequency of 14 times (70 days of rearing) resulted in a floc volume of 120 mL L^{-1} higher than the frequency of 7 times which is only 80 mL L^{-1}. Based on the results of this study, it can be stated that the more often probiotics are given to the rearing medium, the more volume will increase. The maximum floc volume for catfish farming is 100 mL L^{-1}. When the floc volume exceeds the limit, partial water removal can be done and replaced with new water, around 70-80% of the total water volume of the preservation pond (Zaidy, 2022).

In addition to the factor of providing probiotics from swamps as a starter containing heterotrophic bacteria, the increase in floc volume every week in this study was thought to be due to the C/N ratio. The C/N ratio can affect the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), the C/N ratio >10 in fish farming activities is the optimum ratio for the formation of biofloc. Meanwhile, according to Avnimelech *et al.* (1999) required a C/N ratio >15. Therefore, it is necessary to add organic carbon from outside, such as molasses. This study added extra organic carbon in molasses every seven days at a dose of 200 mL m^{-3} (Putra *et al.*, 2017).

Floc Composition

The composition of the flocs in this study is presented in Table 2.

Table 2. Floc composition

Floc-forming microbes	Treatment	
	P1	P2
Chlorophyta	✓	✓
Cyanophyta	✓	✓
Protozoa	✓	✓
Coelenterata	-	✓
Rotifers	✓	✓
Arthropods	-	✓

Information: (✓) exists, (-) does not exist

Based on observations of floc composition in the treatment of probiotics from swamps with P2 frequencies more diverse than P1, namely chlorophyta, cyanophyta, protozoa, coelenterata, rotifers, and arthropods. Whereas in P1, only chlorophyta, cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), the diversity in the composition of the floc-forming microbes is thought to be due to the effect of giving probiotics, which is in line with the increased volume of floc in the rearing medium so that the microbes in the rearing containers can grow properly. Wati (2021) also produced the same floc composition as arthropods, chlorophyta, cyanophyta, protozoa, and rotifers. The composition of the flocs in the form of zooplankton in this study were protozoa, coelenterata, rotifers, arthropods, and phytoplankton in the form of chlorophyta and cyanophyta. This is following the opinion of De Schryver *et al.* (2008), namely the composition of floc in the form of a heterogeneous combination of microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach sizes <1000 µm.

Total Bacterial Colonies

The total bacterial colonies on the rearing medium are presented in Table 3.

Table 3. Data and analysis of T-test total bacterial colonies in flocs

Days to-	Total bacterial colonies (x 10^9 CFU mL^{-1})	
	P1	**P1**
0	2.77±1.00	2.77±1.00
1	13.23±2.82	13.23±2.82
21	5.56±2.01	5.56±2.01
42	8.42±3.19^a	8.42±3.19^a

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the total bacterial colonies in the P1 treatment were not significantly different from the P2 treatment on days 0, 1, and 21 and were significantly different on the 42nd day. This study's bacterial density range was between 2.77 and 19.73 x 10^9 CFU mL^{-1}. After stocking probiotics from swamps on rearing media in treatments P1 and P2, total bacterial colonies increased on day 1, and day 21 decreased. On the 42nd day, the bacterial density increased again with a higher P2 bacterial density of 19.73 x10^9 CFU mL^{-1} compared to P1, which is 8.42 x10^9 CFU mL^{-1}. The high density of bacteria at P2 was thought to be due to adding probiotics again on the 21st day. In research, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish rearing by adding probiotics up to x10^{14} CFU mL^{-1}.

Meanwhile, according to Sitorus *et al.* (2019), the density of bacteria can reach x10^9 CFU mL^{-1}. Widnyana (2016) states that on biofloc technology, total bacterial colonies ranged from 10^3-10^4 CFU mL^{-1}, while the system has a bacterial range of 10^8-10^{10} CFU mL^{-1}. This is caused by the system having a high intensity of sunlight as one of the supporting factors in the growth of bacteria. The high value of the total density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, which is assisted by their effectiveness in breaking down organic matter (Adharani *et al.*, 2016).

Absolute Growth, Feed Efficiency, Survival, and Biomass Production

Based on the research results, absolute growth data, feed efficiency, and survival of catfish for 42 days of rearing are presented in Table 4.

Table 4. Data and results of absolute growth T-test analysis, feed efficiency, survival, and biomass production during rearing

Parameter	Treatment	
	P1	**P2**
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24^a	151.90 ± 7.98^b
Life sustainability (%)	81.54 ± 1.59	89.33 ±6,21
Biomass production (g)	17439.14 ± 1346.55^a	24639.50 ± 1344.51^b

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the analysis of the T-test showed that the growth in absolute length and absolute weight growth of catfish in treatment P1 was not significantly different from treatment P2. The absolute length growth and weight growth values of probiotics from swamps P2 were 8.18 cm and 19.30 g, higher than P1, which were 7.16 cm and 16.42 g. The results of this study indicate that fish use the floc as additional feed to increase fish growth. Catfish can utilize the available floc in the rearing medium as additional feed because it is high in protein and can increase fish growth (Putra *et al.*, 2017). In research, Wijaya *et al.* (2016) floc contains 42.42% protein, 92.15% water, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract material (BETN). According to Febriyanti *et al.* (2018), heterotrophic bacterial communities that accumulate in the rearing medium will form flocs (clumps) that can be used as a feed source for fish. Therefore, giving probiotics from swamps in the form of heterotrophic bacteria such as *Bacillus* sp.

Furthermore, *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. The bacteria *Streptomyces* sp. in fish farming can also be applied as a growth promoter (Cruz *et al.*, 2012). The results of fish growth will affect the value of feed efficiency.

The analysis of the T-test showed that the efficiency of catfish feed in treatment P1 was significantly different from treatment P2. The feed efficiency value for P2 was higher, namely 151.90%, while P1 was 104.97%. If the feed efficiency value is converted to a value feed conversion ratio (FCR) then FCR P1 is 0.96 while P2 is 0.66. The FCR value in this study was better than the research of Wijayanti *et al.* (2021), which is 0.97. Putra *et al.* (2017) researched that administering probiotics to catfish rearing media with biofloc technology also resulted in feed efficiency between 88.17-110.86%. This

study's high feed efficiency value is also suspected because fish use flocks as additional feed. De Schryver *et al.* (2008) stated that using flocks as additional feed is important in increasing feed efficiency. This shows that swamp probiotics can form flocs, which fish then eat as additional feed for fish.

The analysis of the T-test showed that the survival of catfish in treatment P1 was not significantly different from treatment P2. Survival in this study was quite good. The highest yield was in P2, 89.33%, followed by P1, 81.54%. The results of this study are close to the research of Wijayanti *et al.* (2021), namely 87.57% with a rearing period of 90 days. Administration of probiotics from swamps in the form of a combination of bacteria *Bacillus* sp. and bacteria *Streptomyces* sp., the percentage of fish survival can provide defense against pathogens by increasing non-specific immunity in the fish's immune system and maintaining a balance of water quality so that fish can survive (Wijayanti *et al.*, 2020). Growth and survival will affect the production value of fish biomass.

The analysis of the T-test showed that the production of catfish biomass in treatment P1 was significantly different from treatment P2. The production value of P2's biomass was 24,639.50 g, higher than that of P1, which was 17,439.14 g. Giving probiotics is thought to increase the production value of catfish in this biofloc technology. The biomass determines production at the end of cultivation and the survival of fish at the end of cultivation, so the higher the fish that can survive until the end of rearing, the more fish production will be produced (Anam *et al.*, 2017). Based on the results of absolute growth data and feed efficiency in this study, probiotics from swamps can form flocks used by fish as additional feed. Giving probiotics from swamps is also useful in preventing pathogenic bacteria to prevent death in fish. Therefore, giving probiotics from swamps can increase fish growth and survival, resulting in increased fish production.

Water quality

Temperature and pH

Based on the research results, the temperature and pH data of the rearing media are presented in Table 5.

Table 5. Data and results of temperature and pH T-test analysis

Treatment	Temperature (ºC)	pH
P1	28.85-29.22^{a}	7.27-7.40^{a}
P2	29.48-29.59^{b}	7.39-7.42^{b}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the temperature and pH of the catfish-rearing media in treatment P1 were not significantly different from those in treatment P2. The temperature of the rearing medium in this study ranged from 28.85-29.59ºC. According to BSN (2014), the temperature value for raising catfish is 25-30ºC. Catfish will experience slow growth when the temperature is 16-24ºC or 31-32ºC, and the fish will die if the temperature is below 16ºC or above 32ºC (Pujiharsono & Kurnianto, 2020). The value of the degree of acidity or pH in the rearing medium ranges from 7.27 to 7.42. BSN (2014) states that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth when the pH is 4-6.4 or pH 8.6-11, and the fish will die if the pH is below four or above 11 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The results of the DO T-test analysis during rearing are presented in Table 6.

Table 6. Data and results of DO T-test analysis during rearing

Days to-	DO (mg L^{-1}) P1	DO (mg L^{-1}) P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27^{a}	5.25±0.44^{b}
21	4.47±0.03^{a}	4.98±0.33^{b}
28	4.88±0.12^{a}	5.72±0.42^{b}
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The results of the T-test analysis show that Dissolved Oxygen (DO) or dissolved oxygen in treatment P1 was not significantly different from treatment P2 on days 0,7,35 and 42, but significantly different on days 14, 21 and 28. Based on Table 4.6. the frequency of probiotics from swamp P2 (2 times for 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study ranged from 3.91 to 5.95 mg L^{-1}. According to BSN (2014), the dissolved oxygen value for catfish is >3 mg L^{-1}. Therefore, the DO value in this study is still relatively safe for catfish farming. This is because the rearing container is equipped with aeration of 4 points. Besides that, it also comes from photosynthesis results from phytoplankton and oxygen diffusion from the air (Putra *et al.*, 2014). Based on this, it is suspected that the increase in DO at the end of P1 rearing is better than P2. Based on research by Agustina (2018), phytoplankton from the

chlorophyte division can increase dissolved oxygen levels in swamp water cultivation media by 63.63%. In research, Wijayanti *et al.* (2020) also produced a combination of chlorophyta and probiotics from swamps containing bacteria *Bacillus* sp. Furthermore, *Streptomyces* sp. is the best treatment for dissolved oxygen concentrations in fish culture media> 3 mg L^{-1}. The results of research by Kurniawan and Utama (2018) also yield DO values for catfish rearing >6 mg L^{-1} by administering probiotics *Bacillus* sp. as according to Prihanto *et al.* (2021) bacteria *Bacillus* sp. can improve water quality.

Ammonia

The results of the ammonia T-test analysis during rearing are presented in Table 7.

Table 7. Data and results of ammonia T-test analysis during rearing

Days to-	Ammonia (mg L^{-1})	
	P1	P2
0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23^{a}	0.69±0.47^{b}
35	1.59±0.38^{a}	0.66±0.37^{b}
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the ammonia in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 21, and 42 but was significantly different on days 28 and 35. Based on Table 4.7 shows that the administration of probiotics from swamp P2 (2 times for 42 days of rearing) is proven to reduce ammonia levels in rearing media compared to P1 (1 time for 42 days of rearing). The ammonia value in this study was high, ranging from 0.45 to 1.62 mg L^{-1}, while in research by Wijayanti *et al.* (2021), it is 0.27 mg L^{-1}. The value of ammonia for catfish farming on the formation of biofloc is <1 mg L^{-1} (Wijaya *et al.*, 2016). This high value of ammonia can cause death in fish due to the toxic nature of the ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion results, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). The high ammonia value in this study is likely due to the lack of a C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in water. Meanwhile, according to Hargreaves (2006), at a C/N ratio <10, heterotrophic bacteria will release ammonia into their environment. Research results Bakar *et al.* (2015) also stated that ammonia reduction in catfish farming using biofloc technology using a C/N 15 ratio could eliminate ammonia by 93.56% on the 12th day while the C/N 10 ratio on the 12th day was only 11.01%. Furthermore, only the maximum is on the 30th day, namely 98.51%.

Total Dissolved Solid (TDS)

The results of the TDS T-test analysis during rearing are presented in Table 8.

Table 8. Data and results of TDS T-test analysis during rearing

Days to-	TDS (mg L^{-1})	
	P1	P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76^{b}	872.67±41.55^{a}
28	804.17±59.93	757.67±136.93
35	810.00±57.38^{b}	717.33±21.37^{a}
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show

Based on the results of the T-test analysis, it shows that Total Dissolved Solid (TDS) in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 28, and 42 but significantly different on days 21, and 35. Based on Table 4.8. administration of probiotics from swamps P2 (2 times during 42 days of rearing) can reduce TDS values compared to P1 (1 time during 42 days of rearing). TDS values in this study ranged from 717.33 to 1014.83 mg L^{-1}. Catfish can still tolerate the measurement results of these values to live. PPRI No.82 (2001) states that TDS's standard water quality value in fish farming activities is 1000 mg L^{-1}. The high level of TDS on the 21st day, especially in the P1 treatment, is thought to be due to the administration of commercial drugs to prevent dead fish containing organic and inorganic compounds. This follows the statement of Rinawati *et al.* (2016), who stated that large amounts of organic and inorganic compounds can result in high levels of TDS in water.

Conclusion

Based on the results of the research that has been done, it can be concluded that giving probiotics from swamps with a frequency of 2 times for 42 days of rearing (P2) can form biofloc resulting in a floc volume of 68.33 mg L^{-1}, growth in length and absolute weight of 8.18 cm and 19.30 g, feed

efficiency of 135.24%, survival of 89.33% and biomass production 24,639.50 g with water quality of 28.85-29.59ºC, pH 7.27-7.42, DO 3,91-5,72 mg L^{-1}, ammonia 0,45-1,15 mg L^{-1} and TDS 717,33-885,50 mg L^{-1}.

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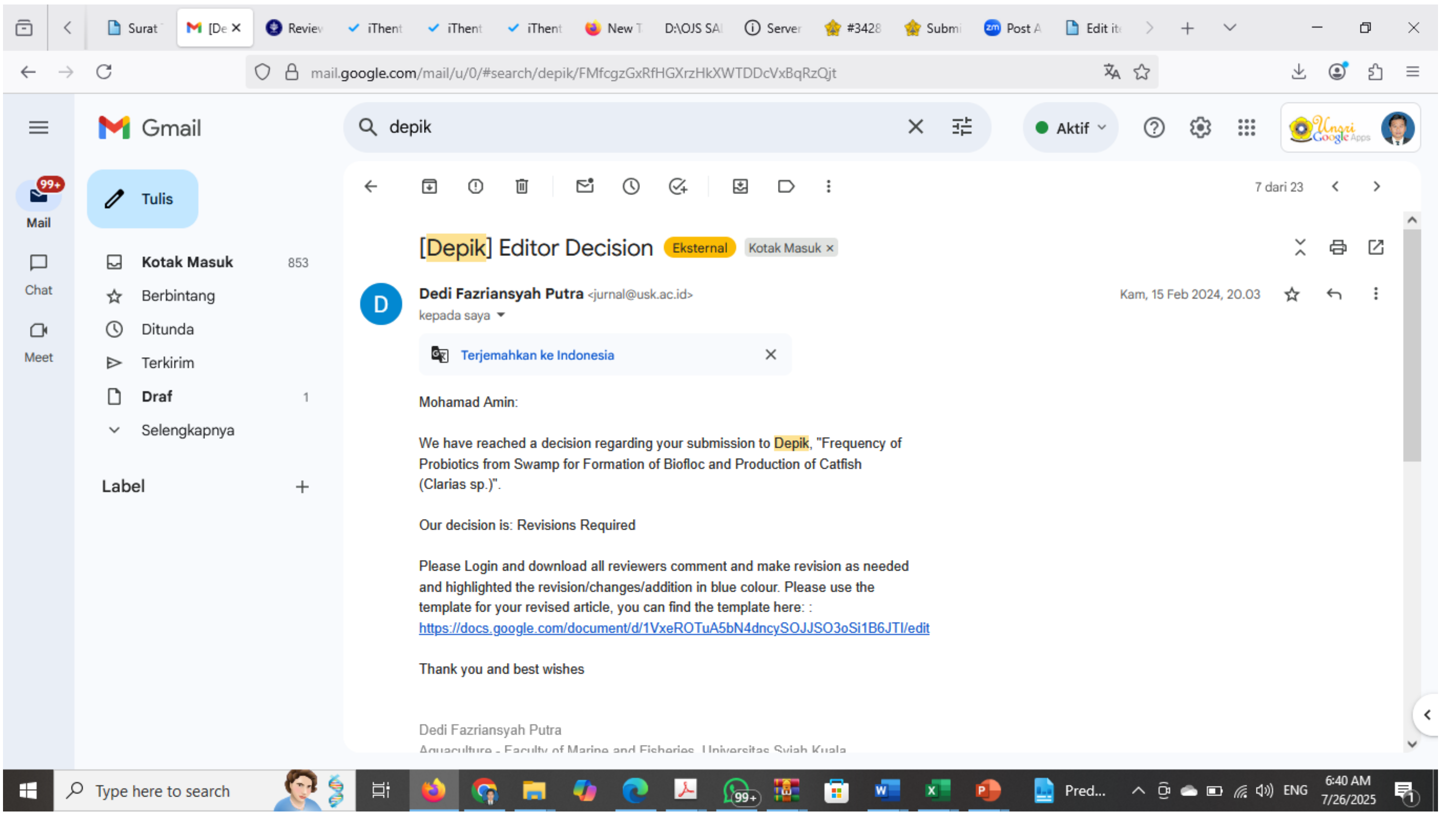

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Frequency of Probiotics from Swamp for Formation of Biofloc and Production of Catfish (*Clarias* sp.)

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ABSTRACT

Catfish (*Clarias* sp.) cultivated with a combination of the addition of probiotics in the biofloc technology are thought to increase production. The frequency of adding probiotics from swamps to cultured water media has never been studied to ensure flocks' availability in rearing media. This study aimed to determine the appropriate frequency of probiotics from swamps in the biofloc technology to improve the parameters of successful cultivation, especially increasing catfish production and biofloc formation. This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given was different in the frequency of giving probiotics from swamps: (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. The results showed that P2 was the best treatment with a floc volume of 68.33 mL L^{-1}, absolute length growth 8.18 cm, absolute weight growth 19.30 g, feed efficiency 135.24 %, survival 89.33%, biomass production 24639.50 g, temperature 28.85-29.59ºC, pH 7.27-7.42, dissolved oxygen (DO) 3.91-5.72 mg L^{-1}, ammonia 0.45-1.15 mg L^{-1} and total dissolved solid (TDS) 717.33-885.50 mg L^{-1}. Therefore, probiotics from swamps should be given to fish culture media with a frequency of 2 times during 42 days of rearing or once every 21 days.

Commented [W1]: explain the parameters that will be observed in this research and the analytical approach used for these parameters.

Introduction

Catfish (*Clarias* sp.) is a freshwater fishery commodity widely cultivated due to high market demand. According to data from the Statistics from the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia (2022), catfish production in Indonesia in the enlargement cultivation sector in 2019 was 289 thousand tons and reached 384 thousand tons in 2020, while in 2021, it was 360 thousand tons. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and with an environmental perspective by minimizing waste disposal in the surrounding waters (Fauzi *et al*., 2022). According to Putra *et al*. (2017), technology biofloc by utilizing probiotics in the form of heterotrophic bacteria can produce natural feed from the flocs that are formed, thereby increasing the value of feed efficiency and improving water quality.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the media. Swamp microorganisms identified are *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti*et al*., 2018). Giving probiotics from swamps (*Bacillus* sp. and *Streptomyces* sp.) has been studied in feeding the agility fish (Tanbiyaskur *et al*., 2022) and the rearing medium with biofloc technology in catfish (Wijayanti *et al*., 2021) and snakehead fish (Wati, 2021; Anil, 2022). In this study, swamp probiotics can increase growth, feed efficiency, fish survival, and aquaculture water quality (Wijayanti *et al*., 2020).

Commented [W2]: Im another study has been reported previously

Probiotics from swamps containing bacteria *Bacillus* sp. and *Streptomyces* sp. can be used as a starter in the biofloc system for floc formation. In research by Wijayanti *et al*. (2021), administration of probiotics from swamps with a frequency of 1 time for 42 days of rearing resulted in a floc volume of 40 mL L^{-1}

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(42nd day). Using these flocs for 60 days of rearing resulted in the final average weight and length growth from the initial average of 7.16 g and 9.50 cm to 36.95 g and 18.50 cm and FCR 0.97. Floc volume results (Wijayanti *et al*., 2021) are lower than Bakar *et al.* (2015) research results, whose floc volume reached 92.5 mL L^{-1}. According to Zaidy (2022), the maximum floc volume for catfish is 100 mL L^{-1}. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water, around 70-80% of the water volume. These flocks must continue to be available in the rearing medium in sufficient quantities as natural food for fish to increase fish growth and improve feed efficiency and water quality. According to Feroza *et al.* (2021), the flocs in the rearing media will decrease because they are consumed by fish every day, so it is necessary to have the availability of flocs in the rearing medium or not cause blooming microorganisms due to excess floc volume. Therefore, it is necessary to research the effect of the frequency of probiotics from swamps on the formation of biofloc and catfish production.

Commented [W4]: dentified from swamp waters there are several types of probiotics such as Bacillus sp. and Streptomyces sp. This type can be used as a starter in a biofloc system for floc formation. Reported by Wijayanti et al. (2021), administering probiotics from swamps with a frequency of 1 time for 42 days of maintenance resulted in a floc volume of 40 mL L-1 (42nd day)

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Materials and Methods

Location and time of research

This research was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University in December 2022–January 2023.

Research Materials

The materials used were catfish seeds (7 ± 0.5 cm in length) and bacteria *Bacillus* sp., *Streptomyces* sp., molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tool used is a tarpaulin pool with a round diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, imhoff cone, loop needle, erlenmeyer, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter and ammonia test kit.

Research Design

This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given is the difference in the frequency of giving probiotics from swamps, which are as follows:
P1 = 1 time during 42 days of rearing
P2 = 2 times during 42 days of rearing

Work Procedure

Swamp Origin Probiotic Culture

Pure culture obtained from the isolation of bacteria from swamps *Bacillus* sp. and *Streptomyces* sp. results of previous studies (Wijayanti *et al*., 2018) liquid culture was carried out. Bacterial liquid culture *Bacillus* sp. was done on the media Nutrient Broth (NB) liquid by taking one ose of bacteria for deep culture Erlenmeyer, which already contains NB 20 mL media. In contrast, *Streptomyces* sp. was done on the media Yeast Malt (YM) liquid by taking one ose of bacteria and culturing in liquid YM media 20 mL deep Erlenmeyer. The two bacteria were agitated with a magnetic hot plate stirrer for three days for *Bacillus* sp. and five days for *Streptomyces* sp. The density of bacteria obtained from liquid culture results was calculated using the cup counting or TPC method (Total Plate Count). Then, do the mixing of bacteria with the formulation. Liquid media for storage media formulations in 5% molasses and filled with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish were reared in 6 round ponds made of tarpaulin with a diameter of 2 m and a height of 1.2 m. Rearing begins with pond preparation, including cleaning, water filling, and pond incubation. The pond is cleaned by brushing the entire inside of the pond and drying it for one day to kill the pathogens. Then, fill with water as high as 0.7 m and incubate for three days (Ma'ruf, 2016). Then salt at a dose of 1 kg m^{-3} and camphor dolomite 50 g m^{-3} (Sucipto *et al*., 2018) were added to the rearing medium and incubated for one day (Wijayanti *et al*., 2021). Installation of aerators is carried out at 4 points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process uses catfish measuring 7 ± 0.5 cm with a stocking density of 500 m^{-3} (Ma'ruf, 2016) and maintained for 42 days. Fish stocking was carried out in the morning when water conditions were normal and acclimatized for seven days to reduce stress on the fish. During rearing, probiotics were added to the biofloc pond at a density of bacteria *Bacillus* sp. 10^5 CFU mL^{-1} and *Streptomyces* sp. 10^5 CFU mL^{-1} (Wijayanti *et al*., 2020) with a frequency according to treatment, namely (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. During rearing, the addition of a carbon source in the form of molasses was also carried out at a dose of 200 mL m^{-3} with a frequency of once every seven days (days 0, 7, 14, 21, 28, 35) (Putra *et al*., 2017). Fish are fed using commercial feed with a

39-41% protein content. The feeding frequency is three times daily, namely at 08.00 WIB, 12.00, and 16.00 WIB with the at satiation. If there are dead fish, the fish are weighed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental pond unit.

Research Parameters

Volume floc and Floc Composition

Floc volume was measured using an Imhoff cone by taking media water maintenance at 1 point of collection as much as 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on days 7, 14, 21, 28, 35 and 42. After measuring the floc's volume, the precipitated floc is taken to observe the composition of floc microorganisms. Floc composition was microscopically observed (40x magnification) on days 0, 1, 21, and 42.

Total Bacterial Colonies

Total bacterial colonies formed in the rearing medium were counted with the cup count method by taking water as a maintenance medium. After The sample water is diluted through dilution steps 10-5, 10-7, 10-9. Bacterial density calculations were done on days 0 (before adding probiotics from swamps), 1, 21, and 42. Growing bacterial colonies were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1) = Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth

The formula used to measure absolute weight growth, according to Hopkins (1992) is:

$$W = Wt - Wo$$

Information:

W	=	Growth in absolute weight of fish kept (g)
Wt	=	Average fish weight at the end of the study (g)
Wo	=	Average fish weight at the start of the study (g)

The formula used to measure absolute length growth, according to Hopkins (1992), is:

$$L = Lt - Lo$$

Information:

L	=	Absolute length growth of reared fish (cm)
Lt	=	Average length of fish at the end of the study (cm)
Lo	=	Average fish length at the start of the study (cm)

Feed Efficiency

Feed efficiency (FE) is calculated based on a formula based on Afrianto & Liviawaty (2005):

$$FE\ (\%) = \frac{(Wt+D)-Wo}{F}\ x\ 100$$

Information:

FE	=	Feed efficiency (%)
Wt	=	Fish biomass at the end of the study (g)
Wo	=	Fish biomass at the start of the study (g)
D	=	Biomass of fish that died during the study (g)
F	=	Amount of fish feed given during the study (g)

Survival Rate (SR)

The survival rate is calculated using a formula based on Aliyu-Paiko*et al.* (2010) as follows:

$$SR\ (\%) = \frac{Nt}{No}\ x\ 100$$

Information:

SR	=	Survival (%)
Nt	=	The final quantity of fish at the end of rearing (g)
No	=	The initial quantity of fish at initial rearing (g)

Biomass Production

The formula can calculate the level of biomass production according to Shang (1982):

$$P\ (g) = W\ x\ N$$

Information:

P	=	Production of biomass (g)
W	=	Average weight of fish at the end of rearing (g)
N	=	The final quantity of fish at the end of rearing (g)

Water quality

Water quality parameters included temperature and pH, measured every day at 08.00 WIB and 16.00 WIB. Dissolved oxygen level, TDS (*Total Dissolved solid),* and ammonia were measured every seven days (day 0, 7, 14, 21, 28, 35, 42) once in 42 days of the rearing period.

Data analysis

Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively.

Results and Discussion

Volume Floc

The results of the T-test analysis of flock volume during rearing are presented in Table 1.

Table 1. Data and results of the T-test analysis of flock volume during rearing

Days to-	Volume flock (mL L^{-1}) P1	P2
7	6.00±2.00	5.33±3.06
14	11.67±2.89	13.00±3.00
21	17.00±2.65	21.67±2.89
28	35.00±5.00^a	53.33±5.77^b
35	43.33±7.64^a	66.67±11.55^b
42	48.33±2.89^a	68.33±10.41^b

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the results of the T-test analysis showed that floc volume on the Based on the results of the T-test analysis showed that the floc volume in treatment P1 was not significantly different from treatment P2 on days 7, 14, and 21 and was significantly different on days 28, 35, and 42. Floc volume is the amount of suspended solids over a certain period in an inverted conical container (Effendi, 2003). The highest floc volume was found in the P2 treatment given probiotics with a frequency of 2 times during 42 days of rearing, reaching 68.33 mL $L^{-1,}$ while P1 is 48.33 mL L^{-1}. The floc volume obtained in this study was higher than that in the study by Wijayanti *et al.* (2021), which is 40 mL L^{-1} (42nd day). This is presumably due to the influence of the time-frequency of giving probiotics to the rearing medium. According to De Schryver *et al.* (2008), the factors that influence the formation of bio floc are the administration of probiotics (starter) with floc-forming microbial composition, agitation intensity by aeration device, organic carbon source, and water quality. This is also reinforced by the results of Malaputra *et al.* (2016), namely the administration of commercial probiotics with a frequency of 14 times (70 days of rearing) resulted in a floc volume of 120 mL L^{-1} higher than the frequency of 7 times which is only 80 mL L^{-1}. Based on the results of this study, it can be stated that the more often probiotics are given to the rearing medium, the more volume will increase. The maximum floc volume for catfish farming is 100 mL L^{-1}. When the floc volume exceeds the limit, partial water removal can be done and replaced with new water, around 70-80% of the total water volume of the preservation pond (Zaidy, 2022).

In addition to the factor of providing probiotics from swamps as a starter containing heterotrophic bacteria, the increase in floc volume every week in this study was thought to be due to the C/N ratio. The C/N ratio can affect the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), the C/N ratio >10 in fish farming activities is the optimum ratio for the formation of biofloc. Meanwhile, according to Avnimelech *et al.* (1999) required a C/N ratio >15. Therefore, it is necessary to add organic carbon from outside, such as molasses. This study added extra organic carbon in molasses every seven days at a dose of 200 mL m^{-3} (Putra *et al.*, 2017).

Floc Composition

The composition of the flocs in this study is presented in Table 2.

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Table 2. Floc composition

Floc-forming microbes	Treatment P1	P2
Chlorophyta	✓	✓
Cyanophyta	✓	✓
Protozoa	✓	✓
Coelenterata	-	✓
Rotifers	✓	✓
Arthropods	-	✓

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Based on observations of floc composition in the treatment of probiotics from swamps with P2 frequencies more diverse than P1, namely chlorophyta, cyanophyta, protozoa, coelenterata, rotifers, and arthropods. Whereas in P1, only chlorophyta, cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), the diversity in the composition of the floc-forming microbes is thought to be due to the effect of giving probiotics, which is in line with the increased volume of floc in the rearing medium so that the microbes in the rearing containers can grow properly. Wati (2021) also produced the same floc composition as arthropods, chlorophyta, cyanophyta, protozoa, and rotifers. The composition of the flocs in the form of zooplankton in this study were protozoa, coelenterata, rotifers, arthropods, and phytoplankton in the form of chlorophyta and cyanophyta. This is following the opinion of De Schryver *et al.* (2008), namely the composition of floc in the form of a heterogeneous combination of microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach sizes <1000 µm.

Total Bacterial Colonies

The total bacterial colonies on the rearing medium are presented in Table 3.

Table 3. Data and analysis of T-test total bacterial colonies in flocs

Days to-	Total bacterial colonies (x 10^9 CFU mL^{-1})	
	P1	P1
0	2.77±1.00	2.77±1.00
1	13.23±2.82	13.23±2.82
21	5.56±2.01	5.56±2.01
42	8.42±3.19^{a}	8.42±3.19^{a}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the total bacterial colonies in the P1 treatment were not significantly different from the P2 treatment on days 0, 1, and 21 and were significantly different on the 42nd day. This study's bacterial density range was between 2.77 and 19.73 x 10^9 CFU mL^{-1}. After stocking probiotics from swamps on rearing media in treatments P1 and P2, total bacterial colonies increased on day 1, and day 21 decreased. On the 42nd day, the bacterial density increased again with a higher P2 bacterial density of 19.73 x10^9 CFU mL^{-1} compared to P1, which is 8.42 x10^9 CFU mL^{-1}. The high density of bacteria at P2 was thought to be due to adding probiotics again on the 21st day. In research, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish rearing by adding probiotics up to x10^{14} CFU mL^{-1}.

Meanwhile, according to Sitorus *et al.* (2019), the density of bacteria can reach x10^9 CFU mL^{-1}. Widnyana (2016) states that on biofloc technology, total bacterial colonies ranged from 10^3-10^4 CFU mL^{-1}, while the system has a bacterial range of 10^8-10^{10} CFU mL^{-1}. This is caused by the system having a high intensity of sunlight as one of the supporting factors in the growth of bacteria. The high value of the total density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, which is assisted by their effectiveness in breaking down organic matter (Adharani *et al.*, 2016).

Absolute Growth, Feed Efficiency, Survival, and Biomass Production

Based on the research results, absolute growth data, feed efficiency, and survival of catfish for 42 days of rearing are presented in Table 4.

Table 4. Data and results of absolute growth T-test analysis, feed efficiency, survival, and biomass production during rearing

Parameter	Treatment	
	P1	P2
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24^{a}	151.90 ± 7.98^{b}
Life sustainability (%)	81.54 ± 1.59	89.33 ±6,21
Biomass production (g)	17439.14 ± 1346.55^{a}	24639.50 ± 1344.51^{b}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the analysis of the T-test showed that the growth in absolute length and absolute weight growth of catfish in treatment P1 was not significantly different from treatment P2. The absolute length growth and weight growth values of probiotics from swamps P2 were 8.18 cm and 19.30 g, higher than P1, which were 7.16 cm and 16.42 g. The results of this study indicate that fish use the floc as additional feed to increase fish growth. Catfish can utilize the available floc in the rearing medium as additional feed because it is high in protein and can increase fish growth (Putra *et al.*, 2017). In research, Wijaya *et al.* (2016) floc contains 42.42% protein, 92.15% water, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract material (BETN). According to Febriyanti *et al.* (2018), heterotrophic bacterial communities that accumulate in the rearing medium will form flocs (clumps) that can be used as a feed source for fish. Therefore, giving probiotics from swamps in the form of heterotrophic bacteria such as *Bacillus* sp.

Furthermore, *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. The bacteria *Streptomyces* sp. in fish farming can also be applied as a growth promoter (Cruz *et al.*, 2012). The results of fish growth will affect the value of feed efficiency.

The analysis of the T-test showed that the efficiency of catfish feed in treatment P1 was significantly different from treatment P2. The feed efficiency value for P2 was higher, namely 151.90%, while P1 was 104.97%. If the feed efficiency value is converted to a value feed conversion ratio (FCR) then FCR P1 is 0.96 while P2 is 0.66. The FCR value in this study was better than the research of Wijayanti *et al.* (2021), which is 0.97. Putra *et al.* (2017) researched that administering probiotics to catfish rearing media with biofloc technology also resulted in feed efficiency between 88.17-110.86%. This

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study's high feed efficiency value is also suspected because fish use flocks as additional feed. De Schryver *et al.* (2008) stated that using flocks as additional feed is important in increasing feed efficiency. This shows that swamp probiotics can form flocs, which fish then eat as additional feed for fish.

The analysis of the T-test showed that the survival of catfish in treatment P1 was not significantly different from treatment P2. Survival in this study was quite good. The highest yield was in P2, 89.33%, followed by P1, 81.54%. The results of this study are close to the research of Wijayanti *et al.* (2021), namely 87.57% with a rearing period of 90 days. Administration of probiotics from swamps in the form of a combination of bacteria *Bacillus* sp. and bacteria *Streptomyces* sp., the percentage of fish survival can provide defense against pathogens by increasing non-specific immunity in the fish's immune system and maintaining a balance of water quality so that fish can survive (Wijayanti *et al.*, 2020). Growth and survival will affect the production value of fish biomass.

The analysis of the T-test showed that the production of catfish biomass in treatment P1 was significantly different from treatment P2. The production value of P2's biomass was 24,639.50 g, higher than that of P1, which was 17,439.14 g. Giving probiotics is thought to increase the production value of catfish in this biofloc technology. The biomass determines production at the end of cultivation and the survival of fish at the end of cultivation, so the higher the fish that can survive until the end of rearing, the more fish production will be produced (Anam *et al.*, 2017). Based on the results of absolute growth data and feed efficiency in this study, probiotics from swamps can form flocks used by fish as additional feed. Giving probiotics from swamps is also useful in preventing pathogenic bacteria to prevent death in fish. Therefore, giving probiotics from swamps can increase fish growth and survival, resulting in increased fish production.

Water quality

Temperature and pH

Based on the research results, the temperature and pH data of the rearing media are presented in Table 5.

Table 5. Data and results of temperature and pH T-test analysis

Treatment	Temperature (ºC)	pH
P1	28.85-29.22^{a}	7.27-7.40^{a}
P2	29.48-29.59^{b}	7.39-7.42^{b}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the temperature and pH of the catfish-rearing media in treatment P1 were not significantly different from those in treatment P2. The temperature of the rearing medium in this study ranged from 28.85-29.59ºC. According to BSN (2014), the temperature value for raising catfish is 25-30ºC. Catfish will experience slow growth when the temperature is 16-24ºC or 31-32ºC, and the fish will die if the temperature is below 16ºC or above 32ºC (Pujiharsono & Kurnianto, 2020). The value of the degree of acidity or pH in the rearing medium ranges from 7.27 to 7.42. BSN (2014) states that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth when the pH is 4-6.4 or pH 8.6-11, and the fish will die if the pH is below four or above 11 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The results of the DO T-test analysis during rearing are presented in Table 6.

Table 6. Data and results of DO T-test analysis during rearing

Days to-	DO (mg L^{-1})	
	P1	P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27^{a}	5.25±0.44^{b}
21	4.47±0.03^{a}	4.98±0.33^{b}
28	4.88±0.12^{a}	5.72±0.42^{b}
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The results of the T-test analysis show that Dissolved Oxygen (DO) or dissolved oxygen in treatment P1 was not significantly different from treatment P2 on days 0,7,35 and 42, but significantly different on days 14, 21 and 28. Based on Table 4.6. the frequency of probiotics from swamp P2 (2 times for 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study ranged from 3.91 to 5.95 mg L^{-1}. According to BSN (2014), the dissolved oxygen value for catfish is >3 mg L^{-1}. Therefore, the DO value in this study is still relatively safe for catfish farming. This is because the rearing container is equipped with aeration of 4 points. Besides that, it also comes from photosynthesis results from phytoplankton and oxygen diffusion from the air (Putra *et al.*, 2014). Based on this, it is suspected that the increase in DO at the end of P1 rearing is better than P2. Based on research by Agustina (2018), phytoplankton from the

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chlorophyte division can increase dissolved oxygen levels in swamp water cultivation media by 63.63%. In research, Wijayanti *et al.* (2020) also produced a combination of chlorophyta and probiotics from swamps containing bacteria *Bacillus* sp. Furthermore, *Streptomyces* sp. is the best treatment for dissolved oxygen concentrations in fish culture media> 3 mg L^{-1}. The results of research by Kurniawan and Utama (2018) also yield DO values for catfish rearing >6 mg L^{-1} by administering probiotics *Bacillus* sp. as according to Prihanto *et al.* (2021) bacteria *Bacillus* sp. can improve water quality.

Ammonia

The results of the ammonia T-test analysis during rearing are presented in Table 7.

Table 7. Data and results of ammonia T-test analysis during rearing

Days to-	Ammonia (mg L^{-1})	
	P1	P2
0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23^{a}	0.69±0.47^{b}
35	1.59±0.38^{a}	0.66±0.37^{b}
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the ammonia in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 21, and 42 but was significantly different on days 28 and 35. Based on Table 4.7 shows that the administration of probiotics from swamp P2 (2 times for 42 days of rearing) is proven to reduce ammonia levels in rearing media compared to P1 (1 time for 42 days of rearing). The ammonia value in this study was high, ranging from 0.45 to 1.62 mg L^{-1}, while in research by Wijayanti *et al.* (2021), it is 0.27 mg L^{-1}. The value of ammonia for catfish farming on the formation of biofloc is <1 mg L^{-1} (Wijaya *et al.*, 2016). This high value of ammonia can cause death in fish due to the toxic nature of the ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion results, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). The high ammonia value in this study is likely due to the lack of a C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in water. Meanwhile, according to Hargreaves (2006), at a C/N ratio <10, heterotrophic bacteria will release ammonia into their environment. Research results Bakar *et al.* (2015) also stated that ammonia reduction in catfish farming using biofloc technology using a C/N 15 ratio could eliminate ammonia by 93.56% on the 12th day while the C/N 10 ratio on the 12th day was only 11.01%. Furthermore, only the maximum is on the 30th day, namely 98.51%.

Total Dissolved Solid (TDS)

The results of the TDS T-test analysis during rearing are presented in Table 8.

Table 8. Data and results of TDS T-test analysis during rearing

Days to-	TDS (mg L^{-1})	
	P1	P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76^{b}	872.67±41.55^{a}
28	804.17±59.93	757.67±136.93
35	810.00±57.38^{b}	717.33±21.37^{a}
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show

Based on the results of the T-test analysis, it shows that Total Dissolved Solid (TDS) in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 28, and 42 but significantly different on days 21, and 35. Based on Table 4.8. administration of probiotics from swamps P2 (2 times during 42 days of rearing) can reduce TDS values compared to P1 (1 time during 42 days of rearing). TDS values in this study ranged from 717.33 to 1014.83 mg L^{-1}. Catfish can still tolerate the measurement results of these values to live. PPRI No.82 (2001) states that TDS's standard water quality value in fish farming activities is 1000 mg L^{-1}. The high level of TDS on the 21st day, especially in the P1 treatment, is thought to be due to the administration of commercial drugs to prevent dead fish containing organic and inorganic compounds. This follows the statement of Rinawati *et al.* (2016), who stated that large amounts of organic and inorganic compounds can result in high levels of TDS in water.

Conclusion

Based on the results of the research that has been done, it can be concluded that giving probiotics from swamps with a frequency of 2 times for 42 days of rearing (P2) can form biofloc resulting in a floc volume of 68.33 mg L^{-1}, growth in length and absolute weight of 8.18 cm and 19.30 g, feed

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efficiency of 135.24%, survival of 89.33% and biomass production 24,639.50 g with water quality of 28.85-29.59ºC, pH 7.27-7.42, DO 3,91-5,72 mg L^{-1}, ammonia 0,45-1,15 mg L^{-1} and TDS 717,33-885,50 mg L^{-1}.

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Frequency of Probiotics from Swamp for Formation of Biofloc and Production of Catfish (*Clarias* sp.)

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ABSTRACT

Catfish (*Clarias* sp.) cultivated with a combination ~~of the addition~~ of probiotics in ~~the~~ biofloc technology are thought to increase production. The frequency of adding probiotics from swamps to cultured water media has never been studied to ensure flocks' availability in rearing media. This study aimed to determine the appropriate frequency of probiotics from swamps in ~~the~~ biofloc technology to improve the parameters of successful cultivation, especially ~~increasing~~ catfish production and biofloc formation. This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given was different in the frequency of giving probiotics from swamps: (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. The results showed that P2 was the best treatment with a floc volume of 68.33 mL L^{-1}, absolute length growth 8.18 cm, absolute weight growth 19.30 g, feed efficiency 135.24 %, survival 89.33%, biomass production 24639.50 g, temperature 28.85-29.59ºC, pH 7.27-7.42, dissolved oxygen (DO) 3.91-5.72 mg L^{-1}, ammonia 0.45-1.15 mg L^{-1} and total dissolved solid (TDS) 717.33-885.50 mg L^{-1}. Therefore, probiotics from swamps should be given to fish culture media with a frequency of 2 times during 42 days of rearing or once every 21 days.

Introduction

Catfish (*Clarias* sp.) is a freshwater fishery commodity widely cultivated due to high market demand. According to data from the Statistics from the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia (2022), catfish production in Indonesia in the enlargement cultivation sector in 2019 was 289 thousand tons and reached 384 thousand tons in 2020, while in 2021, it was 360 thousand tons. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and with an environmental perspective by minimizing waste disposal in the surrounding waters (Fauzi *et al.*, 2022). According to Putra *et al.* (2017), technology biofloc by utilizing probiotics in the form of heterotrophic bacteria can produce natural feed from the flocs that are formed, thereby increasing the value of feed efficiency and improving water quality.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the media. Swamp microorganisms identified are *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti*et al.*, 2018). Giving probiotics from swamps (*Bacillus* sp. and *Streptomyces* sp.) has been studied in feeding the agility fish (Tanbiyaskur *et al.*, 2022) and the rearing medium with biofloc technology in catfish (Wijayanti *et al.*, 2021) and snakehead fish (Wati, 2021; Anil, 2022). In this study, swamp probiotics can increase growth, feed efficiency, fish survival, and aquaculture water quality (Wijayanti *et al.*, 2020).

Probiotics from swamps containing bacteria *Bacillus* sp. and *Streptomyces* sp. can be used as a starter in the biofloc system for floc formation. In research by Wijayanti *et al.* (2021), administration of probiotics from swamps with a frequency of 1 time for 42 days of rearing resulted in a floc volume of 40 mL L^{-1}

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(42nd day). Using these flocs for 60 days of rearing resulted in the final average weight and length growth from the initial average of 7.16 g and 9.50 cm to 36.95 g and 18.50 cm and FCR 0.97. Floc volume results (Wijayanti *et al*., 2021) are lower than Bakar *et al*. (2015) research results, whose floc volume reached 92.5 mL L^{-1}. According to Zaidy (2022), the maximum floc volume for catfish is 100 mL L^{-1}. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water, around 70-80% of the water volume. These flocks must continue to be available in the rearing medium in sufficient quantities as natural food for fish to increase fish growth and improve feed efficiency and water quality. According to Feroza *et al*. (2021), the flocs in the rearing media will decrease because they are consumed by fish every day, so it is necessary to have the availability of flocs in the rearing medium or not cause blooming microorganisms due to excess floc volume. Therefore, it is necessary to research the effect of the frequency of probiotics from swamps on the formation of biofloc and catfish production.

Materials and Methods

Location and time of research

This research was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University in December 2022–January 2023.

Research Materials

The materials used were catfish seeds (7 ± 0.5 cm in length) and bacteria *Bacillus* sp., *Streptomyces* sp., molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tool used is a tarpaulin pool with a round diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, imhoff cone, loop needle, erlenmeyer, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter and ammonia test kit.

Research Design

This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given is the difference in the frequency of giving probiotics from swamps, which are as follows:

P1 = 1 time during 42 days of rearing
P2 = 2 times during 42 days of rearing

Work Procedure

Swamp Origin Probiotic Culture

Pure culture obtained from the isolation of bacteria from swamps *Bacillus* sp. and *Streptomyces* sp. results of previous studies (Wijayanti *et al*., 2018) liquid culture was carried out. Bacterial liquid culture *Bacillus* sp. was done on the media Nutrient Broth (NB) liquid by taking one ose of bacteria for deep culture Erlenmeyer, which already contains NB 20 mL media. In contrast, *Streptomyces* sp. was done on the media Yeast Malt (YM) liquid by taking one ose of bacteria and culturing in liquid YM media 20 mL deep Erlenmeyer. The two bacteria were agitated with a magnetic hot plate stirrer for three days for *Bacillus* sp. and five days for *Streptomyces* sp. The density of bacteria obtained from liquid culture results was calculated using the cup counting or TPC method (Total Plate Count). Then, do the mixing of bacteria with the formulation. Liquid media for storage media formulations in 5% molasses and filled with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish were reared in 6 round ponds made of tarpaulin with a diameter of 2 m and a height of 1.2 m. Rearing begins with pond preparation, including cleaning, water filling, and pond incubation. The pond is cleaned by brushing the entire inside of the pond and drying it for one day to kill the pathogens. Then, fill with water as high as 0.7 m and incubate for three days (Ma'ruf, 2016). Then salt at a dose of 1 kg m^{-3} and camphor dolomite 50 g m^{-3} (Sucipto *et al*., 2018) were added to the rearing medium and incubated for one day (Wijayanti *et al*., 2021). Installation of aerators is carried out at 4 points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process uses catfish measuring 7 ± 0.5 cm with a stocking density of 500 m^{-3} (Ma'ruf, 2016) and maintained for 42 days. Fish stocking was carried out in the morning when water conditions were normal and acclimatized for seven days to reduce stress on the fish. During rearing, probiotics were added to the biofloc pond at a density of bacteria *Bacillus* sp. 10^5 CFU mL^{-1} and *Streptomyces* sp. 10^5 CFU mL^{-1} (Wijayanti *et al*., 2020) with a frequency according to treatment, namely (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. During rearing, the addition of a carbon source in the form of molasses was also carried out at a dose of 200 mL m^{-3} with a frequency of once every seven days (days 0, 7, 14, 21, 28, 35) (Putra *et al*., 2017). Fish are fed using commercial feed with a

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39-41% protein content. The feeding frequency is three times daily, namely at 08.00 WIB, 12.00, and 16.00 WIB with the at satiation. If there are dead fish, the fish are weighed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental pond unit.

Research Parameters

Volume floc and Floc Composition

Floc volume was measured using an Imhoff cone by taking media water maintenance at 1 point of collection as much as 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on days 7, 14, 21, 28, 35 and 42. After measuring the floc's volume, the precipitated floc is taken to observe the composition of floc microorganisms. Floc composition was microscopically observed (40x magnification) on days 0, 1, 21, and 42.

Total Bacterial Colonies

Total bacterial colonies formed in the rearing medium were counted with the cup count method by taking water as a maintenance medium. After The sample water is diluted through dilution steps 10-5, 10-7, 10-9. Bacterial density calculations were done on days 0 (before adding probiotics from swamps), 1, 21, and 42. Growing bacterial colonies were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1) = Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth

The formula used to measure absolute weight growth, according to Hopkins (1992) is:

W= Wt – Wo

Information:

W	=	Growth in absolute weight of fish kept (g)
Wt	=	Average fish weight at the end of the study (g)
Wo	=	Average fish weight at the start of the study (g)

The formula used to measure absolute length growth, according to Hopkins (1992), is:

L= Lt – Lo

Information:

L	=	Absolute length growth of reared fish (cm)
Lt	=	Average length of fish at the end of the study (cm)
Lo	=	Average fish length at the start of the study (cm)

Feed Efficiency

Feed efficiency (FE) is calculated based on a formula based on Afrianto & Liviawaty (2005):

$$FE\ (\%) = \frac{(Wt+D)-Wo}{F}\ x\ 100$$

Information:

FE	=	Feed efficiency (%)
Wt	=	Fish biomass at the end of the study (g)
Wo	=	Fish biomass at the start of the study (g)
D	=	Biomass of fish that died during the study (g)
F	=	Amount of fish feed given during the study (g)

Survival Rate (SR)

The survival rate is calculated using a formula based on Aliyu-Paiko*et al.* (2010) as follows:

$$SR\ (\%) = \frac{Nt}{No}\ x\ 100$$

Information:

SR	=	Survival (%)
Nt	=	The final quantity of fish at the end of rearing (g)
No	=	The initial quantity of fish at initial rearing (g)

Biomass Production

The formula can calculate the level of biomass production according to Shang (1982):

$$P\ (g) = W\ x\ N$$

Information:

P	=	Production of biomass (g)
W	=	Average weight of fish at the end of rearing (g)
N	=	The final quantity of fish at the end of rearing (g)

Water quality

Water quality parameters included temperature and pH, measured every day at 08.00 WIB and 16.00 WIB. Dissolved oxygen level, TDS (*Total Dissolved solid),* and ammonia were measured every seven days (day 0, 7, 14, 21, 28, 35, 42) once in 42 days of the rearing period.

Data analysis

Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively.

Results and Discussion

Volume Floc

The results of the T-test analysis of flock volume during rearing are presented in Table 1.

Table 1. Data and results of the T-test analysis of flock volume during rearing

Days to-	Volume flock (mL L^{-1})	
	P1	P2
7	6.00±2.00	5.33±3.06
14	11.67±2.89	13.00±3.00
21	17.00±2.65	21.67±2.89
28	35.00±5.00[a]	53.33±5.77[b]
35	43.33±7.64[a]	66.67±11.55[b]
42	48.33±2.89[a]	68.33±10.41[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the results of the T-test analysis showed that floc volume on the Based on the results of the T-test analysis showed that the floc volume in treatment P1 was not significantly different from treatment P2 on days 7, 14, and 21 and was significantly different on days 28, 35, and 42. Floc volume is the amount of suspended solids ~~over a certain period in an inverted conical container~~ an inverted conical container over a certain period (Effendi, 2003). The highest floc volume was found in the P2 treatment given probiotics with a frequency of 2 times during 42 days of rearing, reaching 68.33 mL $L^{-1,}$ while P1 is 48.33 mL L^{-1}. The floc volume obtained in this study was higher than that in the study by Wijayanti *et al.* (2021), which is 40 mL L^{-1} (42nd day). This is presumably due to the influence of the time-frequency of giving probiotics to the rearing medium. According to De Schryver *et al.* (2008), the factors that influence the formation of bio floc are the administration of probiotics (starter) with floc-forming microbial composition, agitation intensity by aeration device, organic carbon source, and water quality. This is also reinforced by the results of Malaputra *et al.* (2016), namely the administration of commercial probiotics with a frequency of 14 times (70 days of rearing) resulted in a floc volume of 120 mL L^{-1} higher than the frequency of 7 times which is only 80 mL L^{-1}. Based on the results of this study, it can be stated that the more often probiotics are given to the rearing medium, the more volume will increase. The maximum floc volume for catfish farming is 100 mL L^{-1}. When the floc volume exceeds the limit, partial water removal can be done and replaced with new water, around 70-80% of the total water volume of the preservation pond (Zaidy, 2022).

In addition to the factor of providing probiotics from swamps as a starter containing heterotrophic bacteria, the increase in floc volume every week in this study was thought to be due to the C/N ratio. The C/N ratio can affect the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), the C/N ratio >10 in fish farming activities is the optimum ratio for the formation of biofloc. Meanwhile, according to Avnimelech *et al.* (1999) ~~required a C/N ratio >15~~, a C/N ratio >15 is required. Therefore, it is necessary to add organic carbon from outside, such as molasses. This study added extra organic carbon in molasses every seven days at a dose of 200 mL m^{-3} (Putra *et al.*, 2017).

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Floc Composition

The composition of the flocs in this study is presented in Table 2.

Table 2. Floc composition

Floc-forming microbes	Treatment	
	P1	P2
Chlorophyta	✓	✓
Cyanophyta	✓	✓
Protozoa	✓	✓
Coelenterata	-	✓
Rotifers	✓	✓
Arthropods	-	✓

Information: (✓) exists, (-) does not exist

Based on observations of floc composition in the treatment of probiotics from swamps with P2 frequencies more diverse than P1, namely chlorophyta, cyanophyta, protozoa, coelenterata, rotifers, and arthropods. Whereas in P1, only chlorophyta, cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), the diversity in the composition of the floc-forming microbes is thought to be due to the effect of giving probiotics, which is in line with the increased volume of floc in the rearing medium so that the microbes in the rearing containers can grow properly. Wati (2021) also produced the same floc composition as arthropods, chlorophyta, cyanophyta, protozoa, and rotifers. The composition of the flocs in the form of zooplankton in this study were protozoa, coelenterata, rotifers, arthropods, and phytoplankton in the form of chlorophyta and cyanophyta. This is following the opinion of De Schryver *et al.* (2008), namely the composition of floc in the form of a heterogeneous combination of microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach sizes <1000 μm.

Total Bacterial Colonies

The total bacterial colonies on the rearing medium are presented in Table 3.

Table 3. Data and analysis of T-test total bacterial colonies in flocs

Days to-	Total bacterial colonies (x 10^9 CFU mL^{-1})	
	P1	P1
0	2.77±1.00	2.77±1.00
1	13.23±2.82	13.23±2.82
21	5.56±2.01	5.56±2.01
42	8.42±3.19[a]	8.42±3.19[a]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the total bacterial colonies in the P1 treatment were not significantly different from the P2 treatment on days 0, 1, and 21 and were significantly different on the 42nd day. This study's bacterial density range was between 2.77 and 19.73 x 10^9 CFU mL^{-1}. After stocking probiotics from swamps on rearing media in treatments P1 and P2, total bacterial colonies increased on day 1, and day 21 decreased. On the 42nd day, the bacterial density increased again with a higher P2 bacterial density of 19.73 x10^9 CFU mL^{-1} compared to P1, which is 8.42 x10^9 CFU mL^{-1}. The high density of bacteria at P2 was thought to be due to adding probiotics again on the 21st day. In research, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish rearing by adding probiotics up to x10^{14} CFU mL^{-1}.

Meanwhile, according to Sitorus *et al.* (2019), the density of bacteria can reach x10^9 CFU mL^{-1}. Widnyana (2016) states that on biofloc technology, total bacterial colonies ranged from 10^3-10^4 CFU mL^{-1}, while the system has a bacterial range of 10^8-10^{10} CFU mL^{-1}. This is caused by the system having a high intensity of sunlight as one of the supporting factors in the growth of bacteria. The high value of the total density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, which is assisted by their effectiveness in breaking down organic matter (Adharani *et al.*, 2016).

Absolute Growth, Feed Efficiency, Survival, and Biomass Production

Based on the research results, absolute growth data, feed efficiency, and survival of catfish for 42 days of rearing are presented in Table 4.

Table 4. Data and results of absolute growth T-test analysis, feed efficiency, survival, and biomass production during rearing

Parameter	Treatment	
	P1	P2
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24[a]	151.90 ± 7.98[b]
Life sustainability (%)	81.54 ± 1.59	89.33 ±6,21
Biomass production (g)	17439.14 ± 1346.55[a]	24639.50 ± 1344.51[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the analysis of the T-test showed that the growth in absolute length and absolute weight growth of catfish in treatment P1 was not significantly different from treatment P2. The absolute length growth and weight growth values of probiotics from swamps P2 were 8.18 cm and 19.30 g, higher than P1, which were 7.16 cm and 16.42 g. The results of this study indicate that fish use the floc as additional feed to increase fish growth. Catfish can utilize the available floc in the rearing medium as additional feed because it is high in protein and can increase fish growth (Putra *et al.*, 2017). In research, Wijaya *et al.* (2016) floc contains 42.42% protein, 92.15% water, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract material (BETN). According to Febriyanti *et al.* (2018), heterotrophic bacterial communities that accumulate in the rearing medium will form flocs (clumps) that can be used as a feed source for fish. Therefore, giving probiotics from swamps in the form of heterotrophic bacteria such as *Bacillus* sp.

Furthermore, *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. The bacteria *Streptomyces* sp. in fish farming can also be applied as a growth promoter (Cruz *et al.*, 2012). The results of fish growth will affect the value of feed efficiency.

The analysis of the T-test showed that the efficiency of catfish feed in treatment P1 was significantly different from treatment P2. The feed efficiency value for P2 was higher, namely 151.90%, while P1 was 104.97%. If the feed efficiency value is converted to a value feed conversion ratio (FCR) then FCR P1 is 0.96 while P2 is 0.66. The FCR value in this study was better than the research of Wijayanti *et al.* (2021), which is 0.97. Putra *et al.* (2017) researched that administering probiotics to catfish rearing media with biofloc technology also resulted in feed efficiency between 88.17-110.86%. This

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study's high feed efficiency value is also suspected because fish use flocks as additional feed. De Schryver *et al.* (2008) stated that using flocks as additional feed is important in increasing feed efficiency. This shows that swamp probiotics can form flocs, which fish then eat as additional feed for fish.

The analysis of the T-test showed that the survival of catfish in treatment P1 was not significantly different from treatment P2. Survival in this study was quite good. The highest yield was in P2, 89.33%, followed by P1, 81.54%. The results of this study are close to the research of Wijayanti *et al.* (2021), namely 87.57% with a rearing period of 90 days. Administration of probiotics from swamps in the form of a combination of bacteria *Bacillus* sp. and bacteria *Streptomyces* sp., the percentage of fish survival can provide defense against pathogens by increasing non-specific immunity in the fish's immune system and maintaining a balance of water quality so that fish can survive (Wijayanti *et al.*, 2020). Growth and survival will affect the production value of fish biomass.

The analysis of the T-test showed that the production of catfish biomass in treatment P1 was significantly different from treatment P2. The production value of P2's biomass was 24,639.50 g, higher than that of P1, which was 17,439.14 g. Giving probiotics is thought to increase the production value of catfish in this biofloc technology. The biomass determines production at the end of cultivation and the survival of fish at the end of cultivation, so the higher the fish that can survive until the end of rearing, the more fish production will be produced (Anam *et al.*, 2017). Based on the results of absolute growth data and feed efficiency in this study, probiotics from swamps can form flocks used by fish as additional feed. Giving probiotics from swamps is also useful in preventing pathogenic bacteria to prevent death in fish. Therefore, giving probiotics from swamps can increase fish growth and survival, resulting in increased fish production.

Water quality

Temperature and pH

Based on the research results, the temperature and pH data of the rearing media are presented in Table 5.

Table 5. Data and results of temperature and pH T-test analysis

Treatment	Temperature (ºC)	pH
P1	28.85-29.22[a]	7.27-7.40[a]
P2	29.48-29.59[b]	7.39-7.42[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the temperature and pH of the catfish-rearing media in treatment P1 were not significantly different from those in treatment P2. The temperature of the rearing medium in this study ranged from 28.85-29.59ºC. According to BSN (2014), the temperature value for raising catfish is 25-30ºC. Catfish will experience slow growth when the temperature is 16-24ºC or 31-32ºC, and the fish will die if the temperature is below 16ºC or above 32ºC (Pujiharsono & Kurnianto, 2020). The value of the degree of acidity or pH in the rearing medium ranges from 7.27 to 7.42. BSN (2014) states that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth when the pH is 4-6.4 or pH 8.6-11, and the fish will die if the pH is below four or above 11 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The results of the DO T-test analysis during rearing are presented in Table 6.

Table 6. Data and results of DO T-test analysis during rearing

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Days to-	DO (mg L^{-1}) P1	DO (mg L^{-1}) P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27[a]	5.25±0.44[b]
21	4.47±0.03[a]	4.98±0.33[b]
28	4.88±0.12[a]	5.72±0.42[b]
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The results of the T-test analysis show that Dissolved Oxygen (DO) or dissolved oxygen in treatment P1 was not significantly different from treatment P2 on days 0,7,35 and 42, but significantly different on days 14, 21 and 28. Based on Table 4.6. the frequency of probiotics from swamp P2 (2 times for 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study ranged from 3.91 to 5.95 mg L^{-1}. According to BSN (2014), the dissolved oxygen value for catfish is >3 mg L^{-1}. Therefore, the DO value in this study is still relatively safe for catfish farming. This is because the rearing container is equipped with aeration of 4 points. Besides that, it also comes from photosynthesis results from phytoplankton and oxygen diffusion from the air (Putra *et al.*, 2014). Based on this, it is suspected that the increase in DO at the end of P1 rearing is better than P2. Based on research by Agustina (2018), phytoplankton from the

chlorophyte division can increase dissolved oxygen levels in swamp water cultivation media by 63.63%. In research, Wijayanti *et al.* (2020) also produced a combination of chlorophyta and probiotics from swamps containing bacteria *Bacillus* sp. Furthermore, *Streptomyces* sp. is the best treatment for dissolved oxygen concentrations in fish culture media> 3 mg L^{-1}. The results of research by Kurniawan and Utama (2018) also yield DO values for catfish rearing >6 mg L^{-1} by administering probiotics *Bacillus* sp. as according to Prihanto *et al.* (2021) bacteria *Bacillus* sp. can improve water quality.

Ammonia

The results of the ammonia T-test analysis during rearing are presented in Table 7.

Table 7. Data and results of ammonia T-test analysis during rearing

Days to-	Ammonia (mg L^{-1})	
	P1	P2
0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23[a]	0.69±0.47[b]
35	1.59±0.38[a]	0.66±0.37[b]
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the ammonia in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 21, and 42 but was significantly different on days 28 and 35. Based on Table 4.7 shows that the administration of probiotics from swamp P2 (2 times for 42 days of rearing) is proven to reduce ammonia levels in rearing media compared to P1 (1 time for 42 days of rearing). The ammonia value in this study was high, ranging from 0.45 to 1.62 mg L^{-1}, while in research by Wijayanti *et al.* (2021), it is 0.27 mg L^{-1}. The value of ammonia for catfish farming on the formation of biofloc is <1 mg L^{-1} (Wijaya *et al.*, 2016). This high value of ammonia can cause death in fish due to the toxic nature of the ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion results, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). The high ammonia value in this study is likely due to the lack of a C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in water. Meanwhile, according to Hargreaves (2006), at a C/N ratio <10, heterotrophic bacteria will release ammonia into their environment. Research results Bakar *et al.* (2015) also stated that ammonia reduction in catfish farming using biofloc technology using a C/N 15 ratio could eliminate ammonia by 93.56% on the 12th day while the C/N 10 ratio on the 12th day was only 11.01%. Furthermore, only the maximum is on the 30th day, namely 98.51%.

Total Dissolved Solid (TDS)

The results of the TDS T-test analysis during rearing are presented in Table 8.

Table 8. Data and results of TDS T-test analysis during rearing

Days to-	TDS (mg L^{-1})	
	P1	P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76[b]	872.67±41.55[a]
28	804.17±59.93	757.67±136.93
35	810.00±57.38[b]	717.33±21.37[a]
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show

Commented [Reviewer6]: maybe in the form of a graph

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Based on the results of the T-test analysis, it shows that Total Dissolved Solid (TDS) in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 28, and 42 but significantly different on days 21, and 35. Based on Table 4.8. administration of probiotics from swamps P2 (2 times during 42 days of rearing) can reduce TDS values compared to P1 (1 time during 42 days of rearing). TDS values in this study ranged from 717.33 to 1014.83 mg L^{-1}. Catfish can still tolerate the measurement results of these values to live. PPRI No.82 (2001) states that TDS's standard water quality value in fish farming activities is 1000 mg L^{-1}. The high level of TDS on the 21st day, especially in the P1 treatment, is thought to be due to the administration of commercial drugs to prevent dead fish containing organic and inorganic compounds. This follows the statement of Rinawati *et al.* (2016), who stated that large amounts of organic and inorganic compounds can result in high levels of TDS in water.

Conclusion

Commented [Reviewer7]: Provide recommendations on probiotics

Based on the results of the research that has been done, it can be concluded that giving probiotics from swamps with a frequency of 2 times for 42 days of rearing (P2) can form biofloc resulting in a floc volume of 68.33 mg L^{-1}, growth in length and absolute weight of 8.18 cm and 19.30 g, feed

efficiency of 135.24%, survival of 89.33% and biomass production 24,639.50 g with water quality of 28.85-29.59ºC, pH 7.27-7.42, DO 3,91-5,72 mg L^{-1}, ammonia 0,45-1,15 mg L^{-1} and TDS 717,33-885,50 mg L^{-1}.

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Frequency of Probiotics from Swamp for Formation of Biofloc and Production of Catfish (*Clarias* sp.)

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ABSTRACT

Catfish (*Clarias* sp.) cultivated with a combination of the addition of probiotics in biofloc technology are thought to increase production. The frequency of adding probiotics from swamps to cultured water media has never been studied to ensure flocks' availability in rearing media. This study aimed to determine the appropriate frequency of probiotics from swamps in biofloc technology to improve the parameters of successful cultivation, especially increasing catfish production and biofloc formation. This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given was different in the frequency of giving probiotics from swamps: (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively. The results showed that P2 was the best treatment with a floc volume of 68.33 mL L^{-1}, absolute length growth of 8.18 cm, absolute weight growth of 19.30 g, feed efficiency of 135.24 %, survival of 89.33%, biomass production of 24639.50 g, temperature of 28.85-29.59°C, pH of 7.27-7.42, dissolved oxygen (DO) of 3.91-5.72 mg L^{-1}, ammonia of 0.45-1.15 mg L^{-1}, and total dissolved solids (TDS) of 717.33-885.50 mg L^{-1}. Therefore, probiotics from swamps should be given to fish culture media with a frequency of 2 times during 42 days of rearing or once every 21 days.

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Introduction

Catfish (*Clarias* sp.) is a freshwater fishery commodity widely cultivated due to high market demand. According to data from the Statistics from the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia (2022), catfish production in Indonesia in the enlargement cultivation sector in 2019 was 289 thousand tons and reached 384 thousand tons in 2020, while in 2021, it was 360 thousand tons. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and with an environmental perspective by minimizing waste disposal in the surrounding waters (Fauzi *et al*., 2022). According to Putra *et al.* (2017), technology biofloc by utilizing probiotics in the form of heterotrophic bacteria can produce natural feed from the flocs that are formed, thereby increasing the value of feed efficiency and improving water quality.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the media. Swamp microorganisms identified are *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti*et al*., 2018). Giving probiotics from swamps (*Bacillus* sp. and *Streptomyces* sp.) has been studied in feeding the agility fish (Tanbiyaskur *et al*., 2022) and the rearing medium with biofloc technology in catfish (Wijayanti *et al*., 2021) and snakehead fish (Wati, 2021; Anil, 2022). Im another study has been reported previously that swamp probiotics can increase growth, feed efficiency, fish

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survival, and aquaculture water quality (Wijayanti *et al.*, 2020).

Identified from swamp waters there are several types of probiotics such as Bacillus sp. and Streptomyces sp. This type can be used as a starter in a biofloc system for floc formation. Reported by Wijayanti et al. (2021), administering probiotics from swamps with a frequency of 1 time for 42 days of maintenance resulted in a floc volume of 40 mL L-1 (42nd day). Using these flocs for 60 days of rearing resulted in the final average weight and length growth from the initial average of 7.16 g to 36.95 g and 9.50 cm to 18.50 cm. Floc volume results (Wijayanti *et al.*, 2021) are lower than Bakar *et al.* (2015) research results, whose floc volume reached 92.5 mL L^{-1}. According to Zaidy (2022), the maximum floc volume for catfish is 100 mL L^{-1}. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water, around 70-80% of the water volume. These flocks must continue to be available in the rearing medium in sufficient quantities as natural food for fish to increase fish growth and improve feed efficiency and water quality. According to Feroza *et al.* (2021), the flocs in the rearing media will decrease because they are consumed by fish every day, so it is necessary to have the availability of flocs in the rearing medium or not cause blooming microorganisms due to excess floc volume. Therefore, it is necessary to research the effect of the frequency of probiotics from swamps on the formation of biofloc and catfish production.

Materials and Methods

Location and time of research

This research was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University in December 2022–January 2023.

Research Materials

The materials used were catfish seeds (7 ± 0.5 cm in length) and bacteria *Bacillus* sp., *Streptomyces* sp., molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tool used is a tarpaulin pool with a round diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, imhoff cone, loop needle, erlenmeyer, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter and ammonia test kit.

Research Design

This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given is the difference in the frequency of giving probiotics from swamps, which are as follows:

P1 = 1 time during 42 days of rearing
P2 = 2 times during 42 days of rearing

Work Procedure

Swamp Origin Probiotic Culture

Pure culture obtained from the isolation of bacteria from swamps *Bacillus* sp. and *Streptomyces* sp. results of previous studies (Wijayanti *et al.*, 2018) liquid culture was carried out. Bacterial liquid culture *Bacillus* sp. was done on the media Nutrient Broth (NB) liquid by taking one ose of bacteria for deep culture Erlenmeyer, which already contains NB 20 mL media. In contrast, *Streptomyces* sp. was done on the media Yeast Malt (YM) liquid by taking one ose of bacteria and culturing in liquid YM media 20 mL deep Erlenmeyer. The two bacteria were agitated with a magnetic hot plate stirrer for three days for *Bacillus* sp. and five days for *Streptomyces* sp. The density of bacteria obtained from liquid culture results was calculated using the cup counting or Total Plate Count (TPC) method. Then, do the mixing of bacteria with the formulation. Liquid media for storage media formulations in 5% molasses and filled with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish were reared in 6 round ponds made of tarpaulin with a diameter of 2 m and a height of 1.2 m. Rearing begins with pond preparation, including cleaning, water filling, and pond incubation. The pond is cleaned by brushing the entire inside of the pond and drying it for one day to kill the pathogens. Then, fill with water as high as 0.7 m and incubate for three days (Ma'ruf, 2016). Then salt at a dose of 1 kg m^{-3} and camphor dolomite 50 g m^{-3} (Sucipto *et al.*, 2018) were added to the rearing medium and incubated for one day (Wijayanti *et al.*, 2021). Installation of aerators is carried out at 4 points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process uses catfish measuring 7 ± 0.5 cm with a stocking density of 500 m^{-3} (Ma'ruf, 2016) and maintained for 42 days. Fish stocking was carried out in the morning when water conditions were normal and acclimatized for seven days to reduce stress on the fish. During rearing, probiotics were added to the biofloc pond at a density of bacteria

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Bacillus sp. 10^5 CFU mL^{-1} and *Streptomyces* sp. 10^5 CFU mL^{-1} (Wijayanti *et al*., 2020) with a frequency according to treatment, namely (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. During rearing, the addition of a carbon source in the form of molasses was also carried out at a dose of 200 mL m^{-3} with a frequency of once every seven days (days 0, 7, 14, 21, 28, 35) (Putra *et al*., 2017). Fish are fed using commercial feed with a 39-41% protein content. The feeding frequency is three times daily, namely at 08.00 a.m, 12.00 a.m, and 04.00 p.m with the at satiation. If there are dead fish, the fish are weighed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental pond unit.

Research Parameters

Volume floc and Floc Composition

Floc volume was measured using an Imhoff cone by taking media water maintenance at 1 point of collection as much as 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on days 7, 14, 21, 28, 35 and 42. After measuring the floc's volume, the precipitated floc is taken to observe the composition of floc microorganisms. Floc composition was microscopically observed (40x magnification) on days 0, 1, 21, and 42.

Total Bacterial Colonies

Total bacterial colonies formed in the rearing medium were counted with the cup count method by taking water as a rearing medium, and counting the bacterial colonies using a colony counter. After The sample water is diluted through dilution steps 10^{-5}, 10^{-7}, 10^{-9}. Bacterial density calculations were done on days 0 (before adding probiotics from swamps), 1, 21, and 42. Growing bacterial colonies were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1) = Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth

The formula used to measure absolute weight growth, according to Hopkins (1992) is:

$$W = Wt - Wo$$

Information:

W = Growth in absolute weight of fish kept (g)
Wt = Average fish weight at the end of the study (g)
Wo = Average fish weight at the start of the study (g)

The formula used to measure absolute length growth, according to Hopkins (1992), is:

$$L = Lt - Lo$$

Information:

L = Absolute length growth of reared fish (cm)
Lt = Average length of fish at the end of the study (cm)
Lo = Average fish length at the start of the study (cm)

Feed Efficiency

Feed efficiency (FE) is calculated based on a formula based on Afrianto & Liviawaty (2005):

$$FE\ (\%) = \frac{(Wt+D)-Wo}{F} \times 100$$

Information:

FE = Feed efficiency (%)
Wt = Fish biomass at the end of the study (g)
Wo = Fish biomass at the start of the study (g)
D = Biomass of fish that died during the study (g)
F = Amount of fish feed given during the study (g)

Survival Rate (SR)

The survival rate is calculated using a formula based on Aliyu-Paiko*et al*. (2010) as follows:

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

Information:

SR = Survival (%)
Nt = The final quantity of fish at the end of rearing (g)
No = The initial quantity of fish at initial rearing (g)

Biomass Production

The formula can calculate the level of biomass production according to Shang (1982):

$$P\ (g) = W \times N$$

Information:

P = Production of biomass (g)
W = Average weight of fish at the end of rearing (g)
N = The final quantity of fish at the end of rearing (g)

Water quality

Water quality parameters included temperature and pH, measured every day at 08.00 a.m and 04.00 p.m. Temperature measurements (°C) using a thermometer, pH using a pH meter, Dissolved oxygen (DO) using a DO meter (mg L^{-1}), *Total Dissolved solid* (TDS) (mg L^{-1}) using a TDS meter, and ammonia (mg L^{-1}) using a spectrophotometer.

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Dissolved oxygen level, TDS, and ammonia were measured every seven days (day 0, 7, 14, 21, 28, 35, and 42) once in 42 days of the rearing period.

Data analysis

Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively.

Results and Discussion

Volume Floc

The results of the T-test analysis of flock volume during rearing are presented in Table 1.

Table 1. The results of the T-test analysis of flock of catfish (*Clarias* sp.) in biofloc system

Days to-	Volume flock (mL L^{-1}) P1	Volume flock (mL L^{-1}) P2
7	6.00±2.00	5.33±3.06
14	11.67±2.89	13.00±3.00
21	17.00±2.65	21.67±2.89
28	35.00±5.00^{a}	53.33±5.77^{b}
35	43.33±7.64^{a}	66.67±11.55^{b}
42	48.33±2.89^{a}	68.33±10.41^{b}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the results of the analysis showed that the floc volume in treatment P1 was not significantly different from treatment P2 on days 7, 14, and 21 and was significantly different on days 28, 35, and 42. Floc volume is the amount of suspended solids over a certain period in an inverted conical container (Effendi, 2003). The highest floc volume was found in the P2 treatment given probiotics with a frequency of 2 times during 42 days of rearing, reaching 68.33 mL $L^{-1,}$ while P1 is 48.33 mL L^{-1}. The floc volume obtained in this study was higher than that in the study by Wijayanti *et al.* (2021), which is 40 mL L^{-1} (42nd day). This is presumably due to the influence of the time-frequency of giving probiotics to the rearing medium. According to De Schryver *et al.* (2008), the factors that influence the formation of bio floc are the administration of probiotics (starter) with floc-forming microbial composition, agitation intensity by aeration device, organic carbon source, and water quality. This is also reinforced by the results of Malaputra *et al.* (2016), namely the administration of commercial probiotics with a frequency of 14 times (70 days of rearing) resulted in a floc volume of 120 mL L^{-1} higher than the frequency of 7 times which is only 80 mL L^{-1}. Based on the results of this study, it can be stated that the more often probiotics are given to the rearing medium, the more volume will increase. The maximum floc volume for catfish farming is 100 mL L^{-1}. When the floc volume exceeds the limit, partial water removal can be done and replaced with new water, around 70-80% of the total water volume of the preservation pond (Zaidy, 2022).

In addition to the factor of providing probiotics from swamps as a starter containing heterotrophic bacteria, the increase in floc volume every week in this study was thought to be due to the C/N ratio. The C/N ratio can affect the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), the C/N ratio >10 in fish farming activities is the optimum ratio for the formation of biofloc. Meanwhile, according to Avnimelech *et al.* (1999) required a C/N ratio >15. Therefore, it is necessary to add organic carbon from outside, such as molasses. This study added extra organic carbon in molasses every seven days at a dose of 200 mL m^{-3} (Putra *et al.*, 2017).

Floc Composition

The composition of the flocs in this study is presented in Table 2.

Table 2. Composition of catfish (*Clarias* sp.) flocs maintended in biofloc system

Floc-forming microbes	Treatment P1	Treatment P2
Chlorophyta	✓	✓
Cyanophyta	✓	✓
Protozoa	✓	✓
Coelenterata	-	✓
Rotifers	✓	✓
Arthropods	-	✓

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Based on observations of floc composition in the treatment of probiotics from swamps with P2 frequencies more diverse than P1, namely chlorophyta, cyanophyta, protozoa, coelenterata, rotifers, and arthropods. Whereas in P1, only chlorophyta, cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), the diversity in the composition of the floc-forming microbes is thought to be due to the effect of giving probiotics, which is in line with the increased volume of floc in the rearing medium so that the microbes in the rearing containers can grow properly. Wati (2021) also produced the same floc composition as arthropods, chlorophyta, cyanophyta, protozoa, and rotifers. The composition of the flocs in the form of zooplankton

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in this study were protozoa, coelenterata, rotifers, arthropods, and phytoplankton in the form of chlorophyta and cyanophyta. This is following the opinion of De Schryver *et al.* (2008), namely the composition of floc in the form of a heterogeneous combination of microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach sizes <1000 µm.

Total Bacterial Colonies

The total bacterial colonies on the rearing medium are presented in Table 3.

Table 3. The total bacterial colonies in the flocs of catfish (*Clarias* sp) in the biofloc system

Days to-	Total bacterial colonies ($x\ 10^9$ CFU mL^{-1})	
	P1	P2
0	2.77±1.00	2.77±1.00
1	13.23±2.82	14.89±3.88
21	5.56±2.01	6.65±3.07
42	8.42±3.19[a]	19.73±6.19[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the total bacterial colonies in the P1 treatment were not significantly different from the P2 treatment on days 0, 1, and 21 and were significantly different on the 42nd day. This study's bacterial density range was between 2.77 and 19.73 x 10^9 CFU mL^{-1}. After stocking probiotics from swamps on rearing media in treatments P1 and P2, total bacterial colonies increased on day 1, and day 21 decreased. The decrease in total bacterial colonies on day 21 is thought to be a lack of nutritional sources (macronutrient or micronutrient) for bacteria (Wijayanti *et al.*, 2020). According to Nasmia and Rifai (2020) the decrease in the number of bacterial colonies can be caused by a decrease in the number of carbon sources. On the 42nd day, the bacterial density increased again with a higher P2 bacterial density of 19.73 x10^9 CFU mL^{-1} compared to P1, which is 8.42 x10^9 CFU mL^{-1}. The high density of bacteria at P2 was thought to be due to adding probiotics again on the 21st day. In research, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish rearing by adding probiotics up to x10^{14} CFU mL^{-1}.

Meanwhile, according to Sitorus *et al.* (2019), the density of bacteria can reach with the number of cells is 10^9 CFU mL^{-1}. Widnyana (2016) states that on biofloc technology, total bacterial colonies ranged from 10^3-10^4 CFU mL^{-1}, while the system has a bacterial range of 10^8-10^{10} CFU mL^{-1}. This is caused by the system having a high intensity of sunlight as one of the supporting factors in the growth of bacteria. The high value of the total density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, which is assisted by their effectiveness in breaking down organic matter (Adharani *et al.*, 2016).

Absolute Growth, Feed Efficiency, Survival, and Biomass Production

Based on the research results, absolute growth data, feed efficiency, and survival of catfish for 42 days of rearing are presented in Table 4.

Table 4. The results of absolute growth, analysis, feed efficiency, survival, and biomass production during the rearing of catfish (*Clarias* sp.) in the biofloc system

Parameter	Treatment	
	P1	P2
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24[a]	151.90 ± 7.98[b]
Life sustainability (%)	81.54 ± 1.59	89.33 ±6,21
Biomass production (g)	17439.14 ± 1346.55[a]	24639.50 ± 1344.51[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the analysis showed that the growth in absolute length and absolute weight growth of catfish in treatment P1 was not significantly different from treatment P2. The absolute length growth and weight growth values of probiotics from swamps P2 were 8.18 cm and 19.30 g, higher than P1, which were 7.16 cm and 16.42 g. The results of this study indicate that fish use the floc as additional feed to increase fish growth. Catfish can utilize the available floc in the rearing medium as additional feed because it is high in protein and can increase fish growth (Putra *et al.*, 2017). In research, Wijaya *et al.* (2016) floc contains 42.42% protein, 92.15% water, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract material (NFE). According to Febriyanti *et al.* (2018), heterotrophic bacterial communities that accumulate in the rearing medium will form flocs (clumps) that can be used as a feed source for fish. Therefore, giving probiotics from swamps in the form of heterotrophic bacteria such as *Bacillus* sp.

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Furthermore, *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. The bacteria *Streptomyces* sp. in fish farming can also be applied as a growth promoter (Cruz *et al.*, 2012). The results of fish growth will affect the value of feed efficiency.

The result of the analysis showed that the efficiency of catfish feed in treatment P1 was significantly different from treatment P2. The feed efficiency value for P2 was higher, namely 151.90%, while P1 was 104.97%. If the feed efficiency value is converted to a value feed conversion ratio (FCR) then FCR P1 is 0.96 while P2 is 0.66. The FCR value in this study was better than the research of Wijayanti *et al.* (2021), which is 0.97. Putra *et al.* (2017) researched that administering probiotics to catfish rearing media with biofloc technology also resulted in feed efficiency between 88.17-110.86%. This study's high feed efficiency value is also suspected because fish use flocks as additional feed. De Schryver *et al.* (2008) stated that using flocks as additional feed is important in increasing feed efficiency. This shows that swamp probiotics can form flocs, which fish then eat as additional feed for fish.

The analysis of in this riset showed that the survival of catfish in treatment P1 was not significantly different from treatment P2. Survival in this study was quite good. The highest yield was in P2, 89.33%, followed by P1, 81.54%. The results of this study are close to the research of Wijayanti *et al.* (2021), namely 87.57% with a rearing period of 90 days. Administration of probiotics from swamps in the form of a combination of bacteria *Bacillus* sp. and bacteria *Streptomyces* sp., the percentage of fish survival can provide defense against pathogens by increasing non-specific immunity in the fish's immune system and maintaining a balance of water quality so that fish can survive (Wijayanti *et al.*, 2020). Growth and survival will affect the production value of fish biomass.

The analysis of in the riset showed that the production of catfish biomass in treatment P1 was significantly different from treatment P2. The production value of P2's biomass was 24,639.50 g, higher than that of P1, which was 17,439.14 g. Giving probiotics is thought to increase the production value of catfish in this biofloc technology. The biomass determines production at the end of cultivation and the survival of fish at the end of cultivation, so the higher the fish that can survive until the end of rearing, the more fish production will be produced (Anam *et al.*, 2017). Based on the results of absolute growth data and feed efficiency in this study, probiotics from swamps can form flocks used by fish as additional feed. Giving probiotics from swamps is also useful in preventing pathogenic bacteria to prevent death in fish. Therefore, giving probiotics from swamps can increase fish growth and survival, resulting in increased fish production.

Water quality

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Temperature and pH

Based on the research results, the temperature and pH data of the rearing media are presented in Table 5.

Table 5. The results of the temperature and pH analysis of catfish (*Clarias* sp.) in the biofloc system

Treatment	Temperature (ºC)	pH
P1	28.85-29.22[a]	7.27-7.40[a]
P2	29.48-29.59[b]	7.39-7.42[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the temperature and pH of the catfish-rearing media in treatment P1 were not significantly different from those in treatment P2. The temperature of the rearing medium in this study ranged from 28.85-29.59ºC. According to BSN (2014), the temperature value for raising catfish is 25-30ºC. Catfish will experience slow growth when the temperature is 16-24ºC or 31-32ºC, and the fish will die if the temperature is below 16ºC or above 32ºC (Pujiharsono & Kurnianto, 2020). The value of the degree of acidity or pH in the rearing medium ranges from 7.27 to 7.42. BSN (2014) states that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth when the pH is 4-6.4 or pH 8.6-11, and the fish will die if the pH is below four or above 11 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The results of the DO T-test analysis during rearing are presented in Table 6.

Table 6. The results of the DO analysis during rearing of catfish (*Clarias* sp.) in the biofloc system

Days to-	DO (mg L^{-1}) P1	DO (mg L^{-1}) P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27[a]	5.25±0.44[b]

21	4.47±0.03ᵃ	4.98±0.33ᵇ
28	4.88±0.12ᵃ	5.72±0.42ᵇ
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The results of the analysis show that Dissolved Oxygen (DO) or dissolved oxygen in treatment P1 was not significantly different from treatment P2 on days 0,7,35 and 42, but significantly different on days 14, 21 and 28. Based on Table 4.6. the frequency of probiotics from swamp P2 (2 times for 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study ranged from 3.91 to 5.95 mg L^{-1}. According to BSN (2014), the dissolved oxygen value for catfish is >3 mg L^{-1}. Therefore, the DO value in this study is still relatively safe for catfish farming. This is because the rearing container is equipped with aeration of 4 points. Besides that, it also comes from photosynthesis results from phytoplankton and oxygen diffusion from the air (Putra *et al*., 2014). Based on this, it is suspected that the increase in DO at the end of P1 rearing is better than P2. Based on research by Agustina (2018), phytoplankton from the chlorophyte division can increase dissolved oxygen levels in swamp water cultivation media by 63.63%. In research, Wijayanti *et al.* (2020) also produced a combination of chlorophyta and probiotics from swamps containing bacteria *Bacillus* sp. Furthermore, *Streptomyces* sp. is the best treatment for dissolved oxygen concentrations in fish culture media> 3 mg L^{-1}. The results of research by Kurniawan and Utama (2018) also yield DO values for catfish rearing >6 mg L^{-1} by administering probiotics *Bacillus* sp. as according to Prihanto *et al.* (2021) bacteria *Bacillus* sp. can improve water quality.

Ammonia

The results of the ammonia T-test analysis during rearing are presented in Table 7.

Table 7. The results of ammonia analysis during rearing of catfish (*Catfish* sp.) in the biofloc system

Days to-	Ammonia (mg L^{-1})	
	P1	P2
0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23ᵃ	0.69±0.47ᵇ
35	1.59±0.38ᵃ	0.66±0.37ᵇ
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the ammonia in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 21, and 42 but was significantly different on days 28 and 35. Based on Table 4.7 shows that the administration of probiotics from swamp P2 (2 times for 42 days of rearing) is proven to reduce ammonia levels in rearing media compared to P1 (1 time for 42 days of rearing). The ammonia value in this study was high, ranging from 0.45 to 1.62 mg L^{-1}, while in research by Wijayanti *et al.* (2021), it is 0.27 mg L^{-1}. The value of ammonia for catfish farming on the formation of biofloc is <1 mg L^{-1} (Wijaya *et al*., 2016). This high value of ammonia can cause death in fish due to the toxic nature of the ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion results, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). The high ammonia value in this study is likely due to the lack of a C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in water. Meanwhile, according to Hargreaves (2006), at a C/N ratio <10, heterotrophic bacteria will release ammonia into their environment. Research results Bakar *et al.* (2015) also stated that ammonia reduction in catfish farming using biofloc technology using a C/N 15 ratio could eliminate ammonia by 93.56% on the 12th day while the C/N 10 ratio on the 12th day was only 11.01%. Furthermore, only the maximum is on the 30th day, namely 98.51%.

Total Dissolved Solid (TDS)

The results of the TDS analysis during rearing are presented in Table 8.

Table 8. The results of TDS analysis during the rearing of catfish (*Clarias* sp.) in the biofloc system

Days to-	TDS (mg L^{-1})	
	P1	P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76ᵇ	872.67±41.55ᵃ
28	804.17±59.93	757.67±136.93
35	810.00±57.38ᵇ	717.33±21.37ᵃ
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show

Based on the results of the T-test analysis, it shows that Total Dissolved Solid (TDS) in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 28, and 42 but significantly different on days 21, and 35. Based on Table 4.8. administration of probiotics from swamps P2 (2 times during 42 days of rearing) can reduce TDS values compared to P1 (1 time during 42 days of rearing). TDS values in this study ranged from 717.33 to 1014.83 mg L^{-1}. Catfish can still tolerate the measurement results of these values to live. PPRI No.82 (2001) states that TDS's standard water quality value in fish farming activities is 1000 mg L^{-1}. The high level of TDS on the 21st day, especially in the P1 treatment, is thought to be due to the administration of commercial drugs to prevent dead fish containing organic and inorganic compounds. This follows the statement of Rinawati *et al.* (2016), who stated that large amounts of organic and inorganic compounds can result in high levels of TDS in water.

Conclusion

~~Based on the results of the research that has been done, it can be concluded that giving probiotics from swamps with a frequency of 2 times for 42 days of rearing (P2) can form biofloc resulting in a floc volume of 68.33 mg L^{-1}, growth in length and absolute weight of 8.18 cm and 19.30 g, feed efficiency of 135.24%, survival rate of 89.33% and biomass production of 24,639.50 g with water quality of 28.85 – 29.59°C, pH 7.27 – 7.42, DO 3,91-5,72 mg L^{-1}, ammonia 0,45 – 1,15 mg L^{-1} and TDS 717,33 – 885,50 mg L^{-1}.~~ Based on the results of the research that has been carried out, it can be concluded that giving probiotics from swamp with a frequency of 2 times for 42 days of rearing (P2) gives the best results on flock volume, fish growth, fish feed. fish efficiency, fish survival rate, fish biomass production, and water quality.

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Respon penulis ke reviewer tahap pertama dan artikel yg di resubmit

Tanggapan Hasil Review Tahap 1

No	Komenter Review	Tanggapan
A	Review 1	
1	Abstract	
	explain the parameters that will be observed in this research and the analytical approach used for these parameters.	We've added it Parameter are flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively
2	Introduction	
	identified from swamp waters there are several types of probiotics such as Bacillus sp. and Streptomyces sp. This type can be used as a starter in a biofloc system for floc formation. Reported by Wijayanti et al. (2021), administering probiotics from swamps with a frequency of 1 time for 42 days of maintenance resulted in a floc volume of 40 mL L-1 (42nd day)	We've fixed it Identified from swamp waters there are several types of probiotics such as *Bacillus* sp. and *Streptomyces* sp. This type can be used as a starter in a biofloc system for floc formation. In previous research conducted by Wijayanti et al. (2021), administering probiotics from swamps with a frequency of 1 time for 42 days of maintenance resulted in a floc volume of 40 mL/L (42^{nd} day). Using these flocs for 60 days of rearing resulted in the final average weight and length growth from the initial average of 7.16 g to 36.95 g and 9.50 cm to 18.50 cm. The resulting floc volume is relatively low.
	In another study has been reported previously	We've fixed it
	it is better that the data presented is the difference between the beginning and the end	The reference is not written in the source but only the beginning and end are written
	Resulths and discussion	
	in methode, Floc composition was microscopically observed (40x magnification) on days 0, 1, 21, and 42, but ini result only find 1 data series, please cek again.	The data we present is floc composition data during the observation period, and the results of each observation period are found to be the same floc composition.
	please cek…contain repetition	We've fixed it

	Please cek	We've fixed it
	Not match with data number ini table please cek	We've fixed it
	Conclusion	
	Make statement in simple sentence its better	We've fixed it Based on the results of the research that has been carried out, it can be concluded that giving probiotics from swamp with a frequency of 2 times for 42 days of rearing (P2) gives the best results on flock volume, fish growth, fish feed. fish efficiency, fish survival rate, fish biomass production, and water quality.
B	Reviewer 2	
1	Material and method	
	How many concentrations (salt, nutrient broth, yeast malt, yeast extract, CaCl2, yeast) were added to the culture medium?	We've fixed it bacteria were mixed with 5% molasses for biofloc formation. For stock culture, bacteria were mixed with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.
2	Conclusion	
	Provide recommendations on probiotics	We've fixed it
C	Reviewer 3	
1	Method	
	What growing medium to use?	In calculating the total bacterial colonies, not grown on any media. The technical calculation has been explained in this section, by taking a water sample from the media and directly counting the bacterial colonies using a colony counter.
2	Resulth and Discussion	
	why the 21st day has decreased? It would be interesting if you could explain why the 21st day decreased?.	The decrease in total bacterial colonies on day 21 is thought to be a lack of nutritional sources (macronutrient or micronutrient) for bacteria (Wijayanti et al., 2020). According to Nasmia and Rifai (2020) the decrease in the number of bacterial colonies can be caused by a decrease in the number of carbon sources.

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Frequency of Probiotics from Swamp for Formation of Biofloc and Production of Catfish (*Clarias* sp.)

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ABSTRACT

Catfish (*Clarias* sp.) cultivated with a combination of the addition of probiotics in biofloc technology are thought to increase production. The frequency of adding probiotics from swamps to cultured water media has never been studied to ensure flocks' availability in rearing media. This study aimed to determine the appropriate frequency of probiotics from swamps in biofloc technology to improve the parameters of successful cultivation, especially increasing catfish production and biofloc formation. This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given was different in the frequency of giving probiotics from swamps: (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively. The results showed that P2 was the best treatment with a floc volume of 68.33 mL L^{-1}, absolute length growth of 8.18 cm, absolute weight growth of 19.30 g, feed efficiency of 135.24 %, survival of 89.33%, biomass production of 24639.50 g, temperature of 28.85-29.59°C, pH of 7.27-7.42, dissolved oxygen (DO) of 3.91-5.72 mg L^{-1}, ammonia of 0.45-1.15 mg L^{-1}, and total dissolved solids (TDS) of 717.33-885.50 mg L^{-1}. Therefore, probiotics from swamps should be given to fish culture media with a frequency of 2 times during 42 days of rearing or once every 21 days.

Introduction

Catfish (*Clarias* sp.) is a freshwater fishery commodity widely cultivated due to high market demand. According to data from the Statistics from the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia (2022), catfish production in Indonesia in the enlargement cultivation sector in 2019 was 289 thousand tons and reached 384 thousand tons in 2020, while in 2021, it was 360 thousand tons. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and with an environmental perspective by minimizing waste disposal in the surrounding waters (Fauzi *et al*., 2022). According to Putra *et al.* (2017), technology biofloc by utilizing probiotics in the form of heterotrophic bacteria can produce natural feed from the flocs that are formed, thereby increasing the value of feed efficiency and improving water quality.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the media. Swamp microorganisms identified are *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti*et al*., 2018). Giving probiotics from swamps (*Bacillus* sp. and *Streptomyces* sp.) has been studied in feeding the agility fish (Tanbiyaskur *et al*., 2022) and the rearing medium with biofloc technology in catfish (Wijayanti *et al*., 2021) and snakehead fish (Wati, 2021; Anil, 2022). Im another study has been reported previously that swamp probiotics can increase growth, feed efficiency, fish

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survival, and aquaculture water quality (Wijayanti *et al.*, 2020).

Identified from swamp waters there are several types of probiotics such as Bacillus sp. and Streptomyces sp. This type can be used as a starter in a biofloc system for floc formation. Reported by Wijayanti et al. (2021), administering probiotics from swamps with a frequency of 1 time for 42 days of maintenance resulted in a floc volume of 40 mL L-1 (42nd day). Using these flocs for 60 days of rearing resulted in the final average weight and length growth from the initial average of 7.16 g to 36.95 g and 9.50 cm to 18.50 cm. Floc volume results (Wijayanti *et al.*, 2021) are lower than Bakar *et al.* (2015) research results, whose floc volume reached 92.5 mL L^{-1}. According to Zaidy (2022), the maximum floc volume for catfish is 100 mL L^{-1}. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water, around 70-80% of the water volume. These flocks must continue to be available in the rearing medium in sufficient quantities as natural food for fish to increase fish growth and improve feed efficiency and water quality. According to Feroza *et al.* (2021), the flocs in the rearing media will decrease because they are consumed by fish every day, so it is necessary to have the availability of flocs in the rearing medium or not cause blooming microorganisms due to excess floc volume. Therefore, it is necessary to research the effect of the frequency of probiotics from swamps on the formation of biofloc and catfish production.

Materials and Methods

Location and time of research

This research was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University in December 2022–January 2023.

Research Materials

The materials used were catfish seeds (7 ± 0.5 cm in length) and bacteria *Bacillus* sp., *Streptomyces* sp., molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tool used is a tarpaulin pool with a round diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, imhoff cone, loop needle, erlenmeyer, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter and ammonia test kit.

Research Design

This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given is the difference in the frequency of giving probiotics from swamps, which are as follows:

P1 = 1 time during 42 days of rearing
P2 = 2 times during 42 days of rearing

Work Procedure

Swamp Origin Probiotic Culture

Pure culture obtained from the isolation of bacteria from swamps *Bacillus* sp. and *Streptomyces* sp. results of previous studies (Wijayanti *et al.*, 2018) liquid culture was carried out. Bacterial liquid culture *Bacillus* sp. was done on the media Nutrient Broth (NB) liquid by taking one ose of bacteria for deep culture Erlenmeyer, which already contains NB 20 mL media. In contrast, *Streptomyces* sp. was done on the media Yeast Malt (YM) liquid by taking one ose of bacteria and culturing in liquid YM media 20 mL deep Erlenmeyer. The two bacteria were agitated with a magnetic hot plate stirrer for three days for *Bacillus* sp. and five days for *Streptomyces* sp. The density of bacteria obtained from liquid culture results was calculated using the cup counting or Total Plate Count (TPC) method. Then, do the mixing of bacteria with the formulation. Liquid media for storage media formulations in 5% molasses and filled with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish were reared in 6 round ponds made of tarpaulin with a diameter of 2 m and a height of 1.2 m. Rearing begins with pond preparation, including cleaning, water filling, and pond incubation. The pond is cleaned by brushing the entire inside of the pond and drying it for one day to kill the pathogens. Then, fill with water as high as 0.7 m and incubate for three days (Ma'ruf, 2016). Then salt at a dose of 1 kg m^{-3} and camphor dolomite 50 g m^{-3} (Sucipto *et al.*, 2018) were added to the rearing medium and incubated for one day (Wijayanti *et al.*, 2021). Installation of aerators is carried out at 4 points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process uses catfish measuring 7 ± 0.5 cm with a stocking density of 500 m^{-3} (Ma'ruf, 2016) and maintained for 42 days. Fish stocking was carried out in the morning when water conditions were normal and acclimatized for seven days to reduce stress on the fish. During rearing, probiotics were added to the biofloc pond at a density of bacteria

Bacillus sp. 10^5 CFU mL^{-1} and *Streptomyces* sp. 10^5 CFU mL^{-1} (Wijayanti *et al*., 2020) with a frequency according to treatment, namely (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. During rearing, the addition of a carbon source in the form of molasses was also carried out at a dose of 200 mL m^{-3} with a frequency of once every seven days (days 0, 7, 14, 21, 28, 35) (Putra *et al*., 2017). Fish are fed using commercial feed with a 39-41% protein content. The feeding frequency is three times daily, namely at 08.00 a.m, 12.00 a.m, and 04.00 p.m with the at satiation. If there are dead fish, the fish are weighed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental pond unit.

Research Parameters

Volume floc and Floc Composition

Floc volume was measured using an Imhoff cone by taking media water maintenance at 1 point of collection as much as 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on days 7, 14, 21, 28, 35 and 42. After measuring the floc's volume, the precipitated floc is taken to observe the composition of floc microorganisms. Floc composition was microscopically observed (40x magnification) on days 0, 1, 21, and 42.

Total Bacterial Colonies

Total bacterial colonies formed in the rearing medium were counted with the cup count method by taking water as a rearing medium, and counting the bacterial colonies using a colony counter. After The sample water is diluted through dilution steps 10^{-5}, 10^{-7}, 10^{-9}. Bacterial density calculations were done on days 0 (before adding probiotics from swamps), 1, 21, and 42. Growing bacterial colonies were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1)\ =\ Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth

The formula used to measure absolute weight growth, according to Hopkins (1992) is:

$$W = Wt - Wo$$

Information:

W	=	Growth in absolute weight of fish kept (g)
Wt	=	Average fish weight at the end of the study (g)
Wo	=	Average fish weight at the start of the study (g)

The formula used to measure absolute length growth, according to Hopkins (1992), is:

$$L = Lt - Lo$$

Information:

L	=	Absolute length growth of reared fish (cm)
Lt	=	Average length of fish at the end of the study (cm)
Lo	=	Average fish length at the start of the study (cm)

Feed Efficiency

Feed efficiency (FE) is calculated based on a formula based on Afrianto & Liviawaty (2005):

$$FE\ (\%) = \frac{(Wt+D)-Wo}{F}\ x\ 100$$

Information:

FE	=	Feed efficiency (%)
Wt	=	Fish biomass at the end of the study (g)
Wo	=	Fish biomass at the start of the study (g)
D	=	Biomass of fish that died during the study (g)
F	=	Amount of fish feed given during the study (g)

Survival Rate (SR)

The survival rate is calculated using a formula based on Aliyu-Paiko*et al.* (2010) as follows:

$$SR\ (\%) = \frac{Nt}{No}\ x\ 100$$

Information:

SR	=	Survival (%)
Nt	=	The final quantity of fish at the end of rearing (g)
No	=	The initial quantity of fish at initial rearing (g)

Biomass Production

The formula can calculate the level of biomass production according to Shang (1982):

$$P\ (g) = W\ x\ N$$

Information:

P	=	Production of biomass (g)
W	=	Average weight of fish at the end of rearing (g)
N	=	The final quantity of fish at the end of rearing (g)

Water quality

Water quality parameters included temperature and pH, measured every day at 08.00 a.m and 04.00 p.m. Temperature measurements (°C) using a thermometer, pH using a pH meter, Dissolved oxygen (DO) using a DO meter (mg L^{-1}), *Total Dissolved solid* (TDS) (mg L^{-1}) using a TDS meter, and ammonia (mg L^{-1}) using a spectrophotometer.

Dissolved oxygen level, TDS, and ammonia were measured every seven days (day 0, 7, 14, 21, 28, 35, and 42) once in 42 days of the rearing period.

Data analysis

Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively.

Results and Discussion

Volume Floc

The results of the T-test analysis of flock volume during rearing are presented in Table 1.

Table 1. The results of the T-test analysis of flock of catfish (*Clarias* sp.) in biofloc system

Days to-	Volume flock (mL L^{-1})	
	P1	P2
7	6.00±2.00	5.33±3.06
14	11.67±2.89	13.00±3.00
21	17.00±2.65	21.67±2.89
28	35.00±5.00^{a}	53.33±5.77^{b}
35	43.33±7.64^{a}	66.67±11.55^{b}
42	48.33±2.89^{a}	68.33±10.41^{b}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the results of the analysis showed that the floc volume in treatment P1 was not significantly different from treatment P2 on days 7, 14, and 21 and was significantly different on days 28, 35, and 42. Floc volume is the amount of suspended solids over a certain period in an inverted conical container (Effendi, 2003). The highest floc volume was found in the P2 treatment given probiotics with a frequency of 2 times during 42 days of rearing, reaching 68.33 mL $L^{-1,}$ while P1 is 48.33 mL L^{-1}. The floc volume obtained in this study was higher than that in the study by Wijayanti *et al.* (2021), which is 40 mL L^{-1} (42nd day). This is presumably due to the influence of the time-frequency of giving probiotics to the rearing medium. According to De Schryver *et al.* (2008), the factors that influence the formation of bio floc are the administration of probiotics (starter) with floc-forming microbial composition, agitation intensity by aeration device, organic carbon source, and water quality. This is also reinforced by the results of Malaputra *et al.* (2016), namely the administration of commercial probiotics with a frequency of 14 times (70 days of rearing) resulted in a floc volume of 120 mL L^{-1} higher than the frequency of 7 times which is only 80 mL L^{-1}. Based on the results of this study, it can be stated that the more often probiotics are given to the rearing medium, the more volume will increase. The maximum floc volume for catfish farming is 100 mL L^{-1}. When the floc volume exceeds the limit, partial water removal can be done and replaced with new water, around 70-80% of the total water volume of the preservation pond (Zaidy, 2022).

In addition to the factor of providing probiotics from swamps as a starter containing heterotrophic bacteria, the increase in floc volume every week in this study was thought to be due to the C/N ratio. The C/N ratio can affect the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), the C/N ratio >10 in fish farming activities is the optimum ratio for the formation of biofloc. Meanwhile, according to Avnimelech *et al.* (1999) required a C/N ratio >15. Therefore, it is necessary to add organic carbon from outside, such as molasses. This study added extra organic carbon in molasses every seven days at a dose of 200 mL m^{-3} (Putra *et al.*, 2017).

Floc Composition

The composition of the flocs in this study is presented in Table 2.

Table 2. Composition of catfish (*Clarias* sp.) flocs maintened in biofloc system

Floc-forming microbes	Treatment	
	P1	P2
Chlorophyta	✓	✓
Cyanophyta	✓	✓
Protozoa	✓	✓
Coelenterata	-	✓
Rotifers	✓	✓
Arthropods	-	✓

Information: (✓) exists, (-) does not exist

Based on observations of floc composition in the treatment of probiotics from swamps with P2 frequencies more diverse than P1, namely chlorophyta, cyanophyta, protozoa, coelenterata, rotifers, and arthropods. Whereas in P1, only chlorophyta, cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), the diversity in the composition of the floc-forming microbes is thought to be due to the effect of giving probiotics, which is in line with the increased volume of floc in the rearing medium so that the microbes in the rearing containers can grow properly. Wati (2021) also produced the same floc composition as arthropods, chlorophyta, cyanophyta, protozoa, and rotifers. The composition of the flocs in the form of zooplankton in this study were protozoa, coelenterata, rotifers, arthropods, and phytoplankton in the form of chlorophyta and cyanophyta. This is following the opinion of De Schryver *et al.* (2008), namely the composition of floc in the form of a heterogeneous combination of microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach sizes <1000 μm.

Total Bacterial Colonies

The total bacterial colonies on the rearing medium are presented in Table 3.

Table 3. The total bacterial colonies in the flocs of catfish (*Clarias* sp) in the biofloc system

Days to-	Total bacterial colonies ($\times 10^9$ CFU mL^{-1})	
	P1	P2
0	2.77±1.00	2.77±1.00
1	13.23±2.82	14.89±3.88
21	5.56±2.01	6.65±3.07
42	8.42±3.19^{a}	19.73±6.19^{b}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the total bacterial colonies in the P1 treatment were not significantly different from the P2 treatment on days 0, 1, and 21 and were significantly different on the 42nd day. This study's bacterial density range was between 2.77 and 19.73 x 10^9 CFU mL^{-1}. After stocking probiotics from swamps on rearing media in treatments P1 and P2, total bacterial colonies increased on day 1, and day 21 decreased. The decrease in total bacterial colonies on day 21 is thought to be a lack of nutritional sources (macronutrient or micronutrient) for bacteria (Wijayanti *et al.*, 2020). According to Nasmia and Rifai (2020) the decrease in the number of bacterial colonies can be caused by a decrease in the number of carbon sources. On the 42nd day, the bacterial density increased again with a higher P2 bacterial density of 19.73 x10^9 CFU mL^{-1} compared to P1, which is 8.42 x10^9 CFU mL^{-1}. The high density of bacteria at P2 was thought to be due to adding probiotics again on the 21st day. In research, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish rearing by adding probiotics up to x10^{14} CFU mL^{-1}.

Meanwhile, according to Sitorus *et al.* (2019), the density of bacteria can reach with the number of cells is 10^9 CFU mL^{-1}. Widnyana (2016) states that on biofloc technology, total bacterial colonies ranged from 10^3-10^4 CFU mL^{-1}, while the system has a bacterial range of 10^8-10^{10} CFU mL^{-1}. This is caused by the system having a high intensity of sunlight as one of the supporting factors in the growth of bacteria. The high value of the total density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, which is assisted by their effectiveness in breaking down organic matter (Adharani *et al.*, 2016).

Absolute Growth, Feed Efficiency, Survival, and Biomass Production

Based on the research results, absolute growth data, feed efficiency, and survival of catfish for 42 days of rearing are presented in Table 4.

Table 4. The results of absolute growth, analysis, feed efficiency, survival, and biomass production during the rearing of catfish (*Clarias* sp.) in the biofloc system

Parameter	Treatment	
	P1	P2
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24^{a}	151.90 ± 7.98^{b}
Life sustainability (%)	81.54 ± 1.59	89.33 ±6,21
Biomass production (g)	17439.14 ± 1346.55^{a}	24639.50 ± 1344.51^{b}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the analysis showed that the growth in absolute length and absolute weight growth of catfish in treatment P1 was not significantly different from treatment P2. The absolute length growth and weight growth values of probiotics from swamps P2 were 8.18 cm and 19.30 g, higher than P1, which were 7.16 cm and 16.42 g. The results of this study indicate that fish use the floc as additional feed to increase fish growth. Catfish can utilize the available floc in the rearing medium as additional feed because it is high in protein and can increase fish growth (Putra *et al.*, 2017). In research, Wijaya *et al.* (2016) floc contains 42.42% protein, 92.15% water, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract material (NFE). According to Febriyanti *et al.* (2018), heterotrophic bacterial communities that accumulate in the rearing medium will form flocs (clumps) that can be used as a feed source for fish. Therefore, giving probiotics from swamps in the form of heterotrophic bacteria such as *Bacillus* sp.

Furthermore, *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. The bacteria *Streptomyces* sp. in fish farming can also be applied as a growth promoter (Cruz *et al*., 2012). The results of fish growth will affect the value of feed efficiency.

The result of the analysis showed that the efficiency of catfish feed in treatment P1 was significantly different from treatment P2. The feed efficiency value for P2 was higher, namely 151.90%, while P1 was 104.97%. If the feed efficiency value is converted to a value feed conversion ratio (FCR) then FCR P1 is 0.96 while P2 is 0.66. The FCR value in this study was better than the research of Wijayanti *et al.* (2021), which is 0.97. Putra *et al.* (2017) researched that administering probiotics to catfish rearing media with biofloc technology also resulted in feed efficiency between 88.17-110.86%. This study's high feed efficiency value is also suspected because fish use flocks as additional feed. De Schryver *et al.* (2008) stated that using flocks as additional feed is important in increasing feed efficiency. This shows that swamp probiotics can form flocs, which fish then eat as additional feed for fish.

The analysis of in this riset showed that the survival of catfish in treatment P1 was not significantly different from treatment P2. Survival in this study was quite good. The highest yield was in P2, 89.33%, followed by P1, 81.54%. The results of this study are close to the research of Wijayanti *et al.* (2021), namely 87.57% with a rearing period of 90 days. Administration of probiotics from swamps in the form of a combination of bacteria *Bacillus* sp. and bacteria *Streptomyces* sp., the percentage of fish survival can provide defense against pathogens by increasing non-specific immunity in the fish's immune system and maintaining a balance of water quality so that fish can survive (Wijayanti *et al*., 2020). Growth and survival will affect the production value of fish biomass.

The analysis of in the riset showed that the production of catfish biomass in treatment P1 was significantly different from treatment P2. The production value of P2's biomass was 24,639.50 g, higher than that of P1, which was 17,439.14 g. Giving probiotics is thought to increase the production value of catfish in this biofloc technology. The biomass determines production at the end of cultivation and the survival of fish at the end of cultivation, so the higher the fish that can survive until the end of rearing, the more fish production will be produced (Anam *et al*., 2017). Based on the results of absolute growth data and feed efficiency in this study, probiotics from swamps can form flocks used by fish as additional feed. Giving probiotics from swamps is also useful in preventing pathogenic bacteria to prevent death in fish. Therefore, giving probiotics from swamps can increase fish growth and survival, resulting in increased fish production.

Water quality

Temperature and pH

Based on the research results, the temperature and pH data of the rearing media are presented in Table 5.

Table 5. The results of the temperature and pH analysis of catfish (*Clarias* sp.) in the biofloc system

Treatment	Temperature (°C)	pH
P1	28.85-29.22[a]	7.27-7.40[a]
P2	29.48-29.59[b]	7.39-7.42[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the temperature and pH of the catfish-rearing media in treatment P1 were not significantly different from those in treatment P2. The temperature of the rearing medium in this study ranged from 28.85-29.59°C. According to BSN (2014), the temperature value for raising catfish is 25-30°C. Catfish will experience slow growth when the temperature is 16-24°C or 31-32°C, and the fish will die if the temperature is below 16°C or above 32°C (Pujiharsono & Kurnianto, 2020). The value of the degree of acidity or pH in the rearing medium ranges from 7.27 to 7.42. BSN (2014) states that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth when the pH is 4-6.4 or pH 8.6-11, and the fish will die if the pH is below four or above 11 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The results of the DO T-test analysis during rearing are presented in Table 6.

Table 6. The results of the DO analysis during rearing of catfish (*Clarias* sp.) in the biofloc system

Days to-	DO (mg L^{-1})	
	P1	P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27[a]	5.25±0.44[b]

21	4.47±0.03[a]	4.98±0.33[b]
28	4.88±0.12[a]	5.72±0.42[b]
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The results of the analysis show that Dissolved Oxygen (DO) or dissolved oxygen in treatment P1 was not significantly different from treatment P2 on days 0,7,35 and 42, but significantly different on days 14, 21 and 28. Based on Table 4.6. the frequency of probiotics from swamp P2 (2 times for 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study ranged from 3.91 to 5.95 mg L^{-1}. According to BSN (2014), the dissolved oxygen value for catfish is >3 mg L^{-1}. Therefore, the DO value in this study is still relatively safe for catfish farming. This is because the rearing container is equipped with aeration of 4 points. Besides that, it also comes from photosynthesis results from phytoplankton and oxygen diffusion from the air (Putra *et al*., 2014). Based on this, it is suspected that the increase in DO at the end of P1 rearing is better than P2. Based on research by Agustina (2018), phytoplankton from the chlorophyte division can increase dissolved oxygen levels in swamp water cultivation media by 63.63%. In research, Wijayanti *et al.* (2020) also produced a combination of chlorophyta and probiotics from swamps containing bacteria *Bacillus* sp. Furthermore, *Streptomyces* sp. is the best treatment for dissolved oxygen concentrations in fish culture media> 3 mg L^{-1}. The results of research by Kurniawan and Utama (2018) also yield DO values for catfish rearing >6 mg L^{-1} by administering probiotics *Bacillus* sp. as according to Prihanto *et al.* (2021) bacteria *Bacillus* sp. can improve water quality.

Ammonia

The results of the ammonia T-test analysis during rearing are presented in Table 7.

Table 7. The results of ammonia analysis during rearing of catfish (*Catfish* sp.) in the biofloc system

Days to-	Ammonia (mg L^{-1})	
	P1	P2
0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23[a]	0.69±0.47[b]
35	1.59±0.38[a]	0.66±0.37[b]
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the ammonia in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 21, and 42 but was significantly different on days 28 and 35. Based on Table 4.7 shows that the administration of probiotics from swamp P2 (2 times for 42 days of rearing) is proven to reduce ammonia levels in rearing media compared to P1 (1 time for 42 days of rearing). The ammonia value in this study was high, ranging from 0.45 to 1.62 mg L^{-1}, while in research by Wijayanti *et al.* (2021), it is 0.27 mg L^{-1}. The value of ammonia for catfish farming on the formation of biofloc is <1 mg L^{-1} (Wijaya *et al*., 2016). This high value of ammonia can cause death in fish due to the toxic nature of the ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion results, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). The high ammonia value in this study is likely due to the lack of a C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in water. Meanwhile, according to Hargreaves (2006), at a C/N ratio <10, heterotrophic bacteria will release ammonia into their environment. Research results Bakar *et al.* (2015) also stated that ammonia reduction in catfish farming using biofloc technology using a C/N 15 ratio could eliminate ammonia by 93.56% on the 12^{th} day while the C/N 10 ratio on the 12^{th} day was only 11.01%. Furthermore, only the maximum is on the 30th day, namely 98.51%.

Total Dissolved Solid (TDS)

The results of the TDS analysis during rearing are presented in Table 8.

Table 8. The results of TDS analysis during the rearing of catfish (*Clarias* sp.) in the biofloc system

Days to-	TDS (mg L^{-1})	
	P1	P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76[b]	872.67±41.55[a]
28	804.17±59.93	757.67±136.93
35	810.00±57.38[b]	717.33±21.37[a]
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show

Based on the results of the T-test analysis, it shows that Total Dissolved Solid (TDS) in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 28, and 42 but significantly different on days 21, and 35. Based on Table 4.8. administration of probiotics from swamps P2 (2 times during 42 days of rearing) can reduce TDS values compared to P1 (1 time during 42 days of rearing). TDS values in this study ranged from 717.33 to 1014.83 mg L^{-1}. Catfish can still tolerate the measurement results of these values to live. PPRI No.82 (2001) states that TDS's standard water quality value in fish farming activities is 1000 mg L^{-1}. The high level of TDS on the 21st day, especially in the P1 treatment, is thought to be due to the administration of commercial drugs to prevent dead fish containing organic and inorganic compounds. This follows the statement of Rinawati *et al.* (2016), who stated that large amounts of organic and inorganic compounds can result in high levels of TDS in water.

Conclusion

Based on the results of the research that has been done, it can be concluded that giving probiotics from swamps with a frequency of 2 times for 42 days of rearing (P2) can form biofloc resulting in a floc volume of 68.33 mg L^{-1}, growth in length and absolute weight of 8.18 cm and 19.30 g, feed efficiency of 135.24%, survival rate of 89.33% and biomass production of 24,639.50 g with water quality of 28.85 – 29.59°C, pH 7.27 – 7.42, DO 3,91-5,72 mg L^{-1}, ammonia 0,45 – 1,15 mg L^{-1} and TDS 717,33 – 885,50 mg L^{-1}. Based on the results of the research that has been carried out, it can be concluded that giving probiotics from swamp with a frequency of 2 times for 42 days of rearing (P2) gives the best results on flock volume, fish growth, fish feed. fish efficiency, fish survival rate, fish biomass production, and water quality.

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Hasil review tahap kedua

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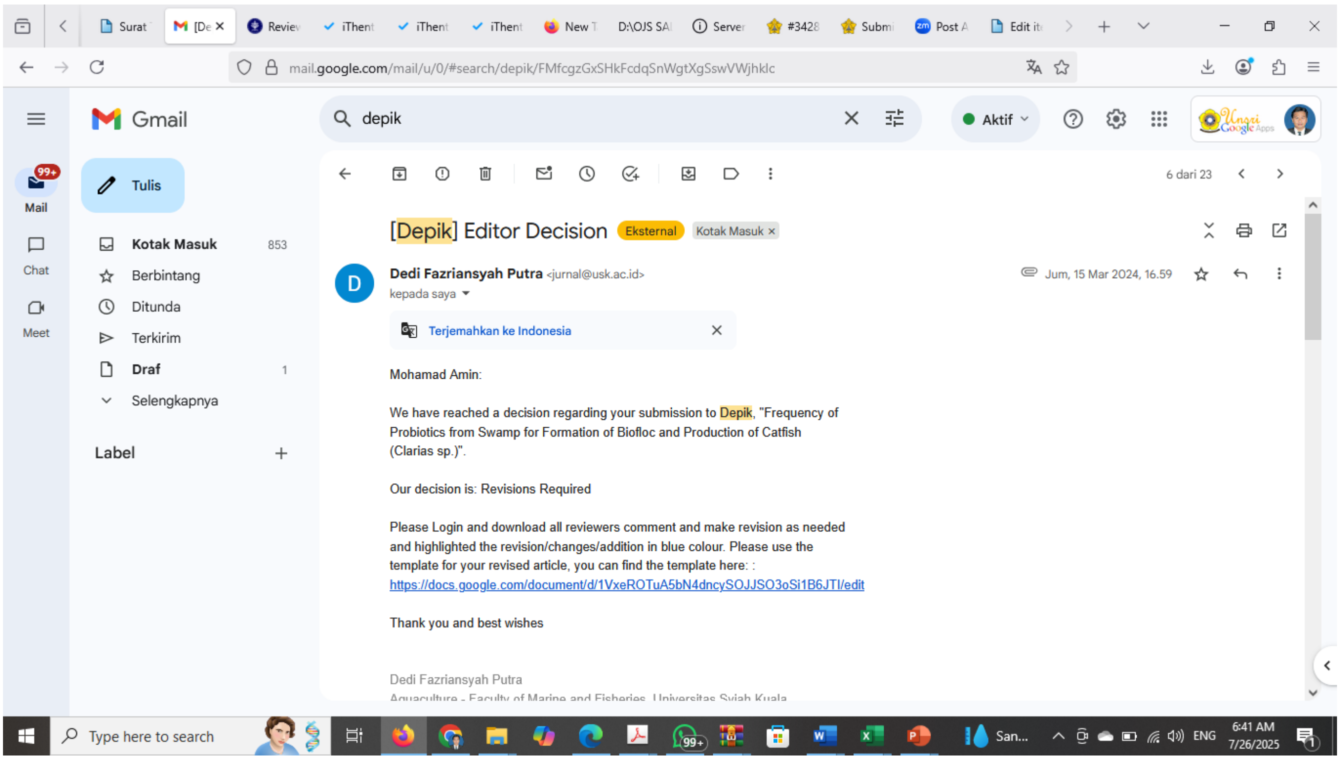

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Frequency of Probiotics from Swamp for Formation of Biofloc and Production of Catfish (*Clarias gariepinus*

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ABSTRACT

Catfish (*Clarias gariepinus*) cultivated with a combination of the addition of probiotics in biofloc technology are thought to increase production. The frequency of adding probiotics from swamps to cultured water media has never been studied to ensure flocks' availability in rearing media. This study aimed to determine the appropriate frequency of probiotics from swamps in biofloc technology to improve the parameters of successful cultivation, especially increasing catfish production and biofloc formation. This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given was different in the frequency of giving probiotics from swamps: (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively. The results showed that P2 was the best treatment with a floc volume of 68.33 ± 10.41 mL/L, absolute length growth of 8.18 ± 1.03 cm, absolute weight growth of 19.30 ± 3.12 g, feed efficiency of 135.24 ± 7.98%, survival of 89.33 ± 6.21%, biomass production of 24639.50 ± 1344.51 g, temperature of 28.85-29.59ºC, pH of 7.27-7.42, dissolved oxygen (DO) of 3.91-5.72 mg/L, ammonia of 0.45-1.15 mg/L, and total dissolved solids (TDS) of 717.33-885.50 mg/L. Therefore, probiotics from swamps should be given to fish culture media with a frequency of 2 times during 42 days of rearing or once every 21 days.

Introduction

Catfish (*Clarias gariepinus*) is a freshwater fishery commodity widely cultivated due to high market demand. According to data from the Statistics from the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia (2022), catfish production in Indonesia in the enlargement cultivation sector in 2019 was 289 thousand tons and reached 384 thousand tons in 2020, while in 2021, it was 360 thousand tons. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and with an environmental perspective by minimizing waste disposal in the surrounding waters (Fauzi *et al.*, 2022). According to Putra *et al.* (2017), technology biofloc by utilizing probiotics in the form of heterotrophic bacteria can produce natural feed from the flocs that are formed, thereby increasing the value of feed efficiency and improving water quality.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the media. Swamp microorganisms identified are *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti *et al.*, 2018). Giving probiotics from swamps (*Bacillus* sp. and *Streptomyces* sp.) has been studied in feeding the agility fish (Tanbiyaskur *et al.*, ~~2021~~ 2022) and the rearing medium with biofloc technology in catfish (Wijayanti *et al.*, 2021) and snakehead fish (Wati, 2021; Anil, 2022). In another study has been reported previously that swamp

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probiotics can increase growth, feed efficiency, fish survival, and aquaculture water quality (Wijayanti *et al.*, 2020).

Identified from swamp waters there are several types of probiotics such as Bacillus sp. and Streptomyces sp. This type can be used as a starter in a biofloc system for floc formation. Reported by Wijayanti et al. (2021), administering probiotics from swamps with a frequency of 1 time for 42 days of maintenance resulted in a floc volume of 40 mL/L (42nd day). Using these flocs for 60 days of rearing resulted in the final average weight and length growth from the initial average of 7.16 g to 36.95 g and 9.50 cm to 18.50 cm. Floc volume results (Wijayanti *et al.*, 2021) are lower than Bakar *et al.* (2015) research results, whose floc volume reached 92.5 mL/L. According to Zaidy (2022), the maximum floc volume for catfish is 100 mL/L. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water, around 70-80% of the water volume. These flocks must continue to be available in the rearing medium in sufficient quantities as natural food for fish to increase fish growth and improve feed efficiency and water quality. According to Feroza *et al.* (2021), the flocs in the rearing media will decrease because they are consumed by fish every day, so it is necessary to have the availability of flocs in the rearing medium or not cause blooming microorganisms due to excess floc volume. Therefore, it is necessary to research the effect of the frequency of probiotics from swamps on the formation of biofloc and catfish production.

Materials and Methods

Location and time of research

This research was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University in December 2022–January 2023.

Research Materials

The materials used were catfish seeds (7 ± 0.5 cm in length) and bacteria *Bacillus* sp., *Streptomyces* sp., molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tool used is a tarpaulin pool with a round diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, imhoff cone, loop needle, erlenmeyer, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter and ammonia test kit.

Research Design

This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given is the difference in the frequency of giving probiotics from swamps, which are as follows:
P1 = 1 time during 42 days of rearing
P2 = 2 times during 42 days of rearing

Work Procedure

Swamp Origin Probiotic Culture

Pure culture obtained from the isolation of bacteria from swamps *Bacillus* sp. and *Streptomyces* sp. results of previous studies (Wijayanti *et al.*, 2018) liquid culture was carried out. Bacterial liquid culture *Bacillus* sp. was done on the media Nutrient Broth (NB) liquid by taking one ose of bacteria for deep culture Erlenmeyer, which already contains NB 20 mL media. In contrast, *Streptomyces* sp. was done on the media Yeast Malt (YM) liquid by taking one ose of bacteria and culturing in liquid YM media 20 mL deep Erlenmeyer. The two bacteria were agitated with a magnetic hot plate stirrer for three days for *Bacillus* sp. and five days for *Streptomyces* sp. The density of bacteria obtained from liquid culture results was calculated using the cup counting or Total Plate Count (TPC) method. Then, do the mixing of bacteria with the formulation. Liquid media for storage media formulations in 5% molasses and filled with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish (*Clarias gariepinus*) were reared in 6 round ponds made of tarpaulin with a diameter of 2 m and a height of 1.2 m. Rearing begins with pond preparation, including cleaning, water filling, and pond incubation. The pond is cleaned by brushing the entire inside of the pond and drying it for one day to kill the pathogens. Then, fill with water as high as 0.7 m (volume 2198 L) and incubate for three days (Ma'ruf, 2016). Then salt at a dose of 1 kg m^{-3} and camphor dolomite 50 g m^{-3} (Sucipto *et al.*, 2018) were added to the rearing medium and incubated for one day (Wijayanti *et al.*, 2021). Installation of aerators is carried out at 4 points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process uses catfish measuring 7 ± 0.5 cm with a stocking density of 500 m^{-3} ~~(Ma'ruf, 2006)~~ (BSN, 2018) and maintained for 42 days. Fish stocking was carried out in the morning when water

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Commented [Ma18R17]: we have repaired conditions were normal and acclimatized for seven days to reduce stress on the fish. During rearing, probiotics were added to the biofloc pond at a density of bacteria *Bacillus* sp. 10^5 CFU/mL and *Streptomyces* sp. 10^5 CFU/mL (Wijayanti *et al.*, 2020) with a frequency according to treatment, namely (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. During rearing, the addition of a carbon source in the form of molasses was also carried out at a dose of 200 mL m^{-3} with a frequency of once every seven days (days 0, 7, 14, 21, 28, 35) (Putra *et al.*, 2017). Fish are fed using commercial feed with a 39-41% protein content. The feeding frequency is three times daily, namely at 08.00 am, 12.00 am, and 04.00 pm with the at satiation. If there are dead fish, the fish are weighed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental pond unit.

Research Parameters

Volume floc and Floc Composition

Floc volume was measured using an Imhoff cone by taking media water maintenance at 1 point of collection as much as 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on days 7, 14, 21, 28, 35 and 42. After measuring the floc's volume, the precipitated floc is taken to observe the composition of floc microorganisms. Floc composition was microscopically observed (40x magnification) on days 0, 1, 21, and 42.

Total Bacterial Colonies

Total bacterial colonies formed in the rearing medium were counted with the cup count method by taking water as a rearing medium, and counting the bacterial colonies using a colony counter. After The sample water is diluted through dilution steps 10^{-5}, 10^{-7}, 10^{-9}. Bacterial density calculations were done on days 0 (before adding probiotics from swamps), 1, 21, and 42. Growing bacterial colonies were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1) = Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth

The formula used to measure absolute weight growth, according to Hopkins (1992) is:

$$W = Wt - Wo$$

Information:

W = Growth in absolute weight of fish kept (g)
Wt = Average fish weight at the end of the study (g)
Wo = Average fish weight at the start of the study (g)

The formula used to measure absolute length growth, according to Hopkins (1992), is:

$$L = Lt - Lo$$

Information:

L = Absolute length growth of reared fish (cm)
Lt = Average length of fish at the end of the study (cm)
Lo = Average fish length at the start of the study (cm)

Feed Efficiency

Feed efficiency (FE) is calculated based on a formula based on Afrianto & Liviawaty (2005):

$$FE\ (\%) = \frac{(Wt+D)-Wo}{F}\ x\ 100$$

Information:

FE = Feed efficiency (%)
Wt = Fish biomass at the end of the study (g)
Wo = Fish biomass at the start of the study (g)
D = Biomass of fish that died during the study (g)
F = Amount of fish feed given during the study (g)

Survival Rate (SR)

The survival rate is calculated using a formula based on Aliyu-Paiko*et al.* (2010) as follows:

$$SR\ (\%) = \frac{Nt}{No}\ x\ 100$$

Information:

SR = Survival (%)
Nt = The final quantity of fish at the end of rearing (g)
No = The initial quantity of fish at initial rearing (g)

Biomass Production

The formula can calculate the level of biomass production according to Shang (1982):

$$P\ (g) = W\ x\ N$$

Information:

P = Production of biomass (g)
W = Average weight of fish at the end of rearing (g)
N = The final quantity of fish at the end of rearing (g)

Water quality

Water quality parameters included temperature and pH, measured every day at 08.00 a.m and 04.00 p.m. Temperature measurements (°C) using a thermometer, pH using a pH meter, Dissolved oxygen (DO) using a DO meter (mg/L), *Total Dissolved solid* (TDS) (mg/L) using a TDS meter, and ammonia (mg/L) using a spectrophotometer.

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Dissolved oxygen level, TDS, and ammonia were measured every seven days (day 0, 7, 14, 21, 28, 35, and 42) once in 42 days of the rearing period.

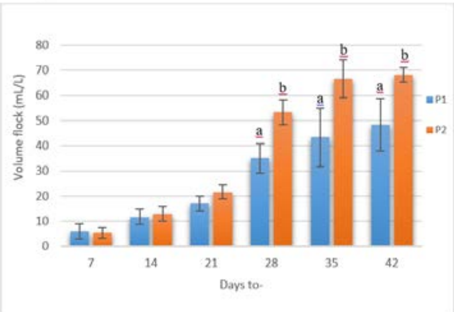
Data analysis

Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively.

Results and Discussion

Volume Floc

The results of the T-test analysis of flock volume during rearing are presented in Figure 1.



Note: Superscript of diferent letters in diagram in same days show significant differences on the 5% level T-test

Figure 1. Diagram of flock volume

Based on the results of the analysis showed that the floc volume in treatment P1 was not significantly different from treatment P2 on days 7, 14, and 21 and was significantly different on days 28, 35, and 42. Floc volume is the amount of suspended solids over a certain period in an inverted conical container (Effendi, 2003). The highest floc volume was found in the P2 treatment given probiotics with a frequency of 2 times during 42 days of rearing, reaching 68.33 mL/L while P1 is 48.33 mL/L. The floc volume obtained in this study was higher than that in the study by Wijayanti *et al.* (2021), which is 40 mL/L (42nd day). This is presumably due to the influence of the time-frequency of giving probiotics to the rearing medium. According to De Schryver *et al.* (2008), the factors that influence the formation of biofloc are the administration of probiotics (starter) with floc-forming microbial composition, agitation intensity by aeration device, organic carbon source, and water quality. This is also reinforced by the results of Malaputra *et al.* (2016), namely the administration of commercial probiotics with a frequency of 14 times (70 days of rearing) resulted in a floc volume of 120 mL/L higher than the frequency of 7 times which is only 80 mL/L. Based on the results of this study, it can be stated that the more often probiotics are given to the rearing medium, the more volume will increase. The maximum floc volume for catfish farming is 100 mL/L. High volume of flock can cause fish death (Rofianingrum *et al.*, 2022). When the floc volume exceeds the limit, partial water removal can be done and replaced with new water, around 70-80% of the total water volume of the preservation pond (Zaidy, 2022).

In addition to the factor of providing probiotics from swamps as a starter containing heterotrophic bacteria, the increase in floc volume every week in this study was thought to be due to the C/N ratio. The C/N ratio can affect the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), the C/N ratio >10 in fish farming activities is the optimum ratio for the formation of biofloc. Meanwhile, according to Avnimelech *et al.* (1999) required a C/N ratio >15. Therefore, it is necessary to add organic carbon from outside, such as molasses. This study added extra organic carbon in molasses every seven days at a dose of 200 mL m^{-3} (Putra *et al.*, 2017).

Floc Composition

The composition of the flocs in this study is presented in Table 2.

~~**Table 2.** Composition of catfish (*Clarias gariepinus*) flocs maintened in biofloc system~~

~~Floc-forming microbes~~	~~Treatment~~	
	~~P1~~	~~P2~~
~~Chlorophyta~~	✓	✓
~~Cyanophyta~~	✓	✓
~~Protozoa~~	✓	✓
~~Coelenterata~~	-	✓
~~Rotifers~~	✓	✓
~~Arthropods~~	-	✓

~~Information: (✓) exists, (-) does not exist~~

Table 2. Composition of catfish (*Clarias gariepinus*) flocs maintened in biofloc system

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Floc-forming microbes	Treatment							
	H0		H1		H21		H42	
	P1	P2	P1	P2	P1	P2	P1	P2
Chlorophyta	✓	✓	✓	✓	✓	✓	✓	✓
Cyanophyta	✓	✓	✓	✓	✓	✓	✓	✓
Protozoa	✓	✓	✓	✓	✓	✓	✓	✓
Coelenterata	-	✓	-	✓	-	✓	-	✓
Rotifers	✓	✓	✓	✓	✓	✓	✓	✓
Arthropods	-	✓	-	✓	-	✓	-	✓

Information: (✓) exists, (-) does not exist

Based on observations of floc composition in the treatment of probiotics from swamps with P2 frequencies more diverse than P1, namely chlorophyta, cyanophyta, protozoa, coelenterata, rotifers, and arthropods. Whereas in P1, only chlorophyta, cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), the diversity in the composition of the floc-forming microbes is thought to be due to the effect of giving probiotics, which is in line with the increased volume of floc in the rearing medium so that the microbes in the rearing containers can grow properly. Wati (2021) also produced the same floc composition as arthropods, chlorophyta, cyanophyta, protozoa, and rotifers. The composition of the flocs in the form of zooplankton in this study were protozoa, coelenterata, rotifers, arthropods, and phytoplankton in the form of chlorophyta and cyanophyta. This is following the opinion of De Schryver *et al.* (2008), namely the composition of floc in the form of a heterogeneous combination of microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach sizes <1000 μm.

Total Bacterial Colonies

The total bacterial colonies on the rearing medium are presented in Table 3.

Table 3. The total bacterial colonies in the flocs of catfish (*Clarias gariepinus*) in the biofloc system

Days to-	Total bacterial colonies ($\times 10^9$ CFU/mL)	
	P1	P2
0	2.77±1.00	2.77±1.00
1	13.23±2.82	14.89±3.88
21	5.56±2.01	6.65±3.07
42	8.42±3.19^a	19.73±6.19^b

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the total bacterial colonies in the P1 treatment were not significantly different from the P2 treatment on days 0, 1, and 21 and were significantly different on the 42nd day. This study's bacterial density range was between 2.77 and 19.73 x 10^9 CFU/mL. After stocking probiotics from swamps on rearing media in treatments P1 and P2, total bacterial colonies increased on day 1, and day 21 decreased. The decrease in total bacterial colonies on day 21 is thought to be a lack of nutritional sources (macronutrient or micronutrient) for bacteria (Wijayanti *et al.*, 2020). The lack nutrition for bacterial growth because nutrients have not been added in the form of a carbon source, namely molasses. According to Nasmia and Rifai (2020) the decrease in the number of bacterial colonies can be caused by a decrease in the number of carbon sources. On the 42nd day, the bacterial density increased again with a higher P2 bacterial density of 19.73 x10^9 CFU/mL compared to P1, which is 8.42 x10^9 CFU/mL. The high density of bacteria at P2 was thought to be due to adding probiotics again on the 21st day. In research, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish rearing by adding probiotics up to x10^{14} CFU/mL.

Meanwhile, according to Sitorus *et al.* (2019), the density of bacteria can reach with the number of cells is 10^9 CFU/mL. Widnyana (2016) states that on biofloc technology, total bacterial colonies ranged from 10^3-10^4 CFU/mL, while the system has a bacterial range of 10^8-10^{10} CFU/mL. This is caused by the system having a high intensity of sunlight as one of the supporting factors in the growth of bacteria. The high value of the total density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, which is assisted by their effectiveness in breaking down organic matter (Adharani *et al.*, 2016).

Absolute Growth, Feed Efficiency, Survival, and Biomass Production

Based on the research results, absolute growth data, feed efficiency, and survival of catfish for 42 days of rearing are presented in Table 4.

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Table 4. The results of absolute growth, analysis, feed efficiency, survival, and biomass production during the rearing of catfish (*Clarias gariepinus*) in the biofloc system

Parameter	Treatment	
	P1	P2
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24^{a}	151.90 ± 7.98^{b}
Survival rate (%)	81.54 ± 1.59	89.33 ±6.21
Biomass production (g)	17439.14 ± 1346.55^{a}	24639.50 ± 1344.51^{b}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the analysis showed that the growth in absolute length and absolute weight growth of catfish in treatment P1 was not significantly different from treatment P2. The absolute length growth and weight growth values of probiotics from swamps P2 were 8.18 ± 1.03 cm and 19.30 ± 3.12 g, higher than P1, which were 7.16 ± 0.12 cm and 16.42 ± 1.06 g. The results of this study indicate that fish use the floc as additional feed to increase fish growth. Catfish can utilize the available flock in the rearing medium as additional feed because it is high in protein and can increase fish growth (Putra *et al*., 2017). In research, Wijaya *et al.* (2016) floc contains 42.42% protein, 92.15% water, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract material (NFE). According to Febriyanti *et al.* (2018), heterotrophic bacterial communities that accumulate in the rearing medium will form flocs (clumps) that can be used as a feed source for fish. Therefore, giving probiotics from swamps in the form of heterotrophic bacteria such as *Bacillus* sp.

Furthermore, *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. The bacteria *Streptomyces* sp. in fish farming can also be applied as a growth promoter (Cruz *et al*., 2012). The results of fish growth will affect the value of feed efficiency.

The result of the analysis showed that the efficiency of catfish feed in treatment P1 was significantly different from treatment P2. The feed efficiency value for P2 was higher, namely 151.90%, while P1 was 104.97%. If the feed efficiency value is converted to a value feed conversion ratio (FCR) then FCR P1 is 0.96 while P2 is 0.66. The FCR value in this study was better than the research of Wijayanti *et al.* (2021), which is 0.97. Putra *et al.* (2017) researched that administering probiotics to catfish rearing media with biofloc technology also resulted in feed efficiency between 88.17-110.86%. This study's high feed efficiency value is also suspected because fish use flocks as additional feed. De Schryver *et al.* (2008) stated that using flocks as additional feed is important in increasing feed efficiency. This shows that swamp probiotics can form flocs, which fish then eat as additional feed for fish.

The analysis of in this research showed that the survival of catfish in treatment P1 was not significantly different from treatment P2. Survival in this study was quite good. The highest yield was in P2, 89.33%, followed by P1, 81.54%. The results of this study are close to the research of Wijayanti *et al.* (2021), namely 87.57% with a rearing period of 90 days. Administration of probiotics from swamps in the form of a combination of bacteria *Bacillus* sp. and bacteria *Streptomyces* sp., the percentage of fish survival can provide defense against pathogens by increasing non-specific immunity in the fish's immune system and maintaining a balance of water quality so that fish can survive (Wijayanti *et al*., 2020). Growth and survival will affect the production value of fish biomass.

The analysis of in the research ~~riset~~ showed that the production of catfish biomass in treatment P1 was significantly different from treatment P2. The production value of P2's biomass was 24,639.50 g, higher than that of P1, which was 17,439.14 g. Giving probiotics is thought to increase the production value of catfish in this biofloc technology. The biomass determines production at the end of cultivation and the survival of fish at the end of cultivation, so the higher the fish that can survive until the end of rearing, the more fish production will be produced (Anam *et al*., 2017). Based on the results of absolute growth data and feed efficiency in this study, probiotics from swamps can form flocks used by fish as additional feed. Giving probiotics from swamps is also useful in preventing pathogenic bacteria to prevent death in fish. Therefore, giving probiotics from swamps can increase fish growth and survival, resulting in increased fish production.

Water quality

Temperature and pH

Based on the research results, the temperature and pH data of the rearing media are presented in Table 5.

Table 5. The results of the temperature and pH analysis of catfish (*Clarias gariepinus*) in the biofloc system

Treatment	Temperature (°C)	pH
P1	28.85-29.22^{a}	7.27-7.40^{a}

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P2	29.48-29.59[b]	7.39-7.42[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the temperature and pH of the catfish-rearing media in treatment P1 were not significantly different from those in treatment P2. The temperature of the rearing medium in this study ranged from 28.85-29.59ºC. According to BSN (2014), the temperature value for raising catfish is 25-30ºC. Catfish will experience slow growth when the temperature is 16-24ºC or 31-32ºC, and the fish will die if the temperature is below 16ºC or above 32ºC (Pujiharsono & Kurnianto, 2020). The value of the degree of acidity or pH in the rearing medium ranges from 7.27 to 7.42. BSN (2014) states that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth when the pH is 4-6.4 or pH 8.6-11, and the fish will die if the pH is below four or above 11 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The results of the DO T-test analysis during rearing are presented in Table 6.

Table 6. The results of the DO analysis during rearing of catfish (*Clarias gariepinus*) in the biofloc system

Days to-	DO (mg/L)	
	P1	P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27[a]	5.25±0.44[b]
21	4.47±0.03[a]	4.98±0.33[b]
28	4.88±0.12[a]	5.72±0.42[b]
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The results of the analysis show that Dissolved Oxygen (DO) or dissolved oxygen in treatment P1 was not significantly different from treatment P2 on days 0,7,35 and 42, but significantly different on days 14, 21 and 28. Based on Table 4.6. the frequency of probiotics from swamp P2 (2 times for 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study ranged from 3.91 to 5.95 mg/L. According to BSN (2014), the dissolved oxygen value for catfish is >3 mg/L. Therefore, the DO value in this study is still relatively safe for catfish farming. This is because the rearing container is equipped with aeration of 4 points. Besides that, it also comes from photosynthesis results from phytoplankton and oxygen diffusion from the air (Putra *et al*., 2014). Based on this, it is suspected that the increase in DO at the end of P1 rearing is better than P2. Based on research by Agustina (2018), phytoplankton from the chlorophyte division can increase dissolved oxygen levels in swamp water cultivation media by 63.63%. In research, Wijayanti *et al.* (2020) also produced a combination of chlorophyta and probiotics from swamps containing bacteria *Bacillus* sp. Furthermore, *Streptomyces* sp. is the best treatment for dissolved oxygen concentrations in fish culture media> 3 mg/L. The results of research by Kurniawan and Utama (2018) also yield DO values for catfish rearing >6 mg/L by administering probiotics *Bacillus* sp. as according to Prihanto *et al.* (2021) bacteria *Bacillus* sp. can improve water quality.

Ammonia

The results of the ammonia T-test analysis during rearing are presented in Table 7.

Table 7. The results of ammonia analysis during rearing of catfish (*Catfish gariepinus*) in the biofloc system

Days to-	Ammonia (mg/L)	
	P1	P2
0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23[a]	0.69±0.47[b]
35	1.59±0.38[a]	0.66±0.37[b]
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the ammonia in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 21, and 42 but was significantly different on days 28 and 35. Based on Table 4.7 shows that the administration of probiotics from swamp P2 (2 times for 42 days of rearing) is proven to reduce ammonia levels in rearing media compared to P1 (1 time for 42 days of rearing). The ammonia value in this study was high, ranging from 0.45 to 1.62 mg/L, while in research by Wijayanti *et al.* (2021), it is 0.27 mg/L. The value of ammonia for catfish farming on the formation of biofloc is <1 mg/L (Wijaya *et al*., 2016). This high value of ammonia can cause death in fish due to the toxic nature of the ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion results, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). The high ammonia value in this study is likely due to the lack of a C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in water. Meanwhile, according to Hargreaves (2006), at a C/N ratio <10, heterotrophic bacteria will release ammonia into their environment. Research results Bakar *et al.* (2015) also stated that ammonia reduction in catfish farming using biofloc technology using a C/N 15 ratio could eliminate ammonia by 93.56% on the 12th day while the C/N 10 ratio on the 12th day was only 11.01%. Furthermore, only the maximum is on the 30th day, namely 98.51%.

Total Dissolved Solid (TDS)

The results of the TDS analysis during rearing are presented in Table 8.

Table 8. The results of TDS analysis during the rearing of catfish (*Clarias gariepinus*) in the biofloc system

Days to-	TDS (mg/L) P1	TDS (mg/L) P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76^{b}	872.67±41.55^{a}
28	804.17±59.93	757.67±136.93
35	810.00±57.38^{b}	717.33±21.37^{a}
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show

Based on the results of the T-test analysis, it shows that Total Dissolved Solid (TDS) in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 28, and 42 but significantly different on days 21, and 35. Based on Table 4.8. administration of probiotics from swamps P2 (2 times during 42 days of rearing) can reduce TDS values compared to P1 (1 time during 42 days of rearing). TDS values in this study ranged from 717.33 to 1014.83 mg/L. Catfish can still tolerate the measurement results of these values to live ~~(check SNI also)~~ (BSN, 2018). states that TDS's standard water quality value in fish farming activities is 1000 mg/L. The high level of TDS on the 21st day, especially in the P1 treatment, is thought to be due to the administration of commercial drugs to prevent dead fish containing organic and inorganic compounds. This follows the statement of Rinawati *et al.* (2016), who stated that large amounts of organic and inorganic compounds can result in high levels of TDS in water.

Conclusion

Based on the results of the research that has been carried out, it can be concluded that giving probiotics from swamp with a frequency of 2 times for 42 days of rearing (P2) gives the best results on flock volume, fish growth, fish feed. fish efficiency, fish survival rate, fish biomass production, and water quality. As a suggestion, probiotics from swamps can be used for biofloc formation and catfish production using biofloc technology with a frequency of 2 times for 42 days of cultivation or once every 21 days.

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Acknowledgments

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Respon penulis dan artikel yang diresubmit

Respon penulis terhadap revisi tahap 2

No	Komenter Review	Tanggapan
	Review 1	
1	Introduction	
	Check the official English name for MMF	Ministry of Marine and Fisheries Affairs of the Republic of Indonesia
	Wijayanti was lower because not pure biofloc (also have aquaponic). Bakar use pure biofloc. Not apple to apple to compare. Suggest change the sentence.	<u>We have repaired</u> According to Agusta *et al.* (2022) the addition of a carbon source in the form of molasses in the biofloc system catfish cultivation with the addition of a carbon source produces a floc volume of 111 mL/L. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water, around 70-80% of the water volume
	The sentences is not relatable. Suggest add previous reports related your study. Find the gap, determine the novelty of your current study.	<u>We have added</u> These flocks must continue to be available in the rearing medium in sufficient quantities as natural food for fish to increase fish growth and improve feed efficiency and water quality. According to Feroza et al. (2021), the flocs in the rearing media will decrease because they are consumed by fish every day, so it is necessary to have the availability of flocs in the rearing medium or not cause blooming microorganisms due to excess floc volume. Therefore, it is necessary to research the effect of the frequency of probiotics from swamps on the formation of biofloc and catfish production
2	Material and Method	
	Determine the species	<u>We've fixed it</u> Catfish (*Clarias gariepinus*)
	Mention the total volume of water	<u>We've fixed it</u>

		volume 2198 L
	Why do u choose satiation? Why not based on FR	we want to see the level of fish feed consumption in the biofloc system, the feeding response of fish in the resulting floc,
3	Resulth and Discussion	
	Why 100 ml/L, how if more than 100 ml/L? source?	We've fixed it The maximum floc volume for catfish farming is 100 mL/L. High volume of flock can cause fish death (Rofianingrum *et al.,* 2022).
	Why lack nutrition at 21 day	We've fixed it The lack nutrition for bacterial growth because nutrients have not been added in the form of a carbon source, namely molasses. According to Nasmia and Rifai (2020) the decrease in the number of bacterial colonies can be caused by a decrease in the number of carbon sources.
4	Conclusion	
	Add the future study suggestion.	Suggestions for further research on giving swamp probiotics with different frequencies to other types of fish in the biofloc system.

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Frequency of Probiotics from Swamp for Formation of Biofloc and Production of Catfish (*Clarias gariepinus*

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ABSTRACT

Catfish (*Clarias gariepinus*) cultivated with a combination of the addition of probiotics in biofloc technology are thought to increase production. The frequency of adding probiotics from swamps to cultured water media has never been studied to ensure flocks' availability in rearing media. This study aimed to determine the appropriate frequency of probiotics from swamps in biofloc technology to improve the parameters of successful cultivation, especially increasing catfish production and biofloc formation. This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given was different in the frequency of giving probiotics from swamps: (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively. The results showed that P2 was the best treatment with a floc volume of 68.33 ± 10.41 mL/L, absolute length growth of 8.18 ± 1.03 cm, absolute weight growth of 19.30 ± 3.12 g, feed efficiency of 135.24 ± 7.98%, survival of 89.33 ± 6.21%, biomass production of 24639.50 ± 1344.51 g, temperature of 28.85-29.59ºC, pH of 7.27-7.42, dissolved oxygen (DO) of 3.91-5.72 mg/L, ammonia of 0.45-1.15 mg/L, and total dissolved solids (TDS) of 717.33-885.50 mg/L. Therefore, probiotics from swamps should be given to fish culture media with a frequency of 2 times during 42 days of rearing or once every 21 days.

Introduction

Catfish (*Clarias gariepinus*) is a freshwater fishery commodity widely cultivated due to high market demand. According to data from the Statistics from the Ministry of Maritime ~~Affairs~~ and Fisheries Affairs of the Republic of Indonesia (2022), catfish production in Indonesia in the enlargement cultivation sector in 2019 was 289 thousand tons and reached 384 thousand tons in 2020, while in 2021, it was 360 thousand tons. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and with an environmental perspective by minimizing waste disposal in the surrounding waters (Fauzi *et al*., 2022). According to Putra *et al*. (2017), technology biofloc by utilizing probiotics in the form of heterotrophic bacteria can produce natural feed from the flocs that are formed, thereby increasing the value of feed efficiency and improving water quality.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the media. Swamp microorganisms identified are *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti *et al*., 2018). Giving probiotics from swamps (*Bacillus* sp. and *Streptomyces* sp.) has been studied in feeding the agility fish (Tanbiyaskur *et al*., 2022) and the rearing medium with biofloc technology in catfish (Wijayanti *et al*., 2021) and snakehead fish (Wati, 2021). In another study has been reported previously that swamp probiotics can

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increase growth, feed efficiency, fish survival, and aquaculture water quality (Wijayanti *et al*., 2020).

Identified from swamp waters there are several types of probiotics such as Bacillus sp. and Streptomyces sp. This type can be used as a starter in a biofloc system for floc formation. In previous research conducted by Wijayanti et al. (2021), administering probiotics from swamps with a frequency of 1 time for 42 days of maintenance resulted in a floc volume of 40 mL/L (42nd day). Using these flocs for 60 days of rearing resulted in the final average weight and length growth from the initial average of 7.16 g to 36.95 g and 9.50 cm to 18.50 cm. The resulting floc volume is relatively low. ~~Floc volume results (Wijayanti *et al*., 2021) are lower than Bakar *et al*. (2015) research results, whose floc volume reached 92.5 mL/L. According to Zaidy (2022), the maximum floc volume for catfish is 100 mL/L.~~ According to Agusta *et al.* (2022) the addition of a carbon source in the form of molasses in the biofloc system catfish cultivation with the addition of a carbon source produces a floc volume of 111 mL/L. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water, around 70-80% of the water volume. These flocks must continue to be available in the rearing medium in sufficient quantities as natural food for fish to increase fish growth and improve feed efficiency and water quality. According to Feroza *et al.* (2021), the flocs in the rearing media will decrease because they are consumed by fish every day, so it is necessary to have the availability of flocs in the rearing medium or not cause blooming microorganisms due to excess floc volume. Therefore, it is necessary to research the effect of the frequency of probiotics from swamps on the formation of biofloc and catfish production.

Materials and Methods

Location and time of research

This research was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University in December 2022–January 2023.

Research Materials

The materials used were catfish seeds (7 ± 0.5 cm in length) and bacteria *Bacillus* sp., *Streptomyces* sp., molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tool used is a tarpaulin pool with a round diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, imhoff cone, loop needle, erlenmeyer, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter and ammonia test kit.

Research Design

This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given is the difference in the frequency of giving probiotics from swamps, which are as follows:
P1 = 1 time during 42 days of rearing
P2 = 2 times during 42 days of rearing

Work Procedure

Swamp Origin Probiotic Culture

Pure culture obtained from the isolation of bacteria from swamps *Bacillus* sp. and *Streptomyces* sp. results of previous studies (Wijayanti *et al*., 2018) liquid culture was carried out. Bacterial liquid culture *Bacillus* sp. was done on the media Nutrient Broth (NB) liquid by taking one ose of bacteria for deep culture Erlenmeyer, which already contains NB 20 mL media. In contrast, *Streptomyces* sp. was done on the media Yeast Malt (YM) liquid by taking one ose of bacteria and culturing in liquid YM media 20 mL deep Erlenmeyer. The two bacteria were agitated with a magnetic hot plate stirrer for three days for *Bacillus* sp. and five days for *Streptomyces* sp. The density of bacteria obtained from liquid culture results was calculated using the cup counting or Total Plate Count (TPC) method. Then, do the mixing of bacteria with the formulation. Liquid media for storage media formulations in 5% molasses and filled with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish (*Clarias gariepinus*) were reared in 6 round ponds made of tarpaulin with a diameter of 2 m and a height of 1.2 m. Rearing begins with pond preparation, including cleaning, water filling, and pond incubation. The pond is cleaned by brushing the entire inside of the pond and drying it for one day to kill the pathogens. Then, fill with water as high as 0.7 m (volume 2198 L) and incubate for three days (Ma'ruf, 2016). Then salt at a dose of 1 kg m^{-3} and camphor dolomite 50 g m^{-3} (Sucipto *et al*., 2018) were added to the rearing medium and incubated for one day (Wijayanti *et al*., 2021). Installation of aerators is carried out at 4 points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process uses catfish measuring 7 ± 0.5 cm with a stocking density of 500 m^{-3} ~~(Ma'ruf, 2006)~~ (BSN, 2018) and maintained for 42 days. Fish stocking was carried out in the morning when water conditions were normal and acclimatized for seven days to reduce stress on the fish. During rearing, probiotics were added to the biofloc pond at a density of bacteria *Bacillus* sp. 10^5 CFU/mL and *Streptomyces* sp. 10^5 CFU/mL (Wijayanti *et al*., 2020) with a frequency according to treatment, namely (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. During rearing, the addition of a carbon source in the form of molasses was also carried out at a dose of 200 mL m^{-3} with a frequency of once every seven days (days 0, 7, 14, 21, 28, 35) (Putra *et al*., 2017). Fish are fed using commercial feed with a 39-41% protein content. The feeding frequency is three times daily, namely at 08.00 am, 12.00 am, and 04.00 pm with the at satiation. If there are dead fish, the fish are weighed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental pond unit.

Research Parameters

Volume floc and Floc Composition

Floc volume was measured using an Imhoff cone by taking media water maintenance at 1 point of collection as much as 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on days 7, 14, 21, 28, 35 and 42. After measuring the floc's volume, the precipitated floc is taken to observe the composition of floc microorganisms. Floc composition was microscopically observed (40x magnification) on days 0, 1, 21, and 42.

Total Bacterial Colonies

Total bacterial colonies formed in the rearing medium were counted with the cup count method by taking water as a rearing medium, and counting the bacterial colonies using a colony counter. After The sample water is diluted through dilution steps 10^{-5}, 10^{-7}, 10^{-9}. Bacterial density calculations were done on days 0 (before adding probiotics from swamps), 1, 21, and 42. Growing bacterial colonies were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1) = Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth

The formula used to measure absolute weight growth, according to Hopkins (1992) is:

$$W = Wt - Wo$$

Information:

W	=	Growth in absolute weight of fish kept (g)
Wt	=	Average fish weight at the end of the study (g)
Wo	=	Average fish weight at the start of the study (g)

The formula used to measure absolute length growth, according to Hopkins (1992), is:

$$L = Lt - Lo$$

Information:

L	=	Absolute length growth of reared fish (cm)
Lt	=	Average length of fish at the end of the study (cm)
Lo	=	Average fish length at the start of the study (cm)

Feed Efficiency

Feed efficiency (FE) is calculated based on a formula based on Afrianto & Liviawaty (2005):

$$FE\ (\%) = \frac{(Wt+D)-Wo}{F} \times 100$$

Information:

FE	=	Feed efficiency (%)
Wt	=	Fish biomass at the end of the study (g)
Wo	=	Fish biomass at the start of the study (g)
D	=	Biomass of fish that died during the study (g)
F	=	Amount of fish feed given during the study (g)

Survival Rate (SR)

The survival rate is calculated using a formula based on Aliyu-Paikoet *al.* (2010) as follows:

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

Information:

SR	=	Survival (%)
Nt	=	The final quantity of fish at the end of rearing (g)
No	=	The initial quantity of fish at initial rearing (g)

Biomass Production

The formula can calculate the level of biomass production according to Shang (1982):

$$P\ (g) = W \times N$$

Information:

P	=	Production of biomass (g)
W	=	Average weight of fish at the end of rearing (g)
N	=	The final quantity of fish at the end of rearing (g)

Water quality

Water quality parameters included temperature and pH, measured every day at 08.00 a.m and 04.00 p.m. Temperature measurements (°C) using a thermometer, pH using a pH meter, Dissolved oxygen (DO) using a DO meter (mg/L), *Total Dissolved solid* (TDS) (mg/L) using a TDS meter, and ammonia (mg/L) using a spectrophotometer. Dissolved oxygen level, TDS, and ammonia were measured every seven days (day 0, 7, 14, 21, 28, 35, and 42) once in 42 days of the rearing period.

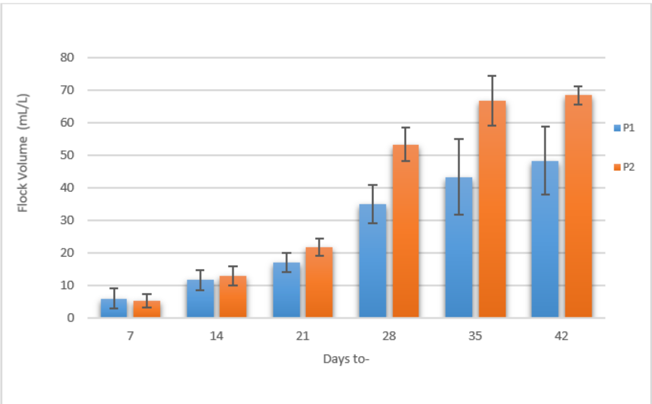
Data analysis

Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively.

Results and Discussion

Volume Floc

The results of the T-test analysis of flock volume during rearing are presented in Figure 1.



Note: Superscript of diferent letters in diagram in same days show significant differences on the 5% level T-test

Figure 1. Diagram of flock volume

Based on the results of the analysis showed that the floc volume in treatment P1 was not significantly different from treatment P2 on days 7, 14, and 21 and was significantly different on days 28, 35, and 42. Floc volume is the amount of suspended solids over a certain period in an inverted conical container (Effendi, 2003). The highest floc volume was found in the P2 treatment given probiotics with a frequency of 2 times during 42 days of rearing, reaching 68.33 mL/L while P1 is 48.33 mL/L. The floc volume obtained in this study was higher than that in the study by Wijayanti *et al.* (2021), which is 40 mL/L (42nd day). This is presumably due to the influence of the time-frequency of giving probiotics to the rearing medium. According to De Schryver *et al.* (2008), the factors that influence the formation of biofloc are the administration of probiotics (starter) with floc-forming microbial composition, agitation intensity by aeration device, organic carbon source, and water quality. This is also reinforced by the results of Malaputra *et al.* (2016), namely the administration of commercial probiotics with a frequency of 14 times (70 days of rearing) resulted in a floc volume of 120 mL/L higher than the frequency of 7 times which is only 80 mL/L. Based on the results of this study, it can be stated that the more often probiotics are given to the rearing medium, the more volume will increase. The maximum floc volume for catfish farming is 100 mL/L. According to Agusta *et al.* (2022) the addition of a carbon source in the form of molasses in the biofloc system catfish cultivation with the addition of a carbon source produces a floc volume of 111 mL/L. When the floc volume exceeds the limit, partial water removal can be done and replaced with new water, around 70-80% of the total water volume of the preservation pond (Zaidy, 2022). High volume of flock can cause fish death (Rofianingrum *et al.*, 2022).

In addition to the factor of providing probiotics from swamps as a starter containing heterotrophic bacteria, the increase in floc volume every week in this study was thought to be due to the C/N ratio. The C/N ratio can affect the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), the C/N ratio >10 in fish farming activities is the optimum ratio for the formation of biofloc. Meanwhile, according to Avnimelech *et al.* (1999) required a C/N ratio >15. Therefore, it is necessary to add organic carbon from outside, such as molasses. This study added extra organic carbon in molasses every seven days at a dose of 200 mL m^{-3} (Putra *et al.*, 2017).

Floc Composition

The composition of the flocs in this study is presented in Table 2.

Table 2. Composition of catfish (*Clarias gariepinus*) flocs maintened in biofloc system

Floc-forming microbes	Treatment							
	H0		H1		H21		H42	
	P1	P2	P1	P2	P1	P2	P1	P2
Chlorophyta	✓	✓	✓	✓	✓	✓	✓	✓
Cyanophyta	✓	✓	✓	✓	✓	✓	✓	✓
Protozoa	✓	✓	✓	✓	✓	✓	✓	✓

Coelenterata	-	✓	-	✓	-	✓	-	✓
Rotifers	✓	✓	✓	✓	✓	✓	✓	✓
Arthropods	-	✓	-	✓	-	✓	-	✓

Information: (✓) exists, (-) does not exist

Based on observations of floc composition in the treatment of probiotics from swamps with P2 frequencies more diverse than P1, namely chlorophyta, cyanophyta, protozoa, coelenterata, rotifers, and arthropods. Whereas in P1, only chlorophyta, cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), the diversity in the composition of the floc-forming microbes is thought to be due to the effect of giving probiotics, which is in line with the increased volume of floc in the rearing medium so that the microbes in the rearing containers can grow properly. Wati (2021) also produced the same floc composition as arthropods, chlorophyta, cyanophyta, protozoa, and rotifers. The composition of the flocs in the form of zooplankton in this study were protozoa, coelenterata, rotifers, arthropods, and phytoplankton in the form of chlorophyta and cyanophyta. This is following the opinion of De Schryver *et al.* (2008), namely the composition of floc in the form of a heterogeneous combination of microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach sizes <1000 µm.

Total Bacterial Colonies

The total bacterial colonies on the rearing medium are presented in Table 3.

Table 3. The total bacterial colonies in the flocs of catfish (*Clarias gariepinus*) in the biofloc system

Days to-	Total bacterial colonies (x 10^9 CFU/mL)	
	P1	**P2**
0	2.77±1.00	2.77±1.00
1	13.23±2.82	14.89±3.88
21	5.56±2.01	6.65±3.07
42	8.42±3.19^a	19.73±6.19^b

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the total bacterial colonies in the P1 treatment were not significantly different from the P2 treatment on days 0, 1, and 21 and were significantly different on the 42nd day. This study's bacterial density range was between 2.77 and 19.73 x 10^9 CFU/mL. After stocking probiotics from swamps on rearing media in treatments P1 and P2, total bacterial colonies increased on day 1, and day 21 decreased. The decrease in total bacterial colonies on day 21 is thought to be a lack of nutritional sources (macronutrient or micronutrient) for bacteria (Wijayanti *et al.*, 2020). The lack nutrition for bacterial growth because nutrients have not been added in the form of a carbon source, namely molasses. According to Nasmia and Rifai (2020) the decrease in the number of bacterial colonies can be caused by a decrease in the number of carbon sources. On the 42nd day, the bacterial density increased again with a higher P2 bacterial density of 19.73 x10^9 CFU/mL compared to P1, which is 8.42 x10^9 CFU/mL. The high density of bacteria at P2 was thought to be due to adding probiotics again on the 21st day. In research, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish rearing by adding probiotics up to x10^{14} CFU/mL.

Meanwhile, according to Sitorus *et al.* (2019), the density of bacteria can reach with the number of cells is 10^9 CFU/mL. Widnyana (2016) states that on biofloc technology, total bacterial colonies ranged from 10^3-10^4 CFU/mL, while the system has a bacterial range of 10^8-10^{10} CFU/mL. This is caused by the system having a high intensity of sunlight as one of the supporting factors in the growth of bacteria. The high value of the total density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, which is assisted by their effectiveness in breaking down organic matter (Adharani *et al.*, 2016).

Absolute Growth, Feed Efficiency, Survival, and Biomass Production

Based on the research results, absolute growth data, feed efficiency, and survival of catfish for 42 days of rearing are presented in Table 4.

Table 4. The results of absolute growth, analysis, feed efficiency, survival, and biomass production during the rearing of catfish (*Clarias gariepinus*) in the biofloc system

Parameter	Treatment	
	P1	**P2**
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24^a	151.90 ± 7.98^b
Survival rate (%)	81.54 ± 1.59	89.33 ±6.21
Biomass production (g)	17439.14 ± 1346.55^a	24639.50 ± 1344.51^b

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the analysis showed that the growth in absolute length and absolute weight growth of catfish in treatment P1 was not significantly different from treatment P2. The absolute length growth and weight growth values of probiotics from swamps P2 were 8.18 ± 1.03 cm and 19.30 ± 3.12 g, higher than P1, which were 7.16 ± 0.12 cm and 16.42 ± 1.06 g. The results of this study indicate that fish use the floc as additional feed to increase fish growth. Catfish can utilize the available flock in the rearing medium as additional feed because it is high in protein and can increase fish growth (Putra *et al*., 2017). In research, Wijaya *et al.* (2016) floc contains 42.42% protein, 92.15% water, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract material (NFE). According to Febriyanti *et al.* (2018), heterotrophic bacterial communities that accumulate in the rearing medium will form flocs (clumps) that can be used as a feed source for fish. Therefore, giving probiotics from swamps in the form of heterotrophic bacteria such as *Bacillus* sp.

Furthermore, *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. The bacteria *Streptomyces* sp. in fish farming can also be applied as a growth promoter (Cruz *et al*., 2012). The results of fish growth will affect the value of feed efficiency.

The result of the analysis showed that the efficiency of catfish feed in treatment P1 was significantly different from treatment P2. The feed efficiency value for P2 was higher, namely 151.90%, while P1 was 104.97%. If the feed efficiency value is converted to a value feed conversion ratio (FCR) then FCR P1 is 0.96 while P2 is 0.66. The FCR value in this study was better than the research of Wijayanti *et al.* (2021), which is 0.97. Putra *et al.* (2017) researched that administering probiotics to catfish rearing media with biofloc technology also resulted in feed efficiency between 88.17-110.86%. This study's high feed efficiency value is also suspected because fish use flocks as additional feed. De Schryver *et al.* (2008) stated that using flocks as additional feed is important in increasing feed efficiency. This shows that swamp probiotics can form flocs, which fish then eat as additional feed for fish.

The analysis of in this research showed that the survival of catfish in treatment P1 was not significantly different from treatment P2. Survival in this study was quite good. The highest yield was in P2, 89.33%, followed by P1, 81.54%. The results of this study are close to the research of Wijayanti *et al.* (2021), namely 87.57% with a rearing period of 90 days. Administration of probiotics from swamps in the form of a combination of bacteria *Bacillus* sp. and bacteria *Streptomyces* sp., the percentage of fish survival can provide defense against pathogens by increasing non-specific immunity in the fish's immune system and maintaining a balance of water quality so that fish can survive (Wijayanti *et al*., 2020). Growth and survival will affect the production value of fish biomass.

The analysis of in the research showed that the production of catfish biomass in treatment P1 was significantly different from treatment P2. The production value of P2's biomass was 24,639.50 g, higher than that of P1, which was 17,439.14 g. Giving probiotics is thought to increase the production value of catfish in this biofloc technology. The biomass determines production at the end of cultivation and the survival of fish at the end of cultivation, so the higher the fish that can survive until the end of rearing, the more fish production will be produced (Anam *et al*., 2017). Based on the results of absolute growth data and feed efficiency in this study, probiotics from swamps can form flocks used by fish as additional feed. Giving probiotics from swamps is also useful in preventing pathogenic bacteria to prevent death in fish. Therefore, giving probiotics from swamps can increase fish growth and survival, resulting in increased fish production.

Water quality

Temperature and pH

Based on the research results, the temperature and pH data of the rearing media are presented in Table 5.

Table 5. The results of the temperature and pH analysis of catfish (*Clarias gariepinus*) in the biofloc system

Treatment	Temperature (°C)	pH
P1	28.85-29.22^{a}	7.27-7.40^{a}
P2	29.48-29.59^{b}	7.39-7.42^{b}

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the temperature and pH of the catfish-rearing media in treatment P1 were not significantly different from those in treatment P2. The temperature of the rearing medium in this study ranged from 28.85-29.59ºC. According to BSN (2014), the temperature value for raising catfish is 25-30ºC. Catfish will experience slow growth when the temperature is 16-24ºC or 31-32ºC, and the fish will die if the temperature is below

16ºC or above 32ºC (Pujiharsono & Kurnianto, 2020). The value of the degree of acidity or pH in the rearing medium ranges from 7.27 to 7.42. BSN (2014) states that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth when the pH is 4-6.4 or pH 8.6-11, and the fish will die if the pH is below four or above 11 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The results of the DO T-test analysis during rearing are presented in Table 6.

Table 6. The results of the DO analysis during rearing of catfish (*Clarias gariepinus*) in the biofloc system

Days to-	DO (mg/L)	
	P1	P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27[a]	5.25±0.44[b]
21	4.47±0.03[a]	4.98±0.33[b]
28	4.88±0.12[a]	5.72±0.42[b]
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The results of the analysis show that Dissolved Oxygen (DO) or dissolved oxygen in treatment P1 was not significantly different from treatment P2 on days 0,7,35 and 42, but significantly different on days 14, 21 and 28. Based on Table 4.6. the frequency of probiotics from swamp P2 (2 times for 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study ranged from 3.91 to 5.95 mg/L. According to BSN (2014), the dissolved oxygen value for catfish is >3 mg/L. Therefore, the DO value in this study is still relatively safe for catfish farming. This is because the rearing container is equipped with aeration of 4 points. Besides that, it also comes from photosynthesis results from phytoplankton and oxygen diffusion from the air (Putra *et al.*, 2014). Based on this, it is suspected that the increase in DO at the end of P1 rearing is better than P2. Based on research by Hargreaves (2013), the biofloc system consists of algae, bacteria, protozoa, zooplankton, and other microorganisms. In research, Wijayanti *et al.* (2020) also produced a combination of chlorophyta and probiotics from swamps containing bacteria *Bacillus* sp. Furthermore, *Streptomyces* sp. is the best treatment for dissolved oxygen concentrations in fish culture media> 3 mg/L. The results of research by Kurniawan and Utama (2018) also yield DO values for catfish rearing >6 mg/L by administering probiotics *Bacillus* sp. as according to Prihanto *et al.* (2021) bacteria *Bacillus* sp. can improve water quality.

Ammonia

The results of the ammonia T-test analysis during rearing are presented in Table 7.

Table 7. The results of ammonia analysis during rearing of catfish (*Catfish gariepinus*) in the biofloc system

Days to-	Ammonia (mg/L)	
	P1	P2
0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23[a]	0.69±0.47[b]
35	1.59±0.38[a]	0.66±0.37[b]
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the ammonia in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 21, and 42 but was significantly different on days 28 and 35. Based on Table 4.7 shows that the administration of probiotics from swamp P2 (2 times for 42 days of rearing) is proven to reduce ammonia levels in rearing media compared to P1 (1 time for 42 days of rearing). The ammonia value in this study was high, ranging from 0.45 to 1.62 mg/L, while in research by Wijayanti *et al.* (2021), it is 0.27 mg/L. The value of ammonia for catfish farming on the formation of biofloc is <1 mg/L (Wijaya *et al.*, 2016). This high value of ammonia can cause death in fish due to the toxic nature of the ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion results, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). The high ammonia value in this study is likely due to the lack of a C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in water. Meanwhile, according to Hargreaves (2006), at a C/N ratio <10, heterotrophic bacteria will release ammonia into their environment. Research results Bakar *et al.* (2015) also stated that ammonia reduction in catfish farming using biofloc technology using a C/N 15 ratio could eliminate ammonia by 93.56% on the 12th day while the C/N 10 ratio on the 12th day was only 11.01%. Furthermore, only the maximum is on the 30th day, namely 98.51%.

Total Dissolved Solid (TDS)

The results of the TDS analysis during rearing are presented in Table 8.

Table 8. The results of TDS analysis during the rearing of catfish (*Clarias gariepinus*) in the biofloc system

Days to-	TDS (mg/L)	
	P1	P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76[b]	872.67±41.55[a]
28	804.17±59.93	757.67±136.93
35	810.00±57.38[b]	717.33±21.37[a]
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show

Based on the results of the T-test analysis, it shows that Total Dissolved Solid (TDS) in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 28, and 42 but significantly different on days 21, and 35. Based on Table 4.8. administration of probiotics from swamps P2 (2 times during 42 days of rearing) can reduce TDS values compared to P1 (1 time during 42 days of rearing). TDS values in this study ranged from 717.33 to 1014.83 mg/L. Catfish can still tolerate the measurement results of these values to live ~~(check SNI also)~~ (BSN, 2018). states that TDS's standard water quality value in fish farming activities is 1000 mg/L. The high level of TDS on the 21st day, especially in the P1 treatment, is thought to be due to the administration of commercial drugs to prevent dead fish containing organic and inorganic compounds. This follows the statement of Rinawati *et al.* (2016), who stated that large amounts of organic and inorganic compounds can result in high levels of TDS in water.

Conclusion

Based on the results of the research that has been carried out, it can be concluded that giving probiotics from swamp with a frequency of 2 times for 42 days of rearing (P2) gives the best results on flock volume, fish growth, fish feed. fish efficiency, fish survival rate, fish biomass production, and water quality. The suggestion from this research is that it is necessary to add immunostimulants to catfish cultivation in the biofloc system.

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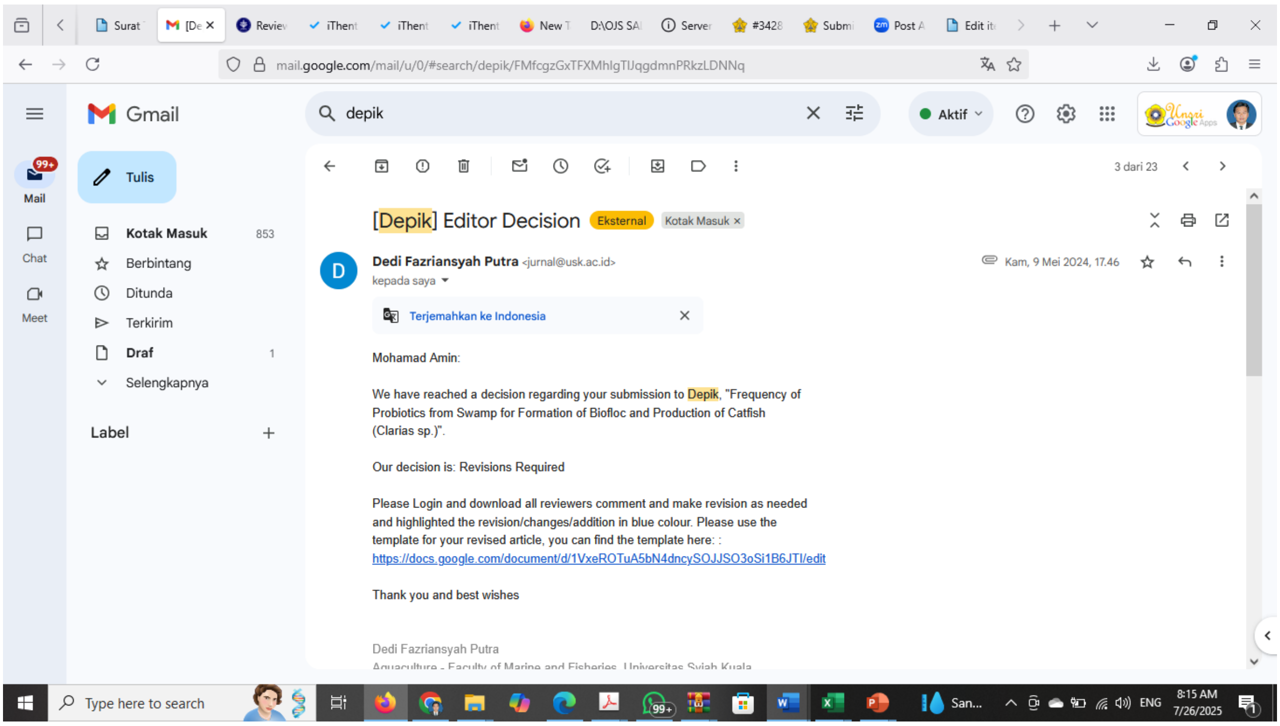

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Frequency of Probiotics from Swamp for Formation of Biofloc and Production of Catfish (*Clarias gariepinus*)

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ABSTRACT

Catfish (*Clarias gariepinus*) cultivated with a combination of the addition of probiotics in biofloc technology are thought to increase production. The frequency of adding probiotics from swamps to cultured water media has never been studied to ensure flocks' availability in rearing media. This study aimed to determine the appropriate frequency of probiotics from swamps in biofloc technology to improve the parameters of successful cultivation, especially increasing catfish production and biofloc formation. This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given was different in the frequency of giving probiotics from swamps: (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively. The results showed that P2 was the best treatment with a floc volume of 68.33 ± 10.41 mL/L, absolute length growth of 8.18 ± 1.03 cm, absolute weight growth of 19.30 ± 3.12 g, feed efficiency of 135.24 ± 7.98%, survival of 89.33 ± 6.21%, biomass production of 24639.50 ± 1344.51 g, temperature of 28.85-29.59°C, pH of 7.27-7.42, dissolved oxygen (DO) of 3.91-5.72 mg/L, ammonia of 0.45-1.15 mg/L, and total dissolved solids (TDS) of 717.33-885.50 mg/L. Therefore, probiotics from swamps should be given to fish culture media with a frequency of 2 times during 42 days of rearing or once every 21 days.

Introduction

Catfish (*Clarias gariepinus*) is a freshwater fishery commodity widely cultivated due to high market demand. According to data from the Statistics from the Ministry of Marine and Fisheries Affairs of the Republic of Indonesia (2022), catfish production in Indonesia in the enlargement cultivation sector in 2019 was 289 thousand tons and reached 384 thousand tons in 2020, while in 2021, it was 360 thousand tons. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and with an environmental perspective by minimizing waste disposal in the surrounding waters (Fauzi *et al*., 2022). According to Putra *et al.* (2017), technology biofloc by utilizing probiotics in the form of heterotrophic bacteria can produce natural feed from the flocs that are formed, thereby increasing the value of feed efficiency and improving water quality.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the media. Swamp microorganisms identified are *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti *et al*., 2018). Giving probiotics from swamps (*Bacillus* sp. and *Streptomyces* sp.) has been studied in feeding the agility fish (Tanbiyaskur *et al*., 2022) and the rearing medium with biofloc technology in catfish (Wijayanti *et al*., 2021). In another study has been reported previously that swamp probiotics can increase growth, feed

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efficiency, fish survival, and aquaculture water quality (Wijayanti *et al*., 2020).

Identified from swamp waters there are several types of probiotics such as *Bacillus* sp. and *Streptomyces* sp. This type can be used as a starter in a biofloc system for floc formation. In previous research conducted by Wijayanti et al. (2021), administering probiotics from swamps with a frequency of 1 time for 42 days of maintenance resulted in a floc volume of 40 mL/L (42^{nd} day). Using these flocs for 60 days of rearing resulted in the final average weight and length growth from the initial average of 7.16 g to 36.95 g and 9.50 cm to 18.50 cm. The resulting floc volume is relatively low. According to Agusta *et al.* (2022) the addition of a carbon source in the form of molasses in the biofloc system catfish cultivation with the addition of a carbon source produces a floc volume of 111 mL/L. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water, around 70-80% of the water volume. These flocks must continue to be available in the rearing medium in sufficient quantities as natural food for fish to increase fish growth and improve feed efficiency and water quality. According to Feroza *et al.* (2021), the flocs in the rearing media will decrease because they are consumed by fish every day, so it is necessary to have the availability of flocs in the rearing medium or not cause blooming microorganisms due to excess floc volume. Therefore, it is necessary to research the effect of the frequency of probiotics from swamps on the formation of biofloc and catfish production.

Materials and Methods

Location and time of research

This research was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University in December 2022–January 2023.

Research Materials

The materials used were catfish seeds (7 ± 0.5 cm in length) and bacteria *Bacillus* sp., *Streptomyces* sp., molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tool used is a tarpaulin pool with a round diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, imhoff cone, loop needle, erlenmeyer, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter and ammonia test kit.

Research Design

This study used a completely randomized design (CRD) consisting of two treatments and three replications. The treatment given is the difference in the frequency of giving probiotics from swamps, which are as follows:

P1 = 1 time during 42 days of rearing
P2 = 2 times during 42 days of rearing

Work Procedure

Swamp Origin Probiotic Culture

Pure culture obtained from the isolation of bacteria from swamps *Bacillus* sp. and *Streptomyces* sp. results of previous studies (Wijayanti *et al*., 2018) liquid culture was carried out. Bacterial liquid culture *Bacillus* sp. was done on the media Nutrient Broth (NB) liquid by taking one ose of bacteria for deep culture Erlenmeyer, which already contains NB 20 mL media. In contrast, *Streptomyces* sp. was done on the media Yeast Malt (YM) liquid by taking one ose of bacteria and culturing in liquid YM media 20 mL deep Erlenmeyer. The two bacteria were agitated with a magnetic hot plate stirrer for three days for *Bacillus* sp. and five days for *Streptomyces* sp. The density of bacteria obtained from liquid culture results was calculated using the cup counting or Total Plate Count (TPC) method. Then, do the mixing of bacteria with the formulation. Liquid media for storage media formulations in 5% molasses and filled with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish (*Clarias gariepinus*) were reared in 6 round ponds made of tarpaulin with a diameter of 2 m and a height of 1.2 m. Rearing begins with pond preparation, including cleaning, water filling, and pond incubation. The pond is cleaned by brushing the entire inside of the pond and drying it for one day to kill the pathogens. Then, fill with water as high as 0.7 m (volume 2198 L) and incubate for three days (Ma'ruf, 2016). Then salt at a dose of 1 kg m^{-3} and camphor dolomite 50 g m^{-3} (Sucipto *et al*., 2018) were added to the rearing medium and incubated for one day (Wijayanti *et al*., 2021). Installation of aerators is carried out at 4 points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process uses catfish measuring 7 ± 0.5 cm with a stocking density of 500 m^{-3} (BSN, 2018) and maintained for 42 days. Fish stocking was carried out in the morning when water conditions were normal and acclimatized for seven days to reduce stress on the fish. During rearing, probiotics were added to the biofloc pond at a density of bacteria *Bacillus* sp. 10^5 CFU/mL and *Streptomyces* sp. 10^5 CFU/mL (Wijayanti *et al.*, 2020) with a frequency according to treatment, namely (P1) 1 time for 42 days of rearing and (P2) 2 times for 42 days of rearing. During rearing, the addition of a carbon source in the form of molasses was also carried out at a dose of 200 mL m^{-3} with a frequency of once every seven days (days 0, 7, 14, 21, 28, 35) (Putra *et al.*, 2017). Fish are fed using commercial feed with a 39-41% protein content. The feeding frequency is three times daily, namely at 08.00 am, 12.00 am, and 04.00 pm with the at satiation. If there are dead fish, the fish are weighed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental pond unit.

Research Parameters

Volume floc and Floc Composition

Floc volume was measured using an Imhoff cone by taking media water maintenance at 1 point of collection as much as 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on days 7, 14, 21, 28, 35 and 42. After measuring the floc's volume, the precipitated floc is taken to observe the composition of floc microorganisms. Floc composition was microscopically observed (40x magnification) on days 0, 1, 21, and 42.

Total Bacterial Colonies

Total bacterial colonies formed in the rearing medium were counted with the cup count method by taking water as a rearing medium, and counting the bacterial colonies using a colony counter. After The sample water is diluted through dilution steps 10^{-5}, 10^{-7}, 10^{-9}. Bacterial density calculations were done on days 0 (before adding probiotics from swamps), 1, 21, and 42. Growing bacterial colonies were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1) = Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth

The formula used to measure absolute weight growth, according to Hopkins (1992) is:

$$W = Wt - Wo$$

Information:

W = Growth in absolute weight of fish kept (g)

Wt = Average fish weight at the end of the study (g)

Wo = Average fish weight at the start of the study (g)

The formula used to measure absolute length growth, according to Hopkins (1992), is:

$$L = Lt - Lo$$

Information:

L = Absolute length growth of reared fish (cm)

Lt = Average length of fish at the end of the study (cm)

Lo = Average fish length at the start of the study (cm)

Feed Efficiency

Feed efficiency (FE) is calculated based on a formula based on Afrianto & Liviawaty (2005):

$$FE\ (\%) = \frac{(Wt+D)-Wo}{F}\ x\ 100$$

Information:

FE = Feed efficiency (%)

Wt = Fish biomass at the end of the study (g)

Wo = Fish biomass at the start of the study (g)

D = Biomass of fish that died during the study (g)

F = Amount of fish feed given during the study (g)

Survival Rate (SR)

The survival rate is calculated using a formula based on Aliyu-Paiko*et al.* (2010) as follows:

$$SR\ (\%) = \frac{Nt}{No}\ x\ 100$$

Information:

SR = Survival (%)

Nt = The final quantity of fish at the end of rearing (g)

No = The initial quantity of fish at initial rearing (g)

Biomass Production

The formula can calculate the level of biomass production according to Shang (1982):

$$P\ (g) = W\ x\ N$$

Information:

P = Production of biomass (g)

W = Average weight of fish at the end of rearing (g)

N = The final quantity of fish at the end of rearing (g)

Water quality

Water quality parameters included temperature and pH, measured every day at 08.00 a.m and 04.00 p.m. Temperature measurements (°C) using a thermometer, pH using a pH meter, Dissolved oxygen (DO) using a DO meter (mg/L), *Total Dissolved solid* (TDS) (mg/L) using a TDS meter, and ammonia (mg/L) using a spectrophotometer.

Dissolved oxygen level, TDS, and ammonia were measured every seven days (day 0, 7, 14, 21, 28, 35, and 42) once in 42 days of the rearing period.

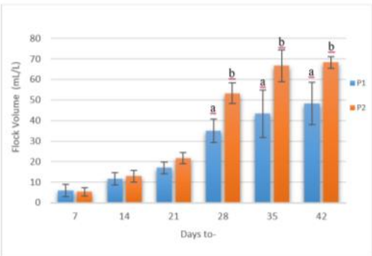
Data analysis

Data on flock volume, total bacterial colonies, absolute growth, feed efficiency, survival, and water quality were analyzed by T-test with a 95% confidence level. In comparison, the data on flock composition was analyzed descriptively.

Results and Discussion

Volume Floc

The results of the T-test analysis of flock volume during rearing are presented in Figure 1.



Note: Superscript of diferent letters in diagram in same days show significant differences on the 5% level T-test

Figure 1. Diagram of flock volume

Based on the results of the analysis showed that the floc volume in treatment P1 was not significantly different from treatment P2 on days 7, 14, and 21 and was significantly different on days 28, 35, and 42. Floc volume is the amount of suspended solids over a certain period in an inverted conical container (Effendi, 2003). The highest floc volume was found in the P2 treatment given probiotics with a frequency of 2 times during 42 days of rearing, reaching 68.33 mL/L while P1 is 48.33 mL/L. The floc volume obtained in this study was higher than that in the study by Wijayanti *et al.* (2021), which is 40 mL/L (42nd day). This is presumably due to the influence of the time-frequency of giving probiotics to the rearing medium. According to De Schryver *et al.* (2008), the factors that influence the formation of biofloc are the administration of probiotics (starter) with floc-forming microbial composition, agitation intensity by aeration device, organic carbon source, and water quality. This is also reinforced by the results of Malaputra *et al.* (2016), namely the administration of commercial probiotics with a frequency of 14 times (70 days of rearing) resulted in a floc volume of 120 mL/L higher than the frequency of 7 times which is only 80 mL/L. Based on the results of this study, it can be stated that the more often probiotics are given to the rearing medium, the more volume will increase. The maximum floc volume for catfish farming is 100 mL/L. According to Agusta *et al.* (2022) the addition of a carbon source in the form of molasses in the biofloc system catfish cultivation with the addition of a carbon source produces a floc volume of 111 mL/L. When the floc volume exceeds the limit, partial water removal can be done and replaced with new water, around 70-80% of the total water volume of the preservation pond (Zaidy, 2022). High volume of flock can cause fish death (Rofianingrum *et al.,* 2022).

In addition to the factor of providing probiotics from swamps as a starter containing heterotrophic bacteria, the increase in floc volume every week in this study was thought to be due to the C/N ratio. The C/N ratio can affect the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), the C/N ratio >10 in fish farming activities is the optimum ratio for the formation of biofloc. Meanwhile, according to Avnimelech *et al.* (1999) required a C/N ratio >15. Therefore, it is necessary to add organic carbon from outside, such as molasses. This study added extra organic carbon in molasses every seven days at a dose of 200 mL m^{-3} (Putra *et al.*, 2017).

Floc Composition

The composition of the flocs in this study is presented in Table 2.

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Table 2. Composition of catfish (*Clarias gariepinus*) flocs maintened in biofloc system

Floc-forming microbes	Treatment							
	H0		H1		H21		H42	
	P1	P2	P1	P2	P1	P2	P1	P2
Chlorophyta	✓	✓	✓	✓	✓	✓	✓	✓
Cyanophyta	✓	✓	✓	✓	✓	✓	✓	✓
Protozoa	✓	✓	✓	✓	✓	✓	✓	✓
Coelenterata	-	✓	-	✓	-	✓	-	✓
Rotifers	✓	✓	✓	✓	✓	✓	✓	✓

Arthropods	-	✓	-	✓	-	✓	-	✓

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Based on observations of floc composition in the treatment of probiotics from swamps with P2 frequencies more diverse than P1, namely chlorophyta, cyanophyta, protozoa, coelenterata, rotifers, and arthropods. Whereas in P1, only chlorophyta, cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), the diversity in the composition of the floc-forming microbes is thought to be due to the effect of giving probiotics, which is in line with the increased volume of floc in the rearing medium so that the microbes in the rearing containers can grow properly. Based on research by Hargreaves (2013), the biofloc system consists of algae, bacteria, protozoa, zooplankton, and other microorganisms. In research, Wijayanti *et al.* (2020) also produced a combination of chlorophyta and probiotics from swamps containing bacteria *Bacillus* sp. This is following the opinion of De Schryver *et al.* (2008), namely the composition of floc in the form of a heterogeneous combination of microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach sizes <1000 µm.

Total Bacterial Colonies

The total bacterial colonies on the rearing medium are presented in Table 3.

Table 3. The total bacterial colonies in the flocs of catfish (*Clarias gariepinus*) in the biofloc system

Days to-	Total bacterial colonies (x 10^9 CFU/mL)	
	P1	**P2**
0	2.77±1.00	2.77±1.00
1	13.23±2.82	14.89±3.88
21	5.56±2.01	6.65±3.07
42	8.42±3.19[a]	19.73±6.19[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the total bacterial colonies in the P1 treatment were not significantly different from the P2 treatment on days 0, 1, and 21 and were significantly different on the 42nd day. This study's bacterial density range was between 2.77 and 19.73 x 10^9 CFU/mL. After stocking probiotics from swamps on rearing media in treatments P1 and P2, total bacterial colonies increased on day 1, and day 21 decreased. The decrease in total bacterial colonies on day 21 is thought to be a lack of nutritional sources (macronutrient or micronutrient) for bacteria (Wijayanti *et al.*, 2020). The lack nutrition for bacterial growth because nutrients have not been added in the form of a carbon source, namely molasses. According to Nasmia and Rifai (2020) the decrease in the number of bacterial colonies can be caused by a decrease in the number of carbon sources. On the 42nd day, the bacterial density increased again with a higher P2 bacterial density of 19.73 x10^9 CFU/mL compared to P1, which is 8.42 x10^9 CFU/mL. The high density of bacteria at P2 was thought to be due to adding probiotics again on the 21st day. In research, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish rearing by adding probiotics up to x10^{14} CFU/mL.

Meanwhile, according to Sitorus *et al.* (2019), the density of bacteria can reach with the number of cells is 10^9 CFU/mL. Widnyana (2016) states that on biofloc technology, total bacterial colonies ranged from 10^3-10^4 CFU/mL, while the system has a bacterial range of 10^8-10^{10} CFU/mL. This is caused by the system having a high intensity of sunlight as one of the supporting factors in the growth of bacteria. The high value of the total density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, which is assisted by their effectiveness in breaking down organic matter (Adharani *et al.*, 2016).

Absolute Growth, Feed Efficiency, Survival, and Biomass Production

Based on the research results, absolute growth data, feed efficiency, and survival of catfish for 42 days of rearing are presented in Table 4.

Table 4. The results of absolute growth, analysis, feed efficiency, survival, and biomass production during the rearing of catfish (*Clarias gariepinus*) in the biofloc system

Parameter	Treatment	
	P1	**P2**
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24[a]	151.90 ± 7.98[b]
Survival rate (%)	81.54 ± 1.59	89.33 ±6.21
Biomass production (g)	17439.14 ± 1346.55[a]	24639.50 ± 1344.51[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

Based on the analysis showed that the growth in absolute length and absolute weight growth of catfish in treatment P1 was not significantly different from treatment P2. The absolute length growth and weight growth values of probiotics from swamps P2 were 8.18 ± 1.03 cm and 19.30 ± 3.12 g, higher than P1, which were 7.16 ± 0.12 cm and 16.42 ± 1.06 g. The results of this study indicate that fish use the floc as additional feed to increase fish growth. Catfish can utilize the available flock in the rearing medium as additional feed because it is high in protein and can increase fish growth (Putra *et al*., 2017). In research, Wijaya *et al.* (2016) floc contains 42.42% protein, 92.15% water, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract material (NFE). According to Febriyanti *et al.* (2018), heterotrophic bacterial communities that accumulate in the rearing medium will form flocs (clumps) that can be used as a feed source for fish. Therefore, giving probiotics from swamps in the form of heterotrophic bacteria such as *Bacillus* sp.

Furthermore, *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. The bacteria *Streptomyces* sp. in fish farming can also be applied as a growth promoter (Cruz *et al*., 2012). The results of fish growth will affect the value of feed efficiency.

The result of the analysis showed that the efficiency of catfish feed in treatment P1 was significantly different from treatment P2. The feed efficiency value for P2 was higher, namely 151.90%, while P1 was 104.97%. If the feed efficiency value is converted to a value feed conversion ratio (FCR) then FCR P1 is 0.96 while P2 is 0.66. The FCR value in this study was better than the research of Wijayanti *et al.* (2021), which is 0.97. Putra *et al.* (2017) researched that administering probiotics to catfish rearing media with biofloc technology also resulted in feed efficiency between 88.17-110.86%. This study's high feed efficiency value is also suspected because fish use flocks as additional feed. De Schryver *et al.* (2008) stated that using flocks as additional feed is important in increasing feed efficiency. This shows that swamp probiotics can form flocs, which fish then eat as additional feed for fish.

The analysis of in this research showed that the survival of catfish in treatment P1 was not significantly different from treatment P2. Survival in this study was quite good. The highest yield was in P2, 89.33%, followed by P1, 81.54%. The results of this study are close to the research of Wijayanti *et al.* (2021), namely 87.57% with a rearing period of 90 days. Administration of probiotics from swamps in the form of a combination of bacteria *Bacillus* sp. and bacteria *Streptomyces* sp., the percentage of fish survival can provide defense against pathogens by increasing non-specific immunity in the fish's immune system and maintaining a balance of water quality so that fish can survive (Wijayanti *et al*., 2020). Growth and survival will affect the production value of fish biomass.

The analysis of in the research showed that the production of catfish biomass in treatment P1 was significantly different from treatment P2. The production value of P2's biomass was 24,639.50 g, higher than that of P1, which was 17,439.14 g. Giving probiotics is thought to increase the production value of catfish in this biofloc technology. The biomass determines production at the end of cultivation and the survival of fish at the end of cultivation, so the higher the fish that can survive until the end of rearing, the more fish production will be produced (Anam *et al*., 2017). Based on the results of absolute growth data and feed efficiency in this study, probiotics from swamps can form flocks used by fish as additional feed. Giving probiotics from swamps is also useful in preventing pathogenic bacteria to prevent death in fish. Therefore, giving probiotics from swamps can increase fish growth and survival, resulting in increased fish production.

Water quality

Temperature and pH

Based on the research results, the temperature and pH data of the rearing media are presented in Table 5.

Table 5. The results of the temperature and pH analysis of catfish (*Clarias gariepinus*) in the biofloc system

Treatment	Temperature (ºC)	pH
P1	28.85-29.22[a]	7.27-7.40[a]
P2	29.48-29.59[b]	7.39-7.42[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The result of the analysis showed that the temperature and pH of the catfish-rearing media in treatment P1 were not significantly different from those in treatment P2. The temperature of the rearing medium in this study ranged from 28.85-29.59ºC. According to BSN (2014), the temperature value for raising catfish is 25-30ºC. Catfish will experience slow growth when the temperature is 16-24ºC or 31-32ºC, and the fish will die if the temperature is below 16ºC or above 32ºC (Pujiharsono & Kurnianto, 2020). The value of the degree of acidity or pH in the rearing medium ranges from 7.27 to 7.42. BSN

(2014) states that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth when the pH is 4-6.4 or pH 8.6-11, and the fish will die if the pH is below four or above 11 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The results of the DO T-test analysis during rearing are presented in Table 6.

Table 6. The results of the DO analysis during rearing of catfish (*Clarias gariepinus*) in the biofloc system

Days to-	DO (mg/L) P1	DO (mg/L) P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27[a]	5.25±0.44[b]
21	4.47±0.03[a]	4.98±0.33[b]
28	4.88±0.12[a]	5.72±0.42[b]
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The results of the analysis show that Dissolved Oxygen (DO) or dissolved oxygen in treatment P1 was not significantly different from treatment P2 on days 0,7,35 and 42, but significantly different on days 14, 21 and 28. Based on Table 4.6. the frequency of probiotics from swamp P2 (2 times for 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study ranged from 3.91 to 5.95 mg/L. According to BSN (2014), the dissolved oxygen value for catfish is >3 mg/L. Therefore, the DO value in this study is still relatively safe for catfish farming. This is because the rearing container is equipped with aeration of 4 points. Besides that, it also comes from photosynthesis results from phytoplankton and oxygen diffusion from the air (Putra *et al*., 2014). Based on this, it is suspected that the increase in DO at the end of P1 rearing is better than P2. Furthermore, *Streptomyces* sp. is the best treatment for dissolved oxygen concentrations in fish culture media> 3 mg/L. The results of research by Kurniawan and Utama (2018) also yield DO values for catfish rearing >6 mg/L by administering probiotics *Bacillus* sp. as according to Prihanto *et al.* (2021) bacteria *Bacillus* sp. can improve water quality.

Ammonia

The results of the ammonia T-test analysis during rearing are presented in Table 7.

Table 7. The results of ammonia analysis during rearing of catfish (*Catfish gariepinus*) in the biofloc system

Days to-	Ammonia (mg/L) P1	Ammonia (mg/L) P2
0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23[a]	0.69±0.47[b]
35	1.59±0.38[a]	0.66±0.37[b]
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level T-test

The T-test analysis showed that the ammonia in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 21, and 42 but was significantly different on days 28 and 35. Based on Table 4.7 shows that the administration of probiotics from swamp P2 (2 times for 42 days of rearing) is proven to reduce ammonia levels in rearing media compared to P1 (1 time for 42 days of rearing). The ammonia value in this study was high, ranging from 0.45 to 1.62 mg/L, while in research by Wijayanti *et al.* (2021), it is 0.27 mg/L. The value of ammonia for catfish farming on the formation of biofloc is <1 mg/L (Wijaya *et al*., 2016). This high value of ammonia can cause death in fish due to the toxic nature of the ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion results, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). The high ammonia value in this study is likely due to the lack of a C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in water. Meanwhile, according to Hargreaves (2006), at a C/N ratio <10, heterotrophic bacteria will release ammonia into their environment. Research results Bakar *et al.* (2015) also stated that ammonia reduction in catfish farming using biofloc technology using a C/N 15 ratio could eliminate ammonia by 93.56% on the 12th day while the C/N 10 ratio on the 12th day was only 11.01%. Furthermore, only the maximum is on the 30th day, namely 98.51%.

Total Dissolved Solid (TDS)

The results of the TDS analysis during rearing are presented in Table 8.

Table 8. The results of TDS analysis during the rearing of catfish (*Clarias gariepinus*) in the biofloc system

Days to-	TDS (mg/L)	
	P1	P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76[b]	872.67±41.55[a]
28	804.17±59.93	757.67±136.93
35	810.00±57.38[b]	717.33±21.37[a]
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show

Based on the results of the T-test analysis, it shows that Total Dissolved Solid (TDS) in treatment P1 was not significantly different from treatment P2 on days 0, 7, 14, 28, and 42 but significantly different on days 21, and 35. Based on Table 4.8. administration of probiotics from swamps P2 (2 times during 42 days of rearing) can reduce TDS values compared to P1 (1 time during 42 days of rearing). TDS values in this study ranged from 717.33 to 1014.83 mg/L. Catfish can still tolerate the measurement results of these values to live (BSN, 2018). states that TDS's standard water quality value in fish farming activities is 1000 mg/L. The high level of TDS on the 21st day, especially in the P1 treatment, is thought to be due to the administration of commercial drugs to prevent dead fish containing organic and inorganic compounds. This follows the statement of Rinawati *et al.* (2016), who stated that large amounts of organic and inorganic compounds can result in high levels of TDS in water.

Conclusion

Based on the results of the research that has been carried out, it can be concluded that giving probiotics from swamp with a frequency of 2 times for 42 days of rearing (P2) gives the best results on flock volume, fish growth, fish feed. fish efficiency, fish survival rate, fish biomass production, and water quality. ~~The suggestion for future study is from this research is that it is necessary to add immunostimulants to catfish cultivation in the biofloc system.~~ Suggestions for further research on giving swamp probiotics with different frequencies to other types of fish in the biofloc system.

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Respon penulis terhadap review tahap ketiga dan artikel yang di resubmit

Respon penulis terhadap revisi tahap 3

No	Komenter Review	Tanggapan
	Review 1	
1	Resulth and Discussion	
	P1 P2? Write the description. (tulis deskripsi kodenya pada grafik	<u>We've fixed it</u>
2	Conclusion	
	Add the future study suggestion.	<u>We've fixed it</u>

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Different Effects of Swamp Probiotics Application Frequency as a Biofloc-forming Agent on The Production of Catfish (*Clarias gariepinus)*

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ABSTRACT

Catfish (*Clarias gariepinus*) that are reared with probiotics as biofloc-forming agent is thought to increase the fish production. Applying swamp probiotics to the water media has never been studied to ensure the flocks' availability in the rearing media. This study aimed to determine the appropriate frequency of probiotics application collected from swamps for biofloc formation to improve the catfish production. This study used a completely randomized design with two treatments and three replications. The treatments were composed of different application frequency of swamp probiotics: (P1) once in 42 days of rearing and (P2) twice in 42 days of rearing. Data on flock volume, total bacterial colonies, absolute growth rate, feed efficiency, survival rate, and water quality were analyzed by T-test with a 95% confidence level. Meanwhile, the flock composition data were analyzed descriptively. The results showed that P2 obtained the best treatment with a floc volume of 68.33 ± 10.41 mL/L, absolute length growth of 8.18 ± 1.03 cm, absolute weight growth of 19.30 ± 3.12 g, feed efficiency of 135.24 ± 7.98%, survival rate of 89.33 ± 6.21%, biomass production of 24639.50 ± 1344.51 g, temperature of 28.85-29.59ºC, pH of 7.27-7.42, dissolved oxygen (DO) of 3.91-5.72 mg/L, ammonia of 0.45-1.15 mg/L, and total dissolved solids (TDS) of 717.33-885.50 mg/L. Therefore, swamp probiotics should be applied to catfish culture media twice for 42 days of rearing or once every 21 days.

Introduction

Catfish (*Clarias gariepinus*) is a widely-cultivated freshwater fish commodity due to high market demand. In Indonesia, the Ministry of Marine and Fisheries Affairs of the Republic of Indonesia (2022) claimed, that the catfish production in grow-out phase in 2019 was 289,000 tons, then reached to 384,000 thousand tons in 2020, before falling to 360,000 tons in 2021. To meet this high market demand, increasing the production of catfish farming must be carried out intensively, efficiently, and minimizing the waste disposal in the surrounding waters (Fauzi *et al*., 2022). According to Putra *et al.* (2017), biofloc technology with probiotics application as heterotrophic bacteria can produce natural feed from the flocs to increase the feed efficiency and improve the water quality of the rearing media.

Swamps have high biodiversity, including microbes that can improve the physical and chemical properties of the rearing media. Microorganisms found in swamp contain *Chlorophyta*, *Bacillus* sp., and *Streptomyces* sp. (Wijayanti *et al*., 2018). The application of swamp probiotics (*Bacillus* sp. and *Streptomyces* sp.) has been studied either as an aquafeed supplement (Tanbiyaskur *et al*., 2022) or a biofloc-forming agent in catfish rearing (Wijayanti *et al*., 2021). Swamp probiotics have also been proved to increase growth, feed efficiency, fish survival rate, and water quality of the rearing media (Wijayanti *et al*., 2020).

Several types of swamp probiotics, such as *Bacillus* sp. and *Streptomyces* sp. can be used as a biofloc-

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forming agent. Previously, Wijayanti et al. (2021) applied the swamp probiotics once in 42 days of rearing, which resulted in a floc volume of 40 mL/L on the final day of rearing (42nd day). Using these flocs for 60 days of rearing resulted in an improved weight and length growth from 7.16 g to 36.95 g and 9.50 cm to 18.50 cm, although producing a relatively low floc volume. According to Agusta *et al.* (2022), the addition of a carbon source (molasses) in catfish culture with biofloc system produced a floc volume of 111 mL/L. If the floc volume exceeds the limit, part of the water can be removed and replaced with new water at about 70-80% of the water volume. According to Feroza *et al.* (2021), flocs in the rearing media will decrease due to frequently consumed by fish every day, thus requiring a sufficient floc formation to control the microorganisms blooming due to excess floc volume. Therefore, it is necessary to evaluate the different application frequency of swamp probiotics as a biofloc-forming agent on the catfish production.

Materials and Methods

Location and time

This study was conducted at the Laboratory of Aquaculture and Experimental Ponds, Aquaculture Study Program and Laboratory of Microbiology and Fishery Products Biotechnology, Fisheries Product Technology Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University on December, 2022–January, 2023.

Materials and Tools

The materials used were catfish fingerlings (7 ± 0.5 cm in length), swamp bacteria (*Bacillus* sp. and *Streptomyces* sp.), molasses, salt, dolomite lime, nutrient broth, yeast malt, yeast extract, $CaCl_2$, yeast, and fish feed (39-41% protein). The tools used were a tarpaulin pool with a diameter of 2 m and a height of 1.2 m, blower, aeration stone, aeration hose, ruler, digital scale, Imhoff cone, loop needle, Erlenmeyer flask, hot plate stirrer, magnetic stirrer, pH meter, thermometer, TDS meter, DO meter, and ammonia test kit.

Study Design

This study used a completely randomized design with two treatments and three replications. The treatments were composed of different frequency application of swamp probiotics, namely:

P1 = once in 42 days of rearing
P2 = twice in 42 days of rearing

Procedures

Swamp Probiotic Culture

Previously, a pure culture of the probiotics contained *Bacillus* sp. and *Streptomyces* sp. was successfully isolated from swamps (Wijayanti *et al.*, 2018) in liquid media. The *Bacillus* sp. were cultured in Erlenmeyer flasks filled with 20 mL of Nutrient Broth (NB) at one Ose needle. In contrast, *Streptomyces* sp. was cultured on the 20 mL of Yeast Malt (YM) liquid media in Erlenmeyer flasks at one Ose needle. These bacteria were agitated with a magnetic hot plate stirrer for three days (*Bacillus* sp.) and five days (*Streptomyces* sp.). The bacterial density was calculated using the Total Plate Count (TPC) method. Then, bacteria were mixed with 5% molasses for biofloc formation. For stock culture, bacteria were mixed with yeast extract, $CaCl_2$, and yeast with a composition of 2%, 1%, and 1%, respectively.

Rearing Media Preparation

Catfish (*Clarias gariepinus*) were reared in six rounded tarpaulin tanks with a diameter of 2 m and a height of 1.2 m. The tank was cleaned by brushing the entire tank and drying for a day to kill the pathogens. Then, the tank was filled with water as high as 0.7 m (volume 2198 L) and incubated for three days (Ma'ruf, 2016). Then, the tank was added with salt at 1 kg m^{-3} and camphor dolomite at 50 g m^{-3} (Sucipto *et al.*, 2018) and incubated for a day (Wijayanti *et al.*, 2021). Aeration was installed at four points of rearing ponds (Ma'ruf, 2016).

Stocking and rearing of catfish

The rearing process used catfish at 7 ± 0.5 cm with a stocking density of 500 fish m^{-3} (BSN, 2018) and reared for 42 days. Fish stocking was carried out in the morning when water conditions were normal and fish were acclimatized for seven days to reduce stress. Probiotics were applied during the rearing period at a density of 10^5 CFU/mL for each bacteria (Wijayanti *et al.*, 2020). The application frequency followed the treatments, namely (P1) once in 42 days of rearing and (P2) twice in 42 days of rearing. A carbon source in the form of molasses was also administered at 200 mL m^{-3} with a frequency of once every seven days (0, 7, 14, 21, 28, 35 days) (Putra *et al.*, 2017). Fish were fed daily using a commercial feed with 39-41% protein content. The feeding frequency was three times, namely at 08.00 am, 12.00 am, and 04.00 pm under apparent satiation. Dead fish were weighed, when existed. Harvesting was done on the 43rd day. Fish weight and length were sampled at the beginning and end of the rearing, with a total sample of 30 fish for each experimental tank unit.

Parameters

Floc Volume and Composition

Floc volume was measured using an Imhoff cone by taking the water at 1000 mL and allowed to stand for 20 minutes to settle the floc (Ombong & Salindeho, 2016). Measurement floc volumes were carried out on 7th, 14th, 21st, 28th, 35th, and 42nd day of rearing. After measuring the floc volume, the precipitated floc was taken to observe the composition of floc microorganisms. Floc composition was microscopically observed with microscope at 40x magnification on 0, 1st, 21st , and 42nd day of rearing.

Total Bacterial Colonies

The total bacterial colonies formed in the rearing media were counted using a colony counter. After The water sample was diluted at 10^{-5}, 10^{-7}, and 10^{-9}, the bacterial density was calculated on 0th (before probiotics application), 1st, 21st, and 42nd day of rearing. The bacterial colonies proliferation were determined in the Colony Forming Unit (CFU) and calculated using the formula (Damongilala, 2009):

$$Total\ Bacteria\ (CFUmL-1) = Number\ of\ colonies \times \frac{1}{dilution\ factoran}$$

Absolute Growth Rate

The formula used to measure the absolute weight growth, according to Hopkins (1992) was:

$$W = Wt - Wo$$

Note:

W	=	Absolute Weight Growth Rate (g)
Wt	=	Average fish weight at the final period of the study (g)
Wo	=	Average fish weight at the initial period of the study (g)

The formula used to measure the absolute length growth, according to Hopkins (1992), was:

$$L = Lt - Lo$$

Note:

L	=	Absolute length growth rate (cm)
Lt	=	Average length of fish at the final period of the study (cm)
Lo	=	Average fish length at the initial period of the study (cm)

Feed Efficiency

Feed efficiency (FE) was calculated based on a formula of Afrianto & Liviawaty (2005):

$$FE\ (\%) = \frac{(Wt+D)-Wo}{F} x\ 100$$

Note:

FE	=	Feed efficiency (%)
Wt	=	Fish biomass at the end of the study (g)
Wo	=	Fish biomass at the start of the study (g)
D	=	Fish dead biomass during the study (g)
F	=	Amount of feed applied during the study (g)

Survival Rate (SR)

The survival rate was calculated using a formula based on Aliyu-Paiko*et al.* (2010):

$$SR\ (\%) = \frac{Nt}{No} x\ 100$$

Note:

SR	=	Survival rate (%)
Nt	=	The final quantity of fish at the end of rearing (g)
No	=	The initial quantity of fish at initial rearing (g)

Biomass Production

The biomass production was calculated, according to Shang (1982):

$$P\ (g) = W\ x\ N$$

Note:

P	=	Biomass Production (g)
W	=	Average weight of fish at the end of rearing (g)
N	=	The final number of fish at the end of rearing (fish)

Water quality

Water quality parameters, including temperature and pH, were measured every day at 08.00 a.m. and 04.00 p.m. Meanwhile, dissolved oxygen level, TDS, and ammonia were measured every seven days (0, 7, 14, 21, 28, 35, and 42 days). Temperature (ºC) was measured using a thermometer, pH was measured using a pH meter, Dissolved oxygen (DO) was measured using a DO meter (mg/L), the Total Dissolved solid (TDS) (mg/L) was measured using a TDS meter, and ammonia (mg/L) was measured using a spectrophotometer method.

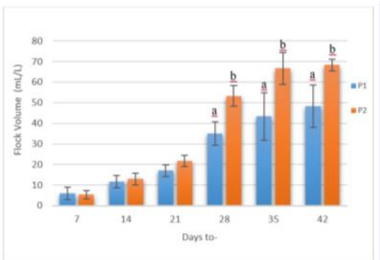
Data analysis

Data on flock volume, total bacterial colonies, absolute growth rate, feed efficiency, survival rate, and water quality were analyzed with T-test at 95% confidence level. Meanwhile, the flock composition data were analyzed descriptively.

Results and Discussion

Floc Volume

The T-test analysis results of flock volume are presented in Figure 1.



Commented [W1]: P1 dan P2 ditulis keterangannya

Note: Different superscript letters in the same days show significant differences at 5% level of T-test

Figure 1. Flock volume

The floc volume in the P1 treatment was significantly different from the P2 treatment on 28th, 35th, and 42nd days. The floc volume is the amount number of suspended solid over a certain period in an inverted conical container (Effendi, 2003). The highest floc volume was found in the P2 treatment at 68.33 mL/L. The floc volume obtained in this study was higher than in Wijayanti *et al.* (2021) at 40 mL/L (42nd day of rearing period). This was presumably due to the influence of the application frequency of probiotics to the rearing medium. According to De Schryver *et al.* (2008), factors that influence the biofloc formation are the administration of probiotics as a floc-forming agent, agitation intensity by aeration device, organic carbon source, and water quality. Malaputra *et al.* (2016) reported, that the administration of commercial probiotics for 14 times (70 days of rearing) produced a floc volume of 120 mL/L, which was higher than for seven times at 80 mL/L. Therefore, the more often probiotics applied to the rearing medium, the more floc volume obtained. The maximum floc volume for catfish culture is 100 mL/L. According to Agusta *et al.* (2022), the addition of molasses as a carbon source in the catfish culture with biofloc system produces a floc volume of 111 mL/L. When the floc volume exceeds the limit threshold, partial water removal should be performed at about 70-80% of the total water volume (Zaidy, 2022). High floc volume can cause fish death (Rofianingrum *et al.*, 2022).

In addition to providing swamp probiotics as a biofloc starter that contain heterotrophic bacteria, the floc volume increase in this study was thought to be due to the C/N ratio, which affected the conversion of aquaculture waste into heterotrophic bacterial biomass. According to Hargreaves (2006), when the C/N ratio >10, the formation of biofloc reaches its optimum level. According to Avnimelech *et al.* (1999), the C/N ratio required for biofloc formation should be >15. Therefore, essential organic carbon addition is necessary such as molasses, which can be added every seven days at 200 mL m^{-3} (Putra *et al.*, 2017).

Floc Composition

The floc composition in this study is presented in Table 2.

Table 2. The floc composition of catfish (*Clarias gariepinus*) reared in biofloc system

Floc-forming microbes	Treatment							
	H0		H1		H21		H42	
	P1	P2	P1	P2	P1	P2	P1	P2
Chlorophyta	✓	✓	✓	✓	✓	✓	✓	✓
Cyanophyta	✓	✓	✓	✓	✓	✓	✓	✓
Protozoa	✓	✓	✓	✓	✓	✓	✓	✓
Coelenterates	-	✓	-	✓	-	✓	-	✓
Rotifers	✓	✓	✓	✓	✓	✓	✓	✓
Arthropods	-	✓	-	✓	-	✓	-	✓

Information: (✓) present, (-) absent

Based on the observations of floc composition, the P2 treatment presents more diverse microbes than P1 treatment, namely Chlorophyta, Cyanophyta, protozoa, coelenterate, rotifers, and arthropods. In P1, the microbes were only Chlorophyta, Cyanophyta, protozoa, and rotifers. According to Feroza *et al.* (2021), diverse microbes in the floc composition was thought due to the effect of probiotics application, which was in line with the increased floc volume in the rearing medium, so the microbes could proliferate properly. Based on Hargreaves (2013), the biofloc system consists of algae, bacteria, protozoa, zooplankton, and other microorganisms. Previously, Wijayanti *et al.* (2020) produced a combination of Chlorophyta and swamp probiotics, containing bacteria *Bacillus* sp. In addition, De Schryver *et al.* (2008) mentioned, that the floc is composed of heterogeneous combination of microbes (filamentous bacteria, fungi, algae, protozoa, rotifers, arthropods, nematodes) with particles, colloids, organic polymers, and cations that are well interconnected in water and can reach <1000 µm in size.

Total Bacterial Colonies

The total bacterial colonies on each rearing medium are presented in Table 3.

Table 3. The total bacterial colonies in the biofloc system on each tank

Days (-th)	Total bacterial colonies ($\times 10^9$ CFU/mL)	
	P1	P2
0	2.77±1.00	2.77±1.00
1	13.23±2.82	14.89±3.88
21	5.56±2.01	6.65±3.07
42	8.42±3.19[a]	19.73±6.19[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The total bacterial colonies in the P1 treatment were only significantly different on the 42nd day. In this study, the bacterial density range was among 2.77-19.73 $\times 10^9$ CFU/mL. After applying the swamp probiotics on the rearing media, the total bacterial colonies increased on the 1st day, then decreased on the 21st day. This condition was thought due to a lack of nutrients (macronutrient or micronutrient) for bacteria (Wijayanti *et al.*, 2020), which could occur as nutrients, like carbons in molasses, have not been added. According to Nasmia and Rifai (2020), the number of bacterial colonies decreased due to carbon source reduction. On the 42nd day, the bacterial density increased again with a higher bacterial density was obtained from the P2 treatment at 19.73 x10^9 CFU/mL, while the P1 treatment only produced the bacterial density of 8.42 x10^9 CFU/mL. . This condition was occurred due to probiotics re-administration on the 21st day of rearing period. Similarly, Adharani *et al.* (2016) produced the total density of bacterial colonies in catfish culture by applying probiotics up to 10^{14} CFU/mL.

According to Sitorus *et al.* (2019), the bacterial density in floc formation can reach approximately 10^9 CFU/mL. Widnyana (2016) stated that the total bacterial colonies in flocs ranged from 10^3-10^4 CFU/mL, while the system has a bacterial range of 10^8-10^{10} CFU/mL. This was because the system had a high intensity of sunlight as one of the supporting factors in bacterial growth. High density of bacterial colonies indicates that the provision of probiotics greatly influences the high density of bacteria, assisted by their effectiveness in breaking down organic matters (Adharani *et al.*, 2016).

Absolute Growth Rate, Feed Efficiency, Survival Rate, and Biomass Production

The absolute growth rate, feed efficiency, and survival rate of catfish for 42 days of rearing period are presented in Table 4.

Table 4. The absolute growth rate, feed efficiency, survival rate, and biomass production of catfish (*Clarias gariepinus*) in the biofloc system

Parameter	Treatment	
	P1	P2
Absolute length growth (cm)	7.16 ± 0.12	8.18 ± 1.03
Absolute weight growth (g)	16.42 ± 1.06	19.30 ± 3.12
Feed efficiency (%)	104.97 ± 8.24[a]	151.90 ± 7.98[b]
Survival rate (%)	81.54 ± 1.59	89.33 ±6.21
Biomass production (g)	17439.14 ± 1346.55[a]	24639.50 ± 1344.51[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The absolute length and weight growth rates of catfish in P1 treatment was insignificantly different from P2 treatment. The absolute length and weight growth rates in P2 treatment were higher than P1 treatment. These results indicate that catfish can utilize the available floc in the rearing medium as additional feed due to high protein content, that can increase the fish growth (Putra *et al.*, 2017). In addition, Wijaya *et al.* (2016) reported that a floc contains 42.42% protein, 92.15% moisture, 1.5% crude fat, 7.09% crude fiber, 8.36% ash, and 40.63% nitrogen-free extract (NFE). According to Febriyanti *et al.* (2018), heterotrophic bacteria such as *Bacillus* sp., can accumulate in the rearing medium and form flocs that can be used as an aquafeed source for fish.

Furthermore, *Bacillus* sp. and *Streptomyces* sp. can increase the growth of catfish. According to Sukoco *et al.* (2016), *Bacillus* sp. can promote digestive enzyme activity and feed absorption to promote fish growth. *Streptomyces* sp. in fish culture can also be applied as a growth-promoter (Cruz *et al.*, 2012).

The feed efficiency in P1 treatment was significantly different from P2 treatment. The feed efficiency of P2 treatment was higher at 151.90%, while P1 treatment was 104.97%. If the feed efficiency value is converted to feed conversion ratio (FCR) value, then the FCR value for P1 is 0.96 while P2 is 0.66. The FCR values in this study were better than Wijayanti *et al.* (2021) at 0.97. Putra *et al.* (2017) administered probiotics to the catfish rearing media with biofloc technology also resulted in high feed efficiency at 88.17-110.86%. In this study, high feed efficiency value was suspected as fish used flocs as additional feed. De Schryver *et al.* (2008) stated that flocs as additional feed is important in increasing the feed efficiency. This shows that swamp probiotics can form flocs, which fish then consume them as additional feed.

The survival rate of catfish culture in P1 treatment was insignificantly different from P2 treatment. The survival rate in P2 treatment was higher than P1 treatment (89.33% vs 81.54%). These results were closed to Wijayanti *et al.* (2021) at 87.57% with a rearing period of 90 days. The administration of swamp probiotics in the form of a *Bacillus* sp. and *Streptomyces* sp. combination provides a defense mechanism against pathogens by increasing the non-specific immunity in the fish immune system and maintaining a water quality balance, so the fish can survive (Wijayanti *et al.*, 2020). Growth and survival rates will affect the production value of fish biomass.

The catfish biomass production in P1 treatment was significantly different from P2 treatment. The biomass production of P2 biomass was higher than P1 (24,639.50 g vs 17,439.14 g). Probiotics application is thought to increase the biomass production of catfish in the biofloc technology. The biomass determines the production value at the end of culture period and the survival rate of fish at the end of culture period, so the more fish survives, the more fish production obtained (Anam *et al.*, 2017). Based on the results of absolute growth rates and feed efficiency in this study, the swamp probiotics can form floc used by fish as additional feed. Applying swamp probiotics is also useful in preventing pathogenic bacteria to prevent mass fish death. Therefore, swamp probiotics can increase the fish growth and survival rate, thus increasing the fish biomass production.

Water quality

Temperature and pH

The temperature and pH of the rearing media are presented in Table 5.

Table 5. The temperature and pH of rearing media with biofloc system

Treatment	Temperature (ºC)	pH
P1	28.85-29.22[a]	7.27-7.40[a]
P2	29.48-29.59[b]	7.39-7.42[b]

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The temperature and pH of the catfish-rearing media in P1 treatment were insignificantly different from P2 treatment. The temperature of the rearing medium in this study was 28.85-29.59ºC. According to BSN (2014), the temperature standard for catfish rearing is 25-30ºC. Catfish will experience slow growth when the temperature is 16-24ºC or 31-32ºC, while catfish will die if the temperature is below 16ºC or above 32ºC (Pujiharsono & Kurnianto, 2020). The degree of acidity or pH was among 7.27-7.42. BSN (2014) stated that the pH value for catfish rearing is 6.5-8. Catfish will experience slow growth, when the pH is 4-6.4 or 8.6-11, and the fish will die if the pH is below 4.0 or above 11.0 (Pujiharsono & Kurnianto, 2020).

Dissolved Oxygen (DO)

The DO value during the rearing period is presented in Table 6.

Table 6. The DO contents of the rearing tank with the biofloc system

Days (-th)	DO (mg/L)	
	P1	P2
0	5.95±0.30	5.53±0.88
7	4.33±0.47	3.91±0.07
14	4.13±0.27[a]	5.25±0.44[b]
21	4.47±0.03[a]	4.98±0.33[b]
28	4.88±0.12[a]	5.72±0.42[b]
35	5.18±0.75	5.48±1.27
42	5.38±0.52	5.22±0.34

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The Dissolved Oxygen (DO) in P1 treatment was significantly different from P2 treatment on 14th, 21th, and 28th days. High frequency of swamp probiotics application as presented in P2 treatment (twice in 42 days of rearing) can increase the value of dissolved oxygen (DO). DO values in this study were 3.91 -5.95 mg/L. According to BSN (2014), the DO value for catfish culture is >3 mg/L. Therefore, the DO value in this study is still relatively safe for catfish, because the rearing container is equipped with four aeration source points, besides photosynthesis from phytoplankton and oxygen diffusion from the air (Putra *et al.*, 2014). Thus, the increased DO value in P1 treatment is better than P2 treatment. Furthermore, *Bacillus* sp. is suspected to regulate the dissolved oxygen concentrations in fish culture media up to > 3 mg/L. The results of Kurniawan and Utama (2018) also yielded DO values for catfish rearing >6 mg/L by administering *Bacillus* sp. treatment, which was in accordance to Prihanto *et al.* (2021) as *Bacillus* sp. bacteria can improve the water quality.

Ammonia

The results of the ammonia contents in the rearing tank are presented in Table 7.

Table 7. The ammonia contents in rearing tanks with the biofloc system

Days (-th)	Ammonia (mg/L)	
	P1	P2

0	0.47±0.02	0.45±0.06
7	0.56±0.65	0.66±0.47
14	1.35±0.08	1.11±0.42
21	1.34±0.13	1.15±0.33
28	1.62±0.23[a]	0.69±0.47[b]
35	1.59±0.38[a]	0.66±0.37[b]
42	1.20±0.21	0.99±0.46

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

The T-test analysis showed that the ammonia in treatment P1 was significantly different on only 28th and 35th days. The administration frequency of swamp probiotics in P2 treatment (twice in 42 days of rearing) is proven to reduce the ammonia levels in rearing media, compared to P1 treatment (once in 42 days of rearing). The ammonia values in this study were high, namely at 0.45 - 1.62 mg/L, while Wijayanti *et al.* (2021) reported the ammonia content was only 0.27 mg/L. The ammonia content for catfish culture with biofloc system should be <1 mg/L (Wijaya *et al.*, 2016). High ammonia value can cause fish death due to the toxic nature of ammonia. The main sources of ammonia in aquaculture ponds come from feces, excretion matter, leftover feed, and dead biota (fish, algae, plants) that experience mineralization (Wahyuningsih & Gitarama, 2020). High ammonia value in this study is likely due to the lack of C/N ratio to convert ammonia into floc-forming bacterial biomass. Avnimelech *et al.* (1999) required a C/N ratio >15 to limit the accumulation of TAN in the water. Meanwhile, Hargreaves (2006) stated that a C/N ratio <10 causes heterotrophic bacteria to release ammonia into the environment. Bakar *et al.* (2015) also stated that ammonia reduction in catfish culture using biofloc technology using a C/N ratio of 15 could eliminate ammonia by 93.56% on the 12^{th} day, while the C/N ratio of 10 on the 12^{th} day was only 11.01%, and the maximum ammonia elimination level was found on the 30th day at 98.51%.

Total Dissolved Solid (TDS)

The TDS analysis results in the rearing tanks are presented in Table 8.

Table 8. The TDS analysis results in the rearing tank with the biofloc system

Days (-th)	TDS (mg/L)	
	P1	P2
0	897.50±6.95	885.50±28.16
7	868.50±59.01	845.83±38.83
14	796.17±5.62	785.00±56.51
21	1014.83±75.76[b]	872.67±41.55[a]
28	804.17±59.93	757.67±136.93
35	810.00±57.38[b]	717.33±21.37[a]
42	879.00±45.90	869.17±85.15

Note: Numbers followed by different superscript letters in the same line show significant differences on the 5% level of T-test

Based on the results of the T-test analysis, the Total Dissolved Solid (TDS) in P1 treatment was only significantly different on 21th and 35th days. The administration of swamp probiotics with P2 treatment can highly reduce the TDS values, compared to P1 treatment. The TDS values in this study was among 717.33 - 1014.83 mg/L. Catfish could still tolerate the TDS values in this study. BSN (2018) stated that TDS standard for water quality of fish culture activities is 1000 mg/L. The high TDS level on the 21st day, especially in the P1 treatment, is thought due to the administration of commercial drugs to prevent dead fish, which contained organic and inorganic compounds. This condition followed the statement of Rinawati *et al.* (2016), that large amounts of organic and inorganic compounds could result in high levels of TDS in the water.

Conclusion

This study concludes that swamp probiotics application twice in 42 days of rearing (P2) provides the best results on flock volume, fish growth rate, feed efficiency, survival rate, biomass production, and water quality. We suggest to evaluate further regarding the swamp probiotic applications in other types of fish with the biofloc system.

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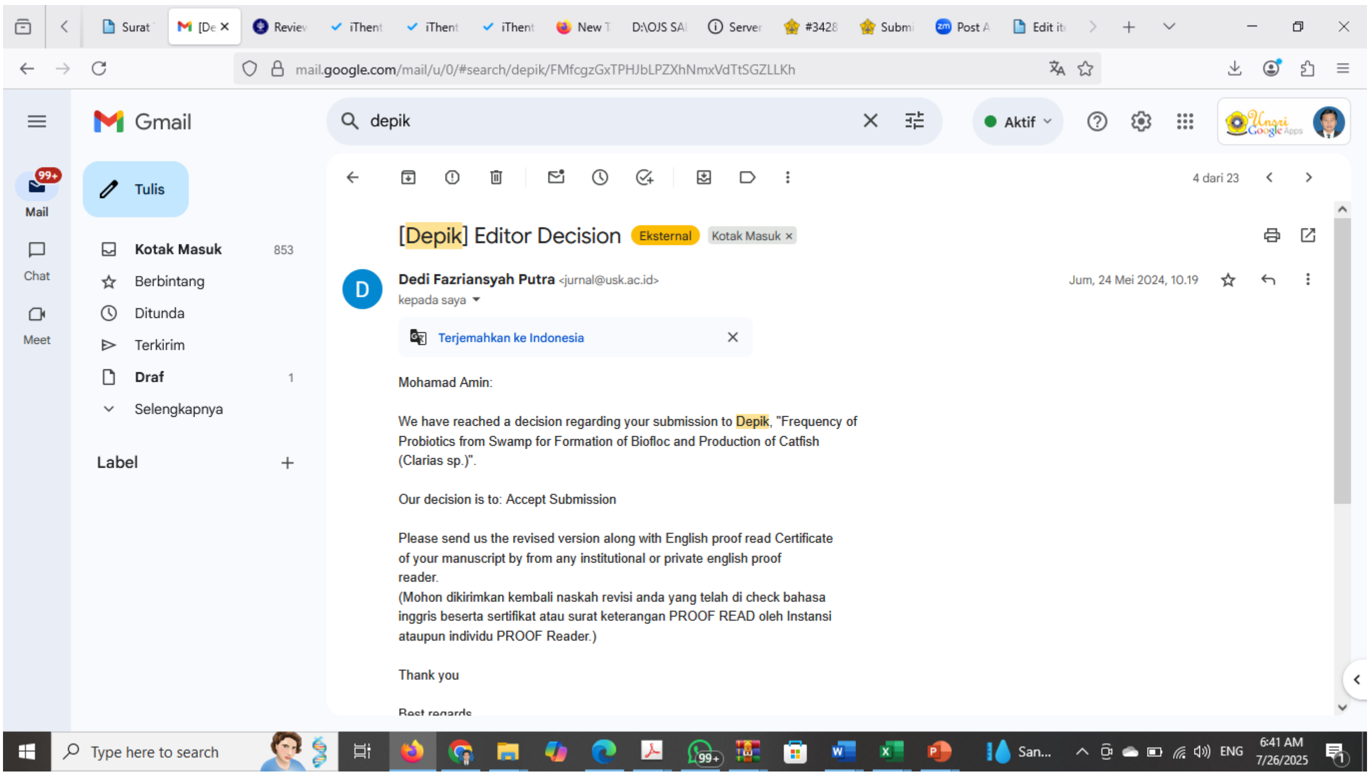

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Acceptance Letter

Dear **Mohamad Amin, Retno Cahya Mukti, Ferdinand Hukama Taqwa, Andini Andini, Marsi Marsi, and Langgeng Priyanto, Marini Wijayanti**

MS: **Different Effects of Swamp Probiotics Application Frequency as a Biofloc-forming Agent on The Production of Catfish (*Clarias gariepinus*)**

I am pleased to inform you that as per the recommendation of the reviewer and editorial board, your above-mentioned manuscript has been **ACCEPTED** for publication in Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Kelautan and will be published in Volume 13 No. 2 August 2024.

Thank you very much for your cooperation.

Sincerely yours,
Banda Aceh, 2nd July, 2024
Editor in Chief,

Dr. Ichsan Setiawan, S.Si, M.Si