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MARINI WLIWIANTI, S.PL, M.S. fp --



Dear Editor-in-Chief, Dr. Joan Valentin Petrescu-Mag

We hereby submit a manuscript entitled "Optimization of Snakehead fish (Channe strate) culture using swamp microbial combination and netrification bacteria" by Wgayanti M, Jubaedah D, Yulistya Q, Tanbiyaskur, and Sasanti AD, to be considered for publication as an original article. We hope our manuscript will be published on AACL Bioflax. The research reported in this manuscript has been funded by Universitas Srieljaya funded by Competitive Grant research in 2018-2018 with Number: 108.223 / UNV / <u>SH2 UP2H PT</u> / 2018 jn and Number: 0015 / UNV / <u>SH2 UP2H PT</u> / 2019.

This article showed the effect of sweep bacteria as probletic candidates for water quality and performance of snakehead culture. We used Bacillus, Streptomyces, and Chlorophyta combined with Nitrifler bacteria (PRIOBAC). We found that the constituum of Bacillus and Streptomyces was the best of snakehead culture in the rearing media for fish performance and water quality. We believe these findings will be of interest to the readers of the journal.

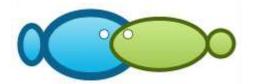
We iteclare that this manuscript is original, has not been published before and is not currently being cursidered for publication elsewhere. We wish to confirm that there no known conflicts of interest associated with this publication. The manuscript has been read and approved by all named authors before submitting it.

We hope you find our manuscript suitable for publication and look forward to hearing from you. Thank you.

and the second second

Best regards,

Marini Wijayanti Aguaculture Study Program Department of Fisheries Faculty of Agriculture Universitas Scheljaya



Optimization of snakehead fish (*Channa striata*) culture using swamp microbial combination and nitrification bacteria

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Abstract. Utilization of swamp area as a fish culture location will cause a decrease in water quality. Therefore, it is necessary to improve the water quality with environmental friendly of biological treatment, one of addition is microbes as probiotics in media culture. The purpose of this study was to determine the combination of microbes from swamps that can improve the water quality of media culture and production of swamp fish culture. The research used Completely Randomized Design (CRD) factorial with two factors consisting of the first factor with two treatments and the second factor with five treatments and three replications. The first factor is without the addition of nitrification bacteria (N1) and the addition of nitrification bacteria (PROBAC) 5×10^6 CFU.mL⁻¹ (N2). The second factor is without the addition of swamp microbes (P1), addition of *Chlorophyta* $(3.43 \times 10^7 \text{ sel.L}^{-1})$ and *Bacillus* sp. $(10^5 \text{ CFU.mL}^{-1})$ (P2), addition of *Chlorophyta* $(3.43 \times 10^7 \text{ sel.L}^{-1})$, *Bacillus* sp. $(10^5 \text{ CFU.mL}^{-1})$ (P2). The result showed that the addition microbes from swamps with combination of N1P4 able to improve the water quality value better than treatment without the addition of microbes (N1P1) and only the addition of nitrification bacteria (N1P2). Combination of N1P4 produces the best survival rate of 63.94%, feed efficiency of 59.65%, absolute weight growth of 2.32 g and absolute length growth of 2.27 cm.

Key Words: probiotic, swamp microbes, snakehead fish, nitrification bacteria.

Introduction. The swamp aquaculture must be improving and maintaining the water quality for fish rearing media. The wastewater of fish rearing on swamps will reduce the quality of water from swamps. So it is necessary to improve water quality with biological treatment environmentally friendly. One of the treatments is adding probiotics in the rearing media. Irianto and Austin (2002), states that environmental degradation can be prevented with probiotics, which aims to degrade the organic materials in the habitat. Hartini *et al.* (2013) showed that the addition of probiotics at a dose of 10 μ l.l⁻¹.week⁻¹ can improve and maintain optimal water quality. In other studies, the addition of the effective microorganism 4 probiotics can reduce ammonia levels and suppress the population of pathogenic microorganisms that exist in culture media (Trisna *et al.*, 2013).

Swamps have high biodiversity, including sediment microbes. Many swamp microbes are able to improve the physical and chemical properties of swamps. Swamp microbes that have been found include Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. (Wijayanti *et al.*, 2018). Bacteria from the swamp (*Bacillus* sp.) can be used as environmental probiotics with concentrations of 10⁵ CFU.ml⁻¹ (Khotimah, 2018) and microalgae Chlorophyta with the optimum concentration 10% of the maximum density are able to grow in the fish culture media (Utami, 2019). Chlorophyta is a microorganism that can be used as Green Water in aquaculture media. Wijayanti *et.al.* (2018) showed that the use of Chlorophyta increased the

level of dissolved oxygen in the culture pond culture media 60.52% and swamp water culture media 63.63%. The increasing dissolved oxygen is caused by Chlorophyta carrying out photosynthetic activity which produces dissolved oxygen in the culture media. Bacillus sp. and Streptomyces sp. obtained are proteolytic bacteria that can increase the content of NH₃, NO₂⁻ , and NO₃⁻ to the media (Yuliani, 2017; Saraswati, 2018). Balcazar et al. (2006) states that Bacillus sp. is an example of an efficient probiotic bacteria used in aquaculture because it is able to convert organic matter into CO₂ used in cell metabolism. Gram-positive bacteria, such as *Bacillus* sp. can increase the animal's immune system and also act favorably in improving the quality of the water system (Mohapatra et al., 2013). Bernal et al. (2017) states that the combination of *Streptomyces* sp. and *Bacillus* sp. demonstrated a significant immunomodulatory activity by increasing the total number of hemocytes and the activity of superoxide dismutase (SOD) which provides a protective effect against Vibrio harveyi bacteria by increasing the immunological status of *Penaeus monodon*. Nitrifying bacteria are chemolithoautotrophic bacteria (ex: Nitrosomonas sp., Nitrobacter sp.), which are able to meet their carbon needs through CO₂ fixation (Calvin cycle), and their energy source comes from the oxidation process of reducing ammonia to nitrate. With the addition of nitrifying bacteria, denitrification and molasses with a C/N ratio of 10 could decrease in ammonia levels by 28.5% (Yuniasari, 2009).

Each of the swamp microbes and nitrifying bacteria has their own advantages and disadvantages, so we need a consortium of swamp microbes and nitrifying bacteria. The consortium is expected to form cooperative, commensal and mutualistic relationships between microbes. The emergence of a swamp microbial consortium and nitrification bacteria resulted in the need for optimization of a combination of *Bacillus* sp., *Streptomyces* sp., Chlorophyta and commercial nitrification bacteria in an effort to improve water quality in media of swamp fish production. The purpose of this study was to determine the combination of swamp microbes and nitrifying bacteria that improve the water quality of media in swamp fish production. This study is expected to get a combination of swamp microbes that can improve media water quality and swamp fish culture.

Material and Method

The experimental design used was a completely randomized factorial design (RAL) consisting of 2 factors. The first factor with 2 treatments and the second factor with 4 treatments and 3 replications. The first factor is the addition of nitrifying bacteria (PROBAC), namely:

N1: Without the addition of nitrifying bacteria (PROBAC)

N2: Addition of nitrifying bacteria (PROBAC) 5×10⁶ CFU.mL⁻¹

The second factor is the addition of swamp microbes, namely:

P1: Without the addition of swamp microbes

P2: Provision of 100 ml Chlorophyta $(3.43 \times 10^7 \text{ Cell.L}^{-1})$ and *Bacillus* sp. $(10^5 \text{ CFU.mL}^{-1})$

P3: Provision of 100 ml Chlorophyta $(3.43 \times 10^7 \text{ Cell.L}^{-1})$ and *Streptomyces* sp. $(10^5 \text{ CFU.mL}^{-1})$

P4: Provision of 100 ml Chlorophyta (3.43×10^7 Cell.L⁻¹), *Bacillus* sp. (10^5 CFU.mL⁻¹) and *Streptomyces* sp. (10^5 CFU.mL⁻¹)

Bacteria Cultivation and Propagation

Pure bacterial colonies were obtained from the results of previous studies. The colony was cultivated using NA (Nutrient Agar) media for *Bacillus* sp and YM (Yeast Malt Agar) for *Streptomyces* sp. Bacterial colonies were scratched in a petri dish containing NA (Nutrient Agar) for *Bacillus* sp and YM (Yeast Malt Agar) media for Streptomyces sp. using the quadrant streak method. Petri dishes were wrapped in wrapping paper and incubated for 2 days at room temperature (28°C-30°C). A single colony formed on a petri dish was transferred to the agar media and incubated until bacteria grow.

Swamp bacteria that grow on NA and YM agar media were multiplied by NB (Nutrient Broth) media for *Bacillus* sp. and liquid YM for *Streptomyces* sp. The suspension was taken as much

as 1 ose to be cultured in the medium as much as 5 mL in a test tube, homogenized with a shaker for approximately 2 days for *Bacillus* sp. and 5 days for *Streptomyces* sp., then multiplied from 5 mL to 500 mL.

Chlorophyta culture

Chlorophyta sp. culture media. used was a technical fertilizer media consisting of ZA, Urea, TSP, and Gandasil B. All technical fertilizer ingredients were mixed in a 250 mL erlenmeyer and added to 100 mL of distilled water and then homogenized on a hot plate using a magnetic stirer and sufficient heating until all ingredients dissolved. The technical fertilizer media in the erlenmeyer was sterilized using an autoclave 121°C for 0.25 hour. *Chlorophyta* isolates (about 10⁷cell.ml⁻¹ in 10 ml stock culture) put into an erlenmeyer containing technical fertilizer media for liquid culture. They cultured during 9 days in room temperature for scaling up to 1 Liter.

Preparation of Fish Rearing media

The container used in rearing was in the form of an aquarium with a size of $30 \times 30 \times 30 \text{ cm}^3$ as many as 24 units. The aquariums were cleaned using potassium permanganate to sterilize diseases or parasites. The aquarium was filled with 20 liters of swamp water.

Fish Culture Test

The test organism used in this study was snakehead fish of 5 ± 1 cm each with 12 heads in 20 liters of water (Mulyadi, 2016). Before stocking, acclimatize as an adaptation to the new environment to reduce stress on the test organism. After 7 days of stocking, Chlorophyta isolate (3.43×10^7 Cell.L⁻¹), *Bacillus* sp. (10^5 CFU.mL⁻¹), *Streptomyces* sp. (10^5 CFU.mL⁻¹) as well as the "PROBAC" Nitrification bacteria (5×10^6 CFU.mL⁻¹) were added in combination with the treatment.

Rearing

The fish culture maintained for 40 days which was calculated after the addition of the treatment. During rearing, they were fed at satiation with a frequency of three times a day. Pellets used are commercial pellets with 40% protein content.

Chlorophyta abundance

Samplings were carried out at the beginning and end of the study by subcomposite methods in each treatment. It used a 25 μ m mesh size mesh plankton net for 5 Liters of rearing media each unit experiment to 25 ml sample. Observation of Chlorophyta samples were used a microscope and textbook The Marine and Fresh Water Plankton (Davis, 1955). Chlorophyta abundance calculation was done using the Leackey Drop Microtransect method (American Public Health Association, 1989) as follows:

$$N = Z \times \frac{X}{V} \times \frac{1}{V}$$

Information:

 $N = Total number (cell.L^{-1})$

Z = Number of individuals found

X = volume of filtered water (25 mL)

Y = Volume 1 drop of sample water (0.05 mL)

V = volume of filtered water (5 liters)

Bacteria Population

Counting of bacterial populations performed at the beginning and end of rearing with plate count method was to perform multilevel dilution were then incubated at a temperature of 28-30°C for 24 hours. The growing population was determined in a colony forming unit (CFU) and calculated using the following formula:

Total of Bacteria = Total of colonies
$$\times \frac{1}{dilution factor} \times \frac{1}{mL sample}$$

Biofloc Volume

Biofloc volume measurements were done on the 10 and 40 days after rearing. Floc volume was obtained by taking a rearing media using glass cone 1L volume, then floc in the water

media was left to settle in the tube for 15-20 minutes. **Survival Rate**

The percentage of fish survival was calculated using the following formula:

Survival rate =
$$\frac{Nt}{No} \times 100\%$$

 N_0 = Number of fish at the beginning of rearing (individuals) N = Number of fish at the end of rearing (individuals)

Absolute Weight Growth

Growth of fish weight during rearing wass calculated using the following formula: $W = Wt - W_0$

W = Growth of weight of fish for rearing (grams)
 Wt = Weight of fish at the end of rearing (grams)
 W₀ = Weight of fish at the beginning of rearing (grams)

Absolute Length Growth

The absolute length growth of fish during rearing was determined by doing the following calculation:

 $L = Lt-L_0$

L = Growth of absolute length of fish for rearing (cm)

Lt = Length of fish at the end of rearing (cm)

 L_0 = Length of fish at the beginning of rearing (cm)

Feed Efficiency

According to NRC (1977) feed efficiency can be calculated by the formula: ((Wt + D) - Wo)

 $\mathsf{EP} = \qquad \mathbf{F} \qquad \times 100\%$

Note: EP = Feed Efficiency (%)

Wt = Weight of fish at the end of rearing (gram)

Wo = initial fish rearing weight (gram)

D = Weight of fish that died during rearing (gram)

F = Amount of feed given (grams)

Water Quality

Measurement of water quality data for snakehead fish rearing media includes pH (pH meter), dissolved oxygen (DO meter), ammonia (spectrophotometry), and biological oxygen demand (DO meter) at the beginning and end of rearing for 40 days.

Data analysis

Research data including biofloc volume, survival, growth, feed efficiency, water quality were statistically analyzed using analysis of variance. If the results of the analysis of the variance show that the treatment has a significant effect, then it is continued with the LSD test (the Least Significance Difference) 5%. Chlorophyta abundance data and the total bacterial population were analyzed descriptively.

Result and Discussion

Chlorophyta abundance, total bacterial population and floc volume

Chlorophyta abundance data on rearing media are presented in Table 1. Chlorophyta density

at each treatment decreased after 40 days of rearing. The addition of Chlorophyta in the rearing media experiences death or predation. In rearing media, a food chain system occurs between Chlorophyta and zooplankton (Figure 1), resulting in a decrease in population of Chlorophyta due to predation.



Figure 1. Biofloc and Chlorophyta profile in the rearing media of snakehead culture in this study (40 magnificaton scale of microscope)

The pattern of relationships between zooplankton and phytoplankton is a series of eating and prey relationships. That relationship forms the path of the food chain. Phytoplankton as primary producers are eaten by zooplanktons; in turn zooplanktons are eaten by small fish at higher tropic levels (Bouman et al., 2003).

Chlorophyta Abundance (Cell.L⁻¹) Commercial Nitrification Swamp microbes 40th day 10th day 0 day Bacteria $2,1 \times 10^{3}$ Ν1 P1 $3,2 \times 10^{3}$ $3,2 \times 10^{3}$ $3,6 \times 10^{3}$ P2 $3,43 \times 10^{7}$ $4,1 \times 10^{3}$ Р3 $4,1 \times 10^{3}$ $3,43 \times 10^7$ $4,1 \times 10^{3}$ P4 $3,7 \times 10^{3}$ $3,43 \times 10^7$ $4,46 \times 10^{3}$ $4,0 \times 10^{3}$ 4.0×10^{3} N2 P1 $2,1 \times 10^{3}$ $3,43 \times 10^{7}$ P2 $3,6 \times 10^{3}$ $2,41 \times 10^{3}$ P3 $3,9 \times 10^{3}$ $3,43 \times 10^7$ $2,34 \times 10^{3}$ P4 $3,4 \times 10^{3}$ $3,43 \times 10^7$ $4,03 \times 10^{3}$

Table 1. Chlorophyta abundance in snakehead rearing media at 0, 10, 40th day

The total bacterial population on rearing media is presented in Table 2, the results of the LSD test of the floc volume at ten and forty days after rearing are showed in Table 3 and Table 4, respectively.

Commercial	Swamp	Total bacterial population (CFU.mL ⁻¹)					
Nitrification Bacteria	microbes	0 day	1st day	20th day	40th day		
N1	P1	$6,60 \times 10^4$	$6,78 \times 10^4$	$1,55 \times 10^{5}$	$6,20 \times 10^3$		
	P2	$6,20 \times 10^4$	$3,95 \times 10^{6}$	$6,93 \times 10^{6}$	$2,77 \times 10^{5}$		
	P3	$7,00 \times 10^4$	$3,28 \times 10^{6}$	$7,53 \times 10^{6}$	$3,01 \times 10^{5}$		
	P4	$4,70 \times 10^4$	$5,59 \times 10^{7}$	$1,00 \times 10^{8}$	$2,99 \times 10^{6}$		
N2	P1	$7,10 \times 10^4$	$2,01 \times 10^7$	$3,54 \times 10^{7}$	$1,42 \times 10^{6}$		
	P2	$4,50 \times 10^4$	$3,29 \times 10^{7}$	$5,59 \times 10^{7}$	$1,68 \times 10^{6}$		
	P3	$4,30 \times 10^4$	$4,99 \times 10^{7}$	$6,41 \times 10^{7}$	$1,93 \times 10^{6}$		
	P4	$4,95 \times 10^4$	$4,70 \times 10^{7}$	$8,75 \times 10^7$	$4,06 \times 10^{6}$		

Table 2. Total bacterial population in rearing media

Based on Table 2, the total bacterial population increased on 20th day and decreased until 40th days. The increase in population on 20th day can be caused by adequate nutrition in the rearing media, so that bacteria and Actinomycetes can use these nutrients for metabolic activity and growth. Whereas, the decline of bacteria population on 40th day could be caused by reducing nutrient (macronutrient and micronutrient) in the water. The bacteria couldn't enough to take their nutrition because of nutrition depletion.

The results of LSD at 10 and 40 days after rearing showed that in the factor of addition of commercial nitrification bacteria, the volume of floc on media without commercial nitrification bacteria was significantly higher compared to the treatment given nitrification bacteria. The addition of nitrifying bacteria can increase the volume of floc, because one of the constituent components of floc is a bacterium. In the factor of microbial addition from swamps, the volume of floc on the media which was given a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. significantly showed higher compared to other treatments.

The Single Effect of Nitrifying Bacteria (N)	The Sin Microbes	igle Influ s (P)	The Main Effects of Nitrifying Bacteria (N)		
	P1	P2	P3	P4	(LSD _{0,05} =4,386)
N1	11,111	16,666	13,332	26,668	16,944 ^b
N2	10,000	10,000	13,332	16,667	12,500ª
The Main Effects of Swamp Microbes (P) (LSD _{0,05} =3,102)	10,556 ª	13,333 ª	13,332 ª	21,667 ^b	

Table 4. The results of LSD test floc volume of rearing media at 40 days after rearing

The Single Effect of Nitrifying Bacteria (N)	The Single (LSD _{0,05} =)	e Influence (6,631)	The Main Effects of Nitrifying Bacteria (N)		
	P1	P2	P3	P4	(LSD _{0,05} =3,315)
N1	11,112ª	26,667 ^b	16,667ª	38,889°	25,834 ^b
N2	13,333ª	13,333ª	23,333 ^b	33,333°	20,833ª
The Main Effects of Swamp Microbes (P) (LSD _{0,05} =4,689)	12,223ª	20,000 ^b	20,000 ^b	41,111 ^c	

It is suspected that the types of microorganisms are easier for forming flocs. On the influence of interactions between factors at 40 days after rearing showed that treatment combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without nitrifying bacteria are the highest of floc volume 38.89 mL.L⁻¹, but not significantly different from the treatment combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and nitrifying bacteria. The volume of floc in this study is lower than the study from Mulyadi *et al.* (2016), where in treatment with stocking density of 450 snakehead fish m⁻³ which was kept for 41 days resulted in a floc volume of 40.7mL.L⁻¹. This is presumed that the rearing media lacks carbon source which bacteria use for floc formation. According to Panigrahi *et al.* (2019), vaname shrimp cultivation without a biofloc system can produce a volume of floc of 4.53 mL. L⁻¹ that is lower than the cultivation of vaname shrimp with a biofloc system by adding molasses. Floc thickness will continue to increase along with the provision of bacteria and carbon sources that are continuously balanced with feed metabolic waste and it produce ammonia. The bacteria could bind to ammonia and will a biofloc (Sitohang *et al.*, 2018).

The results of the analysis of variance showed that the interaction between factors and

the factors of addition of swamp origin microbes to the survival of snakehead fish significantly affected between treatments, but the factor of adding commercial nitrification bacteria had no significant effect. Based on further testing of LSD on microbial factors from swamps, the rearing media given a combination of Chlorophyta, *Bacillus* sp. and Streptomyces sp. were significantly higher than other treatments and interactions between factors showed that the rearing media given a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria significantly different compared to other treatments with a percentage of 63.94%.

Based on the results of survival percentage, it showed that the combination of swamp microbe is able to suppress unfavorable microbes and decreasing water quality in the rearing media, so that snakehead fish can survive well. This is shown in the treatment of rearing media given a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria that provide snakehead fish survival rate of 63.94%. The combination of *Bacillus* sp. and *Streptomyces* sp. was able to provide more protection against unfavorable microbes in the media, where the presence of *Bacillus* sp. gives effect to *Streptomyces* sp. to produce antimicrobial compounds. Luti and Mavituna (2011) explained that *Bacillus* was cultured together with *Streptomyces* can increased the production of antimicrobial compounds when it was compared with the single genus culture.

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The Single Effect of Nitrifying Bacteria (N)		The Single Influence of Swamps Microbes (P) (LSD _{0,05} =6,02)				Main of (N)			
	P1	P2	Р3	P4					
N1	26,06ª	36,91 ^b	28,03ª	63,94 ^d	38,74				
N2	31,75 ^{ab}	28,03ª	35,16 ^b	48,20 ^c	35,79				
The Main Effects of Swamp Microbes (P) $(LSD_{0,05}=4,25)$	28,91ª	32,47ª	31,59ª	56,07 ^b					

Table 5. LSD test of Survival Rate of Snakehead fish

The N1P1 treatment (without the addition of microbes from swamps and commercial nitrification bacteria) was the treatment with the lowest survival value of 26.06% compared to other treatments. These results prove that the addition of swamp origin microbes to rearing media can increase the survival rate of snakehead fish. This is in line with the results of Hartini et al. (2013) that the addition of EM-4 probiotics to rearing media had a significant influence on the survival of snakehead fish. The average survival of snakehead fish with EM-4 probiotics (28.88-96.66%) tended to be higher compared to control treatments (without EM-4 probiotics) which was 8.89%. Budianto and Heny (2017) that the bacteria Bacillus sp. has bacteriocin compounds with specific characters so that it can inhibit the growth of S. iniae and P. fluorescens. Streptomyces bacteria has the potential to control pathogenic bacteria by means of competition, parasitism or producing secondary metabolite compounds (Lutfi, 2018). The combination of the two microbes can provide a high percentage of survival compared to without a combination. According to Irianto and Austin (2002), probiotic bacteria can increase survival or reduce mortality through the development of the immune system, such as increasing phagocyte and lysozyme activity thereby suppressing pathogenic bacterial colonies. Sanchez et al. (2014) states that probiotics can increase immune stimulation in fish as protection against pathogenic bacteria that causes death in fish culture.

Feed Efficiency, Absolute Weight and Length Growth

The results of the analysis of the variety of snakehead fish feed efficiency showed that the interaction between factors, microbial addition factors from swamps and the addition of commercial nitrification bacteria to the value of fish feed efficiency significantly affected between treatments. LSD test results of the efficiency of fish feed for 40 days of rearing are presented in Table 6.

The Single Effect of Nitrifying Bacteria (N)		le Influenc _{0,05} =3,32)	The Main Effects of Nitrifying Bacteria (N)		
	P1	P2	Р3	P4	(LSD _{0,05} =1,66)
N1	18,93ª	47,34 ^d	37,97°	59,65 ^e	40,97 ^b
N2	22,00ª	29,52 ^b	34,89°	44,11 ^d	32,63ª
The Main Effects of Swamp Microbes (P) (LSD _{0,05} = 2.35)	20,47ª	38,43 ^b	36,43 ^b	51,88 ^c	

Table 6. LSD test result of the efficiency of snakehead fish feed for 40 da	lays of rearing
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Based on the results of the LSD test on the main effect of the addition of commercial nitrification bacteria, the value of the snakehead fish feed efficiency in the treatment media of rearing without commercial nitrification bacteria was significantly higher than the treatment given nitrification bacteria. On the influence of the addition of microbes from swamps, the value of snakehead fish feed efficiency in the treatment of rearing media that were given a combination of Chlorophyta, Bacillus sp. and Streptomyces sp. significantly different compared to other treatments and interactions between factors. The value of snakehead fish feed efficiency in the treatment of rearing media that were given a combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without commercial nitrification bacteria were significantly higher than the results of other treatments. It is thought that the origin of swamp microbes added to the media enter the digestive tract of snakehead fish during the process of respiration and when eating. Chlorophyta that enters the digestive tract could be a natural food source, while Bacillus sp. and Streptomyces sp. which entered the digestive tract could be working together to improve their digestibility. High digestibility in the feed will increase the absorption of nutrient. The nutrients were need for the fish and the absorption of nutrients runs optimally. The fish will grow well and increase the value of feed efficiency. Bacillus sp. is a bacterium that can increase digestibility because it can secrete protease, lipase and amylase enzymes (Fardiaz, 1992).

These bacteria can also produce antimicrobial compounds (bacteriocin and antibiotics) that can suppress pathogenic bacterial colonies (Findy, 2009). *Streptomyces* sp. is a genus of Actinomycetes that can produce various antibiotic compounds. Streptomyces has the potential to control pathogenic bacteria by conducting competition, parasitism or producing secondary metabolites (Lutfi, 2018). In increasing the value of feed efficiency, the bacterium *Bacillus* sp. secreting enzymes that can increase digestion while *Streptomyces* sp. secretes antibiotics to be able to suppress pathogens so that the two bacteria work together to improve the digestibility and immunity of snakehead fish which ultimately results in high feed efficiency. Bacterial activity in the digestive tract will experience changes rapidly when there are microbes that enter through feed or water that cause changes in the microbial balance of intestinal origin with incoming microbes. The entry of these microbes is antagonistic to pathogenic microbes in digestion so that the digestive tract of fish will be better at digesting and absorbing feed nutrients and the use of feed will be more efficient (Mulyadi *et al.* 2011).

Swamp microbial addition factor, commercial nitrification bacteria addition factor, and their interaction between factors on the feed efficiency showed significantly different between treatments. Based on the results of the $LSD_{0.05}$ test (Table 7) on the main effect of the addition of commercial nitrification bacteria, the growth of absolute weight of fish in the treatment of rearing media without commercial nitrification bacteria was significantly higher than the

treatment given commercial nitrification bacteria. On the main influence of the addition of microbes from swamps, the growth of the absolute weight of snakehead fish in the treatment of rearing media that were given a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. significantly different than the other treatments. In the interaction between factors, the growth of the absolute weight of snakehead fish in the treatment of rearing media that were given a combination sp., Streptomyces sp. and without commercial nitrification bacteria significantly higher than other treatment results, but not significantly different from the treatment results given a combination of Chlorophyta, Bacillus sp. and without commercial nitrification bacteria significantly higher than other treatment results, but not significantly different from the treatment results given a combination of Chlorophyta, Bacillus sp. and without commercial nitrifying bacteria.

Table	Table 7. LSD 0.05 test results of growth in absolute weight of Snakehead fish								
	The Single Effect of Nitrifying Bacteria (N)	The Single Influence of Swamps Microbe (P) (LSD _{0,05} =0,08)				The Main Effects of Nitrifying Bacteria (N)			
	, , , , , , , , , , , , , , , , , , , ,	P1	P2	Р3	P4	(LSD _{0,05} =0,04)			
	N1	1,30ª	2,26 ^f	1,70°	2,32 ^f	1,90 ^b			
	N2	1,73°	1,41 ^b	1,88 ^d	2,08 ^e	1,78ª			
	The Main Effects of Swamp Microbes (P) (LSD _{0,05} =0,05)	1,51ª	1,84 ^b	1,79 ^b	2,20°				

Based on the results of analysis of variance, microbial addition factor from swamps and interactions between factors significantly influence the growth of absolute length, but the factor of adding commercial nitrification bacteria has no significant effect between treatments. The LSD results of growth in absolute length of snakehead fish are presented in Table 8.

The Single Effect of Nitrifying Bacteria (N)		gle Influen _{0,05} =0,10)	The Main Effects of Nitrifying Bacteria (N)		
	P1	P2	P3	P4	
N1	0,69ª	1,79 ^e	0,91 ^b	2,27 ^f	2,12
N2	1,13 ^c	1,08°	1,60 ^d	1,74 ^e	2,09
The Main Effects of Swamp Microbes (P) (LSD _{0,05} =0,07)	0,91ª	1,44°	1,26 ^b	2,00 ^d	

Table 8. LSD test results for growth in the absolute length of snakehead fish

The main influence of the addition of microbes, the growth of the absolute length of snakehead fish in the treatment of rearing media which were given a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. were significantly different than the other treatments. On the influence of interactions between factors, the absolute length growth of snakehead fish in the treatment of rearing media that were given a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria were significantly higher than the results of other treatments.

The addition of a combination of swamp microbes and commercial nitrification bacteria gave significant results on the growth of absolute weight and absolute length growth. The highest absolute weight and length growth in the treatment of rearing media were given a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria and lowest in the treatment without the addition of microbes. This is thought to be the treatment without the addition of microbes less effective in the performance of absorption of nutrients in the feed which causes less optimal growth compared to other treatments. In the treatment of N1P4, swamp microbes added to the media could be entered into the digestive tract of snakehead fish during the respiration process and when eating.

These microbes break down complex compounds into simple and increase digestibility of feed and accelerate the process of absorption of food by the fish's body. The basic principle of the work of probiotics in aquaculture is the ability of microorganisms to break down long chains of protein, carbohydrates and fats in feed (Feliatra and Suryadi, 2004). The addition of 10⁴ CFU.mL⁻¹ probiotics to rearing media gave the growth and weight of tiger shrimp larvae that were higher than controls (Widarnani *et al.*, 2010).

Dissolved Oxygen and Ammonia in the Rearing Media

The results of the analysis of variance on day 0 showed that the factor of commercial nitrification bacteria, swamp microbes and the interaction between the factors did not significantly influence the dissolved oxygen content (Table 9). At 5 and 10 days after rearing, the factor of commercial nitrification bacteria and swamp microbes significantly affected the dissolved oxygen content, but the interaction between the factors had no significant effect.

The results of dissolved oxygen in the rearing media at 5 and 10 days after rearing on the main effect of the addition of commercial nitrification bacteria showed that they were not given commercial nitrification bacteria were significantly higher than those treated with commercial nitrification bacteria.

The main influence of the addition of microbes from swamp showed that dissolved oxygen in the rearing media were given a combination of Chlorophyta, *Bacillus* sp., and *Streptomyces* sp. (P4) significantly different than other treatments.

Dissolved Oxygen (mg.L ⁻¹)										
	Days a	fter rearin	g							
	0	5	10	15	20	25	30	35	40	
LSD				0,18	0,14	0,14	0,18	0,18	0,18	
N1P1	3,60	3,53	3,53	3,40°	3,10 ^c	3,00 ^c	2,97°	2,77°	2,67°	
INTLT	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	
N1P2	3,63	3,53	3,53	3,23 ^{ab}	2,93 ^{ab}	2,83 ^{ab}	2,77 ^{ab}	2,57 ^{ab}	2,47 ^{ab}	
NIFZ	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	
N1P3	3,67	3,57	3,47	3,33 ^{bc}	3,03 ^{bc}	2,93 ^{bc}	2,87 ^{bc}	2,67 ^{bc}	2,57 ^{bc}	
MILD	±0,1	±0,2	±0,2	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	
N1P4	3,57	3,70	3,60	3,60 ^d	3,50 ^e	3,40 ^e	3,40 ^e	3,20 ^e	3,10 ^e	
INTLA	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	
N2P1	3,63	3,43	3,43	3,13ª	2,83ª	2,73ª	2,67ª	2,47ª	2,37ª	
INZFI	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	
N2P2	3,57	3,47	3,47	3,33 ^{bc}	3,03°	2,93 ^{bc}	2,87 ^{bc}	2,67 ^{bc}	2,57 ^{bc}	
INZI Z	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	
N2P3	3,53	3,43	3,33	3,27 ^{ab}	3,10 ^c	3,00°	2,90 ^{bc}	2,70 ^{bc}	2,60°	
INZF J	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	
N2P4	3,67	3,63	3,53	3,40°	3,30 ^d	3,20 ^d	3,20 ^d	3,00 ^d	2,90 ^d	
	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	
LSD		0,08	0,08	0,09	0,07	0,07	0,09	0,09	0,09	
N1	3,62	3,58 ^b	3,53 ^b	5,09 ^b	3,14 ^b	3,04 ^b	3,00 ^b	2,80 ^b	2,70 ^b	
N2	3,60	3,49ª	3,44ª	4,93ª	3,07ª	2,97ª	2,91ª	2,71ª	2,61ª	
LSD		0,11	0,11	0,13	0,10	0,10	0,13	0,13	0,13	
P1	3,62	3,48ª	3,48 ^{ab}	3,27ª	2,97ª	2,87ª	2,82ª	2,62ª	2,52ª	
P2	3,60	3,50ª	3,50 ^{ab}	3,28ª	2,98ª	2,88ª	2,82ª	2,62ª	2,52ª	
P3	3,60	3,50ª	3,40ª	3,30ª	3,07ª	2,97ª	2,88ª	2,68ª	2,58ª	
P4	3,62	3,67 ^b	3,57 ^b	3,50 ^b	3,40 ^b	3,30 ^b	3,30 ^b	3,10 ^b	3,00 ^b	

Table 9. LSD test result of dissolved oxygen in the rearing media

On 10 days after rearing, the P4 treatment had significantly higher dissolved oxygen content than the P3 treatment, but it was not significantly different from the P1 and P2 treatments. The factor of commercial nitrification bacteria, microbial origin and the interaction between

factors significantly influence the dissolved oxygen content. The dissolved oxygen test on the 15th, 20th, 25th, 30th, 35th and 40th day are presented in Table 9.

Ammonia analysis results on day 0 showed that the factor of commercial nitrification bacteria, microbial origin of the swamp and the interaction between factors did not significantly influence. The variance analysis results on days 5, 10, 15, 20, 25, 30, 35 and 40 showed that the factor of commercial nitrification bacteria, microbial origin of swamp and the interaction between factors significantly affected ammonia content. They are presented in Table 10.

		Ammonia C			_,,		2		
	Days aft	er rearing							
	0	5	10	15	20	25	30	35	40
LSD		0,013	0,013	0,014	0,013	0,020	0,021	0,019	0,022
N1P1	0,290	0,383 ^e	0,393 ^d	0,410 ^d	0,327 ^e	0,540 ^d	0,674 ^e	0,691 ^f	0,948 ^e
	±0,07	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,02
N1P2	0,323	0,273 ^d	0,283 ^c	0,223 ^c	0,203 ^c	0,293 ^b	0,314 ^b	0,321 ^c	0,324 ^b
	±0,03	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01
N1P3	0,283	0,257 ^c	0,267 ^b	0,207 ^b	0,197 ^{bc}	0,363 ^c	0,482 ^d	0,585 ^e	0,692 ^d
	±0,01	±0,01	±0,01	±0,01	±0,01	±0,02	±0,02	±0,01	±0,02
N1P4	0,267	0,220ª	0,230 ^a	0,170 ^a	0,147ª	0,243ª	0,260ª	0,265ª	0,270 ^a
	±0,01	±0,01	±0,01	±0,01	±0,01	±0,02	±0,02	±0,02	±0,02
N2P1	0,230	0,253 ^c	0,263 ^b	0,203 ^b	0,193 ^{bc}	0,282 ^b	0,363 ^c	0,385 ^d	0,392 ^c
	±0,01	±0,01	±0,01	±0,01	±0,01	±0,02	±0,02	±0,01	±0,02
N2P2	0,290	0,233 ^{ab}	0,243ª	0,183ª	0,160ª	0,250ª	0,268ª	0,273ª	0,277ª
	±0,04	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01
N2P3	0,250	0,277 ^d	0,287 ^c	0,227 ^c	0,187 ^b	0,277 ^b	0,296 ^b	0,300 ^b	0,306 ^b
	±0,02	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01
N2P4	0,303	0,237 ^b	0,247 ^b	0,187 ^b	0,163 ^b	0,250ª	0,268ª	0,273 ª	0,277ª
	±0,03	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01
LSD		0,006	0,006	0,007	0,006	0,010	0,011	0,010	0,011
N1	0,291	0,283 ^b	0,293 ^b	0,253 ^b	0,218 ^b	0,360 ^b	0,432 ^b	0,466 ^b	0,558 ^b
N2	0,268	0,250ª	0,260ª	0,200ª	0,176 ^a	0,265ª	0,299ª	0,308ª	0,313ª
LSD		0,009	0,009	0,010	0,009	0,014	0,015	0,013	0,016
P1	0,260	0,318 ^d	0,328 ^d	0,307 ^d	0,260 ^d	0,411 ^d	0,518 ^d	0,538 ^d	0,670 ^d
P2	0,307	0,253 ^b	0,263 ^b	0,203 ^b	0,182 ^b	0,272 ^b	0,291 ^b	0,297 ^b	0,300 ^b
P3	0,267	0,267 ^c	0,277 ^c	0,217 ^c	0,192 ^c	0,320 ^c	0,389°	0,443°	0,499 ^c
P4	0,285	0,228ª	0,238ª	0,178ª	0,155ª	0,247ª	0,264ª	0,269ª	0,274ª

Table 10. LSD test results for ammonia every 5 days on rearing media

The results of LSD test on 5, 10, 15, 20, 25, 30, 35 and 40th days on the main effect of the addition of commercial nitrification bacteria showed that the ammonia content in the rearing media which given commercial nitrification bacteria. The main effect of the addition of microbes from the swamp shows that the ammonia content in the rearing media that was given a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. was significantly different compared to other treatments. The results of LSD on 5, 10, 15 and 20th day, in the interaction between factors, N1P4 treatment had significantly lower ammonia levels compared to other treatments different from N2P2 treatment. The results of LSD on 25, 30, 35 and 40th day in the interaction between factors, N1P4 treatment between factors, N1P4 treatment from N2P2 treatment had significantly lower ammonia levels compared to other treatments. N1P4 treatment factors, N1P4 treatment had significantly different from N2P2 treatment. The results of LSD on 25, 30, 35 and 40th day in the interaction between factors, N1P4 treatment factors, N1P4 treatment from N2P2 treatment had significantly lower ammonia levels compared to other treatments, but not significantly lower ammonia levels compared to other treatments, but not significantly lower ammonia levels compared to other treatments, but not significantly lower ammonia levels compared to other treatments, but not significantly lower ammonia levels compared to other treatments, but not significantly lower ammonia levels compared to other treatments, but not significantly different from N2P2 and N2P4 treatments.

On the factor of adding nitrifying bacteria, the lowest ammonia content in the treatment was given nitrifying bacteria. It is suspected that the added nitrification bacteria are able to carry out the process of nitrification on the rearing media. According to Rurangwa *et al.* (2014), nitrification takes place through 2 reaction stages, where in the first stage the oxidation of ammonium to nitrite is carried out by ammonium oxidizing microbes

(*Nitrosomonas* sp.) and in the second stage nitrite oxidation by nitrite oxidizing microbes (*Nitrobacter* sp.). In the addition of swamp origin microbial factors, the lowest ammonia content in the treatment was given a combination of swamp origin microbes in the form of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. It is suspected that the three microbes from which the swamp was added could break down ammonia. Ammonia is the main source of nitrogen in addition to nitrate which could be used by microalgae for their metabolic processes (Nurhayati *et al.*, 2014). *Bacillus* sp. could oxidize ammonia to nitrite through heterotrophic and chemotrophic processes (Edwards, 2011).

In the interaction between factors, N1P4 treatment had the lowest ammonia levels on the 5th day until the end of rearing, it was suspected that microbes from the swamp provided were able to break down organic material derived from feces or feed into compounds that were not harmful to fish. The N1P4 results were not significantly different from the N2P2 and N2P4 results. The commercial nitrification bacteria in the same ecosystem as *Bacillus* sp. and *Streptomyces* sp., nitrification process activity and growth of nitrifying bacteria are inhibited. Nitrifying bacteria (autotrophic bacteria) produce very small biomass and slow growth. Nitrifying bacteria takes 12 hours to regenerate, while heterotrophic bacteria Bacillus sp. and Streptomyces sp. only need 30 minutes (Ebiling *et al.*, 2006). If there is a limited nitrogen to carbon (high C: N ratio), the nitrification process is inhibited and heterotrophic bacteria develop rapidly. Heterotrophic bacteria will assimilate ammonia directly into bacterial proteins, when heterotrophic bacteria develop rapidly. It will cause competition in the struggle for oxygen, space and nutrients with autotrophic bacteria (Blancheton *et al.*, 2013).

Ammonia levels in all treatments gave microbes on 10th day to 20th day of rearing, decreased and increased until the 40th day. On the 10th to 20th day, it was suspected that the ammonia accumulation from metabolic waste had not yet occurred so that the microbes given to the rearing media were able to work optimally. On the 25th to 40th day, it was suspected that the ammonia accumulation from metabolic waste had been accumulated in the rearing media, so that the microbes given couldn't optimally reduce their ammonia levels. Meanwhile, the treatment without the addition of microbes (N1P1) experienced an increase in ammonia along with the increase in the rearing time. Increasing ammonia levels in the N1P1 treatment was caused of the rearing without siphoning during 40 days, It resulted in a buildup of organic material derived from metabolic waste and levels of ammonia in the rearing media. The decomposing bacteria from natural swamp water on the media have not been able to make an optimal decomposition.

Conclusion

Addition of swamp microbes in the form of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. without the addition of commercial nitrification bacteria on the snakehead rearing media, it provides better water quality values. The best results are obtained in the treatment with the addition of swamp microbes Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. without the addition of commercial nitrification bacteria in the rearing media that gave in the survival rate, feed efficiency, and growth of snake head fish in swamp aquaculture.

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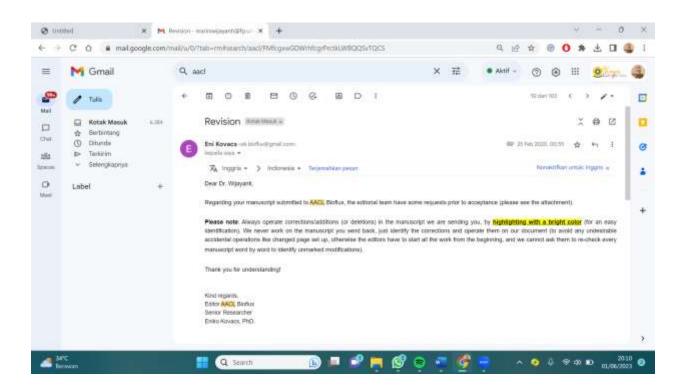
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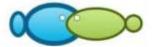
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Optimization of striped snakehead fish (Channa striata) culture using swamp microbial combination and nitrification bacteria

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Abstract. Utilization of swamp area; as a fish culture location; will construct a stress water quality. Therefore, it is necessary to improve the water quality with environmental friendly if-biological treatments, energiation in the addition wat microbes as probletics in media culture. The purpose of this study was to determine the combination of microbes from average that can improve the water quality of study was to determine the combination of microbes from swamps that can improve the water quality of the seamp flat, mode culture and production mindle and production of seamp flat, estimation. The research used Completely Randomized Design (CRD) factorial with two factors consisting of the first factor with two treatments and the second factor with five treatments and three replications. The first factor with two treatments and the second factor with five treatments and three replications. The first factor with two treatments and the second factor with five treatments and three replications. The first factor consistent in a fund according (PRDBAC) $\leq 10^{\circ}$ CFU_mit.¹ (N2). The second factor e-consistent is defined and the addition of swamp microbes (P1) and such addition of: (21) Chevophyta (3.43×10³ set_et_1) and Bachus sp. (10⁶ CFU_mit.¹) (P2). (11) - addition = Chicrophyta (3.43×10³ set_et_1) and Streptomyces sp. (10⁶ CFU_mit.¹) (P3). (11) - addition = Chicrophyta (3.43×10³ set_et_1) and Streptomyces sp. (10⁶ CFU_mit.¹) (P3). (11) - addition = Chicrophyta (3.43×10³ set_et_1) and Streptomyces sp. (10⁶ CFU_mit.¹) (P3). (14) addition = Chicrophyta (3.43×10³ set_et_1) (10⁶ CFU_mit.¹) and Streptomyces sp. (10⁶ CFU_mit.¹) (P4). The result showed that the addition microbes from swamps with public combination of N1 and P4 treatment scenarios as bile to improve the water quality value better than the other treatment scenarios and the addition of microbes from the addition of microbes from the best survival microbes from swamps with public treatment scenarios and bile to improve the water quality value better than the other microbes from swamps mither better survival microbes from the back of the microbes from the sector of the sector of the sector survival microbes from the back of the microbes from the microbes from the back survival microbes from the microbes the microbes from the microbes from the baddition of microbes from the microbes from the microbes fr and any like oddition of minimum metanic (http:// Commention of http:// productions the best survival rate-ut_63.94%), feed efficiency ut_59.65%, absolute weight growth ut_2.32 g] and absolute length growth of (2.27 cm) Key Words: problotic, swamp microbes, snakehead fish, nitrification bacteria.

Introduction. The swamp aquaculture must be-improveing and maintaining the water quality for fish rearing media, through environmentally friendly biological treatments, since, the wastewater of fish rearing on swamps will-reduces the quality of swamps water. from swamps. So it is treatment environmentally friendly One of the treatments is adding problotics in the rearing media. Irianto and <u>§</u> Austin (2002), states that environmental <u>damaging</u> degradation can be prevented with problotics <u>able</u> which aims to degrade the organic materials in the habitat. Hartini et al- (2013) showed that the addition of probiotics at a dose of 10 µl.11.week1 can improve and maintain optimal water quality. In other studies, the addition of the effective probiotic microorganism 4 probioties can reduce ammonia levels and suppress the population of pathogenic microorganisms from thethat exist in culture media (Trisna et al-7 2013). Swamps have high biodiversity, including sediment microbes. Many swamp

are _able to improve the physical and chemical properties of awampstheir media. The identified Sywamp microbes that he include Chlorophyta, Bacillus sp. and Streptomyces sp. (Wijayanti et al., 2018). Bacteria-fre (Bacillus sp.) can be used as environmental probletics with concentrations of 10⁵ CFU.ml⁻¹ (Khotimah₇ 2018) and microelgae. Chlorophyta microelgae with the optimum concentration 10% of the maximum density (Utami 2019) are able to grow in the fish iotics with concentrations of 105 CFU.ml culture media and they can be used as environment orobiot

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Chlorophyta is a microorganism that can be used as Green Water in aquaculture media. Wijayanti et_-al- (2018) showed that the use of Chlorophyta increased the level of dissolved oxygen in the culture pond culture media 60.52% and swamp water culture media 63.63% .- The increasing dissolved oxygen is caused by Chlorophyta carrying out photosynthetic activity which produces dissolved oxygen in the culture media. Bacillus sp. and Streptomyces sp. obtained are proteolytic bacteria that can increase the content of NH3, NO2, and NO3 into the media (Yuliani- 2017; Saraswati- 2018). Balcazar et al-(2006) states that Bacillus sp. is an example of an efficient probiotic bacteria, used in aquaculture because it is able to convert organic matter into CO₂ used in cell metabolism. Gram-positive bacteria, such as Bacillus sp. can increase the animal's immune system capabilities and also act favorably in improving the quality of the water system (Mohapatra et al- 2013). Bernal et al- (2017) states that the combination of Streptomyces sp. and Bacillus sp. demonstrated a significant immunomodulatory activity by increasing the total number of hemocytes and the activity of superoxide dismutase (SOD), which provides a protective effect against Vibrio harvey/ bacteria by increasing the immunological status of Penaeus monodon. Nitrifying bacteria are chemolithoautotrophic bacteria (ex: Nitrosomonas sp., Nitrobacter sp.), which are able to meet their carbon needs through CO2 fixation (Calvin cycle), and their energy source comes from the oxidation process of reducing ammonia to nitrate. With As an example, through the addition of nitrifying bacteria_and, denitrification, and the molasses with a -- C/N ratio of 10 could decrease in ammonia levels by 28.5% (Yuniasari, 2009).

Each of the swamp microbes and nitrifying bacteria has their own advantages and disadvantages, so we needtherefore a consortium of swamp microbes and nitrifying bacteria would be more effective. The consortium is The emergence of a consortium, expected to form cooperative, commensal and mutualistic relationships between microbes__ The emergence of a swamp microbial the needcalls for an optimization optimal of a combination of Bacillus sp., Streptomyces sp., Chlorophyta and commercial nitrification bacteria,dia of swamp fish production. The purpose of this study was to determine the combinations of swamp microbes and nitrifying bacteria that can improve the water quality of media in swamp fish production. on microhes that can in

Material and Method

The experimental design used was a completely randomized factorial design (RAL) consisting of 2 factors-, The the first factor with 2 treatments and the second factor with 4 treatments and 3 replications. The first factor is the addition of nitrifying bacteria (PROBAC), namely:

- N1: Without the addition of nitrifying bacteria (PROBAC)
- N2: Addition of nitrifying bacteria (PROBAC) 5×10⁶ CFU_mL⁻¹
- The second factor is the addition of swamp microbes, namely:

P1: Without the addition of swamp microbes

P2: Provision of 100 ml Chlorophyta (3.43 × 10⁷ Gel_{CCL}+L⁻¹) and Bacillus sp. (10⁵ CFU₂-mL⁻¹) P3: Provision of 100 ml Chlorophyta (3.43 × 10⁷ Gel_{CCL}+L⁻¹) and Streptomyces sp. (10⁵ CFU₂-mL⁻¹)

P4: Provision of 100 ml Chlorophyta (3.43 × 107 Gelicell -L-1), Bacillus sp. (105 CFU_-mL-1) and Streptomyces sp. (105 CFU -mL-1)

Bacteria cultivation and propagation. Pure bacterial colonies were obtained from the results of previous studies. The colony was cultivated using <u>nutrient agar NA (NA nutragar</u>) media for Bacilius sp and <u>yeast mait 4M (YM yeast mait agar</u>) agar agar for Streptomyces sp. Bacterial colonies were scratched in a petri dish containing NA (Nut ar) for Bacillus sp, and YM (Yeast Malt Agar)-media for Streptomyces sp. using the quadrant streak method. Petri dishes were wrapped in wrapping paper and incubated for

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2 days at room temperature (28%-30°C). A single colony formed on a petri dish was transferred to the agar media and incubated until bacteria greew.

Swamp bacteria that grow-prown on NA and YM agar media were multiplied by putrient broth 44B (NB nutrient broth) media for Bacillus sp. and liquid YM for Streptomyces sp. As much as 5 mL of The suspension were collected in a test tube was ken as much as 1 ose in order to be cultured in the medium as muc tube, then homogenized with a shaker for approximately 2 days for Bacillus sp. and 5 days for Streptomyces sp., then multiplied from 5 mL to 500 mL.

Chlorophyta culture, Chlorophyta sp. The culture media: used for Chlorophyta sp. was a technical fertilizer media consisting of ZA, Urea, TSP, and Gandasil B. All technical fertilizer ingredients were mixed in a 250 mL Serlenmeyer and added to 100 mL of distilled water and then homogenized on a hot plate using a magnetic stirrer and sufficient heating, until all ingredients dissolved. The technical fertilizer media in the Eerlenmeyer was sterilized using an autoclave at 121°C for 0.25 hour. *Chlorophyta* isolates (about 10⁷cell_sml⁻¹ in 10 ml stock culture) were put into an Eerlenmeyer containing a technical fertilizer media for liquid culture. They cultured during 9 days-inst the room temperature for scaling up to 1 Liter.

Preparation of fish rearing media. The container used in rearing was in the form of an aquarium with a size of 30 x 30 x 30 cm² as many as 24 units. The aquariums were cleaned using potassium permanganate to be sterilized of diseases or parasites. The aquarium was filled with 20 liters of swamp water.

Fish culture test, The test organisms used in this study was were 12 snakehead fish specimens of 5 ± 1 cm each with 12 heads in for 20 liters of water (Mulyadi, 2016). Before stocking, acclimatize as an adaptation to the new environment to reduce stress on the test organism. After 7 days of stocking, a treatment was applied, consisting in a combination of Chlorophyta isolate (3.43-x-10⁷ Cellcell -L⁻¹), Bacillus sp. (10⁵ CFU_-mL⁻¹), Streptomyces sp. (10⁵ CFU_-mL⁻¹) as well as the "PROBAC" phitrification bacteria (5-x 10" CFU -mL'1), ++ added in co with the treat

Rearing The fish culture was maintained for 40 days which was calculated after the addition of the treatment. During rearing, they were fed at satiation with a frequency of three times a day, by usingliets used are commercial pellets with 40% protein content.

Chlorophyta abundance. Samplings were carried out at the beginning and end of the Chicrophyta abundance, Samplings were carried out at the beginning and end of the study by subcomposite methods, in each treatment. It used a A plankton net with 25 µm mesh size mesh plankton net was used for 5. Liters of rearing media each by unit experimental unit (sample of 25 mL)to 36 ml sample. Observation of Chicrophyta samples A microscope and "The Marine and Fresh Water Plankton" textbook were used a microscope and textbook The Marine and Fresh Water Plankton for the observation of the Chicken Plankton. Chlorophyta samples (Davis, 1955). Chlorophyta abundance calculation was done performed by using the Leackey Drop Microtransect method (American Public Health Association, 1989) as follows: $N = Z \times \frac{X}{v} \times \frac{1}{v}$

InformationWhere:

- N = Total number (cell_L⁻¹) Z = Number of individuals found
- X = volume of filtered water (25 mL) Y = Volume 1 drop of sample water (0.05 mL)
- V = volume of filtered water (5 Hers)

Bacteria population. The Counting counting of bacterial populations was performed at the beginning and end of rearing with the plate count method was to perform on a

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Gurvival rate -	
Survival rateThe percentage of fish survival was calculated by using the following Formatted: Font: Italc	
glass cone 1L volume, then the floc in the water media was left to settle in the tube for 15-20 minutes.	
Biofloc volume_Biofloc volume measurements were done on the 10 and 40 days after Formatted: Font Italic rearing. The fFloc volume was obtained by taking collecting a rearing media, by using a Formatted: Font Not Bold	
[2] 역상 가슴 '가슴 '가슴 이번 것 같은 것 4~1 전 16~1 이번 역간에 이 가슴 영양 것이 좋아? 것이 같은 것을 것 수 있는 것 수 있는 것 2~2 이 가슴 이 있는 것 수 있는 것 같이 없다. 이 것 것 같은 것 같은 것 같이 없는 것 같이 없는 것 같이 없는 것 같이 없는 것 같이 없다. 이 없는 것 같이 없다. 것 같이 없는 것 같이 없는 것 같이 없는 것 같이 없다. 이 없는 것 같이 없다. 것 같이 없는 것 같이 없다. 것 같이 없는 것 같이 없다. 것 같이 없는 것 같이 없다. 것 같이 없는 것 같이 없는 것 같이 없는 것 같이 없다.	
the following formula: Total of Bacteria = Total of colonies $\times \frac{1}{dilution factor} \times \frac{1}{mL sample}$	
multilevel dilution were then incubated at a temperature of 28-30°C for 24 hours. The growing population was determined in a colony forming unit (CFU) and calculated using	
multilevel dilution ware then incubated at a temperature of 29,200° for 24 hours. The	

difference) 5%. Chlorophyta abundance data and the total bacterial population were analyzed descriptively.

Result and Discussion

Chlorophyta abundance, total bacterial population and floc volume. Chlorophyta abundance data on rearing media are presented in Table 1. *Chlorophyta* density at each treatment decreased after 40 days of rearing. The addition of *Chlorophyta* added in the rearing media experiences death or predation. In rearing media, a food chain system occurs between *Chlorophyta* and zooplankton (Figure 1), resulting in a decrease in the population of *Chlorophyta* due to predation.



Figure 1. Biofloc and Chlorophyta profile in the rearing media of snakehead culture in thisstudy (40 magnificaton scale of microscope).

The pattern of <u>the interaction-relationships</u> between zooplankton and phytoplankton is a series of eating and prey relationships. <u>forming</u> That relationship forms the path of the food chain. Phytoplankton as primary producers are eaten by zooplanktons; in turn zooplanktons are eaten by small fish at higher trop<u>h</u>ic levels (Bouman et al., 2003).

Table 1- Chlorophyta abundance in snakehead rearing media at 0, 10, 40th day

Commercial	<u>10</u> (9 (2))	Chiorop	phyta Abundance	: (Cell.L ⁻¹)
nitrification bacteria	Swamp microbes	0 day	10th day	40 th day
	P1	32×10^{3}	3.72 × 103	21×10^{3}
	P2	36×10^{3}	$3,43 \times 10^{7}$	$4,1 \times 10^{3}$
N1	P3	4.1×10^{3}	$3,43 \times 10^{7}$	4.1×10^{3}
	P4	3.7×10^{3}	$3,43 \times 10^{7}$	$4,46 \times 10^{3}$
	P1	4.0×10^{3}	$4,0 \times 10^{3}$	2,1× 103
	P2	$3,6 \times 10^{3}$	$3,43 \times 10^{7}$	$2,41 \times 10^{3}$
N2	P3	$3,9 \times 10^{3}$	$3,43 \times 10^{7}$	$2,34 \times 10^{3}$
	P4	$3,4 \times 10^{3}$	$3,43 \times 10^{7}$	$4,03 \times 10^{3}$

The total bacterial population on-in the rearing media is presented in Table 2, the results of the LSD test of the floc volume at ten and forty days <u>after-of</u> rearing are showed in Table 3 and Table 4, respectively.

Table 2.	Total	bacterial	population	in	rearing	media
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Commercial	Swamp	Total bacterial population (CFU.mL ⁻¹)							
nitrification bacteria	microbes	0 day	Ist day	20th day	40th day				
	P1	6.60×10^{4}	6.78×10^{4}	1.55×10^{5}	$6,20 \times 10^3$				
N1	P2	$6,20 \times 10^{4}$	3.95×10^{6}	6,93 × 10 ⁶	2,77 × 105				
INT.	P3	$7,00 \times 10^{4}$	$3,28 \times 10^{6}$	$7,53 \times 10^{6}$	$3,01 \times 10^{5}$				
	P4	$4,70 \times 10^{4}$	$5,59 \times 10^{7}$	1.00×10^{8}	2,99 × 10 ⁶				
N2	P1	$7,10 \times 10^{4}$	2.01×10^{7}	3,54 × 107	$1,42 \times 10^{6}$				
WZ	P2	$4,50 \times 10^{4}$	$3,29 \times 10^{7}$	5,59 × 107	1,68 × 10 ⁶				

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Table 2+

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P3 P4	4,30 × 10 ⁴ 4,95 × 10 ⁴		1,93 × 10 ⁶ 4,06 × 10 ⁸	Commented [WU9]: Please use "," instead of "," in the entire
				manuscript

Based on Table 2, the total bacterial population increased on 20th day and decreased until the 40th days. The increase in population on the 20th day can be caused by adequate mutrition-mutrients addition in the rearing media, see that bacteria and Actinomycetes can use these nutrients for stimulating the metabolic activity and growth of the bacteria and Actinomycetes. Wwhile reas, the decline of bacteria population observed on the n-40th day could be caused by reducing the nutrient depletion (macronutrient and micronutrient) in the water. The bacteria couldn't enough to take their nutrition because of nutrition depletion.

Related to the factor of nitrification bacteria, the results of LSD at 10 and 40 days after rearing start showed that in the factor of addition of commercial nitrification bacteria, the volume of floc on- in the media without commercial nitrification bacteria, the volume of floc on in the media without commercial nitrification bacteria, the volume of floc on intrifying bacteria can increase the volume of floc, because one of the constituent components of floc is a bacterium. Related to the in the factor of microbial addition from swamps, the volume of floc on the media treated with which was given a combination of *Chlorophyta*, *Bacillus* sp. and *Streptomyces* sp. significantly showed higher levels compared to other treatments.

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Table 3- The results of LSD test of the floc volume in the rearing media at 10 days afterof rearing

The single effect of	The single	influence (The main effects of nitrifying bacteria (N			
nitrifying bacteria (N)	P1	P2 P3		P4	(LSD2.05=4,386)	
N1 N2	11,111 10,000	16,666 10,000	13,332 13,332	26,668 16,667	16,944 ^b 12,500 ^a	
The main effects of swamp microbes (P) (LSD0.05=3,102)	10,556*	13,333*	13,332°	21,6675		

Table 4-

- The results of LSD test floc volume of rearing media at 40 days often of rearing

The single effect of	The singl	e influence (P) (LSD ₀ ,	The main effects of nitrifying bacteria (N		
nitrifying bacteria (N)	P1	P2	P3	P4	(LSD0.05=3,315)
N1 N2	11,112° 13,333°	26,667° 13,333°	16,667ª 23,333º	38,889° 33,333°	25,834 ^b 20,833°
The main effects of swamp microbes (P) (LSD0.05=4,689)	12,223ª	20,000°	20,000 ^b	41,111¢	

It is suspected presumed that certain the types of microorganisms are easier-predisposed for forming-flocs forming. On-Related to the influence of interactions between factors at 40 days ofter of rearing, the observations showed that the treatment combination of *Chlorophyta*, Bacillus sp., Streptomyces sp. and without nitrifying bacteria are determines the highest of floe-volume of floe. 38.89 mL_sL⁻¹, but it is not significantly different from the treatment combination of Chlorophyta, Bacillus sp., Streptomyces sp. and with nitrifying bacteria. The volume of floe in this study is lower than the study from Mulyadi et al. (2016), where in treatment with stocking density of 450 snakehead fish m: ³ which was kept for 41 days resulted in a floe volume of 40.7_mL_sL⁻¹. This-IL is presumed that the rearing media lacks a carbon source used by which bacteria use for the

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floc formation. According to Panigrahi et al= (2019), vaname shrimp cultivation without a biofloc system can produce a volume of floc of 4.53 mL= L⁻¹, which that is lower than the cultivation of vaname shrimp with a biofloc system, by adding molasses. Floc thickness will continue to increase along with the provision of bacteria and carbon sources that are continuously balanced with feed metabolic waste and the producinge ammonia. The bacteria could bind to ammonia and will form a biofloc (Sitohang et al-; 2018).

The results of the analysis of variance showed that the <u>effect of the</u> interaction between factors <u>and</u> <u>and</u> the <u>effect of the</u> factors <u>ef-defined by the</u> addition of swamp origin<u>ated</u> microbes <u>to on</u> the survival of snakehead fish <u>varied</u> significantly <u>effected</u> between treatments, but the factor <u>ef-defined by the</u> adding <u>of</u> commercial nitrification bacteria had no significant effect. Based on further testing of LSD on microbial factors from swamps, in the rearing media <u>eiven-treated</u> with a combination of Chlorophyta, *Bacillus* sp. and Streptomyces sp., <u>the survival rate wasere</u> significantly higher than for the other treatments, <u>and-il</u> Interactions between factors showed that <u>in the</u> rearing media <u>eiven-with</u> a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, <u>the survival rate reached 63.94%</u>, a significantly <u>higher-different performance</u> compared to <u>the</u> other treatments-with a percentage of <u>63.94%</u>.

Based on the results of the survival percentage, it is showed that the combination of swamp microbes is able to suppress unfavorable microbes, and decreasing to improve the water quality in the rearing media and, to increase so that the snakehead fish survival rate can survive well. This is shown in the treatment of rearing media given consisting in a combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without commercial nitrification bacteria, which that provided a snakehead fish survival rate of 63.94%. The combination of Bacillus sp. and Streptomyces sp. was able to provide more protection against unfavorable microbes in the media, where the presence of Bacillus sp. givinges effect to Streptomyces sp. to produce antimicrobial compounds. Luti and & Mavituna (2011) explained that Bacillus wai-cultured together with Streptomyces com increased the production of antimicrobial compounds when it was compared with the single genus culture.

Table 5. LSD test of Survival Rate of Snakehead fish

The Single Effect of Nitrifying Bacteria (N)	The Sin Microber	The Main Effects of Nitrifying Bacteria (N)			
100	P1	P2	P3	P4	-
N1	26,06*	36,915	28,03*	63,94*	38,74
N2	31,75%	28,03*	35,16 ^b	48,20	35,79
The Main Effects of Swamp Microbes (P) (LSD _{0.05} =4,25)	28,91*	32,47*	31,59*	56,075	

The N1P1 treatment (without the addition of microbes from swamps, neither of and commercial nitrification bacteria) was the treatment with the lowest survival value of 26.06% compared to other treatments. These results prove that the addition of swamp origin microbes to rearing media can increase the survival rate of snakehead fish. This is in line with the results of Hartini et al- (2013), suggesting that the addition of EM-4 probiotics to rearing media had a significant influence on the survival of snakehead fish. The average survival of snakehead fish with EM-4 probiotics (28.88-96.66%) tended to be higher, compared to control treatments (without EM-4 probiotics) which was 8.89%. Budianto and 8 Heny (2017) state that the bacteria Bacillus sp. has bacteriocin compounds with specific characters so that it can inhibiting action on the growth of *S. iniae* and *P. fluorescens. Streptomyces* bacteria has the potential to control pathogenic bacteria by means of competition, parasitism or <u>by producing secondary metabolite</u> compounds (Luttf- 2018). The combination of the two microbes can provide a high

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percentage of survival compared to without athe no combination scenario. According to Irianto and <u>S</u> Austin (2002), probiotic bacteria can increase survival or reduce mortality through the development of the immune system, such as due to an increasing phagocyte and lysozyme activity, thereby suppressing pathogenic bacterial colonies. Sanchez et al-(2014) states that probiotics can increase immune stimulation in fish, as a protection against pathogenic bacteria that causes death in fish culture.

Feed efficiency, absolute weight and length growth. The results of the analysis of the <u>efficiency of variousety of feed on the</u> snakehead fish feed efficiency showed that the interaction between factors, the microbial addition factors from swamps and the addition of commercial nitrification bacteria, <u>can to increase</u> the value of fish feed efficiency, which is significantly affected between by the treatment type and concentrations. LSD test results of the efficiency of fish feed for 40 days of rearing are presented in Table 6.

The Single Effect of Nitrifying Bacteria (N)		gle Influenc nun=3,32)	The Main Effects of Nitrifying Bacteria (N)		
	P1	P2	P3	P4	(LSD0,01=1,66)
N1	18,93*	47,344	37,97=	59,65*	40,975
N2	22,00*	29,52	34,89	44,11 ^d	32,63*
The Main Effects of Swamp Microbes (P) (LSD _{0.05} = 2.35)	20,47*	38,43*	36,430	51,88	

Based on these results, the effects of the different combinations on the snakehead fishfeed efficiency could be observed, of the LCD toct commercial nitrification bacteria, The value of the snakehead fish feed efficiency in the treatment of the media of rearing was: (1) significantly higher without commercial nitrification bacteria was significantly higher than for the treatment given with nitrification bacteria (2) significantly different. On the influence of the addition of microbes from swamps, the value of snakehead fi rearing media that were given with with a combination of Chlorophyta, Bacillus sp. and Streptomyces sp., significantly different compared to other treatments and interactions between factors-: (3)were significantly higher The val treatment of rearing media that were given with a combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without commercial nitrification bacteria were higher-than the results of other treatments. It is thought that the swamp microbes added to the media enter the digestive tract of snakehead fish during the process of respiration and when while eating. Chlorophyta that enters the digestive tract could be a natural food source, while Bacillus sp. and Streptomyces sp. which entered the digestive tract could be working together to improve their digestibility. High digestibility in the feed will increase the absorption of nutrient, which at optimal levels-HS FURS O he nutri nts wara and- will increase the value of feed efficiency, favorising fish growth. Pich. Bacillus sp. is a bacterium that can increase digestibility because it can secrete protease, lipase and amylase enzymes (Fardiaz, 1992). These bacteria can also produce antimicrobial compounds (bacteriocin and

These bacteria can also produce antimicrobial compounds (bacteriocin and antibiotics) that can suppress pathogenic bacterial colonies (Findy, 2009). Streptomyces sp. is a genus of <u>Actinomycetes actinomycetes</u> that can produce various antibiotic compounds. Streptomyces has the potential to control pathogenic bacteria by conducting competition, parasitism or <u>by</u> producing secondary metabolites (Luffir 2018). In increasing the value of feed efficiency. The bacterium Bacillus sp. increases the value of feed efficiency by secreting enzymes that can <u>increase stimulate</u> digestion, while Streptomyces sp. secretes antibiotics to be able to suppress pathogens, so that the tweBoth bacteria work together to improve the digestibility and immunity of snakehead fish, which ultimately results in high feed efficiency. Bacterial activity in the digestive

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tract will experience changes rapid fluctuationsiv when with there are microbes that entering through feed or water and that cause causing changes in the intestinal microbial balance of intestinal origin with incoming microbes. The entry of these microbes is antagonistic to the pathogenic microbes in digestion so that the digestive tract of fish will be better of, facilitating the digestioning and absorptionbing of the feed nutrients, and the use of and making feed will be more efficient (Mulyadi et al. 2011).

Swamp microbial addition factor, commercial nitrification bacteria addition factor, and their interactions between factors influenceden the feed efficiency showed in a significantly different manner, depending on thebetween treatments. Based on the results of the LSD_{0.05} test (Table 7) on the main effect of the addition of commercial nitrification bacteria, the growth increase of the absolute weight of the snakehead fish in following the treatment of the rearing media was: (1) without commercial nitrification bacteria was significantly higher without commercial nitrification bacteria than the treatment given with commercial nitrification bacteria than the treatment given with the addition of microbes from swamps, the growth of the absolute weight of anakchead fish in the treatment of rearing media that were given a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. significantly different than with the other treatments (-3) significantly higher than the other treatments' results in the interaction between factors, the growth of the absolute weight of rearing media that were given a combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without commercial nitrification bacteria significantly higher than other treatment results, but (4) not significantly different from the treatment results given a combination of Chlorophyta, *Bacillus* sp. and without commercial nitrification bacteria significantly higher than other treatment results, but (4) not significantly different from the treatment results given a combination of Chlorophyta, *Bacillus* sp. and without commercial nitrification bacteria intrification bacteria.

Table 7. LSD 0.05 test results of growth in absolute weight of Snakehead fish The Main Effects of The Single Influence of Swamps Microbe The Single Effect of Nitrifying Bacteria (P) (LSD_{0.05}=0,08) Nitrifying Bacteria (N) (N) (LSDo.m=0,04) P1 P2 P3 p4 1,30 2,26 1,70 2,32 1.90 N1 1.73 1.41 1.88 2.08* 1.78 N2 Main Effects of The Swamp Microbes (P) 1,51* 1,84* 1,79 2,20

Based on the results of the variance analysis of variance, swamps microbial addition factor from swamps and interactions between factors significantly influenced the growth increase of the absolute length, but the factor of adding commercial nitrification bacteria has no significant-effect-between treatments. The LSD results of growth in absolute length of snakehead fish are presented in Table 8.

Table 8. LSD test results for growth in the absolute length of snakehead fish

The Single Effect of Nitrifying Bacteria (N)		gle Influen _{0.05} =0,10)	The Main Effects of Nitrifying Bacteria (N)		
turn hing success (re)	P1	P2	P3	P4	
NI	D,69*	1,79*	0,91 ^b	2,27	2,12
NZ	1,13	1,06°	1,60*	1,74*	2,09
The Main Effects of Swamp Microbes (P) (LSD _{0.02} =0,07)	0,91*	1,44	1,26 [±]	2,004	

———The main influence of the addition of microbes_on- the growth-increase of the absolute length of snakehead fish in the treatment of rearing media which were given with a combination of Chlorophyta, Bacillus sp. and Streptomyces sp. were was significantly

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Commented [WU17]: Please make the necessary adjustments using the previous tables as models. different than the in other treatments. IOn the influence of interactions between factors, on the increase of the absolute length growth of snakehead fish was significantly higher in the treatment of rearing media with a that were given a combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without commercial nitrification bacteria were significantly higher than the in the results of the other treatments.

The addition of a combination of swamp microbes and commercial nitrification bacteria gave significant results on the growth increase of absolute weight and absolute length growth. The highest absolute weight and length growth increase in the way produced by the treatment of rearing media were given awith a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, and lowest in by the treatment without the addition of microbes. This is thought to be the treatment without the addition of microbes. This is thought to be the treatment without the addition of microbes. This is thought to be the treatment without the addition of nutrients in the feed, which courses cousing a lease sub-optimal growth, compared to the other treatments. In the treatment of N1P4, swamp microbes added to the media could be entered into the digestive tract of snakehead fish during the respiration process and when eating. These microbes break down complex compounds into simpler ones and increase digestibility of feed—and, by accelerate accelerating the process of absorption of the food by the fish's body. The basic principle of the work of probiotics in aquaculture is the ability of microorganisms to break down long chains of proteing, carbohydrates and fats in feed (Feliatra and Suryadi, 2004). The addition of 10⁴ CFU.mL⁻¹ problotics to rearing media gave the growthincreased the length and weight of tiger shrimp larvae, compared to the hat were higher than controls (Widarnani et al., 2010).

Dissolved oxygen and ammonia in the rearing media. The results of the analysis of variance on day 0 showed that the factor of commercial nitrification bacteria, swamp microbes and the interaction between the factors did not significantly influence the dissolved oxygen content (Table 9). At 5 and 10 days after rearing start, the factor of commercial nitrification bacteria and swamp microbes significantly affected the dissolved oxygen content, but the interaction between the factors had no significant effect.

The results of dissolved oxygen in the rearing media at 5 and 10 days afterrearing <u>start showed thaten</u> the main effect of the addition of commercial nitrification bacteria was significantly lower showed that they were not given commercial nitrification bacteria were significantly higher than those treated without commercial nitrification bacteria.

The main influence of the addition of microbes from swamp chowed thaton the dissolved oxygen in the rearing media were given a combination of Chlorophyta, Bacillus op., and Streptomyces sp. (P4) was significantly different with a combination of Chlorophyta, Bacillus sp., and Streptomyces sp. (P4) than in other treatments.

Table 9, LSD test result of dissolved oxygen in the rearing media

8	Dissolv	red Oxyge	n (mg.L"	3					
8	Days a	fter rearing	pig	Contractory and the second	5.400			102-0	0.00
5.00	0	5	10	15	20	25	30	35	40
LSD	90 - E		G	0,18	0,14	0,14	0,18	0,18	0,18
N1P1	3,60 ±0,1	3,53 ±0,1	3,53 ±0,1	3,40° ±0,1	3,10 ^c ±0,1	3,00 ⁴ ±0,1	2,97 ±0,1	2,77 ⁴ ±0,1	2,67° ±0,1
N1P2	3,63 ±0,1	3,53 ±0,1	3,53 ±0,1	3,23** ±0,1	2,93** ±0,1	2,83m ±0,1	2,77# ±0,1	2,57 ^{sh} ±0,1	2,47** ±0,1
N1P3	3,67 ±0,1	3,57 ±0,2	3,47 ±0,2	3,33tc ±0,1	3,03 ^{bit} ±0,1	2,93× ±0,1	2,87% ±0,1	2,67 ±0.1	2,57% ±0,1
N1P4	3,57	3,70 ±0,1	3,60 ±0,1	3,60° ±0,1	3,50° ±0,1	3,40 ^e ±0,1	3,40 [#] ±0,1	3,20 ⁴ ±0,1	3,10" ±0,1
N2P1	3,63 ±0,1	3,43 ±0,1	3,43 ±0,1	3,13* ±0,1	2,83* ±0,1	2,73* ±0,1	2,67* ±0,1	2,47* ±0,1	2,37* ±0,1
N2P2	3,57 ±0,1	3,47 ±0,1	3,47 ±0,1	3,33 ⁵⁴ ±0,1	3,03° ±0,1	2,93 [%] ±0,1	2,87% ±0,1	2,67 th ±0,1	2,57% ±0,1
N2P3	3,53	3,43	3,33	3,27**	3,10	3,00	2,90 ^{bc}	2,70 ^{bc}	2,60*

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N2P4	±0,1 3,67 ±0,1	±0,1 3,63 ±0,1	±0,1 3,53 ±0,1	±0,1 3,40 ±0,1	±0,1 3,30 ⁴ ±0,1	±0,1 3,20 ⁴ ±0,1	±0,1 3,20 ±0,1	±0,1 3,004 ±0,1	±0,1 2,90° ±0,1
LSD	1.56166	0,08	0,08	0,09	0,07	0,07	0,09	0,09	0,09
N1	3,62	3,58*	3,53*	5,09 ^b	3,149	3,04*	3,00*	2,80*	2,70
N2	3,60	3,49*	3,44*	4,93*	3,07*	2,97*	2,91*	2,71*	2,61*
LSD		0,11	0,11	0,13	0,10	0,10	0,13	0,13	0,13
P1	3,62	3,48 ^a	3,48%	3,27*	2,97*	2,87*	2,82*	2,62*	2,52*
P2	3,60	3,50*	3,50**	3,28*	2,98*	2,88*	2,82*	2,62*	2,52*
P3	3,60	3,50*	3,40*	3,30*	3,07*	2,97*	2,88*	2,68*	2,58
P4	3,62	3,67%	3,57%	3,50*	3,404	3,30*	3,30*	3,10 ^b	3,009

On 10Ter days after rearing start, the P4 treatment had a significantly higher dissolved oxygen content than the P3 treatment, but it was not significantly different from the P1 and P2 treatments. The factors of commercial nitrification bacteria, microbial origin addition and their interaction between factors significantly influenced the dissolved oxygen content. The dissolved oxygen test on the 15th, 20th, 35th, 35th and 40th days are presented in Table 9.

-Ammonia analysis results on day 0 were not significantly influenced showed thatby the factor of commercial nitrification bacteria, addition and their interaction microbial origin of the swamp and the interaction between factors did not significantly influence. The variance analysis results on days 5, 10, 15, 20, 25, 30, 35 and 40 showed that the factor of commercial nitrification bacteria, microbial addition and their interactionmicrobial origin of swamp and the interaction between factors significantly affected ammonia content. They are presented in Table 10.

Table 10.	LSD	test	results for	r ammonia	every	5 days	on rearing	media

	Mean of	Ammonia C	concentratio	s (mg.L-1)	- 46° - 5	1			
	Days aft	er rearing	server really.						
	0	5	10	15	20	25	30	35	40
LSD		0,013	0,013	0,014	0,013	0,020	0,021	0,019	0,022
N1P1	0,290	0,383*	0,3934	0,410 ^d	0,327*	0,540	0,674	0,691'	0,948*
	±0,07	±0,01	+0,01	±0,01	±0,01	±0,01	±0,01	±0,01	+0,02
N1P2	0,323	0,2734	0,283	0,223	0,203	0,293*	0,314	0,321	0,324
	±0,03	±0,01	±0,01	±0,01	±0,01	±0,01	#0,01	±0,01	±0,01
N1P3	0,283	0,257	0,267	0,207%	0,197=	0,363	0,482*	0,585*	0,692
	±0,01	±0,01	± 0.01	±0,01	±0,01	±0,02	±0,02	±0,01	±0,02
N1P4	0,267	0,220*	0,230*	0,170*	0,147*	0,243*	0,260*	0,265*	0,270*
	±0,01	±0,01	±0,01	±0,01	±0,01	±0,02	±0,02	±0,02	±0,02
N2P1	0,230	0,253	0,263	0,2039	0,193*	0,2820	0,353	0,385	0,3925
	±0,01	±0,01	±0,01	#0,01	#0,01	±0,02	±0,02	#0,01	±0,02
N2P2	0,290	0,233**	0,243*	0,183	0,160*	0,250*	0,268	0,273*	0,277
	±0,04	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01	±0,01
N2P3	0,250	0,277	0,287	0,227	0,187	0,277	0,2967	0,300*	0,306
	±0,02	±0,01	±0,01	±0,01	#0,01	±0,01	±0,01	±0,01	±0,01
N2P4	0,303	0,237	0,2470	0,1879	0,163	0,250°	0,268*	0,273*	0,277*
	±0,03	±0,01	± 0.01	±0,01	±0,01	±0,01	±0,01	±0,01	$\pm 0,01$
LSD		0,006	0,006	0,007	0,006	0,010	0,011	0,010	0,011
N1	0,291	0,283 ⁹	0,293°	0,253	0,218°	0,360*	0,432	0,466*	0,558*
N2	0,268	0,250%	0,260*	0,200	0,176*	0,2659	0,299	0,306	0,313
LSD		0,009	0,009	0,010	0,009	0,014	0,015	0,013	0,016
P1	0,260	0,3181	0,326°	0,3074	0,260	0,411"	0,518°	0,538*	0,6704
P2	0,307	0,253°	0,263 ^b	0,203 ^b	0,182	0,2720	0,291*	0,297*	0,300 ^b
P3	0,267	0,267	0,277	0,217	0,192	0,320	0,389	0,443	0,499
P4	0,285	0,2289	0,238*	0,178*	0,155	0,247*	0,2649	0,269*	0,2749

The results of LSD test on the 5, 10, 15, 20, 25, 30, 35 and 40th days on the main effect of the addition of commercial nitrification bacteria showed that the ammonia content in the rearing media which given with commercial nitrification bacteria was significantly lower than in the treatments not given without commercial nitrification bacteria. The main

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Commonted [WU20]: Please make the necessary adjustments using the previous tables as models. effect of the addition of microbes from the swamp shows suggested that the ammonia content in the rearing media that was givenvilth a combination of Chlorophyta, *Bacilius* sp. and *Streptomyces* sp. was significantly different compared to the other treatments. The results of LSD on the 5, 10, 15 and 20th days, in the scenario of the interaction between factors, demonstrated that the NIP4 treatment had significantly lower ammonia levels compared to the other treatments, but not significantly different from N2P2 treatment. The results of LSD on the 25, 30, 35 and 40th days in the scenario of the interaction between factors, suggested that the NIP4 treatment had significantly lower ammonia levels compared to other treatments, but not significantly different from N2P2 and N2P4 treatments.

On Related to the factor of adding nitrifying bacteria, the lowest ammonia content was observed in the treatment was given with nitrifying bacteria. It is supported assumed that the added nitrification bacteria are able to carry out the process of nitrification on the rearing media. According to Rurangwa et al. (2014), nitrification takes place through 2 reaction stages, where in the first stage the oxidation of ammonium to nitrite is carried out by ammonium oxidizing microbes (*Nitrosomonas* sp.) and in the second stage nitrite oxidation is performed by nitrite oxidizing microbes (*Nitrosomens* sp.). In Among the addition of swamp origin microbal factors, the lowest ammonia content in was observed in the treatment was givenwith a combination of swamp origin microbes in the form of Chlorophyta, Bacillus sp. and Streptomyces sp. It is suspected that the three microbes from which the swamp was added could break down ammonia. Ammonia is the main source of nitrogen in addition to nitrate, which could be used by microlage for their netabolic processes (Nurhayati et al., 2014). Bacillus sp. could oxidize ammonia to nitrite through heterotrophic and chemotrophic processes (Edwards, 2011).

Ammonia levels in all treatments <u>ave-with</u> microbes on 10th day to 20th day of rearing, decreased and increased until the 40th day. On the 10th to 20th day, it was suspected <u>presumed</u> that the ammonia accumulation from metabolic waste had not yet occurred <u>so-that therefore the</u> microbes given to the rearing media were able to work optimally. On the 25th to 40th day, it was <u>supposed</u> that the ammonia accumulation from metabolic waste had been accumulated in the rearing media, so that the <u>added</u> microbes given couldn't optimally reduce their ammonia levels. Meanwhile, the treatment without the addition of microbes (N1P1) experienced an increase in ammonia along with the increase in the rearing time. Increasing ammonia levels in the N1P1 treatment was caused of the rearing without siphoning during 40 days... It resulted in a buildup of organic material derived from metabolic waste and levels of ammonia in the rearing media. The decomposing bacteria from natural swamp water <u>en-added to</u> the media have not been able to make an optimal decomposition.

Conclusion. Addition of swamp microbes in the form of Chiorophyta, Bacillus sp. and Streptomyces sp., without the addition of commercial nitrification bacteria on the snakehead rearing media, *H* provides better water quality values. The best results are obtained in the treatment with the addition of swamp microbes Chiorophyta, Bacillus sp.

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and Streptomyces sp. without the addition of commercial nitrification bacteria in the rearing media that gave in the survival rate, feed efficiency, and growth of snake head fish in swamp aquaculture.

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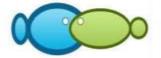
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Optimization of <u>striped</u> snakehead fish (Channa striata) culture using swamp microbial combination and nitrification bacteria

Marini Wijayanti, Dade Jubaedah, Ocktin Yulistya, Tanbiyaskur, Ade Dwi-D. Sasanti

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Abstract. Utilization of swamp areas as a-fish culture locations will couse a quality. Therefore, it is necessary to improve the water quality with environmental friendly ef-biological treatments, one of such as the addition is of microbes as probiotics in media culture. The purpose of this study was to determine the combination of microbes from swamps that can improve the water quality of the swamp fish media culture and production media and produ of swamp fish e. The research used Completely Randomized Design (CRD) factorial with two factors consisting of the first factor with two treatments and the second factor with fouries treatments and three replications. The first factor consists in a two scenarios: (1) without the addition of nitrification bacteria (N1) and (2) with the addition of nitrification bacteria (PROBAC) 5×10⁶ CFU_mL⁻¹ (N2). The second factor 4-00 sists in four scenarios: (1) without the addition of swamp microbes (P1) and, with the addition of: (2) Chlorophyta (3.43×10⁷ sel_L⁻¹) and Bacillus sp. (10⁵ CFU_mL⁻¹) (P2): (3) - oddition of Chlorophyta (3.43×10⁷ sel_L⁻¹) (1.1.5) Server and Streptomyces sp. (10⁵ CFU_mL⁻¹) (P3)₂ (1) deficient of Chlorophyta (3.43×10⁵ set L⁻¹), Bacillos sp. (10⁵ CFU_mL⁻¹) and Streptomyces sp. (10⁵ CFU_mL⁻¹) (P4). The result showed that the addition microbes from swamps with in the combination of N1 and P4 treatment scenarios is able to improve the water quality value better than the other treatment scenarios without the addition of microbes (N1P1) of N104, producinges the best survival rate-of (-63.94%), feed efficiency of (59.65%), absolute weight growth of (2.32 g) and absolute length growth of (2.27 cm).

Key Words: problotic, swamp-members, snakehead fish, nitrification bacteria_biofloc.

Introduction. The swamp aquaculture must be improvaing and maintaining the water quality for fish rearing media, through environmentally friendly biological treatments, sincethe wastewater of fish rearing on swamps will-reduces the quality of swamps water....from swamps. So it is necessary to improve water quality with biological treatment environmentally friendly...One of the treatments is adding probiotics in the rearing media. Irianto and & Austin (2002), states that environmental damaging degradation can be prevented with probiotics able , which aims to degrade the organic materials in the habitat. Hartini et al- (2013) showed that the addition of probiotics at a dose of 10 µL⁻¹.week⁻¹ can improve and maintain optimal water quality. In other studies, the addition of the effective probletic microorganisms from thethat exist in culture media (Trisna et al-, 2013).

Swamps have high biodiversity, including sediment microbes. Many swamp microbes are _able to improve the physical and chemical properties of swampstheir media. The identified Symamp microbes that have been found include Chlorophyta, Bacillus sp. and Streptomyces sp. (Wijayanti et al-7 2018). Bacteria-from the swamp (Bacillus sp.) can be used as environmental probiotics with concentrations of 10⁵ CFU.ml⁻¹ (Khotimah₇ 2018) and microalgae_Chlorophyta microalgae_with the optimum concentration 10% of the maximum density.(Utami 2019) are able to grow in the fish culture media and they can be used as environmental probiotics(Utami, 2019).

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Chlorophyta is a microorganism that can be used as Green Water in aquaculture media. Wijayanti et -al- (2018) showed that the use of Chlorophyta increased the level of dissolved oxygen in the culture-pond culture media 60.52% and swamp water culture media 63.63% .- The increasing dissolved oxygen is caused by Chlorophyta carrying out photosynthetic activity which produces dissolved oxygen in the culture media. Bacillus sp. and Streptomyces sp. obtained are proteolytic bacteria that can increase the content of NH3, NO2, and NO3 into the media (Yuliani, 2017; Saraswati, 2018). Balcazar et al-(2006) states that Bacillus sp. is an example of an efficient probiotic bacteria, used in aquaculture because it is able to convert organic matter into CO2 used in cell metabolism. Gram-positive bacteria, such as Bacillus sp. can increase the animalis immune system capabilities and also act favorably in improving the quality of the water system (Mohapatra et al-, 2013). Bernal et al- (2017) states that the combination of Streptomyces sp. and Bacillus sp. demonstrated a significant immunomodulatory activity by increasing the total number of hemocytes and the activity of superoxide dismutase (SOD), which provides a protective effect against Vibrio harveyi bacteria by increasing the immunological status of Penaeus monodon. Nitrifying bacteria are chemolitho: autotrophic bacteria (ex: Nitrosomonas sp., Nitrobacter sp.), which are able to meet their carbon needs through CO2 fixation (Calvin cycle), and their energy source comes from the oxidation process of reducing ammonia to nitrate. With As an example, through the addition of nitrifying bacteria and, denitrification, and the molasses with a -C/N ratio of 10 could decrease in ammonia levels by 28.5% (Yuniasari, 2009).

Each of the swamp microbes and nitrifying bacteria has their own advantages and disadvantages, so we needtherefore a consortium of swamp microbes and nitrifying bacteria would be more effective. The consortium is <u>The emergence of a consortium</u>, expected to form cooperative, commensal and mutualistic relationships between microbes... The emergence of a swamp microbial consortium and nitrification bacteria resulted in the needcalls for an optimization optimal of a combination of Bacillus sp., Streptomyces sp., Chlorophyta and commercial nitrification bacteria_in an effort to improve water quality in media of swamp microbes and nitrifying bacteria that can improve the water quality of media in swamp fish production. This study is expected to get a combination of swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve media water quality and swamp microbes that can improve the water quality and swamp microbes that can improve media water quality and swamp microbes that can improve the water quality and swamp microbes that can improve the water quality and swamp microbes that can improve the water quality and swamp microbes and m

Material and Method

The experimental design used was a completely randomized factorial design (RAL) consisting of 2 factors—<u>: The the</u> first factor with 2 treatments and the second factor with 4 treatments and 3 replications. The first factor is the addition of nitrifying bacteria (PROBAC), namely:

N1: Without the addition of nitrifying bacteria (PROBAC)

N2: Addition of nitrifying bacteria (PROBAC) 5×10⁶ CFU_mL⁻¹

The second factor is the addition of swamp microbes, namely:

P1: Without the addition of swamp microbes

P2: Provision of 100 ml Chlorophyta (3.43 × 10⁷ Cellcell -L⁻¹) and Bacillus sp. (10⁵ CFU_-mL⁻¹)

P3: Provision of 100 ml Chlorophyta (3.43 × 10⁷ Cellcell -L⁻¹) and Streptomyces sp. (10⁵ CFU_-mL⁻¹)

P4: Provision of 100 ml Chlorophyta (3.43 \times 10⁷ Cellcell -L-1), Bacillus sp. (10⁵ CFU_-mL-1) and Streptomyces sp. (10⁵ CFU_-mL-1)

Bacteria cultivation and propagation. Pure bacterial colonies were obtained from the results of previous studies. The colony was cultivated using <u>nutrient agar NA-(NA nutrient agar</u>) media for *Bacillus* sp and <u>yeast malt YM (YM yeast malt agar</u>) agar for *Streptomyces* sp. Bacterial colonies were scratched in a petri dish containing NA (Nutrient Agar) for *Bacillus* sp_ and YM (Yeast Malt Agar) media for *Streptomyces* sp. using the quadrant streak method. Petri dishes were wrapped in wrapping paper and incubated for

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Bacteria population. The Counting counting of bacterial populations was performed at Formatted: Font: Italic the beginning and end of rearing with the plate count method was to multilevel dilution were then-incubated at a temperature of 28-30°C for 24 hours. The growing population was determined in a colony forming unit (CFU) and calculated using the following formula: Total of Bacteria = Total of colonies $\times \frac{1}{dilution factor} \times \frac{1}{mL sample}$ Biofloc volume_Biofloc volume measurements were done on the 10 and 40 days after Formatted: Font: Italic rearing. The fFloc volume was obtained by taking collecting a rearing media, by using a Formatted: Font: Not Bold glass cone 1L volume, then the floc in the water media was left to settle in the tube for 15-20 minutes. Survival rate. The percentage of fish survival was calculated by using the following Formatted: Font: Italic formula: Survival rate -Nt ×100% Formatted: Centered Where: No = Number of fish at the beginning of rearing (individuals) N = Number of fish at the end of rearing (individuals) Absolute weight growth, Growth of fish weight during rearing wass calculated by using Formatted: Font: Italic the following formula: Formatted: Font: Not Bold $W = Wt - W_0$ Formatted: Centered Where: W = Growth of weight of fish for rearing (grams) Wt = Weight of fish at the end of rearing (grams) W₀ = Weight of fish at the beginning of rearing (grams) Absolute length growth. The absolute length growth of fish during rearing was Formatted: Fort: Italic determined by doing the following calculation: Formatted: Font: Not Bold l = lt - loFormatted: Centered Where: L = Growth of absolute length of fish for rearing (cm) Lt = Length of fish at the end of rearing (cm) L_0 = Length of fish at the beginning of rearing (cm) Formatted: Font: Italic Feed efficiency_According to NRC (1977) feed efficiency can be calculated by using the Formatted: Font: Italic formula: EP = ((Wt + D) - Wo) Formatted: Font: Not Bold × 100% Formatted: Font color: Black NoteWhere: -EP = Feed Efficiency efficiency (%) Formatted: Space After: 0 pt, Line spacing: single Wt = Weight of fish at the end of rearing (gram) Commented [WU7]: Please correct the formatting. Wo = initial fish rearing weight (gram) D = Weight of fish that died during rearing (gram) It's done, thank you F = Amount of feed given (grams) Formatted: Font color: Black, English (Indonesia) Water quality_Measurement of water quality data for snakehead fish rearing media Formatted: Font: Italic includes pH (pH meter), dissolved oxygen (DO meter), ammonia (spectrophotometry), Formatted: Font: Not Bold and biological oxygen demand (DO meter) at the beginning and 40 days later, at the end of the rearing, for 40 days. Data analysis, Research data including biofloc volume, survival, growth, feed efficiency, Formatted: Font: Italic water quality were was statistically analyzed processed by using the variance analysis of Formatted: Font: Not Bold variance. If the results of the variance analysis of the variance show that the treatment has a significant effect, then it is continued with the LSD test (the Least significance AACL Bioflux, 2020, Volume 13, Issue X. 4 http://www.bioflux.com.ro/aad

difference) 5%. Chlorophyta abundance data and the total bacterial population were analyzed descriptively.

Result and Discussion

Chlorophyta abundance, total bacterial population and floc volume. Chlorophyta abundance data on rearing media are presented in Table 1. Chlorophyta density at each treatment decreased after 40 days of rearing. The addition of Chlorophyta added in the rearing media experiences death or predation. In rearing media, a food chain system occurs between Chlorophyta and zooplankton (Figure 1), resulting in a decrease in the population of Chlorophyta due to predation.



Figure 1. Biofloc and Chlorophyta profile in the rearing media of snakehead culture in this study (40 magnification scale of microscope).

The pattern of <u>the interaction-relationships</u> between zooplankton and phytoplankton is a series of eating and prey relationships—<u>forming That relationship forms</u> the path of the food chain. Phytoplankton as primary producers are eaten by zooplanktons; in turn zooplanktons are eaten by small fish at higher trophic levels (Bourna et al., 2003).

Table 1-Table 1-Chlorophyta abundance in snakehead rearing media at 0, 10, 40th day

Commercial		Chlorophyta Abundance (Cell.L ⁻¹)				
nitrification bacteria	Swamp microbes	0 day	10 th day	40 th day		
	P1	$3-20 \times 10^{3}$	$3_{17}20 \times 10^{3}$	210×10^{3}		
	P2	360×10^3	$3-43 \times 10^{7}$	410×10^{3}		
N1	P3	4.10×10^{3}	$3-43 \times 10^{7}$	4.10×10^{3}		
	P4	$3_{-}.70 \times 10^{3}$	$3-43 \times 10^{7}$	4.46×10^{3}		
	P1	$4-00 \times 10^{3}$	$4 - 0 \times 10^3$	$2_{7.10} \times 10^{3}$		
N2	P2	$3-60 \times 10^{3}$	$3,43 \times 10^{7}$	$2-41 \times 10^{3}$		
	P3	$3-90 \times 10^{3}$	$3-43 \times 10^{7}$	$2-34 \times 10^{3}$		
	P4	3-40 x 103	3-43 x 107	4-03 x 103		

The total bacterial population on-in the rearing media is presented in Table 2, the results of the LSD test of the floc volume at ten and forty days after of rearing are showed in Table 3 and Table 4, respectively.

Commercial	Swamp	1	otal bacterial p	opulation (CFU.r	nL-1)
nitrification bacteria	microbes	0 day	1 st day	20th day	40 th day
	P1	6. , 60 × 10 ⁴	6.78 × 104	1_55 × 105	620 × 103
N1	P2	6.720 × 104	395 × 106	693 × 106	2.77 × 105
INI	P3	700 × 10 ⁴	328 × 106	753 × 106	301×10^{5}
	P4	4.70 × 104	559 × 107	100×10^{8}	299×10^{6}
117	P1	710×10^{4}	201×10^{7}	$3,54 \times 10^{7}$	1.42×10^{6}
N2	P2	450×10^{4}	329×10^{7}	5.59×10^{7}	168×10^{6}

P2 4_50 × 10⁴ 3_29 × 10⁷ 5,59 × 10

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Table 2+

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P3	$4_{-30} \times 10^{4}$	$4_{-99} \times 10^{7}$	$6,41 \times 10^{7}$	193×10^{6}
P4	4_795 × 104	4_70 × 107	8,75 × 107	$4,06 \times 10^{6}$

Based on Table 2, the total bacterial population increased on 20th day and decreased until the 40th days. The increase in population on the 20th day can be caused by adequate nutrition -nutrients addition in the rearing media, so that bacteria and Actinomycetes can use these nutrients for stimulating the metabolic activity and growth of the bacteria and Actinomycetes, www.hileereas, the decline of bacteria population observed on the n-40th day could be caused by reducing the nutrient depletion (macronutrient and micronutrient) in the water. The bacteria couldn't enough to take their nutrition because of nutrition depletion.

Related to the factor of nitrification bacteria. t#he results of LSD at 10 and 40+ days after rearing start showed that in the factor of addition of commercial nitrification bacteria, the volume of floc on in the media without commercial nitrification bacteriatreatment was significantly higher compared to the treatment given with nitrification bacteria. The addition of nitrifying bacteria can<u>not</u> increase the volume of floc, because there were less exhausted bacteria in the media. The bacteria need more substrate from unconsumed feed of fishes, while the nitrifying bacteria accelerated the process of dissolving nitrogenous organic matter from the waste. one of the constituent components of floc is a bacterium, Related to the In the factor of microbial addition from swamps, the volume of floc on the media treated with which was given a combination of *Chlorophyta, Bacillus* sp. and *Streptomyces* sp. significantly showed higher <u>levels</u> compared to other treatments.

Table 3					
I dDie 3	-	-	1	-	-
	- 1	d	D	е	- 3

Table 3. The results of LSD test of the floc volume in theor rearing media at 10 days after-

The single effect of	The single	influence (F	2.1.2.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1	microbes	The main effects of nitrifying bacteria (N)
nitrifying bacteria (N)	P1	P2	P3	P4	(LSD0.05=4-386)
N1	11111	16, 666	13-332	26668	16944 ^b
N2	10000	10000	13332	16667	12_500ª
The main effects of swamp microbes (P) (LSD0_05=3_7102)	10 ₂₇ 556ª	13 ₂₇ 333ª	13 <u>.</u> ,332°	21 ₂₇ 667 ⁶	

Table A.			
		1.1	

- The results of LSD test floc volume of rearing media at 40 days after of rearing

The single effect of nitrifying bacteria (N)	The singl	e influence (P) (LSDog	of swamps 05=6 <u>-</u> 631)	microbes	The main effects of nitrifying bacteria (N)
muniying bacteria (iv)	P1	P2	P3	P4	(LSD0_05=3-315)
N1	11112ª	26_667b	16667ª	38, 889	25834 ^b
N2	13333ª	13333ª	23,-3330	33333c	20833ª
The main effects of swamp microbes (P) (LSD _{0.05} =4689)	12 ₅₇ 223ª	20 <u>.</u> 7000 ^b	20 <mark>_7</mark> 000 ⁶	41 <u>.</u> 111 ^c	1

It is suspected presumed that certain the types of microorganisms are easier predisposed for forming-flocs forming. On Related to the influence of interactions between factors at 40 days after of rearing, the observations showed that the treatment combination of *Chlorophyta*, *Bacillus* sp., *Streptomyces* sp. and without nitrifying bacteria are determines the highest of floc. volume of floc. 38.89 mL₋L⁻¹, but it is not significantly different from the treatment combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and with nitrifying bacteria. The volume of floc in this study is lower than the study from

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Mulyadi et al- (2016), where in treatment with stocking density of 450 snakehead fish m⁻³ which was kept for 41 days resulted in a floc volume of 40.7_mL_rL⁻¹. This_It is presumed that the rearing media lacks a carbon source <u>used by which</u> bacteria <u>use for the</u> floc formation. According to Panigrahi et al- (2019), <u>Wanname</u>, shrimp cultivation without a biofloc system can produce a volume of floc of 4.53 mLr L⁻¹. <u>which that</u> is lower than the cultivation of <u>Wanname</u> shrimp with a biofloc system, by adding molasses. Floc thickness will continue to increase along with the provision of bacteria and carbon sources that are continuously balanced with feed metabolic waste and it producinge ammonia. The bacteria could bind to ammonia and will_form a biofloc (Sitohang et al-7 2018).

The results of the analysis of variance showed that the <u>effect of the</u> interaction between factors <u>and</u> <u>and</u> the <u>effect of the</u> factors <u>of defined by the</u> addition of swamp origin<u>ated</u> microbes <u>to on</u> the survival of snakehead fish <u>varied</u> significantly effected between treatments, but the factor of defined by the adding <u>of</u> commercial nitrification bacteria had no significant effect. Based on further testing of LSD on microbial factors from swamps, <u>in</u> the rearing media given treated with a combination of Chlorophyta, *Bacillus* sp. and Streptomyces sp., <u>the survival rate</u> wasere significantly higher than <u>for</u> the other treatments, <u>and if</u> Interactions between factors showed that <u>in</u> the rearing media <u>given with</u> a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, <u>the survival rate reached 63.94%</u>, <u>a</u> significantly <u>higher different performance</u> compared to <u>the</u> other treatments with a <u>percentage of 63.94%</u>.

Based on the results of <u>the</u> survival percentage, it <u>is</u> showed that the combination of swamp microbes is able to suppress unfavorable microbes, <u>and decreasing to improve</u> the water quality in the rearing media <u>and</u> to increase so that the snakehead fish <u>survival rate can survive well</u>. This is shown in the treatment of rearing media given consisting in a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, <u>which that</u> provided a snakehead fish survival rate of 63.94%. The combination of *Bacillus* sp. and *Streptomyces* sp. was able to provide more protection against unfavorable microbes in the media, <u>where</u> the presence of *Bacillus* sp. givinges-effect to *Streptomyces* sp. to produce antimicrobial compounds. Luti and <u>&</u> Mavituna (2011) explained that *Bacillus was*-cultured together with *Streptomyces* can increased the production of antimicrobial compounds when it was compared with the single genus culture.

Table 5.4 LSD test of Survival Rate of Snakehead fish The Main The Single Effect of The Single Influence of Swamps Effects of Nitrifying Bacteria Microbes (P) (LSD0_05=6_02) Nitrifying Bacteria (N) (N) P1 P2 P3 P4 26_-06* 36_-91^b 28_-03ª 63_94^d 38_74 N1 31.75* 28.-03ª 35.-16^b 48.-20^c 35.-79 N2 The Main Effects of Swamp Microbes (P) 28-91* 32-47* 31-59* 56-07* (LSD0_05=4-25)

The N1P1 treatment (without the addition of microbes from swamps, neither of and commercial nitrification bacteria) was the treatment with the lowest survival value of 26.06% compared to other treatments. These results prove that the addition of swamp origin microbes to rearing media can increase the survival rate of snakehead fish. This is in line with the results of Hartini et al. (2013), suggesting that the addition of EM-4 probiotics to rearing media had a significant influence on the survival of snakehead fish.

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The average survival of snakehead fish with <u>10 ull11 week1 EM-4 probiotics (28.88-96.66%)</u> tended to be higher, compared to control treatments (without EM-4 probiotics) which was 8.89%. Budianto and <u>8 HenySuprastvani</u> (2017) <u>state</u> that the bacteria *Bacillus* sp. has bacteriocin compounds with specific characters so that it can inhibiting action on the growth of *S. iniae* and *P. fluorescens. Streptomyces* bacteria has the potential to control pathogenic bacteria by means of competition, parasitism or <u>by</u> producing secondary metabolite compounds (Lutfi₇ 2018). The combination of the two microbes can provide a high percentage of survival compared to without a the no combination <u>scenario</u>. According to Irianto and <u>8</u> Austin (2002), probiotic bacteria can increase survival or reduce mortality through the development of the immune system, <u>such as due to an</u> increasing phagocyte and lysozyme activity, thereby suppressing pathogenic bacterial colonies. Sanchez et al- (2014) states that probiotics can increase death in fish culture.

Feed efficiency, absolute weight and length growth. The results of the analysis of the <u>efficiency of variousety of feed on the</u> snakehead fish feed efficiency showed that the interaction between factors, <u>the</u> microbial addition factors from swamps and the addition of commercial nitrification bacteria, <u>can to increase</u> the value of fish feed efficiency, <u>which is</u> significantly affected between by the treatment type and concentrations. LSD test results of the efficiency of fish feed for 40 days of rearing are presented in Table 6.

ISD test result of the efficiency of snakehead fish feed for 40 days of rearing

The Single Effect of Nitrifying Bacteria (N)	The Sing (P) (LSD	The M Effects Nitrifying Bacteria (N)				
	P1	P2	P3	P4	(LSD0_05=	1_66)
N1	1893ª	47_34 [#]	37_ - 97ª	59 <u>-</u> 65ª	4097*	
N2	2200ª	29_52 ^b	3489°	4411 ^d	32,,63ª	
The Main Effects of Swamp Microbes (P) (LSD _{0.455} = 2 -35)	20 <u>.</u> ,47ª	38 <u>.</u> 43 ^b	36 <u>.</u> 43 ^b	51 <u>.</u> -88°		

Based on these results, the effects of the different combinations on the snakehead fish. feed efficiency could be observed. of the LSD test on the main effect of the addition of commercial nitrification bacteria. The value of the snakehead fish feed efficiency in the treatment of the media of rearing was: (1) significantly higher without commercial nitrification bacteria was significantly higher than for the treatment given with nitrification bacteria .--; (2) significantly higher On the influence of the addition of microbes from swamps, the value of snakehead fish feed efficiency in the treatment of rearing media that were given with with a combination of Chlorophyta, Bacillus sp. and Streptomyces sp., significantly different compared to other treatments and interactions between factors ...; (3) were significantly higher The value of snakehead fish feed efficiency in the treatment of rearing media that were given with a combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without commercial nitrification bacteria were significantly higher than the results of other treatments. It is thought that the ori swamp microbes added to the media enter the digestive tract of snakehead fish during the process of respiration and when while eating. Chlorophyta that enters the digestive tract could be a natural food source, while Bacillus sp. and Streptomyces sp. which entered the digestive tract could be working together to improve their digestibility. High digestibility in the feed will increase the absorption of nutrient, which at optimal levelsand for the fich and the abe fish will grow well and will increase the value of feed efficiency, favorising fish growth. Bacillus sp. is a bacterium that can increase digestibility because it can secrete protease, lipase and amylase enzymes (Fardiaz, 1992).

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These bacteria can also produce antimicrobial compounds (bacteriocin and antibiotics) that can suppress pathogenic bacterial colonies (Findy, 2009). Streptomyces sp. is a genus of Actinomycetes actinomycetes that can produce various antibiotic compounds. Streptomyces has the potential to control pathogenic bacteria by conducting competition, parasitism or by producing secondary metabolites (Lutfi, 2018). increasing the value of feed efficiency, Tthe bacterium Bacillus sp. increases the value of feed efficiency by secreting enzymes that can increase stimulate digestion, while Streptomyces sp. secretes antibiotics to be able to suppress pathogens, so that the twoBoth bacteria work together to improve the digestibility and immunity of snakehead fish, which ultimately results in high feed efficiency. Bacterial activity in the digestive tract will experience changes rapid fluctuationsly when with there are microbes that entering through feed or water and that cause causing changes in the intestinal microbial balance of intestinal origin with incoming microbes. The entry of these microbes is antagonistic to the pathogenic microbes of in digestion so thatin the digestive tract of fish will be better at, digesting and absorbing feed nutrients and the use of feed will be more efficient Improving growth, protein efficiency ratio and feed efficiency (Mulyadi et al. 2011Midhun et al, 2018; Nargesi et al, 2019).

Swamp microbial addition factor, commercial nitrification bacteria addition factor, and their interactions between factors influencedon the feed efficiency showed in a significantly different manner, depending on thebetween treatments. Based on the results of the LSD_{0.05} test (Table 7) on the main effect of the addition of commercial nitrification bacteria, the growth-increase of the absolute weight of the snakehead fish in following the treatment of the rearing media was: (1) without commercial nitrit bacteria was significantly higher without commercial nitrification bacteria than the treatment given with commercial nitrification bacteria; (2)- more triggers growth On the main influence of with the addition of microbes from swamps, the growth of the absolute weight of snakehead fish in the treatment of rearing media that were given a combination of Chlorophyta, Bacillus sp. and Streptomyces sp. significantly different than with the other treatments; (3) the best combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without commercial nitrification bacteria than the other treatments' results In the interaction between factors, the growth of the absolute weig snakehead fish in the treatment of rearing media that were given with a combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without co significantly higher than other treatment results, but (4) not significantly different from the treatment results given a combination of Chlorophyta, Bacillus sp. and without commercial nitrifying bacteria.

The Single Effect of Nitrifying Bacteria (N)		ngle Influer Do _{utos} =0 <u>ut</u> 08	The Main Effects of Nitrifying Bacteria (N)		
turn hing costonia (11)	P1	P2	P3	P4	(LSD _{0_05} =004)
N1	1_30*	2_26	1,704	2_32	1_90*
N2	1.730	1_41 ^b	1_ , 88 ^d	208*	1_78ª
The Main Effects of Swamp Microbes (P) (LSD _{0.405} =0.,05)	1 ₂₇ 51 ²	184*	1 ₂₇ 79°	2_205	

LSD 0.05 test results of growth in absolute weight of Snakehead fish

Based on the results of <u>the variance</u> analysis<u>of variance</u>, <u>swamps</u> microbial addition factor <u>from swamps</u> and interactions between factors significantly influence<u>d</u> the <u>growth</u> <u>increase</u> of <u>the</u> absolute length, but the factor of adding commercial nitrification bacteria has no significant<u>effectbetween treatments</u>. The LSD results of growth in absolute length of snakehead fish are presented in Table 8.

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LSD test results for growth in the absolute length of snakehead fish

The Single Effect of Nitrifying Bacteria (N)		gle Influen 1 _{0_05} =010)	The Main Effects of Nitrifying Bacteria (N)		
intering second (ii)	P1	P2	P3	P4	
N1	069*	1 _{.7} 79*	0 <u>.</u> -91 ^b	2 <u>.7</u> 27 ^t	2_12
N2	1.7134	1.708°	1_760 ^d	1. 74 ^e	2,,09
The Main Effects of Swamp Microbes (P) (LSD ₀₋₀₅ =0_07)	0 _{.7} 91*	1,,44*	1_726 ^b	2_7004	

The addition of a combination of swamp microbes and commercial nitrification bacteria gave significant results on the growth-increase of absolute weight and absolute length growth. The highest absolute weight and length growth increase in thewas produced by the treatment of rearing media were given awith a combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without commercial nitrification bacteria. and lowest in-by the treatment without the addition of microbes. This is thought to be the treatment without the addition of microbes is thought to be the less effective in the terms performance of absorption of nutrients in the feed, which causes causing a less suboptimal growth, compared to the other treatments. In the treatment of N1P4, swamp microbes added to the media could be entered into the digestive tract of snakehead fish during the respiration process and when eating. These microbes break down complex compounds into simpler ones and increase digestibility of feed-and, by accelerate accelerating the process of absorption of the food by the fish's body. The basic principle of the work of probiotics in aquaculture is the ability of microbesorganisms to break down long chains of of proteins, carbohydrates polysacarides, and fatslipids and stress resistance in feedaquaculture system (de melo Pereira et al., 2018)(Feliatra and Survac 2004). The addition of 10⁴ CFU.mL⁻¹ probiotics to rearing media gave the growthincreased the length and weight of tiger shrimp larvae, compared to the hat were higher than controls (Widarnani et al., 2010). B. licheniformis at 10⁵ cfu/mL⁻¹ in the rearing media of P. hypophthalmus showed significant increase in the growth, immune and antioxidant responses compared to 107 cfu/mL⁻¹ (Gobi et al, 2016).

Dissolved oxygen and ammonia in the rearing media. The results of the analysis of variance on day 0 showed that the factor of commercial nitrification bacteria, swamp microbes and the interaction between the factors did not significantly influence the dissolved oxygen content (Table 9). At 5 and 10 days after rearing <u>start</u>, the factor of commercial nitrification bacteria and swamp microbes significantly affected the dissolved oxygen content, but the interaction between the factors had no significant effect.

The results of dissolved oxygen in the rearing media at 5 and 10 days afterrearing <u>start showed thaten</u> the main effect of the addition of commercial nitrification bacteria <u>was significantly lower</u> showed that they were not given commercial nitrification bacteria were cignificantly higher than those treated without commercial nitrification bacteria.

The main influence of the addition of microbes from swamp showed thaton the dissolved oxygen in the rearing media were given a combination of Chlorophyta, Bacillus sp., and Streptomyces sp. (P4) was the best for dissolved oxygen concentration in

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culture mediasignificantly different with a combination of Chlorophyta, Bacillus sp., and Streptomyces sp. (P4) than between other treatments.

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LSD test result of dissolved oxygen in the rearing media

	Dissolv	ed Oxyge	n (mg.L-1)						
	Days a	fter rearin	g						
	0	5	10	15	20	25	30	35	40
LSD				018	0_14	014	018	018	018
N1P1	360	353	3_53	3_40°	3_10 ^c	300*	2_97°	2_77°	2_67
WIFT .	±01	±01	±01	±01	±0_71	±0_71	±01	±01	±01
N1P2	363	3,-53	3_53	323ab	293ab	283 ^{ab}	277=b	257ab	2_47ab
N1P2	±01	±01	±01	±01	±0_71	±01	±0_71	±01	±01
N1P3	3_67	3_57	3_47	333bc	303bc	293 ^{bc}	287tc	267bc	2 -57bc
N1P3	±01	±02	±02	±01	±01	±01	±0_71	±01	±0_71
NIDA	357	3.70	360	3_60 ^d	3,-50*	3.40*	3_40=	3_720e	310 ^e
N1P4	±01	±01	±0,-1	±01	±0.71	±01	±0.71	±01	±01
11201	363	3,43	3_43	313ª	283ª	273*	2_67*	247ª	237*
N2P1	±01	±01	±01	±01	±0,-1	±0,-1	±01	±01	$\pm 0_{-1}$
N2P2	357	3.47	3.47	3 -33bc	3,-034	2-93tx	287tc	267bc	2 -57to
NZPZ	±01	±01	±0_1	±01	±0,-1	±0,-1	±0,-1	±01	±01
N2P3	353	343	3 - 33	3 -27ab	3104	300*	290bc	270bc	260*
NZP3	±01	±01	±01	±01	±0,-1	±01	±0,-1	±01	±01
N2P4	367	363	353	340°	330 ^d	320 ^d	320 ^d	300 ^d	2 -90 ^d
112294	±01	±01	±0_71	±01	±01	±01	±01	±01	±01
LSD		0,-08	008	009	0_07	007	009	0,-09	0_09
N1	362	358b	353 ^b	509 ^b	3-140	304°	300 ^b	280 ^b	2 -70 ^b
N2	360	3.49*	3-44*	4-93*	307ª	297ª	291=	2_71ª	2 -61ª
LSD		011	011	013	010	010	013	013	0_13
P1	3,-62	348ª	348 ^{ab}	327*	297ª	287ª	282°	262ª	252*
P2	360	350ª	3 -50 ^{ab}	3 -28ª	298*	288ª	282*	262*	2 -52*
P3	3 -60	350ª	340ª	330ª	307*	2 97ª	288ª	2_68°	2 -58°
P4	3 -62	367 ^b	3 -57 ^b	3 -50 ^b	340°	330 ^b	330 ^b	3-10 ^b	300 ^b

On 10Ten days after rearing start, the P4 treatment had a significantly higher dissolved oxygen content than the P3 treatment, but it was not significantly different from the P1 and P2 treatments. The factors of commercial nitrification bacteria, microbial origin addition and their interaction between factors significantly influenced the dissolved oxygen content. The dissolved oxygen test on the 15th, 20th, 25th, 30th, 35th and 40th days are presented in Table 9.

Ammonia analysis results on day 0 were not significantly influenced showed thatby the factor of commercial nitrification bacteria, addition and their interaction microbial origin of the swamp and the interaction between factors did not significantly influence. The variance analysis results on days 5, 10, 15, 20, 25, 30, 35 and 40 showed that the factor of commercial nitrification bacteria, <u>microbial</u> addition and their interactionmicrobial origin of swamp and the interaction between factors significantly affected ammonia content. They are presented in Table 10.

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	Mean of	Ammonia C	concentratin	(mg.L ⁻¹)					
	Days after	er rearing							
	0	5	10	15	20	25	30	35	40
LSD		0-013	0-013	0.014	0_013	0_020	0-021	0-019	0-022
N1P1	0-290 ±0-07	0-383 ^e ±0_01	0-393 ^d ±0-01	0,410 ⁴ ±0,01	0-327* ±0_01	0_540 ⁴ ±0_01	0_674° ±0_01	0_691 ^r ±0_01	0 <u>948</u> ±0_02
N1P2	0-323 ±0-03	0-273 ^d ±0-01	0-283 ^c ±0-01	0-223 ^e ±0-01	0-203 ^c ±0-01	0_293 ^b ±0_01	0-314 ^b ±0-01	0-321° ±0-01	0-324 ±0-01

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Table 10.+

N1P3	0-283	0-257	0-267	0-207	0-197 ^{tr}	0363	0.482	0-585	0.6924
	±0,-01	±001	±001	±0-01	±001	±0,-02	±0-02	±0,-01	±0-02
N1P4	0-267	0-220*	0-230°	0-170 ^a	0-147	0.243*	0-260°	0-265*	0-270
	±001	±0-01	±001	±0-01	±0-01	±002	±0-02	±002	±0-02
N2P1	0-230	0-253	0-263 ^b	0-203°	0-193 ^{tr}	0.2820	0363	0-3854	0-392
	±0-01	±001	±001	±0-01	±0.01	±0-02	±0-02	±001	±0-02
N2P2	0-290	0,-233*	0-243ª	0.183*	0-160*	0.250*	0_268ª	0-273*	0.277
	±004	±001	±0-01	±001	±001	±001	±0-01	±0-01	±0-01
N2P3	0-250	0,-277 ^d	0-287	0-227	0-187	0-277°	0-296*	0-300 ^p	0,-306 ^b
	±0-02	±001	±0.,01	±001	±001	±001	±0-01	±001	±0-01
N2P4	0-303	0:237	0-247	0.187	0-1639	0-250ª	0268*	0-273	0.277
1	±0-03	±0-01	±0,-01	±0-01	±001	±001	±0-01	±001	±0-01
LSD		0.006	0-006	0.007	0-006	0.010	0-011	0-010	0-011
N1	0-291	0283*	0-293	0-253	0-218	0360*	0.4326	0-466*	0.558
N2	0-268	0-250 ^a	0-260°	0.200	0-175*	0265*	0-299	0.308*	0.313
LSD		0009	0-009	0.010	0-009	0-014	0.015	0-013	0.016
P1	0-260	0-318	0-3284	0-3075	0-260 ^d	0.4114	0-518	0-5384	0-670
P2	0-307	0,-253°	0-263 ^b	0, 203°	0-182 ^b	0272 th	0.291	0-297	0-300 ^b
P3	0-267	0.267	0-277	0.217	0-192	0.320	0-389	0-443	0.499
P4	0-285	0:-228ª	0-238	0178°	0-155	0.247*	0-264*	0-269*	0274

The results of LSD test on the 5, 10, 15, 20, 25, 30, 35 and 40th days on the main effect of the addition of commercial nitrification bacteria showed that the ammonia content in the rearing media which givenwith commercial nitrification bacteria was significantly lower than in the treatments not givenwithout commercial nitrification bacteria. The main effect of the addition of microbes from the swamp shows-suggested that the ammonia content in the rearing media that was givenwith a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. was the best for reducing ammonia concentration of rearing media.significantly different compared to the other treatments. The results of LSD on the 5, 10, 15 and 20th days, in the scenario of the interaction between factors, <u>demonstrated</u> that the NIP4 treatment had significantly lower ammonia levels compared to the other 25, 30, 35 and 40th days in the <u>scenario of the</u> interaction between factors, <u>suggested</u> that the NIP4 treatment had significantly lower ammonia levels compared to other treatments, but not significantly different from N2P2 treatment. The results of LSD on the 25, 30, 35 and 40th days in the <u>scenario of the</u> interaction between factors, <u>suggested</u> that the NIP4 treatment had significantly lower ammonia levels compared to other treatments, but not significantly different from N2P2 and N2P4 treatments.

On-Related to the factor of adding nitrifying bacteria, the lowest ammonia content was observed in the treatment was given with nitrifying bacteria. It is suspected assumed that the added nitrification bacteria are able to carry out the process of nitrification on the rearing media. According to Rurangwa et al- (2014), nitrification takes place through 2 reaction stages, where in the first stage the oxidation of ammonium to nitrite is carried out by ammonium oxidizing microbes (*Nitrosomonas* sp.) and in the second stage nitrite oxidizing microbes (*Nitrobacter* sp.). In-Among the addition of swamp origin-microbial factors, the lowest ammonia content in-was observed in the treatment was given with a combination of swamp origin microbes in the form of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. It is suspected that the three microbes from which the swamp was added could break down ammonia. Ammonia is the main source of nitrogen in addition to nitrate, which could be used by microalgae for their metabolic processes (Nurhayati et al-, 2014). *Bacillus* sp. could oxidize ammonia to nitrite through heterotrophic and chemotrophic processes (Edwards, 2011).

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there is a limited nitrogen to carbon (high C:-N ratio), the nitrification process is inhibited and heterotrophic bacteria develop rapidly. Heterotrophic bacteria will assimilate ammonia directly into bacterial proteins, when heterotrophic bacteria and they will develop rapidly. It will cause competition in the struggle for oxygen, space and nutrients with autotrophic bacteria (Blancheton et al., 2013).

Ammonia levels in all treatments <u>gave-with</u> microbes on 10th day to 20th day of rearing, decreased and increased until the 40th day. On the 10th to 20th day, it was suspected presumed that the ammonia accumulation from metabolic waste had not yet occurred_<u>see_that-therefore the</u> microbes given to the rearing media were able to work optimally. On the 25th to 40th day, it was supposseded that the ammonia accumulation from metabolic waste had been accumulated in the rearing media, so that the <u>added</u> microbes given-couldn't optimally reduce their ammonia levels. Meanwhile, the treatment without the addition of microbes (N1P1) experienced an increase in ammonia along with the increase in the rearing time. Increasing ammonia levels in the N1P1 treatment was caused of the rearing without siphoning during 40 days_{cor}. It resulted in a buildup of organic material derived from metabolic waste and levels of ammonia in the rearing media. The decomposing bacteria from natural swamp water <u>on-added to</u> the media have not been able to make an optimal decomposition.

Conclusion. The Aaddition of swamp microbes in the form of Chlorophyta, Bacillus sp. and Streptomyces sp. without the addition of commercial nitrification bacteria on the striped snakehead rearing media was more efficient than other treatments because they it-provides better water quality values. The best results are obtained in the treatment with the addition of awamp microbes Chlorophyta, Bacillus are obtained in the treatment with the addition of swamp microbes Chlorophyta, Bacillus are obtained in the treatment with the addition of swamp microbes Chlorophyta, Bacillus are obtained in the treatment gave in the best of survival rate, feed efficiency, and growth of <u>striped</u> snake head fish in swamp aquaculture, although there was not used nitrification bacteria. <u>Bacillus and Streetomyces</u> were the best combination of microbial swamp for striped snakehead culture in swamp water aquaculture which used Chlorophyta as green water system.

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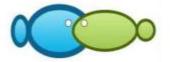
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Optimization of striped snakehead fish (Channa striata) culture using swamp microbial combination and nitrification bacteria

Marini Wijayanti, Dade Jubaedah, Ocktin Yulistya, Tanbiyaskur, Ade D. Sasanti

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Abstract. Utilization of swamp areas as fish culture locations alters water quality. Therefore, it is necessary to improve the water quality with environmental friendly biological treatments, such as the addition of microbes as probiotics in media culture. The purpose of this study was to determine the combination of microbes from swamps that can improve the water quality of the swamp fish culture and production media. The research used Completely Randomized Design (CRD) factorial with two factors consisting of the first factor with two treatments and the second factor with four treatments and three replications. The first factor consisted of two scenarios: (1) without the addition of nitrification bacteria (PROBAC) S×10⁶ CFU mL⁻¹ (N2). The second factor consisted of four scenarios: (1) without the addition of: (2) Chlorophyta (3.43×10⁷ sel L⁻¹) and Bacillus sp. (10⁶ CFU mL⁻¹) (P2); (3) Chlorophyta (3.43×10⁷ sel L⁻¹) and Streptomyces sp. (10⁵ CFU mL⁻¹) (P4). The result showed that the addition microbes from swamps in the combination of N1 and P4 treatment scenarios was able to improve the water quality value better than the other treatments, problotic, water quality.

Introduction. The swamp aquaculture must improve and maintain the water quality for fish rearing media, through environmentally friendly biological treatments, since the wastewater of fish rearing on swamps reduces the quality of swamps water. One of the treatments is adding probiotics in the rearing media. Irianto & Austin (2002) stated that environmental damaging can be prevented with probiotics able to degrade the organic materials in the habitat. Hartini et al (2013) showed that the addition of probiotics at a dose of 10 μ L ^{L-1} week⁻¹ can improve and maintain optimal water quality. In other studies, the addition of effective probiotic microorganisms can reduce ammonia levels and suppress the population of pathogenic microorganisms from the culture media (Trisna et al 2013).

Swamps have high biodiversity, including sediment microbes able to improve the physical and chemical properties of their media. The identified swamp microbes include Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. (Wijayanti et al 2018). Bacteria (*Bacillus* sp.) with concentrations of 10⁵ CFU mL⁻¹ (Khotimah 2018) and Chlorophyta microalgae with the optimum concentration of 10% of the maximum density (Utami 2019) are able to grow in the fish culture media and they can be used as environmental probiotics. Chlorophyta is a microorganism that can be used as green water in aquaculture media. Wijayanti et al (2018) showed that the use of Chlorophyta increased the level of dissolved oxygen in the pond culture media 63.63%. The increasing dissolved oxygen is caused by Chlorophyta carrying out photosynthetic activity which produces dissolved oxygen in the culture media. *Bacillus* sp.

and Streptomyces sp. obtained are proteolytic bacteria that can increase the content of NH₃, NO₂, and NO₃ into the media (Yuliani 2017; Saraswati 2018). Balcazar et al (2006) stated that Bacillus sp. is an example of efficient probiotic bacteria, used in aquaculture because it is able to convert organic matter into CO2 used in cell metabolism. Grampositive bacteria, such as Bacillus sp. can increase the animal immune system capabilities and also act favorably in improving the quality of the water system (Mohapatra et al 2013). Bernal et al (2017) stated that the combination of Streptomyces sp. and Bacillus sp. demonstrated a significant immunomodulatory activity by increasing the total number of hemocytes and the activity of Superoxide Dismutase (SOD), which provides a protective effect against Vibrio harveyi bacteria by increasing the immunological status of Penaeus monodon. Nitrifying bacteria are chemolitho-autotrophic bacteria (for example Nitrosomonas sp., Nitrobacter sp.), which are able to meet their carbon needs through CO2 fixation (Calvin cycle), and their energy source comes from the oxidation process of reducing ammonia to nitrate. As an example, through the addition of nitrifying bacteria and denitrification, the molasses with a C/N ratio of 10 could decrease in ammonia levels by 28.5% (Yuniasari 2009).

Each of the swamp microbes and nitrifying bacteria has their own advantages and disadvantages, therefore a consortium of swamp microbes and nitrifying bacteria would be more effective The emergence of a consortium, expected to form cooperative, commensal and mutualistic relationships between microbes, calls for an optimal combination of Bacillus sp., Streptomyces sp., Chlorophyta and commercial nitrification bacteria. The purpose of this study was to determine combinations of swamp microbes and nitrifying bacteria that can improve the water quality of media in swamp fish production.

Material and Method

The experimental design used was a completely randomized factorial design (RAL) consisting of 2 factors: the first factor with 2 treatments and the second factor with 4 treatments and 3 replications. The first factor is the addition of nitrifying bacteria (PROBAC), namely:

N1- without the addition of nitrifying bacteria (PROBAC);

N2 - with the addition of nitrifying bacteria (PROBAC) 5×10⁶ CFU mL⁻¹.

The second factor is the addition of swamp microbes, namely:

P1- without the addition of swamp microbes;

P2 - provision of 100 ml Chlorophyta (3.43×10⁷ cell L⁻¹) and Bacilius sp. (10⁵ CFU mL⁻¹); P3 - provision of 100 ml Chlorophyta (3.43×10⁷ cell L⁻¹) and Streptomyces sp. (10⁵ CFU mL⁻¹); P4 - provision of 100 ml Chlorophyta (3.43×10⁷ cell L⁻¹), Bacilius sp. (10⁵ CFU mL⁻¹) and Streptomyces sp. (105 CFU mL-1).

Bacteria cultivation and propagation. Pure bacterial colonies were obtained from the results of previous studies. The colony was cultivated using nutrient agar (NA) media for Bacillus sp. and yeast malt (YM) agar for Streptomyces sp. Bacterial colonies were scratched in a petri dish containing NA for Bacillus sp. and YM media for Streptomyces sp. using the quadrant streak method. Petri dishes were wrapped in wrapping paper and incubated for 2 days at room temperature (28-30°C). A single colony formed on a petri dish was transferred to the agar media and incubated until bacteria grew.

Swamp bacteria grown on NA and YM agar media were multiplied by nutrient broth (NB) media for Bacillus sp. and liquid YM for Streptomyces sp. As much as 5 mL of suspension were collected in a test tube in order to be cultured in the medium, and then homogenized with a shaker for approximately 2 days for Bacillus sp. and 5 days for Streptomyces sp., then multiplied from 5 mL to 500 mL. The concentrations of bacteria were measured by Mc Farlan spectrophotometry methods.

Chlorophyta culture. The culture media used for Chlorophyta sp. was a technical fertilizer media consisting of ZA, Urea, TSP, and Gandasil B. All technical fertilizer ingredients were mixed in a 250 mL Erlenmeyer and added to 100 mL of distilled water

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and then homogenized on a hot plate using a magnetic stirrer and sufficient heat, until all ingredients dissolved. The technical fertilizer media in the Erlenmeyer was sterilized using an autoclave at 121°C for 0.25 hour. *Chlorophyta* isolates (about 10⁷cell ml⁻¹ in 10 ml stock culture) were put into an Erlenmeyer containing a technical fertilizer media for liquid culture. They cultured during 9 days at the room temperature for scaling up to 1 L.

Preparation of fish rearing media. The container used in rearing was in the form of an aquarium with a size of 30 x 30 x 30 cm³ as many as 24 units. The aquariums were cleaned using potassium permanganate to be sterilized of diseases or parasites. The aquarium was filled with 20 L of swamp water.

Fish culture test. The test organisms used in this study were 12 Channa striata specimens of 5±1 cm each for 20 L of water (Mulyadi 2016). Before stocking, acclimatization was done as an adaptation to the new environment in order to reduce stress on the test organism. After 7 days of stocking, a treatment was applied, consisting of a combination of Chlorophyta isolate (3.43×10⁷ cell L⁻¹), Bacillus sp. (10⁵ CFU mL⁻¹), Streptomyces sp. (10⁵ CFU mL⁻¹) as well as PROBAC nitrification bacteria (5×10⁶ CPU mL⁻¹).

Rearing. The fish culture was maintained for 40 days calculated after the addition of the treatment. During rearing, they were fed at satiation with a frequency of three times a day, by using commercial pellets with 40% protein content.

Chlorophyta abundance. Samplings were carried out at the beginning and end of the study by sub composite methods, in each treatment. Plankton net with 25 µm mesh size was used for 5 L of rearing media by experimental unit (sample of 25 mL). A microscope and "The Marine and Fresh Water Plankton" textbook were used for the observation of the Chlorophyta samples (Davis 1955). Chlorophyta abundance calculation was performed by using the Leackey Drop Microtransect method (American Public Health Association 1989) as follows:

$$N = Z \times \frac{\pi}{2} \times \frac{1}{2}$$

Where:

- N total number (cell L⁻¹);
- Z number of individuals found;
- X volume of filtered water (25 mL);
- Y volume 1 drop of sample water (0.05 mL);
- V volume of filtered water (5 L).

Bacteria population. The counting of bacterial populations was performed at the beginning and end of rearing with the plate count method on a multilevel dilution incubated at a temperature of 28-30°C for 24 hours. The growing population was determined in a Colony Forming Unit (CFU) and calculated using the following formula (...):

Total of Bacteria = Total of colonies $\times \frac{1}{dilution \ factor} \times \frac{1}{mL \ sample}$

Biofloc volume. The biofloc volume measurements were done on the 10 and 40 days after rearing. The floc volume was obtained by collecting a rearing media, by using a glass cone 1L volume, then the floc in the water media was left to settle in the tube for 15-20 minutes.

Survival rate. The percentage of fish survival was calculated by using the following formula (....):

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	No ×100%	
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	140	
Where:		
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No - number of fish at the beginning of	rearing (Individuals):	
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N - number of fish at the end of rearing (individuals).

Absolute weight growth. Growth of fish weight during rearing was calculated by using the following formula (...):

W = Wt - Wo Where: W - growth of weight of fish for rearing (grams);

Wt - weight of fish at the end of rearing (grams); W₀ - weight of fish at the beginning of rearing (grams).

Absolute length growth. The absolute length growth of fish during rearing was determined by doing the following calculation (...): L = Lt-Lo

Where:

L - growth of absolute length of fish for rearing (cm);

Lt - length of fish at the end of rearing (cm);

Lo - length of fish at the beginning of rearing (cm).

Feed efficiency. According to NRC (1977) feed efficiency can be calculated by using the formula:

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

Where:

EP - Feed efficiency (%);

Wt - Weight of fish at the end of rearing (gram);

Wo - initial fish rearing weight (gram); D - Weight of fish that died during rearing (gram);

F - Amount of feed given (grams).

Water quality. Measurement of water quality data for C. striata rearing media included pH (pH meter), dissolved oxygen (DO meter), amonia (spectrophotometry), and biological oxygen demand (DO meter) at the beginning and 40 days later, at the end of the rearing.

Data analysis. Research data including biofloc volume, survival, growth, feed efficiency, water quality was statistically processed by using the variance analysis. If the results of the variance analysis showed that the treatment has a significant effect, then it was continued with the LSD test (the Least significance difference) 5%. Chlorophyta abundance data and the total bacterial population were analyzed descriptively.

Results and Discussion

Chlorophyta abundance, total bacterial population and floc volume. Chlorophyta abundance data on rearing media are presented in Table 1. Chlorophyta density at each treatment decreased after 40 days of rearing. Chlorophyta added in the rearing media experiences death or predation. In the rearing media, a food chain system occured between Chlorophyta and zooplankton (Figure 1), resulting in a decrease in the population of Chlorophyta due to predation.



Figure 1. Biofloc and Chlorophyta profile in the rearing media of C. striata culture in this study (40 magnificaton scale of microscope).

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The pattern of the interactions between zooplankton and phytoplankton is a series of eating and prey relationships forming the path of the food chain. Phytoplankton as primary producers is eaten by zooplanktons, in turn zooplanktons are eaten by small fish at higher trophic levels (Bouman et al 2003).

Table 1

Table 2

White search other in her cardings are 1.	 Channes attribute 	annuluum mondia at 6	10 40th days
Chlorophyta abundance in	n c <i>hanna striata</i>	rearing media at 0.	10. 40 ^m day

Commercial		Chlorophyta abundance (cell L ⁻¹)					
nitrification bacteria	Swamp microbes	0 day	10 th day	40 th day			
	P1	3.20×10 ³	3.20×10 ³	2.10×10			
N1	P2	3.60×10 ³	3.43×107	4.10×10			
NI	P3	4.10×10 ³	3.43×107	4.10×10			
	P4	3.70×10 ³	3.43×107	4.46×10			
	P1	4.00×103	4.0×103	2.10×10			
117	P2	3.60×10 ³	3.43×107	2.41×10			
N2	P3	3.90×10 ³	3.43×107	2.34×10			
	P4	3.40×103	3.43×107	4.03×10			

The total bacterial population in the rearing media is presented in Table 2, the results of the LSD test of the floc volume at ten and forty days of rearing are showed in Table 3 and Table 4, respectively.

Total bacterial population in the rearing media

Commercial	Swamp -	Total bacterial population (CFU mL ⁻¹)							
nitrification bacteria	microbes	0 day	0 day 1 st day 20 th day		0 day 1 st day 20 th		40 th day		
20020000000	P1	6.60×10 ⁴	6.78×10 ⁴	1.55×105	6.20×103				
N1	P2	6.20×104	3.95×10 ⁶	6.93×10 ⁶	2.77×105				
	P3	7.00×104	3.28×10 ⁶	7.53×10 ⁶	3.01×105				
	P4	4.70×10 ⁴	5.59×107	1.00×10 ⁸	2.99×10 ⁶				
	P1	7.10×10 ⁴	2.01×107	3.54×107	1.42×106				
	P2	4.50×104	3.29×107	5.59×107	1.68×106				
N2	P3	4.30×104	4.99×107	6.41×107	1.93×10 ⁶				
	P4	4.95×104	4.70×107	8.75×107	4.05×10 ⁶				

Based on Table 2, the total bacterial population increased on 20^m day and decreased until the 40th day. The increase in population on the 20th day can be caused by adequate nutrients addition in the rearing media, stimulating the metabolic activity and growth of the bacteria and Actinomycetes, while the decline of bacteria population observed on the 40th day could be caused by the nutrient depletion (macronutrient and micronutrient) in the water.

Related to the factor of nitrification bacteria, the results of LSD at 10 and 40 days after rearing start showed that the volume of floc in the media without treatment was significantly higher compared to the treatment with nitrification bacteria. The addition of nitrifying bacteria cannot increase the volume of floc, because there were less exhausted bacteria in the media. The bacteria need more substrate from unconsumed feed of fishes, while the nitrifying bacteria accelerated the process of dissolving nitrogenous organic matter from the waste. Related to the factor of microbial addition from swamps, the volume of floc on the media treated with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. significantly showed higher levels compared to other treatments.

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The results of LSD test of the floc volume in the rearing media at 10 days of rearing

The single effect of	The single	(P) (LSD)		microbes	The main effects of nitrifying bacteria (N)
nitrifying bacteria (N)	P1	P2	P3	P4	(LSD0.05=4.386)
N1	11.111	16.666	13.332	26.668	16.9445
N2	10.000	10.000	13.332	16.667	12.500*
The main effects of swamp microbes (P) (LSD _{0.05} =3.102)	10.556*	13.333*	13.332*	21.667 ⁶	777

Table 4

The results of LSD test floc volume of rearing media at 40 days of rearing

The single effect of nitrifying bacteria (N)	The single	e influence (P) (LSD ₀)	The main effects of nitrifying bacteria (N		
	P1	P2	P3	P4	(LSD0.05=3.315)
N1	11.112°	26.667 ^b	16.667ª	38.889	25.834
N2	13.333*	13.333*	23.333 ^b	33.3334	20.833*
The main effects of swamp microbes (P) (LSD0.03=4.689)	12.223ª	20.000 ^s	20.000 ^b	41,111 ^c	777

It is presumed that certain types of microorganisms are predisposed for flocs forming. Related to the influence of interactions between factors at 40 days of rearing, the observations showed that the treatment combination of Chlorophyta, Bacillus sp., Streptomyces sp. without nitrifying bacteria determined the highest volume of floc, 38.89 mL L-1, but it was not significantly different from the treatment combination of Chlorophyta, Bacillus sp., Streptomyces sp. with nitrifying bacteria. The volume of floc in this study was lower than the study from Mulyadi et al (2016), where in treatment with stocking density of 450 C. striata m⁻¹ which was kept for 41 days resulted in a floc volume of 40.7 mL L⁻¹. It is presumed that the rearing media lacks a carbon source used by bacteria for the floc formation. According to Panigrahi et al (2019), Litopenaeus vannamei cultivation without a biofloc system can produce a volume of floc of 4.53 mL L 1, which is lower than the cultivation of L. vannamei with a biofloc system, by adding molasses. Floc thickness will continue to increase along with the provision of bacteria and carbon sources that are continuously balanced with feed metabolic waste producing ammonia. The bacteria could bind to ammonia and will form a biofloc (Sitohang et al 2018).

The results of the analysis of variance showed that the effect of the interaction between factors and the effect of the factor defined by the addition of swamp originated microbes on the survival of *C*, striata varied significantly between treatments, but the factor defined by the adding of commercial nitrification bacteria had no significant effect. Based on further testing of LSD on microbial factors from swamps, in the rearing media treated with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp., the survival rate was significantly higher than for the other treatments. Interactions between factors showed that in the rearing media with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, the survival rate reached 63.94%, a significantly higher performance compared to the other treatments.

Based on the results of the survival percentage, it is showed that the combination of swamp microbes is able to suppress unfavorable microbes, to improve the water quality in the rearing media and to increase the *C. striata* survival rate. This is shown in the treatment of rearing media consisting in a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, which provided a *C. striata* survival rate of 63.94%. The combination of *Bacillus* sp. and *Streptomyces* sp.

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was able to provide more protection against unfavorable microbes in the media, the presence of *Bacillus* sp. giving effect to *Streptomyces* sp. to produce antimicrobial compounds. Luti & Mavituna (2011) explained that *Bacillus* cultured together with *Streptomyces* increased the production of antimicrobial compounds when it was compared with the single genus culture.

Table 5

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The single effect of nitrifying bacteria (N)	The single	e influence (P) (LSDo	The main effects of nitrifying bacteria (N)		
	P1	P2	P3	P4	(LSD0.05=)
N1	26.06ª	36.91 ⁿ	28.03ª	63.94 ^d	38.74
N2	31.75 ^{an}	28.03ª	35.16 ^b	48.20 ^c	35.79
The main effects of swamp microbes (P) (LSD0.05=4,25)	28.91*	32.47ª	31.59ª	56.07 ⁵	777

The N1P1 treatment (without the addition of microbes from swamps, neither of commercial nitrification bacteria) was the treatment with the lowest survival value of 26.06% compared to other treatments. These results prove that the addition of swamp origin microbes to rearing media can increase the survival rate of *C. striata*. This is in line with the results of Hartini et al (2013), suggesting that the addition of EM-4 probiotics to the rearing media had a significant influence on the survival of *C. striata*. The average survival of *C. striata* with 10 µL L¹ week¹ EM-4 probiotics 96.66% tended to be higher, compared to control treatments (without EM-4 probiotics) which was 8.89%. Budianto & Heny (2017) stated that the bacteria *Bacillus* sp. has bacteriocin compounds with specific inhibiting action on the growth of *S. iniae* and *P. fluorescens. Streptomyces* bacteria has the potential to control pathogenic bacteria by means of competition, parasitism or by producing secondary metabolite compounds (Lutfi 2018). The combination of the two microbes can provide a high percentage of survival compared to the no combination scenario. According to Irianto & Austin (2002), probiotic bacteria can increase the survival or reduce mortality through the development of the immune system due to an increasing phagocyte and lysozyme activity, thereby suppressing pathogenic bacterial colonies. Sanchez et al (2014) stated that probiotics can increase immune stimulation in fish, as a protection against pathogenic bacteria that causes death in fish culture.

Feed efficiency, absolute weight and length growth. The results of the analysis of the efficiency of various feed on C. striata showed that the interaction between factors, the microbial addition factors from swamps and the addition of commercial nitrification bacteria, can increase the value of fish feed efficiency, which is significantly affected by the treatment type and concentration. LSD test results of the efficiency of fish feed for 40 days of rearing are presented in Table 6.

Table 6

LSD test result of the efficiency of Channa striata feed for 40 days of rearing

The single effect of nitrifying bacteria (N)	The single	e influence (P) (LSD)	microbes	The main effects of nitrifying bacteria (N		
	P1	P2	P3	P4	(LSD0.05=1.66)	
N1	18.93*	47.344	37.97	59.65°	40.97*	
N2	22.00°	29.52 ^b	34.89	44.11 ^d	32.63°	
The main effects of swamp microbes (P) (LSD0.05=2.35)	20.47ª	38.43 ^b	36.43 ^b	51,88¢	20.47*	

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LSD test of survival rate of Channa striata

Based on these results, the effects of the different combinations on the *C. striata* feed efficiency could be observed. The value of the *C. striata* feed efficiency in the treatment of the media of rearing was: (1) significantly higher without commercial nitrification bacteria than for the treatment with nitrification bacteria; (2) significantly higher with a combination of Chiorophyta, *Bacillus* sp. and *Streptomyces* sp., compared to other treatments and interactions between factors; (3) significantly higher with a combination of Chiorophyta, *Bacillus* sp., and without commercial nitrification bacteria than the results of other treatments. It is thought that swamp microbes added to the media enter the digestive tract of *C. striata* during the process of respiration and while eating. Chiorophyta that enters the digestive tract could be a natural food source, while *Bacillus* sp. and *Streptomyces* sp. which entered the digestive tract could be working together to improve their digestibility. High digestibility in the feed will increase the absorption of nutrient, which at optimal levels will increase the value of feed efficiency, favoring fish growth. *Bacillus* sp. is a bacterium that can increase digestibility because it can secrete protease, lipase and amylase enzymes (Fardiaz 1992).

These bacteria can also produce antimicrobial compounds (bacteriocin and antibiotics) that can suppress pathogenic bacterial colonies (Findy 2009). *Streptomyces* sp. is a genus of actinomycetes that can produce various antibiotic compounds. It has the potential to control pathogenic bacteria by conducting competition, parasitism or by producing secondary metabolites (Lutfi 2018). The bacterium *Bacillus* sp. increases the value of feed efficiency by secreting enzymes that can stimulate digestion, while *Streptomyces* sp. secretes antibiotics able to suppress pathogens. Both bacteria work together to improve the digestibility and immunity of *C. striata*, which ultimately results in high feed efficiency. Bacterial activity in the digestive tract will experience rapid fluctuations with microbes entering through feed or water and causing changes in the intestinal microbial balance. The entry of these microbes is antagonistic to the pathogenic microbes in the digestive tract, improving growth, protein efficiency ratio and feed efficiency (Midhun et al 2018; Nargesi et al 2019).

Swamp microbial addition factor, commercial nitrification bacteria addition factor, and interactions between factors influenced the feed efficiency in a significantly different manner, depending on the treatment. Based on the results of the LSD_{0.05} test (Table 7) on the main effect of the addition of commercial nitrification bacteria, the increase of the absolute weight of *C. striata* following the treatment of the rearing media was: (1) significantly higher without commercial nitrification bacteria than with commercial nitrification bacteria; (2) significantly higher with the addition of a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. than with the other treatments; (3) significantly higher than the other treatments; results with a combination of Chlorophyta, *Bacillus* sp. and without commercial nitrification bacteria, but (4) not significantly different from the treatment results given a combination of Chlorophyta, *Bacillus* sp. and without commercial nitrification

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Table 7

The single effect of nitrifying bacteria (N)	The single	e influence (P) (LSDo	of swamps .os=0.08)	microbes	The main effects of nitrifying bacteria (N)
	P1	P2	P3	P4	(LSD0.05=0.04)
N1 N2	1.30ª 1.73°	2.26 ⁷ 1.41 ^b	1.70 ^c 1.88 ^d	2.32' 2.08"	1.90 ^b 1.78 ^a
The main effects of swamp microbes (P) (LSD0.05=0.05)	1.51*	1.845	1.79*	2.20 ^c	1.51ª

LSD 0.05 test results of growth in absolute weight of Channa striata

Based on the results of the variance analysis, swamps microbial addition factor and interactions between factors significantly influenced the increase of the absolute length, but the factor of adding commercial nitrification bacteria has no significant effect. The LSD results of growth in absolute length of *C. striata* are presented in Table 8.

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LSD test results for growth in the absolute length of Channa striata

The single effect of	The single	e influence (P) (LSDo	The main effects of nitrifying bacteria (N)		
nitrifying bacteria (N)	P1	P2	P3	P4	LSD0.05=
N1 N2	0.69 ^a 1.13 ^c	1.79 ^e 1.08 ^c	0.91 ^b 1.60 ^d	2.27 ^r 1.74 ^e	2.12 2.09
The main effects of swamp microbes (P) (LSD _{0.05} =0.07)	0.91*	1.44 ^c	1.26%	2.00 ^d	0.91*

The main influence of the addition of microbes on the increase of the absolute length of snakehead fish in the treatment of rearing media with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. was significantly different than in other treatments. The influence of interactions between factors on the increase of the absolute length of *C*. *striata* was significantly higher in the treatment of rearing media with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria than the in the results of the other treatments.

The addition of a combination of swamp microbes and commercial nitrification bacteria gave significant results on the increase of absolute weight and absolute length. The highest absolute weight and length increase was produced by the treatment of rearing media with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, and the lowest by the treatment without the addition of microbes. The treatment without the addition of microbes is thought to be the less effective in terms of absorption of nutrients in the feed, causing a sub-optimal growth, compared to the other treatments. In the treatment of NIP4, swamp microbes added to the media could be entered into the digestive tract of *C. striata* during the respiration process and when eating. These microbes break down complex compounds into simpler ones and increase digestibility of feed, by accelerating the process of absorption of the food. The work of probiotics in aquaculture is the ability of microbes to break down proteins, polysacarides, lipids and stress resistance in aquaculture system (de melo Pereira et al 2018). The addition of 10⁴ CFU mL⁻¹ probiotics to the controls (Widarnani et al 2010). *B. licheniformis* at 10⁵ CFU mL⁻¹ in the rearing media of *P. hypophthalmus* showed a significant increase in the growth, immune and antioxidant responses compared to 10⁷ CFU mL⁻¹ (Gobi et al 2016).

Dissolved oxygen and ammonia in the rearing media. The results of the analysis of variance on day 0 showed that the factor of commercial nitrification bacteria, swamp microbes and the interaction between the factors did not significantly influence the dissolved oxygen content (Table 9). At 5 and 10 days after rearing start, the factor of commercial nitrification bacteria and swamp microbes significantly affected the dissolved oxygen content, but the interaction between the factors had no significant effect.

The results of dissolved oxygen in the rearing media at 5 and 10 days after rearing start showed that the main effect of the addition of commercial nitrification bacteria was significantly lower than without commercial nitrification bacteria.

The main influence of the addition of microbes from swamp on the dissolved oxygen in the rearing media was the best for dissolved oxygen concentration in culture media with a combination of Chlorophyta, *Bacillus* sp., and *Streptomyces* sp. (P4), than in other treatments.

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LSD test result of dissolved oxygen in the rearing media

				Dissoh	ied oxyge	n (mg L-1)	():		
· .	e		1000	Da	ys after r	earing	24 - Jones -		
	0	5	10	15	20	25	30	35	40
LSD	-		-	0.18	0.14	0.14	0.18	0.18	0.18
N1P1	3.60	3.53	3.53	3.404	3.10	3.00	2,97 ^c	2.77 ^c	2.674
MILT	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
N1P2	3,63	3.53	3.53	3.23%	2.93×b	2.83 th	2,77**	2.57%	2,470
MTL5	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
N1P3	3,67	3.57	3.47	3.33bc	3.03°C	2.93 ^{bc}	2.87°C	2.67bc	2.57%
MIL2	±0.1	±0.2	±0.2	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
N1P4	3.57	3.70	3.60	3.60*	3.50°	3.40 ^e	3,40*	3.20*	3.10 ^e
NILL-4	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
N2P1	3.63	3.43	3.43	3.13*	2.83ª	2.73*	2.67ª	2.47°	2.37*
NZP1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
N2P2	3.57	3.47	3.47	3.33 ^{bc}	3.03°	293 ^{bc}	2.87 ^{bc}	2.67 ^{bc}	2.57%
MZP2	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
N2P3	3,53	3.43	3.33	3.27%	3.10 ^c	3.00	2.90°c	2.70tc	2.60°
NZPS	±0,1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
N2P4	3.67	3.63	3.53	3.40°	3.304	3.20 ^d	3.204	3.00 ^d	2.904
1421-4	±0,1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
LSD		0.08	0.08	0.09	0.07	0.07	0.09	0.09	0.09
N1	3.62	3.58 ^b	3.530	5.09°	3.14 ^b	3.04°	3.00 ^h	2.80 ^b	2.70 ^b
N2	3.60	3.49*	3.44*	4.93*	3.07ª	2.97*	2.91*	2.71*	2.61*
LSD	10000	0.11	0.11	0.13	0.10	0.10	0.13	0.13	0.13
P1	3.62	3.48*	3.48 ^{ab}	3.27*	2.97*	2.87*	2.82*	2.62*	2.52ª
P2	3.60	3.50*	3.50 ^{sb}	3.28*	2.98*	2.88*	2.82*	2.62*	2.52*
P3	3.60	3.50*	3.40*	3.30*	3.07*	2.97*	2.88*	2.68*	2,58*
P4	3.62	3.670	3.57 ^b	3.50°	3.40 ^b	3.30°	3.30 ^h	3.100	3.005

Ten days after rearing start, the P4 treatment had a significantly higher dissolved oxygen content than the P3 treatment, but it was not significantly different from the P1 and P2 treatments. The factors of commercial nitrification bacteria, microbial addition and their interaction significantly influenced the dissolved oxygen content. The dissolved oxygen test on the 15, 20, 25, 30, 35 and 40th days are presented in Table 9. Ammonia analysis results on day 0 were not significantly influenced by the factor

Ammonia analysis results on day 0 were not significantly influenced by the factor of commercial nitrification bacteria, addition and their interaction. The variance analysis results on days 5, 10, 15, 20, 25, 30, 35 and 40 showed that the factor of commercial nitrification bacteria, microbial addition and their interaction significantly affected ammonia content. They are presented in Table 10. The results of LSD test on the 5, 10, 15, 20, 25, 30, 35 and 40th days on the main effect of the addition of commercial nitrification bacteria showed that the ammonia content in the rearing media with commercial nitrification bacteria. The main effect of the addition of microbes from the swamp suggested that the ammonia content in the rearing media without concentration of nearing media compared to the other treatments. The results of LSD on the 5, 10, 15 and 20th days, in the scenario of the interaction between factors, demonstrated that the N1P4 treatment had significantly lower ammonia levels compared to the other treatments, but not significantly different from N2P2 treatment. The results of LSD on the 25, 30, 35 and 40th days in the scenario of the interaction between factors, suggested that the N1P4 treatment had significantly lower ammonia levels compared to the other treatments, but not significantly different from N2P2 treatment. The results of LSD on the 25, 30, 35 and 40th days in the scenario of the interaction between factors, suggested that the N1P4 treatment had significantly lower ammonia levels compared to other treatments, but not significantly different from N2P2 and N2P4 treatments.

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LSD test results for ammonia every 5 days on the rearing media

			Me	and the second se	ionia concer	and the second se	7(-1)		
					ays after rea				
1000	0	5	10	15	20	25	30	35	40
LSD		0.013	0.013	0.014	0.013	0.020	0.021	0.019	0.02
N1P1	0.290	0.383°	0.393*	0.410 [±]	0.327=	0.5404	0.674°	0.691 ^r	0.948
MIP1	±0.07	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.0
ALC: N	0.323	0.273	0.283	0.223	0.203	0.293%	0.314 th	0.321 ^c	0.324
N1P2	±0.03	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.0
N1P3	0.283	0.257	0.267	0.207%	0.197 ^{bc}	0.363	0.4824	0.585*	0.692
NIP3	±0.01	±0.01	±0.01	±0.01	±0.01	±0.02	±0.02	±0.01	±0.0
NHDA	0.267	0.220*	0,230*	0.1704	0.147ª	0.243°	0.260*	0.265*	0.270
N1P4	±0.01	±0.01	±0.01	±0.01	±0.01	±0.02	±0.02	±0.02	±0.0
N2P1	0.230	0.253	0.263%	0.203 ^o	0.193 ^{bc}	0.282°	0.363	0.3850	0.392
N2P1	±0.01	±0.01	±0.01	±0.01	±0.01	±0.02	±0.02	±0.01	±0.0
ALCO D	0.290	0.233 ^{tb}	0.243*	0.183°	0.160°	0.250*	0.268*	0.273°	0.277
N2P2	±0.04	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.0
ALCORD .	0.250	0.277	0.287	0.227-	0.187	0.277	0.296 ^a	0.300 ^p	0.306
N2P3	±0.02	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.0
N2P4	0.303	0.237	0.247	0.1870	0.163 ^b	0.250*	0.268*	0.273°	0.277
N2P4	±0.03	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.0
LSD		0.006	0.006	0.007	0.006	0.010	0.011	0.010	0.01
N1	0.291	0.283°	0.293°	0.253°	0.218°	0.360P	0.432 ⁶	0.4669	0.558
N2	0.268	0.250°	0.260°	0.200°	0.1764	0.265ª	0.299*	0.308ª	0.313
LSD	SUASYAMI	0.009	0.009	0.010	0.009	0.014	0.015	0.013	0.01
P1	0.260	0.3184	0.328	0.3074	0.2604	0.4114	0.5181	0.538*	0.670
P2	0.307	0.253 ^b	0.263°	0.2036	0.182 ^b	0.272°	0.291 ^h	0.297	0.300
P3	0.267	0.267	0.277	0.217-	0.192	0.320	0.389	0.443	0.499
P4	0.285	0.228°	0.238ª	0.178	0.155*	0.247*	0.264*	0.269*	0.274

Related to the factor of adding nitrifying bacteria, the lowest ammonia content was observed in the treatment with nitrifying bacteria. It is assumed that the added nitrification bacteria are able to carry out the process of nitrification on the rearing media. According to Rurangwa et al (2014), nitrification takes place through 2 reaction stages, where in the first stage the oxidation of ammonium to nitrite is carried out by ammonium oxidizing microbes (*Nitrosomonas* sp.) and in the second stage nitrite oxidation is performed by nitrite oxidizing microbes (*Nitrobacter* sp.). Among the addition of swamp microbial factors, the lowest ammonia content was observed in the treatment with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. It is suspected that the three microbes could break down ammonia. Ammonia is the main source of nitrogen in addition to nitrate, which could be used by microalgae for their metabolic processes (Nurhayati et al 2014). *Bacillus* sp. could oxidize ammonia to nitrite through heterotrophic and chemotrophic processes (Edwards 2011).

Among the factors' interactions, N1P4 treatment had the lowest ammonia levels on the 5th day until the end of rearing. It was suspected that microbes from the swamp were able to break down the organic material, derived from feces or feed, into compounds that were not harmful to *C. striata*. The N1P4 results were not significantly different from the N2P2 and N2P4 results. In presence of the commercial nitrification bacteria in the same ecosystem as *Bacillus* sp. and *Streptomyces* sp., the nitrification process activity and the growth of nitrifying bacteria are inhibited. Nitrifying bacteria (autotrophic bacteria) produce very small biomass and slow growth. Nitrifying bacteria takes 12 hours to regenerate, while heterotrophic bacteria *Bacillus* sp. and *Streptomyces* sp. only need 30 minutes (Ebiling et al 2006). If there is a limited nitrogen to carbon (high C:N ratio), the nitrification process is inhibited and heterotrophic bacterial develop rapidly. Heterotrophic bacteria will assimilate ammonia directly into bacterial proteins,

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and they will develop rapidly. It will cause competition in the struggle for oxygen, space and nutrients with autotrophic bacteria (Blancheton et al 2013).

Ammonia levels in all treatments with microbes on 10th day to 20th day of rearing decreased and increased until the 40th day. On the 10th to 20th day, it was presumed that the ammonia accumulation from metabolic waste had not yet occurred, therefore the microbes given to the rearing media were able to work optimally. On the 25th to 40th day, it was supposed that the ammonia accumulation from metabolic waste had been accumulated in the rearing media, so that the added microbes couldn't optimally reduce their ammonia levels. Meanwhile, the treatment without the addition of microbes (NIP1) experienced an increase in ammonia along with the increase in the rearing mitme. Increasing ammonia levels in the NIP1 treatment was caused of the rearing without siphoning during 40 days. It resulted in a buildup of organic material derived from metabolic waste and levels of ammonia in the rearing media. The decomposing bacteria decomposition.

Conclusions. The addition of swamp microbes Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. on the *C. striata* rearing media was more efficient than other treatments because they provided better water quality values and gave the best survival rate, feed efficiency and growth of *C. striata* in swamp aquaculture, although there was no nitrification bacteria used. *Bacillus* and *Streptomyces* were the best combination of microbial swamp for *C. striata* culture in swamp water aquaculture which used Chlorophyta as green water system.

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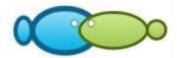
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Optimization of striped snakehead fish (Channa striata) culture using swamp microbial combination and nitrification bacteria

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Abstract. Utilization of swamp areas as fish culture locations alters water quality. Therefore, it is nacessary to improve the water quality with environmental friendly biological treatments, such as the addition of microbes as problotics in media culture. The purpose of this study was to determine the combination of microbes from swamps that can improve the water quality of the swamp fish culture and production media. The research used Completely Randomized Design (CRD) factorial with two factors consisting of the first factor with two treatments and three replications. The first factor consisted of two scenarios: (1) without the addition of nitrification bacteria (N1) and (2) with the addition of nitrification bacteria (PROBAC) 5×10^6 CFU mL⁻¹ (N2). The second factor consisted of four scenarios: (1) without the addition of nitrification bacteria (N2) and streptomyces sp. (10⁵ CFU mL⁻¹) (P3); (4) Chiorophyta (3.43\times10⁷ sel L⁻¹) and with the addition of Swamp microbes (P1) Bacilius sp. (10⁵ CFU mL⁻¹) (P3); (4) Chiorophyta (3.43\times10⁷ sel L⁻¹), Bacilius sp. (10⁵ CFU mL⁻¹) (P4). The result showed that the addition microbes from swamps in the combination of N1 and P4 treatment scenarios was able to improve the water quality value better than the other treatment scenarios, producing the best survival rate (63.94%), feed efficiency (59.65%), absolute weight growth (2.32 g) and absolute length growth of (2.27 cm).

Introduction. The swamp aquaculture must improve and maintain the water quality for fish rearing media, through environmentally friendly biological treatments, since the wastewater of fish rearing on swamps reduces the quality of swamps water. One of the treatments is adding probiotics in the rearing media. Irianto & Austin (2002) stated that environmental damaging can be prevented with probiotics able to degrade the organic materials in the habitat. Hartini et al (2013) showed that the addition of probiotics at a dose of 10 μ L L⁻¹ week⁻¹ can improve and maintain optimal water quality. In other studies, the addition of effective probiotic microorganisms can reduce ammonia levels and suppress the population of pathogenic microorganisms from the culture media (Trisna et al 2013).

Swamps have high biodiversity, including sediment microbes able to improve the physical and chemical properties of their media. The identified swamp microbes include Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. (Wijayanti et al 2018). Bacteria (*Bacillus* sp.) with concentrations of 10⁵ CFU mL⁻¹ (Khotimah 2018) and Chlorophyta microalgae with the optimum concentration of 10% of the maximum density (Utami 2019) are able to grow in the fish culture media and they can be used as environmental probiotics. Chlorophyta is a microorganism that can be used as green water in aquaculture media. Wijayanti et al (2018) showed that the use of Chlorophyta increased the level of dissolved oxygen in the pond culture media 60.52% and swamp water culture media 63.63%. The increasing dissolved oxygen is caused by Chlorophyta carrying out photosynthetic activity which produces dissolved oxygen in the culture media. *Bacillus* sp. and *Streptomyces* sp. obtained are proteolytic bacteria that can increase the content of

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NH₃, NO₂⁻, and NO₃⁻ into the media (Yuliani 2017; Saraswati 2018). Balcazar et al (2006) stated that *Bacillus* sp. is an example of efficient probiotic bacteria, used in aquaculture because it is able to convert organic matter into CO₂ used in cell metabolism. Grampositive bacteria, such as *Bacillus* sp. can increase the animal immune system capabilities and also act favorably in improving the quality of the water system (Mohapatra et al 2013). Bernal et al (2017) stated that the combination of *Streptomyces* sp. and *Bacillus* sp. demonstrated a significant immunomodulatory activity by increasing the total number of hemocytes and the activity of Superoxide Dismutase (SOD), which provides a protective effect against *Vibrio harveyi* bacteria by increasing the immunological status of *Penaeus monodon*. Nitrifying bacteria are chemolitho-autotrophic bacteria (for example Nitrosomonas sp., Nitrobacter sp.), which are able to meet their carbon needs through CO₂ fixation (Calvin cycle), and their energy source comes from the oxidation process of reducing ammonia to nitrate. As an example, through the addition of nitrifying bacteria and denitrification, the molasses with a C/N ratio of 10 could decrease in ammonia levels by 28.5% (Yuniasari 2009).

Each of the swamp microbes and nitrifying bacteria has their own advantages and disadvantages, therefore a consortium of swamp microbes and nitrifying bacteria would be more effective. The emergence of a consortium, expected to form cooperative, commensal and mutualistic relationships between microbes, calls for an optimal combination of *Bacillus* sp., *Streptomyces* sp., Chlorophyta and commercial nitrification bacteria. The purpose of this study was to determine combinations of swamp microbes and nitrifying bacteria that can improve the water quality of media in swamp fish production.

Material and Method

The experimental design used was a completely randomized factorial design (RAL) consisting of 2 factors: the first factor with 2 treatments and the second factor with 4 treatments and 3 replications. The first factor is the addition of nitrifying bacteria (PROBAC), namely:

N1- without the addition of nitrifying bacteria (PROBAC);

N2 - with the addition of nitrifying bacteria (PROBAC) 5×10⁶ CFU mL⁻¹.

The second factor is the addition of swamp microbes, namely:

P1- without the addition of swamp microbes;

P2 - provision of 100 ml Chlorophyta (3.43×10⁷ cell L⁻¹) and Bacillus sp. (10⁵ CFU mL⁻¹); P3 - provision of 100 ml Chlorophyta (3.43×10⁷ cell L⁻¹) and Streptomyces sp. (10⁵ CFU

mL-1);

P4 - provision of 100 ml Chlorophyta (3.43×10⁷ cell L⁻¹), Bacillus sp. (10⁵ CFU mL⁻¹) and Streptomyces sp. (10⁵ CFU mL⁻¹).

Bacteria cultivation and propagation. Pure bacterial colonies were obtained from the results of previous studies. The colony was cultivated using nutrient agar (NA) media for *Bacillus* sp. and yeast malt (YM) agar for *Streptomyces* sp. Bacterial colonies were scratched in a petri dish containing NA for *Bacillus* sp. and YM media for *Streptomyces* sp. using the quadrant streak method. Petri dishes were wrapped in wrapping paper and incubated for 2 days at room temperature (28-30°C). A single colony formed on a petri dish was transferred to the agar media and incubated until bacteria grew.

Swamp bacteria grown on NA and YM agar media were multiplied by nutrient broth (NB) media for *Bacillus* sp. and liquid YM for *Streptomyces* sp. As much as 5 mL of suspension were collected in a test tube in order to be cultured in the medium, and then homogenized with a shaker for approximately 2 days for *Bacillus* sp. and 5 days for *Streptomyces* sp., then multiplied from 5 mL to 500 mL. The concentrations of bacteria were measured by Mc Farlan spectrophotometry methods.

Chlorophyta culture. The culture media used for Chlorophyta was a technical fertilizer media consisting of ZA, Urea, TSP, and Gandasil B. All technical fertilizer ingredients were mixed in a 250 mL Erlenmeyer and added to 100 mL of distilled water and then homogenized on a hot plate using a magnetic stirrer and sufficient heat, until all ingredients dissolved. The technical fertilizer media in the Erlenmeyer was sterilized using an autoclave at 121°C for 0.25 hour. Chlorophyta isolates (about 10⁷cell mL⁻¹ in 10 mL stock culture) were put into an Erlenmeyer containing a technical fertilizer media for liquid culture. They cultured during 9 days at the room temperature for scaling up to 1 L.

Preparation of fish rearing media. The container used in rearing was in the form of an aquarium with a size of 30 x 30 x 30 cm³ as many as 24 units. The aquariums were cleaned using potassium permanganate to be sterilized of diseases or parasites. The aquarium was filled with 20 L of swamp water.

Fish culture test. The test organisms used in this study were 12 Channa striata specimens of 5±1 cm each for 20 L of water (Mulyadi 2016). Before stocking, acclimatization was done as an adaptation to the new environment in order to reduce stress on the test organism. After 7 days of stocking, a treatment was applied, consisting of a combination of Chlorophyta isolate (3.43×10⁷ cell L⁻¹), Bacillus sp. (10⁵ CFU mL⁻¹), Streptomyces sp. (10⁵ CFU mL⁻¹) as well as PROBAC nitrification bacteria (5×10⁶ CFU mL⁻¹).

Rearing. The fish culture was maintained for 40 days calculated after the addition of the treatment. During rearing, they were fed at satiation with a frequency of three times a day, by using commercial pellets with 40% protein content.

Chlorophyta abundance. Samplings were carried out at the beginning and end of the study by sub composite methods, in each treatment. Plankton net with 25 µm mesh size was used for 5 L of rearing media by experimental unit (sample of 25 mL). A microscope and "The Marine and Fresh Water Plankton" textbook were used for the observation of the Chlorophyta samples (Davis 1955). Chlorophyta abundance calculation was performed by using the Leackey Drop Microtransect method (American Public Health Association 1989) as follows:

$$N = Z \times \frac{x}{y} \times \frac{1}{y}$$

Where:

N - total number (cell L-1);

Z - number of individuals found;

X - volume of filtered water (25 mL);

Y - volume 1 drop of sample water (0.05 mL);

V - volume of filtered water (5 L).

Bacteria population. The counting of bacterial populations was performed at the beginning and end of rearing with the plate count method on a multilevel dilution incubated at a temperature of 28-30°C for 24 hours. The growing population was determined in a Colony Forming Unit (CFU) and calculated using the following formula (Pepper & Gerba 2004):

Total of Bacteria = Total of colonies
$$\times \frac{1}{dilution \ factor} \times \frac{1}{mL \ sample}$$

Biofloc volume. The biofloc volume measurements were done on the 10 and 40 days after rearing. The floc volume was obtained by collecting a rearing media, by using a glass cone 1L volume, then the floc in the water media was left to settle in the tube for 15-20 minutes.

Survival rate. The percentage of fish survival was calculated by using the following formula (Aliyu-Paiko et al 2010):

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Nt

Where:

 N_0 - number of fish at the beginning of rearing (individuals); Nt - number of fish at the end of rearing (individuals).

Absolute weight growth. Growth of fish weight during rearing was calculated by using the following formula (Hopkins 1992):

 $W = Wt - W_0$

Where:

W - growth of weight of fish for rearing (grams);

Wt - weight of fish at the end of rearing (grams);

Wo - weight of fish at the beginning of rearing (grams).

Absolute length growth. The absolute length growth of fish during rearing was determined by doing the following calculation (Hopkins 1992): $L = Lt-L_0$

Where:

L - growth of absolute length of fish for rearing (cm); Lt - length of fish at the end of rearing (cm);

Lo - length of fish at the beginning of rearing (cm).

Feed efficiency. According to NRC (1977) feed efficiency can be calculated by using the formula:

$$EP = \frac{((Wt + D) - Wo)}{E} \times 100$$

Where:

EP - feed efficiency (%);

Wt - weight of fish at the end of rearing (gram);

Wo - initial fish rearing weight (gram);

D - weight of fish that died during rearing (gram);

F - amount of feed given (grams).

Water quality. Measurement of water quality data for C. striata rearing media included pH (pH meter), dissolved oxygen (DO meter), ammonia (spectrophotometry), and biological oxygen demand (DO meter) at the beginning and 40 days later, at the end of the rearing.

Data analysis. Research data including biofloc volume, survival, growth, feed efficiency, water quality was statistically processed by using the variance analysis. If the results of the variance analysis showed that the treatment has a significant effect, then it was continued with the LSD test (the Least significance difference) 5%. Chlorophyta abundance data and the total bacterial population were analyzed descriptively.

Results and Discussion

Chlorophyta abundance, total bacterial population and floc volume. Chlorophyta abundance data on rearing media are presented in Table 1. Chlorophyta density at each treatment decreased after 40 days of rearing. Chlorophyta added in the rearing media experiences death or predation. In the rearing media, a food chain system occured between Chlorophyta and zooplankton (Figure 1), resulting in a decrease in the population of Chlorophyta due to predation.



AACL Bioflux, 2020, Volume 13, Issue 2. http://www.bioflux.com.ro/aacl Commented [WU1]: Please delete the %, it is incorrect. It's Done. Thank you Figure 1. Biofloc and Chlorophyta profile in the rearing media of Channa striata culture in this study (40 magnificaton scale of microscope).

The pattern of the interactions between zooplankton and phytoplankton is a series of eating and prey relationships forming the path of the food chain. Phytoplankton as primary producers is eaten by zooplanktons, in turn zooplanktons are eaten by small fish at higher trophic levels (Bouman et al 2003).

Table 1

Chlorophyta abundance in Channa striata rearing media at 0, 10, 40th day

Commercial	and the second decision of the	Chlorophyta abundance (cell L ⁻¹)			
nitrification bacteria	Swamp microbes	0 day	10 th day	40 th day	
	P1	3.20×103	3.20×10 ³	2.10×10 ³	
N1	P2	3.60×103	3.43×107	4.10×103	
	P3	4.10×103	3.43×107	4.10×103	
	P4	3.70×10 ³	3.43×10 ⁷	4.46×10	
	P1	4.00×103	4.00×103	2.10×103	
10	P2	3.60×103	3.43×107	2.41×103	
N2	P3	3.90×103	3.43×107	2.34×103	
	P4	3.40×103	3.43×107	4.03×10 ³	

The total bacterial population in the rearing media is presented in Table 2, the results of the LSD test of the floc volume at ten and forty days of rearing are showed in Table 3 and Table 4, respectively.

Total bacterial population in the rearing media

Table 2

Commercial	Swamp	Total bacterial population (CFU mL ⁻¹)					
nitrification bacteria	microbes	0 day	1 st day	20 th day	40 th day		
	P1	6.60×104	6.78×104	1.55×10 ⁵	6.20×10 ³		
N1	P2	6.20×104	3.95×10 ⁶	6.93×10 ⁶	2.77×105		
	P3	7.00×104	3.28×10 ⁶	7.53×10 ⁶	3.01×10 ⁵		
	P4	4.70×104	5.59×107	1.00×10 ⁸	2.99×10 ⁶		
	P1	7.10×10 ⁴	2.01×107	3.54×107	1.42×10 ⁶		
N2	P2	4.50×104	3.29×107	5.59×107	1.68×10 ⁶		
	P3	4.30×104	4.99×107	6.41×107	1.93×10 ⁶		
	P4	4.95×104	4.70×107	8.75×107	4.06×10 ⁶		

Based on Table 2, the total bacterial population increased on 20th day and decreased until the 40th day. The increase in population on the 20th day can be caused by adequate nutrients addition in the rearing media, stimulating the metabolic activity and growth of the bacteria and Actinomycetes, while the decline of bacteria population observed on the 40th day could be caused by the nutrient depletion (macronutrient and micronutrient) in the water.

Related to the factor of nitrification bacteria, the results of LSD at 10 and 40 days after rearing start showed that the volume of floc in the media without treatment was significantly higher compared to the treatment with nitrification bacteria. The addition of nitrifying bacteria cannot increase the volume of floc, because there were less exhausted bacteria in the media. The bacteria need more substrate from unconsumed feed of fishes, while the nitrifying bacteria accelerated the process of dissolving nitrogenous organic matter from the waste. Related to the factor of microbial addition from swamps, the

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volume of floc on the media treated with a combination of Chlorophyta, Bacillus sp. and Streptomyces sp. significantly showed higher levels compared to other treatments.

Table 3

Table 4

The single effect of	The single	(P) (not si	The main effects of nitrifying bacteria (N)		
nitrifying bacteria (N)	P1	P2	P3	P4	(LSD0.05=4.386)
N1 N2	11.111 10.000	16.666 10.000	13.332 13.332	26.668 16.667	16.944 ^b 12.500°
The main effects of swamp microbes (P) (LSD _{0.05} =3.102)	10.556ª	13.333ª	13.332ª	21.667 ^b	

The results of LSD test of the floc volume in the rearing media at 10 days of rearing

The results of LSD test floc volume of rearing media at 40 days of rearing

The single effect of	The single	e influence (P) (LSDo.	The main effects of nitrifying bacteria (N)		
nitrifying bacteria (N)	P1	P2	P3	P4	(LSD0.05=3.315)
N1	11.112ª	26.667b	16.667ª	38.889°	25.834 ^b
N2	13.333°	13.333ª	23.333 ⁰	33.333°	20.833°
The main effects of swamp microbes (P) (LSD0.05=4.689)	12.223°	20.000 ^b	20.000 ^b	41.111¢	

It is presumed that certain types of microorganisms are predisposed for flocs forming. Related to the influence of interactions between factors at 40 days of rearing, the observations showed that the treatment combination of Chlorophyta, Bacillus sp., Streptomyces sp. without nitrifying bacteria determined the highest volume of floc, 38.89 mL L-1, but it was not significantly different from the treatment combination of Chlorophyta, Bacillus sp., Streptomyces sp. with nitrifying bacteria. The volume of floc in this study was lower than the study from Mulyadi et al (2016), where in treatment with stocking density of 450 C. striata m3 which was kept for 41 days resulted in a floc volume of 40.7 mL L⁻¹. It is presumed that the rearing media lacks a carbon source used by bacteria for the floc formation. According to Panigrahi et al (2019), Litopenaeus vannamei cultivation without a biofloc system can produce a volume of floc of 4.53 mL L1, which is lower than the cultivation of L. vannamei with a biofloc system, by adding molasses. Floc thickness will continue to increase along with the provision of bacteria and carbon sources that are continuously balanced with feed metabolic waste producing ammonia. The bacteria could bind to ammonia and will form a biofloc (Sitohang et al 2018).

The results of the analysis of variance showed that the effect of the interaction between factors and the effect of the factor defined by the addition of swamp originated microbes on the survival of *C. striata* varied significantly between treatments, but the factor defined by the adding of commercial nitrification bacteria had no significant effect. Based on further testing of LSD on microbial factors from swamps, in the rearing media treated with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp., the survival rate was significantly higher than for the other treatments. Interactions between factors showed that in the rearing media with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, the survival rate reached 63.94%, a significantly higher performance compared to the other treatments.

Based on the results of the survival percentage, it is showed that the combination of swamp microbes is able to suppress unfavorable microbes, to improve the water quality in the rearing media and to increase the *C. striata* survival rate. This is shown in the treatment of rearing media consisting in a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, which provided a *C. striata* survival rate of 63.94%. The combination of *Bacillus* sp. and *Streptomyces* sp. was able to provide more protection against unfavorable microbes in the media, the presence of *Bacillus* sp. giving effect to *Streptomyces* sp. to produce antimicrobial compounds. Luti & Mavituna (2011) explained that *Bacillus* cultured together with *Streptomyces* increased the production of antimicrobial compounds when it was compared with the single genus culture.

LSD test of survival rate of Channa striata

Table 5

The single effect of	The single	e influence (P) (LSDo	The main effects of nitrifying bacteria (N)		
nitrifying bacteria (N)	P1	P2	P3	P4	(not significant)
N1 N2	26.06* 31.75*0	36.91 ^b 28.03*	28.03ª 35.16 ^b	63.94 ^d 48.20 ^c	38.74 35.79
The main effects of swamp microbes (P) (LSD0.05=4.25)	28.91*	32.47*	31.59ª	56.07 ^b	

The N1P1 treatment (without the addition of microbes from swamps, neither of commercial nitrification bacteria) was the treatment with the lowest survival value of 26.06% compared to other treatments. These results prove that the addition of swamp origin microbes to rearing media can increase the survival rate of C. striata. This is in line with the results of Hartini et al (2013), suggesting that the addition of EM-4 probiotics to the rearing media had a significant influence on the survival of C. striata. The average survival of C. striata with 10 μ L L⁻¹ week⁻¹ EM-4 probiotics 96.66% tended to be higher, compared to control treatments (without EM-4 probiotics) which was 8.89%. Budianto & Heny (2017) stated that the bacteria Bacillus sp. has bacteriocin compounds with specific inhibiting action on the growth of S. iniae and P. fluorescens. Streptomyces bacteria has the potential to control pathogenic bacteria by means of competition, parasitism or by producing secondary metabolite compounds (Lutfi 2018). The combination of the two microbes can provide a high percentage of survival compared to the no combination scenario. According to Irianto & Austin (2002), probiotic bacteria can increase the survival or reduce mortality through the development of the immune system due to an increasing phagocyte and lysozyme activity, thereby suppressing pathogenic bacterial colonies. Sanchez et al (2014) stated that probiotics can increase immune stimulation in fish, as a protection against pathogenic bacteria that causes death in fish culture.

Feed efficiency, absolute weight and length growth. The results of the analysis of the efficiency of various feed on *C. striata* showed that the interaction between factors, the microbial addition factors from swamps and the addition of commercial nitrification bacteria, can increase the value of fish feed efficiency, which is significantly affected by the treatment type and concentration. LSD test results of the efficiency of fish feed for 40 days of rearing are presented in Table 6.

Table 6

LSD test result of the efficiency of Channa striata feed for 40 days of rearing

The single effect of	The singl	e influence (P) (LSDo	The main effects of nitrifying bacteria (N)		
nitrifying bacteria (N)	P1	P2	P3	P4	(LSD _{0.05} =1.66)
N1	18.93*	47.34 ^d	37.97	59.65*	40.97*
N2	22.00*	29.52 ^b	34.89	44.11 ^d	32.63 ^a

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The main effects of					
swamp microbes (P) (LSD _{0.05} =2.35)	20.47ª	38.43 ^b	36.43 ^b	51.88 ^c	

Based on these results, the effects of the different combinations on the *C. striata* feed efficiency could be observed. The value of the *C. striata* feed efficiency in the treatment of the media of rearing was: (1) significantly higher without commercial nitrification bacteria than for the treatment with nitrification bacteria; (2) significantly higher with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp., compared to other treatments and interactions between factors; (3) significantly higher with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria than the results of other treatments. It is thought that swamp microbes added to the media enter the digestive tract of *C. striata* during the process of respiration and while eating. Chlorophyta that enters the digestive tract could be a natural food source, while *Bacillus* sp. and *Streptomyces* sp. which entered the digestive tract could be working together to improve their digestibility. High digestibility in the feed will increase the absorption of nutrient, which at optimal levels will increase the value of feed efficiency, favoring fish growth. *Bacillus* sp. is a bacterium that can increase digestibility because it can secrete protease, lipase and amylase enzymes (Singh et al 2016).

The bacteria which are members of the genus Bacillus are known to produce a wide variety of antimicrobial substances and bacteriocins that can suppress pathogenic bacteria (Deghhanifar et al 2019). Streptomyces sp. is a genus of actinomycetes that can produce various antibiotic compounds. Common antibiotic compounds produced by Streptomyces have restrictions such as narrow range spectrum, low permeability to specific tissues, and toxicity for the live organisms, as human body (Dehghanifar et al 2019). It has the potential to control pathogenic bacteria by conducting competition, parasitism or by producing secondary metabolites (Lutfi 2018). The bacterium Bacillus sp. increases the value of feed efficiency by secreting enzymes that can stimulate digestion, while Streptomyces sp. secretes antibiotics able to suppress pathogens. Both bacteria work together to improve the digestibility and immunity of C. striata, which ultimately results in high feed efficiency. Bacterial activity in the digestive tract will experience rapid fluctuations with microbes entering through feed or water and causing changes in the intestinal microbial balance. The entry of these microbes is antagonistic to the pathogenic microbes in the digestive tract, improving growth, protein efficiency ratio and feed efficiency (Midhun et al 2018; Nargesi et al 2019).

Swamp microbial addition factor, commercial nitrification bacteria addition factor, and interactions between factors influenced the feed efficiency in a significantly different manner, depending on the treatment. Based on the results of the LSD0.05 test (Table 7) on the main effect of the addition of commercial nitrification bacteria, the increase of the absolute weight of *C. striata* following the treatment of the rearing media was: (1) significantly higher without commercial nitrification bacteria than with commercial nitrification bacteria; (2) significantly higher with the addition of a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. than with the other treatments; (3) significantly higher than the other treatments' results with a combination of Chlorophyta, *Bacillus* sp. and without commercial nitrification bacteria, but (4) not significantly higher from the treatment results given a combination of Chlorophyta, *Bacillus* sp. and without commercial nitrifying bacteria.

Table 7

The single effect of	The single influence of swamps microbes (P) (LSD _{0.05} =0.08)				The main effects of nitrifying bacteria (N)
nitrifying bacteria (N)	P1	P2	P3	P4	$(LSD_{0.05}=0.04)$
N1	1.30ª	2.26 ^f	1.70°	2.32 ^f	1.90 ^b
N2	1.73c	1.41 ^b	1.88 ^d	2.08e	1.78ª
The main effects of	1.51ª	1.84 ^b	1.79 ^b	2.20°	

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LSD 0.05 test results of growth in absolute weight of Channa striata

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swamp microbes (P)	
(LSD0.05=0.05)		

Based on the results of the variance analysis, swamps microbial addition factor and interactions between factors significantly influenced the increase of the absolute length, but the factor of adding commercial nitrification bacteria has no significant effect. The LSD results of growth in absolute length of *C. striata* are presented in Table 8.

Table 8

LSD test results for growth in the absolute length of Channa striata

The single effect of	The single	e influence (P) (LSD ₀	microbes	The main effects of nitrifying bacteria (N)	
nitrifying bacteria (N)	P1	P2	P3	P4	(not significant)
N1 N2	0.69ª 1.13º	1.79 ^e 1.08 ^c	0.91 ^b 1.60 ^d	2.27 ^f 1.74 ^e	2.12 2.09
The main effects of swamp microbes (P) (LSD _{0.05} =0.07)	0.91ª	1.44 ^c	1.265	2.00 ^d	

The main influence of the addition of microbes on the increase of the absolute length of snakehead fish in the treatment of rearing media with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. was significantly different than in other treatments. The influence of interactions between factors on the increase of the absolute length of *C. striata* was significantly higher in the treatment of rearing media with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria than the in the results of the other treatments.

The addition of a combination of swamp microbes and commercial nitrification bacteria gave significant results on the increase of absolute weight and absolute length. The highest absolute weight and length increase was produced by the treatment of rearing media with a combination of Chlorophyta, Bacillus sp., Streptomyces sp. and without commercial nitrification bacteria, and the lowest by the treatment without the addition of microbes. The treatment without the addition of microbes is thought to be the less effective in terms of absorption of nutrients in the feed, causing a sub-optimal growth, compared to the other treatments. In the treatment of N1P4, swamp microbes added to the media could be entered into the digestive tract of C. striata during the respiration process and when eating. These microbes break down complex compounds into simpler ones and increase digestibility of feed, by accelerating the process of absorption of the food. The work of probiotics in aquaculture is the ability of microbes to break down proteins, polysacarides, lipids and stress resistance in aquaculture system (de melo Pereira et al 2018). The addition of 10⁴ CFU mL⁻¹ probiotics to the rearing media increased the length and weight of pacific white shrimp (L. vannamei) larvae, compared to the controls (Widarnani et al 2010). Bacillus licheniformis at 105 CFU mL-1 in the rearing media of Pangasius hypophthalmus showed a significant increase in the growth, immune and antioxidant responses compared to 107 CFU mL⁻¹ (Gobi et al 2016).

Dissolved oxygen and ammonia in the rearing media. The results of the analysis of variance on day 0 showed that the factor of commercial nitrification bacteria, swamp microbes and the interaction between the factors did not significantly influence the dissolved oxygen content (Table 9). At 5 and 10 days after rearing start, the factor of commercial nitrification bacteria and swamp microbes significantly affected the dissolved oxygen content, but the interaction between the factors had no significant effect.

The results of dissolved oxygen in the rearing media at 5 and 10 days after rearing start showed that the main effect of the addition of commercial nitrification bacteria was significantly lower than without commercial nitrification bacteria.

The main influence of the addition of microbes from swamp on the dissolved oxygen in the rearing media was the best for dissolved oxygen concentration in culture

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AACL Bioflux, 2020, Volume 13, Issue 2. http://www.bioflux.com.ro/aacl media with a combination of Chlorophyta, Bacillus sp., and Streptomyces sp. (P4), than in other treatments.

Table 9

LSD test result of dissolved oxygen in the rearing media

				Dissoh	ied oxyge	$n (mg L^{-1})$	6			
	Days after rearing									
-	0	5	10	15	20	25	30	35	40	
LSD				0.18	0.14	0.14	0.18	0.18	0.18	
N1P1	3.60	3.53	3.53	3.40	3.10	3.00	2.97	2.77	2.67	
N1P1	±0.1	±0.1	±0.1	±0.1 °	±0.1 °	±0.1 °	±0.1 °	±0.1 °	±0.1	
N1P2	3.63	3.53	3.53	3.23	2.93	2.83	2.77	2.57	2.47	
N1F2	±0.1	±0.1	±0,1	±0.1 **	±0.1 *b	±0.1 ^{ab}	±0.1 ab	±0.1 **	±0.1 ª	
N1P3	3.67	3.57	3.47	3.33	3.03	2.93	2.87±	2.67±	2.57	
MTP3	±0.1	±0.2	±0.2	±0.1 %	±0.1 ™	±0.1 bt	0.1 °C	0.1 bc	±0.1 b	
N1P4	3.57	3.70	3.60	3.60	3.50	3.40	3.40	3.20	3.10	
1471-4	±0.1	±0.1	±0.1	± 0.1 d	±0.1 °	±0.1 *	±0.1 "	±0.1*	±0.1*	
N2P1	3.63	3.43	3.43	3.13	2.83	2.73	2.67	2.47	2.37	
N2F1	±0.1	±0.1	±0.1	±0.1*	±0.1*	±0.1*	±0.1 "	±0.1 *	±0.1	
N2P2	3.57	3.47	3.47	3.33	3.03	293	2.87	2.67	2.57	
NZFZ	±0.1	±0.1	±0.1	±0.1 ^{bc}	±0.1 °	±0.1 bt	±0.1 %	±0.1 bc	±0.1 ^b	
N2P3	3.53	3.43	3.33	3.27	3.10	3.00	2.90	2.70	2.60	
NZP3	±0.1	±0.1	±0.1	±0.1 m	±0.1 °	±0.1 °	±0.1 bc	±0,1 bc	±0.1	
N2P4	3.67	3.63	3.53	3,40	3.30	3.20	3.20	3.00	2.90	
1421-4	±0.1	±0,1	±0.1	±0.1 °	±0.1 d	±0.1 ^d	±0,1 d	±0.1 d	±0.1	
LSD		0.08	0.08	0.09	0.07	0.07	0.09	0.09	0.09	
N1	3,62	3.58°	3.53 ^b	5.09 ^b	3.14 ^b	3.04 ^b	3.00 ^b	2.80 ^b	2.70 ^b	
N2	3.60	3.49*	3.44*	4.93°	3.07ª	2.97°	2.91ª	2.71*	2.61*	
LSD	04000	0.11	0.11	0.13	0.10	0.10	0.13	0.13	0.13	
P1	3.62	3.48ª	3.48 ^{ab}	3.27ª	2.97ª	2.87°	2.82ª	2.62°	2.52ª	
P2	3.60	3.50*	3.50 ⁱⁿ	3.28*	2.98*	2.88ª	2.82*	2.62*	2.52*	
P3	3.60	3.50"	3.40°	3.30°	3.07°	2.97*	2.88ª	2.68"	2.58*	
P4	3.62	3.67	3.570	3.50°	3.40°	3.30 ^p	3.30 ^b	3.10 ⁿ	3.00 ^b	

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Ten days after rearing start, the P4 treatment had a significantly higher dissolved oxygen content than the P3 treatment, but it was not significantly different from the P1 and P2 treatments. The factors of commercial nitrification bacteria, microbial addition and their interaction significantly influenced the dissolved oxygen content. The dissolved oxygen test on the 15, 20, 25, 30, 35 and 40th days are presented in Table 9.

Ammonia analysis results on day 0 were not significantly influenced by the factor of commercial nitrification bacteria, addition and their interaction. The variance analysis results on days 5, 10, 15, 20, 25, 30, 35 and 40 showed that the factor of commercial nitrification bacteria, microbial addition and their interaction significantly affected ammonia content. They are presented in Table 10. The results of LSD test on the 5, 10, 15, 20, 25, 30, 35 and 40th days on the main effect of the addition of commercial nitrification bacteria showed that the ammonia content in the rearing media with commercial nitrification bacteria was significantly lower than in the treatments without commercial nitrification bacteria. The main effect of the addition of microbes from the swamp suggested that the ammonia content in the rearing media with a combination of Chlorophyta, Bacillus sp. and Streptomyces sp. was the best for reducing ammonia concentration of rearing media compared to the other treatments. The results of LSD on the 5, 10, 15 and 20th days, in the scenario of the interaction between factors, demonstrated that the N1P4 treatment had significantly lower ammonia levels compared to the other treatments, but not significantly different from N2P2 treatment. The results of LSD on the 25, 30, 35 and 40th days in the scenario of the interaction between factors,

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suggested that the N1P4 treatment had significantly lower ammonia levels compared to other treatments, but not significantly different from N2P2 and N2P4 treatments.

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LSD test results for ammonia every 5 days on the rearing media

			Me		ionia concer		71.1)			
	Days after rearing									
	0	5	10	15	20	25	30	35	40	
LSD		0.013	0.013	0.014	0.013	0.020	0.021	0.019	0.022	
N1P1	0.290	0.383	0.393	0.410	0.327	0.540	0.674	0.691	0.948	
HTLT.	±0.07	±0.01*	±0.01*	±0.01¢	±0.01*	±0.01*	±0.01=	±0.01'	±0.02	
N1P2	0.323	0.273	0.283	0.223	0.203	0.293	0.314	0.321	0.32	
NIPZ	±0.03	±0.01#	±0.01 °	±0.014	±0.01°	±0.01 ^b	±0.01 ^b	±0.01°	±0.01	
N1P3	0.283	0.257	0.267	0.207	0.197	0.363	0.482	0.585	0.692	
nurs.	±0.01	±0.01¢	±0.01*	±0.01 ^b	±0.01 ^{1sc}	±0.021	±0.02 ^d	±0.01*	±0.02	
N1P4	0.267	0.220	0.230	0.170	0.147	0.243	0.260	0.265	0.27	
MTP4	±0.01	±0.01°	±0.01*	±0.01°	±0.01°	±0.02*	±0.02°	±0.02*	±0.02	
N2P1	0.230	0.253	0.263	0.203	0.193	0.282	0.363	0.385	0.39	
NZP1	±0.01	±0.01¢	±0.01*	±0.01*	±0.01 m	±0.02 [®]	±0.02<	±0.01 °	±0.02	
N2P2	0.290	0.233	0.243	0.183	0.160	0.250	0.268	0.273	0.27	
NZPZ	±0.04	±0.01 ^{ab}	±0.01ª	±0.01*	±0.01*	±0.01*	±0.01 ^a	±0.01*	±0.01	
N2P3	0.250	0.277	0.287	0.227	0.187	0.277	0.296	0.300	0.30	
MZP3	±0.02	±0.01=	±0.01 t	±0.015	±0.01 b	±0.01 ^b	±0.01 ^b	±0.01 b	±0.01	
N2P4	0.303	0.237	0.247	0.187	0.163	0.250	0.268	0.273	0.27	
NZP4	±0.03	±0.01 ³	±0.01*	±0.01°	±0.01 b	±0.01*	±0.01*	±0.01*	±0.01	
LSD		0,006	0.006	0.007	0.006	0.010	0.011	0.010	0.01	
N1	0.291	0.283 ⁰	0.293°	0.253 ^p	0.218 ^p	0.360 ^µ	0.432 ^b	0.466 ^a	0.558	
N2	0.268	0.250*	0.260*	0.200°	0.176°	0.265*	0.299°	0.308*	0.313	
LSD		0.009	0.009	0.010	0.009	0.014	0.015	0.013	0.01	
P1	0.260	0.3184	0.328 ⁴	0.307 ^d	0.260 ⁴	0.411 ^d	0.518 ^d	0.5384	0.670	
P2	0.307	0.253*	0.263 ^b	0.203 ^b	0.182 ^b	0.272	0.291 ^b	0.297	0.300	
P3	0.267	0.267	0.277	0.217	0.192	0.320	0.389	0.443	0.499	
P4	0.285	0.228*	0.238*	0.178°	0.155°	0.247*	0.264*	0.269*	0.274	

Related to the factor of adding nitrifying bacteria, the lowest ammonia content was observed in the treatment with nitrifying bacteria. It is assumed that the added nitrification bacteria are able to carry out the process of nitrification on the rearing media. According to Rurangwa et al (2014), nitrification takes place through 2 reaction stages, where in the first stage the oxidation of ammonium to nitrite is carried out by ammonium oxidizing microbes (*Nitrosomonas* sp.) and in the second stage nitrite oxidation is performed by nitrite oxidizing microbes (*Nitrosomonas* sp.). Among the addition of swamp microbial factors, the lowest ammonia content was observed in the treatment with a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. It is suspected that the three microbes could break down ammonia. Ammonia is the main source of nitrogen in addition to nitrate, which could be used by microalgae for their metabolic processes (Nurhayati et al 2014). *Bacillus* sp. could oxidize ammonia to nitrite through heterotrophic and chemotrophic processes (Edwards 2011).

Among the factors' interactions, N1P4 treatment had the lowest ammonia levels on the 5^m day until the end of rearing. It was suspected that microbes from the swamp were able to break down the organic material, derived from feces or feed, into compounds that were not harmful to *C. striata*. The N1P4 results were not significantly different from the N2P2 and N2P4 results. In presence of the commercial nitrification bacteria in the same ecosystem as *Bacillus* sp. and *Streptomyces* sp., the nitrification process activity and the growth of nitrifying bacteria are inhibited. Nitrifying bacteria (autotrophic bacteria) produce very small biomass and slow growth. Nitrifying bacteria

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takes 12 hours to regenerate, while heterotrophic bacteria *Bacillus* sp. and *Streptomyces* sp. only need 30 minutes (Ebiling et al 2006). If there is a limited nitrogen to carbon (high C:N ratio), the nitrification process is inhibited and heterotrophic bacteria develop rapidly. Heterotrophic bacteria will assimilate ammonia directly into bacterial proteins, and they will develop rapidly. It will cause competition in the struggle for oxygen, space and nutrients with autotrophic bacteria (Blancheton et al 2013).

Ammonia levels in all treatments with microbes on 10th day to 20th day of rearing decreased and increased until the 40th day. On the 10th to 20th day, it was presumed that the ammonia accumulation from metabolic waste had not yet occurred, therefore the microbes given to the rearing media were able to work optimally. On the 25th to 40th day, it was supposed that the ammonia accumulation from metabolic waste had been accumulated in the rearing media, so that the added microbes couldn't optimally reduce their ammonia levels. Meanwhile, the treatment without the addition of microbes (N1P1) experienced an increase in ammonia along with the increase in the rearing time. Increasing ammonia levels in the N1P1 treatment was caused of the rearing without siphoning during 40 days. It resulted in a buildup of organic material derived from metabolic waste and levels of ammonia in the rearing media. The decomposing bacteria from natural swamp water added to the media have not been able to make an optimal decomposition.

Conclusions. The addition of swamp microbes Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. on the *C. striata* rearing media was more efficient than other treatments because they provided better water quality values and gave the best survival rate, feed efficiency and growth of *C. striata* in swamp aquaculture, although there was no nitrification bacteria used. *Bacillus* and *Streptomyces* were the best combination of microbial swamp for *C. striata* culture in swamp water aquaculture which used Chlorophyta as green water system.

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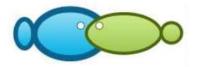
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Optimization of striped snakehead fish (*Channa striata*) culture using swamp microbial combination and nitrification bacteria

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Abstract. Utilization of swamp areas as fish culture locations alters water quality. Therefore, it is necessary to improve the water quality with environmental friendly biological treatments, such as the addition of microbes as probiotics in media culture. The purpose of this study was to determine the combination of microbes from swamps that can improve the water quality of the swamp fish culture and production media. The research used Completely Randomized Design (CRD) factorial with two factors consisting of the first factor with two treatments and the second factor with four treatments and three replications. The first factor consisted of two scenarios: (1) without the addition of nitrification bacteria (NOBAC) $S \times 10^6$ CFU mL⁻¹ (N2). The second factor consisted of four scenarios: (1) without the addition of swamp microbes (P1) and with the addition of: (2) *Chlorophyta* (3.43×10⁷ sel L⁻¹) and *Bacillus* sp. (10⁵ CFU mL⁻¹) (P2); (3) *Chlorophyta* (3.43×10⁷ sel L⁻¹) and *Streptomyces* sp. (10⁵ CFU mL⁻¹) (P4). The result showed that the addition microbes from swamps in the combination of N1 and P4 treatment scenarios was able to improve the water quality value better than the other treatment scenarios, producing the best survival rate (63.94%), feed efficiency (59.65%), absolute weight growth (2.32 g) and absolute length growth of (2.27 cm). **Key Words**: fish culture, biological treatments, problotic, water quality.

Introduction. The swamp aquaculture must improve and maintain the water quality for fish rearing media, through environmentally friendly biological treatments, since the wastewater of fish rearing on swamps reduces the quality of swamps water. One of the treatments is adding probiotics in the rearing media. Irianto & Austin (2002) stated that environmental damaging can be prevented with probiotics able to degrade the organic materials in the habitat. Hartini et al (2013) showed that the addition of probiotics at a dose of 10 μ L L⁻¹ week⁻¹ can improve and maintain optimal water quality. In other studies, the addition of effective probiotic microorganisms can reduce ammonia levels and suppress the population of pathogenic microorganisms from the culture media (Trisna et al 2013).

Swamps have high biodiversity, including sediment microbes able to improve the physical and chemical properties of their media. The identified swamp microbes include Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. (Wijayanti et al 2018). Bacteria (*Bacillus* sp.) with concentrations of 10⁵ CFU mL⁻¹ (Khotimah 2018) and Chlorophyta microalgae with the optimum concentration of 10% of the maximum density (Utami 2019) are able to grow in the fish culture media and they can be used as environmental probiotics. Chlorophyta is a microorganism that can be used as green water in aquaculture media. Wijayanti et al (2018) showed that the use of Chlorophyta increased the level of dissolved oxygen in the pond culture media 60.52% and swamp water culture media 63.63%. The increasing dissolved oxygen is caused by Chlorophyta carrying out photosynthetic activity which produces dissolved oxygen in the culture media. *Bacillus* sp.

and Streptionnors sa, obtained are proteining balance that can increase the content of this, NO₂, and NO₂ into the model (Turani 2017). Survival, 2018; Balance et al. (2016) account of the content of the conte

The experimental deliga used was a completely microardized factorial design (RAL) consisting of 2 factors: the first factor with 2 transmissed and the expense factor with 4 headmarks and 3 replantations. The first factor is the addition of highlying bacterial (PROMC), samely: M1-without weddeen of nethying bacteria (PROMC); M1-without weddeen of nethying bacteria (PROMC); M1-without weddeen of nethying bacteria (PROMC); M1-without weddeen of nethying bacteria, (PROMC); M2-without weddeen of networp microbac; M2-provideo of 100 mic Observations (3.45-10° eff. L¹) and Structures sp. (10° OU mic.¹); M3- provideo of 100 mic Observations (3.45-10° eff. L¹) and Structures sp. (10° OU mic.¹); M3- provideo of 100 mic Observations (3.45-10° eff. L¹) and Structures sp. (10° OU mic.¹); M3- provideo of 100 mic Observations (3.45-10° eff. L¹), Studies sp. (10° OU mic.¹); M3- provideo of 100 mic Observations (3.45-10° eff. L¹), Studies sp. (10° OU mic.¹); M3- provideo of 100 mic Observations (3.45-10° eff. L¹), Studies sp. (10° OU mic.¹); M3- provideo of 100 mic Observations (3.45-10° eff. L¹) and Structures sp. (10° OU mic.¹); M3- provideo of 100 mic Observations (3.45-10° eff. L¹).

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Chlorophyte culture. The nature media used for Chlorophyte up, was a lucticical Antibiae media consisting of 25, Ures, TSP, and Gaussail 8. All bechnical Antibiae impredients ware mixed in a 256 mL Priormeyer and added to 100 mL of childine autor

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Rearing. The fish culture was maintained for 43 time calculated after the addition of the instances. During rearing, they were fiel at solution with a frequency of three times a law, by using conversion petiets with 42% protein content.

Chierophysis absorbance. Samplings were carried out at the beginning and wait of the table to you consolvely nettable, in each treatment, flexation, lew with 25 µm nettable, no wait uses for 52.1 if having models to constraint at all on the order of 25.1.1. A nettremote and "The Manne and Free Wester Paratacer flexations were used for the observation of the Chierophysis tamples (Daving 155). Chierophysis attachates, calculators was and three the user to be too the solution of the observation of the Chierophysis attachates (Daving 155). Chierophysis attachates was and three to user the too the solution of 55.1. Chierophysis attachates was and three to use the too the solution of the solution of the solution of the and the solution of the solu

 $W=T\times \frac{\pi}{2}\times \frac{1}{2}$

have: - total number (cell 1, "), - number of individuals found; - situate of fidewold water (25 mL); - solute is in fidewold water (5 L), - obtaine of fidewold water (5 L).

Becker's populative. The southing of beckeris populations was performed at the segments and and of neurons with the plane cause events on a mathematication incuberier of a secondaria or 2007; for 24 hours. The growing population was distantioned in a Coord Parining Unit (CTU) and calculated using the Moosing formula (Payer 6 Science 1994).

Total of Bostova + Total of solution $r \frac{1}{distant} = \frac{1}{r}$

Biefsc values. The bolics where measurements were done on the 10 and 40 days after moving. The fac values are obtained in collecting a moving media, by using a gives one 11 values, then the fac in the water media was left to write in the table for 15-20 meanies.

Barwhal rate. The percentage of this survival was concerned by using the following formula (Algor-Parko et al 2012): No - 100%

Where: No - rounder of fully at the beginning of rearing (Individuality)

MG Baller, 2008, Natura 23, United 5, 1999

The pattern of the interactions between rooptaneton and phytoplashton is a series sating and pray matiocompa forming the path of the food chain. Phytoplashton primary produces in sultant by coopelestions, in our pooplashtons are eaten by untail fo at higher traphic levels (Bournan et al 2003).

Commercial	24-010100A102230	Chlorop	shyra abundanix	: (ces(2:1)
INDISTRASION Bactoria	Swamp microbes	0 day	10 th day	40° day
NL.	#1 #2 #3 #4	3.20×10 ³ 3.60×10 ³ 4.10×10 ³ 3.70×10 ³	3.20×10 ³ 3.43×10 ³ 3.43×10 ⁷ 3.43×10 ⁷	2.10×10 4.10×10 4.10×10 4.46×10
NZ	#1 #2 #1	4.00×10 ³ 3.60×10 ³ 3.90×10 ³ 3.40×10 ³	4.00+10 ³ 3.43×10 ⁷ 3.43×10 ⁷ 3.43×10 ⁷ 3.43×10 ⁷	2.10+10 2.41×10 2.34×10 4.03×10

The tatal Bacterial population in the rearing mode is presented in Table 2, the results of the LSD test of the floc values at test and furty dwys of rearing are showed in Table 3 and Table 4, respectively.

Table 2

Commercial	Same -	Total Decorrect Decorrect Decorrection (175/ ml.)					
Autofluation Autoria	minutes	ii dey	P ^{at} clay	20 th day	40 ^{rt} day		
	P1	0.00×10*	6.78×10*	1.55×10	6.35+10		
NE	P2	6.30×10*	3.65×10 ⁴	6.93×10 ⁺	2.77×10 ⁵		
141	P3	7.00×10*	3.28×10 [#]	7.53×10 ^o	1.01x10*		
	Pá	4.75×10 ⁴	5.59×10 ⁷	1.00×10^{9}	2.99×10*		
	PT	7.10×10*	2.01×10	3.54×10	1.42 + 101		
	P2	4.50×10*	3.29×10	5.59×10	1.66×10^{5}		
PH/	P3	4.30×10*	4.99x10"	6.41×10 ⁺	1.93×10 ⁵		
	PG	4.95+10*	4.70+107	8.75×10 [*]	4.06+10*		

Based on Table 2, the total bacterial population increased on 20% day and decreased until the 40% day. The increase is population on the 20% day can be caused by adequade nutrients addition in the reacting media, stimulating the netbook activity and growth at the bacteria and Actionmycetes, while the decline at bacteria population observed on the 40% tary cauld be caused by the individe higheritor (macromothies) and incrementing the

40° day could be caused by the nutrient depicter (macronizment and incrementation) or the water. Related to the factor of nonfraction bacteria, the insults of LSO at 10 and 40 days after maining that showed that the volume of fact in the media without the transmission significantly higher compared to the transmiss of short here the media without the bacteria in the media. The factor and makes media and the transmission of the bacteria in the media. The factor and makes there even in substantiation bacteria in the media. The factor and makes there even insurantiation of them, while the nitriving bacteria contereas the higher process of displayments organic matter from the wester. Related to the factor of macrobial addition from severage, the volume of face to this media travial number (substantiate) contranted and Scherquerts, add Scherptorycon sp. significantly showed higher levels compared to other tradments.

A41. Sept.o. 1810. with the 11. interal l. . .

Table 1 Divergetyte abundence in Channe attals nearing media at 0, 10, 90° stay

Commercial	24-010100A100230		ohyta abundance	2 (Cell 27)
INDIVERSION	Swamp relations	0 day	10 th step	40° day
	71	3.20 x 10 ⁴	3.20×10 ²	2.10×10 ⁵
2.NE2	#2	3.60×10 ⁹	3.43×10 ²	4.10x10 ³
0.000	#3	4.10×10^{7}	3.43×10^7	9.10+10*
	P4	3.70 < 107	3.43+10	4,46+107
	172	4.00×10 ³	A.00 ± 10 ¹	2.10+10
1000	#2	3.60×10°	3.43×107	2.41×10^{3}
N2	71	3.90×10 ¹	-3.43×10^{11}	2,34×10 ²
		3.40×201	3.43x107	4.03×18 ²

Total bacterial population in the rearing media

Chorephytic advantance, total Aacterial acqualation and fluc volvess. Dranoghyti abundance data on naring madia an presented in Table 1, Choraphyta abunda at wait instrument decremated after of data of rearring. Oncerephytic abunda in the rearring media regolationes bands or protection. In the rearrog media, a flood shall system occurse between Chorephytic and population. (Figure 1), installing in a decrease in the population of Charaghyte data to protection.



Figure 1. Bofloc and Characterist profile in the rearing media of C. strate output in the study (40 magnitudae scale of managed).

Auc), Solling (1932), returns 11, Store 1, Mpc crosses for first coll (Street)

Where: L - growth of ebsolute length of fait for rearing (cm); L2 - length of fait of the end of neuring (cm); L3 - length of fait of the beginning of rearing (cm). Feed efficiency. According to NRC (1977) feed efficiency can be calculated by using the

 $DP = \frac{\langle (W) + D \rangle_{1} \cdot W \phi |}{V} + D D P \phi$

Absolute weight growth. Growth of fish weight during rearing was collulated by using the following formain (Hispains 1962): W=Wt-Ws.

Absolute Anglfi grounds. The absolute impiri provin of bits during mering was intermined by duing the following calculation (Hopkins 1992): La UNLA

Aldo, Stollar, 2020. Volume 13, Intel 3, 1055 (Terrin Indian colt, Includ)

Haterial and Nethod

N - number of fish at the end of reading (individualit).

Mindre: W - growth of weight of faits for rearing (growte)) M1 - avegat of faits at the end of rearing (growte)) M1 - weight of faits at the beginning of nearing (growte).

Water quality. Newsamment of years quality data for C. attace meaning matter visualed ph (ph meter), deserved servers (DO meter), evenese (spectroperionetry), and biological servers demand (DO meter) at the beginning and 46 anys tater, at the end of the rearing.

Geta analysis. Rescarch data including boths: waters, survives, provids, here efficiency, water quarky was shortloading processed by using the writerior analysis. If here institute of the variance analysis showed from the treatment mas in agentation of the institu-ant of the state of the second state of the state synthesized with the LSD test. One and synthesized with the LSD test the lands: synthesized effectives; 5%. Chienceheta simulation data with the state training parallelance were marked executions; 5%. Chienceheta

Results and Discussion

Illhere: DP - Fred efficiency (%); UII - Weight of faiv at the tool of insamig (grant); Was - action fain nearing weight (grant); D - Weight of fain that alled adming conting (grant); F - Annowell of Feed given (grant);

Table 3 The results of LSD text of the floc volume in the rearing media at 10 stays of re

The single affect of	The single	(P) (not si	The main effects of matrifying bacteria (W)		
nitrifying bacteria (W)	P2	P2	P3	14	(150+++4.386)
N1 N2	11.111 10.000	16.666 10.000	13.332 13.332	26.668 16.667	16.944* 12.500*
The main effects of swamp microbes (P) (LSDs.m=3.102)	10.558*	13.333	13.332*	71.657	

Table 4 The results of LSD test floc volume of rearing media at 40 days of rearing

The single effect of	The single	(P) (LSDs	The main effects of intrifuing bacteria (N)		
nitrifying Secteria (W)	42	P2	P3.	14	(150) == 3.315)
N1 N2	11.112*	26.667*	23.333	38.889 31.315	25.834° 20.833°
The main effects of swamp microbes (P) (15% creat 69%)	12.2230	20.0009	29.000*	*1.111*	

Sector in the sector is a sector in the sector in the sector is a sector in the sector is a sector is a sector is a sector in the sector is a sector in the sector is a sector in the sector is a sector i

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AND Bullet, 2020, Velocity 13, Innet 2, 1986 (Venos and An Long)

was able to provide trare protocitien against unfavorable microbics in the tractile, the presence of Buchas as giving effect to Straptorepose up, is produce anthrecoble compandia, Link A Restruct (2011) explained that absolute contracted together with Straptorecost increased the production of arithmicrobial compaunds when it was compared with the single partice andhrat.

Table 5

	LSO test o	r survival r	ste of Char	ine strute	
The single effect of ribifying bacteria (N)	The sings	(P) (LSD-	The main effects of nitribulog bacteria (S		
	N	12	12	PH	(het significant)
N1 H2	25.09° 31.75°	36.91° 28.01°	28.03° 35.16°	63.54 ⁷ 48.20 ⁴	38.74 35.79
The risks effects of swamp (norsbes (P) (15Damed 25)	28.91*	32.475	31.55*	\$6.07°	Santa

(15Dum+6.25) The MIP1 interferent (without the addition of microbes from swemps, mether of commercial information instarting) was the boatment with the lowest survival wate of 26.00% compared to other treatments. These results prove that the addition of average and the results of whith et al. (2013), suggesting that the addition of 6.4- problems to be many metabolic and any support of the survival rate of C. advats. This is in live with the results of whith et al. (2013), suggesting that the addition of 6.4- problems to be many metabolic and any support of the survival rate of C. advats. This is in live with the results of whith et al. (2013), suggesting that the addition of 6.4- problems to compared to control theorem (whith the survival rate of C. advats. The survival compared to control theorem (whith the survival rate of the survival related that the survival rate of 8.4 minutes the survival rate problems and the gravity of 5.1 minutes of 4.6 minutes (3.6 minutes survival relations) societies introduce to the survival comparation, and the profile relations. According to Theorem (1.6 minutes survival comparation) of the box numerodes can minute relativity outputs advator to the number of the provival at relativity index the provider comparation of the box numerodes and provide in the hyperbruck to the survival comparation of the box numerodes and provide and the provide the development of the intensive system are to intensing phopeyte and psocytic advival, thereby suspensing phopering stationary topics as protection oparity pathwent and provider comparation from a substates. The control control is advival, the development of the intensive system intension phopeyte and psocytic advival, thereby suspensing theorem to an intension phopeyte and psocytic advival, thereby suspensing theorem intensive system topics. Second and the substate and factorial control. The results of the columns.

Feed efficiency, absolute weight and length growth. The results of the analysis of the efficiency of various food in C strated straved that the interaction streteen battory, the manufalk analysis of the scenary and the addition of convertence invitation batterin, can increase the value of this field efficiency, which is algorithmently affected by the treatment type and concentration. USD text results of the efficiency of fain field her 40 bays of resting an protected in T 200 efficiency.

Table 6 150 test result of the efficiency of Channa striata feed for #0 days of rearing

The single effect of	The sings	(P) (150)	The main effects of minihing becturie (R)		
nitrifying dectoris (N).	P1.	- 12	P3	P4	(LSOna=1.66)
81 N2	18.93° 22.00°	47.344 29.520	37.97	58,65 ² 44,11 ²	40,979
The main effects of swamp interables (P) (15Daxi=2.35)	35.471	38.432	36.43*	\$1.00	

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LSD 0.05 best results of growth in absolute weight of Channe striate

The single effect of nitrifying bacturia (N)	The sings	IP1/LSDs	The main effects of intrifying bacteria (h		
	P2.	P2	23		(LSD(u)=0.04)
N1 N2	1.30*	2.56/	1.75*	2.32	1.90*
The main effects of swamp microbes (#) (USDs == 0.05)	1.51*	1.84°	1.79*	2.20	

<u>____</u>8

AALS, Highur, 2020, Vitame JJ, Savar & May / Miler Alaffan, com m/deel

Basici un the results of the variance analysis, avantys recursive addition factor and interactions between factors significantly induced the increase of the abolical length, but the factor of adding commental withfactors bacteria has an us significant effect. The USD results of granth is abolical length of C whate are presented in Table B.

LSD test results for gravely in the absolute length of Owner across

The single effect of advictors bacteria (V)	The south	(P) (LSD)	The man effects at minima taxteria /A		
variation become (A)	- 81	- 72 -	11	74	(not significant)
MI MI	0.69*	1.79	0.91*	2.27	2.12
The mate effects of swarep microbes (P) 3550-mat 600	0.91*	1.49	1.39	2.809	

Descripted asyspen and astroactic is the maxing nuclei. The multiplication of the analysis of latitudes on day 0 whereast that the factor of commercial infinitative lasterial, several microbes will be intensized between the factors of our supplicative plasme. Due dissubled asygen content (Table 5), 4.5 and 10 days after reading table, the factor of commercial infinitative between the intensity significant within the classification and the intensity of the several intensity microbial products and the several analysis of desired aspense in the reading media sits and 10 days after intensity and desired the main effect of the skilders of curversity interfactors matches as significative bottom endow reflect commercial endowed as the several matrix as analysis of the several method commercial endowed in the several matrix excepts in the meaning method with the disadverial endowed aspense in constraints and the several method and the disadverial endowed in curve endowed with the main when the disadverial endowed and excepts in the meaning media was the best first disadverial aspective run. (He), then is other instances.

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had (WER) to reputation

LSD test results for emmorie every 5 days on the moning media. Rean of ammonia concentration (mg.L*) Days after maring

0.327 +0.01 0.297 +0.01 0.197 40.05 0.197 40.05 0.197 40.05 0.197 40.05 0.197 40.05 0.197 40.05 0.197 40.05 0.297 40.05 0.297 40.05 0.297 40.05 0.297 40.05 0.297 40.05 0.297 40.05 0.197 40.05 0.

±0.01 0.163*

0.006

0.155

Pi 0.285 0.239 0.239 0.139 0.159 0.159 0.247 0.209 0.249 0.279 Beisterket to the factor of adding crititying bacteria, the lowest ammonia content was observed in the treatment with rititying bacteria, the is assumed that the addee hardfoeldon bacteria are able to camp out the process of hardfoeldon and the addee hardfoeldon bacteria are able to camp out the process of hardfoeldon on the means media. According to Buarage et al (2014), instruction to these jakes through 2 reactions alarge, where in the treat stage the usodation of armonian to inthe is carried out by ammonian analyzing in the lowest armonia content was observed in the treatment of warm principal factors, the lowest armonia content was observed in the treatment with a combination of Charophyla, Bactila su, and Screptoryces sp. It is suspected that the three microbial factors, the lowest armonia content was observed in the treatment with a combination of Charophyla, Bactila su, and Screptoryces sp. It is suspected that the three microbial factors, the lowest armonia content was observed in the treatment in addition to strate, which coald be used by microalize for their metabolic processes (Narthayel et al 2014). Bactilas up, coald costice armonia to riting through heterotrophic and comentorphic processes (Erwards 2011). Armong the factors interactions, NITA treatment had the lowest armonia level in addition to brank down Armonia, It is presence of the commenties form the same the core interactions. It is presence of the commenties in feed, into process addition to the out of reaving. It was supported that microbes from the saven series able to break down Armonia, It is presence of the commenties in fields. The most break down Armonia, It is presence of the commenties in fields. The same the tory in the addition of the same and size growth, Nithying bacteria (addition the NIZPI and NIZPI mixel). Is present able to break down and the process and the second from the process additin the work of inititying bacteria areak in the l

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±0.00 0.000

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20.01 0.247* ±0.01 0.006 0.297 ±0.01 0.167 ±0.01 0.067 0.255

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0.367 40.01 0.230 40.01 0.290

±0.02 0.303 ±0.03 ±0.01 0.237*

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AND BORG, 1925, Voyag 12, Issue J.

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Table 10

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Table 9 LSD test result of disaslved oxygen in the naming media

_		1.57	1.160		end, oxyge evel after r		-		
	- 10	181	52	15	20	8	30	35	- 40
150				0.18	0.14	0.14	0.18	0.18	0.18
NIPL	3.60	3.53	3.53	3.40*	3.10	3.005	2.97	2.771	2.67
	+5.1	+0.1	+0.5	+0.1	+0.1	+0.1	+0.1	+0.1	#0.1
N1P2	3.63	3.53	3.53	3.23**	2.93*	2.83**	2.77**	3.57**	2.47**
	±0.1	60.1	+0.1	20.1	10.2	±0.1	20.1	±0.1	20.1
NIP3	3.67	3.57	3.47	3,335	3.07	2.554	2.87%	2.67%	2.57%
	+0.1	+0.2	+0.2	1.01	+0.1	#0.1	10.0	+0.1	#0.1
	3.57	1.70	3.60	3.60%	3.50*	3.401	3.40*	3.201	3.10*
WIN4	10.0	80:I	±0.1	20.1	+0.0	±0.1	:±0.1	+0.1	±0.1
N2P1	3.63	1.41	1.43	3.13*	2.83*	2.73*	2.67*	2.47*	2.37%
	±0.1	10.1	20.5	20.1	±0.1	20.1	20.5	±0.1	:10.1
N2P2	1.57	3.47	3.47	3.33**	3.67	293/**	2.67**	3.67%	7.57**
	±0.1	40.1	±0.1	20.1	20.1	20.1	20.1	±0.1	20.1
11293	1.51	100	3.32	3.27%	3.10	3.001	2.90**	2.70%	2.60
	40.1	±0.1	20.5	10.1	±0.1	20.1	20.1	20.1	:10.1
	3.67	3.63	3.53	3.40	3.307	3.35*	3.20*	3.001	1.90
16,014	10.1	80.1	±0.3	20.1	±D.I	#13 I	\$0.1	+0.1	±0.1
LSD		0.08	0.08	0.09	0.07	0.07	0.09	0.09	0.09
N1	3.62	1.58*	3.5.9	5.091	3.145	1.64*	3.00*	2.80*	2,719
MZ	3.60	1.49	3.64*	4.92*	3.074	2.97	2.91*	2.71*	2.614
150		0.11	0.11	0.13	0.10	0.19	0.13	0.13	0.13
P1 -	3.62	3.497	2.407	3.27*	2.971	2.87*	1.62	3.62	3.62*
F2.	3.60	3.99*	3.504	3.284	2.994	2.854	2.829	2.67	2.520
P2.	5.60	1.90*	3.409	3,30%	3.07*	2.971	2.660	2.694	2.581
94	1.62	3.675	1.57	3.500	3.40%	3.35*	3.301	3.10	3.00*

P4 3.63 3.67 3.57 3.57 3.59 3.49 3.09 3.30 3.40 5.00 Tan days after rearing start, the P4 transmith ed a significantly higher dissolved expansion content than the 21 transmost, but it is an an significantly higher three the P1 and P2 treatments. The factors of concentration intrification bacteria, microlian is addition and their interaction significantly influence that dissolve concentrat. This model is a significant in the probability of the probability influence interaction significantly influence that dissolve concentrat. This is a significant in the probability of the probability influence interaction significantly influence that dissolve concentration. The satisfactor simple is a significant in the probability of the probability influence in the probability influence interaction significantly influence and their interaction significantly influence arrange influence to accenter, addition and their interaction significantly influence arrange in the significant in the probability in the factor of commential intrification bacteria instantly that the interaction significantly influence arrange is the instance of the interaction without discussion without commenties instantial the immunity content in the transmit without commenties instantly instant and significantly insert frame the interaction interaction interfloation bacteria instantial the immunity is antised of the teaching media with a commentation in the instantian significantly insert frame the reaction generation concentration for earling media compared to the other interaction factors into the significant the intermention is a significantly insert frame the interaction inter-sion or significant the intermention on the interaction between factors, demonstrate that the NIP4 treatments that significantly invest ammonia texts compared to one other treatments, but not significantly invest ammonia texts compared to some to externeous, but not significantly interviation in the interaction between factors, demonstrate the HIP4 treatments has indinferent tha

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Conclusions. The extrition of second induces Delocophysis, Socialer up, and Simplifying an an end of a strate rearing reading wate more efficient three other trade-more backness they provide their wester quarky values and gover the best survivol rate, final efficiency and govers of C activate in severa parameters, alteruph them was no entrollable backness cost. Activities and Simplower's were the back trademost trademost severa back costs and strategories were the back trademost trademost severa back costs and strategories were the back trademost trademost severa back costs and strategories were the back trademost trademost severa back costs and strategories were the back trademost several several back and the several sector several sector several several back of the several several sector several several back of the several sector several several several several several sector several several several several several sector several several back of the several several sector several several several several several sector several se

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Betereres

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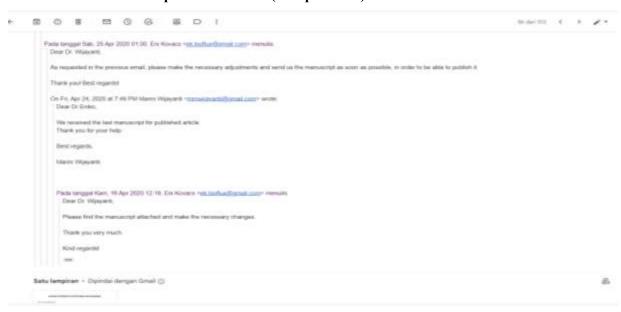
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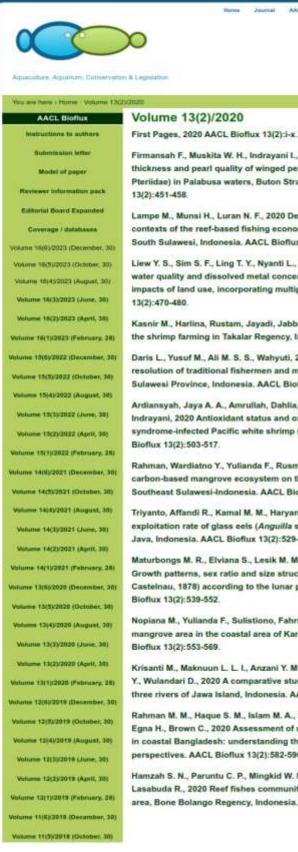
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