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Penulis : Marini Wijayanti, Dade Jubaedah, Ocktin Yulistya, Tanbiyaskur Tanbiyaskur, Ade Dwi Sasanti

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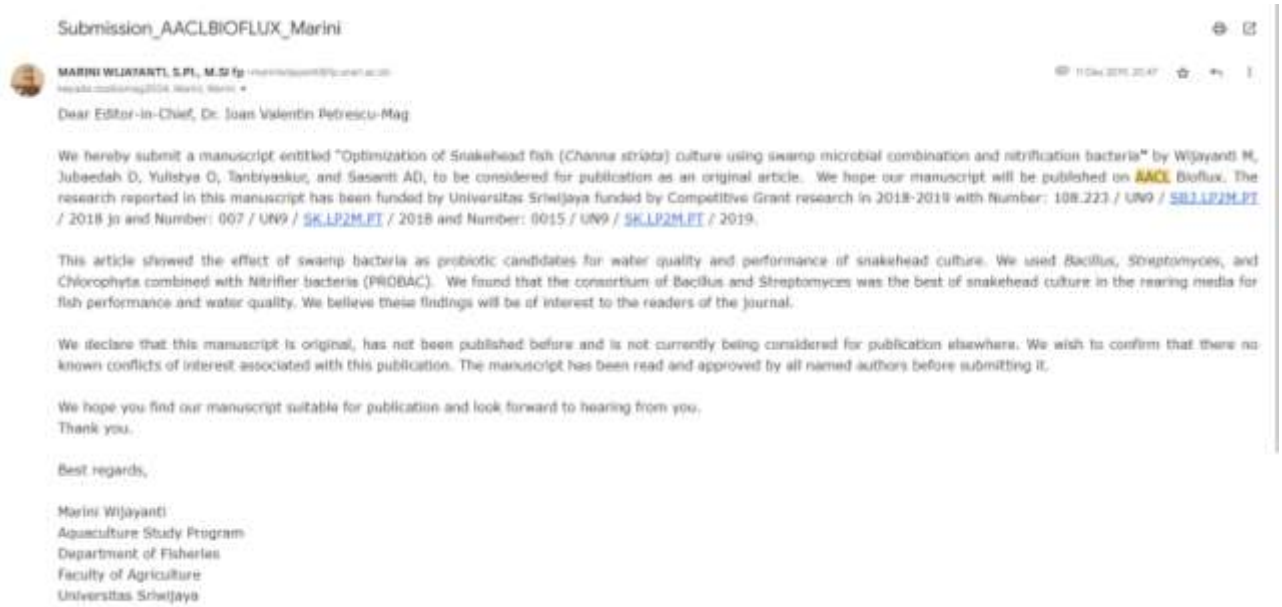


Submission_AACLBIOFLUX_Marini

MARINI WIJAYANTI, S.Pi., M.Si fg

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

We hereby submit a manuscript entitled "Optimization of Snakehead fish (*Channa striata*) culture using swamp microbial combination and nitrification bacteria" by Wijayanti M, Jubaedah D, Yulistya O, Tanbiyaskur, and Sasanti AD, to be considered for publication as an original article. We hope our manuscript will be published on AACL Bioflux. The research reported in this manuscript has been funded by Universitas Sriwijaya funded by Competitive Grant research in 2018-2019 with Number: 108.223 / UN9 / SB3.LP2M.PT / 2018 jo and Number: 007 / UN9 / SK.LP2M.PT / 2018 and Number: 0015 / UN9 / SK.LP2M.PT / 2019.

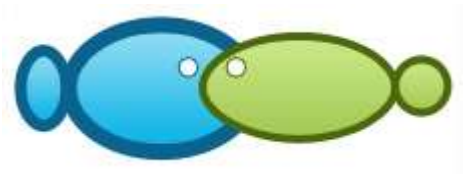
This article showed the effect of swamp bacteria as probiotic candidates for water quality and performance of snakehead culture. We used *Bacillus*, *Streptomyces*, and Chlorophyta combined with Nitrifier bacteria (PROBAC). We found that the consortium of Bacillus and Streptomyces was the best of snakehead 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 declare that this manuscript is original, has not been published before and is not currently being considered 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.

Best regards,

Marini Wijayanti
Aquaculture Study Program
Department of Fisheries
Faculty of Agriculture
Universitas Sriwijaya



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

Marini Wijayanti, Dade Jubaedah, Ocktin Yulistya, Tanbiyaskur, Ade Dwi Sasanti

[1] Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Universitas Sriwijaya, Jl. Palembang Prabumulih Km. 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia
Corresponding author: Marini Wijayanti, mariniwijayanti@fp.unsri.ac.id

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* (3.43×10^7 sel.L^{-1}) and *Bacillus* sp. (10^5 CFU.mL^{-1}) (P2), addition of *Chlorophyta* (3.43×10^7 sel.L^{-1}) and *Streptomyces* sp. (10^5 CFU.mL^{-1}) (P3), addition of *Chlorophyta* (3.43×10^7 sel.L^{-1}), *Bacillus* sp. (10^5 CFU.mL^{-1}) and *Streptomyces* sp. (10^5 CFU.mL^{-1}) (P4). 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 µl.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 10^5 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 NH_3, NO_2^-, and NO_3^- 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 CO_2 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 CO_2 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) 5×10^6 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 (3.43×10^7 Cell.L^{-1}) and *Bacillus* sp. (10^5 CFU.mL^{-1})
P3: Provision of 100 ml Chlorophyta (3.43×10^7 Cell.L^{-1}) and *Streptomyces* sp. (10^5 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^{o}C for 0.25 hour. *Chlorophyta* isolates (about 10^{7}cell.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 x 30 x 30 cm3 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 µm 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:

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

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)
W_0 = 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:

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

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.

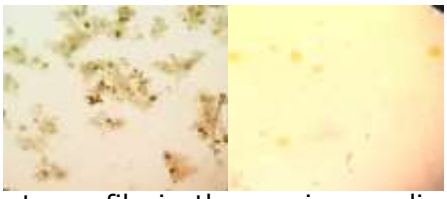


Figure 1. Biofloc and Chlorophyta profile in the rearing media of snakehead culture in this study (40 magnificaton scale of microscope)

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).

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

Commercial Nitrification Bacteria	Swamp microbes	Chlorophyta Abundance (Cell.L^{-1})		
		0 day	10th day	40th day
N1	P1	$3{,}2 \times 10^3$	$3{,}2 \times 10^3$	$2{,}1 \times 10^3$
	P2	$3{,}6 \times 10^3$	$3{,}43 \times 10^7$	$4{,}1 \times 10^3$
	P3	$4{,}1 \times 10^3$	$3{,}43 \times 10^7$	$4{,}1 \times 10^3$
	P4	$3{,}7 \times 10^3$	$3{,}43 \times 10^7$	$4{,}46 \times 10^3$
N2	P1	$4{,}0 \times 10^3$	$4{,}0 \times 10^3$	$2{,}1\times 10^3$
	P2	$3{,}6 \times 10^3$	$3{,}43 \times 10^7$	$2{,}41 \times 10^3$
	P3	$3{,}9 \times 10^3$	$3{,}43 \times 10^7$	$2{,}34 \times 10^3$
	P4	$3{,}4 \times 10^3$	$3{,}43 \times 10^7$	$4{,}03 \times 10^3$

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.

Table 2. Total bacterial population in rearing media

Commercial Nitrification Bacteria	Swamp microbes	Total bacterial population (CFU.mL^{-1})			
		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	$4{,}95 \times 10^4$	$4{,}70 \times 10^7$	$8{,}75 \times 10^7$	$4{,}06 \times 10^6$

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.

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

The Single Effect of Nitrifying Bacteria (N)	The Single Influence of Swamps Microbes (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	12,500[a]
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 Influence of Swamps Microbes (P) ($LSD_{0,05}$=6,631)				The Main Effects of Nitrifying Bacteria (N)
	P1	P2	P3	P4	($LSD_{0,05}$=3,315)
N1	11,112[a]	26,667[b]	16,667[a]	38,889[c]	25,834[b]
N2	13,333[a]	13,333[a]	23,333[b]	33,333[c]	20,833[a]
The Main Effects of Swamp Microbes (P) ($LSD_{0,05}$=4,689)	12,223[a]	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 40.7$mL.L^{-1}$. 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.

Table 5. LSD test of Survival Rate of Snakehead fish

The Single Effect of Nitrifying Bacteria (N)	The Single Influence of Swamps Microbes (P) ($LSD_{0,05}$=6,02)				The Main Effects of Nitrifying Bacteria (N)
	P1	P2	P3	P4	
N1	26,06[a]	36,91[b]	28,03[a]	63,94[d]	38,74
N2	31,75[ab]	28,03[a]	35,16[b]	48,20[c]	35,79
The Main Effects of Swamp Microbes (P) ($LSD_{0,05}$=4,25)	28,91[a]	32,47[a]	31,59[a]	56,07[b]	

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.

Table 6. LSD test result of the efficiency of snakehead fish feed for 40 days of rearing

The Single Effect of Nitrifying Bacteria (N)	The Single Influence of Swamps Microbe (P) ($LSD_{0,05}$=3,32)				The Main Effects of Nitrifying Bacteria (N) ($LSD_{0,05}$=1,66)
	P1	P2	P3	P4	
N1	18,93[a]	47,34[d]	37,97[c]	59,65[e]	40,97[b]
N2	22,00[a]	29,52[b]	34,89[c]	44,11[d]	32,63[a]
The Main Effects of Swamp Microbes (P) ($LSD_{0,05}$= 2.35)	20,47[a]	38,43[b]	36,43[b]	51,88[c]	

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 of Chlorophyta, Bacillus 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 nitrifying bacteria.

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) ($LSD_{0,05}$=0,04)
	P1	P2	P3	P4	
N1	1,30[a]	2,26[f]	1,70[c]	2,32[f]	1,90[b]
N2	1,73[c]	1,41[b]	1,88[d]	2,08[e]	1,78[a]
The Main Effects of Swamp Microbes (P) ($LSD_{0,05}$=0,05)	1,51[a]	1,84[b]	1,79[b]	2,20[c]	

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.

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

The Single Effect of Nitrifying Bacteria (N)	The Single Influence of Swamps Microbe (P) ($LSD_{0,05}$=0,10)				The Main Effects of Nitrifying Bacteria (N)
	P1	P2	P3	P4	
N1	0,69[a]	1,79[e]	0,91[b]	2,27[f]	2,12
N2	1,13[c]	1,08[c]	1,60[d]	1,74[e]	2,09
The Main Effects of Swamp Microbes (P) ($LSD_{0,05}$=0,07)	0,91[a]	1,44[c]	1,26[b]	2,00[d]	

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 10^4 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.

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

	Dissolved Oxygen (mg.L^{-1})								
	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 ±0,1	3,53 ±0,1	3,53 ±0,1	3,40^{c} ±0,1	3,10^{c} ±0,1	3,00^{c} ±0,1	2,97^{c} ±0,1	2,77^{c} ±0,1	2,67^{c} ±0,1
N1P2	3,63 ±0,1	3,53 ±0,1	3,53 ±0,1	3,23^{ab} ±0,1	2,93^{ab} ±0,1	2,83^{ab} ±0,1	2,77^{ab} ±0,1	2,57^{ab} ±0,1	2,47^{ab} ±0,1
N1P3	3,67 ±0,1	3,57 ±0,2	3,47 ±0,2	3,33^{bc} ±0,1	3,03^{bc} ±0,1	2,93^{bc} ±0,1	2,87^{bc} ±0,1	2,67^{bc} ±0,1	2,57^{bc} ±0,1
N1P4	3,57 ±0,1	3,70 ±0,1	3,60 ±0,1	3,60^{d} ±0,1	3,50^{e} ±0,1	3,40^{e} ±0,1	3,40^{e} ±0,1	3,20^{e} ±0,1	3,10^{e} ±0,1
N2P1	3,63 ±0,1	3,43 ±0,1	3,43 ±0,1	3,13^{a} ±0,1	2,83^{a} ±0,1	2,73^{a} ±0,1	2,67^{a} ±0,1	2,47^{a} ±0,1	2,37^{a} ±0,1
N2P2	3,57 ±0,1	3,47 ±0,1	3,47 ±0,1	3,33^{bc} ±0,1	3,03^{c} ±0,1	2,93^{bc} ±0,1	2,87^{bc} ±0,1	2,67^{bc} ±0,1	2,57^{bc} ±0,1
N2P3	3,53 ±0,1	3,43 ±0,1	3,33 ±0,1	3,27^{ab} ±0,1	3,10^{c} ±0,1	3,00^{c} ±0,1	2,90^{bc} ±0,1	2,70^{bc} ±0,1	2,60^{c} ±0,1
N2P4	3,67 ±0,1	3,63 ±0,1	3,53 ±0,1	3,40^{c} ±0,1	3,30^{d} ±0,1	3,20^{d} ±0,1	3,20^{d} ±0,1	3,00^{d} ±0,1	2,90^{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^{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	3,49^{a}	3,44^{a}	4,93^{a}	3,07^{a}	2,97^{a}	2,91^{a}	2,71^{a}	2,61^{a}
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^{ab}	3,27^{a}	2,97^{a}	2,87^{a}	2,82^{a}	2,62^{a}	2,52^{a}
P2	3,60	3,50^{a}	3,50^{ab}	3,28^{a}	2,98^{a}	2,88^{a}	2,82^{a}	2,62^{a}	2,52^{a}
P3	3,60	3,50^{a}	3,40^{a}	3,30^{a}	3,07^{a}	2,97^{a}	2,88^{a}	2,68^{a}	2,58^{a}
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}

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.

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

	Mean of Ammonia Concentratin ($mg.L^{-1}$)								
	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 ±0,07	0,383^{e} ±0,01	0,393^{d} ±0,01	0,410^{d} ±0,01	0,327^{e} ±0,01	0,540^{d} ±0,01	0,674^{e} ±0,01	0,691^{f} ±0,01	0,948^{e} ±0,02
N1P2	0,323 ±0,03	0,273^{d} ±0,01	0,283^{c} ±0,01	0,223^{c} ±0,01	0,203^{c} ±0,01	0,293^{b} ±0,01	0,314^{b} ±0,01	0,321^{c} ±0,01	0,324^{b} ±0,01
N1P3	0,283 ±0,01	0,257^{c} ±0,01	0,267^{b} ±0,01	0,207^{b} ±0,01	0,197^{bc} ±0,01	0,363^{c} ±0,02	0,482^{d} ±0,02	0,585^{e} ±0,01	0,692^{d} ±0,02
N1P4	0,267 ±0,01	0,220^{a} ±0,01	0,230^{a} ±0,01	0,170^{a} ±0,01	0,147^{a} ±0,01	0,243^{a} ±0,02	0,260^{a} ±0,02	0,265^{a} ±0,02	0,270^{a} ±0,02
N2P1	0,230 ±0,01	0,253^{c} ±0,01	0,263^{b} ±0,01	0,203^{b} ±0,01	0,193^{bc} ±0,01	0,282^{b} ±0,02	0,363^{c} ±0,02	0,385^{d} ±0,01	0,392^{c} ±0,02
N2P2	0,290 ±0,04	0,233^{ab} ±0,01	0,243^{a} ±0,01	0,183^{a} ±0,01	0,160^{a} ±0,01	0,250^{a} ±0,01	0,268^{a} ±0,01	0,273^{a} ±0,01	0,277^{a} ±0,01
N2P3	0,250 ±0,02	0,277^{d} ±0,01	0,287^{c} ±0,01	0,227^{c} ±0,01	0,187^{b} ±0,01	0,277^{b} ±0,01	0,296^{b} ±0,01	0,300^{b} ±0,01	0,306^{b} ±0,01
N2P4	0,303 ±0,03	0,237^{b} ±0,01	0,247^{b} ±0,01	0,187^{b} ±0,01	0,163^{b} ±0,01	0,250^{a} ±0,01	0,268^{a} ±0,01	0,273^{a} ±0,01	0,277^{a} ±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	0,250^{a}	0,260^{a}	0,200^{a}	0,176^{a}	0,265^{a}	0,299^{a}	0,308^{a}	0,313^{a}
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^{c}	0,443^{c}	0,499^{c}
P4	0,285	0,228^{a}	0,238^{a}	0,178^{a}	0,155^{a}	0,247^{a}	0,264^{a}	0,269^{a}	0,274^{a}

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 was significantly lower than treatments not 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, but not significantly different from N2P2 treatment. The results of LSD on 25, 30, 35 and 40th day in the interaction between factors, N1P4 treatment had 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 25^{th} to 40^{th} 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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References

Balcazar, J.L., DeBlas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D. And Muzquiz, J.L. 2006. The role of probiotics in aquaculture. Veterinary Microbiology Review, 114, 173-186.

Bernal, M.G., Ricardo, M.M., Angel., I.C. and Jose, M.M., 2017. Probiotic effect of Streptomyces strains alone or in combination with Bacillus and Lactobacillus in juveniles

of the white shrimp Litopenaeus vannamei. Aquaculture International, 25(2), 927-939.
Blancheton, J.P., Attramadal, K.J.K., Michaud L., Roque d'Orbcastel, E. dan Vadstein, O., 2013. Insight into bacterial population in aquaculture systems and its implication. Aquacultural Engineering 53, 30–39.
Bouman, H.A., T. Platt, S. Sathyendranath, W.K.W. Li, V. Stuart, C. Fuentes-Yaco, H. Maass, E.P.W. Horne, O. Ulloa, V. Lutz, and M. Kyewalyanga. 2003. Temperature as indicator of optical properties and community structure of marine phytoplankton: implications for remote sensing. Mar Ecol Prog Ser, 258, 19-30.
Budianto dan Heny, S., 2017. Aktivitas antagonis Bacillus sp. terhadap Streptococcus iniae dan Pseudomonas fluorescens. Jurnal Veteriner, 18(3), 409-415.
Ebeling, J.M., Timmons, M.B. dan Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture, 257, 346-358.
Edwards, V.A., 2011. The nitrogen cycle-Control ammonia, Nitrite in ponds, lakes, Lagoons, Rivers and waste water treatment [Online]. Available at: http://www.biosolve.com.au/index_files [Accessed 20 October 2018].
Fardiaz, D. 1992. Mikrobiologi Pangan I. Jakarta: Gramedia.
Feliatra, I.E., dan Suryadi, E., 2004. Isolasi dan identifikasi bakteri probiotik dari ikan kerapu macan (Ephinephelus fuscogutatus) dalam upaya efisiensi pakan ikan. Jurnal Natur Indonesia. 4(2), 10-14.
Findy, K., 2009. Aktivitas penghambat Bacillus sp. terhadap Xanthomonas oryzae pv. Oryzae, Pseudomonas syringae pv. glycines, dan Pseudomonas fluorescens. Skripsi. IPB.
Hartini, S., Ade, D. S. dan Ferdinand, H. T., 2013. Kualitas air, kelangsungan hidup dan pertumbuhan benih ikan gabus (Channa striata) yang dipelihara dalam media dengan penambahan probiotik. Jurnal Akuakultur Rawa Indonesia, 1(2), 192-202.
Irianto, A and Austin, B., 2002. Probiotics in aquaculture. Journal of Fish Diseases, 25, 633-642.
Khotimah, H., 2019. Uji patogenitas bakteri kandidat probiotik asal sedimen rawa. Skripsi. Universitas Sriwijaya.
Lutfi, M.D., 2018. Eksplorasi Streptomyces Sp. Dari Berbagai Ekosistem Sebagai Penghasil Antibiotik Untuk Penghambat Pertumbuhan B. Subtilis Dan E. Coli. Skripsi. ITB.
Luti, K. J. K. Dan Mavituna, F., 2011. Streptomyces coecolor increases the production of undecylprodigiosin when interacted with Bacillus subtilis. Biotechnol Lett, 33, 113-118.
Mohapatra, S., T. Chakraborty, V. Kumar, G. DeBoeck and K.N. Mohanta, 2013. Aquaculture and stress management: a review of probiotic intervention. J. Anim. Physiol. Anim. Nutr, 97(3), 405-430.
Mulyadi, A.E., 2011. Pengaruh Pemberian Probiotik pada Pakan Komersil terhadap Laju Pertumbuhan Benih Ikan Patin Siam (Pangasius hypotalamus). Skripsi. Universitas Padjajaran.
Mulyadi, G., 2016. Pemeliharaan ikan gabus (Channa striata) dengan padat tebar berbeda dalam media bioflok. Jurnal Akuakultur Rawa Indonesia, 4(2), 159-174.
National Research Council (NRC). 1977. Nutrien Requirement of Warm Water Fishes. National Acad. Press, Washington D.C.
Nurhayati, C., Hamzah, B. Dan Pambayun, R., 2014. Pengaruh pH, konsentrasi isolat Chlorella vulgaris dan waktu pengamatan terhadap tingkat cemaran limbah cair crumb rubber. Jurnal Dinamika Penelitian Industri, 25(2), 97-106.
Panigrahi, A., Sundaram, M., Cakrapani, S., Rajasekar, S., Dayal, J.S. dan Chavali, G., 2019. Effect of carbon and nitrogen ratio (C:N) manipulation on the production performance and immunity of pasific white shrimp Litopenaeus vannamei (Boone, 1931) in a biofloc-based rearing system. Aquaculture Research, 50, 29–41.
Rurangwa, E. dan Verdegem, M.C.J., 2014. Microorganisms in recirculating aquaculture systems and their management. Reviews In Aquaculture, 5, 1-14.
Sanchez, P. T., Ruiz-Zarzuela, I., Blas, I. D., dan Balcazar, J. L. 2014. Probiotics in

aquaculture: A current assessment. Reviews in Aquaculture, 6, 133–146.
Saraswati, N., 2018. Isolasi aktinomiset untuk bioremediasi air rawa yang tercemar bahan organik. Skripsi. Universitas Sriwijaya.
Sitohang, M.L., Fitrani, M. dan Jubaedah, D., 2018. Pemanfaatan campuran buah nanas, air cucian beras, dan gula sebagai sumber karbon pada media pemeliharaan ikan lele (Clarias sp.) Dengan sistem bioflok. Jurnal Akuakultur Rawa Indonesia, 6(1), 51-64.
Trisna, D.E., Sasanti, A.D. dan Muslim, 2013. Populasi bakteri, kualitas air media pemeliharaan dan histologi benih ikan gabus (Channa striata) yang diberi pakan berprobiotik. Jurnal Akuakultur Rawa Indonesia, 1(1), 90-102.
Wijayanti, M., Jubaedah, D., Suhada, J.A., Yuliani, S., Saraswati, N., Tanbiyaskur., Syaifudin, M., and Widjajanti, W., 2018. DNA Barcoding of Swamp Sediment Bacterial Isolates for Swamp Aquaculture Probiotic. E 3S Web of Conferences 1st SRICOENV, https://doi.org/10.1051/e3sconf /20186801068 10.
Yuliani, S., 2017. Isolasi bakteri rawa sebagai agen bioaugmentasi pada air rawa tercemar bahan organik. Skripsi. Universitas Sriwijaya.

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Authors:
Marini Wijayanti, Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Universitas Sriwijaya, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: mariniwijayanti@fp.unsri.ac.id
Dade Jubaedah, Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Universitas Sriwijaya, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: dadejubaedah@fp.unsri.ac.id
Ocktin Yulistya, Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Universitas Sriwijaya, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: yulistyaocktin@gmail.com
Tanbiyaskur, Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Universitas Sriwijaya, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: qurhadi30@gmail.com
Ade Dwi Sasanti, Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Universitas Sriwijaya, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: sasanti.ade@gmail.com

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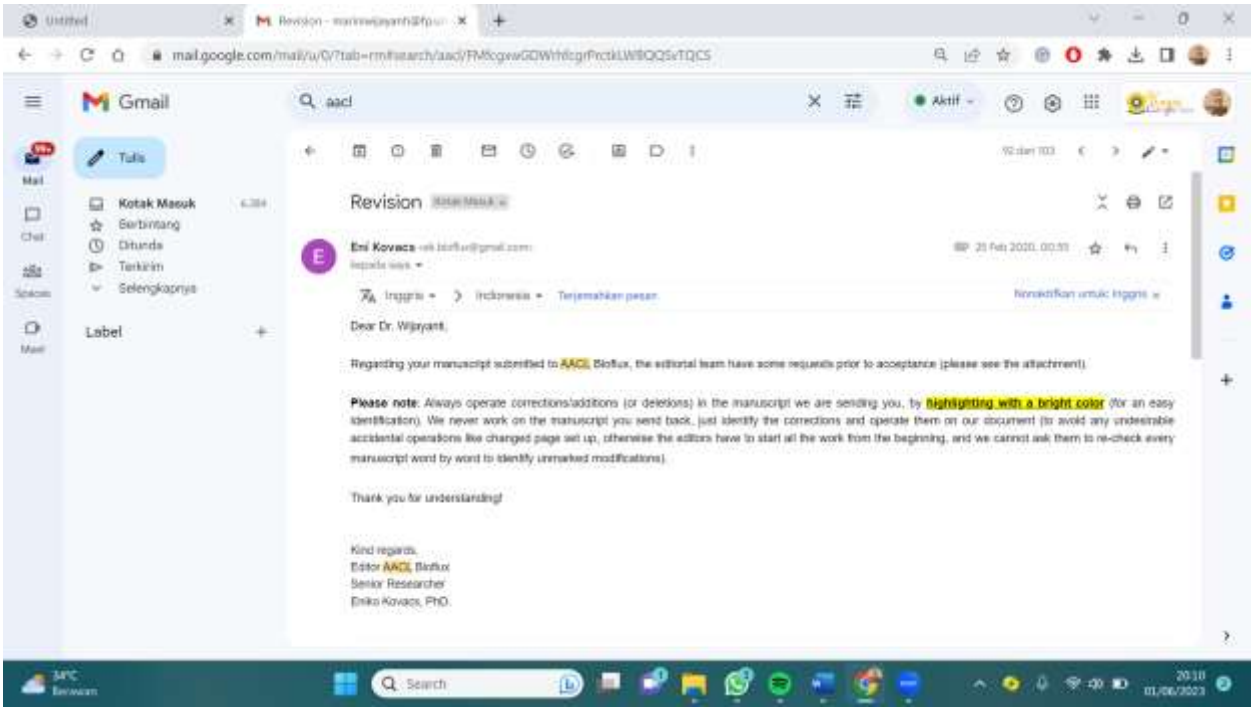

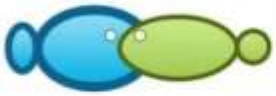

Optimization of striped snakehead fish (*Channa striata*) culture using swamp microbial combination and nitrification bacteria

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

*Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University, Indralaya, Ogan Ilir, South Sumatera, Indonesia. Corresponding author: Marini M. Wijayanti, mariniwijayanti@fp.unsri.ac.id

Abstract. Utilization of swamp area as fish culture location will cause a decrease water water quality. Therefore, it is necessary to improve the water quality with environmental friendly of biological treatments, one ofsuch 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 media culture and production media 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 consistes in 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 is consists in four scenarios: (1) without the addition of swamp microbes (P1) and; with the addition of; (2) *Chlorophyta* (3.43×10^7 sel.L^{-1}) and *Bacillus* sp. (10^5 CFU.mL^{-1}) (P2); (3) addition of *Chlorophyta* (3.43×10^7 sel.L^{-1}) and *Streptomyces* sp. (10^5 CFU.mL^{-1}) (P3); (4) addition of *Chlorophyta* (3.43×10^7 sel.L^{-1}), *Bacillus* sp. (10^5 CFU.mL^{-1}) and *Streptomyces* sp. (10^5 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) and only the addition of nitrification bacteria (N2P1). Combination of N1P4, produceges 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 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 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.l^{-1}.week^{-1} can improve and maintain optimal water quality. In other studies, the addition of the effective probiotic microorganism 4 probiotics can reduce ammonia levels and suppress the population of pathogenic 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 Sswamp 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 10^5 CFU.ml^{-1} (Khotimah, 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 NH_3, NO_2^-, and NO_3^- 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 CO_2 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 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 CO_2 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 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 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. 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 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) 5×10^6 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 (3.43×10^7 Cellcell.L^{-1}) and *Bacillus* sp. (10^5 CFU.mL^{-1})
P3: Provision of 100 ml Chlorophyta (3.43×10^7 Cellcell.L^{-1}) and *Streptomyces* sp. (10^5 CFU.mL^{-1})
P4: Provision of 100 ml Chlorophyta (3.43×10^7 Cellcell.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 nutrient agar NA (NA nutrient agar) media for *Bacillus* sp and yeast malt YM (YM yeast malt agar) 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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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 grew.

Swamp bacteria that grow grown on NA and YM agar media were multiplied by nutrient broth NB (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 taken as much as 1 ose in order to be cultured in the medium as much as 5 mL in a test 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 Erlenmeyer 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 Erlenmeyer was sterilized using an autoclave at 121°C for 0.25 hour. *Chlorophyta* isolates (about 10^7cell·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 in at 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 cm^3 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–10^7 Cellcell·L^{-1}), *Bacillus* sp. (10^5 CFU·mL^{-1}), *Streptomyces* sp. (10^5 CFU·mL^{-1}) as well as the "PROBAC" nNitrification bacteria (5–x 10^6 CFU·mL^{-1}), were added in combination with the treatment.

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 using 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 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 25 ml sample. Observation of Chlorophyta 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 *Chlorophyta* samples (Davis, 1955). *Chlorophyta* abundance calculation was done performed by using the Leackey Drop Microtransect method (American Public Health Association, 1989) as follows:

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

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 litersL)

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

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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. The f~~F~~loc volume was obtained by ~~taking~~ 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:

~~Survival rate =~~

$$\frac{Nt}{No} \times 100\%$$

Where:
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 was~~s~~ calculated by using the following formula:

$$W = Wt - W_0$$

Where:
W = Growth of weight of fish for rearing (grams)
Wt = Weight of fish at the end of rearing (grams)
W_0 = 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:

$$L = Lt - L_0$$

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)

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

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

~~Note~~Where:
-EP = Feed ~~Efficiency~~ 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 40 days later, at the end of the rearing, ~~for 40 days~~.

Data analysis. Research data including biofloc volume, survival, growth, feed efficiency, water quality ~~were~~ was statistically ~~analyzed~~ processed by using the variance analysis ~~of 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

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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 magnificaton scale of microscope).

The pattern of the interaction~~relationship~~s between zooplankton and phytoplankton is a series of eating and prey relationships~~.~~ forming ~~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 trophic levels (Bouman *et al*~~.~~, 2003).

Table 1

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

Commercial nitrification bacteria	*Swamp microbes*	*Chlorophyta Abundance (Cell.L^{-1})*		
		0 day	*10th day*	*40th day*
N1	P1	3,2 × 10^3	3,2 × 10^3	2,1 × 10^3
	P2	3,6 × 10^3	3,43 × 10^7	4,1 × 10^3
	P3	4,1 × 10^3	3,43 × 10^7	4,1 × 10^3
	P4	3,7 × 10^3	3,43 × 10^7	4,46 × 10^3
N2	P1	4,0 × 10^3	4,0 × 10^3	2,1× 10^3
	P2	3,6 × 10^3	3,43 × 10^7	2,41 × 10^3
	P3	3,9 × 10^3	3,43 × 10^7	2,34 × 10^3
	P4	3,4 × 10^3	3,43 × 10^7	4,03 × 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 ~~after~~ of rearing are showed in Table 3 and Table 4, respectively.

Table 2

~~Table 2.~~ Total bacterial population in rearing media

Commercial nitrification bacteria	*Swamp microbes*	*Total bacterial population (CFU.mL^{-1})*			
		0 day	*1st day*	*20th day*	*40th day*
N1	P1	6,60 × 10^4	6,78 × 10^4	1,55 × 10^5	6,20 × 10^3
	P2	6,20 × 10^4	3,95 × 10^6	6,93 × 10^6	2,77 × 10^5
	P3	7,00 × 10^4	3,28 × 10^6	7,53 × 10^6	3,01 × 10^5
	P4	4,70 × 10^4	5,59 × 10^7	1,00 × 10^8	2,99 × 10^6
N2	P1	7,10 × 10^4	2,01 × 10^7	3,54 × 10^7	1,42 × 10^6
	P2	4,50 × 10^4	3,29 × 10^7	5,59 × 10^7	1,68 × 10^6

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	P3	4,30 × 10⁴	4,99 × 10⁷	6,41 × 10⁷	1,93 × 10⁶
	P4	4,95 × 10⁴	4,70 × 10⁷	8,75 × 10⁷	4,06 × 10⁶

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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. ~~While~~ whereas, the decline of bacteria population observed on the ~~a~~ 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~~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 bacteria~~treatment was significantly higher compared to the treatment ~~given~~ with nitrification bacteria. The addition of nitrifying 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.

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

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=4,386)*
	P1	*P2*	*P3*	*P4*	
N1	11,111	16,666	13,332	26,668	16,944[b]
N2	10,000	10,000	13,332	16,667	12,500[a]
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~~ of rearing

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=6,631)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=3,315)*
	P1	*P2*	*P3*	*P4*	
N1	11,112[a]	26,667[b]	16,667[a]	38,889[c]	25,834[b]
N2	13,333[a]	13,333[a]	23,333[b]	33,333[c]	20,833[a]
The main effects of swamp microbes (P) ($LSD_{0.05}$=4,689)	12,223[a]	20,000[b]	20,000[b]	41,111[c]	

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 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·L^{-1}. ~~This~~ It is presumed that the rearing media lacks a carbon source used by~~which~~ bacteria ~~use~~ for the

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 it 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 effect of the interaction between factors and and the effect of the factors of defined by the addition of swamp originated microbes to on the survival of snakehead fish varied significantly affected between treatments, but the factor of 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 given treated with a combination of Chlorophyta, *Bacillus* sp. and Streptomyces sp., the survival rate wasere significantly higher than for the other treatments, and il Interactions between factors showed that in the rearing media given with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, the survival rate reached 63.94%, a significantly higher different performance compared to the other treatments with a percentage of 63.94%.

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* was cultured together with *Streptomyces* can 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 Single Influence of Swamps Microbes (P) ($LSD_{0.05}$=6,02)				The Main Effects of Nitrifying Bacteria (N)
	P1	P2	P3	P4	
N1	26,06^{a}	36,91^{b}	28,03^{a}	63,94^{d}	38,74
N2	31,75^{ab}	28,03^{a}	35,16^{b}	48,20^{c}	35,79
The Main Effects of Swamp Microbes (P) ($LSD_{0.05}$=4,25)	28,91^{a}	32,47^{a}	31,59^{a}	56,07^{b}	

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 & 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 by producing secondary metabolite compounds (Lutfi, 2018). The combination of the two microbes can provide a high

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percentage of survival compared to ~~without a~~the no combination scenario. According to Irianto ~~and~~ & 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 efficiency of variou~~sely of~~ feed on the snakehead fish ~~feed efficiency~~ showed that the interaction between factors, the microbial addition factors from swamps and the addition of commercial nitrification bacteria, can ~~to~~ increase the value of fish feed efficiency, which is significantly affected ~~between~~ by the treatment type and concentration~~s~~. 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 snakehead fish feed for 40 days of rearing

The Single Effect of Nitrifying Bacteria (N)	The Single Influence of Swamps Microbe (P) ($LSD_{0,05}$=3,32)				The Main Effects of Nitrifying Bacteria (N) ($LSD_{0,05}$=1,66)
	P1	P2	P3	P4	
N1	18,93^{a}	47,34^{d}	37,97^{c}	59,65^{e}	40,97^{b}
N2	22,00^{a}	29,52^{b}	34,89^{c}	44,11^{d}	32,63^{a}
The Main Effects of Swamp Microbes (P) ($LSD_{0,05}$= 2.35)	20,47^{a}	38,43^{b}	36,43^{b}	51,88^{c}	

Based on the~~se~~ 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, T~~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 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 origin of~~ 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~~. The nutrients were need for the fish and the absorption of nutrients runs optimally. The 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).

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). ~~In increasing the value of feed efficiency, T~~The 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 two~~Both 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 fluctuations~~ly~~ ~~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~~ in the digestive tract of fish ~~will be better at~~, facilitating the digestion~~ing~~ and absorption~~bing~~ 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~~ interaction~~s~~ between factors influenced~~on~~ the feed efficiency ~~showed~~ in a significantly different manner, depending on the~~between~~ treatment~~s~~. 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 ~~is~~ 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; (2) ~~,~~ significantly different ~~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) significantly higher than the other treatments' results ~~in the interaction between factors, the growth of the absolute weight of snakehead fish in the treatment of rearing media that were given~~with ~~,~~ 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 nitrifying bacteria.

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) ($LSD_{0,05}$=0,04)
	P1	P2	P3	P4	
N1	1,30^a	2,26^f	1,70^c	2,32^f	1,90^b
N2	1,73^c	1,41^b	1,88^d	2,08^e	1,78^a
The Main Effects of Swamp Microbes (P) ($LSD_{0,05}$=0,05)	1,51^a	1,84^b	1,79^b	2,20^c	

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

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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different than the in other treatments. TOn 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 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 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 proteins, carbohydrates and fats in feed (Feliatra and & Suryadi, 2004). The addition of 10^4 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).

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 thaton 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 showed thaton the dissolved oxygen in the rearing media were given a combination of Chlorophyta, *Bacillus* sp., 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

	Dissolved Oxygen (mg.L^{-1})								
	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 ±0,1	3,53 ±0,1	3,53 ±0,1	3,40^{c} ±0,1	3,10^{c} ±0,1	3,00^{c} ±0,1	2,97^{c} ±0,1	2,77^{c} ±0,1	2,67^{c} ±0,1
N1P2	3,63 ±0,1	3,53 ±0,1	3,53 ±0,1	3,23^{ab} ±0,1	2,93^{ab} ±0,1	2,83^{ab} ±0,1	2,77^{ab} ±0,1	2,57^{ab} ±0,1	2,47^{ab} ±0,1
N1P3	3,67 ±0,1	3,57 ±0,2	3,47 ±0,2	3,33^{bc} ±0,1	3,03^{bc} ±0,1	2,93^{bc} ±0,1	2,87^{bc} ±0,1	2,67^{bc} ±0,1	2,57^{bc} ±0,1
N1P4	3,57 ±0,1	3,70 ±0,1	3,60 ±0,1	3,60^{a} ±0,1	3,50^{a} ±0,1	3,40^{a} ±0,1	3,40^{a} ±0,1	3,20^{a} ±0,1	3,10^{a} ±0,1
N2P1	3,63 ±0,1	3,43 ±0,1	3,43 ±0,1	3,13^{a} ±0,1	2,83^{a} ±0,1	2,73^{a} ±0,1	2,67^{a} ±0,1	2,47^{a} ±0,1	2,37^{a} ±0,1
N2P2	3,57 ±0,1	3,47 ±0,1	3,47 ±0,1	3,33^{bc} ±0,1	3,03^{c} ±0,1	2,93^{bc} ±0,1	2,87^{bc} ±0,1	2,67^{bc} ±0,1	2,57^{bc} ±0,1
N2P3	3,53	3,43	3,33	3,27^{ab}	3,10^{c}	3,00^{c}	2,90^{bc}	2,70^{bc}	2,60^{c}

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	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1	±0,1
N2P4	3,67 ±0,1	3,63 ±0,1	3,53 ±0,1	3,40^{c} ±0,1	3,30^{d} ±0,1	3,20^{g} ±0,1	3,20^{g} ±0,1	3,00^{gf} ±0,1	2,90^{g} ±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	3,49^{a}	3,44^{a}	4,93^{a}	3,07^{a}	2,97^{a}	2,91^{a}	2,71^{a}	2,61^{a}
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^{ab}	3,27^{a}	2,97^{a}	2,87^{a}	2,82^{a}	2,62^{a}	2,52^{a}
P2	3,60	3,50^{a}	3,50^{ab}	3,28^{a}	2,98^{a}	2,88^{a}	2,82^{a}	2,62^{a}	2,52^{a}
P3	3,60	3,50^{a}	3,40^{a}	3,30^{a}	3,07^{a}	2,97^{a}	2,88^{a}	2,68^{a}	2,58^{a}
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}

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 that by 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 ammonia every 5 days on rearing media

	Mean of Ammonia Concentratin (mg·L^{-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 ±0,07	0,383^{gf} ±0,01	0,393^{gf} ±0,01	0,410^{gf} ±0,01	0,327^{ef} ±0,01	0,540^{gf} ±0,01	0,674^{gf} ±0,01	0,691^{gf} ±0,01	0,948^{gf} ±0,02
N1P2	0,323 ±0,03	0,273^{bc} ±0,01	0,283^{bc} ±0,01	0,223^{bc} ±0,01	0,203^{bc} ±0,01	0,293^{bc} ±0,01	0,314^{bc} ±0,01	0,321^{bc} ±0,01	0,324^{bc} ±0,01
N1P3	0,283 ±0,01	0,257^{ab} ±0,01	0,267^{ab} ±0,01	0,207^{ab} ±0,01	0,197^{ab} ±0,01	0,363^{cd} ±0,02	0,482^{de} ±0,02	0,585^{ef} ±0,01	0,692^{ef} ±0,02
N1P4	0,267 ±0,01	0,220^{ab} ±0,01	0,230^{ab} ±0,01	0,170^{ab} ±0,01	0,147^{ab} ±0,01	0,243^{ab} ±0,02	0,260^{ab} ±0,02	0,265^{ab} ±0,02	0,270^{ab} ±0,02
N2P1	0,230 ±0,01	0,253^{bc} ±0,01	0,263^{bc} ±0,01	0,203^{bc} ±0,01	0,193^{ab} ±0,01	0,282^{bc} ±0,02	0,363^{cd} ±0,02	0,385^{cd} ±0,01	0,392^{cd} ±0,02
N2P2	0,290 ±0,04	0,233^{ab} ±0,01	0,243^{ab} ±0,01	0,183^{ab} ±0,01	0,160^{ab} ±0,01	0,250^{ab} ±0,01	0,268^{ab} ±0,01	0,273^{ab} ±0,01	0,277^{ab} ±0,01
N2P3	0,250 ±0,02	0,277^{bc} ±0,01	0,287^{bc} ±0,01	0,227^{bc} ±0,01	0,187^{ab} ±0,01	0,277^{bc} ±0,01	0,296^{bc} ±0,01	0,300^{bc} ±0,01	0,306^{bc} ±0,01
N2P4	0,303 ±0,03	0,237^{ab} ±0,01	0,247^{ab} ±0,01	0,187^{ab} ±0,01	0,163^{ab} ±0,01	0,250^{ab} ±0,01	0,268^{ab} ±0,01	0,273^{ab} ±0,01	0,277^{ab} ±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	0,250^{a}	0,260^{a}	0,200^{a}	0,176^{a}	0,265^{a}	0,299^{a}	0,308^{a}	0,313^{a}
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^{ab}	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^{c}	0,443^{c}	0,499^{c}
P4	0,285	0,228^{a}	0,238^{a}	0,178^{a}	0,155^{a}	0,247^{a}	0,264^{a}	0,269^{a}	0,274^{a}

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

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effect of the addition of microbes from the swamp ~~shows~~ suggested that the ammonia content in the rearing media ~~that was given~~with a combination of Chlorophyta, *Bacillus* 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 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.

~~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 oxidation is performed by 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).

~~In~~ Among the ~~interaction between~~ 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 ~~provided~~ were able to break down the 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. In presence of ~~T~~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 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 ~~gave~~ 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 ~~suspected~~ presumed that the ammonia accumulation from metabolic waste had not yet occurred ~~, so that~~ therefore the microbes given to the rearing media were able to work optimally. On the 25th to 40th day, it was ~~suppos~~suspected that the ammonia accumulation from metabolic waste had been accumulated in the rearing media, so that the added 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~~ added to 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.

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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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References

Balcazar, J., L., DeBlas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., And Muzquiz, J., L., 2006. The role of probiotics in aquaculture. Veterinary Microbiology Review, 114, 173-186.

Bernal, M., G., Ricardo, M., M., Angel, I., C., and Jose, M., M., 2017. Probiotic effect of Streptomyces strains alone or in combination with Bacillus and Lactobacillus in juveniles of the white shrimp Litopenaeus vannamei. Aquaculture International, 25(2), 927-939.

Blancheton, J.P., Attramadal, K.J.K., Michaud L., Roque d'Orbcastel, E. dan Vadstein, O., 2013. Insight into bacterial population in aquaculture systems and its implication. Aquacultural Engineering 53, 30–39.

Bouman, H.A., T. Platt, S. Sathyendranath, W.K.W. Li, V. Stuart, C. Fuentes-Yaco, H. Maass, E.P.W. Horne, O. Ulloa, V. Lutz, and M. Kyewalyanga. 2003. Temperature as indicator of optical properties and community structure of marine phytoplankton: implications for remote sensing. Mar Ecol Prog Ser, 258, 19-30.

Budianto dan Heny, S., 2017. [Aktivitas antagonis Bacillus sp. terhadap Streptococcus iniae dan Pseudomonas fluorescens]. Jurnal Veteriner, 18(3), 409-415. [In Indonesian].

Ebeling, J.M., Timmons, M.B. dan Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture, 257, 346-358.

Edwards, V.A., 2011. The nitrogen cycle-Control ammonia, Nitrite in ponds, lakes, Lagoons, Rivers and waste water treatment [Online]. Available at: http://www.biosolve.com.au/index_files [Accessed 20 October 2018].

Fardiaz, D. 1992. Mikrobiologi Pangan I. Jakarta: Gramedia.

Feliatra, I.E., dan Suryadi, E., 2004. Isolasi dan identifikasi bakteri probiotik dari ikan kerapu macan (Ephinephelus fuscogutatus) dalam upaya efisiensi pakan ikan. Jurnal Natur Indonesia. 4(2), 10-14.

Findy, K., 2009. Aktivitas penghambat Bacillus sp. terhadap Xanthomonas oryzae pv. Oryzae, Pseudomonas syringae pv. glycines, dan Pseudomonas fluorescens. Skripsi. IPB.

Hartini, S., Ade, D. S. dan Ferdinand, H. T., 2013. Kualitas air, kelangsungan hidup dan pertumbuhan benih ikan gabus (Channa striata) yang dipelihara dalam media dengan penambahan probiotik. Jurnal Akuakultur Rawa Indonesia, 1(2), 192-202.

Irianto, A and Austin, B., 2002. Probiotics in aquaculture. Journal of Fish Diseases, 25, 633-642.

Khotimah, H., 2019. Uji patogenitas bakteri kandidat probiotik asal sedimen rawa. Skripsi. Universitas Sriwijaya.

Lutfi, M.D., 2018. Eksplorasi Streptomyces Sp. Dari Berbagai Ekosistem Sebagai Penghasil Antibiotik Untuk Penghambat Pertumbuhan B. Subtilis Dan E. Coli. Skripsi. ITB.

Luti, K. J. K. Dan Mavituna, F., 2011. Streptomyces coecolor increases the production of undecylprodigiosin when interacted with Bacillus subtilis. Biotechnol Lett, 33, 113-118.

Mohapatra, S., T. Chakraborty, V. Kumar, G. DeBoeck and K.N. Mohanta, 2013. Aquaculture and stress management: a review of probiotic intervention. J. Anim. Physiol. Anim. Nutr, 97(3), 405-430.

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Mulyadi, A.E., 2011. Pengaruh Pemberian Probiotik pada Pakan Komersil terhadap Laju Pertumbuhan Benih Ikan Patin Siam (Pangasius hypotalamus). Skripsi. Universitas Padjajaran.
Mulyadi, G., 2016. Pemeliharaan ikan gabus (Channa striata) dengan padat tebar berbeda dalam media bioflok. Jurnal Akuakultur Rawa Indonesia, 4(2), 159-174.
National Research Council (NRC). 1977. Nutrien Requirement of Warm Water Fishes. National Acad. Press, Washington D.C.
Nurhayati, C., Hamzah, B. Dan Pambayun, R., 2014. Pengaruh pH, konsentrasi isolat Chlorella vulgaris dan waktu pengamatan terhadap tingkat cemaran limbah cair crumb rubber. Jurnal Dinamika Penelitian Industri, 25(2), 97-106.
Panigrahi, A., Sundaram, M., Cakrapani, S., Rajasekar, S., Dayal, J.S. dan Chavali, G., 2019. Effect of carbon and nitrogen ratio (C:N) manipulation on the production performance and immunity of pasific white shrimp Litopenaeus vannamei (Boone, 1931) in a biofloc-based rearing system. Aquaculture Research, 50, 29–41.
Rurangwa, E. dan Verdegem, M.C.J., 2014. Microorganisms in recirculating aquaculture systems and their management. Reviews In Aquaculture, 5, 1-14.
Sanchez, P. T., Ruiz-Zarzuela, I., Blas, I. D., dan Balcazar, J. L. 2014. Probiotics in aquaculture: A current assessment. Reviews in Aquaculture, 6, 133–146.
Saraswati, N., 2018. Isolasi aktinomiset untuk bioremediasi air rawa yang tercemar bahan organik. Skripsi. Universitas Sriwijaya.
Sitohang, M.L., Fitrani, M. dan Jubaedah, D., 2018. Pemanfaatan campuran buah nanas, air cucian beras, dan gula sebagai sumber karbon pada media pemeliharaan ikan lele (Clarias sp.) Dengan sistem bioflok. Jurnal Akuakultur Rawa Indonesia, 6(1), 51-64.
Trisna, D.E., Sasanti, A.D. dan Muslim, 2013. Populasi bakteri, kualitas air media pemeliharaan dan histologi benih ikan gabus (Channa striata) yang diberi pakan berprobiotik. Jurnal Akuakultur Rawa Indonesia, 1(1), 90-102.
Wijayanti, M., Jubaedah, D., Suhada, J.A., Yuliani, S., Saraswati, N., Tanbiyaskur., Syaifudin, M., and Widjajanti, W., 2018. DNA Barcoding of Swamp Sediment Bacterial Isolates for Swamp Aquaculture Probiotic. E 3S Web of Conferences 1st SRICOENV, https://doi.org/10.1051/e3sconf /20186801068 10.
Yuliani, S., 2017. Isolasi bakteri rawa sebagai agen bioaugmentasi pada air rawa tercemar bahan organik. Skripsi. Universitas Sriwijaya.

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Authors:
Marini Wijayanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: mariniwijayanti@fp.unsri.ac.id
Dade Jubaedah, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program,Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: dadejubaedah@fp.unsri.ac.id
Ocktin Yulistya, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: yulistyaocktin@gmail.com
Tanbiyaskur, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: qurhadi30@gmail.com
Ade Dwi Sasanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: sasanti.ade@gmail.com
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Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit (6 Maret 2020)

MARINI WIJAYANTI, S.PI., M.SI fp <mariniwijayanti@fp.unsri.ac.id> 6 Mar 2020, 11.33

kepada Eni

Dear Editor AACL Bioflux,
Ms. Eni Kovac Ph.D.,

We have revised our manuscript. We hope the revision is in accordance with your suggestion for the publishing process.
Thank you for your help.

Best regards,

Marini Wijayanti

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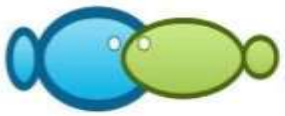

Optimization of striped snakehead fish (*Channa striata*) culture using swamp microbial combination and nitrification bacteria

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

[1]Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya Universitasy Sriwijaya, Indralaya, Ogan Ilir, South Sumatera, Indonesia. Corresponding author: Marini M. Wijayanti, mariniwijayanti@fp.unsri.ac.id

Abstract. Utilization of swamp areas as a fish culture locations will cause a decrease in water quality. Therefore, it is necessary to improve the water quality with environmental friendly of biological treatments, one ofsuch 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 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 fourtwo 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) 5×10^6 CFU mL^{-1} (N2). The second factor is consists in four scenarios: (1) without the addition of swamp microbes (P1) and with the addition of: (2) *Chlorophyta* (3.43×10^7 sel L^{-1}) and *Bacillus* sp. (10^5 CFU mL^{-1}) (P2); (3) addition of *Chlorophyta* (3.43×10^7 sel L^{-1}) and *Streptomyces* sp. (10^5 CFU mL^{-1}) (P3); (4) addition of *Chlorophyta* (3.43×10^7 sel L^{-1}), *Bacillus* sp. (10^5 CFU mL^{-1}) and *Streptomyces* sp. (10^5 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) and only the addition of nitrification bacteria (N1P2). Combination of N1P4 produceces 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, biofloc.

Introduction. The swamp aquaculture must be improveing and maintaining the water quality for fish rearing media, through environmentally friendly biological treatments, since. tThe 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.l^{-1}.week^{-1} can improve and maintain optimal water quality. In other studies, the addition of the effective probiotic microorganism 4 probiotics can reduce ammonia levels and suppress the population of pathogenic 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 Sswamp 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 10^5 CFU.ml^{-1} (Khotimah, 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 NH_3, NO_2^-, and NO_3^- 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 CO_2 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 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 CO_2 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 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 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. 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 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) 5×10^6 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 (3.43×10^7 Cellcell.L^{-1}) and *Bacillus* sp. (10^5 CFU.mL^{-1})

P3: Provision of 100 ml Chlorophyta (3.43×10^7 Cellcell.L^{-1}) and *Streptomyces* sp. (10^5 CFU.mL^{-1})

P4: Provision of 100 ml Chlorophyta (3.43×10^7 Cellcell.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 nutrient agar NA (NA nutrient agar) media for *Bacillus* sp and yeast malt YM (YM yeast malt agar) 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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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 grew.

Swamp bacteria that grew grown on NA and YM agar media were multiplied by nutrient broth NB (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 taken as much as 1 ose in order to be cultured in the mediaum as much as 5 mL in a test tube, then homogenized with a shaker for approximately 2 days for *Bacillus* sp. and 5 days for *Streptomyces* sp., then they were scaled upmultiplied from 5 mL to 500 mL in their liquid media for each cultivation periods. The concentrations of bacteria were measured by Mc Farlan spectrophotometry methods.

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 Eerlenmeyer 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 10^7cell $\cdot$ml^{-1} in 10 ml stock culture) were put into an Eerlenmeyer containing a technical fertilizer media for liquid culture. They cultured during 9 days in at 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 cm^3 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 10^7 Cellcell $\cdot L^{-1}$), *Bacillus* sp. (10^5 CFU $\cdot$mL^{-1}), *Streptomyces* sp. (10^5 CFU $\cdot$mL^{-1}) as well as the "PROBAC" nNitrification bacteria (5 x 10^6 CFU $\cdot$mL^{-1}). were added in combination with the treatment.

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 using. 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 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 25 ml sample. Observation of Chlorophyta 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 *Chlorophyta* samples (Davis, 1955). *Chlorophyta* abundance calculation was done performed by using the Leackey Drop Microtransect method (American Public Health Association, 1989) as follows:

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

InformationWhere:
N = Total number (cell $\cdot L^{-1}$)
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 L)

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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 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. The f~~F~~loc volume was obtained by ~~taking~~ 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:

~~Survival rate =~~

$$\frac{Nt}{No} \times 100\%$$

Where:
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 was~~s~~ calculated by using the following formula:

$$W = Wt - W_0$$

Where:
W = Growth of weight of fish for rearing (grams)
Wt = Weight of fish at the end of rearing (grams)
W_0 = 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:

$$L = Lt\text{-}L_0$$

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)

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

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

~~Note~~Where:
~~-~~EP = Feed ~~Efficiency~~ 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 40 days later, at the end of the rearing, ~~for 40 days~~.

Data analysis. Research data including biofloc volume, survival, growth, feed efficiency, water quality ~~were~~ was statistically ~~analyzed~~ processed by using the variance analysis ~~of 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

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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 the interaction~~relationship~~s between zooplankton and phytoplankton is a series of eating and prey relationships~~.~~ forming ~~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 trophic levels (Bouman et al~~.~~, 2003).

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

Table 1

Commercial nitrification bacteria	*Swamp microbes*	*Chlorophyta Abundance (Cell.L^{-1})*		
		0 day	*10th day*	*40th day*
N1	P1	3,20 × 10^3	3,20 × 10^3	2,10 × 10^3
	P2	3,60 × 10^3	3,43 × 10^7	4,10 × 10^3
	P3	4,10 × 10^3	3,43 × 10^7	4,10 × 10^3
	P4	3,70 × 10^3	3,43 × 10^7	4,46 × 10^3
N2	P1	4,00 × 10^3	4,0 × 10^3	2,10× 10^3
	P2	3,60 × 10^3	3,43 × 10^7	2,41 × 10^3
	P3	3,90 × 10^3	3,43 × 10^7	2,34 × 10^3
	P4	3,40 × 10^3	3,43 × 10^7	4,03 × 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 ~~after~~ of rearing are showed in Table 3 and Table 4, respectively.

~~Table 2.~~ Total bacterial population in rearing media

Table 2

Commercial nitrification bacteria	*Swamp microbes*	*Total bacterial population (CFU.mL^{-1})*			
		0 day	*1st day*	*20th day*	*40th day*
N1	P1	6,60 × 10^4	6,78 × 10^4	1,55 × 10^5	6,20 × 10^3
	P2	6,20 × 10^4	3,95 × 10^6	6,93 × 10^6	2,77 × 10^5
	P3	7,00 × 10^4	3,28 × 10^6	7,53 × 10^6	3,01 × 10^5
	P4	4,70 × 10^4	5,59 × 10^7	1,00 × 10^8	2,99 × 10^6
N2	P1	7,10 × 10^4	2,01 × 10^7	3,54 × 10^7	1,42 × 10^6
	P2	4,50 × 10^4	3,29 × 10^7	5,59 × 10^7	1,68 × 10^6

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	P3	4,30 × 10^4	4,99 × 10^7	6,41 × 10^7	1,93 × 10^6
	P4	4,95 × 10^4	4,70 × 10^7	8,75 × 10^7	4,06 × 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. ~~While~~whileereas, 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~~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 bacteria~~treatment was significantly higher compared to the treatment ~~given~~ 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. ~~, 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.

Table 3

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

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=4,386)*
	P1	*P2*	*P3*	*P4*	
N1	11,111	16,666	13,332	26,668	16,944[b]
N2	10,000	10,000	13,332	16,667	12,500[a]
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~~ of rearing

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=6,631)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=3,315)*
	P1	*P2*	*P3*	*P4*	
N1	11,112[a]	26,667[b]	16,667[a]	38,889[c]	25,834[b]
N2	13,333[a]	13,333[a]	23,333[b]	33,333[c]	20,833[a]
The main effects of swamp microbes (P) ($LSD_{0.05}$=4,689)	12,223[a]	20,000[b]	20,000[b]	41,111[c]	

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 L^{-1}. This It is presumed that the rearing media lacks a carbon source used bywhich bacteria use for the floc formation. According to Panigrahi et al. (2019), vVannamei 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 Vvannamei 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., 2018).

The results of the analysis of variance showed that the effect of the interaction between factors and and the effect of the factors of defined by the addition of swamp originated microbes to on the survival of snakehead fish varied significantly affected between treatments, but the factor of 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 given treated with a combination of Chlorophyta, *Bacillus* sp. and Streptomyces sp., the survival rate wasere significantly higher than for the other treatments, and iI Interactions between factors showed that in the rearing media given with a combination of Chlorophyta, *Bacillus* sp., *Streptomyces* sp. and without commercial nitrification bacteria, the survival rate reached 63.94%, a significantly higher different performance compared to the other treatments with a percentage of 63.94%.

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* was cultured together with *Streptomyces* can 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 Single Influence of Swamps Microbes (P) ($LSD_{0.05}$=6.02)				The Main Effects of Nitrifying Bacteria (N)
	P1	P2	P3	P4	
N1	26.06[a]	36.91[b]	28.03[a]	63.94[d]	38.74
N2	31.75[a b]	28.03[a]	35.16[b]	48.20[c]	35.79
The Main Effects of Swamp Microbes (P) ($LSD_{0.05}$=4.25)	28.91[a]	32.47[a]	31.59[a]	56.07[b]	

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 10 ul.l^{-1} week^{-1} 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~~Suprastyani (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 by producing secondary metabolite compounds (Lutfi~~,~~ 2018). The combination of the two microbes can provide a high percentage of survival compared to ~~without a~~ the no combination scenario. According to Irianto ~~and~~ & 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 efficiency of varioussety ~~of~~ feed on the snakehead fish ~~feed efficiency~~ showed that the interaction between factors, the microbial addition factors from swamps and the addition of commercial nitrification bacteria, can ~~to~~ increase the value of fish feed efficiency, which is significantly affected ~~between~~ by the treatment type and concentration~~s~~. 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 snakehead fish feed for 40 days of rearing

The Single Effect of Nitrifying Bacteria (N)	The Single Influence of Swamps Microbe (P) ($LSD_{0.05}$=3.32)				The Main Effects of Nitrifying Bacteria (N) ($LSD_{0.05}$=1.66)
	P1	P2	P3	P4	
N1	18.93^{a}	47.34^{d}	37.97^{c}	59.65^{e}	40.97^{b}
N2	22.00^{a}	29.52^{b}	34.89^{c}	44.11^{d}	32.63^{a}
The Main Effects of Swamp Microbes (P) ($LSD_{0.05}$= 2.35)	20.47^{a}	38.43^{b}	36.43^{b}	51.88^{c}	

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, T~~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 origin of~~ 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~~.~~ ~~The nutrients were need for the fish and the absorption of nutrients runs optimally. The 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). In 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 ofin 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 nitrification bacteria was significantly higher without commercial nitrification bacteria than the treatment givenwith commercial nitrification bacteria; (2), more triggers growthOn 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 weight of snakehead fish in the treatment of rearing media that were givenwith 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 nitrifying bacteria.

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) ($LSD_{0.05}$=0.04)
	P1	P2	P3	P4	
N1	1.30^a	2.26^f	1.70^c	2.32^f	1.90^b
N2	1.73^c	1.41^a	1.88^d	2.08^e	1.78^a
The Main Effects of Swamp Microbes (P) ($LSD_{0.05}$=0.05)	1.51^a	1.84^b	1.79^b	2.20^c	

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.

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

The Single Effect of Nitrifying Bacteria (N)	The Single Influence of Swamps Microbe (P) ($LSD_{0,05}$=0,10)				The Main Effects of Nitrifying Bacteria (N)
	P1	P2	P3	P4	
N1	0,69[a]	1,79[e]	0,91[b]	2,27[f]	2,12
N2	1,13[c]	1,08[c]	1,60[d]	1,74[e]	2,09
The Main Effects of Swamp Microbes (P) ($LSD_{0,05}$=0,07)	0,91[a]	1,44[c]	1,26[b]	2,00[d]	

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 givenwith a combination of Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. were was significantly different than the in other treatments. TOn 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 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 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 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 Suryadi, 2004). The addition of 10^4 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 10^5 cfu/mL^{-1} in the rearing media of *P. hypophthalmus* showed significant increase in the growth, immune and antioxidant responses compared to 10^7 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 thaten 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 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 media~~significantly different~~ with a combination of Chlorophyta, *Bacillus* sp., and *Streptomyces* sp. (P4) ~~than~~ between other treatments.

Table 9.

LSD test result of dissolved oxygen in the rearing media

	Dissolved Oxygen (mg.L^{-1})								
	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 ±0,1	3,53 ±0,1	3,53 ±0,1	3,40^{c} ±0,1	3,10^{c} ±0,1	3,00^{c} ±0,1	2,97^{c} ±0,1	2,77^{c} ±0,1	2,67^{c} ±0,1
N1P2	3,63 ±0,1	3,53 ±0,1	3,53 ±0,1	3,23^{ab} ±0,1	2,93^{ab} ±0,1	2,83^{ab} ±0,1	2,77^{ab} ±0,1	2,57^{ab} ±0,1	2,47^{ab} ±0,1
N1P3	3,67 ±0,1	3,57 ±0,2	3,47 ±0,2	3,33^{bc} ±0,1	3,03^{bc} ±0,1	2,93^{bc} ±0,1	2,87^{bc} ±0,1	2,67^{bc} ±0,1	2,57^{bc} ±0,1
N1P4	3,57 ±0,1	3,70 ±0,1	3,60 ±0,1	3,60^{d} ±0,1	3,50^{e} ±0,1	3,40^{e} ±0,1	3,40^{e} ±0,1	3,20^{e} ±0,1	3,10^{e} ±0,1
N2P1	3,63 ±0,1	3,43 ±0,1	3,43 ±0,1	3,13^{a} ±0,1	2,83^{a} ±0,1	2,73^{a} ±0,1	2,67^{a} ±0,1	2,47^{a} ±0,1	2,37^{a} ±0,1
N2P2	3,57 ±0,1	3,47 ±0,1	3,47 ±0,1	3,33^{bc} ±0,1	3,03^{c} ±0,1	2,93^{bc} ±0,1	2,87^{bc} ±0,1	2,67^{bc} ±0,1	2,57^{bc} ±0,1
N2P3	3,53 ±0,1	3,43 ±0,1	3,33 ±0,1	3,27^{ab} ±0,1	3,10^{c} ±0,1	3,00^{c} ±0,1	2,90^{bc} ±0,1	2,70^{bc} ±0,1	2,60^{c} ±0,1
N2P4	3,67 ±0,1	3,63 ±0,1	3,53 ±0,1	3,40^{c} ±0,1	3,30^{d} ±0,1	3,20^{d} ±0,1	3,20^{d} ±0,1	3,00^{d} ±0,1	2,90^{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^{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	3,49^{a}	3,44^{a}	4,93^{a}	3,07^{a}	2,97^{a}	2,91^{a}	2,71^{a}	2,61^{a}
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^{ab}	3,27^{a}	2,97^{a}	2,87^{a}	2,82^{a}	2,62^{a}	2,52^{a}
P2	3,60	3,50^{a}	3,50^{ab}	3,28^{a}	2,98^{a}	2,88^{a}	2,82^{a}	2,62^{a}	2,52^{a}
P3	3,60	3,50^{a}	3,40^{a}	3,30^{a}	3,07^{a}	2,97^{a}	2,88^{a}	2,68^{a}	2,58^{a}
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}

~~On 10~~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 ~~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 that~~ by 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 interaction~~microbial origin of swamp and the interaction between factors~~ significantly affected ammonia content. They are presented in Table 10.

Table 10.

LSD test results for ammonia every 5 days on rearing media

	Mean of Ammonia Concentratin (mg.L^{-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 ±0,07	0,383^{e} ±0,01	0,393^{e} ±0,01	0,410^{e} ±0,01	0,327^{e} ±0,01	0,540^{d} ±0,01	0,674^{a} ±0,01	0,691^{f} ±0,01	0,948^{e} ±0,02
N1P2	0,323 ±0,03	0,273^{d} ±0,01	0,283^{c} ±0,01	0,223^{c} ±0,01	0,203^{c} ±0,01	0,293^{b} ±0,01	0,314^{b} ±0,01	0,321^{c} ±0,01	0,324^{b} ±0,01

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N1P3	0.283 ±0.01	0.257^{c} ±0.01	0.267^{b} ±0.01	0.207^{g} ±0.01	0.197^{hi} ±0.01	0.363^{c} ±0.02	0.482^{e} ±0.02	0.585^{g} ±0.01	0.692^{d} ±0.02
N1P4	0.267 ±0.01	0.220^{g} ±0.01	0.230^{g} ±0.01	0.170^{g} ±0.01	0.147^{g} ±0.01	0.243^{g} ±0.02	0.260^{g} ±0.02	0.265^{g} ±0.02	0.270^{g} ±0.02
N2P1	0.230 ±0.01	0.253^{c} ±0.01	0.263^{b} ±0.01	0.203^{g} ±0.01	0.193^{ic} ±0.01	0.282^{b} ±0.02	0.363^{c} ±0.02	0.385^{d} ±0.01	0.392^{c} ±0.02
N2P2	0.290 ±0.04	0.233^{ab} ±0.01	0.243^{a} ±0.01	0.183^{a} ±0.01	0.160^{a} ±0.01	0.250^{a} ±0.01	0.268^{a} ±0.01	0.273^{a} ±0.01	0.277^{a} ±0.01
N2P3	0.250 ±0.02	0.277^{d} ±0.01	0.287^{c} ±0.01	0.227^{c} ±0.01	0.187^{b} ±0.01	0.277^{b} ±0.01	0.296^{b} ±0.01	0.300^{b} ±0.01	0.306^{b} ±0.01
N2P4	0.303 ±0.03	0.237^{ab} ±0.01	0.247^{b} ±0.01	0.187^{b} ±0.01	0.163^{b} ±0.01	0.250^{a} ±0.01	0.268^{a} ±0.01	0.273^{a} ±0.01	0.277^{a} ±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^{a}	0.253^{a}	0.218^{a}	0.360^{a}	0.432^{a}	0.466^{a}	0.558^{a}
N2	0.268	0.250^{a}	0.260^{a}	0.200^{a}	0.176^{a}	0.265^{a}	0.299^{a}	0.308^{a}	0.313^{a}
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^{c}	0.443^{c}	0.499^{c}
P4	0.285	0.228^{a}	0.238^{a}	0.178^{a}	0.155^{a}	0.247^{a}	0.264^{a}	0.269^{a}	0.274^{a}

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 effect of the addition of microbes from the swamp ~~shows~~ suggested that the ammonia content in the rearing media ~~that was given~~with 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, 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 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 oxidation is performed by 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).

~~In~~ Among the ~~interaction between~~ factors' interactions, N1P4 treatment had the lowest ammonia levels on the 5th day until the end of rearing. ~~.~~ ~~I~~It was suspected that microbes from the swamp ~~provided~~ were able to break down the 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. In presence of ~~T~~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

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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 ~~gave~~ 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 ~~suspected~~ presumed that the ammonia accumulation from metabolic waste had not yet occurred~~,so that~~ therefore the microbes given to the rearing media were able to work optimally. On the 25th to 40th day, it was supposspected that the ammonia accumulation from metabolic waste had been accumulated in the rearing media, so that the added 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~~ added to the media have not been able to make an optimal decomposition.

Conclusion. The ~~A~~addition 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 swamp microbes Chlorophyta, *Bacillus* sp. and *Streptomyces* sp. without the addition of commercial nitrification bacteria in the rearing media that~~and gave in the best of survival rate, feed efficiency, and growth of striped snake head fish in swamp aquaculture~~, although there was not used nitrification bacteria. *Bacillus* and *Streptomyces* 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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References

Balcazar~~,~~ J. L., DeBlas~~,~~ I., Ruiz-Zarzuela~~,~~ I., Cunningham~~,~~ D., Vendrell~~,~~ D., ~~And~~ Muzquiz, J. L., 2006. The role of probiotics in aquaculture. Veterinary Microbiology Review~~,~~ 114~~,~~ 173-186.

Bernal~~,~~ M. G., Ricardo~~,~~ M. M., Angel~~,~~ I. C., ~~and~~ Jose~~,~~ M. M., 2017. Probiotic effect of Streptomyces strains alone or in combination with Bacillus and Lactobacillus in juveniles of the white shrimp *Litopenaeus vannamei*. Aquaculture International~~,~~ 25(2)~~,~~ 927-939.

Blancheton, J.P., Attramadal, K.J.K., Michaud L., Roque d'Orbcastel, E. dan Vadstein, O., 2013. Insight into bacterial population in aquaculture systems and its implication. Aquacultural Engineering 53, 30–39.

Bouman, H.A., T. Platt, S. Sathyendranath, W.K.W. Li, V. Stuart, C. Fuentes-Yaco, H. Maass, E.P.W. Horne, O. Ulloa, V. Lutz, and M. Kyewalyanga. 2003. Temperature as indicator of optical properties and community structure of marine phytoplankton: implications for remote sensing. Marine Ecology Progress Series, 258, 19-30.

Budianto dan ~~Heny~~Suprastyani, H~~S~~., 2017. [Aktivitas antagonis Bacillus sp. terhadap Streptococcus iniae dan Pseudomonas fluorescens Antagonist activity of *Bacillus subtilis* against *Streptococcus iniae* and *Pseudomonas fluorescens*]. Jurnal Veteriner~~,~~ 18(3)~~,~~ 409-415. [In Indonesian].

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de Melo Pereira, G.V., de Oliveira Coelho, B., Junior, A.I.M., Thomaz-Soccol, V., Soccol, C.R., 2018. How to select a probiotic? A review and update of methods and criteria. Biotechnology Advances, 36, 2060–2076.

Ebeling, J.M., Timmons, M.B. dan Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture, 257, 346-358.

Edwards, V.A., 2011. The nitrogen cycle-Control ammonia, Nitrite in ponds, lakes, Lagoons, Rivers and waste water treatment [Online]. Available at: http://www.biosolve.com.au/index_files [Accessed 20 October 2018].

Fardiaz, D. 1992. Food MicrobiologyMikrobiologi Pangan I. Jakarta: Gramedia.[In Indonesian]

~~Feliatra, F.E., dan Suryadi, E., 2004. Isolasi dan identifikasi bakteri probiotik dari ikan kerapu macan (Ephinephelus fuscogutatus) dalam upaya efisiensi pakan ikan. Jurnal Natur Indonesia. 4(2), 10-14.~~

Findy, K., 2009. ~~Aktivitas penghambat~~Inhibition activity of *Bacillus* sp. ~~terhadap~~to *Xanthomonas oryzae* pv. *Oryzae*, *Pseudomonas syringae* pv. glycines, ~~dan~~and *Pseudomonas fluorescens*. Undergraduate Thesis~~Skripsi~~. IPB University.[In Indonesian]

Gobi, N., Malaikozhundan, B., Sekar, V., Shanthi, S., Vaseeharan, B., Jayakumar, R., Nazar, A.K. 2016. GFP tagged *Vibrio parahaemolyticus* Dahv2 infection and the protective effects of probiotic *Bacillus licheniformis* Dahb1 on the growth, immune and antioxidant responses in *Pangasius hypophthalmus*. Fish Shellfish Immunology, 52, 230–238

Hartini, S., ~~Ade~~Sasanti, ~~D~~A.D. ~~S.~~ dan ~~Ferdinand~~Tagwa, F. ~~H.~~ ~~T.~~, 2013. Water quality, survival rate, and growth of striped snakehead fish (*Channa striata*) juvenile cultured in media with probiotic. ~~Kualitas air, kelangsungan hidup dan pertumbuhan benih ikan gabus (*Channa striata*) yang dipelihara dalam media dengan penambahan probiotik.~~ Jurnal Akuakultur Rawa Indonesia, 1(2), 192-202.[In Indonesian]

Irianto, A and Austin, B., 2002. Probiotics in aquaculture. Journal of Fish Diseases, 25, 633-642.

Khotimah, H., 2019. Pathogenicity of probiotic candidates bacteria from swamp sediment. ~~Uji patogenitas bakteri kandidat probiotik asal sedimen rawa. Skripsi~~ Undergraduate Thesis. ~~Universitas~~ Sriwijaya University.[In Indonesian]

Lutfi, M.D., 2018. Exploration on Streptomyces sp. Antibiotic Producers from Various Unique Ecosystems in Indonesia~~Eksplorasi Streptomyces Sp. Dari Berbagai Ekosistem Sebagai Penghasil Antibiotik Untuk Penghambat Pertumbuhan B. Subtilis Dan E. Coli. Skripsi. ITB.~~ Undergraduate Thesis. IPB University.

Luti, K. J. K. ~~Dan~~and Mavituna, F., 2011. Streptomyces coecolor increases the production of undecylprodigiosin when interacted with *Bacillus subtilis*. Biotechnology Letter, 33, 113-118.

Midhun, S.J., Neethu, S., Vysakh, A., Radhakrishnan, E.K., Jyothis, M., 2018. Antagonism Against Fish Pathogens by Cellular Components/Preparations of *Bacillus coagulans* (MTCC-9872) and It's In Vitro Probiotic Characterisation. Current Microbiology, 75, 1174–1181.

Mohapatra, S., ~~T.~~ Chakraborty, T~~.~~., Kumar, V., ~~G.~~ DeBoeck, G., ~~and K.N.~~ Mohanta, K.N. 2013. Aquaculture and stress management: a review of probiotic intervention. ~~J. Anim. Physiol. Anim. Nutr~~Journal of Animal Physiology and Animal Nutrition, 97(3), 405-430.

~~Mulyadi, A.E., 2011. Pengaruh Pemberian Probiotik pada Pakan Komersil terhadap Laju Pertumbuhan Benih Ikan Patin Siam (Pangasius hypotalamus). Skripsi. Universitas Padjajaran.~~

Mulyadi, G., 2016. ~~Pemeliharaan ikan gabus (Channa striata) dengan padat tebar berbeda dalam media bioflok~~Rearing of striped snakehead fish (*Channa striata*) with different stocking densities in biofloc media. Jurnal Akuakultur Rawa Indonesia, 4(2), 159-174. [In Indonesia]

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Nargesi, E.A., Falahatkar, B., Sajjadi, M.M., 2019. Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (*Oncorhynchus mykiss*) broodstock. Aquaculture Nutrition, 26(1), 98-108.

National Research Council (NRC). 1977. Nutrien Requirement of Warm Water Fishes. National Acad. Press, Washington D.C.

Nurhayati, C., Hamzah, B. Dan Pambayun, R., 2014. ~~Pengaruh pH, konsentrasi isolat Chlorella vulgaris dan waktu pengamatan terhadap tingkat cemaran limbah cair crumb rubber~~ Effect of pH, chlorella vulgaris isolate concentration and time of observation on the level of contamination of crumb rubber liquid waste. Jurnal Dinamika Penelitian Industri, 25(2), 97-106.[In Indonesian]

Panigrahi, A., Sundaram, M., Cakrapani, S., Rajasekar, S., Dayal, J.S. dan Chavali, G., 2019. Effect of carbon and nitrogen ratio (C:N) manipulation on the production performance and immunity of pasific white shrimp Litopenaeus vannamei (Boone, 1931) in a biofloc-based rearing system. Aquaculture Research, 50, 29–41.

Rurangwa, E. dan Verdegem, M.C.J., 2014. Microorganisms in recirculating aquaculture systems and their management. Reviews In Aquaculture, 5, 1-14.

Sanchez, P. T., Ruiz-Zarzuela, I., Blas, I. D., dan Balcazar, J. L. 2014. Probiotics in aquaculture: A current assessment. Reviews in Aquaculture, 6, 133–146.

Saraswati, N., 2018. ~~Isolasi aktinomiset untuk bioremediasi air rawa yang tercemar bahan organik~~Actinomycetes isolation for bioremediation of swamp water polluted by organic matter. Undergraduate thesis~~Skripsi~~. Universitas Sriwijaya.[In Indonesia]

Sitohang, M.L., Fitrani, M. dan Jubaedah, D., 2018. ~~Pemanfaatan campuran buah nanas, air cucian beras, dan gula sebagai cumber karbon pada media pemeliharaan ikan lele (Clarias sp.) Dengan sistem bioflok~~Utilization of a mixture of pineapple, rice washing water, and sugar as a carbon source in catfish breeding media (Clarias sp.) with a biofloc system. Jurnal Akuakultur Rawa Indonesia, 6(1), 51-64. .[In Indonesia]

Trisna, D.E., Sasanti, A.D. dan Muslim, 2013. ~~Populasi bakteri, kualitas air media pemeliharaan dan histologi benih ikan gabus (Channa striata) yang diberi pakan berprobiotik~~Bacterial population, water quality maintenance media and histology of striped snakehead fish (*Channa striata*) juveniles given antibiotic feed. Jurnal Akuakultur Rawa Indonesia, 1(1), 90-102. [In Indonesia]

Wijayanti, M., Jubaedah, D., Suhada, J.A., Yuliani, S., Saraswati, N., Tanbiyaskur., Syaifudin, M., and Widjajanti, W., 2018. DNA Barcoding of Swamp Sediment Bacterial Isolates for Swamp Aquaculture Probiotic. E 3S Web of Conferences 1st SRICOENV, https://doi.org/10.1051/e3sconf /20186801068 10.

Yuliani, S., 2017. ~~Isolasi bakteri rawa sebagai agen bioaugmentasi pada air rawa tercemar bahan organik~~Isolation of swamp bacteria as a bioaugmentation agent in swamp water polluted by organic matter. ~~Skripsi. Universitas Sriwijaya.~~ Undergraduate thesis. Sriwijaya University.[In Indonesia]

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Authors:
Marini Wijayanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: mariniwijayanti@fp.unsri.ac.id
Dade Jubaedah, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program,Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: dadejubaedah@fp.unsri.ac.id
Ocktin Yulistya, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: yulistyaocktin@gmail.com
Tanbiyaskur, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: qurhadi30@gmail.com

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Ade Dwi Sasanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: sasanti.ade@gmail.com
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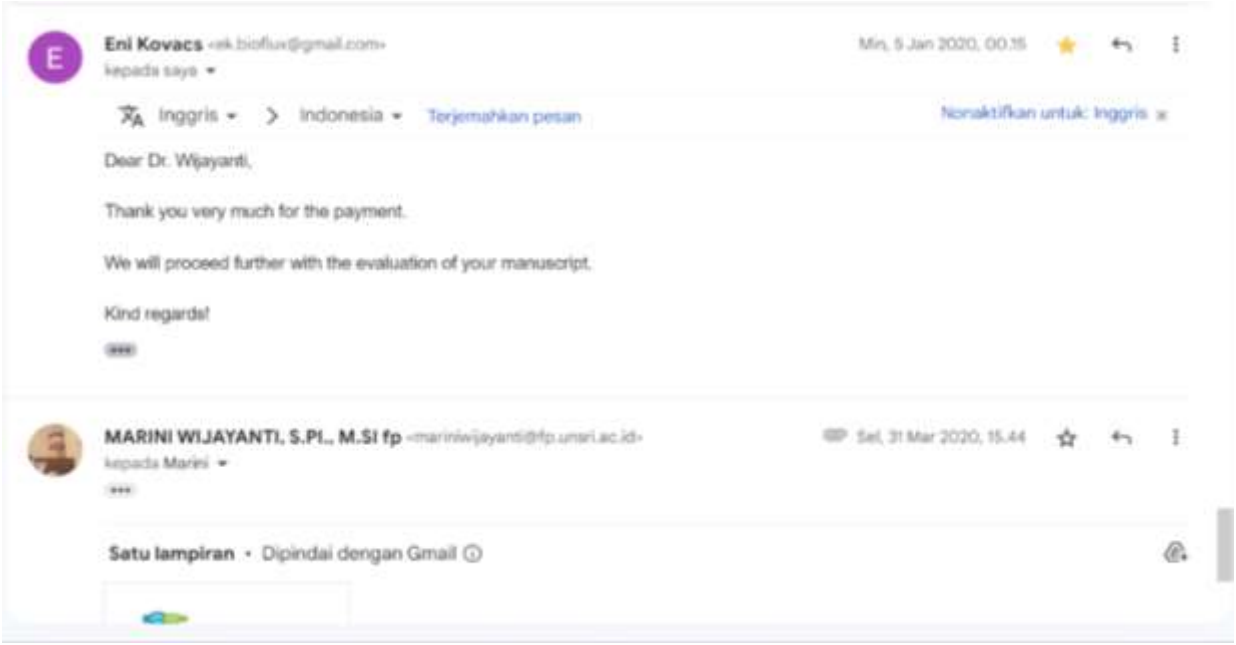

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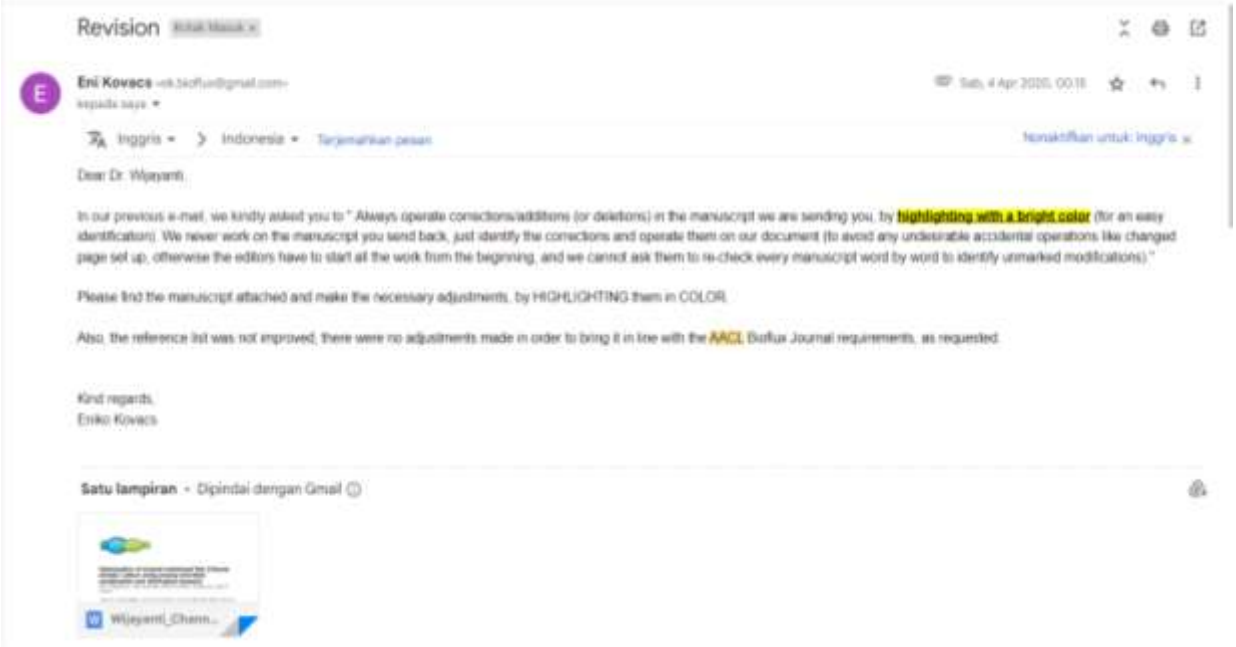

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Optimization of striped snakehead fish (*Channa striata*) culture using swamp microbial combination and nitrification bacteria

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

Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University, Indralaya, Ogan Ilir, South Sumatera, Indonesia. Corresponding author: M. Wijayanti, mariniwijayanti@fp.unsri.ac.id

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 (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 swamp microbes (P1) and with the addition of: (2) *Chlorophyta* (3.43×10^7 sel L^{-1}) and *Bacillus* sp. (10^5 CFU mL^{-1}) (P2); (3) *Chlorophyta* (3.43×10^7 sel L^{-1}) and *Streptomyces* sp. (10^5 CFU mL^{-1}) (P3); (4) *Chlorophyta* (3.43×10^7 sel L^{-1}), *Bacillus* sp. (10^5 CFU mL^{-1}) and *Streptomyces* sp. (10^5 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, probiotic, 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 µL 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 10^5 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 NH_3, NO_2^-, and NO_3^- 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 CO_2 used in cell metabolism. Gram-positive 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 CO_2 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) 5×10^6 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 (3.43×10^7 cell L^{-1}) and *Bacillus* sp. (10^5 CFU mL^{-1});
P3 - provision of 100 ml Chlorophyta (3.43×10^7 cell L^{-1}) and *Streptomyces* sp. (10^5 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 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

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 10^7cell 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 cm^3 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 (3.43×10^7 cell L^{-1}), *Bacillus* sp. (10^5 CFU mL^{-1}), *Streptomyces* sp. (10^5 CFU mL^{-1}) as well as PROBAC nitrification bacteria (5×10^6 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:

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

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 (....):

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

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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 (....):

$$\frac{Nt}{No} \times 100\%$$

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Where:
N_o - 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 was calculated by using the following formula (...):

$$W = Wt - W_0$$

Where:
W - growth of weight of fish for rearing (grams);
Wt - weight of fish at the end of rearing (grams);
W_0 - 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 (...):

$$L = Lt-L_0$$

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).

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

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

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.

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

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

Commercial nitrification bacteria	*Swamp microbes*	*Chlorophyta abundance (cell L^{-1})*		
		0 day	*10th day*	*40th day*
N1	P1	3.20×10^{3}	3.20×10^{3}	2.10×10^{3}
	P2	3.60×10^{3}	3.43×10^{7}	4.10×10^{3}
	P3	4.10×10^{3}	3.43×10^{7}	4.10×10^{3}
	P4	3.70×10^{3}	3.43×10^{7}	4.46×10^{3}
N2	P1	4.00×10^{3}	4.0×10^{3}	2.10×10^{3}
	P2	3.60×10^{3}	3.43×10^{7}	2.41×10^{3}
	P3	3.90×10^{3}	3.43×10^{7}	2.34×10^{3}
	P4	3.40×10^{3}	3.43×10^{7}	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.

Table 2

Total bacterial population in the rearing media

Commercial nitrification bacteria	*Swamp microbes*	*Total bacterial population (CFU mL^{-1})*			
		0 day	*1st day*	*20th day*	*40th day*
N1	P1	6.60×10^{4}	6.78×10^{4}	1.55×10^{5}	6.20×10^{3}
	P2	6.20×10^{4}	3.95×10^{6}	6.93×10^{6}	2.77×10^{5}
	P3	7.00×10^{4}	3.28×10^{6}	7.53×10^{6}	3.01×10^{5}
	P4	4.70×10^{4}	5.59×10^{7}	1.00×10^{8}	2.99×10^{6}
N2	P1	7.10×10^{4}	2.01×10^{7}	3.54×10^{7}	1.42×10^{6}
	P2	4.50×10^{4}	3.29×10^{7}	5.59×10^{7}	1.68×10^{6}
	P3	4.30×10^{4}	4.99×10^{7}	6.41×10^{7}	1.93×10^{6}
	P4	4.95×10^{4}	4.70×10^{7}	8.75×10^{7}	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 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

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

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=???)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=4.386)*
	P1	*P2*	*P3*	*P4*	
N1	11.111	16.666	13.332	26.668	16.944^{b}
N2	10.000	10.000	13.332	16.667	12.500^{a}
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 of rearing

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=6.631)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=3.315)*
	P1	*P2*	*P3*	*P4*	
N1	11.112^{a}	26.667^{b}	16.667^{a}	38.889^{c}	25.834^{b}
N2	13.333^{a}	13.333^{a}	23.333^{b}	33.333^{c}	20.833^{a}
The main effects of swamp microbes (P) ($LSD_{0.05}$=4.689)	12.223^{a}	20.000^{b}	20.000^{b}	41.111^{c}	???

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^{-3} 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

LSD test of survival rate of *Channa striata*

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=6.02)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=.....)*
	P1	*P2*	*P3*	*P4*	
N1	26.06^{a}	36.91^{b}	28.03^{a}	63.94^{d}	38.74
N2	31.75^{ab}	28.03^{a}	35.16^{b}	48.20^{c}	35.79
The main effects of swamp microbes (P) ($LSD_{0.05}$=4.25)	28.91^{a}	32.47^{a}	31.59^{a}	56.07^{b}	???

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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 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 nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=3.32)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=1.66)*
	P1	*P2*	*P3*	*P4*	
N1	18.93^{a}	47.34^{d}	37.97^{c}	59.65^{e}	40.97^{b}
N2	22.00^{a}	29.52^{b}	34.89^{c}	44.11^{d}	32.63^{a}
The main effects of swamp microbes (P) ($LSD_{0.05}$=2.35)	20.47^{a}	38.43^{b}	36.43^{b}	51.88^{c}	20.47^{a}

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 (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., *Streptomyces* 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 nitrifying bacteria.

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Table 7

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

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

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 nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=0.10)*				*The main effects of nitrifying bacteria (N)*
	P1	*P2*	*P3*	*P4*	*$LSD_{0.05}$=....*
N1	0.69^{a}	1.79^{e}	0.91^{b}	2.27^{f}	2.12
N2	1.13^{c}	1.08^{c}	1.60^{d}	1.74^{e}	2.09
The main effects of swamp microbes (P) ($LSD_{0.05}$=0.07)	0.91^{a}	1.44^{c}	1.26^{b}	2.00^{d}	0.91^{a}

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 10^{8} CFU mL^{-1} probiotics to the rearing media increased the length and weight of tiger shrimp larvae, compared to the controls (Widarnani et al 2010). *B. licheniformis* at 10^{5} CFU mL^{-1} in the rearing media of *P. hypophthalmus* showed a significant increase in the growth, immune and antioxidant responses compared to 10^{7} 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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Table 9

LSD test result of dissolved oxygen in the rearing media

	Dissolved oxygen (mg L^{-1})								
	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 ±0.1	3.53 ±0.1	3.53 ±0.1	3.40^{c} ±0.1	3.10^{c} ±0.1	3.00^{c} ±0.1	2.97^{c} ±0.1	2.77^{c} ±0.1	2.67^{c} ±0.1
N1P2	3.63 ±0.1	3.53 ±0.1	3.53 ±0.1	3.23^{ab} ±0.1	2.93^{ab} ±0.1	2.83^{ab} ±0.1	2.77^{ab} ±0.1	2.57^{ab} ±0.1	2.47^{ab} ±0.1
N1P3	3.67 ±0.1	3.57 ±0.2	3.47 ±0.2	3.33^{bc} ±0.1	3.03^{bc} ±0.1	2.93^{bc} ±0.1	2.87^{bc} ±0.1	2.67^{bc} ±0.1	2.57^{bc} ±0.1
N1P4	3.57 ±0.1	3.70 ±0.1	3.60 ±0.1	3.60^{d} ±0.1	3.50^{e} ±0.1	3.40^{e} ±0.1	3.40^{e} ±0.1	3.20^{e} ±0.1	3.10^{e} ±0.1
N2P1	3.63 ±0.1	3.43 ±0.1	3.43 ±0.1	3.13^{a} ±0.1	2.83^{a} ±0.1	2.73^{a} ±0.1	2.67^{a} ±0.1	2.47^{a} ±0.1	2.37^{a} ±0.1
N2P2	3.57 ±0.1	3.47 ±0.1	3.47 ±0.1	3.33^{bc} ±0.1	3.03^{c} ±0.1	293^{bc} ±0.1	2.87^{bc} ±0.1	2.67^{bc} ±0.1	2.57^{bc} ±0.1
N2P3	3.53 ±0.1	3.43 ±0.1	3.33 ±0.1	3.27^{ab} ±0.1	3.10^{c} ±0.1	3.00^{c} ±0.1	2.90^{bc} ±0.1	2.70^{bc} ±0.1	2.60^{c} ±0.1
N2P4	3.67 ±0.1	3.63 ±0.1	3.53 ±0.1	3.40^{c} ±0.1	3.30^{d} ±0.1	3.20^{d} ±0.1	3.20^{d} ±0.1	3.00^{d} ±0.1	2.90^{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^{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	3.49^{a}	3.44^{a}	4.93^{a}	3.07^{a}	2.97^{a}	2.91^{a}	2.71^{a}	2.61^{a}
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^{ab}	3.27^{a}	2.97^{a}	2.87^{a}	2.82^{a}	2.62^{a}	2.52^{a}
P2	3.60	3.50^{a}	3.50^{ab}	3.28^{a}	2.98^{a}	2.88^{a}	2.82^{a}	2.62^{a}	2.52^{a}
P3	3.60	3.50^{a}	3.40^{a}	3.30^{a}	3.07^{a}	2.97^{a}	2.88^{a}	2.68^{a}	2.58^{a}
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}

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

Table 10

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

	Mean of ammonia concentration (mg L^{-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 ±0.07	0.383^{e} ±0.01	0.393^{d} ±0.01	0.410^{d} ±0.01	0.327^{de} ±0.01	0.540^{d} ±0.01	0.674^{de} ±0.01	0.691^{f} ±0.01	0.948^{de} ±0.02
N1P2	0.323 ±0.03	0.273^{d} ±0.01	0.283^{c} ±0.01	0.223^{c} ±0.01	0.203^{c} ±0.01	0.293^{b} ±0.01	0.314^{b} ±0.01	0.321^{c} ±0.01	0.324^{b} ±0.01
N1P3	0.283 ±0.01	0.257^{c} ±0.01	0.267^{b} ±0.01	0.207^{b} ±0.01	0.197^{bc} ±0.01	0.363^{c} ±0.02	0.482^{d} ±0.02	0.585^{e} ±0.01	0.692^{d} ±0.02
N1P4	0.267 ±0.01	0.220^{a} ±0.01	0.230^{a} ±0.01	0.170^{a} ±0.01	0.147^{a} ±0.01	0.243^{a} ±0.02	0.260^{a} ±0.02	0.265^{a} ±0.02	0.270^{a} ±0.02
N2P1	0.230 ±0.01	0.253^{c} ±0.01	0.263^{b} ±0.01	0.203^{b} ±0.01	0.193^{bc} ±0.01	0.282^{b} ±0.02	0.363^{c} ±0.02	0.385^{d} ±0.01	0.392^{c} ±0.02
N2P2	0.290 ±0.04	0.233^{ab} ±0.01	0.243^{a} ±0.01	0.183^{a} ±0.01	0.160^{a} ±0.01	0.250^{a} ±0.01	0.268^{a} ±0.01	0.273^{a} ±0.01	0.277^{a} ±0.01
N2P3	0.250 ±0.02	0.277^{d} ±0.01	0.287^{c} ±0.01	0.227^{c} ±0.01	0.187^{b} ±0.01	0.277^{b} ±0.01	0.296^{b} ±0.01	0.300^{b} ±0.01	0.306^{b} ±0.01
N2P4	0.303 ±0.03	0.237^{b} ±0.01	0.247^{b} ±0.01	0.187^{b} ±0.01	0.163^{b} ±0.01	0.250^{a} ±0.01	0.268^{a} ±0.01	0.273^{a} ±0.01	0.277^{a} ±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	0.250^{a}	0.260^{a}	0.200^{a}	0.176^{a}	0.265^{a}	0.299^{a}	0.308^{a}	0.313^{a}
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^{c}	0.443^{c}	0.499^{c}
P4	0.285	0.228^{a}	0.238^{a}	0.178^{a}	0.155^{a}	0.247^{a}	0.264^{a}	0.269^{a}	0.274^{a}

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 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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References

Balcazar J. L., DeBlas I., Ruiz-Zarzuela I., Cunningham D., Vendrell D., Muzquiz, J. L., 2006 The role of probiotics in aquaculture. Veterinary Microbiology Review 114:173-186.

Bernal M. G., Ricardo M. M., Angel I. C., Jose M. M., 2017 Probiotic effect of Streptomyces strains alone or in combination with Bacillus and Lactobacillus in juveniles of the white shrimp Litopenaeus vannamei. Aquaculture International 25(2):927-939.

Blancheton J. P., Attramadal K. J. K., Michaud L., Roque d'Orbcastel E., Vadstein O., 2013 Insight into bacterial population in aquaculture systems and its implication. Aquacultural Engineering 53:30–39.

Bouman H. A., Platt T., Sathyendranath S., Li W. K. W., Stuart V., Fuentes-Yaco C., Maass H., Horne E. P. W., Ulloa O., Lutz V., Kyewalyanga M., 2003 Temperature as indicator of optical properties and community structure of marine phytoplankton: implications for remote sensing. Marine Ecology Progress Series 258:19-30.

Budianto, Heny S., 2017 [Antagonist activity of *Bacillus subtilis* against *Streptococcus iniae* and *Pseudomonas fluorescens*]. Jurnal Veteriner 18(3):409-415. [In Indonesian].

de Melo Pereira G. V., de Oliveira Coelho B., Junior A. I. M., Thomaz-Soccol V., Soccol C.R., 2018 How to select a probiotic? A review and update of methods and criteria. Biotechnology Advances 36:2060–2076.

Ebeling J. M., Timmons M. B., Bisogni J. J., 2006 Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257:346-358.

Edwards V. A., 2011 The nitrogen cycle-control ammonia, nitrite in ponds, lakes, lagoons, rivers and waste water treatment. Alken Murray Online.

Fardiaz D., 1992 [Food microbiology]. Jakarta, Gramedia, ...

Commented [WU14]: No of pages

Findy K., 2009 [Inhibition activity of *Bacillus* sp. to *Xanthomonas oryzae* pv. *Oryzae*, *Pseudomonas syringae* pv. glycines, and *Pseudomonas fluorescens*]. Undergraduate thesis, IPB University, ... [In Indonesian].
Gobi N., Malaikozhundan B., Sekar V., Shanthi S., Vaseeharan B., Jayakumar R., Nazar A. K., 2016 GFP tagged *Vibrio parahaemolyticus* Dahv2 infection and the protective effects of probiotic *Bacillus licheniformis* Dahb1 on the growth, immune and antioxidant responses in *Pangasius hypophthalmus*. Fish Shellfish Immunology 52: 230–238.
Hartini S., Ade D. S., Ferdinand H. T., 2013 [Water quality, survival rate, and growth of striped snakehead fish (*Channa striata*) juvenile cultured in media with probiotic]. Jurnal Akuakultur Rawa Indonesia 1(2):192-202. [In Indonesian].
Irianto A., Austin B., 2002 Probiotics in aquaculture. Journal of Fish Diseases 25:633-642.
Khotimah H., 2019 [Pathogenicity of probiotic candidates bacteria from swamp sediment]. Undergraduate thesis, Sriwijaya University, ... [In Indonesian].
Lutfi M. D., 2018 [Exploration on *Streptomyces* sp. antibiotic producers from various unique ecosystems in Indonesia]. Undergraduate thesis, IPB University, ... [In Indonesian].
Luti K. J. K., Mavituna F., 2011 Streptomyces coecolor increases the production of undecylprodigiosin when interacted with Bacillus subtilis. Biotechnology Letter 33:113-118.
Midhun S. J., Neethu S., Vysakh A., Radhakrishnan E. K., Jyothis M., 2018 Antagonism against fish pathogens by cellular components/preparations of *Bacillus coagulans* (MTCC-9872) and it's in vitro probiotic characterization. Current Microbiology 75:1174–1181.
Mohapatra S., Chakraborty T., Kumar V., DeBoeck G., Mohanta K. N., 2013 Aquaculture and stress management: a review of probiotic intervention. Journal of Animal Physiology and Animal Nutrition 97(3):405-430.
Mulyadi G., 2016 [Rearing of striped snakehead fish (*Channa striata*) with different stocking densities in biofloc media]. Jurnal Akuakultur Rawa Indonesia 4(2):159-174. [In Indonesian].
Nargesi E. A., Falahatkar B., Sajjadi M. M., 2019 Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (*Oncorhynchus mykiss*) broodstock. Aquaculture Nutrition 26(1):98-108.
National Research Council (NRC). 1977. Nutrien Requirement of Warm Water Fishes. National Acad. Press, Washington D.C.
Nurhayati C., Hamzah B., Pambayun R., 2014 [Effect of pH, chlorella vulgaris isolate concentration and time of observation on the level of contamination of crumb rubber liquid waste]. Jurnal Dinamika Penelitian Industri 25(2):97-106. [In Indonesian].
Panigrahi A., Sundaram M., Cakrapani S., Rajasekar S., Dayal J. S., Chavali G., 2019 Effect of carbon and nitrogen ratio (C:N) manipulation on the production performance and immunity of pasific white shrimp *Litopenaeus vannamei* (Boone, 1931) in a biofloc-based rearing system. Aquaculture Research 50:29–41.
Rurangwa E., Verdegem M. C. J., 2014 Microorganisms in recirculating aquaculture systems and their management. Reviews in Aquaculture 5:1-14.
Sanchez P. T., Ruiz-Zarzuela I., Blas I. D., Balcazar J. L., 2014 Probiotics in aquaculture: A current assessment. Reviews in Aquaculture 6:133–146.
Saraswati N., 2018 [Actinomycetes isolation for bioremediation of swamp water polluted by organic matter]. Undergraduate thesis, Sriwijaya University, ... [In Indonesian].
Sitohang M. L., Fitrani M., Jubaedah D., 2018 [Utilization of a mixture of pineapple, rice washing water, and sugar as a carbon source in catfish breeding media (*Clarias* sp.) with a biofloc system]. Jurnal Akuakultur Rawa Indonesia 6(1):51-64. [In Indonesian].
Trisna D. E., Sasanti A. D., Muslim, 2013 [Bacterial population, water quality maintenance media and histology of striped snakehead fish (*Channa striata*)

Commented [WU15]: No of pages

Commented [WU16]: No of pages

Commented [WU17]: No of pages

Commented [WU18]: Where is it cited?

Commented [WU19]: No of pages

juveniles given antibiotic feed]. Jurnal Akuakultur Rawa Indonesia 1(1):90-102. [In Indonesian].
Wijayanti M., Jubaedah D., Suhada J. A., Yuliani S., Saraswati N., Tanbiyaskur, Syaifudin M., Widjajanti W., 2018 DNA barcoding of swamp sediment bacterial isolates for swamp aquaculture probiotic. E 3S Web of Conferences 68:1-8.
Yuliani S., 2017 [Isolation of swamp bacteria as a bioaugmentation agent in swamp water polluted by organic matter]. Undergraduate thesis, Sriwijaya University, ... [In Indonesian].

Commented [WU20]: No of pages

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Authors:
Marini Wijayanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: mariniwijayanti@fp.unsri.ac.id
Dade Jubaedah, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: dadejubaedah@fp.unsri.ac.id
Ocktin Yulistya, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: yulistyaocktin@gmail.com
Tanbiyaskur, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: qurhadi30@gmail.com
Ade Dwi Sasanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, e-mail: sasanti.ade@gmail.com
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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

Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University, Indralaya, Ogan Ilir, South Sumatera, Indonesia. Corresponding author: M. Wijayanti, mariniwijayanti@fp.unsri.ac.id

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 (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 swamp microbes (P1) and with the addition of: (2) Chlorophyta (3.43×10^7 sel L^{-1}) and *Bacillus* sp. (10^5 CFU mL^{-1}) (P2); (3) Chlorophyta (3.43×10^7 sel L^{-1}) and *Streptomyces* sp. (10^5 CFU mL^{-1}) (P3); (4) Chlorophyta (3.43×10^7 sel L^{-1}), *Bacillus* sp. (10^5 CFU mL^{-1}) and *Streptomyces* sp. (10^5 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, probiotic, 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 µL 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 10^5 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

NH_3, NO_2^-, and NO_3^- 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 CO_2 used in cell metabolism. Gram-positive 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 CO_2 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) 5×10^6 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 (3.43×10^7 cell L^{-1}) and *Bacillus* sp. (10^5 CFU mL^{-1});
P3 - provision of 100 ml Chlorophyta (3.43×10^7 cell L^{-1}) and *Streptomyces* sp. (10^5 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 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 10^7cell 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 cm^3 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 (3.43×10^7 cell L^{-1}), *Bacillus* sp. (10^5 CFU mL^{-1}), *Streptomyces* sp. (10^5 CFU mL^{-1}) as well as PROBAC nitrification bacteria (5×10^6 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:

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

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):

$$\frac{Nt}{N_0} \times 100$$

$$\frac{Nt}{No} \times 100\%$$

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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);
W_0 - 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):

$$L = Lt\text{-}L_0$$

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).

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

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

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.

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 nitrification bacteria	*Swamp microbes*	*Chlorophyta abundance (cell L^{-1})*		
		0 day	*10th day*	*40th day*
N1	P1	3.20×10^{3}	3.20×10^{3}	2.10×10^{3}
	P2	3.60×10^{3}	3.43×10^{7}	4.10×10^{3}
	P3	4.10×10^{3}	3.43×10^{7}	4.10×10^{3}
	P4	3.70×10^{3}	3.43×10^{7}	4.46×10^{3}
N2	P1	4.00×10^{3}	4.00×10^{3}	2.10×10^{3}
	P2	3.60×10^{3}	3.43×10^{7}	2.41×10^{3}
	P3	3.90×10^{3}	3.43×10^{7}	2.34×10^{3}
	P4	3.40×10^{3}	3.43×10^{7}	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.

Table 2

Total bacterial population in the rearing media

Commercial nitrification bacteria	*Swamp microbes*	*Total bacterial population (CFU mL^{-1})*			
		0 day	*1st day*	*20th day*	*40th day*
N1	P1	6.60×10^{4}	6.78×10^{4}	1.55×10^{5}	6.20×10^{3}
	P2	6.20×10^{4}	3.95×10^{6}	6.93×10^{6}	2.77×10^{5}
	P3	7.00×10^{4}	3.28×10^{6}	7.53×10^{6}	3.01×10^{5}
	P4	4.70×10^{4}	5.59×10^{7}	1.00×10^{8}	2.99×10^{6}
N2	P1	7.10×10^{4}	2.01×10^{7}	3.54×10^{7}	1.42×10^{6}
	P2	4.50×10^{4}	3.29×10^{7}	5.59×10^{7}	1.68×10^{6}
	P3	4.30×10^{4}	4.99×10^{7}	6.41×10^{7}	1.93×10^{6}
	P4	4.95×10^{4}	4.70×10^{7}	8.75×10^{7}	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

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

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

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) (not significant)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=4.386)*
	P1	*P2*	*P3*	*P4*	
N1	11.111	16.666	13.332	26.668	16.944^{b}
N2	10.000	10.000	13.332	16.667	12.500^{a}
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 of rearing

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=6.631)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=3.315)*
	P1	*P2*	*P3*	*P4*	
N1	11.112^{a}	26.667^{b}	16.667^{a}	38.889^{c}	25.834^{b}
N2	13.333^{a}	13.333^{a}	23.333^{b}	33.333^{c}	20.833^{a}
The main effects of swamp microbes (P) ($LSD_{0.05}$=4.689)	12.223^{a}	20.000^{b}	20.000^{b}	41.111^{c}	

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^{-3} 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. 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

LSD test of survival rate of *Channa striata*

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=6.02)*				*The main effects of nitrifying bacteria (N) (not significant)*
	P1	*P2*	*P3*	*P4*	
N1	26.06^{a}	36.91^{b}	28.03^{a}	63.94^{d}	38.74
N2	31.75^{ab}	28.03^{a}	35.16^{b}	48.20^{c}	35.79
The main effects of swamp microbes (P) ($LSD_{0.05}$=4.25)	28.91^{a}	32.47^{a}	31.59^{a}	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 µL 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 nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=3.32)*				*The main effects of nitrifying bacteria (N) ($LSD_{0.05}$=1.66)*
	P1	*P2*	*P3*	*P4*	
N1	18.93^{a}	47.34^{d}	37.97^{c}	59.65^{e}	40.97^{b}
N2	22.00^{a}	29.52^{b}	34.89^{c}	44.11^{d}	32.63^{a}

The main effects of swamp microbes (P) ($LSD_{0.05}$=2.35)	20.47[a]	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 $LSD_{0.05}$ 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., *Streptomyces* 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

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

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

swamp microbes (P) ($LSD_{0.05}$=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 nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=0.10)*				*The main effects of nitrifying bacteria (N) (not significant)*
	P1	*P2*	*P3*	*P4*	
N1	0.69^{a}	1.79^{e}	0.91^{b}	2.27^{f}	2.12
N2	1.13^{c}	1.08^{c}	1.60^{d}	1.74^{e}	2.09
The main effects of swamp microbes (P) ($LSD_{0.05}$=0.07)	0.91^{a}	1.44^{c}	1.26^{b}	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 10^4 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 10^5 CFU mL^{-1} in the rearing media of *Pangasius hypophthalmus* showed a significant increase in the growth, immune and antioxidant responses compared to 10^7 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.

Table 9

LSD test result of dissolved oxygen in the rearing media

	Dissolved oxygen (mg L^{-1})								
	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 ±0.1	3.53 ±0.1	3.53 ±0.1	3.40 ±0.1^{c}	3.10 ±0.1^{c}	3.00 ±0.1^{c}	2.97 ±0.1^{c}	2.77 ±0.1^{c}	2.67 ±0.1^{c}
N1P2	3.63 ±0.1	3.53 ±0.1	3.53 ±0.1	3.23 ±0.1^{ab}	2.93 ±0.1^{ab}	2.83 ±0.1^{ab}	2.77 ±0.1^{ab}	2.57 ±0.1^{ab}	2.47 ±0.1^{ab}
N1P3	3.67 ±0.1	3.57 ±0.2	3.47 ±0.2	3.33 ±0.1^{bc}	3.03 ±0.1^{bc}	2.93 ±0.1^{bc}	2.87± 0.1^{bc}	2.67± 0.1^{bc}	2.57 ±0.1^{bc}
N1P4	3.57 ±0.1	3.70 ±0.1	3.60 ±0.1	3.60 ± 0.1^{d}	3.50 ±0.1^{e}	3.40 ±0.1^{e}	3.40 ±0.1^{e}	3.20 ±0.1^{e}	3.10 ±0.1^{e}
N2P1	3.63 ±0.1	3.43 ±0.1	3.43 ±0.1	3.13 ±0.1^{a}	2.83 ±0.1^{a}	2.73 ±0.1^{a}	2.67 ±0.1^{a}	2.47 ±0.1^{a}	2.37 ±0.1^{a}
N2P2	3.57 ±0.1	3.47 ±0.1	3.47 ±0.1	3.33 ±0.1^{bc}	3.03 ±0.1^{c}	293 ±0.1^{bc}	2.87 ±0.1^{bc}	2.67 ±0.1^{bc}	2.57 ±0.1^{bc}
N2P3	3.53 ±0.1	3.43 ±0.1	3.33 ±0.1	3.27 ±0.1^{ab}	3.10 ±0.1^{c}	3.00 ±0.1^{c}	2.90 ±0.1^{bc}	2.70 ±0.1^{bc}	2.60 ±0.1^{c}
N2P4	3.67 ±0.1	3.63 ±0.1	3.53 ±0.1	3.40 ±0.1^{c}	3.30 ±0.1^{d}	3.20 ±0.1^{d}	3.20 ±0.1^{d}	3.00 ±0.1^{d}	2.90 ±0.1^{d}
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^{a}	3.14^{b}	3.04^{b}	3.00^{b}	2.80^{b}	2.70^{b}
N2	3.60	3.49^{a}	3.44^{a}	4.93^{a}	3.07^{a}	2.97^{a}	2.91^{a}	2.71^{a}	2.61^{a}
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^{ab}	3.27^{a}	2.97^{a}	2.87^{a}	2.82^{a}	2.62^{a}	2.52^{a}
P2	3.60	3.50^{a}	3.50^{ab}	3.28^{a}	2.98^{a}	2.88^{a}	2.82^{a}	2.62^{a}	2.52^{a}
P3	3.60	3.50^{a}	3.40^{a}	3.30^{a}	3.07^{a}	2.97^{a}	2.88^{a}	2.68^{a}	2.58^{a}
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}

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.

Table 10

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

	Mean of ammonia concentration (mg L^{-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 ±0.07	0.383 ±0.01^{e}	0.393 ±0.01^{d}	0.410 ±0.01^{d}	0.327 ±0.01^{e}	0.540 ±0.01^{d}	0.674 ±0.01^{e}	0.691 ±0.01^{f}	0.948 ±0.02^{e}
N1P2	0.323 ±0.03	0.273 ±0.01^{d}	0.283 ±0.01^{c}	0.223 ±0.01^{c}	0.203 ±0.01^{c}	0.293 ±0.01^{b}	0.314 ±0.01^{b}	0.321 ±0.01^{c}	0.324 ±0.01^{b}
N1P3	0.283 ±0.01	0.257 ±0.01^{c}	0.267 ±0.01^{b}	0.207 ±0.01^{b}	0.197 ±0.01^{bc}	0.363 ±0.02^{c}	0.482 ±0.02^{d}	0.585 ±0.01^{e}	0.692 ±0.02^{d}
N1P4	0.267 ±0.01	0.220 ±0.01^{a}	0.230 ±0.01^{a}	0.170 ±0.01^{a}	0.147 ±0.01^{a}	0.243 ±0.02^{a}	0.260 ±0.02^{a}	0.265 ±0.02^{a}	0.270 ±0.02^{a}
N2P1	0.230 ±0.01	0.253 ±0.01^{c}	0.263 ±0.01^{b}	0.203 ±0.01^{b}	0.193 ±0.01^{bc}	0.282 ±0.02^{b}	0.363 ±0.02^{c}	0.385 ±0.01^{d}	0.392 ±0.02^{c}
N2P2	0.290 ±0.04	0.233 ±0.01^{ab}	0.243 ±0.01^{a}	0.183 ±0.01^{a}	0.160 ±0.01^{a}	0.250 ±0.01^{a}	0.268 ±0.01^{a}	0.273 ±0.01^{a}	0.277 ±0.01^{a}
N2P3	0.250 ±0.02	0.277 ±0.01^{d}	0.287 ±0.01^{c}	0.227 ±0.01^{c}	0.187 ±0.01^{b}	0.277 ±0.01^{b}	0.296 ±0.01^{b}	0.300 ±0.01^{b}	0.306 ±0.01^{b}
N2P4	0.303 ±0.03	0.237 ±0.01^{b}	0.247 ±0.01^{b}	0.187 ±0.01^{b}	0.163 ±0.01^{b}	0.250 ±0.01^{a}	0.268 ±0.01^{a}	0.273 ±0.01^{a}	0.277 ±0.01^{a}
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	0.250^{a}	0.260^{a}	0.200^{a}	0.176^{a}	0.265^{a}	0.299^{a}	0.308^{a}	0.313^{a}
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^{c}	0.443^{c}	0.499^{c}
P4	0.285	0.228^{a}	0.238^{a}	0.178^{a}	0.155^{a}	0.247^{a}	0.264^{a}	0.269^{a}	0.274^{a}

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

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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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References

Aliyu-Paiko M., Hashim R., Shu-Chien A. C., 2010 Influence of dietary lipid/protein ratio on survival, growth, body indices and digestive lipase activity in snakehead (*Channa striatus*, Bloch 1793) fry reared in re-circulating water system. Aquaculture Nutrition 16:466-474.

Balcazar J. L., DeBlas I., Ruiz-Zarzuela I., Cunningham D., Vendrell D., Muzquiz J. L., 2006 The role of probiotics in aquaculture. Veterinary Microbiology Review 114:173-186.

Bernal M. G., Ricardo M. M., Angel I. C., Jose M. M., 2017 Probiotic effect of Streptomyces strains alone or in combination with Bacillus and Lactobacillus in juveniles of the white shrimp Litopenaeus vannamei. Aquaculture International 25(2):927-939.

Blancheton J. P., Attramadal K. J. K., Michaud L., Roque d'Orbcastel E., Vadstein O., 2013 Insight into bacterial population in aquaculture systems and its implication. Aquacultural Engineering 53:30–39.

Bouman H. A., Platt T., Sathyendranath S., Li W. K. W., Stuart V., Fuentes-Yaco C., Maass H., Horne E. P. W., Ulloa O., Lutz V., Kyewalyanga M., 2003 Temperature as indicator of optical properties and community structure of marine phytoplankton: implications for remote sensing. Marine Ecology Progress Series 258:19-30.

Budianto, Heny S., 2017 [Antagonist activity of *Bacillus subtilis* against *Streptococcus iniae* and *Pseudomonas fluorescens*]. Jurnal Veteriner 18(3):409-415. [In Indonesian].

Dehghanifar S., Keyhanfar M., Emtiazi G., 2019 Production and partial purification of thermostable bacteriocins from *Bacillus pumilus* ZED17 and DFAR8 strains with antifungal activity. Molecular Biology Research Communications 8(1):41-49.
de Melo Pereira G. V., de Oliveira Coelho B., Junior A. I. M., Thomaz-Soccol V., Soccol C.R., 2018 How to select a probiotic? A review and update of methods and criteria. Biotechnology Advances 36:2060–2076.
Ebeling J. M., Timmons M. B., Bisogni J. J., 2006 Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257:346-358.
Edwards V. A., 2011 The nitrogen cycle-control ammonia, nitrite in ponds, lakes, lagoons, rivers and waste water treatment. Alken Murray Online. http://www.alken-murray.com/BioInfo2-03.htm
Gobi N., Malaikozhundan B., Sekar V., Shanthi S., Vaseeharan B., Jayakumar R., Nazar A. K., 2016 GFP tagged *Vibrio parahaemolyticus* Dahv2 infection and the protective effects of probiotic *Bacillus licheniformis* Dahb1 on the growth, immune and antioxidant responses in *Pangasius hypophthalmus*. Fish Shellfish Immunology 52: 230–238.
Hartini S., Ade D. S., Ferdinand H. T., 2013 [Water quality, survival rate, and growth of striped snakehead fish (*Channa striata*) juvenile cultured in media with probiotic]. Jurnal Akuakultur Rawa Indonesia 1(2):192-202. [In Indonesian].
Hopkins K. D., 1992 Reporting fish growth: A review of the basics. Journal of The World Aquaculture Society 23(3):173–179.
Irianto A., Austin B., 2002 Probiotics in aquaculture. Journal of Fish Diseases 25:633-642.
Khotimah H., 2019 [Pathogenicity of probiotic candidates bacteria from swamp sediment]. Undergraduate thesis, Sriwijaya University, Palembang, Indonesia, 54 p. [In Indonesian].
Lutfi M. D., 2018 [Exploration on *Streptomyces* sp. antibiotic producers from various unique ecosystems in Indonesia]. Undergraduate thesis, IPB University, Bogor, Indonesia, 23 p. [In Indonesian].
Luti K. J. K., Mavituna F., 2011 Streptomyces coecolor increases the production of undecylprodigiosin when interacted with Bacillus subtilis. Biotechnology Letter 33:113-118.
Midhun S. J., Neethu S., Vysakh A., Radhakrishnan E. K., Jyothis M., 2018 Antagonism against fish pathogens by cellular components/preparations of *Bacillus coagulans* (MTCC-9872) and it's in vitro probiotic characterization. Current Microbiology 75:1174–1181.
Mohapatra S., Chakraborty T., Kumar V., DeBoeck G., Mohanta K. N., 2013 Aquaculture and stress management: a review of probiotic intervention. Journal of Animal Physiology and Animal Nutrition 97(3):405-430.
Mulyadi G., 2016 [Rearing of striped snakehead fish (*Channa striata*) with different stocking densities in biofloc media]. Jurnal Akuakultur Rawa Indonesia 4(2):159-174. [In Indonesian].
Nargesi E. A., Falahatkar B., Sajjadi M. M., 2019 Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (*Oncorhynchus mykiss*) broodstock. Aquaculture Nutrition 26(1):98-108.
Nurhayati C., Hamzah B., Pambayun R., 2014 [Effect of pH, chlorella vulgaris isolate concentration and time of observation on the level of contamination of crumb rubber liquid waste]. Jurnal Dinamika Penelitian Industri 25(2):97-106. [In Indonesian].
Panigrahi A., Sundaram M., Cakrapani S., Rajasekar S., Dayal J. S., Chavali G., 2019 Effect of carbon and nitrogen ratio (C:N) manipulation on the production performance and immunity of pasific white shrimp *Litopenaeus vannamei* (Boone, 1931) in a biofloc-based rearing system. Aquaculture Research 50:29–41.
Pepper I. L., Gerba C. P., 2004 Environmental microbiology a laboratory manual. Elsevier Academic Press, Burlington, USA, 209 p.

Rurangwa E., Verdegem M. C. J., 2014 Microorganisms in recirculating aquaculture systems and their management. Reviews in Aquaculture 5:1-14.
Sanchez P. T., Ruiz-Zarzuela I., Blas I. D., Balcazar J. L., 2014 Probiotics in aquaculture: A current assessment. Reviews in Aquaculture 6:133–146.
Saraswati N., 2018 [Actinomycetes isolation for bioremediation of swamp water polluted by organic matter]. Undergraduate thesis, Sriwijaya University, Palembang, Indonesia, 55 p. [In Indonesian].
Singh R., Kumar M., Mittal A., Mehta P. K., 2016 Microbial enzymes: industrial progress in 21st century. 3 Biotech 6(174):1-15.
Sitohang M. L., Fitrani M., Jubaedah D., 2018 [Utilization of a mixture of pineapple, rice washing water, and sugar as a carbon source in catfish breeding media (*Clarias* sp.) with a biofloc system]. Jurnal Akuakultur Rawa Indonesia 6(1):51-64. [In Indonesian].
Trisna D. E., Sasanti A. D., Muslim, 2013 [Bacterial population, water quality maintenance media and histology of striped snakehead fish (*Channa striata*) juveniles given antibiotic feed]. Jurnal Akuakultur Rawa Indonesia 1(1):90-102. [In Indonesian].
Utami D., 2019 [Optimization of Chlorophyta density to Nile tilapia as a green water system of laboratory scale]. Undergraduate thesis, Sriwijaya University, Palembang, Indonesia, 33 p. [In Indonesian].
Widanarni, Yuniasari D., Sukenda, Ekasari J., 2010 Nursery culture performance of *Litopenaeus vannamei* with probiotics addition and different C/N ratio under laboratory condition. HAYATI Journal of Biosciences 17(3):115-119.
Wijayanti M., Jubaedah D., Suhada J. A., Yuliani S., Saraswati N., Tanbiyaskur, Syaifudin M., Widjajanti H., 2018 DNA barcoding of swamp sediment bacterial isolates for swamp aquaculture probiotic. E 3S Web of Conferences 68:1-8.
Yuliani S., 2017 [Isolation of swamp bacteria as a bioaugmentation agent in swamp water polluted by organic matter]. Undergraduate thesis, Sriwijaya University, Palembang, Indonesia, 53 p. [In Indonesian].
*** NRC (National Research Council), 1977 Nutrient requirement of warm water fishes. National Academy Press, Washington D.C., 71 p.

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Authors:
Marini Wijayanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: mariniwijayanti@fp.unsri.ac.id
Dade Jubaedah, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: dadejubaedah@fp.unsri.ac.id
Ocktin Yulistya, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: yulistyaocktin@gmail.com
Tanbiyaskur, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: qurhadi30@gmail.com
Ade Dwi Sasanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: sasanti.ade@gmail.com
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Marini Wijayanti

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Dear Dr Eniko,

We have done your suggestion. We have changed some words and list of references as your correction.
Thank you.

Best regards,

Marini Wijayanti

Pada tanggal Sel, 14 Apr 2020 03.19, Eni Kinasih <[redacted]> menulis:

Dear Dr. Wijayanti,

Please find the article attached and make the necessary changes.

Thank you.

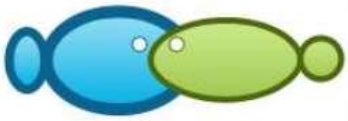
Regards!

On Sun, Apr 12, 2020 at 7:42 PM Marini Wijayanti <[redacted]> wrote:

Dear Dr. Eniko,

We hope it's not too late for sending our revision paper today.
We must correct the wrong value in some tables.
We stripped the wrong values and put the red highlight color at each wrong values in our paper. We are sorry about it. Thank you for your kind attention and help.

Best regards,
Marini Wijayanti
Aquaculture Study Programme
Department of Fisheries



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

Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University, Indralaya, Ogan Ilir, South Sumatera, Indonesia. Corresponding author: M. Wijayanti, mariniwijayanti@fp.unsri.ac.id

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 (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 swamp microbes (P1) and with the addition of: (2) *Chlorophyta* (3.43×10^7 sel L^{-1}) and *Bacillus* sp. (10^5 CFU mL^{-1}) (P2); (3) *Chlorophyta* (3.43×10^7 sel L^{-1}) and *Streptomyces* sp. (10^5 CFU mL^{-1}) (P3); (4) *Chlorophyta* (3.43×10^7 sel L^{-1}), *Bacillus* sp. (10^5 CFU mL^{-1}) and *Streptomyces* sp. (10^5 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, probiotic, 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 µL 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 10^5 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 NH_4, NO_2^-, and NO_3^- 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 CO_2 used in cell metabolism. Gram-positive 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 CO_2 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% (Yuvasari 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) 5×10^6 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 (3.43×10^7 cell L^{-1}) and *Bacillus* sp. (10^8 CFU mL^{-1});
P3 - provision of 100 ml Chlorophyta (3.43×10^7 cell L^{-1}) and *Streptomyces* sp. (10^7 CFU mL^{-1});
P4 - provision of 100 ml Chlorophyta (3.43×10^7 cell L^{-1}), *Bacillus* sp. (10^8 CFU mL^{-1}) and *Streptomyces* sp. (10^7 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 grow.

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

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 10^7cell 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 cm^3 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 (3.43×10^7 cell L^{-1}), *Bacillus* sp. (10^8 CFU mL^{-1}), *Streptomyces* sp. (10^7 CFU mL^{-1}) as well as PROBAC nitrification bacteria (5×10^6 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 Lackey Drop Microtransect method (American Public Health Association 1989) as follows:

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

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):

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

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):

$$\frac{N_t}{N_0} \times 100\%$$

Where:
N_0 - number of fish at the beginning of rearing (individuals);

N_t – 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):

$$W = W_t - W_0$$

Where:
W – growth of weight of fish for rearing (grams);
W_t - weight of fish at the end of rearing (grams);
W_0 - 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):

$$L = L_t - L_0$$

Where:
L – growth of absolute length of fish for rearing (cm);
L_t - 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 using the formula:

$$EP = \frac{(W_t + D) - W_0}{F} \times 100\%$$

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.

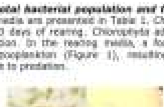


Figure 1. Biofloc and Chlorophyta profile in the rearing media of *C. striata* culture in this study (40 magnification scale of microscope).

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 nitrification bacteria	Swamp microbes	Chlorophyta abundance (cell L^{-1})		
		0 day	*10th day*	*40th day*
N1	P1	3.20×10^7	3.20×10^7	2.10×10^5
	P2	3.60×10^7	3.43×10^7	4.10×10^5
	P3	4.10×10^7	3.43×10^7	4.10×10^5
	P4	3.70×10^7	3.43×10^7	4.46×10^5
N2	P1	4.00×10^7	4.00×10^7	2.10×10^5
	P2	3.60×10^7	3.43×10^7	2.41×10^5
	P3	3.90×10^7	3.43×10^7	2.34×10^5
	P4	3.40×10^7	3.43×10^7	4.03×10^5

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.

Table 2
Total bacterial population in the rearing media

Commercial nitrification bacteria	Swamp microbes	Total bacterial population (CFU mL^{-1})			
		0 day	*1st day*	*20th day*	*40th day*
N1	P1	6.80×10^4	6.78×10^6	1.55×10^7	6.20×10^5
	P2	6.20×10^4	3.95×10^6	6.93×10^6	2.77×10^5
	P3	7.00×10^4	3.28×10^6	7.53×10^6	3.01×10^5
	P4	4.79×10^4	5.59×10^7	1.00×10^8	2.99×10^6
N2	P1	7.10×10^4	2.01×10^7	3.54×10^7	1.42×10^6
	P2	4.50×10^4	3.29×10^7	5.59×10^7	1.68×10^6
	P3	4.30×10^4	4.99×10^7	6.41×10^7	1.93×10^6
	P4	4.95×10^4	4.70×10^7	8.75×10^7	4.08×10^6

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 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

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

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) (not significant)*				*The main effects of nitrifying bacteria (N)* ($LSD_{0.05}$=4.386)
	P1	*P2*	*P3*	*P4*	
N1	11.111	16.666	13.332	26.668	16.994[a]
N2	10.000	10.000	13.332	16.667	12.500[a]
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 of rearing

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=6.631)*				*The main effects of nitrifying bacteria (N)* ($LSD_{0.05}$=3.715)
	P1	*P2*	*P3*	*P4*	
N1	11.112[a]	26.667[b]	16.667[a]	38.889[c]	25.834[a]
N2	13.333[a]	13.333[a]	23.333[b]	33.333[c]	20.833[a]
The main effects of swamp microbes (P) ($LSD_{0.05}$=4.689)	12.223[a]	20.000[b]	20.000[b]	41.111[c]	

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^{-2} 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.

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. Lutfi & Mewitura (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

LSD test of survival rate of *Channa striata*

The single effect of nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=6.02)*				*The main effects of nitrifying bacteria (N) (not significant)*
	P1	*P2*	*P3*	*P4*	
N1	26.06[a]	36.91[b]	28.03[a]	63.94[d]	38.74
N2	31.75[ab]	28.03[a]	35.16[b]	48.20[c]	35.79
The main effects of swamp microbes (P) ($LSD_{0.05}$=4.25)	28.91[a]	32.47[b]	31.59[b]	56.07[c]	

The N(P) 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 Hertini 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 L^{-1} $week^{-1}$ EM-4 probiotics 98.68% 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 Iwanto & 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 influence of swamps microbes (P) ($LSD_{0.05}$=3.72)*				*The main effects of nitrifying bacteria (N)* ($LSD_{0.05}$=1.66)
	P1	*P2*	*P3*	*P4*	
N1	18.93[a]	47.34[d]	37.97[c]	59.65[f]	40.97[b]
N2	22.00[a]	29.52[b]	34.89[c]	44.11[e]	32.63[a]
The main effects of swamp microbes (P) ($LSD_{0.05}$=2.35)	20.47[a]	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 (Deghhanifar et al 2019). It has the potential to control pathogenic bacteria by conducting competition, parasitism or by producing secondary metabolites (Lutfi 2016). 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., *Streptomyces* 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

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

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

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 nitrifying bacteria (N)	*The single influence of swamps microbes (P) ($LSD_{0.05}$=0.10)*				*The main effects of nitrifying bacteria (N) (not significant)*
	P1	*P2*	*P3*	*P4*	
N1	0.69^{a}	1.79^{e}	0.91^{c}	2.27^{f}	2.12
N2	1.13^{c}	1.08^{c}	1.60^{d}	1.74^{e}	2.09
The main effects of swamp microbes (P) ($LSD_{0.05}$=0.07)	0.91^{a}	1.44^{b}	1.26^{b}	2.00^{c}	

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 food, by accelerating the process of absorption of the food. The work of probiotics in aquaculture is the ability of microbes to break down proteins, polysaccarides, lipids and stress resistance in aquaculture system (de melo Pereira et al 2018). The addition of 10^{6} CFU mL^{-1} probiotics to the rearing media increased the length and weight of Pacific white shrimp (*Litopenaeus vannamei*) ~~tiger shrimp~~ larvae, compared to the controls (Widanarni et al 2010). *Bacillus licheniformis* at 10^{8} CFU mL^{-1} in the rearing media of *Pangasius hypophthalmus* showed a significant increase in the growth, immune and antioxidant responses compared to 10^{7} 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.

Table 9

LSD test result of dissolved oxygen in the rearing media

	Dissolved oxygen (mg L^{-1})								
	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 ±0.1	3.53 ±0.1	3.53 ±0.1	3.40^{c} ±0.1	3.10^{c} ±0.1	3.00^{c} ±0.1	2.97^{c} ±0.1	2.77^{c} ±0.1	2.67^{c} ±0.1
N1P2	3.63 ±0.1	3.53 ±0.1	3.53 ±0.1	3.23^{ab} ±0.1	2.93^{ab} ±0.1	2.83^{ab} ±0.1	2.77^{abc} ±0.1	2.57^{abc} ±0.1	2.47^{ab} ±0.1
N1P3	3.67 ±0.1	3.57 ±0.2	3.47 ±0.2	3.33^{bc} ±0.1	3.03^{bc} ±0.1	2.93^{bc} ±0.1	2.87^{bc} ±0.1	2.67^{bc} ±0.1	2.57^{bc} ±0.1
N1P4	3.57 ±0.1	3.70 ±0.1	3.60 ±0.1	3.60^{d} ±0.1	3.50^{d} ±0.1	3.40^{d} ±0.1	3.40^{d} ±0.1	3.20^{d} ±0.1	3.10^{d} ±0.1
N2P1	3.63 ±0.1	3.43 ±0.1	3.43 ±0.1	3.13^{a} ±0.1	2.83^{a} ±0.1	2.73^{a} ±0.1	2.67^{a} ±0.1	2.47^{a} ±0.1	2.37^{a} ±0.1
N2P2	3.57 ±0.1	3.47 ±0.1	3.47 ±0.1	3.33^{bc} ±0.1	3.03^{c} ±0.1	293^{bc} ±0.1	2.87^{bc} ±0.1	2.67^{bc} ±0.1	2.57^{bc} ±0.1
N2P3	3.53 ±0.1	3.43 ±0.1	3.33 ±0.1	3.27^{abc} ±0.1	3.10^{c} ±0.1	3.00^{c} ±0.1	2.90^{bc} ±0.1	2.70^{bc} ±0.1	2.60^{c} ±0.1
N2P4	3.67 ±0.1	3.63 ±0.1	3.53 ±0.1	3.40^{c} ±0.1	3.30^{d} ±0.1	3.20^{d} ±0.1	3.20^{d} ±0.1	3.00^{c} ±0.1	2.90^{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^{a}	3.53^{a}	5.09^{a}	3.14^{a}	3.04^{a}	3.00^{a}	2.80^{a}	2.70^{a}
N2	3.60	3.49^{a}	3.44^{a}	4.93^{a}	3.07^{a}	2.97^{a}	2.91^{a}	2.71^{a}	2.61^{a}
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^{ab}	3.27^{a}	2.97^{a}	2.87^{a}	2.82^{a}	2.62^{a}	2.52^{a}
P2	3.60	3.50^{a}	3.50^{ab}	3.28^{a}	2.98^{a}	2.88^{a}	2.82^{a}	2.62^{a}	2.52^{a}
P3	3.60	3.50^{a}	3.40^{a}	3.30^{a}	3.07^{a}	2.97^{a}	2.88^{a}	2.68^{a}	2.58^{a}
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}

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

Table 10

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

	Mean of ammonia concentration (mg L^{-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 ±0.07	0.383^{e} ±0.01	0.393^{f} ±0.01	0.410^{f} ±0.01	0.327^{h} ±0.01	0.540^{f} ±0.01	0.674^{g} ±0.01	0.691^{f} ±0.01	0.949^{g} ±0.02
N1P2	0.323 ±0.03	0.273^{c} ±0.01	0.283^{c} ±0.01	0.223^{c} ±0.01	0.203^{c} ±0.01	0.293^{c} ±0.01	0.314^{d} ±0.01	0.325^{c} ±0.01	0.324^{d} ±0.01
N1P3	0.283 ±0.01	0.257^{c} ±0.01	0.267^{b} ±0.01	0.207^{b} ±0.01	0.197^{bc} ±0.01	0.363^{c} ±0.02	0.402^{e} ±0.02	0.585^{e} ±0.01	0.692^{f} ±0.02
N1P4	0.267 ±0.01	0.220^{a} ±0.01	0.230^{a} ±0.01	0.170^{a} ±0.01	0.147^{a} ±0.01	0.243^{a} ±0.02	0.260^{a} ±0.02	0.269^{a} ±0.02	0.270^{a} ±0.02
N2P1	0.230 ±0.01	0.253^{c} ±0.01	0.263^{b} ±0.01	0.203^{b} ±0.01	0.193^{bc} ±0.01	0.282^{b} ±0.02	0.363^{c} ±0.02	0.385^{d} ±0.01	0.392^{e} ±0.02
N2P2	0.290 ±0.04	0.233^{ab} ±0.01	0.243^{a} ±0.01	0.183^{a} ±0.01	0.160^{a} ±0.01	0.250^{a} ±0.01	0.268^{a} ±0.01	0.273^{a} ±0.01	0.277^{a} ±0.01
N2P3	0.250 ±0.02	0.277^{b} ±0.01	0.287^{b} ±0.01	0.227^{b} ±0.01	0.187^{b} ±0.01	0.277^{b} ±0.01	0.296^{b} ±0.01	0.300^{b} ±0.01	0.306^{b} ±0.01
N2P4	0.303 ±0.03	0.237^{b} ±0.01	0.247^{b} ±0.01	0.167^{b} ±0.01	0.163^{a} ±0.01	0.250^{a} ±0.01	0.268^{a} ±0.01	0.273^{a} ±0.01	0.277^{a} ±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}	0.490^{b}	0.558^{b}
N2	0.268	0.250^{a}	0.260^{a}	0.200^{a}	0.176^{a}	0.265^{a}	0.299^{a}	0.308^{a}	0.313^{a}
LSD		0.009	0.009	0.010	0.009	0.014	0.015	0.013	0.016
P1	0.260	0.318^{c}	0.328^{d}	0.307^{d}	0.260^{d}	0.411^{c}	0.518^{c}	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^{a}	0.297^{a}	0.300^{a}
P3	0.267	0.267^{c}	0.277^{c}	0.217^{c}	0.192^{c}	0.320^{c}	0.389^{b}	0.443^{c}	0.499^{c}
P4	0.285	0.228^{a}	0.238^{a}	0.178^{a}	0.155^{a}	0.247^{a}	0.264^{a}	0.269^{a}	0.274^{b}

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 food, 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 (Ebling 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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References

Aliyu-Paiko M., Hashim R., Shu-Chien A. C., 2010 Influence of dietary lipid/protein ratio on survival, growth, body indices and digestive lipase activity in snakehead (*Channa striatus*, Bloch 1793) fry reared in re-circulating water system. Aquaculture Nutrition 16:466-474.
Balcázar J. L., DeBlas I., Ruiz-Zarzuela I., Cunningham D., Vendrell D., Muzquiz, J. L., 2006 The role of probiotics in aquaculture. Veterinary Microbiology Review 114:173-186.
Bernal M. G., Ricardo M. M., Angel I. C., Jose M. M., 2017 Probiotic effect of Streptomyces strains alone or in combination with Bacillus and Lactobacillus in juveniles of the white shrimp *Litopenaeus vannamei*. Aquaculture International 25(2):927-939.
Blancheton J. P., Attramadal K. J. K., Michaud L., Roque d'Orbcastel E., Vadstein O., 2013 Insight into bacterial population in aquaculture systems and its implication. Aquacultural Engineering 53:30-39.
Bouman H. A., Platt T., Sathyendranath S., Li W. K. W., Stuart V., Fuentes-Yaco C., Maass H., Horne E. P. W., Ulloa O., Lutz V., Kyewalyanga M., 2003 Temperature as indicator of optical properties and community structure of marine phytoplankton: implications for remote sensing. Marine Ecology Progress Series 258:19-30.
Budiarto, Hera S., 2017 [Antagonist activity of *Bacillus subtilis* against *Streptococcus iniae* and *Pseudomonas fluorescens*]. Jurnal Veteriner 18(3):409-415. [In Indonesian].
Dehghanifar S., Keyhanfar M., Emtiazi G., 2019 Production and partial purification of thermostable bacteriocins from *Bacillus pumilus* ZED17 and DFAR8 strains with antifungal activity. Molecular Biology Research Communications 8(1):41-49.

de Melo Pereira G. V., de Oliveira Coelho B., Junior A. I. M., Thomaz-Soccol V., Soccol C.R., 2018 How to select a probiotic? A review and update of methods and criteria. Biotechnology Advances 36:2060-2076.
Ebeling J. M., Timmons M. B., Bisogni J. J., 2006 Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257:346-358.
Edwards V. A., 2011 The nitrogen cycle-control ammonia, nitrite in ponds, lakes, lagoons, rivers and waste water treatment. Aiken Murray Online.
Gobi N., Malaikozhundan B., Sekar V., Shanthi S., Vaseeharan B., Jayakumar R., Nazar A. K., 2016 GFP tagged *Vibrio parahaemolyticus* Dahv2 infection and the protective effects of probiotic *Bacillus licheniformis* Dahb1 on the growth, immune and antioxidant responses in *Pangasius hypophthalmus*. Fish Shellfish Immunology 52: 230-238.
Hartini S., Ade D. S., Ferdinand H. T., 2013 [Water quality, survival rate, and growth of striped snakehead fish (*Channa striata*) juvenile cultured in media with probiotic]. Jurnal Akuakultur Rawa Indonesia 1(2):192-202. [In Indonesian].
Hopkins K. D., 1992 Reporting fish growth: A review of the basics. Journal of The World Aquaculture Society 23(3):173-179.
Irianto A., Austin B., 2002 Probiotics in aquaculture. Journal of Fish Diseases 25:633-642.
Khotimah H., 2019 [Pathogenicity of probiotic candidates bacteria from swamp sediment]. Undergraduate thesis, Sriwijaya University, 54 p. [In Indonesian].
Lutfi M. D., 2018 [Exploration on *Streptomyces* sp. antibiotic producers from various unique ecosystems in Indonesia]. Undergraduate thesis, IPB University, 23 p. [In Indonesian].
Luti K. J. K., Mavituna F., 2011 *Streptomyces coelicolor* increases the production of undecylprodigiosin when interacted with *Bacillus subtilis*. Biotechnology Letter 33:113-118.
Midhun S. J., Neethu S., Vysakh A., Radhakrishnan E. K., Jyothis M., 2018 Antagonism against fish pathogens by cellular components/preparations of *Bacillus coagulans* (MTCC-9872) and it's in vitro probiotic characterization. Current Microbiology 75:1174-1181.
Mohapatra S., Chakraborty T., Kumar V., DeBoeck G., Mohanta K. N., 2013 Aquaculture and stress management: a review of probiotic intervention. Journal of Animal Physiology and Animal Nutrition 97(3):405-430.
Muhyadi U., 2016 [Rearing of striped snakehead fish (*Channa striata*) with different stocking densities in biofloc media]. Jurnal Akuakultur Rawa Indonesia 4(2):159-174. [In Indonesian].
Nargesi E. A., Falahatkar B., Sajjadi M. M., 2019 Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (*Oncorhynchus mykiss*) broodstock. Aquaculture Nutrition 26(1):98-108.
Nurhayati C., Hamzah B., Pambayun R., 2014 [Effect of pH, chlorella vulgaris isolate concentration and time of observation on the level of contamination of crumb rubber liquid waste]. Jurnal Dinamika Penelitian Industri 25(2):97-106. [In Indonesian].
Panigrahi A., Sundaram M., Saranya C., Rajasekar S., Dayal J. S., Chavali G., 2019 Effect of carbon and nitrogen ratio (C:N) manipulation on the production performance and immunity of pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) in a biofloc-based rearing system. Aquaculture Research 50:29-41.
Pepper I. L., Gerba C. P., 2004 Environmental microbiology a laboratory manual. Elsevier Academic Press, Burlington, USA, 209 p.
Rurangwa E., Verdegem M. C. J., 2014 Microorganisms in recirculating aquaculture systems and their management. Reviews in Aquaculture 5:1-14.
Sanchez P. T., Ruiz-Zarzuela I., Blas I. D., Balcazar J. L., 2014 Probiotics in aquaculture: A current assessment. Reviews in Aquaculture 6:133-146.

Saraswati N., 2018 [Actinomycetes isolation for bioremediation of swamp water polluted by organic matter]. Undergraduate thesis, Sriwijaya University, 55 p. [In Indonesian].
Singh R., Kumar M., Mittal A., Mehta P. K., 2016 Microbial enzymes: industrial progress in 21st century. 3 Biotech 6(174):1-15.
Sitohang M. L., Fitrani M., Jubaedah D., 2018 [Utilization of a mixture of pineapple, rice washing water, and sugar as a carbon source in catfish breeding media (Clarias sp.) with a biofloc system]. Jurnal Akuakultur Rawa Indonesia 6(1):51-64. [In Indonesian].
Triana D. E., Sasanti A. D., Muslim, 2013 [Bacterial population, water quality maintenance media and histology of striped snakehead fish (Channa striata) juveniles given antibiotic feed]. Jurnal Akuakultur Rawa Indonesia 1(1):90-102. [In Indonesian].
Utami D., 2019 [Optimization of Chlorophyta density to nile tilapia as a green water system of laboratory scale]. Undergraduate thesis, Sriwijaya University, 33 p. [In Indonesian].
Widanarni, Yuniasari D., Sukenda, Ekasari J., 2010. Nursery Culture Performance of Litopenaeus vannamei with Probiotics Addition and Different C/N Ratio Under Laboratory Condition. HAYATI Journal of Biosciences 17 (3): 115-119.
Wijayanti M., Jubaedah D., Suhada J. A., Yuliani S., Saraswati N., Tanbiyaskur, Syaifudin M., Widjajanti H., 2018 DNA barcoding of swamp sediment bacterial isolates for swamp aquaculture probiotic. E 3S Web of Conferences 68:1-8.
Yuliani S., 2017 [Isolation of swamp bacteria as a bioaugmentation agent in swamp water polluted by organic matter]. Undergraduate thesis, Sriwijaya University, 53 p. [In Indonesian].
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Authors:
Marini Wijayanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, 30662, e-mail: mariniwijayanti@fp.unsri.ac.id
Dade Jubaedah, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, 30662, e-mail: dadejubaedah@fp.unsri.ac.id
Ocktin Yulistya, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, 30662, e-mail: yulistyaocktin@gmail.com
Tanbiyaskur, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia, 30662, e-mail: gusmadi30@gmail.com

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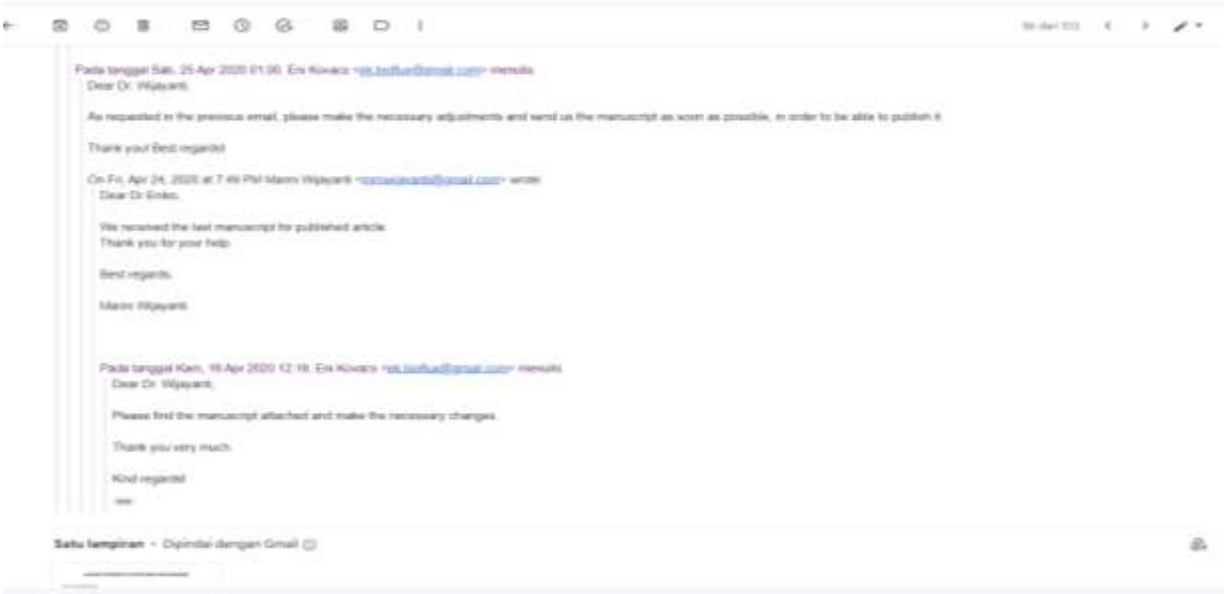

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Marini Wijayanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: mariniwijayanti@fp.unsri.ac.id (+62-813-7313-1117)

Dade Jubaedah, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: dadejubaedah@fp.unsri.ac.id

Ocktin Yulistya, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: yulistyaocktin@gmail.com

Tanbiyaskur, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: qurhadi30@gmail.com

Ade Dwi Sasanti, Sriwijaya University, Faculty of Agriculture, Department of Fisheries, Aquaculture Study Program, Kampus Unsri Indralaya, Jl Palembang Prabumulih km 32, Indralaya, 30662 Ogan Ilir, South Sumatera, Indonesia, e-mail: sasanti.ade@gmail.com

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Fifth author / signature : Ade Dwi Sasanti

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