



Phytoplankton diversity and abundance in biofloc cultivation of African catfish with different stock density

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ABSTRACT

High fish stock density causes a decrease in water quality due to increased organic waste in water media and this can be overcome using biofloc technology. The bacterial consortium in the biofloc system breaks the organic compound into nutrients for phytoplankton growth. This research aims to determine phytoplankton abundance in biofloc ponds with different catfish stocking densities. Furthermore, a completely randomized design (CRD) was used, with four treatments and three replications. The treatments involved 1,000, 1,500, 2,000, and 2,500 catfish per m³, respectively, with a weight of \pm 1-3 grams. Also, the fish was fed 3% of its body weight and reared for 40 days in a tarpaulin pond, with a water volume of \pm 1,974 L. The AMOVA test was used to analyze the data and 10 phytoplankton genera were observed. However, the phytoplankton abundances showed no statistical significance among the treatments. The results showed that the first treatment had the most abundant phytoplankton, with an average number of 13,394 cell/L.

Introduction

The market demand for freshwater fish is rising due to an increased requirement for animal protein in human food. Furthermore, it influenced fish cultivation directly, leading to intensification, including catfish farming (*Clarias gariepinus*) (Strauch *et al.*, 2019; Pruter *et al.*, 2020).

An intensive cultivation system increases, though organic and inorganic wastes adversely impact the environment (Tovar *et al.*, 2000; Cao *et al.*, 2007; Farmaki *et al.*, 2014). The implementation of a biofloc system involves using heterotrophic bacteria to turn the waste into a protein source to feed fish. The biofloc formation is influenced by several factors, such as inorganic nitrogen and organic compound in water. Furthermore, feces and uneaten feed are the sources of nitrogen in water (Avnimelech and Kochba, 2009). The fish density influenced the number of organic matter wastes. Organic matter is important in aquaculture because it helps the growth

of phytoplankton and other aquatic organisms in an extent. The presence of heterotrophic bacteria, phytoplankton, and other microorganisms increases nutrition value in biofloc (Azim and Little, 2008; Ekasari *et al.*, 2010; Emerenciano *et al.*, 2011).

Generally, the concept of biofloc in aquaculture technology is to recycle inorganic nitrogen compounds into microbial protein cells that fish can eat. The organic material in aerated water stimulates the heterotrophic bacteria to stick to organic particles, convert them into organic matter, and absorb toxic minerals. Consequently, the water quality improves, and the organic material is recycled into detritus. The key to a successful biofloc technology involves developing and maintaining the presence of beneficial bacteria in the pond (Farmaki *et al.*, 2014). Additionally, the existence of nutrients such as carbon and nitrogen, and environmental factors like sunlight penetration stimulates plankton growth (Castro *et al.*, 2018). In a biofloc, it is critical

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to know the phytoplankton abundance, as a measurement in determining the floc amount. Also, it is used as an indicator of water quality in the biofloc pond (Pham, 2017).

This research aims to determine the diversity and abundance of phytoplankton on the biofloc system using different African catfish densities. Also, identifies the effect of African catfish stock density on phytoplankton abundance in the biofloc system.

Materials and Methods

Location and time of research

This research was conducted in the experimental pond at the Fishery and Marine Science Faculty, Universitas Jenderal Soedirman, Purwokerto. Furthermore, it was carried out from October to November 2015 in a fish farming laboratory at the Faculty of Fisheries and Marine Science at Jenderal Soedirman University Purwokerto.

Experimental design

A completely randomized design was used with four treatments and replications. The treatments include stock density A, B, C, and D with 1,000, 1,500, 2,000, and 2,500 fish/m³, respectively. In addition, the initial body weight of African catfish was 1.85±0.09, and it was fed using commercial pellets.

Biofloc preparation

The biofloc media preparation was carried out by administering 500 g of salt/pond, which was left for two (2) days in a circular pond. Then 10 mL of EBS (Enviro Balanced System) probiotics/pond was added for the formation of flocs containing *Bacillus* sp. and 50 mL/L molasses. The molasses and probiotics are given daily for two (2) weeks to grow and develop the floc to dominate and maintenance the media. Subsequently, when the color of the water in the rearing media turns brown, it indicates that the microorganisms or bacteria have dominated. Therefore, the rearing media is ready to be stocked seed (Soedibya et al., 2017).

Phytoplankton collection

This research used well water as a source for the biofloc system and the initial phytoplankton samples were collected from this source. The water was taken using a bucket with a volume of 10 liters and filtered using plankton net number 25. In addition, the water was filtered ten times to achieve a total volume of filtered water of approximately 100 liters, while the plankton remained in the collection bottle. Subsequently, the collection bottle was removed from the plankton net. The filtered plankton was transferred into the sample bottles and preserved using formalin until the final concentration was 4%

and two drops of pure Lugol (Sastranegara et al., 2020). However, the phytoplankton samples from the biofloc system were collected at one-week intervals for four weeks of catfish rearing.

Plankton identification

The plankton was observed and identified under a binocular microscope with a magnification of 10 x 40. Furthermore, phytoplankton identification was conducted for both samples from source water and the biofloc system. The stages involve placing a drop of sample water on an object-glass, then the cover glass is blown, and the plankton is observed under a light microscope (Sahlan, 1982). The Sahlan (1982) and Davis (1995) key was used to identify the plankton.

Observations were made in 10 fields of view, where the microscope macro meter was moved randomly with up-down and left-right direction ten times. The phytoplankton was observed in each view filed after the macro meter movement.

Then the phytoplankton abundance was calculated using a formula from Sahlan (1982).

$$\text{Abundance} = [(A/B) \times (C/D) \times (E/F)] \text{ cell/litre}$$

Remarks:

A= Water volume in bottle sample (30 mL)

B= Water volume observed (0.06 mL)

C= Cover glass width (484 mm²)

D= view field number (10)

E= number of phytoplankton individual

F= total volume of filtered water (100 L)

Data analysis

Data on phytoplankton diversity was analyzed descriptively as a total number of species. The AMOVA analysis was used to analyze phytoplankton abundances among the treatment with a confidence level of 95%.

Results

Phytoplankton diversity

Preliminary identification was conducted to evaluate plankton diversity in the water source before being utilized in the biofloc tanks. The identification process discovered 2 phytoplankton divisions in the water, namely Bacillariophyta/Flagilariophyceae (diatoms) and Chlorophyta (green algae). About 5 phytoplankton genera were found, including *Ankistrodesmus*, *Chlamydomonas*, *Closterium*, *Quadrigula*, and *Selenastrum*. Additionally, no undesirable species of cyanobacteria, such as a colonial-like potential toxin producer (*Microcystis* spp.) or filamentous types (*P. perornata*) were observed in the pond water. Therefore, the pond water was selected as biofloc culture media.

The observation on the phytoplankton diversity collected from water media in the biofloc cultivation system of *C. gariepinus* with different stock densities found three classes of phytoplankton. The classes include Cyanophyceae (blue-green algae), Chlorophyceae (green algae), and Flagilariophyceae. Table 1 showed that each class has different phytoplankton genera, respectively.

Phytoplankton abundance

Table 2 depicts the weekly observation, which proved that phytoplankton abundances fluctuated. The phytoplankton genera have different abundance at the end of catfish cultivation. Furthermore, the data showed that *Microcystis* was the most abundant, followed by *Coelosphaerium* (Table 3). Phytoplankton abundance at the end of catfish cultivation was different among treatments or catfish stocking densities. However, variance analysis (ANOVA) showed that phytoplankton abundances were not significantly different among treatments (Table 4).

Nutrient content

Nutrient measurement indicated that the biofloc system had decreased ammonia but increased nitrite (NO_2) and nitrate (NO_3) concentrations. Table 5 showed the nutrient content on water media of catfish cultivation using the biofloc system.

Table 1. Phytoplankton diversity in biofloc system of catfish cultivation.

No.	Classis	Genus
1.	Cyanophiceae	<i>Microcystis</i>
		<i>Oscillatoria</i>
		<i>Coelosphaerium</i>
		<i>Spirulina</i>
		<i>Anabaena</i>
2.	Chlorophyceae	<i>Actinastrum</i>
		<i>Scenedesmus</i>
		<i>Pediastrum</i>
		<i>Characium</i>
3.	Fragilariophyceae	<i>Diatoma</i>

Table 2. Weekly observation of phytoplankton abundance in biofloc system with different catfish stock densities.

Genera	A				B				C				D			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
<i>Microcystis</i>	8774	9416	6313	8845	2746	6135	7026	3210	2818	36	3317	3139	1712	2283	2033	4708
<i>Oscillatoria</i>	0	1676	999	1320	0	214	1890	1213	0	3638	2853	820	0	0	2318	1427
<i>Coelosphaerium</i>	0	2889	5600	3353	0	5814	1962	2425	0	3674	7419	2425	0	3959	5849	1427
<i>Spirulina</i>	0	0	0	0	0	107	749	0	0	0	0	713	0	36	0	0
<i>Anabaena</i>	0	0	0	0	0	0	0	0	0	0	178	0	0	0	0	0
<i>Actinastrum</i>	76	71	606	820	36	0	2853	1605	0	0	4387	1213	0	0	1712	1748
<i>Scenedesmus</i>	36	285	1141	892	0	678	2996	2176	0	0	2211	2532	178	0	5814	2497
<i>Pediastrum</i>	0	36	71	321	143	71	214	571	0	71	1177	250	0	0	1997	1248
<i>Characium</i>	0	0	0	0	0	0	0	0	0	143	0	0	0	0	0	0
<i>Diatoma</i>	0	36	0	0	0	71	0	0	0	0	0	0	0	0	0	0

Table 3. Average abundance of each phytoplankton genus at the end of cultivation periods.

Genera	Abundance (cell/L)				Total
	A	B	C	D	
<i>Microcystis</i>	8,337	4,779	2,328	2,684	18,128
<i>Oscillatoria</i>	999	829	1,828	936	4,592
<i>Coelosphaerium</i>	2,961	2,550	3,380	2,809	11,700
<i>Spirulina</i>	0	214	178	9	401
<i>Anabaena</i>	0	0	45	0	45
<i>Actinastrum</i>	393	1,124	1,400	865	3,782
<i>Scenedesmus</i>	589	1,463	1,186	1,122	4,380
<i>Pediastrum</i>	107	250	375	811	1,543
<i>Characium</i>	0	0	36	0	36
<i>Diatoma</i>	9	18	0	0	27
Total	13,394	11,227	10,756	10,236	

Table 4. ANOVA result showing not significant effect of catfish stocking densities on average abundances of phytoplankton.

Treatment	Abundance (cell/L)	STDEV	Sig.
A	13,394	2,621.35	a
B	11,227	1,525.21	a
C	10,756	1,162.31	a
D	10,236	1,125.55	a

Table 5. Nutrient content on water media of catfish biofloc cultivation.

Time	Nutrient (ppm)		
	NH_3	NO_2	NO_3
Beginning	0.9 – 1.8	0.03 – 0.08	2.18 -5.49
Middle	0.2 – 0.6	0.64 – 3.66	10.9 -12.17
End	0.05 – 0.31	1.99 – 2.11	6.03 -10.19
PP 82/2001	<0.5	<0.06	10-12

Discussion

Phytoplankton diversity

About two groups of phytoplankton were observed in the water source media, such as green algae and diatoms. This was in line with [Green et al. \(2014\)](#), who found green algae and diatoms on their biofloc culture. The similarity indicates that the natural water source for aquaculture contains two groups of phytoplankton, which include green algae and diatoms, though further study is required.

At the initial period, the research found two groups of phytoplankton, and different phytoplankton diversity from a survey by [Roberto et al. \(2017\)](#). Also, [Roberto et al. \(2017\)](#) reported Cyanophyta and Chlorophyta in the biofloc system at their initial research.

Similar phytoplankton diversity and number of genera were observed in the pond cultures with different stock densities. The condition showed that the phytoplankton diversity was not affected by the different fish stock densities ([Table 1](#)). [Table 1](#) showed that 10 phytoplankton species were found after 12 weeks of catfish cultivation in the biofloc systems. However, when compared to preliminary observation, the observed genera in the water source were not found in the biofloc system, rather another genus of Bacillariophyceae was observed, which was *Diatoma*. This is because the five genera cannot adapt to the high ammonia in the biofloc system. According to [Soedibya et al. \(2017\)](#), the observed ammonia ranged from 0.05 to 1.8 mg/L. Also, another possibility is because of losing the competition to new phytoplankton in the biofloc system. These phenomena were observed in previous research, where the biofloc technology increases phytoplankton diversity in the water media ([Green et al., 2014](#); [Nie et al., 2017](#)). However, [Roberto et al. \(2017\)](#) reported different phenomena, where diatoms were observed on the following period of the experiment, while no diatoms were observed at the initial period.

This research obtained a lower number of phytoplankton classes compared to research by [Nie et al. \(2017\)](#), having three and four classes, respectively. The difference is that the present research was conducted in a biofloc system with African catfish cultivation, while [Nie et al. \(2017\)](#) was conducted purely in a biofloc system. Furthermore, the presence of African catfish increased the concentration of ammonia, especially NO_2 ([Soedibya et al., 2017](#)). This concentration is harmful to the aquatic organism in aquaculture water media ([Mishra et al., 2008](#); [Nie et al., 2017](#)). Therefore, it is reasonable

to obtain lower phytoplankton diversity than in biofloc systems, according to [Nie et al. \(2017\)](#).

This research found a higher phytoplankton genus compared to [Castro-Mejia et al. \(2017\)](#), who studied phytoplankton diversity in *Oreochromis niloticus* biofloc cultivation. Also, this research found 10 genera, while [Castro-Mejia et al. \(2017\)](#) found seven. However, this is due to the cultivation of different fishes with various feeding behavior. This research utilized African catfish, which is carnivorous, while [Castro-Mejia et al. \(2017\)](#) used *O. niloticus*, an omnivorous fish. The *Oreochromis niloticus* may consume some phytoplankton in the biofloc system during the cultivation, while the African catfish did not consume phytoplankton. Therefore, this research discovered higher phytoplankton diversity in the biofloc method of culturing African catfish than that reported by [Castro-Mejia et al. \(2017\)](#) in the biofloc system culturing *O. niloticus*.

This results and previous research ([Castro-Mejia et al., 2017](#)) showed that phytoplankton can be variable between researches. This phenomenon is due to different carbon sources utilized during the research. According to [Oehemen et al. \(2014\)](#), various carbon sources affect microorganism diversity and abundances.

Plankton abundance

[Table 3](#) showed that different phytoplankton abundances were observed among genera and treatments. The data provided three important information, where the first is that each genus can adapt to water media in the biofloc system. Furthermore, the second information was that fish density did not affect phytoplankton abundance ([Table 4](#)). Thirdly, each genus showed fluctuating abundance ([Table 2](#)) during the experiment. The phenomenon was commonly reported in biofloc systems ([Green et al., 2014](#); [Castro-Mejia et al., 2017](#); [Nie et al., 2017](#)).

[Table 3](#) depicted that *Mycrosystis* was the most abundant during the research. This differed from a finding by [Green et al. \(2014\)](#), where *Scenedesmus* was the dominant phytoplankton in the biofloc system. Meanwhile, the results were expected because *Mycrosystis* and *Coelosphaerium* adapted well to the intensive biofloc culture with concentrated ammonia.

Intensive aquaculture is characterized by high ammonia originating from feces and food remains not consumed by fish. However, bacteria break ammonia to produce free N in the biofloc system ([Ibeweke et al., 2007](#)), which is an essential micronutrient for phytoplankton. Therefore, it is necessary to observe a high abundance of phytoplankton in the intensive biofloc system.

Additionally, Cyanophyceae or blue-green algae was reported to be highly abundant in the biofloc system, because they optimally use high nutrients (N, P, and K) for better growth (Nie et al., 2017).

Biofloc technology involves floc formation from organic material life, and it is fused into clumps consisting of water microorganisms, including bacteria, algae, fungi, protozoa, metazoan, rotifers, nematodes, Gastrotricha, and other organisms that are suspended by detritus. Furthermore, molasses administration in a biofloc supplies organic carbon in continuity or based on the nitrogen content in the water. Therefore, nutrients like N, P, and K are abundant in the biofloc (Sumitro et al., 2021). This leads to the growth of plankton in the form of Phytoplankton (plants), making the zooplankton development sustainable. Previous research showed that plankton grew rapidly in a pool fertilized by various animal manures. The manures contained nitrogen, phosphorus, and potassium, which are essential for growth (Green et al., 2014).

Generally, biofloc is generated from feed residue, metabolism, and aquaculture feces. The residual feed and fecal wastewater produce inorganic nitrogen, which is converted into a single-cell protein with carbon material water that is used to feed fish, shrimp, and plankton. The condition of the carbon (C) and nitrogen (N) C:N ratio is balanced in cultivation media. Furthermore, heterotrophic bacteria utilize inorganic and inorganic N, for the plankton growth to reduce the concentration of N in the water. The comparison between the C:N ratio is essential in the biofloc system, to enable proper bacteria growth, which affects the growth of phytoplankton (Avnimelech and Kochba, 2009; Crab et al., 2012).

Phytoplankton experiences changes in community composition (succession) due to variations in physical (light intensity and temperature), chemical (nutrients, water quality, and toxins), and biological (competition and predation) conditions. This is justified by Green et al. (2014), who stated that phytoplankton abundance change responds to environmental variables such as temperature, light, nutrient availability, and predator abundance.

The phytoplankton dominance in a biofloc composed of groups of phytoplankton, especially *Microcystis* genus of Cyanophyceae class, and the differences in the plankton dynamics during the treatment period. However, not all phytoplankton can be found in a significant amount in each pool per week. Subsequently, the phytoplankton dynamics in the biofloc pool were diverse, due to the differences

in the amount of phytoplankton abundance weekly. The weekly observation indicated that Cyanophyceae had the highest, which varied among treatments.

Measurement of ammonia, nitrite, and nitrate was conducted at the beginning of the treatment period, to determine if the pond water is ready for the breeding of African catfish. The water quality was measured at the middle and end of the treatment, to reduce the mortality rate of the catfish due to decrease in water quality. Also, it determined the magnitude of ammonia, nitrite, and nitrate suitable to breed African catfish for 40 days of treatment. Table 5 showed that ammonia, nitrite, and nitrate value were 0.9 to 1.8 ppm, 0.0341 to 0.0792 ppm, and 2.167 to 5.492 ppm, respectively, at the beginning of the treatment. However, these results did not follow the Government Regulation No. 82 of 2001, which stated that the value of ammonia, nitrite, and nitrate for water quality management and water pollution control for fisheries is <0.5 ppm, <0.06 ppm, and 10-20 ppm, respectively. Furthermore, the values of ammonia, nitrite, and nitrate in the middle of the treatment were 0.2 to 0.6 ppm, 0.6354 to 3.6579 ppm, and 10.903 to 12.171 ppm, respectively. Then, the value of ammonia, nitrite, and nitrate at the end of the maintenance period were 0.05 to 0.31 ppm, 1.997 to 2.114 ppm, and 6.0260 to 10.1920 ppm, respectively.

The fluctuation of ammonia and nitrite content inhibits the growth of fish. According to Schmittou (1991), the level of ammonia concentration that is > 0.3 ppm disturbs the absorption of nutrients by phytoplankton, hence growth is hampered. Conversely, the nitrite and nitrate level are inversely proportional to the oxygen content in the water. Therefore, for a water pool with an oxygen content that is within the average threshold, the content of nitrite and nitrate can be tolerated by the aquatic organisms (Ohemen et al., 2014). The nitrite and nitrate content in a biofloc was not adequate for fish and plankton growth. Hence, the treatment was conducted for 40 days while maintaining and improving the quality of the water in the biofloc. This was achieved by adding nutrients that helped with phytoplankton growth to increase the productivity of the pond water biofloc, changing the water regularly (once a day as much as 5% of the initial volume), and providing continuous aeration.

Conclusion

About 10 phytoplankton genera were found in the water media of the African catfish biofloc cultivation. Although each genus showed different abundance,

the phytoplankton diversity and abundance are not affected by stock density.

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