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Bacterial Abundance and Identification in Recirculating System of Sumatran Barb Fish Rearing Media with Duckweed as Biofilter

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ABSTRACT

The Sumatran Barb fish (*Puntigrus tetrazona*) is a freshwater ornamental fish which is highly demanded in the ornamental fish export market for its attractive colour patterns. The common problem in ornamental fish farming is the excessive use of water resources, increasing operational costs. A Recirculating Aquaculture System (RAS) is preferred since it can process water repeatedly and is more environmentally friendly. Duckweed plants (*Lemna minor*) can be used as a natural biofilter because they absorb nutrients such as nitrogen and phosphorus and provide a habitat for beneficial bacteria. This study was conducted for 40 days, with the treatments consisting of different plant coverage areas. Duckweed plants were applied with area coverage of P0/control (without duckweed plant), P1 (20% area coverage of duckweed plants), P2 (40% area coverage of duckweed plants), and P3 (60% area coverage of duckweed plants). The bacterial abundance values obtained during the maintenance period ranged from 1.52 to 1.79 x 10^6 CFU mL^{-1} and differed significantly ($P<0.05$) across all treatments. The lowest TPC value was observed in the P0 treatment (1.52 x 10^6 CFU mL^{-1}), and the highest in the P2 treatment (1.79 x 10^6 CFU mL^{-1}). Based on the identification test, bacteria belonging to the genera *Neisseria*, *Acinetobacter*, *Nitrosomonas*, and *Pseudomonas* were identified. Water quality parameters were optimal across all treatments, namely pH between 6.0 and 6.85, dissolved oxygen (3.43-3.95 mg L^{-1}), TAN (0.2-0.4 mg L^{-1}), and phosphate (1.00-2.5 mg L^{-1}). Meanwhile, temperature and nitrate exceeded the optimal limits, with values ranging from 29 to 30 °C and 72 to 210 mg L^{-1} across all treatments.

INTRODUCTION

Ornamental fish is one of Indonesia's fishery commodities having potential to increase the country's foreign exchange income through export activities due to their attractive appearance in terms of color and body shape (Nazhira *et al*., 2017). The export market demand for ornamental fish continues to increase annually, both in terms of type and quantity. According to data of the Central Statistics Agency (BPS) from 2017 to 2021, the value of Indonesian ornamental fish exports increased by USD 27.6 million in 2017 and increased to USD 34.5 million in 2021, with an average growth of 6.11% per year. According to the data of Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia 2024, the production of ornamental fish culture achieved 1.586.492.332 fish in which East Java occupied the highest number of production namely 712.266.621 fish. The number of ornamental fish production in

2024 (1.586.492.332 fish) increased from the previous year namely 2023(1.510.246.438 fish) (KKP, 2025). One of ornamental fish that is highly demanded after in the export market is the Sumatran Barb (*Puntius tetrazona*). The Sumatran Barb fish (*Puntius tetrazona*) is a freshwater ornamental fish originating from Indonesia and is highly sought after in the ornamental fish export market due to its attractive color and body patterns, as well as its high economic and aesthetic value, which has led to increasing market demand (Yuliansyah *et al*., 2021).

One of the frequently encountered and potentially disruptive problems in the cultivation of Sumatran Barb fish is poor water quality of rearing media. Poor water quality is generally influenced by high concentrations of organic and inorganic waste in the fish rearing waters. Among the alternatives, an approach to address the poor water quality is implementing a recirculating aquaculture system (RAS) (Jubaedah *et al*., 2020). A recirculating aquaculture system optimizes water use through a filtration process for continuous use, thus conserving water and managing organic waste effectively. The waste treatment process for recirculating fish cultivation involves mechanical, chemical, and biological filtration (Prayogo *et al*., 2012).

Fish cultivation waste containing organic and inorganic substances, if their content exceeds the threshold, it can cause a decrease in water quality and disrupt the life of aquatic organisms (Nisa, 2022). The effort to reduce the accumulation of fish cultivation waste can be carried out by utilizing phytoremediation methods. Phytoremediation is the use of aquatic plants to remove contamination of aquatic waste. Various plant species frequently used in phytoremediation include *Eichhornia crassipes, Pistia stratiotes, Limnobium laevigatum,* and *Lemna* sp. (Sudiarto *et al*., 2019).

Duckweed (*Lemna minor*) was selected for cultivation of Sumatran Barb fish due to its availability as a phytoremediation agent for rapid nutrient uptake in the form of nitrogen and phosphorus. Duckweed absorbs aquatic waste from fish cultivation through its roots and leaves, which are used as a nutrient source to support its growth (Fuad et al., 2013). Mostly, the species of Lemna indicate the effective ability which contributes to phytoremediation of wastewater in due to their swift vegetative growth, high biomass production, bioaccumulation capacity of varied pollutants including nutrients, organic pollutants, heavy metals and phenols (Mkandawire and Dudel 2007; Pietrini et al. 2016; Ceschin et al., 2020).

Despite its potential for absorbing various forms of nitrogen and phosphorus, duckweed is rarely used in the ornamental fish aquaculture industry. Therefore, the study investigating the interaction among *Lemna minor*, bacterial abundance and bacterial types in rearing media of ornamental fish is necessary. This study aims to determine the interaction among *Lemna minor*, abundance and types of bacteria, as well as water quality in Sumatran Barb fish cultivation media using a recirculation system.

METHOD

Time and Location

This study was carried out over 40 days in the Anatomy and Fish Cultivation, Faculty of Fisheries and Marine (FPK), Universitas Airlangga. Water quality parameter analysis was conducted in the Analysis Chemistry Laboratory, Faculty of Fisheries and Marine, Airlangga University. Bacterial analysis (total abundance test and bacterial identification) was conducted in the Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Airlangga.

Preparation of Rearing Media

This study used 20 aquariums size 40 × 30 × 30 cm with the water volume of 20 L per unit. Before being used, all aquariums and equipment were sterilized with chlorine (25 ppm) for 3 days with strong aeration, then neutralized using sodium thiosulfate (15 ppm). Corner filters with a height of 21 cm and a diameter of 5 cm were installed 7 days before fish rearing to allow the colonization of nitrification bacteria, and filled with filtration media in the form of bioballs, bioforms (coarse sponges), biorings, zeolite, ginger coral, and Malang sand which functioned as mechanical, chemical, and biological filters. The recirculation system was

equipped with a filter pump with a flow capacity of 800 L h-1 (13 L min-1) (Armada AR-650). To determine the area coverage of Duckweed (*Lemna minor*) plants, a barrier was created on the water surface based on the surface area of the aquarium. The study was conducted using a recirculation system. The design of the study can be seen in Figure 1.

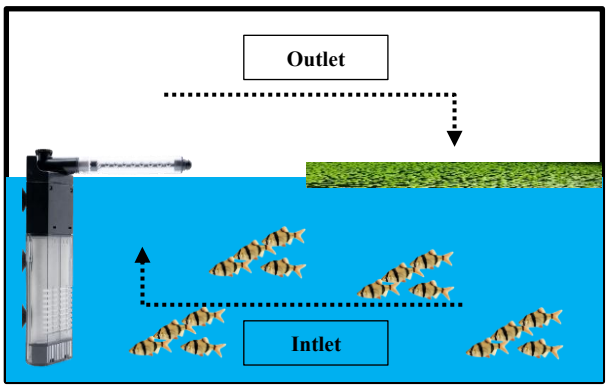

Figure 1. Aquarium design used in the study (control without plants addition)

Preparation of Duckweed Plant and Sumatran Barb Fish

The duckweed (*Lemna minor*) plants used were obtained from a collection pond in the Laboratory. Freshly collected duckweed plants were sterilized by washing them in sterile water for 1 hour and disinfected with 1% sodium hypochlorite solution (0.1 ml or 2 drops per 1 L) for 10 minutes to prevent bacterial contamination and disease (Paterson *et al.,* 2020). Furthermore, the duckweed plants were incubated in clean water for 7 days in the Anatomy and Fish Cultivation Laboratory. The wet weight of the plants used was 7.0±0.5 g (20% coverage area), 14.0±0.5 g (40% coverage area), and 21.0±0.5 g (60% coverage area) (Verma and Suthar, 2015).

Preparation of Sumatran Barb Fish

The Sumatran Barb fish (*Puntigrus tetrazona*) used in the study were size M with a length of 4–6 cm and an average weight of 1.94–2.36 g. Before being used, the fish were adapted for 7 days in a collection pond, followed by a 30-minute acclimatization process in a water-filled profile tank until the fish slowly escaped from the transport bag. After the adaptation period, the fish were reweighed before being placed in the experimental aquarium with a stocking density of 45 fish per 20 L (2.25 fish L^{-1}). Commercial feed namely floating pellet was provided three times a day (at 08.00, 12.00, and 15.00 WIB) at a dose of 3% of the total fish biomass per day. The feed composition contained ±40% crude protein, 5% fat, 6% crude fiber, 16% ash content, and 10% water.

Water Quality Maintenance

Water quality maintenance was carried out through daily siphoning to remove debris settling on the bottom of the aquarium, accompanied by a 2% water change (0.4 L per day). Additionally, the corner filter was cleaned every 10 days, along with a 30% water change (6 L).

Water Quality Measurement

The water quality parameters measured included temperature, pH, DO (dissolved oxygen), TAN (Total Ammonia Nitrogen), nitrate, and phosphate. Temperature, pH, and DO measurements were performed once a day in each aquarium in situ and before feeding time in the morning. Meanwhile, TAN, nitrate, and phosphate concentration measurements were carried out every 10 days and analyzed in the water quality laboratory. The water sampling process was carried out in the morning before morning feeding time. Water samples were taken from the maintenance media column, then placed in a dark sample bottle, and stored at low temperature conditions (0-4^{o}C) until the time of analysis.

Main Parameters

Total Bacterial Abundance Test

The main parameters were the total bacterial abundance test using the Total Plate Count (TPC) method and bacterial identification using biochemical tests. Measurement of total bacterial abundance was measured every 10 days using the spread method on PCA (Plate Count Agar) media. Bacterial colony counts were conducted every 10 days for 40 days. Water sampling for total bacterial abundance analysis and bacterial identification was carried out by taking 60 mL of water and putting it into a sterilized sample bottle. Then, the sample was tested immediately after collection in the Microbiology Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga.

Total bacterial abundance (TKB) can be calculated using the following formula (Madigan *et al.*, 2014):

$$\text{TKB (CFU mL}^{-1}) = \Sigma \text{ colony x } \frac{1}{Vol.\ Spread\ (mL)}\ x\ \frac{1}{fp}$$

in which:

TKB = Total bacterial abundance
Vol. Spread = Volume of Bacteria sample spread
fp = dilution factor

Bacterial Identification Test

Biochemical test was conducted to determine the characteristics and identification of bacteria obtained from the rearing water of Sumatran Barb fish. Biochemical test is a bacterial testing step used to identify bacterial colonies in response to the chemicals administered. Some biochemical tests include oxidase test, catalase test, and sugar fermentation test. Biochemical test results were read based on the table of Cowan and Steel (1974). Bacterial identification tests were conducted on the 40th day, or at the end of the rearing period.

Harvest of *Lemna minor* Plant

To determine the production yield of *Lemna minor* plants, it is calculated using the following formula (Azhar & Memiş, 2025):

$$P = \frac{Fb}{A}$$

In which:

P = Harvest (g m$^{2\ -1}$)
Fb = Harvested biomass (Fb)
A = area (m^2)

Supporting Parameters

The supporting parameters observed were water quality and the production yield of *Lemna minor* plant. The water quality measured included temperature, dissolved oxygen (DO), pH, TAN, nitrate, and phosphate. Water quality measurements were conducted in the morning, afternoon, and evening. Temperature, dissolved oxygen, and pH were measured using a multi-parameter instrument (YSL Pro 20i). TAN, nitrate, and phosphate were analyzed using a spectrophotometer based on the APHA method (2017). The Duckweed harvests were conducted every 10 days.

Data Analysis

The obtained data were processed using Microsoft Excel and then tested for normality and homogeneity using the Shapiro-Wilk Test and Levene's test. Identification data were analyzed descriptively. Meanwhile, data on harvest yields, bacterial abundance, and water quality were analyzed using Analysis of Variance (ANOVA). If the results were significantly different ($P<0.05$), then the Duncan's Multiple Range Test (DRR) was calculated at a 95% confidence interval ($\alpha = 0.05$) to determine the treatment with the best results. Statistical analysis was performed using IBM-SPSS version 25.

RESULT AND DISCUSSION

Observation and Calculation of Bacteria

The results of the study on bacterial counts using the Total Plate Count (TPC) method were obtained through a series of observations and calculations during the incubation period. The data presented includes the results of colony counts for each different treatment and can be seen in Table 1.

Table 1. Average results of total bacterial colony counts (CFU mL^{-1})

Day-	*Dilution 10^6 (Average ± S.Dev)*			
	P0	P1	P2	P3
10	1.52 ± 4.92^{a}	1.58 ± 2.73^{ab}	1.56 ± 2.34^{ab}	1.60 ± 3.64^{c}
20	1.56 ± 9.55^{a}	1.71 ± 12.89^{b}	1.76 ± 7.72^{b}	1.76 ± 7.88^{b}
30	1.60 ± 11.67^{a}	1.73 ± 10.44^{ab}	1.79 ± 12.42^{b}	1.76 ± 11.63^{b}
40	1.64 ± 5.59^{a}	1.49 ± 5.63^{b}	1.58 ± 3.20^{b}	1.60 ± 5.19^{b}

Description: P0 (Treatment without duckweed plants); P1 (Treatment with 20% duckweed plant area coverage); P2 (Treatment with 40% duckweed plant area coverage); P3 (Treatment with 60% duckweed plant area coverage). Different superscript letter notations indicate that there is a significant difference between the treatments ($P<0.05$); while the same superscript letter indicates that the treatments are not significantly different ($P>0.05$).

The values of bacterial colony count obtained during 40 days of study ranged from 1.52 to 1.79 x 10^6 CFU mL^{-1}. The values of total bacterial abundance showed significantly different results ($P<0.05$) in all treatments (Table 1). The lowest value of total bacterial abundance was obtained in the P0 treatment at 1.52 x 10^6 CFU mL^{-1}. Meanwhile, the highest value of total bacterial abundance was found in the P2 treatment at 1.79 x 10^6 CFU mL^{-1}.

The application of duckweed (*Lemna*) treatment can have a positive impact on environment in which the aborption ability of *Lemna* occurs via the roots and leaves and their contribution to the entire absorption of the plant though the gauged kinetic attributes (Cedergreen and Madsen, 2002). Furthermore, Lemna not only contributes to excess nutrients absorption but also supports the development of total bacterial abundance, especially beneficial bacteria. Duckweed plant as a biofilter medium is also indirectly utilized by bacteria as a breeding ground and for the nitrification process. Duckweed which is included in aquatic plant shelters both varied and particular bacteria colonies (Crump and Koch, 2008; Xie et al., 2015; Matsuzawa et al., 2010; Ishizawa et al., 2017). According to the study by Ghozali et al. (2023), duckweed plants have a close relationship with bacterial abundance in an aquatic ecosystem. The presence of bacteria as a decomposer of ammonia into nitrite and then converts nitrite into nitrate, which is then utilized as a nutrient source by the duckweed plant roots (Marda et al., 2015). Meanwhile, the duckweed plant roots produce extrudates, namely enzymes, carbohydrates, and polysaccharides, which can be utilized as an energy source by bacteria (Fitriana & Kuntjoro, 2020).

Bacterial Identification Test

Bacterial identification tests specifically observations of bacterial colony morphology, Gram staining, and a series of biochemical tests which were carried out during the study period can be seen in Table 2.

Table 2. Results of biochemical tests of bacteria in rearing water of Sumatran Barb fish

Treatments	Test Result (Genus)
P0 (Control, without duckweed plant)	*Neisseria*
P1 (20% coverage area of duckweed plants)	*Acinetobacter*

Treatments	Test Result (Genus)
P2 (40% coverage area of duckweed plants)	*Nitrosomonas*
P3 (60% coverage area of duckweed plants)	*Pseudomonas*

Bacterial identification conducted during the 40-day research resulted in several observations, including the genera of *Neisseria, Acinetobacter, Nitrosomonas,* and *Pseudomonas*. The genus of *Neisseria* can be found in aquatic environments, including recirculating aquaculture systems. This bacteria can play a role in assisting the decomposition process of organic matter. Duckweed plants that absorb organic and inorganic compounds are utilized by bacteria to support growth (Ardiansyah and Fotedar, 2016). *Acinetobacter* is a genus of Gram-Negative bacteria commonly found in waters and can act as bioremediation or decomposition of organic matter that helps maintain water quality in recirculating fish farming systems through the denitrification process, in addition to the *Acinetobacter* and *Pseudomonas* genera (Wongkiew et al., 2017). The results of research by Dahal et al. (2023), explain that the *Acinetobacter* bacterial genus can dissolve phosphate content utilized by duckweed plants so that it is more easily absorbed by the roots, which is useful for duckweed plant growth. In line with the study by Ardiansyah and Fotedar (2016), the bacteria found in recirculating aquaculture system (RAS) were *Bacillus, Pseudomonas* and *Acinetobacter* in which *Bacillus* and *Pseudomonas* were more dominant than *Acinetobacter*.

The bacteria genus of *Nitrosomonas* is the most common bacteria found in recirculation fish farming systems because of the role and presence of these bacteria, the nitrification process of converting ammonia to nitrite can run well (Ryan et al., 2017). The bacteria genus of *Nitrosomonas* acts as an ammonia oxidizer in recirculation fish farming systems and is also the main bacteria in the nitrification process. Nitrification process is a key component in recirculation fish farming systems that serves as a remover of nitrogen waste, especially ammonia and nitrite, which are toxic to aquatic organisms (Bartelme et al., 2017). *Pseudomonas* is a genus of heterotrophic bacteria that has the ability to degrade organic compounds and also denitrification that converts nitrate into nitrogen gas (Ardiansyah and Fotedar, 2016). *Pseudomonas* belongs to the *Gammaproteobacteria* family which is capable of carrying out the nitrification and denitrification processes in recirculation fish farming systems. Based on the results of research by Li et al. (2024), explains that the genus *Pseudomonas* can utilize nitrogen (nitrogen assimilation) both in aerobic and anaerobic conditions for cell growth.

Water Quality

Water quality measurements were carried out during the 40-day Sumatran Barb fish culture included temperature, dissolved oxygen, and pH. The range of water quality parameters of each treatment is shown in Table 3.

Table 3. Range of water quality values during the rearing period

Water Quality Parameters	***Treatments (Min-Max)***				
	P0	**P1**	**P2**	**P3**	***References***
Temperature (°C)	29.6-30.0	29.7-30.0	29.7-30.1	29.8-30.2	22.0-28.0[a]
DO (mg L^{-1})	3.43-3.78	3.53-3.81	3.61-3.94	3.55-3.89	>2.0[a]
pH	6.62-6.68	6.69-6.72	6.79-6.85	6.45-7.18	6.5-7.5[a]
TAN (mg L^{-1})	0.234-0.264	0.232-0.372	0.109-0.240	0.088-0.848	<1.0[a]
Nitrate (mg L^{-1})	94.40-217.40	72.20-196.00	75.40-165.60	78.20-179.20	10-30[b]
Phosphate (mg L^{-1})	1.188-3.148	0.778-2.318	0.920-1.930	0.866-1.896	2-3[c]

[a]Tamaru et al. (1997); [b]Estim et al. (2019); [c]Sinha & Pandey (2021).

The results of water quality parameter measurements indicated that all four treatments (P0-P3) were still within the tolerance threshold (Table 3). The water temperature in all treatments ranged from 29.6-30.2°C and was above the optimal value for the growth of

Sumatran Barb fish. The dissolved oxygen content was also within the tolerance limit of 3.43-3.94 mg L-1. The pH values of water were classified as within the tolerance range of 6.0-7.5. However, the P2 treatment showed a high pH value, ranging from 6.62-6.85. Meanwhile, the measurement results of TAN in all treatments were far below the maximum threshold, namely <1 mg L-1. The nitrate values in all treatments showed that the values were above the optimal value, with the highest value found in the P0 (control) treatment. Meanwhile, the values of phosphate in all treatments showed the low values below the optimal limit for ornamental fish cultivation.

Water quality during the rearing period showed optimal values of DO, TAN, and phosphate. However, some water quality parameters such as temperature, pH, and nitrate showed above the optimal values. Temperatures during the rearing period tended to be higher, ranging between 29 and 30°C. However, Sumatran Barb fish were still able to adapt to these conditions. Temperature is an important physical parameter in rearing fish since it affects stress factors in fish (Sinha & Pandey, 2021). In general, fish from the Cyprinidae family can survive in a wide temperature range between 15 and 40°C (Corfield et al., 2008). In most cases, fish grow optimally at temperatures between 20 and 30°C (Effendi, 2003). The pH during rearing period in this study was also still in the range with the pH of rearing media of Sumatran Barb fish in the study by Mukti et al., (2023) which showed 5.7-7.6. Nitrate concentrations during the rearing period showed high values in all treatments. This can be due to the influence of organic matter from leftover feed and the low volume of water changes carried out in the RAS system (Freitag et al., 2015). In RAS, generally nutrient increases, particularly in the form of nitrate due to decreased filtration system performance (Gichana et al., 2018; Lekang, 2008). Nitrate compounds tend to be harmless, but a simultaneous increase in nitrate concentration can limit aquaculture production (Dauda & Akinwole, 2015; Davidson et al., 2014; Martins et al., 2009).

Production Yield of Duckweed Plant

Duckweed production was carried out over a 40-day period, by sampling every 10 days. The average production yield of duckweed plant for each treatment is shown in Table 4 and Figure 2.

Table 4. Data of average production yield of duckweed plant during the rearing period

Parameter	P0	P1	P2	P3
Production Yield (g $m^{2\,-1}$)	0	287^{a}	301^{b}	294^{ab}

Description: P0: No Duckweed coverage area (control), P1: Duckweed coverage area 20%, P2: Duckweed coverage area 40%, P3: Duckweed coverage area 60%

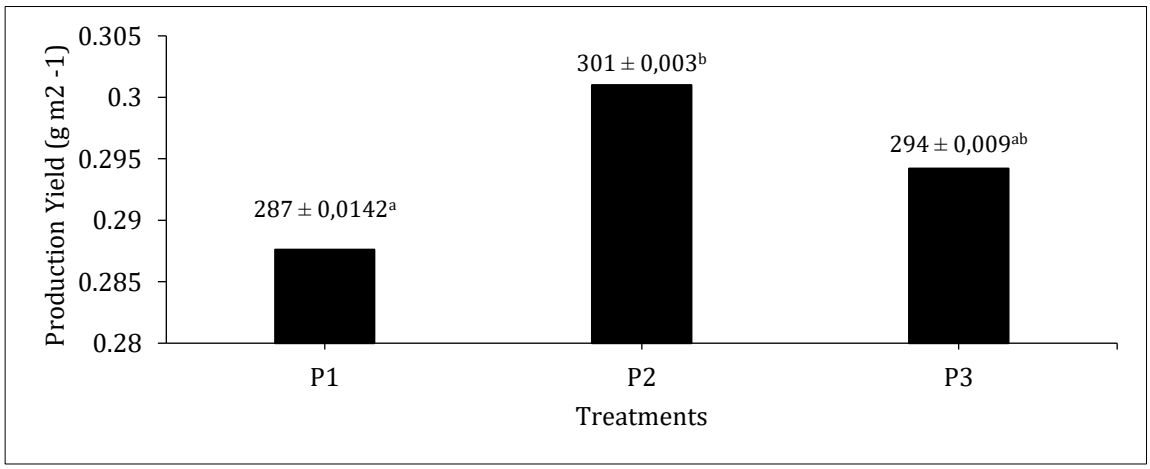

Figure 2. Average number of harvests over 40 days

Based on the average production yield of Duckweed plants in a recirculation fish farming of Sumatran barb fish, the highest production yield were obtained in P2 with the treatment of a 40% coverage area, namely 303 g $m^{2\ -1}$ and the lowest production yield were obtained in P1 with the treatment of a 20% coverage area, namely 295 g $m^{2\ -1}$.

Excessive plant coverage area (in P3 with a 60% coverage area) could lead to intense competition among plants, particularly for light and nutrients, thus limited the potential for duckweed growth. According to Pasos-Panqueva et al. (2024), excessive initial density can cause self-shading and reduced photosynthetic efficiency in duckweed plants, ultimately reducing daily growth rates. This is relevant to the results of this study, in which P3, likely having the most dense coverage (60% coverage area), experienced the harvest rate that was not as good as P2 (40% coverage area). Several factors which influence the growth of duckweed plants are namely abiotic and biotic factors. Abiotic factors that influence the growth of duckweed plants include water temperature, pH value, electrical conductivity, nutrient concentration (nitrogen and phosphorus), light intensity, water depth, and water flow (Hasan and Chakrabarti, 2009; Walsh et al., 2021). The range of pH in this study was 6.62-6.68 which was still in the optimal range (6-9) for growth of Lemna (McLay, 1976; Singh et al., 2022). Meanwhile, biotic factors that influence the growth of duckweed plants include plant density and coverage area (Körner et al., 1998; Verma & Suthar, 2015; Walsh et al. 2021).

CONCLUSION

The use of different area coverage of duckweed (*Lemna minor*) plants as biofilter media in rearing Sumatran Barb fish (*Puntigrus tetrazona*) using a recirculation system has an effect on the total abundance of bacteria with the best treatment in P3 (with an area coverage of duckweed plants of 60%) and also has an effect on bacterial diversity with the identification results in the form of the bacterial genus namely *Neisseria, Acinetobacter, Nitrosomonas*, and *Pseudomonas.*

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