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Research Article

Comparative analysis on the productivity, water quality, and gut microbiome of *Silurus asotus* and *Anguilla japonica* in a biofloc system

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Abstract

This study investigated the effects of biofloc technology (BFT) on water quality, productivity, and intestinal microbial diversity in Far Eastern catfish and Japanese eel. A total of 450 Far Eastern catfish or 50 Japanese eels were housed in separate tanks during a 4-week experiment that compared BFT and the control group. The ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), and nitrate nitrogen (NO_3^--N), along with the growth performance and the gut microbial diversity indices were evaluated. Post-breeding, NH_4^+-N and NO_2^--N levels were consistently maintained below 1 mg/L in BFT tanks, while NO_3^--N levels exhibited continuous increases. Compared to the control group, BFT tanks showed superior growth metrics for both species, particularly Far Eastern catfish, which displayed significantly higher values for body length, body weight, and stocking density ($p<0.05$). Principal coordinates analysis revealed notable similarities in microbial communities across treatments. The Firmicutes phylum was predominant in the gut microflora of both fish, while Proteobacteria, Bacteroidetes, and Fusobacteria predominated in the breeding water. The Firmicutes/Bacteroidetes ratio increased in BFT group, suggesting a positive impact on growth. Operational taxonomic units ranged from 94 to 293 for Japanese eels and 234 to 353 for Far Eastern catfish. Based on Venn diagram analysis, 36 to 75 unique species were identified in Japanese eel gut samples, while Far Eastern catfish gut samples contained 72 to 145 unique species. In conclusion, BFT positively impacts water quality, fish growth, and beneficial intestinal microbes.

Introduction

With the rapid growth of the global population, the demand for aquaculture as a vital food source continues to rise. Projections estimate that aquaculture production will expand by approximately 205%, from 40 million tons in 2008 to 82 million tons by 2050 (FAO, 2022). However, intensive aquaculture can create environmental issues, such as hypoxia, due to the release of nutrient-rich wastewater, which promotes the metabolic activity of aerobic bacteria (Chávez-Crooker and Obreque-Contreras, 2010; Mugwanya *et al*., 2021). Therefore, the expansion of aquaculture must be pursued with a focus on sustainability, taking into account environmental, economic, and social factors (Khanjani and Sharifinia, 2020). Considering the limited availability of land and water resources, improvements in production efficiency and aquaculture technologies are essential to reducing operational costs while meeting production needs (El-Sayed, 2021).

Biofloc technology (BFT) presents an eco-friendly approach that integrates bacteria, algae, and protozoa, along with particulate organic matter, to enhance water quality, manage waste, and control disease in intensive aquaculture environments (Mugwanya *et al*., 2021; Khanjani *et al*., 2023). The system works by maintaining microbial flocs through continuous aeration and the addition of carbon sources, which facilitates aerobic digestion (Ahmad *et al*., 2017; Khanjani *et al*., 2022). BFT helps prevent the build-up of harmful nitrogen compounds through processes such as algae-driven photoautotrophic removal, autotrophic conversion of ammonia to nitrate, and heterotrophic assimilation of ammonia into microbial biomass (Ogello *et al*., 2021). In particular, heterotrophic bacteria play a key role in rapid ammonia removal when sufficient carbon is present (Ferreira *et al*., 2021). Maintaining an optimal carbon-to-nitrogen (C/N) ratio of around 10–20:1 promotes the conversion of nitrogenous waste into microbial biomass, offering a valuable source of protein for aquatic organisms while reducing water treatment costs by up to 30% compared to conventional methods (Crab *et al*., 2012; Ahmad *et al*., 2017; Khanjani and Sharifinia, 2022). Furthermore, bioflocs can enhance the immune response and reproductive performance of fish, leading to improved productivity and seed quality (Bossier and Ekasari, 2017).

Recent studies have demonstrated the positive effects of BFT on growth, gut health, and marketability of species like stinging catfish and olive flounder (Sohel *et al*., 2023; Yu *et al*., 2023). BFT is increasingly viewed as essential for sustainable aquaculture, with ongoing innovations expanding its application (McCusker *et al*., 2023).

However, there remains a research gap on how BFT affects microbial communities in rearing water and fish intestines. The gut microbiome plays a vital role in nutrient absorption, immunity, and disease resistance in fish (Ganguly and Prasad, 2012; Talwar *et al*., 2018), and is influenced by the aquatic environment (Parata *et al*., 2020). Yet, few studies have explored these microbial dynamics in commercially important species like Japanese eel and Far Eastern catfish.

To address this gap, the present study aims to evaluate the effects of BFT system on the microbial composition of both the rearing water and fish intestines, as well as assess growth performance and water quality in Japanese eel and Far Eastern catfish. These species are ideal candidates due to their increasing demand in aquaculture, adaptability to biofloc environments, and potential for improved production outcomes. The findings of this study are expected to provide crucial insights into optimizing aquaculture practices for sustainability and productivity.

Material and methods

Experimental design, fish, and rearing conditions

This research was conducted at the Advanced Aquaculture Research Center, Changwon, Korea. In this study, BFT was applied and three round water tanks measuring 1.8 m in diameter and 0.7 m in height made from fiber-reinforced plastics (FRP) were used for both the control group and BFT treatment group. Groundwater near the Advanced Aquaculture Research Center was used in this study. It was sterilized by an ultraviolet (UV) pipe before the experiment. Water temperature was maintained at 30°C for Japanese eel and 25°C for Far Eastern catfish. One ton of water was maintained during the experiment. No additional water was supplied in BFT treatment except for water from evaporation. In the case of the control group, water was exchanged three times a day. An air blower (Techno Takatsuki, Takatsuki, Japan) was used to aerate water in all tanks. An oxygen generator (Kumho Marine, Busan, Korea) was used in BFT tank to maintain dissolved oxygen (DO) at least 5 mg/L. A water tank cooler (Daeil, Busan, Korea) was used to maintain the water temperature. A total of 100 g of sodium bicarbonate was added to maintain a pH of 6 or more.

Before the experiment, nutrients (BFT-CT, egeeTech, Texas, USA) and probiotics (BFT-ST, egeeTech, USA) helpful for biofloc formation were added at the recommended dose of 300 mg/L to make BFT rearing water. After inoculation, the culture was maintained for 30 days. BFT-ST was composed of 10 strains (*Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Cellulomonas* sp., *Cellulomonas biazotea*, *Nitrosomonas europaea*, *Nitrobacter winogradskyi*, *Pseudomonas stuzeri*, *Pseudomonas dendrificans*, and *Rhodopseudomonas palustris*).

The study was performed for four weeks for both Japanese eels and Far Eastern catfish. During the biofloc preparation phase, a total of 5 L of molasses, serving as the carbon source (C), and 1 kg of a commercial feed (Sajo-Dongaone Co., Seoul, Republic of Korea), which contains 44% protein and acts as the nitrogen source (N), were introduced into the tanks to achieve a C/N ratio of 15:1. For each tank, 50 Japanese eels (*Anguilla Japonica*) with an initial average body length of 390.1±12.86 mm and an initial average body weight of 95.0±4.48 g and 450 Far Eastern catfish (*Silurus asotus*) with an initial body length of 102.5±3.83 mm and an initial body weight of 11.92±1.55 g were used. Both Japanese eels and Far Eastern catfish were put into each tank at an initial stocking density of 5.0 kg/t. Far Eastern catfish and Japanese eels were fed daily at 250 g (5% of initial total weight) at 150 g (3% of initial total weight), respectively. This study proceeded under the approval number 2022-NIFS-IACUC-5 following regulations for the care and use of laboratory animals of the National Institute of Fisheries Science.

Water quality analysis

During the experiment, water quality indicators including water temperature, total ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), and nitrate nitrogen (NO_3^--N) were measured. Water temperature, DO, and pH were measured with a multi-item water quality meter (model YSI-650, Yellow Spring Instruments, Ohio, USA). Total ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen were analyzed with the calorimetric method using a spectrophotometer (Merck KGaA, Darmstadt, Germany).

Fish growth

To estimate the growth performances of Japanese eels and Far Eastern catfish raised for four weeks, they were randomly selected from each tank (n = 10) to measure their body lengths (BLs) and body weights (BWs). Fish growth performance parameters were calculated as follows: Total growth rate=[(final weight-initial weight)/initial weight] x 100, Survival rate (%)=(final individuals/initial individuals) x 100, and FCR (feed coefficient rate)=feed consumption/weight gain.

Analyses of microbes in BFT water and fish guts

BFT water and gut samples at 0 and 4 weeks were gathered for microbial analysis (n=3). Gut samples were collected after the euthanasia of fish using an anesthetic (MS-222, Sigma-Aldrich, St. Louis, MO, USA). PCR conditions, DNA extraction, bioinformatics, and sequencing analysis were carried out using published methods (Niu *et al.*, 2019). In short, a PowerSoil DNA isolation kit (Mobio Laboratories, Inc., Carlsbad, CA, USA) was used for isolating genomic DNAs. The V3-V4 region of the bacterial 16S rRNA gene was amplified using 341F and 785R primers. Sequencing was performed using a MiSeq platform (Illumina) through a commercial service of Macrogen (Seoul, South Korea). To compare alpha diversity, operational taxonomic units (OTUs), Chao1, Shannon, and Gini-Simpson indexes were determined. For beta diversity analysis, principal coordinate analysis (PCoA) and weighted UniFrac distance matrix-based unweighted pair-group mean average (UPGMA) analysis were performed. Venn diagram analysis was used to identify shared and unique species between treatments.

Statistical analysis

Data on fish growth performance were analyzed using an independent t-test. The microbial community in gut and rearing water was analyzed by one-way analysis of variance (ANOVA). Significant differences in each result between treatment groups were determined by the Duncan test at p<0.05 using SPSS program version 25 (SPSS Inc., Chicago, IL, USA).

Results

Water quality for NH_4^+-N, NO_2^--N, and NO_3^--N

In this study, concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N were monitored to assess the effects of BFT system on water quality for both Japanese eel and Far Eastern catfish species over time (Fig. 1). For Japanese eels, the concentration of NH4+-N in BFT treatment group gradually increased, peaking at 0.82 mg/L, whereas the control group showed fluctuations between 0.03 mg/L and 1.17 mg/L (Fig. 1A). The NO_2^--N levels initially rose to 0.81 mg/L in BFT group before declining, while the control group experienced fluctuations and a gradual decrease (Fig. 1B). The concentration of NO_3^--N steadily increased in BFT group, reaching 42.4 mg/L, while remaining relatively constant in the control group (Fig. 1C). For Far Eastern catfish, the NH4+-N levels in BFT group initially decreased, followed by a gradual rise to 0.81 mg/L. In contrast, the control group showed fluctuations between 0.19 mg/L and 0.85 mg/L (Fig. 1D). The NO_2^--N levels remained under 0.59 mg/L in BFT group after day 8. The control group showed a fluctuation over time (Fig. 1E). Lastly, the NO_3^--N levels in BFT group rose steadily to 57.13 mg/L, whereas the control group exhibited more stable concentrations (Fig. 1F).

Growth performance

No mortality was observed in either BFT treatment or control group during the rearing period for Japanese eels. For Far Eastern catfish, the survival rates were 95.08±0.06% in BFT treatment group and 94.18±1.49% in the control group (Table 1). In the case of Japanese eels, the body length in BFT treatment group was significantly greater than that in the control group ($p<0.05$). Similarly, for Far Eastern catfish, BFT treatment group exhibited significantly higher body length, body weight, feed conversion ratio (FCR), and stocking density compared to the control group (all $p<0.05$).

Microbial diversity

The microbial diversity of breeding and fish gut was investigated. Significant differences were observed in all four alpha diversity-related measures (OTUs, Chao1, Shannon, Gini-Simpson) between BFT and control groups for both rearing water and fish gut samples. For Japanese eels, OTUs in rearing water were highest at week 0 for BFT group, followed by those at 4 weeks in BFT group, and then at 4 weeks and 0 week in the control group ($p<0.05$). The OTUs of gut microbiota were highest at week 0, followed by those at 4 weeks in both the control and BFT groups ($p<0.05$). For Far Eastern catfish, microbial diversity in rearing water was significantly higher in BFT samples at week 0 than in other samples ($p<0.05$) (Table 2).

No significant differences were found among the other samples at different time points, although a trend was noted: control at 4 weeks>control at 0 week>BFT at 4 weeks. OTUs showed no significant differences among gut samples, but there was a numerical order of 0 week > BFT at 4 weeks>control at 4 weeks (Table 3). Similar trends were observed for Chao1, Shannon, and Gini-Simpson indices.

Principal coordinates analysis (PCoA) was performed, and an unweighted pair-group method with arithmetic means (UPGMA) tree was constructed to compare microbial similarities between rearing water and fish gut microbes (Fig. 2).

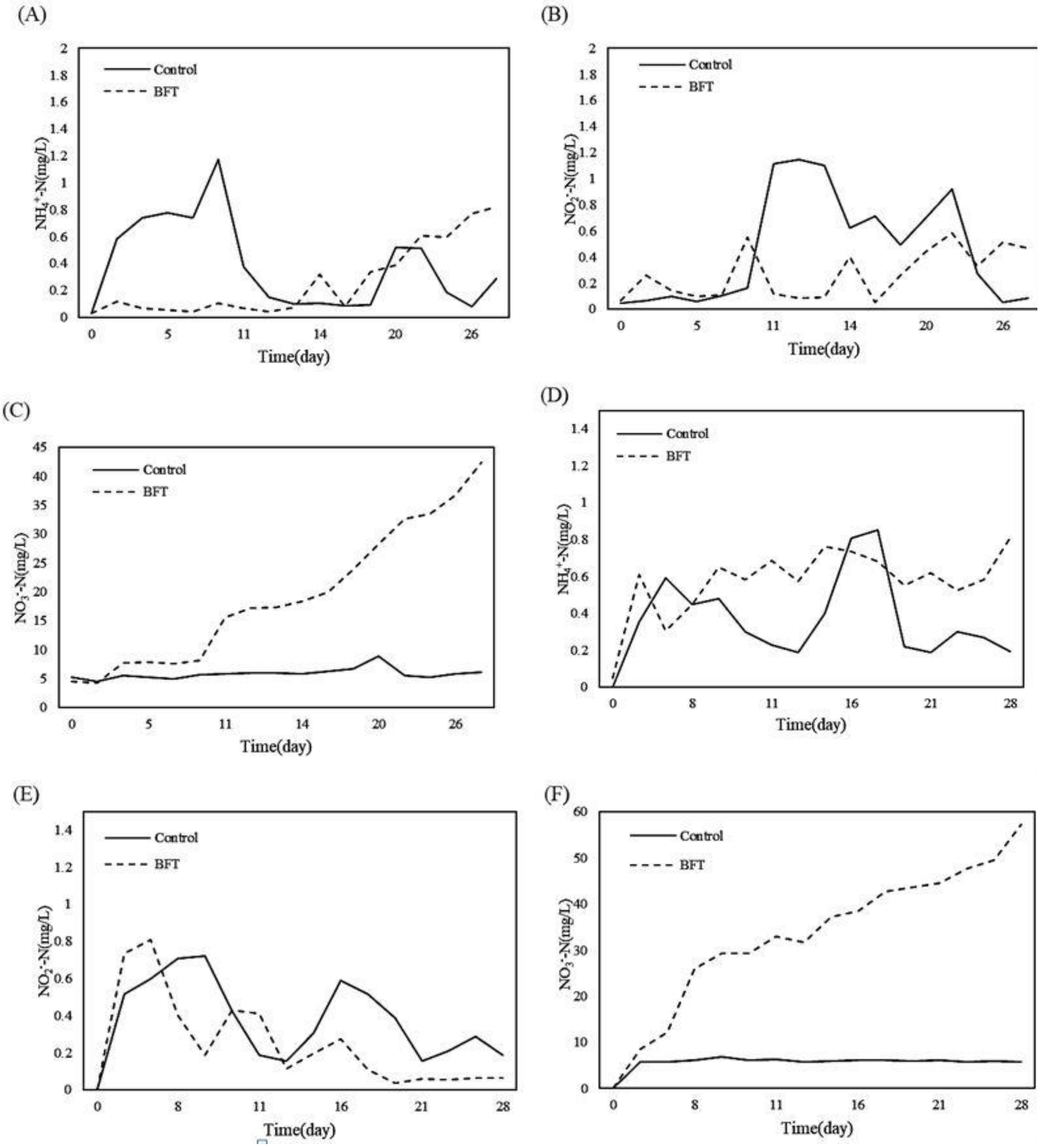

Figure 1: Changes in water quality during the experiment period of rearing Japanese eels and Far Eastern catfish. (A): NH_4^+-N in Japanese eels, (B): NO_2^--N in Japanese eels, (C): NO_3^--N in Japanese eels, (D): NH_4^+-N in Far Eastern catfish, (E): NO_2^--N in Far Eastern catfish, (F): NO_3^--N in Far Eastern catfish.

For Japanese eels, PCoA results indicated considerable microbial similarity in gut samples, regardless of time or treatment. Additionally, BFT rearing water samples at weeks 0 and 4 exhibited similar microbial diversity. In Far Eastern catfish, BFT gut samples and rearing water at week 4 showed close microbial similarities. To analyze shared and unique microbial species, a Venn diagram was employed (Fig. 3).

Table 1: Growth performances of Japanese eel (*Anguilla japonica*) and Far Eastern catfish (*Silurus asotus*) in BFT treatment and control groups.

Species	Treatment	Initial[1]				Final				Total growth rate (%)	Survival rate (%)	FCR[2]
		TW (kg)	BL (mm)	BW (g)	Density (kg/t)	TW (kg)	BL (mm)	BW (g)	Density (kg/t)			
Japanese eel	Control	5.00	390.10 ±12.86	95.00 ±4.48	5.00	6.91 ±0.06	429.50 ±5.80	125.26 ±7.65	6.91 ±0.06	38.20 ±1.11	100	2.20 ±0.06
	BFT	5.00	390.10 ±12.86	95.00 ±4.48	5.00	7.10 ±0.12	444.23* ±12.07	127.83 ±4.46	7.10 ±0.12	42.07 ±2.39	100	2.00 ±0.11
Far Eastern catfish	Control	5.00	102.50 ±3.83	11.92 ±1.55	5.00	10.41 ±0.23	149.07 ±3.44	24.16 ±1.42	10.41 ±0.23	108.13 ±4.58	94.18 ±1.49	1.30 ±0.06
	BFT	5.00	102.50 ±3.83	11.92 ±1.55	5.00	11.83* ±0.42	157.67* ±4.94	28.57* ±1.79	11.83* ±0.42	136.67* ±8.33	95.08 ±0.06	1.03* ±0.07

Values are presented as mean ± standard deviation among treatment groups. Values with * mean significantly different at $p<0.05$.

[1] TW, total weight; BL, body length; BW, body weight.

[2] FCR, feed coefficient rate.

Table 2: Alpha-diversity indices of rearing water of Japanese eel and Far Eastern catfish.

Species	Treatment	Time (week)	OTUs	Chao1	Shannon	Gini-Simpson
Japanese eel	Control	0	97.67 ± 8.39^{a}	98.17 ± 8.43^{a}	2.63 ± 1.06^{a}	0.58 ± 0.24^{a}
		4	128.33 ± 35.00^{a}	129.22 ± 33.83^{a}	3.28 ± 1.12^{a}	0.73 ± 0.16^{ab}
	BFT	0	372.00 ± 11.27^{c}	382.08 ± 11.88^{c}	5.84 ± 0.49^{b}	0.94 ± 0.04^{b}
		4	296.33 ± 29.14^{b}	319.43 ± 35.19^{b}	5.58 ± 0.48^{b}	0.94 ± 0.03^{b}
Far Eastern catfish	Control	0	119.00 ± 44.40^{a}	119.00 ± 44.40^{a}	4.24 ± 0.60^{b}	0.85 ± 0.05^{b}
		4	155.33 ± 26.76^{a}	169.15 ± 20.29^{a}	2.40 ± 0.60^{a}	0.60 ± 0.12^{a}
	BFT	0	372.67 ± 67.09^{b}	390.55 ± 62.00^{b}	6.69 ± 0.24^{c}	0.98 ± 0.00^{b}
		4	67.67 ± 55.58^{a}	76.87 ± 60.92^{a}	1.67 ± 0.65^{a}	0.51 ± 0.14^{a}

$^{a\text{-}c}$Values with different superscripts within a column are significantly different ($p < 0.05$) in the mean score (mean ± standard deviation) among treatment groups (n = 3).

Table 3: Alpha-diversity indices for gut samples of Japanese eel and Far Eastern catfish.

Species	Treatment	Time (week)	OTUs	Chao1	Shannon	Gini-Simpson
Japanese eel	Control	0	293.00 ± 69.63^{c}	343.81 ± 88.85^{b}	3.82 ± 0.75^{b}	0.71 ± 0.08^{ab}
	Control	4	191.00 ± 19.70^{b}	207.44 ± 28.05^{a}	4.39 ± 1.31^{b}	0.86 ± 0.12^{b}
	BFT	4	94.00 ± 35.76^{a}	134.24 ± 44.30^{a}	1.88 ± 0.54^{a}	0.54 ± 0.11^{a}
Far Eastern catfish	Control	0	353.33 ± 159.82^{a}	459.21 ± 276.96^{a}	5.01 ± 0.79^{a}	0.90 ± 0.05^{a}
	Control	4	233.67 ± 125.30^{a}	312.36 ± 180.96^{a}	4.45 ± 1.40^{a}	0.83 ± 0.15^{a}
	BFT	4	258.67 ± 154.37^{a}	364.39 ± 280.99^{a}	3.20 ± 0.91^{a}	0.75 ± 0.10^{a}

$^{a\text{-}c}$Values with different superscripts within a column are significantly different ($p < 0.05$) in the mean score (mean ± standard deviation) among treatment groups (n = 3).

For Japanese eels, 45 common species were detected when comparing week 0 samples between BFT and control groups (Fig.3A), and 43 common species were found in the comparison of week 4 samples (Fig.3B). BFT Japanese eel gut sample contained 36 unique species (Fig. 3B). Moreover, 64 common species were identified when comparing gut samples of BFT and control groups, with BFT gut samples at week 4 having 75 unique species (Fig. 3C).

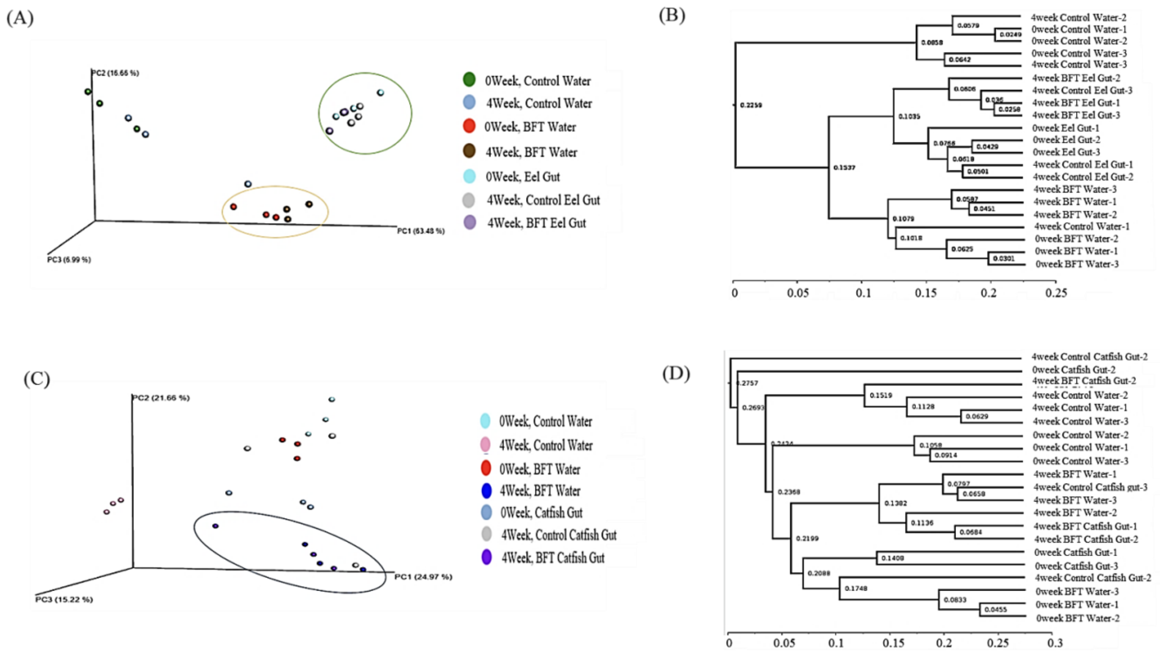

Figure 2: Similarity of bacterial community. (A) Principal coordinate analysis (PCoA) plot based on weighted Uni Frac distance matrix in Japanese eel, (B) UPGMA clustering of samples based on weighted UniFrac distance matrix in Japanese eel, (C) Principal coordinate analysis (PCoA) plot based on weighted UniFrac distance matrix in Far Eastern catfish, (D) UPGMA clustering of samples based on weighted UniFrac distance matrix in Far Eastern catfish.

BFT rearing water contained 124-245 unique species (Fig. 3A and B), depending on the comparison target and time, while BFT gut samples had 36-75 unique species (Fig 3B and 3C). In Far Eastern catfish, 27 common microorganisms were identified when comparing the gut microbiota at week 0 (Fig. 3D). By week 4, 85 common microorganisms were detected among the samples, and BFT-treated Far Eastern catfish at week 4 had 145 unique microorganisms (Fig. 3E). When comparing the gut samples between treatments, 149 common microorganisms were identified, with BFT group at week 4 showing 72 unique microorganisms (Fig. 3F). In BFT rearing water, 7 to 292 unique microorganisms were observed depending on the comparison target and time (Fig. 3D and 3E), while BFT-treated gut samples exhibited 72 to 145 unique microorganisms (Fig. 3E and F).

Microbial communities

Different microbial compositions were observed in breeding water and gut samples between the control and BFT treatment groups (Fig. 4). Generally, Bacteroidetes and Proteobacteria were predominant in Japanese eel breeding water and Firmicutes and Proteobacteria were predominant in Far Eastern catfish breeding water (Fig. 4A and C). In Japanese eel breeding water, the relative abundance of Actinobacteria, Chlamydiae, Chloroflexi, Nitrospirae, and Tenericutes increased significantly over time in BFT treatment ($p<0.05$). Conversely, the abundances of Gemmatimonadetes and Spirochaetes were significantly decreased ($p<0.05$).

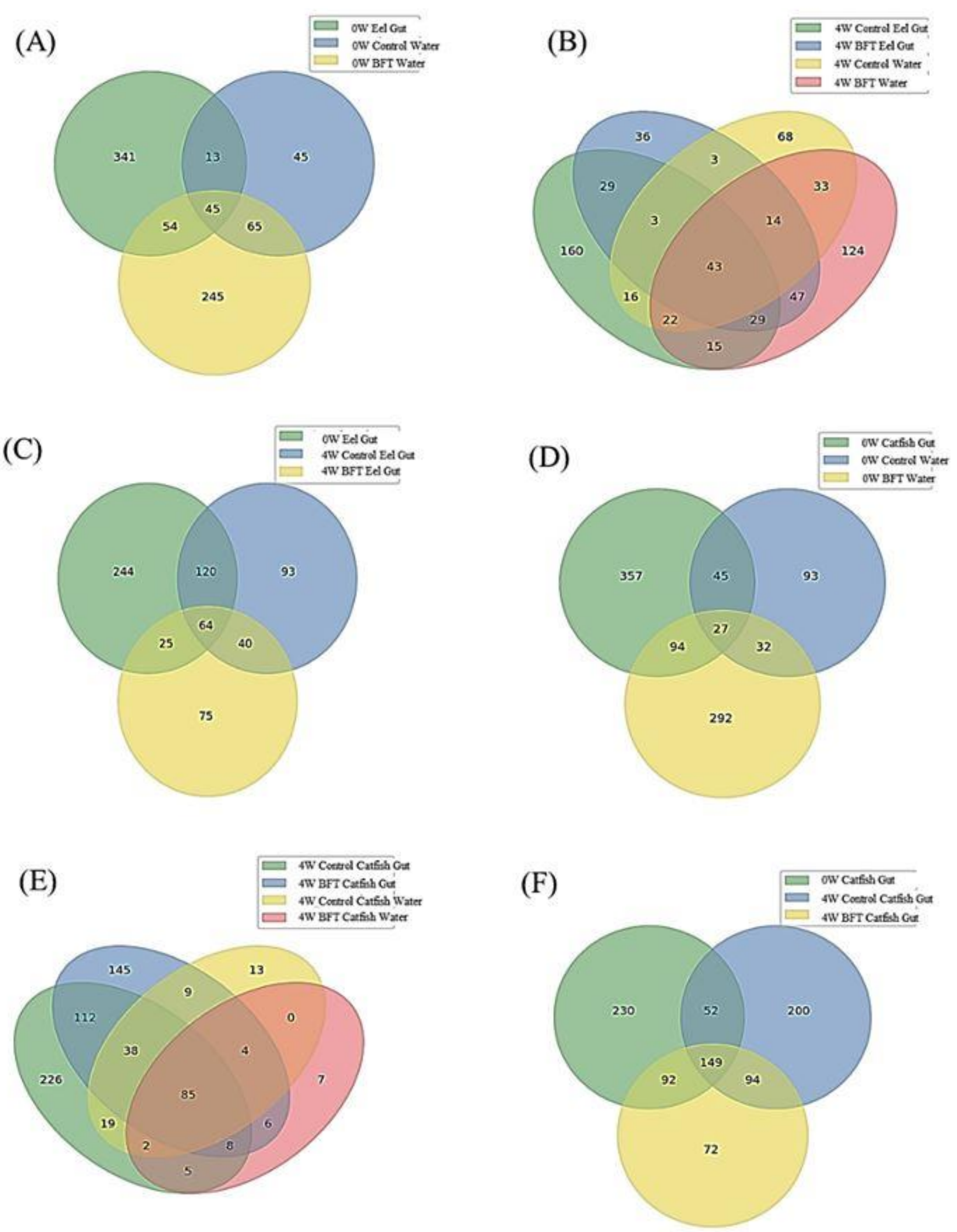

Figure 3: Shared and unique species in Japanese eel and Far Eastern catfish displayed by Venn diagram. (A) 0-week water and gut samples of Japanese eel, (B) 4-week water and gut samples of Japanese eel, (C) 0- and 4-week gut samples of Japanese eel, (D) 0-week water and gut samples of Far Eastern catfish, (E) 4-week water and gut samples of Far Eastern catfish, (F) 0- and 4-week of Far Eastern catfish gut samples.

The relative abundance of Bacteroidetes was significantly lower in the 4 week-BFT treatment compared to that in the control group, whereas that of Firmicutes was significantly higher ($p<0.05$). For Far Eastern catfish, the relative abundance of

Eurychaeota, Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Candidatus Melainabacteria, Chloroflexi, Lentisphaerae, Nitrospirae, Planctomycetes, and Proteobacteria were significantly decreased over time in BFT treatment, whereas Fusobacteria was increased significantly (*p*<0.05). In the gut microbiota of Japanese eels, Firmicutes and Proteobacteria were predominant, whereas Firmicutes, Bacteroidetes, and Fusobacteria were predominant in the gut microbiota of Far Eastern catfish (Fig. 4B and D). For Japanese eel gut microbiota, the relative abundance of Bacteroidetes significantly decreased in BFT treatment, while Spirochaetes significantly increased (*p*<0.05). In Far Eastern catfish reared in BFT system, the relative abundance of Bacteroidetes was significantly lower than in the control group, whereas that of Firmicutes was significantly higher (*p*<0.05).

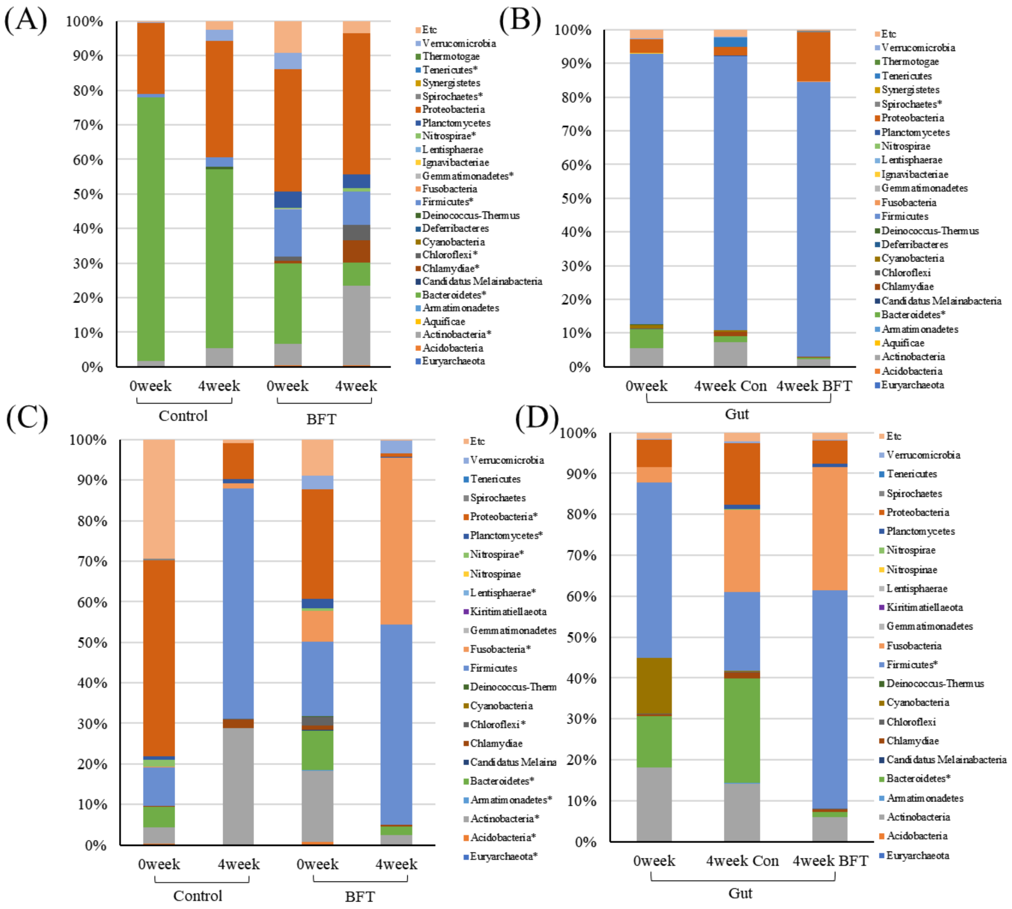

Figure 4: Relative abundance of Japanese eel and Far Eastern catfish at the phylum level. (A) Breeding water samples of Japanese eel, (B) Gut samples of Japanese eel, (C) Breeding water samples of Far Eastern catfish, (D) Gut samples of Far Eastern catfish. n=3 per treatment. Values with * mean significantly different at *p*<0.05.

Discussion

Through the nitrification and denitrification process, the microbial communities of the bioflocs prevent the accumulation of toxic nitrogen compounds for organisms but can also remove organic matter and other compounds (Robles-Porchas *et al.*, 2020). Nitrification is composed of two steps. First is the conversion of NH_4^+-N to NO_2^--N and the second is the conversion of NO_2^--N to NO_3^--N. NO_3^--N is the form of nitrogen that is generally harmless to the fish except for at high levels (>100 mg/L) (Abakari *et al.*, 2021). For both Japanese eels and Far Eastern catfish, concentrations of NH_4^+-N and NO_2^--N in BFT treatment group were maintained stably at lower levels than those in the control group. NO_3^--N concentration was gradually increased in BFT treatment group. This is thought to be because the high oxygen concentration inhibits denitrification (Lu *et al.*, 2020), and oxygen is continuously aerated in BFT treatment. In this study, it is considered that the nitrification process was carried out stably in BFT system.

Several studies have reported that the application of a BFT system has positive effects on growth performance, productivity, and water quality in various aquaculture species (Ekasari *et al.*, 2015; Rajkumar *et al.*, 2016). A significant influence on the growth and survival of Japanese eels using BFT via the improvement of water quality parameters and feed utilization efficiency has been reported (Sadi *et al.*, 2022). In addition, in an experiment to study changes with an increase of stocking density in BFT system, there was no significant difference in any growth performance indicators such as weight or FCR when comparing a high stocking density treatment and a low stocking density treatment (Sukardi *et al.*, 2018). This suggests that biofloc can help improve Japanese eel productivity. Zafar *et al.* (2022) have reported the beneficial effects of BFT on the growth enhancement of Far Eastern catfish. BFT has advantages for Far Eastern catfish breeding, including improvement of water quality, low lipid peroxidation, chymotrypsin activities, and protection from bacterial infection (Dauda *et al.*, 2018). In this study, BFT group showed better productivity, consistent with previous studies.

In the case of shrimp raising using BFT system, it has been observed that the microbial diversity (OTUs) is typically higher in rearing water than in the gut, as the gut environment and immune responses make it challenging for many waterborne microorganisms to survive in the digestive tract (Cardona *et al.*, 2016). However, the results of our study differ from those previously reported, showing that the gut samples of both Japanese eel and Far Eastern catfish had higher operational taxonomic units (OTUs) compared to the rearing water. This suggests that the intestinal environment in these fish species, when reared under BFT conditions, may allow for a more diverse microbiome, potentially due to species-specific differences in immune responses or gut physiology.

In addition, it has been reported that the gut microbes of shrimp in BFT system and microorganisms in BFT-rearing water have a high similarity (Xu *et al.*, 2022). In contrast, the similarity between the gut microbiota and the rearing water increased for Far Eastern catfish under BFT conditions. This suggests that Far Eastern catfish may more effectively integrate or select microorganisms from the surrounding environment into their gut.

Moreover, in both Japanese eel and Far Eastern catfish, the proportion of shared microbes between the gut and rearing water increased over time in both BFT and control groups. This aligns with findings from previous studies indicating that microbial compositions in breeding water can influence intestinal microbiota over time (Talwar *et al*., 2018). This time-dependent microbial exchange suggests that as fish remain in a biofloc-rich environment, the interactions between rearing water and gut microbiomes become more pronounced. Overall, the results highlight that the effects of BFT on microbial diversity and the gut-water microbiome relationship are species-specific. Further research is required to elucidate how these dynamics vary across different fish species and to optimize BFT systems to enhance microbial health and growth performance tailored to the species being cultured.

The phylum Bacteroidetes is commonly abundant in the gut of fish, and certain species within this phylum have been demonstrated to act as pathogens, causing serious infections in both farmed and wild fish populations (Thomas *et al*., 2011). Two genera within this phylum, Prevotella and Flavobacterium, are known opportunistic pathogens that are capable of inducing intestinal inflammation (Ley, 2016; Chhetri *et al*., 2021). Conversely, Firmicutes are prevalent in the intestines of humans and freshwater fish, contributing to gut homeostasis by interacting with the intestinal mucosa (Zeng *et al*., 2020; Sun *et al*., 2022). It is noteworthy that species such as *Romboutsia sedimentorum* within this phylum are involved in the production of short-chain fatty acids (SCFAs), which aid in energy metabolism by breaking down carbohydrates (Chen *et al*., 2022). It is well-documented that the Firmicutes/Bacteroidetes (F/B) ratio in the gut can influence the host's energy absorption and storage. A higher F/B ratio is linked to improved energy efficiency and subsequent weight gain (Kong *et al*., 2021). Bacteroidetes species are known to encode carbohydrate-related enzymes that contribute to glycoconjugate hydrolysis, whereas Firmicutes promote the absorption and metabolism of fatty acids (Zhou *et al*., 2021). In shrimp aquaculture, a high Firmicutes-to-Bacteroidetes ratio has been linked to enhanced nutrient absorption and improved growth (Fan and Li, 2019). Similarly, a high Firmicutes-to-Bacteroidetes ratio has been observed to correlate with faster growth rates in transgenic carp (Sylvain *et al*., 2016).

The present study revealed a reduction in the abundance of Bacteroidetes in BFT treatment group, when compared to the control, for both Japanese eel and Far Eastern catfish. Conversely, the abundance of Firmicutes was significantly higher in BFT Far Eastern Far Eastern catfish group than in the control. This resulted in an increased F/B ratio in both species under BFT treatment, which is consistent with the observed superior growth performance in BFT groups. Prior research has demonstrated that microbial communities in rearing water can influence the gut microbiota of fish (Wang *et al.,* 2018). For example, the gut microbiota of tilapia larvae can be manipulated by managing the microbial composition of the rearing water (Giatsis *et al*., 2015). Similarly, the addition of probiotics to tilapia breeding water has been demonstrated to reduce nitrogenous waste, improve productivity, enhance gut microbiota, and strengthen immune function (Kord *et al*., 2022). In shrimp, the introduction of probiotics into BFT rearing water has also been associated with enhanced immunity (Miao *et al*., 2017).

The present study revealed a notable reduction in the abundance of the Proteobacteria phylum in the rearing water of Far Eastern catfish treated with BFT. While Proteobacteria is a dominant bacterial phylum in the intestines of various marine and freshwater fish, it also contains numerous pathogenic bacteria (Liu *et al*., 2016; Chen *et al*., 2020). The phylum Fusobacteria, which is known for fermenting amino acids and carbohydrates to produce butyric acid, a compound with immunomodulatory and anti-inflammatory effects in the gut (Bereded *et al*., 2021), was also observed in the gut microbiota.

The findings of this study demonstrate that BFT systems are effective in reducing harmful bacteria while promoting beneficial bacteria in both rearing water and fish gut microbiota over time. These microbial shifts support the potential of BFT systems to enhance immune function and improve growth performance in aquaculture species.

Conclusions

This study demonstrates that BFT system significantly improves growth performance, microbial diversity, and water quality management in Japanese eel and Far Eastern catfish aquaculture. BFT treatment resulted in improved growth metrics, higher survival rates, and a more diverse and stable microbial community compared to control conditions. Importantly, concentrations of NH_4^+-N and NO_2^--N were maintained at lower levels in BFT treatment, while NO_3^--N levels increased steadily, indicating effective nitrification processes. These results suggest that the implementation of BFT systems can optimize nutrient utilization, promote fish health, and enhance the overall resilience of aquaculture. Further research is encouraged to explore the long-term benefits and scalability of BFT technology in different aquaculture contexts.

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Conflicts of interest

The authors declare that no conflicts of interest among us.

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