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"Impact of biofloc bacterial consortia on growth performance and quality of *Catla catla* fingerlings"

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Abstract

The sustainable aquaculture practice is Biofloc technology (BFT) which is enhancing fish growth and nutritional quality by the activity of significant microbial consortia. The present study evaluated the impact of biofloc-associated bacterial consortia on water quality, growth performance, survival, as well as muscle proximate composition of *Catla catla* fingerlings. These 50 days investigation was carried out in the Zoology Department of Shri Shivaji College, Parbhani, situated in Marathwada region of Maharashtra, India, using control tanks fed with conventional feed and biofloc tanks supplemented with biofloc containing heterotrophic bacterial consortia. Bacterial isolates were obtained from biofloc samples and identified up to species level as through 16S rRNA gene sequencing and constructed phylogeny revealed DPBF16 showed maximum similarity with *Nitrosomonas oligotropha* BKP NS27, DPBF19 with *Lactobacillus plantarum* strain, DPBF21 with *Nitrobacter vulgaris* SGUV-3, DPBF23 with *Bacillus cereus* BF2 and DPBF27 showed maximum similarity with *Pseudomonas aeruginosa* CP1. Submission of obtained sequences to NCBI GeneBank were carried out and got the accession numbers as DPBF16: PX875990, DPBF19: PX 875986, DPBF21: PX875988, DPBF23: PX875983 and DPBF27: PX875991 from the NCBI GenBank.

Comparatively with traditional method, *Catla catla* in the aquaculture enabled with BFT exhibited significantly enhanced growth performance, as evidenced by increased final body weight, specific growth rate, better feed conversion ratio, also increased survival rate compared to the control group. The BFT also succeed in stabilization of water quality parameters by facilitating efficient nitrogen assimilation and reduction of toxic metabolites. Proximate composition analysis of fish muscle revealed higher crude protein (18.9%) and crude lipid (3.0%) contents in biofloc-reared fish compared to the control group (15.3% and 2.5%, respectively), while moisture and ash contents remained comparable. The improved nutritional profile is attributed to the multifunctional roles of biofloc-associated bacteria, including microbial biomass development by conversion of organic waste, provision of SCP: single-cell protein, enhanced nutrient bioavailability, and detoxification of the rearing environment.

Overall, the findings demonstrate that biofloc-based bacterial consortia significantly improve growth performance and fish flesh quality in *Catla catla*, highlighting BFT as an eco-friendly and nutritionally efficient strategy for sustainable freshwater aquaculture.

1. Topic Introduction

To fulfill the increasing global need for animal protein, aquaculture is playing a significant role; however, conventional intensive culture systems often face challenges related to poor water quality, inefficient feed utilization, and environmental pollution due to nitrogenous waste accumulation (Datta, 2012). Indian major carps, particularly *Catla catla*, contribute

significantly to freshwater aquaculture production, yet their culture efficiency remains constrained by reliance on commercial feeds and frequent water exchange (FAO, 2022).

As Abakari (2021) and Salati (2026) mentioned about the era of sustainable aquaculture practices, the BFT promotes in situ nutrient recycling through microbial manipulation by maintaining an appropriate ratio of carbon: nitrogen (C: N). In biofloc systems, organic carbon supplementation promotes the heterotrophic bacterial proliferation, which assimilate N_2 into biomass in the form of microorganisms, thereby reducing toxic ammonia and nitrite concentrations (Raza et al., 2024).

The resulting biofloc comprise dense aggregates of bacteria, organic matter, and extracellular polymers, which can be directly consumed by fish. Bacterial consortia within biofloc systems typically include heterotrophic bacteria such as *Bacillus* and *Pseudomonas*, nitrifying bacteria including *Nitrosomonas* and *Nitrobacter*, and probiotic bacteria such as *Lactobacillus* spp. (Robles-Porchas et al., 2020). Sharif et al., (2021) reported that these bacteria perform complementary functions, including nitrogen transformation, detoxification of harmful metabolites, enhancement of nutrient availability, and provision of protein in the form of microbial single cells enrich with vital amino acids, vitamins, and minerals.

Although biofloc systems have been extensively studied in shrimp and tilapia culture, limited information is available on the role of bacterial consortia in improving growth performance and fish quality of *Catla catla*, particularly with molecular-level identification of biofloc-associated bacteria (Huang et al., 2023). Therefore, this investigation aims to assess the impacts of biofloc bacterial consortia on performance of growth as well as *C. catla* fingerlings nutritional quality, supported by isolation and species-level identification of dominant biofloc bacteria using 16S rRNA gene sequencing.

Previous studies have demonstrated that biofloc consumption improves growth performance, feed conversion efficiency, and immune responses in several fish species (Gustilatov et al., 2026). However, systematic investigations focusing on bacterial consortia role in *Catla catla* fingerlings growth and fish quality of remain limited.

2. Materials and Methods

2.1 Experimental Site

The experiment was executed in the Shri Shivaji College's Zoology departmental laboratories situated in Parbhani, Maharashtra, India.

2.2 Experimental Design

The investigation is distributed into two experimental groups:

Fish A. Control group: Where conventional commercial feed used.

B. Biofloc group: fed with commercial feed supplemented with biofloc containing bacterial consortia.

20 *Catla catla* fingerlings were introduced in each tank and each treatment was maintained in replicated tanks under identical environmental conditions for 50 Days.

2.3 Experimental Animals

Healthy fingerlings of *Catla catla* of uniform size were procured from regional Govt. Hatchery Center and acclimatized prior experimentation. Fish stocked at a uniform density in all experimental tanks.

2.4 Development of Biofloc System and its Management

By adding an external carbon source to maintain the desired C:N ratio (typically 12–15:1), the biofloc system was produced to (Xu et al., 2016 and Panigrahi et al., 2018). Continuous aeration was provided to keep flocs in suspension. Biofloc development was monitored visually and through floc volume index (FVI) measurements (Shamsuddin et al., 2022).

2.5 Feeding Regime

In the test (Biofloc treatment) as well as control tanks, *Catla catla* fingerlings fed a commercial pelleted diet formulated for carp culture. The food was provided at a fixed percentage of the total biomass per day, divided into two equal meals administered in the morning and evening. Fish biomass was periodically assessed to adjust the feeding rate in the period of experimentation. In the control group, fish depended exclusively on the supplied commercial feed. In contrast, fish reared under biofloc conditions received the same quantity of commercial feed but additionally consumed bio-floc particles naturally present in the culture water. The biofloc biomass served as a supplementary nutritional source containing bacterial protein and associated nutrients. Continuous aeration ensured uniform suspension and availability of biofloc particles in the water column. Feed consumption and fish behavior were monitored daily to ensure effective feeding and to avoid excessive feed wastage. This feeding strategy enabled a direct comparison between conventional feeding and biofloc-supplemented culture conditions.

2.6 Isolation and Identification of Biofloc Bacteria

2.6.1 Bacterial isolation

Biofloc samples were collected aseptically from experimental tanks during the active floc development phase. Homogenized samples were proceeded for serial dilution using presterilized saline solution containing 0.85% Sodium Chloride followed by plating on selective and

non-selective media to isolate dominant bacterial populations. Nutrient agar media plates (Nutrient agar supplemented with growth factors eg. Ammonia and mineral salts for nitrifying bacteria) were used for bacterial isolation using spread plate technique for maximum bacterial isolation. Inoculated petripates were placed in incubator maintained at 28–30°C for 24–48 hours, and morphologically distinct colonies were repeatedly subcultured to obtain pure isolates.

2.6.2 Extraction of Genomic DNA and Amplification of 16S rRNA gene

Pure bacterial isolates were cultured in nutrient broth, and standard phenol–chloroform extraction method as well as a commercial bacterial DNA extraction kit (HiMedia India Pvt. Ltd) was implemented for genomic DNA was extraction. For amplification of the 16S rRNA gene, universal bacterial primers namely: 27F: 5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R: 5′-TACGGYTACCTTGTTACGACTT-3′ were implemented and amplification were performed as per Acharya et al., (2025).

2.6.4 Sequencing and Phylogenetic Analysis

Sanger sequencing of the purified PCR products was performed using both reverse and forward primers. For preliminary isolate identification, similar search of obtained gene sequences was proceeded with the help of BLASTn algorithm of NCBI GenBank database for.

Phylogeny was constructed and analyzed by aligning sequences with closely related reference sequences with the help of Clustal-ω, and phylogeny was constructed using the Neighbor-Joining method in MEGA11 software. Bacterial isolates were identified up to species level based on ≥97% sequence similarity.

The accession numbers were obtained on behalf of submitter 16S rRNA gene sequences to the NCBI GenBank database.

2.7 Water Quality Analysis

According to water quality parameters monitored APHA (2017) standard protocols:

Catla catla fingerlings growth ratio was examined by measuring changes in body weight at regular intervals throughout the investigation duration of 50 Days. Specimens from every tank were sampled carefully to minimize handling stress, blotted dry, and weighed using a digital balance with appropriate precision. Initial and final body weights were recorded to assess overall growth response under control and biofloc conditions.

The following growth performance indices were calculated using standard equations commonly employed in aquaculture studies:

2.8 Growth Performance Parameters

Growth indices were calculated using standard equations:

2.8.1 Weight Gain (WG)

Changes in the weight were estimated by eliminating the mean values (initial from final) in each treatment group:

$$WG\ (g) = W_f - W_i$$

Where W_f: final value

W_i: initial value

2.8.2 Specific Growth Rate (SGR)

For estimating daily growth efficiency and calculating using the natural logarithm of body weight:

$$SGR\ (\%\ day^{-1}) = \frac{\ln W_f - \ln W_i}{t} \times 100$$

Where t is the experimental period in days.

2.8.3 Feed Conversion Ratio (FCR)

It is estimated to assess food utilization efficiency:

$$FCR = \frac{Total\ feed\ consumed\ (g)}{Total\ weight\ gain\ (g)}$$

Lower FCR values indicated better food efficiency.

2.8.4 Survival Rate (SR)

At the final stage of the investigation SR calculated to assess overall health and adaptability of fish under different culture conditions:

$$Survival\ (\%) = \frac{Number\ of\ fish\ harvested}{Number\ of\ fish\ stocked} \times$$

2.9 Analysis of Proximate Composition

At terminal experimental period, *C. catla* samples out of every treatment group was randomly caught to analyze the proximate composition. Fishes then euthanized following standard ethical procedures, and muscle tissues were carefully dissected, washed with distilled water, and kept in deep fridge by maintaining −20 °C temperature until examination.

2.9.1 Moisture data

Moisture in fish muscle was calculated with the help of oven-drying method. A known mass of homogenized muscle part was exposed to heat (105°C) using a hot-air oven till a stable weight was noted. Moisture% was estimated by correlating the initial and final mass values.

2.9.2 Estimation of Protein (Crude)

Calculation of crude protein content using the Kjeldahl procedure, following A.O.A.C. (2019) guidelines. Total N of the muscle sample was calculated through acid digestion, distillation, and titration. Crude protein % was determined by formula:

$$Crude\ protein\ (\%) = Total\ nitrogen\ (\%)\ x\ 6.25$$

2.9.3 Estimation of Lipid (Crude)

Soxhlet extraction method was equipped for crude lipid content estimation. Dried muscle samples were extracted with petroleum ether as the solvent for a specified duration. Lipid content was calculated as % extracted fat with respect to the dry weight.

2.9.4 Total Ash Content

Incineration method used for estimating the ash content in which dried muscle sample was incinerated at 550 °C in muffle furnace until a constant weight was obtained. Ash% was calculated based on the residual inorganic matter.

3. Observations and Results

3.1 Site of Investigation

All the research was performed at research laboratory in zoology department situated in Shri Shivaji Mahavidhyalaya, Parbhani, Maharashtra, India.

Figure 1. Experimental site

3.2 Experimental design and Culture conditions

Two tanks were dedicated for the experimentation, one for Biofloc system and another one for conventional commercial feed.

3.3 Biofloc Development and Water Quality

Biofloc Development

Visible biofloc formation was observed within the initial phase of the experimental period following carbon source supplementation and continuous aeration. The biofloc volume gradually increased and stabilized as the culture progressed, indicating successful establishment of heterotrophic bacterial communities. Floc particles appeared as suspended aggregates with brownish coloration, suggesting active microbial growth and organic matter assimilation.

The stabilization of biofloc volume reflected perfect transformation of dissolved nitrogenous material to biomass in the form of microorganisms. No excessive sludge accumulation was observed, indicating proper system management and aeration efficiency.

Water Quality Parameters

It maintained at the optimum range for *Catla catla* fingerlings throughout the experimental

period. In comparative study, the biofloc system showed improved nitrogen dynamics. Total ammonia nitrogen (TAN) concentrations were consistently decreased in the biofloc tank, indicating effective inorganic nitrogen assimilation with the help of heterotrophic and nitrifying microbes (Table 1).

Nitrite levels remained low, suggesting stable nitrification activity, while nitrate accumulation was gradual and non-toxic. Dissolved oxygen concentrations were maintained at optimal levels due to continuous aeration, supporting both fish metabolism and microbial activity. Biofloc system's constant pH indicates buffering capacity of microbial processes (Table 2).

Table 1. Biofloc development indicators

Parameter	Observed range
Biofloc appearance	Brownish, suspended aggregates
Floc size	100–500 µm
Biofloc volume (mL L^{-1})	10–30
Total suspended solids (mg L^{-1})	300–600
Settling ability	Moderate to good

Table 2. Water quality parameters under biofloc system

Parameter	Control	Biofloc
Temperature (°C)	28 ± 2.5	28 ± 2.5
pH	7.2 ± 0.4	7.2 ± 0.5
Dissolved oxygen (mg L^{-1})	5.0 ± 1.0	5.5 ± 1.8
TAN (mg L^{-1})	1.1 ± 0.5	0.5 ± 0.2
NO_2 (mg L^{-1})	0.4–0.2	0.12 ± 0.06
Nitrate (mg L^{-1})	17 ± 7	30 ± 10

3.4 Feeding Regime

Feeding regime is segregated into two groups: A. Conventional commercial fish food and B. Biofloc System as shown in Figure 2.

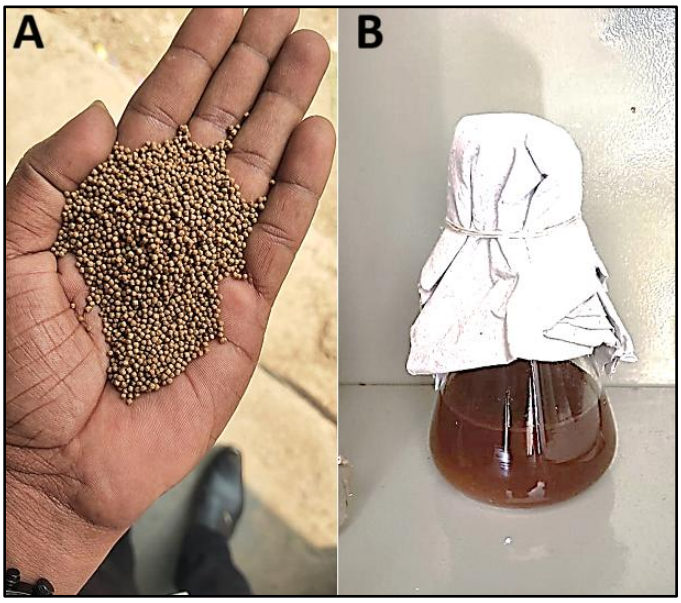

Figure 2. Feeding regime (: A. Conventional commercial fish food and B. Biofloc System)

3.5 Isolation and Identification of Bacteria from Biofloc

Bacterial consortium of about 31 colonies was developed on the nutrient agar plate denoted as DPBF01 to DPBF31 as shown in. All the isolates were divided into 5 bacterial groups and were on the basis of morphological and microscopic characters as shown in Table 3. The 5 isolates (DPBF16, DPBF19, DPBF21, DPBF23 and DPBF27) from each group were proceed for biochemical characterization and were identified as DPBF16: *Nitrosomonas* sp., DPBF19: *Lactobacillus* sp., DPBF21: *Nitrobacter* sp., DPBF23: *Bacillus* sp. and DPBF27: *Pseudomonas* sp.

Table 3. Morphological and Microscopic features of Isolates

Sr. No.	Isolate	Morphological Features								
		Size	Shape	Colour	Margin	Elevation	Opacity	Consistency	Grams's Nature	Motility
1.	DPBF16	Pinpoint	Round	Brown	Entire	Convex	Translucent	Sticky	Negative Rods	No
2.	DPBF19	3mm	Round	Off white	Entire	Convex	Opaque	Mucoid	Positive Rods	No
3.	DPBF21	0.5mm	Round	Brown	Entire	Convex	Opaque	Mucoid	Negative Rods	No
4.	DPBF23	4mm	Oval	White	Entire	Convex	Opaque	Sticky	Positive Rods	Yes
5.	DPBF27	4mm	Round	Pale	Irregular	Elevated	Translucent	Mucoid	Negative Rods	Yes

On the basis of phylogenetic tree analysis, 16S rRNA gene sequence of DPBF16 isolate showed maximum matching ratio with *Nitrosomonas oligotropha* BKP NS27, DPBF19 showed maximum similarity with

Lactobacillus plantarum strain, DPBF21 with *Nitrobacter vulgaris* SGUV-3, DPBF23 with *Bacillus cereus* BF2 and DPBF27 showed maximum similarity with *Pseudomonas aeruginosa* CP1 (Figure 3). Accession numbers of strains DPBF16: PX875990, DPBF19: PX 875986, DPBF21: PX875988, DPBF23: PX875983 and DPBF27: PX875991 were obtained from the NCBI GeneBank as following the deposition of 16S rRNA gene sequences in FASTA format.

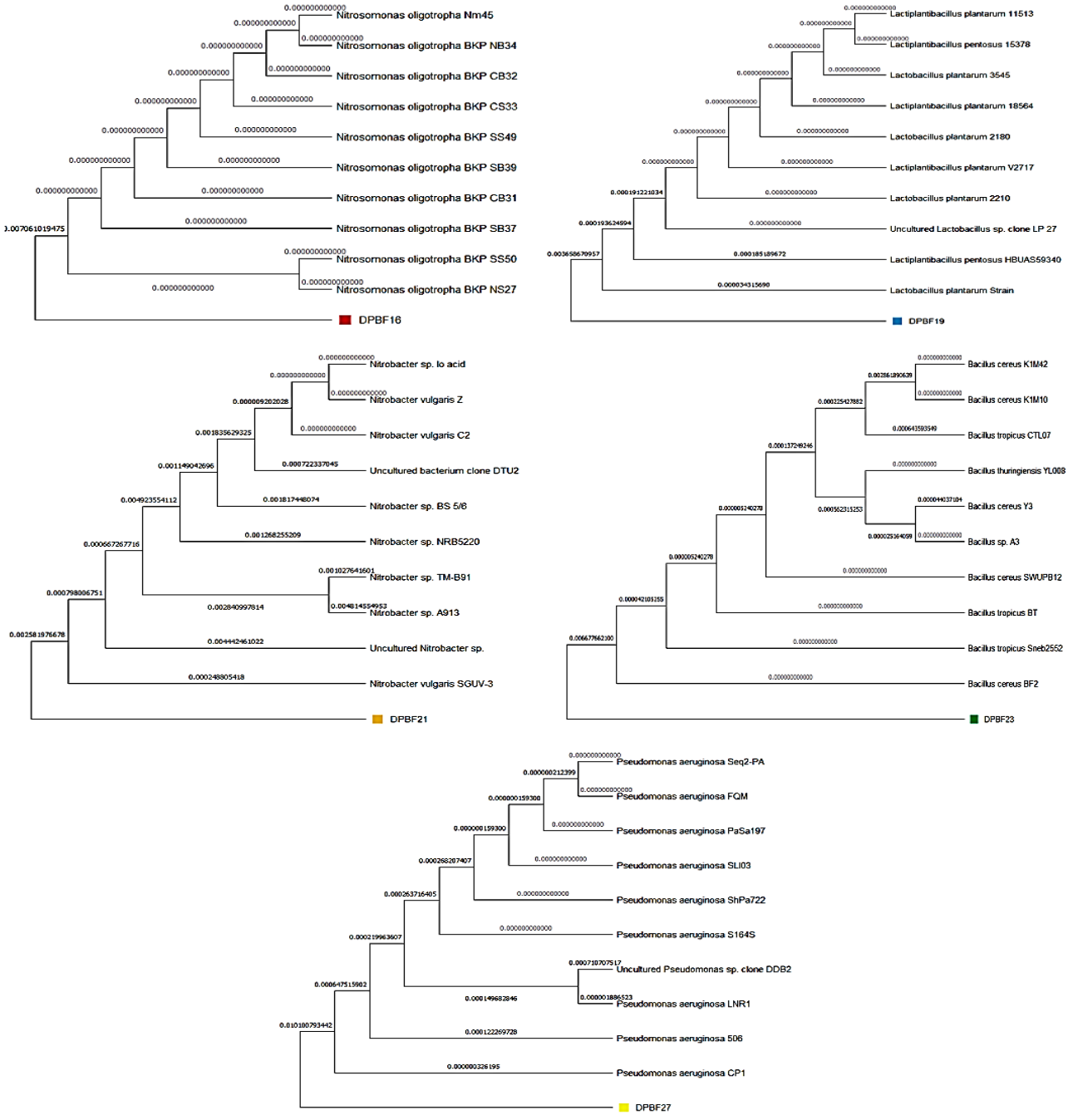

Figure 3. Phylogeny of the DPBF16, DPBF19, DPBF21, DPBF23 and DPBF27 strains constructed using neighbor joining method.

3.7 Growth Performance of *Catla catla* Fingerlings

Fish reared in the BFT tank proved enhanced growth performance while the low growth in another group (Figure 4). Total 20 fingerlings were introduced to each tank of each group for 50 days. Initial weight of fish for each group was 02 ± 0.5 gms. After completion of the experiments, in control group, final weight was 42 ± 03 while in Biofloc group it was 57 ± 04 gms. Natural logarithm control group calculated are $ln W_f$= ln(10.0)=2.3026, $ln W_i$=ln(2.0)=0.6931 and for Biofloc group is $ln W_f$= ln(18)=2.8904, $ln W_i$=ln(2.0)= 0.6931. Food consumption for control group is 14 and for Biofloc group is 19 (Table 3).

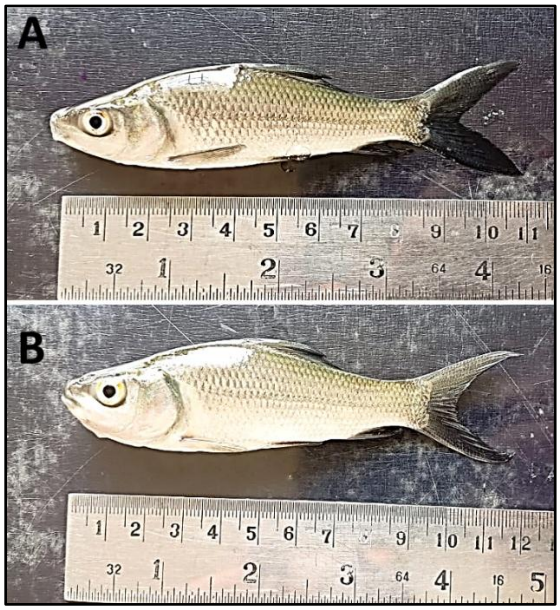

Figure 4. Comparative analysis of fish growth (A: Control Group, B: Biofloc Group)

Table 3. Growth performance parameters of *Catla catla* fingerlings in 50 Days

Sr. No.	Parameter	Control	Biofloc
1.	Initial number of fish	20	20
2.	Final number of fish	14	16
3.	Survival rate (%)	70	80
4.	Initial mass (g)	2.0 ± 0.5	2.0 ± 0.5

5.	Final mass (g)	10.0 ± 3.5	18.0 ± 4.0
6.	Weight gain (g)	8.0	16.0
7.	Feed consumed (g)	14	19
8.	Feed conversion ratio (FCR)	1.75	1.19
9.	Specific growth rate (SGR, % day^{-1})	3.22	4.39

3.8 Proximate Composition of Fish Muscle

Table 4. Moisture content of fish muscle

Group	Wet weight of sample (g)	Dry weight after oven drying (g)	Moisture Content (%)
Control	10.0 ± 3.5	2.6 ± 0.3	76%
Biofloc	18.0 ± 4.0	5.1 ± 0.7	75%

Table 5. Crude protein content of *Catla catla* muscle (Kjeldahl method)

Group	Total nitrogen (%)	Crude protein (%)
Control	2.45%	15.31%
Biofloc	3.03%	18.94%

Table 6. Raw data for crude lipid estimation (Soxhlet extraction)

Group	Dry weight of sample (g)	Weight of Empty flask (g)	Weight of Flask + lipid (g)	Crude Lipid (%)
Control	2.00gm	50.00gm	50.05gm	2.5
Biofloc	2.00gm	50.00gm	50.06gm	3.0

Table 7. Raw data for ash content estimation

Group	Dry weight of sample (g)	Weight of Ash residue (g)	Ash (%)
Control	2.00	0.0278	1.39
Biofloc	2.00	0.0306	1.53

Table 8. Proximate composition of *Catla catla* muscle

Parameter (%)	Control	Biofloc

Moisture	76.0	75.0
Crude protein	15.3	18.9
Crude lipid	2.6	2.9
Ash	1.5	1.6

4. Discussion

The present study demonstrates that biofloc bacterial consortia positively influence growth performance and fish quality of *Catla catla* fingerlings. Improved growth noted in biofloc tank reveals enhanced nutrition recycling, availability of microbial protein, and improved water quality, as reported earlier by Kumar et al., (2018) and Liu et al. (2019).

Heterotrophic bacteria like *Bacillus* spp. rapidly assimilate nitrogenous wastes into microbial biomass, reducing ammonia toxicity and providing a continuous protein source to fish. Nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) are essential in keeping nitrogen balance, while probiotic bacteria (*Lactobacillus* spp.) enhance gut health and digestive efficiency (Robles-Porchas et al., 2020, Qiu et al., 2023, Chattaraj et al., 2024 and Mang et al, 2024).

Enhanced biomolecule content in fish muscle under biofloc conditions further supports the nutritional benefits of bacterial biomass consumption (Klanian et al., 2020). Similar improvements in proximate composition have been reported in carp and tilapia cultured in biofloc systems (Minabi et al., 2020 and Zablon et al., 2022).

5. Conclusion

Study highlights effectiveness of biofloc bacterial consortia in improving growth-performance and quality of *Catla catla* fingerlings. BFT provides a sustainable, cost-effective alternative to conventional aquaculture by enhancing nutrient utilization, reducing environmental pollution, and improving fish nutritional value. Adoption of bacterial consortia–based biofloc technology can significantly contribute to sustainable carp aquaculture.

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