



Enhancing the growth of Gulsha Tengra (*Mystus cavasius*) in biofloc technology systems: Roles of probiotic supplementation, feeding rate and aeration

Anand Basumatary^{1*}

Anuck Islary²

^{1,2}Department of Food Engineering and Technology, Central Institute of Technology Kokrajhar, Kokrajhar 783370, Assam, India.

¹Email: ph20fet1004@cit.ac.in

²Email: a.islary@cit.ac.in



(+ Corresponding author)

ABSTRACT

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The purpose of this study was to investigate the growth performance of Gulsha Tengra (*Mystus cavasius*), a nutritionally and economically important freshwater fish native to South Asia, for sustainable culture under biofloc technology (BFT). A five-month controlled experimental study was conducted using biofloc-treated culture tanks in which fish were reared under different management conditions designed to simulate practical aquaculture environments. Key water quality parameters, including temperature, pH, dissolved oxygen (DO), nitrite, nitrate, and total ammonia nitrogen (TAN), were carefully monitored and maintained within predetermined ranges, while growth performance was assessed biweekly through measurements of body weight, length increment, specific growth rate, and survival. Periodic water quality analyses were also performed to ensure environmental stability throughout the culture period. The findings demonstrated that *Mystus cavasius* achieved significantly improved growth performance in probiotic-treated BFT systems with appropriate feeding rates and aeration, showing higher body weight gain, greater length increment, enhanced specific growth rate, and improved survival under optimal conditions of temperature (27–31 °C), pH (7.0–8.4), and dissolved oxygen (6.5–8.5 mg/L). In contrast, deviations from these optimal environmental conditions, particularly increases in nitrite, nitrate, and TAN concentrations, resulted in reduced growth performance and higher mortality, highlighting the species' sensitivity to changes in water quality. These findings provide practical guidance for aquaculture practitioners by demonstrating that maintaining suitable water quality through effective probiotic application, balanced feeding strategies, and adequate aeration can substantially improve fish health, growth, and production efficiency, thereby supporting the sustainable and profitable culture of *Mystus cavasius* under a BFT system.

Contribution/Originality: This study contributes to the existing literature by identifying optimal BFT conditions for *Mystus cavasius*. It uses a controlled experimental approach to evaluate physicochemical water-quality parameters through the combined assessment of probiotic application, feeding rate, and aeration. It provides practical recommendations for sustainable BFT aquaculture.

1. INTRODUCTION

Gulsha Tengra (*Mystus cavasius*) is a freshwater catfish species belonging to the family Bagridae. It is considered one of the most valuable small indigenous species because of its wide distribution across freshwater ecosystems, including canals, ditches, lakes, ponds, and floodplains (Rahman, Rahman, Khan, & Hussain, 2004). Gulsha Tengra is widely known in India, Bangladesh, Indochina, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, and Thailand for its

high nutritional value, including fat, micronutrients, minerals, protein, and vitamins (Gupta, 2014; Roos, Islam, & Thilsted, 2003).

Biofloc technology (BFT) is an innovative and sustainable approach that has emerged to improve the environmental performance of aquaculture production systems. Designed to provide both ecological and economic benefits, BFT minimizes water consumption and wastewater discharge, reduces dependence on commercial feed, and enhances biosecurity within culture systems (Avnimelech, 2007; Wasielesky, Atwood, Stokes, & Browdy, 2006). In addition, effective management of microbial communities in BFT systems plays a crucial role in maintaining water quality by recycling nutrients and naturally suppressing pathogenic microorganisms, thereby reducing the need for harmful chemicals and antimicrobial agents (Bentzon-Tilia, Sonnenschein, & Gram, 2016; Prindle et al., 2012). In addition, the application of probiotics to modify the microbiota has been shown to improve the health of aquaculture species and water quality (Pandiyan et al., 2013).

Biofloc is characterized as a complex assembly of microorganisms, detritus, algae, and other decomposed materials, encompassing bacteria, diatoms, feces, macroalgae, protozoa, uneaten feed, zooplankton, and exoskeletons from extinct species (Decamp, Moriarty, & Lavens, 2008; Hargreaves, 2006). The formation of microbial cell-matrix flocs occurs through sophisticated flocculation processes driven by a synergy of biological, chemical, and physical factors (De Schryver, Crab, Defoirdt, Boon, & Verstraete, 2008). This process highlights the significance of extracellular polymeric substances in binding biofloc components, thereby facilitating optimal conditions for the development of microbial biomass with slow-sinking properties beneficial for juvenile fish (Sears, Alleman, Barnard, & Oleszkiewicz, 2006).

Probiotics used in aquaculture may lead to a breakthrough because of their potency as nutritional supplements, their enhancement of beneficial microbiota that maintain intestinal and liver health, their efficiency in improving feed intake, their acceleration of growth performance, their ability to balance water-quality parameters, and their strengthening of the immune system to combat pathogens, as reported by El-Saadony et al. (2021); Hai (2015); and Ringø (2020). Sohel et al. (2023) evaluated a multispecies probiotic in a biofloc-based culture system for the Asian stinging catfish, *Heteropneustes fossilis*. The probiotic-enriched biofloc environment supported better growth, haematological condition, intestinal structure, and overall gut health than conventional culture conditions. These findings indicate that probiotics may complement the naturally occurring microbial community of biofloc and create a healthier culture environment for indigenous catfish. Similarly, Kumar et al. (2023) reported that the use of microbial inoculums in a BFT system improved the growth and health of *Heteropneustes fossilis*. The inoculated microbial community helped recycle nutrients, supported beneficial microbial activity, and contributed to improved physiological performance. Gaffar et al. (2023) found that dietary probiotics positively influenced the growth, survival, and intestinal and liver structure of *Mystus cavasius*. Although the experiment was not focused exclusively on the BFT system, its findings provide a strong scientific basis for testing probiotics in a biofloc-based production system for this species. More recently, studies on symbiotic supplementation in *Mystus cavasius* have shown improvements in immune response and disease resistance, further indicating that beneficial microorganisms and supportive prebiotic substrates could be incorporated into biofloc systems to reduce dependence on antibiotics. Majhi et al. (2023) demonstrated that stocking density strongly influences stress, immunity, growth, and welfare in butter catfish, *Ompok bimaculatus*, reared under biofloc conditions. Behera et al. (2025) further showed that the type of carbon source used in BFT systems affects growth and digestive-enzyme activity in *Ompok bimaculatus*. These findings are important because the effectiveness of an added probiotic may depend not only on its bacterial composition and dose but also on the carbon source, carbon-to-nitrogen ratio, stocking density, feeding level, aeration, and existing microbial community.

Hung, Tuan, and Lazard (2001) claimed that feed intake, feeding type, feeding frequency, and nutrient absorption capacity all play major roles in controlling fish growth at different stages and survival rates. Achieving a balance between rapid fish development and making the best use of the provided feed is a significant challenge in fish culture.

BFT has emerged as an important intensive aquaculture method because it converts dissolved nitrogenous waste and residual organic matter into microbial aggregates. These aggregates contain bacteria, microalgae, protozoans, detritus, and other microorganisms that can be consumed by cultured fish. Consequently, fish reared in a mature BFT system obtain nutrients from both formulated feed and naturally produced microbial biomass. This supplementary food contribution may improve feed conversion and reduce dependence on commercial diets, although biofloc should not be treated as a complete replacement for nutritionally balanced feed (Yu et al., 2023).

Feeding rate is particularly important in BFT systems because excessive feed input increases the amount of nitrogen and organic matter entering the water. Uneaten feed and faecal material stimulate microbial respiration, increase oxygen consumption, and may cause the accumulation of suspended solids. In contrast, severe feed restriction can reduce fish growth if the nutritional contribution of the biofloc is insufficient. Feeding rates must therefore be adjusted according to fish size, standing biomass, water quality, floc availability, and feeding behaviour, rather than maintaining one fixed rate throughout the production cycle (Raza, Zheng, & Yang, 2024; Yu et al., 2023).

Saha, Hossain, Mamun, Islam, and Alam (2022) investigated the effects of manipulating the carbon-to-nitrogen ratio on the performance of *Heteropneustes fossilis* reared in a BFT system. The authors reported that suitable carbon-to-nitrogen ratios improved the specific growth rate, protein-efficiency ratio, and feed-conversion efficiency. Carbon-to-nitrogen ratios of approximately 15:1–21:1 produced favourable growth and feed-utilization responses. These findings indicate that effective nutrient recycling and stable microbial floc formation can improve the utilization of formulated feed by stinging catfish. More recent research using jaggery as the carbon source showed that the carbon-to-nitrogen ratio significantly affected water quality, growth, feed efficiency, microbial abundance, and the physiological condition of *Heteropneustes fossilis*. Lower or moderate ratios promoted beneficial microbial communities and improved the nutritional value of the biofloc, whereas excessively high ratios adversely affected intestinal and liver condition. This indicates that merely adding more carbon or producing a greater volume of floc does not necessarily justify reducing formulated feed. Feed reduction should be based on fish growth, floc quality, and health indicators rather than floc volume alone (Mali, Ghosh, Dash, & Chowdhury, 2024).

A practical production study by Sasmal, Das, and Roy (2024) evaluated the culture of Singhi, *Heteropneustes fossilis*, in 10,000-L freshwater BFT tanks. Advanced fry, with an initial average weight of approximately 1.3 g, were stocked at 1,500 fish per tank and fed a floating diet containing 40–45% crude protein twice daily, according to a standard feeding chart. Molasses was applied to maintain microbial growth and the desired carbon-to-nitrogen ratio. The study recorded approximately 92% survival, an average final weight of around 154 g, and a feed-conversion ratio close to 1.3, showing that twice-daily feeding can support satisfactory production in a properly managed Singhi BFT system.

Although the study by Sasmal et al. (2024) provides valuable farm-level information, it did not clearly report the daily ration as a percentage of standing fish biomass. Therefore, the study supports the use of a protein-rich floating feed and twice-daily feeding but cannot be used to establish a precise optimum ration, such as 2%, 3%, or 4% of biomass per day. This limitation demonstrates the need for controlled experiments comparing graded feeding levels in indigenous catfish BFT systems. Majhi et al. (2023) studied the seed rearing of butter catfish, *Ompok bimaculatus*, under different stocking densities in a BFT system. The lower-density treatments produced better individual growth performance and altered the economic returns. The results demonstrate that the quantity of feed offered must be calculated from the actual standing biomass while also considering competition for food, dissolved oxygen availability, and the capacity of the BFT system to process the resulting organic load. Raza et al. (2024) and Yu et al. (2023) indicate that feed reduction should not begin immediately after fish are stocked into a newly prepared BFT tank. During the initial culture period, the microbial community may not be sufficiently developed to provide a significant supplementary food source. The full calculated ration should generally be supplied until floc formation becomes stable. After the fish are observed actively consuming biofloc and acceptable growth is confirmed, formulated feed may be reduced gradually by approximately 5–10% at each adjustment, with a possible overall reduction of around

10–25% compared with the conventional ration. Any reduction should be reversed if growth, condition, or feeding response declines.

Raza et al. (2024) and Yu et al. (2023) note that water-quality monitoring must accompany feeding management because feed input is the principal source of nitrogen and organic matter in an intensive BFT system. Dissolved oxygen, pH, temperature, total ammonia nitrogen, nitrite, alkalinity, floc volume, and total suspended solids should be monitored regularly. High feed input combined with adequate aeration can increase microbial oxygen consumption, whereas excessive microbial activity can reduce alkalinity and pH. Feed should therefore be temporarily reduced when dissolved oxygen becomes low, ammonia or nitrite increases, or suspended solids exceed manageable levels. Sasmal et al. (2024) claimed that floating feed is generally useful for indigenous catfish BFT culture because it allows the farmer to observe appetite and identify uneaten feed. The diet should contain adequate protein and energy for the relevant life stage. However, the most suitable protein concentration may decrease as the fish become larger, provided that essential amino acids and dietary energy remain adequate.

Overall, the available literature indicates that biofloc can improve growth and feed utilization in indigenous catfish, particularly when the carbon-to-nitrogen ratio, aeration, stocking density, and suspended solids are properly managed. However, there is currently insufficient direct evidence to recommend one universal optimum feeding rate for *Heteropneustes fossilis*, *Ompok bimaculatus*, *Clarias batrachus*, or *Mystus cavasius*. Feeding should consequently be adjusted according to life stage, fish biomass, appetite, floc maturity, and water quality rather than following a rigid feeding chart (Majhi et al., 2023; Saha et al., 2022; Sasmal et al., 2024).

Aeration is considered one of the most critical management components in a BFT system because the entire system depends on continuous oxygen availability for fish, heterotrophic bacteria, nitrifying microorganisms, and the suspension of biofloc particles. Unlike conventional pond culture, BFT systems require vigorous and uninterrupted aeration to maintain dissolved oxygen, prevent floc sedimentation, and facilitate the microbial degradation of organic wastes. Insufficient aeration results in oxygen depletion, ammonia accumulation, the collapse of microbial communities, and deterioration of fish health. Therefore, aeration directly determines the biological stability and productivity of BFT systems rather than serving only as a source of dissolved oxygen (Raza et al., 2024; Yu et al., 2023).

Kumar et al. (2023) evaluated microbial inoculum-assisted biofloc production in Asian stinging catfish cultured in indoor fiberglass tanks. Continuous aeration was maintained throughout the 91-day experiment to keep bioflocs suspended and provide sufficient oxygen for microbial metabolism. Fish cultured under properly aerated BFT conditions exhibited significantly higher weight gain, an improved feed-conversion ratio, enhanced digestive enzyme activity, better antioxidant responses, and stronger immune function than fish maintained in conventional clear-water culture. These findings demonstrate that efficient aeration is indispensable for sustaining both microbial activity and fish performance in BFT systems.

A recent investigation by Mali et al. (2024) on *Heteropneustes fossilis* cultured under different carbon-to-nitrogen ratios further highlighted the importance of adequate aeration. Although the primary objective was to optimize the carbon source, continuous aeration was maintained throughout the experiment to ensure homogeneous suspension of bioflocs and sufficient oxygen availability. The authors reported that stable aeration, together with an optimum carbon ratio of approximately 12:1, improved water quality, growth performance, feed efficiency, and fish welfare. Conversely, excessive organic loading at higher carbon ratios increased suspended solids, emphasizing that aeration capacity must increase proportionally with biofloc biomass. Another recent study by Banu, Swain, Das, Velmani, and Kumari (2024) evaluated a freshwater biofloc-based polyculture involving *Heteropneustes fossilis*, *Pangasianodon hypophthalmus*, and *Puntius gonionotus*. Continuous aeration maintained biofloc suspension and favorable microbial development throughout the experiment. The biofloc treatment significantly improved growth performance, digestive enzyme activities, gut microbial diversity, and intestinal histology compared with conventional culture. The authors noted that proper aeration supported microbial proliferation and maintained water

quality, thereby enhancing nutrient availability for all cultured species. Das, Ferozekhan, Choudhary, Kumari, and Mohanty (2025) investigated intensive fingerling production of *Heteropneustes fossilis* under BFT conditions. Continuous aeration maintained floc volumes of approximately 8.3 mL L⁻¹ while improving survival, biomass yield, feed utilization, and innate immune responses. Fingerlings reared in the BFT system exhibited significantly lower feed conversion ratios, higher digestive enzyme activities, and improved serum immune parameters compared with those in the control system. The authors concluded that maintaining stable aeration was essential for sustaining microbial activity and maximizing the benefits of intensive biofloc production.

In BFT systems, maintaining high levels of dissolved oxygen and preventing the settling of solids necessitate vigorous mixing. This turbulence helps prevent anoxic dead zones, which could prove fatal to the organisms being cultivated. Avoiding the accumulation of solid particles in stagnant areas requires a carefully designed configuration of aerators that should be periodically adjusted. BFT systems may require up to 6 mg L⁻¹ h⁻¹ of oxygen, corresponding to roughly 30 hp of aerator capacity per acre of pond area. It is important to maintain higher oxygen concentrations for two reasons: first, cultured fish and other planktonic species need oxygen to breathe; second, the bacterial population needs it to break down wastes and continue to grow, as reported by Ogello, Outa, Obiero, Kyule, and Munguti (2021).

From a practical perspective, aeration requirements increase as stocking density, feeding rate, and microbial biomass increase. Farmers should continuously monitor dissolved oxygen, fish behavior, floc suspension, and water circulation. Surface gasping, reduced feeding activity, excessive floc settlement, or foul odors may indicate inadequate aeration. Additional air blowers or diffusers should be installed before increasing stocking density or feed input. Backup electrical power is equally important because even short interruptions in aeration may rapidly reduce dissolved oxygen and destabilize the microbial community in intensive BFT systems.

Overall, recent studies consistently demonstrate that successful biofloc culture of indigenous catfish depends on uninterrupted and efficient aeration. Adequate aeration supports microbial nitrification, maintains suspended bioflocs, enhances nutrient recycling, improves feed utilization, promotes digestive enzyme activity, strengthens immune responses, and sustains high-density fish production.

Although BFT has been widely recognized as an environmentally sustainable aquaculture system, information on the influence of water quality dynamics on the culture performance of indigenous freshwater catfishes remains limited, particularly under the agro-climatic conditions of Assam, India. *Mystus cavasius*, an economically important indigenous species, requires species-specific management practices to optimize production under BFT conditions. A better understanding of the relationship between water quality parameters and fish growth is therefore essential for improving production efficiency, maintaining fish health, and promoting sustainable aquaculture in the region. The study aims to:

1. Monitor temporal variations in the major physicochemical water-quality parameters, including temperature, pH, dissolved oxygen, total ammonia nitrogen, nitrite, nitrate, alkalinity, and biofloc volume throughout the culture period.
2. Assess the growth performance of *Mystus cavasius* cultured under the BFT system using growth indices such as weight gain, specific growth rate (SGR), feed conversion ratio (FCR), survival rate, and biomass production.
3. Examine the relationships between water-quality parameters and growth performance to identify environmental factors that significantly influence fish growth.
4. Evaluate whether the water quality maintained in the BFT system remained within acceptable limits for the successful culture of *Mystus cavasius* throughout the experimental period.
5. Generate scientific evidence to contribute to developing sustainable biofloc-based culture practices for indigenous catfish species in Northeast India.

To achieve these objectives, the study addressed the following research questions:

- How do the physicochemical characteristics of water change during the culture of *Mystus cavasius* in a BFT system?
- Which water quality parameters exhibit significant relationships with the growth performance of *Mystus cavasius*?
- Does the BFT system provide a stable aquatic environment capable of supporting the optimum growth and survival of *Mystus cavasius* under Assam's climatic conditions?
- Which water quality variables can serve as the most reliable indicators of improved culture performance in BFT systems?

The rest of this paper is organized as follows. Section 2 describes the study area, experimental design, biofloc management practices, water quality monitoring procedures, growth assessment methods, and statistical analyses. Section 3 presents the experimental results on water quality and growth performance and discusses the findings in relation to previous studies and highlights their implications for sustainable biofloc aquaculture. Finally, Section 4 summarizes the major conclusions and provides recommendations for future research and practical applications in indigenous catfish farming.

2. MATERIALS AND METHODS

2.1. Experimental Design

Eight BFT tanks (T1–T8), along with one control tank at the Central Institute of Technology Kokrajhar in Kokrajhar district, Assam, India, were utilized for a five-month study spanning 1 July 2022 to 30 November 2022. The circular BFT tanks, measuring approximately 4 m in width and 1.5 m in height, formed part of this experimental setup. Gulsha Tengra (*Mystus cavasius*) specimens, each measuring approximately 3.77 cm in length and weighing 0.68 g, were stocked. An air pump (SPB-1-80-S) was installed at floor level to provide continuous aeration to the BFT system through a network of air tubes, ensuring adequate dissolved oxygen throughout the culture period. Air was delivered at a flow rate of 17.7 m³ h⁻¹. A uniform stocking density of 1500 fish m⁻³ was maintained in all nine experimental tanks, including the control, to ensure comparable rearing conditions across all treatments.

The experiment was designed to evaluate the individual and combined effects of probiotic concentration, feeding rate, and aeration timing on the growth performance of Gulsha Tengra (*Mystus cavasius*) cultured in a BFT system in Assam, India. A completely randomized 2 × 2 × 2 full factorial design was adopted. The factors and levels were:

Factor A – Probiotic concentration: 2.5 % and 5 %.

Factor B – Feeding rate 2 % and 5 % of fish biomass per day.

Factor C – Aeration timing: 10 hours during daytime and 10 hours during nighttime.

Table 1. Design treatment combination for an experimental study on the growth performance of *Mystus cavasius*.

BFT Tanks	Probiotic Concentration (%)	Feeding Rate (%)	Aeration Timing (10 hours)
T1	5	5	Night
T2	5	5	Day
T3	5	2	Day
T4	5	2	Night
T5	2.5	2	Night
T6	2.5	2	Day
T7	2.5	5	Day
T8	2.5	5	Night

Table 1 summarizes the experimental treatments applied to the eight BFT tanks (T1–T8). Tanks T1 and T2 received a 5% probiotic concentration and a 5% feeding rate, with aeration provided at night in T1 and during the day in T2. Similarly, T3 and T4 were maintained with a 5% probiotic concentration and a 2% feeding rate, with aeration provided during the day and at night, respectively. Likewise, T5 and T6 were maintained with a 2.5%

probiotic concentration and a 2% feeding rate, with aeration provided at night and during the day, respectively. Finally, T7 and T8 received 2.5% probiotics and a 5% feeding rate, with daytime aeration in T7 and nighttime aeration in T8. This factorial arrangement enabled a systematic evaluation of the individual and combined effects of probiotic concentration, feeding rate, and aeration timing on the culture environment and the performance of fish reared under the BFT system.

2.1.1. Illustration of the Factorial Design

Figure 1 presents the experimental arrangement used to study the combined effects of probiotic concentration, feeding rate, and aeration timing. Under a 2.5% probiotic concentration, the 2% feeding rate was combined with two different aeration timings: day (T6) and night (T5), while the 5% feeding rate was combined with day (T7) and night (T8) aeration timings. Under a 5% probiotic concentration, the 2% feeding rate was combined with day (T3) and night (T4) aeration timings, whereas the 5% feeding rate was combined with day (T2) and night (T1) aeration timings.

Thus, T1–T8 represent the different treatment combinations used in the experiment. This arrangement allows the responses of the experimental fish to be compared not only across different probiotic concentrations and feeding rates but also across aeration timings. This type of design helps determine which combination of probiotic concentration, feeding rate, and aeration timing produces the most favorable result.

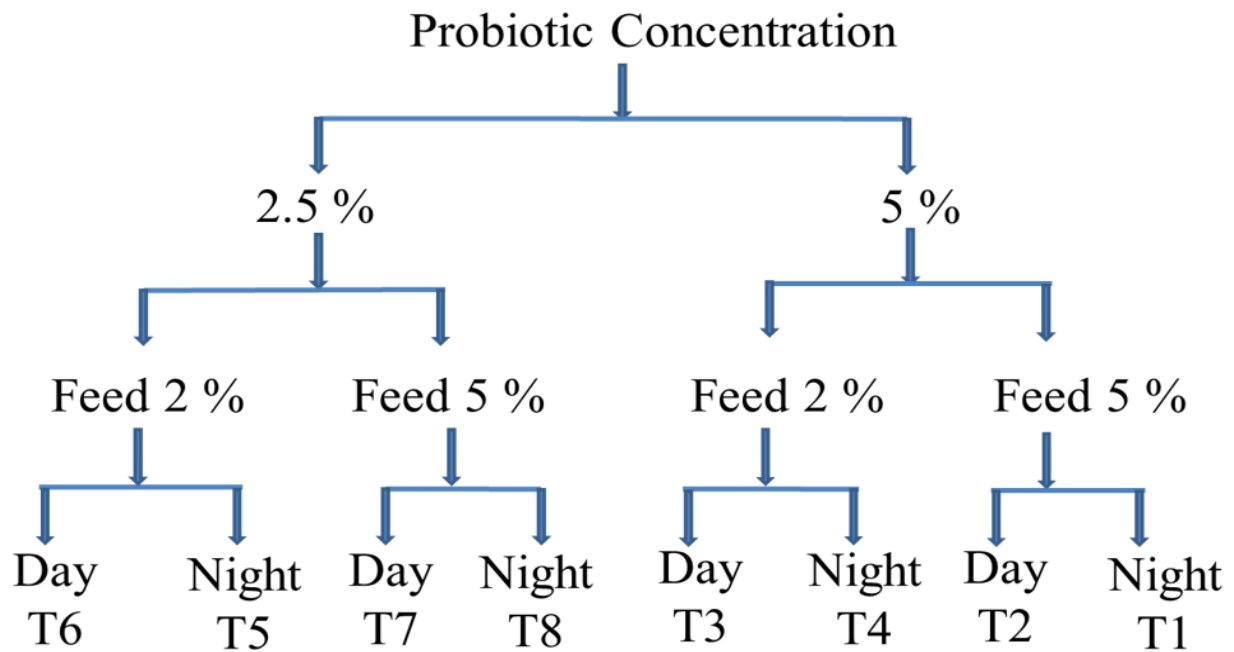


Figure 1. Illustrates the experimental arrangement showing the different combinations of probiotic concentration, feeding rate, and aeration timing.

2.2. Probiotics Content

Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus polymyxa, Rhodobacter sp., Rhodococcus sp., Nitrobacter sp., Nitrosomonas sp., Lactobacillus sp.

2.3. Feed Content

Protein (min): 32 %

Fat (min): 5 %

Crude fiber (max): 8 %

Moisture (max): 10 %

2.4. Number of Biological Replicates

The individual tanks, rather than individual fish within the tanks, were considered the primary experimental units because all fish in the same tank received the same probiotic concentration, feed rate, aeration schedule, and water environment. Fish sampled from the same tank were subsamples and were not fully independent biological replicates. Treating every fish as an independent replicate would artificially increase the sample size and could result in pseudoreplication. Aquaculture growth experiments therefore included replication across independent tanks, and treatment effects were primarily tested using tank-level means.

2.5. Randomization Procedure

Following acclimatization, the fish were deprived of feed for approximately 12–24 hours before initial weighing to reduce variation associated with gut contents. Each fish was weighed individually and inspected for visible injuries, abnormal swimming behavior, and signs of disease. The eight treatment combinations were subsequently assigned randomly to the tanks. Tank positions were balanced across the experimental area to minimize possible effects of light, temperature, water supply, and routine management.

2.6. Power Analysis

An a priori power analysis was conducted for a fixed-effects factorial ANOVA at a significance level of 0.05 and a target power of 80%. Because limited preliminary information was available regarding between-tank variation in BFT-cultured *Mystus cavasius*, the calculation was based on detecting a large and biologically relevant treatment effect.

2.7. Sample-Size Justification

The proposed sample sizes were selected by balancing statistical reliability, the ethical use of experimental animals, available infrastructure, and the risk of pseudoreplication. The use of multiple fish in each tank improves the precision of the tank mean but does not replace the need for multiple tanks. Increasing the number of fish within one tank mainly improves the estimation of that particular tank's response, whereas increasing the number of independent tanks improves the ability to estimate treatment effects across the experimental population.

2.8. Mortality Handling

All tanks were inspected at least twice daily, preferably during morning and evening management periods. Dead fish were removed immediately, counted, weighed where feasible, and recorded against the relevant tank and treatment.

2.9. Physico-Chemical Parameters

Different physical and chemical characteristics affecting water quality include dissolved oxygen (DO), temperature, total dissolved solids (TDS), total ammonia nitrogen (TAN), and floc volume, as reported by the American Public Health Association (1998), as well as pH and turbidity, according to IS 3025 (American Public Health Association, 1998; Bureau of Indian Standards, 2017).

2.10. Growth Parameters

The following formulas will be used to calculate growth parameters (Khanjani, Sajjadi, Alizadeh, & Sourinejad, 2017; Tacon et al., 2002).

$$\text{Final body weight (g)} \quad (1)$$

$$\text{Body length gain (cm)} = \text{final length} - \text{initial length} \quad (2)$$

$$\text{Body weight gain (g)} = \text{final weight} - \text{initial weight} \quad (3)$$

$$\text{Body weight index (BWI) (\%)} = \frac{[(\text{final weight} - \text{initial weight}) / \text{initial weight}] \times 100}{100} \quad (4)$$

$$\text{Survival rate (\%)} = \frac{(\text{Number of individuals at the end of testing period} / \text{initial number of individuals stocked}) \times 100}{100} \quad (5)$$

$$\text{Specific growth rate (SGR in weight) (\%/day)} = \frac{[(\ln \text{ final weight} - \ln \text{ initial weight}) \times 100]}{\text{days of experiment}} \quad (6)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed consumed (dry weight)}}{\text{live weight gain (wet weight)}} \quad (7)$$

2.11. Statistical Analysis

The present study examined how probiotic supplementation, feeding rate, and aeration schedule enhanced the growth and production performance of *Mystus cavasius* cultured in a BFT. These three management practices were selected because they act through closely related biological and environmental pathways. Probiotic microorganisms may support nutrient digestion, stabilize the microbial community, and assist in maintaining water quality. Feeding rate directly determines nutrient availability and organic loading, while the timing of aeration influences dissolved oxygen, biofloc suspension, and microbial utilization of nitrogenous wastes.

The original treatment arrangement included every possible combination of two probiotic concentrations, two feeding rates, and two aeration schedules. It was therefore a $2 \times 2 \times 2$ full-factorial experiment, rather than a simple comparison of eight unrelated treatments. In a factorial experiment, treatment combinations are analyzed according to the underlying factors that created them. This approach makes it possible to distinguish the effect of each factor and determine whether the response to one factor changes according to the level of another factor. A factorial experiment is defined by the inclusion of all possible combinations of the selected levels of two or more factors.

Independence was addressed through the experimental design. Tanks were independently assigned to treatments and used for analysis, while fish within a tank were not treated as statistically independent.

Model residuals were assessed for normality using normal Q-Q plots and the Shapiro-Wilk test. Normality was considered reasonably satisfied when no substantial departure from the Q-Q line was observed and the Shapiro-Wilk test was nonsignificant at $p > 0.05$. Homogeneity of variance was evaluated using Levene's test. The assumption was regarded as acceptable when $p > 0.05$ and the residual-versus-fitted plot did not show a systematic increase or decrease in residual spread.

A three-way factorial analysis of variance (ANOVA) was used to determine whether growth and production responses were influenced by probiotic supplementation, feeding rate, aeration schedule, or interactions among these factors.

When ANOVA identified a significant effect, Tukey's Honestly Significant Difference test was used to determine which treatment means differed. Tukey's test is suitable for multiple pairwise comparisons because it controls the overall probability of making a Type I error.

3. RESULTS AND DISCUSSION

3.1. Water Quality Assessment and Physicochemical Variations

3.1.1. Temperature

Differences in the temperature parameter under various interventions, as displayed in Table 2, show that the highest mean temperature was 30.99 °C in treated tank T4 in August, while the lowest mean temperature was 27.95 °C in the control in November. The values in the control group differed significantly ($P < 0.05$) in September, October, and November. In the study, temperature ranged from 27.95 °C to 30.99 °C. Kohinoor, Begum, and Hussain (2004) claimed that the temperature range during their study was 25.62 ± 0.28 °C to 31.31 ± 1.57 °C.

Overall, the consistently favorable water temperature observed across all treatments demonstrates that the BFT system provided a thermally stable culture environment throughout the experimental period. The gradual seasonal decline from August to November followed the normal climatic pattern of Assam and did not reach levels detrimental

to *Mystus cavasius*. Since the temperature remained within the optimum range reported for tropical freshwater catfish (approximately 28–31 °C), it is unlikely to have imposed physiological stress on the fish. Consequently, the superior growth observed in certain treatments can be attributed mainly to the combined effects of probiotic supplementation, feed rate, and aeration regime rather than to differences in water temperature. Stable temperature also favors the proliferation of beneficial microbial communities, enhances nutrient mineralization, improves feed conversion, and contributes to better overall performance of BFT systems.

The present findings agree with previous studies reporting that maintaining water temperatures between 28–31 °C promotes efficient metabolism, feed utilization, immune function, and microbial activity in BFT systems. Stable temperatures have been shown to enhance the performance of probiotic bacteria and heterotrophic microbial communities, leading to improved water quality and better fish growth. Therefore, the observed temperature profile confirms that the environmental conditions during the experiment were suitable for evaluating the individual and combined effects of probiotic concentration, feed rate, and aeration schedule on the growth performance of *Mystus cavasius* cultured under BFT.

Table 2. Temperature (°C) parameter levels under various interventions over five months (July to November).

Treatments	Months				
	July	August	September	October	November
Control	29.44±0.109 ^{ab}	30.61±0.121 ^{ab}	29.44±0.109 ^b	28.54±0.112 ^d	27.95±0.112 ^c
T1	29.65±0.113 ^a	30.83±0.108 ^{ab}	30.16±0.114 ^a	29.83±0.118 ^a	29.04±0.115 ^a
T2	28.97±0.112 ^b	30.31±0.113 ^b	29.64±0.122 ^{ab}	28.85±0.115 ^{bcd}	28.35±0.122 ^{bc}
T3	29.17±0.108 ^{ab}	30.31±0.116 ^b	29.78±0.124 ^{ab}	28.67±0.124 ^{cd}	28.18±0.118 ^{bc}
T4	29.67±0.114 ^a	30.99±0.111 ^a	29.94±0.117 ^{ab}	29.29±0.119 ^{ab}	28.56±0.119 ^{ab}
T5	29.45±0.115 ^{ab}	30.83±0.110 ^{ab}	30.00±0.125 ^{ab}	29.20±0.111 ^{bc}	28.72±0.121 ^{ab}
T6	29.26±0.120 ^{ab}	30.42±0.116 ^b	30.07±0.112 ^a	28.86±0.122 ^{bcd}	28.25±0.124 ^{bc}
T7	29.56±0.122 ^a	30.58±0.113 ^{ab}	29.98±0.121 ^{ab}	29.03±0.114 ^{bcd}	28.45±0.113 ^{abc}
T8	29.66±0.117 ^a	30.65±0.122 ^{ab}	30.06±0.123 ^a	29.18±0.113 ^{bc}	28.76±0.116 ^{ab}

Note: Values are presented as mean ± SD. Different superscript letters (a, b, c and d) within the same column indicate significant differences among treatments at $p < 0.05$. Values sharing one or more common superscript letters are not significantly different.

3.1.2. pH

Variations in pH under different treatments (Table 3) indicate that the highest pH value was 8.39, recorded in treated tank T8 in November, while the lowest pH value was 7.37, observed in the control tank in July. The control-tank data increased sequentially from July to November (7.37, 7.40, 7.44, 7.48, and 7.51, respectively). Similarly, pH values in treated tanks T5, T6, T7, and T8 showed an increasing trend. In treated tank T8, differences from July to November were not statistically significant ($P < 0.05$). In this study, pH values ranged from 7.37 to 8.39. Hosen, Pervin, and Shahriar (2017) reported that pH values ranged from 6.92 ± 0.10 to 8.20 ± 0.05 .

Among all treatments, T8 consistently exhibited the highest pH values each month, followed by T2 and T7, indicating that this treatment combination provided the most stable water chemistry. The superior pH stability in T8 likely reflects a balanced interaction among probiotic microorganisms, nutrient availability from feed, and efficient aeration, all of which supported active microbial floc development and enhanced nutrient recycling. Such conditions not only improve water quality but also increase the availability of natural microbial protein, thereby supporting better feed utilization and fish growth.

Overall, the results demonstrate that pH remained within the acceptable range for the BFT system throughout the experimental period. The gradual increase from July to November reflects the maturation of the biofloc microbial community rather than harmful alkalization of the culture water. Stable and slightly alkaline conditions favor nitrification, suppress the accumulation of toxic ammonia, improve microbial productivity, and enhance fish health. Therefore, the improved growth performance observed in the probiotic-treated tanks can be attributed, in part, to the maintenance of a stable and biologically optimal pH environment facilitated by the combined effects of probiotic concentration, feeding rate, and aeration management.

Table 3. The monthly pH levels recorded under different treatments from July to November.

Treatments	Months				
	July	August	September	October	November
Control	7.37±0.117 ^d	7.40±0.116 ^c	7.44±0.111 ^d	7.48±0.112 ^c	7.51±0.115 ^b
T1	7.90±0.115 ^{abcd}	7.93±0.120 ^{abc}	7.92±0.113 ^{abcd}	7.93±0.111 ^{abc}	8.14±0.116 ^a
T2	8.16±0.111 ^{ab}	8.15±0.119 ^{ab}	8.15±0.111 ^{ab}	8.19±0.119 ^{ab}	8.23±0.111 ^a
T3	7.48±0.116 ^{cd}	7.56±0.114 ^c	7.52±0.109 ^{cd}	7.69±0.118 ^{bc}	7.96±0.121 ^{ab}
T4	7.49±0.119 ^{cd}	7.57±0.118 ^c	7.56±0.108 ^{cd}	7.74±0.113 ^{bc}	7.99±0.118 ^{ab}
T5	7.56±0.109 ^{cd}	7.60±0.110 ^{bc}	7.60±0.116 ^{cd}	7.77±0.110 ^{bc}	8.03±0.119 ^{ab}
T6	7.60±0.113 ^{bcd}	7.61±0.112 ^{bc}	7.64±0.115 ^{bcd}	7.81±0.115 ^{abc}	8.06±0.112 ^{ab}
T7	7.94±0.110 ^{abc}	7.95±0.111 ^{abc}	8.01±0.110 ^{abc}	8.06±0.108 ^{ab}	8.18±0.113 ^a
T8	8.30±0.114 ^a	8.31±0.115 ^a	8.34±0.118 ^a	8.36±0.109 ^a	8.39±0.109 ^a

Note: Values are presented as mean ± SD. Different superscript letters (a, b, c and d) within the same column indicate significant differences among treatments at $p < 0.05$. Values sharing one or more common superscript letters are not significantly different.

3.1.3. Dissolved Oxygen (DO)

Table 4 showed that different treatments led to variations in dissolved oxygen levels. The highest dissolved oxygen value, recorded in treated tank T8, was 8.31 mg/L in September, while the lowest was 6.92 mg/L in the control tank in November. In the control tank, the data were not significantly different ($P < 0.05$) in July, August, and November, but were significantly different ($P < 0.05$) in September and October. In treated tank T8, the data were not significantly different ($P < 0.05$) across all months: July, August, September, October, and November. Dissolved oxygen ranged from 6.92 mg/L to 8.31 mg/L. Hosen et al. (2017) claimed that DO levels ranged from 5.65 ± 0.15 to 7.80 ± 0.10 mg/L during their study.

Among all treatments, T8 consistently maintained the highest dissolved oxygen concentration throughout the experimental period, followed closely by T2 and T7. These results suggest that the combination of probiotic application, appropriate feed management, and an effective aeration schedule created the most favorable conditions for microbial balance and oxygen availability. Conversely, the comparatively lower DO observed in the control indicates that the absence of probiotic supplementation reduced the efficiency of microbial nutrient cycling, leading to greater oxygen consumption during the decomposition of accumulated organic matter. Nevertheless, even the lowest recorded DO remained above the critical threshold generally required by tropical freshwater fish, indicating that none of the treatments experienced severe oxygen stress.

The present findings demonstrate that maintaining high dissolved oxygen is fundamental for the successful biofloc culture of *Mystus cavasius*. Stable oxygen concentrations enhance aerobic microbial activity, improve nitrification efficiency, reduce ammonia toxicity, and support the development of nutritious microbial flocs that supplement the formulated diet. Consequently, the superior growth performance observed in treatments receiving probiotics, particularly T8 and T2, can be partly attributed to improved dissolved oxygen conditions that optimized both fish physiology and microbial ecosystem function.

The observed results are consistent with previous reports on BFT systems. Avnimelech (2015) reported that continuous aeration is indispensable in BFT systems because it maintains dissolved oxygen, keeps biofloc particles suspended, and supports heterotrophic bacterial activity responsible for nutrient recycling. Similarly, Crab, Defoirdt, Bossier, and Verstraete (2012) demonstrated that maintaining dissolved oxygen above approximately 6 mg L⁻¹ improves microbial efficiency, minimizes ammonia accumulation, and enhances fish growth under intensive BFT systems. Emerenciano, Martínez-Córdova, Martínez-Porchas, and Miranda-Baeza (2017) further emphasized that balanced microbial communities established through BFT systems improve water quality and provide additional microbial protein, resulting in better feed conversion and overall production performance. More recently, Kumar et al. (2023) reported that probiotic-based microbial inoculants significantly enhanced water quality, dissolved oxygen stability, digestive enzyme activity, and the growth of freshwater catfish reared in BFT systems. Likewise, Banu et al. (2024) observed that efficient aeration and microbial community development maintained favorable dissolved oxygen levels and significantly improved the growth and health indices of freshwater fish cultured under BFT

systems. The agreement between the present findings and these earlier studies confirms that the effective integration of probiotics, balanced feeding, and adequate aeration plays a decisive role in maintaining optimal dissolved oxygen and maximizing the growth performance of *Mystus cavasius* in BFT systems.

Table 4. Dissolved oxygen (DO) (mg/L) levels across various treatments over different months (July to November).

Treatments	Months				
	July	August	September	October	November
Control	7.01±0.114 ^d	7.10±0.109 ^d	6.96±0.112 ^c	7.10±0.116 ^e	6.92±0.116 ^d
T1	7.81±0.119 ^{abc}	7.73±0.114 ^{abc}	7.80±0.113 ^{ab}	7.90±0.114 ^{abcd}	7.80±0.112 ^{ab}
T2	8.00±0.113 ^a	7.91±0.115 ^a	8.07±0.115 ^a	8.07±0.113 ^{ab}	8.01±0.108 ^{ab}
T3	7.10±0.110 ^d	7.10±0.118 ^d	7.12±0.116 ^c	7.11±0.117 ^e	7.12±0.118 ^{cd}
T4	7.21±0.116 ^d	7.14±0.113 ^d	7.20±0.108 ^c	7.34±0.115 ^{de}	7.49±0.113 ^{bc}
T5	7.28±0.115 ^{cd}	7.24±0.112 ^{cd}	7.28±0.111 ^{bc}	7.43±0.111 ^{cde}	7.51±0.111 ^{bc}
T6	7.41±0.117 ^{bcd}	7.29±0.117 ^{bcd}	7.29±0.109 ^{bc}	7.51±0.114 ^{bcd}	7.57±0.112 ^{bc}
T7	7.97±0.112 ^{ab}	7.84±0.119 ^{ab}	8.00±0.110 ^a	7.92±0.109 ^{abc}	8.00±0.110 ^{ab}
T8	8.13±0.111 ^a	8.10±0.111 ^a	8.31±0.114 ^a	8.23±0.112 ^a	8.18±0.114 ^a

Note: Values are presented as mean ± SD. Different superscript letters (a, b, c, d and e) within the same column indicate significant differences among treatments at $p < 0.05$. Values sharing one or more common superscript letters are not significantly different.

3.1.4. Turbidity

Changes in turbidity with various treatments are presented in Table 5. The highest turbidity value, 72.10, was recorded in treated tank T8 in September, whereas the lowest value, 1.05, was recorded in the control in September. In the control, the values observed in August, September, October, and November were not significantly different ($P < 0.05$). However, the data for July, August, September, October, and November were significantly different ($P < 0.05$). The data observed in treated tank T8 were not significantly different ($P < 0.05$) during July, August, September, October, and November. Panigrahi et al. (2017) reported that the turbidity value in their study of *Penaeus vannamei* cultivated in a BFT system was 26.98 ± 3.68 .

Among all treatments, T8 consistently maintained the highest turbidity, followed by T2 and T7, suggesting that these treatment combinations provided the most favorable conditions for biofloc development. The combination of probiotic supplementation, suitable feed input, and efficient aeration likely accelerated heterotrophic bacterial growth, increased microbial biomass, and enhanced the formation of compact flocs. These bioflocs continuously assimilate inorganic nitrogen, improve water quality, and provide additional nutrition through microbial protein and bioactive compounds. Consequently, fish cultured in these treatments are likely to exhibit improved feed utilization, enhanced digestive activity, stronger immunity, and better overall growth performance.

It is important to note that the turbidity observed in the biofloc treatments should not be interpreted as poor water quality. In conventional aquaculture, elevated turbidity often indicates suspended soil particles or excessive organic pollution. However, in BFT, turbidity primarily reflects the abundance of beneficial microbial flocs. Therefore, maintaining moderate to high turbidity within an acceptable range is essential for sustaining microbial productivity and maximizing the benefits of the BFT system. Conversely, the very low turbidity observed in the control demonstrates the absence of a well-developed microbial community, reducing the potential for nutrient recycling and natural feed production.

The present findings agree with earlier studies on BFT systems. Avnimelech (2015) reported that increased turbidity in BFT systems reflects active microbial floc development, which enhances nitrogen assimilation, improves water quality, and provides supplementary nutrition for cultured fish. Crab et al. (2012) similarly observed that heterotrophic bacteria convert dissolved nitrogenous compounds into microbial biomass, increasing suspended biofloc particles while reducing toxic ammonia accumulation. Emerenciano, Gaxiola, and Cuzon (2013) further demonstrated that properly managed BFT systems maintain nutrient recycling, digestive efficiency, and fish growth. More recently, Kumar et al. (2023) reported that probiotic-based microbial inoculants significantly enhanced microbial floc formation, improved water quality, and increased the growth performance of *Heteropneustes fossilis* cultured in BFT

systems. The present results are therefore in close agreement with these earlier investigations, confirming that probiotic supplementation, together with appropriate feed management and aeration, promotes optimal biofloc formation and creates a biologically favorable environment for the culture of *Mystus cavasius*.

Overall, the turbidity profile observed during the study indicates the successful establishment and maintenance of the BFT system. The consistently higher turbidity in probiotic-treated tanks reflects enhanced microbial productivity and efficient nutrient recycling rather than deterioration of water quality. These favorable conditions likely contributed to improved fish health, better feed conversion, enhanced immune status, and superior growth performance of *Mystus cavasius* cultured under the BFT system.

Table 5. Varying turbidity (NTU) levels across different treatments over five months (July to November).

Treatments	Months				
	July	August	September	October	November
Control	1.12±0.118 ⁱ	1.11±0.115 ^h	1.05±0.109 ^h	1.11±0.114 ^g	1.07±0.119 ^g
T1	67.09±0.111 ^d	66.11±0.116 ^d	67.15±0.117 ^d	68.07±0.115 ^c	67.12±0.113 ^c
T2	69.12±0.113 ^b	68.07±0.110 ^b	70.18±0.118 ^b	70.11±0.114 ^b	70.09±0.112 ^a
T3	50.10±0.110 ^h	60.03±0.112 ^g	59.11±0.116 ^g	60.13±0.118 ^f	58.06±0.116 ^f
T4	61.11±0.112 ^g	61.09±0.113 ^f	61.07±0.113 ^f	63.10±0.111 ^e	64.05±0.110 ^e
T5	62.11±0.117 ^f	61.06±0.114 ^f	62.09±0.110 ^e	63.08±0.113 ^e	64.15±0.114 ^e
T6	63.13±0.119 ^e	62.11±0.117 ^e	62.11±0.115 ^e	64.20±0.116 ^d	65.09±0.115 ^d
T7	68.12±0.114 ^c	67.11±0.109 ^c	69.09±0.113 ^c	68.11±0.109 ^c	69.13±0.118 ^b
T8	70.11±0.116 ^a	70.10±0.111 ^a	72.10±0.111 ^a	71.07±0.110 ^a	70.14±0.111 ^a

Note: Values are presented as mean ± SD. Different superscript letters (a, b, c, d, e, f, g, h and i) within the same column indicate significant differences among treatments at $p < 0.05$. Values sharing one or more common superscript letters are not significantly different.

3.1.5. Total Dissolved Solids (TDS)

Variations in total dissolved solids levels under different treatments are shown in Table 6. The highest value recorded was 230.03 mg/L in treated tank T8 in September, and the lowest was 106.05 mg/L in the control tank during the same month. The data observed in July, August, September, October, and November differed significantly ($P < 0.05$). In the control tank, the data did not differ significantly across these months. Some treated tanks, including T1, T3, T4, T5, T6, and T8, also showed no significant differences ($P < 0.05$). In this study, TDS ranged from 106.05 mg/L to 230.03 mg/L. In a study by Panigrahi et al. (2017) on *Penaeus vannamei* cultivated in a BFT system, the total dissolved solids value was reported as 575.8 ± 3.9 mg/L.

These findings suggest that the combination of probiotics, higher feed input, and suitable aeration promoted the formation of suspended microbial biomass and organic particulates, thereby increasing dissolved solids in the culture water. Similar observations have been reported by Avnimelech (2015), who explained that elevated TDS is a characteristic feature of active BFT systems due to continuous microbial growth and nutrient recycling. Likewise, Emerenciano et al. (2013) noted that increased microbial abundance and floc development contribute substantially to dissolved and suspended solids in BFT systems.

Among the probiotic treatments, T8 consistently maintained the highest TDS throughout the experiment, while intermediate values were recorded in T4, T5, and T6, and comparatively lower values were observed in T3. The gradual increase in TDS from July to November in several treatments indicates progressive biofloc maturation and continuous mineralization of organic matter. Although excessive TDS may impair water quality, the recorded values remained within the acceptable range generally reported for freshwater biofloc culture and were associated with improved microbial productivity rather than water quality deterioration. This observation agrees with the findings of Crab et al. (2012), who reported that moderate increases in dissolved solids enhance nutrient retention and microbial stability in BFT systems. Similarly, Hargreaves (2013) concluded that elevated TDS in BFT tanks reflects efficient nutrient transformation and supports a stable microbial ecosystem, ultimately contributing to improved fish production when maintained within optimum limits.

Table 6. Monthly (July to November) variation in total dissolved solids (mg/L) levels for different treatments.

Treatments	Months				
	July	August	September	October	November
Control	110.07±0.114 ⁱ	119.93±0.115 ⁱ	106.05±0.117 ⁱ	120.92±0.119 ⁱ	109.98±0.116 ⁱ
T1	192.13±0.118 ^d	180.15±0.117 ^d	193.96±0.115 ^d	195.99±0.114 ^d	191.78±0.113 ^d
T2	212.03±0.113 ^b	198.19±0.108 ^b	208.99±0.111 ^c	224.14±0.113 ^b	213.58±0.112 ^b
T3	120.81±0.109 ^h	120.81±0.116 ^h	120.83±0.118 ^h	123.78±0.110 ^h	120.78±0.115 ^h
T4	128.04±0.110 ^g	126.05±0.114 ^g	131.89±0.116 ^g	143.42±0.116 ^g	157.98±0.110 ^g
T5	136.20±0.117 ^f	134.27±0.112 ^f	135.51±0.115 ^f	150.08±0.111 ^f	160.08±0.111 ^f
T6	154.12±0.115 ^e	135.52±0.110 ^e	139.85±0.112 ^e	161.27±0.118 ^e	162.70±0.109 ^e
T7	201.93±0.112 ^c	195.01±0.113 ^c	212.09±0.110 ^b	203.94±0.112 ^c	205.05±0.114 ^c
T8	220.08±0.111 ^a	218.11±0.111 ^a	240.15±0.114 ^a	230.03±0.115 ^a	221.67±0.119 ^a

Note: Values are presented as mean ± SD. Different superscript letters (a, b, c, d, e, f, g, h and i) within the same column indicate significant differences among treatments at $p < 0.05$. Values sharing one or more common superscript letters are not significantly different.

3.1.6. Nitrite

Changes in nitrite levels across different treatments over five months are presented in Table 7, indicating that the highest value recorded was 2.76 mg/L in treated tank T8 in September, while the lowest was 0.47 mg/L in treated tank T3 in August. In the control group, the data (1.08, 1.09, 1.15, 1.42, and 1.81) showed an increasing trend from July to November, respectively, and were not significantly different ($P < 0.05$) in July, September, and October. The observed values in treated tank T3 were significantly different ($P < 0.05$) in all five months. In treated tank T8, the values were not significantly different ($P < 0.05$) in any month. Emerenciano, Ballester, Cavalli, and Wasielesky (2012) reported that, in their study of *Farfantepenaeus brasiliensis* cultivated in a BFT system, the nitrite value was 0.9 ± 0.6 .

The comparatively lower nitrite concentration in T3 indicates that an appropriate balance of probiotic dosage, feed input, and aeration enhanced the activity of beneficial nitrifying bacteria, thereby improving water quality. Similar findings were reported by Avnimelech (2015), who stated that well-established biofloc microbial communities efficiently regulate nitrogen compounds through microbial uptake and nitrification. Crab et al. (2012) also observed that balanced BFT systems reduce the accumulation of toxic nitrogenous metabolites by promoting beneficial microbial activity.

Table 7. Variation in nitrite (mg/L) levels under different treatments over five months (July to November).

Treatments	Months				
	July	August	September	October	November
Control	1.08±0.110 ^b	1.09±0.113 ^{cd}	1.15±0.119 ^b	1.42±0.110 ^b	1.81±0.113 ^{cd}
T1	2.03±0.109 ^a	1.40±0.110 ^{bc}	2.25±0.114 ^a	2.24±0.112 ^a	2.10±0.114 ^{bc}
T2	2.40±0.123 ^a	1.81±0.114 ^{ab}	2.68±0.113 ^a	2.42±0.120 ^a	2.61±0.120 ^{ab}
T3	0.72±0.120 ^b	0.47±0.114 ^e	0.62±0.116 ^b	0.76±0.109 ^c	0.58±0.111 ^f
T4	0.89±0.124 ^b	0.68±0.129 ^{de}	0.84±0.112 ^b	1.05±0.113 ^{bc}	1.07±0.121 ^{ef}
T5	0.91±0.128 ^b	0.70±0.119 ^{de}	0.90±0.108 ^b	1.21±0.115 ^{bc}	1.47±0.109 ^{de}
T6	1.23±0.111 ^b	0.80±0.126 ^{de}	1.09±0.110 ^b	1.23±0.114 ^{bc}	1.51±0.112 ^{de}
T7	2.22±0.114 ^a	1.54±0.112 ^{bc}	2.43±0.115 ^a	2.33±0.118 ^a	2.40±0.119 ^{ab}
T8	2.61±0.117 ^a	2.36±0.120 ^a	2.76±0.122 ^a	2.47±0.123 ^a	2.74±0.123 ^a

Note: Values are presented as mean ± SD. Different superscript letters (a, b, c, d, e and f) within the same column indicate significant differences among treatments at $p < 0.05$. Values sharing one or more common superscript letters are not significantly different.

The relatively higher nitrite concentrations recorded in T2, T7, and T8 may be attributed to greater feed loading, which increased ammonia production beyond the immediate nitrification capacity of the microbial community. Nevertheless, these values remained within the range commonly observed in actively functioning BFT systems and reflected intensified nitrogen cycling rather than system failure. Emerenciano et al. (2013) reported that temporary fluctuations in nitrite are common during biofloc development because of continuous microbial mineralization and nutrient recycling. Similarly, Hargreaves (2013) concluded that efficient aeration and microbial management are essential to maintain nitrite at safe levels, ensure stable water quality, and promote better fish performance in BFT.

3.1.7. Nitrate

Variations in nitrate levels under different treatments over the months are presented in Table 8. The highest value recorded was 4.42 mg/L in treated tank T8 in August, while the lowest was 0.52 mg/L in treated tank T3 in July. Control-tank values were not significantly different ($P < 0.05$) in July, September, October, or November. Similarly, values in treated tank T8 were not significantly different ($P < 0.05$) from July through November. Panigrahi et al. (2017) claimed that, in their study of *Penaeus vannamei* cultivated in a BFT system, the nitrate value was 0.204 ± 0.05 .

The higher nitrate concentrations in T8, T2, and T7 indicate more active nitrification, during which ammonia and nitrite were efficiently converted into the less toxic nitrate form by nitrifying bacteria. This suggests that the combination of probiotics, higher feed input, and effective aeration enhanced microbial activity and promoted a stable nitrogen cycle within the BFT system. Similar observations have been reported by Kumar et al. (2023), who found that microbial inoculants improved nitrification efficiency and water quality in freshwater BFT culture. Likewise, Yu et al. (2023) emphasized that increased nitrate levels in well-managed biofloc systems are generally associated with efficient nitrogen transformation rather than poor water quality.

Table 8. Monthly (July to November) variation in nitrate (mg/L) levels across different treatments.

Treatments	Months				
	July	August	September	October	November
Control	1.81 ± 0.115^b	1.34 ± 0.112^c	1.66 ± 0.113^b	2.04 ± 0.112^b	2.24 ± 0.112^b
T1	2.68 ± 0.107^a	2.06 ± 0.111^b	2.82 ± 0.112^a	2.88 ± 0.108^a	2.84 ± 0.119^a
T2	3.02 ± 0.113^a	2.46 ± 0.110^b	3.25 ± 0.115^a	2.96 ± 0.111^a	3.24 ± 0.109^a
T3	0.52 ± 0.125^c	0.67 ± 0.115^d	0.68 ± 0.118^c	0.69 ± 0.115^c	0.57 ± 0.116^c
T4	0.68 ± 0.119^c	1.13 ± 0.114^{cd}	0.71 ± 0.111^c	1.49 ± 0.121^b	0.68 ± 0.118^c
T5	1.34 ± 0.115^b	1.20 ± 0.118^{cd}	1.58 ± 0.119^b	1.88 ± 0.118^b	2.17 ± 0.113^b
T6	1.50 ± 0.120^b	1.30 ± 0.121^c	1.62 ± 0.122^b	1.90 ± 0.124^b	2.22 ± 0.110^b
T7	2.78 ± 0.105^a	2.22 ± 0.109^b	3.03 ± 0.109^a	2.93 ± 0.119^a	3.03 ± 0.121^a
T8	3.10 ± 0.110^a	3.42 ± 0.113^a	3.29 ± 0.125^a	3.05 ± 0.122^a	3.41 ± 0.123^a

Note: Values are presented as mean $\pm$ SD. Different superscript letters (a, b, c and d) within the same column indicate significant differences among treatments at $p < 0.05$. Values sharing one or more common superscript letters are not significantly different.

The consistently lower nitrate concentrations in T3 indicate reduced nitrogen loading due to lower feed input and greater assimilation of inorganic nitrogen into microbial biomass. These findings suggest that balanced management of probiotics, feed, and aeration plays a vital role in maintaining nitrogen equilibrium and supporting fish growth. Recent studies by Banu et al. (2024) demonstrated that probiotic-driven BFT systems improve microbial community structure, enhance nutrient recycling, and stabilize water quality in freshwater fish culture. Similarly, Raza et al. (2024) concluded that effective biofloc management promotes the biological conversion of toxic nitrogen compounds into relatively harmless nitrate, thereby creating a healthier environment for cultured fish and improving production efficiency.

3.1.8. Total Ammonia Nitrogen (TAN)

Monthly variations in TAN levels under different treatments are illustrated in Table 9. The highest recorded value was 4.43 mg/L in treated tank T8 during November, whereas the lowest was 0.91 mg/L in treated tank T3 during July and August. Control-group values did not differ significantly ($P < 0.05$) during August, September, and October. Similarly, T8 values did not differ significantly ($P < 0.05$) during July through November. The TAN value was 0.4 ± 0.5 during the study of *Farfantapenaeus brasiliensis* cultivated in BFT system, as reported by Emerenciano et al. (2012).

The comparatively low TAN levels in T3 indicate that an appropriate combination of probiotic supplementation, controlled feed input, and efficient aeration enhanced the activity of ammonia-assimilating and nitrifying microorganisms, resulting in improved removal of toxic nitrogenous waste. In contrast, the higher TAN

concentrations observed in T2, T7, and T8 were likely associated with greater feed loading, which increased ammonia production beyond the immediate microbial assimilation capacity. Similar findings were reported by Kumar et al. (2023), who demonstrated that microbial inoculants in BFT systems significantly improved nitrogen utilization and reduced ammonia toxicity. Likewise, Yu et al. (2023) reported that balanced biofloc management promotes efficient microbial conversion of ammonia, thereby improving water quality and fish health.

The progressive increase in TAN observed in some treatments during the later months suggests increasing organic loading as fish biomass and feed demand increased. Nevertheless, the lower TAN concentrations maintained in T3, T4, and T5 indicate that effective probiotic activity, together with appropriate feeding and aeration, can stabilize nitrogen cycling and minimize ammonia accumulation. Recent work by Banu et al. (2024) showed that probiotic-enriched BFT systems enhance microbial diversity, improve nutrient recycling, and maintain favorable water quality for freshwater fish culture. Similarly, Raza et al. (2024) concluded that efficient biofloc management through optimized microbial communities, feeding practices, and aeration is essential for controlling ammonia accumulation and sustaining productive aquaculture systems.

Table 9. Variation in total ammonia nitrogen (TAN) (mg/L) levels under different treatments over five months (July to November).

Treatments	Months				
	July	August	September	October	November
Control	2.81±0.124 ^c	3.08±0.116 ^b	2.76±0.122 ^b	3.11±0.113 ^b	3.21±0.117 ^{cd}
T1	3.58±0.113 ^b	3.16±0.120 ^b	3.05±0.116 ^b	3.79±0.121 ^a	3.76±0.122 ^{bc}
T2	4.16±0.126 ^{ab}	3.46±0.122 ^{ab}	4.22±0.123 ^a	3.90±0.119 ^a	4.18±0.112 ^{ab}
T3	0.91±0.127 ^e	0.91±0.110 ^d	0.95±0.120 ^d	0.95±0.128 ^d	0.94±0.113 ^f
T4	1.79±0.121 ^d	2.30±0.113 ^c	1.67±0.113 ^c	2.45±0.123 ^c	1.59±0.119 ^e
T5	2.40±0.111 ^c	2.33±0.115 ^c	2.48±0.118 ^b	2.90±0.124 ^{bc}	3.09±0.116 ^d
T6	2.55±0.107 ^c	2.35±0.112 ^c	2.53±0.110 ^b	3.01±0.118 ^{bc}	3.20±0.114 ^{cd}
T7	3.77±0.119 ^{ab}	3.21±0.122 ^b	3.72±0.119 ^a	3.90±0.114 ^a	4.00±0.110 ^{ab}
T8	4.31±0.117 ^a	3.99±0.119 ^a	4.28±0.121 ^a	4.00±0.127 ^a	4.43±0.125 ^a

Note: Values are presented as mean ± SD. Different superscript letters (a, b, c, d, e and f) within the same column indicate significant differences among treatments at $p < 0.05$. Values sharing one or more common superscript letters are not significantly different.

3.1.9. Floc Volume

Table 10 showed the variation in floc volume levels under different treatments over the months showed that the highest value was 26.09 mL in treated tank T8 during November, whereas the lowest value was 1.49 mL in the control during October. In the control, values did not differ significantly ($P < 0.05$) across July, August, October, and November. Similarly, values in T8 did not differ significantly ($P < 0.05$) from July through November. The floc volume of *Penaeus vannamei* cultivated in a BFT system was reported as 18.6 ± 2.2 by Panigrahi et al. (2017).

The greater floc volume observed in T8 suggests that the combined effects of probiotics, higher feed input, and adequate aeration promoted rapid microbial proliferation and aggregation of suspended organic particles, resulting in a stable and productive biofloc environment. Similar findings were reported by Kumar et al. (2023), who demonstrated that microbial inoculants significantly enhanced biofloc formation, nutrient recycling, and water quality in BFT systems. Likewise, Yu et al. (2023) reported that increased floc volume reflects active microbial biomass and efficient utilization of organic waste, which contributes to improved culture performance.

The gradual increase in floc volume from July to November in most treatments indicates continuous maturation of the microbial community as the culture progressed. Although excessive floc accumulation may require careful management, the values observed in T8, T2, and T7 are characteristic of well-functioning BFT systems, where nutrients are effectively recycled into microbial biomass. Recent research by Banu et al. (2024) showed that probiotic-enriched BFT systems promote beneficial microbial communities and improve nutrient conversion, resulting in higher floc production and better fish growth. Similarly, Raza et al. (2024) concluded that optimized probiotic application, balanced feeding, and sufficient aeration are the key drivers of stable floc development, efficient nutrient cycling, and sustainable aquaculture production.

Table 10. Variation in floc volume (mL) levels under different treatments over five months (July to November).

Treatments	Months				
	July	August	September	October	November
Control	1.72±0.125 ^b	1.50±0.122 ^h	1.50±0.117 ^g	1.49±0.116 ^h	1.71±0.120 ^h
T1	21.08±0.118 ^c	20.08±0.113 ^d	19.05±0.111 ^c	20.04±0.120 ^d	21.01±0.121 ^d
T2	22.10±0.123 ^b	22.03±0.125 ^b	21.07±0.118 ^b	22.08±0.124 ^b	23.00±0.111 ^b
T3	16.09±0.121 ^g	15.03±0.121 ^g	13.01±0.125 ^f	16.03±0.129 ^g	17.20±0.112 ^g
T4	17.10±0.119 ^f	15.10±0.123 ^g	14.06±0.128 ^e	17.09±0.127 ^f	18.11±0.115 ^f
T5	18.11±0.124 ^e	16.07±0.127 ^f	14.12±0.122 ^e	17.11±0.122 ^f	18.11±0.124 ^f
T6	19.05±0.118 ^d	17.05±0.119 ^e	16.14±0.127 ^d	18.03±0.125 ^e	19.06±0.119 ^e
T7	22.04±0.122 ^b	21.09±0.110 ^c	19.11±0.128 ^c	21.08±0.121 ^c	22.04±0.126 ^c
T8	24.02±0.116 ^a	24.09±0.116 ^a	23.08±0.121 ^a	24.05±0.119 ^a	26.09±0.125 ^a

Note: Values are presented as mean ± SD. Different superscript letters (a, b, c, d, e, f, g and h) within the same column indicate significant differences among treatments at $p < 0.05$. Values sharing one or more common superscript letters are not significantly different.

3.2. Growth Performance of *Mystus cavasius* Under Different Treatments

3.2.1. Final Body Weight

In July, the starting weights of the fish were similar across all treatments and the control, ranging from 1.42 to 1.67 g, as depicted in Figure 2. As the months progressed, there was a general increase in the final body weight across all treatments and the control group, which was expected as the fish grew over time. The difference in weight between the control and treatment groups became more pronounced over time.

By November, the control group's final body weight was 7.19 g, while the treatment groups ranged from 8.12 to 11.83 g. This suggested that some treatments might have been more effective at promoting growth than the control condition. For example, in November, treatments T1, T7, and T8 had significantly different weights from the control. In conclusion, the data suggested that there were differences in the effectiveness of the various treatments applied in the BFT system on the growth of *Mystus cavasius*, with some treatments resulting in higher final body weights than the control group.

The superior performance of these treatments suggests that the combined effects of probiotic supplementation, an appropriate feeding regime, and optimized aeration created a more favorable culture environment for growth. Improved microbial activity in the BFT system likely enhanced nutrient recycling, maintained better water quality, and supplied additional microbial protein, thereby increasing nutrient availability and feed utilization efficiency. These conditions would have reduced metabolic stress and enabled fish to allocate more energy toward somatic growth rather than maintenance.

The observed trend is consistent with previous findings that probiotics and BFT systems improve growth performance by stimulating digestive enzyme activity, enhancing gut microbial balance, and increasing nutrient digestibility. In *Mystus cavasius*, probiotic application has been shown to significantly increase final body weight, specific growth rate, and feed efficiency compared with untreated controls, with laboratory-developed probiotic formulations producing the highest growth response Gaffar et al. (2023).

Similarly, comprehensive reviews of BFT have reported that biofloc serves not only as a natural supplementary feed rich in protein, vitamins, and essential amino acids but also improves water quality and reduces the feed conversion ratio, ultimately promoting faster growth and higher biomass production in cultured fish (Yu et al., 2023). Therefore, the higher final body weight recorded in the best-performing treatments of the present study indicates that integrating a suitable probiotic dosage, balanced feed rate, and effective aeration can substantially enhance the growth performance of *Mystus cavasius* under BFT culture conditions in Assam.

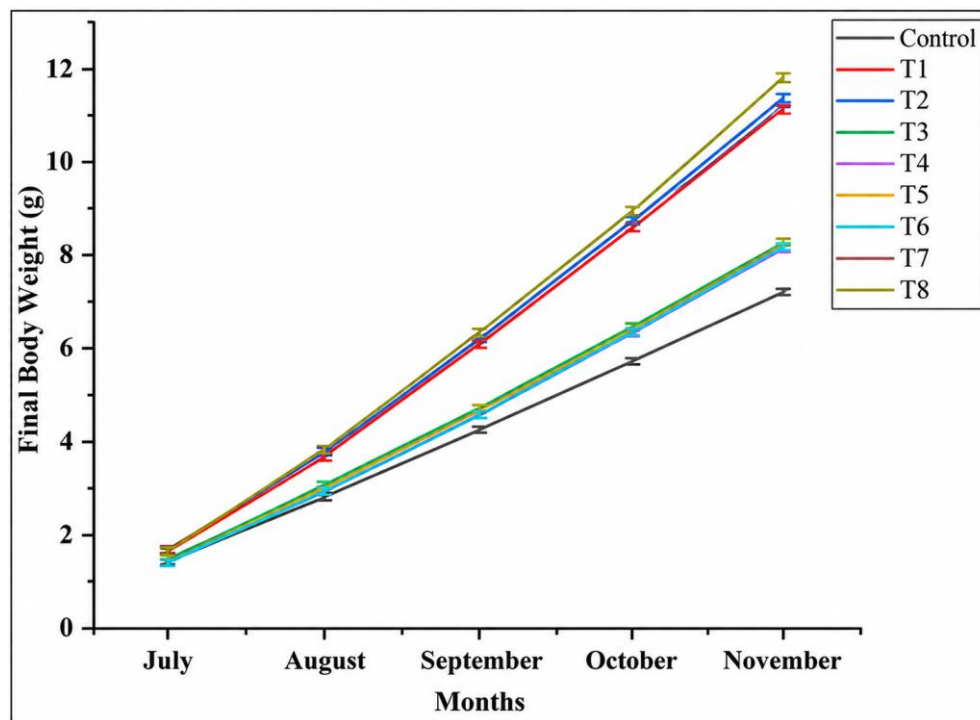


Figure 2. Final body weight trends across different treatments of *Mystus cavasius* over months.

3.2.2. Body Length Gain

The body length gain of *Mystus cavasius*, which appears to be the subject of the study, was assessed in a BFT system over the period from July to November. In July, the control group showed a body length gain of 0.37 cm, with a standard deviation of ± 0.176 . The length gain for the treatment groups T1 through T8 ranged from 0.33 cm (T5) to 0.6 cm (T8), as shown in the graph (Figure 3). In August, the control group had a length gain of 0.67 cm (± 0.260). The treatment groups showed a range from 0.63 cm (T4, T5, and T6) to 0.93 cm (T2 and T8). By September, the control group's gain increased to 0.7 cm (± 0.289). The treatment groups displayed a further increase, with T1 at 1.03 cm and T8 at 1.23 cm. In October, the control group's length gain was 0.73 cm (± 0.260), with the treatment groups' gains peaking at 1.3 cm for T8. In November, the final month recorded, the control group had a length gain of 0.77 cm (± 0.260), and the treatment groups reached up to 1.43 cm in T8, the highest recorded gain across all months and treatments. The superior performance of these treatments suggests that the combined influence of probiotic supplementation, an appropriate feed rate, and effective aeration created favorable conditions for skeletal and muscular development. Probiotics likely improved intestinal microbial balance and digestive efficiency, while biofloc particles provided an additional natural source of protein, vitamins, and essential nutrients. Adequate aeration further maintained dissolved oxygen at optimal levels, reducing physiological stress and allowing more dietary energy to be utilized for tissue growth rather than maintenance. The gradual rise in body length gain over the culture period also indicates that the biofloc ecosystem became more stable over time, enhancing nutrient recycling and supporting sustained fish growth.

The findings of the present study agree with those reported by Gaffar et al. (2023), who observed that probiotic supplementation significantly enhanced body length, weight gain, and overall growth performance of *Mystus cavasius* by improving feed digestion, nutrient absorption, and intestinal health. Likewise, Kumar et al. (2023) demonstrated that microbial inoculants in freshwater BFT systems enhanced digestive enzyme activity, promoted efficient nutrient utilization, and improved the growth of the indigenous catfish *Heteropneustes fossilis*. In a comprehensive review, Yu et al. (2023) concluded that BFT enhances fish growth by maintaining superior water quality while providing a continuous source of microbial biomass rich in protein and bioactive compounds. Therefore, the greater body length gain observed in treatments receiving suitable probiotic levels, balanced feeding, and proper aeration confirms that

integrating these management practices can effectively improve the growth performance of *Mystus cavasius* cultured under BFT conditions in Assam.

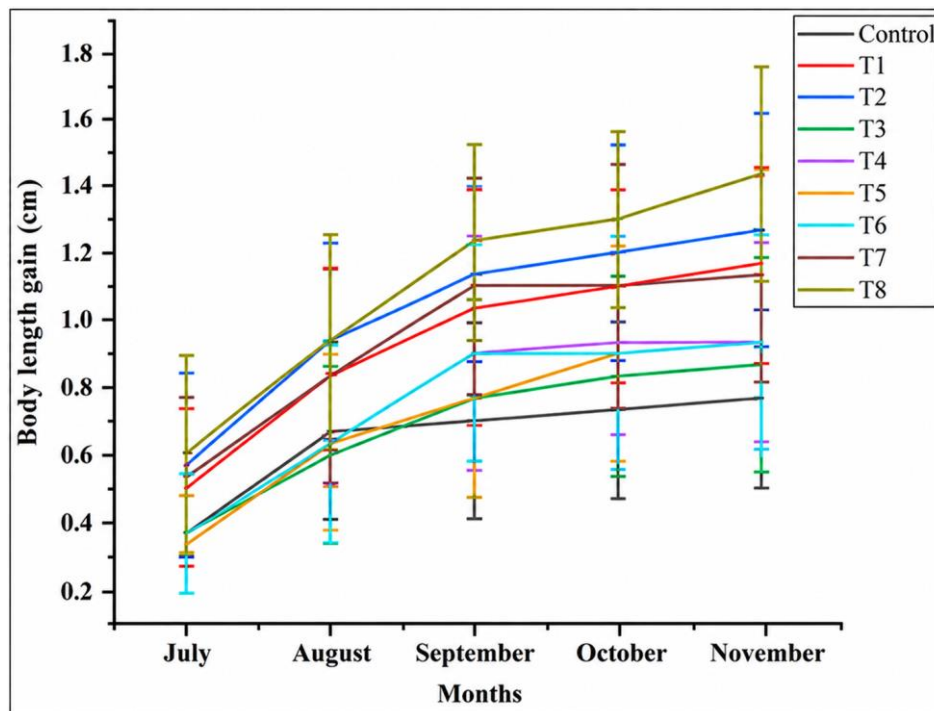


Figure 3. Body length gain trends of *Mystus cavasius* under different treatments by month.

3.2.3. Body Weight Gain

The body weight gain parameter was measured monthly from July to November for a control group and several treatment groups labeled T1 to T8. The results show consistent body weight gain over the months, starting at 0.73 g in July and ending at 0.73 g in November, with slight variations during the intermediate months. All treatment groups started with higher body weight gains in July than the control group, except T6, which started lower at 0.66 g. There seems to be an increasing trend as the months progressed, with T8 showing the most significant body weight gain, starting at 0.96 g in July and reaching 1.48 g in November. August and October showed particularly high gains across most treatment groups, with T2 reaching 1.05 g in August and T8 reaching 1.35 g in October. September and November also showed substantial gains, with T8 reaching 1.30 g and 1.48 g, respectively, as shown in the graph (Figure 4). The marked improvement in weight gain under these treatments indicates that the combined effects of probiotic supplementation, an appropriate feeding rate, and efficient aeration created a favorable rearing environment. The presence of beneficial microorganisms in the BFT system likely enhanced the decomposition of organic matter, improved water quality, and provided an additional source of microbial protein and essential nutrients. These conditions enhanced digestive efficiency and nutrient assimilation, allowing fish to convert feed into body mass more effectively while minimizing energy losses associated with environmental stress. The steady increase in body weight gain observed from September onwards also suggests that the biofloc community became well established over time, thereby providing continuous nutritional support and promoting sustained growth.

The present findings are in close agreement with those of Gaffar et al. (2023), who reported that probiotic supplementation significantly increased body weight gain and growth performance in *Mystus cavasius* by improving gut microbial balance, digestive enzyme activity, and nutrient utilization. Similarly, Kumar et al. (2023) demonstrated that microbial inoculants incorporated into freshwater biofloc systems enhanced weight gain, feed utilization efficiency, and overall health in *Heteropneustes fossilis* through improved microbial activity and digestive metabolism. Furthermore, Yu et al. (2023) concluded that BFT promotes superior growth by recycling nutrients into

microbial biomass, improving water quality, and providing a continuous natural food source rich in protein and bioactive compounds. Comparable observations were also reported by Khanjani, Mozanzadeh, Sharifinia, and Emerenciano (2023), who highlighted that the nutritional value of biofloc contributes significantly to increased growth and biomass production in cultured fish. Therefore, the higher body weight gain recorded in the present study confirms that integrating a suitable probiotic dosage, balanced feeding practices, and adequate aeration can substantially enhance the growth performance of *Mystus cavasius* cultured in BFT systems under the agro-climatic conditions of Assam.

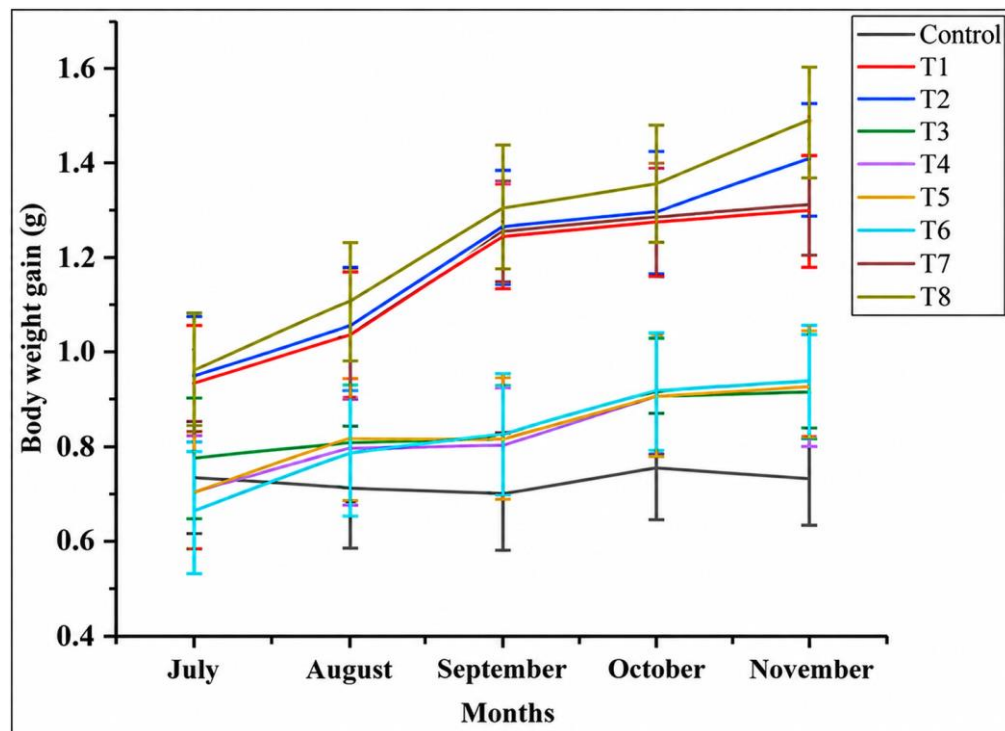


Figure 4. Body weight gain trends of *Mystus cavasius* across different groups over months.

3.2.4. Body Weight Index (BWI)

The BWI was measured for a control group and eight different treatment groups (T1 to T8) from July to November. The values are presented as means with standard deviations, indicating the variability around the mean. The BWI started at 109.90 in July and decreased each month steadily, reaching 10.18 by November, as shown in the graph (Figure 5). This decrease is expected, as the fish's body weight index generally decreases over time as conditions stabilize and fish growth rates slow after an initial period of rapid growth. All treatments started with higher BWI values in July than the control, indicating a possible initial positive effect of the treatments on fish growth. However, the extent of the decline varied among treatments, indicating that differences in probiotic supplementation, feeding rate, and aeration influenced fish growth and body condition. Treatments T7 and T8 consistently maintained comparatively higher BWI values than the control during the culture period, suggesting that these management combinations were more effective in supporting growth and improving the overall physiological condition of the fish. In contrast, treatments such as T4 and T5 recorded relatively lower BWI values by the end of the experiment, indicating that the growth-promoting effects under these conditions were comparatively limited. Probiotic bacteria improve the intestinal microbial balance, enhance digestive enzyme activity, and increase nutrient absorption, while the BFT system provides an additional natural protein source that supports fish growth and physiological condition. Similar improvements in growth performance and body condition of *Mystus cavasius* following probiotic supplementation have been reported by Banu, Akter, Islam, Mondol, and Hossain (2020), who observed significantly

higher weight gain, feed efficiency, and disease resistance in probiotic-treated fish. Likewise, Gaffar et al. (2023) demonstrated that dietary probiotics improved growth performance, survival, and intestinal morphology in *Mystus cavasius*, resulting in better nutrient utilization and overall health.

The gradual reduction in BWI during the later months of culture may be associated with normal ontogenetic changes, during which increases in body weight become proportionally balanced with body length as fish approach a more stable growth phase. Seasonal fluctuations in water quality, natural metabolic adjustments, and increased energy allocation towards maintenance rather than rapid somatic growth may also contribute to this decline. Nevertheless, the probiotic-treated BFT systems consistently maintained a superior body condition compared with the control, indicating that the combined effects of microbial flocs, balanced feeding, and adequate aeration created a more favorable culture environment. Similar observations have been reported by Kumar et al. (2023), who found that microbial inoculants in BFT systems significantly enhanced nutrient assimilation, digestive enzyme activity, and growth performance in freshwater catfish, while Yu et al. (2023) concluded that BFT improves fish condition by enhancing water quality, recycling nutrients, and providing continuous microbial protein for cultured fish. Therefore, the present findings suggest that integrating probiotics with an appropriate feed rate and aeration regime is an effective strategy for improving the body condition and growth efficiency of *Mystus cavasius* cultured under BFT conditions.

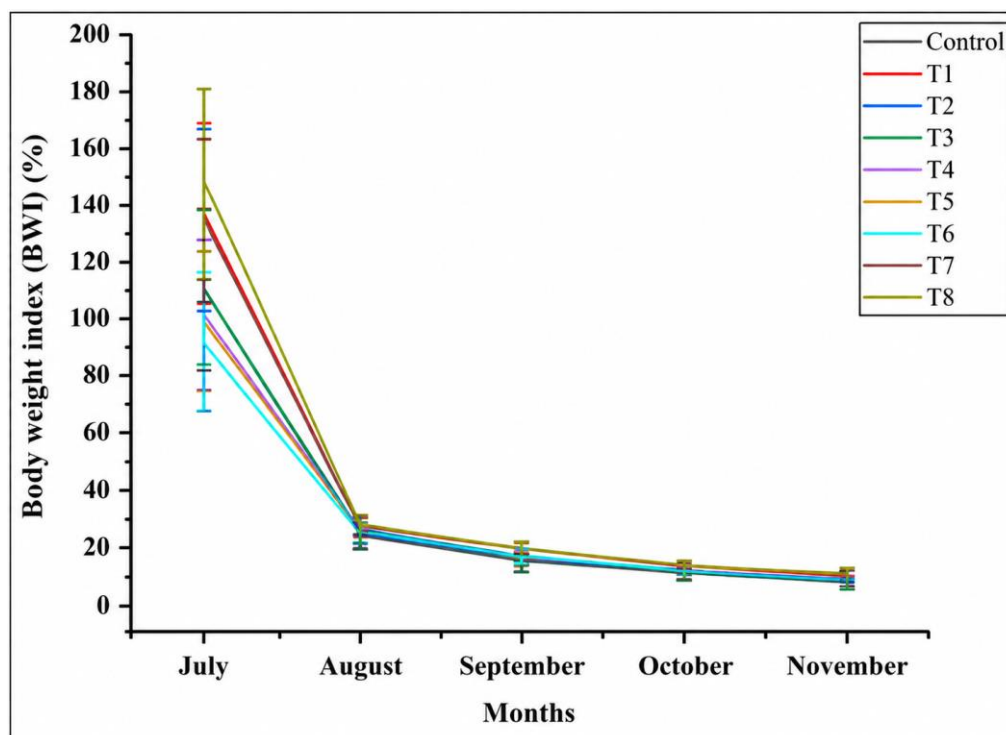


Figure 5. Body weight index trends of *Mystus cavasius* under different treatments by months.

3.2.5. Survival Rate

The survival rate of *Mystus cavasius* gradually declined from July to November in all experimental treatments; however, fish reared in the probiotic-based BFT systems consistently exhibited significantly higher survival than those in the control throughout the culture period. In July, the survival rate started high for all treatments, including the control, with the rates slightly higher in the treatments than in the control. This suggests that the BFT system may have provided a beneficial environment for the fish during this month. In August, there was a noticeable drop in survival rates across all treatments, including the control, but the treatments still maintained higher rates than the control. This drop could have been due to many factors, such as changes in weather, water quality, or fish adaptation

to the BFT system. From September to November, the trend of the treatments outperforming the control continued, but the overall survival rates for all groups declined as the months progressed, as shown in the graph (Figure 6). This decline could have been due to lower temperatures, which are typical during the later months of the year, or other environmental stresses. The improved survival observed in the probiotic-treated BFT systems indicates that the combined effects of probiotics, an optimized feed rate, and adequate aeration created a more favorable culture environment by maintaining better water quality, enhancing immune competence, and reducing physiological stress. Probiotic microorganisms are known to suppress opportunistic pathogens, improve gut microbial balance, stimulate digestive enzyme activity, and strengthen the innate immune response, thereby increasing fish resistance to environmental stress and disease. Likewise, Gaffar et al. (2023) demonstrated that probiotic supplementation significantly improved the survival, growth performance, and intestinal morphology of *Mystus cavasius*, confirming the beneficial role of probiotics in maintaining fish health under intensive culture conditions.

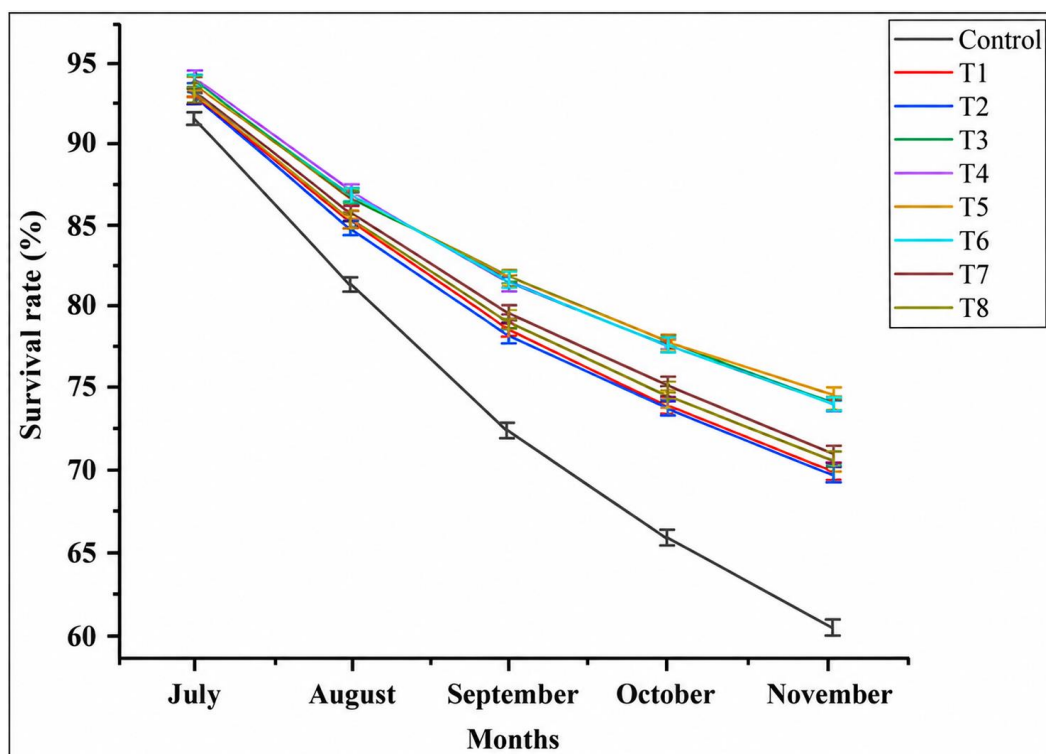


Figure 6. Survival rate trends of *Mystus cavasius* across different treatments over month.

The consistently higher survival recorded in the BFT treatments also highlights the importance of proper aeration and balanced feeding in maintaining stable microbial communities and favorable water quality. Continuous microbial activity in the BFT system converts toxic nitrogenous wastes into microbial biomass, while adequate aeration ensures sufficient dissolved oxygen for both fish and beneficial microorganisms. These conditions reduce environmental stress and improve fish resilience during prolonged culture. Similar observations have been reported by Kumar et al. (2023), who found that microbial inoculants in BFT systems significantly enhanced survival, digestive enzyme activity, and overall health in freshwater catfish through improved microbial balance and immune stimulation. Furthermore, Yu et al. (2023) concluded that BFT enhances fish survival by improving water quality, recycling nutrients, and providing natural microbial protein, thereby reducing stress and disease incidence under intensive aquaculture systems. Therefore, the present findings demonstrate that the integration of probiotics with an appropriate feed rate and efficient aeration is an effective management strategy for maximizing the survival and overall culture performance of *Mystus cavasius* under BFT conditions.

3.2.6. Specific Growth Rate (Weight)

The Specific Growth Rate (SGR) of *Mystus cavasius* decreased progressively from July to November in all experimental treatments, with the highest values recorded during the initial phase of culture and gradually declining as the fish matured. Treatments typically represent different conditions or variables being tested to assess their effects on fish growth. These could include different biofloc densities, varying feed compositions, water-quality conditions, and other factors. In July, the growth rate was highest across all treatments and the control, indicating that this may be a peak growth period for *Mystus cavasius* in the BFT system. T8 showed the highest SGR, at 5.91%. From August to November, as shown in the graph (Figure 7), there was a noticeable decline in growth rates for all groups. This trend is a normal biological phenomenon because younger fish exhibit rapid tissue deposition and higher metabolic activity, whereas the growth rate decreases with increasing body size. This pattern could also suggest that several biological or environmental factors are at play, such as the fish reaching a certain maturity stage, changes in temperature, or the system's carrying capacity. Comparing the control with the treatments, it is evident that some treatments outperformed the control in certain months, suggesting that the variables tested in these treatments may have had a beneficial impact on the growth of *Mystus cavasius*. The standard deviation values indicate the variability of growth rates within each group. A lower standard deviation suggests greater consistency in growth rates among the fish in that particular group.

Among the treatments, T8, T5, and T6 recorded comparatively superior SGR values, indicating that the combined application of probiotics, an appropriate feed rate, and adequate aeration enhanced growth efficiency. Probiotics improve digestive enzyme secretion, intestinal microbial balance, and nutrient absorption, while biofloc particles provide an additional source of microbial protein and essential nutrients that support continuous growth. Similar observations were reported by Gaffar et al. (2023), who found that dietary probiotics significantly improved the growth performance, survival, and intestinal morphology of *Mystus cavasius*. Likewise, Banu et al. (2020) demonstrated that probiotic supplementation enhanced specific growth rate, feed conversion efficiency, and disease resistance in *Mystus cavasius*, confirming the positive influence of probiotics on fish growth under intensive culture conditions..

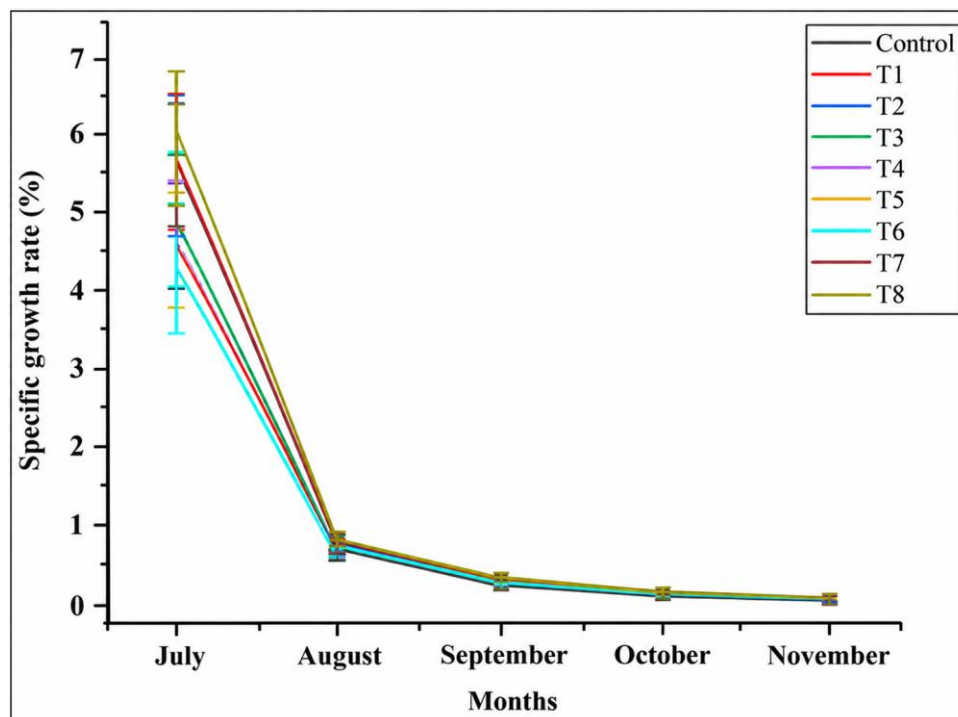


Figure 7. Specific growth rate trends of *Mystus cavasius* under different treatments over months.

The gradual reduction in SGR during the later months is also associated with increasing fish size, a reduced relative metabolic rate, and greater energy allocation towards body maintenance rather than rapid somatic growth. In BFT systems, however, the decline was less pronounced than in the control because microbial flocs continuously recycled nutrients, stabilized water quality, and supplied natural live feed, thereby reducing physiological stress and improving feed utilization. Adequate aeration further supported aerobic microbial activity and maintained sufficient dissolved oxygen, which is essential for efficient metabolism and growth. Similar findings were reported by Kumar et al. (2023), who observed that microbial inoculants in BFT systems significantly improved specific growth rate, digestive enzyme activity, and nutrient utilization in freshwater catfish. Furthermore, Yu et al. (2023) concluded that BFT enhances growth performance by improving water quality, increasing nutrient recycling, and supplying microbial biomass as a supplementary feed source. Therefore, the present findings indicate that integrating probiotics with an optimized feeding regime and effective aeration is an efficient strategy for sustaining higher specific growth rates and improving the overall production performance of *Mystus cavasius* cultured under BFT.

3.2.7. Feed Conversion Ratio (FCR)

The Feed Conversion Ratio (FCR) of *Mystus cavasius* increased gradually from July to November in all treatments, as shown in Figure 8, reflecting the normal reduction in feed utilization efficiency as fish grew larger and their metabolic demands changed over the culture period. Although FCR increased with time, fish reared in the probiotic-based BFT systems consistently exhibited lower FCR values than the control, indicating more efficient conversion of feed into body biomass. Among the experimental groups, treatments T6 and T7 maintained the lowest FCR throughout the study, whereas the control recorded the highest values during the later months of culture. A lower FCR is considered a positive indicator of efficient feed utilization and improved production performance, suggesting that the combined application of probiotics, an appropriate feeding regime, and adequate aeration enhanced nutrient digestibility and growth. Similar findings were reported by Banu et al. (2020), who observed significantly lower FCR and improved growth performance in *Mystus cavasius* supplemented with probiotic yeast, while Gaffar et al. (2023) demonstrated that dietary probiotics enhanced digestive efficiency, intestinal health, and feed utilization, resulting in superior growth and lower feed conversion ratios in *Mystus cavasius*.

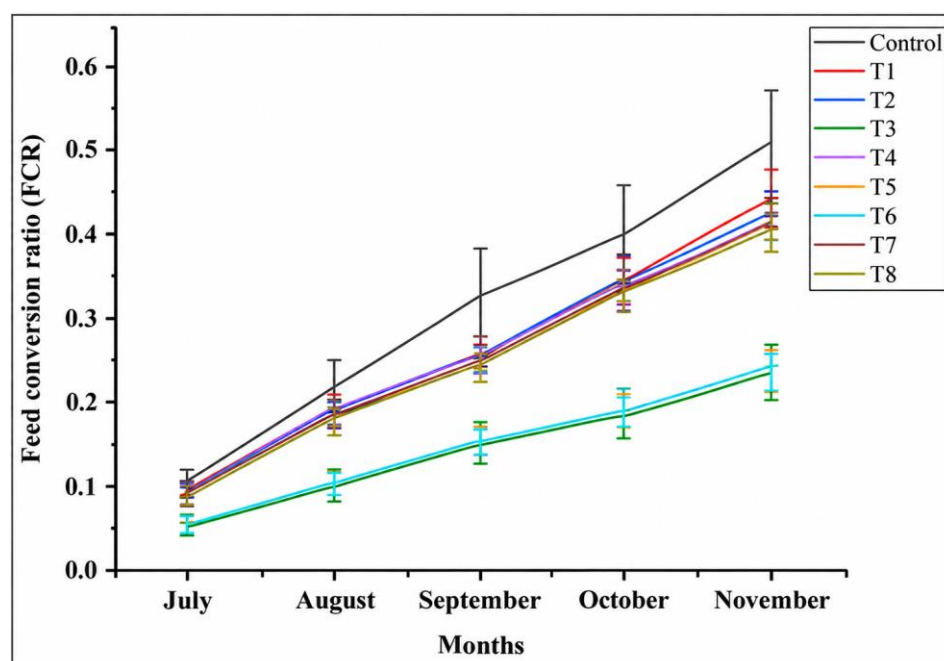


Figure 8. Feed conversion ratio trends of *Mystus cavasius* under different treatments by month.

The improved feed efficiency observed in the BFT treatments can be attributed to the synergistic effects of beneficial microorganisms, continuous nutrient recycling, and stable water quality. Biofloc particles serve as a natural supplementary feed rich in microbial protein, amino acids, vitamins, and digestive enzymes, reducing dependence on formulated feed while improving nutrient assimilation. At the same time, adequate aeration maintains high dissolved oxygen levels and supports the activity of nitrifying bacteria, thereby minimizing environmental stress and promoting efficient metabolism. These conditions collectively contribute to better feed conversion and sustained growth under intensive culture systems. Comparable results were reported by Kumar et al. (2023), who found that microbial inoculants significantly reduced FCR and improved growth performance of freshwater catfish cultured in BFT systems through enhanced digestive enzyme activity and nutrient utilization. Similarly, Yu et al. (2023) concluded that the BFT system improves feed efficiency by recycling nutrients, maintaining water quality, and providing natural microbial biomass as an additional food source, leading to lower feed costs and higher production efficiency. Therefore, the present findings indicate that integrating probiotics with an optimized feed rate and effective aeration significantly improves feed conversion efficiency and contributes to the sustainable culture of *Mystus cavasius* under the BFT system.

4. CONCLUSION

The present study clearly demonstrates that integrating probiotic supplements, appropriate feeding rates, and optimized aeration significantly enhances the growth performance of *Mystus cavasius* cultured under a BFT system. Compared with the control, fish reared in the treated BFT tanks exhibited superior growth, feed utilization, and survival, highlighting the effectiveness of combining microbial management with suitable feeding and aeration practices. Among the experimental treatments, T8 produced the highest body length gain (1.43 ± 0.318 cm), body weight gain (1.48 ± 0.120 g), and specific growth rate ($0.10 \pm 0.006\%$), indicating that this treatment provided the most favorable conditions for somatic growth. In contrast, T5 recorded the highest survival rate ($74.62 \pm 0.370\%$), suggesting that this treatment created a culture environment that effectively minimized stress and supported fish health throughout the experimental period. These findings indicate that, while T8 was the most effective treatment for maximizing growth, T5 was particularly successful in maintaining fish survival, emphasizing the importance of balancing production efficiency with stock health under intensive BFT culture.

The improved performance observed in the treated BFT systems can be attributed to the synergistic effects of probiotics, optimized feed management, and adequate aeration. Probiotic microorganisms enhanced nutrient digestion and absorption, while the biofloc community continuously recycled nitrogenous wastes into microbial biomass that served as an additional natural food source. Simultaneously, adequate aeration maintained dissolved oxygen concentrations necessary for fish metabolism and microbial activity, resulting in improved feed conversion, higher growth rates, and better overall culture performance. These results confirm that the BFT system is an effective and environmentally sustainable production system for the culture of indigenous *Mystus cavasius* in Assam.

The outcomes of this research have significant implications for fisheries development and sustainable aquaculture policy in India, particularly in the North-Eastern region. Government agencies, fisheries departments, and extension organizations should promote probiotic-assisted BFT as a recommended production system for indigenous catfish culture through training programmes, field demonstrations, and financial assistance under schemes such as the Pradhan Mantri Matsya Sampada Yojana (PMMSY) and state fisheries development initiatives. Standard operating protocols should be developed for probiotic application, feed management, and aeration to ensure uniform adoption and improve production efficiency across farms. In addition, the certification and quality control of commercial probiotics should be strengthened to ensure the availability of reliable microbial products for farmers. Establishing regional BFT centres and strengthening collaboration among research institutions, universities, and fisheries extension agencies will facilitate technology transfer and encourage wider adoption of scientifically validated culture practices. These measures can improve fish productivity, reduce environmental impacts through lower water

exchange and nutrient discharge, increase farmers' incomes, enhance food and nutritional security, and support the conservation and commercial farming of indigenous fish species such as *Mystus cavasius*.

Although the present investigation provides valuable information on the roles of probiotic supplements, feeding rate, and aeration in enhancing the growth of *Mystus cavasius* under BFT culture conditions, several limitations should be considered. The experiment was conducted at a single location in Assam during one production cycle; therefore, the findings may not fully capture seasonal or regional variations in culture performance. Only two probiotic levels, two feed rates, and two aeration schedules were evaluated, which may not represent the complete range of management options suitable for commercial aquaculture. Furthermore, the study primarily focused on growth performance, survival, and water quality, while analyses of digestive enzyme activity, immune responses, gut microbiota, microbial community dynamics, and molecular biomarkers were beyond its scope. Finally, because the experiment involved three management factors, future studies should employ a full-factorial statistical approach with larger sample sizes, multi-location validation, and year-round evaluations to better quantify the individual and interactive effects of probiotic supplements, feeding rate, and aeration. Such investigations will further strengthen scientific recommendations and facilitate the large-scale adoption of the BFT system for the sustainable cultivation of *Mystus cavasius* in India and other tropical regions.

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Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: Both authors contributed equally to the conception and design of the study. Both authors have read and agreed to the published version of the manuscript.

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