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Influence of probiotics, bioflocs, and their synergistic use on plankton community dynamics in *Litopenaeus vannamei* culture system

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Abstract
The present study investigates the influence of probiotics and biofloc technology on the dynamics of phytoplankton and zooplankton communities in *Litopenaeus vannamei* culture systems over an 80-day experimental period. The trial was conducted using four treatment groups: T1 (Control - standard commercial diet), T2 (Basal diet supplemented with probiotics), T3 (Basal diet with bioflocs), and T4 (Basal diet supplemented with both probiotics and bioflocs). The aim was to assess the impact of these dietary and microbial interventions on the natural plankton productivity, which plays a critical role in nutrient cycling, water quality maintenance, and provision of live feed for shrimp, especially during early developmental stages.
Phytoplankton and zooplankton densities were quantified at five sampling intervals: Days 0, 20, 40, 60, and 80. The planktonic communities were identified and enumerated using standard taxonomic keys and microscopic techniques. Results demonstrated a marked increase in both phytoplankton and zooplankton abundance in all experimental treatments compared to the control, with the highest proliferation observed in T4. The application of probiotics improved nutrient assimilation and altered microbial communities, while biofloc systems enhanced microbial biomass and nitrogen recycling, together creating a synergistic effect on plankton development.
Statistical analysis using one-way ANOVA followed by Tukey's HSD test confirmed significant differences ($p<0.05$) in plankton densities among treatments and over time. The findings underscore the ecological and nutritional benefits of integrating probiotics and bioflocs into shrimp culture systems, promoting the development of a self-sustaining, nutrient-rich environment. This dual approach has the potential to reduce reliance on artificial feeds, improve culture water quality, and enhance shrimp growth performance through natural food web enrichment. This study highlights the potential of integrating probiotics and biofloc systems as a sustainable, cost-effective strategy for improving ecological balance and output efficiency in intensive shrimp aquaculture.

Keywords: *L. vannamei*, probiotics, bioflocs, phytoplankton, zooplankton

1. Introduction

Aquaculture is one of the fastest-growing food-producing sectors globally, with shrimp farming-particularly of *Litopenaeus vannamei* (Pacific whiteleg shrimp)-playing a significant role in meeting the rising global demand for seafood. However, intensive shrimp culture systems often face serious challenges such as deterioration in water quality, accumulation of organic waste, disease outbreaks, and reduced feed efficiency [1, 2]. These issues are particularly pronounced in high-density systems where traditional water exchange methods are often insufficient or environmentally unsustainable.

The rapid expansion of aquaculture as a means of meeting global seafood demand has necessitated the development of sustainable and environmentally responsible production practices. Among the various species cultured worldwide, *Litopenaeus vannamei* (Pacific white shrimp) has emerged as one of the most economically important species due to its fast growth rate, tolerance to high stocking densities, and adaptability to a wide range of salinities [1, 3]. However, the intensification of shrimp culture systems often leads to several ecological and operational challenges, including water quality deterioration, accumulation of organic waste, nutrient imbalances, and increased disease incidence. Traditionally, chemical treatments and antibiotics were used to manage water quality and disease outbreaks. However, growing concerns regarding antimicrobial resistance, environmental contamination, and regulatory restrictions have prompted the exploration of eco-friendly alternatives [4]. In this context, the use of probiotics and biofloc technology (BFT) has gained substantial attention. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host by enhancing gut microbiota, improving digestion, and stimulating immune responses. In aquaculture, probiotics not only benefit shrimp health but also play a crucial role in improving water quality through the biodegradation of organic matter and suppression of pathogenic microbes [5, 6]. Biofloc technology, on the other hand, relies on the development of microbial flocs in the culture water by manipulating the carbon-to-nitrogen (C:N) ratio. The addition of carbon sources (e.g., molasses) stimulates heterotrophic bacterial growth, which assimilates inorganic nitrogen and converts it into microbial biomass [7, 8, 9, 10]. These bioflocs act as natural bioremediators and supplementary protein-rich feed for shrimp. In addition, bioflocs contribute to improved water quality by reducing ammonia, nitrite, and nitrate levels.

Probiotics and biofloc technologies are widely adopted to improve water quality, promote beneficial microbial communities, and enhance feed utilization. Probiotics stabilize gut microbiota and improve immunity, while bioflocs provide an in-situ protein-rich feed source and modulate nutrient dynamics [7, 8, 9]. However, limited studies have explored their effects on plankton communities-key biological indicators of aquatic health. Both probiotics and bioflocs positively influence the planktonic community, particularly phytoplankton and zooplankton, which are vital components of aquatic ecosystems. Phytoplankton serves as the primary producers, while zooplankton act as a crucial link between primary producers and higher trophic levels, including shrimp. Plankton abundance and diversity are often used as indicators of aquatic health, nutrient cycling, and productivity in culture systems. Despite the recognized individual benefits of probiotics and bioflocs, there is limited scientific understanding of their synergistic effects on plankton communities, especially in *L. vannamei* culture systems. An integrated approach that combines both could potentially harness the strengths of each technology, enhancing biological interactions within the system to create a more resilient and productive environment.

Therefore, the present study was designed to assess the individual and combined effects of probiotics and bioflocs on phytoplankton and zooplankton abundance and diversity over the culture period of *L. vannamei*. The study also aimed to correlate these plankton dynamics with shrimp growth performance under different treatment regimes, thereby contributing to the development of sustainable shrimp farming practices that rely on natural ecological processes. This study aimed to evaluate the individual and combined impacts of probiotics and bioflocs on phytoplankton and zooplankton communities and shrimp growth across four treatment regimes over an 80-day culture period.

2. Materials and Methods

2.1. Experimental Site and Animal Husbandry

The present study was conducted at shrimp culture units located in Allur (Latitude 15.00120° N; Longitude 80.05200° E), Nellore District, Andhra Pradesh, India. Specimens of *Litopenaeus vannamei* (Penaeid shrimp) were procured from local aquaculture ponds and transported to the experimental facility in oxygenated, double-layered polythene bags to minimize handling stress. Shrimp of uniform size were selected following thorough disease screening to ensure a pathogen-free stock. The selected individuals were transferred to acclimatization tanks containing seawater adjusted to the desired salinity. A one-week acclimatization period was maintained under field conditions, during which the shrimp were fed twice daily with a commercial formulated diet containing 35% crude protein (CP).

2.2. Experimental Setup

The feeding trial was conducted in cement tanks (5×10 m) embedded in submerged earth crust with earthen bottoms. Each tank had a water depth maintained at 1 meter, with a total water volume capacity of 3000 liters. Seawater with a salinity of 10 ± 0.5 ppt was used, and water for the experiment was drawn from the Buckingham Canal, treated in a designated pond, and subsequently distributed to all experimental tanks. Aeration was continuously provided using electric blowers connected to air compressors to ensure optimal dissolved oxygen levels. All tanks were covered with black netlon plastic sheets to regulate photoperiod and temperature, and shrimp were maintained under a natural 12 h light: 12 h dark cycle. Water exchange was carried out twice weekly, and dechlorinated seawater was added periodically to compensate for evaporation losses.

2.3. Experimental Design and Feeding Protocol

The present experiment was conducted to evaluate the individual and combined effects of probiotics and bioflocs on the Phytoplankton and Zooplankton populations during the culture operation of *Litopenaeus vannamei*. The study was conducted over a period of 80 days in a controlled outdoor aquaculture research facility. A total of 12 rectangular cement tanks (capacity: 3,000 litres each) were used, arranged in a completely randomized design (CRD) with four treatment groups and three replicates per treatment

- **T1 (Control):** No additives; traditional water management only.
- **T2 (Probiotics only):** Daily addition of a commercial probiotic mix.
- **T3 (Biofloc only):** Induction of biofloc formation via carbon supplementation.
- **T4 (Probiotics + Biofloc):** Combined application of probiotics and biofloc induction.

All tanks were aerated continuously using air stones connected to a central blower to maintain adequate dissolved oxygen and to keep flocs in suspension.

The study was arranged using a completely randomized design (CRD) involving various experimental treatments as independent variables. Acclimatized shrimp of uniform body weight (0.88 ± 0.06 g) were randomly stocked in the experimental tanks. Shrimp were initially fed experimental diets at 10% of their body weight, with the feeding rate gradually adjusted to 3-4% by the end of the 80-day feeding trial experimental period. The daily ration for each treatment group was calculated based on the weekly average body mass of shrimp obtained from subsampling. Feed was distributed in two equal portions daily at 06:00 and 18:00 hours.

2.4 Preparation of Probiotic Feed and Supplementation

Probiotic-enriched Feeds have been prepared following the method outlined by Naresh [11] and Aparna [8]. Bacterial species *Lactobacillus rhamnosus* as well as *Bacillus licheniformis* have been cultured in nutritional broth, extracted by centrifugation at the speed of 10,000 rpm for the time of 10 mins, washed with phosphate buffer, and re-suspended in PBS (pH 7.4). The re-suspended bacteria have been evenly mixed with the meal pellets using a spraying approach. The Probiotic mixed feed has been dried at the temp of 40°C, then sealed in airtight polythene coverings, and kept in a refrigerator for future usage. The Probiotic feed containing *B. licheniformis* and *L. rhamnosus* at a concentration of 10 billion cfu/kg was made weekly.

The basal diet was formulated to meet the nutritional requirements of *L. vannamei*, with the following approximate composition: 35% protein, 7% lipids, 15% carbohydrates, and 10% fiber. The ingredients included fishmeal, soybean meal, wheat flour, and vitamin-mineral premix. The feed was formulated to provide a balanced nutritional profile for optimal shrimp growth [8].

2.5 Biofloc Induction

Biofloc formation was induced in T3 and T4 groups by adjusting the C:N ratio to 15:1 using molasses as the carbon source. Carbon input was calculated based on estimated nitrogen input from feed and was added daily after water quality assessment. Water exchange was avoided to preserve biofloc integrity, and sludge was minimally siphoned only to remove excess solid waste as needed.

2.6 Water Quality Monitoring

Water quality parameters including temperature, pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite, and nitrate were monitored every 5 days using standard protocols:

- **Temperature and DO:** Measured using a multiparameter YSI probe.
- **Ph:** Determined using a portable digital pH meter.
- **TAN, nitrite, nitrate:** Analyzed using spectrophotometric methods (APHA, 2017) [12].

The C:N ratio was recalculated weekly and carbon input adjusted accordingly in T3 and T4.

2.7 Plankton Sampling and Analysis

Plankton samples were collected from each tank on days 0, 20, 40, 60, and 80. One-liter water samples were collected from mid-depth using a sterile container and preserved immediately

- **Phytoplankton:** Preserved in Lugol's iodine (1%) and analyzed under a light microscope using a Sedgwick-Rafter counting chamber. Identification was done up to the genus level using standard identification keys (e.g., Prescott, 1978; APHA, 2017) [13, 12].
- **Zooplankton:** Preserved in 4% formalin, filtered using a 50 µm mesh, and enumerated under a dissecting microscope. Identification followed Edmondson (1959) [15] and Dhanapathi (2000) [14].
- **Data were expressed as**
    - Phytoplankton: Cells/mL
    - Zooplankton: Individuals/L

Diversity indices (Shannon-Wiener index, species richness) were also calculated using the PAST software.

Statistical Analysis

Data were analyzed using one-way ANOVA with post-hoc Tukey HSD test ($p<0.05$). Software: SPSS v26. Plankton count data were log-transformed where necessary to meet assumptions.

3. Results and Discussion

The results presented in Tables. 1-6 depict the distribution and progression of phytoplankton and zooplankton biomass under different feeding treatments over an 80-day culture period. The treatments include Control, Probiotic Added, Bioflocs Added, and a combination of Probiotic and Bioflocs (PB + BF), with values compared based on absolute counts and percentage changes over Control (PDC) and over their respective experimental baselines (PDE).

Phytoplankton dynamics

Phytoplankton levels in the control group exhibited a gradual increase from Day 0 (679) to Day 80 (897), reflecting a total rise of approximately 32% over the culture period. This modest change suggests limited natural enhancement of phytoplankton in the absence of treatment. In contrast, the probiotic-treated group showed a marked increase in phytoplankton levels, from 679 at Day 0 to 4390 at Day 80-corresponding to a 547% increase over the control (PDC) and a 389% increase relative to its baseline (PDE). The consistent upward trend across all sampling intervals indicates that probiotic supplementation significantly promotes phytoplankton productivity, likely through improved microbial balance and nutrient cycling. Similarly, the group supplemented with bioflocs experienced a substantial rise in phytoplankton concentration, increasing from 679 at Day 0 to 4821 at Day 80. This represents a 610% increase over the control and a 437% increase from baseline. The enhanced phytoplankton biomass is attributable to the nutrient recycling and natural food enrichment properties inherent in biofloc systems. The most pronounced effect was observed in the group receiving both probiotics and bioflocs (PB + BF). Phytoplankton levels in this group peaked at 5623 on Day 60 and, despite a slight decline to 5122 by Day 80, remained significantly higher than in all other groups. At Day 80, this treatment resulted in a 654% increase over the control (PDC) and a 471% increase over its baseline value (PDE). The initial peak suggests a synergistic interaction between probiotics and bioflocs, creating an optimal environment for phytoplankton proliferation. However, the slight reduction by Day 80 may indicate the onset of nutrient limitation or system saturation, warranting further investigation.

Statistical Significance: Superscript letters denote statistically significant differences between treatments at each sampling interval ($P<0.05$). From Day 20 onward, the control group consistently differed significantly from all experimental groups. Among treatments, the PB + BF group exhibited significantly higher phytoplankton levels on both Day 60 and Day 80, underscoring the enhanced effectiveness of combining probiotics and bioflocs in maximizing phytoplankton biomass.

Phytoplankton Group Composition and Temporal Trends

The temporal dynamics of phytoplankton communities under varying feeding strategies revealed distinct patterns in both composition and abundance, profoundly influenced by the supplementation of probiotics, bioflocs, or their synergistic combination (Tables 2 & 3).

Control Group (T1): Limited Natural Enhancement

In the control treatment, *Bacillariophyceae* consistently dominated, accounting for 52% at Day 0 and peaking at 69% by Day 60, before a slight decline to 61% at Day 80. *Chlorophyceae* exhibited a downward trend, decreasing from 27% to 14% by Day 80. Meanwhile, Cyanophyceae and *Euglenophyceae* remained minor constituents throughout the culture period, never exceeding 11% and 6%, respectively. Total phytoplankton numbers rose modestly from 679 to 897 cells mL^{-1}, reflecting a percent difference change (PDC) of +32%. This modest increase underscores the limited phytoplankton productivity in the absence of microbial intervention.

Probiotic-Supplemented Group (T2): Enhanced Diversity and Balance

The addition of probiotics resulted in a dramatic rise in total phytoplankton abundance, reaching 4390 cells mL^{-1} by Day 80 +547% increase over the control. While *Bacillariophyceae* remained the predominant group, its relative proportion declined to 35% by Day 80. In contrast, there was a notable rise in the abundance and evenness of *Dinophyceae*, *Euglenophyceae*, and Cyanophyceae, each comprising 10-15% of the total population. This shift indicates that probiotics support a more heterogeneous phytoplankton community, potentially by enhancing organic matter decomposition and nutrient remineralization, which together foster a dynamic microbial ecosystem.

Biofloc-Supplemented Group (T3): Diatom and Green Algae Enrichment

In the biofloc-enriched group, phytoplankton counts surged to 4821 cells mL^{-1} by Day 80, marking a +610% PDC. *Bacillariophyceae* sustained dominance (~50%) throughout, accompanied by a robust *Chlorophyceae* contribution (27%). Unlike the probiotic treatment, the biofloc group exhibited minimal increases in *Dinophyceae* and *Euglenophyceae* (1-3%), suggesting that biofloc particles-rich in organic carbon and microbial biomass-preferentially support fast-growing primary producers like diatoms and green algae that efficiently assimilate regenerated nutrients.

Combined Probiotics + Biofloc Group (T4): Synergistic Peak in Biomass and Diversity

The combination treatment (PB + BF) produced the highest phytoplankton biomass, reaching a peak of 5623 cells mL^{-1} at Day 60, corresponding to a +728% PDC over control. *Bacillariophyceae* remained the dominant group (46-54%), but diversity indices improved substantially with significant contributions from *Dinophyceae* (13%) and *Euglenophyceae* (12%). Initially, *Chlorophyceae* showed an upward trend but declined to 19% by Day 80, indicating a successional shift within the phytoplankton community. A minor decline in total numbers at Day 80 (5122 cells mL^{-1}) may suggest ecological stabilization or nutrient limitation in the later stages of culture.

Comparative Analysis: Patterns and Implications

Dominance Pattern

Across all treatments, *Bacillariophyceae* was the primary contributor. However, in probiotic and PB + BF treatments, its dominance was moderated, reflecting a more balanced phytoplankton assemblage. This shift suggests improved ecological equilibrium and trophic structuring under microbial enhancement.

Diversity Induction

Probiotic-containing treatments-especially the combined PB + BF group-stimulated the emergence of less dominant phytoplankton such as *Euglenophyceae* and *Dinophyceae*. This enhanced diversity is likely due to improved nutrient cycling, organic matter degradation, and stimulation of niche-specific growth conditions.

Productivity Enhancement

The most pronounced increases in total phytoplankton biomass were observed in biofloc and PB + BF groups. These findings reaffirm the role of bioflocs as nutrient-rich matrices that boost primary productivity by recycling organic wastes and promoting microbial trophic interactions.

Ecological and Aquacultural Implications

This study underscores the transformative impact of microbial-based interventions on aquatic ecosystem functioning. Probiotic and biofloc strategies not only elevated phytoplankton biomass but restructured community compositions, offering multifaceted benefits:

- **Ecological Stability:** A diverse and balanced phytoplankton community supports resilience, efficient nutrient turnover, and reduced risk of monoculture dominance.
- **Nutritional Value:** *Bacillariophyceae*, the consistent dominants, serve as high-quality feed for shrimp, rich in essential fatty acids.
- **Immunological Support:** The rise in niche phytoplankton groups under probiotic influence could contribute bioactive compounds beneficial for shrimp gut health and immunity.
- **Synergistic Effects:** The combined use of probiotics and bioflocs orchestrates a robust microbial loop, enhancing biodiversity, improving water quality, and elevating natural productivity.

In conclusion, integrating probiotics and biofloc technology presents a promising avenue for reimagined, ecologically grounded aquaculture practices, fostering both sustainable biomass production and ecosystem integrity.

Phytoplankton Group Composition and Trends

The temporal analysis of phytoplankton communities under various feeding strategies revealed substantial differences in both abundance and taxonomic composition, driven by the supplementation of probiotics, bioflocs, or their synergistic use (Tables 2 & 3).

In the control group, *Bacillariophyceae* consistently dominated the phytoplankton assemblage throughout the culture period, increasing from 52% on Day 0 to 69% by Day 60, before slightly decreasing to 61% at Day 80. *Chlorophyceae* demonstrated a declining trend, dropping from 27% to 14% over the same period. Cyanophyceae and *Euglenophyceae* remained minor constituents, never exceeding 11% and 6%, respectively. Total phytoplankton abundance increased modestly from 679 to 897 cells/mL, reflecting a percent difference change (PDC) of +32%, indicative of limited natural productivity in the absence of microbial enhancements. In the probiotic-treated group, total phytoplankton abundance increased significantly, from 679 (Day 0) to 4390 (Day 80), reflecting a +547% PDC over the control. Although *Bacillariophyceae* remained the dominant group, its relative proportion declined to 35% by Day 80. Notably, there was a marked increase in *Dinophyceae*, *Euglenophyceae*, and Cyanophyceae, each contributing 10-15% to the total phytoplankton composition. This increased diversity suggests that probiotics facilitated a more balanced and heterogeneous microbial environment, likely enhancing nutrient cycling and promoting niche diversification within the phytoplankton community. In the biofloc-only treatment, phytoplankton abundance peaked at 4821 by Day 80, representing a +610% PDC. *Bacillariophyceae* maintained dominance (~50%) throughout, with *Chlorophyceae* contributing a stable 27%. In contrast to the probiotic treatment, *Dinophyceae* and *Euglenophyceae* remained negligible (1-3%), implying that biofloc environments primarily favored the proliferation of diatoms and green algae. The nutrient-rich organic aggregates in bioflocs appeared to selectively support fast-growing primary producers with efficient nutrient uptake strategies. The combined treatment with Probiotics + Bioflocs (PB + BF) resulted in the highest phytoplankton biomass, peaking at 5623 cells/mL on Day 60, with a remarkable PDC of +728%. *Bacillariophyceae* remained dominant (46-54%), but overall diversity increased markedly, with *Dinophyceae* and *Euglenophyceae* reaching 13% and 12%, respectively, at peak levels. *Chlorophyceae* initially increased but declined to 19% by Day 80, indicating a dynamic shift in community structure. A slight reduction in total phytoplankton from 5623 to 5122 at Day 80 may reflect nutrient depletion, ecological feedback, or the onset of equilibrium within the microbial loop. Despite this dip, the PB + BF treatment consistently yielded the highest phytoplankton density and diversity throughout the study.

Comparative Analysis of Phytoplankton Dynamics

- **Dominance Pattern:** *Bacillariophyceae* emerged as the primary phytoplankton group across all treatments. However, its relative dominance was more balanced in the experimental groups, indicating more equitable community composition under probiotic and biofloc influence.
- **Diversity Induction:** Probiotic supplementation, alone or in combination, facilitated the emergence of less-represented groups such as *Euglenophyceae* and *Dinophyceae*. This may be attributed to enhanced organic matter decomposition and efficient nutrient remineralization, favoring a broader range of ecological niches.
- **Productivity Enhancement:** Biofloc-based systems, both alone and in synergy with probiotics, showed the greatest increases in phytoplankton biomass. This demonstrates the role of microbial floc aggregates in recycling nutrients and supporting robust primary production.

Ecological and Aquacultural Implications

The observed shifts in phytoplankton dynamics underscore the ecological benefits and aquacultural value of microbial-based interventions

- **Microbial Stimulation:** Both probiotics and bioflocs significantly enhanced not only phytoplankton biomass but also altered community composition, improving ecological complexity and nutrient cycling.
- **Stable Autotrophic Base:** *Bacillariophyceae*, being consistently dominant, provided a reliable base of nutritional support, particularly in biofloc-dominated systems where their stability is advantageous for trophic transfer to shrimp.
- **Enhanced Functional Diversity:** Probiotic-enriched systems promoted higher diversity, including groups potentially associated with immune stimulation and gut health enhancement in shrimp.
- **Synergistic Efficacy:** The combined PB + BF treatment emerged as the most effective strategy, suggesting complementary mechanisms that promote biodiversity, ecosystem stability, and enhanced natural productivity in shrimp aquaculture systems.

Zooplankton Community Dynamics under Different Treatments

Zooplankton play a pivotal role in aquaculture systems by acting as live feed of high protein and lipid content, particularly valuable during the early developmental stages of *Litopenaeus vannamei*. The present study evaluated zooplankton community dynamics across four treatments-Control, Probiotics, Bioflocs, and Probiotics + Bioflocs (PB + BF)-over an 80-day culture period, as presented in Tables 4-6. Quantitative and qualitative shifts in zooplankton abundance and composition highlighted the influence of microbial interventions on secondary productivity and trophic structuring.

Control Group: Baseline Productivity with Limited Microbial Influence

In the absence of microbial additives, the control tanks exhibited a modest rise in zooplankton from 88 individuals at Day 0 to 301 by Day 80 (+242% PDC). Copepods were predominant (34-48%), with protozoa (20-31%) and rotifers (13-23%) as secondary contributors. Cladocerans and larval forms remained minimal. This limited diversity and abundance indicate constrained nutrient recycling and a lack of microbial stimulation. The dominance of copepods reflects dependency on naturally occurring phytoplankton blooms, but the absence of organic enrichment and microbial loop activity restricted broader zooplankton proliferation.

Probiotic-Treated Group: Enhanced Nutrient Cycling and Trophic Support

Probiotic application led to a marked increase in zooplankton, peaking at 583 individuals at Day 60 and stabilizing at 430 by Day 80 (+389% PDC). The group maintained copepod dominance (33-40%), with increased presence of protozoa (23-28%), rotifers (13-19%), and Cladocerans (up to 10%). Probiotics contributed to improved water quality, enzymatic decomposition of organic matter, and enhanced nutrient mineralization. These processes boosted phytoplankton growth, forming a strong base for zooplankton development. The higher abundance of rotifers and protozoa points to intensified microbial loop activity, with better bacterial biomass recycling and transfer to higher trophic levels, benefiting shrimp digestion and nutrient assimilation.

Biofloc-Treated Group: Particulate Nutrition and Microhabitat Enrichment

Biofloc systems generated a substantial increase in zooplankton numbers, reaching 778 individuals at Day 60 and

518 at Day 80 (+489% PDC). Copepods (48-53%) remained dominant, while protozoa (17-32%) and rotifers (13-14%) were well-represented. Biofloc environments provide suspended aggregates of microbes, detritus, and algae that serve as both food and refuge for zooplankton. The flocs enhanced microbial colonization and nutrient retention, leading to a detritus-based food web. The simultaneous rise in protozoa and Cladocerans reflects a complex, high-nutrient ecosystem that supports diversified zooplankton populations and improved shrimp performance.

PB + BF Group: Synergistic Ecosystem Optimization

The combined treatment of probiotics and bioflocs yielded the highest zooplankton abundance, peaking at 908 individuals at Day 60 and maintaining 651 at Day 80 (+640% PDC). Copepods were the major group (46-58%), followed by protozoa (15-27%), rotifers (12-14%), Cladocerans (9-10%), and larval forms (up to 10%). This synergistic system optimized both autotrophic and heterotrophic nutrient cycling. Probiotics enhanced microbial diversity and enzyme production, while bioflocs provided a constant supply of particulate nutrients and microbial substrates. The co-existence of microalgae and bacterial flocs established a stable food base, supporting diverse zooplankton groups. The prevalence of protozoa and larval forms highlights efficient energy transfer to shrimp, making PB + BF treatments superior for both ecological balance and nutritional output.

Comparative Trends and Implications for Shrimp Culture

- **Control:** Demonstrated slow zooplankton succession with limited diversity due to absence of microbial stimulation.
- **Probiotics:** Enhanced nutrient turnover and phytoplankton support, moderately boosting zooplankton proliferation.
- **Bioflocs:** Provided nutrient-rich microhabitats and diverse food sources, significantly increasing zooplankton abundance.
- **PB + BF:** Created a synergistic ecosystem with the highest zooplankton diversity and density, optimizing shrimp feed availability and water quality.

The increased zooplankton biomass in PB + BF treatments supports

- Reduced dependence on commercial feeds via continuous live feed availability.
- Improved feed conversion efficiency and shrimp growth.
- Enhanced pond ecology with lower disease susceptibility and better resilience.

The following Table shows the Comparative Overview of Taxonomic Trends during *L.vannamei* culture operation.

Group	Highest in	Functional Significance
Copepods	PB + BF > Biofloc	Rich in lipids; vital for post-larval shrimp nutrition
Rotifers	PB + BF > Probiotic	Easy to digest; excellent for early larval stages
Cladocerans	PB + BF > Biofloc	High reproduction rate; water quality indicators
Protozoa	PB + BF > Probiotic	Key in microbial loop; bacterial grazers
Larval Forms	PB + BF	Indicate healthy trophic succession and ecosystem productivity
Others	Moderate across	Diverse contributors including ostracods and nematodes

The diversity and abundance of zooplankton are crucial determinants of feed efficiency, growth performance, and immune competence in *Litopenaeus vannamei*. In this study, it was observed that the integration of probiotics and biofloc systems (PB + BF) established a dynamic, multi-trophic aquatic environment, significantly reducing reliance on artificial feeds. Notably, protozoans and copepods, due to their high protein content and appropriate size, provided ideal natural prey for shrimp larvae. Additionally, rotifers and Cladocerans, recognized for their soft-bodied structure and rapid reproduction, ensured a consistent supply of natural feed throughout the culture period. The increased presence of larval zooplankton in the PB + BF treatments indicates robust ecological succession and efficient nutrient recycling. These factors collectively contributed to improved survival rates, enhanced feed conversion ratio (FCR), and overall sustainability of the aquaculture operation. Data from Table 5 clearly demonstrate that microbial supplementation significantly enhanced both the quantitative and qualitative aspects of the zooplankton community. The control group showed only a slow, steady increase in total zooplankton with limited diversity. In contrast, all treatments involving probiotics, bioflocs, or their combination led to substantial increases in zooplankton, particularly in key groups such as rotifers, Cladocerans, copepods, and protozoans, which are vital live feeds for shrimp juveniles.

Among all treatments, the PB + BF combination consistently yielded the highest zooplankton counts, peaking at 908 individuals by day 60, representing a 932% increase from the baseline and a 188% increase over the control. Copepods emerged as the dominant group, particularly in the PB + BF group, where their proportion exceeded 50% at peak culture stages. This dominance reflects the favorable environmental conditions facilitated by microbial interventions, promoting the proliferation of nutritionally rich zooplankton species. The improved availability of zooplankton in the biofloc-based systems can be attributed to enhanced organic matter recycling, microbial colonization, and the establishment of stable trophic interactions. Probiotics likely contributed by enriching microbial diversity and improving water quality, thereby creating favorable conditions for zooplankton growth. The synergistic effects of probiotics and bioflocs supported both the nutritional demands and microbial prey availability, enhancing the reproductive efficiency of zooplankton. Sustained growth of protozoans and larval forms in the PB + BF treatment further underscores the establishment of a balanced, multi-level trophic system. Beyond their role as feed, these organisms also serve as bioindicators of water quality and ecosystem stability.

Phytoplankton Dynamics and Ecological Implications

As primary producers, phytoplankton play a pivotal role in aquaculture ecosystems by facilitating nutrient cycling, oxygen production, and serving as a foundational food source. Table 1 presents clear trends indicating that phytoplankton abundance and diversity were significantly enhanced by microbial interventions across all experimental treatments. In the control group, phytoplankton counts increased modestly from 679 to 897 cells over the 80-day period (a 32% increase), with Bacillariophyceae (diatoms) consistently dominating the community (52-69%). However, this increase was limited and reflected only background productivity, with minimal ecological enrichment. In contrast, probiotic supplementation led to a notable rise in phytoplankton density, reaching 4390 cells by day 80, 547% increase over the control. Although Bacillariophyceae initially remained dominant, there was a marked rise in Cyanophyceae, *Dinophyceae*, and *Euglenophyceae*, reflecting improved microbial mineralization and nutrient cycling, possibly facilitated by the probiotics' enzymatic and metabolic activity. The biofloc-only treatment further elevated phytoplankton abundance to 4821 cells, marking a 610% increase over the control. In this group, Bacillariophyceae and *Chlorophyceae* were predominant, but the system also supported the growth of more diverse phytoplankton groups due to enhanced nutrient availability and microbial loop functionality provided by biofloc particles. The combined PB + BF treatment demonstrated the most profound effects, achieving a peak of 5623 cells by day 60 and a final count of 5122 cells at day 80, equivalent to a 654% increase. This treatment fostered a well-balanced phytoplankton community, with elevated levels of *Euglenophyceae* and *Dinophyceae*, both associated with improved water quality and heterotrophic interactions. The ecological and nutritional relevance of these findings is significant. Bacillariophyceae, due to their high protein and polyunsaturated fatty acid (PUFA) content, are essential to shrimp health. The increased representation of *Cyanophyceae* and *Dinophyceae* in probiotic and PB + BF treatments indicates a more complex and productive microbial food web.

Discussion

The present study evaluated the temporal changes in phytoplankton and zooplankton communities under four distinct culture treatments-Control, Probiotics, Bioflocs, and the combined Probiotics + Bioflocs (PB+BF)-across a shrimp culture period of 80 days. The results underscore the ecological significance of microbial-based interventions in enhancing plankton productivity and community dynamics, ultimately contributing to the nutritional ecology of *L. vannamei*.

1. Phytoplankton Community Dynamics

The phytoplankton population exhibited a significant upward trend across all treatments compared to the Control, with the most pronounced increase in the PB+BF group (Table 1). The Control treatment showed a modest increase of 32% over the 80-day period, indicating limited autochthonous productivity in the absence of stimulatory inputs.

In contrast, the Probiotics and Biofloc treatments stimulated phytoplankton blooms by 547% and 610% respectively by Day 80, aligning with earlier studies that attributed such enhancement to nutrient recycling, organic matter mineralization, and microbial nutrient conversion [16, 17].

Notably, the PB+BF treatment recorded a 654% increase, suggesting a synergistic interaction wherein probiotics likely facilitated the conversion of organic substrates (via enzyme secretion and bioavailability of nitrogen/phosphorus), while bioflocs served as a substrate for microbial colonization and nutrient retention [18].

Taxonomic analysis (Table.2) revealed a sustained dominance of *Bacillariophyceae* (diatoms) across all treatments, especially in PB+BF and Biofloc groups. Diatoms are known for their high nutritional value, providing essential fatty acids (EPA, DHA) and being readily assimilable by shrimp[19]. The presence of *Euglenoids*, *Dinoflagellates*, and *Chlorophyceae* also increased under the experimental treatments, contributing to planktonic diversity. Their proliferation under probiotic influence can be attributed to the improved water quality and microbiome balance that probiotics impart [20].

2. Zooplankton Community Dynamics

Zooplankton counts followed a pattern similar to phytoplankton (Tables 4-6). The initial zooplankton numbers were uniform across all treatments (88 individuals), yet by Day 80, PB+BF treatments peaked at 651 individuals, a 640% increase from baseline, and 116% increase over PDE. The peak abundance occurred consistently on Day 60 across all treatments (Table 6), indicating a predictable trophic build-up coinciding with mid-culture stages.

Among zooplankton taxa, *Copepods* and *Protozoans* were dominant (Table 5), particularly in PB+BF systems. *Copepods* are of paramount importance in shrimp larviculture due to their rich protein and fatty acid profile, which are essential for larval metamorphosis and growth [21]. Their enhanced abundance in biofloc-rich environments could be due to detrital food chains supported by microbial flocs, which serve both as feed and habitat [22]. Similarly, *Protozoa* showed notable proliferation, particularly in PB+BF (175 individuals at Day 80), suggesting their role as primary consumers of microbial biomass and phytoplankton. *Rotifers* and Cladocerans, known for their soft-bodied structure and high reproductive rate, were more abundant in biofloc and probiotic groups, likely favored by enriched organic substrates and stable microbial interactions (Dhanapathi, 2000) [14].

3. Synergistic Interactions and Trophic Efficiency

The combined PB+BF treatment consistently outperformed singular applications of either probiotics or bioflocs. This synergism likely arises from complementary mechanisms: while probiotics enhance water quality and nutrient transformation through microbial metabolism, bioflocs contribute organic substrates and stabilize trophic cascades by supporting a detritus-based food web [23]. The resulting microbial loop supports both autotrophic and heterotrophic plankton, leading to diversified and sustained blooms beneficial to shrimp.

This is corroborated by earlier findings where integrated microbial strategies led to improved feed conversion ratio, water quality, and plankton availability [24, 25]. The trophic transfer of microbial biomass via protozoa, rotifers, and copepods to shrimp represents a cost-effective, ecological alternative to traditional feed-heavy systems.

Conclusion and Implications for Sustainable Aquaculture

This study clearly demonstrates that microbial dietary amendments, particularly the synergistic application of probiotics and biofloc technology, lead to significant improvements in both phytoplankton and zooplankton dynamics. The enhanced abundance and diversity of plankton communities translate into:

- Increased availability of natural food,
- Improved shrimp digestion and immunity,
- Reduced dependency on commercial feeds,
- Better water quality and nutrient recycling,
- Enhanced survival, growth performance, and FCR.

These improvements collectively reinforce the ecological integrity and economic viability of *L. vannamei* farming systems.

Thus, the integrated use of probiotics and biofloc technology (PB + BF) emerges as a key innovation for advancing sustainable, intensive shrimp aquaculture. By fostering a resilient and self-sustaining planktonic ecosystem, this approach aligns with the principles of ecological aquaculture-minimizing environmental impact while maximizing productivity and health outcomes in shrimp culture systems.

The current study highlights the positive influence of microbial-based interventions on plankton community structure and abundance in *L. vannamei* culture systems. Notably, the synergistic application of probiotics and bioflocs (PB+BF) led to the highest diversity and abundance of both phytoplankton and zooplankton. These enhancements not only improve the natural feed availability but also promote better water quality and ecological stability, suggesting a promising sustainable strategy for intensive shrimp aquaculture.

Table1: Consolidated Table showing Phytoplankton Distribution

Type of Feeding Trail	Days of Culture				
	0	20	40	60	80
Control	679^{a} PDC	687^{a} (-12)	814^{b} (+20)	870^{b} (+28)	897^{c} (+32)
Probiotic Added	679^{a} PDC PDE	2567^{b} (+278) (+274)	3911^{b} (+476) (+380)	4167^{b} (+514) (+379)	4390^{c} (+547) (+389)
Bioflocs Added	679^{a} PDC PDE	3145^{b} (+363) (+358)	4590^{b} +576 (+464)	4636^{b} (+583) (+433)	4821^{c} (+610) (+437)
PB + BF Added	679^{a} PDC	3597^{b} (+430) (+424)	4941^{b} (+628) (+507)	5623^{c} (+728) (+546)	5122^{c} (+654) (+471)

Values presented in parenthesis are PDC: Percent Change over their respective Control
Values presented in parenthesis are PDE: Percent Change over their respective Experimental
Values with different superscripts are significantly different from each other @ $P<0.05$.

Table 2: Phytoplankton distribution in *L. vannamei* culture operation under different feeding trails

Control	Duration of Culture Operation (DOC) (in days)									
	0		20		40		60		80	
	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
Bacilliriophyceae	353	52	477	70	534	66	602	69	543	61
Chlorophyceae	187	27	153	21	165	20	143	17	121	14
Cyanophyceae	75	11	12	2	34	4	24	3	58	6
Dinophyceae	22	3	15	2	46	6	44	5	28	3
Eugleophyceae	4	1	5	1	23	3	28	3	58	6
Others	38	6	25	4	12	1	29	3	89	10
Total	679		687		814		870		897	
	PDC		+1.2		+20		+28		+32	
Probiotics Added										
Bacilliriophyceae	353	52	1254	49	1628	41	1724	41	1528	35
Chlorophyceae	187	27	675	26	579	15	469	11	657	15
Cyanophyceae	75	11	236	9	542	14	578	14	658	15
Dinophyceae	22	3	124	5	268	7	437	11	679	15
Eugleophyceae	4	1	126	5	437	11	417	10	431	10
Others	38	6	152	6	457	12	542	13	437	10
Total	679		2567		3911		4167		4390	
	PDC		+278		+476		+514		+547	
Bioflocs Added										
Bacilliriophyceae	353	52	1494	47	2104	46	2321	50	2402	50
Chlorophyceae	187	27	868	28	1396	30	1094	24	1322	27
Cyanophyceae	75	11	395	12	549	12	645	14	554	12
Dinophyceae	22	3	112	4	185	4	159	3	151	3
Eugleophyceae	4	1	23	1	28	1	39	1	31	1
Others	38	6	253	8	328	7	378	8	361	7
Total	679		3145		4590		4636		4821	
	PDC		+363		+576		+583		+610	
Probiotic + Biofloc Added										
Bacilliriophyceae	353	52	1542	43	2345	47	2589	46	2785	54
Chlorophyceae	187	27	945	26	1542	31	1213	21	987	19
Cyanophyceae	75	11	284	8	234	5	268	5	248	5
Dinophyceae	22	3	268	7	345	7	548	10	651	13
Eugleophyceae	4	1	234	7	236	5	678	12	237	5
Others	38	6	324	9	239	5	327	6	214	4
Total	679		3597		4941		5623		5122	
	PDC		+430		+628		+728		+654	

Table 3: Comparative Overview of Phytoplankton at DOC 80

Treatment	Total Phytoplankton at Day 80	% Change over Control (PDC)	Notable Dominant Groups
Control	897	-	Bacillariophyceae (61%)
Probiotic	4390	+547%	Bacillariophyceae, Euglenoids, Dinoflagellates
Biofloc	4821	+610%	Bacillariophyceae, *Chlorophyceae*
PB + BF	5122	+654%	Bacillariophyceae, Dinoflagellates, Euglenoids

Table 4: Consolidated Table showing Zooplankton Distribution

	0	20	40	60	80
Control	88 PDC	144 (+64) +PDE	185 (+110) (+29)	315 (+258) (+119)	301 (+242) (+109)
Probiotic Added	88 PDC	207 (+135) (+44)	315 (+258) (+70)	583 (+563) (+85)	430 (+389) (+43)
Bioflocs Added	88 PDC	271 (+208) (+88)	508 (+477) (+175)	778 (+784) (+147)	518 (+489) (+72)
PB + BF Added	88 PDC	395 (+349) (+174)	607 (+590) (+228)	908 (+932) (+188)	651 (+640) (+116)

Values presented in parenthesis are PDC: Percent Change over their respective Control
Values presented in parenthesis are PDE: Percent Change over their respective Experimental
Values with different superscripts are significantly different from each other @ $P<0.05$.

Table 5: Zooplankton distribution in *L. vannamei* culture operation under different feeding trails

	Duration of Culture Operation (DOC) (in days)									
Control	**0**		**20**		**40**		**60**		**80**	
	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
Rotifera	13	15	14	10	43	23	47	15	39	13
Cladocerans	4	4	12	8	26	14	36	11	38	13
Copepods	42	48	56	39	36	19	137	43	102	34
Protozoa	18	20	38	25	54	29	78	25	95	31
Larval forms	4	5	17	12	12	7	12	4	13	4
Others	7	8	7	5	14	8	5	2	14	5
Total	88		144		185		315		301	
	PDC		+64		+110		+258		+242	
Probiotics Added										
Rotifera	13	15	28	14	59	19	73	13	54	12
Cladocerans	4	4	17	8	32	10	59	10	43	10
Copepods	42	48	79	38	105	33	234	40	167	39
Protozoa	18	20	47	23	72	23	158	27	121	28
Larval forms	4	5	21	10	26	8	36	6	21	5
Others	7	8	15	7	21	7	23	4	24	6
Total	88		207		315		583		430	
	PDC		+135		+258		+563		+389	
Bioflocs Added										
Rotifera	13	15	39	14	69	14	98	13	25	5
Cladocerans	4	4	22	8	42	8	78	10	57	11
Copepods	42	48	102	31	243	48	415	53	198	38
Protozoa	18	20	55	20	89	17	143	18	164	32
Larval forms	4	5	32	12	31	6	27	4	29	5
Others	7	8	21	8	34	7	17	2	45	9
Total	88		271		508		778		518	
	PDC		+208		+477		+784		+489	
Probiotic + Biofloc Added										
Rotifera	13	15	49	12	86	14	123	14	68	10
Cladocerans	4	4	35	9	63	10	85	9	54	8
Copepods	42	48	187	47	279	46	527	58	258	40
Protozoa	18	20	67	17	98	16	138	15	175	27
Larval forms	4	5	42	11	34	6	21	2	67	10
Others	7	8	15	4	47	8	14	2	29	5
Total	88		395		607		908		651	
	PDC		+349		+590		+932		+640	

Table 6: Zooplankton dynamics during *L. vannamei* culture operation.

Treatment	Day 80 Zooplankton Count	% Change from Day 0	Peak Abundance (DOC)	Peak Count
Control	301	+242%	Day 60	315
Probiotics	430	+389%	Day 60	583
Bioflocs	518	+489%	Day 60	778
PB + BF	651	+640%	Day 60	908

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