

Bioencapsulated *Moina macrocopa* Enhances Growth Performance, Nutritional Quality, and Disease Resistance in *Macrobrachium rosenbergii* Cultured in Substrate-Based Systems

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Abstract

Sustainable aquaculture production depends on nutritionally enriched live feed, particularly during early developmental stages. This study evaluated the effects of bioencapsulated *Moina macrocopa* enriched in a substrate-based culture system (SbCS) on growth performance, nutritional composition, microbial profile, and disease resistance of *Macrobrachium rosenbergii* post-larvae. A 26-day feeding trial was conducted with six treatments under two culture systems, SbCS and clear water system (CWS), using encapsulated and non-encapsulated *M. macrocopa* and *Artemia*. The results showed that prawns reared in SbCS and fed encapsulated *M. macrocopa* (SbCS-E) exhibited significantly higher ($P < 0.05$) growth performance, with weight gain of $1037.9 \pm 0.14\%$, specific growth rate (SGR) of $9.36 \pm 0.03\% \text{ day}^{-1}$, and survival of $91.65 \pm 0.67\%$ compared to other treatments. Nutritional composition was also significantly improved, with higher protein ($77.14 \pm 0.14\%$) and lipid ($11.89 \pm 0.04\%$) contents in SbCS-E compared to other treatments. Enhanced fatty acid profiles were observed in SbCS-E, including increased levels of arachidonic acid ($5.16 \pm 1.21\%$), eicosapentaenoic acid ($9.06 \pm 0.25\%$), docosahexaenoic acid ($6.49 \pm 0.56\%$), and total polyunsaturated fatty acids ($72.49 \pm 0.55\%$). Furthermore, the whole-body microbial community of treated *M. rosenbergii* was dominated by *Bacilli*, *Gammaproteobacteria*, and *Clostridia*. A significantly higher abundance ($P < 0.05$) of bacilli (92%) was observed in SbCS-E, indicating improved microbial balance. Histological observations likely enhanced resistance to *Aeromonas hydrophila*, with all treatments except SbCS-A and CWS-A exhibiting normal histological structure. In conclusion, bioencapsulation of *M. macrocopa* within a substrate-based culture system enhances its nutritional and functional value, improving growth, survival, microbial balance, and disease resistance of *M. rosenbergii* and offering a promising strategy for sustainable aquaculture intensification.

Categories: Aquaculture for sustainable food production, Aquatic animal health management, Food security and sustainability

Keywords: bioencapsulation, aquaculture live-food, macrobrachium rosenbergii, substrate-based culture system, moina macrocopa, disease resistance

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Introduction

Aquaculture has emerged as one of the fastest-growing sectors in global food production, contributing substantially to food security and meeting the increasing demand for high-quality protein [1,2]. Recent reports indicate that global aquaculture production has exceeded 130 million tons, highlighting its critical role in sustaining future food systems [3]. Despite this rapid expansion, the larval and post-larval stages of aquatic organisms remain critical bottlenecks due to their heightened sensitivity to environmental fluctuations and nutritional deficiencies [4]. Ensuring the availability of nutritionally adequate and biologically functional live feed is, therefore, essential to support optimal growth, survival, and physiological development during these early life stages. Recent advances highlight the importance of optimizing live feed and micro-diet strategies to enhance larval rearing efficiency and overall aquaculture productivity [5].

Among commonly used live feeds, cladocerans such as *M. macrocopa* have gained considerable attention owing to their appropriate size, ease of mass culture, and favorable nutritional profile [6,7]. Previous studies have demonstrated that *M. macrocopa* can be nutritionally improved under controlled culture conditions, leading to enhanced protein, lipid, and fatty acid composition [8,9]. However, conventional live feeds often remain deficient in essential nutrients, particularly long-chain polyunsaturated fatty acids (PUFAs), which play a critical role in membrane integrity, growth regulation, and immune function [10]. To address these limitations, bioencapsulation using microalgae and functional microbial inputs has been increasingly explored as an effective strategy to enhance both the nutritional quality and delivery efficiency of live feeds [11]. Recent studies further highlight that microalgae serve not only as nutrient sources but also as functional ingredients rich in bioactive compounds that improve immunity and metabolic performance in aquatic organisms [12].

In parallel, there is growing recognition of the importance of microbial processes in aquaculture systems. Probiotics have been widely recognized as sustainable alternatives to antibiotics, improving growth, feed utilization, immune response, and disease resistance in aquatic organisms [13]. In particular, genera such as *Bacillus*, *Lactobacillus*, and other beneficial microbes have demonstrated significant potential in enhancing host health and modulating gut microbiota [14]. Recent advances also indicate that probiotics, synbiotics, and postbiotics function as key components of functional feeds, contributing to improved water quality, microbial balance, and overall aquaculture sustainability [15].

Substrate-based culture systems (SbCS), which integrate microalgae with beneficial microorganisms, have emerged as an innovative approach to enhance aquaculture productivity. These systems facilitate nutrient recycling, improve water quality, and establish a stable microbial environment that supports the proliferation of beneficial microbial communities [16,17]. Recent studies highlight that host-microbiome-diet interactions are central to aquaculture performance, with gut microbiota playing a pivotal role in digestion, nutrient assimilation, and immune modulation [18]. Despite these advances, microbiome-driven approaches remain underexplored in many aquaculture species, particularly crustaceans.

The integration of bioencapsulation with SbCS represents a promising yet insufficiently investigated strategy for simultaneously improving feed quality and environmental conditions. Enriching a live feed within a microbially active system may enhance nutrient bioavailability, promote efficient nutrient transfer, and facilitate beneficial gut microbiota colonization, thereby improving disease resistance and overall performance. However, limited studies have systematically evaluated the combined effects of SbCS-based bioencapsulation on growth performance, fatty acid composition, microbial dynamics, and disease resistance in freshwater aquaculture species.

Macrobrachium rosenbergii, a commercially important freshwater prawn, is widely cultured due to its rapid growth rate, adaptability, and high market demand [19]. However, disease outbreaks, particularly those caused by opportunistic pathogens such as *A. hydrophila*, continue to constrain sustainable production. Probiotics and functional feeding strategies have been increasingly recognized as effective approaches to enhance immunity, improve gut health, and mitigate disease risks in aquaculture systems [13,20]. Given that the immune system of *M. rosenbergii* is predominantly innate, strategies that enhance nutritional status and modulate gut microbiota are particularly important for improving resilience against pathogens.

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Therefore, the present study aimed to evaluate the effects of bioencapsulated *M. macrocopa* enriched within a substrate-based culture system on growth performance, nutritional composition, fatty acid profile, microbial balance, and disease resistance of *M. rosenbergii* post-larvae. It was hypothesized that the integration of bioencapsulation with a substrate-based culture system would synergistically enhance the nutritional quality of live feed, resulting in improved growth performance, favorable modulation of gut microbiota, enhanced immune response, and increased resistance to *A. hydrophila* compared to conventional feeding and clear water culture systems. Furthermore, we propose that these improvements are driven by the combined effects of enhanced nutrient delivery and microbial-mediated environmental and gut-level interactions within the integrated system.

Materials And Methods

Experimental design

A 26-day feeding trial was conducted to evaluate the effects of bioencapsulated *M. macrocopa* and culture system on the growth performance, nutritional composition, microbiome, and disease resistance of *M. rosenbergii* post-larvae. The experiment followed a two-factor factorial design, consisting of two culture systems: SbCS and CWS, and three feed types: encapsulated *M. macrocopa* (E), non-encapsulated *M. macrocopa* (NE), and *Artemia* (A). This resulted in six treatments (SbCS-E, SbCS-NE, SbCS-A, CWS-E, CWS-NE, and CWS-A), each performed in triplicates.

Culture of microalgae and probiotic bacteria

The probiotic bacterium *Bacillus amyloliquefaciens* was obtained from I-AQUAS, COSMOS-SATREPS, Universiti Putra Malaysia and cultured in Luria-Bertani (LB) broth at 37°C. The freshwater microalga *Chlorella vulgaris* was obtained from the Institute of Bioscience, Universiti Putra Malaysia, and maintained in Bold's Basal Medium (BBM). Microalgal cultures were maintained at room temperature under continuous illumination (70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) with a 12:12 light:dark photoperiod. Stock cultures were maintained at a lower light intensity (<50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Cultures were initially grown in 50 mL volumes and subsequently scaled up to 6 L. The pH of BBM was adjusted to 7.0 [21,22].

Co-culture preparation followed the method of Ramlee et al. [23]. Briefly, *C. vulgaris* (3.0×10^5 cells mL^{-1}) was inoculated into 400 mL BBM and incubated for 24 hours. Subsequently, *B. amyloliquefaciens* (3.0×10^6 CFU mL^{-1}) (grown in LB broth) was added at 1% (v/v), and the co-culture was maintained at $30 \pm 2^\circ\text{C}$ under natural light (12:12 light:dark cycle) for 48 hours.

Effects of microalgae and probiotic bacteria on *M. macrocopa*

A preliminary experiment was conducted to evaluate the effects of *C. vulgaris*, *B. amyloliquefaciens*, and their co-culture on *M. macrocopa* growth. Neonates were stocked at five individuals per liter in 1 L beakers containing 800 mL of sterilized freshwater. Three treatments (microalgae, bacteria, and co-culture) were tested in triplicates under controlled conditions ($30 \pm 2^\circ\text{C}$; 12:12 light:dark cycle). Population growth was monitored over 15 days.

The rate of population increase (r) was calculated using the exponential equation:

$$r = (\ln N_t - \ln N_0) / t$$

Where N_t and N_0 are the final and initial population densities at time t , respectively, and t is time in days, and r = specific growth rate (day^{-1}).

Bioencapsulation of *M. macrocopa*

Bioencapsulation was performed using a substrate-based enrichment system. *Moina macrocopa* was cultured in 25-L tanks containing 20 L filtered pond water. A consortium system was established using maize starch as a carbon source and bamboo as an organic substrate. Maize starch was added initially at a ratio of 1:5 (w/v) and gradually increased up to 3:5 (w/v) as population density increased. The solution was fermented for 24 hours prior to application [24]. Six bamboo poles (1 $\times$ 1 cm) were installed per tank to support microbial colonization [25].

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For the encapsulated group, after 28 days, *M. macrocopa* was harvested from the rearing tanks and transferred to enrichment medium containing microalgae-bacteria co-culture. Bioencapsulation was carried out for 24 hours under continuous aeration prior to feeding [23]. The non-encapsulated group was cultured identically to the encapsulated group but without exposure of *M. macrocopa* to the microalgae-bacterial co-culture. The whole experimental setup was maintained at $30 \pm 2^\circ\text{C}$ under natural sunlight for a 12:12 (light:dark) cycle. Bioencapsulation efficiency was inferred through subsequent biochemical analysis of enriched *M. macrocopa*.

Nutritional profile analyses of encapsulated *M. macrocopa*

Moina macrocopa was collected from each experimental tank at the end of the experiment and oven-dried at 103°C until it reached a constant weight. The dried samples were then analyzed for their proximate profiles: crude protein, total lipids, and fatty acid. The crude protein was extracted using the salt/alkaline extraction method and estimated by the Bradford method [26]. Total lipids and fatty acids were estimated according to the methodology of Suhaimi et al. [27].

Artemia decapsulation and hatching

Artemia cysts were purchased from the local aquarium shop and decapsulated by soaking 5 g of cysts in freshwater for 1–2 hours and transferred to the mixture solution containing 100 mL chilled chlorox solution, 100 mL chilled 25 ppt seawater, 1 g sodium hydroxide (NaOH), and 3 g sodium thiosulfate. The cysts were then aerated for 5 minutes until they changed from brown to orange, filtered, and rinsed with seawater thrice and stored at 4°C . When needed, the stored cysts were washed with freshwater and transferred to the hatching cone filled with 25 ppt seawater and allowed to hatch under light illumination of $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 24 hours and harvested using a 200 μm filter [28].

Preparation of rearing culture systems for *M. rosenbergii*

Two culture systems were established: SbCS and CWS. The SbCS was developed by adding fermented maize starch and installing bamboo substrates to promote microbial growth and biofilm formation, as mentioned in the section "Bioencapsulation of *M. macrocopa*." The system was maintained for 3 days prior to stocking to establish microbial communities. The concentration of maize starch was increased according to the growth and development of *M. rosenbergii*. The CWS consisted of dechlorinated freshwater without the addition of a carbon source or substrate.

Rearing of *M. rosenbergii*

The post-larvae (PL) of *M. rosenbergii* were stocked at 1 PL L^{-1} and were maintained in eighteen 25 L tanks, each filled with 20 L of filter-sterilized and aerated freshwater pond water. All PL and sampling procedures were in accordance with the approved International Animal Care and Use Committee (IACUC) guidelines number UPM/IACUC/AUP-R034/2022. All feeding trials were conducted for 26 days. The test experiments were randomly assigned in triplicate. Feeding of PL was started immediately after 5 days of assimilation. Feeding was done two times per day (08:30 and 15:30 hours). The feed was initially supplied at $15\% \text{ body weight day}^{-1}$ and subsequently adjusted based on feed consumption observations while maintaining equal feeding schedules among treatments. For CCS-A and CWS-A treatments, the *Artemia* was hatched as mentioned in the section "Artemia decapsulation and hatching" and fed directly to *M. rosenbergii*. The whole culture system was maintained at $30 \pm 2^\circ\text{C}$ under natural sunlight for a 12:12 (light:dark) cycle, and the optimum pH, temperature, and dissolved oxygen (DO) levels were maintained throughout the study.

Growth performance and survival

The growth performance (percentage weight gain and specific growth rate (SGR)) and survival of *M. rosenbergii* in all treatments were determined according to the method by Jagruthi et al. [29]. The formulas for calculating the above growth performance and survival were as follows:

Weight gain % = (Final weight – Initial weight)/initial weight $\times 100$

$\text{SGR } (\% \text{ day}^{-1}) = [(\ln W_t - \ln W_0)/t] \times 100$

where W_0 is the initial mean body weight (g), W_t is the final mean body weight (g), and t is the culture period (days).

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Survival% = No. of PL survived $\times$ 100/No. of PL stocked

Water quality analyses

The water quality in terms of pH, temperature, and DO were determined using a YSI multiparameter (Yellow Spring Instrument 556, YSI Incorporated, USA). Concentrations of ammonia-N, nitrate-N, and nitrite-N were determined according to the standard methods for the examination of water (APHA 2005) [30]. For determining ammonia-N, nitrite-N, and nitrate-N, the water samples were collected from all culture tanks every week of the experimental period, filtered using vacuum filtration, and analyzed immediately after filtration.

Nutritive profile analyses

The PL were collected from each tank at the end of the experiment and were oven-dried at 103°C until they reached a constant weight. The dried post-larval samples were then analyzed for crude protein, total lipids, and fatty acids as described in the section "Effects of microalgae and probiotic bacteria on *M. macrocopa*."

Total bacterial load analysis

Three prawns were collected from each experimental tank after the experiment for whole-body microbiota evaluation. Each prawn was deactivated in the freezer at -20°C for 10 minutes and sterilized using formalin (50 ppm) and weighed into sterile universal bottles containing peptone water (0.1%) to release the available bacteria for a period of 2 hours. An aliquot of 1 mL was taken from each sample bottle and diluted 10-fold and subsequently serially diluted with a dilution factor of 10^{-6} . After dilution, 1 mL was taken from each diluted sample and dispensed into two petri dishes containing the following agar: For total aerobic bacteria (TAB), total enteric bacteria (TEB), and total probiotic bacteria (TPB), the diluted samples were poured into Tryptic soy agar (TSA), MacConkey and de Man, Rogosa and Sharpe agar (MRS) agar, respectively, and incubated for 24 hours at 37°C to allow distinguishable colonies to form. The colonies formed were then counted. The TAB, TEB, and TPB were expressed in $\log_{10}$ CFU g^{-1} [31].

Amplicon sequencing of the bacterial community in treated *M. rosenbergii*

Total Bacterial DNA Extraction from Treated *M. rosenbergii*

Due to resource constraints and the substantially lower performance of *Artemia*-fed groups, amplicon sequencing was restricted to *M. macrocopa*-fed treatments. Three prawns per tank were collected from the other four experiments (SbCS-E, SbCS-NE, CWS-E, and CWS-NE) after the trial. Each prawn was deactivated in a freezer at -20°C for 10 minutes and sterilized using formalin (50 ppm) and weighed into sterile universal bottles containing peptone water (0.1%) to release the available bacteria for a period of 2 hours. A 1 mL sample was taken from each bottle in an Eppendorf tube and centrifuged at 12,000 rpm for 2 minutes. The supernatant was removed, and 480 μL of 50 mM EDTA (ethylenediamine tetra-acetic acid) + 120 μL of lysozyme (10 mg mL^{-1}) was added and incubated at 37°C for 30-60 minutes. The mixture was again centrifuged at 12,000 rpm for 2 minutes, and the supernatant was removed. To each sample, 600 μL of nuclei lysis solution was added, mixed gently, and incubated at 80°C for 5 minutes. After incubation, once the sample got cooled down, 3 μL of RNase was added and incubated at 37°C for 30 minutes and cooled to room temperature. A 200 μL of protein precipitation solution was added to the sample, vortexed, incubated in ice for 5 minutes, and centrifuged at 12,000 rpm for 3 minutes. The supernatant obtained was transferred to the new Eppendorf tube, and 600 μL of isopropanol was added, gently mixed, and centrifuged again at $14,000 g$ for 2 min. The supernatant was then discarded, and 600 μL of 70% ethanol was added and centrifuged at $14,000 g$ for 2 mins, and the ethanol was aspirated and air-dried for 10-15 minutes. To the air-dried sample, 50 μL of DNA rehydration solution was added and incubated at 65°C for 1 hour.

The extracted DNA concentration was measured using a spectrophotometer (Implen NanoPhotometer® N60/N50, USA) and fluorometric quantification using iQuant™ Broad Range dsDNA Quantification Kit. DNA samples that have passed QC were sent to Novogene Biological Information Technology Co. (Tianjin, China) through Apical Scientific Sdn. Bhd. (Seri Kembangan, Malaysia). The libraries were normalized and pooled according to the Illumina protocol before being sequenced on the MiSeq platform with 300 PE.

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The gDNA was submitted to amplicon library preparation using two-step PCR in accordance with the Illumina 16S metagenomic library preparation recommendations. Initially, 16S rRNA gene PCR primers of the V3-V4 region were added to amplify 16S rRNA, while the Illumina adapter overhang nucleotide was added to gene-specific sequences. The resulting 16S rRNA amplicon PCR forward and reverse primers were as follows: forward (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and reverse (5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'). In the second stage PCR, dual indices were attached to the amplicon PCR using the Illumina Nextera XT Index Kit v2 according to the manufacturer's guidelines. All PCR reactions were performed using KOD-Multi & Epi® (Toyobo).

16S rRNA Amplicon Sequencing Data Processing and Bioinformatics Analysis

All the raw data from MiSeq sequencing were obtained and processed using Quantitative Insights into Microbial Ecology (QIIME) software (version 1.9.1 64-bit). All paired-end reads were trimmed using BBDuk of the BBTools package (<https://sourceforge.net/projects/bbmap>). The forward and reverse reads were merged with USEARCH v11.0.667 (http://hannonlab.cshl.edu/fastx_toolkit/) with a Phred Quality Score of $Q = 20$ and $P = 70$. The sequences shorter than 150 bp or longer than 600 bp were removed along with the low-quality bases ($Q \leq 30$) to avoid accuracy interference caused by unspecific matches. The chimeric errors were detected using VSEARCH v2.6.2 against the 16S rRNA (SILVA Release 132) or UNITE ITS database and removed from the downstream. Non-chimeric clean sequences were known as effective tags. After the quality assessment, the effective tags were clustered into the operation taxonomic unit (OTU) with at least a 97% similarity threshold using UPARSE v11.0.667. The rate of OTUs with less than 2 reads, which are often spurious, was deleted from downstream processing. The representative sequence for each OTU was picked, and the taxonomic information was annotated using QIIME v1.9.1 against the SILVA database, a 16S rRNA database. The sequences that were not identified as bacteria in the OTU abundance matrix were regarded as contaminating sequences and were eliminated before further downstream analysis. The bacterial taxonomic classification was carried out up to the genus level, and features of unrelated cells were removed through a filtering process for downstream statistical analysis. The filtering process was set on a minimum of four counts with a prevalence of over 20% in the entire dataset. The 10% low variance filter was performed using interquartile range (IQR), and the data were normalized through sum scaling (TSS) or by the total number of readings. The alpha diversity metrics and relative abundance were studied using Microbiome Analyst software with a two-way analysis of variance (ANOVA) at a 0.05 significance level.

Bacterial challenge test

To evaluate prawn resistance to disease infection, a challenge test using the pathogenic bacteria *A. hydrophila* was performed following a 15-day feeding trial. Prior to the challenge test, a preliminary experiment was performed to determine the LD50 (lethal dose) of the pathogenic bacteria. Briefly, 10 prawns were selected from each replicate tank from experiment 1 and are randomly transferred into fifty-four 2 L beakers filled with 1,800 mL dechlorinated freshwater and aerated. The prawns were then exposed to a bacterial solution of *A. hydrophila* at different immersion concentrations of 1×10^5 , 1×10^6 , and 1×10^7 CFU mL⁻¹ for 40 mins and returned into the experimental setup. Hence, there were eighteen treatments in triplicates. *Aeromonas hydrophila* was cultured in tryptic soy broth and incubated for 24 hours at 35°C. The cells were then centrifuged at 3,000 *g* for 30 minutes to form pellets. The pellets were re-suspended in 1 mL of PBS, absorbance was observed at OD 660 nm, and the OD value was adjusted to 1. The prawns were fasted for 24 hours before infection, and feeding with experimental diets resumed 12 hours later. The median lethal dose, LD50, was determined by the Reed and Munch (1938) method. It was determined by calculating the daily mortality and survival of all prawn groups for 3 days.

Post feeding trial, the PL of *M. rosenbergii* were subjected to a bacterial challenge test. For the challenge test, 10 prawns were selected from each replicate tank from experiment 1 and transferred into another eighteen 2 L beakers filled with dechlorinated freshwater with continuous aeration. *Aeromonas hydrophila* was introduced into the treatment tanks at 1.1×10^6 CFU mL⁻¹ based on the LD50 value. Feeding was then resumed with the same experimental diets. All prawn groups were kept under observation for 15 days to record any abnormal clinical signs and the daily mortality. Mortality was determined for an interval of every 12 hours. The survived PL at the end of the challenge test were then processed for histological analysis.

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Physical Deformities Examination by Histopathology

Three PL were taken from each experimental tank before and after the challenge and were fixed in Davidson's solution for 24 hours for histology. After 24 hours, PL was transferred to 70% alcohol and kept for 2 days. The processing of PL, embedding, sectioning, and eosin staining has been performed by the Veterinary Histopathology Laboratory, Veterinary Laboratory Services Unit (VLSU), Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Malaysia. Each histology section was carefully observed at $\times 40$ magnification under a microscope (Leica DM 750, Germany) attached to a camera lens (Leica ICC 50, Germany).

Statistical analysis

All data are presented as mean $\pm$ standard error (SE). Prior to statistical analyses, data were tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. Statistical significance was accepted at $P < 0.05$. The experimental tank was considered the experimental unit for all growth, survival, water quality, microbiological, and nutritional analyses.

Data obtained from "Effects of microalgae and probiotic bacteria on *M. macrocopa*" section (effects of microalgae and probiotic bacteria on the growth of *M. macrocopa*) were analyzed using one-way ANOVA, followed by Tukey's Honest Significant Difference (HSD) test for multiple comparisons.

Data from the sections "Bioencapsulation of *M. macrocopa*" and "Artemia decapsulation and hatching" (bioencapsulation and nutritional profile analyses of *M. macrocopa*) were analyzed using an independent samples *t*-test to compare encapsulated and non-encapsulated groups.

Data from sections "Rearing of *M. rosenbergii*" to "Bacterial challenge test" were analyzed using two-way ANOVA to determine the main effects of feed type, culture system, and their interaction. When significant differences were detected, Tukey's HSD test was used for post hoc multiple comparisons.

For microbiome analyses (Section "Amplicon sequencing of the bacterial community in treated *M. rosenbergii*"), alpha-diversity indices (Chao1, Shannon, and Simpson indices) were compared among treatments using one-way ANOVA followed by Tukey's HSD test. Relative abundance data of dominant bacterial taxa were analyzed using two-way ANOVA to assess the effects of feed type, culture system, and their interaction.

Results And Discussion

Synergistic effects of microalgae-bacteria co-culture on *M. macrocopa*

The population dynamics of *M. macrocopa* were significantly influenced by dietary treatments ($P < 0.05$). Among all groups, the co-culture treatment (*C. vulgaris* + *B. amyloliquefaciens*) exhibited the highest population density (43.2 ± 2 ind mL⁻¹), followed by *B. amyloliquefaciens* alone (40.3 ± 2 ind mL⁻¹), while the lowest density was observed in *C. vulgaris* (25.5 ± 3 ind mL⁻¹) (Figure 1). The specific growth rate (*r*) also followed the same pattern, and hence the co-culture treatment had a significantly higher ($P < 0.05$) growth rate among treatments (Figure 2).

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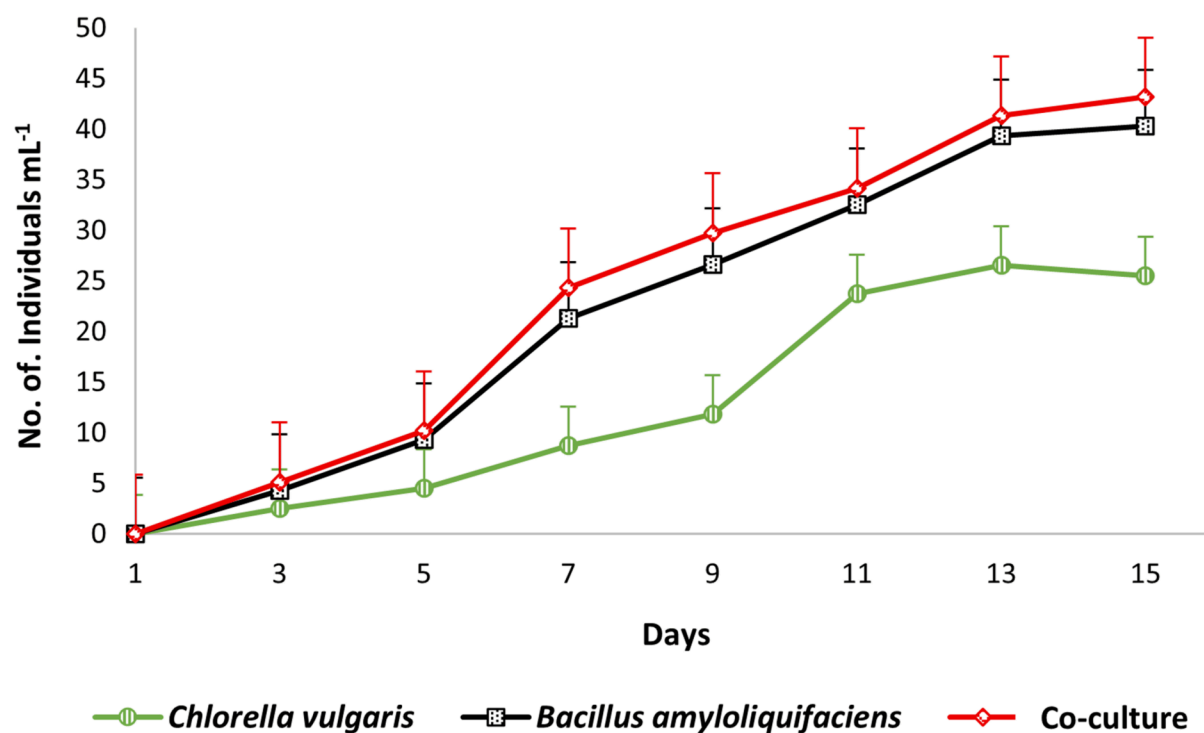


FIGURE 1: Effects of *Chlorella vulgaris* (Treatment 1), *Bacillus amyloliquifaciens* (Treatment 2) and co-culture (Treatment 3) on the growth of *Moina macrocopa*.

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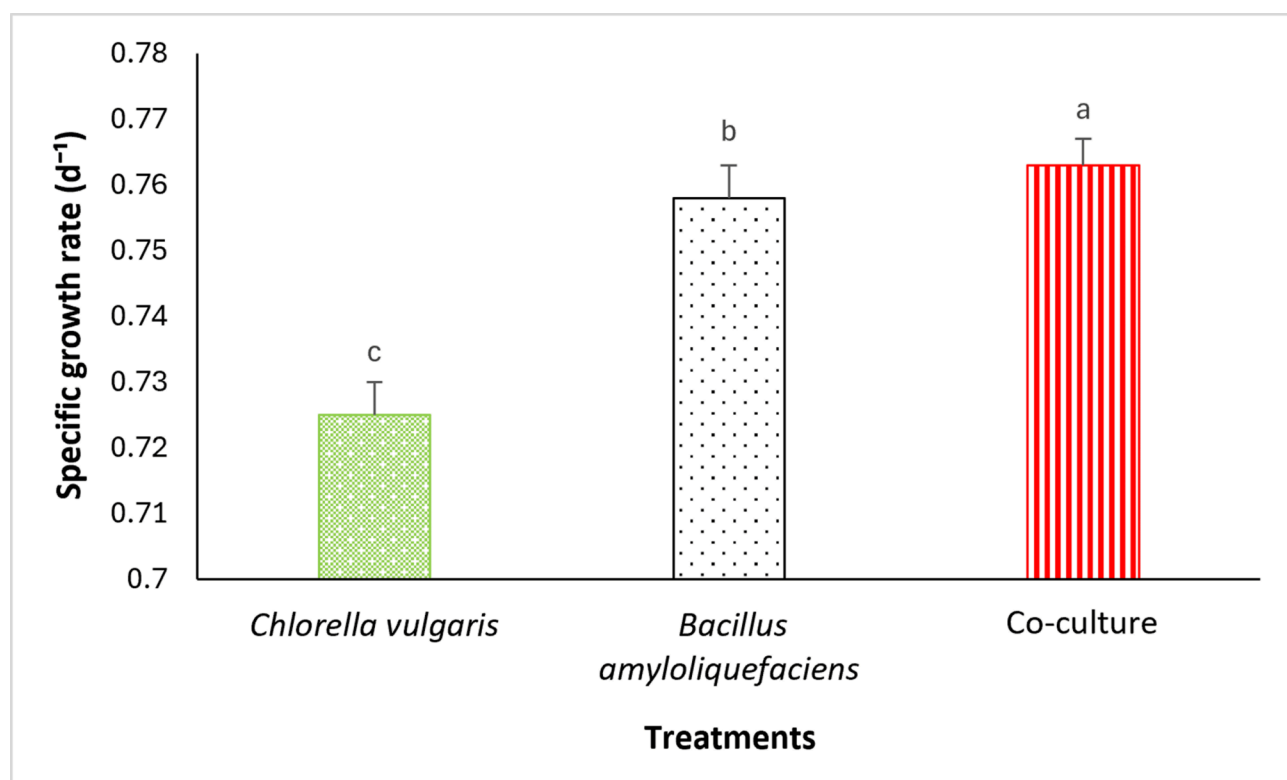


FIGURE 2: Mean $\pm$ SE specific growth rate (r) of *Chlorella vulgaris* (Treatment 1), *Bacillus amyloliquefaciens* (Treatment 2) and co-culture (Treatment 3) on *Moina macrocopa*.

Vertical bars are standard errors of the means. Means with different letters are significantly different at $P < 0.05$.

Zooplankton grazing rate represents a critical determinant in the selection of appropriate algal feed and probiotic supplementation strategies. Quantitative assessment of grazing dynamics and dietary selectivity, particularly in relation to the physicochemical and morphological properties of feed particles, is essential for optimizing zooplankton nutritional efficiency [32]. Previous studies have demonstrated that although cladocerans are capable of utilizing a broad spectrum of algal species, their ingestion rates and growth performance are strongly influenced by algal size, structure, and digestibility [33]. For example, *M. macrocopa* exhibited significantly higher ingestion rates when fed *C. vulgaris* compared to *Scenedesmus obliquus*, *Microcystis flos-aquae*, and *Ankistrodesmus falcatus* [34]. In addition, increasing evidence highlights the functional compatibility between *C. vulgaris* and *Bacillus* spp., with documented synergistic effects on cladoceran growth and reproductive performance [23,35,36]. Accordingly, *C. vulgaris* and *B. amyloliquefaciens* were selected as enrichment agents in the present study.

The significantly elevated population density observed in the co-culture treatment (*C. vulgaris* + *B. amyloliquefaciens*) relative to mono-treatments indicates a strong synergistic interaction between microalgae and probiotic bacteria. Microalgae such as *C. vulgaris* function as a primary nutritional source, providing essential fatty acids, amino acids, vitamins, and bioactive compounds that enhance growth, stress tolerance, and immune function while also contributing to bioremediation and modulation of bacterial quorum sensing [37]. In parallel, *Bacillus* spp. are widely recognized as effective alternatives to antibiotics in aquaculture systems, owing to their capacity to improve water quality, enhance digestive processes, and stimulate innate immunity through the production of antimicrobial compounds, extracellular enzymes, and growth-promoting metabolites [38-41].

How to cite this article:

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The combined microalgae-bacteria system likely facilitated enhanced nutrient cycling through organic matter decomposition and nutrient remineralization, thereby increasing the bioavailability of dissolved nutrients and stabilizing the microbial environment. This integrated effect plausibly contributed to the observed enhancement in cladoceran reproductive output and population density.

These findings are consistent with previous reports demonstrating that probiotic supplementation significantly improves zooplankton survival and population dynamics. For instance, Pérez-Sánchez et al. [42] and Pimental et al. [43] documented increased cladoceran density in probiotic-enriched biofloc systems. Similarly, the higher population density observed in the *B. amyloliquefaciens* treatment compared to *C. vulgaris* alone in the present study aligns with earlier observations. Notably, however, the co-culture approach yielded substantially greater population densities than previously reported dietary combinations, including *Chlorella* with yeast [44] and *C. vulgaris* supplemented with canola oil [45], underscoring the superior efficacy of the microalgae-probiotic consortium strategy.

Protein and lipid profiles of bioencapsulated *M. macrocopa*

Bioencapsulation significantly improved the nutritional profile of *M. macrocopa* ($P < 0.05$). Encapsulated individuals (E) showed a marked increase in crude protein ($85.86 \pm 0.45\%$) and total lipid content ($21.2 \pm 0.40\%$) compared to the non-encapsulated group, NE ($83.15 \pm 0.95\%$ and $19.36 \pm 0.61\%$, respectively) (Figures 3 and Figure 4).

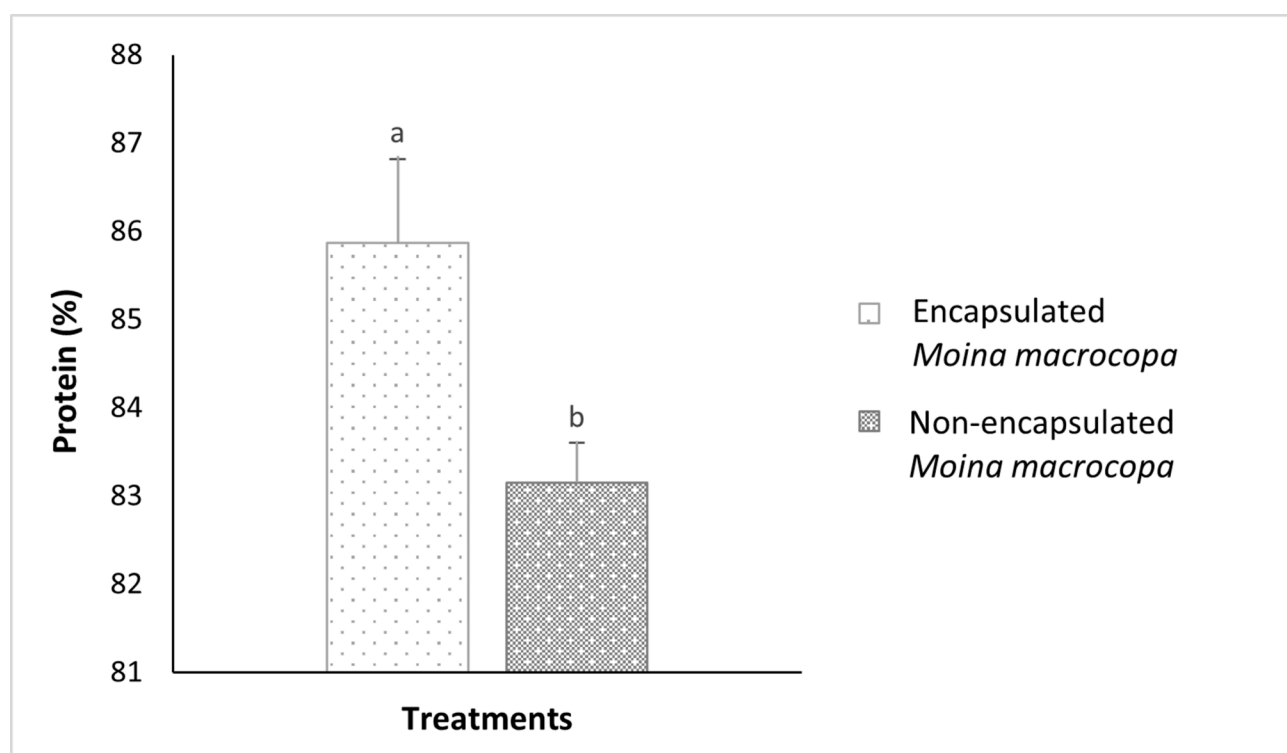


FIGURE 3: Protein profile (%) of encapsulated *M. macrocopa* (E) and non-encapsulated *M. macrocopa* (NE).

Vertical bars are standard errors of the means. Means with different letters are significantly different at $P < 0.05$.

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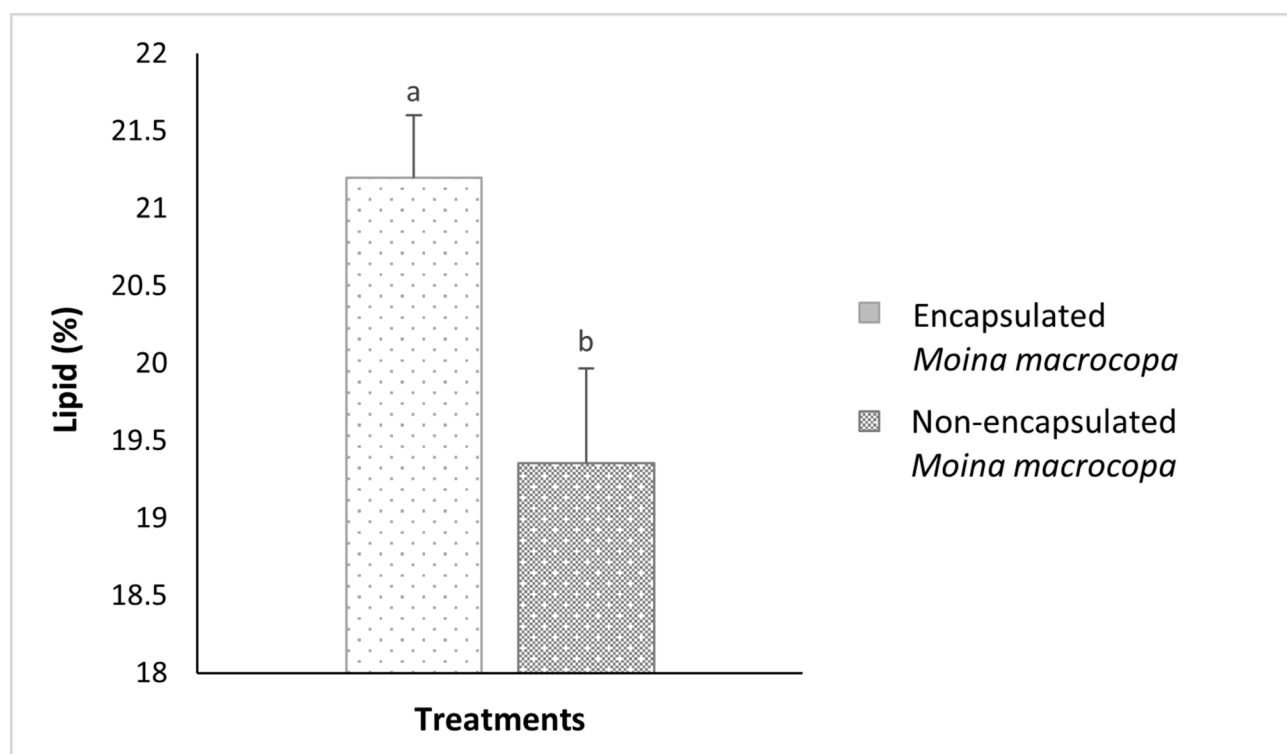


FIGURE 4: Lipid profile (%) of encapsulated *M. macrocopa* (E) and non-encapsulated *M. macrocopa* (NE).

Vertical bars are standard errors of the means. Means with different letters are significantly different at $P < 0.05$.

Bioencapsulation markedly improved the proximate composition of *M. macrocopa*, particularly protein and lipid content, demonstrating the efficient assimilation of microalgal and bacterial biomass within the substrate-based system. The results suggest that bioencapsulation may function as more than a passive enrichment process [46]. The incorporation of microbial biomass, including both microalgae and probiotic bacteria, may have facilitated the transfer of bioavailable nutrients and metabolites to higher trophic levels [47,48].

The observed increase in protein content suggests enhanced nitrogen assimilation and incorporation into structural and functional biomolecules, which is critical for rapid tissue synthesis during early developmental stages of crustaceans [1,10]. Similarly, the elevated lipid content reflects improved energy reserves and membrane biosynthesis capacity, both of which are essential for larval growth, molting, and stress resilience [49,50]. These findings support the concept that nutritionally enriched live feed can directly influence metabolic efficiency and developmental performance in aquaculture species.

However, the magnitude of improvement observed in this study exceeds that reported in earlier works using single-source enrichment strategies. For instance, protein and lipid levels recorded here were higher than those documented by Manklinniam et al. [44] and Hasan et al. [9] suggesting that the integration of probiotic bacteria within the enrichment system enhances nutrient assimilation beyond conventional approaches. This may be attributed to microbial-mediated processes such as enzymatic breakdown of organic matter, synthesis of bioactive compounds, and improved digestibility of feed components.

Fatty acid profiles of *M. macrocopa*

Encapsulated *M. macrocopa* (E) exhibited significantly higher levels of key fatty acids, including α -linolenic acid (ALA), arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), compared to the non-encapsulated group (NE) ($P < 0.05$). Total PUFAs were also elevated in the encapsulated treatment (Table 7).

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Fatty acid (%)	Treatments	
	E	NE
C18:2n6t	0.26 ± 0.2 ^a	0.24 ± 0.01 ^b
C18:2n6c	0.46 ± 0.1 ^a	0.44 ± 0.01 ^a
C18:3n6	0.33 ± 0.01 ^a	0.30 ± 0.01 ^b
C18:3n3 (ALA)	8.84 ± 0.03 ^a	8.81 ± 0.01 ^b
C20:2	0.00	0.00
C20:3n6	0.00	0.00
C20:3n3	0.00	0.00
C20:4n6 (ARA)	5.16 ± 0.01 ^a	5.10 ± 0.01 ^b
C20:5n3 (EPA)	5.92 ± 0.1 ^a	5.84 ± 0.05 ^b
C22:2	0.41 ± 0.01 ^a	0.39 ± 0.01 ^a
C22:6n3 (DHA)	1.87 ± 0.3 ^a	1.80 ± 0.01 ^b
SUM PUFAs	23.25 ± 0.78 ^a	22.92 ± 0.15 ^b

TABLE 1: Fatty acid composition (%) of encapsulated *M. macrocopa* (E) and non-encapsulated *M. macrocopa* (NE)

Values are presented as mean ± standard deviation (SD). Means with different superscripts within the same row indicates significant differences between treatments (*P* < 0.05)

PUFA, Polyunsaturated Fatty Acids; EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid; ARA, Arachidonic Acid

Importantly, the co-culture system resulted in a substantial improvement in essential fatty acid composition, particularly EPA, ARA, and, to a lesser extent, DHA. These fatty acids are known to play pivotal roles in cellular signaling, membrane fluidity, and immune modulation in crustaceans. The enhancement of these compounds in *M. macrocopa* suggests trophic transfer from *C. vulgaris* and associated microbial communities. Previous studies have demonstrated that microalgae-based enrichment can significantly improve PUFA profiles in zooplankton [51,52], and the present findings are consistent with this trend.

Although DHA levels remained lower than those achieved using commercial enrichment products [53,54], the simultaneous increase in EPA, ARA, and total PUFA content indicates a more balanced and functionally relevant fatty acid profile. This is particularly important, as the synergistic interaction among these fatty acids often determines physiological outcomes such as growth, stress tolerance, and immune competence. These findings are consistent with

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recent reports indicating that nutrient- and probiotic-enriched zooplankton can improve the nutritional quality of live feeds and enhance nutrient transfer to cultured aquatic organisms [7,35,55,56]. Recent reviews have further emphasized the growing importance of probiotic and microalgal enrichment strategies for improving larval nutrition, growth, and disease resistance in aquaculture systems [57].

Collectively, these findings highlight a key advancement over existing enrichment strategies. Unlike conventional methods that rely solely on microalgal or commercial lipid supplementation, the substrate-based bioencapsulation approach integrates nutritional and microbial components, resulting in a more efficient and biologically functional enrichment system. This possible dual mechanism not only enhances the biochemical composition of live feed but also potentially improves its digestibility and physiological impact when consumed by higher trophic organisms.

Growth performances of *M. rosenbergii*

Growth parameters of *M. rosenbergii* PL were significantly affected by both feeding regime and culture system ($P < 0.05$). Prawns reared in the substrate-based culture system and fed encapsulated *M. macrocopa* (SbCS-E) showed the highest weight gain ($1037.9 \pm 0.14\%$), specific growth rate ($9.36 \pm 0.03\% \text{ day}^{-1}$), and survival ($91.65 \pm 0.67\%$). This was followed by SbCS-NE and SbCS-A treatments, while all CWS groups showed comparatively reduced growth performance (Table 2).

Two-way ANOVA revealed significant effects of feed type, culture system, and their interaction on all growth performance parameters of *M. rosenbergii* post-larvae. For weight gain, significant effects were observed for feed type ($F = 282,196.20$, $P < 0.001$), culture system ($F = 1503258.42$, $P < 0.001$), and feed $\times$ culture system interaction ($F = 136,502.71$, $P < 0.001$). Similarly, SGR was significantly influenced by culture system ($F_{1,12} = 301.89$, $P < 0.001$), feed type ($F_{2,12} = 129.70$, $P < 0.001$), and their interaction ($F_{2,12} = 40.97$, $P < 0.001$). Survival was also significantly affected by feed type ($F_{1,12} = 105.13$, $P < 0.001$), culture system ($F_{2,12} = 63.88$, $P < 0.001$), and the interaction between these factors ($F_{2,12} = 4.63$, $P = 0.032$), indicating that both dietary treatment and culture environment contributed significantly to the observed growth and survival responses.

In general, prawns fed *M. macrocopa* (encapsulated or non-encapsulated) exhibited significantly ($P < 0.05$) higher growth and survival compared to those fed *Artemia*. Additionally, the SbCS consistently resulted in better performance than the CWS across all feeding treatments.

How to cite this article:

Parameters	Treatments					
	SbCS-E	SbCS-NE	SbCS-A	CWS-E	CWS-NE	CWS-A
Weight gain (%)	1037.9 ± 0.14 ^a	700.2 ± 0.22 ^b	648.4 ± 0.60 ^c	496.4 ± 0.08 ^d	481.8 ± 0.07 ^e	392.6 ± 0.09 ^f
Final weight (mg)	35.3 ± 0.45 ^a	24.8 ± 0.08 ^b	18.7 ± 0.63 ^d	16.7 ± 0.04 ^e	19.2 ± 0.02 ^c	13.5 ± 0.13 ^f
Specific growth rate (% day ⁻¹)	9.36 ± 0.03 ^a	7.76 ± 0.01 ^b	6.91 ± 0.05 ^c	6.87 ± 0.03 ^d	6.77 ± 0.02 ^e	6.05 ± 0.02 ^f
Survival (%)	91.65 ± 0.67 ^a	85.00 ± 0.23 ^b	77.76 ± 0.16 ^c	73.75 ± 0.1 ^d	63.35 ± 0.2 ^e	56.65 ± 0.03 ^f

TABLE 2: Means ± SE of growth and survival rates of *M. rosenbergii* cultured in different culture systems and given different feeds.

Values are expressed as mean ± standard deviation (SD). Within each row, means with different superscripts indicate significant differences among treatments ($P < 0.05$). *Macrobrachium rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A).

The enhanced nutritional quality of encapsulated *M. macrocopa* was associated with improved growth performance of *M. rosenbergii*, as evidenced by significantly higher weight gain, SGR, and survival in the SbCS-E treatment. The observed SGR (9.36% day⁻¹) and survival (>91%) represent a substantial improvement over conventional live feed-based systems, where SGR values typically range between 6% and 8% day⁻¹ and survival between 70% and 85% [58,59]. This corresponds to an approximate 15-30% increase in growth rate and 10-20% improvement in survival, highlighting the enhanced performance of the integrated SbCS-based feeding strategy.

While previous studies have demonstrated enhanced growth in biofloc systems due to microbial protein availability and improved water quality, the present study achieved comparatively higher performance metrics [60-62]. This suggests that the addition of nutritionally enriched cladocerans provides an additional trophic advantage beyond the benefits of microbial flocs alone. Unlike conventional biofloc systems that rely primarily on suspended microbial aggregates, the SbCS employed here integrates both high-quality live feed and microbially enriched environmental inputs, resulting in a more efficient nutrient transfer pathway [63,64].

The enhanced growth observed in the SbCS-E treatment may reflect the combined effects of nutritional enrichment and environmental optimization. The SbCS likely functioned as a biofloc-like environment, characterized by continuous microbial production and nutrient recycling. This system not only improves water quality but also provides a constant supply of microbial biomass, which can serve as a supplementary protein and energy source [64,65]. Such dual feeding pathways, direct ingestion of enriched *M. macrocopa* and indirect consumption of microbial aggregates, likely contributed to the enhanced growth performance.

In addition to external nutritional inputs, the microbial communities associated with SbCS may have played a critical role in improving digestive efficiency. Probiotic bacteria, particularly *Bacillus* spp., are known to produce extracellular enzymes such as proteases, lipases, and amylases, which enhance feed digestibility and nutrient absorption [63,66]. This

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is consistent with previous findings that microbial enrichment improves feed utilization efficiency and growth in crustaceans [67,68]. The combined effects of improved feed quality and enhanced gut microbial activity likely resulted in higher nutrient assimilation efficiency, thereby supporting increased weight gain and SGR.

The comparatively lower growth performance observed in clear water systems further underscores the limitations of conventional rearing approaches. In the absence of microbial complexity and nutrient recycling, these systems rely solely on external feed inputs, leading to reduced nutrient utilization efficiency and suboptimal growth [69]. In contrast, the SbCS provides a dynamic and biologically active environment that supports both nutritional and microbial interactions, resulting in significantly improved production outcomes. The improved growth performance observed in the present study is comparable to recent findings in shrimp culture systems, where probiotic-based and synbiotic approaches promoted growth, feed utilization, and overall production performance. Such improvements have been attributed to enhanced nutrient availability and favorable microbial interactions within the culture environment [70-72].

Overall, the present findings demonstrate that the integration of bioencapsulated live feed with a substrate-based culture system produces a synergistic effect, enhancing growth performance beyond levels reported in conventional biofloc or clear water systems. This suggests that SbCS may represent a promising culture strategy deserving further evaluation.

Role of microbial communities in water quality regulation

The physical parameters remained within optimal ranges throughout the experimental period: pH (6.21-7.60), temperature (26.19-28.98°C), and DO (5.11-6.41 mg L⁻¹) recommended by FAO (2014) in both the culture systems of *M. rosenbergii* (Table 3). In case of chemical parameters, SbCS-E treatment exhibited significantly ($P < 0.05$) lower ammonia (NH₃-N) (0.57 ± 0.07 mg L⁻¹) and nitrite (NO₂-N) (0.13 ± 0.01 mg L⁻¹) concentrations and higher nitrate (NO₃-N) (24.72 ± 0.03 mg L⁻¹) levels compared to the other treatments ($P < 0.05$) (Table 4).

Two-way ANOVA revealed significant effects of feed type, culture system, and their interaction on all measured nitrogenous waste parameters. Total ammonia nitrogen (TAN) concentrations were significantly influenced by feed type ($F_{2,12} = 200.32$, $P < 0.001$), culture system ($F_{1,12} = 15,720.31$, $P < 0.001$), and their interaction ($F_{2,12} = 25.78$, $P < 0.001$), with lower TAN levels consistently observed in SbCS treatments, particularly in SbCS-E. Similarly, nitrite concentrations were significantly affected by feed type ($F_{2,12} = 411.68$, $P < 0.001$), culture system ($F_{1,12} = 2,608.37$, $P < 0.001$), and feed $\times$ culture system interaction ($F_{2,12} = 9.64$, $P = 0.003$), with the lowest values recorded in SbCS-E and the highest in CWS-A. In contrast, nitrate concentrations were significantly influenced by feed type ($F_{2,12} = 19,361.65$, $P < 0.001$), culture system ($F_{1,12} = 514,798.31$, $P < 0.001$), and their interaction ($F_{2,12} = 395.75$, $P < 0.001$), with substantially higher nitrate levels observed in SbCS treatments, particularly SbCS-E. These results indicate that both dietary treatment and culture system significantly influenced nitrogen dynamics, with the substrate-based culture system promoting the conversion of ammonia and nitrite into nitrate, thereby contributing to improved water quality.

How to cite this article:

Treatments	Parameters		
	pH	DO (mg L ⁻¹)	Temperature (°C)
SbCS-E	6.54-7.60	5.59-6.41	26.49-28.20
SbCS-NE	6.57-7.59	5.54-6.32	26.31-28.15
SbCS-A	6.59-7.51	5.53-6.25	26.22-28.26
CWS-E	6.21-7.40	5.22-6.01	26.20-28.98
CWS-NE	6.32-7.26	5.34-6.31	26.19-28.97
CWS-A	6.37-7.28	5.11-6.28	26.24-28.88

TABLE 3: Ranges of physical parameters, pH, DO (mg L⁻¹), temperature (°C) of *M. rosenbergii* reared in different culture systems and given different feeds.

DO, Dissolved Oxygen

How to cite this article:

Treatments	Parameters		
	NH ₃ -N (mg L ⁻¹)	NO ₂ -N (mg L ⁻¹)	NO ₃ -N (mg L ⁻¹)
SbCS-E	0.57 ± 0.07 ^f	0.13 ± 0.01 ^f	24.72 ± 0.03 ^a
SbCS-NE	0.65 ± 0.01 ^e	0.27 ± 0.02 ^e	21.36 ± 0.04 ^b
SbCS-A	0.71 ± 0.04 ^d	0.43 ± 0.02 ^d	19.50 ± 0.03 ^c
CWS-E	1.55 ± 0.02 ^c	0.85 ± 0.08 ^c	7.51 ± 0.03 ^d
CWS-NE	1.76 ± 0.02 ^b	1.56 ± 0.06 ^b	5.06 ± 0.06 ^e
CWS-A	1.81 ± 0.03 ^a	1.84 ± 0.02 ^a	4.84 ± 0.002 ^f

TABLE 4: Chemical parameters, ammonia-N (NH₃-N), nitrite-N (NO₂-N), nitrate-N (NO₃-N) (mg L⁻¹) of *M. rosenbergii* reared in different culture systems and given different feeds.

The mean values in the same column with different superscripts are significantly different ($P < 0.05$). *Macrobrachium rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A).

A key functional advantage of the consortium culture system was the marked reduction in ammonia (NH₃-N) and nitrite (NO₂-N) concentrations, indicating efficient nitrogen transformation within the system. Compared to conventional clear water systems, where ammonia levels frequently exceed 1.0 mg L⁻¹ under intensive conditions, the SbCS maintained substantially lower concentrations (e.g., ~0.57 mg L⁻¹). Similar improvements in nitrogen dynamics have been reported in biofloc-based systems [37,73], although the present system achieved comparable or improved stability under integrated feeding conditions.

The observed reduction in nitrogenous waste can be attributed to enhanced microbial assimilation driven by the addition of an external carbon source (maize starch). Carbon supplementation likely stimulated heterotrophic bacterial proliferation, increasing the C:N ratio and promoting the rapid conversion of dissolved inorganic nitrogen into microbial biomass [74-76]. This mechanism not only detoxifies the culture environment but also facilitates nutrient recycling, whereby microbial biomass becomes an additional trophic resource for cultured organisms [77,78].

In contrast to conventional systems that rely heavily on water exchange for waste removal, the SbCS appeared to function as a biologically self-regulating system. This internal nutrient cycling significantly reduces the need for water replacement, offering a potentially more sustainable approach to aquaculture management [74,79,80]. Similar principles have been demonstrated in heterotrophic biofloc systems, where carbon-driven microbial assimilation improves water quality and production efficiency; however, the integration of live feed enrichment in the present study provides an added functional dimension.

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Beyond nitrogen removal, the SbCS maintained stable dissolved oxygen and pH levels throughout the experimental period, indicating overall system stability. Such physicochemical consistency is essential for minimizing physiological stress and optimizing metabolic performance in cultured organisms [81]. The presence of diverse microbial consortia likely supported both heterotrophic assimilation and autotrophic nitrification pathways, contributing to efficient nitrogen turnover and reduced accumulation of toxic intermediates.

The magnitude and consistency of water quality improvement observed in this study highlight the effectiveness of microbial management strategies in aquaculture systems. Compared to traditional clear water approaches, which often exhibit fluctuations in nitrogenous waste and require frequent intervention, the SbCS provides a more resilient and balanced environment [80,81]. This environmental stability is a critical factor underpinning the enhanced growth and survival observed in the present study, reinforcing the importance of integrating microbial processes into aquaculture system design. Recent studies have similarly demonstrated that biologically active aquaculture systems enriched with beneficial microorganisms can improve nitrogen transformation processes, reduce ammonia accumulation, and promote more stable water quality conditions. These findings support the role of microbial-mediated nutrient cycling in maintaining favorable rearing environments [72,82-84].

Protein and lipid profiles of *M. rosenbergii*

The proximate composition of *M. rosenbergii* was significantly affected by both culture system and feed type ($P < 0.05$). The highest protein ($77.14 \pm 0.14\%$) and lipid ($11.89 \pm 0.04\%$) contents were observed in the SbCS-E treatment, whereas the lowest values were recorded in CWS-A (Table 5).

Two-way ANOVA revealed significant effects of feed type, culture system, and their interaction on both the crude protein and lipid contents of *M. rosenbergii*. For crude protein content, significant effects were observed for feed type ($F_{2,12} = 2155.53$, $P < 0.001$), culture system ($F_{1,12} = 40251.12$, $P < 0.001$), and their interaction ($F_{2,12} = 1857.78$, $P < 0.001$). Similarly, lipid content was significantly influenced by feed type ($F_{2,12} = 682.72$, $P < 0.001$), culture system ($F_{2,12} = 1844.16$, $P < 0.001$), and the interaction between these factors ($F_{2,12} = 287.29$, $P < 0.001$). Prawns receiving bioencapsulated feed exhibited significantly higher crude protein and lipid contents than those fed non-bioencapsulated feed, while both nutritional parameters also varied significantly among culture systems. The significant feed type $\times$ culture system interactions indicate that the effects of feed on the proximate composition of *M. rosenbergii* were dependent on the culture system employed.

How to cite this article:

Treatments	Proximate analysis (%)	
	Protein	Lipid
SbCS-E	77.14 ± 0.14 ^a	11.89 ± 0.04 ^a
SbCS-NE	74.03 ± 0.17 ^b	11.66 ± 0.08 ^b
SbCS-A	69.34 ± 0.09 ^c	11.31 ± 0.03 ^c
CWS-E	63.85 ± 0.06 ^d	11.05 ± 0.01 ^d
CWS-NE	63.24 ± 0.05 ^e	10.66 ± 0.07 ^e
CWS-A	61.48 ± 0.12 ^f	8.59 ± 0.03 ^f

TABLE 5: Protein and lipid compositions (%) of *M. rosenbergii* reared in different culture systems and given different feeds.

The mean values in the same column with different superscripts are significantly different ($P < 0.05$). *Macrobrachium rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A).

The present study demonstrated that both culture system and feed type significantly influenced the proximate and fatty acid composition of *M. rosenbergii*. Prawns reared in the substrate-based consortium culture system and fed encapsulated *M. macrocopa* (SbCS-E) exhibited the highest protein and lipid contents, indicating that the combined effects of the consortium culture environment and nutritionally enriched live feed substantially improved the nutritional quality of the prawns. In contrast, the lowest protein and lipid levels observed in the CWS fed with *Artemia* (CWS-A) suggest that conventional rearing systems without microbial enrichment are less effective in promoting nutrient deposition.

The elevated protein and lipid levels in SbCS-E may be attributed to the nutritional enhancement of *M. macrocopa* through bioencapsulation with the *C. vulgaris*-*B. amyloliquefaciens* co-culture. Microalgae such as *C. vulgaris* are rich in proteins, essential amino acids, vitamins, sterols, and PUFAs, improve the nutritional quality of live feed organisms [85]. Simultaneously, probiotic bacteria contribute digestive enzymes, antimicrobial compounds, and growth-promoting metabolites that enhance nutrient assimilation and feed utilization in aquatic organisms [86]. The synergistic interaction between microalgae and probiotics likely increased nutrient bioavailability and promoted efficient transfer of nutrients through the food chain, ultimately resulting in higher nutrient accumulation in *M. rosenbergii*.

The superior nutritional performance observed in the consortium culture system further suggests that the microbial-rich environment enhanced the availability of natural food particles and microbial protein. Previous studies have reported that biofloc- and consortium-based systems can improve crude protein and lipid deposition in crustaceans due to the continuous availability of microbial biomass and enhanced nutrient recycling [87,88]. The present findings are consistent with these reports and indicate that substrate-supported consortium systems may function similarly to biofloc systems by promoting microbial productivity and nutrient retention.

How to cite this article:

Fatty acid profiles of *M. rosenbergii*

Significant differences in fatty acid composition were observed among treatments ($P < 0.05$). The SbCS -E group showed the highest levels of ARA ($5.16 \pm 1.21\%$), EPA ($9.06 \pm 0.25\%$), DHA ($6.49 \pm 0.56\%$), and total PUFA ($72.49 \pm 0.55\%$). Notably, DHA was detected only in prawns fed encapsulated *M. macrocopa* (Table 6).

Fatty acid (%)	SbCS-E	SbCS-NE	SbCS-A	CWS-E	CWS-NE	CWS-A
C18	17.71 ± 0.64^a	15.45 ± 0.78^c	15.38 ± 0.25^c	16.66 ± 0.23^b	14.79 ± 0.38^d	13.89 ± 0.03^e
C18:1n-9	24.94 ± 0.87^a	21.03 ± 0.33^c	19.71 ± 0.25^e	20.33 ± 0.48^d	21.42 ± 0.43^b	20.32 ± 0.02^d
C18:2n-6	6.50 ± 0.26^a	5.25 ± 0.41^e	5.86 ± 0.46^b	5.52 ± 0.26^d	5.83 ± 0.47^c	5.18 ± 0.02^f
C19	-	0.28 ± 0.01	-	-	-	-
C20	1.09 ± 0.13^d	0.90 ± 0.05^e	1.46 ± 0.09^a	1.34 ± 0.02^b	1.15 ± 0.03^c	0.76 ± 0.01^f
C20:2n-9	-	-	-	-	2.36 ± 0.03	
C20:4n-6 (ARA)	5.16 ± 1.21^a	4.05 ± 0.89^c	4.80 ± 0.38^b	3.41 ± 0.36^d	0.15 ± 0.01^e	0.12 ± 0.12^e
C20:5n-3 (EPA)	9.06 ± 0.25^a	8.63 ± 0.08^b	6.74 ± 1.37^c	6.28 ± 0.22^d	5.94 ± 0.42^e	4.88 ± 0.57^f
C21:3	-	-	1.72 ± 0.12^a	-	-	1.53 ± 0.2^b
C21:4	6.79 ± 0.5^b	7.33 ± 0.36^a	4.73 ± 0.31^e	4.88 ± 0.11^d	6.68 ± 0.03^c	4.62 ± 0.12^f
C22	1.24 ± 0.28^d	1.01 ± 0.01^e	2.00 ± 0.39^a	1.56 ± 0.00^c	1.45 ± 0.04^c	1.62 ± 0.05^b
C22:6n-3 (DHA)	6.49 ± 0.56^a	-	-	4.15 ± 0.42^b	-	-
C24	-	-	1.66 ± 0.14^a	1.30 ± 0.16^b	-	
PUFA	72.49 ± 0.55^a	70.41 ± 3.48^b	64.06 ± 3.76^c	61.28 ± 1.84^e	63.92 ± 1.84^d	52.92 ± 1.16^f

TABLE 6: Fatty acid profile (%) of *M. rosenbergii* reared in different culture systems and given different feeds.

The mean values in the same row with different superscripts are significantly different ($P < 0.05$). *Macrobrachium rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A). PUFA, Polyunsaturated Fatty Acids; EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid; ARA, Arachidonic Acid

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Significant differences in fatty acid composition were also detected among treatments, particularly in the levels of essential fatty acids such as ARA, EPA, DHA, and total PUFA. The highest concentrations of these fatty acids were recorded in the SbCS-E treatment, indicating that encapsulated *M. macrocopa* served as an efficient carrier of highly unsaturated fatty acids (HUFAs) to *M. rosenbergii*. Notably, DHA was detected only in prawns fed encapsulated *M. macrocopa*, suggesting the effectiveness of the bioencapsulation strategy in enhancing the transfer of essential fatty acids to the cultured prawns.

Essential fatty acids, particularly EPA and DHA, play vital roles in crustacean growth, membrane integrity, stress resistance, immune modulation, and reproductive performance [89]. The significantly higher levels of EPA and DHA observed in SbCS-E likely contributed to the enhanced growth and survival performance recorded in this study. Similar improvements in fatty acid composition following enrichment with microalgae-based diets have been reported in previous studies involving freshwater cladocerans and crustaceans [51,54]. The enrichment effect observed in the present study may be associated with the high PUFA content of *C. vulgaris* and the improved metabolic activity induced by probiotic supplementation. The enrichment of essential fatty acids observed in the present study agrees with recent reports demonstrating that microalgae-based enrichment can substantially improve the fatty acid composition of live feeds, thereby enhancing the transfer of nutritionally important compounds to cultured aquatic species during early developmental stages [57,90-92].

The lower PUFA, EPA, and DHA contents observed in the clear water treatments, particularly CWS-A, indicate that conventional feeding systems without enrichment provide insufficient levels of essential fatty acids for optimal prawn nutrition. In contrast, the consortium culture system appeared to facilitate enhanced fatty acid retention, possibly through improved microbial-mediated nutrient transformation and continuous supplementation of natural microbial biomass. The increased PUFA content in SbCS treatments also suggests improved lipid metabolism and incorporation of dietary fatty acids into prawn tissues.

Overall, the findings demonstrate that the integration of a substrate-based consortium culture system with encapsulated *M. macrocopa* was associated with improvements in the proximate composition and fatty acid profile of *M. rosenbergii*. The enhanced deposition of protein, lipid, EPA, DHA, and total PUFA highlights the potential of microalgae-probiotic enrichment strategies as sustainable alternatives for improving the nutritional quality and culture performance of freshwater prawns.

Microbiome modulation and *M. rosenbergii* health

Total Bacterial Load

Microbial analysis revealed significantly ($P < 0.05$) higher TPB, lower TAB, and TEB counts in SbCS treatments, particularly in the SbCS-E group compared to other groups (Table 7).

Two-way ANOVA revealed significant effects of feed type ($F = 388.26$, $P < 0.001$), culture system ($F = 2128.86$, $P < 0.001$), and feed $\times$ culture system interaction ($F = 19.06$, $P < 0.001$) on total bacterial abundance.

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Treatments	TAB (10 ⁵ CFU/g)	TEB (10 ⁵ CFU/g)	TPB (10 ⁵ CFU/g)
SbCS-E	3.71 ± 0.64 ^f	2.12 ± 0.87 ^f	350.33 ± 0.26 ^a
SbCS-NE	4.46 ± 0.78 ^d	3.58 ± 0.33 ^e	229.54 ± 0.41 ^b
SbCS-A	4.21 ± 0.25 ^e	4.34 ± 0.25 ^d	156.29 ± 0.46 ^c
CWS-E	7.67 ± 0.23 ^a	5.13 ± 0.48 ^c	116.34 ± 0.26 ^d
CWS-NE	6.15 ± 0.38 ^b	5.56 ± 0.43 ^b	44.9 ± 0.47 ^e
CWS-A	5.46 ± 0.03 ^c	5.67 ± 0.02 ^a	18.69 ± 0.02 ^f

TABLE 7: The total counts of aerobic (TAB), enteric (TEB) and probiotic bacteria (TPB) (10⁵ CFU g⁻¹) in the whole-body microflora of *M. rosenbergii* reared in different culture systems and given different feeds.

The mean values in the same column with different superscripts are significantly different ($P < 0.05$). *Macrobrachium rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *Moina macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A). CFU, Colony-Forming Units

Data Acquisition and Alpha Diversity from Amplicon Sequencing of the Bacterial Community

A total of 414,528, 440,673, 482,233, and 422,569 paired-end reads were generated from *M. rosenbergii* treated in SbCS-E, SbCS-NE, CWS-E, and CWS-NE, respectively (Table 8). After cleaning and chiral removal, a total of 301,593, 293,835, 351,006, and 380,301 non-chimeric reads (effective tags) were obtained from *M. rosenbergii* treated in SbCS-E, SbCS-NE, CWS-E, and CWS-NE, respectively. Based on the 97% similarity cut-off, a range of 8-16 OTUs for SbCS-E, 9-13 OTUs for SbCS-NE, 8-12 OTUs for CWS-E, and 11-14 OTUs for CWS-NE were listed for their corresponding effective tags.

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Treatments	Number of paired-end reads	Number of clean tags	Number of effective tags	Number of OTUs
SbCS-E	138176 ± 12740.07	130466 ± 11933.03	100531 ± 14089.69	13 ± 3.77
SbCS-NE	146891 ± 19640.82	138657 ± 18433.24	97945 ± 6764.16	11 ± 2.08
CWS-E	160744 ± 16372.63	151948 ± 15237.44	117002 ± 5187.93	10 ± 1.81
CWS-NE	140856 ± 18989.73	133001 ± 18008.79	106872 ± 21178.82	13 ± 1.53

TABLE 8: Mean ± SE 16S rRNA sequencing summary for *M. rosenbergii* reared in different culture systems and given different feeds.

OTU, Observation Taxonomic Units

Alpha diversity was quantified by the Shannon and Simpson indices, which relate both the OTU richness and evenness, and by the observed OTUs and Chao 1 index (Figure 5). The Chao 1 index was between 12 and 13 for SbCS-E, 11 and 12 for SbCS-NE, 10 and 11 for CWS-E, and 12 and 13 for CWS-NE. There were no significant differences ($P > 0.05$) between the Chao 1 indices of SbCS-E and CWS-NE. As per Shannon indices, OTUs varied from 0.75 to 1.50 for CCS-E, 0.52 to 1.06 for SbCS-NE, 0.52 to 1.70 for CWS-E, and 1.52 to 1.62 for CWS-NE, and there were significant differences ($P < 0.05$) among all treatments. In Simpson, the OUTs were 0.35-0.7 for SbCS-E, 0.25-0.59 for SbCS-NE, 0.3-0.8 for CWS-E, and 0.7-0.8 for CWS-NE, and again, there were significant differences ($P < 0.05$) in the diversities between the treatments.

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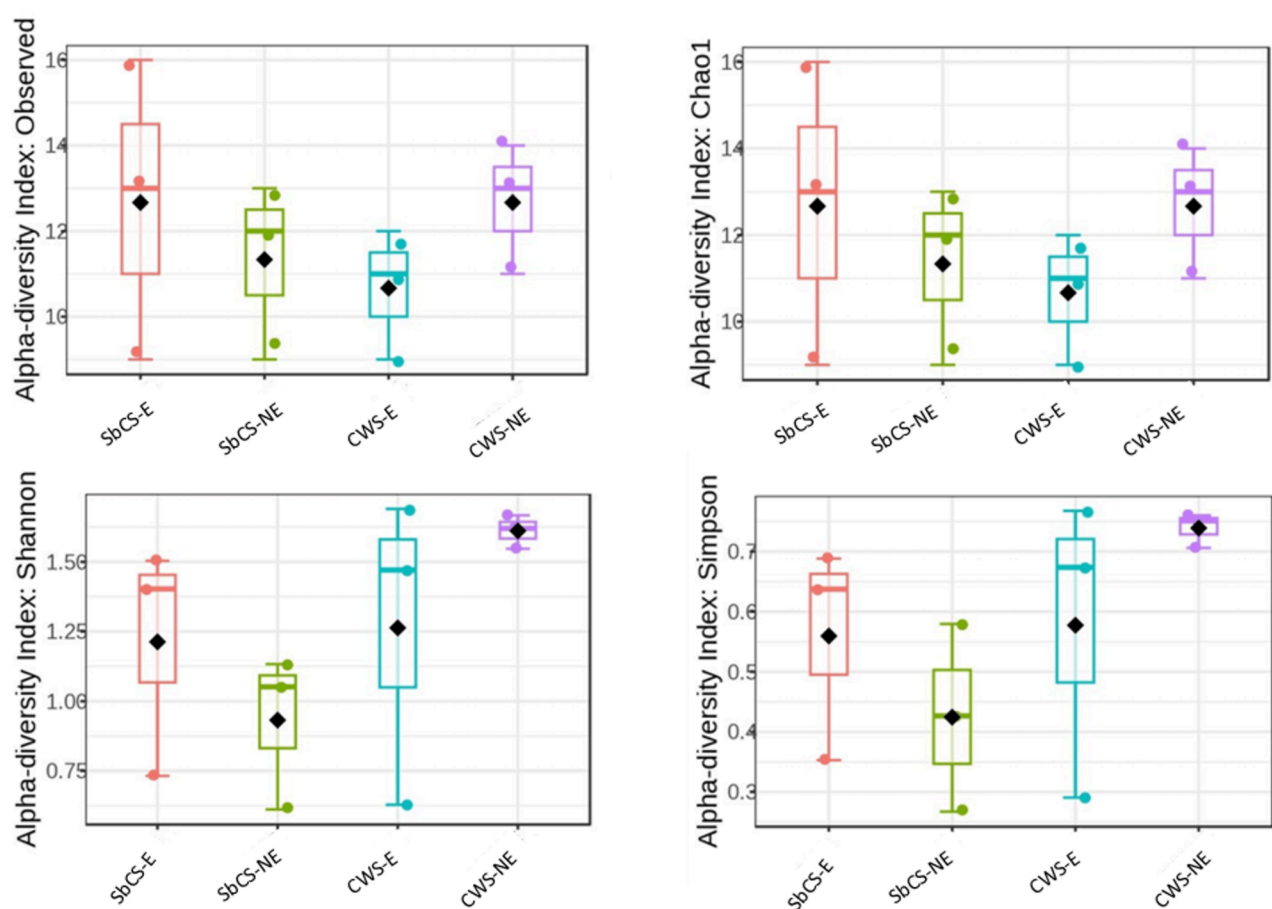


FIGURE 5: Alpha diversity metrics for (a) Observed OTUs; (b) Chao 1 C; (c) Shannon; (d) Simpson. *Macrobrachium rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A).

Microbiome Composition

Microbiome analysis demonstrated clear shifts in bacterial community structure between treatments. At the class level, the microbial composition of *M. rosenbergii* varied significantly among treatments ($P < 0.05$). In the SbCS-E group, the microbiome was predominantly composed of Bacilli (92%), which was significantly higher than Gammaproteobacteria (5%) and Clostridia (3%). Similarly, Bacilli was the dominant class in SbCS-NE (71%), followed by Gammaproteobacteria (25%) and Clostridia (5%), with significant differences among classes ($P < 0.05$).

CWS-E also showed a higher relative abundance of Bacilli (63%), which was significantly greater than Gammaproteobacteria (35%) and Clostridia (3%) ($P < 0.05$). In contrast, CWS-NE exhibited a different pattern, with Gammaproteobacteria (57%) significantly exceeding Bacilli (43%) ($P < 0.05$). Overall, the abundance of the bacilli group in SbCS-E represents a substantial enrichment compared to all other treatments (Figure 6).

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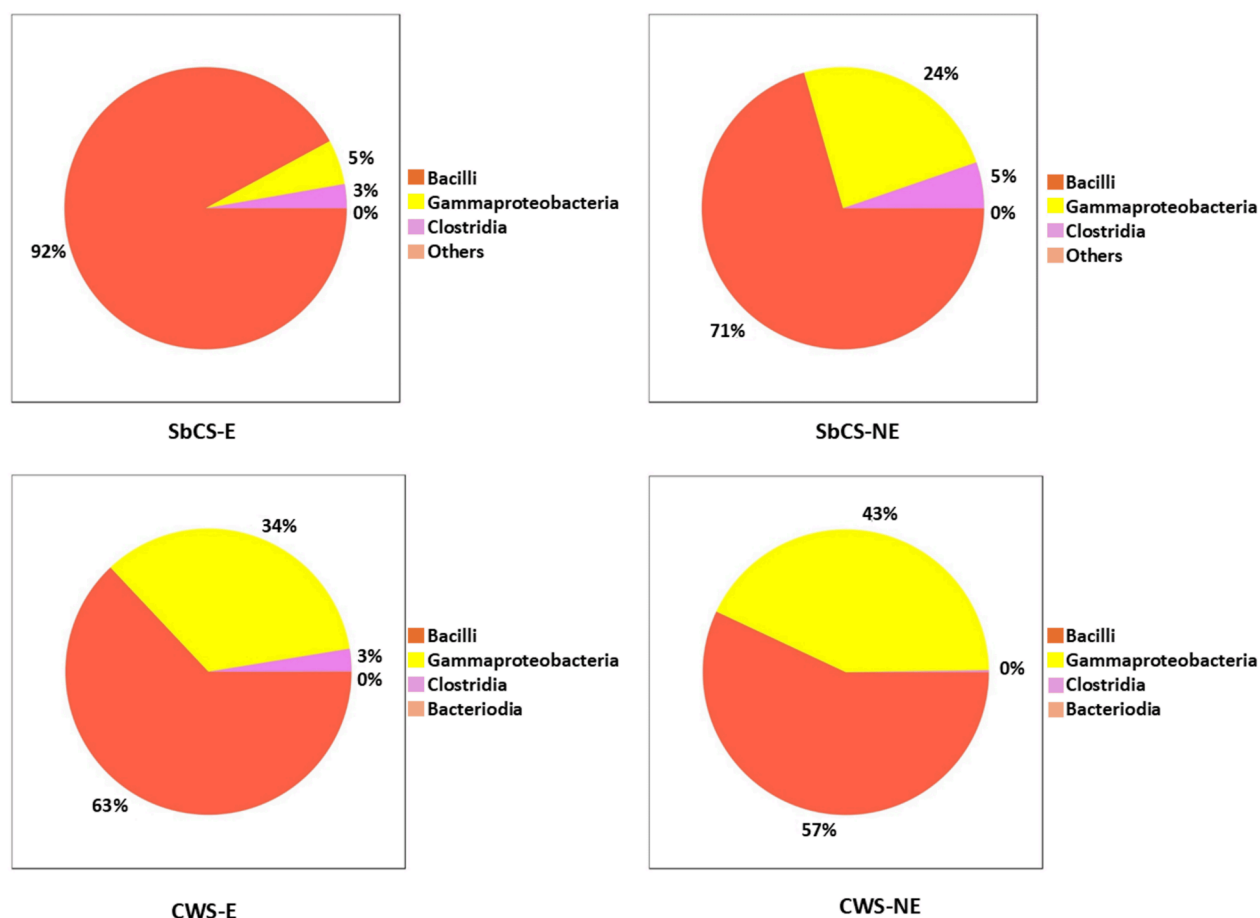


FIGURE 6: Class-level bacterial community in *M. rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A).

Bacterial genera belonging to the four dominant classes were identified across all treatments and are summarized in Figure 7. A total of eight genera were detected in both SbCS-E and SbCS-NE treatments. Among these, two genera (*Bacillus* and *Lactococcus*) belonged to Bacilli, while three genera (*Acinetobacter*, *Enterobacter*, and unclassified *Incertae sedis*) were affiliated with Gammaproteobacteria. One genus (*Clostridium*) was assigned to Clostridia, and the remaining two genera were unclassified.

In the CWS-E treatment, seven genera were identified, including one genus from Bacilli (*Bacillus*), two from Gammaproteobacteria (*Acinetobacter* and *Incertae sedis*), and two from Clostridia (*Clostridium* and *Pseudonocardia*), with two additional genera remaining unclassified. Similarly, the CWS-NE treatment comprised seven genera, including two Bacilli (*Bacillus* and *Lactococcus*), two Gammaproteobacteria (*Enterobacter* and *Incertae sedis*), and one Clostridia (*Clostridium*), along with two unclassified genera.

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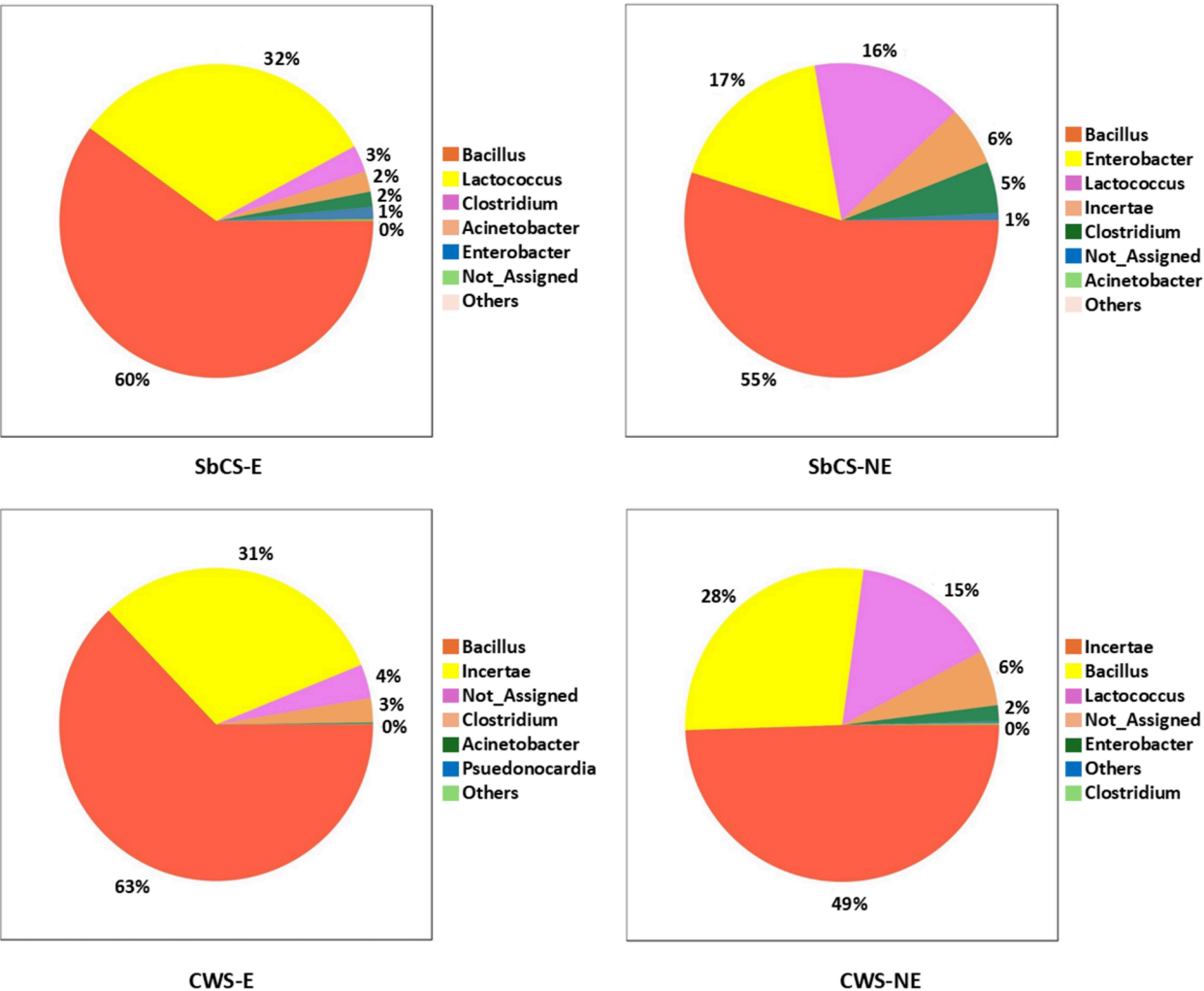


FIGURE 7: Genus-level bacterial community in *M. rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A).

The pronounced dominance of beneficial bacteria, particularly *Bacillus*, in SbCS treatments indicates effective microbial modulation driven by the integrated culture system. The high relative abundance of bacilli (up to 92% in SbCS-E) represents a substantial shift compared to clear water treatments, where more heterogeneous and potentially less stable microbial communities were observed. This selective enrichment suggests that the substrate-based culture system creates conditions favorable for probiotic taxa, thereby restructuring the microbial ecosystem toward a functionally beneficial state [93].

Members of the genus *Bacillus* are well-established probiotics in aquaculture, known to exert multiple beneficial effects, including competitive exclusion of pathogens, secretion of antimicrobial compounds, and stimulation of host immune responses [66,94]. In addition, *Bacillus* spp. produces extracellular enzymes such as proteases, lipases, and amylases, which enhance digestive efficiency and nutrient assimilation. The concurrent enrichment of *Lactococcus* further strengthens this probiotic profile, as lactic acid bacteria are associated with improved microbial composition, pathogen

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inhibition, and modulation of host immunity [95,96]. Similar probiotic-driven microbial shifts have been reported in aquaculture systems supplemented with beneficial bacteria [68,97], although the magnitude of dominance observed in the present study suggests a more pronounced selection pressure within the substrate-based culture systems.

The reduction in enteric and opportunistic bacterial groups in SbCS treatments further indicates improved microbial balance and reduced pathogen risk. In intensive aquaculture systems, the proliferation of opportunistic taxa often associated with Gammaproteobacteria has been linked to disease outbreaks and reduced host performance [98,99]. The comparatively lower abundance of these groups in SbCS suggests that probiotic dominance may suppress undesirable microbial populations through competitive and antagonistic interactions. This aligns with previous findings demonstrating that probiotic enrichment can reduce pathogen load and stabilize microbial communities in aquatic organisms [100-102].

At the broader community level, the predominance of Bacilli, alongside moderate representation of Gammaproteobacteria and Clostridia, is consistent with reported gut microbiota profiles in crustaceans [103,104]. However, the relative abundance of Bacilli observed in this study is notably higher than typical values reported under conventional rearing conditions, where microbial communities are often more evenly distributed across multiple taxa. This suggests that the combined effects of enriched live feed and microbial-based culture conditions exert a stronger selective influence on whole-body microbial composition.

It is also noteworthy that minor discrepancies were observed between culture-dependent and sequencing-based analyses. Such differences are well documented and arise from the inherent limitations of conventional culturing techniques, which capture only a fraction of total microbial diversity [105,106]. Despite these methodological differences, both approaches consistently indicated a shift toward beneficial microbial taxa under SbCS conditions, reinforcing the robustness of the observed trend.

The predominance of *Bacillus* observed in the present study is consistent with recent aquaculture studies reporting successful establishment of *Bacillus* populations following probiotic supplementation. Increased abundance of *Bacillus* has frequently been associated with favorable shifts in microbial community structure and improved culture performance, although direct causal relationships require further investigation [107,108]. Although increased abundance of *Bacillus* spp. and improved microbial composition were observed in the SbCS-E treatment, the present study does not establish direct causal relationships between specific microbial taxa and growth or health outcomes. Future investigations integrating metagenomic, transcriptomic, and functional analyses will be necessary to clarify the mechanisms underlying these associations.

Overall, the results suggest that compared to conventional feeding or single-probiotic approaches, the integrated SbCS strategy appears to exert a more comprehensive and sustained influence on microbial community structure, highlighting its potential as a next-generation tool for microbiome management in aquaculture. Also, due to resource limitations, sequencing analyses were restricted to *Moina*-fed treatments. Future studies should include all dietary groups to allow a complete factorial assessment of microbiome responses.

Disease resistance

Bacterial Challenge Test

Following *A. hydrophila* challenge, survival patterns clearly differentiated treatments. The SbCS-E group exhibited the highest resistance, maintaining significantly greater survival over the experimental period compared to all other groups ($P < 0.05$) according to Kaplan-Meier survival curve (Figure 8). In contrast, *Artemia*-fed prawns, particularly under clear water conditions (CWS-A), showed rapid mortality and reduced resilience.

The bacterial challenge assay using *A. hydrophila* clearly demonstrated enhanced disease resistance in *M. rosenbergii* reared under consortium culture conditions and fed encapsulated *M. macrocopa*. The SbCS-E treatment exhibited the highest survival following infection, exceeding survival levels typically reported in conventional systems, where post-challenge survival often declines to below 60-70% [109,110]. This indicates a substantial improvement in host resilience under the integrated feeding and culture strategy. Similar improvements in host resilience and disease resistance have

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been reported in recent studies evaluating probiotic-supplemented aquaculture systems. Enhanced resistance has been associated with improved nutritional status, beneficial microbial modulation, and stimulation of innate immune responses in cultured aquatic organisms [70,111-113].

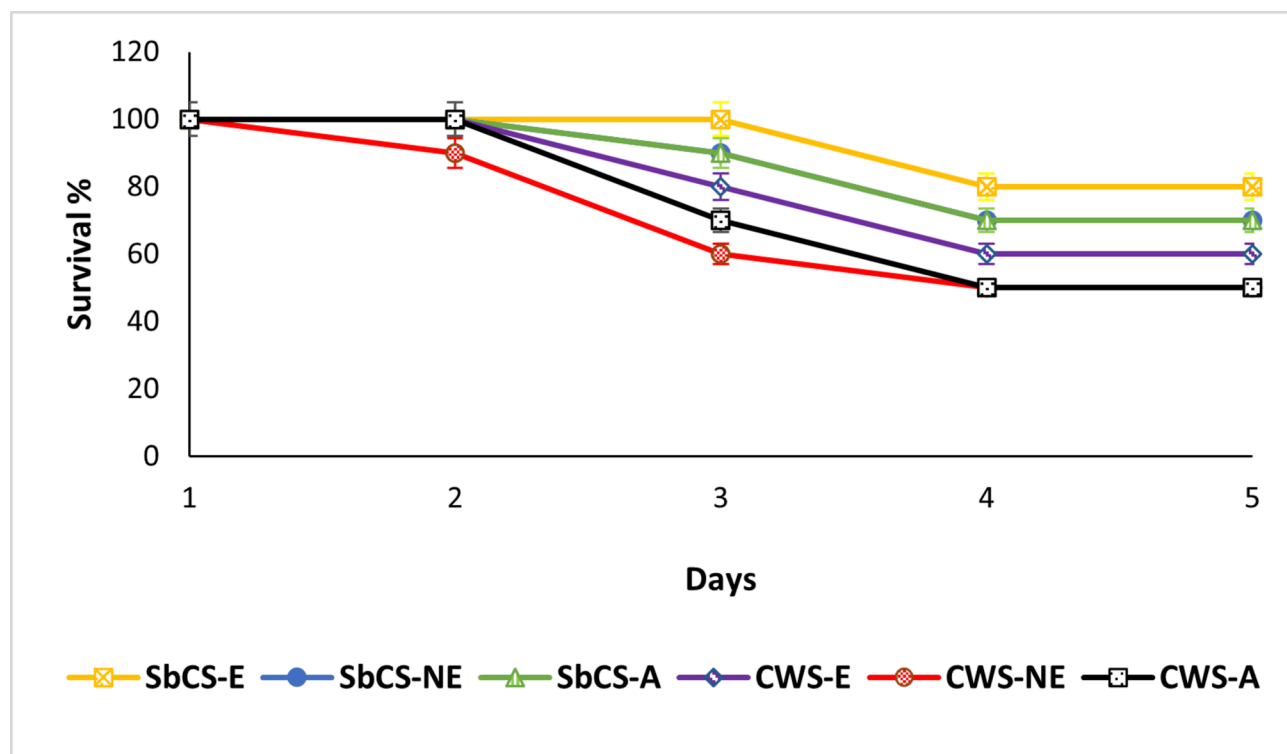


FIGURE 8: Mean ($\pm$ SE) Kaplan–Meier survival curve of *M. rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A).

The enhanced resistance observed in the present study is likely driven by a combination of improved nutritional status and probiotic-mediated immune modulation. Bioencapsulation facilitates the direct delivery of beneficial microorganisms and bioactive compounds into the host, promoting effective gut colonization and sustained interaction with the host immune system. In particular, *Bacillus* spp., which dominated the whole-body microbiota in SbCS treatments, are known to stimulate key innate immune responses in crustaceans, including phenoloxidase activity, lysozyme production, and antimicrobial peptide expression [114]. These immune enhancements have been widely documented in probiotic-based aquaculture systems and are associated with increased resistance to bacterial pathogens.

In addition to immunostimulation, the improved fatty acid profile observed in encapsulated *M. macrocopa*, particularly the enrichment of ARA, EPA, and DHA, may have contributed to enhanced immune competence. These fatty acids play critical roles in regulating inflammatory responses and maintaining cellular membrane integrity, thereby strengthening host defense mechanisms [115,116]. The combined effects of nutritional enrichment and probiotic activity likely resulted in a more robust and responsive immune system.

Histopathological Observations

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Histological analysis supported the survival and challenge test results. Prawns from SbCS-E treatment exhibited normal hepatopancreatic tissue architecture. In contrast, pathological alterations such as vacuolation and necrosis were observed in *Artemia*-fed groups after infection (Figure 9 and figure 10). Histopathological observations were descriptive and did not include quantitative lesion scoring or blinded evaluation.

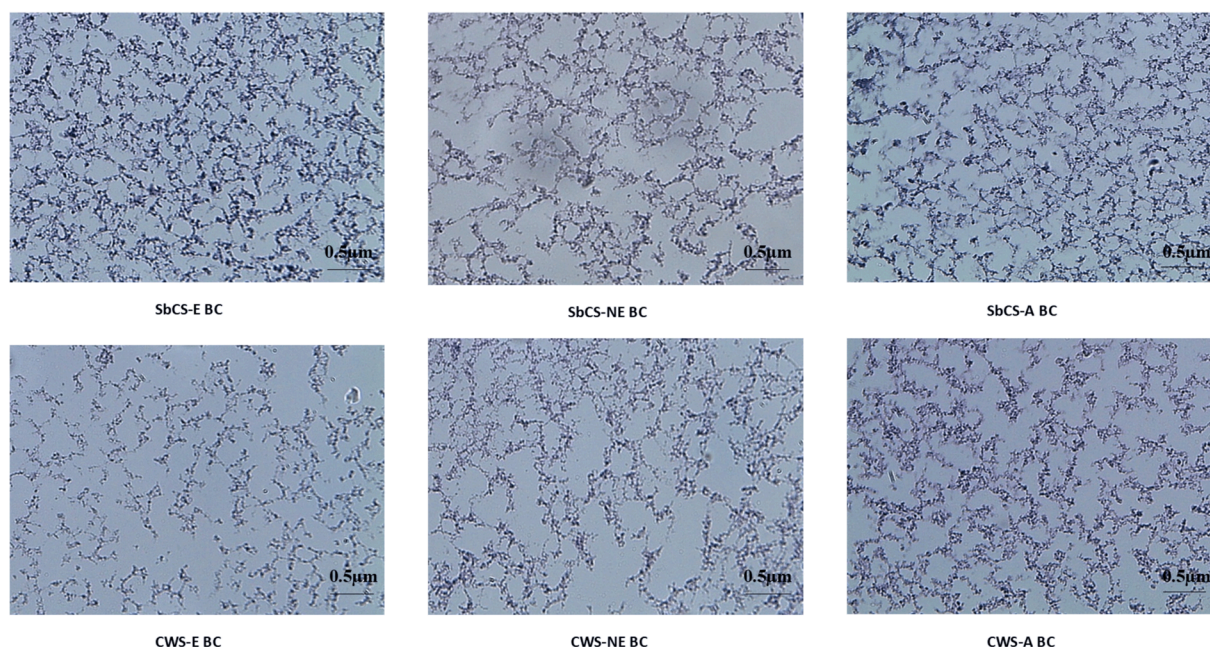


FIGURE 9: Histological appearance of the hepatopancreas section of *M. rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A) before challenge (BC). Magnification $\times 40$ (H&E).

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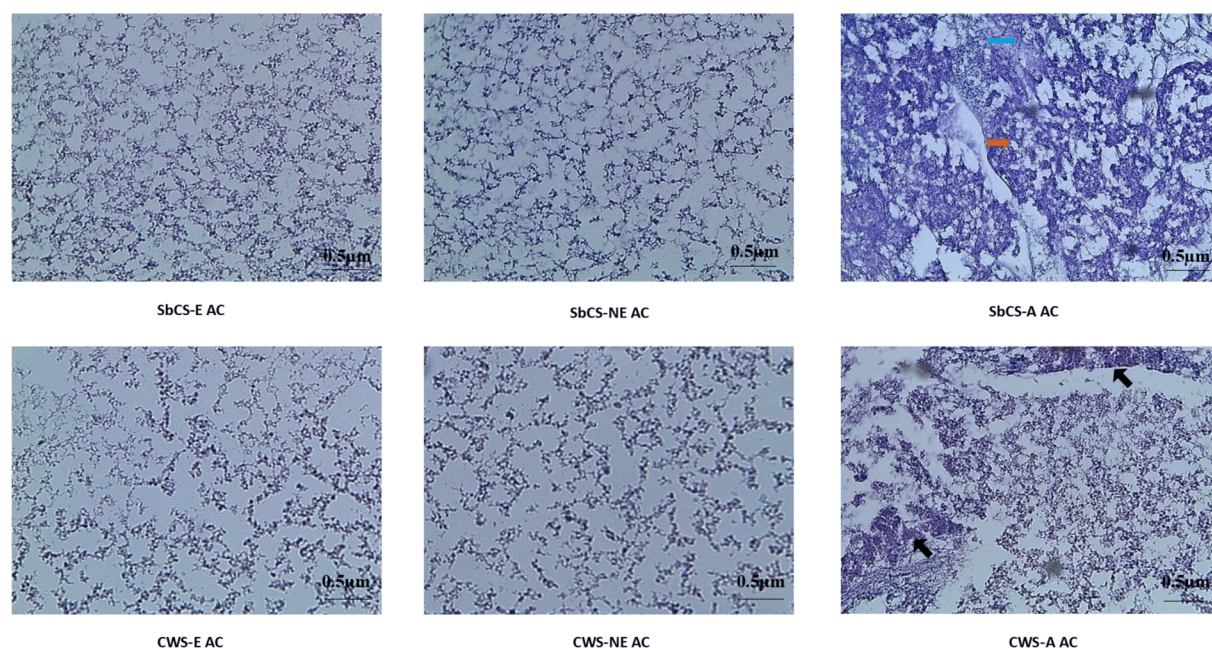


FIGURE 10: Histological appearance of the hepatopancreas section of *M. rosenbergii* reared in substrate-based culture system and fed with encapsulated *M. macrocopa* (SbCS-E), non-encapsulated *M. macrocopa* (SbCS-NE), non-encapsulated *Artemia* (SbCS-A) and *M. rosenbergii* reared in clear water system and fed with encapsulated *M. macrocopa* (CWS-E), non-encapsulated *M. macrocopa* (CWS-NE), non-encapsulated *Artemia* (CWS-A) after challenge (AC). Vacuole (Orange arrow), shrunken hepatocytes (yellow arrow), inflammation with focal necrosis (black arrow). Magnification $\times 40$ (H&E).

The reduced pathological damage in SbCS treatments confirms the protective role of the substrate-based culture system and bioencapsulation strategy. Histopathological observations further corroborated these findings. Prawns in SbCS-E exhibited no alterations following infection, with largely intact hepatopancreatic structure, whereas severe pathological changes, including vacuolation, cellular degeneration, and necrosis, were evident in control groups, particularly those reared under clear water conditions. Similar protective effects of probiotics against tissue damage have been reported in previous studies [68,95]; however, the extent of protection observed here suggests a more effective mitigation of pathogen-induced stress.

Compared to earlier studies that focused primarily on probiotic supplementation, nutritional enhancement, or microbial management as individual strategies, the present study evaluated the combined application of enriched live feed and a substrate-based culture system in *M. rosenbergii* post-larvae under controlled laboratory conditions. Previous studies have independently demonstrated the benefits of probiotic supplementation, microbial community manipulation, and biologically active culture systems for improving water quality, growth performance, and disease resistance [72,117]. The enhanced disease resistance observed in the present study suggests that integrating bioencapsulation with a substrate-based culture system may provide complementary nutritional, microbial, and environmental benefits. Such an integrated approach may contribute to pathogen suppression and improved host resilience through multiple pathways.

Overall, these findings highlight the potential of SbCS-based bioencapsulation strategies to significantly improve disease resilience in aquaculture species. By simultaneously enhancing whole-body microbial balance, nutritional status, and immune function, this approach offers a robust and sustainable alternative to conventional disease management strategies.

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Limitations of the study

This study was conducted under laboratory conditions for 26 days using triplicate tanks. While the results demonstrate promising effects of bioencapsulated *M. macrocopa* and substrate-based culture systems, longer-term trials under commercial production conditions are necessary. In addition, microbiome analyses in *M. rosenbergii* were limited to selected treatments, and histopathological observations were qualitative rather than quantitatively scored. These factors should be addressed in future investigations.

Conclusions

The present study indicates that the use of bioencapsulated *M. macrocopa* within a SbCS can improve the growth performance, nutritional quality, microbial composition, water quality, and disease resistance of *M. rosenbergii* PL under laboratory conditions. Bioencapsulation enhanced the nutritional value of *M. macrocopa*, particularly with respect to protein, lipid, and essential fatty acid contents, which was associated with improved growth, survival, and nutrient deposition in prawns receiving the enriched live feed. The substrate-based culture system was associated with improved water quality parameters and increased abundance of beneficial bacterial populations such as *Bacillus* and *Lactococcus* compared with conventional clear-water culture. Furthermore, *M. rosenbergii* reared in the SbCS and fed bioencapsulated *M. macrocopa* exhibited enhanced resistance to *A. hydrophila* challenge, as reflected by higher post-challenge survival and reduced histopathological alterations. Although shifts in the host-associated microbial community were observed, the present study does not establish direct causal relationships between specific microbial taxa and the observed improvements in growth or health status.

Overall, the findings suggest that the combination of bioencapsulated live feed and a substrate-based culture system may provide a promising approach for improving freshwater prawn production. However, the study was conducted over a relatively short culture period under laboratory conditions, and microbiome analyses were limited to selected treatments. Therefore, longer-term studies under commercial farming conditions are required to validate the scalability, consistency, and economic feasibility of this approach. Future research should also investigate the functional mechanisms underlying microbial modulation and evaluate the applicability of this strategy across different aquaculture species and production systems.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Fatimah M. Yusoff, A Ashwitha, F M. Ikshan Natrah, Murni Karim, Chou Min Chong

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Critical review of the manuscript for important intellectual content: Fatimah M. Yusoff, A Ashwitha, Hui Teng Tan, Yaw Sim Khaw, F M. Ikshan Natrah, Murni Karim, Chou Min Chong

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Drafting of the manuscript: A Ashwitha

Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen. **Material Transfer Agreement:** The study involves transfer of Biological Material, and a Material Transfer Agreement was signed by the author. **Human subjects:** All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** The study involves the use of animal subjects, International Animal Care and Use Committee (IACUC) guidelines

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Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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