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Gut microbiota in *Macrobrachium Rosenbergii* a comparative analysis associated with water quality parameters

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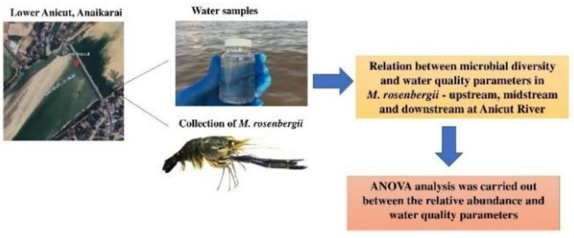
Graphical abstract



Highlights

- Comprehensive analysis of the gut microbiota of M. rosenbergii
- Vibrio (Proteobacteria) was the dominant bacterial species
- Assessment of various water quality parameters were observed with significant variations.
- ANOVA analysis were preformed between the microbial community and environmental factors.

Abstract

This study examines the microbial diversity and environmental gradients of aquatic habitats from upstream, midstream, and downstream locations near the anicut. Using relative abundance and diversity indices, these investigations revealed a decrease in downstream aquifer microbial diversity, with *Vibrio* (Proteobacteria) becoming more frequent. Anthropogenic activities and natural processes are reflected in the significant geographical variability ($p < 0.05$) of environmental parameters as pH, DO, salinity, and nutrient levels (ammonia, nitrate, and phosphate). In the downstream regions, nutrient enrichment is high and DO is low, which could suggest that aquatic ecosystems may be disrupted by eutrophication. The dominance of the Vibrio genus in certain pathogenic individuals suggests poor water quality and compromised ecological integrity. These findings highlight the connection of microbial communities and environmental conditions, underscoring the significance of sustainable management approaches. Restoring riparian habitat, managing nitrogen loads, and conducting frequent monitoring are some suggestions to lessen ecological difficulties.This study thereby integrates environmental analysis and microbial profiling as an important discovery for ecosystem dynamics, which therefore stresses the urgent need for well-targeted intervention measures to prevent the loss of biodiversity and disruption of ecological equilibrium in river systems.

Keywords: Freshwater, water quality, microbiota, *M. rosenbergii,* statistical analysis

1. Introduction

Aquatic ecosystems depend on freshwater prawns like *Macrobrachium rosenbergii*afor transfer of energy and recycling of nutrients. Eating algae, debris, and small invertebrates keeps the environment in balance and provides larger aquatic animals with an essential sources of food. In artisanal fisheries and small-scale fish farming, provides food and livelihoods in many places it is economically very useful. For *M. rosenbergii* to survive, grow, and be healthy, its gut microbiome is essential. Gut microbial populations produce vital metabolites, enhance nutrition absorption, facilitate food digestion and improve defences against illness (Zhang *et al.* 2022). Water quality parameters like pH, salinity, and DO have a major impact

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on the variety and activity of the gut microbiota, which in turn affects the production and wellbeing of the prawn.

The gut, a complex ecosystem, serves to be the richest site for absorption and digestion in mammals. Gut microbiota has been reported to play a fundamental role in determining the physical characteristics of aquatic species, including immunity, disease resistance, development, metabolism, energy consumption, and nutrient absorption (Sun and Xu 2021). Bacteria abundance in the digestive tracts of aquatic animals is very diverse. It was demonstrated previously that host factors like growth, development, and dietary characteristics have a positive correlation with the intestinal flora population. In the intestine of the host, gut immune mechanisms and conditions for the environment help maintain relatively consistent gut bacterial richness. For example, aquatic organisms with greater growth rate had higher relative levels of intestinal probiotics. Aquatic animals mostly possess facultative anaerobic, obligate anaerobic, and aerobic microbes in their gut microbiota. Host genetic assets, consumption patterns, food availability, and aquaculture system are a few of the factors that are responsible for greatly influencing the gut microflora structure and composition of aquatic animals, in addition to the tissue, wall, and contents of the gut (Shi *et al.* 2020). Moreover, the aquatic species' living environment, nutritional source, feeding style, and genetic composition have a great bearing on the gut microbiota composition and structure. These microorganisms exist in the gut walls and the contents of digestive tracts (Garibay-Valdez *et al.* 2020).

Many investigations have shown that the gut microbiota's symbiotic adaptive balance during shrimp development depends on the developmental characteristics of the host, enabling the diversity of gut bacteria and the host to promote one another's growth (Liu *et al.* 2021).In fact, the immunology, gut development, and host development of various aquatic species depend on the gut microbiota. Furthermore, the metabolites produced are the main channel of communication between the gut microbiota and the host. The dynamics of the gut flora may also influence the intestinal metabolic network. There have been numerous studies over the past years exploring the associations of aquatic creatures with their gut microbiotas. Studies show that gut bacteria in aquatic animals help the host's immunological and digestive tracts function (Shi *et al.* 2020). Most of the current research on shrimp gut microbiota focuses on seawater shrimp. For instance, research on black tiger shrimp showed how the development of probiotics may enhance growth and disease resistance. In his investigation, Sujaya analyzed the bacterial community within the gut of Penaeus monodon domesticated and wild animals. He found out that the interior habitat of the shrimp host does apply selective pressure to the population of bacteria (Zhao *et al.* 2018).

Temperature, pH, salinity, dissolved oxygen, and nutrient levels are the environmental variables affecting the gut microbiota of shrimp and prawns in river water. These factors determine the composition and activity of microbial populations in the gut, influencing immunity, digestion, and general health. For example, ideal pH and salinity stimulate the growth of beneficial bacteria, though deviations or extremes can lead to dysbiosis and make the body prone to various diseases. Pollution-induced low dissolved oxygen and high nutrient loads can change the makeup of microorganisms, favouring dangerous or opportunistic ones. Therefore, alterations in the environment, whether man-made or natural, have a direct impact on the host's ability to grow, survive, and withstand stress (Liu *et al.* 2022). Management of ecosystem health and sustainable aquaculture depends on an understanding of these relationships. An investigative study is currently proposed, which primarily devotes its attention to understanding the interplay that water quality indices would have in relation to the diversity of gut microbes found in Macrobrachium rosenbergii species dwelling across the different regions, i.e., upper, middle, and lower regions of the Lower Anicut River. The work is about 16S rRNA sequencing. The prominent microbial taxa will be collected, derived from various microbial diversity indices such as Shannon and Simpson, and correlated to several water quality parameters such as pH, DO, salinity, ammonia, nitrate, and phosphate. This study will help give teeth to sustainable aquaculture practices as well as prawn health determined by quality of the environment. Pollution and nutrient loading due to anthropogenic activities put aquatic ecosystems in jeopardy while modifying the microbial community indispensable in host metabolism and immunity. A microbial indicator study will further be made to show them as very sensitive measures of the environmental health. The study of these shifts among microbes is to throw light on proper conditions optimal for growth along with risk conditions. This work will emphasize spatial analysis across river gradient and links in microbial ecological studies with environmental data. This holistic approach would be valuable for water quality management, aquaculture sustainability, and ecosystem health. Ultimately, this research would fine-tune strategic interventions to strengthen prawn farming through microbial and environmental monitoring.

2. Review of Literature

(Gao *et al.* 2022) observed the relationship between the abdominal microbial community and phenotypic qualities in *M. rosenbergii* by usingculture-dependent and high-throughput sequencing approaches. The outcomes of the study reveal that the predominant bacterial species was *Lactococcus garvieae.* And the statistical analysis shows there is a significant correlation between the gut microbiota and phenotypic traits. Furthermore, the outcomes of the conventional culture method further supported the finding that *L. garvieae* was substantially more prevalent across all groups, indicating that it plays a significant role in the growth of Giant Freshwater Prawns (GFP). Because of its impact on food metabolism in the gut, this study strongly implies that the intestinal microbiota of GFP is closely associated with morphotype differentiation. (Sun *et al.* 2021) observed the raising of fish performance requires an understanding of fish gut microbial populations and the variables affecting community composition. The study analyzed the gut microbiota of young black sea bream, *Acanthopagrus schlegeliias*, using 16s rRNA gene sequencing in cage, pond, and wild cultures. It analyzed the relationship of gut microbiota with associated ecological parameters. Major findings in the study concerning gut microbiota of young *A. schlegelii* brought about differences majorly as influenced by habitat. Gut microbiome of juvenile *A. schlegelii* was more diverse in wild populations than in individuals that were raised in pond environments because they are opportunistic feeders and have many natural food sources.

(Lan *et al.* 2023) utilized LC-MS metabolomic and 16S rRNA sequencing techniques to study the critical gut microbiota and metabolites associated with the growth rate of M. rosenbergii individuals. For this study, 90 M. rosenbergii families were built to evaluate the growth performance. After ninety-two days of culture, three experimental groups representing three levels of growth performancehigh (H), moderate (M), and low (L) were collected based on the weight gain and specific growth rate of the families.According to the gut microbiota composition, Group H had significantly greater relative abundances of *Firmicutes, Lachnospiraceae, Lactobacillus*, and *Blautia* than did the M and L groups. According to correlation analysis, alpha-ketoglutaric acid and L-arginine levels were favourably connected with *Lactobacillus* and *Blautia* abundances. Additionally, there was a favourable correlation between spermidine, taurine, and adenosine levels with the abundance of*Blautia*. (Omogbai and Onokwai 2014) studied microbial loads and chemical composition of freshwater prawns were kept at various temperatures for five days using standard microbiological techniques. The finding of the study isolated various bacteria like *Staphylococcus aureus, Bacillus spp., Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella spp*. and *Aspergillus flavus*. The results concluded that the microbial loads of the stored prawns were higher when compared with the fresh sample prawns. Species like *Staphylococcus aureus, Proteus mirabilis* and *Escherichia coli* had the highest occurrence while *Salmonella spp*. had the lowest occurrence in the overall samples. (Zhuang *et al.* 2022) investigated the effect of bovine lactoferricin (LFcinB) on growth and non-specific resistance in *M. rosenbergii*. A total of five diet experiments were conducted, in the control group the basal part was lacking bovine lactoferricin. For an 8-week feeding trial, six hundred prawns were randomly assigned to five groups, each in triplicate in fifteen tanks. The outcome demonstrated that the final mass, weight increase rate, exact growth rate, and existence rate of prawns in the treatment groups were significantly better than the control. The current study concludes that feeding Bovine lactoferricin may considerably increase the growth performance and antioxidative status of *M. rosenbergii*. (Gao *et al.* 2022) acquired gut and hemolymphatic samples of big (ML), moderate (MM), and small (MS) male GFPs and utilized sequencing of 16S rRNA and liquid chromatography-mass spectrometry-based metabolomic approaches to examine gut microbiota and metabolites linked with GFP growth.Firmicutes and Proteobacteria were the most common bacteria; higher growth rates were associated with a higher Firmicutes/Bacteroides ratio. Serum metabolite levels varied considerably between the ML and MS groups. The findings demonstrated that *Faecalibacterium* and *Roseburia* may improve gut health in GFP by releasing butyrate, which affects physiological homeostasis and causes metabolic changes associated with GFP growth disparities. (Sharma *et al.* 2021) reported to determine the proportion of antibiotic-resistant bacteria and gut microbiota populations in commercially accessible shrimp. 31 raw and baked shrimp samples were acquired from stores in Florida and Georgia (USA). The obtained results were compared to species caught in the wild shrimp, imported farm-raised shrimp have unique gut microbial communities and a higher prevalence of antibiotic-resistant genes in their gut. The presence of antibiotic-resistant bacteria in cooked shrimp necessitates a change in processing to mitigate their spread.

Recent research has shown the incorporation of IoT with intelligent algorithms for enhanced water management and agricultural sustainability. A hybrid HG-RNN model coupled with IoT sensors for real-time prediction and treatment of polluted pond water has been proposed by (Maruthai *et al.* 2025). The focus of this model is on the health of the aquatic ecosystem. Similarly, (Raveena and Surendran 2024) presented a bi-directional RNN-based system for reclaimed water irrigation in coffee plantations with an accuracy of 95.66 in optimizing water consumption. The model (Venkatraman *et al.* 2025) proposed ODD-RecurFlowNet, which synergistically combined global attention, feature selection through GArO, and deep learning modules to handle the intricacy of the data from water, thus achieving very high prediction accuracy (98.01%). In the agriculture domain, (Jegan *et al.* 2024) employed the Random Forest method to optimize hydroponics lettuce cultivation by forecasting the ideal growth conditions from environmental parameters. Expanding on this, (Selvanarayanan *et al.* 2024) proposed a fuzzy logic-based FRNN system that dealt with wastewater reuse in coffee plantations, accounting for temporal and uncertain data and hence enhancing treatment efficiency. Furthermore, (Venkatraman *et al.* 2023) considered sustainable aquaponics by utilizing IoT, robotics, and virtual simulation for real-time monitoring of plant and aquatic health and precision agriculture with minimal resource use. Collectively, these studies outline the potential of smart technologies in water quality management, wastewater recycling, and sustainable crop production, thereby setting the base for further eco-efficient agricultural innovations.

3. Study area

In the Thanjavur District of Tamil Nadu, Sir Arthur Cotton built the historic masonry dam known as Lower Anicut (Anaikarai) in 1850 to bridge the Kollidam River, a distributary of the Cauvery River. Situated around 25 km downstream of the Grand Anicut (Kallanai), it is an essential irrigation system that sustains agriculture in the verdant Cauvery Delta (Keerthan *et al.* 2023). The region is well known for its diverse aquatic life, riparian flora, and tropical climate, all of which promote ecological balance and human way of life. The dam increases the productivity of rice and sugarcane by regulating water and preventing flooding. Its ecological value is further enhanced by the fact that many bird species frequent the surrounding area.

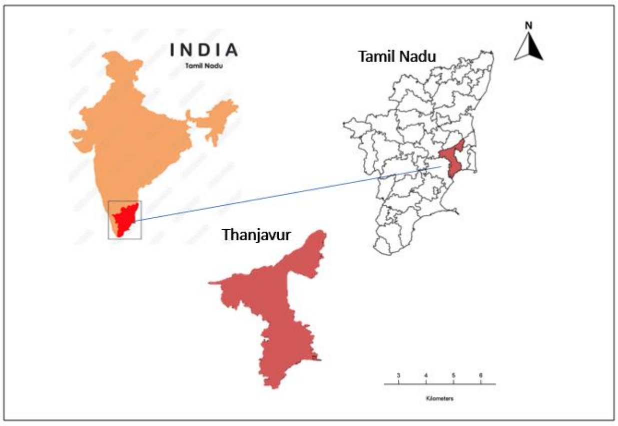


Figure 1. Study area map

Figure 2. Google Earth image of the Lower Anaicut

Figure 1 shows the map of the study area. With hot summers (28°C to 40°C), a monsoon season with moderate to high rainfall from both southwest and northeast monsoons (1,000–1,200 mm yearly) and warm winters (20°C to 30°C), the Lower Anicut (Anaikarai) region has a tropical climate. Due to the possibility of pollution from pollutants and agricultural runoff, the Kollidam River is not usually used directly for drinking purposes without treatment, even though it supplies water for agricultural irrigation throughout the lush Cauvery Delta. Its irrigation supports crops like rice, sugarcane, and bananas in parts of the districts of Thanjavur, Ariyalur, and Cuddalore. **Figure 2** represents the Google Earth image of the Lower Anicut which flows from the Kollidam River. The Lower Anicut is a dam across the Kollidam River. Its primary goal is to regulate water flow and ensure consistent irrigation across the fertile plains of the Cauvery Delta. The structural design of the dam ensures efficient waterdistribution and protection from seasonal floods.

4. Materials and methods

4.1. Water sample collection

Water samples were collected from the Lower Anicut, Anaikarai, Thanjavur, Tamil Nadu, based on the different environmental parameters such as upstream (clear water area), midstream (less polluted area) and downstream (polluted area) locations. The water samples were collected in all three sites using sterile 1-litre bottles to analyse physiochemical parameters like pH, temperature, DO, salinity, nitrate, phosphate and ammonia. The parameters like pH, temperature, and DO were measured during the sampling sites and samples were carried out to the laboratory immediately for further analysis.

4.2. Collection of M rosenbergii

An adult *M. rosenbergii*was collected using cast nets during the early morning to avoid the stress of organisms. The collected samples were transferred to a sterile container with river water and transported to the laboratory at the optimum temperature to preserve the microbiota integrity. All the collected samples were labelled according to the sample sites and stored at 4°C until it brought to the laboratory (**Figure 3**).

Figure 3. Image of the Macrobrachium rosenbergii

4.3. Analysis of gut microbiota

4.3.1. Sample preparation

The prawns were carefully dissected in a clean environment. By using sterilised instruments, the foregut, midgut and hindgut were extracted from the prawns. The gut samples were transferred to the sterile tube with a preservative buffer and brought to the lab at a cold temperature to prevent DNA degradation.

4.3.2. DNA extraction

To isolate the microbial cells from the host tissue, the gut was homogenized in a phosphate buffer saline solution. This process was done using the cleaned mortar and pestle. For the extraction of genomic DNA from the gut microbiota, a commercial DNA extraction kit was used. The name of the kit was Qiagen Power soil DNA Isolation kit were utilized for microbial studies. Once the isolation was done the samples needed to be qualified. For this, the extracted DNA was quantified using the fluorometric or spectrophotometer methods. Gel electrophoresis was used to evaluate the DNA's purity and to check for any contamination in the samples.

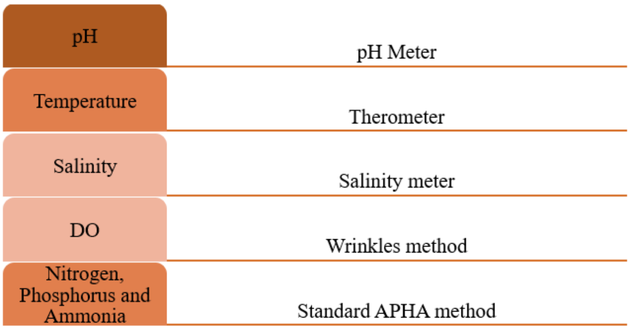
4.3.3. DNA Amplification and Sequencing

The DNA was amplified using definite primers targeting the 16s ribosomal RNA gene, which is commonly used in bacterial studies. The regions of V3-V4 of the 16s rRNA gene were targeted to identify the bacterial species from the samples. Then the amplified DNA was processed into the library by attaching the specific barcodes and followed by this quality control was done to check the quality for sequencing the DNA samples. For sequencing high-throughput sequencing platforms were used to generate large amounts of raw sequencing data.

```
Pseudocode for Microbiome Diversity Assessment and Water Quality Analysis
BEGIN
Input: Sample collected from Lower Anicut River.
FOR each site DO
    Collect water samples and record parameters
    Collect multiple gut samples
FOR each gut sample DO
    Extract DNA by standard protocol.
    Amplifying through PCR 16S rRNA gene (V3-V4 region).
    Sequence it using any high-throughput sequencing methods
Preprocess sequencing data
    Remove low-quality reads Filter chimeras and noise
    Cluster into OTUs or ASVs
Assign taxonomy to OTUs/ASVs using reference database (e.g., SILVA).
Calculate diversity indices as Shannon, Simpson for each site:
Calculate Relative abundance of dominant phyla and genera
FOR each site DO
    Correlating microbial diversity against parameters of water quality
    Visualize data using heatmaps, bar plots, and diversity box plots
    Analyze PCA on microbial community structure and water quality impacts
END
```

4.4. Water quality assessment

Environmental variables were chosen for this study based on water parameters and microbial diversity factors. Numerous physicochemical characteristics, such as water temperature, pH, salinity, DO, ammonia, nitrogen, and phosphorous were selected to analyse the quality of the river water (**Figure 4**). Using a pH meter, a salinity meter, a Dual-purpose package tool and the warmth of a water condition analyser with reading ranges from 0–14 pH and 0-9999 ppm, several parameters, including water temperature, were examined at the sample location. Each site's samples of water were gathered in a 1000 ml sample bottle and right away placed in the fridge for additional physiochemical analysis. After that, the collected samples were delivered to the lab and analysis of ammonia,nitrogen, and phosphorous, was done using standard (APHA) techniques (American Public Health Association (APHA) 2022).



pH	pH Meter
Temperature	Therometer
Salinity	Salinity meter
DO	Wrinkles method
Nitrogen, Phosphorus and Ammonia	Standard APHA method

Figure 4. Methods to Assess Water Quality Parameter

4.5. Statistical Analysis

A statistical analysis or technique called analysis of variance (ANOVA) compares the means of two or more groups to determine their significant differences. When examining the effects of a classified independent variable on a continuously dependent variable it is very beneficial. Microboilogical diversity indexes like Simpson and Shannon variation, which vary greatly depending on the environment or collecting sites, can be found using ANOVA (Chakraborty *et al.* 2023). The following equations (1-3) were used to calculate the ANOVA are,

$$F = \frac{\text{Mean square between groups(MSB)}}{\text{Mean square withi groups(MSW)}} \qquad (1)$$

Where,

$$\text{MSB} = \frac{\text{Sum of squares between the groups}}{\text{Degree of freedom between groups(df B)}} \qquad (2)$$

$$\text{MSW} = \frac{\text{Sum of squares within groups}}{\text{Degree of freedom within the groups(df W)}} \qquad (3)$$

To calculate the degree of freedom,

- B = k – 1, k is the number of groups,
- W = n- k, n is the total amount of observations.

5. Results and discussion

5.1. Data of 16s rRNA Sequencing

The data from the upstream location show a lower level of microbial diversity with a Shannon Index of 2.0 and a Simpson Index of 0.75 (**Table 1**). Compared to downstream, *Vibrio* has a reduced relative abundance, suggesting that potentially dangerous bacteria are less prevalent. Greater variety indicates a more stable gut microbial ecology, which could be influenced by the moderate water quality of the upstream region. This had the highest diversities for midstream regions: a Simpson Index of 0.8, and Shannon Index of 2.2. The presence of beneficial bacteria of relatively higher relative abundance such as *Bacillus* and *Flavobacterium* that enhances the immune responses and metabolism can be utilized with better food will possibly increase during these environmental conditions at midstream, as proposed from the study conducted. The downstream results display lower microbial diversity, with a Simpson Index of 0.72 and a Shannon Index of 1.8. Although the most represented genus is *Vibrio*, with 35.1% relative abundance, this result may indicate an environmental stress or less-than-optimal water quality of this region and could influence both health and growth of *M. rosenbergii*.

Table1. Results of microbial diversity in *M. rosenbergii*- upstream, midstream and downstream at Anicut River

Location	Phylum	Genus	Relative Abundance (%)	Shannon Index	Simpson Index
Upstream	Proteobacteria	*Vibrio*	30.2	2.4	0.85
Upstream	Firmicutes	*Bacillus*	25.3		
Upstream	Bacteroidetes	*Flavobacterium*	18.1		
Upstream	Actinobacteria	*Arthrobacter*	12.4		
Midstream	Proteobacteria	*Vibrio*	35.6	2.1	0.78
Midstream	Firmicutes	*Bacillus*	22.4		
Midstream	Bacteroidetes	*Flavobacterium*	15.7		
Midstream	Actinobacteria	*Arthrobacter*	8.2		
Downstream	Proteobacteria	*Vibrio*	40.1	1.8	0.73
Downstream	Firmicutes	*Bacillus*	18.5		
Downstream	Bacteroidetes	*Flavobacterium*	10.3		
Downstream	Actinobacteria	*Arthrobacter*	5.2		

Table 2. Water quality parameters in upstream, midstream and downstream at Anicut River.

Location	pH	Temperature (°C)	DO (mg/L)	Salinity (ppt)	Ammonia (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
Upstream	7.4	27.8	6	0.5	0.3	1.1	0.04
Midstream	7.8	27.5	6.5	0.6	0.2	0.9	0.03
Downstream	7.1	28.1	5.2	0.8	0.5	1.3	0.07

5.2. Associated water quality parameters

From the **Table 2** the results show that the water quality statistics show considerable differences between the upstream, midstream, and downstream sections of Lower Anicut. Upstreamconditions indicate moderate water quality, with a near-neutral pH (7.4), moderate DO (6.0 mg/L), and low ammonia levels (0.3 mg/L). Phosphate and salt levels are likewise modest (0.04 mg/L and 0.5 ppt, respectively), indicating minor nutrient pollution and restricted salinity intrusion, most likely due to the effect of natural freshwater inflow. Midstream regions have the best overall water quality, with a slightly alkaline pH of 7.8, the maximum dissolved oxygen (6.5 mg/L), and the lowest levels of ammonia (0.2 mg/L) and phosphate (0.03 mg/L). These conditions, together with moderate salinity (0.6 ppt), provide the ideal setting for prawn health and microbial diversity. Downstream areas exhibit signs of environmental stress, which include higher levels of phosphate (0.07 mg/L), ammonia (0.5 mg/L), and lower DO (5.2 mg/L) that are indicative of fertilizer runoff or pollution from human or agricultural sources. Higher salinity (0.8 ppt) is indicated downstream by increased evaporation rates or saltwater intrusion that could stress the prawns and influence the dynamics of their gut microbiota. These results reveal the requirement for maintaining well-balanced water quality for good health and productivity in *M. rosenbergii*. (Chakraborty *et al.* 2023) applied 16S rRNA genes along with Illumina sequencing technologies for analysis of stomach and midgut bacterial population composition of two species of shrimps, that were receiving probiotic, *Bacillus amyloliquefaciens* treatment. Downstream areas, on the other hand, show an indication of environmental stress such as high phosphate levels (0.07 mg/L), high ammonia (0.5 mg/L), and low DO of 5.2 mg/L that might be due to runoff from fertilizer or pollution through man or agricultural sources. A high salinity of 0.8 ppt downstream indicates higher evaporation rates or saltwater intrusion, which could stress the prawns and alter their gut microbiome dynamically. These results highlight how important it is to keep the water quality balanced in order to generate healthy *M. rosenbergii*. (Imaizumi *et al.* 2021) studied bacterial composition of two shrimp species during probiotic, *Bacillus amyloliquefaciens*, treatment using stomach and midgut samples and Illumina sequencing technology and 16S rRNA genes. On the other hand, downstream areas showed manifestations of environmental stress, such as a higher phosphate level with 0.07 mg/L, a higher ammonia content with 0.5 mg/L, and a lower DO with 5.2 mg/L, which may point to fertilizer runoff or pollution from human or agricultural origins. Higher salinity, that is, 0.8 ppt measured downstream, would imply increased evaporation rates or saltwater intrusion, possibly stressing the prawns themselves and altering the dynamics of their gut microbiota. These results point out the importance of maintaining balanced water quality for *M. rosenbergii* productivity and health. Applied 16S rRNA genes and Illumina sequencing technologies to investigate the bacterial composition of the stomach and midgut of two shrimp species during probiotic treatment with a *Bacillus amyloliquefaciens*.

The gut microbiome of *M. rosenbergii's* indicated a clear connection between the microbial diversity and water quality of upstream, middle and downstream area. The upstream area samples showed higher abundance of microbial as reflected by higher Simpson and Shannon indices, better parameters for water quality as DO increased, and ammonia content dropped with the pH remaining neutral (Satyanarayana *et al.* 2024; Vijayalakshmi *et al.* 2023). The presence of dominant taxa like *Bacillus* and *Flavobacterium* in the upstream samples suggests that the environment supports a functional, well-balanced microbiota. Conversely, downstream samples had a lower diversity of microbiota, including *Vibrio* and were probably caused by stresses such as rising in ammonia, decreasing DO, and acidic pH.

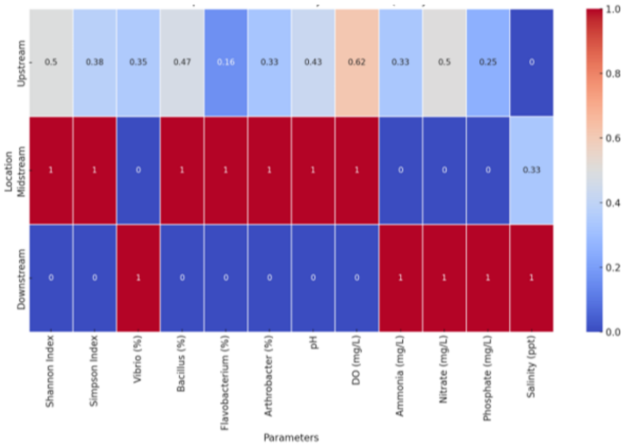


Figure 5. Heatmap integrates both microbial diversity and water quality parameters

Figure 5 shows the heat map of the correlation between microbial diversity and water quality parameters at midstream, downstream, and upstream locations in Lower Anicut (Anaikarai). Midstream areas show the best conditions with enhanced diversity of microbes and balanced water quality parameters like a high level of DO, low ammonia and phosphate concentration, etc. Upstream has mild conditions with lower levels of microbial diversity and water quality indexes showing the least influence from the anthropogenic effects. In contrast, downstream shows signs of environmental stress with lower microbial diversity and a high dominance of *Vibrio*, in addition to higher concentrations of ammonia, phosphate, and salinity. These results thus highlight the importance of midstream regions to prawn health and downstream pollution as a potential risk to the balance of ecosystems and sustainability of aquaculture (Sharma *et al.* 2024; Karpagavalli *et al.* 2024; Kumar *et al.* 2024)

5.3. Data analysis

The results of the ANOVA show the microbiological and environmental parameters at Upstream, Midstream, and Downstream locations revealed significant differences in various variables (**Table 3**). There was no significant difference in relative abundance(p = 0.939), indicating that the general microbial distribution remains rather consistent across locales. Significant differences were identified in environmental factors, including pH, temperature, DO, salinity, ammonia, nitrate, and phosphate levels (all p < 0.05). These findings show that, whereas microbial abundance remains stable, environmental gradients change dramatically along the river. This shift in environmental circumstances is expected to alter microbial diversity, as evidenced by the falling Shannon and Simpson indices downstream, which indicate reduced diversity and increased dominance of certain taxa, such as *Vibrio*. These findings emphasize the substantial link between environmental conditions and microbial community structure.

Table 3. Results of ANOVA between the relative abundance, different genera and locations.

Location	Genera	Relative Abundance (%)	pH	Temperature (°C)	DO (mg/L)	Salinity (ppt)	Ammonia (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
Upstream	*Vibrio*	30.2	7.4	27.8	6	0.5	0.3	1.1	0.04
Upstream	*Bacillus*	25.3	7.4	27.8	6	0.5	0.3	1.1	0.04
Upstream	*Flavobacterium*	18.1	7.4	27.8	6	0.5	0.3	1.1	0.04
Upstream	*Arthrobacter*	12.4	7.4	27.8	6	0.5	0.3	1.1	0.04
Midstream	*Vibrio*	35.6	7.8	27.5	6.5	0.6	0.2	0.9	0.03
Midstream	*Bacillus*	22.4	7.8	27.5	6.5	0.6	0.2	0.9	0.03
Midstream	*Flavobacterium*	15.7	7.8	27.5	6.5	0.6	0.2	0.9	0.03
Midstream	*Arthrobacter*	8.2	7.8	27.5	6.5	0.6	0.2	0.9	0.03
Downstream	*Vibrio*	40.1	7.1	28.1	5.2	0.8	0.5	1.3	0.07
Downstream	*Bacillus*	18.5	7.1	28.1	5.2	0.8	0.5	1.3	0.07
Downstream	*Flavobacterium*	10.3	7.1	28.1	5.2	0.8	0.5	1.3	0.07
Downstream	*Arthrobacter*	5.2	7.1	28.1	5.2	0.8	0.5	1.3	0.07

(Qiu *et al.* 2023) investigated on impacts of probiotics on *M. rosenbergii's* gut flora, development performance, nonspecific immunity, absorption, and water quality in the biofloc culture system. They were divided into three groups (control group, Con), *Bacillus subtilis* (BS), and effective microorganisms (EM). The finding reveals that Both the EM and BS groups considerably lowered the concentration of total nitrogen. While the feed conversion rate of *M. rosenbergii* was dramatically reduced, the final mean body weight, weight gain, and specific growth rate were all significantly boosted by the BS and EM groups.

6. Conclusion

This study highlights the strong connection between the environmental factors and microbial diversity in many areas located near the lower Anicut region. Due to significant variations in pH, DO, nutrients and salinity, microbial abundance decreases downstream and taxa like

Vibrio become more prevalent. Concern regarding eutrophication, deteriorating water quality and the growth of harmful microorganisms are raised by these changes. Restoring riparian habitats, implementing nutrient management strategies, and conducting continuous monitoring to maintain ecological balance are essential to addressing these issues. To ensure lasting benefits for local communities and aquatic living organisms, the management of the lower Anicut should requires similar focus on water regulation, conservation and wellness of the ecosystem. In this study, recorded a maximum Shannon Index of 2.4 (upstream), down to a minimum of 1.8 (downstream), which indicates that microbial diversity decreased as pollution levels increased. Existing studies indicate that the Shannon Index fell within 2.5 and 3.2 under probiotic or controlled conditions, indicating some level of ecological stress. Also, Vibrio had a peak abundance of 40.1% downstream, which was higher than the 25 - 30% ideal ranges of abundance reported in cleaner aquaculture systems showing a sign of poor water quality. A potential limitation to this study was its limited temporal nature, as we only sampled in one season, which could be responsible for limited seasonal variations in microbial diversity and water quality. The study also analyzed a limited number of selected physicochemical parameters, and ignored other potential environmental stressors. The study was limited to just one species, M. rosenbergii, and did not consider broader potential ecological impacts. Further work should integrate year-round monitoring, and broader microbial profiling to include metagenomic analysis to obtain functional information on the microbial communities. More comprehensive ecological assessments could occur by experimenting with more than one aquatic species and incorporating advanced pollution source tracking.

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