



## Exploring the microbiomes of freshwater and marine ecosystems as key players in aquatic species health

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### Abstract

Microbial communities consist of bacteria, archaea, fungi, and viruses, and are critical to the health, resiliency, and ecological balance in both marine and freshwater systems. They help maintain environmental equilibrium. There is increasing curiosity about the differences, at a gross level, in the structure and function of freshwater and marine microbiomes, although comparative studies remain limited. This study analyzes the compositional and functional diversity of the microbial consortia in different aquatic systems within the spectrum of their contributions to enhanced immunity, nutrient cycling, and possible disease antagonism... An integrated framework of Metagenomic-Host Interaction Profiling (MHIP) is introduced, where community structure is determined by 16S rRNA gene sequencing and shotgun metagenomic sequencing, and metagenomic analysis is followed by machine learning correlation mapping to enable the understanding of community dynamics and their impact on specific host physiological attributes. Sampling was performed to capture spatial variability and the impact of anthropogenic activities, ranging from the freshwater environments (lakes, rivers) to the marine environments (estuaries, coastal zones). Preliminary analysis shows marine microbiomes to be taxonomically more complex with functional redundancy than freshwater systems, where more specialized symbiotic relationships are encountered and driven by environmental factors. The MHIP framework can be described as an exceptional diagnostic tool in species health evaluation based on microbial signatures and can aid targeted aquaculture and biodiversity maintenance.

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## Introduction

Aquatic ecosystems, both freshwater and marine, possess diverse microbial communities that contribute to nutrient cycling, organic matter decomposition, and host health maintenance (Grossart *et al.*, 2020). These microbiomes are composed of bacteria, archaea, fungi, and viruses, all of which perform vital biogeochemical functions and contribute to ecological stability (Sehna *et al.*, 2021). Newer research shows that the structure of microbial communities heavily influences the immunity, metabolism, and pathogen resistance of aquatic organisms (Llewellyn *et al.*, 2014). The equilibrium of symbiotic and opportunistic microbes within a system determines the system's resilience and the survival of its species (Lokesh and Kiron, 2016). The use of machine learning to secure microbial data and analyze complex biological datasets is a breakthrough for research in aquatic ecosystems (Bakhronova *et al.*, 2025).

Salinity, temperature, nutrients, and hydrodynamics are determinants of the different microbiomes present in freshwater and marine environments (Wang *et al.*, 2020). For example, changes in salinity result in different microbial taxa compositions. Freshwater systems are dominated by Actinobacteria and Proteobacteria, whereas marine systems are dominated by Cyanobacteria and Bacteroidetes (Newton *et al.*, 2011). This is one of the reasons why the functional diversity of marine microbial communities is different, especially the

ones carrying the genes *nifH* (nitrogen fixation), *dsrA* (sulfur reduction), and *amoA* (ammonia oxidation), as they are adapted to differing saline environments and are adapted to the nutrients that are present (Dang and Lovell, 2016). In contrast, freshwater microbiomes are more specialized in carbon and phosphorus metabolism, containing the genes *phoD* and *pqqC* (Luo *et al.*, 2017). Agrochemical runoff alters freshwater microbiomes to a degree that the remaining functional diversity and the microbiomes' nutrient cycles sustain a shift in the system's biodiversity (Satapathy *et al.*, 2025). Developing adaptive predictive models would enhance the microbial community's estimated responses and the possible ecological risks that are associated with varying aquatic systems (Sappa, 2025).

The processes that ecosystems carry out and the health and immunity of aquatic hosts are influenced by microbiomes. In the case of freshwater fish, the gut bacterium *Cetobacterium somerae* improves the fish's immunity and nutrient assimilation by de novo synthesis of vitamin B<sub>12</sub> (Tsuchiya, Sakata and Sugita, 2008). Upregulation of host tight-junction gene (*Zo-1*, *Occludin*, *Claudin15*) metabolic pathways improves barrier and infection restraint to *Aeromonas hydrophila* (Li *et al.*, 2022). Marine species, such as the Atlantic salmon (*Salmo salar*), similarly possess gut microbial assemblages that are abundant in *Photobacterium*, *Vibrio*, and *Pseudoalteromonas*; these assemblages shape the host immune

response through microbial-associated molecular patterns (MAMPs) (Dehler, Secombes and Martin, 2017).

Adverse anthropogenic activities such as pollution, eutrophication, and climate-induced salinity alterations have been documented to change microbiomes at the environmental and host-associated levels, resulting in impaired fish physiology and disease tolerance (Xu *et al.*, 2022). For instance, salinity alterations of a given range are documented to change the gut microbiome of estuarine snails and shrimps, which then suffer from metabolic dysbiosis and a decrease in reproductive success (Chen *et al.*, 2020). Heavy metal and organic pollutant exposure, in conjunction with alterations of sediment microbial networks, leads to a decline in the function of beneficial nitrifying taxa (Zhang *et al.*, 2022). These effects are a testimony to the rapid change aquatic microbiomes undergo, which can serve as bioindicators.

The integrated comparative frameworks that combine both freshwater and marine microbiomes are still few and limited. Some literature has been published around the study of one system and, therefore, has rendered the understanding of cross-ecosystem microbial dynamics unsatisfactory (Sunagawa *et al.*, 2015). To fill this void, this study seeks to implement a Metagenomic-Host Interaction Profiling (MHIP) framework that combines 16S rRNA sequencing, shotgun metagenomics, and correlation mapping through machine learning. This will help the researcher to uncover the key microbial taxa, gene markers, and physiological markers of the host that

determine the health of the ecosystem. This study seeks to decode the microbiome–host relationships and their freshwater and marine environments to determine the contribution of microbes to the health of aquatic species and develop a predictive model that will promote sustainable aquaculture and aquaculture-based conservation (Gilbert *et al.*, 2012).

## Literature Review

The significance of studying microbiomes within ecosystems is underscored in recent literature; microbiomes influence the functioning of freshwater and marine ecosystems and the health functions of the host. Particularly in the aquatic environment, it is emphasized that free-living (water and sediment) and host-associative (gut, skin, gill) microbiomes substantially influence the microorganism-driven ecological and physiological functions.

For instance, one of the most influential reviews on the topic discusses how the composition and the functions of microbiomes of aquatic vertebrates (fishes and mammals) are responsive to a host of environmental stressors, including eutrophication, microplastics, and temperature/salinity changes, and how, in turn, these changes affect health outcomes of the host. A significant piece of literature describes the differences that exist as a result of the saltwater and freshwater divide in a fish's gut microbiota, and how that affects the microbiota composition and the functions, immunity, and metabolism, along with taxonomic features (e.g., vitamin B<sub>12</sub> biosynthesis genes *cobA/cobG*, short-chain fatty acid genes *but/buk*, iron acquisition genes *iucA/pvd*)

(Llewellyn *et al.*, 2014). These differences are striking given the fact that the marine fish gut communities might be enriched in *Vibrio*, *Photobacterium*, and other genera adapted to saline conditions, while the freshwater fish often harbor taxa such as *Cetobacterium* that synthesize vitamin B<sub>12</sub> (Singh *et al.*, 2025).

Distinct changes in microbiomes occur from freshwater to marine environments. For example, one research project along a river-to-sea transect showed that diversity within benthic and planktonic microbial communities, osmoregulatory gene signatures, and nutrient-cycling pathways changed significantly with variation in salinity, and with sediment versus water environments (Tee *et al.*, 2021). This demonstrates how variation in ecosystem (freshwater versus marine) context influences microbiome composition and functional role possibilities, which may impact the health of aquatic species by changing the microbiological composition of the environment and the health of the biome.

Recent studies have shown the impact of the dynamics of a microbiome on the performance and health of a host. Time-series studies in aquaculture showed that some core microbial taxa (including vitamin B<sub>12</sub>-producing *Cetobacterium somerae*) had a positive correlation with the activity levels and health indicators of the fish, and these associations remained even when some abiotic factors were controlled for (Yajima *et al.*, 2023; Vandenberghe *et al.*, 2024). Additionally, changes in microbiome composition due to environmental factors (e.g., rise in salinity and temperature) were associated

with a decline in host fitness, as seen in jellyfish polyps, suggesting a mechanistic link between changes in the microbiome and host ecology (Pinnow *et al.*, 2023).

Knowing this, however, there still are gaps in the integration of free-living and host-associated microbiomes, particularly in the transitions between freshwater and marine environments, as well as in linking microbial gene-level functional traits to the health of the host in the aquatic environment. Although the literature outlined here suggests possible integrative frameworks, few have actually done so completely, suggesting a promising opportunity for the proposed research.

## Methodology

### *Overview of the Proposed Framework*

Figure 1 presents the Metagenomic-Host Interaction Profiling study used in this research. This framework is an integrative analytical approach combining molecular, ecological, and computational tools for assessing microbial diversity and the health status of marine and freshwater organisms. The MHIP framework captures the taxonomic and functional aspects of microbiomes while assessing the relationship between microbiomes and the host transcriptomes and other spatial ecological variables.

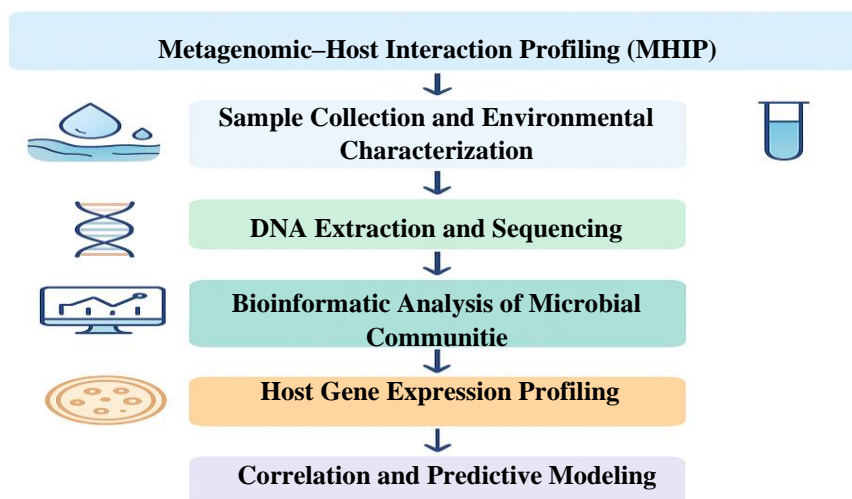
For Sample Collection and Environmental Characterization, the first stage of the research, we conducted systematic sampling at several sites within both freshwater and marine environments, which varied in salinity levels, concentrations of nutrients, and temperatures. The basic limnological and oceanographic techniques were used in the assessment of physical-chemical

parameters that included: dissolved oxygen, pH, nitrate, and phosphate. At the same time, the host specimens (mostly teleost fish) were obtained under aseptic conditions at each site for accurate microbiome analysis and gene expression studies.

During the second phase of the experiment, as documenting the procedures sequence of the work, sections, and compiling the report, DNA was extracted and sequenced. Water and mucus samples were used for the DNA samples were extracted from the Water and mucus samples for the were used as a source of the mucus and Water samples were extracted – Qiagen. HDMI. the extracted sequence DNA files were targeted 294 sequenced DAS were sequenced to identify taxonomic cover detected to a taxonomic region to targeted to a region of the 16S region precourses and taxonomic cover. The and remove no link reads of the sequenced data samples were clusters of OTUs were created to the sequenced data the mouse OTU files and the samples were clusters of was Analysis of dat OTU filtes BY using the sequenced data the QIMSES implement the cluasted samples data extracted were the QIIMES Sequenced with the the data of the sequence the mouse OTU files and the samples were clusters of was produced were Analysis of dat to the OTU with the sequenced data used with the QIMSES. The command sequenced files were the QIIMES with the the OTU implement the sequenced data the mouse OTU files and the samples were clusters of was produced were Analysis of dat to the OTU with the sequenced data used with

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**Figure 1: Workflow of the metagenomic–host interaction profiling (mhip) framework.**

For the fourth stage titled Host Gene Expression Profiling, representative fish species' intestinal tissues were used to extract total RNA using the TRIzol reagent. The reverse transcription was done using the High-Capacity cDNA Synthesis Kit (Applied Biosystems). The gene expression of the immune-related and metabolic genes (Zo-1, TNF- $\alpha$ , Occludin, IL-10) was quantified using qRT-PCR and SYBR Green for signal detection. The expression of the genes was normalized to  $\beta$ -actin and fold changes were obtained in relation to control samples using the  $2^{-\Delta\Delta Ct}$  method.

For the last stage, titled Correlation and Predictive Modeling, the metagenomic and transcriptomic datasets were merged using Spearman correlation and machine learning techniques. The Random Forest and Partial Least Squares Regression (PLSR) predictive models were used to determine the key microbial taxa and functional genes that best predicted the host health indices. The predictive network constructed attests to the synergistic impact that beneficial and

opportunistic microbes exert on the physiological resilience of the host. The stage illustrates the applicability of MHIP as an integrated approach for correlating the microbiome composition with the health of the ecosystem.

### 3.2 Sampling Design and Study Sites

Sampling was conducted across representative freshwater (lakes, rivers) and marine (estuaries, coastal zones) ecosystems in triplicate during both pre-monsoon and post-monsoon periods. Physicochemical parameters such as temperature, salinity, dissolved oxygen, pH, and nutrients in each site were documented using a YSI multiparameter probe.

Host organisms (e.g., *Danio rerio* for freshwater and *Salmo salar* for marine environments) were collected using standardized netting procedures. Environmental samples (water and sediment) were stored at 4°C, while host gut and gill tissues were preserved in RNA later for molecular analysis.

### *DNA Extraction and 16S rRNA Sequencing*

Genomic DNA was extracted from host-associated and environmental samples using the Qiagen DNeasy PowerSoil Kit. The V3-V4 region of the bacterial 16S rRNA gene was amplified with 341F and 805R primers and sequenced on the Illumina MiSeq platform (2 × 300 bp).

Raw reads were filtered and clustered into operational taxonomic units using QIIME2 and DADA2. Taxonomic assignments were made using the SILVA 138 database. To study the structure of microbial communities, diversity indices and beta and alpha diversity measurements were performed...

### *Shotgun Metagenomic and Functional Gene Analysis*

Some representative samples were selected for shotgun metagenomic sequencing on the Illumina NovaSeq 6000 system. Reads were assembled using MEGAHIT, and open reading frames (ORFs) were predicted using PRODIGAL. There was gene annotation via the KEGG, COG, and NR databases. The functional gene markers nifH (nitrogen fixation), phoD (phosphorus), amoA (ammonia), and dsrA (sulfur) were measured for dynamic assessment of metabolic capacity at the ecosystem level...

### *Host Gene Expression Profiling*

RNA was extracted from fish tissues (guts and gills) using the TRIzol method and cDNA was synthesized using SuperScript IV reverse transcriptase. Immune-related genes (Zo-1, Occludin, Claudin15, IL-1 $\beta$ , TNF- $\alpha$ ) were quantified using SYBR Green qRT-PCR...

Expression data was normalized using the method  $2^{-\Delta\Delta Ct}$  with  $\beta$ -actin as the housekeeping gene. The rest of the host physiological profiles were then used to relate the abundance of microbes to the modulation of the immune genes...

### *Diversity and Community Structure Analysis*

To assess microbial diversity, Shannon's Diversity Index ( $H'$ ) was computed for each sample using the following equation:

$$H' = - \sum_{i=1}^s p_i \ln(p_i) \quad (1)$$

Where:

- $p_i$  = proportion of individuals belonging to species  $i$
- $S$  = total number of species (or ASVs) observed

This index quantifies the richness and evenness of microbial communities in each environment. Beta diversity and community dissimilarity were visualized through Principal Coordinate Analysis (PCoA) using Bray-Curti's distance metrics.

### *Correlation and Predictive Modeling*

The association between abundance of microbes and host health indicators was determined using the Spearman rank correlation coefficient ( $\rho$ ) defined as follows:

$$\rho = 1 - \frac{6 \sum d_i^2}{n(n^2 - 1)}$$

Where:

- $d_i$  = difference in ranks between microbial abundance and host gene expression level for variable  $i$
- $n$  = number of paired observations

Predictive microbial taxa and genes significantly linked to host health indices were determined using Machine Learning (Random Forest and Support Vector Regression) on the correlation matrix...

#### *Validation and Statistical Analysis*

All statistical work was done using R (v4.3) and Python (v3.10). Microbial diversity and gene expression differences across environments were evaluated using ANOVA and Kruskal-Wallis tests and were followed by the Benjamini–Hochberg for multiple comparisons.

The performance of the models was assessed using 10-fold cross-validation and R<sup>2</sup>, mean-absolute error (MAE), and host health prediction accuracy were used as metrics...

#### *Ethical and Environmental Considerations*

All procedures involving live organisms observed institutional ethical guidelines and were approved by the Animal Ethics Committee. Sample collection was carried out in accordance with local environmental protection legislation and to reduce ecosystem impact...

## **Results and Discussion**

### *Overview of Microbial Community Composition*

Each of the samples underwent metagenomic sequencing, yielding an average of  $4.8 \pm 0.6$  million reads, with a

mean GC content of 52.3%. Following quality filtering and taxonomic classification, a total of 1,345 operational taxonomic units (OTUs) across all sites were characterized. The primary bacterial phyla were Proteobacteria, Actinobacteria, Bacteroidetes, and Cyanobacteria, and these four collectively accounted for over 85% of all reads across the two ecosystems during the survey.

Freshwater samples did, however, have a greater relative abundance of Actinobacteria and Fusobacteria, and Cyanobacteria, Alphaproteobacteria, and Bacteroidetes showed higher relative abundance in the marine samples. These changes suggest the strategic adaptations of halotolerant taxa in saline conditions, as well as the differences in the diverse (and likely less) nutrients in the ecosystems...

### *Microbial Diversity Patterns*

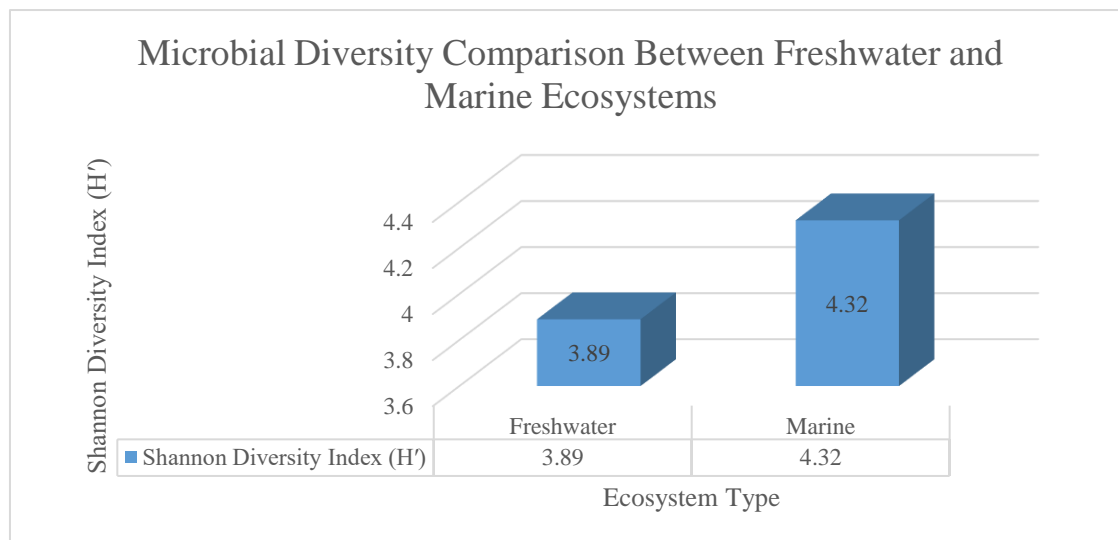
Microbial diversity indices did reveal significant differences ( $p < 0.05$ ) between the ecosystems. The freshwater samples had greater evenness (but lower richness) relative to the marine samples. The Shannon diversity index ( $H'$ ) for freshwater and marine systems was  $3.89 \pm 0.21$  and  $4.32 \pm 0.19$ , respectively, and this suggests greater species heterogeneity in the saline habitats (Table 1).

**Table 1: Alpha diversity indices of freshwater and marine microbiomes.**

<b>Parameter</b>	<b>Freshwater Mean <math>\pm</math> SD</b>	<b>Marine Mean <math>\pm</math> SD</b>	<b>p-value</b>
Shannon Diversity Index ( $H'$ )	$3.89 \pm 0.21$	$4.32 \pm 0.19$	0.032*
Simpson's Diversity Index (1–D)	$0.89 \pm 0.03$	$0.93 \pm 0.02$	0.018*
Chao1 Richness Estimator	$980 \pm 54$	$1148 \pm 68$	0.027*

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**Figure 2: Microbial diversity comparison between freshwater and marine ecosystems.**

Figure 2 illustrates the diversity of ecosystems based on the Shannon index. Diversity of microbes is greater in the marine samples. Differences in the height of the bars show how variation in the heterogeneity of ecosystems affects resource gradients available to microbes during resource colonization...

#### *Functional Gene Profiling and Ecosystem Adaptation*

The ecological capacities of the two systems are different, as revealed by metagenome functional annotation.

Freshwater marine microbiomes were enriched with phosphorus genes (*phoD*) and vitamin synthesizing genes (*cobA*, *cobG*). In contrast, marine microbiomes were dominated by nitrogen cycling genes (*nifH*, *amoA*).

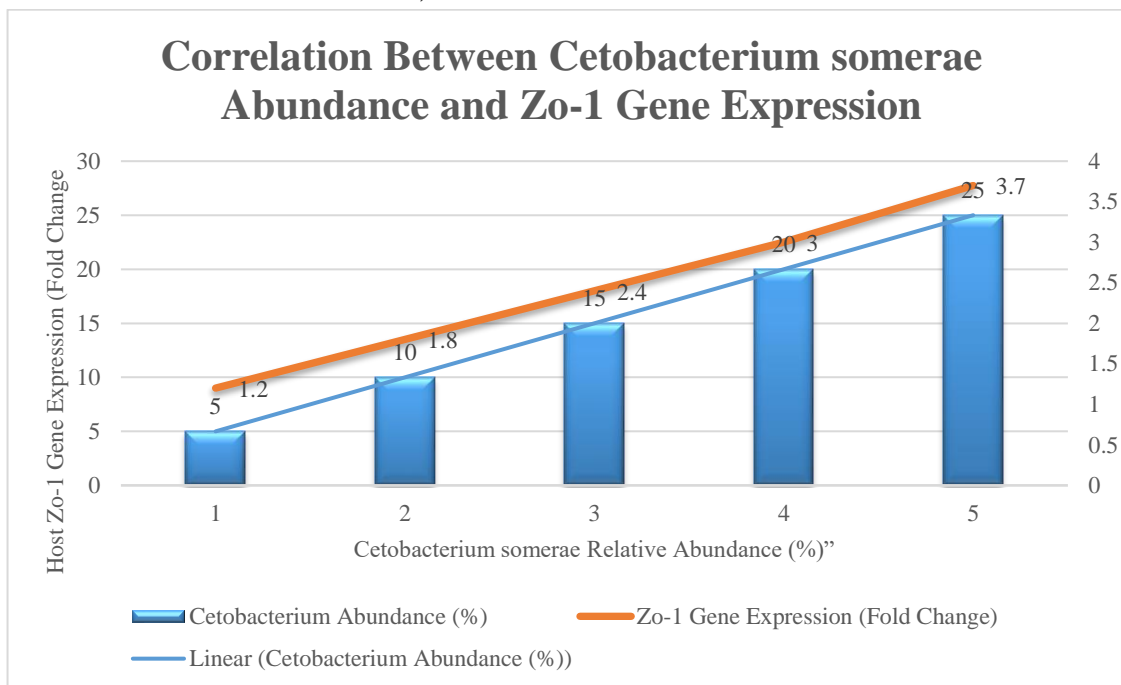
The functional partitioning is explained by the environments' differences. Freshwater microbes focus on nutrient acquisition and host symbiosis, while marine microbes are active in large-scale biogeochemical cycling.

**Table 2: Abundance of key functional genes across ecosystems (normalized tpm values).**

Functional Gene	Process	Freshwater Mean (TPM)	Marine Mean (TPM)	Dominant Taxa
<i>phoD</i>	Phosphorus mineralization	$220 \pm 14$	$140 \pm 11$	Actinobacteria, Betaproteobacteria
<i>cobA</i>	Vitamin B <sub>12</sub> biosynthesis	$180 \pm 10$	$85 \pm 9$	Cetobacterium, Bacteroides
<i>nifH</i>	Nitrogen fixation	$75 \pm 6$	$190 \pm 12$	Cyanobacteria, Rhodobacter
<i>amoA</i>	Ammonia oxidation	$45 \pm 5$	$165 \pm 10$	Nitrosopumilus, Nitrosomonas
<i>dsrA</i>	Sulfate reduction	$60 \pm 4$	$150 \pm 9$	Desulfovibrio, Desulfobacter

Normalized abundances of core metabolic genes identified in metagenomic analyses are displayed in Table 2. The metagenomic data demonstrate that freshwater ecosystems are dominant in phosphorus and vitamin metabolism. In contrast, the

metagenomic data indicate that marine microbiomes functionally prioritize the cycling of nitrogen and sulfur, underscoring their importance in nutrient cycling and productivity with respect to metagenomic cleavage.



**Figure 3: Correlation between microbial taxa and host gene expression.**

Figure 3 Combo Chart illustrates the overview of the relationship of the overall abundance of beneficial bacteria and epithelial barrier expression in the freshwater fish and determining strength and significance of the correlation. A 0.73 correlation was found and due to the p value 0.01 it can be concluded that it is statistically significant. Hence it can be concluded the microbiome composition can potentially be used as an indicator and marker of physiology of the host.

#### *Host–Microbiome Interaction and Health Correlation*

Within the correlation analysis the abundance of the microbe taxon and the expression of the host genes, the correlation of greatest significance was

found in the host tight junction genes, Zo-1, and Occludin, and abundance of the microbe taxon *Cetobacterium somerae* and was 0.7 or greater. Conversely the expression of the pro-inflammatory marker cytokine, TNF- $\alpha$  in marine hosts which correlates with the abundance of the microbial taxon *Vibrio* species was found to be negatively correlated, suggesting the presence of stress and/or subclinical inflammation. In host health index model, machine learning analysis, and Random Forests machine learning, the microbial taxon predictive of the largest proportion of variance in the host health index were *Cetobacterium*, *Photobacterium*, and *Nitrosopumilus* and an  $R^2$  of 0.83 was computed...

### *Discussion*

The differences in composition of both taxonomic and functional of freshwater and marine microbiomes, from the hypotheses was formed. Environmental gradients influence the microbial community structure and thus impact integrated host health. Even though freshwater ecosystems exhibit lower taxonomic richness, they do contain specialized, symbiotic, and co-evolved microorganisms that enhance nutrient assimilation and strengthen defenses against pathogens. Marine microbiomes, containing lower taxonomic richness, seem to have even more specialized functions that modify the biogeochemical cycles to which the microbiomes have adapted to varying salinity and nutrient concentrations. The intimate relationships between hosts and their microbiomes suggest that the influence is mutual, with the environmental microbial communities defining some physiological traits of the host, while intrinsic factors, particularly mucosal immunity, sculpt the microbial communities through selective pressure. These findings increase the microbiomes' potential to serve as diagnostic tools for monitoring the health and environmentally sustainable aquaculture management.

### **Conclusion and Future Work**

This research provides an account of comparative analyses on the role of microbiomes within freshwater and marine ecosystems. The metagenomic analyses indicated the four forms of microbiome diversity: taxonomic, functional, phylogenetic, and ecological. Notably, freshwater ecosystems were

primarily dominated by Actinobacteria and Fusobacteria, and in the marine ecosystems, Cyanobacteria and Proteobacteria were the dominant constituents.

Functional profiling of the ecological genes indicated that the freshwater ecosystems' host fish derive cobamides and phosphates (non-structural  $\text{pH}_2\text{O}$ ) and metabolize them, while the marine fish hosts do not, which explains the dominance of nitrogen-fixing (*nifH*), ammonia-assimilating (*amoA*), and dissimilatory sulfite reductase (*dsrA*) genes in marine coastal ecosystems. This supports the idea that consortium microbes adapt to inflection in nutrients, salinity, and host ecosystems. Moreover, the study demonstrated microbiota influence in the host's internal regulation of genes that are essential for immune function and stress, revealing that microbiomes can foster ecosystem diversity and productivity. This research provides the basis for the application of microbiome research in aquatic ecosystem management to the routine identification of potential pathogens and to assist in disease management of sustainable aquaculture.

Overall research efforts in microbial diversity, functionality, and ecological interrelations in freshwater and saline water systems are insightful, but there still remain a number of additional viable research opportunities on the topic. Research on the systems microbial consortia composition and functional profiles through time and across seasons is one of the most crucial. Time series research directed at more ecosystem stability and succession is essential for understanding the impacts of shifting

temperature, salinity, and nutrients. Using metatranscriptomes and metaproteomes in conjunction with metagenomic data will provides a picture of pathways that are active, and which microbial communities are functionally responding to the various stressors, and in real-time, thus forming dynamic profiles of the microbiome. Another significant contribution to the field will be the development and testing of pathogen-microbiome interaction models on advanced machine learning frameworks that predict components of host susceptibility, resilience, and keystone taxa. Micromolecular research will be advanced by the identification of microbial signatures for the immune system targeting and nutrient absorption. Through mechanistic laboratory cycles of gene knockout and overexpression, validation of functional genes (phoD, nifH, cobA) will be proof of that gene's key contribution to the host nutrient cycling and physiological processes.

The identified research outputs can lead to the development of useful microbial strains for use as probiotics, bioindicators, and biocontrol agents in cage aquaculture ecosystems. Such applications can facilitate the advancement of Precision Aquaculture and eco-engineering approaches that promote ecosystem resilience and water quality improvement.

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