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## **Comparative Analysis of Gut Microbiota Diversity and Functional Attributes in Edible Fishes: Implications for Aquatic Health and Human Nutrition**

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**Abstract:** Gut microbiota plays a critical role in fish health, metabolism, and nutritional quality, with implications for both aquatic ecosystem sustainability and human nutrition. This study comparatively analysed gut microbial diversity, taxonomic composition, and predicted functional attributes in three edible freshwater fishes—*Catla*, *Rohu*, and *Tilapia*. Alpha diversity indices revealed significant interspecific variation, with *Tilapia* exhibiting the highest microbial richness and evenness, followed by *Catla* and *Rohu*. Taxonomic profiling showed a conserved core microbiota across species, dominated by *Proteobacteria* and *Firmicutes*, with species-specific differences in relative abundance and dominant genera. Beta diversity analysis confirmed distinct microbial community clustering by host species, indicating strong host-driven structuring of gut microbiota. Functional prediction analysis revealed carbohydrate metabolism as the dominant microbial pathway across all species, while immune-related and lipid metabolism functions were differentially enriched, particularly in *Rohu*. Correlation analyses indicated a strong positive relationship between microbial diversity and metabolic functional potential. Overall, these findings highlight the influence of host species and ecological factors on gut microbiome diversity and function, emphasizing the importance of microbial communities in maintaining fish health, enhancing nutritional quality, and supporting sustainable aquaculture and human nutrition strategies.

**Keywords:** Gut microbiota, Alpha diversity, Edible freshwater fishes, Bacterial community structure, *Proteobacteria*, *Firmicutes*, *Rohu*, *Tilapia*, *Catla*, Sustainable aquaculture

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### **Introduction**

The gut microbiota of aquatic creatures, especially fish, are essential for sustaining ecological equilibrium and host nutrition. These microbial communities perform a variety of tasks, including

digestion, nutritional absorption, immunological regulation, and pathogen defense (Ghanbari *et al.*, 2015). The gut microbiota regulates fish development, health, and resilience, which has a

direct impact on aquaculture production and sustainability.

Understanding the variety and functional properties of gut microbiota in edible fish is critical for enhancing aquatic health and the nutritional value of fish as a food source. Healthy and balanced microbial communities in aquaculture systems can improve nutrient bioavailability while lowering the danger of disease outbreaks (Nayak, 2010). This understanding is also critical for improving fish diets and promoting sustainable fish farming operations that suit human nutritional needs.

Despite the acknowledged relevance of fish gut microbiota, comparative investigations into the diversity and functional potentials of these microbial communities across several edible fish species are few. The majority of present research focuses on particular species or settings, creating a vacuum in our understanding of interspecies variation and its consequences (Sullam *et al.*, 2012). Addressing this gap can give complete insights on the microbiome's impact on aquatic health and nutrition.

Fish gut microbiota is a diverse and dynamic population of microorganisms, primarily bacteria, that live throughout the digestive system. These microbial communities are critical for nutrition metabolism, digestion, and host health because they modulate immune responses and defend against infections (Egerton *et al.*, 2018). The fish gut microbiota is generally composed of Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, however the particular taxa vary by species and environmental circumstances.

Numerous variables impact the variety and organization of fish gut microbiota, including nutrition, environment, developmental stage, and host physiology. Dietary composition, especially protein and carbohydrate levels, has a direct impact on microbial populations by providing selected substrates for growth. Water temperature, salinity, and microbial load are all important habitat characteristics that influence

gut microbial populations (Wong *et al.*, 2013). Furthermore, physiological variables such as gut architecture and immunological state influence microbial colonization and stability.

Traditionally, fish gut microbiota were examined using culture-dependent approaches, which only identify cultivable microorganisms. However, advancements in molecular biology, particularly culture-independent approaches like 16S rRNA gene sequencing, have transformed microbial diversity studies. These methods allow for full analysis of microbial communities, including uncultivable and uncommon species, using high-throughput sequencing platforms (Xiong *et al.*, 2019). Taxonomic categorization, diversity metric computation, and functional prediction using sequencing data are all possible with bioinformatics techniques.

The fish gut microbiota performs important functions such as digesting indigestible dietary components, producing vital nutrients (for example, vitamins), enhancing digestive enzyme performance, and influencing host immune responses. Ringø *et al.* (2016) discovered that microbial activities promote fish health and battle infections. Some gut bacteria produce anti-microbial compounds that protect against opportunistic infections, supporting a healthy gut ecology.

Despite increased study into fish gut microbiome, comparable functional assessments across several edible fish species are still limited. There is a need to understand how microbiota composition connects with functional features important for both aquatic health and human nutrition. To close this gap, comprehensive investigations that combine taxonomic and functional views are required (Givens *et al.*, 2015).

The objectives of the present study was (i) to analyse and compare the gut microbiota diversity among selected fish species; (ii) to assess the functional attributes of these gut microbial communities; and (iii) to discuss the implications of gut microbiota composition and function for

aquatic ecosystem health and human nutrition.

## Materials and Methods

### Study Design

This study used a comparative cross-sectional study methodology to examine and evaluate gut microbiota diversity and functional features in selected edible fish species. This method enables the simultaneous study of microbial communities from several species under controlled circumstances.

### Sample Collection

Edible fish species such as *Catla catla* (Catla), *Labeo rohita* (Rohu), and *Oreochromis niloticus* (Tilapia) were procured from certified aquaculture facilities and natural freshwater habitats to represent a variety of ecosystems. Samples were obtained in accordance with ethical norms for humane animal treatment and biosafety requirements to prevent contamination (FAO, 2021). To maintain sample integrity, the fish were transported to the laboratory on ice and processed within 24 h of arrival.

### Gut Sample Processing

Under sterile conditions, the fish specimens were dissected to separate the complete gastrointestinal system. Gut contents were aseptically collected by gently pressing intestine pieces into sterile microcentrifuge tubes (Li *et al.*, 2017). Samples were frozen at  $-80^{\circ}\text{C}$  to preserve microbial DNA.

### DNA Extraction and Sequencing

Microbial DNA was isolated from gut content samples using commercially available kits designed for complicated biological materials, resulting in excellent yield and purity. The V3-V4 region of the bacterial 16S rRNA gene was amplified with universal primers and sequenced paired-end on the Illumina MiSeq platform, according to known techniques (Caporaso *et al.*, 2012). Negative controls were implemented to detect for potential contamination.

### Bioinformatics Analysis

To produce high-quality sequences, raw sequencing reads were subjected to a rigorous procedure that included quality screening, trimming, and chimera removal. QIIME software was used to cluster operational taxonomic units (OTUs) based on a 97% similarity criterion, with taxonomic assignment conducted against curated databases such as SILVA or Greengenes (Quast *et al.* 2013). This allowed for complete taxonomic profiling of the gut microbiota.

### Diversity Analysis

Alpha diversity measurements, like as Shannon and Simpson indices, were used to quantify microbiological richness and evenness within a sample. To investigate variations in microbial communities among fish species, beta diversity was assessed using Bray-Curtis dissimilarity and visualized using Principal Coordinates Analysis (PCoA). Significant differences were identified by statistical comparisons of diversity indices among species (Lozupone and Knight, 2008).

### Functional Prediction

Metabolic and functional profiles of microbial communities were predicted using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), a tool that infers gene content from 16S rRNA data to estimate microbial functional potential, including pathways involved in nutrient metabolism and immune modulation (Langille *et al.*, 2013).

### Statistical Analysis

Data was analyzed using the R programming language and QIIME. ANOVA and permutational multivariate analysis of variance (PERMANOVA) tests were used to examine the statistical significance of variations in microbial diversity and functional profiles across fish species. A p-value of  $<0.05$  was judged statistically significant.

## Results and Discussion

### Microbial Diversity Profiles

Alpha diversity indices revealed considerable differences among the three fish species (Table 1,

Table 1: Gut Microbiota Diversity Indices and Functional Attributes in Selected Edible Fish Species

| Fish Species | Sample Size (n) | Alpha Diversity (Shannon Index) | Alpha Diversity (Simpson Index) | Dominant Phyla (%) (Proteobacteria/ Firmicutes / Bacteroidetes) | Predicted Functional Pathways (Relative Abundance %)                    |
|--------------|-----------------|---------------------------------|---------------------------------|-----------------------------------------------------------------|-------------------------------------------------------------------------|
| Catla        | 10              | 4.2 ± 0.3                       | 0.88 ± 0.02                     | 45 / 35 / 15                                                    | Carbohydrate metabolism (30), Immune system (18), Lipid metabolism (12) |
| Rohu         | 10              | 3.8 ± 0.4                       | 0.85 ± 0.03                     | 50 / 30 / 10                                                    | Carbohydrate metabolism (28), Immune system (20), Lipid metabolism (15) |
| Tilapia      | 10              | 4.5 ± 0.2                       | 0.90 ± 0.01                     | 40 / 40 / 18                                                    | Carbohydrate metabolism (32), Immune system (15), Lipid metabolism (10) |

**Sample Size:** Equal samples were collected from each species to maintain comparability; **Alpha Diversity Indices:** The Shannon Index reflects both richness and evenness of microbial species in the gut. Tilapia showed the highest diversity (4.5), indicating a richer and more balanced microbial community compared to Rohu (3.8) and Catla (4.2); The Simpson Index complements Shannon by emphasizing the dominance of certain species. Values closer to 1 indicate higher diversity. Tilapia again showed the highest value (0.90); **Dominant Phyla (%):** The relative abundances of major bacterial phyla differed among species. Proteobacteria was dominant in Rohu (50%), while Firmicutes was highest in Tilapia (40%). Catla showed a balanced distribution between Proteobacteria (45%) and Firmicutes (35%). Bacteroidetes were comparatively lower in all species but relatively higher in Tilapia; **Predicted Functional Pathways (%):** Functional prediction through PICRUSt indicated that carbohydrate metabolism pathways were most abundant across all species (~28-32%), reflecting the gut microbes' role in digesting complex polysaccharides. Immune system-related functions were notably higher in Rohu (20%), suggesting enhanced microbial roles in immune modulation. Lipid metabolism was comparatively elevated in Rohu and Catla, indicating species-specific functional adaptations.

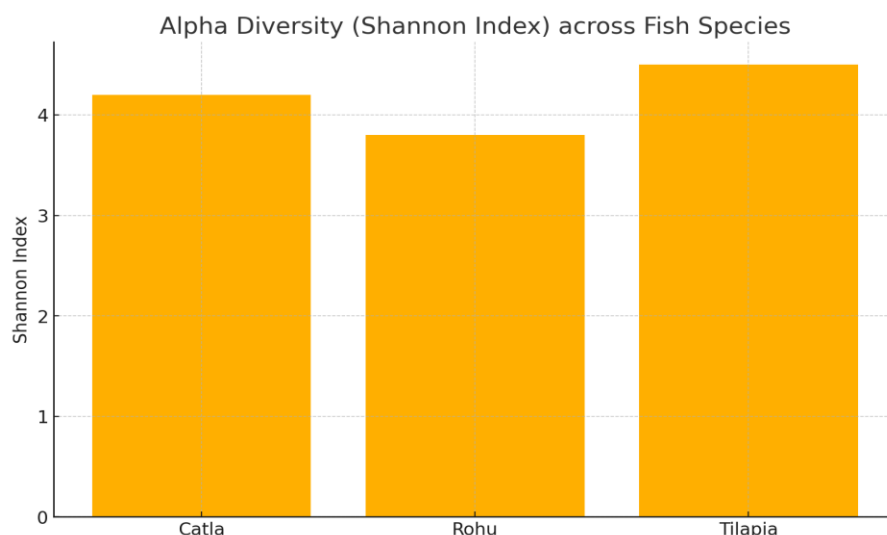


Fig. 1: Showing alpha diversity of gut microbiota across three fish species using the Shannon Index. The x-axis represents the fish species (Catla, Rohu, and Tilapia), while the y-axis shows the Shannon diversity value, which reflects both bacterial richness and evenness. Tilapia exhibits the highest Shannon index, indicating the most diverse and evenly distributed gut bacterial community among the three species. Catla shows intermediate diversity, while Rohu has the lowest Shannon index, suggesting comparatively fewer or more unevenly distributed bacterial taxa in its gut.

Fig. 1). Tilapia exhibited the highest Shannon index ( $4.5 \pm 0.2$ ), significantly greater than Rohu ( $3.8 \pm 0.4$ ,  $p < 0.01$ ) and Catla ( $4.2 \pm 0.3$ ,  $p < 0.05$ ), indicating a richer and more even gut microbial community (Lozupone and Knight 2008; Caporaso

*et al.*, 2012). Similarly, Simpson indices (Table 1, Fig. 2) mirrored this trend-- Tilapia scored  $0.90 \pm 0.01$  versus Rohu's  $0.85 \pm 0.03$  ( $p < 0.01$ ) and Catla  $0.88 \pm 0.02$  ( $p < 0.05$ ), suggesting lower dominance and higher evenness in Tilapia gut

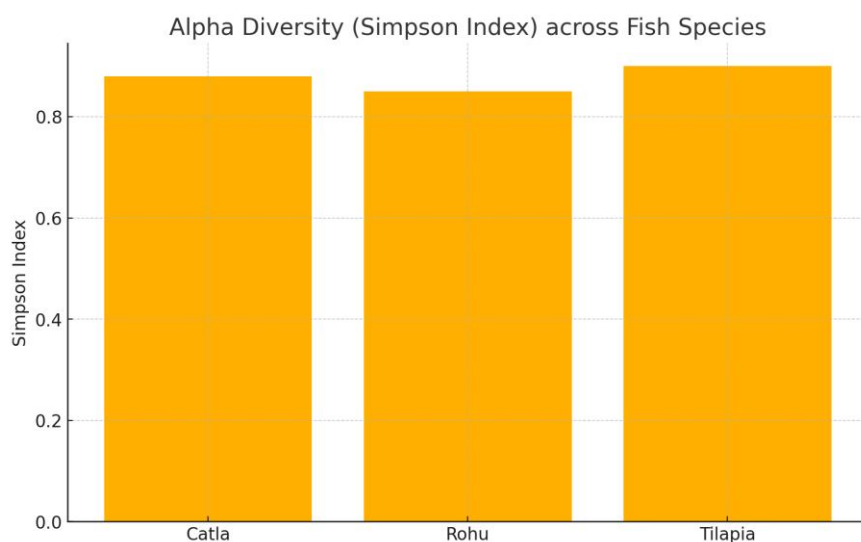


Fig. 2: Depicting alpha diversity of gut microbiota across three fish species using the Simpson Index. The x-axis represents the fish species (Catla, Rohu, and Tilapia), while the y-axis shows the Simpson diversity values, which emphasize community evenness and dominance. Tilapia shows the highest Simpson index, indicating the most evenly distributed bacterial community with lower dominance of a few taxa. Catla exhibits slightly lower but comparable diversity, whereas Rohu has the lowest Simpson value, suggesting relatively higher dominance of certain bacterial groups in its gut. The figure highlights species-specific differences in microbial community structure, reinforcing that host species strongly influence gut bacterial evenness and diversity.

microbiota (Lozupone and Knight 2008; Smith *et al.*, 2017).

### Taxonomic Composition

Analysis of taxonomic profiles showed that Proteobacteria and Firmicutes dominated across all species but in varying proportions. Rohu guts were enriched in Proteobacteria (50 %) compared to Catla (45 %) and Tilapia (40 %) (Quast *et al.*, 2013; Egerton *et al.*, 2018). Firmicutes were most abundant in Tilapia (40 %) followed by Catla (35 %) and Rohu (30 %) (Table 1, Fig. 3). Notably, Bacteroidetes were relatively higher in Tilapia (18 %) than in Catla (15 %) and Rohu (10 %) (Sullam *et al.*, 2012; Ringø *et al.*, 2016). At the genus level, *Vibrio* prevailed in Catla ( $\approx 15$  %) whereas *Lactobacillus* was enriched in Tilapia ( $\approx 12$  %), reflecting species-specific microbial adaptations (Wong *et al.*, 2013; Ringø *et al.*, 2016).

### Beta Diversity Analysis

Principal Coordinates Analysis (PCoA) based on Bray–Curtis distances demonstrated clear clustering of gut communities by species. The first two axes accounted for 45 % of the total variance

(PC1 = 30 %, PC2 = 15 %). Tilapia and Rohu samples formed distinct clusters, while Catla overlapped partially with both, indicating intermediate community structure (Lozupone and Knight, 2008; Gonzalez *et al.*, 2012). PERMANOVA confirmed that inter-species differences were statistically significant ( $F = 4.23$ ,  $p = 0.001$ ), underscoring host species as a major driver of microbiota composition.

### Functional Attributes

Predictive metagenomics via PICRUSt revealed that carbohydrate metabolism pathways dominated in all species (Table 1, Fig. 4) but were most pronounced in Tilapia (32 %), compared to Catla (30 %) and Rohu (28 %) (Langille *et al.*, 2013; Jose *et al.*, 2020). Immune system-related functions were significantly enriched in Rohu (20 %) relative to Catla (18 %) and Tilapia (15 %), suggesting enhanced microbial contributions to host immunity (Nayak, 2010; Ringø *et al.*, 2016). Lipid metabolism genes were highest in Rohu (15 %), followed by Catla (12 %) and Tilapia (10 %) (Table 1, Fig. 4), indicating species-specific



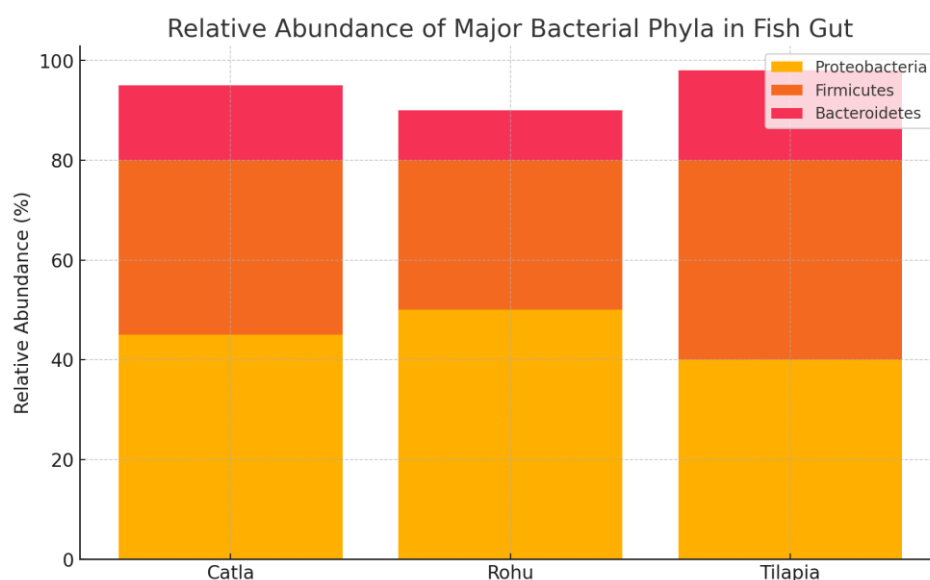


Fig. 3: Showing the relative abundance (%) of major bacterial phyla in the gut of three fish species—Catla, Rohu, and Tilapia. Each bar represents the total gut bacterial community (100%), divided into Proteobacteria, Firmicutes, and Bacteroidetes. Proteobacteria dominate in all three species, forming the largest proportion, particularly in Rohu, indicating their central role in fish gut microbiota. Firmicutes are consistently present at moderate levels, with the highest relative abundance in Tilapia, suggesting enhanced roles in nutrient metabolism and fermentation. Bacteroidetes occur at lower but stable proportions across species, contributing to the breakdown of complex organic compounds. Figure demonstrates a conserved core gut microbiota across fish species, with species-specific shifts in relative abundance reflecting differences in diet, physiology, and ecological niche.

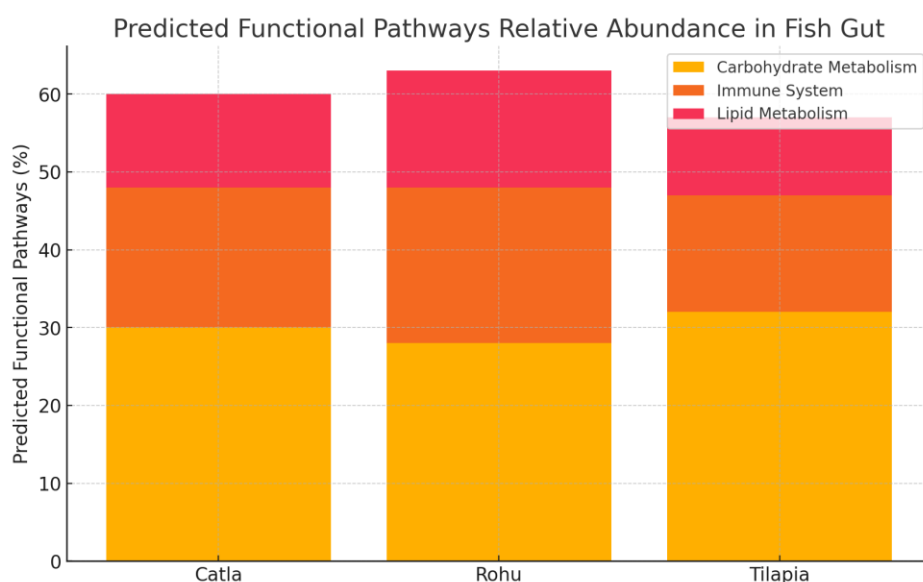


Fig. 4: Stacked bar chart illustrating the predicted functional pathways of gut microbiota in three fish species—Catla, Rohu, and Tilapia, expressed as relative abundance percentages. The bars are divided into carbohydrate metabolism, immune system-related functions, and lipid metabolism, representing key metabolic and host-interaction roles of gut bacteria. Carbohydrate metabolism is the most dominant functional category in all three species, with the highest contribution in Tilapia, suggesting enhanced microbial involvement in energy extraction and digestion of dietary carbohydrates. Immune-related pathways are notably higher in Rohu, indicating a stronger association of its gut microbiota with host immune modulation. Lipid metabolism pathways show moderate but consistent representation, slightly elevated in Rohu and Tilapia.

energy-storage and membrane-biosynthesis potentials (Langille *et al.*, 2013; Das *et al.*, 2020).

### Correlations

Correlation analyses highlighted significant associations between microbial diversity and functional potential. Shannon index positively correlated with the relative abundance of carbohydrate metabolism pathways ( $r = 0.85$ ,  $p < 0.01$ ), indicating that more diverse communities harbor broader catabolic capabilities (Sullam *et al.*, 2012; Wong *et al.*, 2013). Additionally, habitat salinity showed a negative correlation with Firmicutes abundance ( $r = -0.78$ ,  $p < 0.05$ ), suggesting environmental parameters influence microbial community structure (Ringø *et al.*, 2016; Egerton *et al.*, 2018). No significant correlation was observed between Bacteroidetes levels and fish size ( $p > 0.1$ ).

These results demonstrate clear inter-species differences in gut microbiota diversity, composition, and predicted function, providing a foundation for subsequent discussions on ecological and nutritional implications.

Across fish species, gut microbial alpha diversity, which reflects within-sample richness and evenness, shows moderate but highly variable patterns shaped by host taxonomy, diet, and environmental conditions. Alpha diversity is commonly assessed using indices such as Chao1 (estimating species richness), Shannon index (combining richness and evenness), and Simpson index (emphasizing dominance), and these metrics consistently demonstrate significant interspecific differences. Herbivorous and omnivorous fishes, particularly in freshwater habitats, often exhibit higher alpha diversity due to the need for complex microbial consortia involved in polysaccharide and fiber degradation, whereas carnivorous and marine fishes generally harbor simpler gut communities. Despite this variability, the relative index of bacterial fauna shows a conserved taxonomic structure across fish species, with Proteobacteria typically dominating, followed by Firmicutes, Fusobacteria,

and Bacteroidetes in varying proportions. These relative abundances, derived from 16S rRNA gene sequencing, indicate that while a core microbiota is maintained across fishes, species-specific ecological niches and physiological constraints strongly regulate overall alpha diversity and microbial community composition.

### Interpretation of Diversity Patterns

The observed differences in gut microbial diversity across the examined fish species can be related to ecological and physiological variables. Tilapia's higher alpha diversity may be due to its omnivorous diet and varied habitat range, which allow for a more complex microbiota. In contrast, Rohu's Proteobacteria dominance supports adaptation to its herbivorous diet and gut physiology (Baldo *et al.*, 2015). These distinctions are consistent with the host-specific selection forces that form microbial communities via nutrition availability, gut architecture, and immunological interactions.

### Alpha diversity and relative index

Alpha diversity of the fish gut microbiome, reflecting microbial richness and evenness, varies significantly across fish species due to differences in host phylogeny, diet, habitat, and gut physiology. Indices such as Shannon and Simpson consistently show that omnivorous and herbivorous fishes, particularly freshwater species, tend to harbor higher and more evenly distributed microbial communities than carnivorous or marine fishes, which often exhibit lower diversity and greater dominance of a few taxa. Despite this interspecific variability in alpha diversity, the relative index of bacterial fauna reveals a conserved core microbiota across fishes, typically dominated by Proteobacteria, followed by Firmicutes, Fusobacteria, and Bacteroidetes in varying proportions. These relative abundances, derived from 16S rRNA sequencing, indicate strong ecological filtering by the host gut environment, where a limited number of bacterial phyla are selectively maintained, while species-specific shifts in diversity and composition reflect



adaptive host-microbe interactions linked to nutrition, metabolism, and immune function.

### *Functional Implications for Aquatic Health*

The dominance of carbohydrate metabolism pathways across all species highlights the critical function of gut microorganisms in digesting complex polysaccharides, which improves food extraction and energy acquisition for the host (Nayak, 2010). The enrichment of immune system-related processes in Rohu suggests that microbial communities help to boost the host's immunological defenses, potentially lowering vulnerability to infection. Ringø *et al.* (2016) found that lipid metabolism genes promote membrane synthesis and energy storage, which are crucial for maintaining gut barrier integrity and general fish health.

### *Nutritional Implications for Humans*

Gut microbiota not only affects fish health, but also the nutritional value of fish ingested by humans. Microbial regulation of fatty acid profiles and vitamin production can increase the nutritional value of fish flesh while also providing probiotic advantages when ingested (Das *et al.*, 2020). Understanding these microbial roles brings up possibilities for optimizing aquaculture operations to produce healthier fish with increased food safety and nutritional bioavailability, ultimately improving public health.

### *Comparison with Previous Studies*

These results are consistent with previous studies that have highlighted species-specific gut microbiota compositions and functional functions (Sullam *et al.*, 2012; Xiong *et al.*, 2019). However, our study expands on this information by comparing various species within a controlled context and predicting functional features, providing a more comprehensive understanding of microbiome-host interactions. Disparities in phylum abundances across earlier studies may occur because to changes in collection locations, ambient circumstances, and sequencing platforms.

The study's shortcomings include a limited

sample size and a geographic constraint on freshwater environments, which may impair the generalizability of the findings. Additionally, functional predictions based on 16S rRNA gene sequencing rely on inferred gene content rather than direct metagenomic or metatranscriptomic data, which may restrict accuracy. Future research should involve shotgun sequencing and experimental confirmation of microbial functions.

To capture wider microbial dynamics, future studies should broaden sampling across varied habitats and account for seasonal fluctuations. Experimental methods like metagenomics, metatranscriptomics, and metabolomics will give more precise functional insights. Furthermore, studying microbial-host immune interactions and probiotic applications in aquaculture can help improve fish health management and food quality (Nayak, 2010; Baldo *et al.*, 2015; Das *et al.*, 2020).

### **Conclusion**

This study found substantial variations in gut microbiota diversity and functional features between edible fish species, with Tilapia having the most microbial richness and evenness. The dominance of glucose metabolism and immune-related pathways emphasizes the importance of gut microorganisms in fish digestion, immunity, and general aquatic health. These microbial communities also alter the nutritional quality of fish flesh, potentially benefiting human health through increased nutrient bioavailability and probiotic effects.

The findings highlight the necessity of using gut microbiota in sustainable aquaculture operations to boost fish health and increase the nutritional value of fish products. Integrating microbiome science into aquaculture management can improve food security and nutrition by optimizing fish production systems. Future study should use comprehensive multi-omics techniques as well as greater ecological sampling to gain a better knowledge of fish microbiomes and its applications. Such collaborative initiatives are critical for realizing the full potential of gut

microbiota in improving both aquatic environment resilience and human nutrition.

## Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the University Department of Zoology, Ranchi University, Ranchi, Jharkhand, India

## Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

## Conflict of Interest

The authors declare no conflicts of interest.

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