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First Insight into the Gut Microbiome of Rohu Fish from Halda River and Kaptai Lake Using 16S rRNA Sequencing

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Article

Keywords: Gut bacteriome, LAB, Probiotic, 16S rRNA metagenome, Sustainable aquaculture

Posted Date: August 12th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7192852/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on February 13th, 2026. See the published version at https://doi.org/10.1038/s41598-025-33754-5.

Abstract

Freshwater ecosystems are vital for biodiversity and livelihoods in Bangladesh, where interest in fish gut microbiota via amplicon sequencing is growing to aid aquaculture sustainability through microbial interventions. Therefore, this research investigated the bacteriomes of the gut of Rohu from the Halda River and Kaptai Lake, using 16S rRNA amplicon sequencing. The evaluation of diversity demonstrated notable variations in both alpha and beta diversity indices ($p < 0.05$). The fish in the Halda River had a varied bacteriome, mostly composed of Pirellulaceae_uncultured (9.26%), with environmentally tolerant taxa such as *Exiguobacterium* (5.48%). In contrast, the Kaptai Lake fish have a bacteriome that is abundant in probiotics, including *Lactiplantibacillus* (48.84%) and *Pediococcus* (8.82%). Water samples exhibited unique microbiological signatures: Halda River water was mostly characterized by *Exiguobacterium* (41.93%), while Kaptai Lake water was primarily composed of *Acinetobacter* (71.24%). Furthermore, functional analysis indicated that fish from the Halda River comprised metabolically diverse communities involved in nitrogen cycling, whereas the Kaptai Lake fish demonstrated a strong capacity for ammonia oxidation and pollutant breakdown. The research offers significant insights into the relationship between the host, microbiome, and environment, with implications for enhancing fish health and promoting sustainable aquaculture practices.

Introduction

In riverine nations such as Bangladesh, where inland fisheries provide a substantial amount of protein and employment alternatives, freshwater ecosystems are vital for the preservation of biodiversity, the maintenance of livelihoods, and the contribution to national economies [1]. The Halda River and Kaptai Lake are notable freshwater bodies owing to their unique biological features and socio-economic significance [2,3]. The Halda River, situated in southern Bangladesh, is uniquely recognized as the only natural breeding habitat for Indian major carps (*Labeo rohita, Catla catla*, and *Cirrhinus cirrhosus*) [2,4,5]. This river functions as both a biodiversity hotspot and a vital genetic reservoir essential for aquaculture and conservation initiatives across South Asia [4,5]. The river's abundant nutrient supply and consistent flow patterns create an optimal environment for natural reproduction, making it a crucial asset for sustainable aquaculture and biodiversity preservation in the area [2,6,7]. Meanwhile, the Karnaphuli River was dammed in 1961 to form Kaptai Lake, one of the largest artificial freshwater reservoirs in South-east Asia [8–10]. In natural environments such as the Halda River, Indian Major Carps spawn during the monsoon season in flowing rivers, spurred by water flow, turbidity, and temperature. However, Kaptai Lake's stagnant environment lacks these cues, hindering natural spawning. The lack of vigorous currents and appropriate substrates, along with constrained migration routes due to the dam, impedes reproduction [10,11]. Consequently, Carp populations in the lake depend on artificial breeding and stocking programs. While the lake supports growth with abundant plankton and foraging areas, it serves more as a rearing ground than a natural spawning habitat for Indian Major Carps [10,11]. There are growing concerns about ecosystem deterioration and fish quality due to their artificial origin and increasing

human pressures, such as agricultural runoff and uncontrolled fishing. Despite this, the waterway supports a diverse range of fish species, providing food and income for thousands of residents [11,12].

Rohu (*Labeo rohita*), a prominent species of Indian large carp, has considerable ecological and commercial significance in Bangladesh. The Halda River and Kaptai Lake are notable among other freshwater systems for their distinct biological dynamics and significant contributions to fisheries resources [10,13]. The microbiota in the fish gut plays a crucial role in their development, physiology, and overall well-being. Similar to land animals, fish possess a sophisticated gut microbiome that aids in digestion, immune processes, and various ecological relationships [14–16]. Microbiota colonization starts soon after hatching, influenced by factors such as water microbiota, diet, host genetics, and environmental conditions [17]. Although vertical transmission through egg surfaces can occur [18], the primary method of acquisition remains horizontal, primarily through water and feed [19].

Gut microbiome facilitate digestion by breaking down complex carbohydrates, producing vitamins such as B12 and K, and generating short-chain fatty acids [20–22]. Enhancing nutrient absorption and feed efficiency is vital in aquaculture. Additionally, the gut microbiome plays a role in immune development by aiding gut-associated lymphoid tissues and influencing immune responses [23–25]. Such beneficial bacteria also inhibit pathogen colonization by producing antimicrobial compounds and through competitive exclusion [26,27]. The impact of fish gut microbiome significantly reaches into the surrounding environment [15]. Fish release microbes and metabolites that influence nearby microbial communities and the cycling of nutrients [28]. Species that feed on plants and decomposing matter are essential for breaking down organic materials and recycling nutrients, thus connecting the health of hosts with the dynamics of ecosystems [29]. Comparative gut microbiome analyses of Rohu populations from these two aquatic environments provide essential insights into the effects of varying environmental circumstances, ecological constraints, and human interventions on fish health, and microbial relationships [30]. Understanding the microbiome the structure of Rohu fish from these two distinct freshwater environments is also crucial for the sustainable management of fishery resources [31]. In this context, utilizing 16S rRNA amplicon sequencing provides a robust method for investigating the bacterial communities associated with fish and their environments, shedding light on both natural diversity and human impact [32].

This study aims to investigate the largely unexplored microbial communities in the gastrointestinal tracts of Rohu from the Halda River and Kaptai Lake through 16S rRNA metagenomic sequencing, aiming to enhance our comprehension of the complex interactions between fish hosts and their aquatic environments.

Methods

Ethical statement

The Ethics Approval Committee (EAC) of Chattogram Veterinary and Animal Sciences University (CVASU) reviewed and authorized the study protocol (Approval no. CVASU/Dir (R&E) EC/2025/881/15. The fish sampling approach rigorously complied with the ARRIVE 2.0 criteria [33]. The research was conducted by the protocols specified in the "Guide for the Care and Use of Laboratory Animals" issued by the National Institutes of Health. All methods were carried out in accordance with relevant guidelines and regulations. The research site was neither privately owned nor protected, and the study was not conducted in an area with endangered or protected species.

Sample collection and processing

A total of eight mature *Labeo rohita* (Rohu) fish were collected during the period of March to April 2025 from two ecologically distinct freshwater bodies in Bangladesh: Halda River (n=4) and Kaptai Lake (n=4) (S1 Fig). Concurrently, eight corresponding water samples were collected from the same sites (Halda River = 4, Kaptai Lake = 4) to enable comparative microbiome analysis. The fish specimens were obtained by local fishermen using traditional fishing methods. Following capture, the fish were stored in chilled containers and transferred at 4°C to the Central Genomics Laboratory, Department of Genomics and Bioinformatics, Chattogram Veterinary and Animal Sciences University (CVASU), ensuring that processing commenced within 12 hours of collection. Upon arrival, the physical characteristics of each fish—including height, width, and weight—were recorded (Supplementary figure 1). Gut content samples (~100 mg) were then aseptically isolated to minimize external contamination (Supplementary figure 2). The collected gut samples were stored at −80 °C until DNA extraction and subsequent microbial profiling were conducted.

DNA extraction and 16S rRNA gene sequencing

The genomic DNA from fish and water samples of both the Halda River and Kaptai Lake was extracted for 16 representative metagenome samples using the DNeasy PowerSoil Pro Kit (Cat. No. 47014), following the manufacturer's guidelines. The pure DNA samples were then preserved at -80°C for future use. The V1-V9 region of the 16S rRNA gene was amplified using Q5 High-Fidelity 2X Master Mix, according to the manufacturer's instructions [34]. Amplification was conducted under the following PCR parameters: initial denaturation at 95 °C for 3 minutes, followed by 30 cycles of 95 °C for 15 seconds, 60 °C for 15 seconds, and 72 °C for 60 seconds, concluding with a final extension at 72 °C for 10 minutes. Amplified DNA was purified using AMPure XP reagent (Beckman Coulter, Inc.) and quantified using Qubit 4 with Qubit™ 1X dsDNA High Sensitivity (HS) reagent (Thermo Fisher Scientific, USA). A total of 200 ng of DNA was used for end preparation and dA tailing, subsequently followed by the ligation of barcodes using the Native Barcoding Kit 24 V14 (SQK-NBD114.24) from Oxford Nanopore. The NEBNext® Quick Ligation Module (NEB, E6056) was used to ligate the sequencing adaptor, and the resultant DNA libraries were consolidated. The fully adapter-ligated and purified library, together with 37.5 µL of Sequencing Buffer and 25.5 µL of Loading Beads, was loaded into the R10.4.1 flow cell (FLO-MIN106; Oxford Nanopore Technologies) and sequenced using the MinION Mk1C. Data collection was performed using MINKNOW software version 1.11.5 from Oxford Nanopore Technologies.

Bioinformatic analysis

The Guppy version 6.3.2 (Oxford Nanopore Technologies) was used in high accuracy mode for basecalling the MinION Mk1C sequencing data (FAST5 files), providing pass reads (FASTQ format) with a mean quality score above 9. The adaptor and barcode sequences were trimmed using Porechop v0.2.4 [35], whereas low-quality bases were removed with Nanofilt [36]. The quality-trimmed reads were filtered by size, preserving sequences of 1300–1600 bp for the V1-V9 area, according to the size distribution of 16S rRNA gene sequences. The taxonomic assignment of the reads was performed using the wf-16s process in Epi2me software (Oxford Nanopore Technologies) [37], using SILVA [38] as the reference database and Kraken2 as the classifier. Additionally, all other parameters were configured to their default settings [39].

Statistical analysis

The Phyloseq (V 4.2) [40], Vegan [41], ggplot2, ggpubr, and microbiomeutilities packages for R (v 4.3.0) was used for the downstream analysis including alpha-beta diversity, microbial composition, differential abundance calculation, and statistical comparison. The OTU data were standardized using total sum scaling (TSS) methods, and the normalized data were used to assess alpha and beta diversity. To assess within-sample diversity (Alpha (α)-diversity), we computed the observed OTUs, Chao1, ACE, Shannon, Simpson, and InvSimpson diversity indices using the phyloseq and vegan packages. The Wilcoxon rank-sum test from the microbiomeutilities R package was used to evaluate the differences in microbial diversity and abundance across the groups. To illustrate variations in microbial diversity (β-diversity) among the sample groups, β-diversity between groups was assessed using Principal Coordinate Analysis (PCoA) and non-metric multidimensional scaling (NMDS) based on the Bray distance metric. At the same time, permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was employed to calculate a p-value for group differences. The METAGENassist tool was used to forecast functional annotations [42].

Results

Bacteriome diversity and composition of fish and water samples

We analyzed eight fish and eight water samples from the Halda River and Kaptai Lake, categorized into four groups—Halda River fish, Halda River water, Kaptai Lake fish, and Kaptai Lake water—to elucidate the bacteriome signature and diversity using 16S rRNA amplicon sequencing. Supplementary table 1 contains the details of the study's sampling methodology. This information includes sample descriptions, sampling sources and locations, amplicon sequence data, operational taxonomic units (OTUs) assigned to each sample, and the corresponding sequence read archive (SRA) accession numbers.

The study found 263 OTUs among the sample groups. To determine whether the diversity of fish and water bacteriome in the Halda River and Kaptai Lake differs across sample categories, we examined

both within-sample (alpha) and between-sample (beta) diversities of the bacterial communities. Alpha diversity, evaluated through Observed species (p-value = 0.0079), Chao1 (p-value = 0.0079), ACE (p-value = 0.0079), Shannon (p-value = 0.0059), Simpson (p-value = 0.0055), and InvSimpson (p-value = 0.0055) indices, demonstrated significant disparities in bacterial community richness (p-value <0.05, Kruskal-Wallis rank sum test) (**Figure 1A**). The principal coordinate analysis (PCoA), and non-metric multidimensional scaling (NMDS) using Bray–Curtis dissimilarity distance revealed a substantial difference in bacteriome composition across sample groups (p-value = 0.001, R^2 = 1.0, PERMANOVA test) (**Figure 1B & C**).

The observed OTUs included 16 phyla, 27 classes, 75 orders, 109 families, and 263 genera of bacteria. The distinct and overlapping distribution of bacterial species in the four sample groups is shown in Venn diagram (**Figure 1D, Supplementary table 2**).

Relative abundances of bacteriomes in fish and water samples

The gut bacteriome of Halda River fish mostly consisted of six bacterial phyla. Firmicutes had the greatest relative abundance (33.09%), followed by Planctomycetota (29.82%) and Proteobacteria (18.14%). However, Verrucomicrobiota (4.81%), Actinobacteriota (3.54%), and Bdellovibrionota (3.17%) were less prevalent, although still significant. The Halda River water samples were predominantly composed of Firmicutes (70.96%), followed by Proteobacteria (12.33%) as the second most prevalent phylum. Other phyla including, Planctomycetota (6.33%), Cyanobacteria (2.48%), and Actinobacteriota (2.29%) were less prevalent (**Figure 2, Supplementary data 1**). The gut bacteriome of Kaptai Lake fish was predominantly composed of Firmicutes (92.16%), with Proteobacteria accounting for 3.36% of the overall bacterial population. Contrarily, the microbial population of Kaptai Lake water was mostly comprised of Proteobacteria (85.88%), followed by Planctomycetota (3.23%) and Actinobacteriota (2.08%). Nevertheless, the prevalence of all other phyla remained below 2% and varied among the four sample groups (**Supplementary data 1**).

The gut bacteriome of Halda River fish exhibited a varied composition at the class level. In this sample group, Planctomycetes (29.82%) was found as the predominant class, followed by Bacilli (19.10%), Clostridia (15.22%), Gammaproteobacteria (9.18%), and Alphaproteobacteria (8.95%). Additionally, other significant classes were Verrucomicrobiae (4.81%), Oligoflexia (3.17%), and Actinobacteria (3.31%). On the other hand, the Halda River water was mostly composed of Bacilli (65.26%), followed by Gammaproteobacteria (9.02%), Planctomycetes (6.33%), and Clostridia (5.69%). Additional identified classes were Alphaproteobacteria (2.91%) and Cyanobacteria (2.48%) (**Figure 3, Supplementary data 1**). The gut bacterial community of Kaptai Lake fish was mostly composed of Bacilli, accounting for 91.53%. However, the bacterial population in the Kaptai Lake water samples was mostly composed of Gammaproteobacteria (77.98%), followed by Alphaproteobacteria (7.59%) and Planctomycetes (3.17%). The prevalence of all other classes remained below 2% and varied among the four sample groups (**Supplementary data 1**).

The bacterial community structure associated with fish from the Halda River exhibits significant taxonomic diversity at the order level. The predominant order detected was Pirellulales (20.79%), followed by Clostridiales (11.70%) and Rhizobiales (8.12%). Additional orders with moderate participation were Bacillales (7.09%), Pseudomonadales (6.92%), Lactobacillales (6.41%), Gemmatales (6.31%), Chthoniobacterales (4.33%), Oligoflexales (3.17%), Exiguobacterales (2.81%), and Corynebacteriales (2.18%). The Halda River water had a pronounced predominance of the order Exiguobacterales (41.93%), with Bacillales (23.63%) being the second most prevalent species. Pseudomonadales (7.13%) exhibited a notable prevalence, followed by Pirellulales (4.22%), Clostridiales (4.03%), Synechococcales (2.48%), and Rhizobiales (2.46%) (**Figure 4, Supplementary data 1**). Further, bacterial examination of fish samples from Kaptai Lake revealed a predominant presence of the bacterial order Lactobacillales (89.81%) of the total identified population. Where, the Kaptai Lake water samples indicated a significant predominance of the order Pseudomonadales (72.21%), with Sphingomonadales (6.48%) as the second most prevalent order, followed by Burkholderiales (5.36%). However, the prevalence of all other orders remained below 2% and varied among the four sample groups (**Supplementary data 1**).

The microbial profiling of fish samples from the Halda River revealed a taxonomically diverse bacterial population, characterized by a significant presence of various bacterial families. The predominant family detected was Pirellulaceae (20.79%), followed by Clostridiaceae (11.70%), Moraxellaceae (6.92%), Bacillaceae (6.61%), Gemmataceae (6.31%), and Lactobacillaceae (4.55%). Additional families with significant contributions were Oligoflexaceae (3.17%), Exiguobacteraceae (2.81%), Beijerinckiaceae (3.23%), Xanthobacteraceae (2.61%), Chthoniobacteraceae (3.07%), Peptostreptococcaceae (2.07%), and Mycobacteriaceae (2.18%). However, the bacterial community composition of Halda River water were primarily characterized by the family Exiguobacteraceae (41.93%). Planococcaceae (13.21%), Bacillaceae (10.34%), Moraxellaceae (7.13%), Pirellulaceae (4.22%), Clostridiaceae (4.03%), and Cyanobiaceae (2.48%) (**Figure 5, Supplementary data 1**). Besides, the analysis of the bacterial community associated with fish from Kaptai Lake indicated a significant predominance of the Lactobacillaceae family, comprising almost 77.50% of the overall bacterial abundance. Besides, Enterococcaceae (5.43%) and Streptococcaceae (3.64%) were significant constituents of the community. However, the Kaptai Lake water were mostly composed of Moraxellaceae (72.08%), followed by Sphingomonadaceae (6.48%) and Comamonadaceae (3.44%). The prevalence of all other families remained below 2% and varied among the four sample groups (**Supplementary data 1**).

The study at the genus level of the bacterial communities observed in Halda River fish suggested a diverse set of microbial community that represents both environmental and host-associated species. The predominant genus identified was Pirellulaceae_uncultured (9.26%), followed by other genera including *Clostridium sensu stricto* 1 (7.92%), *Acinetobacter* (6.92%), *Bacillus* (6.61%), Gemmataceae_uncultured (6.31%), *Rhodopirellula* (5.62%), and *Pirellula* (5.48%). Genera of lower abundance, nevertheless, with ecological significance were *Sarcina* (3.57%), *Lactiplantibacillus* (3.55%), *Exiguobacterium* (2.81%), Oligoflexaceae_uncultured (2.64%), and *Mycobacterium* (2.18%). However, the bacterial community in Halda River water samples indicated a significant predominance of

Exiguobacterium, accounting for roughly 41.93% of the overall abundance. In this sample group, *Bacillus* (10.09%) ranked as the second most prevalent genus, followed by *Acinetobacter* (6.92%), *Planococcus* (6.56%), Pirellulaceae_uncultured (2.58%), *Cyanobium* PCC-6307 (2.35%), and *Clostridium sensu stricto* 1 (2.23%) (**Figure 6, Supplementary data 1**). Besides, the bacterial community associated with fish from Kaptai Lake indicated that *Lactiplantibacillus* (48.84%) was the most predominant bacterial genus, followed by *Pediococcus* (8.82%), *Enterococcus* (5.43%). *Lactococcus* (3.34%) and *Limosilactobacillus* (3.20%). Contrarily, the bacterial community of Kaptai Lake water was primarily composed of the species *Acinetobacter* (71.24%), followed by the genus *Novosphingobium* (5.37%). The relative abundace of all other genera remained below 2% and varied among the four sample groups (**Supplementary data 1**).

Functional profile of bacterial communities in Halda River and Kaptai Lake

The functional analysis of bacterial communities from fish and water samples of Halda River and Kaptai Lake demonstrated unique metabolic capacities linked to environmental adaptability and biogeochemical cycling (**Figure 7, Supplementary data 2**). The bacterial community in Halda River fish had significant functional potential with Dehalogenation (20.71%) demonstrated the highest activity, succeeded by sulfate-reducing bacteria (15.53%), xylan degraders (12.58%), ammonia oxidizers (10.65%), nitrite reducers (8.41%), chitin degraders (8.20%), sulfide oxidizers (5.87%), aromatic hydrocarbons degrader (3.40%), streptomycin-producers (3.90%), and nitrogen-fixing bacteria (3.92%). The data also suggested that dehalogenation activity constitutes the predominant microbial function in Halda River water (25.27%), followed by sulfide oxidization (19.20%), sulfate reduction (9.90%), xylan degradation (8.63%), ammonia oxidation (8.59%), nitrite reduction (7.28%), chitin degradation (7.20%), streptomycin production (3.60%), and aromatic hydrocarbons degradation (3.36%) The prevalence of all other families remained below 2% and varied among the four sample groups (**Figure 7, Supplementary data 2**).

The microbial communities of Kaptai River fish had a distinct functional profile, characterized by a notably high capability for ammonia oxidizers (36.48%), followed by dehalogenating bacteria (34.73%). However, chitin degrader (7.77%), xylan degrader (5.69%), sulfate-reducing bacteria (3.16%), and sulfide-oxidizing bacteria (4.50%) were present in significant quantities. Additionally, the Kaptai River water community had more equitable functional distribution, with significant contributions from ammonia oxidation (11.52%), dehalogenation (11.96%), chitin degradation (10.09%), sulfate reduction (12.04%), and nitrite reduction (11.43%). This community had significant potential for xylan degrader (10.71%), and aromatic hydrocarbon degraders (10.68%) (**Figure 7, Supplementary data 2**).

Discussion

The emergence of intensive aquaculture has led to modifications in gut microbiota through the use of probiotics, prebiotics, and nutritional treatments, which represents a potential approach to enhance fish growth, disease resistance, and environmental sustainability [43]. A comprehensive knowledge of

microbial-host interactions may provide more precise and effective strategies in aquaculture, reducing dependence on antibiotics and alleviating environmental consequences [44-46]. This work aimed to delineate and contrast the bacteriome signatures of fish and water samples from two ecologically diverse freshwater systems in Bangladesh—Halda River and Kaptai Lake—utilizing 16S rRNA amplicon sequencing. Eight fish and eight water samples were gathered and categorized into four categories: Halda River fish, Halda River water, Kaptai Lake fish, and Kaptai Lake water. The analysis revealed 263 OTUs, reflecting the overall bacterial diversity found in all sample groups. The alpha diversity indices, including Observed species, Chao1, ACE, Shannon, Simpson, and Inverse Simpson, demonstrated statistically significant variations ($p < 0.05$) among the four sample groups. The richness-related indices (Observed, Chao1, ACE) indicated that certain groups—presumably Halda River samples—possess more diverse bacterial communities. In contrast, evenness-focused indices (Shannon, Simpson) suggested variations in compositional balance, potentially reflecting differing environmental pressures or host-specific microbial structure [47,48]. Furthermore, beta diversity analysis using PCoA and NMDS, based on Bray–Curtis dissimilarity, revealed a distinct segregation of microbial communities across the sample groups (PERMANOVA, p-value = 0.001, $R^2 = 1.0$). The pronounced disparity suggested that each group—characterized by habitat (river versus lake) and supply (fish versus water)—exhibits a distinct microbial signature [48-50].

The comparative analysis of bacterial communities in Halda River and Kaptai Lake samples revealed unique microbial fingerprints influenced by their distinct aquatic habitats and host interactions. The bacterial population in Halda River fish exhibits significant variety, including both environmental and host-associated taxa. The predominant taxon (genus), Pirellulaceae_uncultured (9.26%), comprising *Rhodopirellula* and *Pirellula*, indicates a significant presence of the phylum Planctomycetota, often associated with aquatic environments and recognized for their involvement in nitrogen cycling and the breakdown of complex carbohydrates [51,52]. The existence of taxa such as *Sarcina*, *Lactiplantibacillus*, and *Exiguobacterium*—albeit in lower quantities—suggests a potential probiotic effect and environmental resilience, especially under varying temperature and nutritional conditions [53-55]. Conversely, the bacterial community of Kaptai Lake fish is predominantly composed of lactic acid bacteria (LAB), with Lactiplantibacillus (48.84%), Pediococcus (8.82%), Enterococcus (5.43%), Lactococcus (3.34%), and Limosilactobacillus (3.20%) [56]. This community structure is characteristic of a host-associated microbiota, particularly in the intestines of fish, where LAB play a crucial role in digestion, immunological regulation, and pathogen exclusion [56-58]. The significant prevalence of LAB indicates a comparatively healthy and possibly probiotic-rich microbiome in Kaptai Lake fish, which dietary factors, ambient microbial load, or aquaculture methods may influence [59]. Finally, the microbial composition of Kaptai Lake fish indicates a superior gut microbiome, distinguished by an elevated presence of advantageous, host-associated probiotics. This indicates a potentially more stable and health-enhancing gut environment, probably shaped by uniform environmental conditions or aquaculture methodologies.

Additionally, the microbial composition of water samples from the Halda River and Kaptai Lake demonstrates significant disparities in community structure, indicative of varying environmental

conditions and potential anthropogenic impacts. The Halda River water has a notable prevalence of *Exiguobacterium* (41.93%), a genus recognized for its robustness to environmental stresses, such as temperature fluctuations, salinity, and pollution. Its prevalence indicates a microbial adaptation to variable riverine environments or contamination [60,61]. *Bacillus* (10.09%) and *Acinetobacter* (6.92%) reappear as predominant genera, underscoring their ecological adaptability and potential functions in organic matter breakdown and pollution degradation [62,63]. On the other hand, the water samples from Kaptai Lake are mostly composed of *Acinetobacter* (71.24%), suggesting a considerable human impact, as *Acinetobacter* is often found in wastewater and contaminated aquatic ecosystems [63]. Its prevalence indicates probable eutrophication, pollution, or other types of environmental stress within the lake ecology [64,65]. The presence of *Novosphingobium* (5.37%), further substantiates the probability of pollutant exposure in the water column due to its ability to degrade aromatic chemicals and complex xenobiotics [64,66,67]. The unique microbial compositions identified in gut and water samples from both the Halda River and Kaptai Lake indicate that the analyses of fish gut bacteriomes are devoid of waterborne microbial cross-contamination. The gut samples are primarily composed of host-associated lactic acid bacteria (e.g., *Lactiplantibacillus, Pediococcus, Enterococcus*) [43], while the water samples exhibit markedly distinct microbial profiles, featuring environmental and pollutant-tolerant taxa such as *Acinetobacter*, *Exiguobacterium*, and *Novosphingobium* [60,64,66]. This distinct variance in community structure verifies that the gut-associated microbiota accurately represents genuine endogenous microbial communities, rather than artifacts from adjacent aquatic habitats [68].

The comparative functional analysis of bacterial communities in fish from the Halda River and Kaptai Lake demonstrate unique ecological strategies and adaptations that influence fish health and environmental interactions. The Halda River fish possess bacteriome with varied functional capabilities, notably engaged in dehalogenation, sulfur cycling, and the decomposition of organic materials [69,70]. The existence of ammonia and nitrite-processing bacteria, in conjunction with streptomycin-producing and nitrogen-fixing bacteria, signifies a comprehensive microbial ecology that facilitates detoxification, nutrient cycling, and perhaps immunological defense [71,72]. Conversely, the bacteriome of Kaptai Lake fish is functionally imbalanced, exhibiting a predominant presence of ammonia oxidizers (36.48%) and dehalogenating bacteria (34.73%). This suggests robust nitrogen processing capacity and potential for pollutant degradation; however, the reduced diversity in other ecological functions—such as sulfur cycling and comprehensive organic matter decomposition—indicates a more specialized microbiome, potentially as a result of aquaculture-related nutrient influx or environmental stressors [73,74].

The comparative microbial analysis of fish from the Halda River and Kaptai Lake revealed significant disparities in taxonomic composition and functional potential, which are influenced by their distinct aquatic habitats and ecological conditions. In summary, the fish from Kaptai Lake have a microbiome dominated by probiotics that benefited the host. In contrast, the fish from the Halda River displayed a functionally diversified and ecologically robust microbial community. The Halda fish microbiome may offer extensive physiological and environmental benefits, underscoring its potential significance for fish health and ecological balance in more variable or stressed riverine ecosystems.

Conclusion

This work emphasizes the unique bacteriome profiles in fish and water samples from two ecologically varied freshwater systems in Bangladesh—Halda River and Kaptai Lake—utilizing 16S rRNA amplicon sequencing. Metrics of richness and evenness, in conjunction with ordination analysis (PCoA, NMDS), validated distinct microbial compositions influenced by environmental and host-related variables. Fish from the Halda River had a varied and functionally adaptable microbiome, mostly characterized by Planctomycetota (specifically Pirellulaceae_uncultured), including taxa associated with nitrogen cycle, sulfur metabolism, and pollution degradation. Conversely, the fish from Kaptai Lake have a microbiome abundant in probiotics, mostly consisting of lactic acid bacteria, suggesting a more host-oriented and stable gut ecology. The Halda fish microbiome exhibited extensive ecological functions, including nitrogen and sulfur cycling, detoxification, and the synthesis of antimicrobial compounds. Kaptai fish microbiomes exhibited a bias towards ammonia oxidation and dehalogenation, indicating a more specialized functional niche. Overall, Kaptai Lake fish have a health-enhancing probiotic profile, but Halda River fish feature a functionally diversified microbiome, which may provide superior resistance to environmental fluctuations. These results highlight habitat-specific microbial adaptations that have ramifications for fish health, environmental monitoring, and sustainable aquaculture management.

Declarations

Acknowledgments

We are thankful to the Research and Extension, and Department of Genomics and Bioinformatics, Chattogram Veterinary and Animal Sciences University.

Ethical declarations

The Ethics Approval Committee (EAC) of Chattogram Veterinary and Animal Sciences University (CVASU) reviewed and authorized the study protocol (Approval no. CVASU/Dir (R&E) EC/2025/881/15.

Author contributions

M. S. U., K. C. and M. H. U. M. Conceptualized and designed the study outline, performed all the analyses, prepared the figures, wrote and revised the manuscript; **M. R. N., S. C., and I. H.** performed the analyses, wrote the manuscript; **M. H. U. M. and ASM. L. A.** Supervised the study and revised the manuscript; All authors have agreed with the manuscript and provided their consent for publication.

Data availability

Sequence data that support the findings of this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) with the primary accession code PRJNA1270017.

Competing Interests Statement

The authors declare no competing interests

Funding Declaration

This research is funded by the Research and Extension (Grant ID: 830-75) Division of Chattogram Veterinary and Animal Sciences University, Chattogram.

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Figures

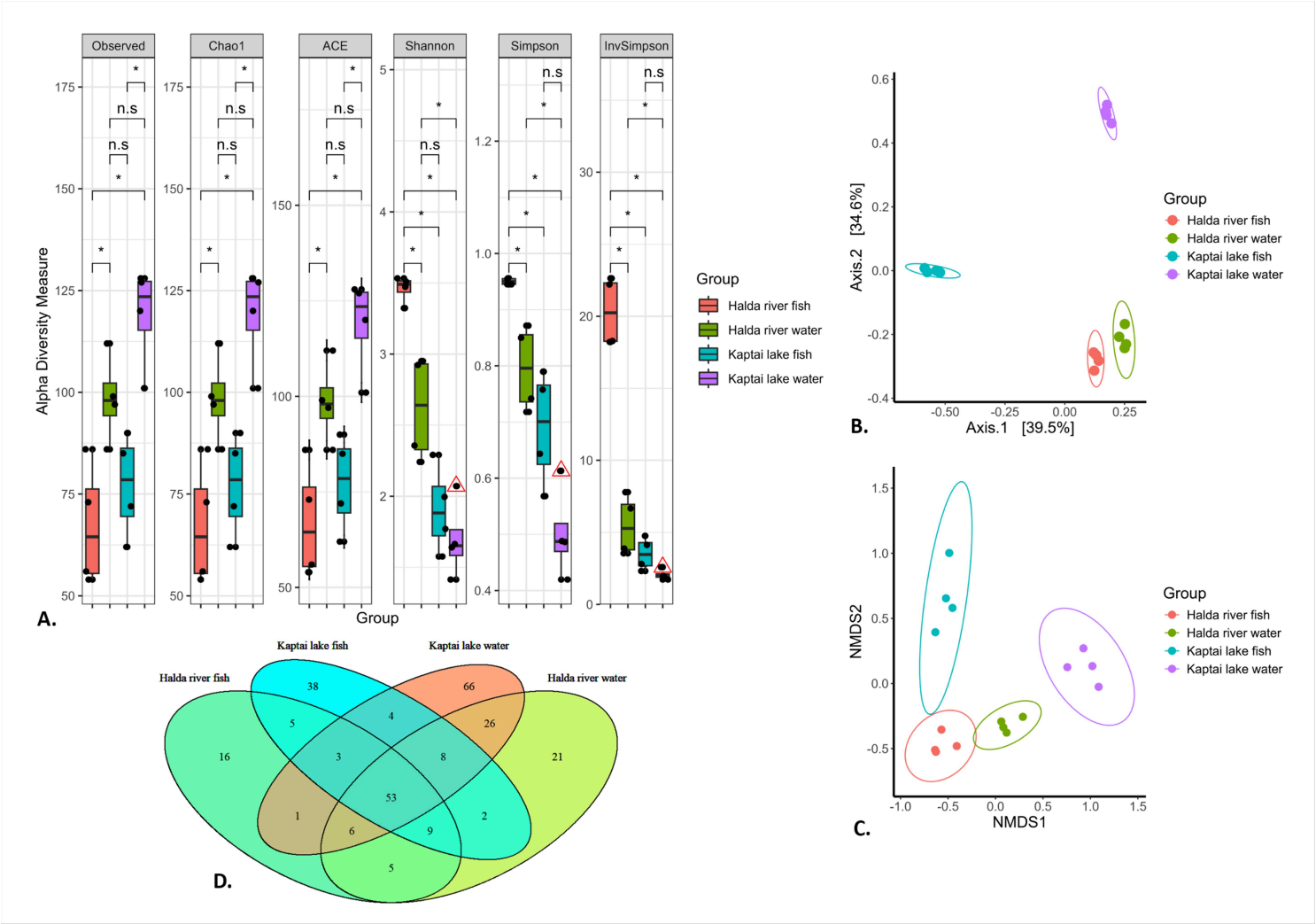


Figure 1

Bacteriome diversity. (A) Alpha variation measure within the subjects. We examined the diversity of bacteria in the Halda River fish, Halda River water, Kaptai Lake fish, and Kaptai Lake water samples using the Observed, Chao1, ACE, Shannon, Simpson, and InvSimpson measures. The data are presented in the form of boxplots, and Kruskal-Wallis rank sum tests are used to compare them**. (B & C)** An evaluation of a variety (beta) values for various types of samples. The Bray-Curtis dissimilarity distance method was used to determine bacterial beta diversity. The results were shown using principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) plots. The samples are color-coded by category and linked to the corresponding ellipses. A PERMANOVA test with a simplified model and pairwise comparisons on a distance matrix shows that the bacterial communities of the different sample groups are significantly different (p-value = 0.001, R^2 = 1.0, PERMANOVA test). **(D)** The observed number of operational taxonomic units (OTUs), including 16 phyla, 27 classes, 75 orders, 109 families, and 263 genera of bacteria.

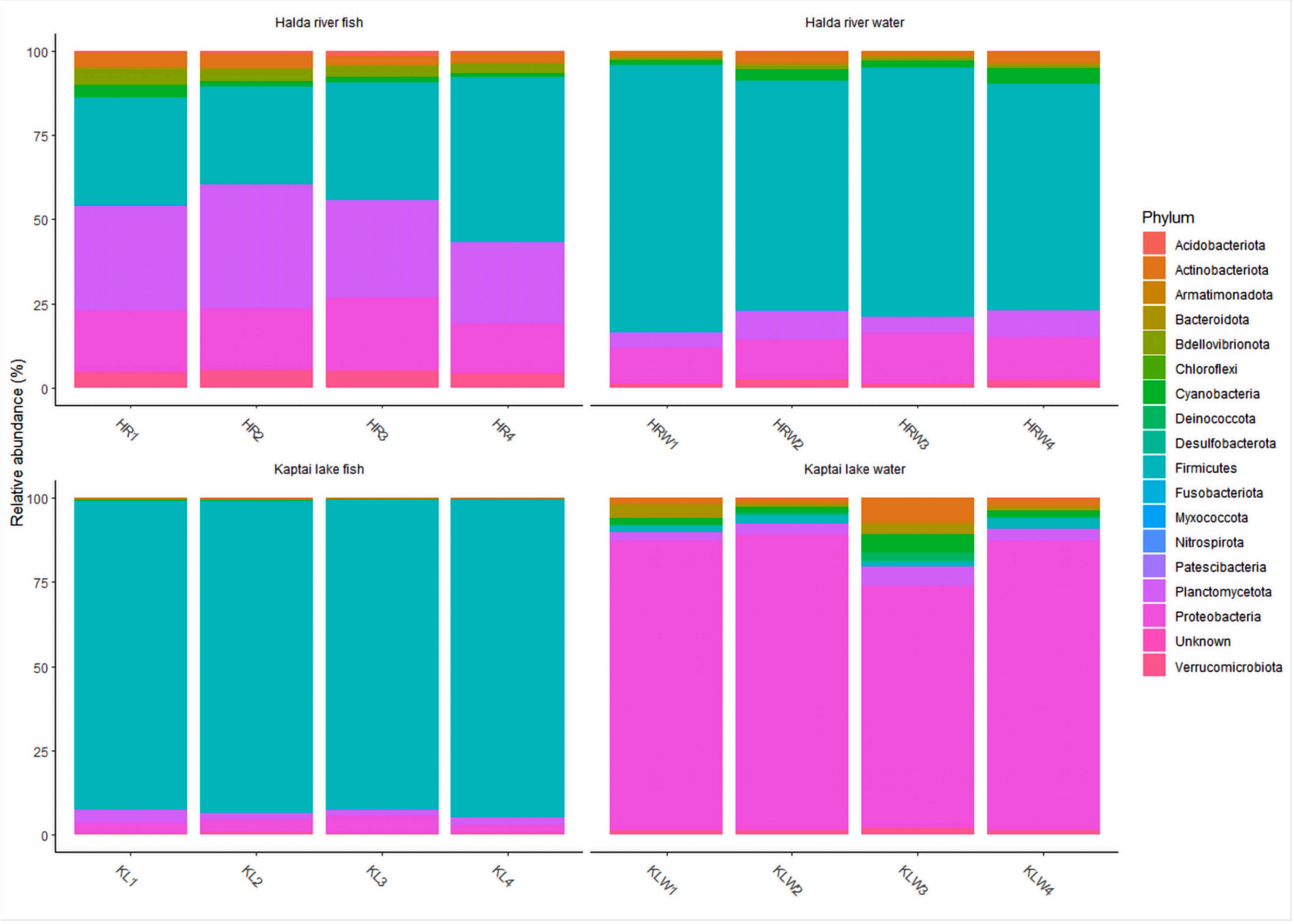


Figure 2

The relative abundance of bacteriome in fish and water samples at phylum-level. Each stacked bar plot illustrates the abundance of bacterial phyla within each sample in the respective categories.

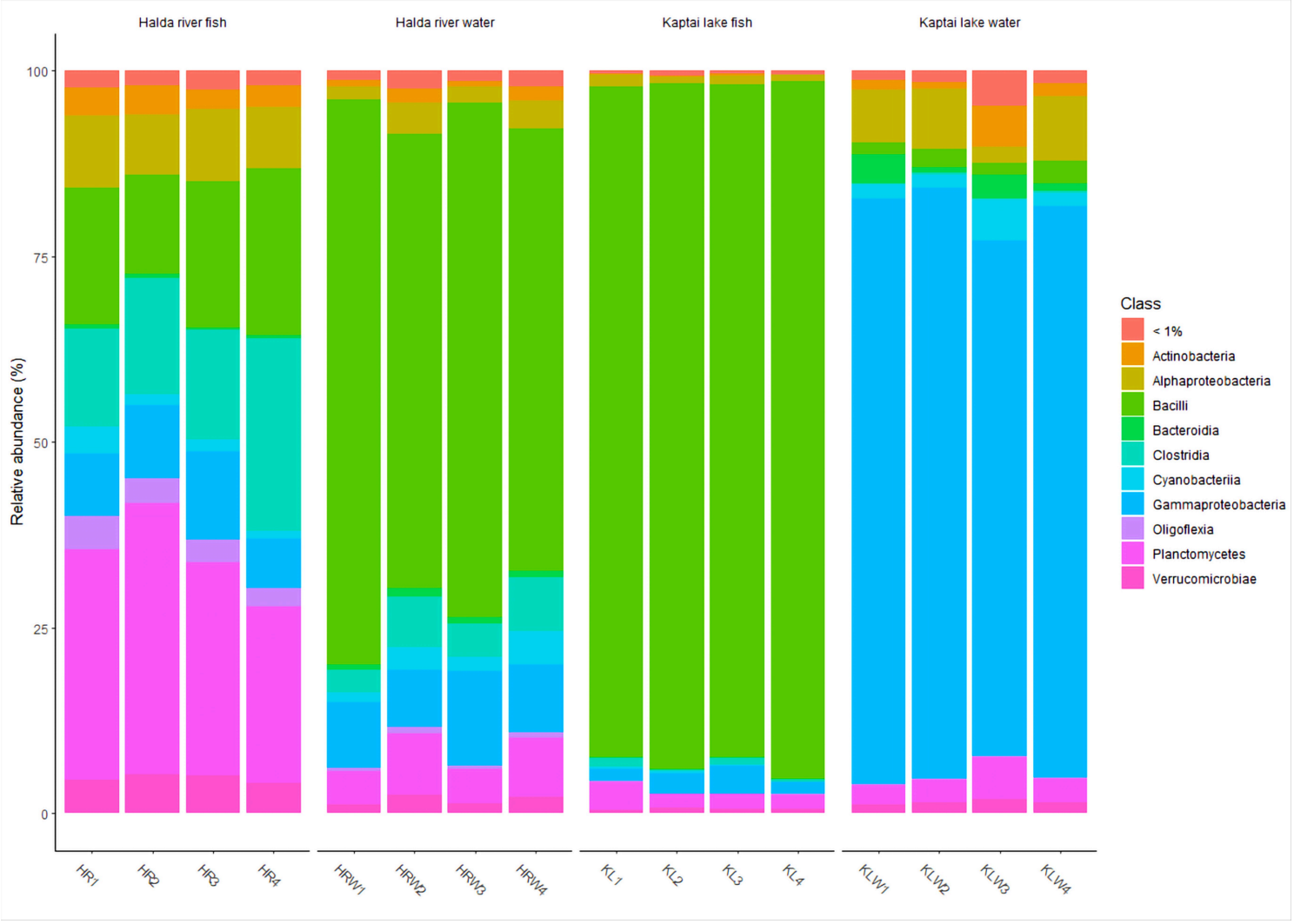


Figure 3

The relative abundance of bacteriome in fish and water samples at class-level. Each stacked bar plot illustrates the abundance of bacterial classes within each sample in the respective categories.

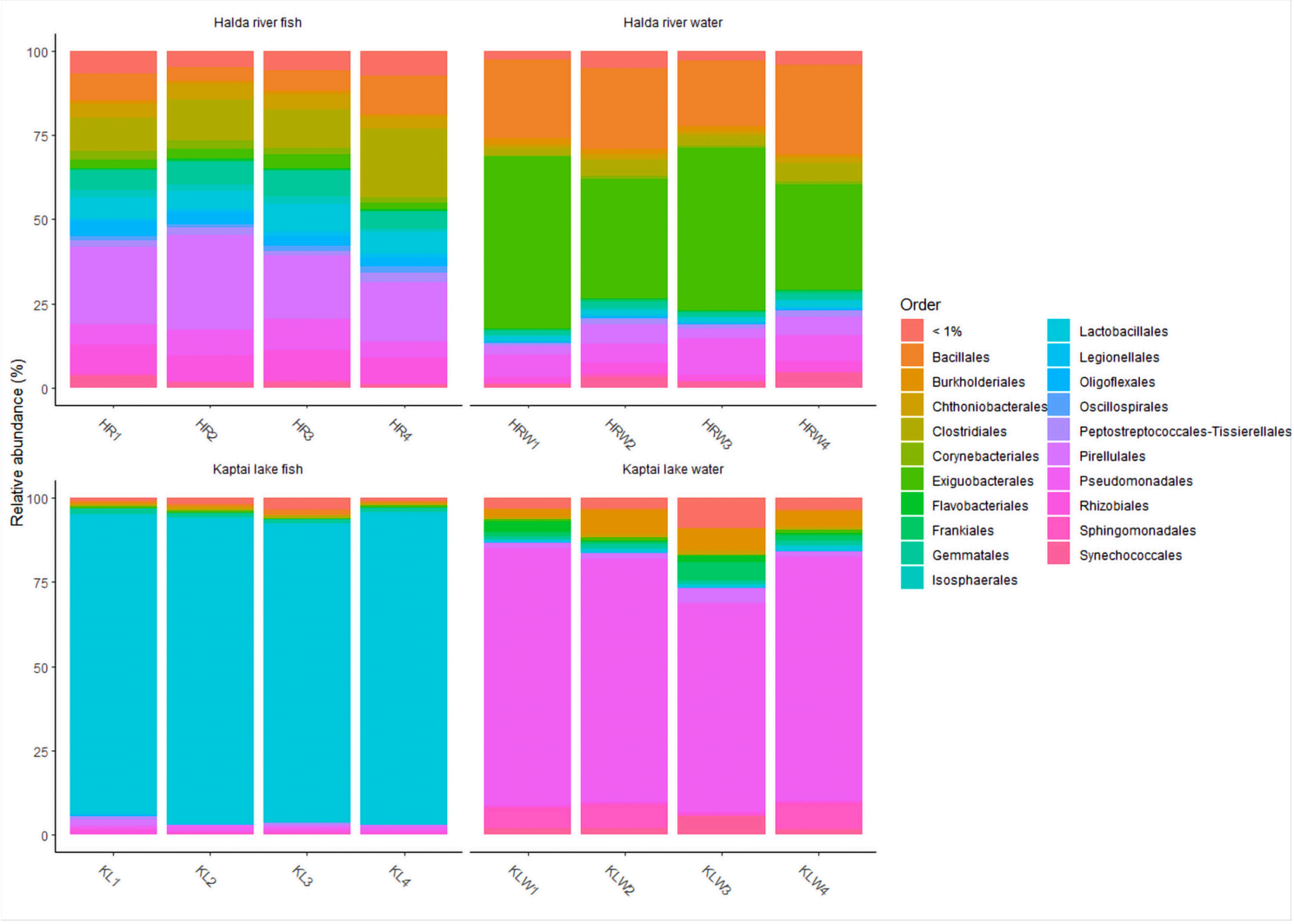


Figure 4

The relative abundance of bacteriome in fish and water samples at order-level. Each stacked bar plot illustrates the abundance of bacterial orders within each sample in the respective categories.

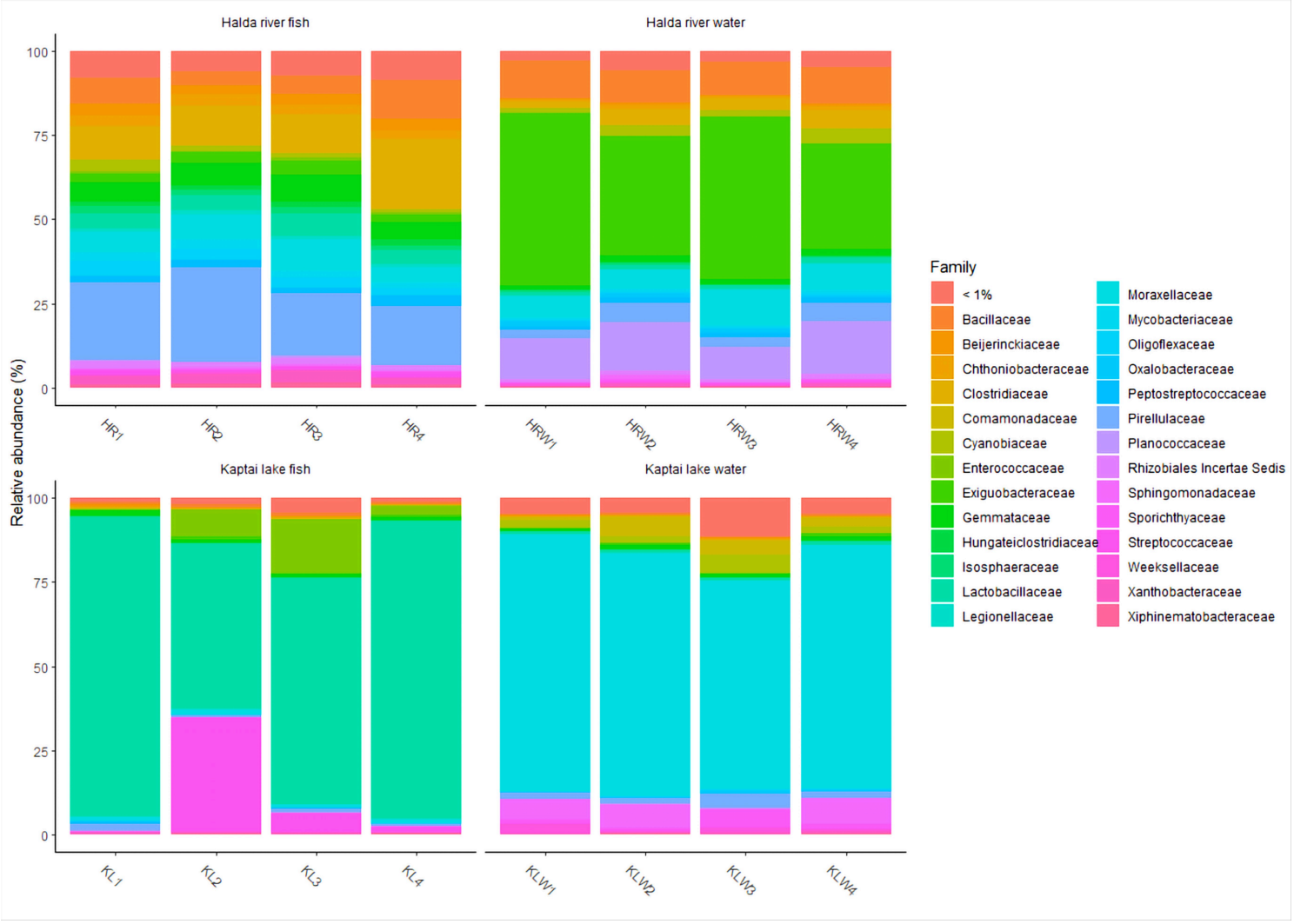


Figure 5

The relative abundance of bacteriome in fish and water samples at family-level. Each stacked bar plot illustrates the abundance of bacterial families within each sample in the respective categories.

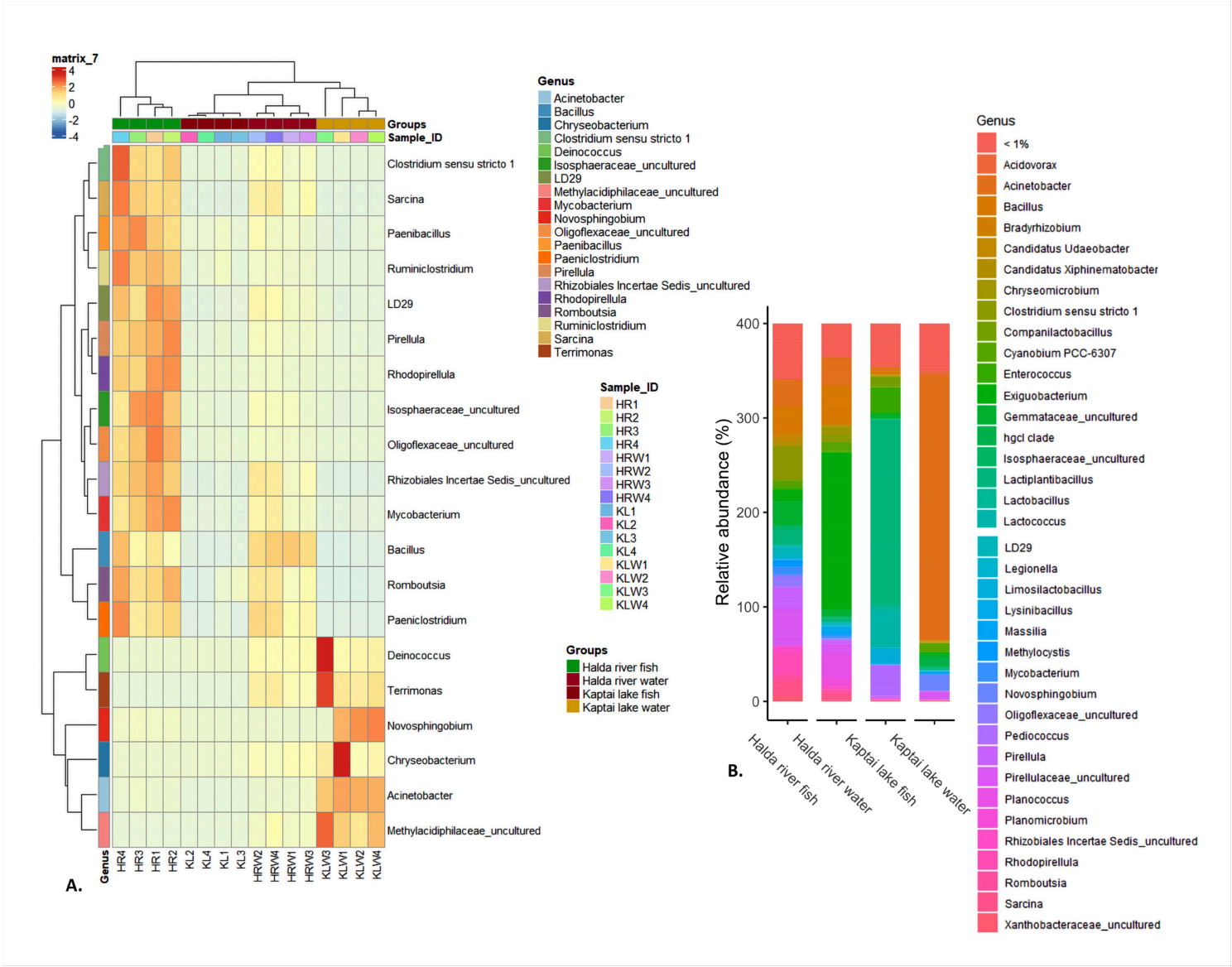


Figure 6

Taxonomic profile of bacteriome at the genus level. (A) Heatmap illustrating the average relative abundances and hierarchical grouping of the bacterial genera (n = 20) in the research samples. The color bar (matrix_7 score) at the top indicates the relative abundance of bacterial species in the respective samples. The color codes denote the existence and completeness of each bacterial genus, represented by a number ranging from -4 (lowest abundance) to 4 (maximum abundance). The red color signifies the more prevalent patterns, whilst blue cells represent the less numerous taxa in that specific sample. (B) The stacked bar plot illustrates the abundance of bacterial families within each sample in the respective categories.

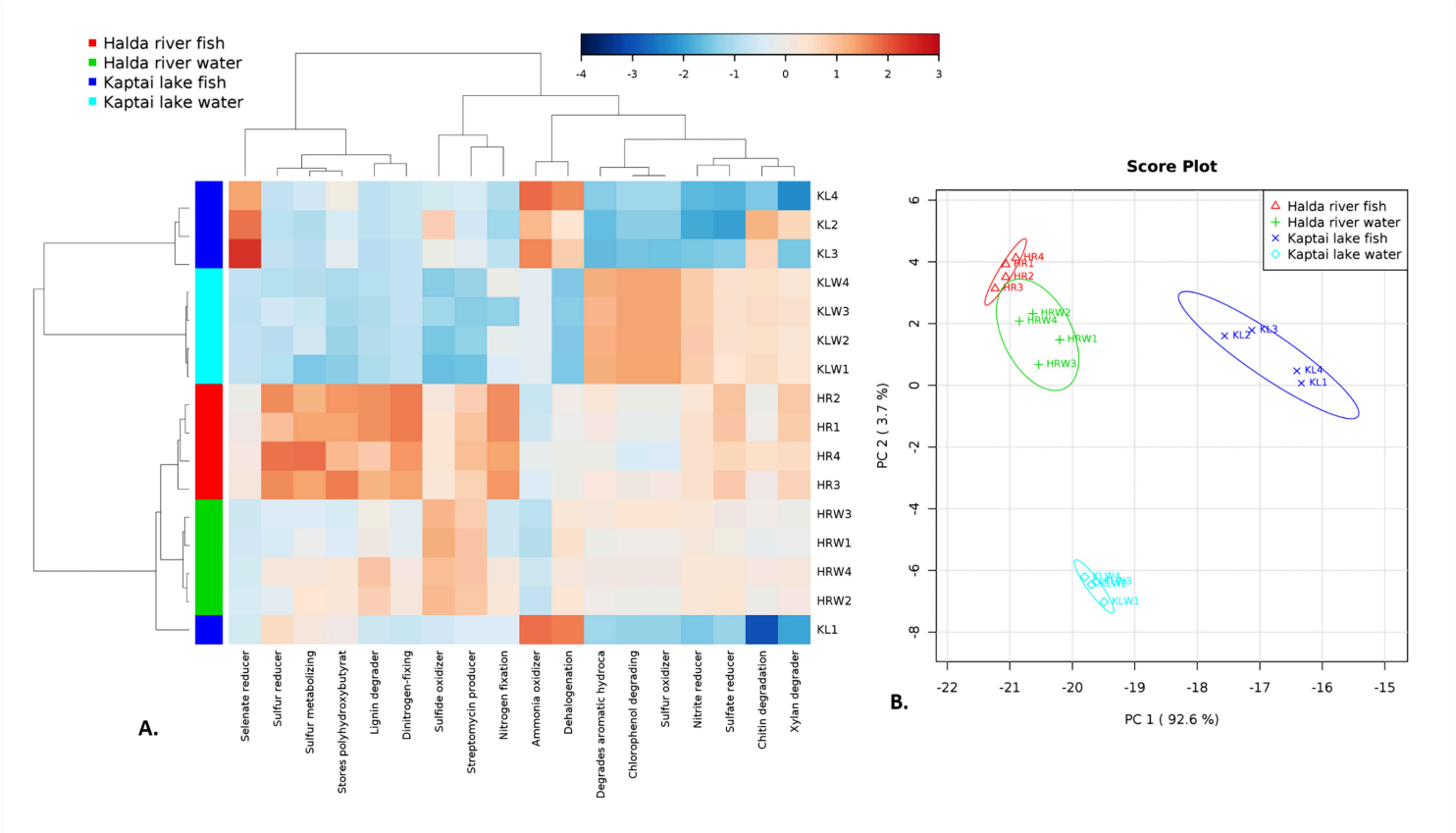


Figure 7

Functional profile of bacteriome among sample groups. (A) Heatmap illustrating the average relative abundances and hierarchical grouping of the functional profile (n = 18) in the research samples. The red color signifies the more prevalent patterns, whilst blue cells represent the less numerous taxa in that specific sample. (B) The PCA score plot illustrates the functional profile of bacteriome within each sample in the respective categories.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata1.xlsx
- Supplementarytable1.docx
- Supplementarydata2.xlsx
- Supplementarytable2.docx
- Supplementaryfigure1.tif
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