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# Comparative Metagenomic Insights into Gut Microbiota of Farmed and Wild *Channa striata* (Bloch, 1793)

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## ABSTRACT

**Background:** Gut microbiota plays a pivotal role in regulating fish nutrition, metabolism, immune regulation and biological homeostasis. However, to date, environmentally influenced gut microbial communities of *Channa striata* (Striped murrel) from wild and farmed habitats remain poorly understood.

**Methods:** Gut microbial communities of wild (WG) and farm (FG) *C. striata* were investigated using 16S rRNA gene-based metagenomic sequencing. A total of six gut samples were subjected using paired-end sequencing on the Illumina MiSeq platform. The bioinformatic analyses was performed using mothur pipeline and taxonomic assignment base on SILVA database. Microbial diversity was assessed using alpha and beta analyses, whereas functional pathways were predicted using MEGAN software based on KEGG pathway annotations.

**Result:** A total of 651,352 raw reads were generated, of which 174,526 high-quality sequences were retained and 2,357 operational taxonomic units (OTUs) identified. At the phylum level, Proteobacteria dominated in both groups; however, marked differences in the microbial composition were observed at the genus level between habitats. The α-diversity analysis indicated that FG had significantly higher species richness (Sobs, $p$ = 0.022). The β-diversity analysis using Bray-Curtis dissimilarity and NMDS ordination showed a clear and statistically significant difference between the two groups (PERMANOVA, $p$-value <0.001). Predicted functional profiling indicated comparatively higher representation of metabolism-related pathways in wild *C. striata*. This study highlights notable habitat-driven variation in the gut microbiota of *C. striata* and provides insights into microbial diversity as well as functional potential and may aid in the identification and application of beneficial microbes for improving productivity in murrel aquaculture.

**Key words:** *Channa striata*, Gut microbiome, Metagenomics, Microbial diversity, Wild vs farmed fish.

## INTRODUCTION

*Channa striata*, the striped murrel, is an economically important freshwater fish species in Southeast Asia and inhabits both lotic and lentic ecosystems (Kumar *et al*., 2021; 2022). It is in high demand as a food fish due to its good taste, high nutrient content, fewer muscular spines and notable pharmacological properties (Sahu *et al*., 2012). Consumers meet their demand for *C. striata* either from culture systems or from wild-caught sources. The species, being an air-breathing fish, exhibits remarkable tolerance to hypoxic conditions and is considered one of the climate-resilient candidates for sustainable aquaculture.

Healthy fish promote greater consumer preference and the gut microbiota also determines the well-being of the fish (Butt and Volkoff, 2019). The gut microbiome benefits the animal host through nutrient assimilation and absorption, digestion, metabolism, pathogen exclusion and immune response, thereby aiding various essential physiological and biological functions (Egerton *et al*., 2018). Factors such as host genetics, ecology, environmental conditions and feeding behaviours are potential drivers of changes in gut microbiota (Wang *et al*., 2018). In fact, the presence or absence of certain groups of microbiota can serve as health indicators of a host animal (Evariste *et al*., 2019). In recent years, the exploration and isolation of beneficial bacteria from the fish gut have been steadily reported, paving the way for research on the development of probiotic-based products (Hai, 2015). The application of probiotics can improve gut health in fish (Ringø *et al*., 2018). In this context, gut microbiome study is of immense importance and recent advances in next-generation sequencing (NGS), particularly 16S rRNA gene sequencing

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through platforms such as Illumina, PacBio, Oxford Nanopore and Ion Torrent, have created a new dimension in gut metagenomics research (Johnson *et al*., 2019).

Recent studies on *C. striata* have increasingly focused on molecular, microbial and omics-based approaches to better understand the physiology, health, nutrition and reproductive biology. Emerging research has characterized gut microbial diversity and its association with fatty acid profiles in *C. striata*, highlighting the importance of host-microbiome interactions in fish health and metabolism (Sharma *et al.,* 2025). Using a metagenomic approach, Rasal *et al.* (2023) investigated host-specific gut and skin mucus microbiota in *C. striata*, demonstrating the utility of next-generation sequencing for profiling microbial communities associated with aquaculture-relevant tissues. Similarly, Behera *et al.* (2025) explored microRNA-mediated regulation of gonadal development and reproduction across different reproductive stages, reporting stage- and sex-specific expression of several key miRNAs associated with gametogenesis, steroidogenesis, sperm maturation and reproductive recovery. These findings collectively advance current understanding of the molecular and microbial mechanisms underlying health and reproduction in this commercially important aquaculture species. Furthermore, Iyyappan *et al.* (2026) employed an integrated metagenomic and transcriptomic approach to evaluate the influence of plant oil-based diets on juvenile *C. striata*, further emphasizing the growing application of multi-omics tools in nutritional and functional studies of this species. Despite this growing research on comparative metagenomic investigations examining gut microbial communities of wild (WG) and farmed (FG) *C. striata* across different habitat conditions remain largely unexplored. Therefore, considering the ecological and biological importance of *C. striata* and its gut microbiota, the present study was aimed to (i) compare gut microbial diversity between WG and FG *C. striata* group, (ii) characterize the taxonomic composition at phylum and genus levels and (iii) examine predicted functional pathways differentiating the two groups.

## MATERIALS AND METHODS

### Study area and sample collection

Live and healthy striped murrel, yearling size (mean body weight: 190.0±15.0 g; total length: 26.0±3.0 cm), were collected from both wild and farm environments during April-May 2022. The wild population was collected from natural freshwater bodies in Jenapur, Puri District, Odisha, India (19°44′45″N; 85°34′28″E), whereas the farm population was collected from murrel culture ponds located at the Air-Breathing Fish Unit, Aquaculture Production and Environment Division, ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India (20°11′36″N; 85°51′16″E), as shown in Fig 1. Fish were subsequently transported live into the laboratory and processed within 2-3 hours of collection. Experimental procedures were carried out following the guidelines approved by the institute animal ethical committee (IAEC) of ICAR-Central Institute of Freshwater Aquaculture. (F.No.ICAR-CIFA/Eth.Comm./2025-26/11).

### Sample processing for metagenomic analysis

For the metagenomics study, the live fish samples (*n* = 6, collected from wild and farm habitats) were anaesthetized using Tricaine Methanesulfonate (MS-222; 100 mg/L) prior to dissection. Similar conservative sampling designs have been adopted in comparable 16S rRNA-based fish gut microbiota studies (Iwatsuki *et al.,* 2021; Rasal *et al.,* 2023).




(a) Farmed fish (FG) sampling site at the murrel culture pond, air-breathing fish unit, APED, ICAR-CIFA, Bhubaneswar (20°11′36″N, 85°51′16″E); (b) Wild fish (WG) sampling site at Jenapur, Puri (19°44′45″N, 85°34′28″E). The map was generated using google earth and illustrates the spatial distribution of sampling locations.

**Fig 1:** Geographical locations of sampling sites in Odisha, India.

The euthanised fish were dissected using sterile laboratory materials under aseptic conditions and the gut was removed using sterilized dissection tools. The gut was made digesta-free by gently rinsing with sterile normal saline solution (0.85% NaCl) to obtain gut tissue-associated microbiota. The cleaned gut samples were immediately transferred into sterile cryovials containing 100% molecular-grade ethanol (Merck, India) to preserve microbial DNA integrity. The wild group samples were marked as WG1, WG2 and WG3 and the farmed group samples were marked as FG1, FG2 and FG3, respectively. The vials were stored at -80°C in a deep freezer. The preserved samples were sent to BioXplore Lab Facilities, Chennai, India, under cold-chain conditions for downstream metagenomics analysis.

**DNA extraction and 16S rRNA gene sequencing**

Total microbial DNA was extracted using the phenol-chloroform method following the protocol described by Sambrook and Russell (2001). This method is mainly suitable for environmental and gut-associated samples due to its effectiveness in recovering high-quality total microbial DNA across diverse microbial communities. Negative controls were run through a similar process alongside the samples in order to detect contamination. The gut samples were homogenised and lysed using lysis buffer containing detergent and proteinase K. It was followed by extraction using phenol: chloroform:isoamyl alcohol. The extracted microbial DNA was precipitated in cold ethanol, washed and resuspended in nuclease-free water (NFW). The DNA concentration and quality were checked using spectrophotometry and gel electrophoresis.

The V3-V4 region of the microbial 16S rRNA was amplified using region-specific primers 341F: 5′-CCTAC GGGNGGCWGCAG-3′ and 785R: 5′-GACTAC HVGGGTAT CTAATCC-3′. The PCR amplification was carried out in a final reaction volume of 25 µL, containing template DNA (20 ng), 2× PCR master mix (12.5 µL), 0.5 µM of each primer and NFW. The amplification was performed in a thermal cycler under the following conditions: initial denaturation at 95°C for 3 minutes; 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 5 minutes. No-template controls were included during the PCR amplification to ensure the absence of reagent contamination. No amplification was observed in the negative control, ensuring the reliability of the procedure.

PCR amplicons were verified by agarose gel electrophoresis and visualized under UV illumination. The confirmed amplicons were subsequently subjected to library preparation and paired-end sequencing on the Illumina MiSeq platform employing paired-end (PE) 2 × 250 bp chemistry. The raw paired-end data were subjected to quality-control filtering to remove low-quality sequences and chimeras. The processed datasets were subsequently utilised for the comparative analysis of gut microbial communities between wild and cultured *C. striata*. The raw FASTQ sequences were submitted to the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1437389.

**Bioinformatic and statistical analysis of sequencing data**

Raw paired-end reads generated by the Illumina MiSeq platform were demultiplexed according to the MiSeq pipeline guidelines, yielding 651,352 sequences. The resultant sequences were subjected to removal of low-quality and ambiguous reads (Q > 25), using Mothur (v1.48.0) following MiSeq Standard Operating Procedure (SOP) and were aligned against the SILVA database (v138.2). Non-bacterial, poorly aligned and chimeric sequences(identified using UCHIME) were removed before downstream analysis. Taxonomic classification was performed using the RDP classifier (Release 19) with an 80% bootstrap confidence threshold. Sequences were clustered into operational taxonomic units(OTUs) at 97% similarity using VSEARCH/ Low-abundance OTUs (<10 reads) were removed to minimize sequencing noise. Datasets were rarefied to the minimum library size (*i.e*., the depth of the shallowest sample) before $\alpha$ and $\beta$diversity analyses. Relative abundance normalisation was additionally applied for taxonomic composition comparisons to account for the large disparity in total reads between the FG and WG groups. Mothur software was used to calculate the alpha diversity indices (Sobs, Shannon, Simpson) and beta diversity metrics (Bray-Curtis dissimilarity and visualised using non-metric multidimensional scaling, NMDS) of the gut microbiota between the wild and farmed fish samples (Schloss *et al.,* 2014).

The Alpha diversity between groups was assessed using the Kruskal-Wallis test considering the small sample size and non-normal distribution of microbiome data. Pairwise comparisons were performed using Benjamini-Hochberg false discovery rate (FDR) correction to adjust for multiple testing. Statistical interpretation was strengthened by the calculation of effect sizes and corresponding confidence intervals. Beta diversity was evaluated using PERMANOVA (Permutational Multivariate Analysis of Variance) based on Bray-Curtis dissimilarity to judge alterations in community composition between groups. The core microbiome analysis was done using microbiome R package with defined prevalence (20%) and abundance (0.01%) thresholds. Functional prediction of microbial pathways was conducted using MEGAN based on KEGG databases. MEGAN was employed for functional inference as it supports direct integration with the SILVA-based taxonomic assignments used in the pipeline and provides KEGG-based pathway annotation without the need for reference genome databases required by PICRUSt2 and Tax4Fun2. Statistical analyses and visualisations were performed in R using *vegan, phyloseq, microbiome* and *ggplot2* packages, along with Microbiome Analyst.

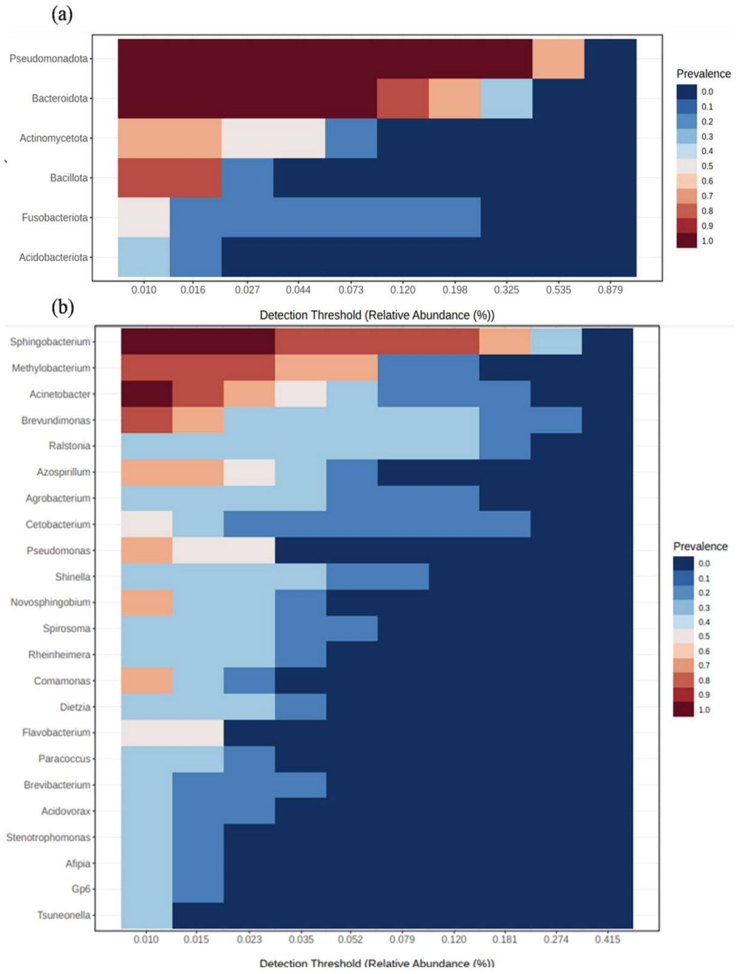
## RESULTS AND DISCUSSION

### Comparison of gut microbiota profiling from farm and wild fish habitat

Gut profiling of the two groups, wild (WG) and farmed (FG) *C. striata*, supported by taxonomic distribution plots and prevalence heatmaps, revealed distinct compositional patterns between the groups. A total of 174,526 high-quality sequences were retained, out of which FG showed higher read abundance (157,479 reads; 90.23%) than WG (17,047 reads; 9.77%). A total of 2,357 OTUs were obtained; FG contained 1,859 OTUs whereas WG contained 513 OTUs.

A total of six phyla comprising 22 genera were identified from the analysis (Fig 2a, 2b). At the phylum level, the gut microbiota of farmed *C. striata* (FG) was dominated by Pseudomonadota (Proteobacteria), followed by Bacteroidota, Spirochaetota, Actinomycetota and Fusobacteriota (Fig 3a). In contrast, wild fish (WG) exhibited dominance of proteobacteria, followed by bacteroidota, bacillota and actinomycetota. Notably, proteobacteria predominated in both groups, with higher relative abundance in WG (74.38%) compared to FG (47.89%). Similar dominance of Proteobacteria has been reported in *C. striata* under dietary modulation, such as soya oil-enriched feeding, highlighting the strong influence of diet on gut microbial composition (Iyyappan *et al*., 2026).

Consistent with previous studies, Proteobacteria, Bacillota (Firmicutes), Fusobacteriota, and Bacteroidota constitute a conserved core gut microbiota across diverse fish species, although their relative abundances vary



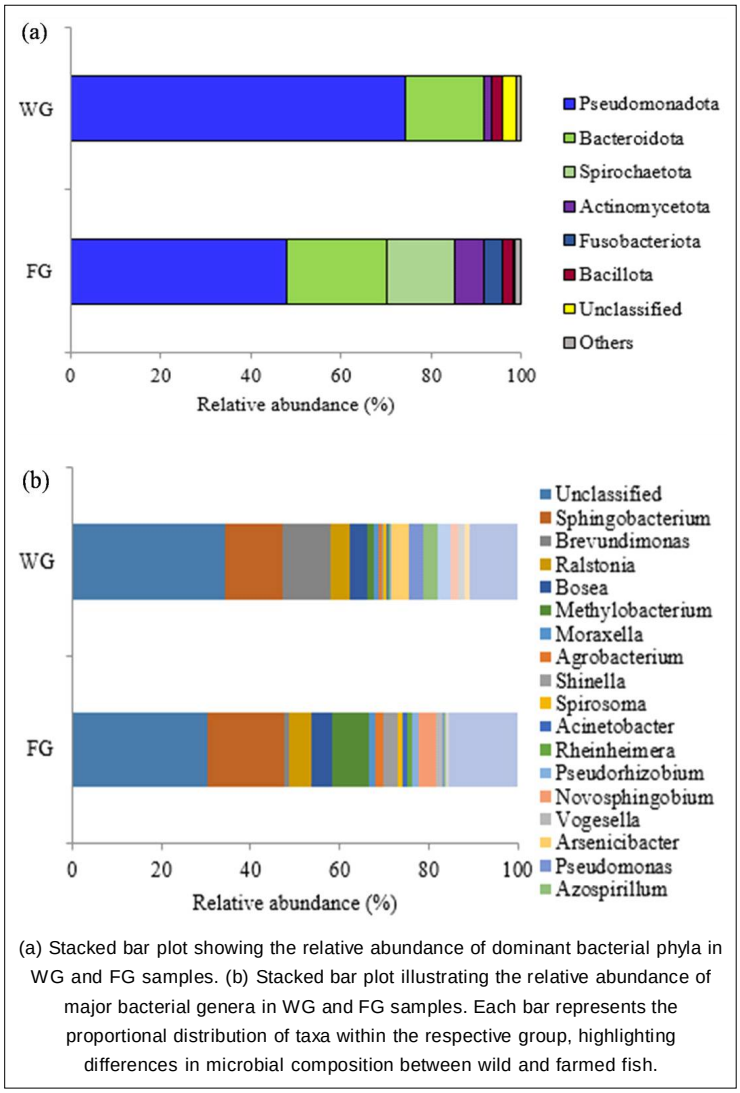

(a) Heatmap representing the core bacterial phyla across all samples, showing prevalence patterns at varying detection thresholds (Relative abundance, %). (b) Heatmap illustrating the core bacterial genera shared among all samples, indicating their prevalence distribution across increasing detection thresholds. Color gradients represent the prevalence of taxa (Proportion of samples in which a taxon is detected), highlighting consistently occurring microbial groups within the gut microbiota.

**Fig 2:** Core microbiota analysis based on prevalence and detection thresholds.

between wild and farmed populations owing to differences in diet, habitat, and culture conditions (Mugetti *et al.*, 2023; Liu *et al.*, 2023; Huang *et al.*, 2024; Kanika *et al.*, 2025). Along with the persistent occurrence of these taxa, together with Actinomycetota, aids in a stable fish gut core microbiome (Egerton *et al.*, 2018). From a functional perspective, Proteobacteria enrichment in aquaculture systems is linked to stress-related opportunistic traits such as virulence and antibiotic resistance potential (Liu *et al.*, 2023; Huang *et al.*, 2024). In contrast, Bacillota and Fusobacteriota are associated with various beneficial host functions, such as enzyme production, protein digestion, short-chain fatty acids production, vitamin B12 and antibacterial and immune-modulatory activities (Ringø *et al.*, 2018; Kuebutornye *et al.*, 2019; Butt and Volkoff, 2019). Furthermore, the proliferation of Bacillus, Sphingomonas and Pseudomonas species in farmed fish indicates their acclimation to rearing conditions and feeding regimes potentially conferring probiotic or ecological fitness advantages (Liu e*t al.*, 2023; Pingle and Khandagle, 2023; Hrabar *et al.*, 2025).

At the genus level, a total of 23 core genera were identified across all the samples (Fig 2b). Dominant genera included *Sphingobacterium*, *Methylobacterium*, *Ralstonia* and *Brevundimonas* (Fig 3b), indicating active microbial turnover and nutrient cycling within the gut ecosystem. The notable abundance of *Sphingobacterium* suggests ecological stability, whereas enrichment of *Methylobacterium*, *Bosea* and *Novosphingobium* in FG reflects microbial exchange between the host and aquaculture environment. In contrast, WG samples exhibited relatively higher representation of environmentally associated taxa, suggesting greater microbial diversity and resilience in natural habitats (Sylvain *et al.,* 2020).

Overall, diet and environmental conditions emerge as key drivers shaping gut microbiota composition. FG typically show enrichment of taxa associated with formulated feeds,

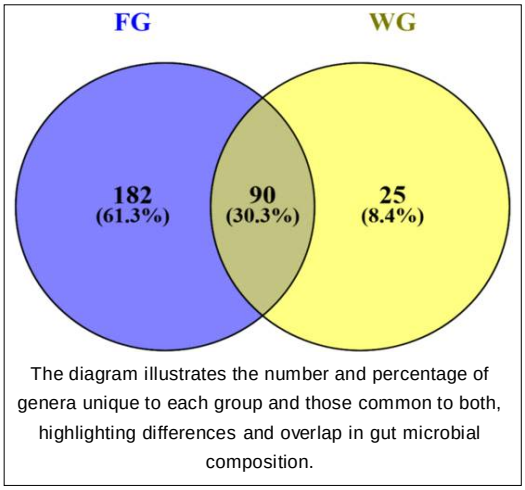



(a) Stacked bar plot showing the relative abundance of dominant bacterial phyla in WG and FG samples. (b) Stacked bar plot illustrating the relative abundance of major bacterial genera in WG and FG samples. Each bar represents the proportional distribution of taxa within the respective group, highlighting differences in microbial composition between wild and farmed fish.

**Fig 3:** Taxonomic composition of gut microbiota in WG and FG *C. striata*.

whereas WG harbor more diverse, environmentally derived microbial communities with broader metabolic potential (Yukgehnaish *et al.,* 2020). Despite these differences, a stable core microbiome persists across both conditions, supporting essential functions such as digestion, nutrient synthesis and immune regulation, while environment-specific taxa reflect continuous microbial exchange with water and diet (Mugetti *et al.,* 2023; Kanika *et al.,* 2025; Hrabar *et al.,* 2025). Furthermore, studies in *C. striata* have shown that dietary interventions and habitat modifications significantly influence growth, health, stress tolerance, reproductive performance and overall physiological status, highlighting the critical role of nutrition and environmental conditions in shaping host-microbe interactions and fish health (Damle *et al*., 2023; Ajidhaslin *et al*., 2025; Dheeran *et al*., 2025).

Venn diagram (Fig 4) analysis identified 90 shared genera; FG (182) exhibited greater unique diversity than WG (25). These results imply that the culture conditions can trigger microbiome dynamics through feed and rearing water in FG, while WG reflects niche specialization and ecological filtering (Risely, 2020). The presence of core microbial taxa across the sample group indicates their important role in host physiology and maintaining gut homeostatis, however the variation in WG samples may be indicative of individual specific microbial heterogeneity influenced by environment and dietary factor. The presence of core taxa across all samples highlights their role in host physiology and maintaining gut homeostasis, however variation among WG samples can be related to individual-specific microbial heterogeneity influenced by environmental and dietary factors (Butt and Volkoff, 2019). Recent advances further confirm that habitat and environmental factors are key drivers of fish gut microbiome structure and function (Kanika *et al.,* 2025).




The diagram illustrates the number and percentage of genera unique to each group and those common to both, highlighting differences and overlap in gut microbial composition.

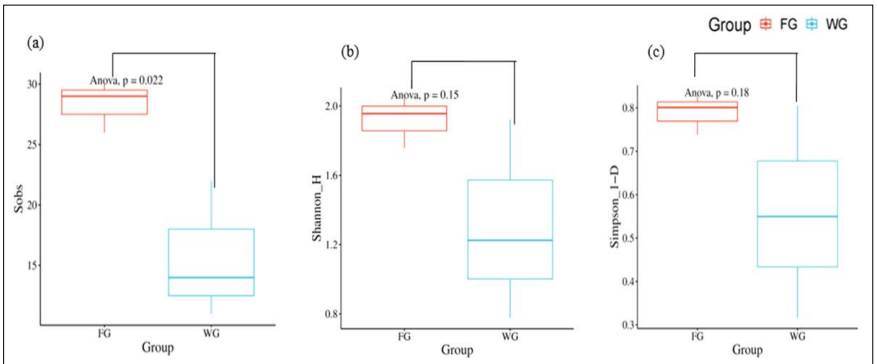
**Fig 4:** Venn diagram showing shared and unique bacterial genera between FG and WG *C. striata*.

### Alpha diversity of the gut microbiota

Alpha diversity analysis showed significantly higher microbial richness (Sobs) in FG than WG ($p$ = 0.022), indicating species richness in farmed fish (Fig 5). Shannon and Simpson indices showed no significant difference between the two groups, indicating diversity evenness in the samples (Fig 5). This suggests that the additional species in FG are largely rare taxa recruited from the aquaculture environment (feed, rearing water), while the core evenness structure remains similar. Rarefaction curves approached saturation in all the samples, hence indicating sufficient sequencing depth for reliable diversity estimation (Fig 6).

### Beta diversity and differential abundance analysis of the gut microbiota

Beta diversity analysis based on bray-curtis dissimilarity and NMDS demonstrated distinct clustering patterns between the FG and WG (Fig 7a, b). The microbial communities of FG and WG formed clearly separated clusters, indicating substantial differences in gut microbial composition between the two groups. PERMANOVA analysis further confirmed that the observed separation was statistically significant (F = 164.1, df = 5; $p$<0.001). The clear separation of FG and WG microbial communities observed in NMDS and Bray-Curtis analyses indicates




Comparison of the Sob index (a), Shannon index (b) and Simpson index (c) of the gut microbiota between the FG and WG groups. $P \leq 0.05$ indicates significant differences.

**Fig 5:** Comparison of diversity indices in the gut microbiota of FG and WG groups.

strong habitat-specific microbial structuring in the gut microbiome. Such clustering patterns suggest that environmental conditions, feeding regimes and aquaculture practices play a major role in shaping microbial community composition in FG, whereas WG microbiota are more strongly influenced by natural habitat variability and ecological interactions. Similar habitat-driven differences in fish gut microbial communities have been reported previously in both wild and cultured fish species (Talwar *et al.,* 2018). The significant PERMANOVA result further indicate that the gut microbiota of FG and WG are compositionally distinct and shaped by their respective environmental conditions.

Differential abundance analysis revealed distinct microbial signatures between the FG and WG. The FG exhibited significant enrichment of several genera, including *Phenylobacterium*, *Dietzia*, *Sporocytophaga*, *Sphingobium*, *Enterobacter*, *Azospirillum*, *Cloacibacterium*, *Gordonia*, *Exiguobacterium*, *Micrococcus*, *Bacillus*, *Ravibacter*, *Fluviicola* and *Pyxidicoccus*. In contrast, WG was characterized by a higher abundance of *Pseudorhizobium* , which exhibited the highest positive fold change among all detected genera (Fig 7c). Furthermore, higher species abundance and tighter microbial clustering were observed in FG compared to WG.

The differential enrichment of bacterial genera between FG and WG highlights the influence of rearing environment, dietary inputs and habitat conditions on gut microbial assembly. The broader enrichment of genera in the FG suggests that aquaculture conditions promote microbial homogenization and increased microbial exchange through formulated feed, shared water systems and controlled husbandry practices. Several enriched genera in FG, such as *Bacillus*, *Exiguobacterium* and *Sphingobium*, are commonly associated with nutrient metabolism, environmental resilience and aquaculture-associated microbial communities. Similar results have been stated in farmed fish species where diet and culture conditions strongly shaped gut microbial composition (Talwar *et al.,* 2018).

In contrast, the strong enrichment of *Pseudorhizobium* in WG suggests adaptation to natural environmental conditions. WG used in the present study were collected from habitats influenced by seasonal brackish water influx, resulting in fluctuating salinity regimes. Environmental salinity, together with diverse natural feeding habits, can significantly influence fish gut microbiota composition and select for habitat-specific bacterial taxa. Comparable salinity-driven microbial shifts have been documented in Atlantic salmon and grass carp (Liu *et al.,* 2023). Therefore, the microbiota of WG likely reflects ecological adaptation to variable environmental conditions, whereas the FG microbiota primarily reflects culture-associated selective pressures.

**Predicted functional profiles of farmed and wild fish gut microbiota**

Comparative KEGG level 3 functional profiling of the gut microbiota revealed distinct functional differences between the FG and WG of *C. striata* (Fig 8). The predicted functional pathways were primarily associated with metabolism, environmental information processing, genetic information processing and cellular processes. A clear functional differentiation between the two groups was observed, with WG exhibiting comparatively higher enrichment across multiple pathways than FG.

Among the predicted metabolic functions, pathways related to general metabolism (34.0), energy metabolism (16.0), sulfur metabolism (16.0), amino acid metabolism (18.0) and valine, leucine and isoleucine degradation (18.0) were more abundant in WG. Additionally, pathways associated with environmental information processing and host-microbe interactions, including signaling molecules and interaction, neuroactive ligand-receptor interaction, cellular processes, cell-motility, regulation of actin cytoskeleton, organismal systems, immune system and complement and coagulation cascades, were predominantly enriched in WG. In contrast, FG displayed comparatively lower functional diversity, with functional representation mainly restricted to metabolism-related pathways, particularly energy metabolism and sulfur metabolism. Variability among WG samples was also comparatively higher, indicating greater heterogeneity in microbial functional composition.

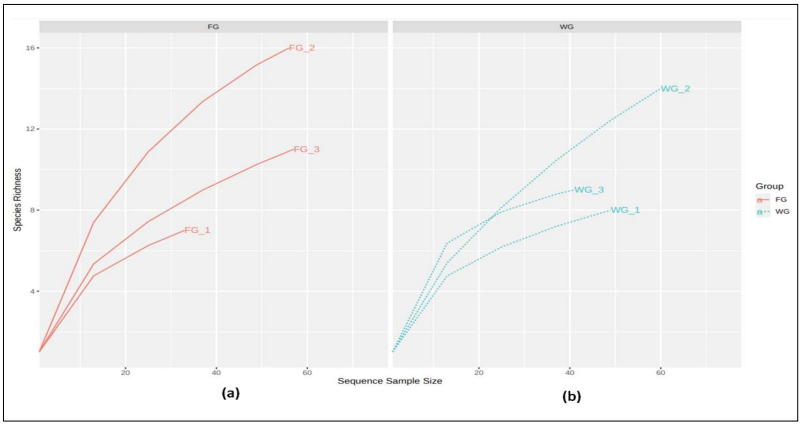



**Fig 6:** Rarefaction curve sequences showing the microbial community complexities in the guts of FG (a) and WG *C. striata* (b).

KEGG-based functional profiling demonstrated that the gut microbiota of WG *C. striata* possess greater functional diversity and ecological adaptability. The enrichment of pathways related to amino acid metabolism and branched-chain amino acid degradation in WG suggests enhanced microbial capacity for nutrient utilization and metabolic flexibility, likely reflecting adaptation to diverse and fluctuating natural food resources. Similar associations between habitat variability, diet and gut microbial functionality have been reported in previous fish microbiome studies (Sylvain *et al.,* 2020).

The higher abundance of pathways related to environmental information processing, cellular processes and host-microbe interactions in WG further indicates enhanced microbial adaptive responsiveness to environmental variability. Enrichment of signaling molecules and interaction pathways, immune system functions and complement and coagulation cascades indicates that the

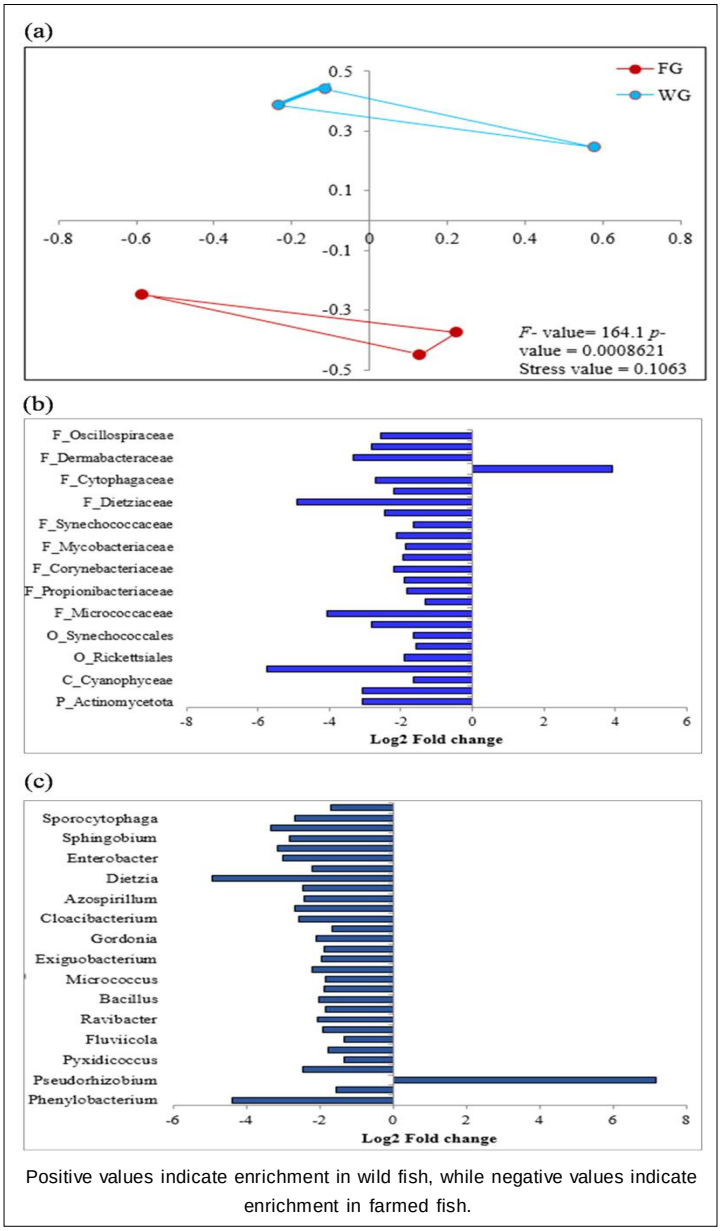



**Fig 7:** (a) Beta diversity analysis of gut microbiota in FG and WG *C. striata* based on NMDS using bray-curtis dissimilarity, showing clear separation between groups (PERMANOVA, *p*-value <0.001). (b) Differential abundance of microbial taxa at higher taxonomic levels (family/order) based on log, fold change. (c) Genus-level differential abundance of gut microbiota, indicating distinct microbial signatures associated with farmed and wild habitats.

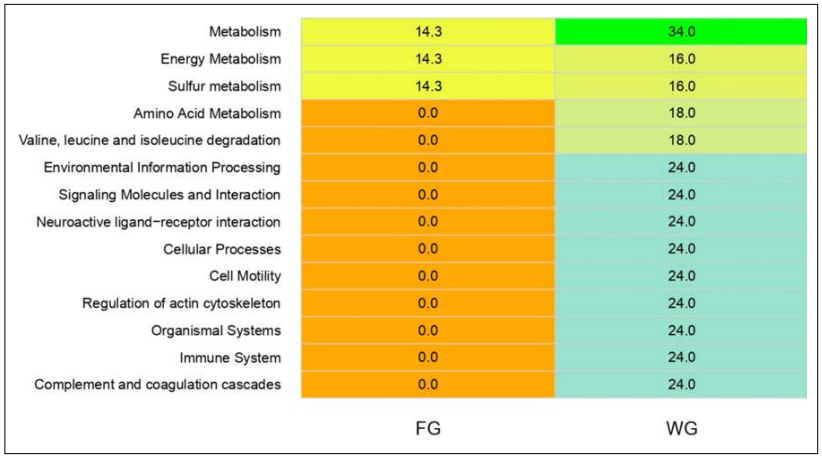



**Fig 8:** Comparative heatmap of KEGG (level 3) functional profiling of gut microbiota between FG and WG.

gut microbiota of WG may play a crucial role in immune modulation and ecological adaptation. Such functional complexity is mainly driven by exposure to heterogeneous environmental conditions, seasonal salinity fluctuations and diverse natural diets. In contrast, the comparatively restricted predicted functional repertoire observed in FG indicates that aquaculture-associated conditions may favor microbiota specialized toward metabolic efficiency rather than functional diversity. The higher representation of energy and sulfur metabolism pathways in FG likely reflects adaptation to controlled feeding regimes and stable rearing environments (Egerton *et al.,* 2018; Ringø *et al.,* 2018). Similar reductions in microbial functional diversity under captive and aquaculture conditions have been documented previously, where standardized diets and husbandry practices constrained microbial metabolic potential. Overall, the present findings demonstrate that habitat conditions and feeding ecology strongly influence not only the taxonomic composition but also the functional capabilities of the gut microbiota in *C. striata*. WG harbor more functionally diverse and environmentally adaptive microbial communities, whereas FG microbiota appear more metabolically specialized under controlled culture conditions.

**Limitations and future directions**

The present study provides an exploratory comparison of gut microbial communities in wild and farmed *C. striata*. Although sequencing-depth normalization and rarefaction procedures were employed to minimize analytical bias, the relatively small sample size (n = 3 per group) and restricted geographic coverage may limit statistical power and constrain broader conclusions regarding habitat-associated microbial variation in *C. striata*. Similar sample size pertaining to 16S rRNA-based fish gut microbiota research was reported in studies on the same species (Rasal *et al*., 2023) and on deep-sea teleosts (Iwatsuki *et al*., 2021). However, despite of high-depth sequencing with rarefaction normalization was applied to maximize the reliability of diversity estimates, the statistical power of the study is inherently constrained. In particular, the PERMANOVA findings should be interpreted cautiously, since the limited sample size may affect statistical power and could lead to an overestimation of the F-value. Therefore, findings of the study should be treated as preliminary and hypothesis driven and should not be considered definitive, especially considering the probiotic potential and metabolic efficiency. The limitation of adequate sample size of *C. striata* due to seasonal and ecological constraints have necessitated to this conservative sampling design.

In addition, while the DNA extraction protocol applied in the present study was effective, the use of standardized commercial DNA extraction kits may further improve reproducibility and comparability across studies and this will be considered in future investigations. Moreover, larger independently cohort spanning across seasons, habitats and geographical locations can be undertaken for more validation and generating more robust datasets for future validation. Further, integrating multi-omics approaches including metagenomics, metaproteomics, metatranscriptomics and metabolomics along with controlled probiotic trials may facilitate the identification of the functionally beneficial microbial candidates conferring health benefits and improving production performance of *C. striata* and steering sustainable aquaculture.

## CONCLUSION

The findings of the current study reflect differences in gut microbiota composition and functional potential between wild and farmed *C. striata*, highlighting the strong influence of habitat conditions and feeding ecology on microbial community structure. The farmed *C. striata* showed higher gut microbial richness and clustering, likely driven by uniform diet and controlled rearing environments, whereas Wild fish exhibited a broader predicted functional potential. Beta diversity analyses further confirmed significant habitat-specific microbial structuring between the two groups.

The findings collectively indicate that environmental variability appeared to influence the predicted functional capabilities of the gut microbiota of wild fish, while aquaculture practices promote microbiota specialized toward metabolic efficiency. A proper understanding of habitat-driven variation in the gut microbiota of *C. striata* is important for developing microbiome-based strategies for sustainable aquaculture. Moreover, Several bacterial taxa identified in wild *C. striata* may represent potential sources of beneficial microbes for future probiotic evaluation and validation studies.

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### Disclaimers

The opinions, interpretations and inferences presented in this manuscript are those of the authors alone and may not essentially reflect the official positions of their affiliated organisations. While every effort has been made to ensure the accuracy and completeness of the information provided, the authors disclaim accountability for any direct or indirect consequences arising from the use of the content presented herein.

### Informed consent

The use of animal and its research undertaken complies international, national and/or institutional guidelines and current animal welfare norms in India. All the procedures were followed according to the guidelines set forth by Institute Animal Ethical Committee (IAEC), ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha, India.

### Data availability

The raw sequences data of the samples were submitted in FASTQ format to the Sequence Read Archive (SRA), NCBI, under the BioProject accession number-PRJNA1 437389.

### Conflict of interest

The authors have no conflicts of interest regarding the publication.

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