Change in dissolved oxygen of water bodies is the major driver of alteration in intestinal bacterial community of the bottom-feeding fishCirrhinusmrigala

Chandana Basak
University of North Bengal
Nibendu Mondal
Bose Institute
Sumit Chatterjee
Bose Institute
Wriddhiman Ghosh
Bose Institute
Ranadhir Chakraborty (rcnbusiliguri@gmail.com)
University of North Bengal

Research Article

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Abstract

Oxygen depletion, or hypoxia, a common consequence of eutrophication, has adverse effect on aquatic flora and fauna. Bacteria living within or around aquatic macroorganisms struggle to thrive in low dissolved oxygen (DO_2) conditions. The goal of our study was to see how the bacterial population in the intestine of a bottom-feeding fish, *Cirrhinus mrigala*, changed when the DO_2 level in the water decreased from 7 ± 0.5 ppm to 0.35 ± 0.05 ppm. The V3 regions of 16S rRNA genes derived out of metagenome extracted from the intestines of control and experimental fishes were sequenced and analysed. The findings revealed that during DO_2 stress (0.35 ± 0.05ppm), the aerobic bacterial population in the gut (existing when fish lived in 7 ± 0.5ppm DO_2) changed significantly, transforming the microbial community into a facultative and/or obligate anaerobic community with dominance of microaerophilic bacterial populations; density of *Cetobacterium* increased in intestinal samples, which is linked to the prevention of paradoxical anaerobism in fish tissues. The current research advances our understanding of the microbial ecology of intestinal bacterial flora under DO_2 stress, as well as their interactions with changing environmental parameters within the host body.

Introduction

Temperature, pH, oxygen availability, salinity, desiccation, anthropogenic pressure, and activities such as xenobiotic compound disposal, including pesticides, and other environmental challenges have an impact on both terrestrial and aquatic wildlife. As a result, these challenges in fish are associated with physiological, behavioural, and anatomical changes, and have a significant impact on gut microbial populations that are either permanent or temporary residents of the host organism. Because both hosts and bacteria have been undergoing reciprocal evolutionary changes for millions of years, the gut microbiota may be regarded as a fundamental component of the host body.The feeding environment, digestion, absorption, metabolism, immune response, energy homeostasis, and overall health of the host have all been shown to interact with the gut-brain axis, which is mediated by hormonal, immunological, and neurological signals (Butt and Volkoff 2019). Water body desiccation or eutrophication can cause dissolved oxygen (DO_2) concentrations to fall to unacceptably low levels, which can have a negative impact on fish physiology by interfering with the type of available feed and impairing fish growth and development (Abdel-Tawwabet al. 2014; Roy et al. 2018). DO_2-stress alters the gut bacterial ecology, resulting in metabolic changes, a weakened immune system, and an increased risk of disease (Brown et al. 2012).

Cirrhinus mrigala (also known as 'Mrigal'), a Cyprinidae family member, is the third most popular and widely consumed Indian carp fish. It is also widely cultivated in other Asian countries. This carp used to live in the rivers and backwaters of Northern India, Pakistan, and Burma. They, like *C. molitorella*, the Chinese mud carp, are bottom feeders that eat mostly decaying plants. In manipulative experiments, *C. mrigala* was found to be able to sustain five times the amount of eutrophic water conditions [soluble phosphate (TP) = 5 X 1.3 = 6.5 mg L^{-1}; soluble inorganic nitrogen (TN) = 5 X 16 = 80.0 mg L^{-1}] and have a

higher survival rate (33.33%) in the lowest DO_2 (1.7–1.8 mg L-1) than *C. molitorella* (Yu et al. 2019).Firmicutes (25.6%), Bacteroidetes (34.3%), Betaproteobacteria (17.4%), Gammaproteobacteria (13.4%), Parcubacteria (3.4%), and Actinobacteria (2.1%) were previously shown to be the major contributing bacterial phyla present in hypoxic waters of Taihu Lake in China, but the dominant phyla in oxic water were significantly different, including Firmicutes (52.9%), Gammaproteobacteria (13.8%), Actinobacteria (22.5%), Alphaproteobacteria (5.2%), Betaproteobacteria (2.1%), and Bacteroidetes (1.0%) (Cai et al. 2018). According to another study, Proteobacteria, Firmicutes, Bacteroidetes, and Fusobacteria dominate the gut microbiome of several carp species (Li et al. 2018).

The goal of this research is to learn how the fish gut microbiota changes in response to DO_2 stress, and how this shift in microbial population may reduce the population of obligately aerobic bacteria while increasing the population of facultative and obligate anaerobes. To define and compare the intestinal microbiota of *C. mrigala*, meta-taxonomic data analyses were performed using sequences of the V3 region of bacterial 16S rRNA genes amplified from DNA extracted from the fish gut.

Materials And Methods

Design of experiment

The hatchery provided ten live *C. mrigala* with a size distribution of 17±2 cm and a weight of 48±6 g, and two experimental tanks (length, 3 ft; height, 1.5 ft; width, 1 ft; volume, 127.4 l) were kept in the laboratory, each filled with 100 l of fresh water. Both tanks were kept at 24±2 °C, pH 7.0±0.3and no mechanical surface agitation was used. The DO_2 level was 4±0.5 ppm without aeration and 7±0.5ppm after aeration (the traditional Winkler's method was used to estimate DO2). At this point, five fish were placed in each of two aquariums.The fishes were acclimatized for 5 days in the tanksbefore beginning the experiments.The oxygen pump in the experimental tank was turned off, causing a gradual decrease in the amount of dissolved oxygen over time. Fish activity was monitored every hour, and DO_2 levels were measured every three hours. After 24 and 36 hours, the DO_2 level of water in the non-aerated (test) tank dropped to 5.5±0.5 and 2.5±0.3 ppm, respectively. At 2±0.3ppm DO_2, the fish in the test tank began swimming on the water's surface and gasping at the air-water interface, displaying disturbed behavior. On further incubation for 4 -5 h from the point when DO_2 was 2±0.3 ppm, the fish stopped moving and overturned, elevating their ventral position on the water surface, but they did not die; DO_2 was measured at this point to be 0.35±0.05 ppm.These fish were caught with nets and anaesthetized with clove oil before being dissected under aseptic conditions to extract the digestive tract (Deriggi et al.2006). The DO_2 in the control tank was kept at 7±0.5 ppm throughout the experiment. Fish from the control tank were similarly collected, euthanized, and their guts dissected under aseptic conditions after 38 h, as per standard protocol (Basak et al. 2021).

Each fish's dissected gut, whether control or experimental, was further chopped into two parts, representing the anterior and posterior regions. The control fish's anterior and posterior guts were

designated as MCA and MCP, respectively. Similarly, the test/experimental fish's anterior and posterior gut regions were designated as MTA and MTP, respectively.Metagenomic DNA was extracted from all four samples using the DNeasyPowerSoil Pro Kit (Qiagen, Germany) according to the manufacturer's protocol. The final elution volume of each sample was 80 µl, and it was stored at -20°C until further processing.

PCR amplification and amplicon sequencing

Following the fusion primer methodology, universal forward primer341f (5'-CCTACGGGAGGCAGCAG-3') and universal reverse primer 515r (5'-TATTACCGCGGCTGCTGG-3'), were used to amplify the V3 region of 16S rRNA genes from each of the four samples (MCA, MCP, MTA, and MTP) (Roy et al. 2020a; 2020b). Prior to sequencing, the amplified PCR products were quantified with a Qubitfluorometer (Thermo Fisher Scientific, USA) and then sequenced on an Ion S5 next-generation DNA sequencing machine (Thermo Fisher Scientific) (Roy et al. 2020a; 2020b).

Analysis of meta-taxonomic data

Using the Ion S5 DNA sequencing machine's inbuilt Torrent Suite 5.8.0 software, all V3 sequence reads were filtered for low quality reads, polyclonal reads, barcode sequences, and adaptor sequences. To improve accuracy and diversity estimates, the resulting sequence was filtered for quality value 20 once more, and reads shorter than 100 bp were trimmed using FASTX-Toolkit predictions (v0.0.13.2). USEARCH was used for clustering, and operational taxonomic units (OTUs) were created with a cut-off of 97 % 16S rRNA gene sequence identity (Edgar 2013; Mondal et al. 2022).Singletons were removed before clustering into OTUs. The Simpson and Shannon index methods were used to calculate the alpha diversity of each sample. Rarefaction curves were used to confirm that read sequences are adequate for identifying microbial diversity in samples. RDP Classifier (http://rdp.cme.msu.edu/classifer/classifer.jsp) was used with an 80 % confidence level to classify the consensus sequence of each OTU at the taxonomic level. MicrobiomeAnalyst was used for additional analysis, and data was deposited in the form of an OTU abundance table for marker data profiling. Low count features were filtered using 20% sample prevalence to remove sequencing errors or low level contaminations, and low variance data was filtered using 10% inter-quantile range to remove sequencing errors or low level contaminations.The data was scaled using total sum scaling, and it was rarefied to the smallest library size possible. The 2D PCoA map was created to assess beta diversity using the PERMANOVA statistical methodology and the Bray-Curtis distance method. Actual abundance profiles and interactive pie charts were created at the phylum, class, and genus levels, using samples organized by gastrointestinal region. The Euclidean distance measure and the ward clustering algorithm were used to perform hierarchical clustering and heatmap visualization at the family level. A dendogram analysis at the feature level was performed using the Bray-Curtis index. To look for patterns at the family and genus levels, the Pearson r distance method was used (Dhariwalet al. 2017).

Results

Sequence evaluation and assessment of microbial diversity

For all four samples, a total of 1446597 raw sequences were collected, with 1378928 good quality sequences recovered after quality and length filtering. Following that, the sequences were divided into 974 OTUs, with 339, 254, 328, and 225 OTUs covering the MCA, MTA, MCP, and MTP, respectively. Unclassified bacterial OTUs account for 5.6 % and 8.26 % of total OTUs in MCA and MTA, respectively; while 8.54 % and 8.0 % of bacterial OTUs remain unclassified in MCP and MTP, respectively.Rarefaction curves for all four samples revealed that the number of observed species initially increased with sequencing depth, and then plateaued, resembling general rarefaction curves tapering off at the end. The saturation plateau on the rarefaction curves indicated that the amount of sequencing was sufficient for further analysis (Fig. S1).

The microbial community in *C. mrigala*'s foregut and hindgut is more abundant and diverse under control (DO_2 = 7±0.5ppm) conditions. In response to low dissolved oxygen concentrations, the anterior gut shows a 3.6 times (Chao1 value =33) and posterior gut shows a 2.9 times (Chao1 value =40) drop in gut bacterial population abundance (Chao1 value =120 or 114 in the foregut or hindgutof the control)(Fig. 1). The presence of changes in the bacterial community in *C. mrigala* anterior and posterior gut samples was revealed by beta diversity analysis (Fig. 2 a).In both control and experimental conditions, gut samples have clearly distinct microbial compositions. The Venn diagram depicts sharing of 107 OTUsby all four samples (Fig. 2 b). Control and experimental anterior gut samples shared 10 more OTUs with these 107 OTUs, and control and experimental posterior gut samples shared 22 more OTUs. In the control sample, the anterior guts of *C.mrigala* contained 143 unique OTUs, but only 47 in the experimental sample. The posterior gut of control fish, on the other hand, contained 112 unique OTUs, whereas the experimental sample contained 20 unique taxa.

Differences in gut microbial community among control and experimental samples

In the study of actual abundance, nine phyla were found to be significant contributors to gut bacterial populations. Proteobacteria, Bacteroidetes, Firmicutes, Fusobacteria, and Actinobacteria were the most common phyla found in all four intestinal samples. According to a phylum-based study, when the dissolved oxygen content dropped from 7±0.5ppm to 0.35±0.05 ppm, *C. mrigala* had a lower population of Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria in both the anterior and posterior guts(Fig. 3). Surprisingly, the population of Fusobacteria increases in both the anterior and posterior guts under the experimentalconditions described above. Dendrogram analyses revealed that the anterior gut microbiota of control *C. mrigala* is more similar to that of its posterior gut microbiota (Fig. S2).

Other bacterial classes, besides Fusobacteria, Bacteroidia, Erysipelotrichia, and Cyanobacteria, showed a decrease in bacterial abundance during experimental conditions compared to control conditions in both gut areas, according to a class-based analysis (Fig. 4). Furthermore, only MTAdid Chloroplast and Mollicutes show a small increase in bacterial population abundance. MTA and MTP had significantly lower concentrations of Betaproteobacteria, Gammaproteobacteria, Flavobacteriia, and Alphaproteobacteria than MCA and MCP control samples. The population of Sphingobacteria, Cytophagia, and Deinococci was reduced to nil under dissolved oxygen stress conditions.

Verrucomicrobiae were only found in experimental conditions. Under experimental conditions, the abundance of Clostridia and Bacilli decreases in both guts, while Erysipelotrichia increases.

OTUs were distributed in 32 different orders in MCA, with Flavobacteriales and Burkholderiales dominating at the top, followed by Fusobacteriales, Clostridiales, Enterobacteriales, and Actinomycetales, Neisseriales, and Pseudomonadale (all with the same number of OTUs); in the experimental anterior gut (MTA), Fusobacteriales were dominant, followed by Erysipelotrich (in descending order). OTUs in MTP, on the other hand, were distributed in 30 different orders, with Flavobacteriales dominating the list followed by Burkholderiales, Fusobacteriales, Actinomycetales, Pseudomonadales, Clostridiales, and Bacillales at the bottom, while Enterobacteriales, Bacteroidales, Actinomycetales, Erysipelotrichales, and Clostridiales dominated(Fig. S3).

Flavobacteriaceae and *Fusobacteriaceae* were almost equally prevalent among OTUs distributed across 49 families in the MCA sample, whereas *Flavobacteriaceae* was most prevalent among OTUs classified across 51 families in the MCP sample. *Fusobacteriaceae* was found to be the most prevalent family among OTUs sorted from MTA and MTP sequence reads, respectively, for 25 and 27 families.

As shown in Fig. 5, the abundance of the gut microbial community was significantly influenced by the amount of dissolved oxygen in the water. Both the anterior and posterior guts had a low abundance of microbial diversity under dissolved oxygen stress (0.35±0.05 ppm). All other genera, with the exception of *Cetobacterium*, *Pseudomonas*, *Aeromonas*, and *Staphylococcus*, vanished completely in the experimental situations, as shown in Fig. 5. *Mycobacterium*and *Turibacter* were found in both the anterior and posterior guts of *C. mrigala* during the DO_2-stress experiment. *Cetobacterium* was the most abundant genus in both the anterior and posterior guts of *C.mrigala*, with a dramatic increase in abundance under DO_2 stress conditions. In addition, the relative abundance of *Aeromonas*in MTA was higher than that of MCA.

Community profiling, Clustering and correlation of control vs. experimental gut microbiota

According to the microbiome investigation of *C. mrigala*, less availability of DO_2 concentration in water altered bacterial groups constituting key contributors of core intestinal microbiota during control conditions. DO_2 stress caused the main contributing groups to shift, as well as the appearance and disappearance of a few microbial populations in the core intestinal microbiome (Fig. 6).

At the family level, heatmap analysis of all four samples was performed to reflect microbiological complexity and measure similarity or dissimilarity among and among samples. The heatmap pattern shows that bacterial diversity and relative abundance patterns for *C. mrigala*gut samples (both anterior and posterior) diverged significantly under normal and stressed DO_2concentrations (Fig. 7). In terms of the overall heatmap abundance pattern, the anterior and posterior gut samples of *C. mrigala* (MCA and MCP) do not closely resemble the gut sample under DO_2 stress (MTA and MTP).The bacterial complexity of the control fish's anterior gut (MCA) resembled that of the fish's posterior gut (MCP). Similarly, the bacterial complexity of the anterior gut (MTA) of the DO_2 stressed fish was more similar to that of the

posterior gut (MTP). Furthermore, many of the bacterial species found in MCA and MCP were reduced to zero in MTA and MTP (under DO_2 stress conditions). According to a pattern study in MicrobiomeAnalyst, *Erysipelotrichaceae* has a positive relationship with *Mycobacteriaceae*, *Enterobacteriaceae* (*Plesiomonas*), *Fusobacteriaceae*, and *Aeromonadaceae*, but a negative relationship with other family members (Fig. 8). Similarly, the genus *Cetobacterium* had a positive relationship with *Turicibacter* and *Mycobacterium* but a negative correlation with the remaining genera.

Discussion

The microbial community in a fish gut is an indicator of the animal's response to environmental conditions, and changes in gut microbial composition under environmental stress are the focal point for tracking microbial ecology shifts. The purpose of this study was to see how the microbial population in the gut of *C. mrigala* changed as the dissolved oxygen concentration in the water decreased (from 7 ± 0.5 to 0.35 ± 0.05 ppm). According to the results of an alpha-diversity analysis, the gut regions of *C. mrigala* fishes (MCA and MCP) in normal DO_2 conditions (7 ± 0.5 ppm) have more microbial variety and richness than DO_2 stressed (0.35 ± 0.05ppm) fishes (MTA and MTP).Furthermore, beta-diversity analysis revealed that *C. mrigala*'s anterior and posterior gut samples from fish at 7 ± 0.5 ppm DO_2 (MCA and MCP) are clearly separated and highly non-overlapping when compared to gut samples from DO_2 stressed fish (MTA and MTP). The difference in the anterior guts of control (DO_2 = 7 ± 0.5 ppm) and experimental (DO_2 = 0.35 ± 0.05ppm) samples is caused by 143 and 47 distinct OTUs found in MCA and MTA, respectively. The presence of 112 and 20 distinct OTUs in MCP and MTP, respectively, contributes to the dissimilarity between the posterior guts of control and experimental samples.These findings were confirmed by dendrogram analyses, which revealed that the anterior gut microbiota of control *C. mrigala* is more similar to that of its posterior gut microbiota (Fig S2). The intersection of the four sets MCA, MCP, MTA, and MTP forms the middle of the Venn diagram, which contains 107OTUs (Fig. 2b). These OTUs are expected to be keystone taxa in a specific microbial community, playing critical roles in microbiome structure and function, either individually or collectively.

Previous research in different carp fishes (common carp, grass carp, crucian carp, and bighead carp) using both culture-based and culture-independent methods revealed that Fusobacteria, Firmicutes, Proteobacteria, and Bacteroidetes dominate the carp fish intestinal microbial community, while Actinobacteria, Verrucomicrobia, Spirochaetes, and Deinococcus-Thermus remainless dominant (Li et al. 2014; Mulyaniet al. 2018). This pattern was observed in this study as well (Fig. 3). Members of the phylum Proteobacteria, classified as Burkholderiales, Methylophilales, and Neisseriales in our study, must have been exposed to a stressful level of oxygen concentration in the gut, resulting in a decrease in MTA and MTP abundance, as shown in Fig. S3.Pseudomonadales, which had higher OTU counts in MCA and MCP (control), had lower OTU counts in the experimental condition (Fig. S3). Except for a few *Pseudomonas* species, all other genera in Pseudomonadales have disappeared due to dissolved oxygen stress in water. In experimental conditions (DO2 = 0.35 ± 0.05ppm), the OTU counts of Enterobacteriales, which have been shown to survive in both aerobic and anaerobic environments, were nearly identical in

the anterior gut but increased in the posterior gut (Rogers 2020).The number of OTUs of Aeromonadales did not change in MTP of DO2 stressed fishes, but it did decrease significantly in MTA (Fig. S3).This finding suggests that as the amount of soluble oxygen in water and the gut decreased, several aerobic bacterial communities suffered, allowing only anaerobic and facultative aerobic bacteria to thrive. Some of the benefited bacteria start colonizing more effectively, as evidenced by higher OTU counts in experimental conditions.

Fusobacteriia, which encompassed the genus *Cetobacterium*, was shown to be a microaerotolerant bacteria (Finegoldet al. 2003), resulting in a significant increase in OTU count in MTA and MTP (Fig. 5). In both cases, the anterior gut contains more *Cetobacterium* than the posterior gut, indicating that this genus is involved in the digestion process. *Cetobacterium* has been extensively studied in fish guts due to its ability to breakdown proteins and produce vitamin B12 (Tsuchiya et al. 2008).They can also help toprevent paradoxical anaerobism in the fish gut by acting as an ethanol sink, lowering the amount of ethanol in the fish tissues and thus preserving aerobic metabolism (Bhuteet al. 2020). Because the fish gut was subjectedto anaerobic conditions during the experiment, increasing the concentration of *Cetobacterium* in MTA and MTP was critical.Another study on the intestinal microbiota of the scaleless catfish *Pelteobagrus vachelli* found that 24 h of hypoxia caused significant alterations at the phylum level of the microbial community, with the number of Bacteroidetes increasing in the hypoxic condition compared to the control condition (Zheng et al.2021). This is in contrast to our present study, which found that the concentration of Bacteroidetes in *C. mrigala* decreased under hypoxic conditions.

Under experimental conditions, Bacteroidia, which encompassed*Porphyromonadaceae*, was found in greater abundance in both gut areas. Because this family contains anaerobic bacteria (Sakamoto 2014), an inversely proportional relationship between bacterial population and dissolved oxygen in water is warranted. Similarly, *Erysipelotrichia* was classified to the family level, and members of the *Erysipelotrichaceae* were discovered in mammalian intestines, where they could increase their population in the host during metabolic and inflammatory conditions (Kaakoush 2015). This family includes members who are anaerobic, facultatively anaerobic, and aerobic (Verbarget al. 2004; Cox et al. 2017). Although it was not classified to the genus level, its greater abundance in MTA and MTP could be attributed to lower DO_2 levels in the water.

Previous research has suggested that not only diet (Bolnicket al. 2014; Miyake et al. 2015), but also environmental microbiota (Wong and Rawls 2012) can have a significant impact on the microbial compositions of aquatic animals due to their close association with water and sediment (Del'Duca et al. 2013; Huanget al. 2018). Because microbial populations have different oxygen requirements, oxygen stress in water must have played a significant role in changing gut microbial composition by removing strict aerobic species and encouraging the population of microaerophilic, aerotolerant, and anaerobic bacteria.In our current study, the core gut microbial population changed during stressful DO_2, with Fusobacteria and Bacteroidia appearing as the second and third significant contributors, respectively, along with two more classes, Erysipelotrichia and Mollicutes, in third and fourth place.

Deltaproteobacteria, Sphingobacteria, Cytophagia, and Deinococci were eliminated as core contributors in DO_2 stressed samples when compared to control samples (Fig. 6).

According to a heatmap created at the family level, several populations that were dominant in both gut regions during the control condition vanished completely in the experimental condition. Presence of aerobic bacteria, with a few exceptions, noted are members of the families *Comamonadaceae* (Willems 2014), *Flavobacteriaceae* (McBride 2014), *Moraxellaceae* (Teixeira and Merquior 2014),*Neisseriaceae* (Bennett et al. 2014), and *Deinococcaceae* (Rainey and Oren 2011). Members of the *Rhodocyclaceae* (Oren 2014) and *Oxalobacteriaceae* (Baldaniet al. 2014) families,reported to have aerobic, anaerobic, and facultative anaerobic populations, were reduced to zero in experimental conditions. *Fusobacteriaceae* (Olsen 2014), *Enterobacteriaceae* (Octavia and Lan 2014), *Erysipelotrichaceae* (Verbarget al. 2004), *Aeromonadaceae* (Huys 2014), and *Porphyromonadaceae* (Sakamoto 2014) are primarily strict and facultative anaerobic families.

This is the first study to look at how low dissolved oxygen in water affects the diversity and structure of the gut microbiome of a gill breather carp fish, *C. mrigala*. When DO_2 is reduced from 7 ± 0.5 to 0.35 ± 0.05ppm, the microbial ecology of the fish gut shows a significant shift in bacterial number and diversity. We used a negative correlation feature (Fig. 8) to assess the trend of a few microorganisms that were abundant in low DO_2, which supports our theory yet again.These massive population shifts could help aquaculture managers detect water quality warnings. As a result, we hope that the current study will help us understand how the gut microbial population reacts to changes in water quality, as well as the involvement of specific taxa in the presence of low dissolved oxygen in water.

Declarations

Data availability

The raw sequence files of all the four samples were submitted to the National Center for Biotechnology Information (NCBI) in the United States and accession numbers were assigned (Table1).

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Competing interest

Authors have no competing interest to declare.

Author Contributions

Ranadhir Chakraborty and Chandana Basak contributed to the study conception,methodology, data curation, data analysis and visualization.Sequencing and data curation was done by Nibendu Mondal and Sumit Chatterjee. The first draft of the manuscript was written by Chandana Basak; reviewed and edited by Ranadhir Chakraborty and Wriddhiman Ghosh. The work was done under the supervision of Ranadhir Chakraborty and all authors read and approved the final manuscript.

Ethics Declaration

The study was conducted according to the guideline of the CPCSEA (Committee for the purpose of control and supervision of experiments on animals) for experimentation on fishes (http://cpcsea.nic.in/WriteReadData/LnPdf/GuidelinesofCPCSEAforExperimentationonFishes-2021.pdf) through Institutional Animal Ethics Committee (840/GO/Re/S/04/CPCSEA; 25-02-2021).

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Tables

Table 1 Biosample, Bioproject and SRA accession numbers of control and experimental metataxonomic samples; MCA, MCP, MTA and MTP.

Sample	*Biosample Accession No.*	BioProject Accession No.	*SRA* Accession No.
MCA	SAMN19268787	**PRJNA731121**	SRR14588795
MCP	SAMN19269177	**PRJNA731129**	SRR14588844
MTA	SAMN26937392	PRJNA819835	SRR18475209
MTP	SAMN26937855	PRJNA819842	SRR18475964

Figures

Fig. 1

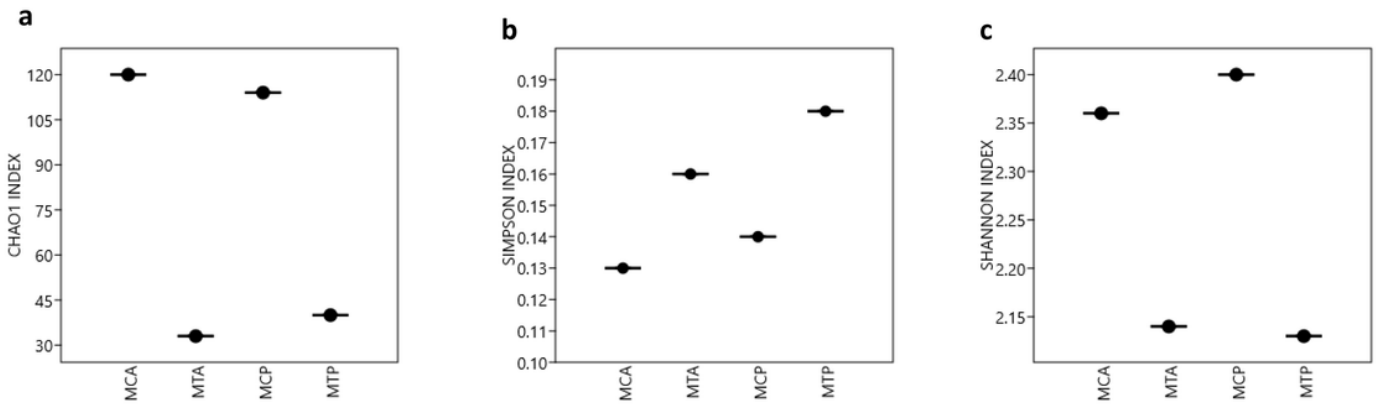

Figure 1

Boxplot of Alpha-diversity indices reflecting the abundance and diversity of gut microbial populations: **a.** Chao1 index, **b.** Simpson index and **c.** Shannon index

Fig. 2

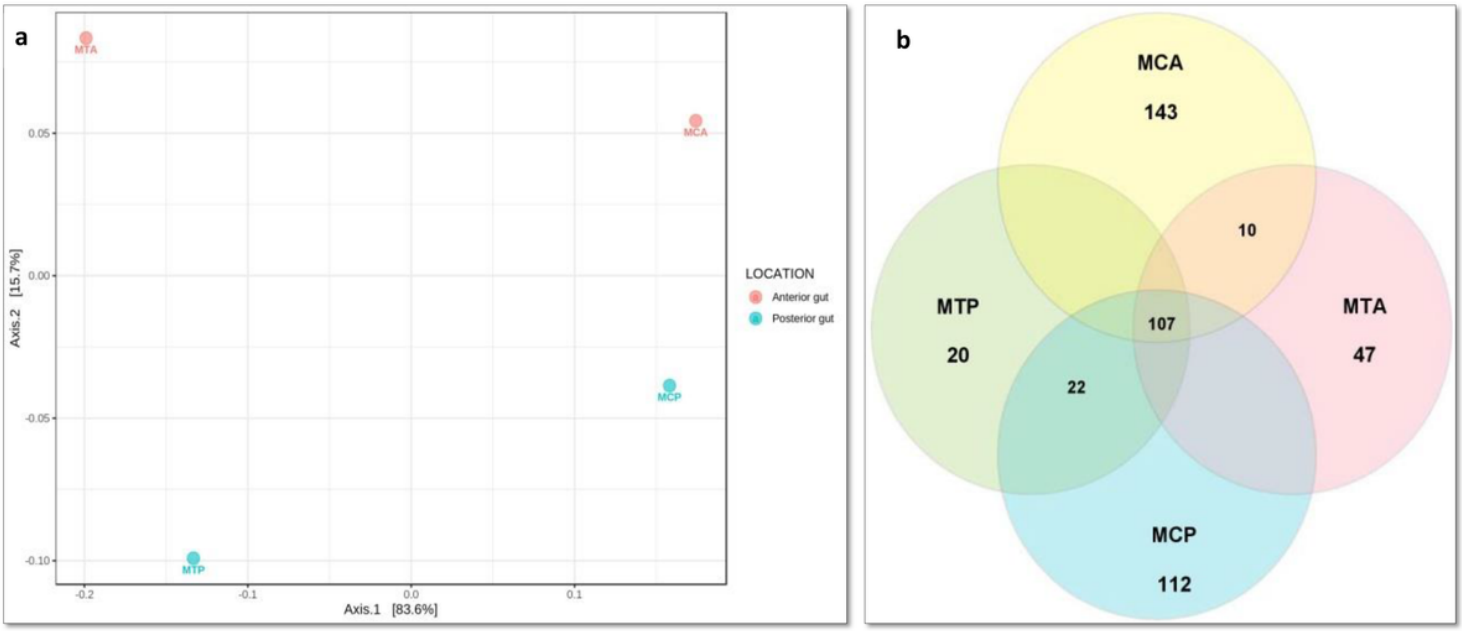

Figure 2

a. The beta diversity of the anterior and posterior gut microbiomes of *C. mrigala* visualized using principal coordinate analysis (PCoA) based on the Bray-Curtis index distance method. MCA: Anterior gut of *C. mrigala* under control condition, MCP: Posterior gut of *C. mrigala* under control condition, MTA: Anterior gut of *C. mrigala* under test condition, MTP: Posterior gut of *C. mrigala* under test condition

b. Venn diagram displaying the number of common and unique bacterial genera found in all four gut microbiota (MCA, MCP, MTA and MTP)

Fig. 3

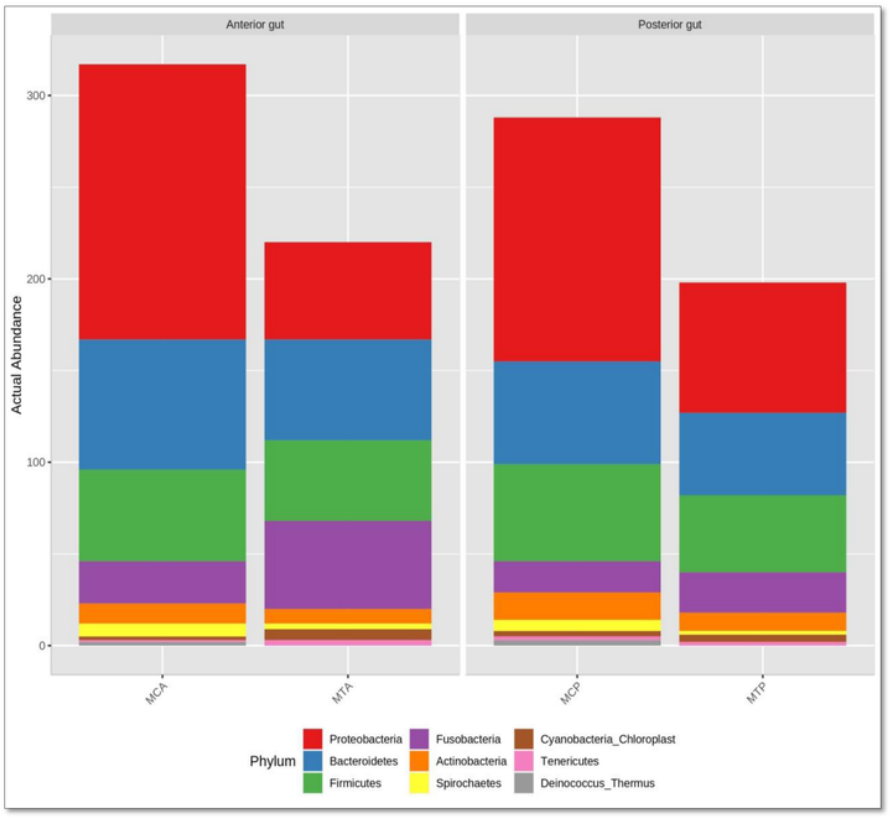

Figure 3

Stacked bar depicting abundance of diverse bacteria (at phylum level) in anterior and posterior gut samples *C. mrigala* under control (MCA and MCP) vs experimental (MTA and MTP) condition

Fig. 4

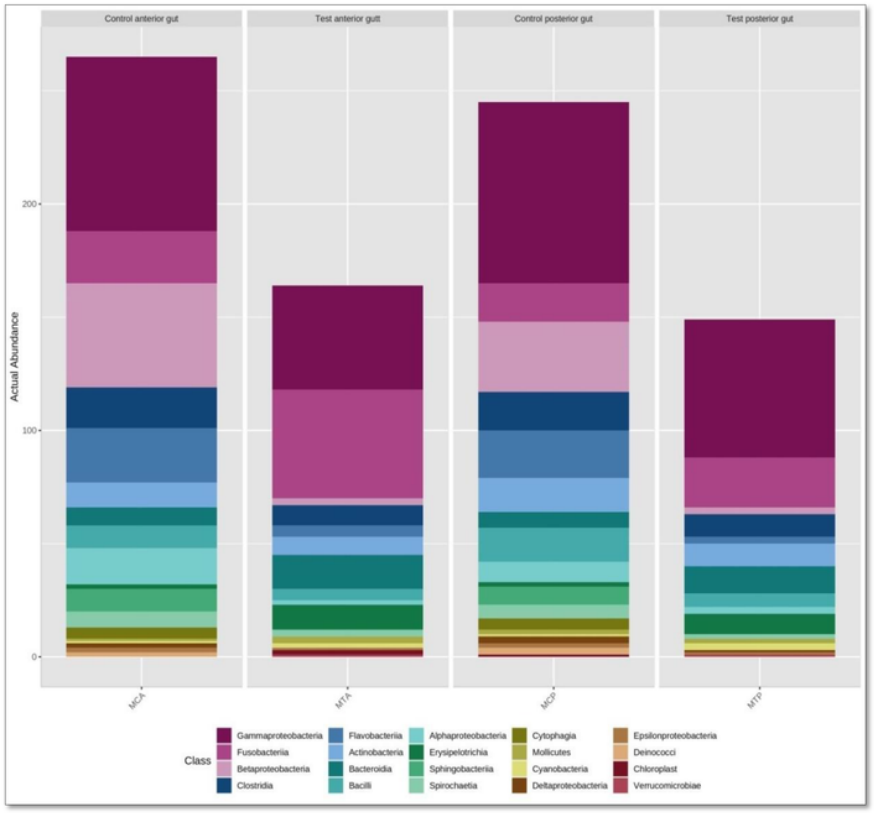

Figure 4

Stacked bar depicting abundance of diverse bacteria (at class level) in anterior and posterior gut samples *C. mrigala* under control (MCA and MCP) vs experimental (MTA and MTP) condition

Fig. 5

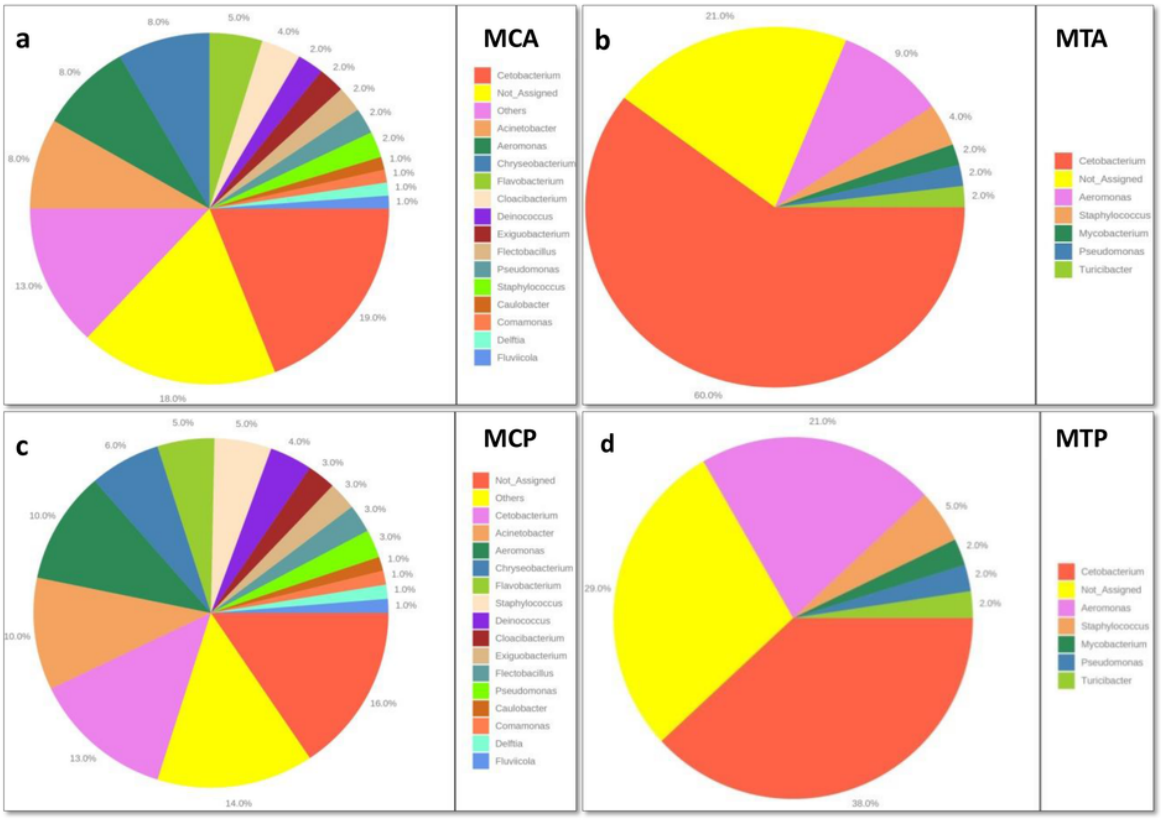

Figure 5

Distribution of different intestinal microbial composition of *Cirrhinus mrigala* at genus level:

a.MCA: Distribution of intestinal microbial composition in anterior gut under control condition

b.MTA: Distribution of intestinal microbial composition in anterior gut under experimental condition

c.MCP: Distribution of intestinal microbial composition in posterior gut under control condition

d.MTP: Distribution of intestinal microbial composition in posterior gut under experimental condition

Fig. 6

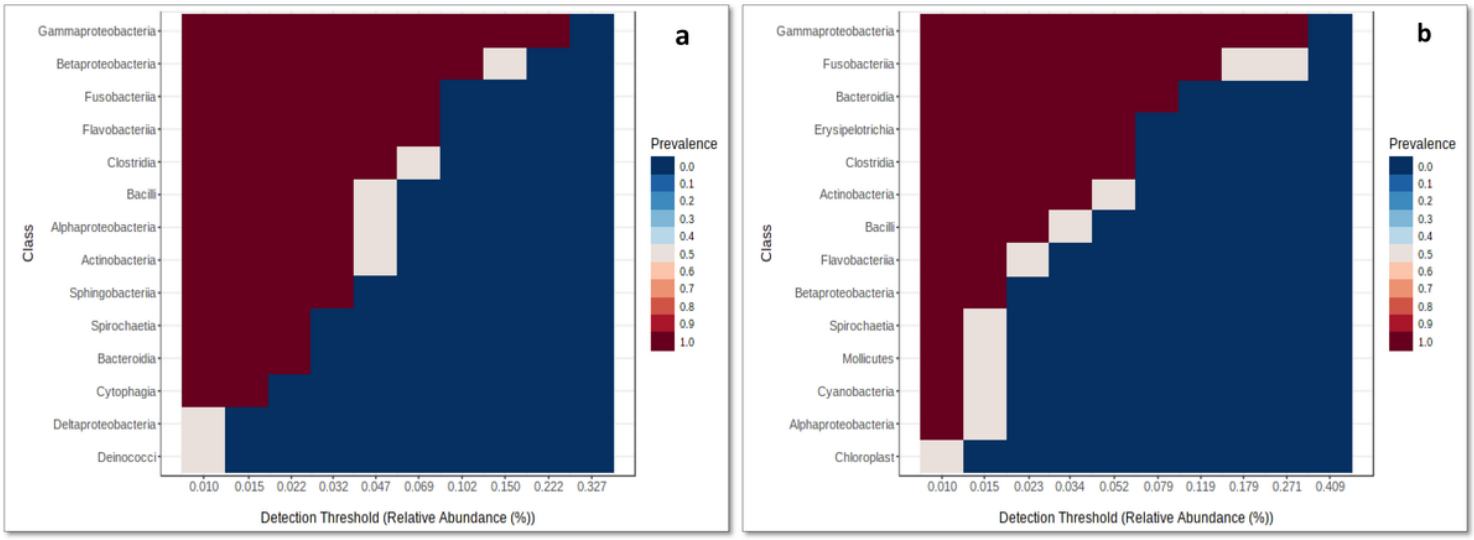

Figure 6

Core intestinal bacterial community of *C. mrigala*: **a.** under control condition and **b.** under experimental condition

Fig. 7

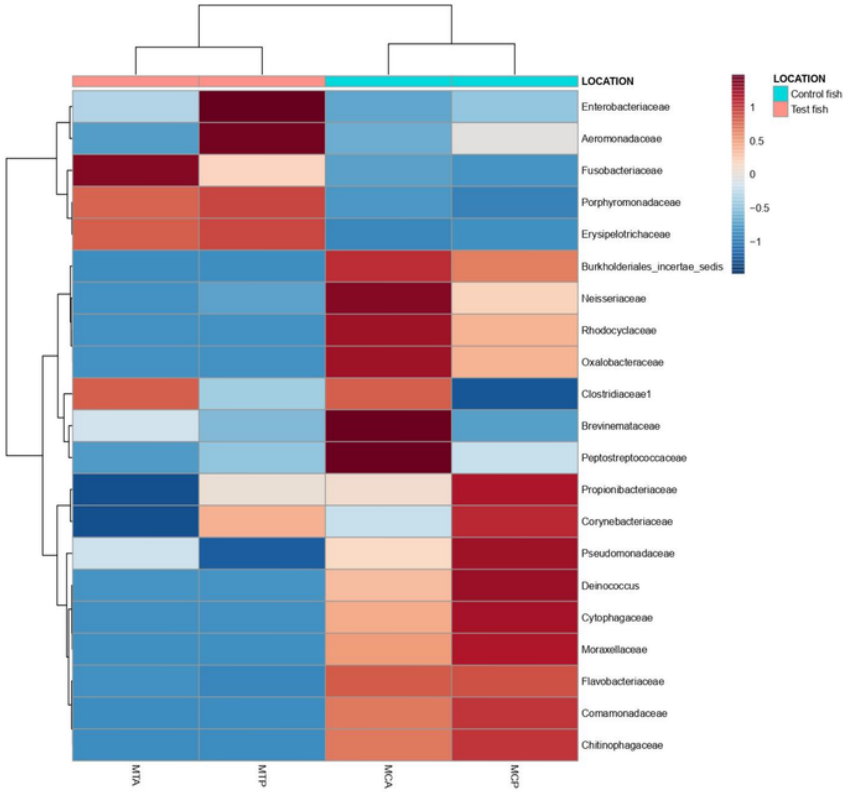

Figure 7

Heatmap showing the distribution of the bacterial community at the family level in four gut microbiota samples under control (MCA and MCP) vs experimental (MTA and MTP) condition

Fig. 8

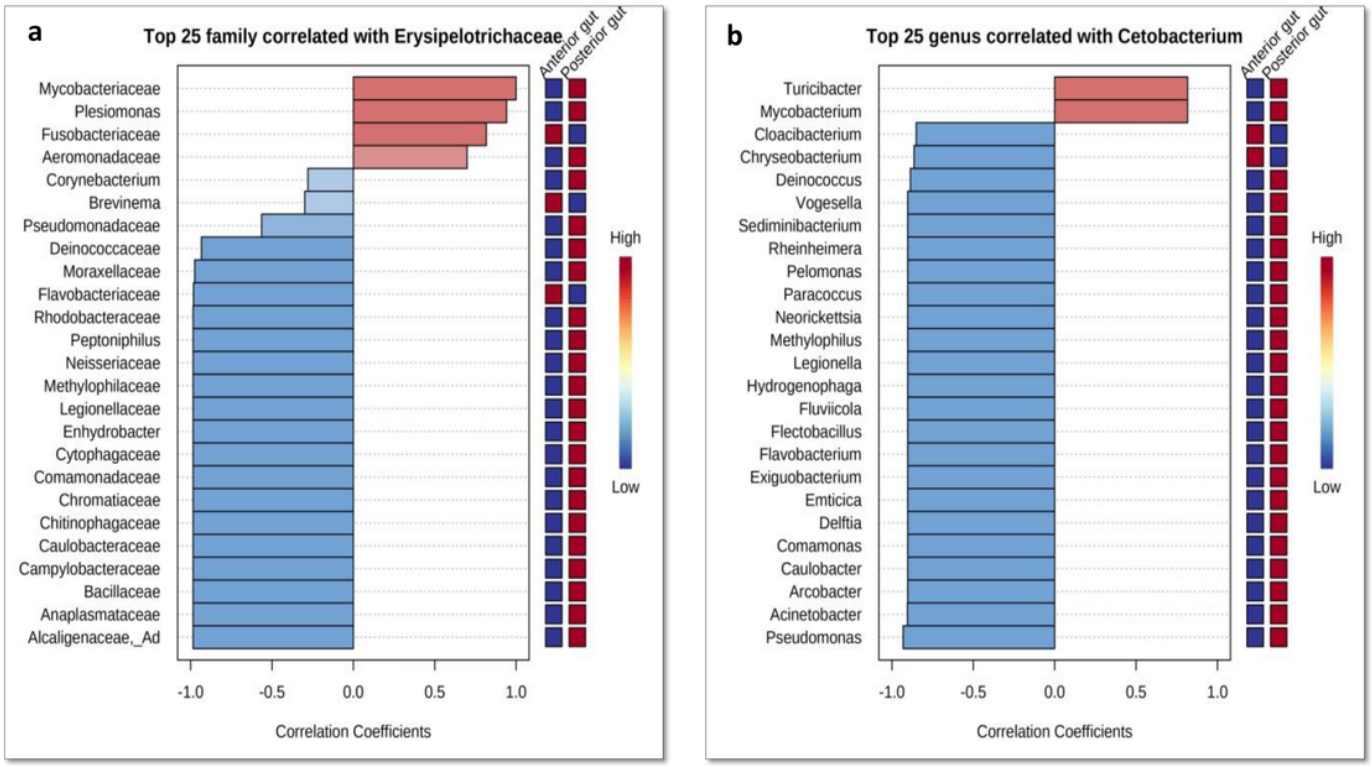

Figure 8

Negative correlation feature established within intestinal microbial population using MicrobiomeAnalyst:

a.Top 25 family correlated with *Erysipelotrichaceae*

b.Top 25 genus correlated with *Cetobacterium*

Supplementary Files

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