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Original Article

Dietary gold nanoparticles modulate the gut microbiota of *Mystus vittatus* (Bagridae): evidence from 16S rRNA amplicon sequencing

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Abstract: We evaluated whether biosynthesized gold nanoparticles (AuNPs) used as a dietary supplement reshape the gut microbiota of *Mystus vittatus* using 16S rRNA (V3-V4) amplicon sequencing. Across all samples, we resolved 716 amplicon sequence variants (ASVs) spanning 20 phyla, 33 classes, 75 orders, 129 families, and 190 genera. AuNP-fed fish showed numerically higher richness and evenness than controls; however, alpha-diversity indices (Chao1, Shannon, and Simpson) and beta-diversity (unweighted UniFrac PCoA) did not differ significantly (P>0.05). Despite the lack of whole-community separation, several taxa responded to AuNPs. Genera detected as candidate biomarkers in univariate testing included *Ralstonia*, *Rikenellaceae_RC9_gut_group*, *Candidatus arthromitus*, *Family_XIII_AD3011_group*, and *Undibacterium*, alongside a higher relative abundance of *Bacillus*—consistent with putative probiotic activity reported in fish. Community-function predictions with PICRUSt2 indicated enrichment of pathways related to amino acid and energy metabolism, nucleotide metabolism, and replication/repair in the AuNP group, whereas carbohydrate-focused pathways (for example, glycolysis and fructose/mannose metabolism) were relatively higher in controls. These results suggest that dietary AuNPs can shift microbiome composition and predicted functional potential toward profiles compatible with improved nutrient processing and immune readiness. Because functional inferences were derived from 16S marker data rather than shotgun metagenomes, they should be interpreted cautiously. We also note that community-level diversity differences represented trends rather than statistical separations. Overall, AuNP supplementation at 50 mg/kg produced measurable changes in the microbiome without disrupting the core phylum-level structure. These findings support further evaluation of AuNPs as a feed additive in aquaculture, including dose–response testing, validation using metagenomic or metatranscriptomic sequencing, and long-term safety assessments that track performance, disease outcomes, and environmental release.

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Introduction

The striped dwarf catfish, *Mystus vittatus* (Bagridae), is an indigenous, commercially valuable species in Bangladesh, prized for its nutrient-dense flesh, rapid growth, and suitability for intensive culture (Mondal et al., 2017; Paul et al., 2019; Shahariar et al., 2024). To sustain high productivity, hatchery and grow-out operations depend on optimized stocking density, balanced feeds, and strategies that enhance health and immunity (Mou et al., 2018). Conventional additives—such as probiotics, prebiotics/synbiotics, plant extracts, and antibiotics—can improve performance; however, non-native probiotic strains often underperform in local systems, and routine antibiotic use raises concerns about resistance and environmental impacts (Harikrishnan et al., 2019; Rohani et al., 2022; Hossain et al., 2023). Safe, sustainable alternatives are therefore needed. Nanotechnology offers one such alternative. Due to their small size (1-100 nm) and large surface-area-to-volume ratios, nanoparticles exhibit enhanced bioavailability and antimicrobial activity, which are relevant to feed technology, disease prevention, and water quality (Nasrollahi et al., 2011; Rafique et al.,

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2017; Khan et al., 2019). In aquaculture, several studies have reported improved growth, feed utilization, and disease resistance when nano-formulations (e.g., chitosan nanoparticles) are incorporated into diets (Abd El-Naby et al., 2020; Younus et al., 2020). Among candidate materials, gold nanoparticles (AuNPs) are particularly attractive due to their perceived biocompatibility and emerging evidence suggesting that they can modulate host–microbe interactions in the gut.

The fish gut microbiome underpins nutrient digestion, energy harvest, epithelial development, and immune priming, with community shifts translating to measurable effects on growth and resilience (Segata et al., 2013; Moszak et al., 2020; Bhat et al., 2023). Culture-independent sequencing approaches now enable high-resolution profiling of these communities, moving beyond cultivation to detect low-abundance and fastidious taxa, while facilitating comparative functional inference (Handelsman et al., 1998). In diverse fishes—including zebrafish, yellowfin sea bream, Pacific bluefin tuna, and rainbow trout—dietary interventions consistently reshape gut community structure and predict metabolic outcomes (Udayangani et al., 2017; Kodama et al., 2020; Pan et al., 2021; Rasmussen et al., 2022). Recent work on economically important regional species, such as hilsa (*Tenualosa ilisha*), further underscores the value of microbiome-aware feed design in South Asian aquaculture (Kawser et al., 2024).

Despite this momentum, the effects of dietary AuNPs on the gut microbiota of *M. vittatus* remain unknown. Building on our previous feeding trial that optimized AuNP dosage for growth performance in this species (Shahariar et al., 2024), we tested whether biosynthesized AuNPs at 50 mg/kg would measurably alter gut community composition and predicted functions without compromising within-sample diversity. To capture taxonomic resolution appropriate for community-level inference while remaining cost-effective for replicated designs, we employed 16S rRNA (V3-V4) amplicon sequencing. Given prior reports that diet can enrich taxa involved in amino-acid and energy metabolism and alter immune-relevant pathways, we further used PICRUSt2 to predict functional capacities from amplicon sequence variants (ASVs).

Accordingly, our objectives were to: (i) characterize the gut microbiota of *M. vittatus* under a standard commercial diet versus the same diet supplemented with biosynthesized AuNPs; (ii) assess alpha- and beta-diversity to determine whether AuNPs alter richness/evenness or community structure; (iii) identify differentially abundant taxa that may serve as candidate biomarkers of AuNP exposure; and (iv) infer putative functional shifts in KEGG pathways related to nutrient metabolism and immune readiness. By linking a practical feed additive (AuNPs) to microbiome composition and predicted function in a regionally important model catfish, this study aims to inform rational, nanotechnology-enabled strategies for sustainable aquafeed development.

Materials and Methods

Experimental setup and feeding trial: This study investigated alterations in the gut microbiome of *M. vittatus* following dietary supplementation with biosynthesized gold nanoparticles (AuNPs). The experimental design was based on our previous study (Shahariar et al., 2024). Experimental fish were assigned to two dietary groups: a control group receiving a commercial diet without AuNPs (0 mg/kg) and a treatment group receiving a diet enriched with 50 mg/kg of AuNPs. The experiment was conducted in triplicate for each treatment to ensure statistical robustness, with fish distributed across three separate tanks per group.

Experimental diet formulation: This study used gut samples collected from our previously reported experiment (Shahariar et al., 2024). In that study, striped dwarf catfish were fed diets supplemented with biosynthesized AuNPs. The detailed feeding protocol and diet formulation were described previously (Shahariar et al., 2024). In the present study, we focus exclusively on metagenomic analysis of the gut samples. The 50 mg/kg dose was selected based on our previous trial (Shahariar et al., 2024) and other studies reporting optimal growth and gut microbiota

modulation at similar concentrations. Preliminary trials in our laboratory further confirmed that this concentration resulted in improved feed conversion efficiency without adverse effects on fish health. Specifically, AuNPs were ultrasonically dispersed in distilled water using an Elma ultrasonic bath (Germany) at 35 kHz for 10 min (Banaee et al., 2016) to ensure even nanoparticle distribution. The treated solution was thoroughly mixed with ground basal feed, pelleted, and sun-dried for 6 hours. The diets were stored at -20°C until use.

Aquarium setup and experimental design: The fish were sourced from a commercial hatchery and acclimated in a controlled laboratory environment before the experiment. The trial used six rectangular glass aquaria (60×45×45 cm; ~70 L each), with three per group (control vs. AuNPs), and 60 fish per tank, for a total of 360 fish (n = 360). To ensure experimental consistency, each dietary group was maintained in three replicate aquaria. A total of 360 fish (60 per tank) were evenly distributed across the six treatments (three AuNPs and three controls). Fish were fed to apparent satiation three times daily, and water quality parameters were monitored to maintain optimal conditions.

Sample collection and processing: For gut microbiome analysis, six biological replicates were collected, with two fish randomly selected from each of the three replicate tanks per treatment group. Prior to gut collection, all dissection tools, including tweezers, scalpels, and scissors, were sterilized with 70% ethanol. The fish surface was initially rinsed with distilled water, followed by 70% ethanol for disinfection, and subsequently rinsed again with distilled water to remove residual ethanol. Under aseptic conditions, the fish were dissected, and the entire gut was extracted. Using sterile scalpels, the liver and bile ducts were carefully separated to prevent contamination. The gut samples were immediately transferred into sterilized Eppendorf tubes, flash-frozen in liquid nitrogen, and stored at -80°C for subsequent metagenomic analysis.

Extraction of eDNA and amplicon generation: The phenol-chloroform-isoamyl alcohol (PCI) extraction technique was applied to isolate total genomic DNA from the samples. The concentration and purity of the DNA were evaluated using 1% agarose gel electrophoresis. Subsequently, the DNA was diluted to ~1 μg/μL in sterile water (Liu et al., 2020). The hypervariable V3–V4 region of the 16S rRNA gene was amplified using the primers 515F (5′-GTG CCA GCM GCC GCG GTAA-3′) and 806R (5′-GGA CTA CHV GGG TWT CTA AT-3′). Each PCR reaction consisted of 15 μL of Phusion High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of each primer, and 10 ng of template DNA. The thermal cycling protocol consisted of an initial denaturation step at 98°C for 1 minute, followed by 30 cycles of denaturation at 98°C for 10 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds, concluding with a final extension at 72°C for 5 minutes. The PCR products were then combined with an equal volume of 1X loading buffer containing SYBR Green and analyzed on a 2% agarose gel (Liu et al., 2020; Liu et al., 2021; Xing et al., 2023). Equimolar amounts of the PCR products were pooled and purified using the Qiagen Gel Extraction Kit (Qiagen, Germany).

Library preparation, sequencing, and quality control: Amplicon-based metagenomic analysis was performed by Novogene (China). Library construction was performed using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer's instructions, incorporating index codes for sample identification. Measurements were taken using the Qubit® 2.0 Fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 system to assess the quality of the prepared libraries. Sequencing was performed on an Illumina NovaSeq 6000 platform (PE250 mode), generating 250 × 2 bp paired-end reads corresponding to the V3–V4 hypervariable region of the 16S rRNA gene. Sample demultiplexing was performed by identifying unique barcode sequences, followed by trimming barcode and primer sequences from the raw reads (Liu et al., 2021; Liu et al., 2020; Xing et al., 2023). Paired-end reads were merged using FLASH (v1.2.7), a bioinformatics tool that efficiently aligns overlapping sequences from

opposite ends of the same DNA fragment (Magoč and Salzberg, 2011). The resulting merged sequences, referred to as raw tags, were subjected to quality filtering under stringent parameters to produce high-quality clean tags, as described by Bokulich et al. (2013) and implemented in QIIME 2 (version 2019.4). The UCHIME algorithm (Edgar et al., 2011) was used to remove potential chimera sequences by comparing sequences against the SILVA reference database. Any identified chimeric sequences were subsequently filtered using an approach similar to that described by Haas et al. (2011), retaining only effective tags for downstream analyses.

Data filtering and analysis process: The raw data were merged and filtered to produce clean, valid data. The valid data were then denoised using DADA2, and sequences with an abundance of less than 5 were filtered out to get the final amplicon sequence variants (ASVs) (Li et al., 2020). Species annotation was performed for each ASV to obtain the corresponding species information, and a species-based abundance distribution was generated (Callahan et al., 2017). ASVs were analyzed by abundance. Alpha diversity was calculated to explore species richness and evenness, and a Venn diagram was constructed to visualize the common and unique ASVs. To visualize variation in community structure and species composition (beta diversity) among samples, PCoA and bar plots were constructed in R.

ASV analysis: The DADA2 method was mainly used for noise reduction. It no longer clusters by similarity; instead, it performs dereplication (i.e., at 100% similarity) (Callahan et al., 2016). Each deduplicated sequence generated after denoising using DADA2 is referred to as an ASV. The DADA2 method is more sensitive and specific than the traditional OTU method and can detect biological variation missed by the OTU method while producing fewer false sequences (Callahan et al., 2019). Replacing OTUs with ASVs improves the accuracy, comprehensiveness, and reproducibility of marker-gene data analysis (Amir et al., 2017). The classify-sklearn algorithm (Bokulich, 2018a; Bolyen, 2019) of QIIME 2 (version 2019.4) was used to annotate taxa using a pre-trained Naive Bayes classifier for each ASV. Based on ASV annotation results and feature tables for each sample, species abundance tables were generated at the kingdom, phylum, class, order, family, genus, and species levels. Relative-abundance plots were generated with QIIME 2 plugins.

Statistical analysis: Group differences in α-diversity (Chao1, Shannon, and Simpson) were tested with two-sided t-tests (or Wilcoxon rank-sum tests when normality was not met). β-diversity differences were evaluated with PERMANOVA on unweighted UniFrac distance matrices (999 permutations). Differentially abundant taxa were identified with LEfSe (Kruskal–Wallis $P<0.05$; linear discriminant analysis [LDA] score > 2.0), restricting the analysis to taxa present in ≥20% of samples; false-discovery rate control (Benjamini–Hochberg) was applied where appropriate. Predicted functional profiles were inferred from ASV tables with PICRUSt2 to estimate KEGG pathway abundances.

Data deposition: Raw 16S rRNA amplicon reads were submitted to the NCBI Sequence Read Archive under BioProject PRJNA1193237 and linked to BioSamples SAMN45134591–SAMN45134596 (Control 1-3; AuNPs 1-3). Additional BioSamples SAMN45134597–SAMN45134599 (silver-nanoparticle group) belong to the same BioProject but are not analyzed here.

Results

Sequencing outcomes: The final body weight was 1.77 g in the control diet and 2.27 g in the AuNPs-treated diet. High-throughput 16S rRNA (V3–V4) amplicon sequencing was used to characterize gut microbial communities in *M. vittatus* fed commercial versus AuNP-enriched diets. At the domain level, 98.99% of reads were assigned to Bacteria and ~1.0% to Archaea. In total, 641,047 raw paired reads were generated; after quality control and chimera removal, 607,676 merged, non-chimeric reads (Q30 = 94.3%; mean length ~256 bp) remained, resolving 716 amplicon sequence variants (ASVs) spanning 20 phyla, 33 classes, 75 orders, 129 families, and 190 genera. The average Phred Q30 score (94.30%)

Table 1. Alpha diversity index of control and AuNPs-treated units

Sample Name	chao1	Goods coverage	Observed OTUs	Shannon	Simpson
CR1	72.666667	0.999963776	71	2.67443878	0.75588318
CR2	73	0.999993963	73	2.79318694	0.804096068
CR3	29	0.999993963	29	1.93370793	0.659884537
AuNPs1	216.3	0.999981888	216	3.69566177	0.848015198
AuNPs2	49	0.999993963	49	2.23923105	0.706878832
AuNPs3	465.11111	0.999987925	465	4.59155928	0.867849372

indicated high data quality.

Analysis of gut microbiome diversity: The Venn diagram showed 574 and 85 ASVs unique to the AuNP-treated and control groups, respectively, with 57 ASVs shared (Fig. 1). Alpha-diversity indices (Chao1, Shannon, Simpson, Pielou's evenness, dominance, Good's coverage) are summarized in Table 1. Good's coverage averaged 0.99 in both groups, indicating adequate sequencing depth. Although mean Chao1 (58.22 control; 243.47 AuNPs), Shannon (2.4611 control; 3.5088 AuNPs), and Simpson (0.7399 control; 0.8076 AuNPs) were numerically higher in the AuNPs group, between-group differences were not significant (P>0.05) (Fig. 2). Beta diversity (unweighted UniFrac, PCoA) showed substantial overlap between groups with no distinct clustering and no significant difference by PERMANOVA (P>0.05) (Fig. 3). The largest pairwise dissimilarity was observed between samples Control-R1 and AuNPs-R2.

Taxonomic characterization: Across both groups, five phyla dominated: Proteobacteria, Bacteroidota, Firmicutes, Fusobacteriota, and Actinobacteriota (Fig. 4). In controls, relative abundances were ~98.20% Proteobacteria, 0.14% Bacteroidota, 0.31% Firmicutes, 1.26% Fusobacteriota, and 0.06% Actinobacteriota. In the AuNPs group, these were 48.55, 19.88, 22.02, 7.44, and 1.34%, respectively. Spirochaetota, Chloroflexi, and Euryarchaeota were detected only in AuNP-treated fish; Crenarchaeota and Myxococcota were absent in both groups.

At the genus level, ~20 abundant genera were observed (Fig. 5). In controls, *Plesiomonas* (1.32%), *Mycoplasma* (0.002%), *Cetobacterium* (1.263%), *Romboutsia* (0.004%), and *Bacillus* (0.065%) were among the most represented. In AuNP-treated fish,

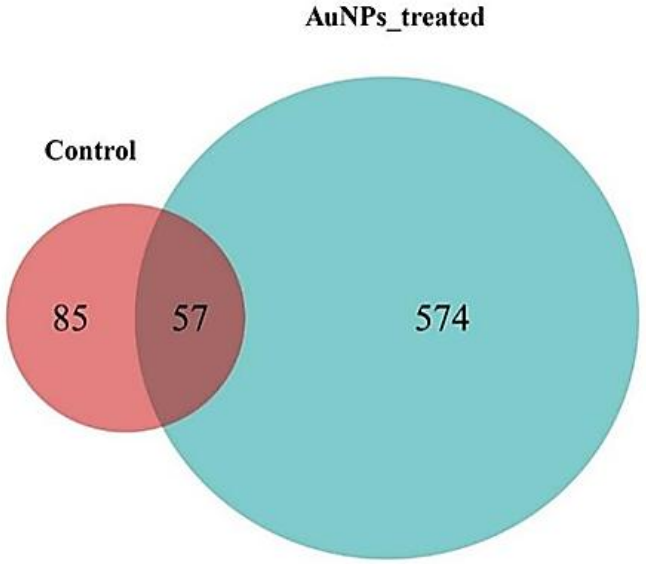

Figure 1. Venn diagram of amplicon sequence variants (ASVs).

these genera increased to 41.66, 10.15, 7.44, 3.095, and 2.204%, respectively. Several genera were detected only in the AuNP group, including *Ralstonia*, *Rikenellaceae*_RC9_gut_group, *Candidatus arthromitus*, *Family_XIII_AD3011_group*, and *Undibacterium*. These taxa exhibited the largest between-group differences and were identified as candidate biomarkers enriched with AuNP supplementation by univariate testing.

Predicted functional profiling: PICRUSt2-based KEGG annotations indicated that Level-1 categories were dominated by Metabolism, followed by Environmental Information Processing and Genetic Information Processing, with a higher proportion of Metabolism and Genetic Information Processing in the AuNPs group (Fig. 6). At Level-2 (41 sub-pathways), membrane transport, carbohydrate metabolism, amino-acid metabolism, and replication/repair were most abundant (Fig. 7). Relative to controls, AuNP-treated fish showed higher

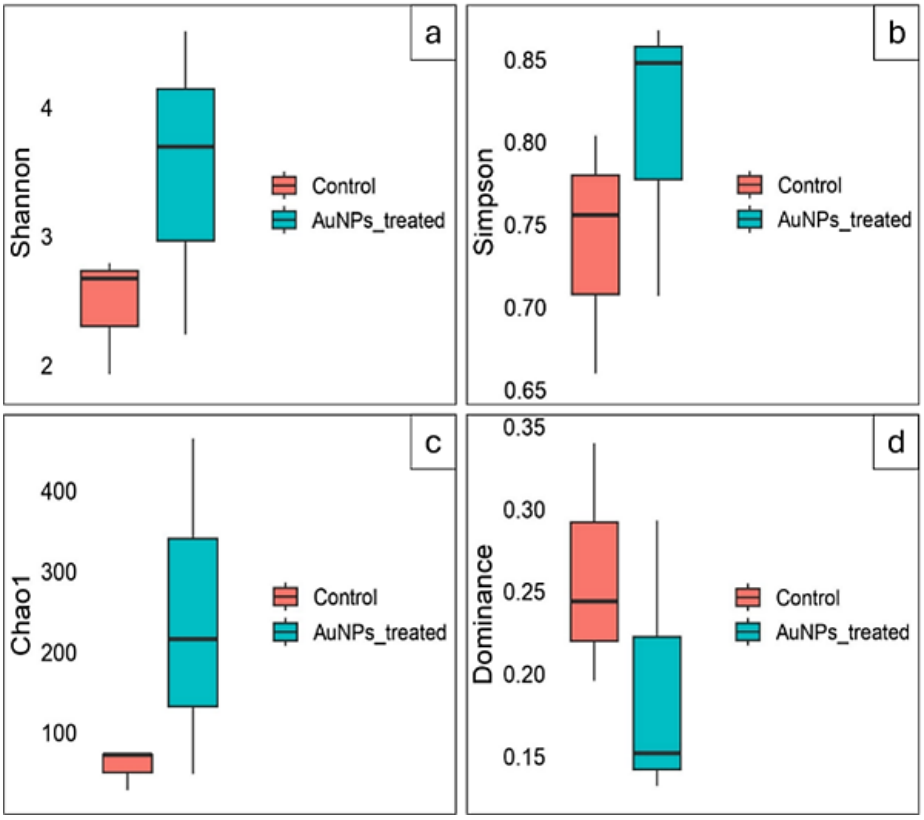

Figure 2. Alpha diversity indices: (a) Shannon, (b) Simpson, (c) Chao1 and (d) Dominance.

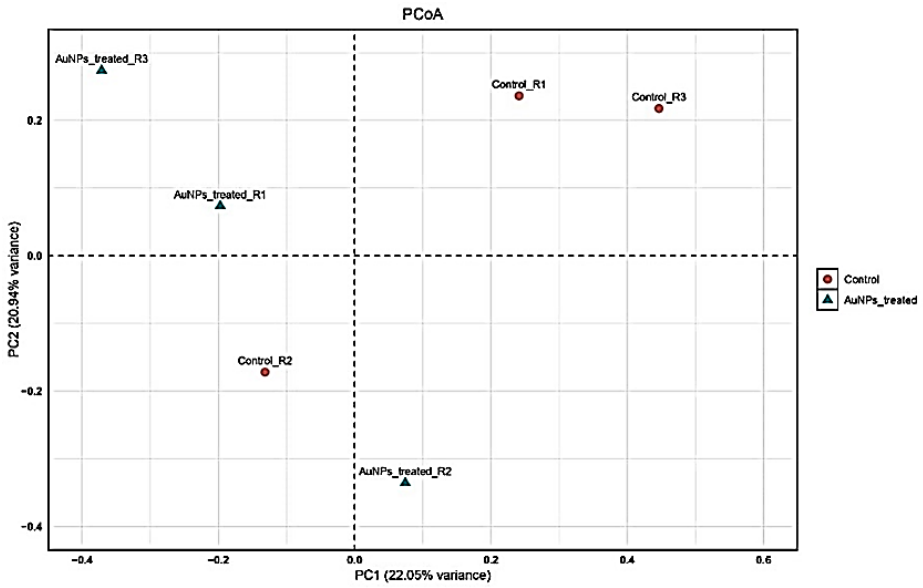

Figure 3. Beta diversity visualized through PCoA plot.

predicted abundances in amino-acid, energy, nucleotide metabolism, and metabolism of cofactors and vitamins, whereas carbohydrate metabolism (e.g., glycolysis, fructose/mannose, starch/sucrose) tended to be higher in controls. At Level-3, pathways such as ribosome and purine metabolism, multiple amino-acid metabolism modules (glycine/serine/threonine; valine/leucine/isoleucine; histidine; phenylalanine; lysine biosynthesis), lipid pathways (glycerophospholipid, lipopolysaccharide, fatty-acid metabolism/biosynthesis, glycolipid), and vitamin/cofactor pathways (folate biosynthesis,

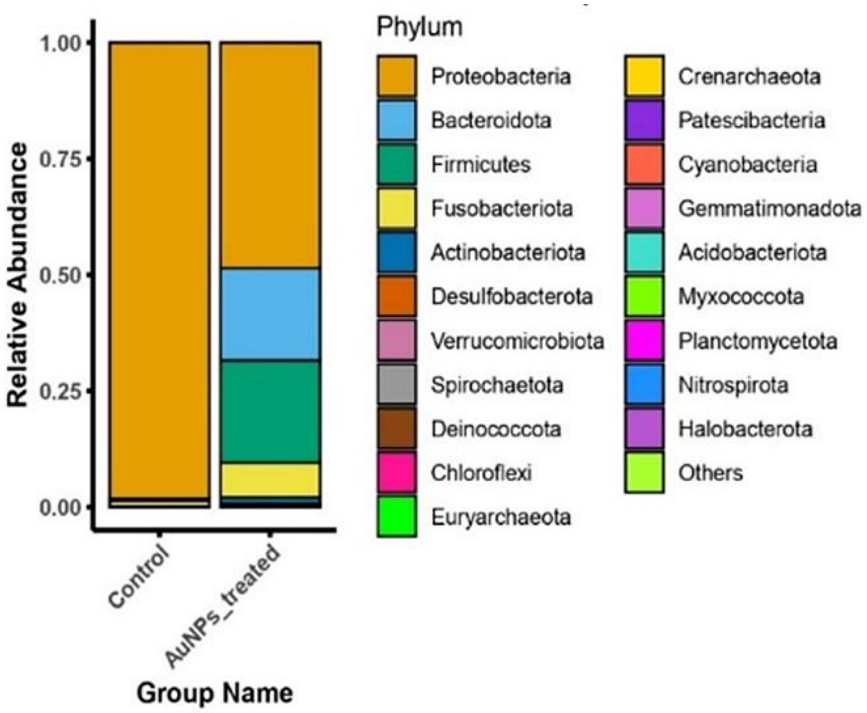

Figure 4. Gut microbiota composition of *Mystus vittatus* at the phylum level. Multi-colored bar plots show the relative abundance of microbial phyla.

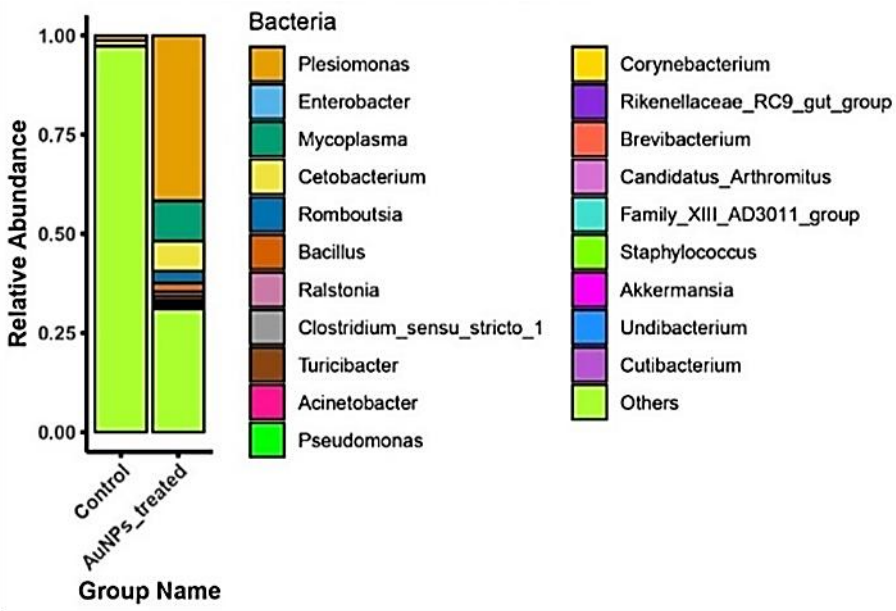

Figure 5. Gut microbiota composition of *Mystus vittatus* at the genus level. Multi-colored bar plots showing the relative abundance of microbial genera.

riboflavin metabolism) were more abundant in the AuNPs group (Fig. 8). Overall, the functional profile in AuNP-treated fish was consistent with enhanced capacities for amino-acid and energy metabolism and for replication/repair.

Discussions

Gut microbiota composition and diversity: This study provides the first 16S rRNA amplicon–based characterization of the gut microbiota of captive *M. vittatus*. Sequencing depth was robust (Goods coverage ~1), and 716 ASVs were resolved, spanning 20 phyla (notably Proteobacteria, Bacteroidota, Firmicutes, Fusobacteriota, and Actinobacteria). These results indicate a diverse gut community in *M. vittatus* consistent with previous work in teleosts.

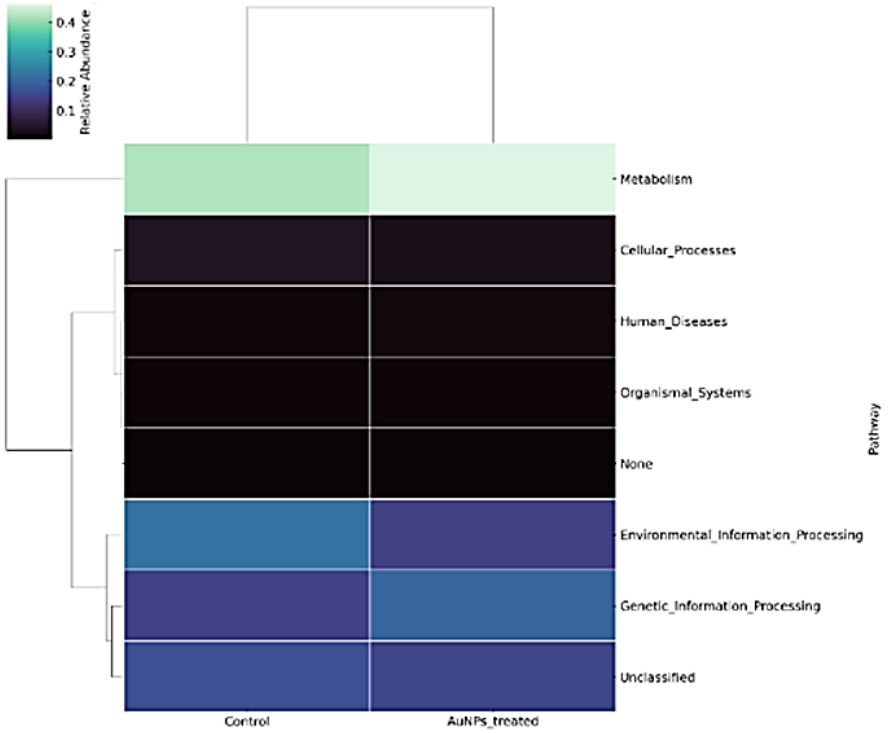

Figure 6. Heatmap of clustering analysis of the KEGG pathway at level 1. Multiple colors showing the relative abundance of microorganisms associated with the function.

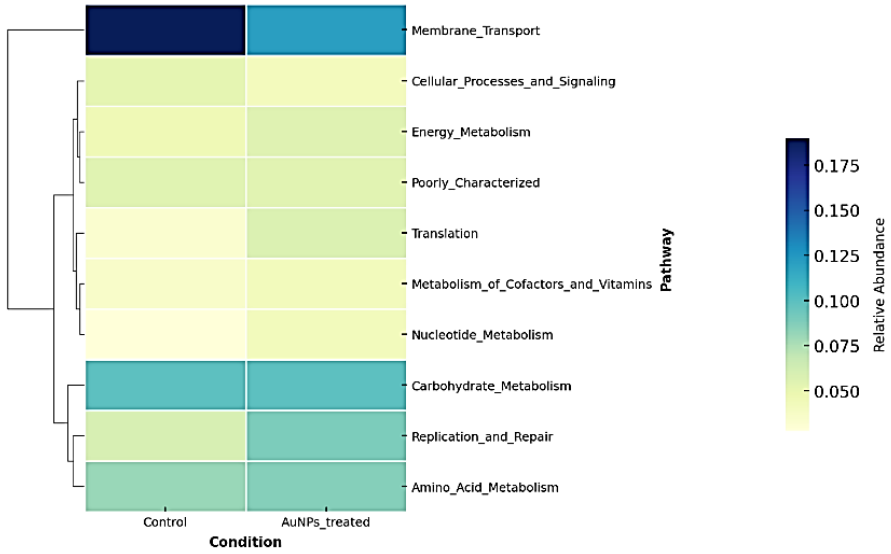

Figure 7. Heatmap of clustering analysis of the top KEGG pathway at level 2. Multiple colors showing the relative abundance of microorganisms associated with the function.

Impact of AuNP supplementation on microbial diversity: We tested a single dietary dose (50 mg/kg) that previously yielded the best growth performance (Shahariar et al., 2024). Alpha-diversity indices (Chao1, Shannon, Simpson) were numerically higher in the AuNP group but not statistically different, indicating a trend rather than a confirmed increase in richness/evenness. Importantly, the higher Simpson value in AuNP-treated fish reflects greater evenness (not decreased diversity). Beta diversity (unweighted UniFrac) also did not differ significantly, suggesting that AuNPs modulated community structure without producing a wholesale community shift.

Functional roles of dominant taxa: Core phyla were broadly similar between groups, but relative abundances shifted: the AuNP diet reduced extreme

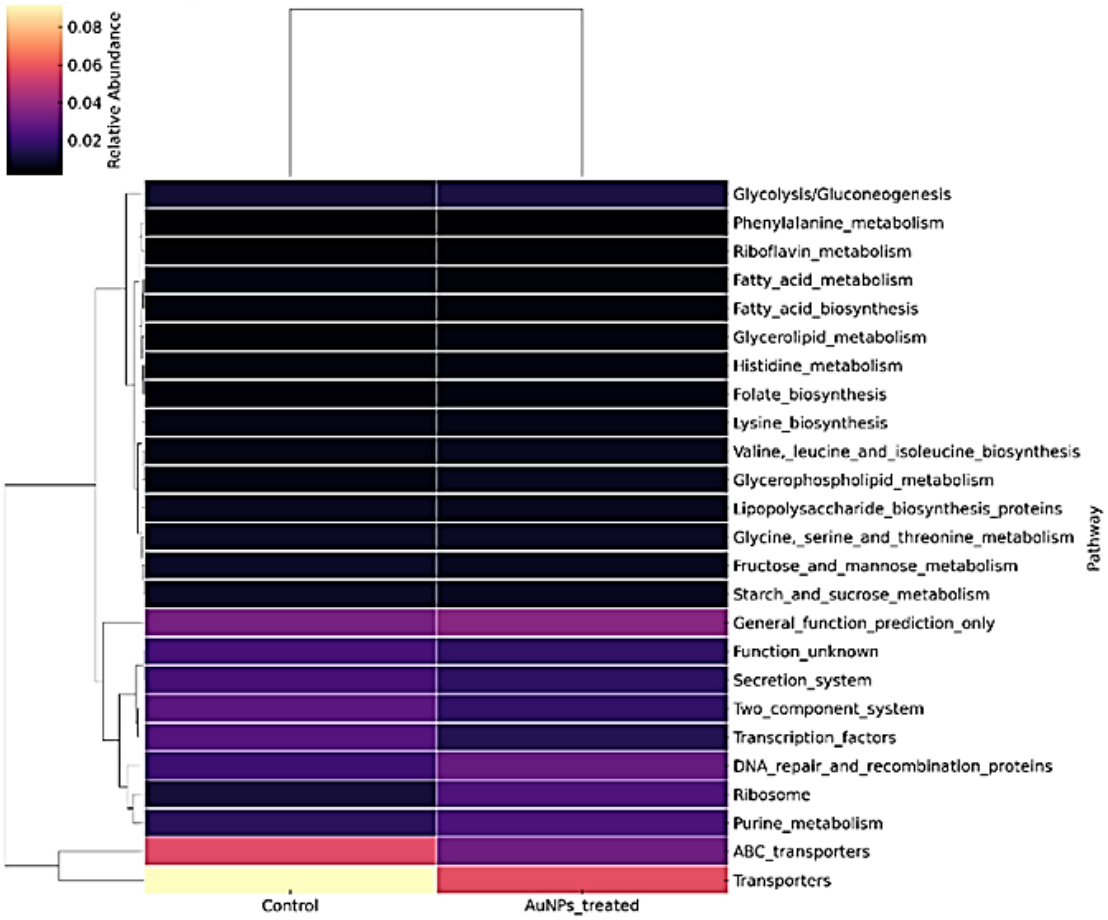

Figure 8. Heatmap of clustering analysis of the top KEGG pathway at level 3. Multiple colors showing the relative abundance of microorganisms associated with the function.

Proteobacteria dominance and increased Bacteroidota and Firmicutes—phyla linked to polysaccharide degradation, SCFA production, and energy harvest (Kersters et al., 2006; Roeselers et al., 2011; Corrigan et al., 2015; Gharechahi and Salekdeh, 2018). Similar phylum-level constellations have been reported in other cultured fishes (e.g., *Spinibarbus sinensis*; Deng et al., 2024) and are consistent with diet-responsive gut ecosystems (Ghanbari et al., 2015).

At the genus level, *Plesiomonas*, *Mycoplasma*, *Cetobacterium*, *Romboutsia*, and *Bacillus* increased in AuNP-fed fish. *Mycoplasma* and *Cetobacterium* have been associated with gut health and growth in teleosts (Rasmussen et al., 2022; Zhang et al., 2022), whereas *Romboutsia* is involved in glycometabolism (Fan et al., 2024). *Bacillus*, a common probiotic in aquaculture (Balcázar et al., 2006; Aly et al., 2008; Nayak, 2010), also increased, suggesting compatibility of AuNP supplementation with probiotic-like taxa. Conversely, *Plesiomonas* includes strains that can be pathogenic in farmed fish (Duman et al., 2023), so its increase warrants monitoring in future trials (e.g., strain typing or targeted qPCR) to rule out adverse effects.

Candidate indicator taxa: Several genera—*Ralstonia*, *Rikenellaceae_RC9_gut_group*, *Candidatus arthromitus*, *Family_XIII_AD3011 _group*, and *Undibacterium*—were detected only in the AuNP group and emerged as candidate biomarkers. Reported functions include complex carbohydrate degradation (*Rikenellaceae*), host-linked SCFA/vitamin contributions (*Candidatus arthromitus*), and amino-acid–related correlations (*Undibacterium*) (Escalas et al., 2022; LeBlanc et al., 2017; Wu et al., 2021). Because some *Ralstonia* species are occasionally reported as low-biomass or reagent contaminants, inclusion of extraction/PCR negatives is advisable in future work to confirm

biological signal. Overall, these taxa merit validation across larger cohorts and with orthogonal differential abundance methods.

Predicted functional shifts: PICRUSt2 predictions indicated higher capacities for amino acid, energy, nucleotide, and cofactor/vitamin metabolism, and for replication/repair in AuNP-fed fish, whereas carbohydrate metabolism modules tended to be higher in controls. Similar feed-responsive shifts in metabolic potential have been observed in other fishes (Ni et al., 2014). Given the higher final body weight observed in the AuNP group, these predicted functions are coherent with improved nutrient processing; however, they remain inferences from 16S data and should be confirmed with shotgun metagenomics or metatranscriptomics.

Implications and future directions: Dietary AuNPs at 50 mg/kg were associated with compositional shifts toward a more even community and with predicted gains in metabolic functions relevant to growth and immune readiness. From an application standpoint, AuNPs could complement probiotic strategies (e.g., *Bacillus*) to support feed efficiency and resilience. Priorities for future work include (i) multi-dose trials to define an optimal window and safety margins; (ii) confirmation of predicted functions by shotgun omics; (iii) strain-level or targeted assays for taxa of interest (e.g., *Plesiomonas*, *Candidatus arthromitus*); and (iv) incorporation of negative controls and environmental/water microbiome profiling to contextualize tank effects.

Limitations: Key limitations include a moderate sample size (three gut samples per group), use of a single AuNP dose, reliance on 16S amplicon data (limited taxonomic/functional resolution), and predictive (not measured) functional profiling. While alpha/beta diversity trends were encouraging, the lack of statistical significance means conclusions about diversity should be interpreted cautiously. These constraints define clear next steps and do not detract from the main conclusion that AuNP supplementation can modulate the *M. vittatus* gut microbiome toward functions consistent with improved nutrient metabolism.

Conclusion

This 16S rRNA (V3–V4) amplicon study provides the first evidence that a dietary dose of biosynthesized gold nanoparticles (50 mg/kg) can modulate the gut microbiota of *M. vittatus*. While α- and β-diversity did not differ significantly between groups, AuNP feeding shifted community composition, including increases in putatively beneficial genera (e.g., *Bacillus*, *Mycoplasma*, *Cetobacterium*) and the appearance of candidate indicator taxa (*Ralstonia*, *Rikenellaceae_RC9_gut_group*, *Candidatus arthromitus*, *Family_XIII_AD3011_group*, *Undibacterium*). Functional predictions (PICRUSt2) indicated enrichment of pathways related to amino-acid and energy metabolism, nucleotide metabolism, cofactor/vitamin metabolism, and replication/repair in the AuNP group—profiles consistent with more efficient nutrient processing and immune readiness. Taken together, these results suggest that AuNP supplementation can steer the *M. vittatus* gut microbiome toward functions that may support growth and resilience, positioning AuNPs as a promising complement to conventional feed additives. Nonetheless, conclusions regarding function remain predictive, and trends in diversity were not statistically significant.

Acknowledgments

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Ethics approval and consent to participants: All experimental procedures followed the guidelines of the Institutional Animal Research Ethics Committee (AREC) of Gazipur Agricultural University (Ref. No. FVMAS/AREC/2023/31).

Data availability: The raw 16S rRNA amplicon reads are deposited in the NCBI Sequence Read Archive under BioProject PRJNA1193237 and BioSamples SAMN45134591–SAMN45134596. Records are scheduled for public release on 2025-12-31 (or earlier upon article acceptance). During peer review, data are available from the corresponding author. SRA-run

accessions (SRRs) will be added upon assignment.

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