Microbial Ecology
https://doi.org/10.1007/s00248-022-01993-y

MICROBIOLOGY OF AQUATIC SYSTEMS

Successional Changes of Microbial Communities and Host-Microbiota Interactions Contribute to Dietary Adaptation in Allodiploid Hybrid Fish

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Received: 22 December 2021 / Accepted: 7 March 2022

Abstract

Host-microbiota interactions play critical roles in host development, immunity, metabolism, and behavior. However, information regarding host-microbiota interactions is limited in fishes due to their complex living environment. In the present study, an allodiploid hybrid fish derived from herbivorous *Megalobrama amblycephala* (♀) × carnivorous *Culter alburnus* (♂) was used to investigate the successional changes of the microbial communities and host-microbiota interactions during herbivorous and carnivorous dietary adaptations. The growth level was not significantly different in any developmental stage between the two diet groups of fish. The diversity and composition of the dominant microbial communities showed similar successional patterns in the early developmental stages, but significantly changed during the two dietary adaptations. A large number of bacterial communities coexisted in all developmental stages, whereas the abundance of some genera associated with metabolism, including *Acinetobacter*, *Gemmobacter*, *Microbacterium*, *Vibrio*, and *Aeromonas*, was higher in either diet groups of fish. Moreover, the abundance of phylum Firmicutes, Actinobacteria, and Chloroflexi was positively correlated with the host growth level. In addition, Spearman's correlation analysis revealed that the differentially expressed homologous genes in the intestine associated with cell growth, immunity, and metabolism were related to the dominant gut microbiota. Our results present evidence that host genetics-gut microbiota interactions contribute to dietary adaptation in hybrid fish, which also provides basic data for understanding the diversity of dietary adaptations and evolution in fish.

Keywords Hybrid fish · Microbial communities · Dietary adaptation · Host development · Host-microbiota interactions

Introduction

The ubiquity and importance of the gut microbiota is supported by its influence on host development, metabolism, immunity, and numerous other processes including behavior and speciation [1–3]. Acquisition of microbiota by animal hosts during development marks the onset of microbial symbiosis, which is followed by the adaptation of these microbial communities to the gut environment for prolonged sustenance. Generally, gut microbial communities can be divided into two groups: resident (autochthonous) and transient (allochthonous) communities. Resident communities can adhere to and colonize mucosal surfaces or occur within epithelial tissues, where they become more intimately associated with host cells [4]. Transient communities are characterized as nonadherent, free-living microorganisms that are generally excluded after some period [5].

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Published online: 02 April 2022

Teleost fishes are the most diverse clade of vertebrates, with over 34,000 living species, and are considered the most successful vertebrates to evolve on Earth [6]. Successful fish evolution may not have been possible without the help of gut microbiota [7]. In recent years, our understanding of the fish gut microbiota has significantly improved with the development of next-generation sequencing (NGS) platforms. Like other animals, it is now clear that fish are able to select and enrich microbial communities from their surrounding environment. Studies from certain fish species have demonstrated that egg surfaces are rapidly colonized by microbiota from the surrounding water, with further microbiota colonizing the outer surfaces and gastrointestinal tract of posthatch larvae through the ingestion of water [8–10]. Then, the successive changes in the diversity and composition of gut microbiota occur throughout host development and interact with various internal and external factors, such as host genetics (genotype, sex, age, immune system, and gene expression), diet source, temperature, pH, and salinity [11–14]. Information regarding the host-microbiota and microbe-microbe systems suggested that a diverse balanced microbiota was critical for healthy functioning in fish, but the mechanisms behind these interactions remain elusive. Indeed, mounting evidence has revealed that host genetics (mainly genotype) and diet source have a larger influence on gut microbiota enrichment and succession than other factors in fish [14–16]. To date, much of the research on fish microbiota has been derived from laboratory models or important farmed species, and the newly formed hybrid lineage fish are still poorly studied.

In our previous studies, reciprocal intergeneric hybrid lineages were successfully obtained from hybridization between the herbivorous blunt snout bream (BSB, *Megalobrama amblycephal*a) and the carnivorous topmouth culter (TC, *Culter alburnus*) [17, 18]. The allodiploid hybrid fish derived from female BSB × male TC was bisexual fertile and contained 48 chromosomes with one set from BSB and one set from TC [17]. The divergent genomes merged in the hybrid somatic cells together present an ideal model to investigate some interesting biological processes, such as genetic stability (gene evolution and regulations), phenotypic variation, development, reproduction, and breeding [18–20]. Interestingly, the hybrid fish was herbivore, which increased germplasm resources of herbivorous fish. Subsequently, two studies documented that the parent host genomic interaction had a sizeable effect on shaping the gut microbiota assemblages [21] and dietary adaptation [22]. However, the interaction between host genetics and microbiota assemblages during dietary adaptation has not been studied. In this study, hybrid F_1 derived from a female BSB × male TC were used to investigate successive changes in gut microbiota and their correlations with differentially expressed homologous genes (DEHs) during herbivorous and carnivorous dietary adaptation. This study may provide basic data for understanding the diversity of dietary adaptations and evolution in fish, and also provide perspectives in fish ecology.

Methods

Experimental Fish

Hybrid F_1 fish were obtained from female BSB × male TC as described in our previous studies [17, 22]. After crossing, the fertilized embryos, larvae, and juveniles were all raised in the Engineering Center of Polyploidy Fish Breeding of the National Education Ministry located at Hunan Normal University, China.

Experimental Setup and Sampling

Experiment 1: The fertilized embryos of hybrid fish were immediately transferred and hatched in a 200-L recirculating water system (25 ± 1.0 °C, 6.5 ± 0.5 mg/L dissolved oxygen). The embryo development hatched into larvae fish after about ~ 40 h. At 5 days post-hatching (dph), the larvae fish freely swam in the water. At 7 dph, approximately 2000 individuals remained and the first feeding was fed with *Artemia*. During the breeding process, the larval fish were exposed to ambient light concentrations and fed *Artemia* routinely three times a day at 8:00, 13:00, and 18:00 o'clock. The amount of food was gradually increased according to the fish body weight gains. Fecal samples were collected at 10, 20, 40, and 60 dph (each time point $n = 3$) (Fig. S1). The bottom feces were carefully scraped within 1 h after feeding, and the collected feces were then immediately stored at − 80 °C. The body weight (BW) of the larval fish at 20, 40, and 60 dph (each time point $n = 100$) were recorded.

Experiment 2: After experiment 1, the fish were transferred to a tank and then raised in two separate cages (each included 200 individuals) (Fig. S1). Moreover, the dietary resources of the two groups of fishes were changed. One group of fish was fed *Chironomid* larvae (defined as carnivorous), and another was fed artificial fodder and duckweed (defined as herbivorous) routinely two times a day at 9:00 and 16:00 o'clock. The artificial fodder included the following components (per 100 g): fish meal 5.00 g, soybean meal 30.00 g, rapeseed meal 20.00 g, rice bran 35.00 g, and fish oil 3.50 g, among others. The duckweed was collected and washed by double distilled water to remove inverts before feed. During the breeding process, the water temperature ranged from 25 ~ 28 °C, and the photoperiod was natural. The two groups of fish were sampled at 90 and 120 dph. Prior to dissection, the experimental fish were deeply anesthetized with 50 mg/L MS-222 (Sigma-Aldrich, St. Louis,

MO, USA). The allodiploid fish were chosen by DNA content analysis [17]. The intestinal contents (each group $n = 3$ individuals) were scraped and immediately stored in a − 80-°C freezer until bacterial DNA extraction. The remaining experimental fish were raised in the same environment. The BW, folk length (FL), and intestinal length (IL) of the experimental fish at 70, 90, 120, and 150 dph ($n = 10$) were recorded.

DNA Extraction and Bacterial 16S rRNA Gene Sequencing

The QIAmp© Fast DNA stool mini kit (Qiagen Inc., Valencia, CA, USA) was used to extract bacterial DNA of a total of 24 samples according to the manufacturer's instructions, and the quality of DNA checked on 1% agarose gel. DNA concentration and purity were determined with NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). Partial DNA fragments of bacterial 16S rRNA genes were amplified by touchdown PCR, as it is the optimal method for avoiding eukaryotic contamination. Variable regions (V3 ~ V4) of the bacterial 16S rRNA genes were amplified with a primer pair (515F: 5′-GTGCCAGCMGCCGCGGTAA-3′ and 806R: 5′-GCACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, USA). PCR products were subsequently quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Equal amounts of each sample were combined and gel-purified using a QIAquick Gel Extraction Kit (QIAGEN, USA) before being re-quantified using PicoGreen. The prepared DNA library was sequenced by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) using the MiSeq platform (2 × 300 bp, Illumina, San Diego, USA). The sequencing data in this study were submitted to the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) (accession number PRJNA788359).

Sequence Data Processing

The raw sequencing reads of each sample were demultiplexed, quality filtered by Trimmomatic, and merged by FLASH [23], and total operational taxonomic units (OTUs) were generated. Then, the OTUs with 97% similarity cut off were clustered using UPARSE (version 7.1, http://drive5.com/uparse/), and chimeric sequences were identified and removed [24]. Then, the taxonomy of each OTU representative sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the 16S rRNA database (SILVA SSU138.1) using confidence threshold of 0.7 [25]. Finally, the OTU sequences less than 5 in each sample or total OTU sequences less than 10 of each group (three samples) was removed, respectively. A combined total of 1,275,319 16S rRNA gene sequences (532.4 Mbp) from 24 samples (12 from the feces and 12 from the gut contents) was generated (Table 1). These sequences represented a total of 1081 effective OTUs, 475 genera, and 23 phyla (Supplemental File 1).

Data Analysis

The intestinal DEHs from carnivorous and herbivorous dietary fish (at 120 dph) used here were derived from our previous study [22]. Namely, total RNA from intestine tissues were extracted; after first (second)-strand cDNA synthesize, the fragment cDNA sequenced on an Illumina HiSeq 2000 platform. The total clean reads of each sample were aligned to the two parent genomes by using HISAT software based on the species-specific SNPs [18]. DEseq2 in R software was used to search for differentially expressed homoeolog genes (DEHs) with a false discovery rate (FDR) < 0.01 and a threshold normalized absolute log twofold change > 1.0. Alpha diversity (including Sobs, Shannon, Simpson and ACE diversity indices) was determined for each sample using QIIME [26]. Principal component analysis (PCA) was visualized via the R Project (http://www.r-project.org/) based on weighted UniFrac distances. A Venn diagram of

Table 1 Basic information of 16S RNA gene sequencing data

Groups	Samples	Seq no	Base no	Stages	Dietary type
Hy_1	F1_1	58,743	24,739,689	10 dph	Carnivorous
	F1_2	54,173	22,771,279		
	F1_3	55,027	23,225,602		
Hy_2	F1_4	34,600	14,469,428	20 dph	Carnivorous
	F1_5	48,748	20,659,338		
	F1_6	58,273	24,277,663		
Hy_3	F1_7	48,941	20,769,747	40 dph	Carnivorous
	F1_8	55,302	23,519,501		
	F1_9	53,189	22,581,649		
Hy_4	F1_10	58,387	24,204,975	60 dph	Carnivorous
	F1_11	57,096	23,568,966		
	F1_12	57,928	23,967,974		
Hy_5	F1_13	48,656	19,970,901	90 dph	Herbivorous
	F1_14	57,811	23,815,309		
	F1_15	51,724	21,356,787		
Hy_6	F1_16	49,094	20,600,526	90 dph	Carnivorous
	F1_17	52,511	22,251,410		
	F1_18	51,911	21,721,458		
Hy_7	F1_19	64,983	26,835,400	120 dph	Herbivorous
	F1_20	52,488	21,639,631		
	F1_21	61,869	25,842,272		
Hy_8	F1_22	50,407	20,813,330	120 dph	Carnivorous
	F1_23	47,723	19,898,621		
	F1_24	45,735	18,901,165		
	Total	1,275,319	532,402,621		

shared and unique genera was used to describe the similarities and differences among the fish in the different dietary groups. Redundancy analysis (RDA) and canonical correspondence analysis (CCA) were used to analyze the relationships between growth factors (including BW, FL, IL) and dominant microbial communities (at the phylum and genus levels, respectively) [27]. Spearman's correlation analysis was used to investigate the relationship between the dominant microbial communities and DEHs (strong correlation cutoff $|r|>0.6$ and $p<0.05$) [28]. Significantly abundant phyla or genera were identified using linear discriminant analysis (LDA) effect size (LEfSe) [29], which detected the significant ($p<$ value cutoff 0.05 and LDA cutoff 2.0) features of the respective groups. Finally, the PICRUSt software package was used to predict the metagenome functional content of microbial communities [30].

Relative gut length (RGL = IL/FL) and Zihler's index (ZI = IL (cm) × BW $(g)^{1/3}$) were used to evaluate the effects of ontogeny and diet on the gut dimensions [31]. All statistically significant differences between groups were determined using Student's *t* test and one-way ANOVA.

Table 2 Gut characteristics of hybrid fish at different developmental stages

Dietary type	90 dph		120 dph	
	RGL	ZI	RGL	ZI
Carnivorous	1.28 ± 0.04	4.08 ± 0.09	1.19 ± 0.03	6.72 ± 0.32
Herbivorous	1.36 ± 0.05	4.04 ± 0.09	1.58 ± 0.04[a]	6.84 ± 0.24

[a]A significant difference between the two dietary groups of fish ($n=10$, $p<0.05$, one-way ANOVA)

Results

Growth Level of the Hybrid Fish

The growth level of the hybrid fish at different developmental stages was recorded, and the results are presented in Table S1. No significant difference was observed in the BW, FL, IL, and ZI indices between the carnivorous and herbivorous fish. However, the RGL index in herbivorous fish was much higher than in carnivorous fish at 120 dph ($p<0.05$) (Table 2).

Successional Changes in the Diversity and Composition of the Microbial Communities of Hybrid Fish at Different Developmental Stages

Functional diversity analysis was calculated based on the distances between microbial communities at different developmental stages. The alpha diversity (Sobs index at the phylum level) of microbial communities decreased from 10 to 120 dph. Gut microbiota diversity in herbivorous groups was higher than that in carnivorous groups (Fig. 1a). Beta diversity (PCA) at the genus level revealed that the microbial communities of all 24 samples could be broadly classified into three main clusters: the fecal groups (including 10, 20 and 40 dph), the herbivorous groups (including 90,

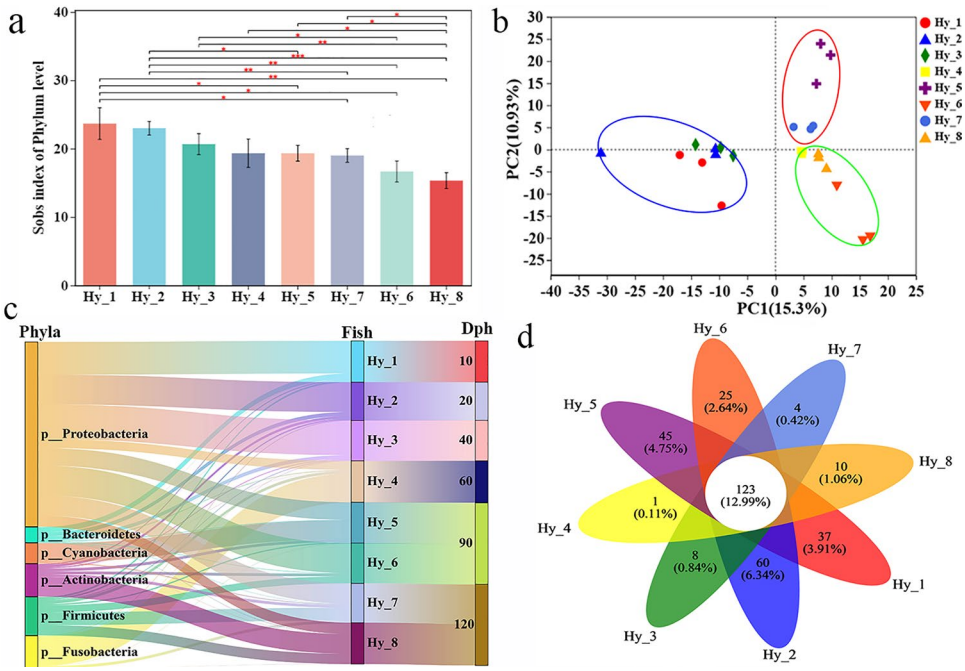

Fig. 1 Successional changes in the microbial communities of the hybrid fish at different developmental stages. **a** Alpha diversity (Sobs index) was estimated among the eight groups of fish. **b** PCA (at the phylum level) was estimates among the eight groups of fish. **c** Relative abundance of microbial communities in the hybrid fish at different developmental stages. **d** Venn diagram showing the shared genera in the hybrid fish at different developmental stages. *Significant difference between groups (*$p<0.05$, **$p<0.01$, ***$p<0.001$, Student's *t*-test). Feces groups: Hy_1, Hy_2, Hy_3, and Hy_4; herbivorous dietary group: Hy_5 and Hy_7; carnivorous diet groups: Hy_6 and Hy_8

120 dph), and carnivorous groups (including 60, 90, and 120 dph) (Fig. 1b).

Proteobacteria, Bacteroidetes, and Firmicutes were the most abundant phyla in feces at 10, 20, and 40 dph. Specifically, high relative abundances of Proteobacteria, which cumulatively accounted for > 70.0% of the microbial abundance, were observed at the early developmental stages (Table 3). At 60 dph, the abundance of Fusobacteria was significantly increased, and the abundances of Proteobacteria and Bacteroidetes were significantly decreased. In carnivorous fish (90 and 120 dph), the dominant gut microbial communities were changed to the phyla Proteobacteria, Actinobacteria, and Firmicutes. In contrast, Proteobacteria and Cyanobacteria were the most abundant phyla at 90 dph in herbivorous fish, while the abundance of Firmicutes was significantly increased and that of Cyanobacteria was significantly decreased at 120 dph (Fig. 1c).

At the genus level, a total of 123 (12.99%) genera were detected in the hybrid fish at all developmental stages, and the most abundant microbial communities included *Aeromonas*, *Cetobacterium*, *Chloroplast*, *Gemmobacter*, *Plesiomonas*, *Lactococcus*, and *Leucobacter* (Fig. 1d). However, the abundance of these dominant microbial communities was significantly changed at different developmental stages (Fig. S2).

Successional Changes in the Composition of Gut Microbiota During Different Dietary Adaptations

To better understand the effect of dietary shift on gut microbiota selection and enrichment from the surrounding environment, unique and shared bacterial taxa were analyzed. The relative abundance of microbial communities showed a significant difference at the phylum level between the two dietary groups of fish (Fig. 2a; Table 3). Interestingly, Proteobacteria was the most stable phylum in all groups of fish. The abundance of dominant microbiota (in genera level) in herbivorous fish was higher than that in carnivorous fish at both 90 and 120 dph (Fig. 2b). At the genus level, a total of 200 (27.40%) genera coexisted in the two dietary groups of fish (Fig. S3). Among these shared microbial communities, *Chloroplast*, *Gemmobacter*, *Leucobacter*, *Lactococcus*, *Aeromonas*, and *Enterobacter* were the dominant genera (Fig. 2c; Supplemental Table 2). In addition, Proteobacteria (phylum level), Alphaproteobacteria (class level), Rhizobiaceca, and Moraxellaceae (family level) were the abundant taxa in herbivorous fish. Firmicutes (phylum level), Clostridia and Fusobacteria (class level), Enterococcaceae and Fusobacteriaceae (family level), *Plesiomonas*, and *Enterococcus* (genus level) were abundant in carnivorous fish (Fig. 2d).

The abundance of some genera, including *Rhizobiales*, *Gemmobacter*, *Acinetobacter*, *Microbacterium*, *Flavobacterium*, and *Burkholderiaceae*, was much higher in fish in herbivorous groups (twofold). In contrast, the abundance of *Vibrio*, *Streptomyces*, *Shewanella*, *Aeromonas*, PeM15 and SJA-15 was much higher in carnivorous groups (Table S2). Moreover, the relative abundances of some genera, including *Chloroplast*, *Plesiomonas*, *Aeromonas*, *Leucobacter*, and *Baillus*, were significantly changed in the two dietary groups of fish at different developmental stages (Fig. S4).

Functional assessment revealed that the dominant microbiota in herbivorous fish was mainly contributed to gluconeogenesis, lysine biosynthesis, isoleucine biosynthesis, fatty acid biosynthesis, and glycolysis compared with those in carnivorous fish (Fig. 3a). In addition, KEGG analysis revealed that the dominant gut microbiota in herbivorous fish were mainly enriched in pyruvate metabolism, fatty acid elongation, carbohydrate digestion and absorption, phosphonate and phosphinate metabolism, etc., while the dominant gut microbiota in carnivorous fish mainly contributed to bile secretion, isoflavonoid biosynthesis, primary bile acid biosynthesis, alpha-linolenic acid metabolism, etc. (Fig. 3b).

Host Genetics-Gut Microbiota Interact in Hybrid Fish During Dietary Adaptation

To better understand the host genetics-gut microbiota interaction, the correlation between the fish growth factors (BW, IL, BL) and the dominant microbial communities

Table 3 Abundance of the microbial communities in different groups of the hybrid fish

Groups	Proteobacteria	Fusobacteria	Firmicutes	Bacteroidetes	Actinobacteria	Cyanobacteria
Hy_1	70.64 ± 4.63%	1.44 ± 1.26%	3.56 ± 1.69%	9.42 ± 2.87%	5.27 ± 3.16%	0.18 ± 0.17%
Hy_2	77.5 ± 7.25%	0.09 ± 0.08%	4.51 ± 1.19%	12.61 ± 5.12%	2.71 ± 0.35%	0.32 ± 0.15%
Hy_3	82.93 ± 2.01%	1.06 ± 0.78%	1.90 ± 1.40%	8.74 ± 0.91%	1.25 ± 0.58	0.17 ± 0.09%
Hy_4	21.1 ± 2.86%	69.53 ± 4.33%	6.80 ± 2.22%	1.98 ± 0.62%	0.38 ± 0.07%	0.02 ± 0.01%
Hy_5	41.82 ± 8.40%	0.54 ± 0.093%	3.15 ± 1.11%	1.38 ± 0.79%	6.68 ± 1.68%	44.93 ± 9.63%
Hy_6	47.70 ± 14.66%	6.55 ± 0.79%	30.61 ± 13.78%	0.85 ± 0.61%	6.63 ± 1.65%	2.55 ± 1.39%
Hy_7	71.95 ± 6.82%	0.01 ± 0.00%	15.70 ± 10.16%	1.07 ± 1.50%	6.71 ± 1.51%	0.01 ± 0.01%
Hy_8	26.25 ± 5.97%	0.18 ± 0.28%	24.41 ± 8.96%	0.42 ± 0.53%	46.87 ± 10.22%	0.18 ± 0.11%

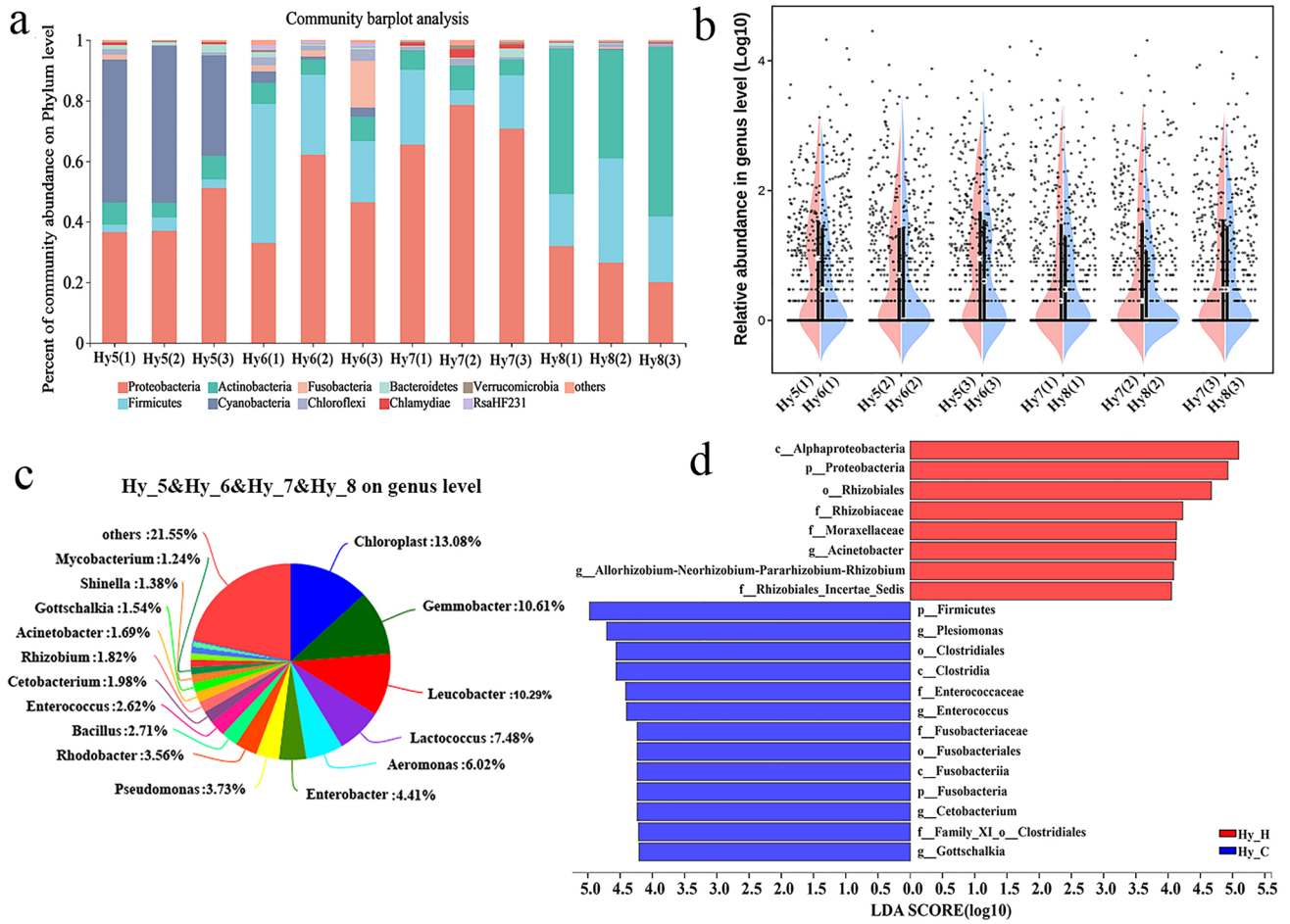

Fig. 2 Effect of dietary shifting on gut microbial communities during dietary adaptation. **a** Relative abundance of microbial communities at the phylum level of herbivorous diet groups (Hy_5 and Hy_7) and carnivorous diet groups (Hy_6 and Hy_8). **b** Relative abundance of the 50 dominant microbiota (in the genus level) between herbivorous diet groups (Hy_5 and Hy_7) and carnivorous diet groups (Hy_6 and Hy_8). **c** Abundance of the shared genera (greater than 1%) in herbivorous diet groups (Hy_5 and Hy_7) and carnivorous diet groups (Hy_6 and Hy_8). **d** Bar chart showing the significantly abundant taxa in each group of fish, identified based on LEfSe analysis (non-parametric factorial Kruskal–Wallis (KW) sum-rank test, $p < 0.05$ and effect size > 4.0). Hy_H: herbivorous diet groups, Hy_C: carnivorous diet groups

was investigated. First, RDA/CCA analysis showed a positive correlation between fish growth factors and dominant genera in the two dietary groups of fish (Fig. 4a). Specifically, a positive correlation between fish body weight and Firmicutes (coefficient = 0.008, $P = 0.0005$), Actinobacteria (coefficient = 0.003, $P = 0.0097$), and Chloroflexi (coefficient = 0.0018, $P = 0.0056$) was detected by MaAslin analysis (Fig. 4b–d). A negative correlation was detected between fish body weight and Bacteroidetes (coefficient = −0.008, $P = 0.0000017$) and Acidobacteria (coefficient = −0.0001, $P = 0.0008$) (Fig. 4e, f).

Based on the analysis above, Spearman's correlation was used to infer the relationship between the composition of the microbial communities in terms of the dominant genera and DEHs. A total of 1776 DEHs (including 838 upregulated and 938 downregulated homologous genes) and 50 dominant genera were explored. The strong relationships between the DEHs and genera are listed in Supplemental Table 3. The upregulated gene insulin-like growth factor binding protein 3 (*igfbp3*) was strongly related to *Bosea*, *Reyranella*, and *IMCC26207*, while immunoglobulin heavy chain (*igh*), fibroblast growth factor receptor 1 (*fgfr1a*), and vascular endothelial growth factor (*figf*) were strongly related to *Acinetobacter*. Specifically, a number of upregulated homologous genes from metabolic pathways (carbon metabolism, biosynthesis of amino acids, protein digestion, and absorption pathways) showed a strong correlation ($r \geq 0.6$, $p < 0.05$) with the dominant microbiota, including *Acinetobacter*, *Arenimonas*, *Enterococcus*, *Lactococcus*, and *Microbacterium*, while other downregulated homologous genes showed a negative correlation ($r \leq -0.6$, $p < 0.05$) with the dominant microbiota, such as *Bacillus*, *Leucobacter*, *Rhodococcus*, and *Streptomyces* (Fig. 5).

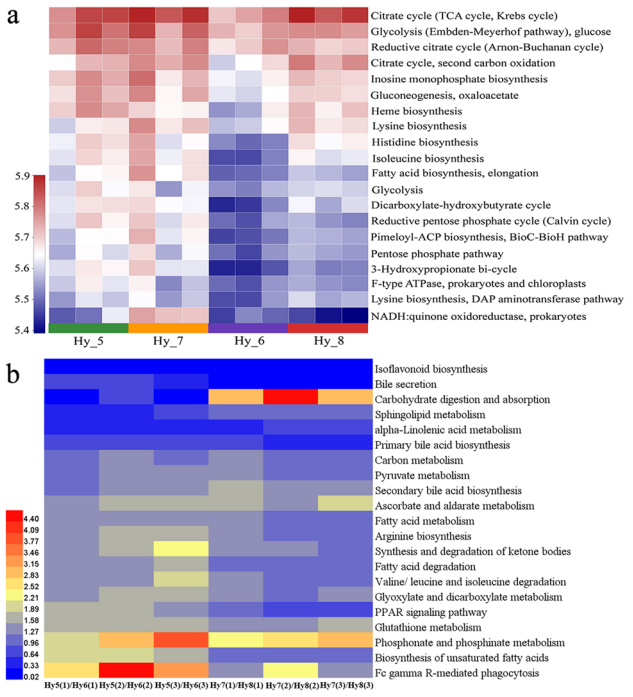

Fig. 3 KEGG functional enrichment of the dominant gut microbiota between the herbivorous and carnivorous fish. **a** Module of the dominant gut microbiota in fish in different diet groups. **b** Pathways of the dominant gut microbiota between the herbivorous and carnivorous fish. Herbivorous dietary group: Hy_5 and Hy_7; carnivorous diet groups: Hy_6 and Hy_8

Discussion

Dynamic Changes in Microbial Communities During Host Development

It is generally recognized that the gut microbiota serves several functions during early fish developmental stages, such as aiding digestion, intestinal and immune system development, and maturation [32, 33]. In this study, the composition of the dominant phyla Proteobacteria, Bacteroidetes, and Firmicutes showed similar successional patterns at the early developmental stages (Fig. 2), suggesting balanced host-microbiota or microbe-microbe interactions in the original environment. Interestingly, phylum Proteobacteria represented the largest portion (> 70%) of the total microbial communities (Table 3), suggesting that the bacteria belonging to the Proteobacteria phylum are especially well adapted to the conditions in the hybrid fish intestine [34]. Proteobacteria, such as *Vibrio*, *Gammaproteobacteria*, and the SAR324 clade marine group, are known to induce important responses in the host [32, 35]. Therefore, we suspected that the Proteobacteria phylum may play critical roles that contribute to intestinal maturation and immunity of hybrid fish at the early developmental stages.

A large number of genera, such as *Aeromonas*, *Cetobacterium*, and *Gemmobacter*, were detected coexisting in the hybrid fish at all developmental stages, indicating that these bacteria are resident communities and may contribute to host health (Fig. 2) [36]. However, the diversity of microbial communities gradually decreased, and the abundance of the dominant phyla and genera significantly changed during hybrid fish development (Fig. 2; Fig. S2). Similar results were also observed in some farmed or laboratory fish, such as Atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*), and zebrafish (*Danio rerio*) [14, 15, 37]. In discus fish (*Symphysodon haraldi*) at different development stages, the community diversity and richness of intestinal microbiota first decreased, then increased and finally decreased with the change of diets [38]. These observations indicated stage-specific signatures in intestinal microbiota assembly and succession. It is worth noting that fish retain some low abundant microorganisms in their surrounding environment which could further evolve to be more prolific colonizers, and dynamic changes in intestinal microbiota might suggest their potential in adapting to surroundings and contribute to host development.

Diet Source Shapes on Gut Morphology and Microbiota Assembly

In the present study, the growth level of the hybrid fish showed no difference after adaptation to the two diets (Table S1). This may result from the same laboratory environment and feeding intensity of the two groups of fish. However, the RGL in herbivorous fish was significantly larger than that in carnivorous fish (Table 2), in accordance with previous studies that documented that herbivorous fish possess relatively longer narrower intestines than carnivorous species [31].

Early life exposure and the establishment of stable, appropriately diverse, and resilient microbiota are likely to be critical to ensure optimum host health and nutrition as adults [9, 39]. Obviously, the gut microbiota can mature throughout host development and with changes in diet. Indeed, studies in fish under both natural and controlled conditions, such as gibel carp (*Carassius aurarius gibelio*), clownfish (*Premnas biaculeatus*), yellowtail kingfish (*Seriola lalandi*), Nile tilapia (*Oreochromis niloticus*), and zebrafish (*Danio rerio*), have revealed that diet has a greater influence on gut microbiota assembly and succession than other environmental factors [15, 21, 40]. Similar results were observed in the hybrid fish. In particular, the composition and structure of the dominant microbial communities were significantly changed during dietary adaptation (Fig. 3). This phenomenon, called a neutral process, which suggests that microbes are transient with no evidence of adaptation to their environment, has

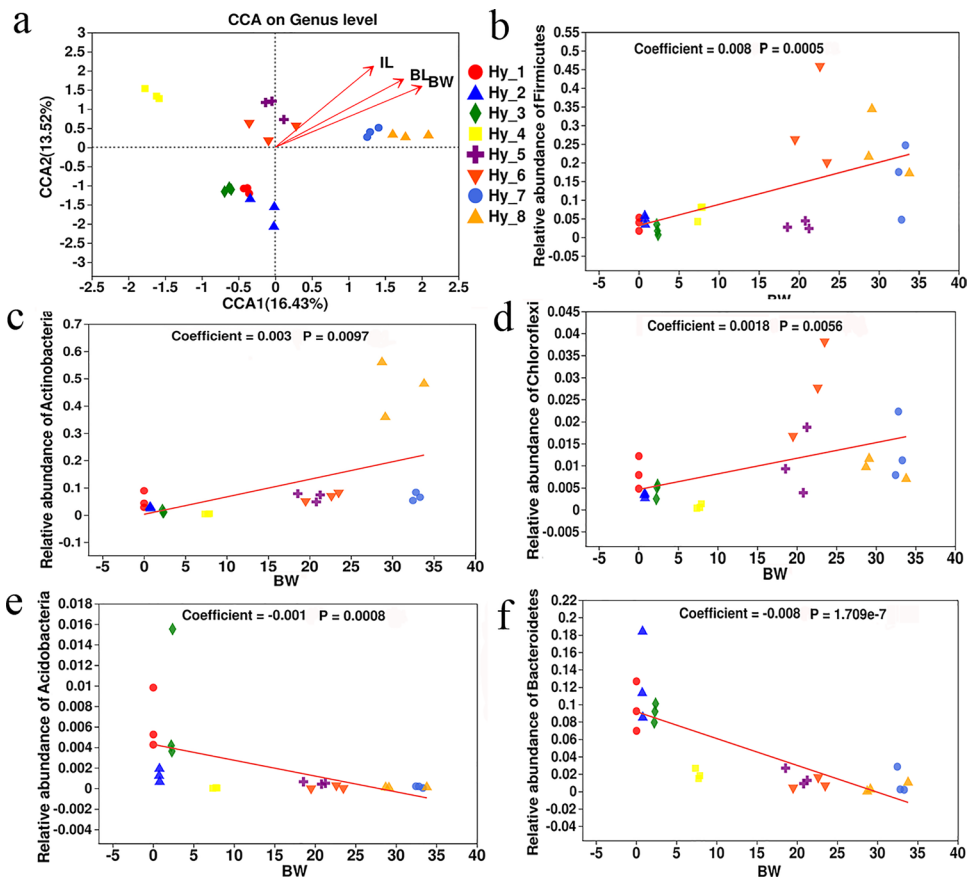

Fig. 4 Relationships between the dominant microbiota and fish growth factors. **a** Redundancy analysis/canonical correspondence analysis (RDA/CCA) showed a positive correlation between fish growth factors and dominant genera. Multivariate association with linear model (MaAslin) analysis showed a positive correlation between fish body weight and Firmicutes (**b**), Actinobacteria (**c**), and Chloroflexi (**d**) and a negative correlation between fish body weight and Bacteroidetes (**e**) and Acidobacteria (**f**). $p < 0.05$ indicates a significant correlation between microbiota and fish growth factors (one-way ANOVA analysis)

also been observed in zebrafish and Atlantic Salmon (*Salmo salar*) [15, 40, 41].

Generally, the composition and metabolic capacity of the fish gut microbiota may differ widely with diet. In the current study, although the microbial communities were dynamically changed during the hybrid fish adaptation to different diets, a number of dominant genera were coexisting in all groups of fish (Fig. 3). These symbiotic bacteria including *Gemmobacter*, *Chloroplast*, *Bacillus*, *Cetobacterium*, and *Aeromonas*, may contribute to host nutrition and health by providing complementary enzymatic activities and synthesizing vitamins [42]. Additionally, some genera with significant abundance, including *Rhizobiales*, *Acinetobacter*, *Gemmobacter*, *Microbacterium*, *Vibrio*, *Streptomyces*, *Shewanella*, and *Aeromonas*, were specifically enriched in herbivorous or carnivorous fish (Table S2), indicating that the hybrid fish can select the microbial communities harbored in their gut during diet adaptation [39]. Studies in wild freshwater fish have revealed that gut microbiota enrichment in carbohydrate pathways (starch and sucrose metabolism, glycolysis/gluconeogenesis) generally occurs in herbivores and omnivores, while protein and amino acid pathways (alanine, aspartate and glutamate metabolism, protein digestion, and absorption) are enriched in carnivores [36, 43]. Our results also showed that the dominant and unique microbial communities in the two dietary groups of fish were enriched in carbohydrate digestion and absorption, glycolysis, fatty acid elongation, and bile secretion pathways (Fig. 4; Fig. S3), suggested that dynamic changes of the gut micorbiota is in adapting to diet [12, 36, 39]. Combined with our previous studies on the intestinal morphology, liver histology and biochemical assays, and intestinal and liver transcriptomes, the results of this study also provide evidence that hybrid fish have the potential to adapt to herbivorous diets more than carnivorous diets [21, 22].

Host-Microbiota Interactions with Fish Development

Host genetics (genotype, development, and immune system) have been documented to be linked to gut microbial composition, which can interact with environmental factors that affect gut microbial enrichment and succession [44, 45]. For example, in brook charr (*Salvelinus fontinalis*), host quantitative trait loci (QTLs) influence microbiota taxonomic composition, and the specific host genomic regions regulate the recruitment of specific bacterial genera which possess antibacterial activity [46]. Our previous study also found that host subgenomic interactions had a sizeable effect on shaping gut microbiota assemblages in the hybrid fish [21].

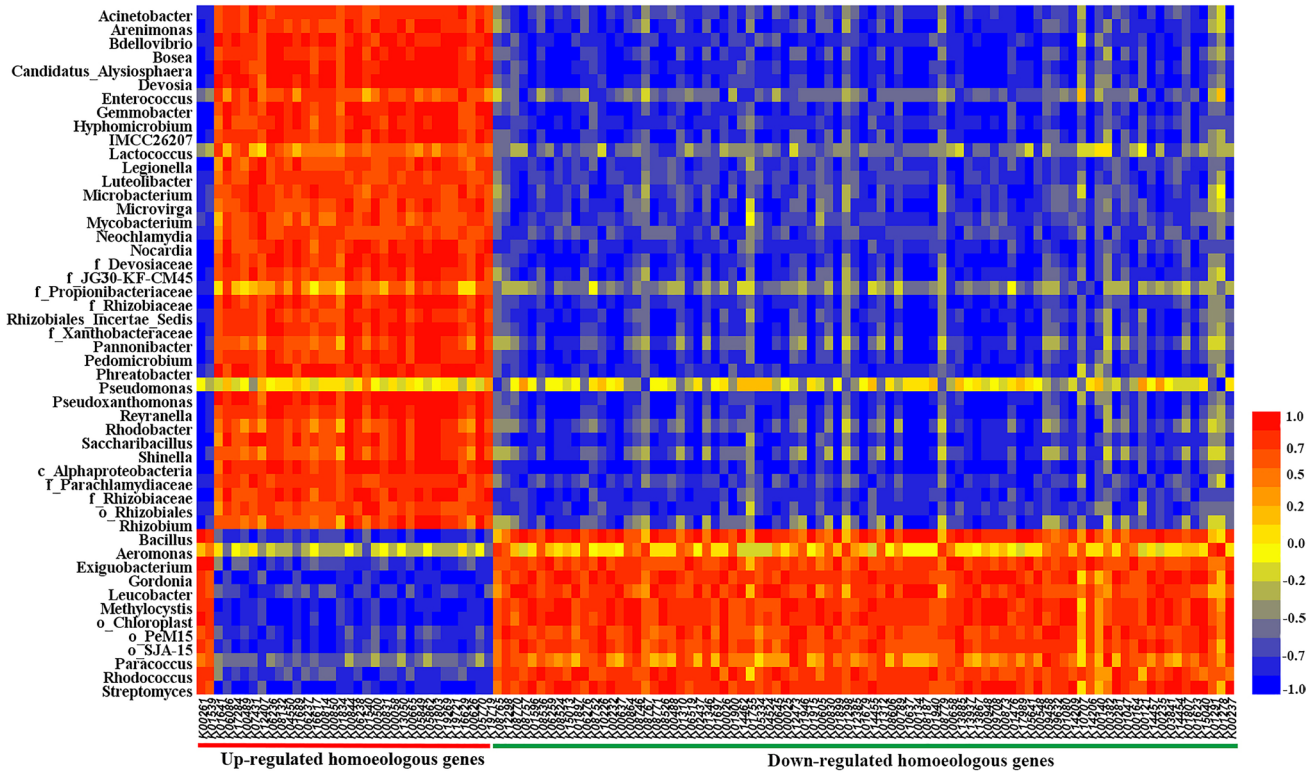

Fig. 5 Correlations between the dominant microbial communities (at the genus level) and DEHs by Spearman's correlation analysis. The DEHs were derived from the PPAR signaling pathway, carbon metabolism, fat digestion and absorption, amino acids biosynthesis, fatty acid biosynthesis, protein digestion, and absorption pathways. Strong correlation cutoff $|r| > 0.6$ and $p < 0.05$

In return, the selected and enriched microbiota especially probiotics confer several beneficial effects to host including enhances immunity, helps in digestion, promotes growth and reproduction [5]. In Huanghe carp new strain and fast-growing transgenic common carp, the percentage of Firmicutes was detected relate to growth performance [43, 47]. In this study, phyla Firmicutes was also detected positively correlated with increasing body weight of the hybrid fish (Fig. 4). Besides, there also detected several phyla were negative related to growth performance. These findings imply that host-microbiota interactions or microbe-microbe interactions (such as competing for space and nutrients) can regulate and help the host select for certain microbial taxa during host development [36, 43].

In animals, current evidence suggests that multiple mechanisms, including endocrine and neurocrine pathways, may be involved in microbiota-gut (liver)-brain signaling and that the brain (liver) can in turn alter microbial composition and behavior via the autonomic nervous system [48]. For example, gut bacteria can interact with host cells and effect the expression of special genes [49]. In Huanghe carp new strain, the abundance of the genera *Aeromonas* and *Roseomonas*, as well as differential expression of *IL12*, was related to anti-disease ability [43]. In olive flounder (*Paralichthys olivaceus*), *Bacillus* sp. supplementation can induce growth performance and growth-related gene (*gh*) expression [34]. In this study, we also found some DEHs associated with intestinal growth and immunity were related to the *Bosea*, *Acinetobacter* (Supplemental Table 3). In addition, some upregulated (*glud1b*, *atp1a1*, *hmgcs1*, *slc8a1a*) and downregulated genes (*cpt1aa*, *apoa1q*) associated with metabolism were related to dominant genera between the herbivorous and carnivorous fish (Fig. 5) [22]. These observations suggested that the differences in gut bacterial community composition and host genetics-microbiota interactions may be important factors contributing to the growth and disease resistance of hybrid fish [43, 50]. How fish hosts and their microbial components can cooperate or interact in response to various environmental factors needs further investigation.

Conclusions

This study presents evidence that host genetics-gut microbiota interactions contribute to dietary adaptation in hybrid fish. The diversity and composition of the dominant

microbial communities showed similar successional patterns during early developmental stages but significantly changed during the two dietary adaptations. Moreover, although the dominant bacterial communities were shared in all developmental stages, some dominant microbiota associated with metabolism specifically colonized the guts of fishes in the two dietary groups. Furthermore, a positive correlation between host growth factors and dominant gut microbiota was detected in both herbivorous and carnivorous fish. In addition, the intestinal DEHs associated with cell growth, immunity, and metabolism showed a strong correlation with dominant microbial communities. The results in this study further provide evidence that hybrid fish have the potential to adapt to herbivorous diets more than carnivorous diets. This study provides basic data for understanding the diversity of dietary adaptations and evolution in fish.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-022-01993-y.

Author contribution This study is conceived and designed by SJL and WHL. Most statistical analyses and writing of the manuscript: WHL, HQL, SW, and ZXZ. Experimental work: JH, FZH, RRZ, and LR. Experimental materials collect: CW, CCT, LZ, and QFL. Manuscript modification: MT, CZ, and QQB. All authors read and approve the final manuscript.

Funding This work was supported by the Laboratory of Lingnan Modern Agriculture Project (NT2021008) (for fish breeding, recipient: Shaojun Liu), the Research Foundation of Education Bureau of Hunan Province (Grant No. 21B0053) (for bacterial 16S rRNA gene sequencing, recipient: Wuhui Li), the National Natural Science Foundation of China (Grant Nos., 31730098, U19A2040), earmarked fund for China Agriculture Research System (Grant No. CARS-45) (for fish hybridization experiments and breeding, recipient: Shaojun Liu), and the National Natural Science Foundation of China (31802287) (for data analysis, recipient: Shi Wang).

Data availability Data sets further supporting the conclusions of this article are included within the article and its supplemental files. The complete clean reads will submit to the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA788359.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate The guidelines established by the Administration of Affairs Concerning Animal Experimentation state that approval from the Science and Technology Bureau of China and the Department of Wildlife Administration is not necessary when the fish in question are neither rare nor near extinction (first- or second-class state protection level). Therefore, approval was not required for the experiments described in this manuscript.

In this study, all experiments were approved by the Animal Care Committee of Hunan Normal University and followed the stated guidelines of the Administration of Affairs Concerning Animal Experimentation of China. Fish collect and crossing were approved by the Animal Care Committee and Protection Station of Polyploidy Fish of Hunan Normal University. All samples were raised in natural ponds, all dissections were performed with 50 mg/L MS-222 (Sigma-Aldrich, St Louis, MO, USA), and all efforts were made to minimize suffering. Specific operation methods were as follows: first, add 4 L of water to a 10-L glass container, then weigh 200 mg of MS-222 solid powder and dissolve in water. After the powder was dissolved, the experiment fish was put into the water. When the body of the experiment fish gradually lost its balance or sank to the bottom of the tank, the frequency of gill motion decreased, and the breathing was weak or stopped. At this time, the experiment fish was under deep anesthesia, so subsequent experiments could be carried out.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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