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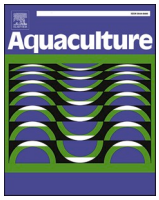

Triploidization modulates intestinal microbiota and promotes growth in *Carassius auratus*

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ABSTRACT

Intestinal microbiota plays a key role in modulating host growth. However, the effect of triploidization on intestinal microflora and fast growth has not been fully explored. Here, autotriploid *Carassius auratus* (3nRR, 3n = 150) derived from *Carassius auratus* red var. (♀, RCC, 2n = 100) × autotetraploid fish (♂, 4nRR, 4n = 200) was established. Intestinal microbial community analysis of RCC and 3nRR was performed by 16S rRNA gene sequencing. Gut characteristics, growth rate, and digestive enzyme activity of the two groups of fish were explored. Significant changes in gut assemblages were observed in 3nRR fish. Differences in intestinal characteristics (structure, relative gut density, relative gut length, relative gut mass, and Zihler's index) were observed between RCC and 3nRR groups. Associations between microbial assemblages and intestinal characteristics were evaluated. Growth rate and activities of intestinal digestive enzymes (cellulase, amylase and trypsin) were higher in 3nRR compared with that in RCC. Spearman's correlation analysis showed a positive correlation between bacteria orders (Clostridiales, Micrococcales and Lactobacillales) and fast growth in 3nRR. In summary, the findings indicated that triploidization caused changes in gut microbiota and characteristics, and digestive enzyme activity. The relative abundance of several bacteria species was positively correlated with fast growth in 3nRR. The present study provided new insights into the growth mechanism of 3nRR and its interaction with intestinal microorganisms.

1. Introduction

Animals harbor a diverse microbial ecosystem, and higher diversity in gut microbiota. Gut microbiota comprises highly diverse microorganisms including bacteria, archaea, viruses, fungi and microeukaryotes that inhabit the host gastrointestinal tract (Sekirov et al., 2010; Brüssow, 2016). Gut microbiome is a source of key enzymes that affect food digestion and nutrient absorption in the host (Turnbaugh et al., 2006, 2009). Advances in next-generation sequencing (NGS) technologies have improved understanding on host diverse and complex communities of microbial symbiont systems. Studies report that the diverse and dynamic microbial community in gastrointestinal tract plays key roles modulation of host health and nutrition (Tremaroli and Bäckhed, 2012), regulation of immunity and defense against pathogens (Thursby and Juge, 2017), promotes growth and development (Panke-Buisse et al., 2015), and affects the host behavior (Diaz Heijtz et al., 2011).

Polyploidy refers to having more than two sets of chromosomes in the cell nucleus (Madlung, 2013), which is a well-known and common phenomenon in the plant kingdom (Adams and Wendel, 2005). Almost all organisms display even ploidy levels, but some species may have uneven ploidy, being triploids, for instance. In mammals, triploidy is lethal (Hassold and Hunt, 2001), whereas in certain vertebrates, such as birds (Knief and Forstmeier, 2016), lizards (Bogart and Bi, 2013), amphibians, and fish (Mable et al., 2011), triploids can survive and display relatively normal traits. In a wide variety of fish species, triploidy has been generated, such as the case of triploid fish derived from *Carassius cuvieri* (♀) × allotetraploid hybrids (♂) (Guo et al., 2006); as well as triploid Atlantic salmon (*Salmo salar*) and triploid brook trout (*Salvelinus fontinalis*) (Piferrer et al., 2009). Triploid fish frequently have a number of enhanced traits over diploid fish, including improved growth rate, delicious taste, lower mortality rate, greater stress tolerance, and sterility (Liu et al., 2018; Wang et al., 2020; Liu et al., 2021). Several

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studies have suggested that energy normally used for gonadal development might be used to develop flesh, allowing sterile triploids to grow faster (Liu et al., 2000, 2003).

Intestinal microfloras are closely associated with the animal growth performance. The composition and changes of intestinal microbe are correlated with numerous direct or indirect factors. Previous studies report that gut microbiota diversity and structure can be influenced by stress (Galley et al., 2014), diet (Schroeder and Bäckhed, 2016), environment (Shin et al., 2015) and host genetics (Turpin et al., 2016). Dietary organic acids improve growth performance of nursery pigs by modulating the composition of gut microbiota (Wei et al., 2021). Plant-based diets have significant effect on the distal intestinal microflora of rainbow trout (*Oncorhynchus mykiss*) (Desai et al., 2012). Moreover, gut microbiota results in differences in behavioral and physiological traits of native American fish and exotic Asian carp (Ye et al., 2014). Microbial communities are associated with the innate immune system of zebrafish (Kanther and Rawls, 2010). In juvenile salmon, triploidy can influence both the community and drug resistance of culturable intestinal microbiota (Cantas et al., 2011). In our previous study, the autotriploid *Carassius auratus* fish (3nRR, 3n =150) are derived from *Carassius auratus* red var. (♀, RCC, 2n = 100) × autotetraploid fish (♂, 4nRR, 4n = 200), of which the male 3nRR are sterile, whereas the females generated dynamic eggs (Wang et al., 2021). Therefore, the 3nRR provide excellent resources for evaluating the relationship between growth performance and gut microbiome of triploid fish.

Here, the composition of intestinal microbiota, gut traits, growth performance, and activities of gut digestive enzymes (cellulase, amylase and trypsin) of RCC and 3nRR were analyzed to assess the effect of triploidization on growth and gut microbiota structure and composition. Furthermore, the results of this research will help to reveal the impact of gut microflora on growth performance in the triploid fish.

2. Materials and methods

2.1. Ethics statement

Approval from the ethics committee of the Institute of Experimental Animals, Hunan Province, China was obtained. The fish used in the experiment were anesthetized with MS-222 (100 mg/L, Western Chemical, Inc., Ferndale, Washington) prior to dissection.

2.2. Fish production and rearing conditions

4nRR and RCC fish were taken from the State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, China. Hybrids (3nRR) of RCC (♀) × 4nRR (♂) and RCC of RCC (♀) × RCC (♂) were generated in April 2020 within the Engineering Research Center of Polyploid Fish Breeding and Reproduction of the State Education Ministry, China, located at Hunan Normal University. The embryos developed in the culture dishes at a freshwater temperature of 22–24 °C. All larvae of RCC and 3nRR (approximately 50 each) were maintained under the same cement pond (4 m^2). The fish of RCC and 3nRR (approximately 35 each) with same weight were transferred into a bigger cement pond (20 m^2) with controlled aquatic environment conditions [atmospheric pressure (0.067 ha), ph (7.0–8.5), water temperature (22–24 °C), oxygen saturation (>70%) and sufficient feed (a mixture of 30% crude protein, 3.5% crude fat and 10% crude fiber)] after 30 days.

2.3. Sample collection, microbial DNA extraction, amplification and sequencing

After 8 h of feeding, the one-year-old fish (n = 3 per RCC and 3nRR group) before surgery were euthanised using 100 mg/L MS-222. The fish intestinal tract was dissected with sterile scissors, and then gut contents were carefully collected to sterile tubes and stored at −80 °C. DNA of the gut microbiome was extracted with the PowerSoil DNA Isolation Kit (MoBio, Carlsbad CA) as the manufacturer's protocol (Gilbert et al., 2010), and the quality and quantity of DNA were analyzed on a NanoDrop spectrophotometer (NanoDrop Technologies). Library construction for Illumina HiSeq 2500 sequencing was performed at Biomarker Technologies Company (Beijing, China). Primers [338-Forward (5′-ACTCCTACGGGAGGCAGCAG) and 806-Reverse (5′-GGACTACHVGGGTWTCTAAT)] targeting the V3 to V4 region of the 16S rRNA were used (Fadrosh et al., 2014).

2.4. Bioinformatics analysis

The raw 16S rRNA sequences were uploaded onto NCBI under BioProject ID PRJNA792731. The original data were quality filtered (Trimmomatic, version 0.33) and primer sequences were removed by means of Cutadapt (version 1.9.1). The paired-end reads were then merged and chimeras were removed using UCHIME (version 8.1) to obtain effective reads. Effective sequences with ≥97% similarity were classified into multiple operational taxonomic units (OTUs) with USEARCH package (version 10.0, http://drive5.com/usearch/). Representative OTU sequences were assigned using naïve Bayesian classifier (QIIME2, version 2020.6, https://qiime2.org) against the SILVA reference database (version 132, https://www.arb-silva.de/). Principal coordinate analysis (PCoA) was performed for analysis of beta diversity to explore the differences in community structure among different groups. Alpha diversity parameters (ACE and Shannon) were calculated through the QIIME2 tool. Abundance statistics of each taxonomy at order level in RCC and 3nRR were determined and visualized using heatmap package with R software (version 3.5.3). The biological function of OTUs was inferred from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database by PICRUSt (http://picrust.github.com/picrust/).

2.5. Analysis of gut characteristics and body weight

Fish (two groups including RCC and 3nRR) were harvested after a year for gut examination. Intestinal characteristics including gut structure, body length (BL) and weight (BW) and intestinal length (IL) and weight (IW) (n = 8 per group) were examined for each fish according to the GB/T 18654.3–2008 patent (Zou et al., 2008). Relative gut length (RGL = IL/BL), Zihler's index [ZI = IL (cm) × $BW(g)^{1/3}$], relative gut mass (RGM = IW/BW) and relative gut density (RGD = IL/IW, cm/g) were applied to investigate the influence of polyploidization on intestinal characteristics. Linear relationships between microbial communities (principal component, at OTUs level) and genetic factors (RGL, ZI, RGM, and RGD) were investigated through linear regression analysis (LRA) on an online OmicShare cloud platform (https://www.omicshare.com/). Furthermore, redundancy analysis (RDA) was conducted using a web page tool (http://www.cloudtutu.com/) to explore the association between genetic factors and intestinal microbial communities.

Body weight (BW) for RCC and 3nRR fish were measured at months 3, 6, 9 and 12 after the start of the trial (n = 20 per group). Correlation between body weight (growth rate) and microbial species was assessed by spearman's correlation analysis using a web page tool (http://www.cloudtutu.com/).

2.6. Analysis of digestive enzymatic activity

One-year-old RCC and 3nRR fish (n = 3 per group) were collected and their intestinal contents were used for the digestive enzymatic activity assay. Cellulase, amylase and trypsin activities were conducted using a Fish Cellulase ELISA Kit, Fish Amylase ELISA Kit and Fish Trypsin ELISA Kit, respectively; both from Servicebio Company (Wuhan, China). Experimental data were recorded at 450 nm using a microplate reader (Rayto, RT-6100, China) and analyzed using Microsoft Excel software.

Table 1
Statistics of species annotation.

Sample	Kindom	Phylum	Class	Order	Family	Genus	Species
RCC_1	1	25	57	137	231	405	435
RCC_2	1	22	49	133	224	405	433
RCC_3	1	26	57	129	221	401	423
3nRR_1	1	25	60	130	209	381	410
3nRR_2	1	24	63	133	211	375	400
3nRR_3	1	28	64	128	200	333	362
Total	1	36	88	204	350	654	708

RCC_1, RCC_2, RCC_3, samples from *Carassius auratus* red var. (RCC); 3nRR_1, 3nRR_2, 3nRR_3, samples from the autotriploid *Carassius auratus* (3nRR). Values in each column represents number of annotated terms in each taxonomic level.

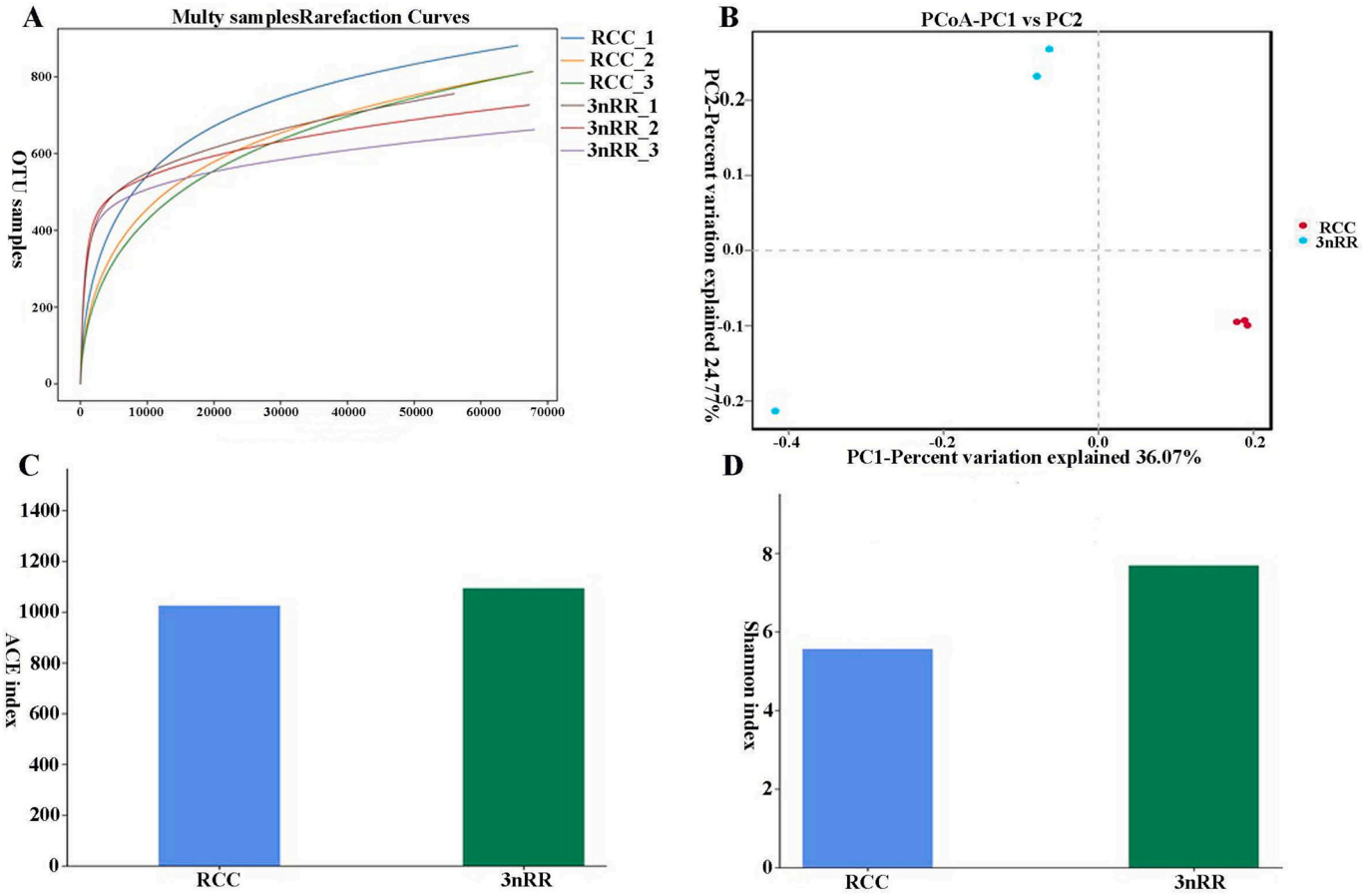

Fig. 1. Rarefaction curve, alpha and beta diversity. (A) Rarefaction curve of each sample. (B) PCoA of samples at the OTU level. (C) Alpha diversity based on the ACE index of the OTU level. (D) Alpha diversity based on the Shannon index of the OTU level.
OTU, operational taxonomic unit; ACE, abundance-based coverage estimator; RCC_1, RCC_2, RCC_3, samples from *Carassius auratus* red var. (RCC); 3nRR_1, 3nRR_2, 3nRR_3, samples from the autotriploid *Carassius auratus* (3nRR). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.7. Statistical analysis

Data were given as the mean ± SD. Comparison between the two groups of fish was conducted using students *t*-test. All statistical analyses were performed with Microsoft Excel 2019.

3. Results

3.1. Analysis of sequencing data

A total of 480,278 good quality 16S rRNA gene sequences (200.03 Mbp) were obtained. Notably, 393,126 sequences (81.85%) were mapped to 1556 OTUs. The number of effective OTUs was identified into 36 phyla, 88 classes, 204 orders, 350 families, 654 genera and 708 species (Table 1).

Rarefaction curve results indicated that the sequencing data of all samples had good quality for analysis of microbial diversity (Fig. 1A). PCoA was conducted to evaluate the roles of polyploidization on gut microbiota dynamics and the results showed that 3nRR and RCC fish were clustered into two categories (Fig. 1B).

Results on ACE index exhibited higher index values in 3nRR fish compared with RCC fish indicating differences in gut microbial richness (Fig. 1C). Diversity of the microbial community was significantly higher in 3nRR group, as indicated by the high Shannon index compared with that of RCC group (Fig. 1D). These results implied that intestinal microbiota from 3nRR group had significantly higher levels of richness

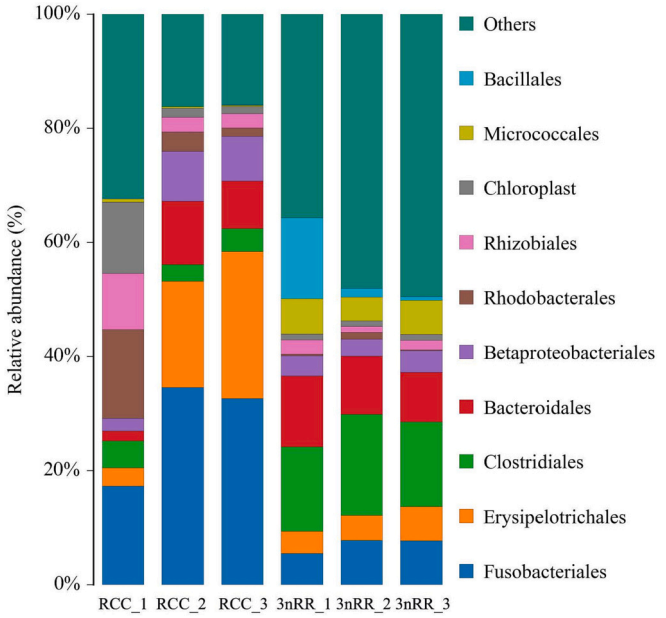

Fig. 2. The relative abundance of top 10 order in the gut microbiota between RCC and 3nRR.
RCC_1, RCC_2, RCC_3, samples from *Carassius auratus* red var. (RCC); 3nRR_1, 3nRR_2, 3nRR_3, samples from the autotriploid *Carassius auratus* (3nRR). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Relative abundance of the top 10 order of gut microbiota in RCC and 3nRR (Mean ± SD $n = 3$).

Order	RCC	3nRR
Fusobacteriales	28.12 ± 9.47	6.96 ± 1.28
Erysipelotrichales	15.86 ± 11.54	4.74 ± 1.09
Clostridiales	3.89 ± 0.92*	15.78 ± 1.67
Bacteroidales	7.08 ± 4.81	10.46 ± 1.86
Betaproteobacteriales	6.27 ± 3.52	3.44 ± 0.39
Rhodobacterales	6.80 ± 7.61	0.56 ± 0.52
Rhizobiales	4.98 ± 4.26	1.73 ± 0.73
Chloroplast	5.13 ± 6.36	1.02 ± 0.02
Micrococcales	0.33 ± 0.20*	5.43 ± 1.13
Lactobacillales	0.29 ± 0.12*	5.21 ± 1.06

RCC, *Carassius auratus* red var.; 3nRR, autotriploid *Carassius auratus*. The * indicates a significant difference between RCC and 3nRR ($p < 0.05$).

and diversity compared with those from RCC group.

3.2. Taxonomic composition of RCC and 3nRR groups

Sequences were correctly identified to the order level and these findings suggested that Fusobacteriales, Erysipelotrichales, Bacteroidales, Rhodobacterales, Betaproteobacteriales, Chloroplast, Rhizobiales, Clostridiales, Micrococcales and Lactobacillales were the ten most dominant microbe in the experimental fish (Fig. 2). Analysis showed that RCC and 3nRR had different proportions of bacterial communities. For example, Fusobacteriales, Erysipelotrichales, Bacteroidales, Rhodobacterales, Betaproteobacteriales, Chloroplast and Rhizobiales showed higher abundance in RCC, however, the bacterial taxa ratios were not significantly different between RCC (74.24%) and 3nRR groups (28.91%) ($p > 0.05$, Student's *t*-test) (Table 2). In addition, the abundance of Clostridiales, Micrococcales and Lactobacillales in RCC (4.51%) was significantly lower compared with the abundance in 3nRR group (26.42%) ($p < 0.05$, Student's t-test) (Table 2).

3.3. Functional prediction of intestinal microflora in 3nRR and RCC

The function of intestinal microflora was performed using PICRUSt tool based on the 16S sequencing data in RCC and 3nRR. The identified genes showed significant enrichment of KEGG level 1 pathways including metabolism (RCC: 77.59%; 3nRR: 78.39%), environmental information processing (RCC: 7.21%; 3nRR: 6.44%), genetic information processing (RCC: 6.69%; 3nRR: 7.91%), cellular processes (RCC: 3.80%; 3nRR: 3.03%), human diseases (RCC:3.21%; 3nRR: 2.57%) and organismal systems (RCC: 1.46%; 3nRR: 1.43%). These predicted genes were further classified into 43 functional categories of KEGG level 2 pathways. Most KEGG level 2 pathways including metabolism, genetic information processing and environmental information processing were significantly enriched ($p < 0.05$, Student's *t*-test) in 3nRR relative to RCC. Metabolism pathways included carbohydrate metabolism, lipid metabolism, nucleotide metabolism, biosynthesis of other secondary metabolites, metabolism of terpenoids and polyketides, glycan biosynthesis and metabolism and global and overview maps. Genetic information processing pathways included translation folding, sorting and degradation, transcription, replication and repair. Moreover, environmental information processing pathways included signal transduction and signaling molecules and interaction (Fig. 3). Notably, the relative abundances of enriched KEGG terms of metabolism were significantly higher in 3nRR group relative to the abundance in RCC group. These findings implyed that triploidization regulates the function of gut microbiota, and is associated with enrichment of pathways related to metabolism.

3.4. Gut characteristics in RCC and 3nRR

The results showed differences in intestinal structures and morphologies between RCC and 3nRR group. RCC and 3nRR groups possessed a “six-loop” intestine and a “five-loop” intestine, respectively (Fig. 4). Several gut parameters (RGL, ZI, RGM, and RGD) were evaluated to assess the effect of triploidization on intestinal characteristics. ZI and RGM were significantly higher in the 3nRR relative to RCC group ($p < 0.05$, Student's *t*-test). On the contrary, RGD and RGL were significantly lower in the triploid fish than in the diploid fish ($p < 0.05$, Student's t-test) (Table 3). These results indicated that triploidization modulated intestinal characteristics.

Fish gut characteristics and microbial composition changed after triploidization, therefore, association between genetic factors and intestinal microbiota assemblages were explored. Linear regression analysis (LRA) was performed based on the genetics factors (RGL, ZI, RGM, and RGD) and intestinal microbial taxa. The squared correlation coefficient of LRA and the genetic factors of the two groups decreased in the following order: RGD > ZI > RGL > RGM. A high correlation coefficient of intestinal characteristics (RGM, RGL, ZI, and RGD) indicated a strong association between the genetics factors and intestinal microbiota taxa (Fig. 5). RDA analysis indicated that the profile of microbial flora was significantly shaped by the genetic factors of RCC and 3nRR groups. Relative abundances of Fusobacteriales and Rhodobacterales were significantly positively correlated with the RGD and RGL in RCC, whereas the relative abundance of Bacteroidales was positively correlated with ZI and RGM in 3nRR. The relative abundance of Bacteroidales showed a negative correlation with RGD and RGL in 3nRR group, whereas the relative abundance of Fusobacteriales and Rhodobacterales showed a negative correlation with ZI and RGM in RCC group (Fig. 6). These results indicated that triploidy significantly modulates community structures of intestinal microbiota.

3.5. Body weight of RCC and 3nRR

Average body weight (BW) of RCC and 3nRR during the study period are shown in Table 4. The average BW of 12-month-old 3nRR fish was 148.9 g, which was significantly higher than that of RCC fish (97.7 g) at

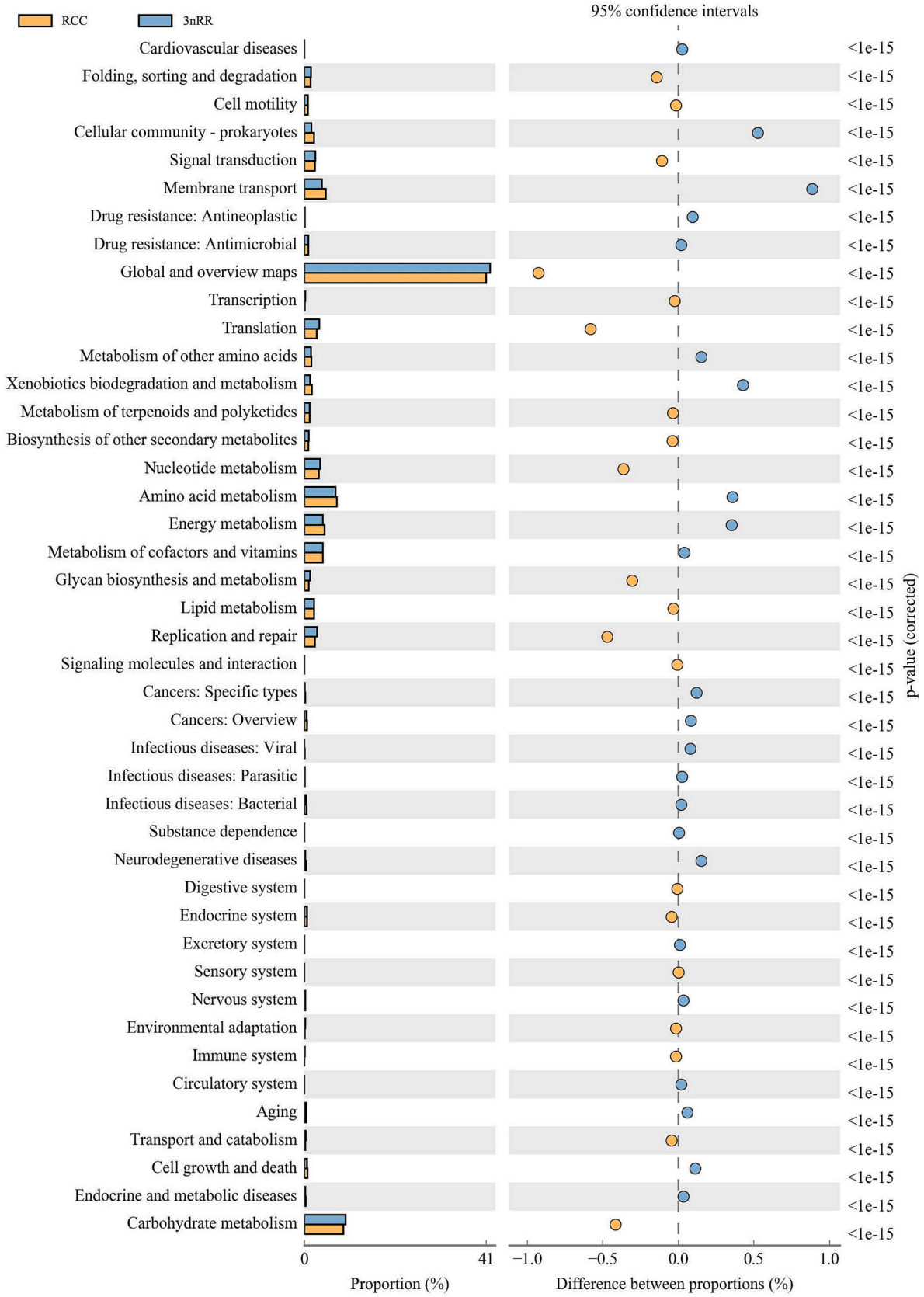

Fig. 3. Comparison of predicted KEGG functions of gut bacteria between RCC and 3nRR.
The graph is the differential analysis diagram of KEGG metabolic pathways at the second level: different colors in the figure represent different groups. Note: the left part of the graph shows the abundance proportion of different functions in two groups of samples, the middle part of the graph shows the difference in bacterial abundance within the 95% confidence interval, and the value on the far right is *p* value. RCC, *Carassius auratus* red var.; 3nRR, autotriploid *Carassius auratus*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

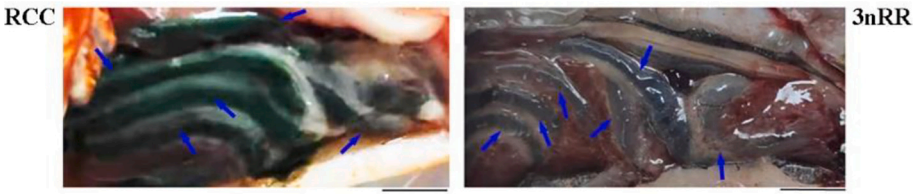

Fig. 4. Gut structures of the RCC and 3nRR.
RCC, *Carassius auratus* red var.; 3nRR, autotriploid *Carassius auratus*; bar = 10 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Summary of basic measurements for RCC and 3nRR (Mean ± SD $n = 8$).

Fish type	RGL	ZI	RGM	RGD
RCC	4.06 ± 0.13*	197.62 ± 13.75*	0.02 ± 0.001*	26.40 ± 0.36*
3nRR	3.62 ± 0.10	262.83 ± 4.71	0.023 ± 0.004	16.17 ± 2.30

RCC, *Carassius auratus* red var.; 3nRR, autotriploid *Carassius auratus*; RGD, relative gut density; RGM, relative gut mass; RGL, relative gut length; ZI, Zihler's index. *Represents statistically significant differences between RCC and 3nRR ($p < 0.05$).

the same age ($p < 0.05$, Student's *t*-test).

3.6. Relationship between growth and intestinal microflora

The association between body weight (growth rate) and indicator species is presented in Fig. 7. The findings indicated that relative abundances of Clostridiales, Micrococcales and Lactobacillales were positively correlated with body weight. These findings indicated that these microorganisms play an essential role in the fast growth of 3nRR fish.

3.7. Digestive enzyme activity

The digestive enzyme (cellulase, amylase and trypsin) activity in RCC and 3nRR intestine was shown in Table 5. Cellulase, amylase and trypsin activities were significantly higher in 3nRR group (1939.30 ± 807.26, 2.00 ± 0.20, 0.29 ± 0.11 U/g) than in RCC group (1492.16 ± 66.12, 1.92 ± 0.15, 0.22 ± 0.02 U/g) ($p < 0.05$, Student's t-test).

4. Discussion

Changes in gut microbial composition are modulated by several factors, including diet (Bibbò et al., 2016), habitat (Sullam et al., 2012), ageing (Maynard and Weinkove, 2018) and health (Milani et al., 2017). Compared with diploid *Carassius auratus*, triploid *Carassius auratus* has different bacteria composition and abundance (Cai and Wei, 2023). In juvenile salmon, triploidy is associated with both community composition and drug resistance in intestinal microbiota (Cantas et al., 2011). The results of this study are like the results of the previous studies. The findings in the present study showed a significant difference in gut microflora composition and structure between RCC and 3nRR fish, which indicated that triploidization modulates abundance and profile of

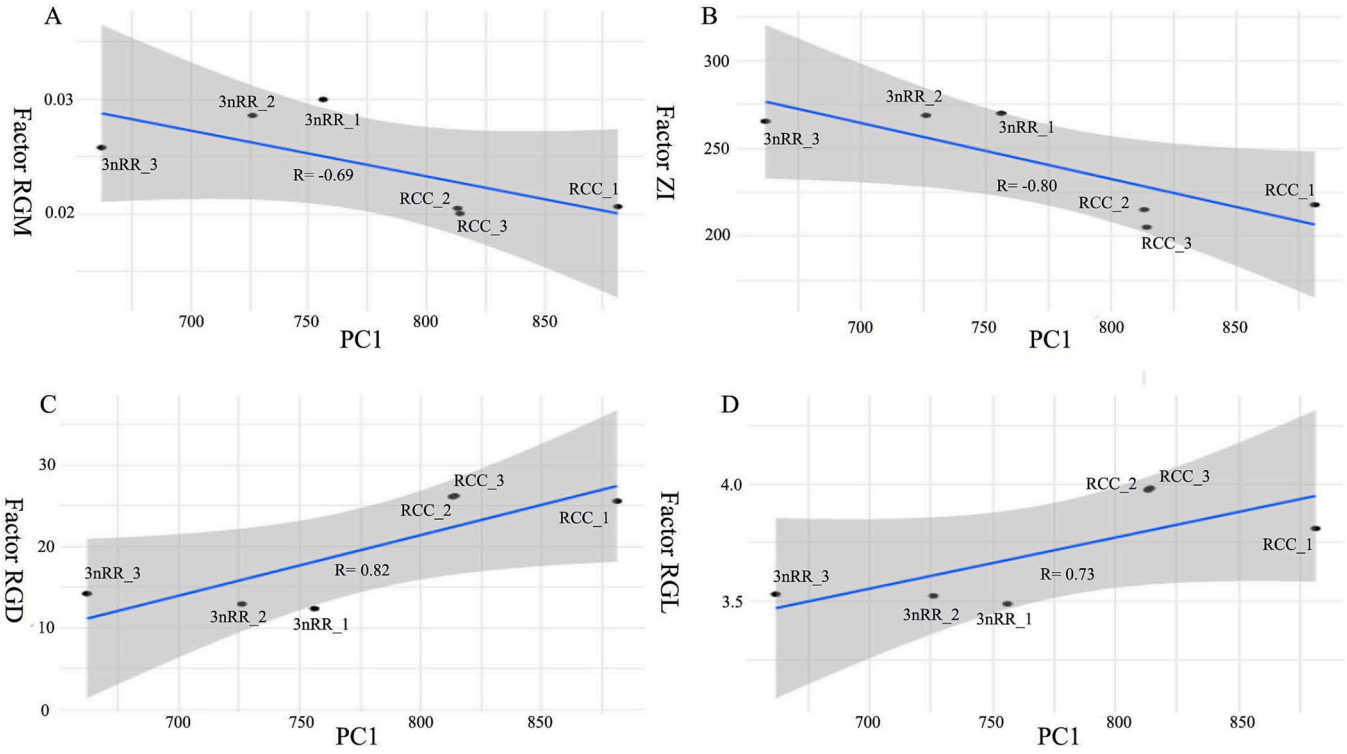

Fig. 5. Relationship between abundance of intestinal traits and microbial taxa (principal component, at the OTUs level) at linear regression analysis. (A) RGD, relative gut density; (B) ZI, Zihler's index, (C) RGL, relative gut length; (D) RGM, relative gut mass. R indicates correlation coefficient; The x axis shows principal component; the y axis shows the genetics factors. RCC_1, RCC_2, RCC_3, samples from *Carassius auratus* red var. (RCC); 3nRR_1, 3nRR_2, 3nRR_3, samples from the autotriploid *Carassius auratus* (3nRR). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

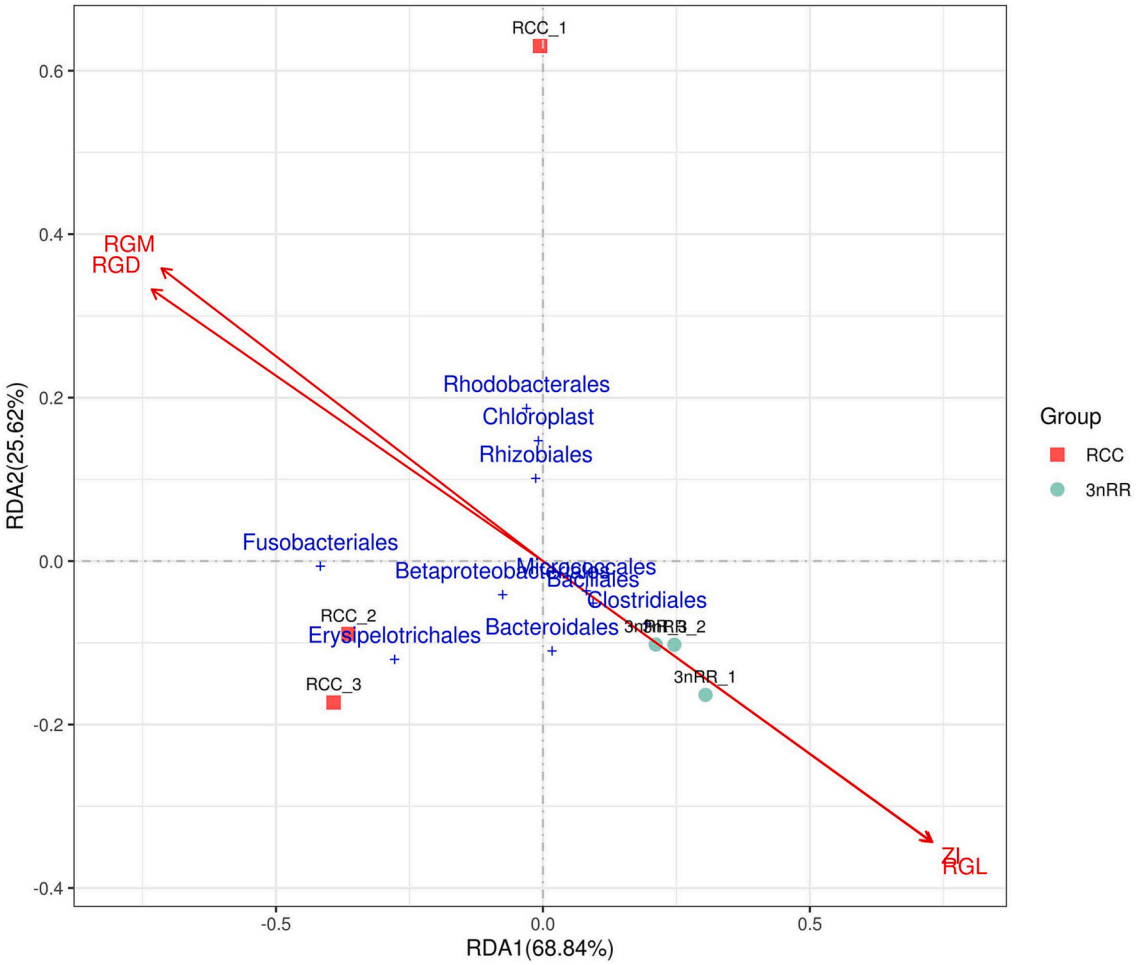

Fig. 6. Redundancy analysis (RDA) of genetics factors and dominant microbial taxa.
The direction of the colour arrow indicates the correlation between intestinal traits and dominant microbial taxa at order level, red arrows indicate genetics factor (intestinal traits), blue plus signs represent the dominant microbial taxa in the two groups of fish. RCC, *Carassius auratus* red var.; 3nRR, autotriploid *Carassius auratus*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
The average body weight of RCC and 3nRR (g) (Mean ± SD $n = 20$).

Fish type	1 month	3 months	6 months	9 months	12 months
RCC	1.76 ± 0.053	8.6 ± 2.1*	26.5 ± 8.1*	55.7 ± 10.5*	97.7 ± 12.3*
3nRR	1.76 ± 0.057	11.2 ± 2.2	39.1 ± 7.4	103.2 ± 9.3	148.9 ± 14.9

RCC, *Carassius auratus* red var.; 3nRR, autotriploid *Carassius auratus*. *Represents statistically significant differences between RCC and 3nRR ($p < 0.05$).

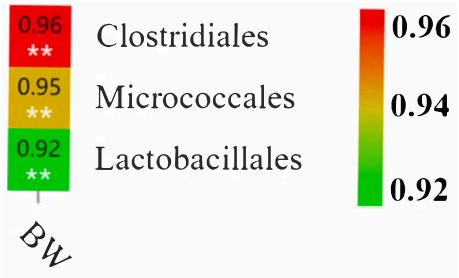

Fig. 7. Correlation analysis of body weight (BW) and three indicator species. ** $p < 0.05$.

Table 5
Digestive enzyme activity in RCC and 3nRR (U/g) (Mean ± SD n = 3).

Enzyme	RCC	3nRR
Cellulase	1492.16 ± 66.12*	1939.30 ± 807.26
Amylase	1.92 ± 0.15*	2.00 ± 0.20
Trypsin	0.22 ± 0.02*	0.29 ± 0.11

RCC, *Carassius auratus* red var.; 3nRR, autotriploid *Carassius auratus*. The * indicates a significant difference between RCC and 3nRR ($p < 0.05$).

symbiotic microorganisms.

Intestinal traits (structure, relative mass and length) are affected by multiple factors, including diet (Elliott and Bellwood, 2010), host genetics (Li et al., 2018a, 2018b), ontogeny and phylogeny (German and Horn, 2006; Davis et al., 2013). However, studies have not fully explored different gut traits during the process of triploidization. Significant differences in gut characteristics (structure, RGL, RGM, ZI, and RGD) were observed between RCC and 3nRR in the current study. These differences indicated that intestinal signature is modulated by triploidization. According to a relevant study, the gut microbiome of Sparidae is strongly influenced by gut morphology and diet (Escalas et al., 2021). It has been discovered that fish gut length and relative gut length are conserved in host phylogeny and are considered to be important specialized traits for the utilization of exogenous resources (Greene et al., 2020). Thus, we speculate that the changes of intestinal

morphology may affect the composition of intestinal flora and improve the absorption and utilization of nutrients, thereby promoting the rapid growth of the triploids.

Triploidization promotes faster growth of the triploid offspring compared with their parents (Shen et al., 2006). For instance, the growth rate of triploid Pacific oyster (*Crassostrea gigas*) is higher than the growth of diploids (Garnier-Géré et al., 2002). Triploid hybrids derived from female grass carp and male blunt snout bream exhibited a faster growth rate (He et al., 2013). Moreover, the growth rate of triploid marine medaka (*Oryzias dancena*) is higher compared with that of diploid marine medaka (Park et al., 2018). In the present study, the average body weight of one-year-old 3nRR fish was 148.9 g, which was higher than that of one-year-old RCC fish (97.7 g). This finding indicates that triploidization can improve the growth of 3nRR. The higher body weight can be due to the fact that the energy provided to gonadal development is used to promote the growth of fish (Ladisa et al., 2021).

Digestive enzyme activity of animals is commonly affected by several factors, such as diet (German et al., 2004), disease (Hurwitz et al., 1972) and probiotics (Balcázar et al., 2006). Digestive enzyme activity plays a key role in digestion and absorption. Previous studies report a positive relationship between digestive enzyme activity and animal growth performance (Yu et al., 2019; Jiang et al., 2020; Long et al., 2020). Similarly, faster growth rate in fish is positively correlated with activities of digestive enzymes (Bélanger et al., 2002). For instance, growth rate is strongly associated with activities of digestive enzymes in juvenile yellow catfish (*Pelteobagrus fulvidraco*) (Zhao et al., 2021). Digestive enzyme activity is correlated with digestive capacity in Atlantic salmon (*Salmo salar L.*) thereby affecting the growth rate (Krogdahl and Bakke-McKellep, 2005). In the current study, 3nRR exhibited higher activities of digestive enzymes (cellulase, amylase and trypsin) compared with the activities of RCC group. This finding indicates that triploid may increase activity of intestinal digestive enzymes in 3nRR further promoting digestion and absorption of nutrients and improve growth.

Previous findings indicate that the composition of gut microbial communities can be altered by the host genetics. Gut microbiota composition and diversity in threespine stickleback (*Gasterosteus aculeatus*) is dependent on host genotype (Smith et al., 2015). Wild and captive Malaysian Mahseer (*Tor tambroides*) exhibits differences in microbiota composition, implying that the host modulates the microbiome composition (Tan et al., 2019). Studies report distinct differences in structure of the gut microbiota of common carp and rainbow trout intestine (Daly et al., 2019). Moreover, the microbiota community structure of grass carp and crucian carp in new lineages exhibited significant differences, indicating that the microbial structure was influenced by host genetics (Zou et al., 2020). Notably, the relationship between microbial communities and host genetics induced by triploidization has not been fully elucidated. The findings of the current study showed an association between different intestinal characteristics and intestinal microbiota taxa. In addition, a significant correlation was observed between dominant microbial taxa at the order level and genetic factors of RCC and 3nRR fish. These results indicated that genome doubling (host genetics) may directly or indirectly affect intestinal microbiota composition assemblages in 3nRR.

Microflora promote host metabolism and are closely associated with animal growth performance (Chapagain et al., 2019; Jang et al., 2019). The Clostridiales order comprises several bacterial species and shows high abundance in intestinal tract (Kawashima et al., 2020). These species are implicated in metabolism of short-chain fatty acid and dietary fiber (Oakley et al., 2014; Niu et al., 2015). Micrococcales promotes biofilm formation (Blakeman et al., 2019), and the members play a key role in metabolism of hydrocarbons (Archaya et al., 2014; Surger et al., 2018). Lactobacillales are members of lactic acid bacteria, and are involved in physiological functions such as digestion, carbohydrate metabolism, and membrane transportation, which can promote host growth (Li et al., 2018a, 2018b; Richards et al., 2019). The relative abundance of Clostridiales, Micrococcales and Lactobacillales in 3nRR was higher than that of RCC in the present study. Notably, a positive correlation was observed between the abundances of Clostridiales, Micrococcales and Lactobacillales and growth rate of fish. Furthermore, PICRUSt prediction analysis showed that 3nRR had significantly enriched metabolism pathway. This finding implies that the increase in abundance of Clostridiales, Micrococcales and Lactobacillales, may be closely related to enrichment of metabolism pathways, thus promoting growth in 3nRR fish.

5. Conclusion

In summary, the findings this study indicated that triploidization modulates the composition and function of gut bacteria community, intestinal characteristics, growth rate and digestive enzyme activity in 3nRR fish. Moreover, Spearman's correlation analysis indicated that the abundance of Clostridiales, Micrococcales and Lactobacillales may promote faster growth in 3nRR. These findings provide an understanding on the underlying relationship between triploidization and intestinal microbiota assemblages, and provide novel insight on the probable mechanism underlying the effect of triploidization on faster growth of triploid fish.

Author statement

Chongqing Wang: design of the study, establishment of the hybrid experiments, collection and analysis the experimental data, wrote and modified the manuscript.

Xiang Luo: establishment of the fish experiments, collection and analysis the experimental data.

Yuxin Zhang: collection and analysis the experimental data.

Yue Zhou: establishment of the fish experiments.

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Shaojun Liu: the conception and design of the study, wrote and modified the manuscript.

Qinbo Qin: the conception and design of the study, wrote and modified the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The obtained raw 16S rRNA gene sequences are available from the NCBI (PRJNA792731) (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA792731).

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