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Research Article

Effects of Low-Salinity Environments on Growth, Antioxidant Response, and Intestinal Microorganisms of the Largemouth Bass (*Micropterus salmoides*)

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This research revealed that low-salinity environments have positive effects on largemouth bass (*Micropterus salmoides*), with the study finding that low-salinity levels can have beneficial impacts on this species. A 8-week experiment was conducted using four salinity gradients S0, S1, S2, S3 (0, 2, 4, 6 psu), and biochemical indicators and 16S rRNA sequencing were analyzed. Results showed relatively good growth performance at S0 and S1, and no significant antioxidant stress response at S3. Na^+–K^+–ATPase activity was significantly lower in the S2 group. The diversity showed no significant differences between the treatment groups. However, with increasing salinity, the proportion of potentially pathogenic bacteria in freshwater decreased significantly while that in the seawater increased. Indicator species analysis showed a significantly higher abundance of positive microbial species in the 4 psu group. In conclusion, although growth performance decreased at 4 psu, antioxidant damage was relatively small and disease risk was significantly reduced, indicating a positive effect on the largemouth bass.

1. Introduction

Salinity is an important environmental factor for aquatic animals, profoundly affecting their physiological activities and physical or chemical properties [1]. For example, a suitable salinity range of 6–12 can promote the growth of Yellowfin seabream (*Acanthopagrus latus*) and Asian sea bass (*Lates calcarifer*), improve their intestinal digestion ability, and allow their nonspecific immune system to respond to different salinity conditions, maintaining overall health [2]. Additionally, the variation in salinities significantly impacts the activity of Na^+–K^+–ATPase in the gill filaments of fine-scaled salmon juveniles and the activity of antioxidant enzymes in their liver. As salinity increases, the activity of Na^+–K^+–ATPase in the gill filaments and liver of sea bass (*Lateolabrax maculatus*) increases [3]. Furthermore, Atlantic salmon exhibit faster growth at lower salinities; however, higher salinities increase their mortality and disease risk [4]. *Micropterus salmoides*, a freshwater aquaculture species native to lakes and rivers in eastern North America, has been widely introduced as an aquaculture species worldwide due to its excellent performance. In China, *Micropterus salmoides* is an important economic aquaculture species due to its delicious meat and rapid growth rate. As a euryhaline fish species, it exhibits tolerance to water bodies with certain salinity levels. Research also indicates that the growth performance and blood physiological indicators of *Micropterus salmoides* fry exhibited different trends in water environments with different salinities [5]. Other studies have shown that high salinities adversely affect *Micropterus salmoides* growth performance [6]. The microbial community structure and diversity of intestinal microorganisms are also influenced by water body salinity. Tian et al. [7] demonstrated that in yellow croaker treated with high salinity, the content of

pathogenic Vibrio in an intestinal microflora increased while the content of probiotics with positive significance for hosts decreased. Another study by Zhang et al. [8] showed that reducing salinity increased the diversity of intestinal microorganisms in Tilapia (*Oreochromis mossambicus*). Induction of detoxification and immune mechanisms was found in the groups transferred from seawater to 50% seawater to freshwater. Changes in the bacterial communities under different environments of osmotic pressure suggest that bacteria may play a role in the promotion of host adaptation. Based on these results, we can conclude that salinity has a certain impact on the antioxidant capacity and intestinal microbial community. Within an acceptable range of oxidative damage, it can reduce the risk of disease in *Micropterus salmoides*.

2. Materials and Methods

2.1. Fish and Experimental Conditions. According to the previous studies, largemouth bass physiological activity is inhibited at 9 psu salinity [6]. Therefore, we chose a salinity of 6 psu as the maximum salinity (S3) for this experiment, which is also the upper limit of the classification standard for lightly saline-alkaline land, and set the gradient by arithmetic progression. The units (2, 4, and 6 psu) were obtained by diluting preprepared artificial seawater and confirmed by a salinometer before use. The breeding experiment was conducted in the aquaculture greenhouse at the Balidian Comprehensive Experimental Base of the Zhejiang Freshwater Fisheries Research Institute. Each experimental fish were artificially reared largemouth bass fry from the base, with an average weight of 48 ± 2.85 g. They were weighed and randomly selected into 12 tanks (water volume = 300 L), with 30 fish per tank. They were fed twice a day with commercial feed. The experiment lasted for 10 weeks, with water temperature at 23–25°C and pH between 7.3 and 8.3.

2.2. Sample Collection and Method of Preparation. After the feeding period, all experimental fish underwent a 24hr fasting period. Subsequently, 10 fish were randomly selected from each tank for sampling. The experimental fish were anesthetized using 20 mg/L Tricaine methanesulfonate (MS-222). Blood samples were obtained from the caudal vein using a 2 mL disposable syringe, followed by centrifugation to separate serum. Furthermore, after blood collection, the fish were sacrificed using a high dose of ethyl-3 aminobenzoate methanesulfonate (100 mg/L).

Subsequently, the bodies of the fish were placed on a superclean worktop and sanitized using 75% alcohol. A longitudinal incision was made along the abdomen, starting from the urogenital opening. The abdomen was rinsed with sterile phosphate buffered saline (PBS) and dried with absorbent paper.

Liver and gill samples were dissected on an ice tray. A volume of 0.86% sodium chloride solution nine times that of the tissue samples, was added to the liver and gill filament tissue samples. The mixture was homogenized and then centrifuged at 3,500 rpm for 10 min to obtain the supernatant, with the sediment being discarded. Ultimately, plasma and tissue samples were stored in liquid nitrogen for subsequent biochemical analysis.

The procedure for gut microbiota sampling involved isolating and cutting the entire fish intestine on a superclean workbench. The intestinal contents were squeezed into a sterile centrifuge tube and any remaining intestinal wall residue was scraped into the tube. The intestinal contents were weighed and stored promptly stored in a −80°C freezer.

2.3. Detection Methods

2.3.1. Biochemical Indicators Detection in Serum, Liver Tissue, and Gill Tissue. Total cholesterol (TC) content was determined using the cholesterol oxidase–peroxidase method [9]. The serum triglyceride (TG) content was determined by glycerol-3-phosphate oxidase-peroxidase method [10]. Malondialdehyde (MDA) was determined using the thiobarbituric acid method [11]. The total activity of superoxide dismutase (SOD) in liver tissue was determined by the hydroxylamine method [12]. Succinate dehydrogenase SDH was determined by measuring the reduction rate of 2, 6-DPIP. Catalase CAT was determined by ammonium molybdate method. Glutamate oxaloacetate transaminase GOT and lactate dehydrogenase LDH were determined by 2, 4-dinitrophenylhydrazine method. Na^+–K^+–ATPase and Ca^+–Mg^+–ATPase were determined by measuring the amount of inorganic phosphorus generated by ATP decomposition. Glutathione peroxidase (GSH-Px) activity was determined by measuring the consumption of reduced glutathione in the reaction. All these indicators were detected according to the test kit (Nanjing Jiancheng Institute of Bioengineering, China) and measured by enzyme-linked immunosorbent assay.

2.3.2. Detection of Gut Microbiota 16SrRNA. PCR was performed in the V3–V4 region. All purified amplicons were combined and subjected to paired-end sequencing. The collected raw data were deposited in the NCBI Sequence Read Archive (SRA) database with the accession number SUB13150398.

Bioinformatic analysis (OTU, community composition, α-diversity, β-diversity analysis and functional prediction) was performed using various software, including UPARSE (version 9.2.64). All sequencing and bioinformatics analysis were performed on the Omicsmart online platform (http://www.omicsmart.com). Also, we would like to thank Genendovo Biotechnology Co., Ltd. (Guangzhou, China) for their assistance with 16SrRNA gene sequencing.

2.4. Calculations and Statistical Analysis. All the data were analyzed by SPSS 25.0 (SPSS, Chicago, IL, USA). The results are presented as means ± SE. After confirmation of normality and homogeneity of data by Kolmogorov–Smirnov and Levene's tests, respectively, one-way ANOVA, and Tukey test's multiple-range as post hoc test were performed. The $P < 0.05$ was considered as significant for all statistical tests.

The calculation formulas for growth performance are as follows:

$$\mathrm{SGR} = (\ln \mathrm{BW}_2 - \ln \mathrm{BW}_1) \times 100/d \quad (\mathrm{BW} = \text{body weight}, \; d = \text{number of days}). \tag{1}$$

Table 1: Growth performance, nutrient utilization, and survival of *Micropterus salmoides* of different salinity for 60 days.

Parameters	Treatments				
	S0	S1	S2	S3	*P*-value
IBW (g)	1002.4 ± 31.9	992.3 ± 26.4	1021.8 ± 24.3	1027.8 ± 12.9	0.692
FBW (g)	2467.3 ± 92.5	2263.1 ± 15.9	2211.7 ± 42.77	2226.8 ± 50.4	0.045
FCR	1.03 ± 0.04^{b}	1.08 ± 0.02^{ab}	1.17 ± 0.01^{a}	1.15 ± 0.02^{a}	0.021
SGR (%/day)	1.76 ± 0.08^{a}	1.66 ± 0.03^{a}	1.53 ± 0.03^{a}	1.53 ± 0.08^{a}	0.102
Survival (%)	100	100	100	100	–

Note: Values are expressed as Mean ± SE ($n = 3$); mean values in each row with different superscripts differ significantly ($P < 0.05$).

$$\text{FCR} = (\text{FI}/d)/((\text{IBW} - \text{FBW})/d)(\text{FI} = \text{feed intake}, \quad \text{IBW} = \text{initial body weight}, \ \text{FBW} = \text{final body weight}). \tag{2}$$

3. Results

3.1. Effect of Different Salinity Water Environments on the Growth Performance of Micropterus Salmoides. Based on the results in Table 1. After 8 weeks of the feeding trial, the FCR coefficients of groups S1, S2, and S3 were higher than that of group S0 ($P < 0.05$). There were no statistical differences in SGR among the groups ($P > 0.05$). The survival rate of all groups was 100%.

Treatment groups having graded levels of salinity, i.e., S0 0 psu; S1 2 psu; S2 4 psu; S3 6 psu IBW, initial body weight; FBW, final body weight; SGR, specific growth rate; FCR, feed conversion ratio;

3.2. Effect of Different Salinity Water Environments on the Biochemical Indicators of Gills, Blood, and Liver in Micropterus salmoides. Liver CAT activity was lower in S0–S2 groups and not significantly different from freshwater ($P < 0.05$). In S3 group, liver CAT activity was higher and significantly different from the other groups ($P > 0.05$). GSH enzyme activity was significantly reduced in S1 and S2 groups ($P < 0.05$). No significant difference in Ca^{+}–Mg^{+}–ATPase among groups under long-term culture ($P > 0.05$). Na–K–ATPase activity was significantly lower in S2 group under long-term culture ($P < 0.05$). No significant difference in AKP, SOD, and ALT enzyme activities among groups ($P < 0.05$). GOT enzyme activity was significantly higher in S2 and S3 groups than in S0 and S1 groups ($P < 0.05$) (Figure 1).

3.3. Effect of Different Salinity Water Environments on the Intestinal Microbial Flora of Micropterus Salmoides

3.3.1. Differences in the Intestinal Microbial Diversity and Composition.

(1) Alpha Diversity. The intestinal microbiota was clustered in 939 OTUs. Alpha diversity, including the Sobs index, Shannon index, and PD (phylogenetic tree). Although there were differences in Sobs, Shannon index, and PD (phylogenetic tree) of *Micropterus salmoides* under different salinity environments, there was no significant change statistically ($P < 0.05$) (Figure 2).

(2) *Beta Diversity*. The beta diversity reflected the composition of the similarity of the bacterial community in different samples (Figure 3). Principal coordinates analysis (PCoA) presents a visualization of the similarity or dissimilarity of research data. It is a nonconstrained data dimensionality reduction analysis method that can be used to study the similarity or dissimilarity of sample community composition. Nonmetric multidimensional scaling (NMDS) is a nonlinear model based on the Bray–Curtis distance, which is among the beta diversity evaluation methods. From the PCoA plot, it is evident that the 95% confidence ellipses of groups S1 and S0 nearly overlap, with their centroids being almost coincident. On the contrary, the centroids of the 95% confidence ellipses of groups S2 and S3 are significantly shifted from those of groups S0 and S1 ($P > 0.05$). The confidence ellipse of group S3 barely overlaps with the 95% confidence ellipses of groups S1 and S0, while the 95% confidence ellipse of group S2 encompasses those of groups S0, S1, and S3. The NMDS plot exhibits a similar pattern.

3.3.2. Relative Abundance of the Prominent Taxa in the Intestinal Microbiota of Micropterus Salmoides. At the phylum level (Figure 4(a)), the top three intestinal microbiota in group S0 were *Firmicutes* (48.9%), *Fusobacteriota* (28.0%), and *Proteobacteria* (13.7%). The same was true for group S1, where the top three intestinal microbiota were *Firmicutes* (54.8%), *Fusobacteriota* (23.4%), and *Proteobacteria* (14.5%). In group S2, the proportion of *Bacteroidota* increased significantly, with the top three gut microbiota being *Firmicutes* (34.6%), *Bacteroidota* (23.6%), and *Proteobacteria* (19.6%). The top three gut microbiota in group S3 were *Firmicutes* (25.5%), *Proteobacteria* (25.5%), and *Fusobacteriota* (23.9%).

At the genus level (Figure 4(b)), in group S0, *Mycoplasma* was the most dominant genus in the gut microbiota of fish in freshwater conditions (47.6%) (Figure 4), followed by several species of *Bacteroides*, *Cetobacterium* (28.3%), and *Ralstonia* (3.3%). In group S2, *Mycoplasma* remained a prominent genus in the gut microbiota (53.3%), followed by several bacteroides species, *Cetobacterium* (24.5%), and *Plesiomonas* (2.5%). When treated with 4 psu salinity in group S2, *Mycoplasma* (27.9%) and *Cetobacterium* (22.0%) were the main genera in the gut microbiota, with a significant increase observed in *Vibrio* (6.5%). In group S3, *Cetobacterium* (27.7%) exceeded *Mycoplasma* (24.8%) as the most dominant genus in the gut microbiota, with *Vibrio* (4.4%) slightly lower than in group S2.

3.3.3. Differential Abundance of the OTUs in Intestinal Microbiota of Striped Micropterus Salmoides. LEfSe was used to determine the intestinal biomarkers (OTUs) in

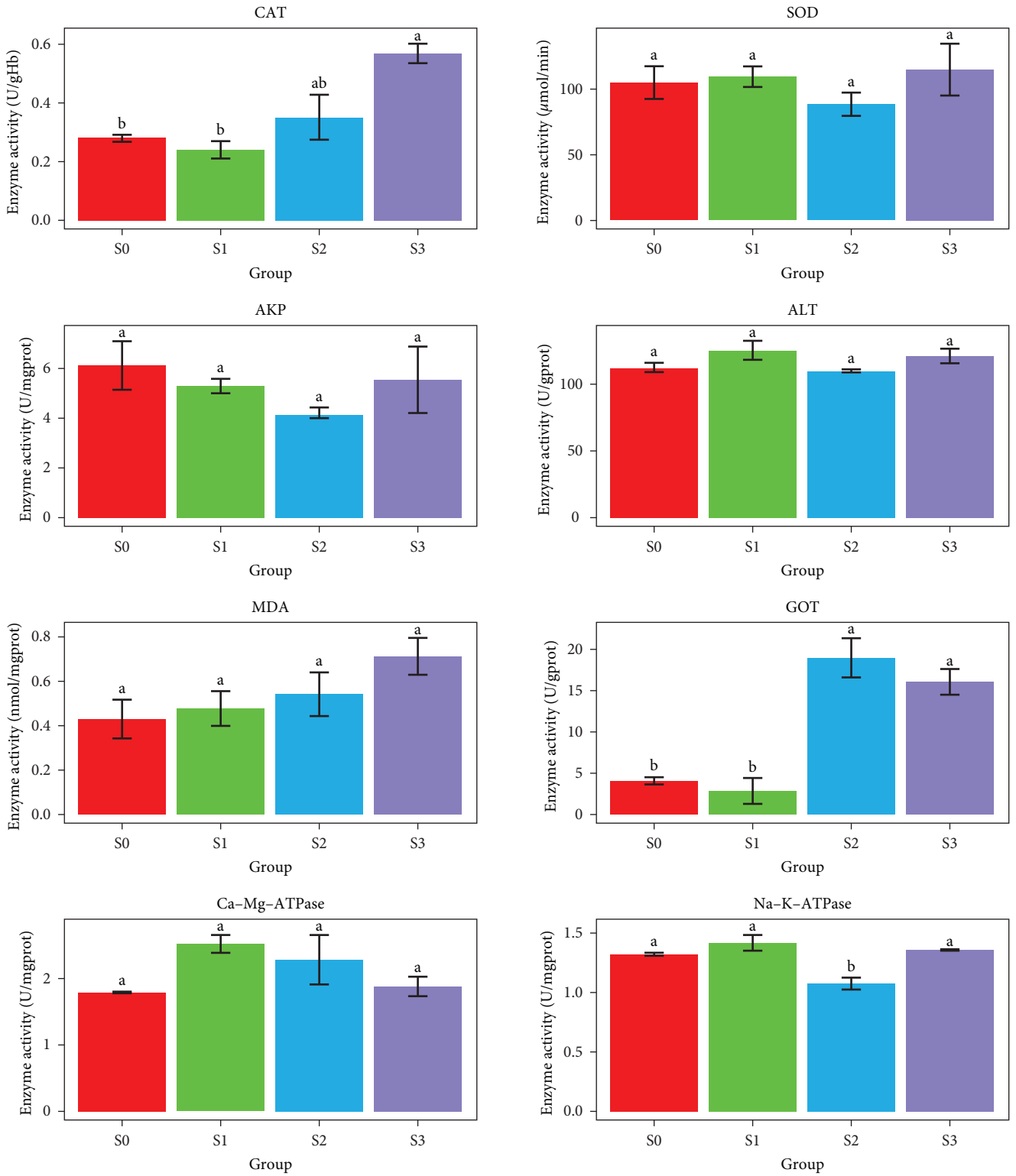

FIGURE 1: Effect of salinity on biochemical indicators of *Micropterus salmoides* gills, blood, and liver. Values are group means; different letters indicate significant differences ($P < 0.05$). *Note*. values are expressed as mean ± SE ($n = 3$). SOD, superoxide dismutase; MDA, malonaldehyde; CAT, catalase; ALT, alanine aminotransferase; GOT, glutamic-oxaloacetic transaminase; Ca–Mg–ATPase, calcium-magnesium adenosine triphosphatase; Na^+–K^+–ATPase, sodiumpotassium adenosine triphosphatase; GSH-Px, glutathione peroxidase.

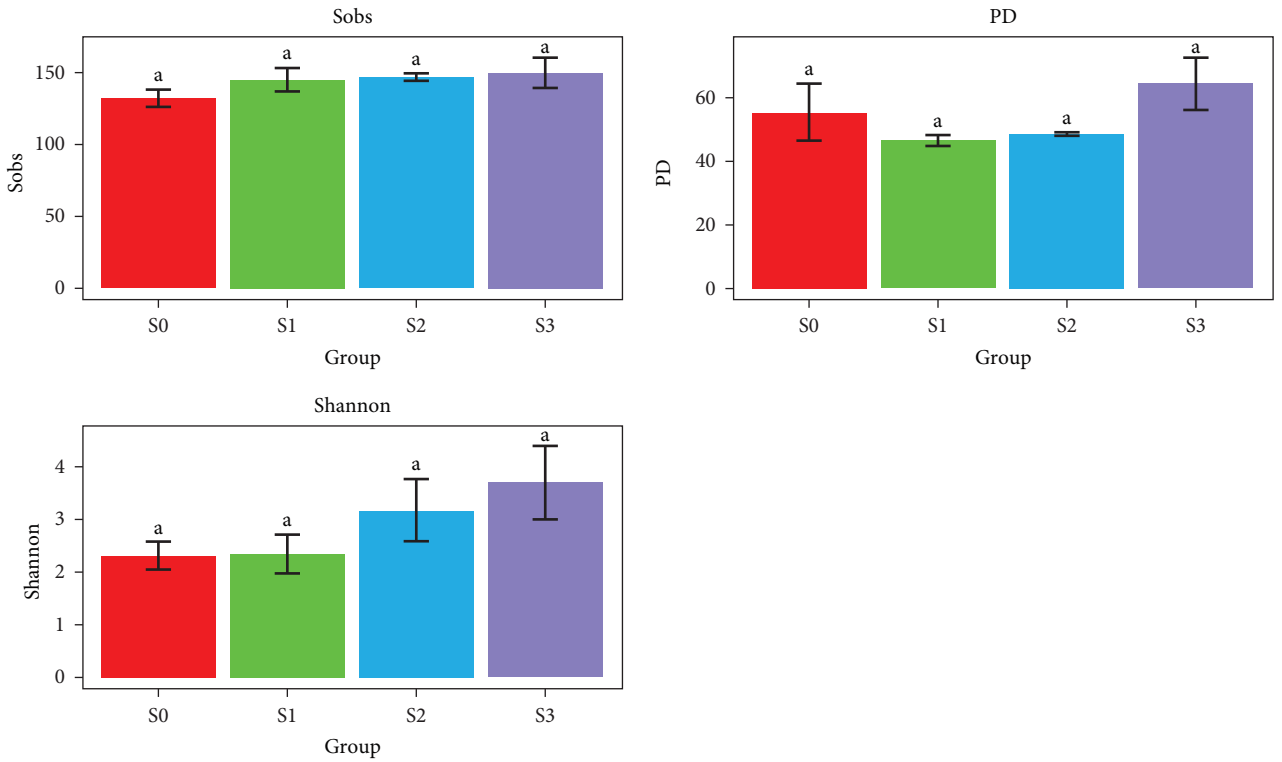

FIGURE 2: Alpha diversity in the intestinal microbiota of *Micropterus salmoides* under different levels of salinity. *Note*. Sobs, observed operational taxonomic units; Shannon index, Shannon diversity index; Pd, phylogenetic diversity. Results are given as mean ± SD. Different lower case letters denote statistically significant differences.

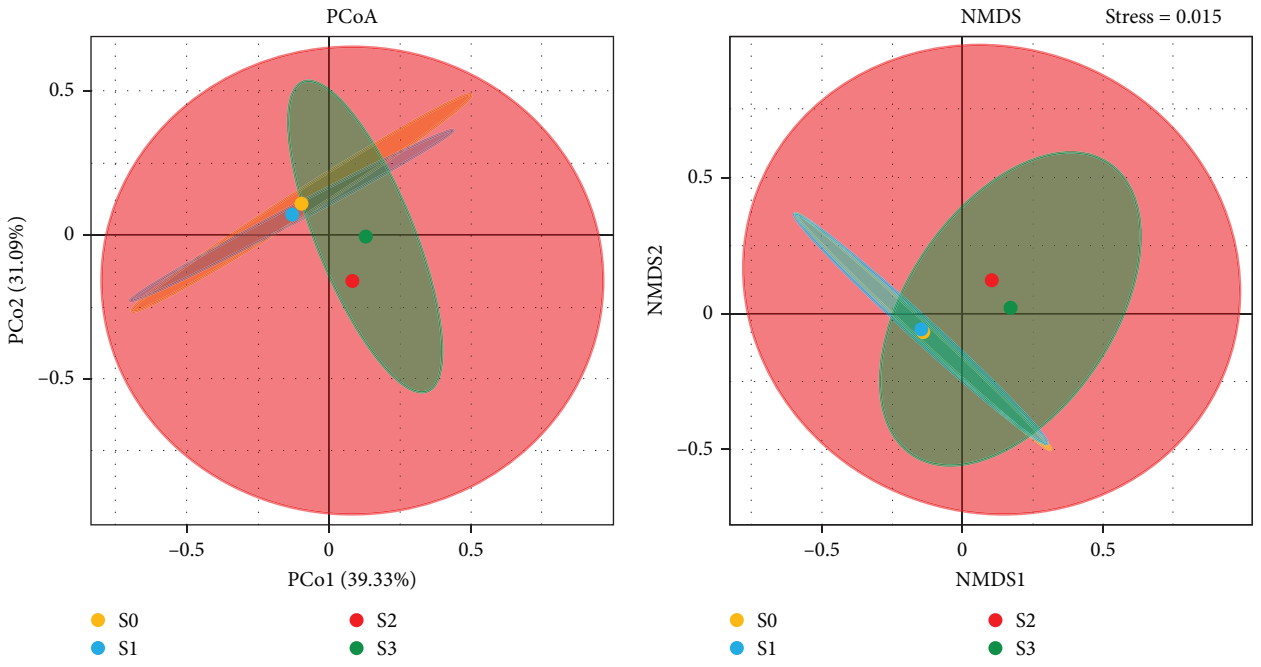

FIGURE 3: Principal coordinate analysis (PCoA) plot and nonmetric multidimensional scaling (NMDS) plot based on Bray–Curtis distance similarities in the intestinal microbiota of *Micropterus salmoides*.

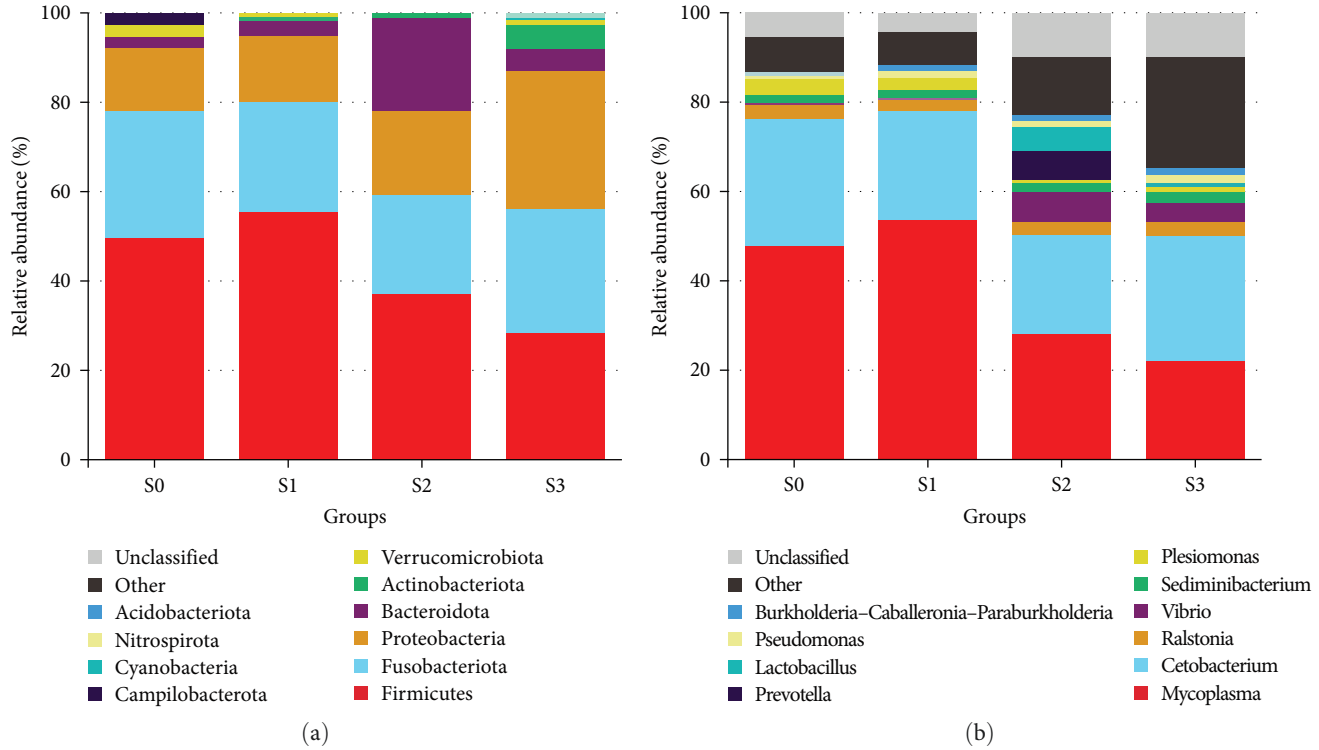

Figure 4: The mean relative abundance of the intestinal microbiota of *Micropterus salmoides* at different salinity level. *Note*. Only the OTUs present at the top 10 rankings are retain. (a) Is the phylum horizontal species distribution stack diagram, and (b) is the genus horizontal species distribution stack diagram.

striped catfish exposed to different levels of salinity (Figure 5). At the family level, the *Bacteroidaceae* and *Rikenellaceae* families are biomarkers in the 4 psu treatments, mainly reflecting the abundance of OTUs in the *Bacteroidales* order. Furthermore, the *Ruminococcaceae* family is enriched under 4-psu treatment, which also indicates a significant abundance of the *Osdillospirales* order. At the same time, under 4-psu treatment, the *Lentisphaerae* phylum also showed significant OTU abundance. Under 0-psu treatment, the *Nostocaceae* family has significant OTU abundance. The abundance of OTU of the *Hyphomicrobiaceae* family has significant differences in 6 psu treatment. There are no groups with significant abundance under 2-psu treatment, reflecting that there is no significant difference in species abundance between 2-psu treatment and other groups. Biomarkers with significant abundance in different salinity environments are different, indicating that biomarkers in each group are affected by the salinity of the water environment.

4. Discussion

4.1. Growth Performance. Salinity plays a crucial role in the growth of aquatic animals. For certain fish species, an optimal salinity level can enhance their growth performance. However, excessively high- or low-salinity levels can have detrimental effects [13, 14]. In this experiment, no significant differences were observed in the specific growth rates of largemouth bass among the various experimental groups. However, the feed conversion ratio was significantly higher in the 4 and 6-psu salinity groups compared to the 2-psu salinity group and the freshwater control group. This can be attributed to the impact of salinity on fish metabolism and energy expenditure. Salinity levels in aquaculture environments are closely associated with fish osmoregulation and energy metabolism. As salinity increases, the energy available for fish growth and development decreases correspondingly. For euryhaline fish species, water environments with salinity levels exceeding a certain threshold can inhibit their growth. For instance, in an Atlantic cod (*Gadus morhua*) salinity gradient culture experiment, a negative correlation was observed between the specific growth rates of the different experimental groups and changes in water environment salinity [15]. Research has shown that rainbow trout exhibit higher growth rates at lower salinities, while excessive salinity levels negatively impact their growth [16]. Similarly, within a safe salinity range, juvenile blue tilapia (*Oreochromis aureus*) exhibits optimal specific growth rates, which decline when salinity levels exceed this range [17]. Eurasian perch (*Perca fluviatilis*) exhibits the fastest growth rates in water environments with salinities ranging from 0 to 4 psu. However, when water environment salinity reaches 10 psu, their growth rate significantly decreases [18]. In this experiment, freshwater group S0 and experimental group S2 with a salinity of 2 psu imposed lower osmotic pressure loads on largemouth bass, resulting in relatively better growth performance under these conditions.

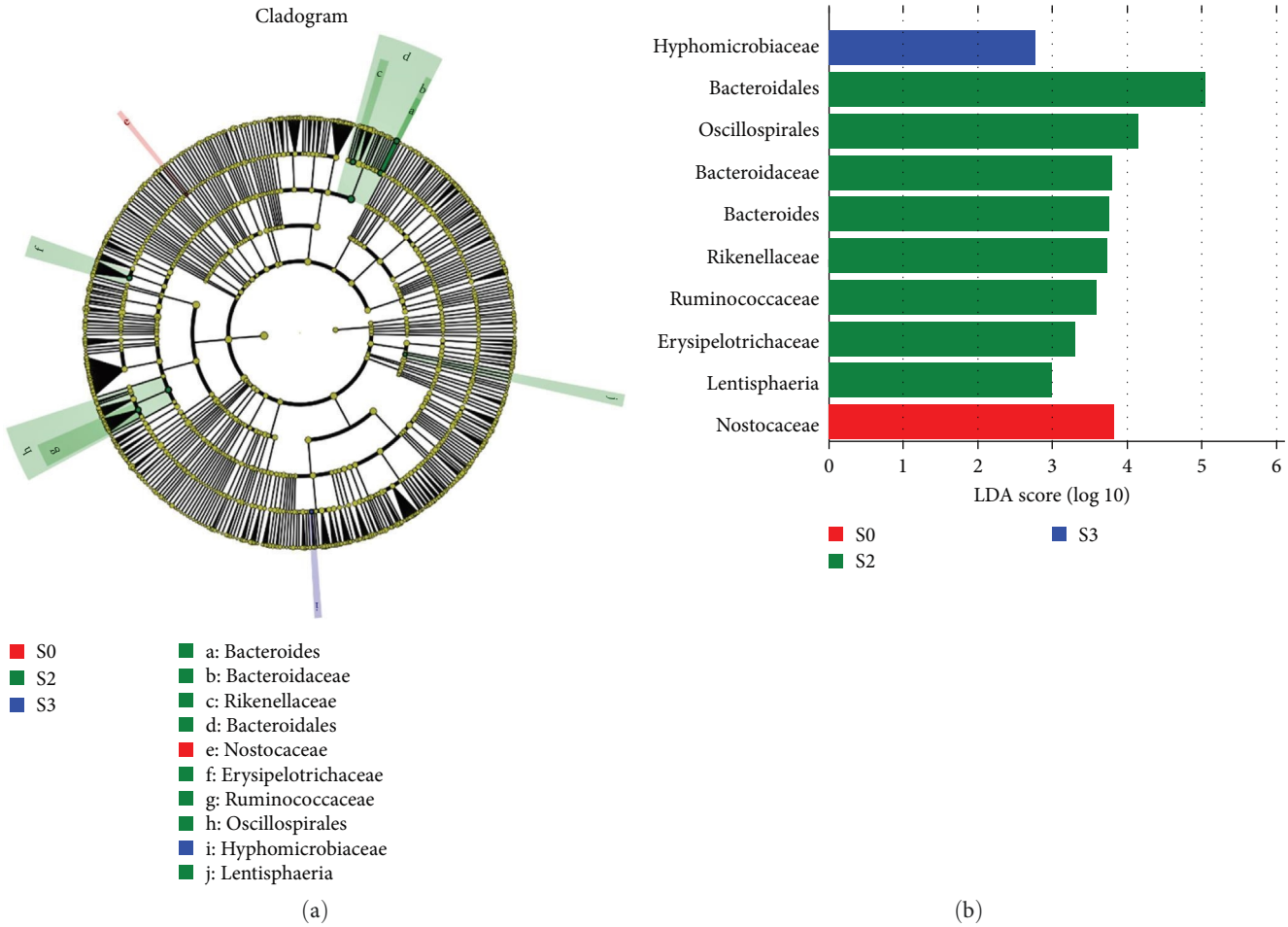

FIGURE 5: Cladogram showing differentially abundant Taxa in the intestinal microbiota of *Micropterus salmoides* at 0, 2, 4, and 6 psu for the various salinity conditions. *Note*. Linear discriminant analysis effect size (LEfSe) was used to validate the statistical significance and the effect size of the different abundances of Taxa in the treatments compared to the control. ((a) Is cladogram, and (b) is LDA score chart).

4.2. Biochemical Indicators. Changes in water salinity can exert physiological stress on fish, triggering a response from their antioxidant systems. When oxidative stress cannot be eliminated, the body will suffer oxidative damage. The results of this experiment indicate that the antioxidant system response of largemouth bass in the low-salinity group S2 at 2 psu was not strong. However, liver CAT activity increased significantly in the medium–high salinity groups S2 and S3, and serum GOT activity also significantly increased. The increase in serum GOT may indicate liver cell damage in fish, resulting in the release of GOT from liver cells into the blood [19]. Studies in European sea bass [20], Yellowfin seabream (*Acanthopagrus latus*), and Asian sea bass (*Lates calcarifer*) [2] have shown that salinity stress can lead to increased CAT and SOD activity in the liver. However, in this experiment, there was no significant change in SOD in liver tissue samples from each group, while CAT activity increased significantly in the high-salinity group. Another largemouth bass salinity stress experiment with salinity gradient groups of 0, 3 and 9 psu showed that both CAT and SOD in the 9-psu group were significantly higher than those of the freshwater group [6]. This may be due to the fact that the highest salinity in this experiment was lower than 9 psu and oxidative stress was not severe enough. As fish were cultured in saline water for a long time, they adapted to the corresponding salinity, resulting in no significant difference in SOD activity. At the same time, GSH-PX is an antioxidant enzyme that removes lipid hydroperoxides and organic hydroperoxides. Related studies have shown that GSH-PX can replace CAT to eliminate hydrogen peroxide when the CAT content is low in tissues [21].

In this experiment, the activity of the GSH-Px enzyme decreased first and then increased with the salinity of the experimental group. At the same time, there was no difference between GSH-Px levels in the high salinity group S3 and the GSH-Px levels of the freshwater group. These results are similar to those of studies on turbot (*Scophthalmus maximus*) [22], where CAT and GSH-Px work together to degrade hydrogen peroxide in high-salinity environments. In low-salinity environments where fish do not experience significant oxidative stress, GSH-PX does not rise to the same level as in freshwater groups. MDA is an important indicator of lipid peroxidation rate and intensity in organisms and reflects the degree of free radical attack on organisms. The results of MDA content in this experiment indicate that although the average MDA content has an upward trend with increasing salinity gradient of experimental groups,

there is no statistically significant difference. This is similar to the research on *Cyprinus carpio*, where MDA content increases due to salinity stress but gradually stabilizes over time [23].

Fish regulate their internal water–salt balance through osmoregulation. The Na^+–K^+–ATPase enzyme in fish gill epithelial cells can maintain the stability of fish osmotic pressure by regulating the permeability of cell membrane ions [24]. Some researchers have studied the effect of salinity on fish Na^+–K^+–ATPase [25]. Our studies have shown that as the duration of stress culture increases, the activity of Na+–K+–ATPase increases, and then decreases to a stable level. When aquatic organisms enter a saline water body, ion transport enzyme activity increases to maintain the osmotic pressure environment within the organism due to changes in osmotic pressure and ion loss from the fish body, and then decreases and stabilizes after a period of time [26–28]. In this experiment, there was no significant difference in Ca^{2+}–Mg^{2+}–ATPase activity between groups, which is consistent with the conclusions of previous studies. And the activity of Na^+–K^+–ATPase in group S2 with a salinity of 4 psu was significantly lower. The lower Na^+–K^+–ATPase activity in *Micropterus salmoides* in 4-psu saline water environment may be due to the small difference in osmotic pressure between the inside and outside of the fish.

4.3. Osmotic Stress Alters the Intestinal Microbial Diversity. Previous studies have investigated the effects of salinity on largemouth bass, but most have focused on physiology and impacts [29–32]. So far, no one has paid attention to the changes in the intestinal microbiota of this species under salinity conditions. Using an amplicon sequencing approach, we obtained 1.56 million raw sequence reads from a Miseq run distributed over 12 samples. Low-quality reads were filtered out and then assembled, with paired-end reads stitched into tags. The tags were then filtered to obtain clean tags and clustered on clean tags. Chimeric tags detected during the clustering alignment process were removed to obtain 1.47 million effective tags. OTU clustering resulted in 2,528 OTUs, and finally, abundance screening (rarefaction, at least two groups with a total tag count of 10) resulted in 210 effective OTUs. This study shows that there was no significant change in the α diversity index of the intestinal microbiota at salinities from 0 to 6 psu, which is similar to the previous studies on some euryhaline aquatic organisms [4, 33].

Fish regulate ion balance in their intestines by increasing water intake and salt excretion, which can lead to changes in the ecological niches within the intestines [4, 34]. The conclusions of the beta analysis in this study show that the bacterial communities of S0 and S1 have a high degree of similarity, while the bacterial communities of S2 and S3 have similarities, but S3 is quite different from S0 to S1. In addition, the results show that the confidence ellipse of group S2 overlaps with the other three groups, but its centroid is close to group S3. Therefore, it can be inferred that although the intestinal microbiota of group S2 is similar to the other three groups, it has a higher similarity to group S3. It can be speculated that while group S2 has distinctiveness from groups S0 and S1, its bacterial community richness is good.

At the same time, LEfse analysis showed that the abundance of species in group S2 was significantly higher compared to treatments 0, 2, and 6 psu. *Erysipelotrichaceae* is a common bacterial family in the gut and is associated with the synthesis of short-chain fatty acids (SCFAs) such as butyrate. *Ruminococcaceae*, as a symbiotic bacterium in the gut microbiota, has high efficiency in the decomposition of cellulose. Meanwhile, *Bacteroides* had a significant abundance in S2. *Bacteroides* are typically symbiotic in animal gut microbiota. Most proteins produced by the bacteroides genome can degrade polysaccharides and metabolize their sugars, playing an important role in processing complex molecules into simpler ones in the host intestine. The bacterial families with significant abundance of species in S0 and S6 were *Hyphomicrobiaceae* and *Nostocaceae*, respectively, and the roles of these two bacterial families in the gut of organisms are not yet clear. Therefore, from the perspective of bacterial species with significant abundance, the gut microbiota under 4 psu treatments is more beneficial to the physiological activities of the large yellow croaker than the other groups.

4.4. Osmotic Stress Alters the Intestinal Microbiota Taxonomic Composition. Bacteria are the dominant group among endogenous microorganisms in freshwater fish [35]. At the phylum level, we found that *Firmicutes*, *Clostridia*, *Proteobacteria*, and *Bacteroidetes* were dominant in all groups of largemouth bass. Previous studies have shown that *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Clostridia* are common dominant groups in the fish intestinal microbiota [36, 37]. Related studies have shown that an increase in Bacteroidetes species and a decrease in Proteobacteria may reflect the stable state of intestinal biota [36]. Among the four experimental groups, only group S2 had a higher proportion of *Bacteroidetes* than *Proteobacteria*, which may indicate that the intestinal microbiota of largemouth bass at 4 psu is more stable than those at 0, 2, and 6 psu. *Bacteroidetes* are generally present under different salinity conditions, indicating their importance in the function of largemouth bass intestines. *Proteobacteria* is the largest phylum among bacteria and is also the dominant phylum in various waters, widely present in various habitats [38]. Comparing the proportion of *Proteobacteria* in the four experimental groups, the proportion of *Proteobacteria* increased significantly to 31.14% in the 6-psu group, indicating that the salinity environment has an impact on the composition of intestinal microbiota.

At the genus level, *Mycoplasma* and *Fusobacterium* were the dominant genera in all four experimental groups. *Fusobacterium* is a common anaerobic genus in the fish intestine. Existing research shows that *Mycoplasma* is a pathogen that aquatic animals are susceptible to, which can damage the host's immune system and participate in the development of other pathological lesions and induce disease exacerbation [39]. In this experiment, the higher the salinity of the group, the lower the proportion of *Mycoplasma*, indicating that as salinity increases, the risk of disease caused by *Mycoplasma* is significantly reduced. At the same time, with the increase of

salinity, the increase of *Proteobacteria* is mainly reflected in the change of *Vibrio* abundance. In groups S1 and S2 with salinities of 4 and 6 psu, respectively, the abundance of *Vibrio* increased significantly, but the proportion was relatively small (6.5% in group S1; 4.4% in group S2). In summary, in environments with a salinity above 4 psu, it is possible to reduce the pathogenicity of pathogens in freshwater. At the same time, the intestinal microbiota of largemouth bass at 4 psu is more stable, so it can be inferred that 4 psu may be the most positive condition for largemouth bass.

5. Conclusion

From the current research, it can be seen that salinity has a significant impact on the cultivation of largemouth bass (*Micropterus salmoides*). Within an acceptable range, largemouth bass can be cultivated in the environments with 0–6 psu without affecting their survival rate. However, because of the impact of salinity, the feed coefficient will increase significantly. At the same time, an increase in the salinity of the water environment will significantly cause liver damage to largemouth bass. However, in environments with salinity higher than 4 psu, the risk of pathogenicity brought by freshwater pathogens is reduced. This research will help farmers make effective risk management decisions when growing largemouth bass in unfavorable saline–alkali environmental conditions. Further research can also be conducted to explore reducing the degree of liver damage to largemouth bass caused by salinity through dietary intervention or further improving the quality of largemouth bass muscle. This study can also provide a preliminary exploration for improving the taste of largemouth bass through short-term salinity cultivation.

Data Availability

The data sets in this study are available from the corresponding author on reasonable request. All data and materials are available for publication.

Ethical Approval

All applicable international, national and/or, institutional guidelines for the care and use of animals were followed. This study was performed according to the Guidelines for the Care and Use of Laboratory Animals developed by the Ministry of Science and Technology (Beijing, China). All experiments were conducted according to the Constitution of Academic Committee of Huzhou University and approved by the Academic Committee of Huzhou University. The annotation number is 20190625.

Disclosure

I hereby declare that I have undergone training in accordance with the academic conduct and regulations of my affiliated institution. All actions and behaviors outlined in this research have been reviewed and approved by my institution to ensure compliance with the international standards of proper academic conduct and regulations.

Furthermore, I affirm that all experimental procedures conducted in this research have been subjected to scrutiny and approval by the ethical review committee of my affiliated institution. This adherence to the ethical norms has been maintained in accordance with the regulations and standards set forth by my institution.

Conflicts of Interest

Authors have no conflicts of interest to declare for the publication of the present work.

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