

Acute temperature stresses trigger liver transcriptome and microbial community remodeling in largemouth bass (*Micropterus salmoides*)

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ABSTRACT

The largemouth bass is an important freshwater fish in China. As global climate changes, mass mortality in largemouth bass due to extremely low/high temperatures has aroused growing concern. In the present study, we investigated the physiological responses of the liver and intestine in largemouth bass to acute low/high-temperature stresses. Acute low/high-temperature stresses caused histological alterations such as structural damage in the liver. Based on liver transcriptomic data, a total of 6678 differentially expressed genes (DEGs) were detected, of which 2106, 5235, 3 and 449 DEGs were identified in fish treated with temperatures of 9 °C (serve cold-stress group, SC group), 18 °C (light cold-stress group, LC group), 30 °C (light heat-stress group, LH group), and 33 °C (serve heat-stress group, SH group), respectively. Further functional analysis found that the apoptosis, amino acid metabolism and lipid metabolism related signaling pathways were significantly enriched in fish after low-temperature stress, and protein processing in endoplasmic reticulum were significantly associated with heat stress. The weighted gene co-expression network analysis (WGCNA) results showed that multiple modules were significantly correlated with the LC or SC or SH groups, in which key signaling pathways were identified. Meanwhile, sequencing of the bacterial 16S rDNA gene demonstrated that acute high temperature stress reshaped the composition of the microbial community. Correlation analysis between bacteria and DEGs showed that bacteria could regulate pathways to affect the healthy status of host. This study provides the first systematic analysis about the adaptative responses of largemouth bass under acute temperature stresses by unveiling microbial, transcriptional, and histological changes, and identify microbial biomarkers/genes to monitor the health status of largemouth bass and to improve fish temperature tolerance, which will lay the foundation for further studies on the environmental stress response in largemouth bass.

1. Introduction

Fish, as the oldest vertebrates, live in almost all aquatic environments. Water temperature is the most important environmental factor for fish, affecting various biological processes, including growth, development, reproduction, and metabolism (Fonds et al., 1992; Handeland et al., 2008; Watson et al., 2018). Most fish are ectothermic, in which the body temperature tracks the ambient water temperature (Haesemeyer, 2020; Zahangir et al., 2022). Within the species-specific thermal tolerance ranges, fish are able to adapt to temperature changes through physiological plasticity or microevolution (Soyano and Mushirobira, 2018). However, the health status of fish is significantly affected when they are exposed to temperatures close to or beyond the limits of thermal tolerance (Fetzer et al., 2011; Takegaki and Takeshita, 2020). With the rapid development of industry and agriculture, the

frequency of extreme weather in the natural environment caused by humans is increasing, with the result that the aquaculture industry faces to more challenges (Yasumoto et al., 2018). Multiple studies have reported that commercial farming of fish is seriously affected by cold and heat stresses, such as largemouth bass, which have brought economic losses to the aquaculture industry (Paukert and Willis, 2004; Li et al., 2016; Phrompanya et al., 2021).

The largemouth bass (*Micropterus salmoides*), a typical eurythermic fish naturally inhabiting North America and Canada, was introduced in China in 1983 (Bai et al., 2008) and has become an important freshwater fish species in China, with annual production levels of over 450,000 tons (China Fishery Statistical Yearbook, 2018). The optimum growth temperature of largemouth bass is from 26 to 29 °C (Diaz et al., 2007). The acute change in water temperature due to global climate change also has an adverse influence on the culture of this kind of fish, which can even

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lead to the occurrence of mass mortality in largemouth bass (Vanlan-deghem et al., 2010; Landsman et al., 2011). Grant et al. (2003) found that the mortality of largemouth bass reached 100% after exposed at 35 °C. Therefore, it is essential to investigate the molecular mechanisms in largemouth bass in response to acute temperature.

In recent years, omics approaches, such as transcriptomes and 16S rDNA sequencing, have played important roles in identifying physiological pathways and microbiota in hosts in response to various conditions, facilitating the exploration of global host changes caused by abiotic stresses. Many studies have been conducted to identify gene expression profiles elicited by external stress in various fish species, such as gilthead sea bream (*Sparus aurata*), turbot *Scophthalmus maximus*, and spotted seabass (*Lateolabrax maculatus*) (Mininni et al., 2014; Zhang and Sun, 2017; Cai et al., 2020). For example, Hu et al. (2016) compared the interspecies transcriptional diversification in cold responses between zebrafish *Danio rerio* and tilapia *Oreochromis niloticus*. Although many studies have been carried out to investigate fish responses to low/high-temperature treatments using transcriptome and 16S rDNA sequencing technologies, few studies have focused on the similarities and differences in fish in response to cold and heat stresses. In addition, previous studies have focused on gene expression differentiation in fish exposed to high/low temperatures for weeks to months, and combining analysis on the transcriptome and microbiome was lacking. The regulatory mechanism of fish in response to acute temperature stress remains unclear.

The present study aimed to reveal the response mechanisms of largemouth bass by unveiling microbial, transcriptional, and histological changes and identify microbial biomarkers/genes to monitor the health status of largemouth bass and to improve fish tolerance ability under acute temperature stresses. The results laid a foundation for better understanding the molecular mechanism of eurythermic fish in response to acute temperature change, and provide a scientific basis for the breeding of heat- or cold-resistant largemouth bass for aquaculture.

2. Materials and methods

2.1. Fish and rearing conditions

A total of 125 healthy juvenile largemouth bass with an average body length of 14.97 ± 3.66 cm were purchased from a local breeding farm in Hancheng (Shaanxi, China). They were reared in a 500 L tank at $27^\circ\text{C} \pm 0.2^\circ\text{C}$ for two weeks before processing. During the acclimation period, the fish were fed twice per day with a commercial pellet diet (Fuxing Organism Feed Co., Ltd.), and two-thirds of the water was changed in each tank every day. During the experiments, water quality parameters, including dissolve oxygen, pH and ammonia, were maintained at about 6 mg L^{-1} , 7.4 ± 0.2 and 0.05 mg L^{-1} , respectively. In addition, natural light cycle in July was adopted.

2.2. Stress exposure and sample collection

After two weeks of acclimation, a total of 120 fish individuals were randomly divided into five groups, which were treated at 9°C (severe cold-stress group, SC group), 18°C (light cold-stress group, LC group), 27°C (control group, CK group), 30°C (light heat-stress group, LH group), and 33°C (severe heat-stress group, SH group). The CK group was the control group for all the groups. Each group is consisted of four replicates, and each replicate included 6 fish. Twenty rectangular tanks (100L) contained 70 L of aerated freshwater were used for low/high-temperature at a density of 6 fish per tank (Park et al., 2015).

Three individuals per tank were randomly sampled after 24 h and 48 h of exposure to low/high temperatures. The sampled fish were anesthetized using tricaine methanesulfonate (MS-222), and the liver and intestine were sampled. The liver tissues were divided into two parts: one part was stored at -80°C for RNA-seq and quantitative real-time PCR, and the other part was stored in 4% paraformaldehyde for

hematoxylin and eosin (HE) staining. For each group, three half-liver tissues were mixed as one replicate for RNA-seq. The intestine tissue was first rinsed with PBS; then, the outer layer and the contents were separately collected using sterile scissors and tweezers. In each group, three intestine tissues were mixed as one replicate (total of four replicates) for 16S rDNA amplicon analysis.

2.3. Liver transcriptome analysis

Total RNA was extracted from the liver mixture of each replicate in five groups (CK, LC, SC, LH, SH groups) after 24 h of low/high-temperature exposure using TRIzol (Invitrogen, USA) following the manufacturer's instructions. Then, the total RNA was quantified and checked for integrity with an Agilent 2100 Bioanalyzer (Agilent Technologies, America), followed by digestion with DNase I (Invitrogen, Life Technologies, Carlsbad, CA, USA) to degrade residual genomic DNA. The RNA quality was assessed using RNA integrity number (RIN) value, and the RIN value of all specimens were >7 , which could be used for further analysis. Sequencing libraries were generated using the NEBNext Ultra™ RNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's instructions, and index codes were added to attribute sequences to each sample. In brief, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads and then fragmented into small pieces using fragmentation reagent and NEBNext First Strand Synthesis Reaction Buffer (5×). Then, double-strand cDNA was synthesized using random hexamer primers and ligated to an adapter. To preferentially select cDNA fragments 240 bp in length, the library fragments were purified with an AMPure XP system (Beckman Coulter, Beverly, USA). Then, PCR was conducted using Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. Finally, PCR products were purified (AMPure XP system), and library quality was assessed on the Agilent Bioanalyzer 2100 system.

The clustering of the index-coded samples was carried out on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) based on the manufacturer's recommendations. Following cluster generation, the library preparations were sequenced on an Illumina HiSeq 4000 platform, and paired-end reads were generated. Clean reads were obtained after filtering reads containing adaptor sequences, $>5\%$ unknown nucleotides, $>50\%$ bases with a Q-value <20 , and low-quality reads. These clean reads were then mapped to the reference genome sequence using HISAT2 software. Only reads with a perfect match or one mismatch were further analyzed and annotated based on the reference genome (ASM1485139v1). The reads that have never been annotated in the reference genome were further analyzed to identify new transcripts and new genes. To acquire comprehensive information on gene functions of above reads, assembled unigenes were searched against the NCBI nonredundant protein sequences (Nr), NCBI nucleotide (Nt), eukaryotic ortholog groups (KOG), a manually annotated and reviewed protein sequence database (SwissProt), Protein family (Pfam), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using BLASTx and BLASTn with an E -value $<10^{-5}$. Blast2GO was used for Gene Ontology (GO) annotation with an E -value $<10^{-5}$ based on the protein annotation results of the Nr database (Conesa et al., 2005).

The gene expression of each read was quantified using RSEM software with default parameters, and the fragments per kilobase of transcript per million fragments mapped (FPKM) value was used to represent the expression abundance of the genes (Li and Dewey, 2011). Then, principal component analysis (PCA) was conducted by the R package to determine the relationships among the transcriptional expression profiles of all samples. Differential expression analysis between two groups, including LC vs. CK, SC vs. CK, LH vs. CK, and SH vs. CK, was performed using DESeq2 R package software (Wang et al., 2010). Genes with adjusted P -values <0.01 and $\log_2(\text{fold_change}) > 1$ between two groups were identified as differentially expressed genes (DEGs). The KEGG enrichment analysis mapped unigenes onto known signaling pathways, while GO enrichment analysis mapped unigenes to functional categories

to define gene product attributes. To better understand the DEGs functions, Go and KEGG enrichment analyses were conducted by the phyper R package, in which signal pathways with false discovery rates (FDRs) <0.05 were considered as significantly enriched.

To identify specific modules in relation to different temperature treatments, a weighted gene co-expression network analysis (WGCNA) was conducted using the WGCNA package. A soft-thresholding power β of 8 was applied to ensure that the networks exhibited an approximate scale-free topology, and the adjacency matrix was transformed into the topological overlap matrix (TOM). Then, the gene dendrogram was generated based on the TOM, which was used to identify modules using the dynamic tree cut method (<https://cran.r-project.org/web/packages/dynamicTreeCut/>). Finally, the expression values of screened genes (FPKM>1) were input into WGCNA, and the coexpression module with setting parameters of mergeCutHeight and minModule Size was 0.25 and 30, respectively.

Pearson's correlation coefficients were used to investigate the relationship between modules and different temperature treatments, and the module most strongly correlated with different temperature treatments was defined as the hub module ($R^2 > 0.7$, $P < 0.05$). To explore the biological functions of genes in different key modules, KEGG enrichment analyses were conducted. Genes with the highest degrees of connectivity within a network were referred to as key regulatory genes (Yang et al., 2014). Relative networks were visualized using Cytoscape v3.6.0 (Shannon et al., 2003).

2.4. Intestinal microbiome analysis

Total microbial genomic DNA collected from the fish intestine mixture of each replicate in the five groups after 24 h and 48 h of exposure was extracted using a MN NucleoSpin 96 Soi kit (MN, Germany), and then the quality and concentration of the extracted DNA were measured using a NanoDrop spectrophotometer (ND – 1000, NanoDrop Technologies, Wilmington, DE, United States) and agarose gel electrophoresis (AGE). We found that the total DNA of each replicate both could be used for further analysis. The V3–V4 region of the bacterial 16S rDNA gene was amplified by PCR, and the PCR conditions were as follows: 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 40 s, and 72 °C for 7 min using the primer pairs 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGAC-TACHVGGGTWTCTAAT-3'), which were modified with a random 6-base oligo barcode tag. The PCR products were checked with 1.8% agarose gel electrophoresis, and then a mixture of PCR products was purified using a Monarch DNA Gel Extraction Kit (Thermo Fisher Scientific, Waltham, MA, United States). Sequence libraries were validated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, United States) and quantified with a Qubit 2.0 Fluorometer (Thermo Fisher). Finally, the library was sequenced to generate paired-end reads using an Illumina NovaSeq 6000 platform (Illumina, Inc., San Diego, CA, United States) at Biomarker Bioinformatics Technology, Co., Ltd. (Beijing, China).

Raw reads were assigned to the samples based on their unique barcodes. FLASH software (Version 1.2.7) was used to merge the overlap of the paired-end reads in the same DNA fragment to obtain the splicing sequences (Magoc and Salzberg, 2011). Trimmomatic v0.33 was used to filter raw tags to obtain high-quality clean tags, and then the primer sequence was removed using cutadapt 1.9.1. (Bolger et al., 2014). The chimera sequences were detected by comparing tags with the reference database (RDP Gold database) using the UCHIME algorithm and then removed (Edgar et al., 2011). The effective sequences were then used in the final analysis. Sequences were clustered into operational taxonomic units (OTUs) against the SILVA bacterial database with $\geq 97\%$ similarity using Usearch software (Edgar, 2013; Quast et al., 2013). They were then taxonomically classified to different levels (phylum, class, order, family, genus, and species) using the Ribosomal Database Program (RDP) classifier with 80% confidence (Wang et al., 2007). Multiple

sequence alignment was performed using MUSCLE software to investigate the phylogenetic relationships among different OTUs.

The alpha diversity indices, including ACE, Chao1, Shannon, and Simpson, were calculated by Mothur v1.30 and used for richness and diversity indicators of the bacterial community (Schloss et al., 2009). Beta diversity based on unweighted UniFrac and nonmetric multidimensional scaling (NMDS) was evaluated using the vegan package in R software, after which intragroup and intergroup beta distance boxplot diagrams were generated (Caporaso et al., 2010). A one-way analysis of similarity (ANOSIM) was conducted to detect the differences in bacterial communities among groups. Linear discriminant analysis (LDA) effect size (LEfSe) analysis was conducted using the Python LEfSe package to identify biomarkers for highly dimensional colonic bacteria in the control and treatment groups (Segata et al., 2011). All statistical analyses were performed in R package software. In addition, the alpha diversity index values were expressed as the mean \pm SE and analyzed using two-way ANOVA (SPSS v20.0), followed by Tukey's multiple comparison tests to analyze the significant differences between independent experimental groups and the control group. $P < 0.05$ was regarded as statistically significant.

2.5. Correlation analysis of intestinal bacteria and liver DEGs

To perform the correlation analysis between liver DEGs and microbial genera in the intestine collected from the five groups after 24 h of low/high- temperature exposure, Pearson correlation analysis was conducted using the "corplot" package. The correlation coefficient threshold was set as 0.8. $P < 0.05$ was regarded as statistically significant, $P < 0.01$ was regarded as very significant, and $P < 0.001$ was regarded as extremely significant.

2.6. Quantitative real-time PCR analysis for RNA-seq validation

Nine randomly selected DEGs were analyzed by qRT-PCR to validate the expression patterns obtained by RNA-seq. The cDNA (CK, LC, SC, LH and SH) applied to RNA-seq was used as the qRT-PCR template. The primer pairs used to amplify the selected gene were designed using Primer Premier 5, and the sequences are listed in Table 1. *Tuba* and *EF1 α* were employed as the internal references to normalize the relative expression levels among samples (Ma et al., 2019). qRT-PCR was performed in a Roche LightCycler 96 Real-time PCR system using SYBR premix (TaKaRa, Japan). Each reaction was performed in triplicate, and the PCR conditions were as follows: 95 °C for 30s and 40 cycles of 95 °C for 5 s, 60 °C for 30 s, followed by melting curve determination. The relative gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). All data are expressed as the mean \pm standard

Table 1
The primer pairs used in this study.

Gene name	Product size	Sequences of the primer 5'-3'
gene-hsp70	155	F: ACCCCTCTCCCTGGAAATT R: GCACGCTCACCTCATAAACC
gene-caspase 6	135	F: AGACTCAGACTGCTTTGCTCR R: TTTCCACAGCTTCTACAC
gene-baxa	174	F: GGGTCAGCGGTATGGAGACA R: TGGGACTGAGTGAAGAGTTGTAT
gene-bcl21	159	F: TTCACTGATCTGCAAACACAG R: CTTCTCACGCACTCCACACAC
gene-c5	128	F: AACATGGAATCCCCTATGCA R: TTCACTCAAATCTGTTCTCGCT
gene-galectin-3	184	F: TCACTGAGGGGGCAACAGG R: CGGAACCTAAACAGCGGGATG
gene-gst	132	F: CTACCTCTACGCCCTCGTCTTTG R: TTCTGGACCTGAACTCCACT
gene-tp53	201	F: GTGGAGAGGTGGTGGCATTG R: GGGAGAAAGGAGTGTCTGGGT
gene-ISP	125	F: TCGCTCCGTTCACACCTGCT R: TCCACAACACACACCCCT

deviation and *F* test was applied. Then, the data were analyzed by *t*-test using SPSS version 21.0 software; when *P* < 0.05, the difference was considered statistically significant.

3. Results

3.1. The influence on liver and intestine histopathology after cold or heat stress

To investigate the histopathological changes in the liver after low/high-temperature stresses, histopathological examination was conducted. After 24 h and 48 h of treatment, no histopathological change was identified in the livers of the CK group (Fig. 1A&F). The hepatocytes exhibited a polygonal shape, granular cytoplasm, and rounded nuclei. Some histological modifications were detected in largemouth bass liver after 24 h and 48 h of low/high-temperature stresses. The boundaries between hepatocytes were blurred and became difficult to characterize. In addition, hepatocyte and nuclear hyperemia into capillaries was also detected in the treatment groups (LC, LH, SC, and SH groups).

3.2. Transcriptome sequencing analysis

3.2.1. Sequencing results and functional annotation

Twenty samples collected from largemouth bass liver that had been subjected to different temperature stresses for 24 h, with each sample consisting of three randomly assigned individuals [PRJNA876793], were used to extract cDNA and were then sequenced. In general, the number of clean bases per library ranged from 5,787,695,922 to 9,493,892,188, and the Q30 in each cDNA library was >93% (Supplementary Table 1). Subsequently, these clean reads were mapped onto the reference genome, and the mapping rates ranged from 81.24% to 96.17%. In addition, 3512 new genes were identified, of which 1681 were functionally annotated in the seven databases (Supplementary material 1).

3.2.2. Identification, analysis and validation of differentially expressed genes

Principal component analysis was conducted to obtain a global view on transcriptome profile of fish under different temperature treatments. The results showed that the first principal component accounted for 76.33% of the variance in the dataset (Fig. 2A). A distinct expression profile was found between the CK group and the low/high temperature groups. Remarkably, the gene expression of LH samples was more similar to that of CK samples. The transcriptome profile of the LC group was much closer to that of the SC group, while the SH group exhibited significantly different expression from the CK, LH, LC and SC groups.

To identify the DEGs, the expression levels of 30,678 genes were compared in fish between the control group and temperature treatment groups, including LC vs. CK, SC vs. CK, LH vs. CK, and SH vs. CK. A total of 6678 DEGs were detected, in which 2106 (1002 upregulated and 1104 downregulated), 5235 (2898 upregulated and 2337 downregulated), 3 (1 upregulated and 2 downregulated) and 449 (203 upregulated and 246 downregulated) DEGs were identified in LC vs. CK, SC vs. CK, LH vs. CK, and SH vs. CK, respectively (Fig. 2B, Supplementary material 2). The number of DEGs in fish liver after low-temperature treatments was higher than that after high-temperature treatments.

qRT-PCR was performed to verify the DEGs obtained by RNA-seq. Nine DEGs were selected and applied to qRT-PCR validation. Real-time PCR yielded a single product for all of the verified DEGs, and the expression patterns of the genes we selected were consistent with those of the RNA-seq data (Fig. S1). Therefore, the qRT-PCR analysis results confirmed that the RNA-seq results were reliable.

To better understand the regulatory mechanism of largemouth bass in response to low/high temperature stresses, the KEGG analyses were conducted on upregulated and downregulated DEGs in five groups, and significantly enriched pathways varied in five groups (Table 2). Among

them, metabolism-related signaling pathways were significantly changed in LC, SC, and SH groups, and immune-related signaling pathways were significantly upregulated in SC groups, such as NOD-like receptor and Toll-like receptor signaling pathways (Fig. S2). A total of 821 DEGs were shared by low-temperature treatment groups, respectively (Fig. 3A). KEGG analysis on low-temperature stressed DEGs found that apoptosis related- (apoptosis and p53 signaling pathway) and amino acid related- (Arginine biosynthesis and Aminoacyl-tRNA biosynthesis) signaling pathways were significantly induced (Fig. 3B&S1&S3A). A total of 216 DEGs were unique in SH group, KEGG analysis found that Protein processing in endoplasmic reticulum and Steroid biosynthesis were top two among them (Fig. 3A&3C). In the protein processing in ER pathway, 9 genes encoding heat shock proteins (Hsps), including *hsp40* (*danJa*, *danJb*, *danJc*), *hsp60*, *hsp30*, *hsp70b*, *hsp90a*, *hsp90b*, and *hspB1*, were upregulated under high temperature treatment, and six genes, such as, *hsp60*, *hsp70*, *danJa* and *hsp75*, were still upregulated under the low temperature (Fig. S3B). In addition, 59 DEGs were shared by all temperature groups except for LH vs. CK. KEGG analysis results showed that these DEGs were involved in fatty acid metabolism, including the genes *sterol regulatory element binding transcription factor 2* (*srebf2*), *solute carrier family 18 member 3b* (*slc18a3b*), *slc18c*, and Citrate cycle (TCA) cycle (Fig. 3D, Supplementary material 3). Interestingly, these genes exhibited opposite expression tendencies between the low-and high-temperature treatment groups.

3.2.3. Weighted gene co-expression network analysis (WGCNA)

A weighted gene co-expression network was constructed for the livers of largemouth bass to explore the underlying mechanisms of fish in response to cold/heat stress. The network contained 2305 unigenes that were classified into 14 modules, with module sizes ranging from 37 to 748 (Fig. 4). In these modules, the cyan, blue and midnight blue modules were significantly positively correlated with the LC group, and the red module was significantly negatively correlated with the LC group. The brown, turquoise, green-yellow, and pink modules were positively correlated with the SC group, while the yellow, magenta, red and midnight blue modules were negatively correlated with the SC group. Three modules, including black, magenta and red, were positively correlated in the SH group. The genes in each module were displayed in Fig. S4. Subsequently, DEGs in temperature-related modules were further investigated by KEGG enrichment analysis (Supplementary Table 2). In general, fatty acids elongation and biosynthesis of unsaturated fatty acid were negatively related with low temperature stresses, in which four DEGs were identified, and three of them were annotated as *acyl-coenzyme A thioesterase 1*. Apoptosis was positively related with low temperature stresses, and *Bcl-2*, *AKT serine/threonine kinase 2* (*Akt2*), and *Micropterus salmoides* new Gene 5074 (serine/threonine-protein kinase/endoribonuclease IRE1) were identified as key regulators (Fig. 5A). In addition, Protein processing in endoplasmic reticulum was significantly related with high temperature stress, in which heat shock related genes were key regulator genes (Fig. 5B).

3.3. Intestinal microbial sequencing analysis

3.3.1. Total Illumina sequence reads, quality trimming and OTU designation

In general, 1,254,279 valid sequence reads classified into 1747 OTUs were retrieved from fish of the control and challenge groups [PRJNA876793]. The rarefaction curves of most samples were nearly asymptotic, indicating that the sequencing depth in each sample was sufficient to reflect most microorganisms (Fig. S5). For the intestines collected from fish treated with low/high temperature for 24 h, a total of 1733 OTUs were shared by all five groups, while 1 OTU was shared by four treatment groups (Fig. 6A). For the samples collected at 48 h, a total of 1600 OTUs were shared by the five groups, whereas 139 OTUs were lost in the SH group, and 1 OTU was unique in the control group (Fig. 6B). In addition, the OTUs in the same group were similar between

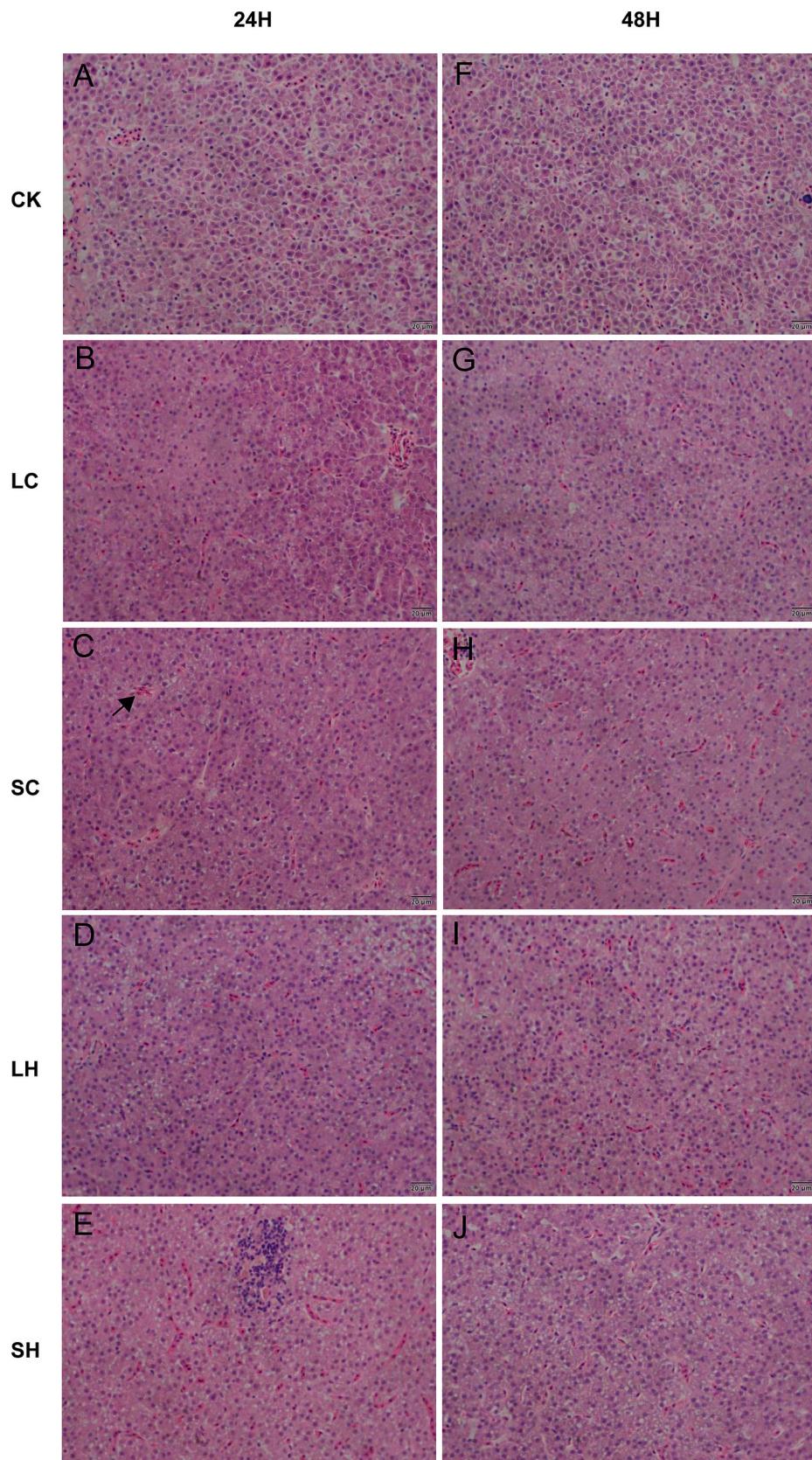


Fig. 1. Representative micrographs of the effects of different treatments (CK, LC, SC, LH, and SH) over time on the liver structure of largemouth bass. A-E Fish in the five groups (CK (A), LC (B), SC (C), LH (D), SH (E)) were treated for 24 h. F-J Fish in five groups (CK (F), LC (G), SC (H), LH (I), SH (J)) were treated for 48 h. The arrow indicates the hepatocyte and nuclear hyperemia into capillaries.

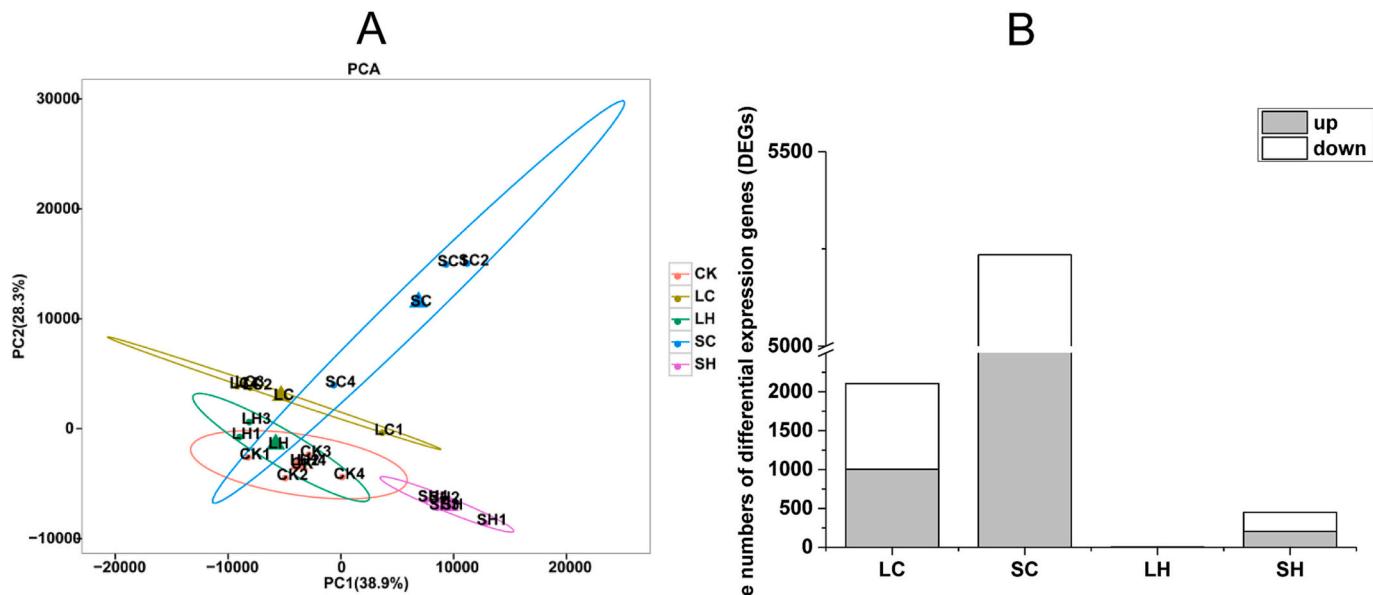


Fig. 2. Overview of the transcriptomic data response to acute temperature stresses. A. A Principal component analysis (PCA) of global gene expression in liver response to acute temperature stresses. B. Numbers of DEGs in the four groups (LC, SC, LH, and SH groups) after 24 h of stress. The DEGs were identified by the DEGseq method. Light grey represents for upregulated genes, while white represents for downregulated genes.

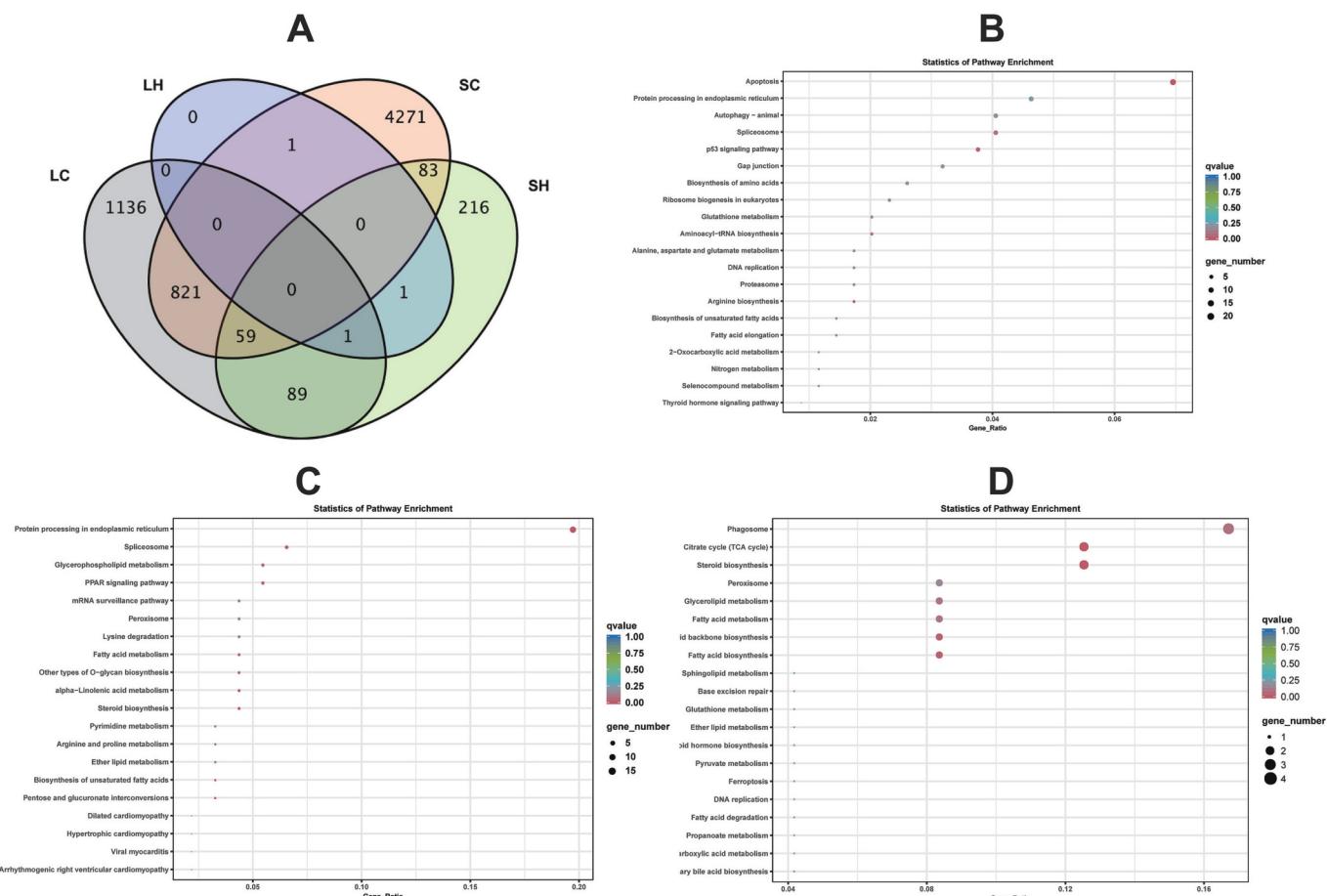


Fig. 3. The KEGG enrichment analysis of DEGs identified from acute temperature stresses. A. Venn diagram showing the number of DEGs identified in four groups (LC, SC, LH and SH groups). B. KEGG enrichment analysis for DEGs identified from acute cold stresses. C. KEGG enrichment analysis for DEGs identified from acute high temperature stresses. D. KEGG enrichment analysis for DEGs shared by the LC, SC, and SH groups.

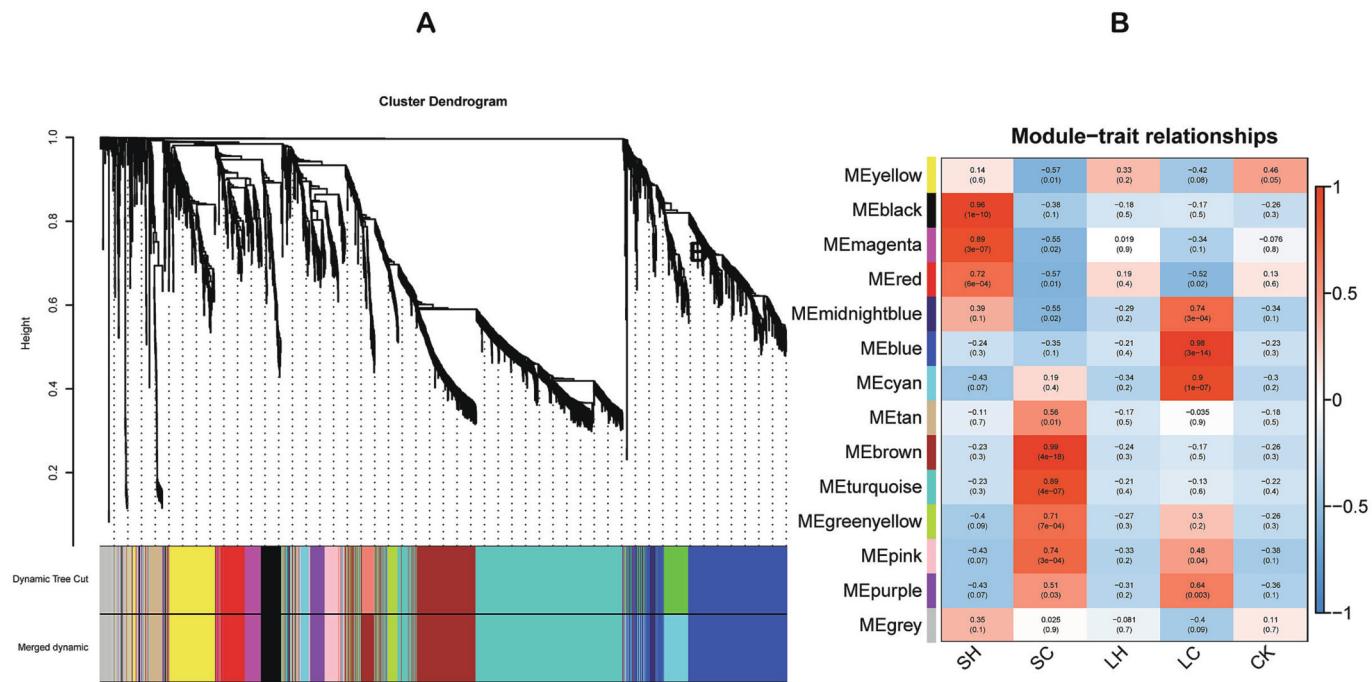


Fig. 4. Weighted gene co-expression network analysis (WGCNA) of genes in all samples. A. Hierarchical cluster tree displaying the coexpression modules identified by WGCNA. The major tree branches were formed by 14 modules ([fragments per kilobase of exon per million reads mapped (FPKM) ≥ 1 , module similarity threshold ≥ 0.25). B. Module-sample association. Each row corresponds to a module labeled with a different color. Each column corresponds to a sample. Each cell contains the corresponding correlation with the *P* value in parentheses.

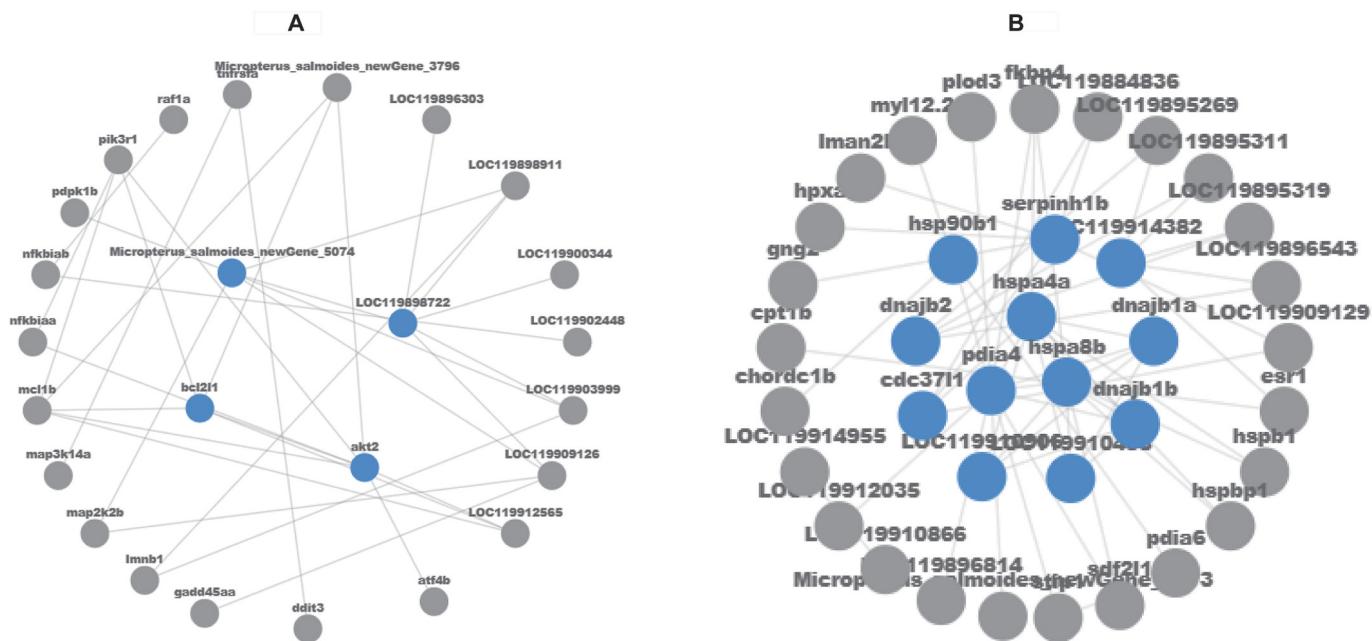


Fig. 5. Network diagrams of apoptosis (A) and Protein processing in endoplasmic reticulum (B) pathways in temperature-related modules. The apoptosis is positively correlated with low temperature, and the Protein processing in endoplasmic reticulum is significantly correlated with high temperature. The black line represents the association between two genes within the network.

24 h and 48 h of low/high- temperature exposure. To assess the intestinal microbial diversity between two groups, the alpha diversity (ACE1, Chao1, Shannon and Simpson indices) was analyzed. At 24 h, no significant difference was found among the five groups (Supplementary Table 3). At 48 h, the diversity indices (Chao1 and ACE) and observed species indicated a relatively low diversity within the SH groups after 48 h of high temperature treatment. Interestingly, the Simpson index in the

CK group was significantly higher than those in the other groups. To further investigate the difference between different groups, the UPGMA were used to construct a sample clustering tree, and samples from high temperature groups after 48 h of exposure was obviously distinct to other groups, indicating that the acute high temperature treatment might have a significant influence on the intestinal microbiome (Fig. 6C). In addition, ANOSIM test was conducted among the five

Table 2

KEGG enrichment analysis of DEGs under acute low/high temperature stresses.

Groups	DEGs_type	KEGG_term	Enrichment Score	Q value
LC group	up	Proteasome	10.81	6.98E-24
LC group	up	Steroid biosynthesis	9.9	1.37E-09
LC group	up	Terpenoid backbone biosynthesis	7.76	7.16E-07
LC group	up	Spliceosome	3.23	2.09E-06
LC group	up	Protein processing in endoplasmic reticulum	2.47	3.68E-05
LC group	up	Aminoacyl-tRNA biosynthesis	4.61	0.000428
LC group	up	Ribosome biogenesis in eukaryotes	3.09	0.001814
LC group	up	Mismatch repair	5.28	0.004449
LC group	up	Protein export	5.66	0.00736
LC group	up	DNA replication	3.33	0.010091
LC group	up	Pyruvate metabolism	3.19	0.012126
LC group	up	Apoptosis	1.98	0.012126
LC group	up	Butanoate metabolism	4.24	0.02542
LC group	up	Biosynthesis of amino acids	2.41	0.027084
LC group	up	RNA transport	1.94	0.029326
LC group	up	Synthesis and degradation of ketone bodies	6.03	0.029326
LC group	up	Citrate cycle (TCA cycle)	3.23	0.042373
LC group	up	Sulfur metabolism	5.32	0.042373
LC group	down	Glycine, serine and threonine metabolism	5.51	6.42E-05
LC group	down	Histidine metabolism	5.82	0.001238
LC group	down	Arginine biosynthesis	5.96	0.002009
LC group	down	Arginine and proline metabolism	3.63	0.002009
LC group	down	Glycolysis / Gluconeogenesis	3.13	0.004282
LC group	down	Thyroid hormone signaling pathway	12.29	0.004282
LC group	down	Tryptophan metabolism	3.61	0.004592
LC group	down	Linoleic acid metabolism	4.68	0.004749
LC group	down	Folate biosynthesis	4.1	0.010901
LC group	down	Biosynthesis of amino acids	2.62	0.021008
LC group	down	Alanine, aspartate and glutamate metabolism	3.16	0.026532
LC group	down	Arachidonic acid metabolism	2.76	0.026532
LC group	down	Carbon metabolism	2.15	0.02837
LC group	down	Purine metabolism	1.97	0.031173
LC group	down	Ascorbate and aldarate metabolism	4.1	0.031173
LC group	down	Fatty acid degradation	3.12	0.037007
SC group	up	Ribosome biogenesis in eukaryotes	3.05	3.71E-08
SC group	up	FoxO signaling pathway	2.25	4.99E-08
SC group	up	Apoptosis	2.05	1.30E-06
SC group	up	Adipocytokine signaling pathway	2.54	5.89E-06
SC group	up	Autophagy - animal	1.94	6.66E-05
SC group	up	Insulin signaling pathway	1.94	8.04E-05
SC group	up	Mitophagy - animal	2.24	0.000759
SC group	up	MAPK signaling pathway	1.52	0.000785
SC group	up	Endocytosis	1.53	0.000799
SC group	up	Cytokine-cytokine receptor interaction	1.62	0.00095
SC group	up	Salmonella infection	1.57	0.00122
SC group	up	mTOR signaling pathway	1.7	0.0014
SC group	up	C-type lectin receptor signaling pathway	1.77	0.001887
SC group	up	Cellular senescence	1.62	0.003598
SC group	up	Toll-like receptor signaling pathway	1.82	0.004613
SC group	up	p53 signaling pathway	1.92	0.004766
SC group	up	Gap junction	1.76	0.011472
SC group	up	Ferroptosis	2.23	0.011472
SC group	up	ErbB signaling pathway	1.8	0.014283
SC group	up	VEGF signaling pathway	1.83	0.041284
SC group	up	NOD-like receptor signaling pathway	1.44	0.041284
SC group	up	Fatty acid biosynthesis	2.63	0.041284
SC group	up	Spliceosome	1.58	0.041284
SC group	up	RNA transport	1.52	0.041284
SC group	down	Herpes simplex virus 1 infection	1.97	5.71E-11
SC group	down	Basal transcription factors	3.9	0.000119
SC group	down	Base excision repair	3.02	0.000562
SC group	down	Nucleotide excision repair	2.53	0.003869
SC group	down	DNA replication	2.56	0.03153
SC group	down	Peroxisome	1.96	0.041054
SH group	up	Protein processing in endoplasmic reticulum	12.67	7.48E-24
SH group	up	Arrhythmogenic right ventricular cardiomyopathy	28.34	0.049529
SH group	up	Viral myocarditis	25.51	0.049529
SH group	up	Spliceosome	4.05	0.049529
SH group	up	Hypertrophic cardiomyopathy	19.62	0.049529
SH group	up	Dilated cardiomyopathy	19.62	0.049529
SH group	down	Steroid biosynthesis	53.82	8.08E-25
SH group	down	Terpenoid backbone biosynthesis	23.16	4.53E-08
SH group	down	Fatty acid metabolism	11.86	4.53E-08
SH group	down	PPAR signaling pathway	7.66	4.71E-05

(continued on next page)

Table 2 (continued)

Groups	DEGs_type	KEGG_term	Enrichment Score	Q value
SH group	down	Fatty acid biosynthesis	16.34	0.000179
SH group	down	Propanoate metabolism	8.73	0.003258
SH group	down	alpha-Linolenic acid metabolism	11.58	0.004019
SH group	down	Pyruvate metabolism	7.13	0.006274
	down	Glycerophospholipid metabolism	4.49	0.00794
SH group	down	Biosynthesis of unsaturated fatty acids	7.79	0.012794
SH group	down	Fatty acid degradation	6.43	0.023557
SH group	down	Butanoate metabolism	9.5	0.023789
SH group	down	Ether lipid metabolism	5.26	0.04077

groups to measure the effect of high/low temperature on the intestinal microbial communities. The difference was significant ($P < 0.01$) between the high temperature treatment group and the control group (Fig. 6D).

3.3.2. Variation in intestinal microbial community composition

The abundance of taxa was identified as the most resolvable taxa (phylum and genus). Firmicutes was the most dominant phylum in all samples, followed by Proteobacteria, Bacteroidetes, and Actinobacteria (Fig. 7A). The four dominant phyla were relatively similarly distributed in each sample, but with different abundance and variation trends. The abundance of Firmicutes increased after exposure to high temperature for 48 h, while the abundance of Actinobacteria was reduced significantly. At the genus level, Muribaculaceae was significantly decreased after 24 h and 48 h of low/high temperature exposure (Fig. 7B). In addition, three genera (Enterobacter, Bacteroides and Faecalibacterium) were obviously increased after 48 h of heat stress in the SH group compared to the CK group, while RB41 and Lactobacillus exhibited the opposite tendency.

3.3.3. Identification of potential intestinal microbial biomarkers

LEfSe analysis was employed to investigate the potential biomarker taxa in acute temperature treatments. A total of 28 discriminatory taxa were identified in the intestine after 24 h of low/high-temperature exposure (Fig. S6A). In phylum level, Patescibacteria, Proteobacteria, and Bacteroidetes was differentially abundant clades. In genus level, five types of bacteria, including Ruminococcaceae UGG 014, Candidatus Saccharimonas, Lachnospiraceae, Muribaculaceae, Staphylococcus, were differentially abundant clades. In addition, a total of 91 taxa (38 taxa in SH, 25 taxa in SC group, 17 taxa in LH group, 31 taxa in LC group, and 8 taxa in CK) displayed significant enrichment in their abundance in single groups after 48 h of low/high temperature stresses (Fig. S6B). In phylum level, 7 types of bacteria were differentially abundant, including Firmicutes, Epsilonbacteraeota, Actinobacteria, Plantctomyces, Acidobacteria, Chloroflexi, Bacteroidetes. In genus level, 28 types of bacteria were differentially abundant clades. In addition, Ruminococcaceae UGG 014 and Muribaculaceae were both unique genera in CK group.

3.4. Analysis of the correlation between the gut microbiota and liver DEGs

Correlation analysis was conducted to reveal the relationships between temperature, intestinal bacteria and DEGs (Supplementary material 4). The results showed that 11 kinds of bacteria showed significantly correlation with low temperature, while 6 kinds of bacteria showed significantly correlation with high temperature (Table 3). Acute high temperature treatment might have a significant influence on the intestinal microbiome, and 273 DEGs showed significantly correlation relationship with these 6 kinds of bacteria (Supplementary material 5). KEGG analysis showed that Protein processing in endoplasmic reticulum was significantly enriched, and Hspa8b, Hypoxia up-regulated 1 (Hyou1) and Hspa4a were key regulatory genes (Fig. 8A&8B). Muribaculaceae was significantly reduced after high/low-temperature stress. For the LC and SH groups, multiple pathways were significantly correlated with it, including Steroid biosynthesis, Terpenoid backbone

biosynthesis (Fig. 8C&8D). Ruminococcaceae UCG 014 was also differentially abundant clades, and KEGG analysis found that PPAR signaling pathway and Ribosome biogenesis in eukaryotes were top two of them (Fig. 8E). In addition, PPI network analysis results indicated that two genes, i.e., pidd and cellular tumor antigen P53 (tp53), might be key to interact with bacteria in largemouth bass after acute low/high temperature stresses (Fig. 8F).

4. Discussion

The largemouth bass is an important freshwater fish species worldwide. Mass mortality events frequently occur due to extremely low or high temperature stresses, which results in huge economic losses and deeply hinders the development of the largemouth bass industry. However, comprehensive studies of the effect of acute thermal stress on largemouth bass are still scarce, and bioindicators for the health status of was few. In the present study, the histological changes in fish liver were investigated after acute temperature change, and then a comparative liver transcriptome and the intestinal microbiota of largemouth bass were analyzed to elucidate the molecular mechanisms underlying heat/cold stress in largemouth bass. The liver is one of the most sensitive organs to show alterations in biochemistry, physiology and structure following exposure to various environmental stresses (Hinton and Couch, 1998). Previous reports have shown that changes in gene expression were relatively larger in the liver than other tissues in response to an extreme environment (Song et al., 2015). Therefore changes in the liver may serve as markers to indicate physiological status of fish prior exposure to environmental stressors (Bernet et al., 1999; Velmurugan et al., 2007). Cell structure changes and cell dysfunction are the base of all diseases, as shown by histopathological studies (Rodrigues et al., 2019; Liu et al., 2021). Our histopathological examination found that the largemouth bass exposed to high/low temperatures for 24 h and 48 h showed severe bleeding in the liver. Then, high-throughput sequencing analysis was conducted in the livers of largemouth bass after 24 h of high/low-temperature exposure, and 2106 DEGs, 5235 DEGs, 449 DEGs, and 3 DEGs were detected in 18 °C, 9 °C, 33 °C, and 30 °C stress groups, respectively. In addition, intestinal microbiome analysis showed that the composition of microbe was reshaped after acute high temperature stress. Taken together, these results indicated that acute temperature stresses have rapid and severe effects on the physiological status of largemouth bass.

Apoptosis plays an essential role in embryologic development, cell turnover, immune response, survival and environmental stresses (Payne et al., 1995). In the present study, apoptosis was positively related with low temperature stresses, implying that the induction of apoptosis in fish after low temperature stress. The upregulation of p53 and upregulation of bcl-2 associated X protein (bax) could promote apoptosis (Sheikh and Fornace, 2000; Pena-Blanco and Garcia-Saez, 2018). Similar results were also found in present study, in which p53 and bax expression increased significantly after acute temperature stresses compared with that in control group. Therefore, hepatocyte damage we observed in low-temperature stresses groups might be related with apoptosis, which eventually lead to the fish death. In addition, WGCNA results showed that bcl-2, akt2, and serine/threonine-protein kinase/endoribonuclease IRE1

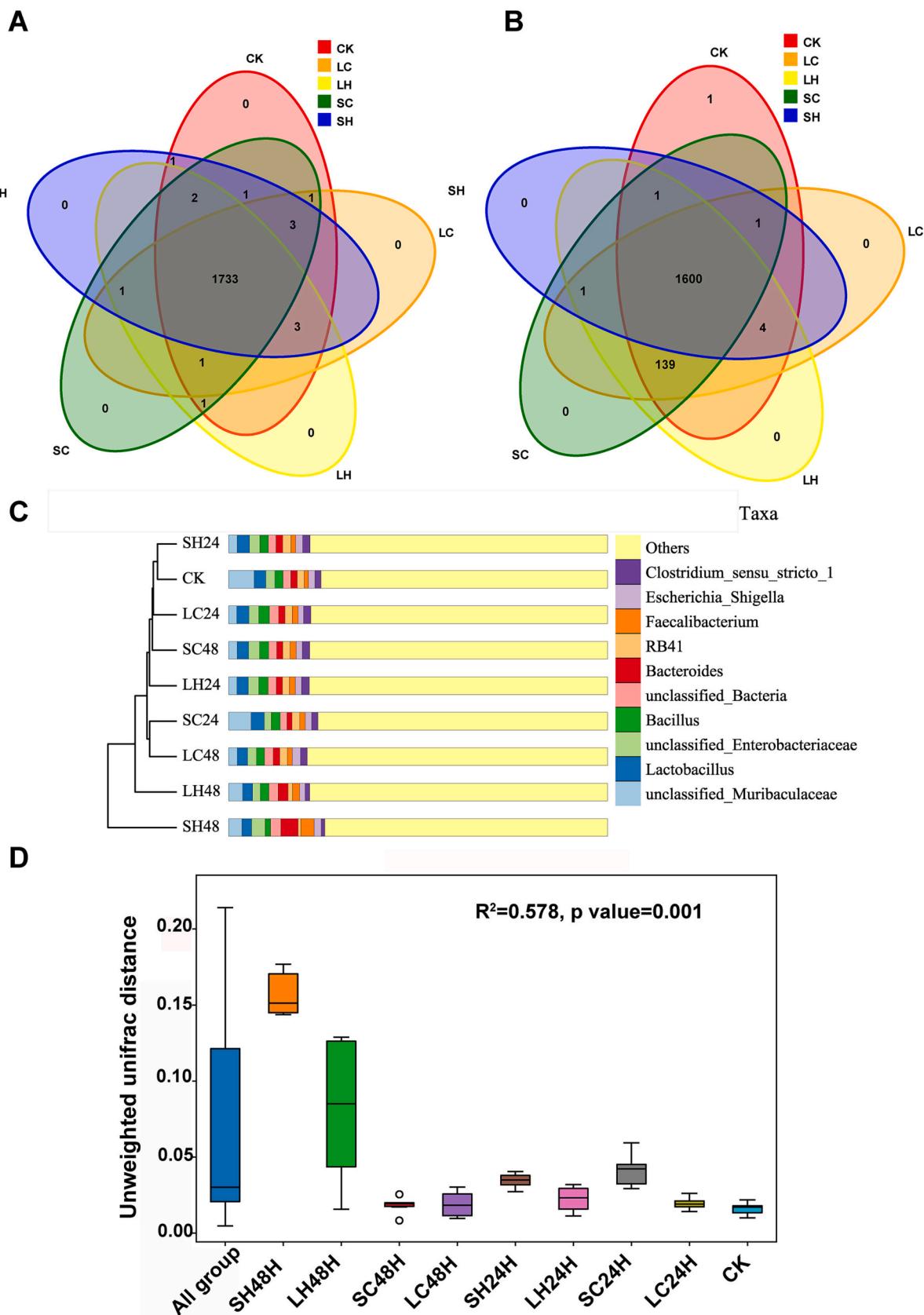


Fig. 6. Overview of the microbial community in the intestine after 24 h and 48 h of low/high temperatures. A&B The Venn results showed that the numbers of shared and specific OTUs in the five groups that exposed to different temperature for 24 h and 48 h, respectively. C. The microbial community composition of intestine samples in different groups of phyla based on UPGMA clustering. D. Analysis of similarity (ANOSIM) test.

Table 3

significantly positive correlations between low temperature and bacteria in largemouth bass.

Temperature	Genus	Pearson's correlation coefficient	p-value
Low temperature	Alloprevotella	1	0
	Agathobacter	-1	0
	Bryobacter	-1	0
	Desulfovibrio	-1	0
	Solirubrobacter	-1	0
	Subdoligranulum	-1	0
	Bacilli	-1	0
	Gemmamimonadaceae	-1	0
	Porphyromonadaceae	-1	0
	Uncultured soil bacterium	-1	0
	Micromonosporaceae	1	0
	Agathobacter	1	0
High temperature	Phascolarctobacterium	1	0
	Serratia	1	0
	Streptococcus	1	0
	Subdoligranulum	1	0
	Candidatus	-1	0
	Saccharimonas	-1	0

were identified as key regulatory genes in apoptosis. Bcl-2 is a mitochondrial or perinuclear-associated oncoprotein that could prolong the cell life span via inhibiting programmed cell death. Akt also could increase cell survival by inhibiting apoptosis, while IRE1 could increase cell apoptosis (Huang et al., 2011). These three genes were significantly changed after low temperature stress, which might be biomarkers for the healthy status of fish. Although hepatocyte damage was found in fish liver after acute heat stress, apoptosis-related genes did not change after heat stress, indicating that hepatocyte damage might not be induced by apoptosis.

Our results also showed that acute low temperature stress provide a response involving in Arginine biosynthesis. Arginine is directly correlated with cold tolerance (Mao et al., 2023). The increase in Arginine biosynthesis in largemouth bass indicated better cold tolerance. Arginine is also involved in the generation of ornithine, the precursor for the synthesis of Proline, which was mediated by ornithine decarboxylase (odc1) (Morris, 2013). The pyrrolidine-5-carboxylate reductase 1 (pycr1) could catalyze the final step in Proline production. The up-regulation of odc1 and pycr1 expression observed in the present study might thus be involved in the accumulation of Proline (Misener et al., 2001). As reported in fruit fly and plants, the prolines are responsible for cold resistance. Therefore, the accumulation of Arginine and Proline might be involved in an adaptive mechanism of fish to low temperature stress, which might be helpful to increase the survival rate of fish under cold stress.

Lipids are essential macromolecules for organisms and have both functional and structural roles in multiple biological processes, including functions as energy supply and signaling molecules. Previous reports have found that lipid metabolism is essential for the survival and propagation of aquatic animals ubiquitously exposed to fluctuating environmental factors, such as salinity, temperature, and toxicants (Lee et al., 2018; Imbs et al., 2021). Our study found that fatty acids elongation and biosynthesis of unsaturated fatty acid were negatively related with low temperature stresses. The lipid is the main component of cell membrane. The level of unsaturated fatty acid in cell membrane is closely linked to cold tolerance. The repression in unsaturated fatty acid biosynthesis indicated that the survival rate of fish might be decreased after cold stress. In addition, lipid metabolism was significantly enriched after heat stress. Integrated metabolome and transcriptome analyses were performed in juvenile turbot, and the results also showed that high temperature altered the mode of lipid metabolism in juvenile turbot liver (Zhao et al., 2021). In our work, the Steroid biosynthesis was significantly changed after acute heat stress. The steroid is closely

related to reproduction, growth, and development (Sanderson, 2006). Therefore, it is plausible that lipid metabolism was reprogrammed after low/high-temperature exposure, and different biological processes might be triggered, though studies on this topic are limited.

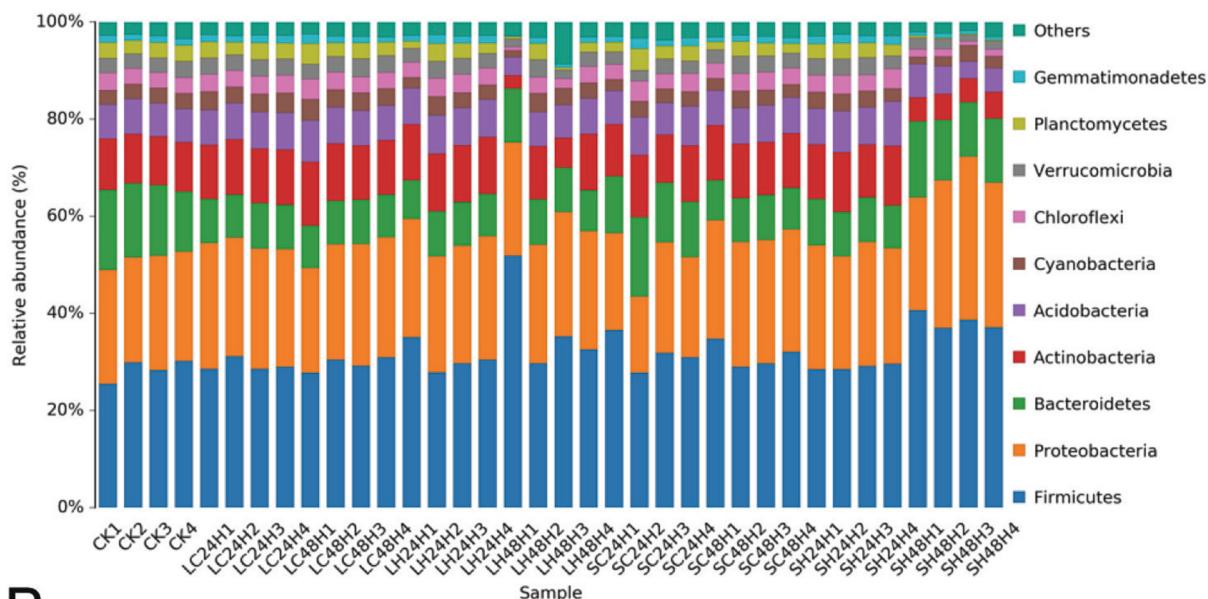
Under high temperature stress, Protein processing in endoplasmic reticulum was significantly changed. Similar results were also found in rainbow trout, in which DEGs identified under heat stress were particularly associated with protein processing in the endoplasmic reticulum (Li et al., 2017). Therefore, the endoplasmic reticulum is an important location in response to acute high temperature stress (Xiong et al., 2021). Heat shock proteins are involved in a variety of cellular processes in response to the shock from environmental stresses (Rendell et al., 2006; Banerjee et al., 2015). In the protein processing in endoplasmic reticulum pathway, 9 genes encoding heat shock proteins, especially *hsp70* and *hsp90*, were significantly upregulated in fish after acute heat stress, which is consistent with previous studies (Lv et al., 2021; Singh et al., 2021). WGCNA results also showed that Hsp70 and their chaperones were significantly correlated with heat stress. The Hsps, mainly Hsp70 and their chaperons, contributes not only to the correct folding of denatured proteins but also to the removal of unfolded proteins (Wang et al., 2015). Hsp90s could prevent protein aggregation and refold stress-denatured proteins to promote endoplasmic reticulum homeostasis. Therefore, Hsp proteins were massively activated to protect largemouth bass from heat stress.

The gut microorganisms occupying the intestine of the host could affect a variety of physiological processes in the host (Burokas et al., 2015). In this study, the high-throughput sequencing analysis of 16S rDNA revealed that 48 h of heat stress but not cold stress markedly disrupted the diversity and abundance, which was verified by the Simpson and Shannon indices. Therefore, the gut microbiota might be more sensitive to heat stress than to cold stress. Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria, microbial biomarkers in five groups, were the main intestinal microbial phyla in largemouth bass, which was consistent with findings in other fish (Li et al., 2016). Firmicutes are the major participants in fatty acid absorption (Semova et al., 2012). Actinobacteria constitute one of the largest phyla among bacteria and are considered excellent elaborators of pharmaceutical products such as antibiotics, antimicrobial agents, and industrial enzymes (Grond et al., 2018; Zothanpuia et al., 2018). An increase in Firmicutes in the intestine of largemouth bass suggested that fatty acid absorption might be increased after 48 h of high-temperature stress. A decrease in Actinobacteria implied that the antibacterial ability decreased in largemouth bass after high temperature stress. Hence, acute heat stress might disrupt the intestinal microbiota balance, leading to the unhealthy status of the intestine even fish death, and different types of bacteria have different responses to stress.

We found that 6 kinds of bacteria showed significantly correlation with high temperature. For example, Phascolarctobacterium exhibited positively correlation with high temperature, which is consistent with that in broilers (Yang et al., 2014). A total of 273 DEGs showed significantly correlation relationship with these 6 kinds of bacteria. KEGG analysis showed that Protein processing in endoplasmic reticulum was significantly enriched, and heat shock-related genes were key regulatory genes. This result further supported that these bacteria could affect the host status via regulating Protein processing in endoplasmic reticulum. However, current study about its' roles was limited, which will be performed in the future.

Muribaculaceae and Ruminococcaceae UCG 014 were unique clades in CK group. Muribaculaceae possesses bile salt hydrolase activity, converting main bile acids to secondary bile acids to increase intestinal permeability (Chen et al., 2019; Zeng et al., 2020). The decrease in Muribaculaceae has been regarded as the key to the development of colitis in mice (Shang et al., 2021). Ruminococcaceae could produce short-chain fatty acids, such as butyrate (Xie et al., 2022). Gut butyrate production could promote the differentiation of Treg cells, which enhance epithelial barrier integrity, inhibiting the pro-inflammatory

A



B

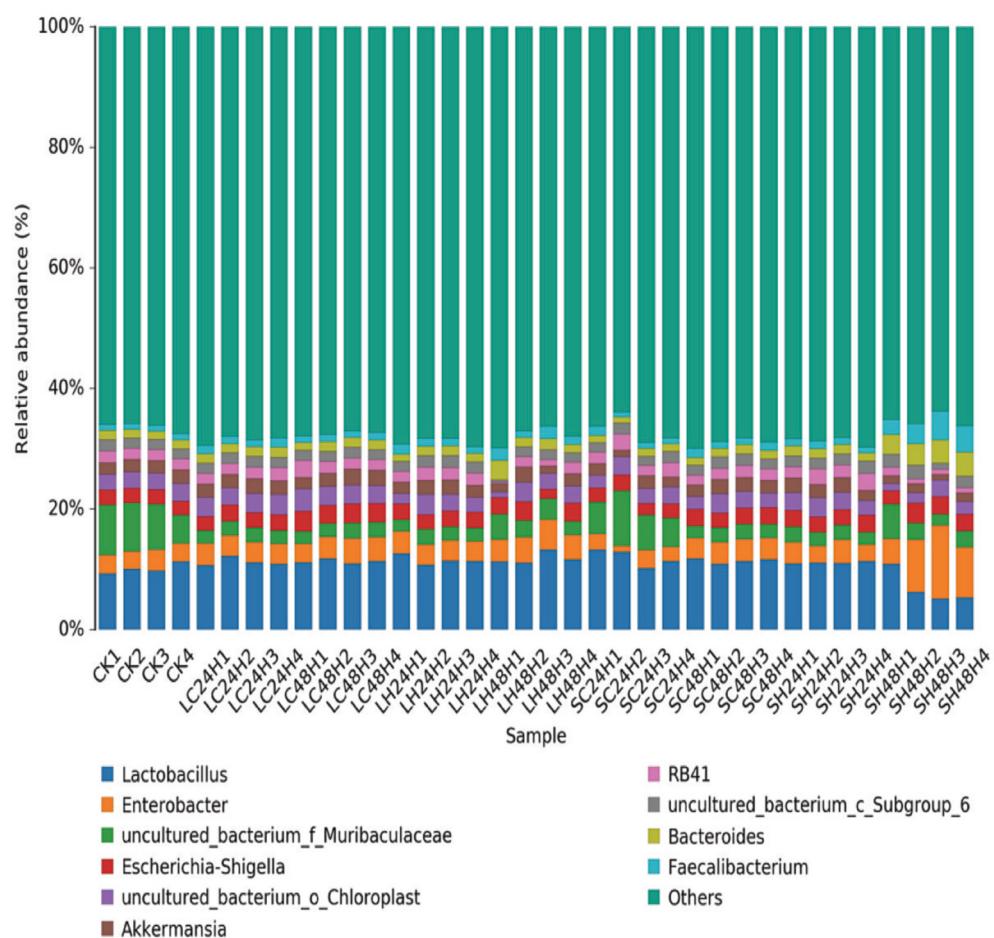


Fig. 7. Relative abundance of the microbial communities at the phylum (A) and genus (B) levels in the intestine in samples in the five groups exposed to different temperatures for 24 h and 48 h, respectively.

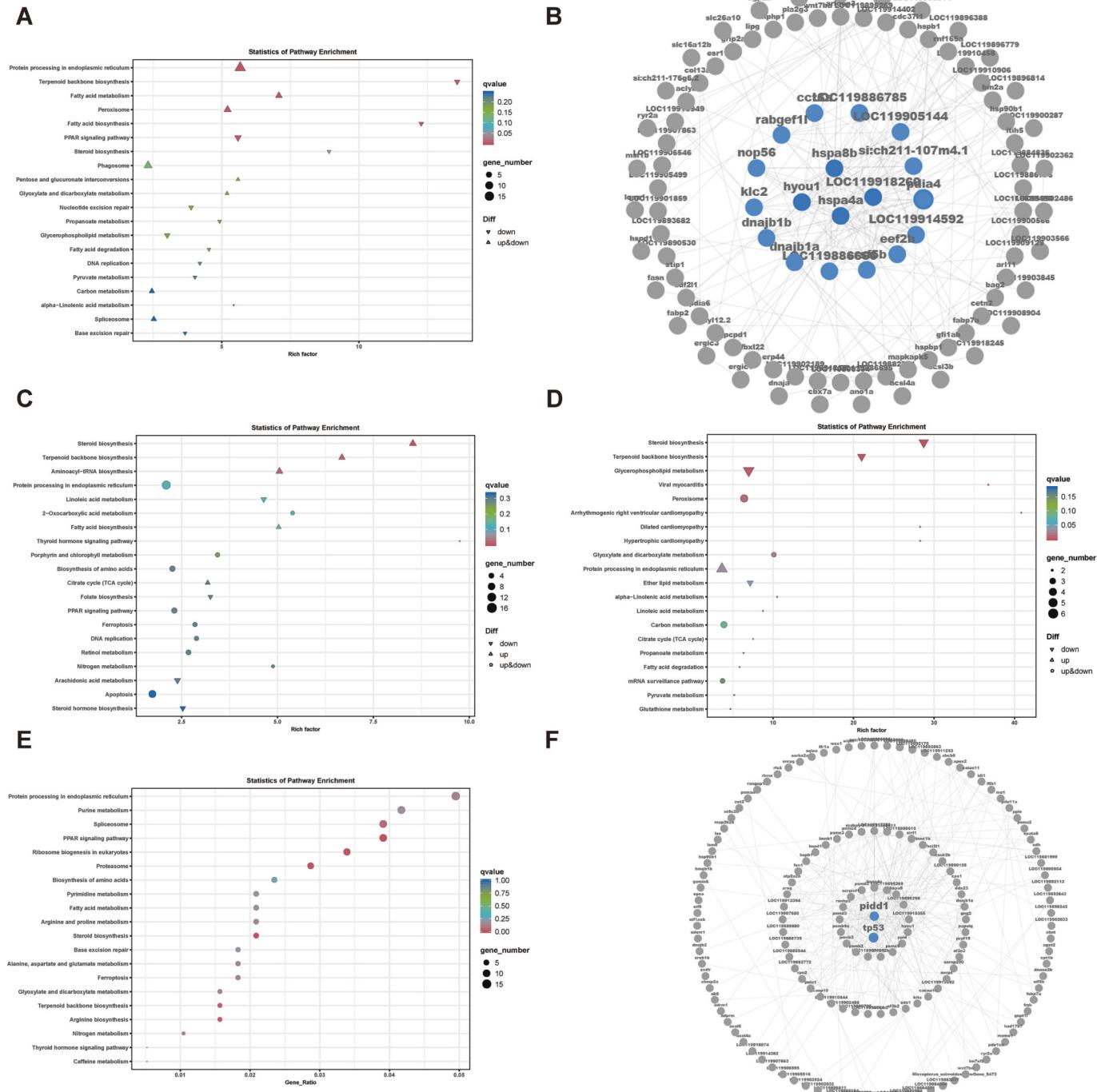


Fig. 8. A. KEGG enrichment analysis for 273 DEGs significantly correlated with 6 kinds of high-temperature related bacteria. B. Correlation network of key genes in Protein processing in endoplasmic reticulum, significantly correlated with 6 kinds of high-temperature related bacteria. C&D. KEGG enrichment analysis for DEGs significantly correlation with Muribaculaceae in LC (C) and SH (D) groups. E. KEGG enrichment analysis for DEGs significantly correlation with Ruminococcaceae UCG 014 in SH group. F. Correlation network of key genes significantly correlated with Ruminococcaceae UCG 014.

(Singh et al., 2021). These two bacteria were both related to intestinal inflammation. In addition, we found that Muribaculaceae was obviously correlated with Steroid biosynthesis, and Ruminococcaceae was significantly correlated with PPAR signaling pathway. Therefore, alterations in the abundance of Muribaculace and Ruminococcaceae may contribute to adverse health effects on largemouth bass, such as increase on intestinal inflammation, via influencing the lipid metabolism homeostasis, and it might indicate the physiological status of fish.

5. Conclusions

The present study integrated microbial, transcriptional, and histological indicators to reveal the response mechanisms of largemouth bass after acute temperature stresses. The results showed that acute temperature stresses have rapid and severe effects on the physiological status of largemouth bass. One hand, acute temperature stresses caused histological alterations and gene expressions variations in the livers. In detail, low temperature could induce apoptosis, Arginine biosynthesis,

and inhibit fatty acids elongation and biosynthesis of unsaturated fatty acid in fish liver, while high temperature could influence Protein processing in endoplasmic reticulum and Steroid biosynthesis. Among these biological processes, multiple key regulatory genes for low/high temperature stresses were identified, such as Hsp90, Hsp8, Bcl-2. On the other hand, acute high temperature stress reshaped the composition of intestinal microbial community, and multiple microbial bioindicators for temperature were identified, such as Muribaculaceae and Ruminococcaceae UCG 014. Correlation analysis between these bacteria and DEGs showed that bacteria could influence the host status via regulating the lipid metabolism or Protein processing in endoplasmic reticulum in fish. Our study could provide practical significance regarding aquaculture application, as largemouth bass could encounter temperature fluctuation when the winter or summer comes. The microbial biomarkers/genes identified in present study could be applied to monitor the health status of largemouth bass under acute temperature stresses, and screened as tolerance-related markers in the fish to improve selective breeding and cultivation practices.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2023.739573>.

Ethics approval and consent to participate

All experiments in this study was conducted with the approval by the Animal Ethical and Welfare Committee of Northwest A&F University.

CRediT authorship contribution statement

Jiajia Yu: Writing – original draft, Formal analysis, Investigation. **Lixin Wang:** Conceptualization, Writing – review & editing, Supervision. **Debin Zhong:** Data curation, Formal analysis, Validation. **Shuai Li:** Data curation, Formal analysis, Validation. **Zhihao Zhang:** Data curation, Formal analysis, Validation. **Haolin Mo:** Data curation, Formal analysis, Validation.

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 data that has been used is confidential.

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