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Effect of temperature on exercise metabolism, hypoxia tolerance, and RNA-seq analysis in *Sinilabeo rendahli* from the Yangtze River, China

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Temperature variation affects the growth performance of fish species due to growing constraints and trade-off on physiological functions. Here we experimentally investigated several metabolic and physiological parameters in *Sinilabeo rendahli*, an endemic fish species found in the Yangtze River in China. After a 14-day acclimation period, we measured routine metabolic rate (RMR), individual metabolic rate (MO_2), temperature quotient (Q_{10}), loss of equilibrium (LOE), and critical oxygen tension (P_{crit}) at three different temperatures (15, 20, and 25°C). Moreover, we sampled the muscle tissue from juvenile *S. rendahli* under experimental conditions after 28 days of acclimation and performed transcriptome-RNA sequencing (RNA-seq). The P_{crit} of the fish at the above acclimation temperatures were determined to be 1.07, 1.28, and 1.33 mg·L⁻¹, respectively, and corresponded with increasing acclimation temperatures (15–25°C). RMR was positively correlated with P_{crit} ($r = 0.4711$, $P = 0.0201$), negatively correlated with LOE ($r = -0.4284$, $P = 0.0367$), and significantly positively correlated with MR_{crit} ($r = 0.8797$, $P < 0.001$) at temperatures ranging from 15 to 25°C. In addition, a total of 4,710 differentially expressed genes (DEGs) were identified. The results of DEG analysis and KEGG clustering analysis indicated that energy metabolism played a central role in thermal stress in *S. rendahli* for the major upregulated genes. This was followed by autophagy, mitophagy, cardiac muscle contraction, extracellular matrix (ECM)-receptor interaction, and protein digestion and absorption. This study is significant for understanding the adaptive response of *S. rendahli* to thermal stress. Even more importantly, this study demonstrates that *S. rendahli* is more suitable for cold-water life.

KEYWORDS

Sinilabeo rendahli, temperature acclimation, critical oxygen tension (P_{crit}), routine metabolic rate (RMR), RNA-seq

1. Introduction

The physiological state of animals under the effects of temperature and dynamic change in the ecological sense is a critical issue (Machac et al., 2012; Morash et al., 2021). Variations in water temperature due to climate change severely challenge the fitness of fish species (Frolicher and Laufkotter, 2018; McKenzie et al., 2021). Water temperature is an environmental factor that affects the survival and distribution of fish. It is generally accepted that the dissolved oxygen level in water decreases with increasing temperature and that the routine metabolic rate (RMR) increases in the appropriate temperature range (Xie and Sun, 1989; Cook et al., 2006; Killen et al., 2010). Temperature changes affect the hypoxia tolerance of fish, such as critical oxygen pressure (P_{crit}) and critical oxygen metabolic rate (MR_{crit}). Temperature acclimation, as a unique life history characteristic of ectotherms such as fish and reptiles, allows them to adjust their physiological functions, biochemical reactions, and behavior patterns to adapt to climate change in the appropriate temperature range (Chen et al., 2019).

Sinilabeo rendahli (Cypriniformes: Labeoninae) is an endemic fish in the middle and upper reaches of the Yangtze River (Zhang and Chen, 2006). This species has recently been listed into local law which encourages artificial breeding as a way to reduce the demand on the wild population and to help wildlife conservation (Yang et al., 2021); however, research on *S. rendahli* is limited to nutritional physiology (Zhang et al., 2010; Zhu et al., 2010), reproductive biology (Li et al., 2005; Ying et al., 2005; Zheng and Wu, 2011), population genetics (Zheng, 2007; Shao et al., 2013), and other aspects. In addition, researchers are dedicated to defining the metabolism and hypoxia tolerance of various fish species of aquaculture importance (Ott et al., 1980; Sørensen et al., 2014; He et al., 2015; Xia et al., 2017), and the response of *S. rendahli* to thermal variations at the physiological and biochemical levels remains unknown. Furthermore, the gene regulatory network of thermal adaptation can be analyzed using transcriptome level analysis.

In the current study, we explored the physiological response of juvenile *S. rendahli* to temperature acclimation from the perspective of routine metabolic rate and hypoxia tolerance. In addition, the adaptation mechanism of *S. rendahli* in response to persistent hyperthermia was further explored at the transcriptome level. This study aimed to elucidate the metabolic pathway of *S. rendahli* under different temperatures and to provide a theoretical basis for constructing signal transduction pathways, discovering temperature tolerance genes, and identifying adaptation mechanisms.

2. Materials and methods

2.1. Experimental conditions

The Institutional Animal Care and Use Committee of Southwest University, China, approved experimental procedures. Experimental *S. rendahli* ($N = 90$) was transported from a local fishery (Pingchang, Sichuan, China) and reared in an indoor aerated recirculating aquaculture system (diameter 3 m, height

1.2 m) at a water temperature of $22 \pm 0.5^\circ\text{C}$. In total, 20% of the water was renewed daily for oxygen supply.

Experimental fish (with the body weight of each fish being ~ 4 g) were randomly divided into three experimental groups: LT (15°C), MT (20°C), and HT (25°C) (which stand for low, medium, and high temperature, respectively), according to the seasonal temperature changes (Wang et al., 2014). The experimental fish were transferred to a 100-L glass tank, and there were 30 fish in each group. The thermal regime protocol used in this study follows that described in Li et al.'s (2021) study with slight modifications due to species. Dissolved oxygen in the experiment was above 7.0 mg L^{-1} , with 10% of the water renewed daily. The fish-housed photoperiod was 12L: 12D, and the experimental fish were fed commercial diets once daily, after which the residual feed was siphoned. The contents of protein, fat, crude ash, and water in fish meals were 35, 4, 15, and 12.5%, respectively. The duration for carrying out the experimental procedures was 28 days.

2.2. Determination of routine metabolic rate

Experimental fish were denied food for 48 h before the determination of routine metabolic rate (Sandblom et al., 2014). After 14 days of acclimation, a streamlined respiratory metabolism instrument was used to determine the routine metabolic rate (RMR; unit: $\text{mg h}^{-1} \text{ kg}^{-1}$). In total, 48 experimental fish were assigned to receive the same three treatments as in the acclimation phase. The body weight and length of the experimental fish were measured before the assignment. Each experimental fish was then individually placed in the respiratory chamber (size: 3 cm diameter, volume: 130 mL) of the metabolic analyzer for 12 h on the night before the measurement. A breathing chamber without fish was set as a blank control (Fu et al., 2007). The oxygen consumption rate (unit: $\text{mg h}^{-1} \text{ kg}^{-1}$) of the experimental fish was measured by a flowing-water respiratory metabolism instrument. With these settings, the dissolved oxygen of the flowing water in each chamber was monitored quantitatively to adjust the velocity for controlling the concentration of dissolved oxygen within $0.5\text{--}1.0 \text{ mg L}^{-1}$ between the chamber with fish and the empty one. The dissolved oxygen of all respiratory rooms in the apparatus was measured six times at the outlet using a dissolved oxygen meter (HQ30d, Hach Company, Loveland, CO, USA), with measurements once every hour. The mean value of dissolved oxygen was taken to calculate the routine metabolic rate of the individual fish in this experiment. In formal testing, the testing environment (including dissolved oxygen, water temperature, light, etc.) was consistent with the acclimation treatment.

The formula for calculating the routine metabolic rate (RMR) of MO_2 is

$$MO_2 = \Delta O_2 \cdot V/M, \quad (1)$$

where ΔO_2 is the difference between the dissolved oxygen level in the respiratory chamber of the blank tank and that in the respiratory chamber of the one with experimental fish (unit: $\text{mg}\cdot\text{L}^{-1}$); V is the water flow rate in the respiratory section

of laboratory fish (unit: $L \cdot h^{-1}$); M is the final body mass of experimental fish (unit: kg).

The calculation formula for the temperature coefficient Q_{10} is as follows:

$$Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}, \quad (2)$$

where R_1 and R_2 are the physiological parameters T_1 and T_2 , respectively.

2.3. Determination of resistance to hypoxia

After the determination of the RMR, eight fish were randomly selected. The P_{crit} of a single experimental fish was determined using a closed respiratory and metabolic instrument (220 mL) made of polymethylmethacrylate (PMMA) (Chen et al., 2019). The experimental fish were individually transferred to the closed respirator for at least 1 h after adjusting velocity to keep them swimming. During the adaptation period, water was allowed to flow into the upper part of the closed breathing chamber to maintain communication with the water environment of the water tank, ensuring sufficient oxygen and preventing experimental fish from escaping. An additional circulating pump was installed outside the breathing room and activated to provide low-speed water flow at selected adaptive temperatures. During the measurement, it was necessary to seal the breathing room port, use the dissolved oxygen meter (HQ30d, Hach Company, Loveland, CO, USA) to seal the top port of the breathing room, and apply petroleum jelly (Vaseline) at the seal to ensure that the breathing room was completely closed. The changes in dissolved oxygen in the respiratory chamber were continuously monitored. Dissolved oxygen and the oxygen percentage were recorded every 3 min. In formal testing, the testing environment (including dissolved oxygen, water temperature, light, etc.) was consistent with the acclimation treatment. P_{crit} and $M_{r,crit}$ were determined using the double-line method (Yeager and Ultsch, 1989).

The formula for calculating the respiration and oxygen consumption rates of the experimental fish at each unit time interval is as follows:

$$MO_2 = ([O_2]_k - [O_2]_{k+1} \cdot V)/(t \cdot M_b), \quad (3)$$

where $[O_2]_k$ is the dissolved oxygen value ($mgO_2 L^{-1}$) at the previous moment; $[O_2]_{k+1}$ is the dissolved oxygen value at the next moment; V represents the volume (L) of fish removed in the breathing room; t represents the interval time (h) between the previous point and the next point; and M_b represents the weight (kg) of the tail fish.

2.4. Sample collection

Experimental fish were deprived of food for 48 h after temperature acclimation for 28 days. Triplicates of 12 fish from each treatment (LT and HT) were euthanized, with the muscle

tissue (white muscle at the dorsal fin) attributed to three biological replicates. Shanghai Paisenuo Company powered the service for digital RNA sequencing (RNA-seq).

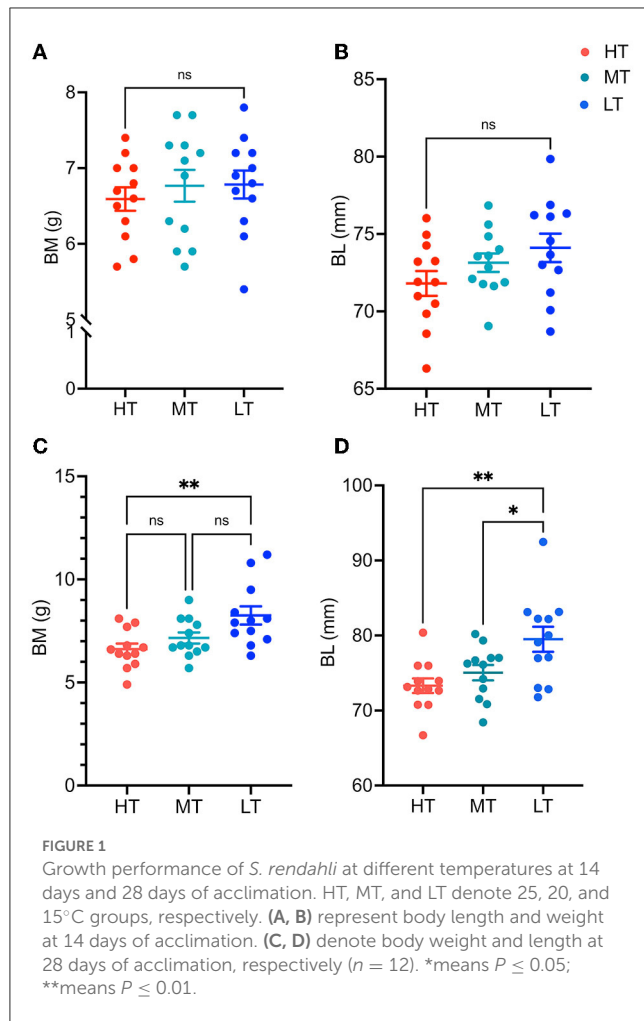
2.5. RNA extraction, cDNA synthesis, and RNA sequencing

An equal amount (~ 1.5 g of each mixed sample) of muscle tissue samples was acquired from the LT and control groups (HT) for the analysis of RNA-seq and expression profiles. Before sample acquisition, total RNA was extracted from *Sinilabeo rendahli* muscle tissues (LT and HT all had four samples) using RNA extraction lysis buffer (RNAiso Plus, TaKaRa, Japan) according to the manufacturer's instructions. The RNA purity and concentration were checked using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, USA). Equal amounts of 6 μ g of qualified RNA were obtained from the LT and HT groups as input material for RNA-seq library preparation. After the library was constructed, library fragments were enriched using PCR amplification. Then, the concentration and the quality of the library were detected using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., America). Then, the effective concentration of the library was accurately quantified using the quantitative real-time PCR (qPCR) technology, and the different libraries that passed the library inspection were sequenced on the Illumina NovaSeq PE150 platform according to the effective concentration (Personal Biotechnology Co., Ltd., Shanghai, China).

2.6. RNA-seq analysis

The raw reads were converted from raw image files using the software of the sequencing platform. Afterward, the clean reads of LT and HT samples were obtained by removing reads containing three terminal sequences with adapters and low-quality reads (quality score < 20) using Cutadapt and then assembled using Trinity for *de novo* assemblies. With these clean reads, the longest transcript of each gene was selected as the representative sequence of the gene, a unigene.

Six public databases, namely, NCBI non-redundant protein sequences (NR), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), evolutionary genealogy of genes: Non-supervised Orthologous Groups (eggNOG), Swiss-Prot (manually annotated and reviewed protein sequence database), and Pfam, were selected to compare with unigene sequences and annotate gene functions using Blastx with an E-value of $< 10^{-5}$. Transcriptome sequences and FPKM values were calculated to quantify gene expression using DESeq (Simon and Wolfgang, 2010; Dewey and Li, 2011) using the software package to analyze differentially expressed genes and gene expression levels that were significant at $|\log_2 \text{FoldChange}| \geq 1$ or a P -value of < 0.05 . Databases such as GO (Gene Ontology Consortium, 2004) and KEGG (Kanehisa et al., 2010) were used for the functional annotation of differentially expressed genes.



2.7. Validation of DEGs using real-time PCR (qPCR)

To further validate the confidence of our RNA-seq sequencing data, 20 DEGs were selected randomly and analyzed for qPCR analysis using the same RNA samples from the muscle RNA-Seq. Total RNA was purified and evaluated according to the above method. Then, 5 μg of total RNA from each test sample was reverse transcribed using the GoScript™ Reverse Transcription System (Promega, USA) following the manufacturer's protocols, synthesizing cDNA for qPCR. The qPCR was carried out with a CFX96 Real-Time PCR Detection System (Bio-Rad, USA). The total reaction volume of 15 μl reactions contained 7.5 μl of GoTaq® qPCR Master Mix (Promega, USA), 1 μl of diluted cDNA or nuclease-free water for the template as a negative control, 5.7 μl of nuclease-free water, and 0.4 μl of 10 mM stock solutions of each primer. The amplification profile was as follows: 95°C for 30 min, 95°C for 30 s, 40 amplification cycles of 95°C for 5 s, and 60°C for 1 min. Each qPCR assay was performed in triplicate for analysis, and target specificity was determined using dissociation curve analysis. Primers were designed according to unigenes obtained by Trinity splicing of

RNA-seq. Software Primer 6.0 was used to design qPCR primers. β -actin genes were selected as the internal reference, and the samples from the control group were used as calibrators. The relative mRNA expression of the target gene was determined using the $2^{-\Delta\Delta\text{CT}}$ method.

2.8. Statistical analyses

Equal variances and a Gaussian distribution of residuals were checked before one-way ANOVA prior to multiple comparisons. The P_{crit} was obtained using the “two-line method” (Yeager and Ultsch, 1989). The qPCR results were converted into the relative expression of each gene calculated by $2^{-\Delta\Delta\text{CT}}$ (Livak and Schmittgen, 2001). The result was expressed as “mean \pm error (Std. Error of Mean, SEM)”. A t-test was used to compare the expression of qPCR on the muscle tissue of *Sinilabeo rendahli* between the LT and HT groups. The obtained data were statistically analyzed and plotted using GraphPad Prism 9.0 software or R, and the significance level was set at a P -value of < 0.05 .

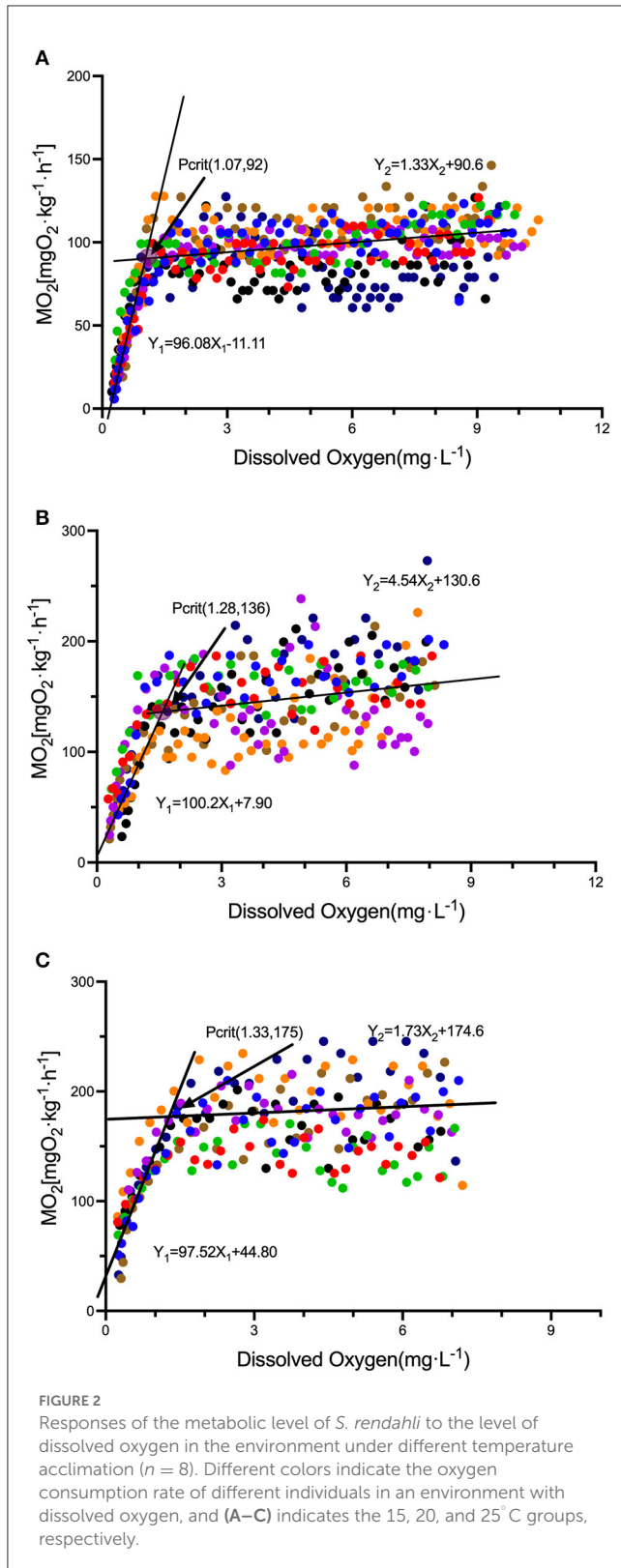
3. Experimental results

3.1. Morphological statistics

After 14 days of temperature acclimation, there was no significant difference in body length and weight among all temperature groups in Supplementary Table 1 ($P > 0.05$; Figures 1A, B). After 28 days of temperature acclimation, the statistical results of body weight and body length showed significant differences between the LT and HT groups ($P < 0.05$; Figures 1C, D).

3.2. Metabolic characteristics

The body length and weight of juvenile *S. rendahli* used in the experiment measuring the resting metabolic rate were not statistically significant among the three temperature groups (Supplementary Table 1). It was observed that, with increasing domestication temperatures, the resting metabolic rate also increased among the different temperature groups. The P_{crit} of the LT, MT, and HT groups was 1.07, 1.28, and 1.33 $\text{mg}\cdot\text{L}^{-1}$, respectively (Figure 2). The MO_2 was 92 (Figure 2A), 136 (Figure 2B), or 175 $\text{mg}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$. The LOE was 0.36, 0.42, and 0.27 $\text{mg}\cdot\text{L}^{-1}$ (Supplementary Table 1), respectively. MR_{crit} was 88.79, 141.1, and 178.0 $\text{mg}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$ (Supplementary Table 1), respectively. The metabolic level of the experimental fish remained at a relatively stable level until the dissolved oxygen concentration in the environment decreased below P_{crit} (the crossing point of the two black lines in Figure 2). The metabolic level decreased with a decline in dissolved oxygen in the environment. Moreover, RMR was positively correlated with P_{crit} ($r = 0.4711$, $P = 0.0201$), negatively correlated with LOE ($r = -0.4284$, $P = 0.0367$), and significantly positively correlated with MR_{crit} ($r = 0.8797$, $P < 0.001$).



3.3. Quality of library sequencing

Two test points, each with three replicates (LT and HT), were analyzed using RNA-Seq. Six digital RNA-seq libraries were

sequenced from the control and treatment groups, generating an average of 47,899,330 raw reads and 44,040,656 clean reads after filtering out low-quality sequences (Table 1). The percentage of Q30 bases in each sample was more than 95% (Table 1). These data indicate that the sequencing depth was sufficient for transcriptome coverage in *S. rendahli*. The sequencing results are shown in Table 1. After sequencing quality control, the percentage of Q30 bases in each sample was not <95%, and the GC base content was ~42%. This finding suggests that the digital RNA-seq data were reliable and sufficient for subsequent bioinformatics analysis. The RNA-Seq data were uploaded to the NCBI Sequence Read Archive (SRA) database in NCBI at <https://submit.ncbi.nlm.nih.gov/subs/sra/> with accession number PRJNA902088.

3.4. Differential expression analysis (DEGs)

Based on differential expression analysis with an adjusted *P*-value of <0.05 as the threshold, 4,710 DEGs were identified from samples collected from the muscle of the fish at different temperatures (Figure 3B). Compared with the LT group, the HT group had 2,215 upregulated genes, including *hspb1*, *hsp70*, *hsp30*, *myh6*, *myoa5*, *myh7*, *c-jun*, *mstnb*, and *ldha* (Table 2). A total of 2,495 genes were downregulated, including *hif1a*, *pgm21l*, *g6pd*, *mapk14a*, *cyp2k1*, *cyp2k4*, and *npy*.

3.5. GO enrichment and KEGG pathway analysis of DEGs

We performed GO and KEGG functional enrichment analyses of the 4,710 DEGs. GO enrichment analysis showed that the 20 most significant GO enrichment items were mainly related to exercise activity and ATP binding (Figure 3A). The results of the KEGG cluster analysis showed that the pathways enriched in the muscles of M_LT and M_HT refer to muscle (M) RNA-Seq on the low temperature (LT) group and high temperature (HT) group respectively after 28 days of temperature treatment were mainly concentrated in the metabolic and biological systems, and several tracks related to muscle function were upregulated in the HT group, such as autophagy, mitophagy-animal, cardiac muscle contraction, extracellular matrix (ECM)-receptor interaction, and protein digestion and absorption (Table 3).

3.6. Real-time PCR analysis

To validate the DEGs identified using RNA-seq analysis, we randomly selected 20 genes (*hspb1*, *hsp70*, *hsp30*, *myh6*, *myoa5*, *myh7*, *c-jun*, *mstnb*, *ldha*, *hif1a*, *g6pd*, *mapk14a*, *cyp2k4*, *hspb2*, *hsp90ab1*, *hspa8*, *hsp90a.1*, *junb*, *g6pc*, and *ucp2*) for qPCR confirmation at different temperatures. The expression levels of these 20 DEGs were evaluated using qPCR and compared with the expression levels obtained from the RNA-Seq analysis. The qPCR results were mostly consistent with those of the RNA-Seq experiments (Figures 3C, D), with similar upregulation and downregulation patterns.

TABLE 1 Summary of illumina sequencing results.

Sample	Raw reads	Clean reads	Bases (bp)	N (%)	Q20 (%)	Q30 (%)
M_HT_1	49366914	44634110	7 405037100	7.7e-05	98.38	95.46
M_HT_2	51509334	47565142	7 726400100	8.0e-05	98.35	95.32
M_HT_3	48813146	45262716	7 321971900	7.9e-05	98.46	95.57
M_LT_1	46669056	42875806	7 000358400	7.9e-05	98.28	95.18
M_LT_2	45940478	42270070	6 891071700	7.6e-05	98.23	95.04
M_LT_3	45097050	41636092	6 764557500	8.3e-05	98.36	95.39

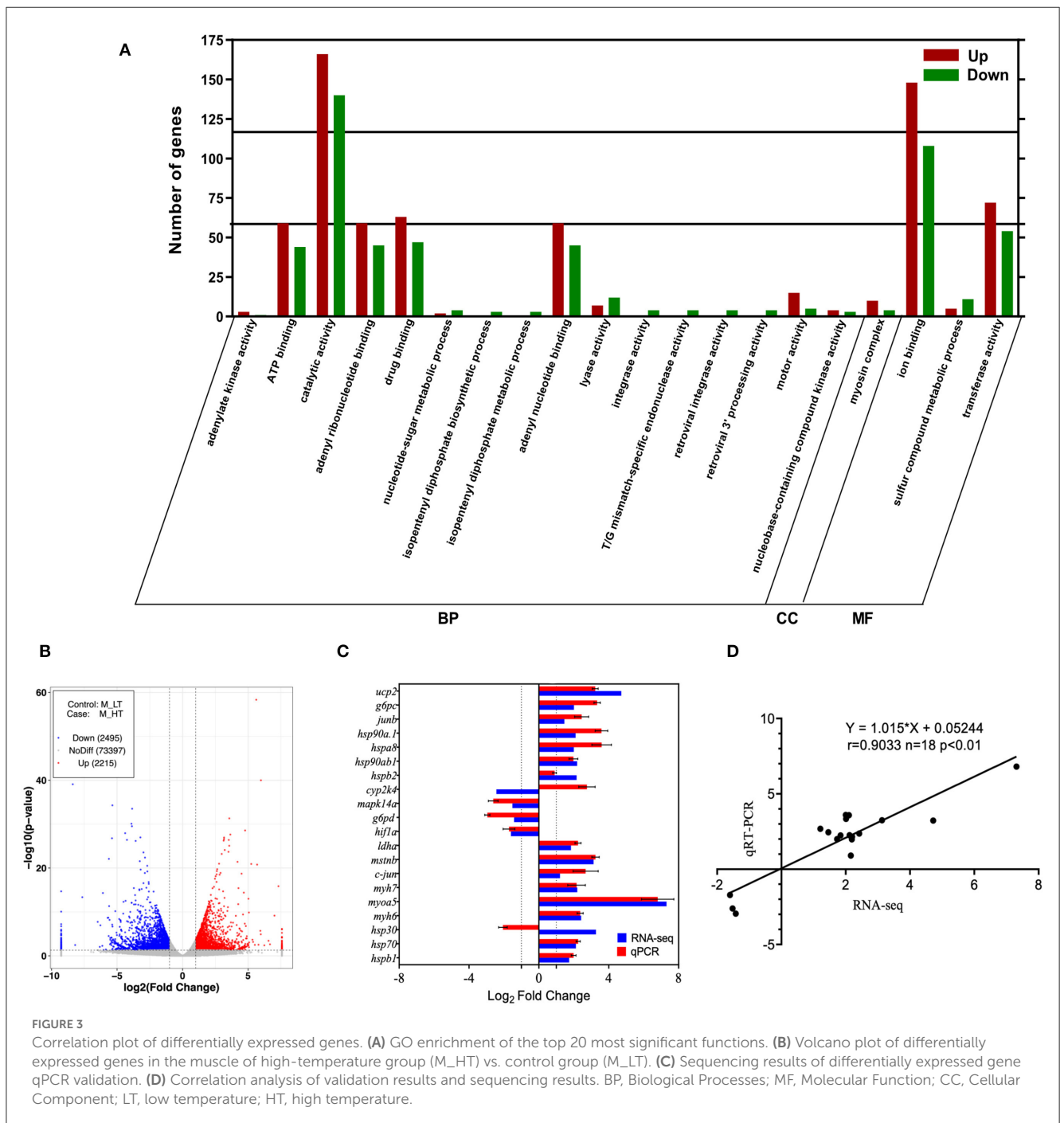


TABLE 2 Several upregulated genes after thermal acclimation (25°C) of *S. rendahli*.

Gene	Gene full name	Log ₂ fold change	P-value
<i>hspb1</i>	Heat shock protein beta-1	1.727	7.62E-03
<i>hsp70</i>	Heat shock 70 kDa protein	2.123	2.40E-05
<i>hsp30</i>	Heat shock protein 30	3.274	9.02E-10
<i>myh6</i>	Myosin-6	2.932	3.02E-03
<i>myoa5</i>	Unconventional myosin-Va	7.314	1.38E-16
<i>myh7</i>	Myosin-7	2.198	2.01E-02
<i>c-jun</i>	Transcription factor AP-1	1.047	1.65E-03
<i>mstnb</i>	Growth/differentiation factor 8	3.130	5.71E-27
<i>ldha</i>	L-lactate dehydrogenase A	1.837	1.00E-07
<i>igfbp2b</i>	Insulin-like growth factor-binding protein 2-B	1.916	3.84E-05

TABLE 3 KEGG pathway of differentially expressed genes.

KEGG pathway	Pathway ID	DEG number	Total number
Terpenoid backbone biosynthesis	ko00900	11	34
Purine metabolism	ko00230	40	254
Arginine and proline metabolism	ko00330	24	135
Mitophagy—animal	ko04137	21	125
Autophagy—other	ko04136	12	60
Other types of O-glycan biosynthesis	ko00514	8	36
Porphyrin and chlorophyll metabolism	ko00860	11	59
Circadian rhythm	ko04710	12	69
Pyrimidine metabolism	ko00240	17	110
Cardiac muscle contraction	ko04260	31	241
ECM-receptor interaction	ko04512	22	160
Metabolism of xenobiotics by cytochrome P450	ko00980	12	74
Cysteine and methionine metabolism	ko00270	17	118
Starch and sucrose metabolism	ko00500	14	93
Chemical carcinogenesis	ko05204	12	77
Protein digestion and absorption	ko04974	25	193
Autophagy—animal	ko04140	35	288
Amino sugar and nucleotide sugar metabolism	ko00520	13	87
p53 signaling pathway	ko04115	15	106
Tight junction	ko04530	48	424

In the current analysis, DEG refers to differentially expressed genes.

4. Discussion

Temperature is a significant environmental factor that has profound effects on all levels of biological organization, ranging from molecules to ecosystems (Dickson and Graham, 2004). Two important functions of fish at high temperatures are exercise metabolism and hypoxia tolerance. Previous studies have demonstrated that variations in water temperature have an impact on the homeostasis of fish behavior and metabolism (Lv and Yuan,

2017). However, there is still a lack of basic biological research on behavioral strategies, metabolic strategies, and other related aspects of *S. rendahli* at different environmental temperatures. Therefore, our primary goal in the study was to better understand the metabolic characteristics, hypoxia tolerance, and molecular regulation mechanisms of *S. rendahli* at different temperatures.

In this study, we measured exercise metabolism and hypoxia tolerance at three temperatures (15, 20, and 25°C) after 14 days of acclimation in *S. rendahli*. At the same time, we conducted digital

RNA-Seq analysis of gene-trait correlations to analyze muscle gene expression at 15 and 25°C after 28 days of acclimation in *S. rendahli*.

4.1. Temperature effects on the metabolism of juvenile *S. rendahli*

The individual cost of maintaining the metabolic level in poikilothermic animals is at most 50% of total (Steyermark et al., 2005). Due to polymorphisms in temperature sensitivity among different species, there are different degrees of interspecific differences in their physiological response parameters. In the current study, we observed that the Q_{10} value of the RMR of juvenile *S. rendahli* was close to or <2 in the temperature ranges of 15–20°C and 20–25°C. However, the Q_{10} of the RMR of widespread temperature fish, such as *Parabramis pekinensis* and *Carassius auratus*, is generally <2 . Salmonids have been reported to adapt to cold-water life year-round, and the Q_{10} of the RMR of low-temperature fish, such as *Oncorhynchus nerka* and *Salmo salar*, is the opposite, that is, >2 . Combined with the temperature coefficient Q_{10} of *S. rendahli*, the results showed that the sensitivity of *S. rendahli* to temperature was more substantial than that of common ectotherm cyprinid fish but weaker than that of salmonids.

The RMR of fish exhibits a thermal dependence in a suitable temperature range (Kelly et al., 2014; Xia et al., 2017). RMR refers to the level of metabolism that a fish needs to maintain its life when it is at rest or on an empty stomach, which reflects its ability to maintain its metabolic characteristics. Studies have shown that, in the temperature range of 4–10°C, the resting metabolic rate of *Gadus macrocephalus* remains relatively stable or even does not increase with increasing temperature (Hölker, 2003; Hanna et al., 2008). In this study, temperature acclimation had a significant effect on the metabolic level of juvenile *S. rendahli*, and RMR increased with increasing temperature. Due to the instability of the natural environment, the metabolic level of *S. rendahli* was correspondingly changed by the ambient temperature. The reason for the instability is that the activities of related respiratory and metabolic enzymes in *S. rendahli* change with temperature, as reflected in swimming ability, reflecting the strength of sports function (He et al., 2015).

4.2. Temperature effects on the hypoxia tolerance of juvenile *S. rendahli*

Different fish strategies for coping with high-temperature and low-oxygen environments were peculiar, showing similarities and differences in plasticity (Dhillon et al., 2013). The continuous temperature generated different degrees of body weight effects in *S. rendahli* after 28 days of treatment compared to that observed after 14 days of treatment. The response pattern of P_{crit} to temperature changes is different in a hypoxic environment. In this study, we observed that, after continuous temperature treatment for 14 days, the metabolism and hypoxia tolerance of *S. rendahli* were significantly affected. RMR and P_{crit} increased with temperature, and the P_{crit} of the experimental fish increased by 24.2%. Studies have shown that hypoxia tolerance in fish species exhibit a stable or increasing pattern even in a narrow

variation (Barrionuevo and Burggren, 1999; Mandic et al., 2009). It is generally understood that, with an increase in ambient temperature, fish metabolism doubles, oxygen demand increases, and anoxic tolerance decreases, and this study provides further evidence for these trends (Sørensen et al., 2014). Therefore, it has been speculated that the physiological plasticity of *S. rendahli* enables it to cope with acute hypoxia. With an increase in temperature, the RMR of *S. rendahli* may be associated with a change in hypoxia tolerance, and its internal adaptation mechanism requires further study.

4.3. Gene expression

Continuous high-temperature acclimation produced a strong stress response in *S. rendahli*. The KEGG enrichment pathway showed a significant upregulation of autophagy-related pathways in the muscle tissues, which may have exacerbated the process of apoptosis. Heat shock proteins are generally synthesized in response to stress caused by environmental treatments and are essential for stabilizing cell structure and maintaining cell function (Fujimoto and Nakai, 2010). In this study, the expression of stress-related genes was upregulated in the muscle tissue, such as *hsp1*, *hsp70*, *hsp30*, and *hsp90ab1*, which are genes in the heat shock protein family. It is believed that the acclimation process may occur primarily through the acceleration of metabolic processes at the molecular and physiological levels in response to the high-temperature environment to regulate life activities and maintain the normal growth activities of the organism.

In this study, GO enrichment showed that the ion binding and transport activities of different genes were significantly enriched at a high temperature (25°C). The permeability of biological membranes may be enhanced during continuous high-temperature acclimation, which accelerates the exchange rate between substances transported across the membrane. It will also increase the storage and turnover of environmental contaminants in the muscles of *S. rendahli* in China (Nikinmaa, 2013). In addition, temperature, as a stress factor, tends to induce the transcription of genes most often associated with toxic effects. At the same time, cytochrome P450-related pathways that concern the metabolism of endogenous substrates and detoxifying heterologous compounds may also activate the immune response of *S. rendahli*.

5. Conclusion

In an aquaculture orientation, this study determined the metabolic characteristics and changes in hypoxia tolerance at different temperatures (15, 20, and 25°C) after 14 days of temperature acclimation in *S. rendahli*. We also measured the effects of different temperatures on the expression of metabolism-related genes in *S. rendahli* at different temperatures (15 and 25°C) after 28 days of temperature acclimation. The main conclusions are given below.

The metabolic rate and hypoxia tolerance of *Siniperca chuatsi* changed with different acclimation temperatures. Under high temperatures, the ability of *S. rendahli* to maintain metabolism, exercise activity, and adaptability was enhanced. Under low temperatures, the individual metabolism of *S. rendahli* was low,

with strong hypoxia tolerance, poor exercise ability, and poor adaptability. Transcriptome gene expression difference analysis indicated that, compared with low temperatures (15°C), high temperatures (25°C) produced a stronger stress response, which affected its normal physiological metabolism. Fatty acids, as energy storage substances, may be used more in muscles to maintain the swimming demand at high temperatures.

Under continuously high temperatures (25°C), the growth rate of *S. rendahli* was inhibited by enhancing metabolism, which was the reason for low oxygen tolerance; therefore, it was inferred that *S. rendahli* is a type of fish that is more adapted to cold-water life.

Data availability statement

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found below: NCBI; PRJNA902088.

Ethics statement

Ethical review and approval was not required for the animal study because SARS-CoV-2 negatively interrupts the normal approval workflow, and in practical terms our study involves less than minimal risk, and therefore not require ethical review.

Author contributions

SL: software, validation, investigation, data curation, writing—review and editing, and visualization. HG: software: validation, investigation, data curation, writing—original draft, writing—review, editing, and visualization. C-YD and Y-XT: software, investigation, and data curation. J-YF: formal analysis, investigation, and data curation. HX: formal analysis and investigation. XP: conceptualization, methodology, resources, funding acquisition, and project administration. YL: conceptualization, methodology, funding acquisition, project administration, supervision, validation, writing—review, and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2023.1159161/full#supplementary-material>

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