Xie Y, Liu Y, Xu D, et al. Multi-omics analysis provides new insights into the adaptive strategies of *Procambarus clarkii* in response to acute hypoxia stress. *Israeli Journal of Aquaculture - Bamidgeh*. 2026;78(3):316-332. doi:10.46989/001c.168524

Original Research Articles

Multi-omics analysis provides new insights into the adaptive strategies of *Procambarus clarkii* in response to acute hypoxia stress

Yuting Xie[1], Yuan Liu[1], Dingxiang Xu[1], Siqin Ouyang[1], Min Xiong[1], Yongjun Dai[2], Fei Liu[1], Yebing Yu[1], Wenping Yang[1], Hongyan Tian[1], Wuxiao Zhang[1], Silei Xia[1], Gongneng Feng[1], Xiaoping Guan[3], Aimin Wang[1a]

[1] School of Ocean and Biological Engineering, Yancheng Institute of Technology, Jiangsu Yancheng, 224051, PR China, [2] Yancheng Aquatic Science Research Institute, Yancheng Agricultural College, Yancheng, 224051, PR China, [3] Yili Yueran Ecological Agriculture Co., Ltd., Xinjiang Uygur Autonomous Region, Ili Kazakh Autonomous Prefecture, 835000, PR China

Keywords: transcriptomics, metabolomics, hypoxic stress, energy metabolism, *Procambarus clarkii*

https://doi.org/10.46989/001c.168524

Israeli Journal of Aquaculture - Bamidgeh

Vol. 78, Issue 3, 2026

Suitable dissolved oxygen levels are crucial for crustacean respiration and energy metabolism. To investigate the molecular mechanisms underlying hypoxia adaptation in the red swamp crayfish (*Procambarus clarkii*), we performed an integrated multi-omics analysis combining RNA-Seq transcriptomics and LC-MS non-targeted metabolomics on gill tissues under acute hypoxic (2.0±0.2 mg/L) and normoxic (7.5±0.2 mg/L) conditions. A total of 1,021 differentially expressed genes (DEGs) and 286 differentially expressed metabolites (DEMs) were identified. KEGG enrichment analysis revealed that DEGs were predominantly associated with lipid metabolism pathways, whereas DEMs were significantly enriched in nucleotide metabolism, purine metabolism, ABC transporter pathways and unsaturated fatty acid biosynthesis. Integrated transcriptomic and metabolomic analysis revealed 47 genes and 47 metabolites significantly associated across multiple key metabolic pathways, including lipid, carbohydrate, amino acid, and nucleotide metabolism. Specifically, genes associated with lipid synthesis and lipid degradation were upregulated, while the pentose phosphate pathway was activated to provide raw materials for fatty acid synthesis and produce NADPH to defend cell membranes from oxidative stress damage, indicating significant adjustments in energy metabolism pathways under hypoxic conditions. These findings provide a theoretical basis for deepening our understanding of hypoxic adaptation strategies in crustaceans. This multi-omics integration systematically revealed a coordinated metabolic reprogramming strategy in the gills of *P. clarkii* under acute hypoxia. These findings highlight the shift toward enhanced lipid turnover and NADPH production pathways (e.g., the pentose phosphate pathway) as key adaptive mechanisms, providing new insights into energy homeostasis and hypoxia tolerance in crustaceans beyond the traditional glycolysis-centered model.

1. INTRODUCTION

In recent years, the global aquaculture industry has experienced rapid growth, generating significant economic benefits for society. However, the environmental degradation caused by the continuous expansion of intensive aquaculture operations has become increasingly prominent. The rearing environment is closely linked to the survival and health of crustaceans.[1] Parameters such as temperature,[2] pH,[3] salinity,[4] ammonia nitrogen,[5] nitrite,[6] dissolved oxygen,[7] and stocking density[8] are key factors affecting crustacean survival and health. Among these, hypoxia stress stands as a major challenge in crustacean aquaculture, negatively impacting growth and development, energy metabolism, immune defense, and antioxidant capacity.[9]

Some crustaceans respond to hypoxic stress through complex behavioral and physiological strategies.[10] The physiological response of crustaceans to hypoxia involves biochemical and molecular changes in energy metabolism, antioxidant defense, and immune response, as well as regulation of apoptosis and cell cycle.[11,12] Hypoxia affects the metabolic patterns of aquatic animals.[13] At the physiological level, crustaceans exposed to low dissolved oxygen initiate the oxygen-carrying protein (hemocyanin) synthesis mechanism to improve oxygen transport efficiency,[14] and activate anaerobic metabolism, manifested as increased

a Corresponding author: E-mail: blueseawam@ycit.cn (Aimin Wang)

lactate production[15] and tissue-specific induction of glycolytic enzymes (Hexokinase, lactate dehydrogenase, and phosphofructokinase).[16-18] Hypoxia may also stimulate lipolysis.[19] At the cellular level, metabolic adaptation of crustaceans is reflected by regulating energy metabolism, lipid and carbon metabolism, and nutrient acquisition.[20] At the molecular level, short-term hypoxia induced the up-regulated expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene in the gills of *Litopenaeus vannamei* in response to stress[21] ; *Eriocheir sinensis* up-regulated HIF-1α during hypoxia, further activated the NF-κB-ALFs pathway, and played an immune defense role in response to hypoxia stress[22] ; HIF-1α regulates key genes in glycolysis and TCA cycle under hypoxic conditions to achieve energy programming.[23] At present, research on the mechanisms of hypoxia stress in crustaceans is deepening and progressing from basic physiological phenotypes to deeper molecular mechanisms.

Procambarus clarkii, commonly known as crayfish, is one of the most important crustacean aquaculture species in the global freshwater economy and plays a vital role in the world economy.[24] According to the 'China Crayfish Industry Development Report (2025)', in 2024, crayfish aquaculture production in China will reach 3.4476 million tons, ranking fourth among freshwater aquaculture varieties. *P. clarkii* is vulnerable to hypoxia stress in aquaculture and commercial transportation, and hypoxia can significantly affect the expression profile and metabolic response of related genes. Previous studies have shown that *P. clarkii* can increase the concentration of lactic acid and glucose in hemolymph under low dissolved oxygen conditions,[25] regulate the dynamic changes of antioxidant enzymes in hepatopancreas, reduce oxidative stress, and up-regulate the expression of key enzymes in glycolysis to activate anaerobic metabolism,[26] experiencing changes in the abundance of metabolites related to energy metabolism.[27] Zeng et al.[28] found that hypoxic stress significantly affected the activities of antioxidant enzymes (SOD, CAT) and anaerobic metabolic enzymes (LDH), as well as the expression of antioxidant and immune-related genes, in the hepatopancreas of juvenile and subadult crayfish. The higher the degree of hypoxia stress, the higher the gene expression level. This indicates that the regulation of the expression of specific enzymes required for metabolic adaptation to hypoxia is achieved through differential transcription. Zhang et al.[29] showed that hypoxia significantly affected glucose metabolism (glycolysis/gluconeogenesis, AMPK, and insulin signaling pathways) and oxygen transport (HIF-1, MAPK, and PI3K-Akt signaling pathways) in a transcriptomic analysis of the hepatopancreas of *Procambarus clarkii*. These metabolic changes are regulated by genes related to glucose metabolism and oxygen transport. The gill is an important organ for crustaceans to breathe, excrete, and regulate osmotic pressure, and is extremely sensitive to changes in dissolved oxygen in water. Because the gills of crustaceans are in direct contact with the external environment, hypoxia stress can directly damage their gill tissues.[30,31] At present, the molecular mechanism of metabolic regulation of key genes and pathways of acute hypoxia stress in the gills of *Procambarus clarkii* is still poorly understood. Therefore, it is of great significance to study the metabolic pattern of the gill tissue of crustaceans in a hypoxic environment.

In a large number of studies on hypoxic environments, aquatic animals do not rely on carbohydrates as the main source but initiate metabolic compensation mechanisms that potentially enhance the metabolism of amino acids, lipids, and other nutrients to maintain energy demand.[19, 32] In the post-genomic era, the widespread use of transcriptomics, metabolomics, and proteomics analyses has brought new insights to biology.[33] In recent years, multi-omics analysis methods have been employed to investigate stress responses in aquatic animals.[34] However, the mechanisms and molecular regulatory networks underlying *P. clarkii* gill responses to acute hypoxia stress have not been clearly elucidated using transcriptomic and metabolomic analyses, an important approach for understanding gene expression and metabolite differences in *P. clarkii* gills under low dissolved oxygen conditions.

Therefore, it was hypothesized that acute hypoxia triggered a comprehensive metabolic reprogramming in the gills of *P. clarkii* to restore energy homeostasis and resist oxidative stress. The purpose of this study was to identify differentially expressed genes and metabolites in the gill tissues of *P. clarkii* under acute hypoxia conditions. Through combined transcriptomic and metabolomic analysis, key pathways and regulatory networks were revealed, and the molecular mechanisms of energy homeostasis and oxidative stress resistance in the gills during hypoxia adaptation were further elucidated. This integrated multi-omics study will deepen our understanding of the hypoxia adaptation strategies of crustaceans and provide a theoretical reference for the sustainable development of the crustacean aquaculture industry.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL ANIMALS AND FEEDING MANAGEMENT

The subadult shrimp (*Procambarus clarkii*) used in this experiment was obtainted from the breeding laboratory of Yancheng Institute of Technology, with an average weight of 7.5±0.5 g. The crayfish were domesticated in a laboratory indoor aquarium (55 cm×25 cm×35 cm) for 7 days. During the domestication, the dissolved oxygen in the aquaculture water was maintained at 7.5±0.2 mg/L, the water temperature was 24±2°C, the pH value was 7.0±0.2, and the ammonia nitrogen content was maintained less than 0.02 mg/L. The photoperiod followed the natural light-dark cycle. The crayfish were fed with special compound feed at 8:00 a.m. and 6:00 p.m., respectively. The feeding rate was 3%-5% of the body weight of crayfish. Residual food and excreta were siphoned daily, and one-third of the water was replaced every two days to maintain water quality and ensure a healthy environment.

2.2. HYPOXIA TREATMENT AND SAMPLE COLLECTION

After the end of the acclimation period, the hypoxic environment was induced. The exposure time is set to 24 hours, which can induce a stable physiological metabolic response in *P. clarkii*, with no deaths, and demonstrates hypoxia adaptability.[28,35] Sixty healthy and lively crayfish (unseparated male and female were in the intermolt period) with normal body color were randomly placed in 6 aquariums (55 cm×25 cm×35 cm), 10 crayfish in each aquarium (aquaria were arranged in a randomized block design to minimize potential positional effects within the laboratory). During the experiment, the breeding environment was fully consistent with the domestication period, except for dissolved oxygen conditions. In this experiment, low dissolved oxygen group (Low, 2.0±0.2 mg/L) and normoxic control group (CK, 7.5±0.2 mg/L) were set up, with 3 replicates in each group, all of which were treated for 24 h. Low dissolved oxygen treatment involves applying low oxygen stress while maintaining normal dissolved oxygen levels. The hypoxia experiment in this study was performed using the Wang et al.[12] method. In short, the air in the CK group was continuously pumped into the water to maintain a normoxic state of about 7.5 mg/L. The low-oxygen treatment maintained a dissolved oxygen level of about 2.0 mg/L by injecting nitrogen and air into the water and controlling the flow rate. A pen dissolved oxygen meter (AR8210, XIMA) was used to monitor the dissolved oxygen concentration of the water in real time throughout the test. Gill tissue samples of *P. clarkii* treated with two dissolved oxygen concentrations were collected after the end of hypoxia treatment. Transcription experiments and metabolic experiments were set up with 3 and 6 parallels for the construction of transcriptome libraries and the analysis of metabolite profiles, respectively. After the shrimp were dissected, the tissue was frozen in liquid nitrogen and stored at −80°C in a refrigerator for further RNA extraction and metabolite assays.

2.3. TRANSCRIPTOME ANALYSIS

2.3.1. RNA EXTRACTION, LIBRARY CONSTRUCTION AND SEQUENCING

According to the manufacturer 's protocol, total RNA was extracted from the gill tissue of *P. clarkii* using magnetic beads connected with Oligo (dT), and the total RNA amount of each sample was 2 μg. RNA purity was measured using a kaiao K5500® spectrophotometer (Kaiao, Beijing, China), and RNA integrity and concentration were assessed using an Agilent 2100 bioanalyzer system. The construction and sequencing of cDNA and RNA Seq libraries were carried out in Wuhan Benard Technology Co., Ltd.in accordance with the manufacturer 's specifications. The library fragments were purified using Ampure XP magnetic beads, and library quality was evaluated using the Agilent Bioanalyzer 2100. Subsequently, these libraries were further amplified with phi29 polymerase to prepare DNA nanospheres (DNB), which were then loaded onto the patterned nanoarrays and generated 150 bp double-ended sequencing reads on the T7 platform of Wuhan Benard Technology Co., Ltd.

2.3.2. QUALITY CONTROL, MAPPING AND ASSEMBLY

The original data were first processed using FastQC,[36] and the joints and low-quality sequences were filtered from the original sequence (raw reads) to obtain high-quality effective sequences (clean reads). The Trinity software (v2.14) was used to generate unigenes.[37] Then, the effective sequences were aligned to the reference genome using HISAT (*Procambarus clarkii* GCA_040958095.1.genome.fa). The RSEM (v1.3.1) software package was used to calculate gene expression levels for each sample,[38] expressed as per-million alignment fragments per thousand base transcripts (FPKM), with a threshold of FPKM > 0.5. The gene expression levels of the hypoxia stress samples were compared with those of the control samples to identify differentially expressed genes (DEGs).

2.3.3. DEGS SCREENING AND FUNCTIONAL PREDICTION

DEseq2 (v1.34.0)[39] was used to screen differentially expressed genes (DEGs) of Low vs CK, and the screening criteria was |log2FoldChange|⩾2 and P<0.05. ClusterProfiler (v3.8)[40] was used to perform gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, and P<0.05 was used as the standard.

2.4. NON-TARGETED METABOLOMICS ANALYSIS

2.4.1. METABOLITE EXTRACTION

25 mg gill tissue samples were weighed and thawed at low temperature, and then 500 μL -40°C pre-cooled extract [methanol:acetonitrile:water=2:2:1(v/v/v)] was added. The extract contained an isotope-labeled internal standard; steel balls were added, and the mixture was homogenized in a homogenizer (35 Hz, 4 min), then transferred to an ice-water bath for ultrasound for 5min. This step was repeated three times. Then it was allowed to stand at −40°C for 1 h, and 300 μL was added to a 96-well filter plate. The 96-well filter plate-collection plate combination was placed in a positive-pressure device and slowly pressurized to 6 psi for 3 min. The filter plate-collection plate combination was removed from the positive pressure device, and the same amount of supernatant was mixed to prepare a quality control (QC) sample. The treatment and analysis methods of QC samples are the same as those of test samples.

2.4.2. LC-MS ANALYSIS

LC-MS analysis of samples was performed using a Vanquish (Thermo Fisher Scientific) ultra-high-performance liquid chromatograph. Chromatographic separation of target compounds was achieved via a Waters ACQUITY UPLC BEH Amide column (2.1 mm×50 mm, 1.7 μm). LC mobile phase A was an aqueous phase containing 25 mmol/L ammonium acetate and 25 mmol/L ammonium hydroxide, while phase

B was acetonitrile. The sample tray temperature was maintained at 4°C, with an injection volume of 2 µL.

2.4.3. *DATA ANALYSIS*

In this study, raw data were converted to mzXML format using ProteoWizard and processed with an internally developed program in R, based on XCMS, for peak detection, extraction, alignment, and integration. The R package and BiotreeDB (V3.0) were used to identify metabolites. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed on the normalized data using SIMCA software (v18.0.1). The OPLS-DA replacement test model was used to identify and screen for differential metabolites between the control and hypoxia groups. Combined with t-test analysis (P1) and the difference fold of metabolites |log2FoldChange|⩾2, they were screened as potential biomarkers and differential metabolites. In addition, the differential metabolites were compared, annotated and analyzed by KEGG (http://www.genome.jp/kegg/) database.[40]

2.5. INTEGRATION ANALYSIS BETWEEN TRANSCRIPTOME AND METABOLOME

Differential genes (DEGs, P<0.05 and |log2FoldChange|>2) and differential metabolites (DEMs, VIP>1 and P<0.05) were used for the integrated analysis of Low vs CK. The Pearson correlation coefficient method was used to integrate correlation coefficient analysis of metabolic groups and transcriptome data. The heat map intuitively shows the relationship between genes and metabolites. R software (R Version 3.5.1) was used to combine the information of each group, KEGG pathway enrichment analysis was performed, and matrix heat map was constructed according to Pearson correlation coefficient. CytoScape software (CytoScape 3.5.1) was used to generate a gene and metabolite interaction network.

2.6. STATISTICAL ANALYSIS

All analyses and charts are produced using R software (v3.5.1). DEGs were identified using DEseq2 (v1.34.0), and GO term annotation and KEGG pathway enrichment were performed using hypergeometric test. After FDR adjustment, adjusted P<0.05 indicated statistical significance.

3. RESULTS

3.1. TRANSCRIPTOME ANALYSIS

The sequencing results of 6 libraries are shown in Table 1. The number of Raw reads and Clean reads was stable between 40.02 Mb and 40.06 Mb. Q20 and Q30 were greater than 97.52 % and 93.58 %, respectively. The GC content of all samples was greater than 36.95 %.

3.1.1. *IDENTIFICATION AND ANNOTATION OF DEGS*

The number of differentially expressed genes of *P. clarkii* under two different dissolved oxygen levels is shown in Fig.1. Compared with the CK group, a total of 1021 differentially expressed genes were identified in the Low group (843 up-regulated and 178 down-regulated).

3.1.2. *GO AND KEGG ENRICHMENT ANALYSIS OF DEGS*

In order to further explore the biological functions of differentially expressed genes in the gill transcriptome under hypoxia stress, GO and KEGG enrichment analysis were performed on the differentially expressed genes detected under two different dissolved oxygen levels. In terms of GO enrichment analysis, most of the differentially expressed genes between Low group and CK group were mainly enriched in cellular anatomical entity (396), cell periphery (118) and extracellular region (117). Structural molecule activity (95), structural component of cuticle (91), and so on were enriched in molecular function. In the biological process, small molecule metabolic process (39), lipid metabolic process (32) and organic acid metabolic process (25) were enriched, such as Fig 2A.

In order to identify the biological pathways activated or inhibited in the response of gill tissue of *Procambarus clarkii* to hypoxia stress, DEGs were annotated into the KEGG database. KEGG enrichment analysis showed that 226 DEGs in Low vs CK were enriched into 116 KEGG pathways. Fig 2B showed the first 20 significantly enriched pathways in the gills, and were significantly enriched in multiple metabolic pathways (P<0.05). It includes unsaturated fatty acid biosynthesis (map01040), fatty acid metabolism (map01212), fatty acid elongation (map00062), glycerolipid metabolism (map00561), glutathione metabolism (map00480), folic acid biosynthesis (map00790), fatty acid degradation (map00071), α-linolenic acid metabolism (map00592), pentose phosphate pathway (map00030), D-amino acid metabolism (map00470) and other lipid metabolism, other amino acid metabolism, cofactor and vitamin metabolism and carbohydrate metabolism pathways. However, we cannot completely prove that these physiological processes occur in organisms. Therefore, we further tested the metabolome to identify metabolic pathways or metabolites related to the transcriptomic results, thereby confirming that hypoxia stress induces changes in physiological activity and energy metabolism in the gill tissue.

3.2. METABOLOME ANALYSIS

3.2.1. *OVERALL CHANGES IN METABOLITES*

To detect changes in metabolites in *Procambarus clarkii* under low dissolved oxygen stress, LC-MS was used for non-targeted metabolomics analysis. Based on orthogonal partial least squares discriminant analysis (OPLS-DA), R^2Y⩾0.99, and Q^2>0.84 (Table 2), indicating that the model stability is better; according to the OPLS-DA score scatter plot in Fig 3, it can be seen that there is a significant differ-

Table 1. Quality control of the RNA-Seq data.

Sample	Raw reads	Clean reads	Q20(%)	Q30(%)	GC(%)	Total Mapped (%)
CK1	40059122	40059100	97.62	93.88	37.17	97.00
CK2	40021564	40021534	97.83	94.45	37.87	95.24
CK3	40049064	40049036	97.90	94.58	38.12	93.77
Low1	40025572	40025540	97.52	93.58	38.45	91.20
Low2	40035958	40035932	97.93	94.62	36.95	96.72
Low3	40052708	40052688	97.58	93.89	38.33	97.05

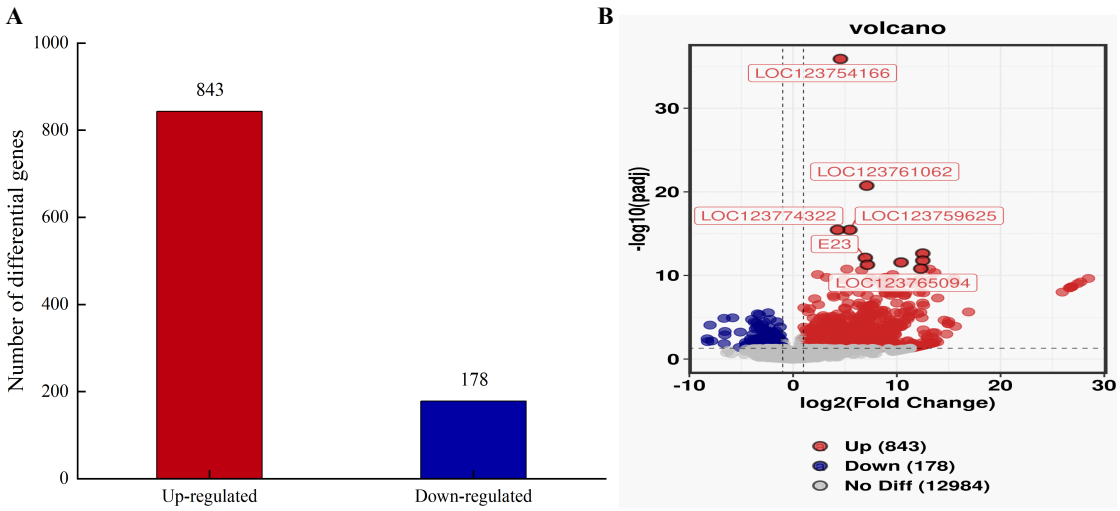

Fig. 1. Transcriptome analysis of Low and CK. The up-regulated and down-regulated DEGs (A), volcano diagram of DEGs (B) between Low and CK.

Note: In the volcanic map, red represents significantly up-regulated differentially expressed genes, blue represents significantly down-regulated differentially expressed genes, and gray represents no significantly differentially expressed genes.

ence between the two groups, which can be used for subsequent analysis.

3.2.2. SCREENING OF DEMS

The metabolic changes of gill tissue of *Procambarus clarkii* under low dissolved oxygen stress were studied by non-targeted metabolomics analysis. According to the criteria of VIP>1.0 and P<0.05, a total of 286 DEMs were identified between the Low group and the CK group, of which 175 increased and 111 decreased (Fig 4).

3.2.3. HIERARCHICAL CLUSTER ANALYSIS OF DEMS

Fig 5 shows the heat map of Low vs CK based on the top 20 DEMs in abundance. Metabolites with similar expression patterns are usually functionally related, further underscoring the differences in expression patterns between the two groups. According to the cluster heat map, 20 DEMs can be distributed into 7 clusters. Beta-Alanine and Aspartate showed different expression trends in the CK and Low groups, both of which belonged to organic acids and their derivatives. In addition, the samples in the Low group and the CK group showed similar colors, but the color difference between the two groups was significant; that is, greater intra-group similarity was observed between the groups.

3.2.4. FUNCTIONAL ENRICHMENT ANALYSIS OF DEMS RELATED TO HYPOXIA STRESS

This study found that the abundance of differential metabolites identified in the Low vs CK group showed a correlation, and a variety of metabolites showed a significant positive correlation (P<0.05, Fig 6A). The KEGG pathway enrichment analysis of differential metabolites showed that the significantly enriched pathways in the Low vs CK group

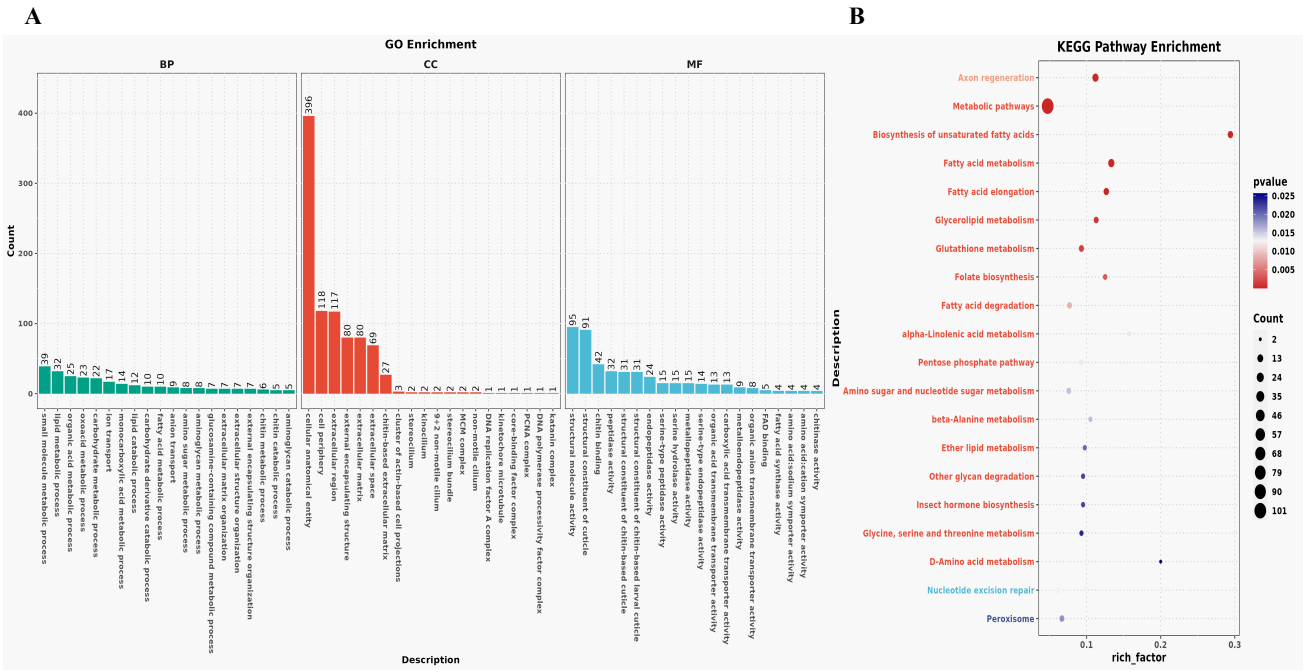

Fig. 2. Functional annotation and signal pathway enrichment analysis of DEGs. The GO enrichment analysis of DEGs in the classification of cell components, biological processes and molecular functions (A), and the enrichment analysis of the top 20 KEGG pathways of DEGs (B) of Low vs CK.

Note: The abscissa in B represents the enrichment factor between the two sample data sets, and the ordinate represents the first 20 KEGG pathways significantly enriched by differentially expressed genes.

Table 2. Evaluation parameters of OPLS-DA model

Groups	Score of PCA		
	R^2X	R^2Y	Q^2
Low vs CK	0.309	0.996	0.849

were mainly: Nucleotide metabolism; purine metabolism; biosynthesis of unsaturated fatty acids; ABC transporters; alanine, aspartate and glutamate metabolism; 2-Oxocarboxylic acid metabolism; citric acid cycle. Among them, there are differences in the degree of enrichment of different pathways (Fig 6B).

3.3. CONJOINT ANALYSIS OF TRANSCRIPTOME AND METABOLOME

To further understand the association between DEGs and DEMs, conjoint analysis of transcriptome and metabolome was performed. Fig 7A shows that a total of 47 genes and 47 metabolites were associated between the 1021 differentially expressed genes identified by transcriptome and the 286 differentially expressed metabolites identified by metabolomics. The circular diagram of the association between differentially expressed genes and metabolites showed that the ABCG2 gene (E23) in the ABC transporter membrane family was associated with three types of metabolites (lipids, organic nitrogen-containing compounds, and organic heterocyclic compounds), synergistically regulating the energy metabolism of *P. clarkii* (Fig.7B). The KEGG pathway of transcription and metabolic association analysis was mainly enriched in lipid, nucleotide, carbohydrate and amino acid metabolic pathways (Table 3). In terms of lipid metabolism, the fatty acid biosynthesis (map00061), fatty acid elongation (map00062) and fatty acid degradation (map00071) pathways were significantly enriched and up-regulated, and the steroid hormone biosynthesis (map00140) pathway was significantly enriched and down-regulated (Fig 7C); in terms of nucleotide metabolism, the pathways of purine metabolism (map00230) and pyrimidine metabolism (map00240) were significantly enriched and up-regulated (Fig 7C). In carbohydrate metabolism, the mutual conversion of pentose and glucuronate (map00040) and the pentose phosphate pathway (map00030) were significantly enriched; in terms of amino acid metabolism, alanine, aspartic acid and glutamic acid metabolism (map00250) and glycine, serine and threonine metabolism (map00260) pathways were significantly enriched; in addition, it is also enriched in biosynthetic pathways of secondary metabolites, such as caffeine metabolism (map00232).

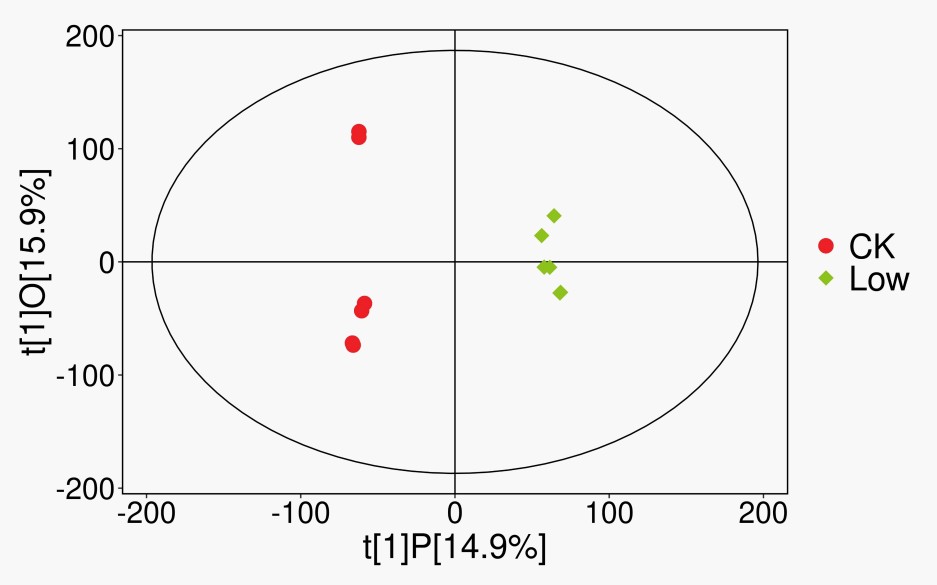

Fig. 3. OPLS-DA score scatter plot of Low vs CK.

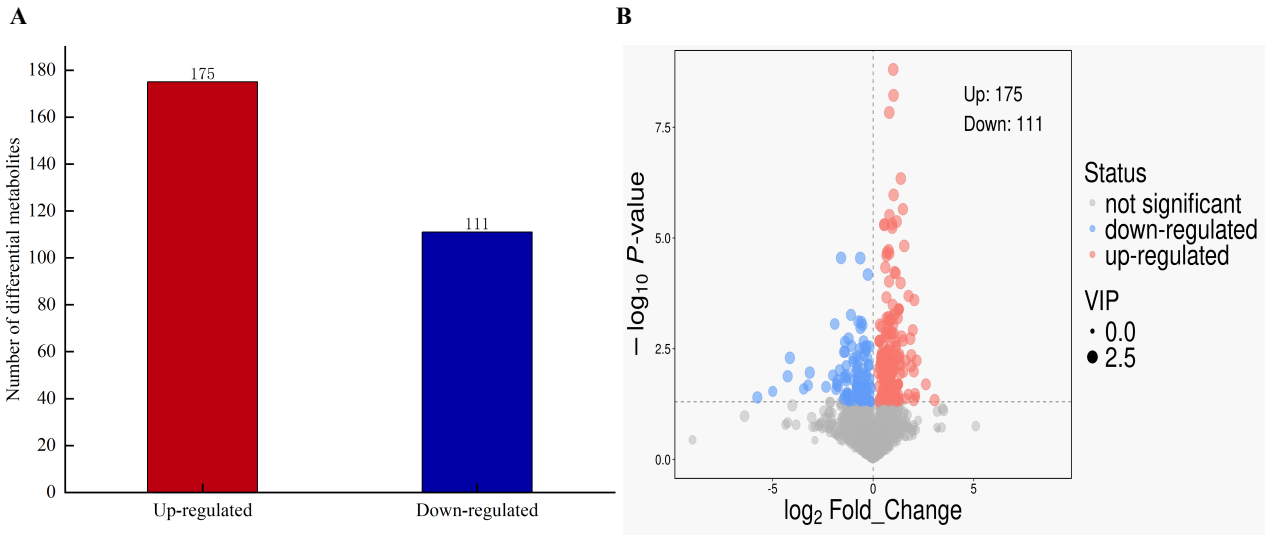

Fig. 4. The up-regulated and down-regulated DEMs (A), differentially expressed metabolite volcano plot (B) between Low and CK.

Note: In the figure, red represents a significantly up-regulated differential metabolite, blue represents a significantly down-regulated differential metabolite, and gray represents a metabolite with no significant difference.

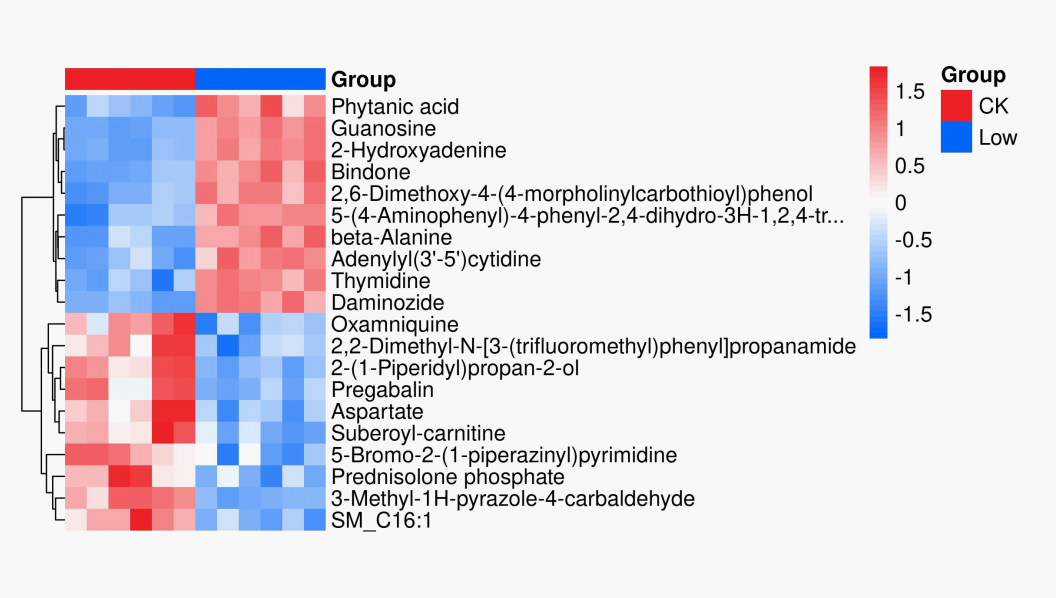

Fig. 5. Hierarchical cluster analysis heat map based on the abundance of the top 20 DEMs of Low vs CK.

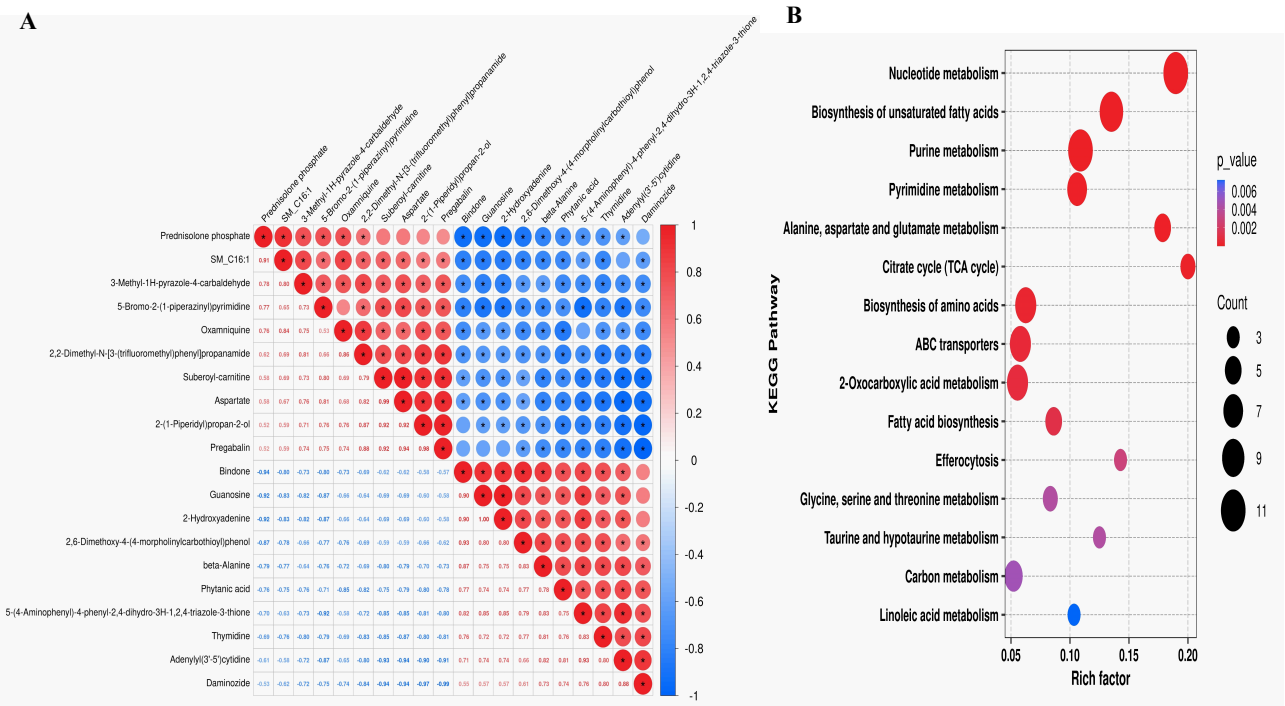

Fig. 6. Relationship analysis and function of differential metabolites. Correlation analysis of differential metabolites between Low group and CK group (A). KEGG pathway enrichment map of differential metabolites between Low group and CK (B).

4. DISCUSSION

The research on the stress response of *P. clarkii* to hypoxia primarily focuses on physiological and biochemical aspects, as well as single transcriptomic analyses related to energy metabolism and immune function, but research on the hypoxia response of key gene functions and regulatory networks remains limited. In particular, the combined transcriptomic and metabolomic analysis of the effects of

Table 3. The key SDEGs and SDEMs of *Procambarus clarkii* associated with hypoxia stress in some important pathway.

Category	Pathway ID	Pathway name	Gene/Metabolite	Description	Up/ Down
Lipid metabolism	map00061	Fatty acid biosynthesis	LOC123773166（FASN）	fatty acid synthase	Up
			LOC123767158（ACSBG）	long-chain-fatty-acid--CoA ligase ACSBG2	Up
			C00249	Palmitic acid	Up
	map00140	Steroid hormone biosynthesis	LOC123767673（DHRS11）	17beta-estradiol 17-dehydrogenase	Down
			LOC123770683（UGT）	glucuronosyltransferase	Up
			LOC123765007	very-long-chain 3-oxoacyl-CoA reductase	Up
			LOC123756166	very-long-chain 3-oxoacyl-CoA reductase	Down
			LOC123756167	very-long-chain 3-oxoacyl-CoA reductase	Down
	map00062	Fatty acid elongation	LOC123764660	elongation of very long chain fatty acids protein 7	Up
			LOC123765007	very-long-chain 3-oxoacyl-CoA reductase	Up
			LOC123754540	acetyl-CoA acyltransferase 2	Up
			Baldspot	elongation of very long chain fatty acids protein 6	Up
			LOC123759372	elongation of very long chain fatty acids protein 7	Up
			LOC123756166	very-long-chain 3-oxoacyl-CoA reductase	Down
			LOC123756167	very-long-chain 3-oxoacyl-CoA reductase	Down
			LOC123764749	elongation of very long chain fatty acids protein 7	Up
			LOC138351039	elongation of very long chain fatty acids protein 7	Up
	map00071	Fatty acid degradation	novel2365	enoyl-CoA hydratase	Down
			LOC123754540（ACAA2）	acetyl-CoA acyltransferase 2	Up
			LOC123767158（ACSBG）	long-chain-fatty-acid--CoA ligase ACSBG	Up
			Acadvl（ACADVL）	very long chain acyl-CoA dehydrogenase	Up
			LOC123771699（ACOX1）	peroxisomal acyl-coenzyme A oxidase 1	Down
			LOC123757282（ACOX3）	peroxisomal acyl-coenzyme A oxidase 3-like	Down
			Whd（CPT1A）	carnitine O-palmitoyltransferase 1	Up
			novel2365（ECHS1）	enoyl-CoA hydratase	Down
			C00249	Palmitic acid	Up
Nucleotide metabolism	map00230	Purine metabolism	LOC123759323	IMP cyclohydrolase	Down
			LOC123757285（PDE8）	high affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8	Up
			LOC123775106（ADK）	adenosine kinase	Up
			LOC123760653（XDH）	xanthine dehydrogenase/oxidase	Down
	map00240	Pyrimidine metabolism	Kri（UPRT）	uracil phosphoribosyltransferase	Up
			C00295	Orotic acid	Up
			C02354	Cytidine 2',3'-cyclic phosphate	Up
			C00214	Thymidine	Up
			C00299	Uridine	Up
			C00099	beta-Alanine	Up
			C00055	Cytidine 5'-monophosphate (CMP)	Up
			C02067	Pseudouridine	Up
Carbohydrate	map00040	Pentose and	LOC123755374（xylA）	xylose isomerase	Down

Category	Pathway ID	Pathway name	Gene/Metabolite	Description	Up/ Down
metabolism	map00030	glucuronate interconversions	LOC123770683（UGT）	glucuronosyltransferase	Up
			LOC123768500（AKR1B）	aldehyde reductase	Down
			LOC123770666（SORD）	sorbitol dehydrogenase	Down
		Pentose phosphate pathway	LOC123774400（SHPK）	Sedoheptulokinase	Down
			Fbp（FBP）	fructose-1,6-bisphosphatase I	Down
			LOC123762457（GLD）	glucose 1-dehydrogenase (FAD, quinone)	Up
			LOC123755442（GLD）	glucose 1-dehydrogenase (FAD, quinone)	Up
			LOC123762291（GLD）	glucose 1-dehydrogenase (FAD, quinone)	Up
			C00022	Pyruvate	Up
Amino acid metabolism	map00250	Alanine, aspartate and glutamate metabolism	LOC123769161（AGXT2）	alanine-glyoxylate transaminase	Down
			LOC123772103（DDO）	D-aspartate oxidase	Down
	map00260	Glycine, serine and threonine metabolism	LOC123772393（PIPOX）	peroxisomal sarcosine oxidase	Down
			Aay（PSPH）	phosphoserine phosphatase	Up
			LOC123769161（AGXT2）	alanine-glyoxylate transaminase	Down
			LOC123771694（DMGDH）	dimethylglycine dehydrogenase	Down

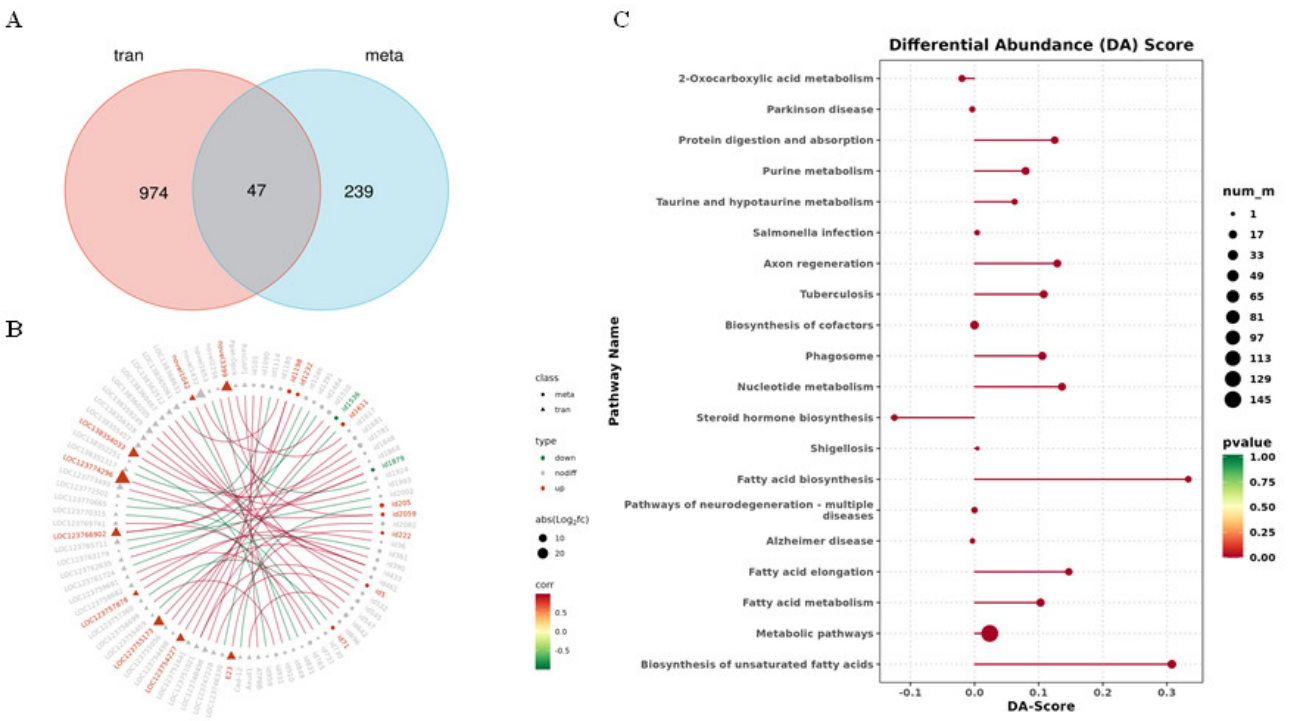

Fig. 7. Transcriptional metabolic correlation Wayne diagram (A), correlation loop diagram (B), DA-Score diagram (C) of Low vs CK

hypoxia stress on P. clarkii remains unclear. In this study, the combined analysis of transcriptional and metabolic KEGG enrichment pathways showed that hypoxia significantly affected lipid, carbohydrate, nucleotide, and amino acid metabolism in *P. clarkii*. This is similar to the metabolic changes of *Takifugu Obscurus* gills after acute hypoxia 6 h stress studied by Zhang et al.[41] It was found that energy synthesis and metabolism-related pathways such as lipid metabolism, amino acid metabolism, and purine metabolism were significantly enriched. However, compared with fish, crustaceans have an open circulatory system and gill tissue is directly exposed to the water environment. Their metabolic reprogramming may entail more urgent physiological needs to resist oxidative stress and maintain ion exchange function. The results of this study will provide a scientific basis for the characteristics of hypoxia adaptation metabolic reprogramming in crustacean gill tissue.

4.1. HYPOXIA STRESS AFFECTS LIPID METABOLISM OF *PROCAMBARUS CLARKII*

Under hypoxic conditions, organisms must adjust their energy requirements to maintain basic physiological processes. Lipids, as important energy sources and precursors of signaling molecules, play key roles in maintaining membrane structure, regulating energy metabolism, and controlling signal transduction.[42] Therefore, lipid homeostasis is essential for maintaining health.[43] More and more studies show that the compensatory mechanism of aquatic animals under hypoxic stress involves enhanced lipid metabolism.[13] Nguyen et al.[44] found that metabolites, such as fatty acids and amino acids, in the hemolymph of *Litopenaeus vannamei* changed significantly after hypoxia exposure by GC-MS metabolomics analysis, suggesting that hypoxia stress led to an energy metabolism disorder and a disturbance of amino acid metabolism. The gills of crustaceans are important sites for lipid storage and metabolism.[45] This study found that under hypoxic conditions, the contents of most fatty acyl and glycerophospholipid metabolites in the significantly enriched differential metabolites in the gills of *P. clarkii* were significantly increased, and the expression of fatty acid biosynthesis-related genes, such as fatty acid synthase (*fasn*), long-chain fatty acid-CoA ligase (*acsbg*), and fatty acid degradation-related gene carnitine palmitoyltransferase 1 (*cpt*-1A), was up-regulated. Among the significantly enriched metabolic pathways, four were directly related to lipid metabolism, indicating that fatty acid synthesis in the gills of *Procambarus clarkii* was significantly enhanced under hypoxia stress. At the same time, polyunsaturated fatty acids (PUFAs) also changed significantly in the gills of *P. clarkii* under hypoxic conditions; metabolites such as arachidonic acid, linolenic acid, palmitic acid, arachidonic acid, and oleic acid were significantly accumulated after 24 h of hypoxia. *Fasn* is a key enzyme involved in the synthesis of endogenous fatty acids. *cpt*-1 is a key enzyme that limits the rate of fatty acid β-oxidation and can transport fatty acids to mitochondria. The *fasn* and *cpt*-1 genes in this study were up-regulated simultaneously, indicating that the crayfish initiated the synthesis and degradation of fatty acids in the gills under hypoxic conditions. This "dynamic lipid turnover" strategy has important physiological significance: on the one hand, the enhancement of fatty acid synthesis can provide raw materials for cell membrane repair and signal molecule generation; on the other hand, moderately accumulated unsaturated fatty acids can participate in cell stress protection through esterification and reduce lipid peroxidation damage, thereby maintaining the integrity of gill epithelial cells. In addition, crustacean molting is a biological process that requires substantial energy.[46,47] Enhancing lipid catabolism under hypoxic conditions may also help conserve protein reserves and allocate limited amino acids to more important physiological processes, such as molting-related structural reconstruction. The above results indicate that lipid metabolism is the primary energy-metabolism adjustment pathway in gill tissue to cope with acute hypoxia.

4.2. HYPOXIA STRESS AFFECTS CARBOHYDRATE METABOLISM OF *PROCAMBARUS CLARKII*

The metabolic adaptation of crustaceans to hypoxic stress means a shift from aerobic metabolism to anaerobic metabolism.[48] In the study on *Litopenaeus vannamei*, HIF-1 signaling pathway has been confirmed to transcriptionally regulate the expression of glycolytic enzymes such as GAPDH in gill tissue under hypoxic conditions, and mediate the activation of anaerobic glycolysis.[49] Carbohydrate metabolism is an important pathway of energy metabolism, serving as a hypoxic metabolic compensation strategy to maintain the balance between oxidation and anaerobic metabolism and energy supply.[50] Studies have shown that during hypoxia, the expression of phosphofructokinase (*pfk*) and fructose 1,6-bisphosphatase (*fbp*) in the hepatopancreas of *Penaeus vannamei* is up-regulated, which activates glycolysis and gluconeogenesis pathways.[18] This study showed that hypoxia stress activated the pentose phosphate pathway in the gill tissue of *P. clarkii*, increased the final metabolite pyruvate in the glycolytic process, and down-regulated the expression of fructose-1,6-bisphosphatase 1 (*fbp*), a key rate-limiting enzyme in the gluconeogenesis pathway, indicating that acute hypoxia may enhance glycolysis and inhibit gluconeogenesis. This may be due to the fact that, under hypoxic conditions, the glycolytic product pyruvate cannot directly enter the mitochondria and is recycled via the TCA cycle, resulting in decreased ATP production. The inhibition of *fbp* leads to the closure of gluconeogenesis, and the accumulation of glycolytic intermediate glucose-6-phosphate (G6P), which in turn promotes the up-regulation of glucose-1-dehydrogenase (GLD) gene expression related to the pentose phosphate pathway (PPP). The carbon flow eventually enters the pentose phosphate pathway, providing a large amount of NADPH for cell energy metabolism. It is worth noting that the pentose phosphate pathway is not only the main source of NADPH,[51] but also provides raw materials for the synthesis of fatty acids, ribonucleic acid, and cholesterol. NADPH, as a reducing agent, can maintain glutathione in its reduced state and effectively remove reactive oxygen species, thereby reducing oxidative damage caused by hypoxia stress.[52] In this study, the synergistic activation of lipid anabolism and the pentose phosphate pathway under hypoxia provided sufficient energy to support *P. clarkii*'s metabolic demands and prevent oxidative damage to gill tissue. This is similar to the multi-omics analysis of tilapia (GIFT, *Oreochromis niloticus*) under hypoxia stress, which is characterized by activation of the pentose phosphate pathway and upregulation of unsaturated fatty acid biosynthesis and fatty acid metabolism.[53] Similar to the results of this study, Sun et al.[54] found that glycolysis was enhanced and gluconeogenesis was inhibited in largemouth bass under acute hypoxia conditions. In this study, similar metabolic patterns were observed in the gill tissues of crustaceans, but their open circulatory system exposed them to greater direct oxidative stress. Therefore, the activation of the PPP pathway may have more urgent physiological significance in protecting the function of gill epithelial cells.

4.3. HYPOXIA STRESS AFFECTS OTHER METABOLISMS OF *PROCAMBARUS CLARKII*

Most of the 286 differential metabolites identified under hypoxia stress were amino acids, peptides, and analogs, and were significantly enriched in alanine, aspartic acid, and glutamic acid metabolism (map00250) and glycine, serine, and threonine metabolism (map00260) pathways. Huo et al.[55] showed that amino acid metabolism is an important process for sea cucumbers to adapt to hypoxia stress, and may play a role in osmotic regulation and energy regulation. Nguyen et al.[44] also found that hypoxia could disrupt amino acid metabolism in *Litopenaeus vannamei*, further supporting the universality of amino acid metabolism in the hypoxia response of crustaceans. In this study, the contents of alanine, β-alanine and glutamic acid increased under hypoxia stress, and the KEGG pathway of transcriptome and metabolome combined analysis was significantly enriched in amino acid metabolic pathway. These results indicated that *P. clarkii* initiated amino acid metabolism to improve its immune response and anti-stress capacity under hypoxic stress.[56]

The final product of purine metabolism is uric acid, which is an important antioxidant that helps maintain stable blood pressure and reduce oxidative stress.[57] Among them, xanthine dehydrogenase (XDH) is the rate-limiting enzyme for hypoxanthine to produce uric acid.[58] This study showed that hypoxia down-regulated the expression of XDH gene and up-regulated the expression of adenosine kinase (ADK) gene, which synergistically regulated the purine metabolism of *P. clarkii* to cope with hypoxia stress. This finding is consistent with the view of synergistic regulation of multiple signaling pathways in crustaceans under hypoxia, as reported in the review by Zheng et al.,[9] suggesting that purine metabolism may play an important role in crustacean antioxidant defense.

ABC transporters are a family of membrane proteins widely distributed in prokaryotes and eukaryotes. The energy generated by ATP hydrolysis is used to transport a variety of substrates (inorganic and organic small molecules and larger organic compounds) across the membrane in an inverse concentration gradient. Kropf et al.[59] demonstrated the existence, diversity and functional activity of ATP-binding cassette (ABC) transporters in the gills of rainbow trout, and may be involved in the active transport of endogenous substances and exogenous compounds through basal side transport. In this study, metabolomic results showed that the ABC transporter pathway was significantly enriched, indicating that the ABC transporter in the gill of *P. clarkii* under hypoxic stress may transport small molecules, such as nucleosides and organic acids, across the membrane to support energy metabolism and maintain normal life activities. In view of the multiple functions of crustacean gill tissue, such as respiration, ion regulation, and excretion, the activation of ABC transporters may play a key compensatory role in maintaining ion homeostasis and metabolite exchange of gill epithelial cells under hypoxic conditions. Therefore, hypoxia stress may lead to metabolic reprogramming of *P. clarkii*. Likewise, short-term temperature stress has been documented to significantly alter key metabolic enzymes in *P. clarkii*, indicating metabolic adjustment/reprogramming under stress conditions.[60]

5. CONCLUSSION

In summary, this study examined the response of *P. clarkii* to low dissolved oxygen stress by analyzing gill transcriptional and metabolic levels and identified the key regulatory pathways involved in the hypoxic stress response, along with differentially expressed genes and differentially abundant metabolites. This work identified 1021 DEGs and 286 DEMs. The combined analysis of transcriptome and metabolome showed that *fasn* and *cpt*-1A genes related to fatty acid synthesis showed a strong correlation with DEMs under hypoxia stress, and were involved in improving the hypoxia tolerance of *P. clarkii*. Hypoxia stress significantly affected the metabolism of lipids, amino acids, nucleotides, and carbohydrates in *P. clarkii*. Lipid metabolism is the primary pathway underlying the response of *P. clarkii* to acute hypoxia stress. Under hypoxia, *P. clarkii* improves hypoxia tolerance by enhancing the synthesis of unsaturated fatty acids and activating the pentose phosphate pathway to provide a large amount of NADPH to respond to hypoxia stress and defend cells and cell membranes from oxidative damage, while remodeling energy metabolism to meet its own needs. In addition, the functional initiation of ABC transporters and the adjustment of amino acid and nucleotide metabolism also provide conditions for metabolic reprogramming of *Procambarus clarkii* in hypoxic environment.

ACKNOWLEDGEMENTS

This work was supported by the transfer and transformation project of scientific and technological achievements of the Science and Technology Department of Xinjiang Uygur Autonomous Region (ZYYD2025CG03) and the special fund project of Jiangsu modern agricultural industry technology system construction (JATS[2023]471).

AUTHORS'CONTRIBUTION

Conceptualization: Yuting Xie (Equal). Data curation: Yuting Xie (Lead). Formal Analysis: Yuting Xie (Equal), Yuan Liu (Equal). Validation: Yuting Xie (Equal), Yongjun Dai (Equal). Writing – original draft: Yuting Xie (Lead). Writing – review & editing: Yuting Xie (Equal). Methodology: Dingxiang Xu (Equal), Siqin Ouyang (Equal). Software: Fei Liu (Equal), Yebing Yu (Equal). Investigation: Wenping Yang (Equal), Hongyan Tian (Equal). Resources: Wuxiao Zhang (Equal), Silei Xia (Equal). Supervision: Gongneng Feng (Equal), Aimin Wang (Equal). Funding acquisition: Xiaoping Guan (Equal), Aimin Wang (Equal). Project administration: Aimin Wang (Lead).

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

The article adheres to the Convention on Biological Diversity and the Convention on Trade in Endangered Species of Wild Fauna and Flora Research.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request.

Submitted: August 02, 2026 CDT. Accepted: September 01, 2026 CDT. Published: September 10, 2026 CDT.

REFERENCES

1. Behringer DC, Duermit-Moreau E. Crustaceans, one health and the changing ocean. *Journal of Invertebrate Pathology*. 2021;186:107500. doi:10.1016/j.jip.2020.107500

2. Bir J, Sarker H, Mita FS, et al. The impact of salinity and temperature stress on survival, behaviour, immune response, and proximate composition of giant freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture International*. 2024;32(5):6333-6352. doi:10.1007/s10499-024-01468-6

3. Yu Q, Xie J, Huang M, et al. Growth and health responses to a long-term pH stress in pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Reports*. 2020;16:100280. doi:10.1016/j.aqrep.2020.100280

4. Zhang D, Qi T, Liu J, et al. Adaptively differential expression analysis in gill of chinese mitten crabs (*Eriocheir japonica sinensis*) associated with salinity changes. *International Journal of Biological Macromolecules*. 2018;120:2242-2246. doi:10.1016/j.ijbiomac.2018.08.054

5. Luo Z, Zhou F, Jiang S, et al. Immune and physiological responses in *Penaeus monodon* to ammonia-N stress: a multi-omics approach. *Frontiers in Immunology*. 2024;15:1510887. doi:10.3389/fimmu.2024.1510887

6. Bian DD, Shi YX, Zhu XR, et al. Effects of nitrite exposure on the oxidative stress, immune response and intestine microbiota of *Procambarus clarkii*. *Aquaculture*. 2024;593:741331. doi:10.1016/j.aquaculture.2024.741331

7. Araujo AN, San Andres CF, Nguyen KQ, et al. Effects of minimum dissolved oxygen setpoints for aeration in semi-intensive pond production of Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture*. 2025;594:741376. doi:10.1016/j.aquaculture.2024.741376

8. Araneda M, Gasca-Leyva E, Vela MA, et al. Effects of temperature and stocking density on intensive culture of pacific white shrimp in freshwater. *Journal of Thermal Biology*. 2020;94:102756. doi:10.1016/j.jtherbio.2020.102756

9. Zheng C, Zhao Q, Li E, et al. Role of hypoxia in the behaviour, physiology, immunity and response mechanisms of crustaceans: a review. *Reviews in Aquaculture*. 2022;14(2):676-687. doi:10.1111/raq.12618

10. Burnett LE, Stickle WB. Physiological responses to hypoxia. In: Rabalais NN, Turner RE, eds. *Coastal and Estuarine Studies*. American Geophysical Union; 2001:101-114. doi:10.1029/CE058p0101

11. Chang F, Li N, Shi X, et al. Physiological and muscle tissue responses in *Litopenaeus vannamei* under hypoxic stress via iTRAQ. *Frontiers in Physiology*. 2022;13. doi:10.3389/fphys.2022.979472

12. Wang P, Liu H, Zhao S, et al. Hypoxia stress affects the physiological responses, apoptosis and innate immunity of Kuruma shrimp, *Marsupenaeus japonicus*. *Fish & Shellfish Immunology*. 2022;122:206-214. doi:10.1016/j.fsi.2022.02.016

13. Zhao LL, Sun JL, Liang J, et al. Enhancing lipid metabolism and inducing antioxidant and immune responses to adapt to acute hypoxic stress in *Schizothorax prenanti*. *Aquaculture*. 2020;519:734933. doi:10.1016/j.aquaculture.2020.734933

14. Tommerdahl AP, Burnett KG, Burnett LE. Respiratory properties of hemocyanin from wild and aquacultured penaeid shrimp and the effects of chronic exposure to hypoxia. *Biological Bulletin*. 2015;228(3):242-252. doi:10.1086/BBLv228n3p242

15. Soñanez-Organis JG, Racotta IS, Yepiz-Plascencia G. Silencing of the hypoxia inducible factor 1 –HIF-1- obliterates the effects of hypoxia on glucose and lactate concentrations in a tissue-specific manner in the shrimp *Litopenaeus vannamei*. *Journal of Experimental Marine Biology and Ecology*. 2010;393(1-2):51-58. doi:10.1016/j.jembe.2010.06.031

16. Soñanez-Organis JG, Peregrino-Uriarte AB, Sotelo-Mundo RR, et al. Hexokinase from the white shrimp *Litopenaeus vannamei*: cDNA sequence, structural protein model and regulation via HIF-1 in response to hypoxia. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*. 2011;158(3):242-249. doi:10.1016/j.cbpb.2010.12.006

17. Soñanez-Organis JG, Rodriguez-Armenta M, Leal-Rubio B, et al. Alternative splicing generates two lactate dehydrogenase subunits differentially expressed during hypoxia via HIF-1 in the shrimp *Litopenaeus vannamei*. *Biochimie*. 2012;94(5):1251-1260. doi:10.1016/j.biochi.2012.02.015

18. Cota-Ruiz K, Peregrino-Uriarte AB, Felix-Portillo M, et al. Expression of fructose 1,6-bisphosphatase and phosphofructokinase is induced in hepatopancreas of the white shrimp *Litopenaeus vannamei* by hypoxia. *Marine Environmental Research*. 2015;106:1-9. doi:10.1016/j.marenvres.2015.02.003

19. Li M, Wang X, Qi C, et al. Metabolic response of nile tilapia (*Oreochromis niloticus*) to acute and chronic hypoxia stress. *Aquaculture*. 2018;495:187-195. doi:10.1016/j.aquaculture.2018.05.031

20. Lee P, Chandel NS, Simon MC. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nature Reviews Molecular Cell Biology*. 2020;21(5):268-283. doi:10.1038/s41580-020-0227-y

21. Camacho-Jiménez L, Peregrino-Uriarte AB, Martínez-Quintana JA, et al. The glyceraldehyde-3-phosphate dehydrogenase of the shrimp *Litopenaeus vannamei*: molecular cloning, characterization and expression during hypoxia. *Marine Environmental Research*. 2018;138:65-75. doi:10.1016/j.marenvres.2018.04.003

22. Ma Y, Wu Z, Tu Y, et al. Hypoxia-inducible factor-1α involves in regulating anti-lipopolysaccharide factors expression via NF-κB under hypoxia stress in Chinese mitten crab (*Eriocheir sinensis*). *Fish & Shellfish Immunology*. 2025:162. doi:10.1016/j.fsi.2025.110328

23. Zhang J, Liu C, Liu B, et al. Hypoxia inducible factor HIF-1α regulates anaerobic metabolism after hypoxia stress in Pacific oyster *Crassostrea gigas*. *Aquaculture International*. 2025;33(5). doi:10.1007/s10499-025-02059-9

24. Fao. *The State of World Fisheries and Aquaculture 2024*. FAO; 2024.

25. Bonvillain CP, Rutherford DA, Kelso WE, et al. Physiological biomarkers of hypoxic stress in red swamp crayfish *Procambarus clarkii* from field and laboratory experiments. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 2012;163(1):15-21. doi:10.1016/j.cbpa.2012.04.015

26. Xu Y, Lin H, Yan W, et al. Full-length transcriptome of red swamp crayfish hepatopancreas reveals candidate genes in hif-1 and antioxidant pathways in response to hypoxia-reoxygenation. *Marine Biotechnology*. 2022;24(1):55-67. doi:10.1007/s10126-021-10086-9

27. Izral NM, Brua RB, Culp JM, et al. Developing metabolomics-based bioassessment: crayfish metabolome sensitivity to food and dissolved oxygen stress. *Environmental Science and Pollution Research*. 2018;25(36):36184-36193. doi:10.1007/s11356-018-3518-5

28. Zeng Q, Luo M, Qin L, et al. Effects of hypoxia stress on survival, antioxidant and anaerobic metabolic enzymes, and related gene expression of red swamp crayfish *Procambarus clarkii*. *Biology*. 2024;13(1):33. doi:10.3390/biology13010033

29. Zhang L, Song Z, Zhong S, et al. Acute hypoxia and reoxygenation induces oxidative stress, glycometabolism, and oxygen transport change in red swamp crayfish (*Procambarus clarkii*): Application of transcriptome profiling in assessment of hypoxia. *Aquaculture Reports*. 2022;23:101029. doi:10.1016/j.aqrep.2022.101029

30. Chen X, Bai Y, Ma J, et al. Comparative transcriptome analysis of gill tissue response to hypoxia stress in the Chinese mitten crab (*Eriocheir sinensis*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 2025;288:110081. doi:10.1016/j.cbpc.2024.110081

31. Sun S, Xuan F, Fu H, et al. Transciptomic and histological analysis of hepatopancreas, muscle and gill tissues of oriental river prawn (*Macrobrachium nipponense*) in response to chronic hypoxia. *BMC Genomics*. 2015;16(1):491. doi:10.1186/s12864-015-1701-3

32. Xiao K, Wang X, Wang MM, et al. Metabolism, antioxidant and immunity in acute and chronic hypoxic stress and the improving effect of vitamin C in the channel catfish (*Ictalurus punctatus*). *Fish Physiology and Biochemistry*. 2024;50(1):183-196. doi:10.1007/s10695-023-01205-5

33. Saito K, Matsuda F. Metabolomics for functional genomics, systems biology, and biotechnology. *Annual Review of Plant Biology*. 2010;61:463-489. doi:10.1146/annurev.arplant.043008.092035

34. Xiao J, Liu QY, Du JH, et al. Integrated analysis of physiological, transcriptomic and metabolomic responses and tolerance mechanism of nitrite exposure in *Litopenaeus vannamei*. *Science of The Total Environment*. 2020;711:134416. doi:10.1016/j.scitotenv.2019.134416

35. Jie YK, Cheng CH, Wang LC, et al. Hypoxia-induced oxidative stress and transcriptome changes in the mud crab (*Scylla paramamosain*). *Comp Biochem Physiol C Toxicol Pharmacol*. 2021;245:109039. doi:10.1016/j.cbpc.2021.109039

36. Chen S, Zhou Y, Chen Y, et al. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. 2018;34(17):i884-i890. doi:10.1093/bioinformatics/bty560

37. Grabherr MG, Haas BJ, Yassour M, et al. Full-length transcriptome assembly from RNA-seq data without a reference genome. *Nature Biotechnology*. 2011;29(7):644-652. doi:10.1038/nbt.1883

38. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-seq data with or without a reference genome. *BMC Bioinformatics*. 2011;12(1):323. doi:10.1186/1471-2105-12-323

39. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*. 2014;15(12):550. doi:10.1186/s13059-014-0550-8

40. Yu G, Wang LG, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS: A Journal of Integrative Biology*. 2012;16(5):284-287. doi:10.1089/omi.2011.0118

41. Zhang H, Hu Z, Li R, et al. Metabolomic analysis of the *Takifugu obscurus* gill under acute hypoxic stress. *Animals*. 2022;12(19):2611. doi:10.3390/ani12192611

42. Loix M, Zelcer N, Bogie JFJ, et al. The ubiquitous role of ubiquitination in lipid metabolism. *Trends in Cell Biology*. 2024;34(5):416-429. doi:10.1016/j.tcb.2023.09.001

43. Loizides-Mangold U. On the future of mass-spectrometry-based lipidomics. *FEBS Journal*. 2013;280(12):2817-2829. doi:10.1111/febs.12202

44. Nguyen TV, Alfaro AC, Rodriguez J, et al. Changes in metabolic profiling of whiteleg shrimp (*Penaeus vannamei*) under hypoxic stress. *Journal of Invertebrate Pathology*. 2022;193. doi:10.1016/j.jip.2022.107798

45. Li WF, Zhang S, Chiu KH, et al. Silencing of crustacean hyperglycemic hormone gene expression reveals the characteristic energy and metabolic changes in the gills and epidermis of crayfish *Procambarus clarkii*. *Frontiers in Physiology*. 2023;14:1349106. doi:10.3389/fphys.2023.1349106

46. Yuan Y, Jin M, Fang F, et al. New Insight Into the Molting and Growth in Crustaceans: Regulation of Energy Homeostasis Through the Lipid Nutrition. *Frontiers in Marine Science*. 2022;9. doi:10.3389/fmars.2022.914590

47. Li Z, Zhang G, Pan K, et al. Functional transcriptome reveals hepatopancreatic lipid metabolism during the molting cycle of the Chinese mitten crab *Eriocheir sinensis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 2023;284:111474. doi:10.1016/j.cbpa.2023.111474

48. Sun S, Fu H, Zhu J, et al. Molecular cloning and expression analysis of lactate dehydrogenase from the oriental river prawn *Macrobrachium nipponense* in response to hypoxia. *International Journal of Molecular Sciences*. 2018;19(7):1990. doi:10.3390/ijms19071990

49. Camacho-Jimenez L, Leyva-Carrillo L, Peregrino-Uriarte AB, et al. Regulation of glyceraldehyde-3-phosphate dehydrogenase by hypoxia inducible factor 1 in the white shrimp *Litopenaeus vannamei* during hypoxia and reoxygenation. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 2019;235:56-65. doi:10.1016/j.cbpa.2019.05.006

50. Ulaje SA, Rojo-Arreola L, Lluch-Cota SE, et al. Gene expression and energetic metabolism changes in the whiteleg shrimp (*Litopenaeus vannamei*) in response to short-term hypoxia. *Aquaculture Research*. 2019;50(4):994-1004. doi:10.1111/are.13960

51. Hu W, Zhi L, Zhuo MQ, et al. Purification and characterization of glucose 6-phosphate dehydrogenase (G6PD) from grass carp (*Ctenopharyngodon idella*) and inhibition effects of several metal ions on G6PD activity in vitro. *Fish Physiology and Biochemistry*. 2013;39(3):637-647. doi:10.1007/s10695-012-9726-x

52. Jin ES, Lee MH, Murphy RE, et al. Pentose phosphate pathway activity parallels lipogenesis but not antioxidant processes in rat liver. *American Journal of Physiology Endocrinology and Metabolism*. 2018;314(6):E543-E551. doi:10.1152/ajpendo.00342.2017

53. Ma JL, Qiang J, Tao YF, et al. Multi-omics analysis reveals the glycolipid metabolism response mechanism in the liver of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) under hypoxia stress. *BMC Genomics*. 2021;22(1):105. doi:10.1186/s12864-021-07410-x

54. Sun JL, Zhao LL, Wu H, et al. Acute hypoxia changes the mode of glucose and lipid utilization in the liver of the largemouth bass (*Micropterus salmoides*). *Science of the Total Environment*. 2020;713:135157. doi:10.1016/j.scitotenv.2019.135157

55. Huo D, Zhang L, Yang H, et al. Adaptation to hypoxic stress involves amino acid metabolism: a case in sea cucumber. *Environmental Pollution*. 2023;330:121766. doi:10.1016/j.envpol.2023.121766

56. Huang Z, Aweya JJ, Zhu C, et al. Modulation of Crustacean Innate Immune Response by Amino Acids and Their Metabolites: Inferences From Other Species. *Frontiers in Immunology*. 2020;11. doi:10.3389/fimmu.2020.574721

57. Nemkov T, Sun K, Reisz JA, et al. Hypoxia modulates the purine salvage pathway and decreases red blood cell and supernatant levels of hypoxanthine during refrigerated storage. *Haematologica*. 2018;103(2):361-372. doi:10.3324/haematol.2017.178608

58. Furuhashi M. New insights into purine metabolism in metabolic diseases: role of xanthine oxidoreductase activity. *American Journal of Physiology Endocrinology and Metabolism*. 2020;319(5):E827-E834. doi:10.1152/ajpendo.00378.2020

59. Kropf C, Fent K, Fischer S, et al. ABC transporters in gills of rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*. 2020;223(15). doi:10.1242/jeb.221069

60. Jiang H, Liu J, Huang Q, Yang D. Effects of Short-Term Temperature Stress on Metabolic and Digestive Enzymes Activities of *Procambarus clarkii*. *Israeli Journal of Aquaculture - Bamidgeh*. 2024;76(4):286-293. doi:10.46989/001c.126179