



Original research article

Temperature stress alters transcriptomic and physiological responses in hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*)

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ABSTRACT

The continuous intensification of climate change exposes hybrid grouper to fluctuating temperatures, affecting physiology, immunity, and overall performance. This study investigates the molecular, biochemical, and histological responses of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) exposed to one day (21–33 °C) and five days (22–31 °C) temperature fluctuations. Sub-adult fish (20.95 ± 0.64 cm) were grouped into acute (A), tolerant (R), and sensitive (S) categories based on behavioural responses such as feeding and swimming behaviours. Skin transcriptome profiling revealed that the DEGs were most significantly enriched in genetic information processing networks, including pathways involved in folding, sorting and degradation, translation, and transcription. Genes involved in protein processing in the endoplasmic reticulum (*skp1*, *ero1a*, *rpn2*) were significantly upregulated in A vs C and R vs C groups, while genes involved in energy metabolism were significantly upregulated in S vs C group. However, genes involved in the ribosome pathway (*rpl22*, *rps15*, *rpl9*, *rps21*, *rpl12*, *rpl19*) were significantly downregulated across all three comparison groups. Biochemical markers, including elevated alanine aminotransferase (ALT) and glucose (GLU) levels and reduced lactate dehydrogenase (LDH) activity indicate metabolic disturbances. Histopathological alterations included hepatocytic vacuolation, inflammatory infiltration in the liver, and melanomacrophage aggregation in the spleen and head kidney, indicating systemic stress and immune activation. This integrated analysis reveals significant molecular and physiological mechanisms underlying thermal stress responses in hybrid grouper. The identified genes, pathways, and biomarkers offer valuable insights for improving stress resilience and guiding management strategies in aquaculture under climate change.

1. Introduction

The urgency of climate change presents significant risks to fisheries and aquaculture, exacerbating pre-existing environmental challenges (FAO, 2022; Maulu et al., 2021). Extensive evidence showed the detrimental effects of climate change on biodiversity loss (Das et al., 2021).

Although the connection between climate-resilient species and their relevance to aquaculture may not be immediately apparent, it is crucial to identify such species. Among the various tropical fishes, groupers are particularly susceptible to temperature increases due to their adaptation to a relatively stable thermal environment (Das et al., 2021). Alterations in temperature have the potential to disrupt fish physiology, impacting

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crucial aspects such as growth rates, reproduction, inhibit spawning activity, and overall survival (Huang et al., 2021; Maulu et al., 2021; Das et al., 2021; Cheung & Frölicher, 2020). Studies indicate that tropical marine species exhibit greater sensitivity to water temperature and pH fluctuations when compared to their sub-tropical or temperate counterparts. Typically, tropical species inhabit environmental temperatures that already push the upper limits of their tolerance, making them highly vulnerable to temperature changes beyond this threshold, which can have severe consequences on their survival (Das et al., 2021). Research studies indicate that coral grouper is highly sensitive to modest increases in ocean temperature, leading to decreased survival, aerobic scope, and activity. Consequently, ongoing ocean warming poses a threat to coral grouper catchability and the long-term viability of reef-based fisheries, particularly in low latitude regions (Pratchett et al., 2017).

Fish skin is fundamental as a protective barrier, separating the internal environment from the external surroundings. It plays a crucial role in safeguarding against physical damage and maintaining the integrity of the body (Bai et al., 2020; Qin et al., 2020). Fish are capable of sensing the temperature of the surrounding water through their skin. When exposed to high temperature stress, it will increase in the secretion of skin mucus (Zhang et al., 2021). Skin transcriptome profiling has therefore been widely used to investigate environmental and physiological stress responses in fish, including temperature change in *Notothenia coriiceps* (Kim et al., 2019), acute hypoxia in *Pelteobagrus fulvidraco* (Wang et al., 2022), infection in *Larimichthys crocea* (Bai et al., 2022), and transport stress in *Oreochromis niloticus* (Heba et al., 2023), because transcriptional changes in skin reflect both barrier integrity and mucosal immune activity.

Temperature affects fish in terms of feeding, digestion, and metabolism, leading to slow growth and a decrease in appetite. Therefore, comprehending the impact of temperature on fish is essential in understanding their physical responses (Liu et al., 2022). Observing a shift to a darker body coloration is a common occurrence in stressed fish and serves a significant function in relation to thermoregulation (Wu et al., 2023). Fish will exhibit decreased ability to feed, diminished swimming vitality and decreased survival rates when the water temperature drops below the ideal range (Chen et al., 2023). Environmental stress can cause fish to undergo internal anatomical changes that can be adaptive or detrimental (Hernandez-Lopez et al., 2018). Temperature stress triggers a cascade of physiological and biochemical responses in fish, often resulting in structural and functional changes in critical organs (Aboka et al., 2017; Liu et al., 2022; Wang et al., 2019). The liver, a central metabolic organ, is particularly sensitive to environmental perturbations (Wang et al., 2019). Histopathological alterations such as hepatocellular vacuolation, and necrosis have been associated with exposure to suboptimal temperatures, as documented in various aquaculture species (He et al., 2023; Zhao et al., 2022). Immune organs such as the spleen and head kidney play vital roles in haematopoiesis and immune regulation (Sun et al., 2019a), similarly exhibit stress-induced changes, including melanomacrophage center (MMC) aggregation, inflammatory infiltration, and necrotic damage (Gao et al., 2022; Xu et al., 2018). These organ-level changes provide valuable insights into the physiological toll of thermal stress and serve as early indicators of compromised health in aquaculture species. In addition to histological assessments, biochemical markers offer critical insights into the metabolic and physiological state of stressed fish (Li et al., 2022).

Transcriptome analysis enhances our understanding of the pathways governing cell fate, development, and disease progression. It provides a rapid and effective means for genome surveying and identification of functional genes and molecular markers. RNA sequencing (RNA-Seq) is an evolving modern technology that utilizes next-generation sequencing (NGS) for transcriptome profiling (Sudhagar et al., 2018). Researchers have characterized the transcriptome changes in response to elevated water temperature in *Oncorhynchus tshawytscha* (Bowen et al., 2020) and *Salmo salar* (Beemelmans et al., 2021; Shi et al., 2019) and to cold stress

in a number of fish species including *Paralichthys olivaceus* (He et al., 2023) and *Epinephelus coioides* (Sun et al., 2019b). In a study conducted by Kim et al. (2019), the transcriptome analysis of the skin in *Notothenia coriiceps* revealed significant upregulation of the actin cytoskeleton regulation pathway in response to temperature changes. Conversely, metabolic pathways such as muscle contraction and choline regulation were found to be downregulated.

Hybrid groupers, while resilient to moderate environmental fluctuations, remain vulnerable to extreme temperature shifts, which can significantly impair their immune and metabolic functions (Fang et al., 2023; Faudzi et al., 2021). Despite their prominence in aquaculture, limited studies have explored the combined molecular, histological and biochemical responses of this species to thermal stress. Therefore, this study aims to comprehensively evaluate the molecular and physiological responses of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) to fluctuating water temperatures by integrating biochemical profiling, histological assessment of important immune organs (liver, spleen, and head kidney), and transcriptomic analysis of skin tissue. Specifically, it seeks to identify heritable temperature resilience traits through differential gene expression profiling between temperature tolerant and temperature sensitive phenotypes. By comparing the transcriptomic profiles of tolerant, sensitive, and acutely stressed individuals, we are able to identify genes and biological pathways closely associated with thermal adaptation and stress response. Combined with biochemical and histological findings, this integrative approach clarifies the major biological processes to elucidate the molecular mechanisms underlying metabolic regulation and cellular homeostasis during temperature fluctuation. The outcomes of this study are expected to provide valuable molecular indicators for the selective breeding of thermotolerant grouper varieties, inform aquaculture management strategies, and contribute to a broader understanding of thermal resilience mechanisms in marine fish.

2. Materials & methods

2.1. Ethical statement

All animal handling procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia (certificate no.: UPM/ACUC/AUP-R054/2022) prior to the initiation of experiments.

2.2. Fish management and maintenance

Healthy hybrid grouper with an average body weight of 158.3 ± 16.22 g and body length of 20.95 ± 0.64 cm were purchased from a local nursery in Sepang, Selangor, Malaysia. The randomly picked fishes were acclimatized for four weeks in a 1.5 tonne tank filled with seawater with recirculating aquaculture system equipped with physical and biological filtration systems, at the Hatchery Unit, Institute of Bioscience, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. Water temperature ranges from 24.4 to 27.8 °C, with a seawater salinity of 22–25 ppt and pH of 7.5–7.7. During the acclimatization period, the fishes were fed twice daily to apparent satiation with a commercial 5 mm pellet (Dindings 920). Total ammonia nitrogen was measured daily using API Test Kit (Mars Fishcare North America Inc., USA) and maintained at 0–1 ppm. Sponge in physical filters was washed twice a week, while 50% water exchanged carried out once a week.

2.3. Experimental design and sampling

After the acclimatization period, all hybrid grouper were colour tagged using cotton threads on their dorsal fins to facilitate individual identification. Then fish were conditioned into two separate 250 L glass aquariums (122 × 46 × 46 cm) with aeration for 48 h. Forty-five fishes were randomly divided into three glass aquariums for the control and

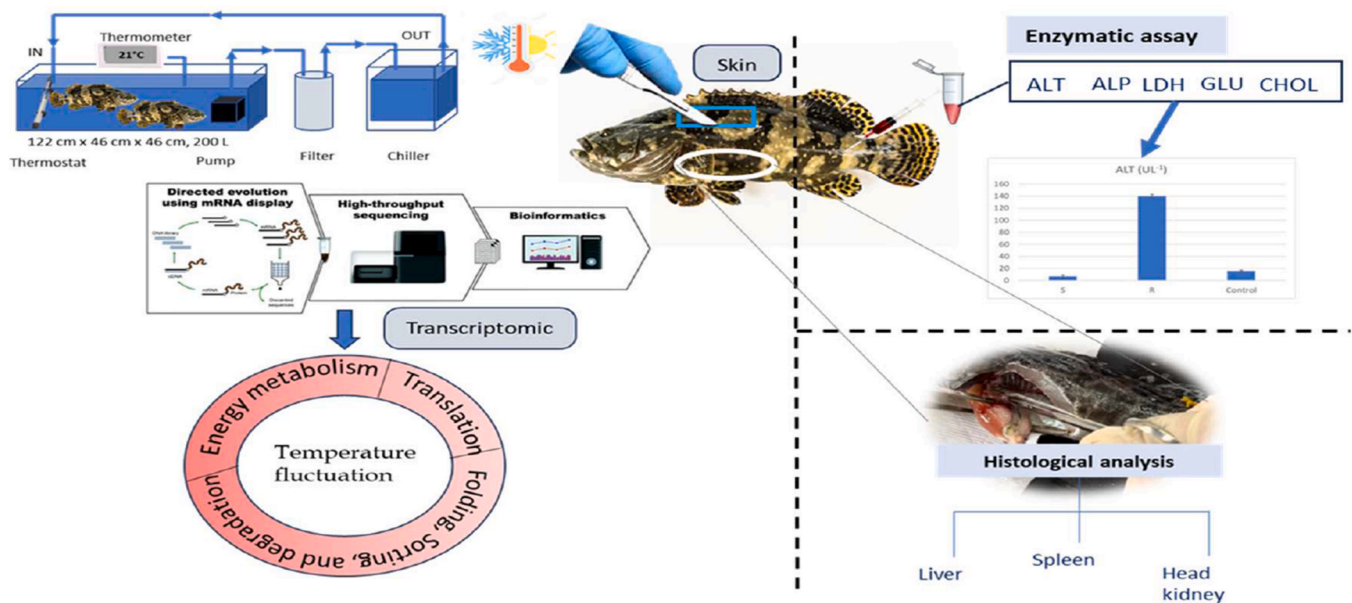


Fig. 1. Experimental workflow for assessing the effects of temperature stress on hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*). Fish were exposed to controlled acute and five days temperature fluctuations, followed by sampling of skin, blood, liver, spleen, and head kidney tissues. Skin samples were used for transcriptomic analysis to investigate gene expression changes associated with thermal stress. Blood plasma samples were analysed through enzymatic assays to assess metabolic and hepatic responses, while liver, spleen, and head kidney tissues underwent histological examination to evaluate tissue-level alterations.

treatment groups, respectively. Prior to the initiation of experiment, two experimental tanks were set up with a 150W thermostat (Aqual, Poland) and a chiller (Hailea HC-500A, China) with sponge filter. While, the fishes in control tank were maintained at ambient temperatures (24.6–27.7 °C). All fishes were fed twice daily to apparent satiation with a commercial 5 mm pellet (Dindings 920). Based on study done by local researcher in Malaysia (De et al., 2019; Faudzi et al., 2021), 21 and 33 °C were selected as the lowest and highest experimental stress temperature to obtain rich gene expression information and sufficient sample size. One batch of fish was exposed to acute temperature fluctuation, ambient water temperature (25.5 °C) was dropped to 21 °C using a chiller and ice packs, maintained for 1 h then increased to 33 °C, at a constant rate of 3 °C per hour similar to Kim et al. (2021) with slight modification. Samples of blood, skin, liver, spleen, and head kidney were collected after 1 h of exposure to acute temperature fluctuation.

Another batch of fish was exposed to five days high and low temperatures fluctuation through heating with thermostat and then follow by cooling with chiller. Water temperature was increased from 27 to 31 °C, set to peak at 1200 h, and decreased to 22 °C, set to peak at 0000 h. Similar to method described by Massey et al. (2022) with slight modification. Alteration of water temperature in the aquarium was at a constant rate of 1 °C per hour. This cycle was repeated for five consecutive days. The physical and behavioural changes were observed and recorded throughout the experimental period at 1100 h when water temperature at 31 °C, and at 2000, when water temperature at 22 °C.

On the sixth day, three fish were randomly sampled from the control tank. For the five days temperature fluctuation tank, colour tagged fish were individually categorized based on observable changes (Sharma, 2019) and interpreted into table as follows: absent ("−") and present ("+"). Each change was given a score indicated by "+", thus the accumulation of "+" will contribute to the numbers of the final score. Similar to behavioural stages described by Mujar et al. (2025) with modification. Fishes were then divided into two distinct groups, those with a total score greater than 4 were classified as "S" (sensitive to temperature fluctuation), while those with a score of 2 or below were classified as "R" (tolerant to temperature fluctuation).

2.4. Samples collection

Fish were fasted for 24 h prior to sampling. Fishes were anaesthetised with tricaine methanesulfonate (MS-222) at 100 mg/L to facilitate blood collection via caudal puncture, with added anticoagulant, and centrifuged at 15 000×g for 10 min at 4 °C to separate the plasma. Resulted plasma was then collected into Eppendorf vials and stored at −80 °C until analyse. The fishes were then euthanised with MS-222 at 200 mg/L and surgically dissected. Scales were first removed, then skin tissues were dissected from the dorsal region on one side of the fish. Skin samples were then wash with 1 × PBS + NaCl and froze in liquid nitrogen immediately while awaiting quantitative transcript analysis. Liver, spleen, and head kidney were collected and fixed in Bouin's solution for 20 h, then rinsed and kept in 70% alcohol until used for histological investigations. All collected samples were subsequently analysed as outlined in Fig. 1.

2.5. Transcriptomic analysis

2.5.1. RNA extraction, library construction, and sequencing

Total RNA was isolated from hybrid grouper skin samples preserved in liquid nitrogen. Triplicate samples of biological replicates were freshly collected from four groups of hybrid grouper: acute (A1, A2, and A3), tolerant (R1, R2, and R3), sensitive (S1, S2, and S3), and control (C1, C2, and C3). Total RNA was extracted using TRIzol reagent (Invitrogen) and treated with DNase on column using the RNeasy Plus Kit (Qiagen) to remove genomic DNA contamination. RNA integrity and degradation were assessed on 1% agarose gels, while RNA purity was evaluated using a NanoPhotometer spectrophotometer (IMPLEN, CA, USA). Messenger RNA was purified from total RNA using poly-T oligo-attached magnetic beads. The mRNA was then fragmented and used for first strand cDNA synthesis using random hexamer primers, followed by the second strand cDNA synthesis. In directional library preparation, dUTP was incorporated during second-strand synthesis, enabling strand-specific sequencing; in non-directional library preparation, dTTP was used. For the non-directional library construction, end repair, A-tailing, adapter ligation, size selection, PCR amplification, and purification were performed. For the directional library construction, USER enzyme

digestion was applied prior to PCR amplification. The libraries were quantified using a Qubit fluorometer and real-time PCR, and size distribution was checked using a Bioanalyzer. Quantified libraries were pooled based on effective concentration and target data yield, then clustered according to the manufacturer's instructions. Sequencing was performed on an Illumina platform to generate paired-end reads, and base calling was conducted using the CASAVA pipeline.

2.5.2. Sequence quality assessment, de novo assembly and functional DEGs annotation

Raw data (raw reads) of FASTQ format were firstly processed using FASTP to obtain high-quality clean reads, where adapter sequences, reads containing poly-N, and low-quality reads were removed. Low-quality reads containing adapter sequences, reads with more than 10% of unknown nucleotide (N), all low-quality nucleotides with more than 50% of the bases below 5 were removed. At the same time, Q20, Q30, GC-content and sequence duplication level of the clean data were calculated. All the downstream analyses were based on these high-quality clean reads. Reference genome and gene annotation files were downloaded from NCBI website (<https://www.ncbi.nlm.nih.gov>). Index of the reference genome was built, and paired-end clean reads were aligned to the reference genome using HISAT2 v2.0.5. The transcript of each sample was *De novo* assembled using StringTie v1.3.3b in a reference-based approach. The featureCounts v1.5.0-p3 was used to count the reads numbers mapped to each gene and the FPKM (Fragments Per Kilobase of transcript sequence per Million base pairs sequenced) of each gene was calculated to identify the gene expression levels.

2.5.3. Differential expressed genes and enrichment analysis

The differential expression analysis between groups was performed using the DESeq2 R package (version 1.20.0). DESeq2 applies statistical models based on the negative binomial distribution to determine differential gene expression from digital gene expression data. P-values were adjusted using the Benjamini-Hochberg approach to control the false discovery rate. Genes with an adjusted p-value (Padj) < 0.05 were considered significantly differentially expressed. Finally, the set of differentially expressed genes (DEGs) was subsequently used for Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis to explore functional differences between groups.

2.5.4. GO and KEGG enrichment analyses of DEG

All DEGs were subjected to the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses to better understand their functional relevance. The GO enrichment analysis of DEG was performed using the clusterProfiler R package, with correction for gene length bias. GO terms with an Padj < 0.05 were considered significantly enriched. Based on GO classification, DEGs were categorized into three major categories: biological processes (BP), cellular component (CC), and molecular functions (MF). Meanwhile, the KEGG database (<https://www.genome.jp/kegg/>) provides a resource to understand high-level biological system functions and interactions based on molecular datasets, particularly those generated by high-throughput technologies (Kanehisa et al., 2017). KEGG pathway enrichment analysis was conducted using the clusterProfiler R package, comparing DEGs against the whole genome background. The KEGG pathways with Padj < 0.05 were considered significantly enriched.

2.5.5. Integration analysis of highly significant pathways

Network analyses were conducted using Cytoscape (version 3.10.4) to visualize the interactions among genes associated with significantly enriched pathways, thereby elucidating the functional relationships of genes responsive to acute and prolonged temperature fluctuations.

Table 1

The primer sequence that used for verified genes were used for reverse transcriptase quantitative PCR (RT-qPCR).

Gene name	Primers	Sequence
NADH ubiquinone oxidoreductase subunit C2, <i>ndufc2</i>	F	GGTTACACACCATGCCCCCTTG
	R	GGACACCTGATTTTAGCGGC
ATP synthase peripheral stalk subunit d, <i>atp5pd</i>	F	CTGAAGACTCGCAGTGTATGC
	R	TGTGTGTCAGTAGGCTCAGG
ribosomal protein L12, <i>rpl12</i>	F	GGAGCGTGACCTTCGATGAG
	R	GACACTCGACTTTGCCACTG
ribosomal protein S15, <i>rps15</i>	F	ATGTGCGGTCACTACTTGGG
	R	TTGTGGCGAGACCAAGTTTTT
malic enzyme 3, <i>me3</i>	F	CACCAAGAAGAGGGGCTACG
	R	CAGAGGTCAGCAAGCGGTAA
endoplasmic reticulum oxidoreductase 1 alpha, <i>ero1a</i>	F	GCAGAGGTAAATGAGCAGCC
	R	TTGGGAATCAGAGGTCGCTT
ribosomal protein S21, <i>rps21</i>	F	CGGTGGGTATCTGTCCGAGC
	R	GCTGGCAGAGCATTTACGTG
peroxisomal biogenesis factor 14, <i>pex14</i>	F	CCAGTCAACCAACCAACAG
	R	TGGCTCACATCATCTCTCTC
hypoxanthine phosphoribosyltransferase 1, <i>hprt1</i>	F	CCACAACCATACAGCAGCAG
	R	TAGTTTTGCGGGCACTTCAC

2.6. Total RNA extraction and real time – quantitative PCR, RT-qPCR

Experiment was repeated and sampling was carried out following the same procedures mentioned above. RNA was isolated from skin samples using Monarch® Total RNA Miniprep Kit, New England Biolabs, USA. The RNA purity and concentration of samples were assessed using microvolume spectrophotometer (Infitek Co. Ltd., China). The cDNA was synthesized using SensiFAST cDNA Synthesis Kit (Bioline, UK) following protocol by manufacturer.

Quantitative PCR analysis was performed using 2x qPCRBIO SyGreen Mix Separate-ROX (PCR Biosystem, UK) following the manufacturer's protocol. The samples were run by using a Real – Time PCR Detection System X60B (Heal Force, China). No template control (NTC) was added also in each run. The threshold (CT) value was analysed and recorded through AriaMx Real-time PCR System (Agilent, USA). The primers used in RT-qPCR were listed in Table 1. The specificity of primer candidates generated by Primer3 and verified by Primer-BLAST on the NCBI website (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). The primers were evaluated using OligoAnalyzer (<https://www.idtdna.com/calculator/analyze>) to check the possibility of Self-Dimer and Hetero-Dimer formation. The primer pairs were efficiently determined by performing serial dilutions of reference cDNA and used to quantify cDNA concentration. The standard cycling parameters included an initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 20 s. Melting curve analysis was performed at the end of each PCR run. A melting curve profile was obtained by heating the mixture to 95 °C, cooling to 65 °C for each 5 s. Primer specificity was evaluated by determining single peak in all melting curves and single targeted band on 1.0% agarose gel electrophoresis of all RT-PCR amplicons indicating absence of primer-dimer and optimal performance of the primers. The mean CT value was used for gene expression analysis.

To validate the transcriptome data, five DEGs from five days temperature stress and six DEGs from acute temperature stress were randomly selected for RT – qPCR described in Table 1. By using three computational programs included geNorm (Vandesompele et al., 2002), NormFinder (Andersen et al., 2004) and BestKeeper (Pfaffl et al., 2004) for analysing stability of housekeeping genes, the most stable peroxisomal biogenesis factor 14, *pex14* was chosen as control gene among other housekeeping genes (*cog5*, *pex11a*, *arf1*, *hprt1*, *rpl8*, *β-actin*, and *GAPDH*) for the immune gene expression study of the hybrid grouper skin (Liang et al., 2022). Relative quantification was applied for determining expression of stress-tolerant and stress-sensitive in the skin of hybrid grouper. The relative expression levels of the genes were calculated by using the efficiency corrected method of Pfaffl method (Pfaffl,

Table 2

Observable changes in hybrid grouper exposed to temperature fluctuations for five days.

Fish sample	Darker body colour	Loss of appetite	Erratic swimming	Agitation	Solitary	Lethargic	Total score
T1	+	+	+	+	-	-	4
T2	-	-	-	-	+	+	2
T3	+	-	+	+	+	+	5
T4	-	-	-	-	-	-	0
T5	-	-	-	+	-	-	1
T6	+	+	+	+	+	+	6
T7	+	-	+	+	-	+	4
T8	+	+	+	+	+	+	6
T9	-	-	-	+	-	-	1
T10	-	+	-	+	-	-	2
T11	-	-	-	-	-	-	0
T12	-	+	-	-	+	-	2
T13	+	+	-	-	+	-	3
T14	+	+	+	+	+	+	6
T15	-	-	-	-	-	-	0

Symbol shows '-', no sign; '+' with sign.

2001). The data of five selected genes were compared and obtained from RT-qPCR and transcriptome sequencing to validate the reliability of transcriptome sequencing data.

2.7. Enzymatic assay

The assessment of blood plasma of control, acute (A), resistant (R), and sensitive (S) groups were conducted through enzymatic activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glucose (GLU), and cholesterol (CHOL) using the enzymatic assay kit (BioREX MANNHEIM Diagnostics, Germany) in a Biolis 24i Premium automatic biochemical analyser.

2.8. Histological analysis

Fixed organ samples, three individuals from each control, R, and S group were processed at the Histopathology Laboratory, Faculty of Veterinary Medicine, UPM. Liver, spleen, and head kidney were processed with one section from each organ, mounted on glass slides at 4 μm thick, and stained with haematoxylin and eosin (H&E). To investigate hemosiderin deposition in macrophages, liver samples were stained with Pearl's Prussian blue. Slides were observed and photographed with a Nikon Eclipse Ci microscope camera (Nikon, Japan) for histological examination followed by histomorphometry analysis using ImageJ

1.54d software (National Institute of Health, USA). The histological images were uploaded to ImageJ, and corresponding areas were calculated (μm^2) based on colour threshold. Necrosis areas (when observed) were manually outlined and measured (μm^2). Quantitative measurements of the histological changes were obtained from ten non-overlapping field views taken from each organ section of each individual.

2.9. Statistical analysis

All data were expressed as means with standard deviation (mean $\pm$ SD). Data were subjected to one-way ANOVA and further analysed using Tukey's Honest Significant Difference (HSD) post-hoc test, where the significance difference was set at 0.05 level ($P < 0.05$). All analyses were conducted by using SPSS software.

3. Results

3.1. Observable changes in fish

Mortality was not observed during the five-day experimental period. Throughout the experiment, the control fish exhibited normal behaviour and maintained their appetite. Fish exposed to five days temperature fluctuations were observed at 1100 h after the water temperature had

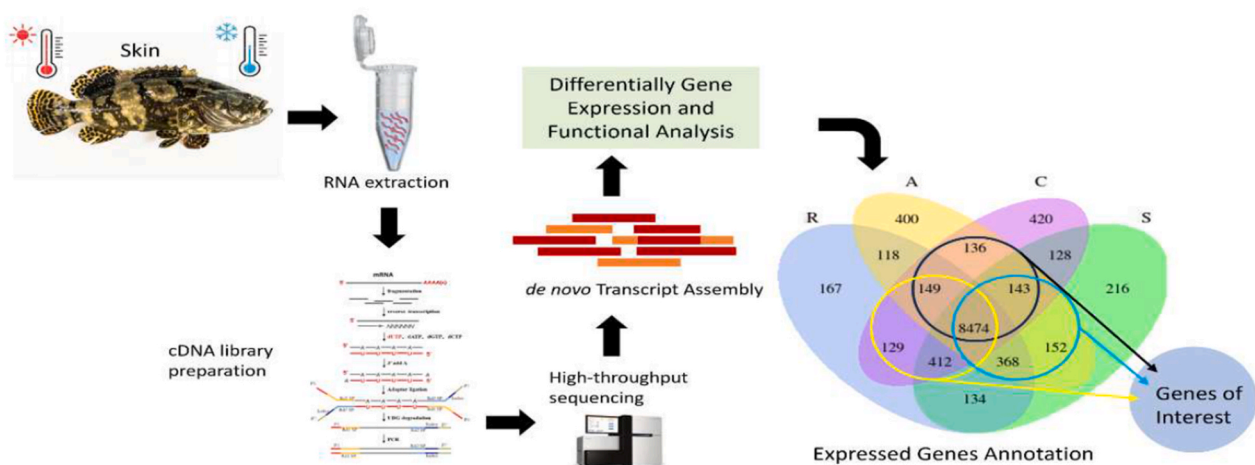


Fig. 2. RNA-sequencing workflow of hybrid grouper skin after exposed to temperature fluctuations. The flow diagram shows transcriptomic analysis begins with the extraction of RNA from the skin tissue of hybrid grouper construction of cDNA libraries followed by the use of high-throughput sequencing technology that produce short fragmented reads. Fragments were then aligned and assembled through a *de novo* assembly of a reference transcriptome or based on homology to the reference genome of a closely related species. Differential expression of transcripts between samples of interest was computed and functionally analysed to elucidate gene interactions and potential implications.

Table 3

Overview of the sequenced data of hybrid grouper skin exposed to temperature fluctuation.

Sample	Raw reads	Clean reads	Clean bases (G)	Q20 %	Q30 %	GC %	Error %	Total mapped rate (%)
C1	161233262	159213728	23.88	97.49	93.44	45.75	0.03	86.02
C2	85192928	84415356	12.66	97.66	93.64	46.31	0.03	88.53
C3	82994728	81974218	12.3	97.21	93.05	44.56	0.03	82.61
A1	97229110	96539862	14.48	98.03	94.37	50.04	0.02	92.75
A2	83480128	82869058	12.43	98.1	94.5	49.8	0.02	93.29
A3	99287306	98567028	14.79	97.86	93.98	49.75	0.03	93.22
R1	112412090	111538314	16.73	97.92	94.16	49.19	0.03	92.0
R2	100409686	99512226	14.93	97.98	94.34	49.9	0.02	92.51
R3	101989016	101245630	15.19	98.08	94.46	50.33	0.02	93.98
S1	86469312	85651336	12.85	97.59	93.53	47.1	0.03	90.12
S2	90900072	90140538	13.52	98.15	94.61	50	0.02	93.73
S3	99654918	98881152	14.83	98	94.34	49.63	0.02	92.9

C: control group, A: acute group, R: tolerant group, S: sensitive group.

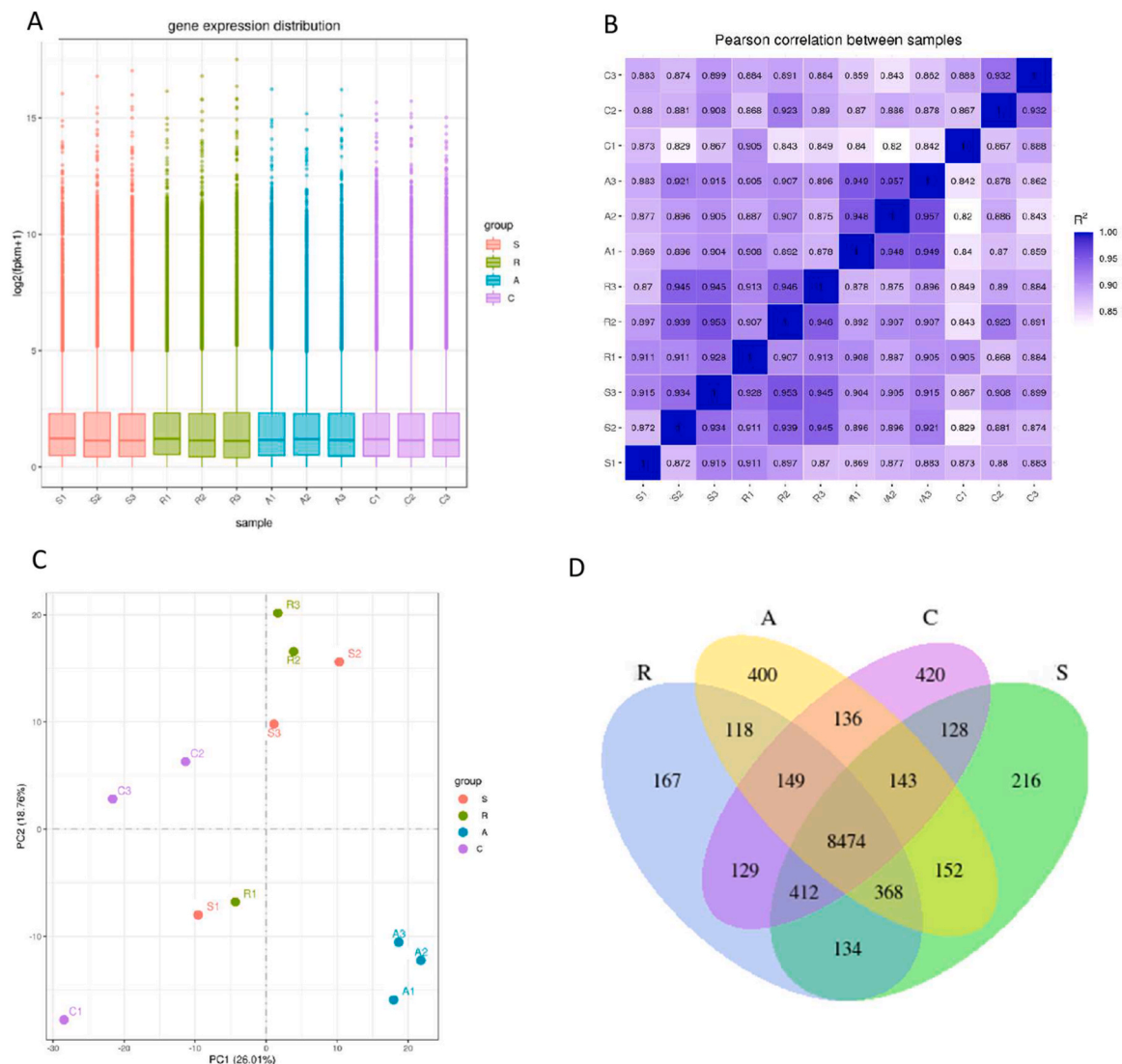


Fig. 3. Sample gene expression analysis of the skin tissues of hybrid grouper exposed to temperature fluctuation. (A) Parameters of box plot were indicated, including maximum, upper quartile, mid-value, lower quartile and minimum; (B) Correlation heatmap summarizes expressed genes identified from different groups. R^2 = square of the Pearson correlation coefficient, the correlation coefficient was calculated based on the FPKM value of all genes in each sample; (C) Principal component analysis (PCA) shows significant separation in the skin samples obtained from different groups; (D) Venn diagram shows the number of expressed genes in different groups.

risen to 31 °C. Adverse effects included reduced swimming activity, settling at the bottom of the tank, darker body coloration, solitary behaviour, lethargy, and loss of appetite in some fish during feeding.

However, symptoms of lethargy and solitary behaviour were observed after two days. Observations conducted at 2000 h, after the water temperature decreased to 22 °C, fish showed darker body coloration,

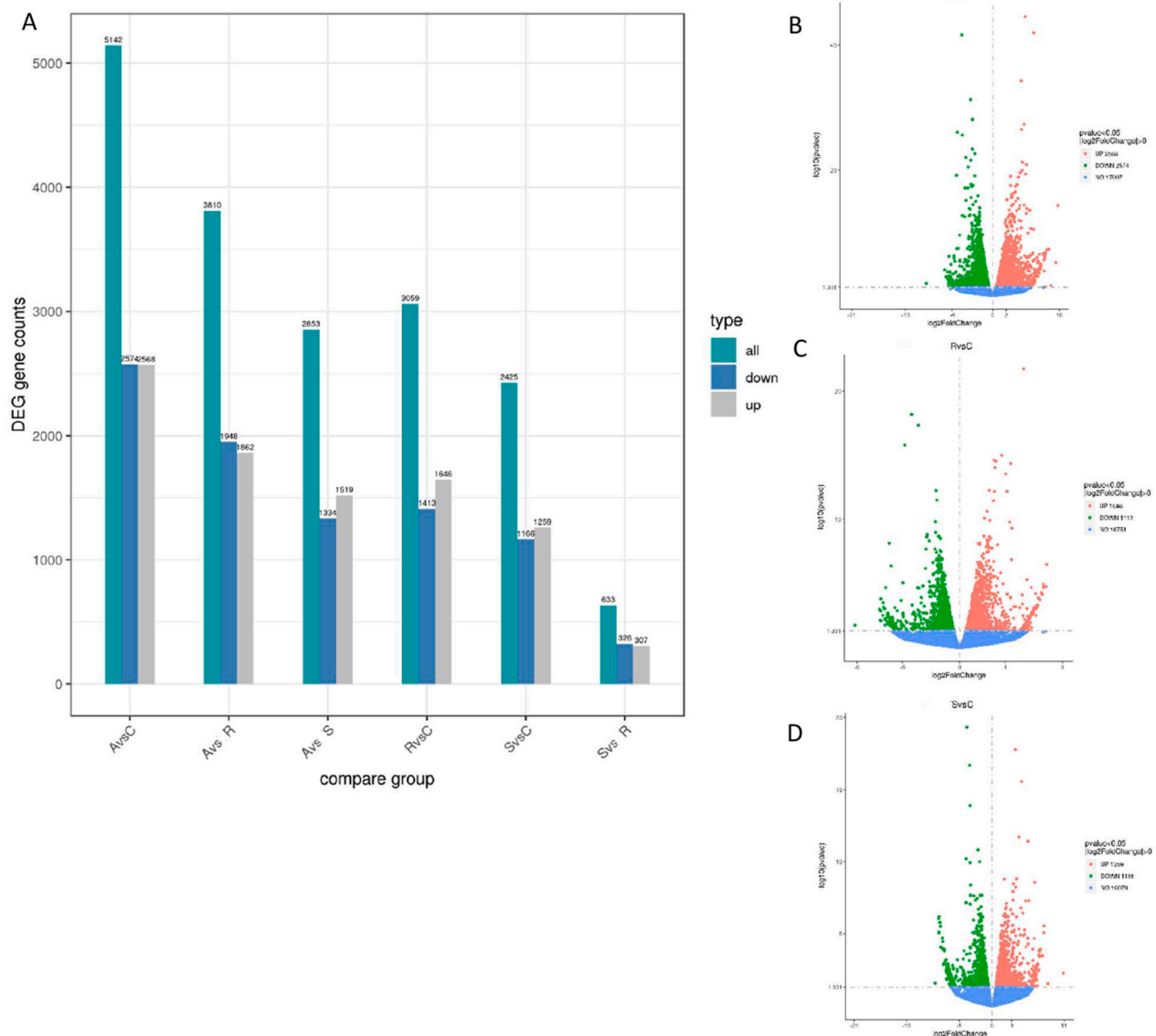


Fig. 4. Comparison of differentially expressed genes (DEGs) in the skin tissue of hybrid grouper subjected to thermal fluctuation. Bar graph (A) and volcano plot for group A vs C (B); R vs C (C); and S vs C (D) showing upregulated and downregulated genes in different group. Red dots indicate up-regulated genes, green dots indicate down-regulated genes, and blue dots indicate genes that are not significantly different ($P < 0.05$).

lack of appetite and increased agitation. Their mobility decreased progressively, and eventually huddled in groups, swimming vertically and erratically while rubbing against each other.

Six observable behavioural changes were identified: darker body coloration, loss of appetite, erratic swimming, agitation, solitary behaviour, and lethargy. The total scores for all observable changes in hybrid groupers exposed to temperature fluctuations are presented in Table 2. In the control fish, the score was consistently “0,” as no observable changes were detected. Fish with a total score of 4 or higher were categorized as “S”, while those with a score of 2 or lower were categorized as “R”.

Hybrid groupers exposed to acute temperature fluctuations were categorized into a single acute group. These fish exhibited agitation, group swimming with body rubbing, and darker body coloration when the temperature dropped to 21 °C. When the temperature increased to 33 °C, the fish returned to a calmer state, staying at the bottom of the tank.

3.2. De novo transcriptome assembly and annotation

To identify genes that are differentially expressed under acute and five days temperature fluctuations, transcriptomes were generated from skin samples of hybrid grouper which group into the Control (C) group, acute (A), and tolerant (R) group, and sensitive (S) group, as indicated in Fig. 2. In total, 329 420 918, 279 996 544, 314 810 792, and 277 024 302 raw reads were obtained from C, A, R, and S respectively. A total of 325 603 302, 277 975 948, 312 296 170, and 274 673 023 clean reads were generated after removing reads containing adapters or read of low quality respectively (Table 3). Overview of quality control is shown in Table 3: Q20 was 97.21%–98.15%, Q30 was 93.05%–94.61%, GC was 44.56%–50.33%, and error rate was 0.02%–0.03%.

3.3. Gene expression level analysis

The distribution of gene expression levels based on FPKM values across different samples was visualized using boxplot to compare gene expression patterns under thermal fluctuation (Fig. 3A). Pearson's

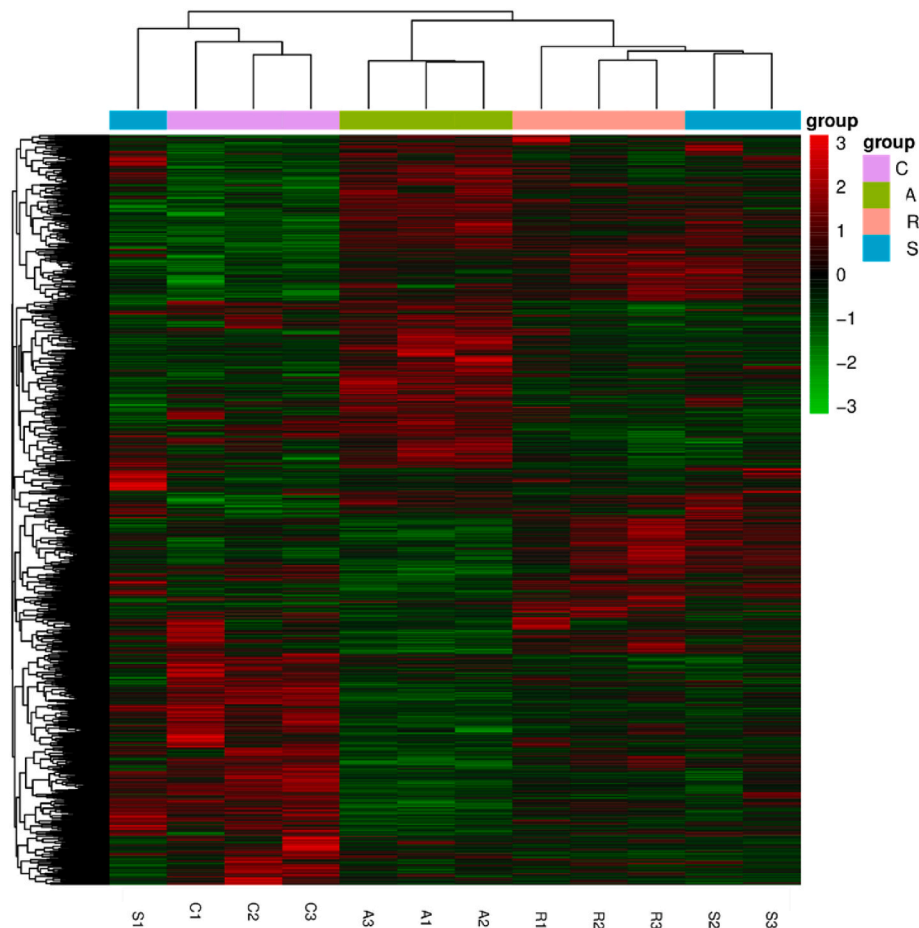


Fig. 5. Cluster heatmap of differentially expressed genes (DEGs) for C1, C2, C3, A1, A2, A3, R1, R2, R3, S1, S2, and S3. Heatmap showing the expression patterns of DEGs in different samples. Red colour represents relatively high expression level, while green represents a relatively low expression level. The DEGs are shown in rows, while the samples are in columns.

correlations coefficients (R^2) among skin sample replicates were 0.867–0.932 for C1, C2, and C3; 0.948–0.957 for A1, A2, and A3; 0.907–0.946 for R1, R2, and R3; and 0.872–0.934 for S1, S2, and S3 (Fig. 3B), indicating good reproducibility among biological replicates. A strong correlation ($R^2 > 0.82$) in gene expression patterns was observed between different groups, suggesting high similarity in the overall transcriptomic profiles. Principal component analysis (PCA) showed that groups A, R, and S were clearly separated from group C along PC1. Group C showed a positive correlation with groups R and S, but an inverse correlation with group A. Although R1 and S1 were dispersed from their respective clusters, they still aligned along PC1, and clustering analysis confirmed that their Pearson correlation coefficients ($R^2 > 0.907$ for R1 and > 0.872 for S1) were acceptable for further analysis (Fig. 3C). A total of 11 546 uniquely expressed genes were identified across the C, A, R, and S groups in skin tissue, as shown in the Venn diagram, with overlapping region representing genes that were co-expressed (Fig. 3D).

3.4. Differential expression analysis

Gene expression levels were quantified using FPKM values, and differential expression analysis was conducted for the comparisons A vs C, R vs C, and S vs C. A total of 10 626 significantly differently expressed genes (DEGs) ($P < 0.05$) were identified from skin tissue across the comparisons of group A vs C, R vs C, and S vs C (Fig. 4A). It was found that 5142 significant DEGs ($P < 0.05$) consists of 2568 upregulated genes and 2574 downregulated genes were identified in the skin tissue of A vs C (Fig. 4B). There were 3059 significant DEGs ($P < 0.05$) in the R

vs C group comparison, consists of 1646 upregulated genes and 1413 downregulated genes (Fig. 4C). A total of 2425 significant DEGs ($P < 0.05$) in the S vs C group, consists of 1259 upregulated genes and 1166 downregulated genes (Fig. 4D). Heatmap was constructed to visualize the expression patterns of DEGs across the four treatment groups (Fig. 5), revealing distinct gene expression profiles associated with each condition.

3.5. GO and KEGG enrichment analysis of DEGs

The DEGs were categorized into three main Gene Ontology (GO) classifications: biological processes (BP), molecular functions (MF), and cellular components (CC). The top 5 most significant enriched functional groups ($P_{adj} < 0.05$) for each comparison (A, R, and S vs C) were listed in Table 4. The significant GO terms ($P_{adj} < 0.05$) in BP, MF, and CC categories, along with their associated DEGs were illustrated in Fig. 6. In the A vs C comparison, the most significant enriched category in CC was ribosome. For the R vs C group comparison, the most significant enriched category in MF was oxidoreductase activity. In the S vs C group comparison, the most significant enriched CC category was cytoplasmic part.

KEGG pathway analysis was conducted to identify functional interactions among the DEGs (Fig. 7). The most significantly enriched pathways ($P_{adj} < 0.05$) for each group were listed in Table 5. In the A vs C group comparison, 4 KEGG pathways were significantly enriched ($P_{adj} < 0.05$), with “ribosome” identified as the most enriched downregulated pathway (59 downregulated DEGs), while “protein processing in endoplasmic reticulum” was the most enriched upregulated pathway (62

Table 4

The top 5 significantly enriched GO analysis in skin tissues of hybrid grouper exposed to temperature fluctuation ($P_{adj} < 0.05$).

Groups	GOID	Description	Term type	P_{adj}
A vs C	GO:0005840	Ribosome	Cellular component	2.00E-09
	GO:0044444	Cytoplasmic part	Cellular component	9.80E-09
	GO:0003735	Structural constituent of ribosome	Molecular function	1.16E-07
	GO:0005198	Structural molecule activity	Molecular function	1.89E-07
	GO:1990904	Ribonucleoprotein complex	Cellular component	5.02E-07
R vs C	GO:0016491	Oxidoreductase activity	Molecular function	6.61E-08
	GO:0044444	Cytoplasmic part	Cellular component	7.19E-07
	GO:0005737	Cytoplasm	Cellular component	4.55E-06
	GO:0055114	Oxidation-reduction process	Biological process	3.45E-06
	GO:0031090	Organelle membrane	Cellular component	0.000597
S vs C	GO:0044444	Cytoplasmic part	Cellular component	4.89E-05
	GO:0005737	Cytoplasm	Cellular component	0.000116
	GO:0006754	ATP biosynthetic process	Biological process	0.00069
	GO:0009142	Nucleoside triphosphate biosynthetic process	Biological process	0.00069
	GO:0009145	Purine nucleoside triphosphate biosynthetic process	Biological process	0.00069

upregulated DEGs). Under five days temperature fluctuation, the R vs C and S vs C group comparisons showed 16 and 13 significantly enriched pathways, respectively. Oxidative phosphorylation pathway was the most significantly upregulated pathways in both R (41 upregulated DEGs) and S (39 upregulated DEGs). Meanwhile, ribosome (29 and 30 downregulated DEGs in R and S groups, respectively) and spliceosome (32 and 26 downregulated DEGs in R and S groups, respectively) were the most enriched downregulated pathways. Based on Orthology (KO) annotations, the significantly enriched pathways were further classified into six major functional networks: metabolism, genetic information processing, environmental information process, cellular processes, organismal systems, and human diseases (Fig. 8).

3.6. Integrated analysis of significantly enriched pathways

Following the functional enrichment analysis, network visualization was performed to further explore the connectivity among genes involved in the most significantly enriched pathways in KEGG. The resulting network graph illustrates the gene-gene interaction architecture across the A vs C, R vs C, and S vs C groups, revealing distinct functional clusters associated with metabolic and cellular responses induced by thermal stress. The detailed gene interaction networks are presented in Fig. 9.

3.7. Validation of RNA-seq by RT-qPCR

To verify the transcriptome sequencing data, five DEGs from five days temperature stress and six DEGs from acute temperature stress involved in metabolism, immune system, and apoptosis were screened for RT-qPCR. Generally, the expression trends of the five DEGs from five days temperature stress and six DEGs from acute temperature stress identified by RT-qPCR were consistent with the expression profiles detected by Illumina RNA-Seq analysis (Fig. 10). These findings indicate that RNA-Seq data were of high quality and yielded reliable gene

expression profiles, supporting the identification of molecular mechanisms involved in the hybrid grouper's response to temperature stress.

3.8. Enzymatic assay

There were no significant differences ($P > 0.05$) in the levels of alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glucose (GLU), or cholesterol (CHOL) among the acute (A), R, and S groups of hybrid groupers after five days of exposure to temperature fluctuations. These findings suggest that short-term or five-day exposure to temperature fluctuations does not significantly ($P > 0.05$) affect blood ALT, ALP, LDH, glucose, or cholesterol levels in hybrid groupers (Fig. 11). Nevertheless, the blood plasma GLU and ALT enzyme levels were comparatively higher in the A group than in the R, control, and S groups. Conversely, ALT, LDH, and GLU levels were comparatively lower in the R group compared to those in the control, S, and A groups. Meanwhile, blood plasma ALP levels were higher in the R group and lowest in the S group.

3.9. Histological examination

3.9.1. Liver

Histological examinations of liver specimens from hybrid groupers exposed to temperature fluctuations revealed varying degrees of histopathological alterations. Fish categorized under the S group exhibited focal areas of inflammatory cell infiltration in hepatic tissue, a phenomenon less frequently observed in the acute (A) and R groups. Notably, the hepatic tissue characteristics in all three experimental groups (A, S, and R) differed significantly from those in the control group ($P < 0.05$, Table 6). The liver specimens from the R group exhibited significantly higher ($P < 0.05$) levels of hepatocytic vacuolation and degenerative vacuolization compared to the control, A, and S groups. Additionally, small foci of golden-brown granular materials, indicative of hemosiderosis, were observed in the R and S groups. Hemosiderosis was significantly more pronounced ($P < 0.05$) in the R and S groups compared to the control group. The presence of hemosiderin was confirmed by Prussian blue staining, which produced a blue coloured cytoplasmic appearance. Hepatopancreatic detachment was also observed in both the R and S groups, characterized by the separation of the hepatopancreas from the surrounding connective tissue. Representative histological features of these alterations are shown in Fig. 12.

3.9.2. Spleen

Histopathological changes were minimal in the spleen of hybrid groupers following exposure to five days temperature fluctuations. However, the accumulation of melanomacrophage aggregates in the splenic parenchyma and infiltrating inflammatory cells was observed (Fig. 13). The total count of melanomacrophage centers (MMC) in the splenic parenchyma of the R and S groups was significantly higher ($P < 0.05$) compared to the control and A groups. No significant difference ($P > 0.05$) was observed between the R and S groups (Table 7). In contrast, hybrid groupers exposed to acute temperature fluctuations exhibited notable histopathological changes in the spleen, with significantly higher ($P < 0.05$) levels of necrosis observed in the A group compared to the control, R, and S groups.

3.9.3. Head kidney

Histological examination revealed infiltration of inflammatory cells and aggregation of melanomacrophage centers (MMC) in the hematopoietic tissue of the head kidney in hybrid groupers exposed to temperature fluctuations. Moderate multifocal areas of necrosis and MMC aggregation were observed in the S group, less prominently in the R and A groups, and were barely visible in the control group (Fig. 14). However, MMC aggregation in the hematopoietic tissue showed no significant differences ($P > 0.05$) between the groups (Table 8).

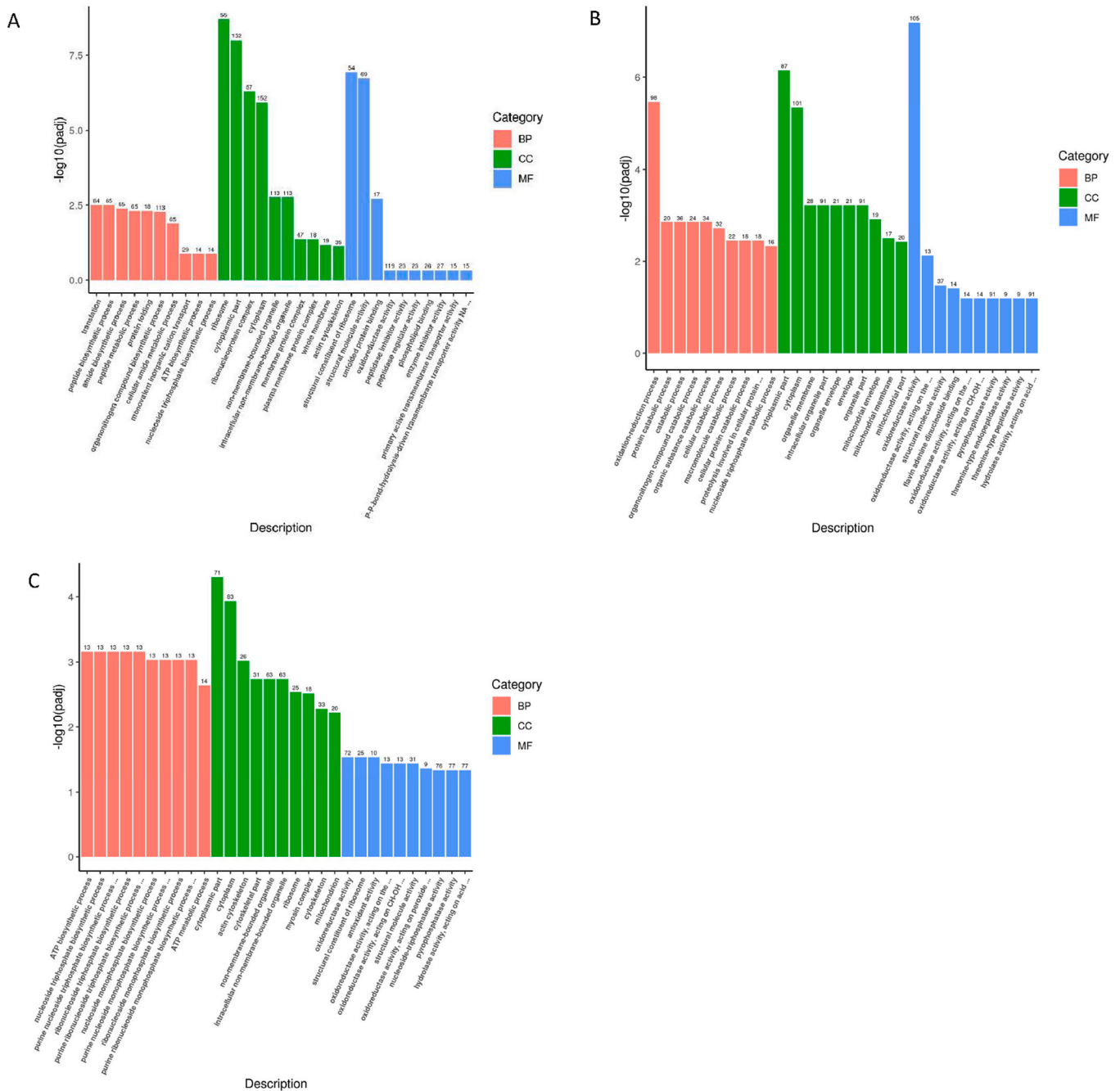


Fig. 6. Gene ontology (GO) enrichment analysis of DEGs for hybrid grouper subjected to temperature fluctuation. The results of GO enrichment analysis of DEGs were classified into three categories: biological process (BP), cellular component (CC), and molecular function (MF). The x-axis is gene functional classification of GO; y-axis is the corresponding number of DEGs. (A) GO pathway analysis of DEGs in the A vs C group; (B) GO pathway analysis of DEGs in the R vs C group; (C) GO pathway analysis of DEGs in the S vs C group.

4. Discussion

4.1. The effect of temperature fluctuation on hybrid grouper

Temperature is a critical environmental factor that significantly affects the physiology, metabolism, and growth of poikilothermic fish (Huang et al., 2022). In the face of climate-induced temperature fluctuations, species such as the hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) when exposed to considerable stress, affecting critical organs involved in metabolism and immunity (Thalib et al., 2021). While various studies have explored thermal stress responses in other aquatic species, such as *Procambarus clarkii*, *Oncorhynchus mykiss*, *Lota*

lota, and *Cynoscion nebulosus* (Li et al., 2022; Luo et al., 2022; Song & McDowell, 2021; Yang et al., 2021), the molecular mechanisms underlying temperature tolerance in hybrid grouper remain unclear. Here, we used skin transcriptome analysis to identify gene expression patterns and regulatory pathways associated with thermal stress, supported by physiological and histological findings that highlight the species' adaptive limits.

4.2. Behavioural and physiological responses to temperature fluctuation

The exposure of different aquatic organisms to temperature fluctuation results in various adverse biochemical and physiological effects

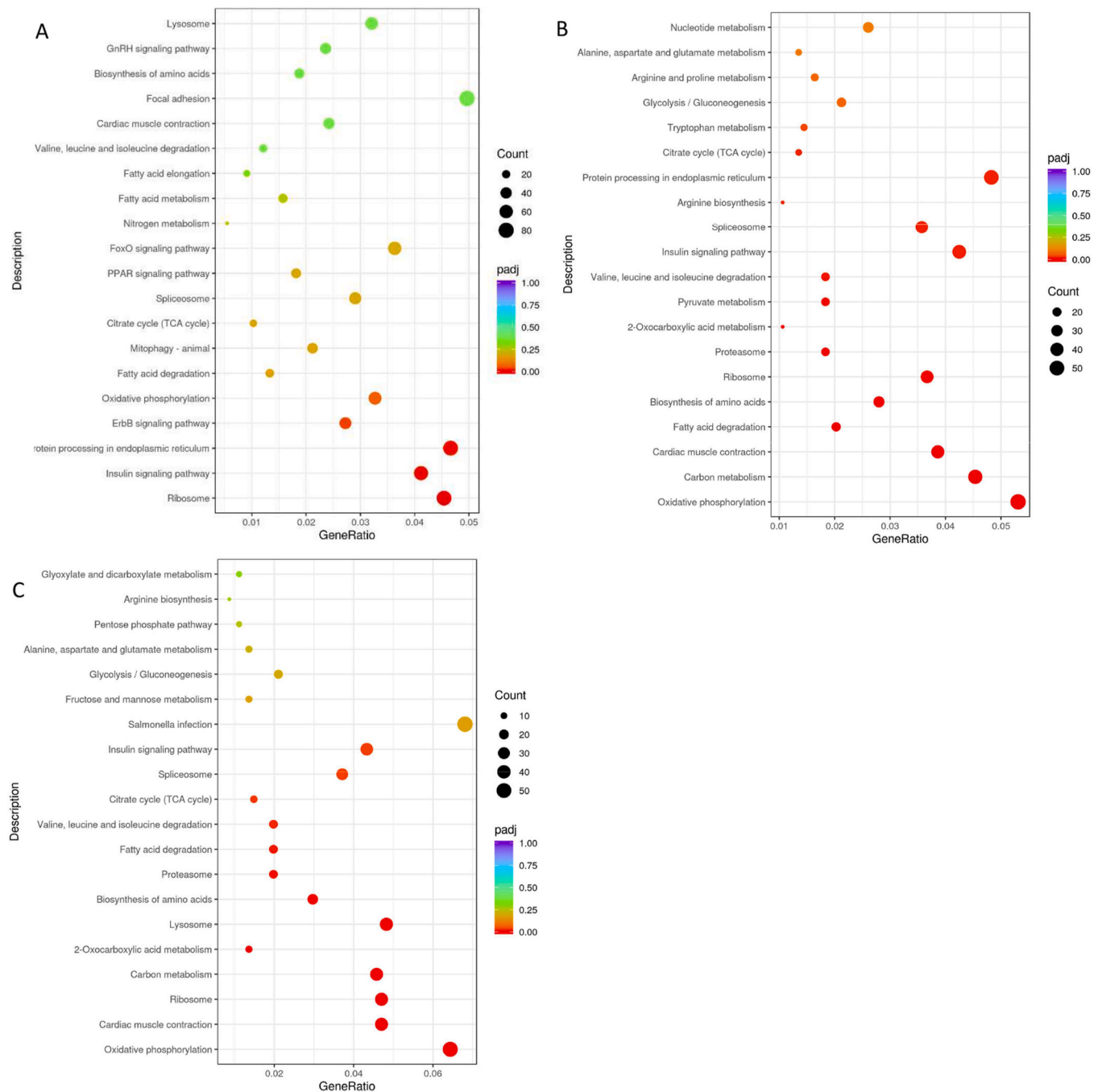


Fig. 7. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment scatter plot of DEGs in the skin tissue of hybrid grouper. (A) Top 20 enriched KEGG pathway of DEGs in the A vs C group; (B) Top 20 enriched KEGG pathway of DEGs in the R vs C group; (C) Top 20 enriched KEGG pathway of DEGs in the A vs C group. The x-axis is the ratio of the number of DEGs on the KEGG pathway to the total number of DEGs, and y-axis is the KEGG pathway. The size of a point represents the number of genes annotated to a specific KEGG pathway. The colour from red to purple represents the significant level of the enrichment ($P_{adj} < 0.05$).

depending upon its range and length of exposure time (Thalib et al., 2021). In the present study, the impact of temperature from 21 to 33 °C for acute stress and 22–31 °C for five days stress was investigated. Optimum temperature range of hybrid grouper is between 26 and 30 °C whereby, temperature below and beyond this range previously reported to have adverse effect on the growth of hybrid grouper, TGGG (Shapawi, Abdullah, Senoo, & Mustafa, 2019). Therefore, the selected temperature was set to evaluate the molecular and physiological responses of hybrid grouper to temperature fluctuations. Temperature stress tolerant fish, R showed no behavioural changes compared to sensitive, S group that showed symptoms of lethargy after five days. Our observations are

consistent with the findings of Mujar et al. (2025), where susceptible hybrid groupers exhibited pronounced behavioural responses when exposed to temperature fluctuations. In the present study, organ studied were skin, liver, spleen, head kidney, and blood plasma. These are the main target organs of temperature stress (Kulczykowska, 2019; Wang et al., 2019; Xu et al., 2018; De et al., 2019). Our results demonstrated that liver and spleen exhibited significant histopathological changes in response to five days temperature fluctuations. Reports by Zhang et al. (2022) also showed that serious tissues damage in the liver of sensitive *Pampus argenteus* and less seen in the resistant group under extreme cold stress. While, acute stress resulted in pronounced necrosis in spleen,

Table 5The significantly enriched KEGG pathway analysis in skin tissues of hybrid grouper exposed to temperature fluctuation ($P_{adj} < 0.05$).

Groups	Pathway term	KEGGID	KEGG Orthology	P_{adj}	Gene count	Up/Down
A vs C	Ribosome	efo03010	Translation	4.07E-19	59	Down
	Protein processing in endoplasmic reticulum	efo04141	Folding, sorting and degradation	1.19E-07	62	Up
	Spliceosome	efo03040	Transcription	6.46E-07	41	Down
	Oxidative phosphorylation	efo00190	Energy metabolism	0.000187	42	Up
	mRNA surveillance pathway	efo03015	Translation	0.001074	22	Down
	Citrate cycle (TCA cycle)	efo00020	Carbohydrate metabolism	0.001157	16	Up
	TGF-beta signalling pathway	efo04350	Signal transduction	0.002836	33	Up
	Fatty acid degradation	efo00071	Lipid metabolism	0.013852	17	Up
	ErbB signalling pathway	efo04012	Signal transduction	0.020746	26	Down
	Insulin signalling pathway	efo04910	Endocrine system	0.016129	36	Down
	Lysosome	efo04142	Transport and catabolism	0.034985	38	Up
R vs C	Oxidative phosphorylation	efo00190	Energy metabolism	6.86E-09	41	Up
	Spliceosome	efo03040	Transcription	1.20E-07	32	Down
	Ribosome	efo03010	Translation	2.04E-06	29	Down
	Proteasome	efo03050	Folding, sorting and degradation	5.47E-06	19	Up
	Carbon metabolism	efo01200	Glycolysis - core module involving three-carbon compounds	5.91E-06	34	Up
	Cardiac muscle contraction	efo04260	Circulatory system	1.41E-05	30	Up
	Fatty acid degradation	efo00071	Lipid metabolism	8.66E-05	17	Up
	Insulin signalling pathway	efo04910	Endocrine system	0.00029	34	Up
	Citrate cycle (TCA cycle)	efo00020	Carbohydrate metabolism	0.000389	11	Up
	Protein processing in endoplasmic reticulum	efo04141	Folding, sorting and degradation	0.000478	34	Up
	Valine, leucine and isoleucine degradation	efo00280	Amino acid metabolism	0.00048	16	Up
	2-Oxocarboxylic acid metabolism	efo01210	Carbohydrate metabolism	0.000632	8	Up
	Fatty acid metabolism	efo01212	Lipid metabolism	0.000681	16	Up
	Biosynthesis of amino acids	efo01230	Proline biosynthesis, glutamate (proline)	0.000848	21	Up
	Pyruvate metabolism	efo00620	Carbohydrate metabolism	0.01132	13	Up
	PPAR signalling pathway	efo03320	Endocrine system	0.022676	16	Up
S vs C	Oxidative phosphorylation	efo00190	Energy metabolism	8.84E-11	39	Up
	Ribosome	efo03010	Translation	1.86E-09	30	Down
	Cardiac muscle contraction	efo04260	Circulatory system	1.01E-06	28	Up
	Spliceosome	efo03040	Transcription	1.10E-06	26	Down
	Proteasome	efo03050	Folding, sorting and degradation	2.48E-05	16	Up
	Carbon metabolism	efo01200	Glycolysis - core module involving three-carbon compounds	3.12E-05	28	Up
	Lysosome	efo04142	Transport and catabolism	0.000228	29	Up
	2-Oxocarboxylic acid metabolism	efo01210	Carbohydrate metabolism – Pyruvate oxidation, Citrate cycle; Amino acid metabolism - Lysine degradation	0.000353	9	Up
	Biosynthesis of amino acids	efo01230	Proline biosynthesis, glutamate (proline)	0.000353	19	Up
	Fatty acid degradation	efo00071	Lipid metabolism	0.000353	14	Up
	Valine, leucine and isoleucine degradation	efo00280	Amino acid metabolism	0.000474	14	Up
	Citrate cycle (TCA cycle)	efo00020	Carbohydrate metabolism	0.000709	11	Up
	Regulation of actin cytoskeleton	efo04810	Cell motility	0.036132	29	Down

suggesting a threshold effect where rapid temperature changes elicit severe structural damage. The enzymatic assay results provide critical insights into the physiological responses of hybrid groupers to acute and five days temperature fluctuations. ALT activity, a biomarker for hepatic damage and metabolic stress, displayed elevated levels in the acute group compared to the control, R, and S groups. The lack of statistical significance may result from the relatively short duration of temperature exposure or compensatory mechanisms during acute stress adaptation. Elevated ALT levels in the acute group likely reflect transient hepatocellular damage as the liver responds to sudden metabolic demands induced by temperature fluctuations. Such elevations are consistent with findings in other teleost species, where thermal stress induces gluconeogenesis, mobilizing amino acids like alanine to maintain glucose homeostasis (Han, Meng, et al., 2022). The comparative increase in GLU levels observed in the acute group aligns with an adaptive response to thermal stress. Hyperglycaemia during acute stress reflects heightened metabolic activity to supply immediate energy requirements (Islam et al., 2022). Conversely, the relatively lower GLU levels in the R group suggest metabolic stabilization or efficient energy utilization. During the initial stages of fasting, glycogenolysis plays a crucial role in maintaining blood glucose homeostasis by releasing stored glucose from liver glycogen into the bloodstream (Dai et al., 2024). These variations highlight the differential coping mechanisms among the experimental

groups, emphasizing the importance of evaluating energy metabolism under stress conditions (Dai et al., 2024; Thalib et al., 2021). LDH activity, an indicator of anaerobic glycolysis, showed no significant differences across groups but was lower in the R group compared to the control and S groups. Reduced LDH activity in the R group may indicate a lesser reliance on anaerobic metabolism, potentially reflecting more effective oxygen utilization or reduced hypoxic stress (Reid et al., 2022). This observation aligns with studies demonstrating that well-adapted fish exhibit stabilized LDH activity under thermal stress (Fang et al., 2023). Interestingly, ALP levels were highest in the R group and lowest in the S group. This pattern suggests potential differences in phosphate metabolism or osmoregulatory demands under five days temperature stress. Higher ALP activity suggesting heightened immune function in tolerant group (Li et al., 2022). This observation is consistent with a previous study on *Thunnus albacares*, which reported an initial decrease in ALP activity under high temperature exposure, followed by a subsequent increase with prolonged stress duration (Liu, Fu, et al., 2022). Similarly, CHOL levels, which remained consistent across groups, indicate that lipid mobilization and energy storage were not significantly impacted during the experimental period (Liu, Fu, et al., 2022). However, the observed trends warrant further investigation over prolonged exposure durations to ascertain their physiological relevance.

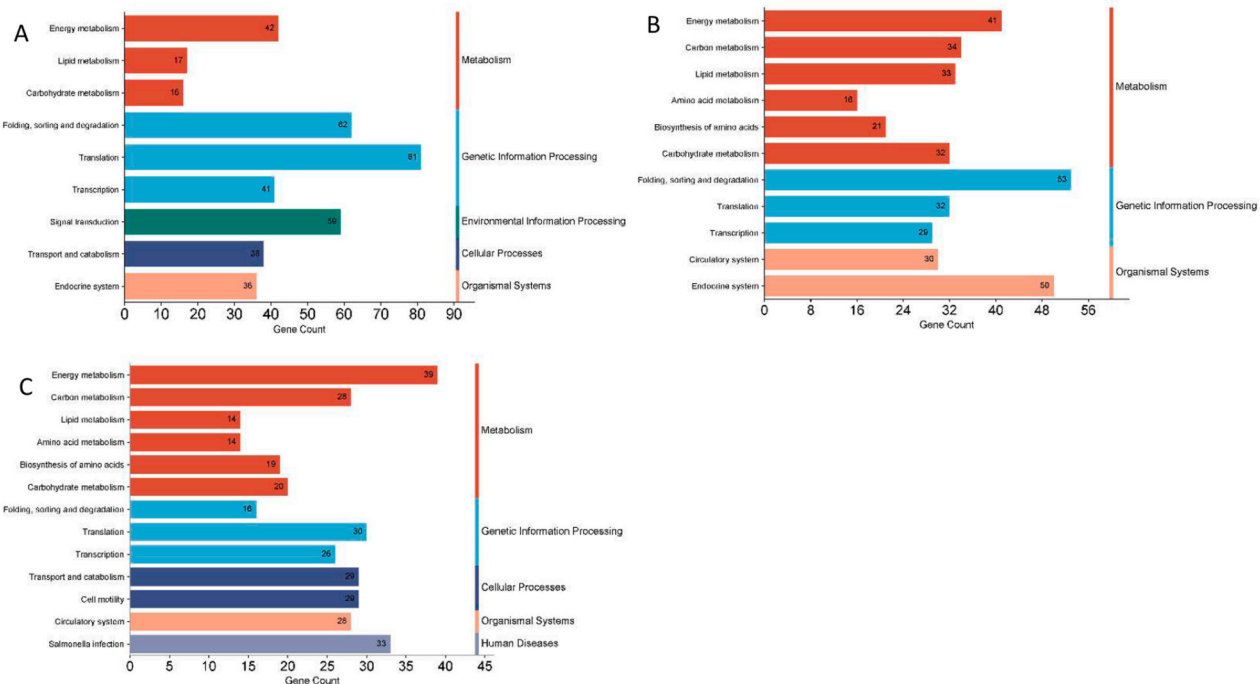


Fig. 8. Functional significance classification of KEGG Orthology (KO) of DEGs ($P_{adj} < 0.05$). (A) The KO for A vs C was summarized in five categories: metabolism, genetic information processing, environmental information processing, cellular processes, and organismal systems; (B) The KO for R vs C was summarized in three categories: metabolism, genetic information processing, and organismal systems; (C) The KO for S vs C was summarized in five categories: metabolism, genetic information processing, cellular processes, organismal systems, and human diseases. The x-axis indicated the number of genes annotated under that pathway. The y-axis indicated the name of the KEGG metabolic pathways.

4.3. Significantly enriched pathway under temperature fluctuation stress

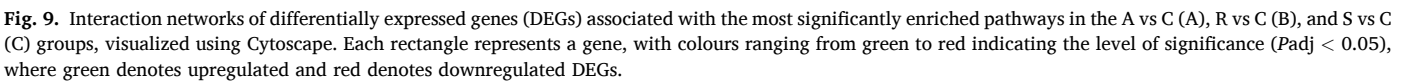
The present study focuses mainly on differentially expressed genes (DEGs) and pathways significantly enriched and expressed in acute (A vs C), tolerant (R vs C), and sensitive (S vs C) groups, the focuses DEGs was circled in Fig. 2. We were looking for genes that were significantly enriched and expressed in R group but not in S or A groups; significantly enriched and expressed in S group but not in R or A groups; and significantly enriched and expressed in A group but not in R or S groups; notably, these genes also significantly expressed in control group. Our findings demonstrated that during both acute and five days temperature fluctuation stress, metabolic and immune function pathways were significantly enriched based on GO and KEGG analyses ($P_{adj} < 0.05$). Under acute stress comparison (A vs C), GO enrichment indicated the DEGs associated with cellular components and molecular functions critical to ribosomal structural integrity, while KEGG pathway analysis indicated predominant enrichment in translation. For five days stress in the R vs C comparison, DEGs were significantly associated with molecular functions, cellular components, and biological processes linked to oxidation reduction reaction and cellular integrity. The most significantly enriched in pathways were folding, sorting, and degradation. In contrast, S vs C comparison under five days stress, DEGs significantly enriched in cellular components and biological processes of cellular integrity and biosynthesis involved in energy metabolism. The most significantly enriched in pathways were energy metabolism. Temperature stress has been widely associated with metabolic disruptions and oxidative stress in fish. For instance, such responses have been documented in *Epinephelus coioides* following temperature shock, in *Pampus argenteus* exposed to prolonged cold stress, and in the Jinhu grouper (*E. fuscoguttatus* × *E. tukula*) subjected to acute temperature stress (Duan et al., 2024; Sun et al., 2019; Zhang et al., 2022). Collectively, our results demonstrate that temperature fluctuations in hybrid grouper impact cellular integrity, oxidative stress, and energy metabolism, which is consistent with the metabolomic study by Mujar et al. (2025),

who reported that temperature fluctuations perturb cellular repair mechanisms, promote oxidative stress, and consequently alter energy metabolism in this species.

4.4. Protein processing in endoplasmic reticulum pathway

Endoplasmic reticulum (ER) stress activates the unfolded protein response (UPR), which reduces protein synthesis and increases the levels of chaperones and markers of protein degradation and autophagy. This process also affects adaptation, metabolism, and remodelling of ER (Marzuca-Nassr et al., 2021). Under stress conditions, cells trigger three defensive mechanisms which are halting further protein synthesis in ribosomes, repairing defective proteins through the mobilization of ER-resident molecular chaperones, and eliminating and degrading defective proteins from the ER (Chen, Wu, et al., 2023). In this study, exposure to acute and five days temperature fluctuations resulted in the upregulation of endoplasmic reticular oxidoreductase-1α (*ero1a*) in both the A vs C and R vs C groups comparisons, which helps mitigate ROS production as a major pro-apoptotic factor during ER stress (Cao & Kaufman, 2014). Significant upregulation of similar gene was also reported in *Boreogadus saida* (Kempf et al., 2024) and *Cyprinus carpio* (Hung et al., 2022) under hypoxic and warming conditions. ER stress and the UPR can also lead to ROS production, playing a crucial role in various cellular processes. Disruptions in redox homeostasis within the ER can lead to ER stress and further ROS production in the ER and mitochondria (Zhao et al., 2022). In *Larimichthys polyactis*, high temperature stress induced the expression of genes involved in ER process, which promoted cell survival by decreasing the accumulation of misfolded proteins (Chu et al., 2020). In contrast, prolonged cold stress in *Pampus argenteus* activated heat shock response genes as a defence mechanism against the accumulation of misfolded proteins in the cytoplasm (Zhang et al., 2022).

Transcriptomic analysis of hybrid grouper skin revealed significant upregulation of numerous differentially expressed genes (DEGs)



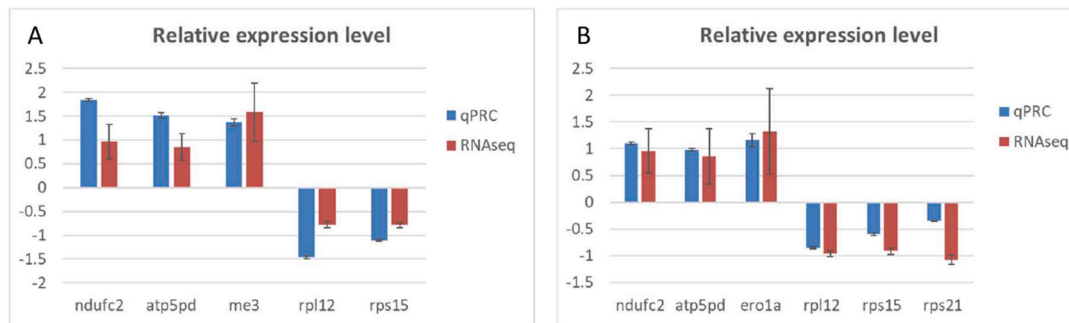


Fig. 10. Validation of the RNA-seq using RT-qPCR. (A) Five days temperature fluctuation stress; (B) Acute temperature fluctuation stress. The x-axis represents the randomly selected DEGs and the y-axis indicates the relative expression values in RNA-seq (red) and qPCR (blue) experiments. The qPCR results were obtained by normalization to the reference gene (*pex14*) and the relative expression between temperature stressed and control fish was calculated using Pfaffl method. Results are mean $\pm$ SD; $n = 3$.

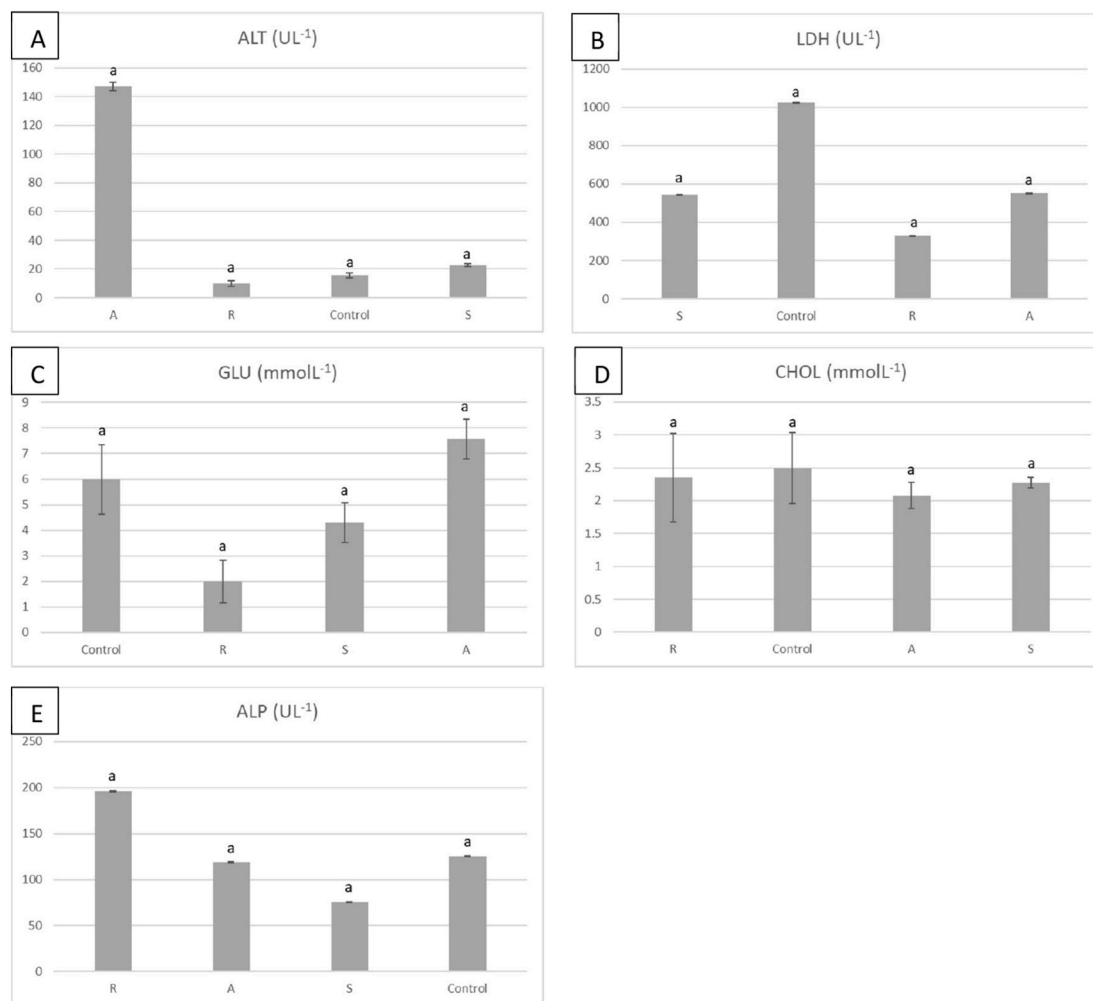


Fig. 11. Alanine transaminase, ALT (A); lactate dehydrogenase, LDH (B); glucose, GLU (C); alkaline phosphatase, ALP (D); and cholesterol, CHOL (D) of hybrid grouper after being exposed to five days and acute temperature fluctuations. Blood plasma was collected from acute (A), resistant (R), sensitive (S), and control groups. The values are mean $\pm$ SD; $n = 3$; $P > 0.05$. Similar letters denote no significant differences between groups.

associated with ER processes in the A vs C group comparison, including DnaJ heat shock protein family (Hsp40) member B1b and A2a, *dnajb1b*, *dnaja2a*; heat shock protein 90 alpha class B member 1, *hsp90ab1*; ribophorin II, *rpn2*; S-phase kinase-associated protein 1, *skp1*; and ubiquitination factor E4B, *ube4b*. Similarly, *skp1*; ubiquitin recognition factor in ER associated degradation 1, *ufd1l*; and *rpn2* were upregulated in the R vs C group comparison. Additionally, genes involved in

proteasome function were significantly upregulated in both the R vs C and S vs C groups, while genes associated with the lysosome were significantly upregulated in the A vs C and S vs C groups. The ubiquitin-proteasome system and the autophagy-lysosome system are the two primary protein degradation pathways in eukaryotic cells. The proteasome degrades ubiquitinated, damaged, or misfolded proteins through proteolytic activity, whereas autophagy targets defective organelles and

Table 6

Statistical analysis of liver with infiltrated inflammatory cells, hemosiderosis, and cytoplasmic area in hybrid grouper for control, A, R, and S groups after being exposed to five days and acute temperature fluctuations. The values are presented as mean $\pm$ SD; n = 3; $P < 0.05$. Different letters denote significant differences between groups within the same column.

Infiltrated inflammatory cells		Hemosiderosis		Cytoplasmic area	
Treatment	Mean $\pm$ SD (μm^2)	Treatment	Mean $\pm$ SD (μm^2)	Treatment	Mean $\pm$ SD (μm^2)
Control	0.00 $\pm$ 0.00 ^a	Control	0.00 $\pm$ 0.00 ^a	R	53.20 $\pm$ 0.49 ^a
A	0.00 $\pm$ 0.00 ^a	A	0.00 $\pm$ 0.00 ^a	Control	62.47 $\pm$ 0.20 ^b
S	10.00 $\pm$ 1.07 ^b	S	10.00 $\pm$ 1.23 ^b	A	63.52 $\pm$ 0.38 ^b
R	10.00 $\pm$ 0.96 ^b	R	10.00 $\pm$ 1.07 ^b	S	69.23 $\pm$ 0.42 ^b

protein aggregates by sequestering them in autophagosomes, which subsequently fuse with lysosomes for degradation (San et al., 2021). These findings suggest that proteasome and autophagy responses are crucial for the recovery of hybrid grouper following acute and five days temperature fluctuations. Temperature stress can disrupt protein folding, leading to the accumulation of misfolded and aggregated proteins, which in turn triggers ER stress. The significant upregulation of DEGs related to the UPR and ER stress pathways indicates an adaptive mechanism to manage proteotoxic stress and maintain ER homeostasis under fluctuating thermal conditions (Raposo de Magalhaes et al., 2024).

Concurrently, the significant downregulation of key genetic information processing pathways, including those involved in translation and transcription, suggests a suppression of ribosome biogenesis across all stress-exposed groups. This suppression possibly indicates that skin cells were experiencing degradation resulting from oxidative damage induced by both acute and five days temperature fluctuations (Quinn et al., 2011).

4.5. Metabolic response pathway

In the present study, genes associated with oxidative phosphorylation, pyruvate metabolism, fatty acid degradation, and the PPAR signalling pathway were significantly enriched in hybrid grouper exposed to temperature fluctuations. Both pyruvate metabolism and PPAR signalling pathway were specifically upregulated and significantly enriched in resistant, R group. The expression of LOC125903262 gene, encodes an acetyl coenzyme A synthetase 2 (ACSS2)-like mitochondrial variant X1, belongs to AMP binding enzyme family, significantly enriched in tolerant group. ACSS2, a key enzyme in the acetyl-CoA synthetase (ACSS) family, catalyses the conversion of acetate into acetyl coenzyme A (acetyl-CoA), a critical intermediate in energy metabolism (Ling et al., 2022). Our findings indicate that temperature fluctuation significantly enhances the expression of LOC125903262, gene related to pyruvate metabolism which in turn promotes cellular survival under metabolic stress (Liu et al., 2022). The upregulation of *ndufc2* and *atp5pd* suggests enhanced mitochondrial electron transport and ATP synthesis, indicating elevated oxidative metabolism under thermal stress. Concurrent enrichment of the citrate cycle (TCA) pathway supports the hypothesis that temperature fluctuation accelerates energy turnover through mitochondrial respiration. Furthermore, PPAR signalling pathway, regulates the homeostasis of lipid metabolism by modulating fatty acid uptake, binding, oxidation, and lipid transport (Liu et al., 2022). Compared to both acute and sensitive groups, the expression of lipid droplet associated proteins perilipin 2, *plin2*; angiotensin related protein 4, LOC125896266; solute carrier family 27 member1a, *slc27a1a*; and very long chain acyl-CoA synthetase, LOC125888110, the genes of PPAR signalling pathway, were

significantly upregulated in resistant group. The upregulation of these genes in R group indicates an adaptive shift toward lipid utilization and β -oxidation for energy production. This pathway provides a more sustainable energy source compared to glycolysis, reducing the accumulation of metabolic by-products such as lactate.

These transcriptomic adjustments collectively highlight a metabolic reprogramming aimed at optimizing energy efficiency and maintaining homeostasis during thermal challenge. This was further corroborated by biochemical analysis discussed in Section 4.2, where elevated ALT activity and plasma GLU levels in the acute group reflect transient hepatic stress and mobilization of glucose reserves to sustain energy demand. ALT catalyses the conversion of alanine into pyruvate, feeding into the TCA cycle and aligning with the observed upregulation of oxidative and carbohydrate metabolic pathways. Conversely, the R group exhibited lower LDH activity, supporting the transcriptomic evidence of reduced anaerobic metabolism and increased reliance on oxidative phosphorylation. Elevated ALP levels in the tolerant group may reflect heightened phosphate turnover and membrane remodelling to support cellular repair and metabolic stabilization under prolonged thermal exposure. These integrated results suggest that hybrid grouper employ a dual metabolic strategy under temperature stress. During acute exposure, energy is primarily derived from carbohydrate catabolism and hepatic gluconeogenesis, while prolonged or repeated fluctuations stimulate lipid oxidation and mitochondrial energy pathways to maintain ATP balance. Such metabolic plasticity enhances resilience by allowing efficient substrate switching and minimizing oxidative burden. Similar mechanisms have been reported in *Takifugu obscurus* (Han et al., 2022), *Trachinotus ovatus* (Li et al., 2023), *E. coioides* (Sun et al., 2019b), where thermal stress induces lipid catabolism and adaptive activation of the AMPK and PPAR pathways to sustain energy homeostasis (Miao et al., 2022; Yang et al., 2024).

The transcriptomic analysis also revealed that cellular integrity was compromised in sensitive hybrid grouper when the regulation of actin cytoskeleton pathway was significantly downregulated. This pathway plays a crucial role in maintaining cellular homeostasis and function during stress, by facilitating adaptive responses and promoting cell survival (Aravena-Canales et al., 2024). Our results showed that genes associated with cytoskeletal regulation were significantly downregulated exclusively in the sensitive hybrid grouper exposed to temperature fluctuation. These included Rho-associated coiled-coil containing protein kinase 1, *rock1*; regulator of cell cycle RGCC-like PF15151, *si dkey-27i16.2*; paxillin-like variant X1, LOC125878539; GTPase HRas variant X1, LOC125887517; actin beta 1, *actb1*; myosin light chain 9b, *myl9b*; and profilin 1, *pf1*. The downregulation of these genes suggests a disruption in cytoskeletal dynamics, which may impair the cellular response to thermal stress in sensitive individuals, ultimately affecting their resilience and survival.

4.6. Translation and signal transduction pathway

In response to acute temperature fluctuations, hybrid groupers exhibited significant enrichment of TGF-beta signalling, ErbB signalling, and mRNA surveillance pathway. Transcriptomic analysis of skin tissue following acute stress revealed significant upregulation of genes associated with the TGF-beta signalling pathway, such as activin receptor type 1-like, LOC125899602; transforming growth factor beta 1a, *tgfb1a*; TGF-beta receptor type 2-like, LOC125904247; gremlin 2-like, LOC125903491; and mothers against decapentaplegic homolog 6-like, LOC125880679. The TGF-beta signalling pathway is fundamental in cellular and tissue growth and differentiation (Wang et al., 2024). The upregulation of *tgfb1a*, an anti-inflammatory cytokine, enhanced the ability of tissue to counteract inflammatory response (Xu et al., 2024). Additionally, both ErbB signalling and mRNA surveillance pathways were significantly downregulated. ErbB signalling pathway is involved in regulating cellular processes including differentiation, proliferation, apoptosis, and maintenance of homeostasis (Sun et al., 2025), were

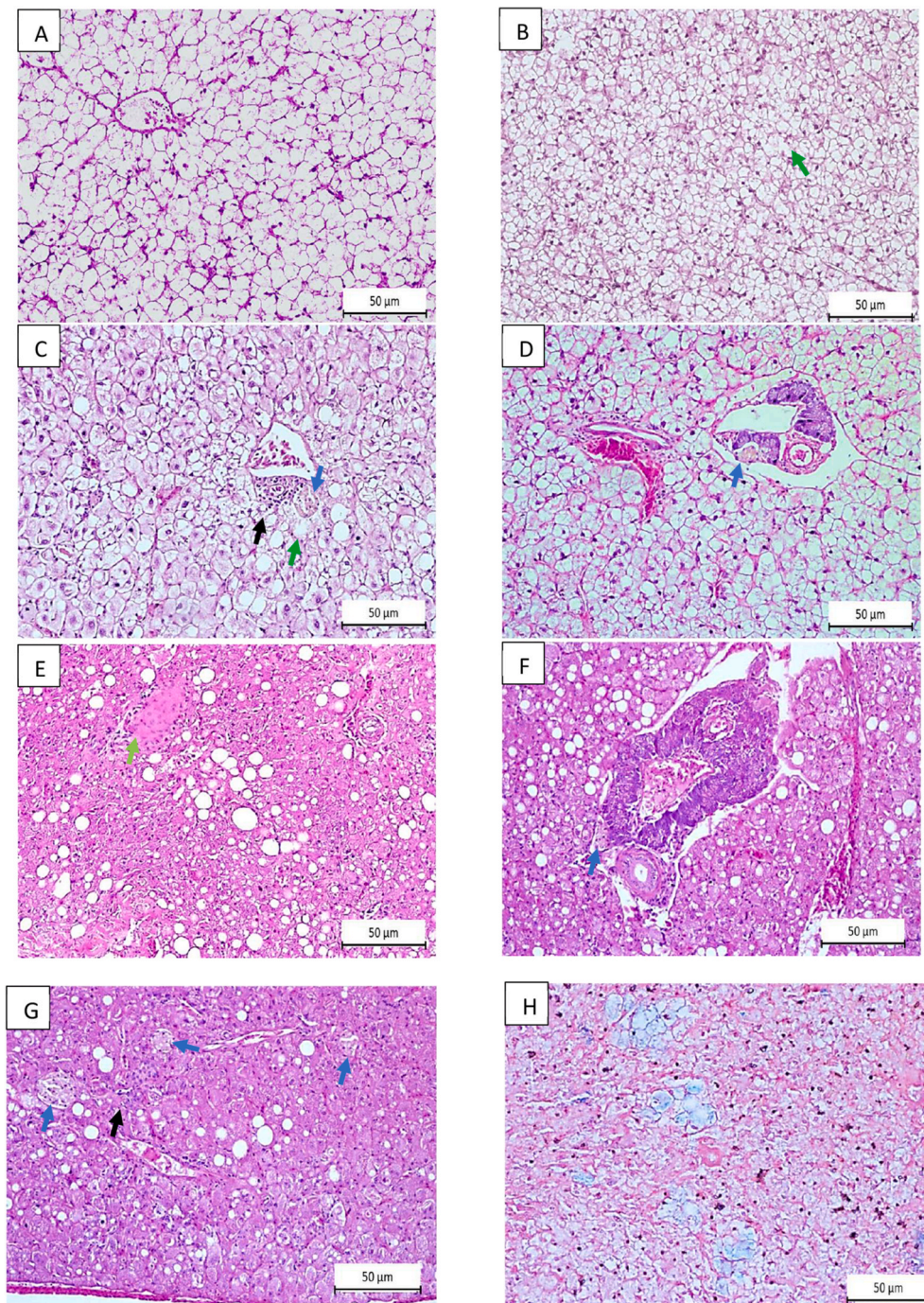


Fig. 12. Histology of liver of control (A), A (B), R (C, D), and S (E, F, G) groups of hybrid grouper after being exposed to five days and acute temperature fluctuations. 20X, H&E. Blue arrow: hemosiderin, green arrow: hepatic degeneration, black arrow: infiltration of inflammatory cells. Hepatocytic vacuolation can be seen in C, D, E, F, and G. Hepatopancreatic hemosiderosis was found in R and S group fish. Pearl's Prussian Blue, the blue colour indicates positive accumulation of iron in the hepatocytes (H). Hepatocytes can store iron in two distinct forms: soluble ferritin or insoluble hemosiderin. When subjected to an iron stain, the cytoplasm of hepatocytes exhibits a faint bluish colour for soluble ferritin, while the non-soluble hemosiderin manifests as coarse blue granules.

disrupted under acute temperature fluctuations. The related genes namely raf-1 proto-oncogene serine/threonine kinase a, *raf1a*; calcium/calmodulin-dependent protein kinase type II subunit gamma-like variant 1, LOC125881099; GRB2-associated binding protein 1, *gab1*; signal transducer and activator of transcription 5B-like, LOC125880992; betacellulin epidermal growth factor family member, *btc*; mitogen-activated protein kinase kinase 4b, *map2k4b*; and phosphatidylinositol 3-kinase regulatory subunit gamma-like, LOC125902071 were significantly downregulated. While, mRNA

surveillance pathway works by maintaining transcript fidelity via monitoring newly synthesized mRNAs and distinguishing aberrant from functional transcripts to ensure proper translation (Das & Panigrahi, 2025). In the present study, the significant downregulation of genes such as serine/arginine repetitive matrix 1, *srrm1*; PCF11 cleavage and polyadenylation factor subunit, *pcf11*; SMG7 nonsense mediated mRNA decay factor, *smg7*; cleavage and polyadenylation specificity factor subunit 7-like, LOC125881746; poly(A) binding protein nuclear variant X1, *pabpn1*; pinin desmosome associated protein, *pnn*; WD repeat

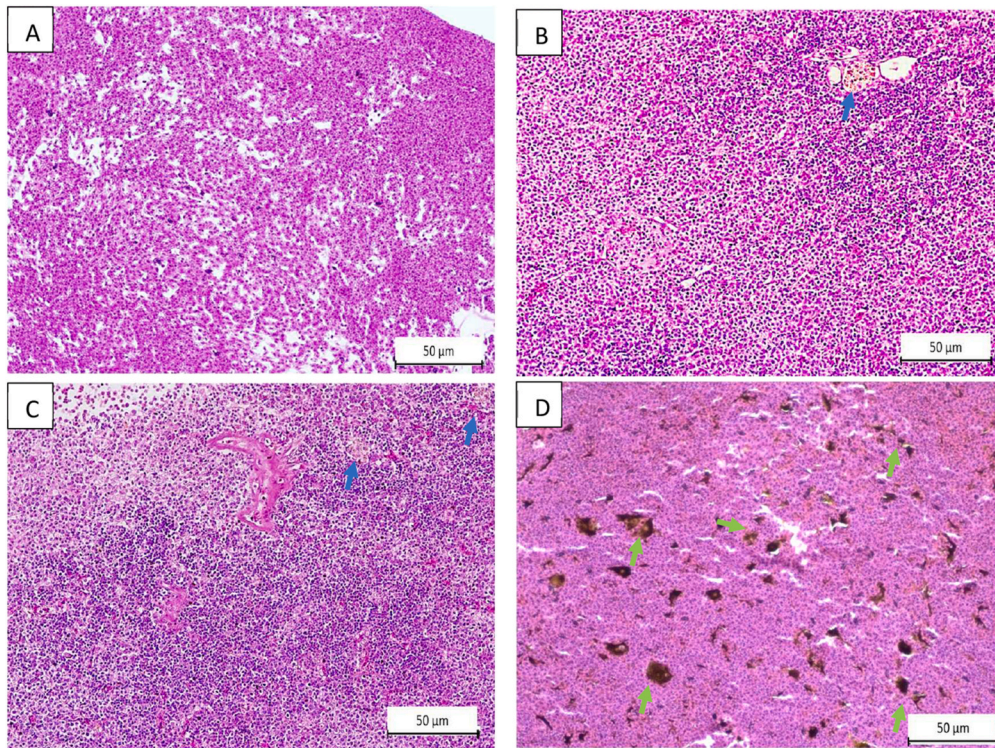


Fig. 13. Histology of spleen in control (A), R (B), S (C), and A (D) groups of hybrid grouper after being exposed to five days and acute temperature fluctuations. 20X, H&E. Blue arrow: melanomacrophages center (MMC); green arrow: necrosis.

Table 7

Statistical analysis of spleen with necrosis and melanomacrophage center (MMC) total counts (μm^2) against total area (μm^2) in control, A, R, and S groups of hybrid grouper after being exposed to five days and acute temperature fluctuations. The values are presented as mean $\pm$ SD; $n = 3$; $P < 0.05$. Different letters denote significant differences between groups within the same column.

Treatment	Total count	Total area	Necrosis
	Mean $\pm$ SD (μm^2)	Mean $\pm$ SD (μm^2)	Mean $\pm$ SD (μm^2)
Control	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
A	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	1.59 $\pm$ 0.63 ^b
S	0.80 $\pm$ 0.89 ^b	64.74 $\pm$ 1.30 ^b	0.00 $\pm$ 0.00 ^a
R	1.13 $\pm$ 0.63 ^b	97.11 $\pm$ 0.77 ^b	0.00 $\pm$ 0.00 ^a

domain 82, *wdr82*; and protein phosphatase 2 regulatory subunit B alpha a, *ppp2r2aa* may represent a cellular adaptation to reduce transcriptional burden and mitigate potential translation errors during acute temperature fluctuations in hybrid grouper (He et al., 2024). At the protein level, this response appears to coincide with increased activity in protein processing within the endoplasmic reticulum (ER) and subsequent transport to the Golgi apparatus. This mechanism likely supports the upregulation of lysosome pathways we discussed in Section 4.4, facilitating the clearance of misfolded proteins and aberrant mRNAs (Kim et al., 2021; Song & McDowell, 2021). These findings highlight the critical role of translation and signal transduction in promoting cellular survival and adaptation of hybrid grouper exposed to acute temperature fluctuations. These pathways are essential for managing important cellular processes such as proliferation, differentiation, motility, stress response, and apoptosis (He et al., 2024; Sun et al., 2019b).

5. Conclusion

This study provides comprehensive insights into the complex impacts of acute and five days temperature fluctuations on hybrid grouper (*Epinephelus fuscoguttatus* $\times$ *Epinephelus lanceolatus*), revealing

significant alterations in both skin transcriptomic profiles and physiological responses. Temperature fluctuations significantly disrupted vital biological processes in the skin of susceptible hybrid groupers, particularly affecting antioxidant defence mechanisms and metabolic function. Resilient hybrid groupers exhibited better adaptability by maintaining homeostasis and modulating metabolic pathways. Adaptation to acute thermal stress appeared to involve the downregulation of mRNA transcription and intracellular signal transduction, while simultaneously supporting cellular development and homeostatic regulation. Furthermore, temperature stress impacted genetic information processing pathways, including protein folding, sorting, degradation, translation, and transcription, underscoring their roles in regulating cellular stress responses and thermal adaptation. Biochemical markers, including elevated ALT and GLU levels and reduced LDH activity, indicate metabolic disturbances. While histopathological changes in vital organs highlight tissue damage and immune challenges. These findings not only outline the species' physiological and molecular thresholds but also emphasize the importance of maintaining stable thermal conditions in aquaculture. Future research should prioritize long-term exposure studies, integrative omics approaches, and selective breeding for thermal resilience. Together, these insights offer a valuable foundation for mitigation strategies and sustainable aquaculture practices in the face of climate-driven environmental variability.

CCRediT authorship contribution statement

Saleema Matusin: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Ellia Kartini Mujaer:** Investigation. **Annie Christianus:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **Norazrin Ariffin:** Investigation. **Annas Salleh:** Writing – review & editing, Validation, Formal analysis. **Chen Fei Low:** Writing – review & editing, Validation. **Chou Min Chong:** Methodology, Data curation, Conceptualization. **Ina Salwany Md Yasin:** Visualization. **Muhammad Hafiz Abu Bakar:** Visualization. **Yuzine Esa:**

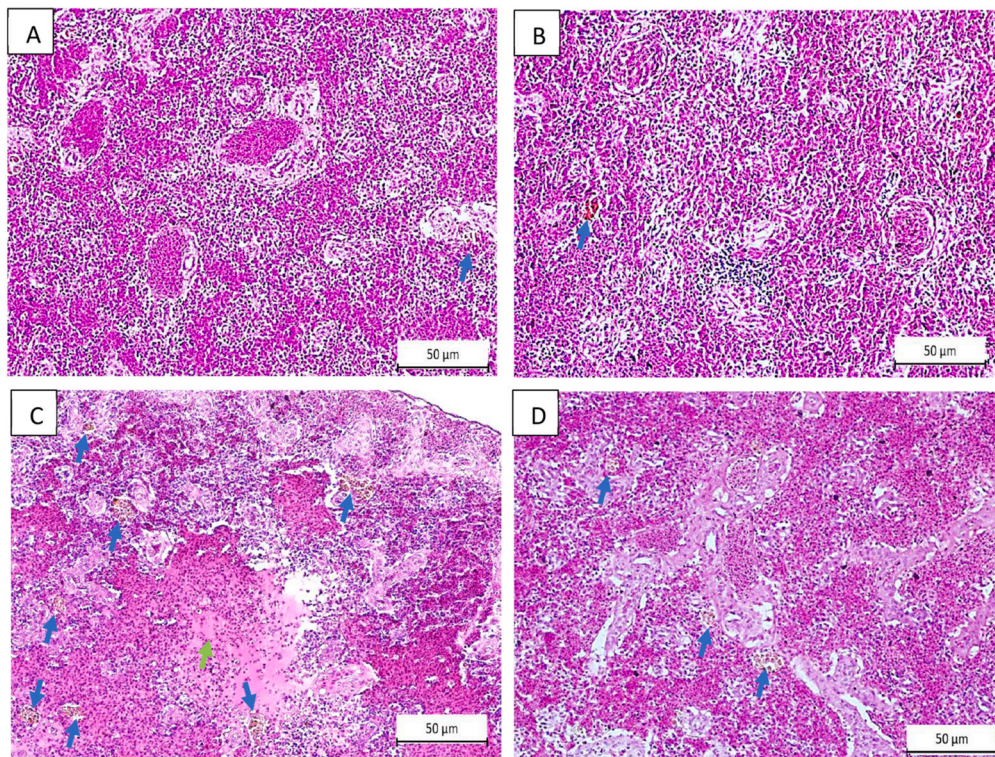


Fig. 14. Histology of head kidney in control (A), R (B), S (C), and A (D) groups of hybrid grouper after being exposed to five days and acute temperature fluctuations. 20X, H&E. Blue arrow: melanomacrophages center (MMC); green arrow: necrosis with degeneration of hematopoietic tissues.

Table 8

Statistical analysis of head kidney with melanomacrophage center (MMC) total counts (μm^2) and total area (μm^2) in hybrid grouper for control, A, R, and S groups after being exposed to five days and acute temperature fluctuations. The values are presented as mean $\pm$ SD; $n = 3$; $P < 0.05$. Different letters denote significant differences between groups within the same column.

Total count		Total area	
Treatment	Mean $\pm$ SD (μm^2)	Treatment	Mean $\pm$ SD (μm^2)
R	3.00 $\pm$ 1.11 ^a	S	135.14 $\pm$ 0.38 ^a
S	3.27 $\pm$ 1.48 ^a	R	117.59 $\pm$ 0.30 ^a
A	3.33 $\pm$ 2.08 ^a	Control	88.21 $\pm$ 0.38 ^a
Control	3.71 $\pm$ 1.78 ^a	A	106.36 $\pm$ 0.61 ^a

Visualization. **Beng Chu Kua:** Visualization.

Ethics statement

All animal handling procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia (certificate no.: UPM/ACUC/AUP-R054/2022) prior to the initiation of experiments.

Declaration of generative AI in scientific writing

During the preparation of this work NO generative AI was use.

Declaration of competing interest

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

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Data availability

All data generated and analysed during this study are included in this published article.

References

- Aboka, E. R., Zhu, J., Sun, S., Zhang, W., Yu, H., Su, Y., ... Zhu, J. (2017). Histopathological changes in gills, liver, and kidney tissues of bighead carp (*Aristichthys nobilis*) due to the effects of acute high-temperature stress. *The Israeli journal of aquaculture - Bamidgeh*, 69, 1372.
- Andersen, C. L., Jensen, J. L., & Ørntoft, T. F. (2004). Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Research*, 64(15), 5245–5250. <https://doi.org/10.1158/0008-5472.Can-04-0496>
- Aravena-Canales, D., Valenzuela-Munoz, V., Gallardo-Escarate, C., Molina, A., & Valdes, J. A. (2024). Transcriptomic and epigenomic responses to cortisol-mediated stress in rainbow trout (*Oncorhynchus mykiss*) skeletal muscle. *International Journal of Molecular Sciences*, 25(14). <https://doi.org/10.3390/ijms25147586>
- Bai, Y., Wang, M., Zhao, J., Bai, H., Zhang, X., Wang, J., Ke, Q., Qu, A., Pu, F., Zheng, W., Zhou, T., & Xu, P. (2022). Comparative transcriptome analysis reveals immunoregulation mechanism of lncRNA-mRNA in gill and skin of large yellow croaker (*Larimichthys crocea*) in response to *Cryptocaryon irritans* infection. *BMC Genomics*, 23(1), 206. <https://doi.org/10.1186/s12864-022-08431-w>
- Bai, H., Zhou, T., Zhao, J., Chen, B., Pu, F., Bai, Y., Wu, Y., Chen, L., Shi, Y., Ke, Q., Yu, X., & Xu, P. (2020). Transcriptome analysis reveals the temporal gene expression patterns in skin of large yellow croaker (*Larimichthys crocea*) in response to *Cryptocaryon irritans* infection. *Fish & Shellfish Immunology*, 99, 462–472. <https://doi.org/10.1016/j.fsi.2020.02.024>
- Beemelmans, A., Zanuzzo, F. S., Xue, X., Sandrelli, R. M., Rise, M. L., & Gamperl, A. K. (2021). The transcriptomic responses of Atlantic salmon (*Salmo salar*) to high temperature stress alone, and in combination with moderate hypoxia. *BioMed Central Genomics*, 22, 1–33. <https://doi.org/10.1186/s12864-021-07464-x>

- Bowen, L., von Biela, V. R., McCormick, S. D., Regish, A. M., Waters, S. C., Durbin-Johnson, B., Britton, M., Settles, M. L., Donnelly, D. S., & Laske, S. M. (2020). Transcriptomic response to elevated water temperatures in adult migrating Yukon River Chinook salmon (*Oncorhynchus tshawytscha*). *Conservation Physiology*, 8(1), Article coaa084. <https://doi.org/10.1093/conphys/coaa084>
- Cao, S. S., & Kaufman, R. J. (2014). Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. *Antioxidants and Redox Signaling*, 21(3), 396–413. <https://doi.org/10.1089/ars.2014.5851>
- Chen, S., Tian, Y., Li, Z., Liu, Y., Li, Z., Duan, P., Li, L., Wang, X., Wang, L., He, X., Zhao, X., Li, W., & Wang, Q. (2023). Heterosis in growth and low temperature tolerance in Jinhui grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus tukula* ♂). *Aquaculture*, 562, Article 738751. <https://doi.org/10.1016/j.aquaculture.2022.738751>
- Chen, Y., Wu, X., Li, P., Liu, Y., Song, M., Li, F., Ou, J., & Lai, J. (2023). Integrated metabolomic and transcriptomic responses to heat stress in a high-altitude fish, *Tripterygion loricatum*. *Fish & Shellfish Immunology*, 142, Article 109118. <https://doi.org/10.1016/j.fsi.2023.109118>
- Cheung, W. W., & Frölicher, T. L. (2020). Marine heatwaves exacerbate climate change impacts for fisheries in the northeast Pacific. *Scientific Reports*, 10(1), 6678. <https://doi.org/10.1038/s41598-020-63650-z>
- Chu, T., Liu, F., Qin, G., Zhan, W., Wang, M., & Lou, B. (2020). Transcriptome analysis of the *Larimichthys polyactis* under heat and cold stress. *Cryobiology*, 96, 175–183. <https://doi.org/10.1016/j.cryobiol.2020.06.014>
- Dai, Y., Shen, Y., Guo, J., Yang, H., Chen, F., Zhang, W., Wu, W., Xu, X., & Li, J. (2024). Glycolysis and gluconeogenesis are involved of glucose metabolism adaptation during fasting and re-feeding in black carp (*Mylopharyngodon piceus*). *Aquaculture and Fisheries*, 9(2), 226–233. <https://doi.org/10.1016/j.aaf.2022.04.003>
- Das, R., & Panigrahi, G. K. (2025). Messenger RNA Surveillance: Current understanding, regulatory mechanisms, and future implications. *Molecular Biotechnology*, 67(2), 393–409. <https://doi.org/10.1007/s12033-024-01062-4>
- Das, S. K., Xiang, T. W., Noor, N. M., De, M., Mazumder, S. K., & Goutham-Bharathi, M. (2021). Temperature physiology in grouper (*Epinephelinae*: Serranidae) aquaculture: A brief review. *Aquaculture Reports*, 20, Article 100682. <https://doi.org/10.1016/j.aqrep.2021.100682>
- De, M., Ghaffar, M. A., Noor, N. M., Cob, Z. C., Bakar, Y., & Das, S. K. (2019). Effects of water temperature and diet on blood parameters and stress levels in hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) juveniles. *Aquaculture Reports*, 15, Article 100219. <https://doi.org/10.1016/j.aqrep.2019.100219>
- Duan, P., Tian, Y., Li, Z., Chen, S., Li, L., Wang, X., Wang, L., Liu, Y., Zhai, J., Li, W., Wang, Q., Ma, W., & Pang, Z. (2024). Comparative transcriptome analysis of hybrid Jinhui grouper (*Epinephelus fuscoguttatus* × *Epinephelus tukula*) and *Epinephelus fuscoguttatus* under temperature stress. *Aquaculture*, 578, Article 740037. <https://doi.org/10.1016/j.aquaculture.2023.740037>
- Fang, D., Mei, J., Xie, J., & Qiu, W. (2023). The effects of transport stress (temperature and vibration) on blood biochemical parameters, oxidative stress, and gill histomorphology of pearl gentian groupers. *Fishes*, 8(4), 218. <https://doi.org/10.3390/fishes8040218>
- FAO. (2022). *The State of world fisheries and aquaculture 2022. Towards blue transformation*. FAO. <https://doi.org/10.4060/cc0461en>
- Faudzi, N. M., Sobri, M. I., Othman, R., Ching, F. F., & Shaleh, S. R. M. (2021). *Water temperature and stocking density for long hour transportation of hybrid grouper Epinephelus fuscoguttatus x E. lanceolatus*. *Aquaculture, Aquarium, Conservation and legislation - International journal of bioflux society*.
- Gao, S., Han, C., Ye, H., Chen, Q., & Huang, J. (2022). Transcriptome analysis of the spleen provides insight into the immunoregulation of *Scortum barcoo* under *Streptococcus agalactiae* infection. *Ecotoxicology and Environmental Safety*, 245, Article 114095. <https://doi.org/10.1016/j.ecoenv.2022.114095>
- Han, B., Meng, Y., Tian, H., Li, C., Li, Y., Gongbao, C., Fan, W., & Ma, R. (2022). Effects of acute hypoxic stress on physiological and hepatic metabolic responses of triploid rainbow trout (*Oncorhynchus mykiss*). *Frontiers in Physiology*, 13, Article 921709. <https://doi.org/10.3389/fphys.2022.921709>
- Han, S., Wei, S., Chen, R., Ni, M., & Chen, L. (2022). Tissue-specific and differential cold responses in the domesticated cold tolerant fugu. *Fishes*, 7(4), 159. <https://doi.org/10.3390/fishes7040159>
- He, X., Liao, Y., Shen, Y., Shao, J., Wang, S., & Bao, Y. (2024). Transcriptomic analysis of mRNA and miRNA reveals new insights into the regulatory mechanisms of *Anadara granosa* responses to heat stress. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 52, Article 101311. <https://doi.org/10.1016/j.cbpd.2024.101311>
- He, J., Zhu, Q., Han, P., Zhou, T., Li, J., Wang, X., & Cheng, J. (2023). Transcriptomic networks reveal the tissue-specific cold shock responses in Japanese flounder (*Paralichthys olivaceus*). *Biology*, 12(6), 784. <https://doi.org/10.3390/biology12060784>
- Heba, H. N., El-Galil, M. A. E.-A. A., Mohamed, M. A., Rasha, A. E.-L. S., Seddek, A., & m. (2023). *Oreochromis niloticus* (Nile tilapia) skin tissues response to 5hrs transportation in fresh and brackish water. *International Journal of Veterinary Medical Sciences*, 1(1), 33–37. <https://doi.org/10.21608/ijv.2023.352859>
- Hernández-López, J. R., Hernández-Rodríguez, M., Rivas-Manzano, P., & Büchle-Ramírez, L. F. (2018). Thermal effect of acute and chronic stress on hepatic and renal tissue of the Pacific sardine, *Sardinops sagax caeruleus* (Jenyns, 1842). *International Journal of Morphology*, 36(1).
- Huang, M., Ding, L., Wang, J., Ding, C., & Tao, J. (2021). The impacts of climate change on fish growth: A summary of conducted studies and current knowledge. *Ecological Indicators*, 121, Article 106976. <https://doi.org/10.1016/j.ecolind.2020.106976>
- Huang, D., Ren, M., Liang, H., Ge, X., Xu, H., & Wu, L. (2022). Transcriptome analysis of the effect of high-temperature on nutrient metabolism in juvenile grass carp (*Ctenopharyngodon idellus*). *Gene*, 809, Article 146035. <https://doi.org/10.1016/j.gene.2021.146035>
- Hung, K.-S., Chen, S.-Y., Hsu, P.-H., Lin, B.-A., Hu, C.-H., Yang, C.-H., Pai, T.-W., Tzou, W.-S., & Chung, H.-Y. (2022). Comparative transcriptome analysis of organ-specific adaptive responses to hypoxia provides insights to human diseases. *Genes*, 13(6), 1096. <https://doi.org/10.3390/genes13061096>
- Islam, M. J., Kunzmann, A., & Slater, M. J. (2022). Responses of aquaculture fish to climate change-induced extreme temperatures: A review. *Journal of the World Aquaculture Society*, 53(2), 314–366. <https://doi.org/10.1111/jwas.12853>
- Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., & Morishima, K. (2017). KEGG: New perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Research*, 45(D1), D353–d361. <https://doi.org/10.1093/nar/gkw1092>
- Kempf, S., Windisch, H. S., Kunz, K. L., Pörtner, H.-O., Mark, F. C., & Lucassen, M. (2024). Hepatic transcriptomic responsiveness of Polar cod, *Boreogadus saida*, to ocean acidification and warming. *Fishes*, 9(7), 271. <https://doi.org/10.3390/fishes9070271>
- Kim, B.-M., Ahn, D.-H., Kang, S., Jeong, J., Jo, E., Kim, B.-K., Kim, J.-H., & Park, H. (2019). Skin transcriptome profiling reveals the distinctive molecular effects of temperature changes on Antarctic bullhead notothen. *Molecular and Cellular Toxicology*, 15(2), 163–172. <https://doi.org/10.1007/s13273-019-0020-1>
- Kim, W.-J., Lee, K., Lee, D., Kim, H.-C., Nam, B.-H., Jung, H., Yi, S.-J., & Kim, K. (2021). Transcriptome profiling of olive flounder responses under acute and chronic heat stress. *Genes and Genomics*, 43, 151–159. <https://doi.org/10.1007/s13258-021-01053-8>
- Kulczykowska, E. (2019). Stress response system in the fish skin—Welfare measures revisited. *Frontiers in Physiology*, 10, 72. <https://doi.org/10.3389/fphys.2019.00072>
- Li, S., Liu, Y., Li, B., Ding, L., Wei, X., Wang, P., Chen, Z., Han, S., Huang, T., & Wang, B. (2022). Physiological responses to heat stress in the liver of rainbow trout (*Oncorhynchus mykiss*) revealed by UPLC-QTOF-MS metabolomics and biochemical assays. *Ecotoxicology and Environmental Safety*, 242, Article 113949. <https://doi.org/10.1016/j.ecoenv.2022.113949>
- Li, Q. Q., Zhang, J., Wang, H. Y., Niu, S. F., Wu, R. X., Tang, B. G., Wang, Q. H., Liang, Z. B., & Liang, Y. S. (2023). Transcriptomic response of the liver tissue in *Trachinotus ovatus* to acute heat stress. *Animals*, 13(13), 2053. <https://doi.org/10.3390/ani13132053>
- Liang, J., Xu, J., Xie, S., Cao, J., & Tan, B. (2022). Selection of appropriate housekeeping genes for gene expression normalization in hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*). *Turkish Journal of Fisheries and Aquatic Sciences*, 22(9). <https://doi.org/10.4194/TRJFAS20646>
- Ling, R., Chen, G., Tang, X., Liu, N., Zhou, Y., & Chen, D. (2022). Acetyl-CoA synthetase 2 (ACSS2): A review with a focus on metabolism and tumor development. *Discover Oncology*, 13(1), 58. <https://doi.org/10.1007/s12672-022-00521-1>
- Liu, H., Fu, Z., Yu, G., Ma, Z., & Zong, H. (2022). Effects of acute high-temperature stress on physical responses of yellowfin tuna (*Thunnus albacares*). *Journal of Marine Science and Engineering*, 10, 1857. <https://doi.org/10.3390/jmse10121857>
- Liu, M., Liu, N., Wang, J., Fu, S., Wang, X., & Chen, D. (2022). Acetyl-CoA Synthetase 2 as a therapeutic target in tumor metabolism. *Cancers*, 14(12). <https://doi.org/10.3390/cancers14122896>
- Liu, Y., Yao, M., Li, S., Wei, X., Ding, L., Han, S., Wang, P., Lv, B., Chen, Z., & Sun, Y. (2022). Integrated application of multi-omics approach and biochemical assays provides insights into physiological responses to saline-alkaline stress in the gills of crucian carp (*Carassius auratus*). *Science of the Total Environment*, 822, Article 153622. <https://doi.org/10.1016/j.scitotenv.2022.153622>
- Liu, E., Zhao, X., Li, C., Wang, Y., Li, L., Zhu, H., & Ling, Q. (2022). Effects of acute heat stress on liver damage, apoptosis and inflammation of pikeperch (*Sander lucioperca*). *Journal of Thermal Biology*, 106, Article 103251. <https://doi.org/10.1016/j.jtherbio.2022.103251>
- Luo, L., Huang, J.-H., Liu, D.-L., Jiang, S.-G., Zhou, F.-L., Jiang, S., Yang, Q.-B., Li, Y.-D., Li, T., & Tan, L.-Q. (2022). Comparative transcriptome analysis of differentially expressed genes and pathways in *Procambarus clarkii* (Louisiana crawfish) at different acute temperature stress. *Genomics*, 114(4), Article 110415. <https://doi.org/10.1016/j.ygeno.2022.110415>
- Marzuca-Nassar, G. N., Kuwabara, W. M. T., Vitzel, K. F., Murata, G. M., Torres, R. P., Mancini-Filho, J., Alba-Loureiro, T. C., & Curi, R. (2021). Endoplasmic reticulum stress and autophagy markers in soleus muscle disuse-induced atrophy of rats treated with fish oil. *Nutrients*, 13(7), 2298. <https://doi.org/10.3390/nu13072298>
- Massey, M. D., Fredericks, M. K., Malloy, D., Arif, S., & Hutchings, J. A. (2022). Differential reproductive plasticity under thermal variability in a freshwater fish. *Danio rerio*. *Proceedings of the Royal Society B*, 289, Article 20220751. <https://doi.org/10.1098/rspb.2022.0751>, 1982.
- Maulu, S., Hasimuna, O. J., Haambiya, L. H., Monde, C., Musuka, C. G., Makorwa, T. H., Munganga, B. P., Phiri, K. J., & Nsekanabo, J. D. (2021). Climate change effects on aquaculture production: Sustainability implications, mitigation, and adaptations. *Frontiers in Sustainable Food Systems*, 5, Article 609097. <https://doi.org/10.3389/fsufs.2021.609097>
- Miao, B. B., Niu, S. F., Wu, R. X., Liang, Z. B., & Zhai, Y. (2022). The microRNAs-transcription factors-mRNA regulatory network plays an important role in resistance to cold stress in the pearl gentian grouper. *Frontiers in Marine Science*, 8, Article 824533. <https://doi.org/10.3389/fmars.2021.824533>
- Mujar, E. K., Matusin, S., Yahya, N. S., Bustamam, M. S. A., Christianus, A., Shaari, K., Kassim, N. K., Abas, F., Tan, J. K., & Ismail, I. S. (2025). Physiological responses of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) to temperature fluctuation stress revealed by LCMS/MS-based mucus and sera metabolomics. *Fish Physiology and Biochemistry*, 51(3), 99. <https://doi.org/10.1007/s10695-025-01510-1>

- Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, 29(9). <https://doi.org/10.1093/nar/29.9.e45>. e45–e45.
- Pfaffl, M. W., Tichopad, A., Prgomet, C., & Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: Bestkeeper–excel-based tool using pair-wise correlations. *Biotechnology Letters*, 26(6), 509–515. <https://doi.org/10.1023/b:biote.0000019559.84305.47>
- Pratchett, M. S., Cameron, D. S., Donelson, J., Evans, L., Frisch, A. J., Hobday, A. J., Hoey, A. S., Marshall, N. A., Messmer, V., & Munday, P. L. (2017). Effects of climate change on coral grouper (*Plectropomus* spp.) and possible adaptation options. *Reviews in Fish Biology and Fisheries*, 27, 297–316. <https://doi.org/10.1007/s11160-016-9455-9>
- Qin, H., Zhu, Z. X., Lin, H. R., Xia, J. H., & Jia, Y. X. (2020). Exploring candidate genes in a major QTL region associated with salinity tolerance in the skin of Nile tilapia based on transcriptomic analysis. *Aquaculture*, 526, Article 735380. <https://doi.org/10.1016/j.aquaculture.2020.735380>
- Quinn, N. L., McGowan, C. R., Cooper, G. A., Koop, B. F., & Davidson, W. S. (2011). Ribosomal genes and heat shock proteins as putative markers for chronic, sublethal heat stress in Arctic charr: Applications for aquaculture and wild fish. *Physiological Genomics*, 43(18), 1056–1064. <https://doi.org/10.1152/physiolgenomics.00090.2011>
- Raposo de Magalhães, C., Sandoval, K., Kagan, F., McCormack, G., Schrama, D., Carrilho, R., Farinha, A. P., Cerqueira, M., & Rodrigues, P. M. (2024). Transcriptomic changes behind *Sparus aurata* hepatic response to different aquaculture challenges: An RNA-seq study and multiomics integration. *PLoS One*, 19(3), Article e0300472. <https://doi.org/10.1371/journal.pone.0300472>
- Reid, C. H., Patrick, P. H., Rytwinski, T., Taylor, J. J., Willmore, W. G., Reesor, B., & Cooke, S. J. (2022). An updated review of cold shock and cold stress in fish. *Journal of Fish Biology*, 100(5), 1102–1137. <https://doi.org/10.1111/jfb.15037>
- San, L., Liu, B., Liu, B., Guo, H., Guo, L., Zhang, N., Zhu, K., Jiang, S., & Zhang, D. (2021). Transcriptome analysis of gills provides insights into translation changes under hypoxic stress and reoxygenation in golden pompano, *Trachinotus ovatus* (Linnaeus 1758). *Frontiers in Marine Science*, 8, Article 763622. <https://doi.org/10.3389/fmars.2021.763622>
- Shapawi, R., Abdullah, F. C., Senoo, S., & Mustafa, S. (2019). Nutrition, growth and resilience of tiger grouper (*Epinephelus fuscoguttatus*) × giant Grouper (*Epinephelus lanceolatus*) hybrid—a review. *Reviews in Aquaculture*, 11(4), 1285–1296. <https://doi.org/10.1111/raq.12292>
- Sharma, M. (2019). Behavioural responses in effect to chemical stress in fish: A review. *International Journal of Fisheries and Aquatic Studies*, 7(1), 1–5.
- Shi, K.-P., Dong, S.-L., Zhou, Y.-G., Li, Y., Gao, Q.-F., & Sun, D.-J. (2019). RNA-seq reveals temporal differences in the transcriptome response to acute heat stress in the Atlantic salmon (*Salmo salar*). *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 30, 169–178. <https://doi.org/10.1016/j.cbd.2018.12.011>
- Song, J., & McDowell, J. R. (2021). Comparative transcriptomics of spotted seatrout (*Cynoscion nebulosus*) populations to cold and heat stress. *Ecology and Evolution*, 11(3), 1352–1367. <https://doi.org/10.1002/ece3.7138>
- Sudhagar, A., Kumar, G., & El-Matbouli, M. (2018). Transcriptome analysis based on RNA-Seq in understanding pathogenic mechanisms of diseases and the immune system of fish: A comprehensive review. *International Journal of Molecular Sciences*, 19(1), 245. <https://doi.org/10.3390/ijms19010245>
- Sun, J., Han, Y., Li, J., Li, H., Meng, Y., Sun, G., Li, C., & Ma, R. (2025). Role of miRNAs in the regulation of the triploid rainbow trout (*Oncorhynchus mykiss*) liver transcriptome in hypoxic environments. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, Article 101515. <https://doi.org/10.1016/j.cbd.2025.101515>
- Sun, Y., Li, Y., An, J., Liu, Z., & Chen, Q. (2019a). Antioxidative and inflammatory responses in spleen and head kidney of Yellow Catfish (*Pelteobagrus fulvidraco*) induced by waterborne cadmium exposure. *Turkish Journal of Fisheries and Aquatic Sciences*, 20(2), 87–96. <https://doi.org/10.4194/1303-2712-v20.02.01>
- Sun, Z., Tan, X., Liu, Q., Ye, H., Zou, C., Xu, M., Zhang, Y., & Ye, C. (2019c). Physiological, immune responses and liver lipid metabolism of orange-spotted grouper (*Epinephelus coioides*) under cold stress. *Aquaculture*, 498, 545–555. <https://doi.org/10.1016/j.aquaculture.2018.08.051>
- Sun, Z., Tan, X., Xu, M., Liu, Q., Ye, H., Zou, C., & Ye, C. (2019b). Liver transcriptome analysis and *de novo* annotation of the orange-spotted groupers (*Epinephelus coioides*) under cold stress. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 29, 264–273. <https://doi.org/10.1016/j.cbd.2018.12.008>
- Thalib, Y. A., Razali, R. S., Mohamad, S., Rahmah, S., Ghaffar, M. A., Nhan, H. T., & Liew, H. J. (2021). Environmental changes affecting physiological responses and growth of hybrid grouper—the interactive impact of low pH and temperature. *Environmental Pollution*, 271, Article 116375. <https://doi.org/10.1016/j.envpol.2020.116375>
- Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, 3, 1–12.
- Wang, Y., Li, C., Pan, C., Liu, E., Zhao, X., & Ling, Q. (2019). Alterations to transcriptomic profile, histopathology, and oxidative stress in liver of pikeperch (*Sander lucioperca*) under heat stress. *Fish & Shellfish Immunology*, 95, 659–669. <https://doi.org/10.1016/j.fsi.2019.11.014>
- Wang, M., Li, B., Wang, J., Xie, S., & Zhang, L. (2022). Skin transcriptome and physiological analyses reveal the metabolic and immune responses of yellow catfish (*Pelteobagrus fulvidraco*) to acute hypoxia. *Aquaculture*, 546, Article 733727. <https://doi.org/10.1016/j.aquaculture.2021.737277>
- Wang, Q. H., Wu, R. X., Ji, J. N., Zhang, J., Niu, S. F., Tang, B. G., Miao, B. B., & Liang, Z. B. (2024). Integrated transcriptomics and metabolomics reveal changes in cell homeostasis and energy metabolism in *Trachinotus ovatus* in response to acute hypoxic stress. *International Journal of Molecular Sciences*, 25(2), 1054. <https://doi.org/10.3390/ijms25021054>
- Wu, S., Huang, J., Li, Y., & Pan, Y. (2023). Dynamic and systemic regulatory mechanisms in rainbow trout (*Oncorhynchus mykiss*) in response to acute hypoxia and reoxygenation stress. *Aquaculture*, 572, Article 739540. <https://doi.org/10.1016/j.aquaculture.2023.739540>
- Xu, C., Li, E., Suo, Y., Su, Y., Lu, M., Zhao, Q., Qin, J. G., & Chen, L. (2018). Histological and transcriptomic responses of two immune organs, the spleen and head kidney, in Nile tilapia (*Oreochromis niloticus*) to long-term hypersaline stress. *Fish & Shellfish Immunology*, 76, 48–57. <https://doi.org/10.1016/j.fsi.2018.02.041>
- Xu, X.-W., Yang, H., Wei, X.-L., Abu-Elala, N., & Luo, Z. (2024). Influences of dietary Cu levels on growth performance, muscle development, antioxidant capacity, and inflammatory response of juvenile yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture Reports*, 36. <https://doi.org/10.1016/j.aqrep.2024.102080>
- Yang, Q. T., Wu, R. X., Liang, Y. S., Niu, S. F., Miao, B. B., Liang, Z. B., & Shen, Y. X. (2024). Liver transcriptome changes in pearl gentian grouper in response to acute high-temperature stress. *Aquaculture*, 593, Article 741336. <https://doi.org/10.1016/j.aquaculture.2024.741336>
- Yang, T., Zhang, Y., Meng, W., Zhong, X., Shan, Y., & Gao, T. (2021). Comparative transcriptomic analysis brings new insights into the response to acute temperature acclimation in burbot (*Lota lota lota*). *Aquaculture Reports*, 20, Article 100657. <https://doi.org/10.1016/j.aqrep.2021.100657>
- Zhang, J., Cai, X., Zhang, X., Lin, L., Zhao, H., & Liu, X. (2021). Proteome analysis and thermal-tolerant protein marker screening in the skin mucus of large yellow croaker *Larimichthys crocea*. *Aquaculture Reports*, 21, Article 100870. <https://doi.org/10.1016/j.aqrep.2021.100870>
- Zhang, M., Hu, J., Zhu, J., Wang, Y., Zhang, Y., Li, Y., Xu, S., Yan, X., & Zhang, D. (2022). Transcriptome, antioxidant enzymes and histological analysis reveal molecular mechanisms responsive to long-term cold stress in silver pomfret (*Pampus argenteus*). *Fish & Shellfish Immunology*, 121, 351–361. <https://doi.org/10.1016/j.fsi.2022.01.017>
- Zhao, X., Li, L., Li, C., Liu, E., Zhu, H., & Ling, Q. (2022). Heat stress-induced endoplasmic reticulum stress promotes liver apoptosis in largemouth bass (*Micropterus salmoides*). *Aquaculture*, 546, Article 737401. <https://doi.org/10.1016/j.aquaculture.2021.737401>