DOI: 10.54083/ResBio/8.3.2026/115-124 *Res. Bio.*, 2026, 8(3):115-124



Research Biotica

e-ISSN: 2582-6743
July-September, 2026

Article ID: RB271 Research Article

Gill Transcriptomic Insights into Adaptive Response of *Clarias magur* to Diurnal Thermal Fluctuations

Mamta Singh* and Ved Prakash Saini

College of Fisheries, Kishanganj (Bihar Animal Sciences University, Patna), DKAC Campus Arrabari, Kishanganj, Bihar (855 107), India

Open Access

Corresponding Author
Mamta Singh
✉: mamta0131@gmail.com

Conflict of interests: The authors have declared that no conflict of interest exists.

How to cite this article?

Singh, M., Saini, V.P., 2026. Gill Transcriptomic Insights into Adaptive Response of *Clarias magur* to Diurnal Thermal Fluctuations. *Research Biotica* 8(3), 115-124. DOI: 10.54083/ResBio/8.3.2026/115-124.

Copyright: © 2026 Singh and Saini. This is an open access article that permits unrestricted use, distribution and reproduction in any medium after the author(s) and source are credited.

Abstract

Climate-induced changes in water temperature, particularly diurnal thermal fluctuations, pose serious challenges to aquatic organisms, especially fish. The present study investigated the gill transcriptomic responses of the catfish *Clarias magur* subjected to diurnal heat stress. Fish maintained at ambient temperature (26 °C) served as the control group, whereas the treatment group was exposed to cyclic diurnal temperature fluctuation (DTF) over a 24-h period (26→28→30→32→34→32→30→28→26 °C), with 3 h at each temperature for 4 days. Gill RNA from control and DTF-exposed fish were analysed by high-throughput sequencing and differentially expressed genes (DEGs) were identified using log2 fold change criteria and adjusted *p*-values. Comparative transcriptome analysis revealed a clear separation between control and treatment groups, indicating extensive transcriptional modulation under diurnal heat stress conditions. Differential expression analysis identified 90,574 significantly regulated transcripts, including 6,629 upregulated and 83,945 downregulated transcripts, indicating a predominance of transcriptional suppression in heat-stressed fish. Pathway enrichment analysis pointed to MAPK signalling, endocytosis, regulation of actin cytoskeleton, mTOR signalling, autophagy and immune-related pathways. The results indicate extensive restructuring of gill gene expression during repeated thermal cycling and suggest that the gill is a major site of molecular adaptation to fluctuating thermal environments. Overall, the present study expands the available transcriptomic resources for *C. magur* and provides a molecular framework for investigating thermal resilience of air-breathing catfish under changing climatic conditions.

Keywords: *Clarias magur*, DEGs, Diurnal heat stress, Gill transcriptome, Heat adaptation

Introduction

Water temperature is a major environmental determinant regulating the biochemical reactions, physiological processes, metabolic response, growth and survival of aquatic organisms. In ectothermic animals such as fish, environmental temperature directly influences cellular and biochemical processes, thereby affecting overall health and productivity. Under the current scenario of climate change, freshwater ecosystems are increasingly experiencing frequent heat waves and irregular thermal fluctuations, posing serious threats to aquaculture sustainability and aquatic biodiversity (Barbarossa *et al.*, 2021). Elevated temperature can disrupt physiological homeostasis, induce oxidative stress, alter metabolic activity, impair immune responses and reduce growth and reproductive performance in fish (Chang *et al.*, 2018; Kim *et al.*, 2019; Genin *et al.*, 2020; Roychowdhury *et al.*, 2021; Khieokhajonkhet *et al.*, 2022; Liu *et al.*, 2022; Mitra *et al.*, 2023; Qin *et al.*, 2023; Singh *et al.*, 2025). Most previous studies investigating thermal stress in fish have focused primarily on constant heat exposure under controlled laboratory conditions. A large proportion of previous experimental works have examined temperature

Article History
RECEIVED on 02nd April 2026 RECEIVED in revised form 20th September 2026 ACCEPTED in final form 27th September 2026

stress using a single sustained elevated temperature. However, natural aquatic environments rarely experience stable temperatures throughout the day. Instead, water bodies undergo continuous diurnal thermal fluctuations (DTF) driven by solar radiation, ambient climatic conditions and seasonal variability. In shallow freshwater ecosystems and aquaculture ponds, temperature may rise sharply during the day and decline at night, exposing fish to repeated cycles of thermal stress and recovery. Such diurnal heat stress conditions may trigger physiological and molecular responses distinct from those induced by constant high-temperature exposure. Unlike chronic constant heat stress, fluctuating thermal regimes impose dynamic metabolic and cellular adjustments that require repeated activation of stress-responsive pathways (de Alba *et al.*, 2021; Clow and Craig, 2026; Guo *et al.*, 2026). Therefore, studying diurnal heat stress provides a more ecologically relevant understanding of thermal adaptation mechanisms in fish under realistic climate-change scenarios. Among different organs, the gill is one of the most sensitive tissues responding to the environmental stressors in fish (Evans *et al.*, 2005; Cappello *et al.*, 2016; Wen *et al.*, 2018; Singh *et al.*, 2019). Gill tissues perform many vital physiological functions including respiration, osmoregulation, ion transport, acid-base balance and nitrogenous waste excretion. Due to their direct contact with the external environment, gills rapidly perceive and respond to temperature fluctuations at the cellular and molecular levels. Thermal stress can alter gill permeability, oxygen uptake efficiency, epithelial integrity and signalling pathways, leading to activation of adaptive mechanisms aimed at maintaining cellular homeostasis and organismal survival. The Indian catfish *Clarias magur* is an economically and nutritionally important freshwater air-breathing fish species endemic to the Indian subcontinent. The species is highly valued because of its rapid growth, high protein content, medicinal value and tolerance to low dissolved oxygen conditions. However, increasing thermal variability associated with climate change may adversely affect its physiology, health and aquaculture productivity. Despite its commercial significance, limited information is available regarding the molecular mechanisms underlying heat stress adaptation in *C. magur*, particularly under fluctuating diurnal thermal conditions. Transcriptome profiling provides insights into differentially expressed genes (DEGs), stress-responsive signalling pathways and cellular processes involved in adaptation and survival. Our previous transcriptomic studies in *C. magur*, revealed that thermal stress modulates genes associated with heat shock response, oxidative stress, apoptosis, immune regulation, protein turnover and energy metabolism (Zhou *et al.*, 2021; Mokhtar *et al.*, 2023; Dettleff *et al.*, 2020; Dettleff *et al.*, 2024; Singh *et al.*, 2025; Duan *et al.*, 2024; Duan *et al.*, 2026). Nevertheless, the molecular responses of gill tissue to cyclic diurnal heat stress remain poorly understood in *C. magur*. Therefore, the present study aimed to investigate the gill transcriptomic responses of *C. magur* exposed to DTF conditions. Transcriptomic profiles of control and DTF-exposed fish were compared to identify regulated transcripts and enriched biological pathways that may participate in adaptation to cyclic thermal conditions, which will contribute to understanding climate-resilient mechanisms in commercially important aquaculture species.

Materials and Methods

Experimental Fish and Ethical Approval

Live *C. magur* were maintained in an indoor experimental facility for the study. Fish were collected from Alta Lake, Kishanganj district, Bihar, India (26.142898° N, 87.855241° E) and subsequently acclimatized at the instructional fish farm of the College of Fisheries, Kishanganj, Bihar Animal Sciences University (BASU), Patna. Fish were anaesthetized before sacrifice. The experimental procedures involving live fish followed the Institutional Animal Ethics Committee (IAEC) guidelines (Approval No. COF/BASU/01; 12 September 2022).

Temperature Exposure Regime and Tissue Sampling

Fish were first acclimated for four weeks in 50-L glass tanks. Feed was withheld for 24 h immediately before thermal exposure. Two experimental conditions were used. In the DTF treatment, fish experienced a daily temperature range of 26-34 °C for 96 h (CMDT), while control fish remained at 26 °C for the same duration (CMC). Each aquarium contained 50 L of dechlorinated tap water and was equipped with thermostatically regulated glass heaters and continuous aeration. Water-quality variables were monitored using the standard procedures of APHA (2005) and remained within the acceptable range during the experiment.

The DTF schedule consisted of sequential temperature steps of 26→28→30→32→34→32 →30→28→26 °C, with a 3-h period at each step; the cycle was repeated for four days (Figure 1). Control and treatment experiments were run in duplicate. Eighty fish were used in total, with 40 fish assigned to each experimental condition and 20 fish maintained in each replicate. Gill sampling was performed after 96 h. For each replicate, equal quantities of gill tissue collected from the fish within that replicate were pooled and treated as one biological sample. Thus, two biological replicates were available for the control and two for DTF exposure. The control RNA-seq data were obtained from the previously published *C. magur* heat-stress study of Singh *et al.* (2025), whereas the treatment libraries were generated for the present investigation.

Transcriptome Profiling (RNA-Seq) of Gill Tissue

Transcriptome profile data of the gill tissue of the control (CMCR1 and CMCR2) were used from our previously published study on the effect of constant heat stress in *C. magur* (Singh *et al.*, 2025) and gill transcriptome data of the treatment were generated and analysed using the steps mentioned below.

1. Library Construction and High-Throughput Sequencing

Total RNA was isolated from gill tissue using the QIAamp RNA Isolation Kit as per the manufacturer's instructions. RNA quantity and quality were assessed using a Qubit® 4.0 fluorometer and integrity was evaluated on a 1% agarose gel. For sequencing, paired-end libraries were prepared with the NEBNext® Ultra™ RNA Library Preparation Kit for Illumina,

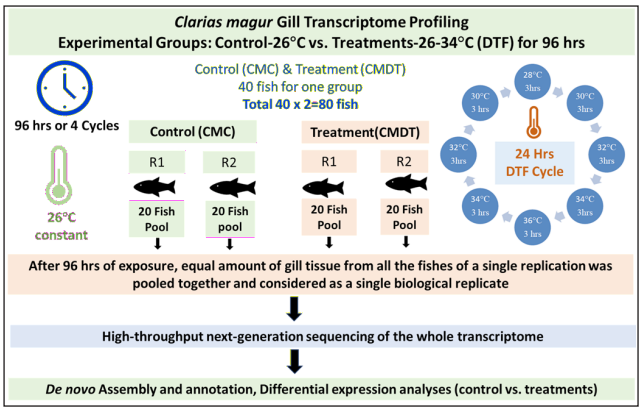


Figure 1: Schematic representation of the experimental design used for gill transcriptome profiling in *Clarias magur*. Fish were exposed either to constant ambient temperature (26 °C; control) or to diurnal thermal fluctuation (DTF; 28-34 °C cyclic exposure at 3 h intervals) for 96 h. Gill samples from pooled biological replicates were processed for RNA sequencing, *de novo* transcript reconstruction and differential-expression analysis.

using 1000 ng of RNA per sample. The workflow included mRNA enrichment, fragmentation, first- and second-strand cDNA synthesis, end repair, 3' adenylation, adapter ligation and PCR enrichment. Library size distribution and quality were evaluated using an Agilent 4150 TapeStation and HSD1000 Screentape®. After validation, libraries were prepared for cluster formation and sequencing. Sequencing was performed on an Illumina NovaSeq 6000 platform with 2 × 150-bp paired-end reads.

2. De novo Assembly and Annotation

Because of the Absence of a reference genome assembly for *C. magur*, the transcripts need to be assembled into transcripts through a *de novo* transcriptome assembly approach. The high-quality reads were assembled into transcripts using the RNA-SPAdes assembler using default parameters. Transcripts were assembled separately for each condition, *i.e.*, control and treatment. The transcripts of both replicates of each condition were merged and identical contigs were removed and unitig assemblies were created using the evidential gene tr2cds workflow. Evidential gene tr2cds workflow used CD-HIT and exonerate in the background with a custom script to do the analysis and to also predict CDS and amino acid sequences from the transcriptome. Also, transcripts with the normalized expression, which were normalised using transcript per million formats (from salmon) of < 2 TPM, were removed from assembly as there were not enough reads to support the transcripts. This produces the final assembly for treatment. Also, transcripts with annotations found in the NCBI's non-redundant (NR) database were retained in the final assembly.

3. Differential Expression Analysis

For differential expression, CMDTR1 and CMDTR2 were compared with CMCR1 and CMCR2. Transcript abundance was estimated with Salmon and the resulting normalized counts were analysed in DESeq2 within the R environment. Genes exhibiting an adjusted *p*-value (false discovery rate, FDR) < 0.05 were considered significantly differentially expressed. Furthermore, transcripts with log2 fold-change (log2 FC) > 2 were categorized as significantly upregulated, whereas transcripts with log2 FC < -2 were considered significantly downregulated. A volcano plot was generated from log2 fold-change values and -log10 adjusted p-values to display the overall distribution of regulated transcripts. Significantly upregulated and downregulated genes were highlighted separately to identify transcripts exhibiting strong transcriptional responses under diurnal heat stress conditions. The volcano plot enabled rapid identification of highly significant DEGs associated with thermal adaptation. To examine expression patterns among samples, a heatmap was generated using the top 50 significantly DEGs identified from DESeq2 analysis. Normalized expression values of selected genes were subjected to hierarchical clustering to evaluate similarities among samples and expression profiles among genes. The heatmap was visualized using the R-based visualization packages, where relative expression levels were represented using a colour gradient ranging from low to high expression.

Functional interpretation of the DEGs included Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. GO enrichment analysis was carried out using annotated transcripts against the Gene Ontology database. The significantly enriched GO terms were categorized under Biological Process (BP) and the top 20 enriched biological processes were selected based on adjusted *p*-value significance. Enrichment results were visualised graphically to identify the major cellular and physiological processes affected by thermal stress. KEGG enrichment analysis was conducted with Cluster Profiler in R. Because *C. magur* lacks a complete reference genome, *Danio rerio* was used as the reference organism for pathway annotation. Over-representation testing was performed separately for upregulated and downregulated transcripts using Fisher's exact test and the 20 pathways with the most significant adjusted p-values were retained for visualization.

Results and Discussion

Comparative transcriptomic analysis of gill tissues revealed pronounced molecular alterations in *C. magur* exposed to DTF compared with the control group maintained at ambient temperature. The results obtained from sequencing quality assessment, differential gene expression and functional enrichment analyses are presented below in a section-wise manner.

RNA Sequencing and Transcriptome Assembly

High-throughput RNA sequencing generated high-quality transcriptomic data from the gill tissues of control and diurnal heat-stressed *Clarias magur*. A total of four samples comprising two control replicates (CMR1 and CMR2) and two treatment replicates (CMDTR1 and CMDTR2) were analysed. Quality filtering revealed that more than 99.9% of the reads in all samples were retained as high-quality reads, indicating excellent sequencing quality. The total number of high-quality reads obtained ranged from 29.8 to 54.6 million

per sample. Due to the absence of a reference genome for *C. magur*, *de novo* transcriptome assembly was performed using the RNA-SPAdes assembler. The initial assembly generated 156,640 transcripts, which were subsequently refined to 103,403 transcripts after removal of redundant and low-expression transcripts using the Evidential Gene tr2cds pipeline. The final transcriptome assembly had an N50 value of 1,622 bp with a GC content of 49.70%, indicating good assembly quality and transcript continuity.

Principal Component Analysis (PCA)

PCA demonstrated a clear distinction between control and DTF-exposed group (Figure 2). The PC1 explained 98% of the total variance and distinctly separated the DTF-exposed samples from the control, indicating that thermal treatment was the major factor influencing transcriptomic variation. Control samples clustered closely together on one side of the PCA axis, whereas DTF-exposed samples formed a separate cluster, demonstrating high reproducibility within groups and marked transcriptional divergence between control and treatment conditions. The PC2 accounted for only 1% of the variance, indicating minimal variation unrelated to treatment effects.

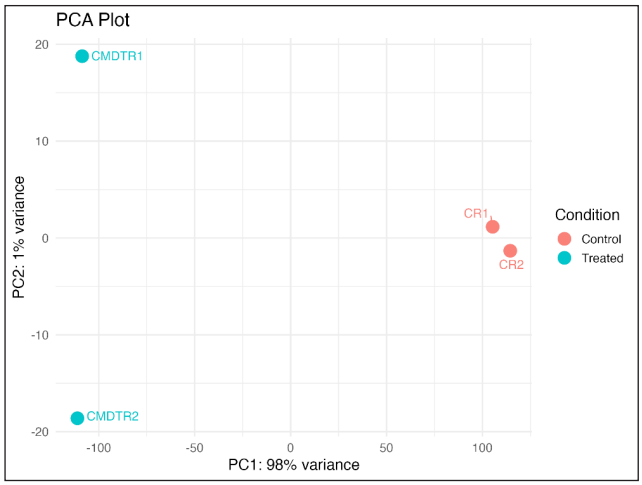


Figure 2: PCA showing the clustering pattern of transcriptomic profiles from gill tissues of control and DTF-stressed *C. magur.* PC1 accounted for 98% of the total variance and clearly separated the control and treatment groups.

Differential Gene Expression Analysis

DESeq2 analysis detected 90,574 significantly regulated transcripts when DTF-exposed fish were compared with controls. Of these, 6,629 showed increased expression and 83,945 showed decreased expression. Within the same dataset, 746 transcripts displayed more than 10-fold upregulation and 36,907 showed more than 10-fold downregulation.

Volcano Plot Analysis

The volcano plot illustrated a strongly asymmetric response to DTF (Figure 3). Regulated transcripts were distributed over a broad range of statistical significance and fold change, with the downregulated component substantially larger than the upregulated component. These findings suggest that diurnal thermal stress predominantly suppressed the transcriptional responses rather than activating them. Several genes displayed extremely high statistical significance along with pronounced expression changes, demonstrating a robust molecular response of gill tissue to DTF.

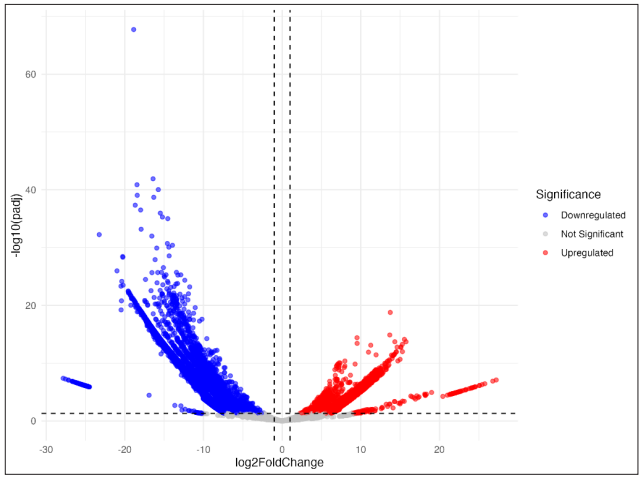


Figure 3: Volcano plot showing DEGs in gill tissues of C. *magur* exposed to DTF stress compared with the control group. The horizontal axis (X) shows log2 fold change and the vertical axis (Y) shows -log10 adjusted p-value; colours distinguish significantly regulated and non-significant transcripts.

Heatmap of Differentially Expressed Genes

Expression profiling of the top 50 differentially expressed genes further highlighted the distinct transcriptional patterns between control and DTF-exposed fish (Figure 4). The heatmap demonstrated strong clustering of samples according to treatment condition. Most of the highly significant genes exhibited low expression levels in the DTF group as compared to the control. Hierarchical clustering analysis showed high similarity among biological replicates within each group and clear separation between control and treatment samples.

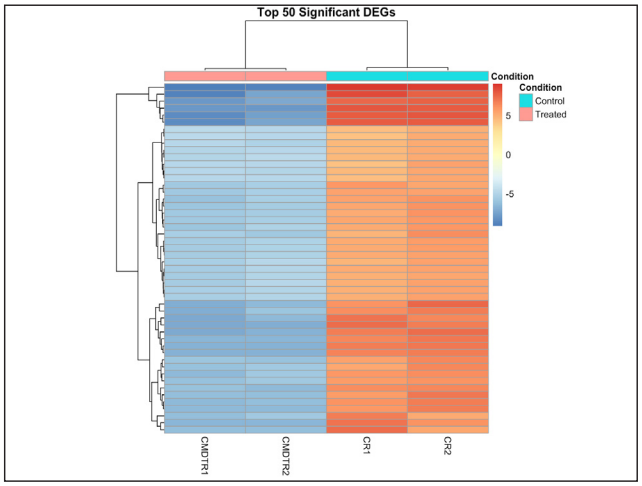


Figure 4: Heatmap of the 50 most significant DEGs in control and DTF-exposed gill samples of C. *magur.* Rows represent transcripts and columns represent biological samples. Hierarchical clustering illustrates the expression separation between the two experimental conditions.

1. Downregulated Transcripts

Analysis of the top 50 downregulated transcripts revealed substantial suppression of genes associated with cellular signalling, cytoskeletal organisation, epithelial integrity, protein turnover, metabolism and membrane transport in the gill tissues of DTF compared with the control group. Several signalling and regulatory genes exhibited reduced expression under DTF conditions, including *fibroblast growth factor receptor 1a (FGFR1a)*, *Rho guanine nucleotide exchange factor 1*, *ral guanine nucleotide dissociation stimulator-like 2*, *Kruppel-like factor 4* and *probable serine/threonine-protein kinase irlA*. Suppression of these transcripts suggests altered intracellular signaling and transcriptional regulation during thermal stress adaptation. A pronounced downregulation of genes related to cytoskeletal organization and structural maintenance was observed in heat-stressed fish. These included *filamin A*, *ankyrin 3b*, *myosin heavy chain 9b*, *myosin heavy polypeptide 1.1-like*, *keratin 5*, *keratin 15*, *keratin 91*, *keratin 94*, *collagen type I alpha 1a*, *collagen type VI alpha 2* and *catenin delta 1*. Reduced expression of these genes indicates substantial remodelling of gill epithelial architecture and cytoskeletal dynamics under fluctuating thermal conditions. Genes associated with membrane transport and epithelial function, such as *aquaporin 3a*, *solute carrier family 4-member 1a*, *mucin 5.1*, *transmembrane serine protease 13a* and *platelet and endothelial cell adhesion molecule 1b*, were also significantly downregulated, suggesting alterations in osmoregulatory processes, epithelial permeability and cell adhesion mechanisms in response to DTF exposure. Several transcripts involved in protein degradation and post-translational regulation showed reduced abundance, including *cathepsin D*, *calpastatin*, *HECT and RLD domain-containing E3 ubiquitin protein ligase 3, F-box protein 46* and *serpin peptidase inhibitor clade B member 14*. Additionally, genes associated with translational regulation and intracellular trafficking such as *eukaryotic translation initiation factor 3 subunit 6 interacting protein*, *vacuolar protein sorting 4 homolog B* and *RAB11 family interacting protein 1a* were downregulated under thermal stress conditions. Metabolism- and stress-related genes, including *malate dehydrogenase 2*, *isocitrate dehydrogenase 2*, *delta (4)-desaturase sphingolipid 2* and *prosaposin*, also showed reduced expression in heat-treated fish, indicating modulation of mitochondrial metabolism and lipid-associated cellular processes during thermal stress. Furthermore, suppression of *major vault protein* and *lymphocyte antigen 6 family member M5* suggests possible alterations in cellular defence and immune-related pathways.

2. Upregulated Transcripts

Analysis of the top upregulated transcripts revealed that diurnal thermal fluctuation (DTF) strongly induced genes associated with protein synthesis, energy metabolism, RNA modification and cellular stress adaptation. Several ribosome-associated transcripts showed marked upregulation under DTF exposure, including *ribosomal protein SA*, *ribosomal protein L10*, *ribosomal protein L18*, *ribosomal protein S13* and *ribosomal protein S4 X-linked*. The elevated expression of these genes indicates enhanced translational activity and increased protein synthesis requirements during thermal stress adaptation. Genes associated with RNA processing and protein folding were also significantly upregulated in heat-stressed fish. These included *dihydrouridine synthase 3-like* and *peptidylprolyl isomerase Ab (cyclophilin A)*, suggesting activation of RNA modification and protein-folding mechanisms to maintain cellular homeostasis under fluctuating thermal conditions. Several metabolic genes exhibited increased expression in response to DTF treatment. Upregulation of *carbamoyl-phosphate synthase 1*, *mitochondrial*, *transketolase a*, *inositol monophosphatase 1* and *hydroxysteroid (17-beta) dehydrogenase 8* indicates enhanced metabolic adjustment, amino acid metabolism, carbohydrate metabolism and energy production in gill tissue during thermal stress exposure.

Gene Ontology (GO) Enrichment Analysis

Gene Ontology (GO) enrichment analysis of differentially expressed genes identified significant enrichment of multiple biological processes in the gill tissues of *Clarias magur* exposed to diurnal thermal fluctuation (DTF) compared with the control group (Figure 5). Among the enriched GO terms, *translation* exhibited the highest gene ratio, followed by *mRNA metabolic process*, *protein modification by small protein conjugation* and *DNA damage response*. Several GO terms associated with protein degradation and turnover were significantly enriched, including *protein ubiquitination*, *ubiquitin-dependent protein catabolic process*, *proteasomal protein catabolic process*, *modification-dependent macromolecule catabolic process* and *modification-dependent protein catabolic process*. Processes related to RNA metabolism and processing such as *mRNA processing*, *RNA splicing*, *rRNA processing*, *rRNA metabolic process*, *tRNA metabolic process* and *RNA splicing via transesterification reactions* were also significantly represented. Additionally, biological processes associated with ribosome assembly and nucleoprotein synthesis, including *ribosome biogenesis* and *ribonucleoprotein complex biogenesis*, were enriched under DTF exposure. Other significantly enriched GO terms included *DNA repair* and *Golgi vesicle transport*. The enriched biological processes exhibited highly significant adjusted *p*-values and substantial gene counts, indicating extensive modulation of transcriptional and protein regulatory processes in response to diurnal heat stress.

KEGG Pathway Enrichment Analysis

KEGG pathway enrichment analysis identified significant enrichment of multiple pathways in the gill tissues of *Clarias magur* exposed to diurnal thermal fluctuation (DTF) compared with the control group (Figure 6). The top enriched pathways included *IgSF CAM signaling*, *MAPK signaling pathway* and *Endocytosis*, which exhibited the highest gene ratios among all significantly enriched pathways. Several pathways associated with cytoskeletal organization and cell adhesion were significantly enriched, including *Cytoskeleton in muscle cells*, *Regulation of actin cytoskeleton*, *Focal*

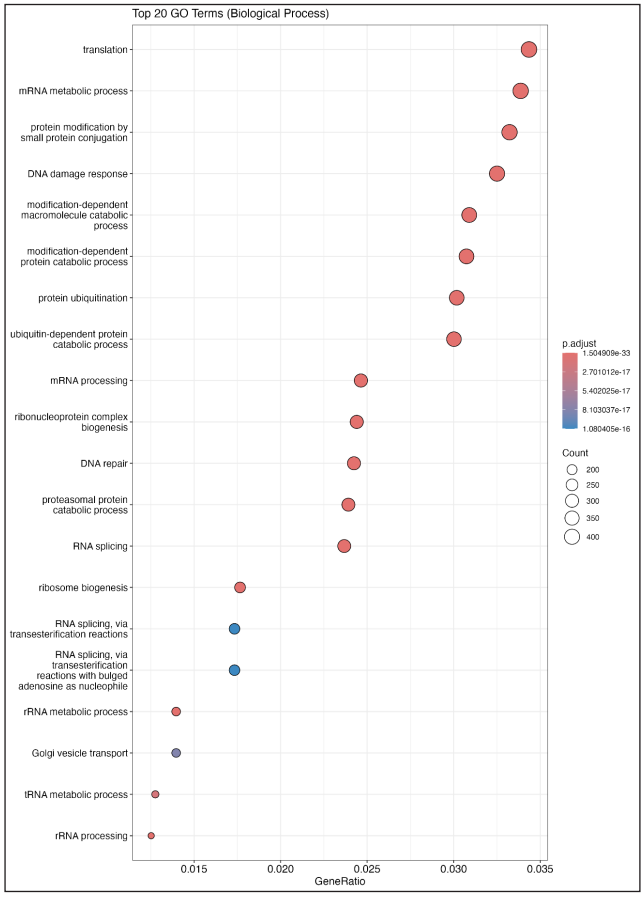


Figure 5: Bubble plot of the 20 most significantly enriched GO Biological Process terms among DTF-responsive transcripts. Bubble size denotes the number of associated genes and colour represents adjusted-p-value significance.

adhesion, *Tight junction* and *Integrin signaling*. Pathways related to intracellular trafficking and protein turnover such as *Autophagy*, *Protein processing in endoplasmic reticulum*, *Endocytosis* and *Ubiquitin-mediated proteolysis* were also significantly represented. Immune- and stress-associated pathways including *Salmonella infection*, *Herpes simplex virus 1 infection*, *NOD-like receptor signaling pathway* and *FoxO signaling pathway* showed significant enrichment in heat-treated fish. Additionally, pathways related to cellular growth and metabolic regulation, including *mTOR signaling pathway*, *Wnt signaling pathway*, *Cell cycle*, *Cellular senescence* and *Efferocytosis*, were enriched under DTF exposure. The enriched pathways exhibited highly significant adjusted *p*-values and gene counts, indicating extensive transcriptomic modulation in response to diurnal heat stress in the gill tissue of *C. magur*.

The present study provides comprehensive transcriptomic insights into the gill-mediated molecular responses of *C. magur* under diurnal thermal fluctuation (DTF) conditions. Comparative analysis between control and DTF-exposed fish revealed extensive transcriptional modulation, indicating that cyclic heat stress strongly alters cellular, metabolic and structural processes in gill tissue. The clear separation between control and treatment groups observed in PCA analysis further confirms that DTF acts as a major driver of transcriptomic variation in *C. magur*. A notable observation in the present study was the predominance of downregulated transcripts compared with upregulated genes under DTF exposure. Such large-scale transcriptional suppression suggests that prolonged cyclic heat exposure may reduce multiple energy-demanding physiological processes to maintain cellular homeostasis during thermal stress. Similar large-scale transcriptomic repression under elevated temperature has been reported in several teleost fishes exposed to heat stress (Singh *et al.*, 2025). In Atlantic salmon, heat stress resulted in extensive suppression of metabolic and structural genes associated with cellular maintenance and tissue organization (Misk *et al.*, 2026). Similarly, heat stress in largemouth bass resulted in suppression of immune-response genes and reduced disease resistance under elevated temperature conditions (Yang *et al.*, 2022). The predominance of downregulated genes observed in the present study, particularly those associated with cytoskeletal organization, epithelial integrity, intracellular signalling, membrane transport and metabolism, suggests that prolonged cyclic thermal exposure may suppress several energy-intensive physiological pathways while reallocating cellular resources toward essential stress adaptation and survival processes. The concurrent enrichment of pathways related to ubiquitin-mediated proteolysis, autophagy and protein processing further indicates that cellular homeostasis during DTF exposure may rely more on protein quality control and stress mitigation mechanisms than on active cellular growth and structural maintenance.

The marked downregulation of cytoskeleton-associated genes, including *filamin A*, *myosin heavy chains*, *keratins*, *collagens* and *catenin delta 1*, indicates substantial remodeling of gill epithelial architecture during DTF exposure. The gill is highly sensitive to environmental stress because of its direct interaction with the surrounding aquatic environment. Structural reorganization of cytoskeletal proteins under heat stress has previously been documented in fish gill tissues and is considered an adaptive response to maintain epithelial integrity and cellular stability under fluctuating environmental conditions. Similar alterations in cytoskeletal and adhesion-related pathways were reported in zebrafish exposed to thermal stress (Dettleff *et al.*, 2024). Several membrane transport and epithelial function-related genes, including *aquaporin 3a*, *solute carrier family 4-member 1a* and *mucin 5.1*, were also significantly downregulated under DTF conditions. Aquaporins are known to regulate water permeability and osmoregulatory balance in fish gills and altered aquaporin expression has been associated with environmental stress adaptation in teleosts (Yang *et al.*, 2023). Downregulation of epithelial transport-associated genes in the present study may therefore reflect physiological adjustments to maintain ionic and osmotic balance during repeated thermal fluctuations.

The present study also identified suppression of several genes involved in immune signaling and cellular defense, including *FGFR1a*, *lymphocyte antigen 6 family member M5*, *major vault protein* and *platelet and endothelial cell adhesion*

molecule 1b. Similar suppression of immune-associated pathways was previously observed in fishes, including *C. magur* (Beemelmanns *et al.*, 2021; Sapozhnikova *et al.*, 2024; Meng *et al.*, 2025; Singh *et al.*, 2025), where heat stress weakened immune responses and increased oxidative stress. Upregulation of ribosomal proteins (*RPL10*, *RPL18*, *RPS13*, *RPS4X*) and RNA-modifying genes such as *dihydrouridine synthase 3-like* and *cyclophilin A* suggests enhanced translational and protein-folding activity in response to DTF. Increased ribosomal biogenesis and translational activation are common cellular responses during stress adaptation and recovery processes (Zhao *et al.*, 2024; Turner *et al.*, 2025). Cyclophilin proteins, in particular, function as molecular chaperones and contribute to protein folding under stressful conditions. Similar induction of ribosome-associated pathways has been reported during heat adaptation in teleost fishes exposed to fluctuating environmental temperatures. GO enrichment analysis further supported the transcript-level observations by demonstrating significant enrichment of biological processes associated with translation, mRNA metabolism, RNA splicing, ubiquitin-dependent protein catabolism, ribosome biogenesis and DNA damage response (Zhou *et al.*, 2023; Zhao *et al.*, 2024). KEGG pathway enrichment analysis revealed significant modulation of stress-responsive signaling pathways including MAPK signaling, mTOR signaling, FoxO signaling, endocytosis, autophagy and ubiquitin-mediated proteolysis. The MAPK pathway is a major regulator of cellular stress responses and has been widely implicated in thermal adaptation, apoptosis and oxidative stress regulation in fish (Yan *et al.*, 2022; Yu *et al.*, 2022). Likewise, mTOR and FoxO signalling pathways are central regulators of metabolism, cellular survival and stress adaptation under adverse environmental conditions (Ma *et al.*, 2023; Liu *et al.*, 2025).

We also compared the present study on diurnal thermal fluctuation (26-34 °C cyclic exposure) with our previous study under constant heat exposure at 34 °C. Heat stress induces oxidative stress and weakens the immune system in catfish *C. magur* (Singh *et al.*, 2025), which primarily demonstrated severe oxidative stress, tissue damage and suppression of immune competence. A major distinction between the two thermal regimes appears to be the nature of cellular adaptation. Under constant heat exposure, fish experienced sustained thermal burden without recovery intervals, leading to persistent oxidative stress, immune dysregulation and physiological impairment. The earlier study reported elevated oxidative stress biomarkers, histopathological alterations and transcriptomic signatures associated with cellular injury and compromised defence mechanisms. In contrast, the diurnal thermal fluctuation (DTF) regime in the present study induced stronger enrichment of adaptive and regulatory pathways involved in protein turnover, RNA processing, translational regulation and signalling networks such as MAPK and mTOR pathways. These findings suggest activation of dynamic compensatory mechanisms that may help maintain cellular homeostasis during cyclic thermal stress. Another important difference lies in the transcriptomic balance between stress injury and stress adaptation. Constant exposure to 34 °C appeared to trigger chronic stress-associated damage pathways, whereas fluctuating temperature conditions induced coordinated transcriptional responses potentially linked with repeated cycles of stress and recovery. The enrichment of pathways related to cytoskeletal organization, endocytosis and ribosome biogenesis in the DTF study indicates active cellular remodeling and metabolic adjustment rather than only pathological stress responses. The predominance of upregulated genes observed during DTF exposure further supports the hypothesis that fluctuating thermal regimes stimulate broad adaptive transcriptional activation. In natural aquatic systems, fish are more frequently exposed to cyclic daily temperature oscillations than to continuous constant temperatures. Therefore, the transcriptomic responses observed under DTF conditions may better represent ecologically relevant thermal adaptation mechanisms in *C. magur*. Collectively, comparison of the two studies indicates that constant heat exposure mainly promotes oxidative damage and immune suppression, whereas diurnal thermal fluctuation induces more complex molecular plasticity involving stress signalling, translational control and cellular adaptation pathways.

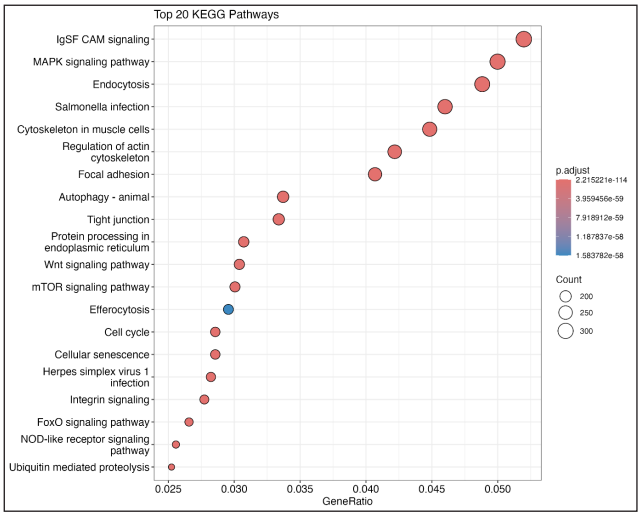


Figure 6: Bubble plot of the 20 most significantly enriched KEGG pathways associated with DTF-responsive transcripts in *C. magur* gill. Bubble size indicates gene count and colour represents enrichment significance based on adjusted p-values.

Conclusion

The present study demonstrates that diurnal thermal fluctuation (DTF) induces extensive transcriptomic reprogramming in the gill tissue of *Clarias magur*, characterized predominantly by large-scale downregulation of genes associated with cytoskeletal organization, membrane transport, metabolism and immune-related functions, along with selective upregulation of genes involved in translation, protein folding and stress adaptation. Enrichment of GO terms and KEGG pathways related to RNA metabolism, ubiquitin-mediated proteolysis, MAPK, mTOR, FoxO signaling, autophagy and endocytosis indicates activation of complex molecular mechanisms

underlying thermal adaptation under cyclic heat exposure. Comparative analysis with our previous constant heat stress study further suggests that DTF induces dynamic adaptive and compensatory responses rather than the severe oxidative damage and immune suppression observed under continuous heat exposure. Overall, the findings provide novel insights into the molecular basis of thermal resilience in *C. magur* and highlight the importance of considering fluctuating thermal regimes in climate change and aquaculture stress biology studies.

Financial Support

The present study was financially supported by ICAR-NICRA (Indian Council of Agricultural Research-National Innovations on Climate Resilient Agriculture), bearing Grant No.: 2-13(44)/21-22/NICRA.

Ethical Statement

The authors declare that no generative artificial intelligence tools were used in drafting or preparing this manuscript. The manuscript was written, interpreted and revised solely by the authors, who take full responsibility for its content.

References

APHA, 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st Edition. American Public Health Association. Standard, Washington, DC, USA. p. 1220.

Barbarossa, V., Bosmans, J., Wanders, N., King, H., Bierkens, M.F.P., Huijbregts, M.A.J., Schipper, A.M., 2021. Threats of global warming to the world's freshwater fishes. *Nature Communication* 12, 1701. DOI: https://doi.org/10.1038/s41467-021-21655-w

Beemelmanns, A., Zanuzzo, F.S., Sandrelli, R.M., Rise, M.L., Gamperl, A.K., 2021. The Atlantic salmon's stress- and immune-related transcriptional responses to moderate hypoxia, an incremental temperature increase, and these challenges combined. *G3 (Bethesda)* 11(7), jkab102. DOI: https://doi.org/10.1093/g3journal/jkab102

Cappello, T., Brandao, F., Guilherme, S., Santos, M.A., Maisano, M., Mauceri, A., Canario, J., Pacheco, M., Pereira, P., 2016. Insights into the mechanisms underlying mercury-induced oxidative stress in gills of wild fish (*Liza aurata*) combining ^{1}H NMR metabolomics and conventional biochemical assays. *Science of The Total Environment* 548-549, 13-24. DOI: https://doi.org/10.1016/j.scitotenv.2016.01.008

Chang, C.H., Huang, J.J, Yeh, C.Y., Tang, C.H., Hwang, L.Y., Lee T.H., 2018. Salinity effects on strategies of glycogen utilization in livers of euryhaline milkfish (*Chanos chanos*) under hypothermal stress. *Frontiers in Physiology* 9, 00081. DOI: https://doi.org/10.3389/fphys.2018.00081

Clow, T.M., Craig, P.M., 2026. Diel temperature fluctuations alter molecular, biochemical and whole-animal responses to thermal stress and hypoxia in adult zebrafish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 318, 112017. DOI: https://doi.org/10.1016/j.cbpa.2026.112017

de Alba, G., Lopez-Olmeda, J.F., Sanchez-Vazquez, F.J., 2021. Rearing temperature conditions (constant vs. thermocycle) affect daily rhythms of thermal tolerance and sensing in zebrafish. *Journal of Thermal Biology* 97, 102880. DOI: https://doi.org/10.1016/j.jtherbio.2021.102880

Dettleff, P., Zuloaga, R., Fuentes, M., Gonzalez, P., Aedo, J., Estrada, J.M., Molina, A., Valdes, J.A., 2020. Physiological and molecular responses to thermal stress in red cusk-eel (*Genypterus chilensis*) juveniles reveals atrophy and oxidative damage in skeletal muscle. *Journal of Thermal Biology* 94, 102750. DOI: https://doi.org/10.1016/j.jtherbio.2020.102750

Dettleff, P., Toloza, C., Fuentes, M., Aedo, J., Zuloaga, R., Estrada, J.M., Molina, A., Valdes, J.A., 2024. Gills de novo assembly reveals oxidative stress, unfolded protein and immune response on red cusk-eel (*Genypterus chilensis*) under thermal stress. *Marine Environmental Research* 196, 106440. DOI: https://doi.org/10.1016/j.marenvres.2024.106440

Duan, C., Zhou, D., Feng, R., Li, X., Yang, L., Li, X., Li, G., Chen, H., Liao, Y., Tian, C., 2024. Long-term thermal acclimation enhances heat resistance of Hong Kong catfish (*Clarias fuscus*) by modulating gill tissue structure, antioxidant capacity and immune metabolic pathways. *Ecotoxicology and Environmental Safety* 284, 116930. DOI: https://doi.org/10.1016/j.ecoenv.2024.116930

Duan, C., Liu, Y., Liu, F., Zhou, D., Zhang, Y., Li, G., Huang, C., Pan, C., Chen, H., Tian, C., 2026. Gill adaptation of the Hong Kong catfish (*Clarias fuscus*) to chronic heat stress: Tissue remodeling, enhanced antioxidant defense and immune metabolism regulation. *Ecology and Evolution* 16(4), e73470. DOI: https://doi.org/10.1002/ece3.73470

Genin, A., Levy, L., Sharon, G., Raitsos, D.E., Diamant, A., 2020. Rapid onsets of warming events trigger mass mortality of coral reef fish. *Proceeding of National Academy of Science (PNAS)* 117(41), 25378-25385. DOI: https://doi.org/10.1073/pnas.2009748117

Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85(1), 97-177. DOI: https://doi.org/10.1152/physrev.00050.2003

Guo, Y., Dong, C., Peng, H., Zhang, J., He, J., Gao, Y., Dai, X., Zhao, S., Chu, Z., Zhao, B., Du, W., 2026. Behavioral responses and transcriptional dynamics of the stream fish (*Acrossocheilus fasciatus*) under temperature change. *Water Biology and Security* 5(1), 100413. DOI: https://doi.org/10.1016/j.watbs.2025.100413

Khieokhajonkhet, A., Sangphrom, S., Aeksiri, N., Tatsapong, P., Wuthijaree, K., Kaneko, G., 2022. Effects of long-term exposure to high temperature on growth performance, chemical composition, hematological and histological changes and physiological responses in hybrid catfish [♂*Clarias gariepinus* (Burchell, 1822) × ♀*C. macrocephalus* (Günther, 1864)]. *Journal of*

Thermal Biology 105, 103226. DOI: https://doi.org/10.1016/j.jtherbio.2022.103226

Kim, J.H., Park, H.J., Kim, D.H., Oh, C.W., Lee, J.S., Kang, J.C., 2019. Changes in hematological parameters and heat shock proteins in juvenile sablefish depending on water temperature stress. *Journal of Aquatic Animal Health* 31(2), 147-153. DOI: https://doi.org/10.1002/aah.10061

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, 103251. DOI: https://doi.org/10.1016/j.jtherbio.2022.103251

Liu, Y., Mai, K., Zhang, W., 2025. mTOR signaling in aquaculture animals: A central nutrient-sensing hub orchestrating growth, metabolism, health and quality. *Reviews in Aquaculture* 17(4), e70068. DOI: https://doi.org/10.1111/raq.70068

Ma, F., Zhao, L., Ma, R., Wang, J., Du, L., 2023. FoxO signaling and mitochondria-related apoptosis pathways mediate tsinlinglenok trout (*Brachymystax lenok tsinlingensis*) liver injury under high temperature stress, *International Journal of Biological Macromolecules* 251, 126404. DOI: https://doi.org/10.1016/j.ijbiomac.2023.126404

Meng, Q., Tao, Y., Peng, Y., Guo, J., Xun, C., Chen, X., Li, F., Huang, H., Zhou, F., Li J., 2025. Long-term heat stress triggers immune activation and cell death remodeling in the brain of largemouth bass (*Micropterus salmoides*). *Animals (Basel)* 15(21), 3067. DOI: https://doi.org/10.3390/ani15213067

Misk, E., Whyte, S.K., Purcell, S.L., Groves, L., Michaud, D., Cai, W.C., Langille, B.L., Garber, A.F., Fast M.D., 2026. Thermal modulation of genetic resistance to infectious salmon anemia virus: Transcriptome analyses of Atlantic salmon families (*Salmo salar*) at 10 °C and 20 °C. *Comparative Immunology Reports* 10, 200274. DOI: https://doi.org/10.1016/j.cirep.2026.200274

Mitra, A., Abdel-Gawad, F.K., Bassem, S., Barua, P., Assisi, L., Parisi, C., Temraz, T.A., Vangone, R., Kajbaf, K., Kumar, V., Guerriero, G., 2023. Climate change and reproductive biocomplexity in fishes: Innovative management approaches towards sustainability of fisheries and aquaculture. *Water* 15(4), 725. DOI: https://doi.org/10.3390/w15040725

Mokhtar, D.M., Zaccone, G., Alesci, A., Kuciel, M., Hussein, M.T., Sayed, R.K.A., 2023. Main components of fish immunity: An overview of the fish immune system. *Fishes* 8(2), 93. DOI: https://doi.org/10.3390/fishes8020093

Qin, H., Long, Z., Huang, Z., Ma, J., Kong, L., Lin, Y., Lin, H., Zhou, S., Li, Z., 2023. A Comparison of the physiological responses to heat stress of two sizes of juvenile spotted seabass (*Lateolabrax maculatus*). *Fishes* 8(7), 340. DOI: https://doi.org/10.3390/fishes8070340

Roychowdhury, P., Aftabuddin, M., Pati, M.K., 2021. Thermal stress-induced oxidative damages in the liver and associated death in fish, *Labeo Rohita*. *Fish Physiology and Biochemistry* 47(1), 21-32. DOI: https://doi.org/10.1007/s10695-020-00880-y

Sapozhnikova, Y.P., Koroleva, A.G., Sidorova, T.V., Potapov, S.A., Epifantsev, A.A., Vakhteeva, E.A., Tolstikova, L.I., Glyzina, O.Y., Yakhnenko, V.M., Cherezova, V.M., Sukhanova, L.V., 2024. Transcriptional rearrangements associated with thermal stress and preadaptation in baikal whitefish (*Coregonus baicalensis*). *Animals* 14(21), 3077. DOI: https://doi.org/10.3390/ani14213077

Singh, M., Barman, A.S., Devi, A.L., Devi, A.G., Pandey, P.K., 2019. Iron mediated hematological, oxidative and histological alterations in freshwater fish *Labeo rohita*. *Ecotoxicology and Environmental Safety* 170, 87-97. DOI: https://doi.org/10.1016/j.ecoenv.2018.11.129

Singh, M., Saini, V.P., Meena, L.L., 2025. Heat stress induces oxidative stress & weakens the immune system in catfish *Clarias magur*: Evidence from physiological, histological, & transcriptomic analyses. *Fish & Shellfish Immunology* 161, 110294. DOI: https://doi.org/10.1016/j.fsi.2025.110294

Turner, L.A., Easton, A.A., Ferguson, M.M., Danzmann, R.G., 2025. Differences in gene expression between high and low tolerance rainbow trout (*Oncorhynchus mykiss*) to acute thermal stress. *PLoS One* 20(1), e0312694. DOI: https://doi.org/10.1371/journal.pone.0312694

Wen, B., Jin, S.R., Chen, Z.Z., Gao, J.Z., 2018. Physiological responses to cold stress in the gills of discus fish (*Symphysodon aequifasciatus*) revealed by conventional biochemical assays and GC-TOF-MS metabolomics. *Science of The Total Environment* 640-641, 1372-1381. DOI: https://doi.org/10.1016/j.scitotenv.2018.05.401

Yan, W., Qiao, Y., He, J., Qu, J., Liu, Y., Zhang, Q., Wang, X., 2022. Molecular mechanism based on histopathology, antioxidant system and transcriptomic profiles in heat stress response in the gills of Japanese flounder. *International Journal of Molecular Sciences* 23(6), 3286. DOI: https://doi.org/10.3390/ijms23063286

Yang, C., Dong, J., Sun, C., Li, W., Tian, Y., Liu, Z., Gao, F., Ye, X., 2022. Exposure to heat stress causes downregulation of immune response genes and weakens the disease resistance of *Micropterus salmoides*. *Comparative Biochemistry and Physiology: Part D Genomics Proteomics* 43, 101011. DOI: https://doi.org/10.1016/j.cbd.2022.101011

Yang, S., Li, D., Feng, L., Zhang, C., Xi, D., Liu, H., Yan, C., Xu, Z., Zhang, Y., Li, Y., Yan, T., He, Z., Wu, J., Gong, Q., Du, J., Huang, X., Du, X., 2023. Transcriptome analysis reveals the high temperature induced damage is a significant factor affecting the osmotic function of gill tissue in Siberian sturgeon (*Acipenser baerii*). *BMC Genomics* 24(1), 2. DOI: https://doi.org/10.1186/s12864-022-08969-9

Yu, J., Li, Y., Zhang, Z., Chen, Y., Yu, H., Mo, H., Wang, L., 2022. Genome-wide identification of MKK and MAPK gene families and their expression analysis under abiotic stress in largemouth bass (*Micropterus salmoides*). *Aquaculture* 561, 738688. DOI: https://doi.org/10.1016/j.aquaculture.2022.738688

Zhao, G., Liu, Z., Quan, J., Lu, J., Li, L., Pan, Y., 2024. Ribosome profiling and RNA sequencing reveal translation and transcription regulation under acute heat stress in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) liver. *International Journal of Molecular Science* 25(16), 8848. DOI: https://doi.org/10.3390/ijms25168848

Zhou, C.Q., Ka, W., Yuan, W.K., Wang, J.L., 2021. The effect of acute heat stress on the innate immune function of rainbow trout based on the transcriptome. *Journal of Thermal Biology* 96, 102834. DOI: https://doi.org/10.1016/j.jtherbio.2021.102834

Zhou, T., Jia, C., Meng, Q., Xu, D., Zhang, Z., Zhu, F., Zhao, Y., Sun, R., Yang, Y., Chen, S., 2023. Transcriptome-based analysis of the liver response mechanism of black porgy (*Acanthopagrus schlegelii*) to stocking density. *Fishes* 8(7), 356. DOI: https://doi.org/10.3390/fishes8070356