

# Differential Responses of Golden Trout and Rainbow Trout Livers to Simulated Transport Stress: A Comparative Analysis of Antioxidant, Energy Metabolism, and Transcriptome Profiles

Zixuan Zhong, Yujun Kang\*, Chunmei Zhang, Donglin Liu, Jianfu Wang, Zhe Liu, Jingqiang Huang College of Animal Science & Technology, Gansu Agricultural University, Lanzhou, China

## Introduction

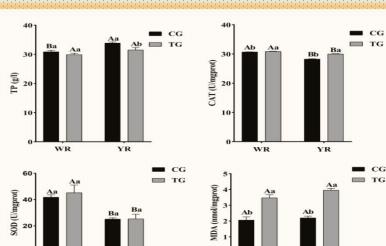
- Live fish transportation is vital for the global aquatic product supply chain but causes stress that impairs fish health and quality, leading to economic losses.
- Golden trout and rainbow trout are key cold-water aquaculture species, yet their phenotypic differences may affect stress adaptation during transport.
- Transportation conditions impact fish antioxidant capacity, energy metabolism, muscle quality, and survival, with coldwater fish having unique stress responses.
- The liver's role in energy metabolism, antioxidant synthesis, and detoxification during stress can reveal adaptive differences between golden trout and rainbow trout, aiding in optimizing transport protocols.

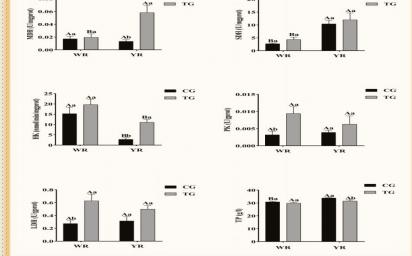
#### Methods

- Experimental Animals and Acclimation: Forty female rainbow trout (20 golden trout and 20 rainbow trout) were acclimated for two weeks in a recirculating aquaculture system with monitored water quality parameters. Feeding was stopped 48 hours before the transportation experiment.
- Experiment Design: After acclimation, 16 fish of each type were divided into control (CG) and treatment groups (TG). The treatment group was subjected to a 6-hour simulated transportation with a density of 300 kg/m³ and horizontal vibrations, while maintaining water quality.
- Biochemical, Histological Analyses and RNA Extraction and Sequencing: Antioxidant and energy metabolism enzyme activities were measured with commercial kits. Liver tissues were HE-stained for histology. Total RNA was extracted from liver tissues for transcriptomic sequencing, and differentially expressed genes were validated by qRT-PCR.

### Results and Conclusion

• Conclusion 1: Transport stress can affect the antioxidant and energy metabolism functions of rainbow trout and golden trout.





**Fig. 1**Changes and differences in the activity indicators of enzymes related to hepatic antioxidant and energy metabolism in YR and WR under CG and TG.

Conclusion 2: In the CG, both golden trout and rainbow trout had normal liver structure. After 6 hours of transportation, both species showed significant liver damage, with more severe effects in golden trout.

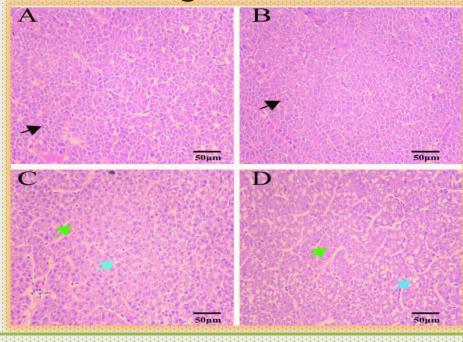
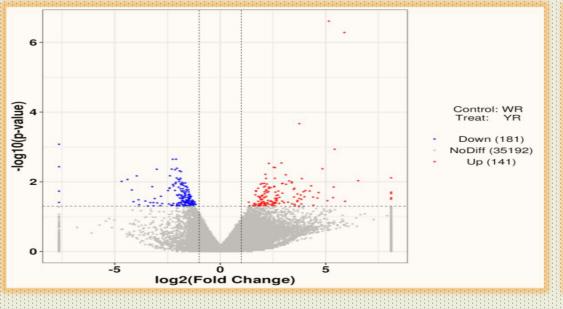


Fig. 2 Fig. 2 HE staining of YR and WR under CG and TG. (A) WR-CG: normal hepatocytes with intact cords; (B) YR-CG: regular boundaries and clear sinusoids; (C) WR-TG: mild vacuolar degeneration and sparse inflammation; (D) YR-TG: severe nuclear dissolution, extensive vacuolation, and dense inflammation. Scale bar: 50 μm.

# **Results and Conclusion**

Sample	Reads No.	Bases (bp)	Q30 (bp)	N (%)	Q20(%)	Q30 (%)
YR-1	46174458	6972343158	6678560647	0.009346	98.43	95.79
YR-2	44892722	6778801022	6516462936	0.009397	98.62	96.13
YR-3	62955930	9506345430	9152260808	0.009286	98.70	96.28
WR-1	52889840	7986365840	7659124127	0.009385	98.52	95.90
WR-2	60721146	9168893046	8823721487	0.009285	98.65	96.24
WR-3	54436038	8219841738	7949300131	0.009522	98.86	96.71

Table.1 Transcription data and comparison with reference genes. Hepatic tissue samples were collected in triplicate from rainbow trout and golden trout at 6 hours post-transportation, creating six cDNA libraries. The raw data are deposited in CNCB (accession number CRA028244). For the transcriptome sequencing, Q20 and Q30 base percentages were >98.43% and >95.79%, respectively. The average GC content was 50.02% in the 0 h group, 48.89% in the 6 h group, and 48.69% in the 24 h group, with combined G and C percentages exceeding 48% (Table 1).



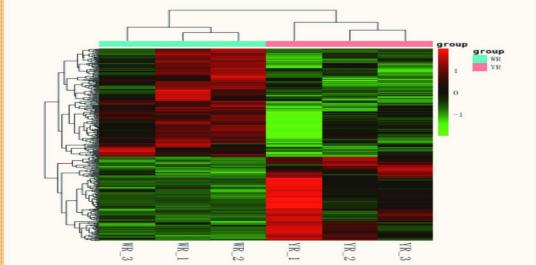
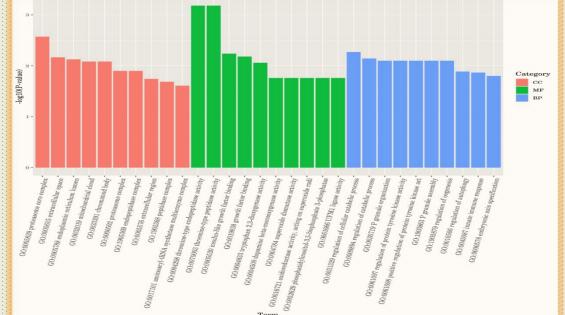


Fig. 3 Volcano plot of DEGs and Differentially expressed gene clustering.



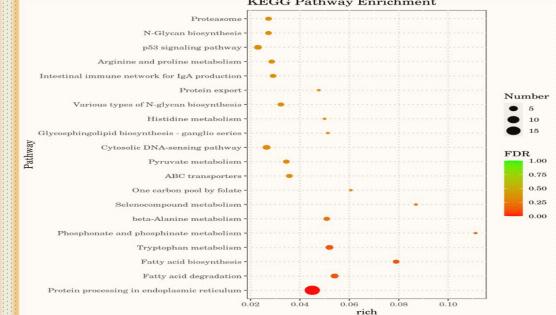
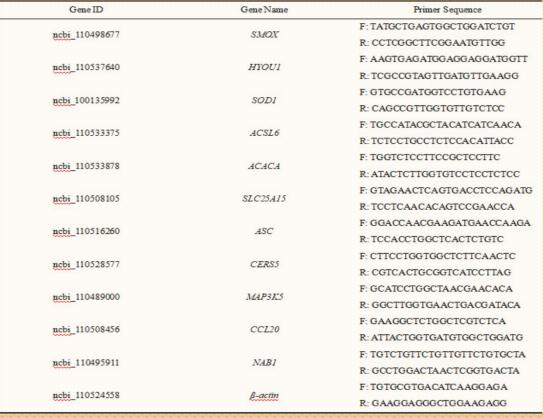
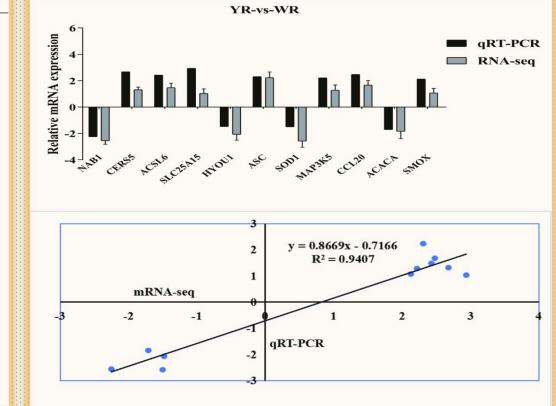


Fig.4 Bar chart of GO enrichment and KEGG enrichment analysis.





**Table.2** To validate the transcriptome analysis, 11 DEGs were selected and verified using qRT-PCR with β-actin as the reference gene. Primers were designed using Primer 6.0, and qRT-PCR was performed using the ChamQ Universal SYBR qPCR Master Mix kit. The relative expression levels were calculated using the  $2-\Delta\Delta$ CT method and matched well with the transcriptome data. Eleven DEGs were selected for qRT-PCR validation. Their expression levels correlated significantly with RNA-seq data (R<sup>2</sup> = 0.9407), confirming the transcriptomic dataset's accuracy in **Fig 5.** 

• Conclusion 3: The results of the transcriptome analysis show that the golden trout has a lower tolerance to stress than the rainbow trout. This is characterized by a stronger oxidative stress response, more severe hepatocyte damage, a preference for energy supply through the tricarboxylic acid cycle, and the activation of the inflammation-apoptosis pathway.

Aknowledgements: This work was supported by the National Natural Science Foundation of China (Grant No. 32260923), Science and Technology Plan Project of Gansu Province (Grant No. 25CXNA033) and Discipline Team Project of Gansu Agricultural university (Grant No. GAU-XKTD-2022-23).

指导教师:康玉军,男,副教授,硕士生导师;研究方向为水产动物抗逆生物学、水产健康养殖。刘哲,男,二级教授,博士生导师;研究方向为水产基础生物学、水产动物抗病、抗逆性状遗传机理学。