

# Blood transcriptome analysis provides adaptive characteristic of ex-situ and captive Yangtze finless porpoises (*Neophocaena asiaorientalis*)

Zhichen Cao, Denghua Yin, Zhanwei Li, Yan Yan



**Abstract:** The Yangtze finless porpoise (*Neophocaena asiaorientalis*, YFP) is a small-toothed whale endemic to the Yangtze River in China and is the only freshwater population in the genus *Neophocaena*. To compare and analyze the adaptive changes of YFPs between ex-situ and captive waters, we performed RNA sequencing of YFPs blood tissues for ex-situ and captive population. A total of 1,201 differentially expressed genes (DEGs) were identified, of which 423 were upregulated in ex-situ population and 778 were upregulated in captive population. Gene Enrichment analysis showed that DEGs were generally enriched for visual, digestive and immune system related pathways. Further analysis revealed that several key immune system pathways, such as chemokine signaling pathway and B cell receptor signal pathway, were activated in ex-situ population. In addition, key pathways related to vision, including phototransduction and inflammatory mediator regulation of TRP channels, as well as pathways related to the digestive system, such as protein digestion and absorption and salivary secretion, were activated in captive population. These results suggest that the ex-situ population may have adapted to the complex environmental conditions of semi-natural waters by enhancing their immune function; and that the visual function and protein digestion of YFPs have been improved compared to the ex-situ population based on the conditions of artificial feeding, such as higher transparency of the water and regular feeding. This study provides a theoretical basis for evaluating the adaptability of the YFPs to different environment and provide useful reference for the ex-situ and captive protection in the future.

**Key words :** Yangtze finless porpoise; environment; blood transcriptome; visual; digestive system; immune system

## 1. Introduction

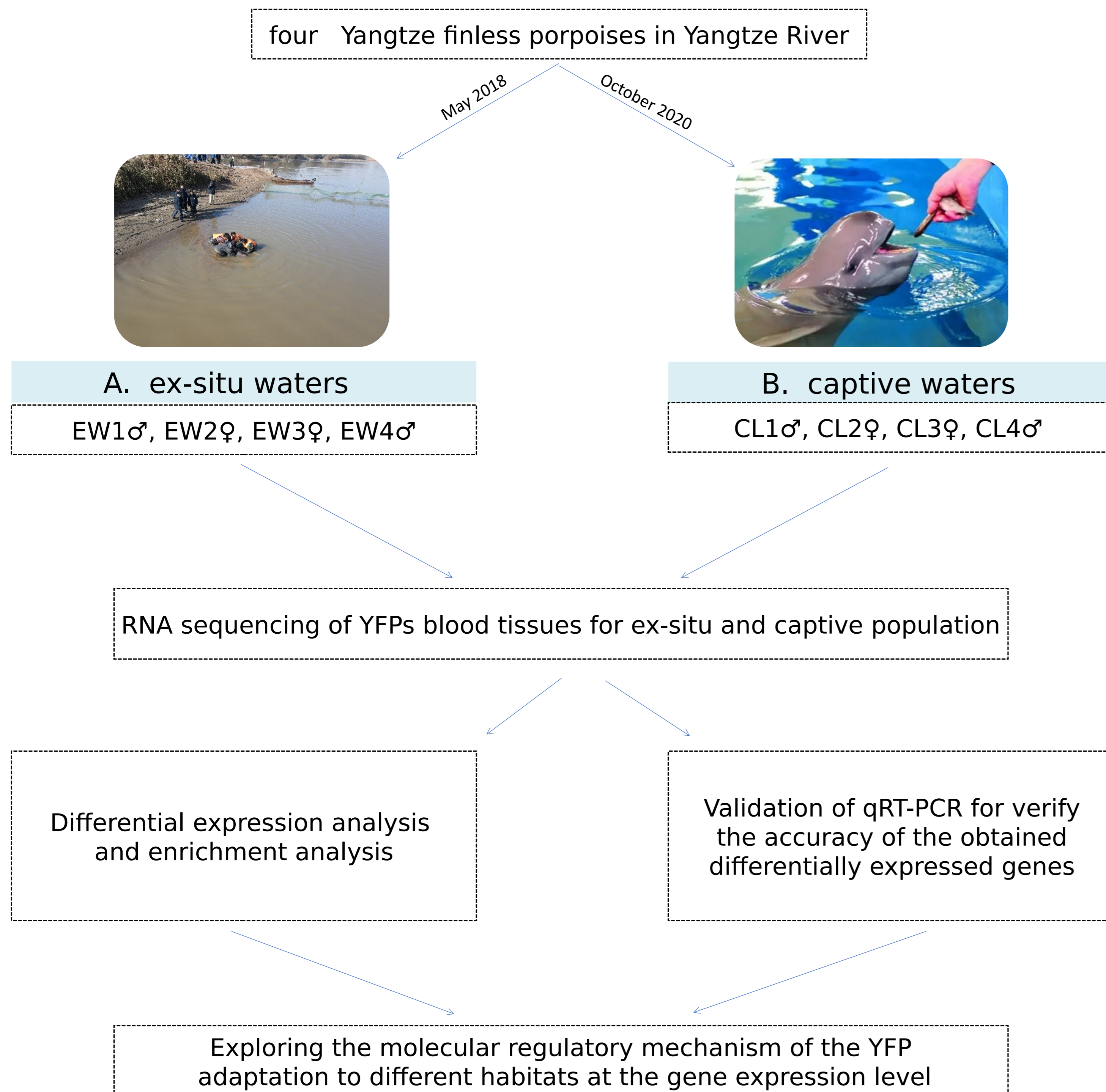
The Yangtze finless porpoise (*Neophocaena asiaorientalis*, YFP) is a small-toothed whale endemic to China, found only in the middle and lower reaches of the Yangtze River, as well as in adjacent Dongting Lake and Poyang Lake. It serves as a crucial indicator species for assessing the health of the freshwater ecosystem of the Yangtze River and the state of their biodiversity[1]. Unfortunately, the YFPs population has faced numerous threats, including threat of intensive shipping, sand mining, dam and other water related engineering construction, and pollution of the Yangtze River water environment. As a result, the YFPs population has declined significantly, from around 2700 in the early 1990s to approximately 1249 in 2022[2]. Given the endangered status of the YFPs population, the International Union for Conservation of Nature's Species Survival Commission (IUCN/SSC) classified the YFPs as "Critically Endangered" (CR) in 2013[3]. On February 5, 2021, the adjusted "List of National Key Protected Wild Animals" listed the YFPs as a national first-class key protected wild animal, further increasing the demand for protection. Although the rapid decline in the population of the YFPs has been curbed, the extremely endangered situation has not changed, and the protection situation remains severe. Therefore, the protection and research of the YFPs are urgently needed.

Since the 1980s, China has gradually explored three major conservation strategies for the YFPs: in-situ conservation, ex-situ conservation, and captive breeding[4]. Establishing suitable ex-situ conservation population in selected water areas has proven to be a direct and effective measure for protecting YFPs[5]. Furthermore, relocating YFPs to aquariums with advanced facilities and expertise in cetacean managed caring is an essential protection measure. Currently, China has set up 5 ex-situ conservation and 2 captive population[6]. In ex-situ waters, there is less human activity and abundant fish resources, but water quality conditions are affected by neighboring residents' lives and farmland drainage. For instance, in the semi-natural protected waters of Tongling, Mi detected triple nitrogen and *E. coli* exceeding the required range for captive pools[7]. In contrast, the captive bases have more comprehensive facilities and the water in the captive pools is continuously treated through a livelihood system. A professional water quality testing team monitors the water daily, ensuring that all water quality indicators meet the required standards for the survival of animals.

Research has shown that different environment can have significant effects on the metabolism[8], immunity[9], and sensory systems[10] of the YFPs. Lin Gang et al. found that the rate of amino acid metabolism in the captive population was significantly higher than that in the wild population[8], which might be mainly due to the fact that the wild YFPs had a high activity level, and a large amount of amino acids in the blood were taken up by the muscle tissues. Nabi et al. found that the environment environment affected the immune system of the YFPs, and that compared with the group from the old channel of the Tian'ezhou National Natural Reserve, the relative expression of immune genes in the group from the Poyang Lake was lower[9]. Additionally, Liu analyzed the blood transcriptome study and found that there were significant differences in auditory functions between the relocated and natural populations of Yangtze finless porpoises[10]. However, there are still fewer studies on the effects of environment on the YFPs, and even fewer studies involving the transcriptomics of the YFPs.

In this study, we conducted mRNA sequencing and analysis on the blood tissues of four YFPs that had their environment converted from semi-natural ex-situ waters to captive waters. By constructing an expression profile of mRNAs in blood tissues and conducting differential gene expression analysis, we identified key DEGs and pathways. This study aimed to explore the adaptive changes of the YFPs living in the two different environments, providing a theoretical basis and reference materials for future ex-situ protection and captive breeding of YFPs.

## 2. Materials and Methods



## 3. Results and Discussion

As can be seen from the gene expression Venn diagram, there was no significant difference in the expression of most of the genes in the EW group and the CL group (Figure 1A), and we identified a total of 1201 differentially expressed genes (DEGs). Among them, 778 DEGs were up-regulated in the CL group, while 423 DEGs were up-regulated in the EW group (Figure 1B). To further understand the functional implications of the DEGs, we conducted GO database analysis for functional annotation and classification. A total of 695 DEGs were annotated, comprising 293 cellular process, 155 immune system process and 140 response to stimulus (Figure 1C). Additionally, 588 DEGs were annotated in the KEGG database, with 17 related to the sensory system, 69 to the digestive system, and 135 to the immune system (Figure 1D). These results indicate that the YFPs might undergo physiological regulation to adapt to different environment.

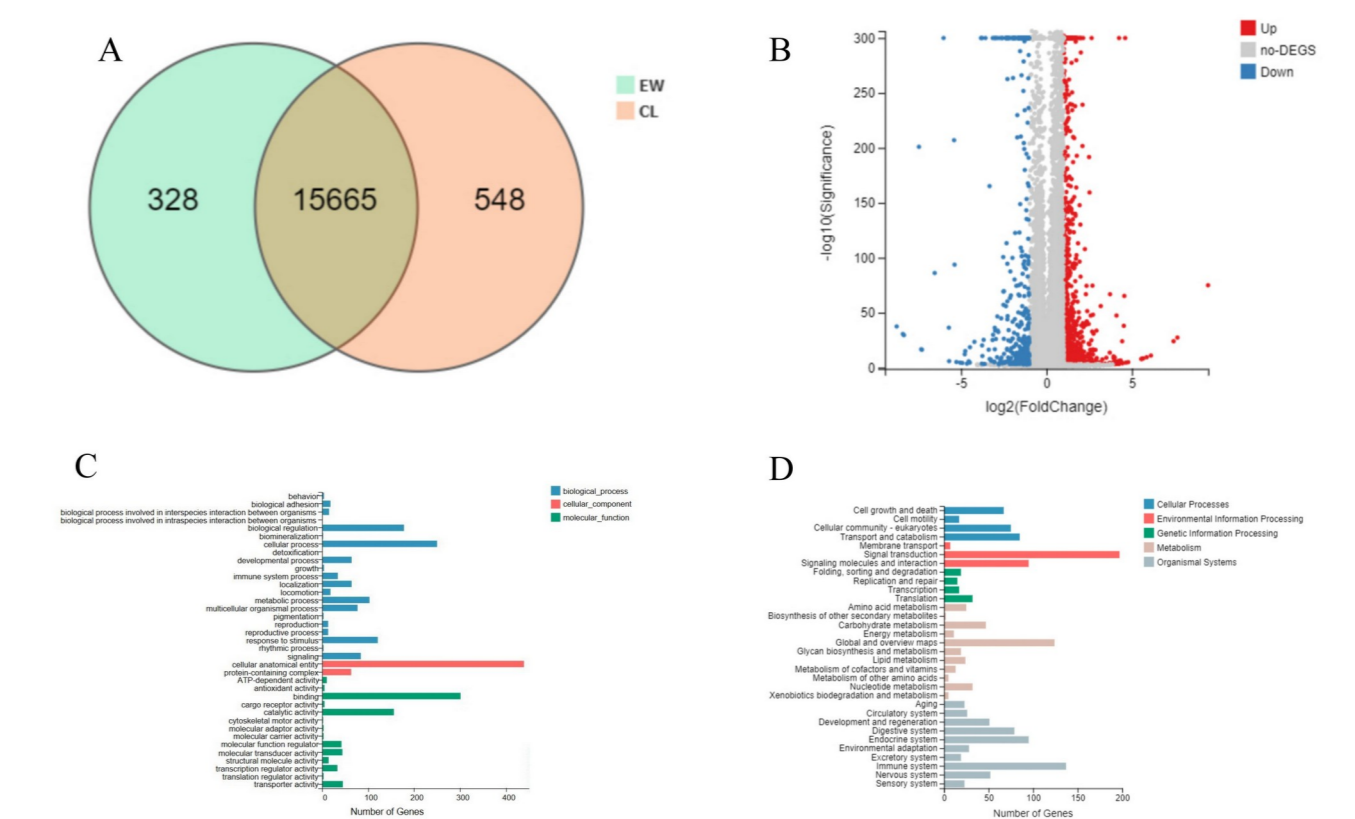


Figure 1

Gene Enrichment analysis showed that DEGs were generally enriched for visual, digestive and immune system related pathways (Figure 2). Several vision-related genes such as RHO (rhodopsin) and EFCAB7 (EF-hand calcium binding domain 7) were significantly up-regulated in the CL group, and activated vision-related pathways such as phototransduction and inflammatory mediator regulation of TRP channels. In addition, several key digestive genes, such as COL4A2 (collagen type IV alpha 2 chain) and COL5A2 (collagen type V alpha 2 chain) were also significantly up-regulated in the CL group, and activated protein digestion and absorption, protein synthesis and absorption and other digestive system-related pathways. Furthermore, several immune-related genes like C1S (complement C1s) and CCR10 (C-C motif chemokine receptor 10) were significantly up-regulated in the EW group. The expression was significantly up-regulated in complement and coagulation cascade, chemokine signaling pathway, B-cell receptor signaling pathway and other immune system-related pathways.

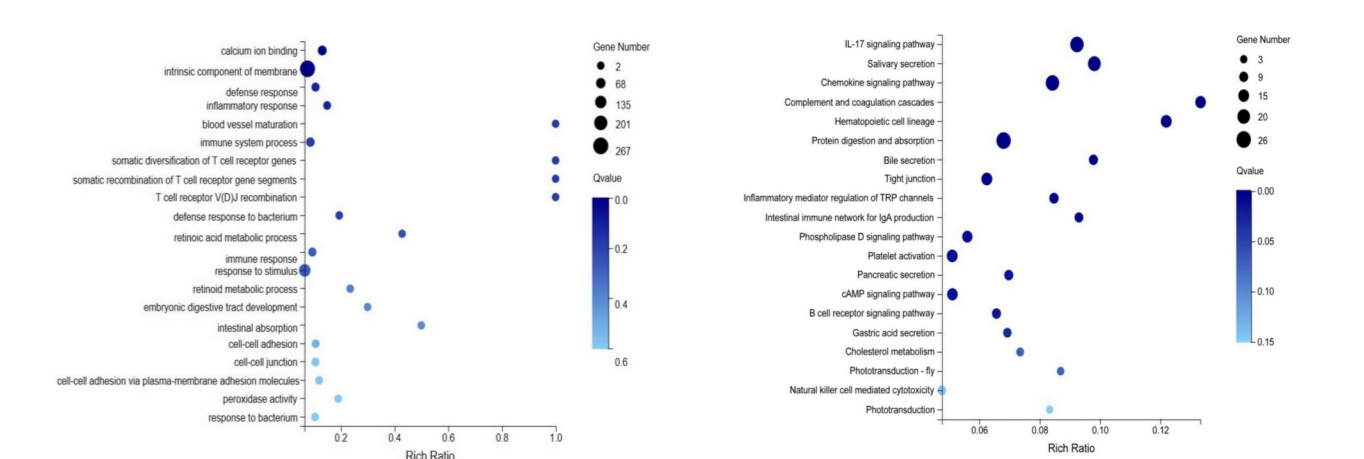


Figure 2

Further analysis revealed that most of the vision-related pathways and digestion-related pathways were activated in the CL group, while most of the immune system-related pathways were activated in the EW group (Figure 3A).

Additionally, a combination of literature review revealed that several visual, digestive and immune system-related genes were significantly different in the gene expression profiles of Yangtze finless porpoises in the EW and CL groups, vision- and digestion-related genes were almost all up-regulated and expressed in the CL group (Figure 3B, Figure 3C), whereas most of the immune system-related genes were up-regulated in the EW group (Figure 3D).

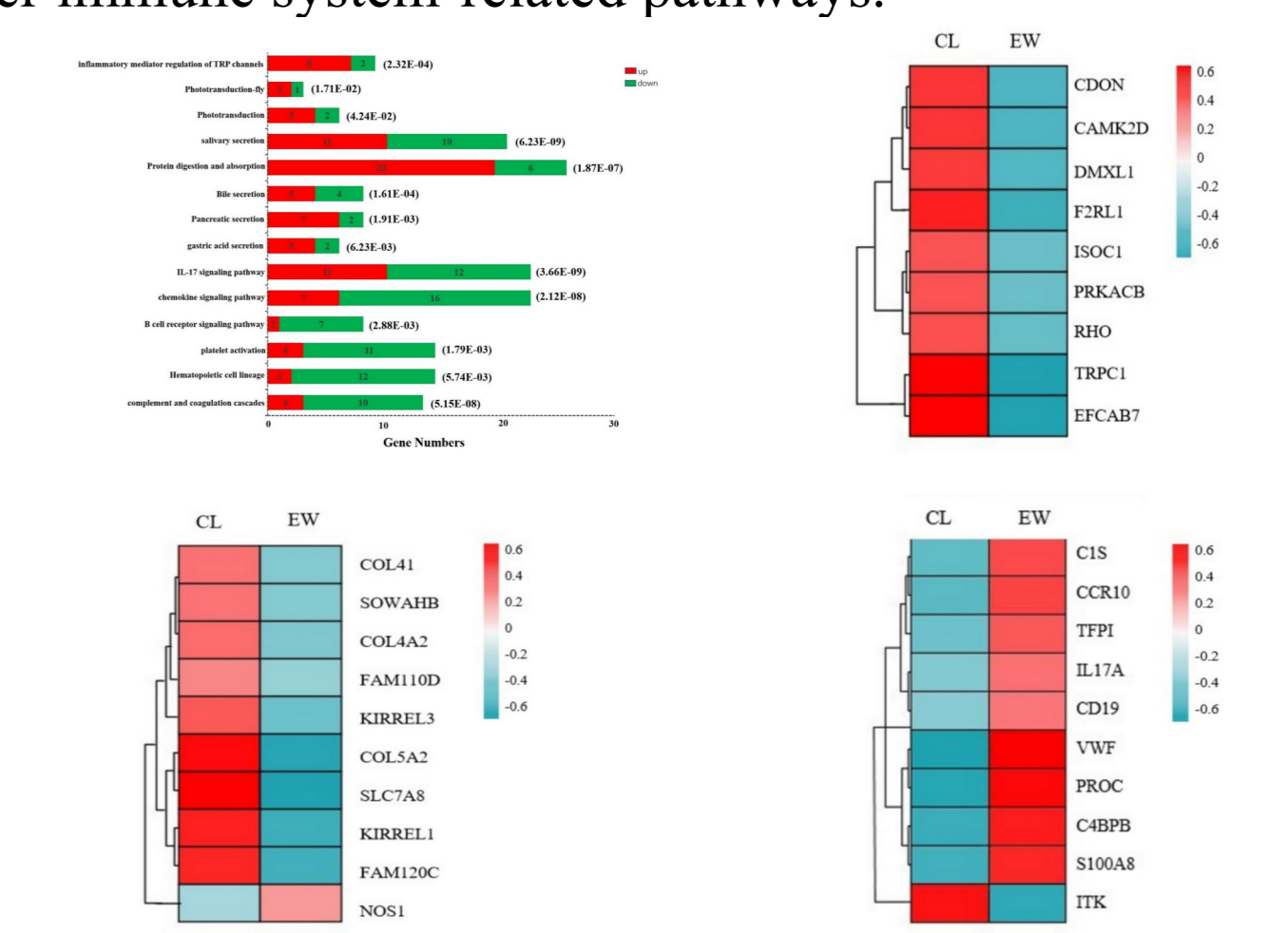


Figure 3

We suggest that vision-related genes and pathways were activated when YFPs were moved from the ex-situ waters with high turbidity to the captive waters with high transparency, which indicated that the visual function of the captive population was improved compared with that of the relocated population. The improvement in digestive function may be related to the fact that regular feeding is beneficial for maintaining the high activity of proteases in the digestive tract of YFP. And the ex-situ population may adapt to the complex environmental conditions of ex-situ waters by activating immune system-related genes and pathways.

## 3. Conclusion

This study reveals the differences in the expression profiles of the blood transcriptome of the Yangtze finless porpoise in ex-situ and captive waters, and illustrates the multiple effects and mechanisms of environmental factors on the YFPs. The results suggest that the visual function and protein digestion function of captive population may be improved compared with that of the ex-situ population, while the ex-situ population may be adapted to the complex environmental conditions of ex-situ waters by enhancing the immune function of the organism. It is worth noting that all the above conclusions are based on blood transcriptome data, which may not accurately reflect the expression of visual, protein digestive and immune functions of the YFPs. In conclusion, ex-situ conservation and captive breeding are two different conservation strategies, each with its own emphasis and equally important. Therefore, the protection of the YFPs requires the selection of appropriate protection methods according to the specific situation, and various factors can be considered comprehensively in order to realize the best protection effect.

## 4. Reference

- Zhou, X., Guang, X., Sun, D., Xu, S., Yang, G. Population genomics of finless porpoises reveal an incipient cetacean species adapted to freshwater. *Nature Communications* 2018, 9, 1276.
- Zhang, X. F., Liu, R. J. The population of finless porpoise in the middle and lower reaches of Yangtze River. *Acta Theriologica Sinica* 1993, 4, 005.
- Wang, D.; Turvey, S.; Zhao, X.; Mei, Z. *Neophocaena asiaorientalis* ssp. *asiaorientalis*, the IUCN red list of threatened species. Version 3.1. 2013.
- Zhao, X.; Barlow, J.; Taylor, B. L.; Pihman, R. L.; Wang, K. Abundance and conservation status of the Yangtze finless porpoise in the Yangtze River, China. *Biological Conservation* 2008, 141, 12.
- Oliveira, M. J.; Aguiar, S. F. H.; Moraes, W. T.; Smaizotti, M.; Banhos, A.; Moreira, N. Ex situ population of the Happy Eagle and its potential for integrated conservation. *Zookeys* 2022, 1083.
- Liu, Z. G. The changes of micro-ecological of diseased Yangtze finless and research on its protection under Ex-situ. *Huachong Agricultural University* 2020.
- Mi, L.; Yu, D. P.; Jiang, W. H.; Zhou, F. Water quality analyzing of rearing Yangtze finless porpoise in semi-nature reserve. *Journal of Anhui University (Natural Sciences)* 2003, 4, 93-97-110.
- Lin, G.; Hao, Y. J.; Wang, D. Determination of serum amino acid concentration in free-ranging and captive Yangtze finless porpoise (*neophocaena phocaenoides asiaorientalis*). *Acta Hydrobiologica Sinica* 2008, 2, 244-251.
- Nabi, G.; Li, Y.; McLaughlin, R. W.; Mei, Z.; Wang, K.; Hao, Y.; Zheng, J.; Wang, D. Immune Responses of the Critically Endangered Yangtze Finless Porpoises (*Neophocaena asiaorientalis* ssp. *asiaorientalis*) to Escalating Anthropogenic Stressors in the Wild and Seminal Environments. *Frontiers in Physiology* 2019, 10.
- Liu, W.; Yin, D. H.; Liu, D. Q.; Yan, Y.; Zhu, X. Y.; Ying, C. P.; Zhang, J. L.; Xu, P.; Liu, K. Blood Transcriptome Analysis Reveals Gene Expression Differences between Yangtze Finless Porpoises from Two Habitats: Natural and Ex Situ Protected Waters. *Fishes* 2022, 7, 3.