

## Research Article

**Bioinformatics analysis of key genes and biological pathways in rainbow trout (*Oncorhynchus mykiss*) affected by proliferative kidney disease (PKD)**Pasandideh R.<sup>1\*</sup>, Pasandideh M.<sup>2</sup>

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**Keywords**

Differentially expressed genes (DEGs),  
Gene network analysis,  
Microarray,  
Proliferative kidney disease (PKD),  
Rainbow trout (*Oncorhynchus mykiss*)

**Abstract**

Proliferative kidney disease (PKD), caused by *Tetracapsuloides bryosalmonae*, is a major challenge in rainbow trout (*Oncorhynchus mykiss*) aquaculture. To better understand the host response at the molecular level, eight microarray datasets from erythrocytes of healthy and infected fish (GSE198859) were analyzed to identify differentially expressed genes (DEGs) using the GEO2R tool. The results revealed that upregulated genes such as *SNRPE*, *miR-301b*, *TNFRSF10B*, *Bckdha*, and *Aqp1* are associated with RNA processing, post-transcriptional regulation, osmoregulation, energy metabolism, and apoptosis, highlighting their essential roles in compensatory responses to kidney damage. Conversely, downregulated genes including *Degs1*, *Ftl1*, *BATF3*, and *RALY* suggested suppression of angiogenesis, inhibition of dendritic cell responses, disruption of immune signaling, and impairment of lipid metabolism and RNA processing, which may represent part of the parasite's strategy to evade host immunity. GO analysis showed significant enrichment in cellular components such as the nucleus, nucleosome, plasma membrane, exosomes, and extracellular regions. In parallel, molecular functions related to kinase activity and protein binding emphasized the importance of signaling and intercellular communication during infection. Overall, these findings suggest that the rainbow trout response to PKD involves a combination of activated defense pathways and simultaneous suppression of some critical immune components by the parasite, which contributes to its survival. The identified genes and pathways may serve as potential molecular markers and support future efforts in selective breeding to improve resistance against PKD.

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## Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important cultured species in freshwater, brackish, and even saltwater aquaculture worldwide. According to the latest FAO statistics, the global production of this species reached approximately 940,000 tons in 2019, reflecting its major role in the aquaculture sector (FAO, 2021). Despite its economic importance, rainbow trout is susceptible to several infectious diseases that can severely impact fish health, farm productivity, and industry sustainability. Proliferative kidney disease (PKD) is caused by *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea), an endoparasitic cnidarian that primarily infects salmonids (Kent and Hedrick, 1985). The life cycle of this parasite involves two hosts, and disease outbreaks typically occur seasonally from late spring to early autumn (Grabner and El-Matbouli, 2008). During this period, bryozoan invertebrates release infectious spores that enter the fish host via gills or skin and subsequently disseminate to organs such as the kidney, spleen, and liver, where proliferation occurs. The disease is characterized by lymphocytic hyperplasia in the kidneys, splenomegaly, and the development of anemia (Carraro *et al.*, 2016). Rainbow trout represents an unusual and terminal host for *T. bryosalmonae* and is generally unable to tolerate severe infections, with mortality rates in aquaculture systems reaching up to 95%. In contrast, the natural host, brown trout (*Salmo trutta*), displays a higher level of resistance, allowing the parasite to complete its life cycle, form spores, and enable transmission (Bailey *et al.*, 2017;

Chan *et al.*, 2023). Although *T. bryosalmonae* has been widely reported in Europe and North America, there are no available reports confirming PKD outbreaks in Iran as of October 2025. However, given Iran's major role in global rainbow trout production and the documented spread of PKD in nearby regions, the potential risk is considerable. This underscores the need for targeted monitoring and surveillance programs.

Research on PKD and other myxozoan infection models has revealed several common features of the host immune response against this group of parasites. For instance, infections caused by *Tetracapsuloides bryosalmonae*, *Sphaerospora molnari*, *Ceratonova shasta*, and *Enteromyxum leei* exhibit common transcriptional patterns, including upregulation of *IL-10*, proliferation of B lymphocytes, and the development of hypergammaglobulinemia (Chan *et al.*, 2023). Although extensive studies have been conducted on PKD and myxozoan infection models in salmonids, most of them have focused on descriptive immunology, while genetic and genomic investigations remain limited. For example, research in brown trout has largely addressed global transcriptomic profiling, with only a few studies focusing on specific immune signaling pathways (Sudhagar *et al.*, 2019). Nevertheless, a comprehensive understanding of the molecular mechanisms and genetic pathways involved in the interaction between *T. bryosalmonae* and rainbow trout is still lacking (Bailey *et al.*, 2020). In this context, bioinformatics-based approaches employing RNA-seq and microarray data are particularly valuable.

These methods enable the identification of differentially expressed genes (DEGs), functional enrichment of biological pathways, assessment of alternative splicing patterns, and construction of protein–protein interaction (PPI) networks. Recent RNA-seq studies in brown trout challenged with PKD have identified dozens of differentially regulated genes and associated biological processes (Sudhagar *et al.*, 2019). Similarly, an RNA-seq study examining the posterior kidney of rainbow trout exposed to both PKD and hormonal stress induced by 17 $\alpha$ -ethinylestradiol (EE2) revealed 444 DEGs, which was substantially higher compared to single-stress conditions (280 genes under PKD infection and only 14 genes under EE2 exposure) (Bailey *et al.*, 2019). Previous studies have demonstrated that immune regulatory genes such as TNF- $\alpha$ , COX-2, and TGF- $\beta$ 1 are significantly expressed in PKD-infected rainbow trout (Holland *et al.*, 2003). Furthermore, recent RNA-Seq analyses have reported thousands of DEGs in various tissues during single or co-infections with *Myxobolus cerebralis* and *T. bryosalmonae* (Akram *et al.*, 2025). On the other hand, Gorgoglione *et al.* (2013) revealed that the adaptive immune response—including antibody and T helper genes—is predominant in this disease.

Investigating host–parasite interactions is essential to understand how protective immunity against pathogens is established and how infection-induced damage can be mitigated. Therefore, the present study was conducted using publicly available microarray data from the GEO database and standard bioinformatics tools to identify key genes and biological pathways

associated with PKD in rainbow trout. This fully bioinformatics-based approach aims to improve our understanding of the molecular mechanisms of the disease and support the identification of potential biomarkers and future management strategies in aquaculture.

## Materials and methods

### *Microarray dataset retrieval*

Microarray datasets from healthy and *Tetracapsuloides bryosalmonae*-infected rainbow trout (*Oncorhynchus mykiss*), the causative agent of proliferative kidney disease (PKD), were retrieved from the Gene Expression Omnibus (GEO) database of the National Center for Biotechnology Information (NCBI) under the accession number GSE198859 (Chan *et al.*, 2023). A total of eight microarray samples corresponding to erythrocytes from four healthy and four infected fish were analyzed. The accession IDs of the datasets used in this study are provided in Figure 1.

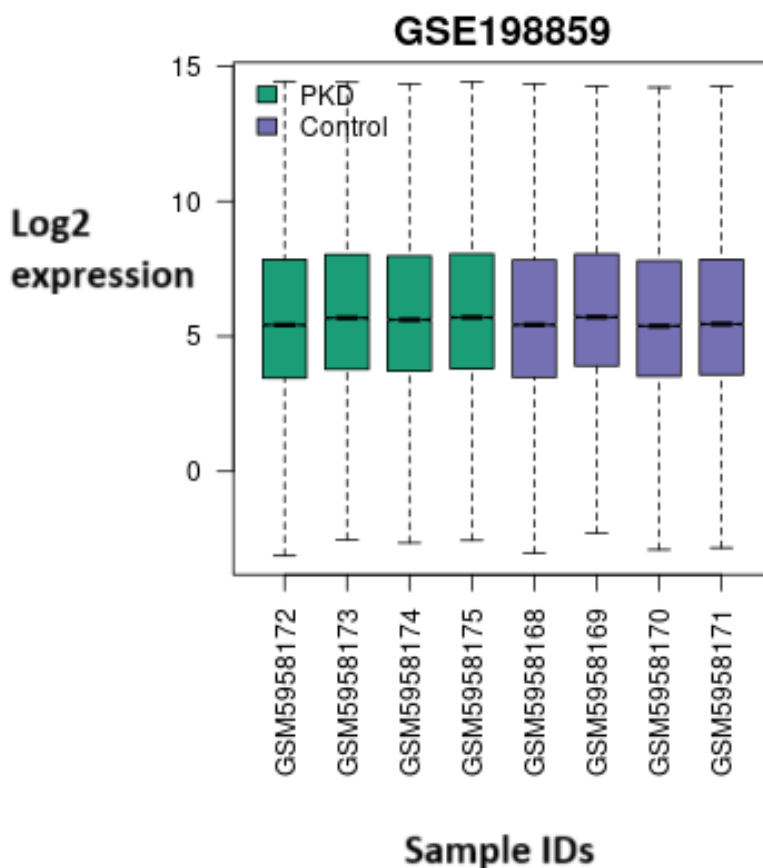
### *Identification of differentially expressed genes (DEGs)*

Microarray data were analyzed using the GEO2R web-based tool (<https://www.ncbi.nlm.nih.gov/geo/>).

GEO2R is a widely used bioinformatics platform that enables comparison of gene expression profiles between two or more sample groups, facilitating the identification of differentially expressed genes (DEGs) that may serve as potential biomarkers associated with specific traits. Data quality was initially assessed, and since the dataset was not normalized, the tool's automatic normalization feature was applied. The processed data were then

exported into Microsoft Excel, where DEGs were identified based on an adjusted  $p$ -value  $<0.05$ . Genes with  $|\text{LogFC}| \geq 2$  were classified as significantly differentially

expressed, with  $\text{LogFC} > 2$  indicating upregulated genes and  $\text{LogFC} < -2$  indicating downregulated genes (Sarathi and Palaniappan, 2019).



**Figure 1:** Accession numbers of microarray datasets used in this study, derived from erythrocytes of healthy and PKD-affected rainbow trout. The X-axis represents the microarray sample accession IDs, and the Y-axis shows the log<sub>2</sub>-normalized expression intensities (unitless).

#### *Protein–protein interaction (PPI) network construction and hub gene identification*

To construct a gene interaction network of DEGs, the STRING database (<https://string-db.org>) was employed. STRING is a comprehensive resource of known and predicted protein–protein interactions, integrating direct (physical) and indirect (functional) associations derived from computational predictions, inter-organism information transfer, and curated datasets from primary sources. Subsequently, protein–protein interaction

(PPI) networks of DEGs were visualized and analyzed using Cytoscape v3.8.2. Key hub genes were identified with the CytoHubba plugin employing the Maximum Clique Centrality (MCC) method, and genes with a score  $> 200$  were selected.

#### *Functional annotation and gene ontology (GO) analysis*

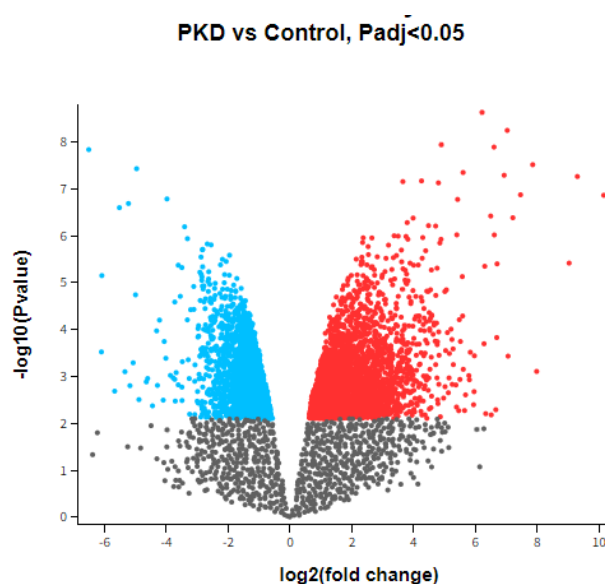
Gene ontology (GO) and functional annotation of DEGs were performed using the DAVID bioinformatics resource

(<https://david.ncifcrf.gov>). Given the challenges of genome annotation in non-model fish species, functional interpretation of DEGs was performed by homology-based annotation using public databases such as GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), Ensembl (<https://www.ensembl.org/index.html>), and UniProt (<https://www.uniprot.org/>). This approach has been widely adopted in genomic studies of aquaculture species (Tso *et al.*, 2020).

## Results

### Identification of DEGs

Comparison of microarray data from healthy and PKD-infected rainbow trout samples using the GEO2R tool revealed a total of 1,518 differentially expressed genes, of which 1,177 were upregulated and 341 were downregulated ( $p < 0.05$ ). The volcano plot of DEGs is presented in Figure 2. Table 1 lists the top 10 upregulated and downregulated genes in rainbow trout following infection with the PKD pathogen ( $p < 0.05$ ).



**Figure 2: Volcano plot illustrating differentially expressed genes (DEGs) in erythrocytes of healthy and PKD-infected rainbow trout. Upregulated genes are represented by red dots, and downregulated genes by blue dots.**

### Gene Ontology (GO) analysis of DEGs

Gene ontology (GO) enrichment analysis revealed that DEGs were mainly associated with immune-related and transcriptional regulatory pathways. In the biological process (BP) category, several key processes such as signal transduction, transcriptional regulation, chromatin remodeling, and the inflammatory response

were highly enriched (Table 2), highlighting the strong immunophysiological modulation triggered by PKD infection. In the cellular component (CC) category, most DEGs were localized to the nucleus, plasma membrane, membrane, and cytosol (Table 3), indicating that infection predominantly affects transcriptional activity and

membrane-associated signaling hubs. For the molecular function (MF) category, the enriched terms were primarily linked to protein binding, DNA binding, and kinase activity (Table 4), emphasizing the central role of transcription factors and phosphorylation-dependent signaling in

host response mechanisms. These combined GO findings suggest that PKD infection in rainbow trout activates complex regulatory networks involved in immune defense, apoptosis, and transcriptional reprogramming.

**Table 1: Differentially expressed genes (DEGs) with the highest and lowest expression levels ( $p < 0.05$ ) in rainbow trout following infection with *T. bryosalmonae***

Upregulated genes (DEGs)			Downregulated genes (DEGs)		
Gene symbol	LogFC	Full gene name	Gene symbol	LogFC	Full gene name
<i>Aqp1</i>	10.15	Aquaporin 1	<i>Phc2</i>	-6.51	Polyhomeotic 2
<i>Bckdha</i>	9.29	Branched chain keto acid dehydrogenase E1 subunit alpha	<i>Flt1</i>	-6.10	Fms related receptor tyrosine kinase 1
<i>TRAPPC6A</i>	9.03	Trafficking protein particle complex 6A	<i>Tpr2</i>	-6.08	Tetratricopeptide repeat protein 2
<i>Zfp148</i>	7.98	Zinc finger protein 148	<i>Il</i>	-5.67	long lived
<i>TNFRSF10B</i>	7.85	TNF receptor superfamily member 10b	<i>Degs1</i>	-5.52	delta(4)-desaturase, sphingolipid 1
<i>FBXL19</i>	7.46	F-box and leucine rich repeat protein 19	<i>Npr3</i>	-5.22	Natriuretic peptide receptor 3
<i>SNRPE</i>	7.21	Small nuclear ribonucleoprotein polypeptide E	<i>ENTPD6</i>	-5.18	Ectonucleoside triphosphate diphosphohydrolase 6
<i>CG11141</i>	7.05	Uncharacterized protein	<i>Rbp3</i>	-5.07	Retinol binding protein 3, interstitial
<i>RPL23P2</i>	7.03	Ribosomal protein L23 pseudogene 2	<i>BATF3</i>	-5.001	Basic leucine zipper ATF-like transcription factor 3
<i>miR-301b</i>	6.70	microRNA 301b	<i>D2Nds1</i>	-4.96	DNA segment, Chr 2, Nuffield Department of Surgery 1

**Table 2: Predicted biological processes associated with differentially expressed genes (DEGs) in rainbow trout**

Biological process	GO ID	Gene count
Signal transduction	GO:0007165	42
Positive regulation of transcription by RNA polymerase II	GO:0045944	36
Negative regulation of DNA-templated transcription	GO:0045892	19
Response to xenobiotic stimulus	GO:0009410	11
Positive regulation of apoptotic process	GO:0043065	12
Cell division	GO:0051301	13
Cell surface receptor signaling pathway	GO:0007166	12
DNA damage response	GO:0006974	11
Chromatin remodeling	GO:0006338	23
Positive regulation of DNA-templated transcription	GO:0045893	19
Inflammatory response	GO:0006954	13

**Table 2 continued**

Regulation of transcription by RNA polymerase II	GO:0006357	37
Protein phosphorylation	GO:0006468	10
Regulation of DNA-templated transcription	GO:0006355	23

Note: A minimum count threshold of 10 genes per annotation term was applied, and a significance level of  $p < 0.05$  was used throughout the enrichment analyses.

**Table 3: Predicted cellular components associated with differentially expressed genes (DEGs) in rainbow trout.**

Cellular component	GO ID	Gene count
Nucleus	GO:0005634	122
Plasma membrane	GO:0005886	110
Nucleoplasm	GO:0005654	84
Membrane	GO:0016020	106
Glutamatergic synapse	GO:0098978	19
Extracellular exosome	GO:0070062	50
Extracellular region	GO:0005576	48
Cell surface	GO:0009986	19
Golgi membrane	GO:0000139	19
External side of plasma membrane	GO:0009897	13
Neuronal cell body	GO:0043025	12
Chromatin	GO:0000785	26
Collagen-containing extracellular matrix	GO:0062023	11
Cytoplasmic vesicle	GO:0031410	10
Dendrite	GO:0030425	13
Endosome	GO:0005768	10
Cytosol	GO:0005829	96

Note: A minimum count threshold of 10 genes per annotation term was applied, and a significance level of  $p < 0.05$  was used throughout the enrichment analyses.

**Table 4: Predicted molecular functions of differentially expressed genes (DEGs) in rainbow trout.**

Molecular Function	GO ID	Gene count
DNA-binding transcription activator activity, RNA polymerase II-specific	GO:0001228	20
Transcription cis-regulatory region binding	GO:0000976	13
Protein binding	GO:0005515	234
Identical protein binding	GO:0042802	42
DNA binding	GO:0003677	33
Protein serine kinase activity	GO:0106310	13
DNA-binding transcription repressor activity, RNA polymerase II-specific	GO:0001227	11
Protein serine/threonine kinase activity	GO:0004674	13
GTPase activity	GO:0003924	12
RNA binding	GO:0003723	34
Sequence-specific double-stranded DNA binding	GO:1990837	16
Signal receptor binding	GO:0005102	12
ATP binding	GO:0005524	34
Protein kinase activity	GO:0004672	11
Protein kinase binding	GO:0019901	14

Note: A minimum count threshold of 10 genes per annotation term was applied, and a significance level of  $p < 0.05$  was used throughout the enrichment analyses.

*Protein-protein interaction network analysis of DEGs and prediction of key gene functions*

Based on the STRING online database results, 445 DEGs formed a co-expression network. To identify hub genes within this

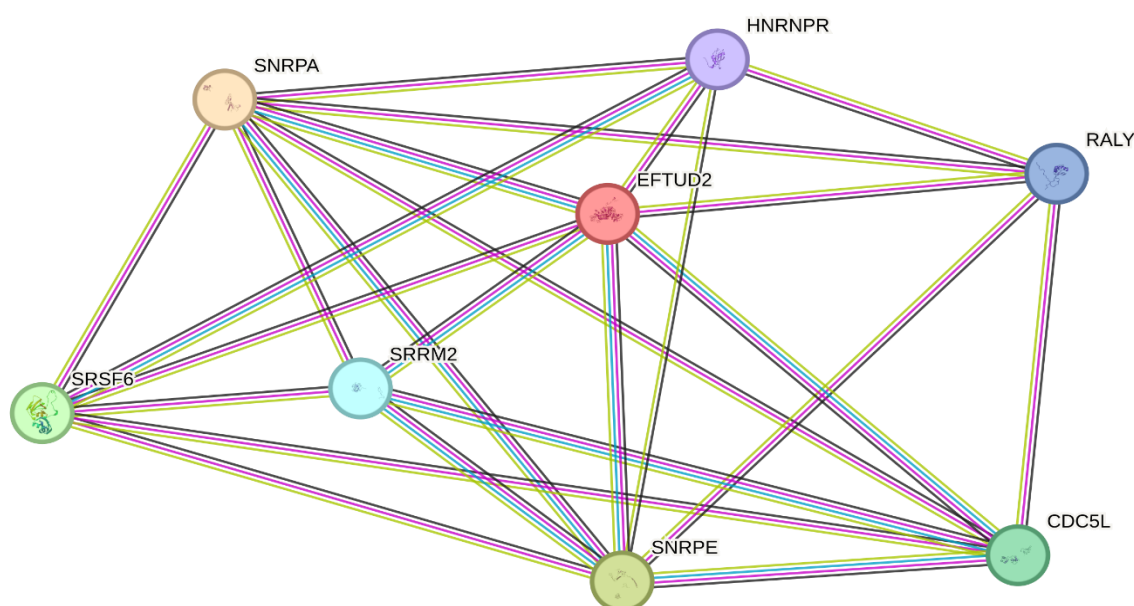
biological network, the protein–protein interaction (PPI) network of DEGs was analyzed using Cytoscape v3.8.2 with the CytoHubba plugin employing the Maximum Clique Centrality (MCC) method. Genes with the highest scores above 200 were selected. Eight genes with the highest homology to well-annotated orthologs were identified as key PKD-related genes in rainbow trout. Based on information from public databases, the corresponding protein names or predicted functions of each gene were retrieved and

are presented in Table 5. As presented in Table 5, the LogFC values of the key genes based on microarray data analysis revealed that six genes were upregulated, while two genes were downregulated (adjusted  $p$ -value < 0.05). Analysis of the PPI network of the identified key genes using STRING showed extensive interactions among them (Fig. 3). The interconnections among these hub genes may indicate their synergistic roles in response to *T. bryosalmonae* in rainbow trout.

**Table 5: Predicted functions and expression levels of key hub genes associated with PKD in rainbow trout**

Rank	Gene symbol	Score	Full gene name	LogFC
1	<i>EFTUD2</i>	1130	Elongation factor Tu GTP binding domain containing 2	2.37 ↑
2	<i>SNRPA</i>	1128	Small nuclear ribonucleoprotein polypeptide A	-2.13 ↓
3	<i>SNRPE</i>	1112	Small nuclear ribonucleoprotein polypeptide E	7.21 ↑
4	<i>SRSF6</i>	859	Serine and arginine rich splicing factor 6	3.65 ↑
5	<i>CDC5L</i>	844	CDC5 cell division cycle 5-like	3.94 ↑
6	<i>SRRM2</i>	727	Serine/arginine repetitive matrix 2	3.97 ↑
7	<i>RALY</i>	244	RALY heterogeneous nuclear ribonucleoprotein	-2.83 ↓
8	<i>HNRNPR</i>	240	Heterogeneous nuclear ribonucleoprotein R	2.79 ↑

Note: Hub genes were identified using CytoHubba with the MCC method in Cytoscape. LogFC values are based on differential expression analysis (adjusted  $p < 0.05$ ).



**Figure 3: Protein–protein interaction network of key genes associated with PKD in rainbow trout, analyzed using STRING.**

## Discussion

The original study by Chan *et al.* (2023), which generated the microarray dataset used in the present work, primarily focused on describing the immunological role of IgM<sup>+</sup> red blood cells during *T. bryosalmonae* infection in rainbow trout. In contrast, our study expands the scope of analysis by conducting a comprehensive transcriptomic investigation aimed at identifying DEGs, their enriched biological processes, molecular functions, and cellular components, as well as hub genes involved in host responses to PKD. This systems-level approach provides complementary insights that were not addressed in the original publication, and highlights regulatory networks and potential biomarkers associated with disease progression. Therefore, our re-analysis not only validates the original dataset but also generates new mechanistic hypotheses and broader biological interpretations, contributing additional value to the understanding of PKD-associated immune modulation in salmonids.

The results of this study recognized 1,518 genes in rainbow trout exhibited differential expression in response to *T. bryosalmonae* infection, with the majority (77.5%) showing upregulation. This indicates extensive activation of cellular and immune pathways in the kidney in response to the PKD-causing parasite. For instance, the upregulation of *Aqp1*, a membrane water channel, may reflect the cells' efforts to restore osmotic and fluid balance under conditions of renal inflammation and damage (Finn and Cerdà, 2011). Additionally, *Bckdha*, a key gene involved in branched-chain amino acid

(BCAA) metabolism, may support the metabolic demands of cells for energy production and tissue repair in the kidney (Du *et al.*, 2022). The upregulation of *TNFRSF10B*, a TNF-dependent apoptosis receptor, may indicate activation of apoptotic pathways to remove damaged renal cells (Zhao *et al.*, 2014).

Furthermore, the presence of genes involved in RNA processing, such as *SNRPE*, and post-transcriptional regulators like *miR-301b*, suggests that widespread changes in RNA processing and gene expression regulation at the post-transcriptional level are integral components of the host response to infection (Wang *et al.*, 2024). Previous studies have demonstrated that *miR-301b* plays a critical role in modulating immune and inflammatory responses under thermal stress conditions in fish (Huang *et al.*, 2022). During heat stress, *miR-301b* targets immune and inflammatory pathways to fine-tune cellular responses (Lin and Meegaskumbura, 2025). Therefore, its upregulation in the present study may reflect the rainbow trout's effort to control excessive inflammation and limit kidney tissue damage caused by parasitic infection. These findings highlight that *miR-301b* not only responds to environmental stressors but also may also contribute to immune regulation during parasitic infection.

In contrast, a subset of genes was downregulated, which may reflect parasite-mediated suppression of key immune and tissue repair pathways. For example, the reduced expression of *Flt1*, a receptor for vascular endothelial growth factor (VEGF), may be associated with impaired angiogenic signaling (Carmeliet, 2005).

Similarly, downregulation of *BATF3*, a critical transcription factor for dendritic cell differentiation, could weaken antigen presentation and suppress adaptive immune responses, thereby favoring parasite survival and proliferation (Hildner *et al.*, 2008). In addition, the decreased expression of *Degs1* may indicate inhibition of sphingolipid biosynthesis. Sphingolipids are not only structural components of membranes but also key bioactive signaling molecules in immune responses; thus, reduced production may impair lipid-mediated immune signaling (Lee *et al.*, 2023). Collectively, these patterns suggest that the parasite simultaneously modulates immune and metabolic pathways, promoting activation of certain host defense mechanisms while silencing critical immune responses, ultimately facilitating parasite persistence and disease progression.

Among the identified DEGs, eight key genes were recognized as hubs within the protein–protein interaction network, mainly involved in RNA splicing, transcriptional regulation, and cell division. For instance, *EFTUD2* and *SNRPE*, which were significantly upregulated, are core components of the spliceosome complex and play essential roles in pre-mRNA processing (Pasternack *et al.*, 2013; Lei *et al.*, 2017). This finding suggests that extensive alterations in gene regulation and RNA processing may constitute part of the molecular response of fish to *T. bryosalmonae* infection. In addition, *SRSF6* and *SRRM2*, also upregulated in this study, belong to the family of serine/arginine-rich RNA-binding factors. These factors are central regulators of alternative splicing, a

key mechanism generating protein diversity and modulating immune responses in fish (Wang *et al.*, 2024). Moreover, *SRRM2* functions as a nuclear scaffold protein, regulating alternative splicing and contributing to innate immune responses (Xu *et al.*, 2022). The upregulation of these genes may therefore reflect the activation of post-transcriptional regulatory pathways and the adaptive remodeling of protein networks to meet cellular demands under PKD-associated stress.

In addition, *CDC5L* was significantly upregulated. This gene is a critical regulator of cell cycle progression from G<sub>2</sub> to M phase and plays a crucial role in DNA repair and genome stability (Zhang *et al.*, 2009). Although direct evidence regarding its function in fish remains limited, transcriptomic studies in flounder responding to lymphocystis disease virus (LCDV) infection have demonstrated that cell cycle and DNA repair pathways are markedly modulated during host–pathogen interactions (Wu *et al.*, 2018). Thus, the upregulation of *CDC5L* in this study likely reflects the activation of cell proliferation and repair mechanisms in hematopoietic and immune cells, compensating for physiological damage in the kidney of rainbow trout during PKD infection.

In this context, the presence of *HNRNPR* as a key hnRNP involved in immune responses further highlights the importance of post-transcriptional regulation during infection. Recent studies have demonstrated that members of the hnRNP family can modulate immune cell function, such as macrophages, by regulating gene expression, alternative splicing, and protein translation, thereby playing crucial roles in

innate immune regulation (Maceratessi and Sampaio, 2024). Although direct evidence on the function of *HNRNPR* in fish is lacking, other hnRNP family members have been reported to regulate immune responses in teleosts. For example, *hnRNPM* interacts with *IRF7* and inhibits interferon signaling in fish (Zhong *et al.*, 2023). This suggests that the upregulation of *HNRNPR* in response to PKD infection may indicate a post-transcriptional regulatory role in immune or anti-parasitic pathways in rainbow trout.

Conversely, *SNRPA* and *RALY* were downregulated in this study. *SNRPA*, a core component of the U1 snRNP, is essential for pre-mRNA splicing and mRNA maturation; its reduced expression may compromise general RNA processing efficiency, which in turn could influence the expression of immune-related genes under pathological conditions such as PKD (Lu *et al.*, 2025). *RALY*, another hnRNP family member, is involved in RNA splicing and stability (Liang *et al.*, 2022). Reduced expression of this gene may reflect defects in post-transcriptional regulation and a loss of protein diversity necessary for an effective host response to PKD. Taken together, the downregulation of *SNRPA* and *RALY* may represent a disruption of RNA processing networks under disease conditions, which could alter immune efficiency or increase the susceptibility of kidney cells to parasitic damage.

The results of gene ontology (GO) analysis further supported these interpretations. Based on the data, pathways related to signal transduction, transcriptional regulation, response to

xenobiotic stimuli, cell division, chromatin remodeling, regulation of apoptosis, and inflammatory response were among the dominant biological processes identified. This suggests that infection with *T. bryosalmonae* not only triggers direct immune responses but also affects fundamental pathways associated with gene regulation and cell cycle control.

Moreover, the analysis of cellular components revealed that a substantial proportion of differentially expressed genes were localized in the nucleus and nucleoplasm, underscoring the central role of transcriptional regulation and RNA processing in the rainbow trout response to *T. bryosalmonae* infection. In addition, the enrichment of genes in the plasma membrane and extracellular components, including exosomes, indicates that intercellular communication and immune signaling through vesicular and membrane-mediated pathways are key mechanisms in host defense (He *et al.*, 2021). Additionally, the presence of genes associated with the extracellular matrix (ECM) and Golgi membrane suggests tissue remodeling and structural adaptations in the infected kidney, consistent with histopathological findings of PKD (Okamura *et al.*, 2011). Another notable finding was the enrichment of genes within components related to neuronal structures, such as the glutamatergic synapse and dendrites. Although this may appear unexpected, recent studies have shown that shared signaling pathways between the nervous and immune systems play important roles in regulating stress and infection responses in fish (Kawasaki and Kawai, 2014).

The results of molecular function analysis revealed that a large proportion of differentially expressed genes were involved in protein, DNA, and RNA binding, highlighting the importance of protein–protein interactions and nucleic acid processing in the response to *T. bryosalmonae* infection. In addition, the enrichment of genes associated with kinase activity, particularly serine/threonine protein kinases, suggests the potential involvement of conserved signaling pathways, such as MAPK/Erk, in the host response to *T. bryosalmonae* infection. Such pathways have been identified as evolutionarily conserved hubs in other fish species, including Nile tilapia, where they play central roles in cell cycle regulation, apoptosis, and T cell function, forming key mechanisms for coping with stress and disease (Wei *et al.*, 2020). Also, genes related to transcriptional regulation by RNA polymerase II and cis-regulatory element binding indicate broad transcriptional reprogramming and cellular preparedness for effective responses to tissue damage and inflammation in the infected kidney. Similar findings in other fish species have shown that extensive changes in the expression of transcriptional regulators represent essential mechanisms underlying immune defense during disease conditions (Zhong and Gao, 2022).

### Conclusion

This study demonstrated that *T. bryosalmonae* infection in rainbow trout is associated with extensive alterations in gene expression and cellular pathways. Upregulation of genes involved in RNA processing (e.g., *SNRPE*), post-

transcriptional regulation (e.g., *miR-301b*), and regulators of cell division and DNA repair (e.g., *CDC5L*), together with the downregulation of metabolic and processing-related genes (e.g., *Degs1* and *RALY*) indicate reciprocal host-parasite mechanisms in regulating immune response and survival. Moreover, the enrichment of genes in kinase-related pathways, plasma membrane components, and extracellular structures emphasizes the critical roles of signaling and intercellular communication in this process. Overall, these findings not only provide a deeper understanding of the molecular mechanisms involved in PKD, but also offer a foundation for future studies aimed at identifying molecular biomarkers, developing novel diagnostic approaches, and designing selective breeding strategies to enhance genetic resistance in freshwater fish.

### Conflicts of 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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