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The identification of toll-like receptor genes in large yellow croaker (*Larimichthys crocea*): provides insights into its environmental adaptation to biological and abiotic stresses

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Large yellow croaker (*Larimichthys crocea*), the most developing fish of China, suffering from both biotic and abiotic stressors. A genome-wide study was performed for the first time to investigate the roles of Toll-like receptor genes in large yellow croaker (*LcTLRs*) working in hypoxia response and *Aeromonas hydrophila* infection. 12 *TLR* genes were identified and annotated, and phylogenetic tree, analysis of structure and motifs demonstrated that *TLRs* were highly conserved. To further investigate the *LcTLRs* under environmental stress, expression pattern analysis of hypoxic response and *Aeromonas hydrophila* infection revealed that there were nine and six members of *LcTLRs* showing significant differential expression, indicating that they were concerned in hypoxia stress and disease responses. Meanwhile, their expression levels were validated utilizing qPCR. Taken together, a greater appreciation and understanding of *TLRs* function in the reaction to biotic and abiotic stress would ultimately lead to more efficiently environmental adaptation in large yellow croaker.

KEYWORDS

large yellow croaker, toll-like receptors genes, stress response, environmental adaptation, innate immune

1 Introduction

Large yellow croaker (*Larimichthys crocea*), the native species of East Asia, has the highest aquaculture yield among marine fish in China (257,683 tons in 2022) (MOA, 2023). However, the intensive net-cage inshore cultivation model, the degrading farming environment, outbreaks of various abiotic and biotic stressors like hypoxia and *Aeromonas hydrophila* infection, and other factors, had caused significant economic losses to the aquaculture industry (Ding et al., 2022; Zhang et al., 2022). Many previous studies had confirmed that Toll-like receptor genes played a vital role in innate immunity (Ni et al., 2022; Yao et al., 2023; Gao et al., 2024), however, research on their role in environmental adaptability was still in its early stages. Therefore, the research of biotic and abiotic environmental factors was on the front-page of *L.crocea*'s survival. This experiment would lay a foundation for the specific role of Toll-like receptor genes in the host environmental adaptation to resistance to hypoxic response and *Aeromonas hydrophila* infection.

Dissolved oxygen (DO), or molecular oxygen dissolved in water, is the primary source of oxygen for a variety of aquatic organisms. As *L. crocea* cultures grow and their densities rise, the culture environment degrades and the water exchange capacity eventually drops, leaving large areas with low DO levels. Marine ecosystems are more vulnerable to hypoxic stress than terrestrial environments are (Lee et al., 2024). Low dissolved oxygen levels have a negative impact on fish physiological and biochemical processes, including apoptosis (Liu et al., 2022b), energy metabolism (Jaworski et al., 2019), and other processes, which can result in a variety of illnesses and injuries (Shun et al., 2024). Reactive oxygen species (ROS) are produced by the organism from part of the oxygen taken up by fish respiration. A tiny percentage of ROS control gene expression and cell activity through their involvement in cell signaling pathways (Shi et al., 2024). According to Abdel-Tawwab et al. (2019), fish that experience oxidative stress due to excessive ROS production during hypoxia eventually undergo apoptosis and sustain tissue damage. Aquatic species' growth, survival, behavior, immunity, and reproduction are all significantly impacted by such hypoxic environments. According to Ding et al. (2020), hypoxia in *L.crocea* causes a rise in mortality and is now the primary barrier to the growth of its sustainable mariculture. On the other hand, the breeding of *L.crocea* was threatened by bacterial diseases (Mu et al., 2018). Previous studies had shown that the diseased *L. crocea* had suffered a variety of severe illness symptoms after Gram-negative bacteria *A. hydrophila* infection (Chen et al., 2010; Mu et al., 2010; Zhang et al., 2022), as well as the verification of *TLRs* differential expression following an *A. hydrophila* challenge in common carp (Gong et al., 2017). Therefore, in order to allow large yellow croak culture population, exploit a better ecological opportunity under the stress of environmental factors such as hypoxia and *A. hydrophila* infection, researchers were committed to exploring the molecular mechanism of resistance regulation of *TLR* genes.

To date, studies have shown that TLRs bear the responsibility of playing important roles in innate immunity in organisms (Verma et al., 2024). Innate immune cells, such as dendritic cells (DCs), are activated when the immune system detects microorganisms through a variety of receptors known as pattern recognition receptors (PRRs), which are encoded by germ-line genes and recognize a wide range of microbial structures (Wang et al., 2024). Up to now, many PRRs have been found, including C-type lectin receptors, scavenger receptors (SR), intracellular receptors, lipopolysaccharides (LPS) and β-1, toll-like receptors (TLRs), and Down syndrome cell adhesion molecules (Dscam) (Tran et al., 2019). Among these PRRs, the role of TLRs in innate immune responses during evolution is thought to be highly conserved, usually acting as transmembrane receptors that recognized different microbial components and directly activated immune cells. Exposure of immune cells to the ligands of receptors can activate intracellular signaling cascades, and then rapidly induce the expression of a variety of overlapping and unique genes involved in inflammation and immune responses. After pathogen infection, pathogen-associated molecular patterns (PAMPs) are recognized, myeloid differentiation factor 88 (MyD88) and adaptors containing TIR domains are recruited by TLRs to induce interferon-beta (IFN-β) level (Dias et al., 2022; Ryan and O'Neill, 2023), thereby leading to the activation of protein 1 (AP-1) transcription factor and nuclear factor kappa-B (NF-κB) (Wang et al., 2017). This induces the production of pro-inflammatory cytokines, such as TNF-α and IL1b, to activate innate and adaptive immune responses (Vidya et al., 2018). Structurally, TLR consists of several domains, including N-terminal signaling domains, multiple extracellular leucine-rich repeat (LRRs) domains, transmembrane domains, and intracellular Toll/interleukin-1 receptor (TIR). The TIR domain functions as a protein scaffold, attracting downstream molecules and initiating signaling cascades that activate the expression of target genes, such as antimicrobial peptides, while the extracellular LRR domain recognizes the PAMP ligands of pathogenic microorganisms.

TLRs are a family of germline-encoded PRRs conserved in function. They are essential for recognizing PAMPs like flagellin, lipoprotein, nucleic acid, LPS, and lipoteichoic acid (Tran et al., 2019). For instance, bacterial flagellin stimulates TLR5, while double-stranded RNA (dsRNA) can activate TLR3 (Adams et al., 2024). Similar in location, structure, and function, TLR7 and TLR8 are recognized to detect single-stranded RNA (ssRNA) from viruses (Wallach et al., 2023). Furthermore, it has been discovered that TLR9 and TLR21 can both identify CpG DNA in fish and that they react preferentially to CpG DNA with distinct CpG (Yeh et al., 2013). Following ligand identification, TLR uses the Toll/Interleukin-1 receptor (TIR) domain and TIR-containing cohesive molecules, such Myd88 and other TIR-domain-containing convergent proteins, to initiate downstream signaling cascades and an immunological response (Sahoo et al., 2012). Activation of the TLR signaling pathway triggers the production of co-stimulatory molecules and inflammatory cytokines, which effectively trigger the host's antiviral or antimicrobial immune responses (Li et al., 2022).

2 Materials and methods

2.1 Identification of TLR genes in large yellow croaker

In order to carry out a thorough identification of the TLR family members in *L. crocea*, all TLR sequences of nine teleosts, including channel catfish (*Ictalurus punctatus*), fugu (*Takifugu rubripes*), grass carp (*Ctenopharyngodon idella*), Japanese flounder (*Paralichthys olivaceus*), medaka (*Oryzias latipes*), Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*), spotted gar (*Lepisosteus oculatus*), spotted sea bass (*Lateolabrax maculatus*) and zebrafish (*Danio rerio*) were downloaded from NCBI (http://www.ncbi.nlm.nih.gov) and Ensembl (http://www.ensembl.org) databases (Yan et al., 2021). The non-redundant TLRs full-length coding sequences were utilized as queries to search against *L. crocea* genomic resources by TBLASTN and BLASTP, with an e-value threshold of e-value < e^{-10} to acquire the candidates, after the redundant and incomplete sequences were manually removed (El-Gebali et al., 2019). The conserved TLR domain was then confirmed and verified by submitting the putative TLR sequences to the Pfam and SMART databases (Letunic and Bork, 2018). The ExPASy ProtParam tool (http://web.expasy.org/protparam/) was utilized to assess the molecular weight, theoretical isoelectric point, and amount of amino acids (Duvaud et al., 2021). Ultimately, every potential TLR gene extracted from the *L. crocea* genome was renamed based on their homology to TLRs found in zebrafish, and then added to the GenBank database.

2.2 Phylogenetic tree construction

A phylogenetic tree was created using all of the amino acid sequences of TLR from *L. crocea* and other teleosts. Multiple sequence alignment was performed by ClustalW with default settings (Edgar, 2004). Using the JTT (Jones-Taylor-Thornton) matrix-based model, phylogenetic analysis was carried out through the MEGA 7 program and the Maximum Likelihood approach (Kumar et al., 2016). The Evolview website (https://evolgenius.info//evolview) was then used to show the phylogenetic tree (Zhang et al., 2012).

2.3 Exon/intron organization and study of conserved motifs

Using the Gene Structure Display Server (GSDS, http://gsds.cbi.pku.edu.cn) online analysis tool, the exon/intron structures were visualized by aligning the coding sequences with the corresponding genomic DNA sequences. To display the gene structure of *L. crocea*, including the length and position of the introns and exons, the gff file was uploaded to the GSDS program (GenBank No. GCA_000972845.2). The conserved protein motif was analyzed using the MEME website (http://meme-suite.org/tools/meme), with the motif number set to 10 and the other parameters left at default (Bailey et al., 2009). The TBtools program was then used to create the phylogenetic tree, conserved motifs, and gene structure (Chen et al., 2020).

2.4 Hypoxia stress

A total of 300 healthy *L. crocea* were obtained and maintained in indoor tanks at the same conditions as the culture cages (25°C, salinity: 23–25, DO concentration: 7.8 ± 0.5 mg/L). The fish were fed compound feed twice a day, at 6:00 and 17:00, up until twelve hours before the trials began. Following a temporary raising period of 14 days, nitrogen gas was blasted into the tanks to conduct hypoxia-exposure studies. Using a DO meter (YSI, Canada), the appropriate concentration of dissolved oxygen was measured. In the control group, the DO concentration was 7.8 ± 0.5 mg/L. Over the course of ten minutes, the oxygen concentration in the tank was reduced in the experimental group from 7.8 ± 0.5 mg/L to 1.6 ± 0.2 mg/L. For the purpose of extracting RNA from the gills, heart, spleen, and head kidney at 0, 6, 24, and 48 hours following the hypoxic test, six subjects were chosen at random. To create templates for additional transcriptome analysis, equivalent molar ratios of RNA from two subjects were combined into one replicate at each time point, for a total of three repetitions. Raw sequencing read data had been deposited in the Sequence Read Archive (SRA) of NCBI under the BioProject accession numbers PRJNA574876 and PRJNA576086.

2.5 Aeromonas hydrophila infection

A total of 300 healthy *L. crocea* were acclimated for 2 weeks, and feeding was stopped one day before the experiment. In a preliminary experiment (DO 7.5–8.5 mg/L, 23 ± 0.5°C), the experimental group was injected intraperitoneally into 60 fish, with three experimental replicates per group, at the 24-hour half-lethal dose (LD50) of *A. hydrophila*, which was found to be 8×10^6 CFU·mL^{-1} (0.5 mL). The control group was injected with 0.5 ml PBS. At 0, 3, 12, and 24 hours, the survival rates of *L. crocea* were 100.0%, 100.0%, 77.5% and 56.0%, respectively. Three individuals from each group were chosen randomly, and the head kidney tissues were taken for RNA sequencing. Fish were put down with 0.05% MS-222 (3-aminobenzoic acid ethyl ester methanesulfonate, Sigma, USA) before to sampling. The NCBI Sequence Read Archive received the raw data and assigned it the accession number PRJNA764439.

2.6 Validation of qRT-PCR

In order to corroborate the *TLR* gene expression patterns after both hypoxic and *A. hydrophila* infection stressors above, using primers created by Primer Premier 5.0 software for qRT-PCR and reported in Supplementary Table 1, we identified genes that were differentially expressed. Utilizing *β-actin* as the internal control, we computed the relative expression level using the $2^{-\triangle\triangle CT}$ technique

10.3389/fmars.2024.1404183

(n = 3). Tukey's multiple range test and one-way ANOVA were used for the statistical analysis (SPSS, version 22.0).

3 Results

3.1 *TLR* gene identification and annotation

Following the *TLR* gene screening and validation in channel catfish, fugu, grass carp, Japanese flounder, medaka, Nile tilapia, rainbow trout, spotted gar, spotted sea bass and zebrafish, 12 *TLR* genes in *L. crocea* (*LcTLRs*) were identified. Table 1 displayed the *TLR* gene copy counts for eleven different teleost species. The probable molecular weights of the proteins ranged from 50,904 kDa (*LcTLR2b*) to 121,457 kDa (*LcTLR9*), and their theoretical isoelectric points (pI) varied from 5.98 (*LcTLR2b*) to 8.94 (*LcTLR5*). The proteins' lengths varied from 439 (*LcTLR2b*) to 1056 (*LcTLR9*) amino acids. Table 2 provided specific information on 12 *LcTLRs*.

3.2 Phylogenetic analysis of the *LcTLR* genes

139 full protein sequences from the teleosts mentioned above were used to create a phylogenetic tree and examine the evolutionary relationships between the eleven teleost *TLR* genes. Figure 1 illustrated the similar genes from these species grouped together, demonstrating the relative conservation of *TLR* genes. *LcTLR3*, *LcTLR5*, *LcTLR21*, *LcTLR22*, and *LcTLR23* were clustered into a branch; *LcTLR1*, *LcTLR2*, and *LcTLR14* were grouped together; *LcTLR7*, *LcTLR8*, and *LcTLR9* were clustered into a branch.

3.3 *LcTLR* gene motif and CDS structure analysis

We examined the CDS structure and conserved motifs of the TLR family to gain a deeper understanding of the structural conservation of the TLR gene family. Ten conserved motifs were predicted from the *TLR*s of *L. crocea*, as Figure 2 illustrated. The numbers and placements of these motifs were more similar in proteins that were closer in relationship. There were more motifs in *LcTLR7*, *LcTLR8*, and *LcTLR9* than in *LcTLR5*, which has the fewest motifs. Motifs 4, 8, and 9 were present in all 12 *LcTLRs*, and motif 5 was present in most *LcTLRs*. Of all the *LcTLRs*, *LcTLR2a* had the most introns, and *LcTLR14* had the longest length of introns.

3.4 Gene expression profiling of *LcTLRs* under hypoxic stress

Based on RNA-seq data, the expression profile of *LcTLRs* in four hypoxia stress-affected tissues (the gill, heart, kidney and spleen) was examined to learn more about the function of the *LcTLRs* gene in the hypoxic response. Eight *LcTLR* genes were

TABLE 1 The *TLR* gene copy numbers in representative species.

Gene /species	*C. idella*	*D. rerio*	*I. punctatus*	*L. maculatus*	*L. oculatus*	*O. latipes*	*O. mykiss*	*O. niloticus*	*T. rubripes*	*P. olivaceus*	*L. crocea*
TLR1	2	1	2	2	2	2	1	1	1	1	1
TLR2	1	1	1	2	1	1	1	1	1	1	2
TLR3	1	1	1	1	1	1	1	1	1	1	1
TLR4	2	2	1	0	1	0	0	0	0	0	0
TLR5	2	2	1	1	1	2	1	1	2	2	1
TLR7	0	1	1	1	1	1	1	1	1	1	1
TLR8	1	1	1	1	2	1	1	1	1	1	1
TLR9	1	1	1	1	2	1	1	1	1	1	1
TLR13	1	1	1	0	0	0	1	0	0	0	0
TLR14	0	0	0	1	1	1	0	0	1	1	1
TLR18	1	1	1	0	0	0	0	0	0	0	0
TLR19	1	1	0	0	0	0	1	0	0	0	0

(*Continued*)

10.3389/fmars.2024.1404183

TABLE 1 Continued

Gene /species	*C. idella*	*D. rerio*	*I. punctatus*	*L. maculatus*	*L. oculatus*	*O. latipes*	*O. mykiss*	*O. niloticus*	*T. rubripes*	*P. olivaceus*	*L. crocea*
TLR21	1	1	1	1	0	1	0	1	1	1	1
TLR22	2	1	1	1	1	1	2	1	1	1	1
TLR23	0	0	0	2	0	0	0	1	1	0	1
All	16	15	13	14	13	12	11	10	12	11	12

involved in gill ($P<0.05$, $|\log_2$ fold change$|>1$), as Figure 3 illustrates. There was a considerable up-regulation of *LcTLR1* at 48 h and a noticeable up-regulation of *LcTLR5* at 24 h. The expression level of *LcTLR2a*, *LcTLR3*, *LcTLR14* and *LcTLR22* showed a decreasing trend at different time points, while *LcTLR9* and *LcTLR21* were increased.

Eight *LcTLR* genes exerted actions in the heart under hypoxia challenge. The results of the examination showed that whereas *LcTLR5* was dramatically up-regulated at 6 h, *LcTLR2a*, *LcTLR3*, and *LcTLR9* were remarkably down-regulated. It is noteworthy that there was a considerable up-regulation of *LcTLR8* expression at 24 hours, but a decrease at 6 hours.

During hypoxic stress, nine *LcTLRs* took involved in the kidney's immunological response. Following a 6-hour drop, *LcTLR2a* increased till 48 hours later. The expression of *LcTLR3, LcTLR5, LcTLR7, LcTLR8* and *LcTLR9* increased persistently, and there was a discernible rise in *LcTLR7* and *LcTLR8* at 48 hours. After 6 hours, *LcTLR14* was down-regulated and continued to decline.

The spleen regulated nine out of the *LcTLR* genes. At 6 h, there was a noticeable drop in *LcTLR2a*, *LcTLR7*, and *LcTLR8* and then showed an increasing trend step by step. *LcTLR5* and *LcTLR14* were signally down-regulated at both 24 h and 48 h. Supplementary Table 2 revealed the *P*-value and $\log_2$ fold change.

3.5 Expression patterns of *LcTLRs* in the kidney after *Aeromonas hydrophila* infection

Using *A. hydrophila* infection transcriptome sequencing data in *L. crocea*, the involvement of *TLR* genes in the kidney was visualized in Figure 4. Supplementary Table 3 displayed *P*-value and $\log_2$ fold change values. The majority of *TLR* genes were markedly down-regulated ($P < 0.05$, $|\log_2$ fold change$|>1$) in bacterial infections. At 3 h after bacterial infection, *LcTLR3*, *LcTLR7*, *LcTLR8*, *LcTLR9* and *LcTLR14* were remarkedly down-regulated; At 12 h after bacterial infection, *LcTLR3*, *LcTLR7*, *LcTLR9* and *LcTLR14* were significantly down-regulated. At 24 h after bacterial infection, the expressions of *LcTLR3*, *LcTLR7*, *LcTLR9* and *LcTLR14* showed similar downward trends like above two-time points. Interestingly, only *LcTLR5* was visibly elevated at 3 h and then started a decreasing trend until it returned to normal at 24 h. Notably, *LcTLR2b* did not participate in immune inflammatory response from beginning to end.

3.6 qRT-PCR validation of RNA-Seq data following *A. hydrophila* infection and hypoxic stress

After *A. hydrophila* infection and hypoxic stress, expression profiles of *LcTLR2a*, *LcTLR3*, *LcTLR5*, *LcTLR8*, *LcTLR9*, and *LcTLR22* were created using qRT-PCR to confirm the differentially expressed *TLR* genes. The expression trends of the qRT-PCR data were essentially in line with the RNA-Seq findings,

TABLE 2 TLR mRNA (complete cds) summary features in the genome of *L. crocea*.

Gene name	NCBI accession number	Number of amino acids	Molecular weight	Theoretical pI
LcTLR1	OR669624	534	61083.55	8.53
LcTLR2a	OR669625	816	93350.30	5.98
LcTLR2b	OR669626	439	50904.72	6.83
LcTLR3	OR669627	919	103369.33	8.78
LcTLR5	OR669628	641	71612.32	8.94
LcTLR7	OR669629	1053	121440.16	8.53
LcTLR8	OR669630	1030	118782.44	8.72
LcTLR9	OR669631	1056	121457.68	6.46
LcTLR14	OR669632	876	100583.29	6.53
LcTLR21	OR669633	981	113751.71	8.58
LcTLR22	OR669634	967	111214.55	8.92
LcTLR23	OR669635	950	108746.23	6.20

as illustrated in Figure 5. In general, the qPCR findings proved that the results of RNA-seq analysis were accurate and reliable.

4 Discussion

It is now evident that TLRs promote and regulate the quality of the adaptive immune response in addition to playing a critical role in the innate identification of microorganisms (Manicassamy and Pulendran, 2009). The discovery of 21 distinct TLR types in a range of fish species in recent years has spurred interest in investigating TLRs' potential as targets for increasing fish immunity and disease resistance (Mahapatra et al., 2023). Twelve TLRs in all were found in the *L. crocea* examined in this investigation. The number was the same as the closely related species *T. rubripes* and *O. latipes* (Xie et al., 2023). The majority of genes were carried by *L. crocea*, which reached a high level of evolutionary conservativeness. Besides, four "fish-specific" family members (Rebl et al., 2010) of *TLRs* were identified in *L. crocea*, including *TLR21*, *22*, *23*. These findings corroborated those of earlier research, which revealed that fish TLRs had a great deal of diversity and distinctive traits, most likely due to their varied evolutionary histories and the varied settings they live in (Palti, 2011).

Members of the TLR family in teleosts were divided into six clades based on sequence homology comparison (Palti, 2011; Fan et al., 2019). Additionally, TLRs 18–20 and 22–28 are thought to be special to fish (Palti, 2011). While the majority of fish TLRs have not been documented to recognize comparable PAMPs, the fish TLRs have unique characteristics resulting from their varied evolutionary backgrounds and intricate aquatic environments. Notably, even though *L. crocea* contained most *TLR* genes, we found that the large yellow croaker's genome lacked the *TLR4* and *TLR13* genes, and we also noticed the similar situation in the genome of *D. rerio*, *P. olivaceus* and *O. niloticus* etc., indicating that gene gain and loss events might occur frequently in fish.

The phylogeny of gene families may be further explained by structural analysis. Here, we found that *LcTLR14* had a longer intron structure while others had shorter introns. Besides, it was discovered that the majority of the TLR genes in *Cyprinus carpio* and *Patinopecten yessoensis* have intron-less architectures (Gong et al., 2017; Xing et al., 2017). The splicing process of post-transcriptional modification will take longer for gene architectures with longer or more introns (Jeffares et al., 2008), which is not good for the efficient regulation of genes. It was discovered that under stress, genes with short or no introns might express themselves quickly (Guo et al., 2015; Liu et al., 2022a). These findings implied that the TLR gene family might have evolved in a significant way toward gene structural simplification as a result of environmental adaption.

Studies have shown that environmental stressors just like hypoxia stress and bacterial infection could affect TLR expression. In the kidneys, spleen, brain, and fish gills, hypoxia typically results in cell necrosis, apoptosis, and inflammation while also encouraging the growth of new blood vessels (Harper and Wolf, 2009). However, little is known about how TLRs function in fish in response to hypoxia. Prior research has demonstrated that in both human and mouse dendritic cells (DCs), hypoxia preferentially upregulated the expressions of TLR2 and TLR6 (Kuhlicke et al., 2007). According to Stridh et al. (2011), hypoxia in neonatal mice led to an increase in TLR1 and TLR2 expression in the brain. Additionally, research on Tibetan schizothoracine fish (*Gymnocypris eckloni*) revealed that while *TLR2* and *TLR3* expressions increased in the head kidney, *TLR8a*, *TLR12* and *TLR19* in the spleen, and *TLR1* expressions increased in the gill, the majority of TLRs' mRNA expressions either significantly decreased or remained unchanged under acute hypoxia (Qi et al., 2017). In this study, the expression of *LcTLR1*, *LcTLR9*, and *LcTLR21* in gill, *LcTLR5* in heart, *LcTLR3, LcTLR5, LcTLR7, LcTLR8* and *LcTLR9* in kidney, and *LcTLR2a*, *LcTLR7*, *LcTLR8* in spleen were significantly up-regulated, whereas the expressions of other *TLRs* in the gill, heart, kidney and spleen declined

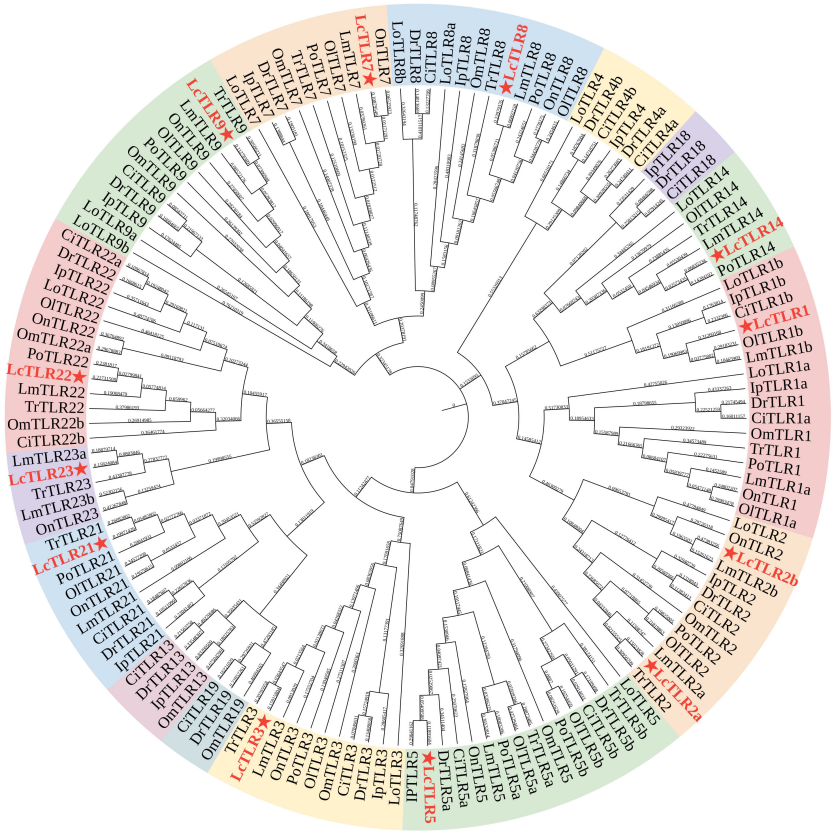

FIGURE 1
Phylogenetic tree of eleven teleost species' *TLR* gene families. A red asterisk was used to identify the *LcTLRs*. The Ip, *Ictalurus punetaus*, Tr, *Takifugu rubripes*; Ci, *Ctenopharyngodon idella*; Po, *Paralichthys olivaceus*; On, *Oreochromis niloticus*; Om, *Oncorhynchus mykiss*; Ol, *Oryzias latipes*; Lo, *Lepisosteus oculatus*; Lm, *Lateolabrax maculatus*; Dr, *Danio rerio* and Lc, *Larimichthys crocea*.

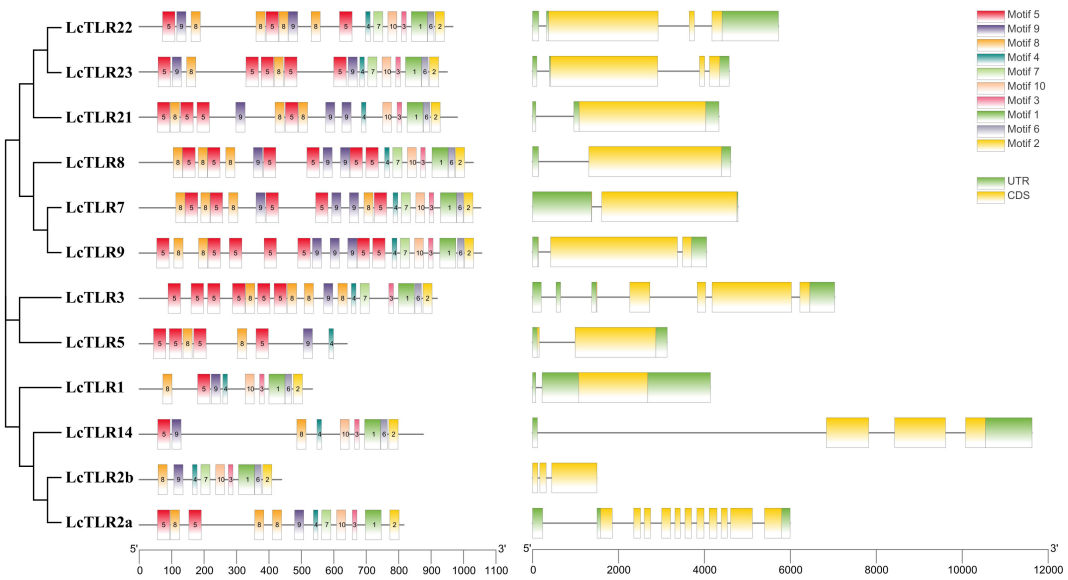

FIGURE 2
The arrangement of 12 *LcTLRs'* conserved motif, gene structure, and evolutionary relationship. The phylogenetic relationships are displayed in the figure on the left. The distribution of conserved motifs is depicted by the middle picture, and each colored rectangular box represents a motif. Rectangular boxes with yellow backgrounds stand for CDSs, whereas green backgrounds stand for UTRs. The scale can be used to infer the length of the exons.

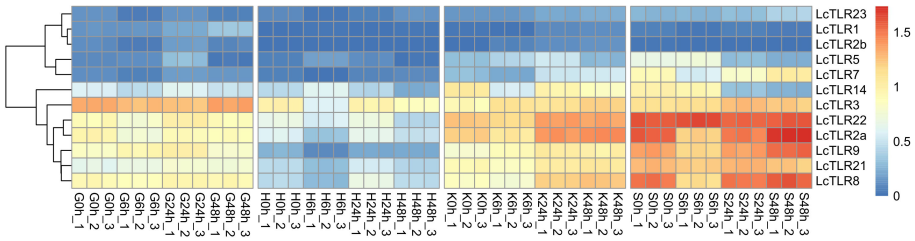

FIGURE 3
Heatmap of *LcTLRs* expression under hypoxia stress. Every cell in the heatmap represents an expression level that has been $\log_{10}$ (FPKM+1) normalized. G stands for gill, H for heart, K for kidney and S for spleen. 0 h as control groups, 6 h, 24 h and 48 h represent after infection treatment 6, 24 and 48 hours. There are three biological replications: X-1, X-2, and X-3.

dramatically or remained unchanged in a severe hypoxic environment. This result was consistent with Tibetan schizothoracine fish *TLRs* expression in response to acute hypoxia. The cessation of the energy-saving mechanism of TLR protein synthesis leads to the reduction of *TLR* mRNA (Roesner et al., 2006), and TLRs are normally involved in innate immune responses regulated by the expression of damage-associated molecular patterns (DAMP), which are secreted by necrotic or damaged cells (Mkaddem et al., 2010). Thus, the unique function of *TLRs* in mediating the innate immune response to host defense against hypoxia-induced tissue damage might be connected to tissue-specific TLR expression under hypoxic settings. TLRs offer a great model for examining the natural selection imposed by pathogenic microorganisms on the organism genome because of their direct placement at the host–environment interface and potential for coevolutionary dynamics with their pathogenic counterparts (Barreiro et al., 2009).

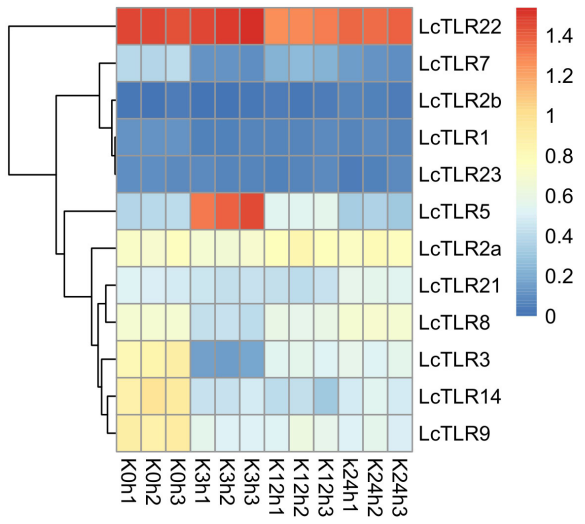

FIGURE 4
Heatmap illustrating the expression of *LcTLRs* following infection with *Aeromonas hydrophila*. Every cell in the heatmap represents an expression level that has been $\log_{10}$ (FPKM+1) normalized. K is kidney. K0h as control groups, K3, K12 and K24 represent after infection treatment 3, 12 and 24 hours, X1, X2 and X3 are the three biological replications.

There had suggested an idea called TLR/TLR cross-talk, which means that, in comparison to DCs stimulated with individual TLR4 ligand and TLR7 ligand, TLR/TLR synergy, such as simultaneous stimulation of human monocyte-derived DCs with LPS (TLR4 agonist) and R484 (TLR7 agonist), leads in a synergistic rise in anti-inflammatory cytokine IL-10 (Napolitani et al., 2005; Manicassamy and Pulendran, 2009). In this study, *A. hydrophila* infection was performed to evaluate *L. crocea TLR* gene alterations, and our results were similar to a research of *L. crocea* in response to *Cryptocaryon irritans* infection, demonstrating that *TLR5* was distinctly up-regulated following infection (Zhang et al., 2020). This might due to the function of *TLR5* responding to PAMP, such as lipids and bacterial proteins (Yang et al., 1999). *TLR* genes that were up-regulated during the bacterial infection were generally thought to be crucial for identifying bacterial ligands (Zhang et al., 2017). Besides, *LcTLR3*, *LcTLR7*, *LcTLR9* and *LcTLR14* were remarkedly down-regulated after 12 h in our study. The role of *TLR3* in immune responses was complex and differed between species. *TLR3* in the spleen did not significantly alter following injection of *Vibrio parahaemolyticus* in the prior research of *Pseudosciaena crocea*, suggesting that bacterial infection of the spleen might not be the initial cause of the *PcTLR3* response (Huang et al., 2011). After bath challenge with a Gram-negative bacterium, rainbow trout's spleen showed a similar outcome (Rodriguez et al., 2005). However, *TLR3* was shown to be significantly down-regulated in the kidney of channel catfish following an infection with *Edwardsiella tarda*. This finding, along with our results, suggested that this protein might be the most susceptible to bacterial infection, as the subpopulation of phagocytes expressing these genes might quickly migrate from the kidney to the infection sites (Zhang et al., 2013). This variation in expression changes of *TLR3* might be due to differences in TLR3-mediated inflammatory responses among different immune organs, further hinting that apart from their recognition of dsRNA, *TLR3* was also engaged in fish immune responses to bacterial infections. *SmTLR7* was markedly down-regulated in the gill and intestine of turbot (*Scophthalmus maximus*) following infection with both *E. tarda* and *Vibrio anguillarum* (Wang et al., 2022). After *A. hydrophila* infection, the expression level of *MaTLR14* was up-regulated in the liver, spleen, foregut, and hindgut of Asian swamp eels (*Monopterus albus*) (Liu et al., 2022b). All of these findings revealed that different Gram-negative bacterial components regulated *TLRs*, suggesting that the immune response to bacterial infection might involve numerous TLR-mediated signaling cascades at the same

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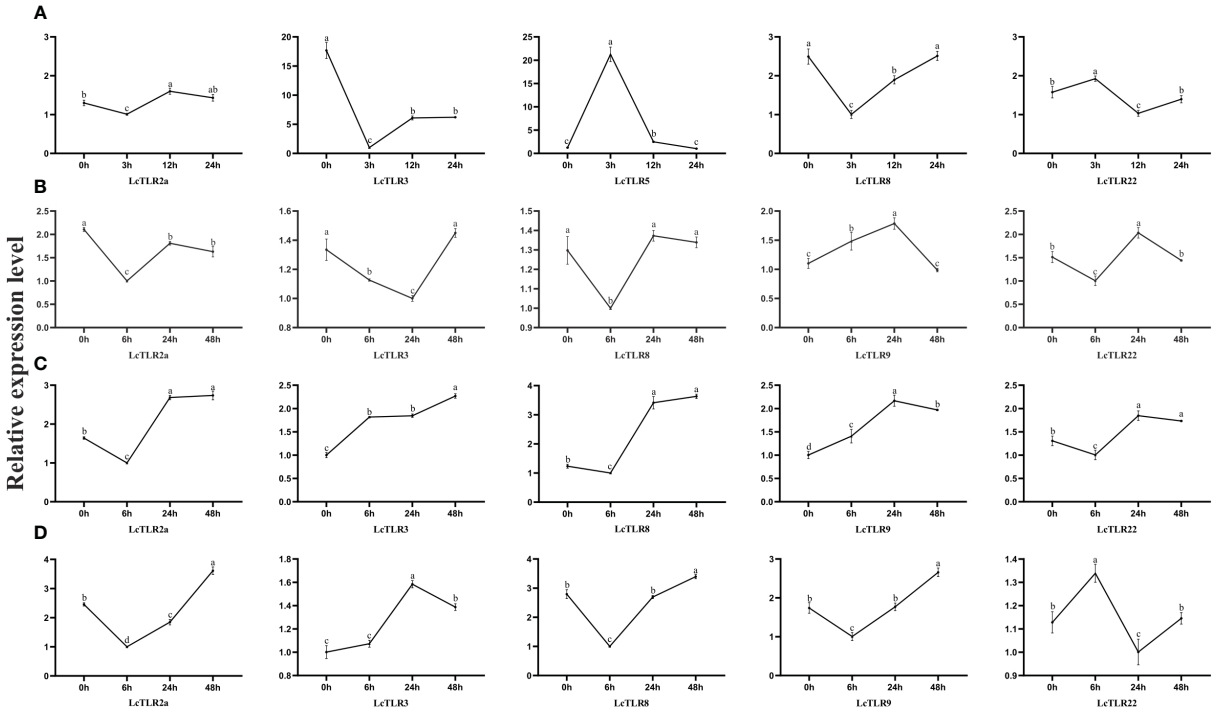

FIGURE 5
Validation of the expression levels of *LcTLRs* after *A. hydrophila* infection and hypoxic stress. **(A–D)** stands for four groups. Group A represents *A. hydrophila* infection, the remaining three groups represent gill, kidney and spleen after hypoxia stress, respectively.

time. Notably, *LcTLR2* had no significant change throughout the process, which was inconsistent with the previous study showing that the *LcTLR2* transcripts increased significantly after *Vibrio parahaemolyticus*, Polyinosinic-polycytidylic acid (Poly(I:C)) and LPS immune challenges ($p < 0.05$) in the spleen, liver and head-kidney (Fan et al., 2015). This might be due to differences in immune response induced by *A. hydrophila* and *V. parahaemolyticus*.

Together with *A. hydrophila* infection and hypoxic stress, this study revealed that there were six and nine members of *LcTLRs* showing significant differential expression in immune responses, respectively. These results provided insights into *TLRs* regulation to biological and abiotic stresses, implying that TLR expressions specific to particular tissues mediated innate immune responses for host defense against tissue damage or physiological changes brought on by bacterial infection and hypoxic stress.

5 Conclusion

The larger yellow croaker (*Larimichthys crocea*) was found to harbor a total of 12 *TLR* genes in this study. Analysis of the *TLR* genes' phylogenetic relationships, gene structures, and motif compositions revealed that these genes were highly conserved among different species. Expression pattern analysis and qPCR validation of *A. hydrophila* infection and hypoxic stress revealed that there were six and nine members of *LcTLRs* showing significant differential expression in immune responses, respectively. Notably, *LcTLR5* was significantly expressed under both biological and abiotic stresses, suggesting that it might play a significant role in the effort to combat environmental stressors. In summary, the identification of TLRs and their roles would advance our knowledge of fish disease resistance mechanisms and open up new avenues for therapeutic intervention to control immune responses.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The animal study was approved by the Institutional Animal Care and Use Committee at the Zhejiang Laboratory Animal Research Center and Ningbo University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

T-YY: Writing – original draft, Methodology, Formal analysis. Q-TM: Writing – original draft, Investigation. X-YS: Writing – original draft, Conceptualization. CR: Writing – original draft, Data curation. Q-PX: Writing – original draft, Formal analysis. X-BW: Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1404183/full#supplementary-material

References

Abdel-Tawwab, M., Monier, M., Hoseinifar, S., and Faggio, C. (2019). Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. *Fish. Physiol. Biochem.* 45, 997–1013. doi: 10.1007/s10695-019-00614-9

Adams, C. S., Kim, H., Burtner, A. E., Lee, D. S., Dobbins, C., Criswell, C., et al. (2024). *De novo* design of protein minibinder agonists of TLR3. *bioRxiv. [Preprint].* 2024.04.17.589973. doi: 10.1101/2024.04.17.589973

Bailey, T., Boden, M., Buske, F., Frith, M., Grant, C., Clementi, L., et al. (2009). MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res.* 37, W202–W208. doi: 10.1093/nar/gkp335

Barreiro, L., Ben-Ali, M., Quach, H., Laval, G., Patin, E., Pickrell, J., et al. (2009). Evolutionary dynamics of human Toll-like receptors and their different contributions to host defense. *PLoS Genet* 5, e1000562. doi: 10.1016/j.molp.2020.06.009

Chen, C., Chen, H., Zhang, Y., Thomas, H., Frank, M., He, Y., et al. (2020). TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* 13, 1194–1202. doi: 10.1016/j.molp.2020.06.009

Chen, X., Wu, Z., Yu, S., Wang, S., and Peng, X. (2010). Beta2-microglobulin is involved in the immune response of large yellow croaker to *Aeromonas hydrophila*: a proteomic based study. *Fish. Shellfish. Immunol.* 28, 151–158. doi: 10.1016/j.fsi.2009.10.015

Dias, B. T., Goundry, A., Vivarini, A. C., Costa, T. F. R., Mottram, J. C., Lopes, U. G., et al. (2022). Toll-like receptor- and protein kinase R-induced type I interferon sustains infection of *leishmania donovani* in macrophages. *Front. Immunol.* 13, 801182. doi: 10.3389/fimmu.2022.801182

Ding, J., Liu, C., Luo, S., Zhang, Y., Gao, X., Wu, X., et al. (2020). Transcriptome and physiology analysis identify key metabolic changes in the liver of the large yellow croaker (*Larimichthys crocea*) in response to acute hypoxia. *Ecotoxicol. Environ. Saf.* 189, 109957. doi: 10.1016/j.ecoenv.2019.109957

Ding, J., Zhang, Y., Wang, J., Liu, C., Gao, X., Wu, Y., et al. (2022). Genome-wide association study identified candidate SNPs and genes associated with hypoxia tolerance in large yellow croaker (*Larimichthys crocea*). *Aquaculture* 560, 738472. doi: 10.1016/j.aquaculture.2022.738472

Duvaud, S., Gabella, C., Lisacek, F., Stockinger, H., Ioannidis, V., and Durinx, C. (2021). Expasy the Swiss bioinformatics resource portal, as designed by its users. *Nucleic Acids Res.* 49, W216–W227. doi: 10.1093/nar/gkab225

Edgar, R. (2004). MUSCLE: multiple sequence alignment with high accuracy and high Throughput. *Nucleic Acids Res.* 32, 1792–1797. doi: 10.1093/nar/gkh340

El-Gebali, S., Mistry, J., Bateman, A., Eddy, S., Luciani, A., Potter, S., et al. (2019). The Pfam protein families database in 2019. *Nucleic Acids Res.* 47, D427–D432. doi: 10.1093/nar/gky995

Fan, H., Wang, L., Wen, H., Wang, K., Qi, X., Li, J., et al. (2019). Genome-wide identification and characterization of toll-like receptor genes in spotted sea bass (*Lateolabrax maculatus*) and their involvement in the host immune response to *Vibrio harveyi* infection. *Fish. Shellfish. Immunol.* 92, 782–791. doi: 10.1016/j.fsi.2019.07.010

Fan, Z., Jia, Q., and Yao, C. (2015). Characterization and expression analysis of Toll-like receptor 2 gene in large yellow croaker, *Larimichthys crocea*. *Fish. Shellfish. Immunol.* 44, 129–137. doi: 10.1016/j.fsi.2015.01.037

Gao, S., Huang, W., Peng, S., Zhou, J., Zhan, H., Lu, T., et al. (2024). Molecular characterization and expression analysis of nine toll like receptor (TLR) genes in *Scortum barcoo* under *Streptococcus agalactiae* infection. *Int. J. Biol. Macromol.* 254, 127667. doi: 10.1016/j.ijbiomac.2023.127667

Gong, Y., Feng, S., Li, S., Zhang, Y., Zhao, Z., Hu, M., et al. (2017). Genome-wide characterization of Toll-like receptor gene family in common carp (*Cyprinus carpio*) and their involvement in host immune response to *Aeromonas hydrophila* infection. *Comp. Biochem. Physiol. Part D. Genomics Proteomics* 24, 89–98. doi: 10.1016/j.cbd.2017.08.003

Guo, M., Liu, H., Lu, P., Zhai, F., Wang, H., Gong, H., et al. (2015). Genome-wide analysis of the CaHsp20 gene family in pepper: comprehensive sequence and expression profile analysis under heat stress. *Front. Plant Sci.* 6, 806. doi: 10.3389/fpls.2015.00806

Harper, C., and Wolf, J. (2009). Morphologic effects of the stress response in fish. *ILAR. J.* 50, 387–396. doi: 10.1093/ilar.50.4.387

Huang, X., Wang, Z., and Yao, C. (2011). Characterization of Toll-like receptor 3 gene in large yellow croaker, *Pseudosciaena crocea*. *Fish. Shellfish. Immunol.* 31, 98–106. doi: 10.1016/j.fsi.2011.04.009

Jaworski, L., Kleinhans, K., and Jackson, A. (2019). Effects of oxygen concentration and culture time on porcine nucleus pulposus cell metabolism: an in *vitro* study. *Front. Bioeng. Biotechnol.* 7. doi: 10.3389/fbioe.2019.00064

Jeffares, D., Penkett, J., and Bahler, J. (2008). Rapidly regulated genes are intron poor. *Trends Genet.* 24, 375–378. doi: 10.1016/j.tig.2008.05.006

Kuhlicke, J., Frick, J., Morote-Garcia, J., Rosenberger, P., and Eltzschig, H. (2007). Hypoxia inducible factor (HIF)-1 coordinates induction of Toll-like receptors TLR2 and TLR6 during hypoxia. *PloS One* 2, 1364. doi: 10.1371/journal.pone.0001364

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. *Biol. Evol.* 33, 1870–1874. doi: 10.1093/molbev/msw054

Lee, Y., Kim, D. H., Lee, J. S., Kim, H. S., Maszczyk, P., Wang, M., et al. (2024). Combined exposure to hypoxia and nanoplastics leads to negative synergistic oxidative stress-mediated effects in the water flea *Daphnia magna*. *Mar. pollut. Bull.* 202, 116306. doi: 10.1016/j.marpolbul.2024.116306

Letunic, I., and Bork, P. (2018). 20 years of the SMART protein domain annotation resource. *Nucleic Acids Res.* 46, D493–D496. doi: 10.1093/nar/gkx922

Li, S., Yang, Z., Tian, H., Ren, S., Zhang, W., and Wang, A. (2022). Effects of dietary carbohydrate/lipid ratios on non-specific immune responses, antioxidant capacity, hepatopancreas and intestines histology, and expression of TLR-MAPK/NF-κB signaling pathway-related genes of *Procambarus clarkia*. *Fish. Shellfish. Immunol.* 124, 219–229. doi: 10.1016/j.fsi.2022.04.005

Liu, R., Qi, Y., Feng, H., Niu, Y., Zhang, F., Yang, G., et al. (2022b). Fish-specific Toll-like receptor 14 (TLR14) from Asian swamp eel (*Monopterus albus*) is involved in immune response to bacterial infection. *Fish. Shellfish. Immunol.* 124, 313–323. doi: 10.1016/j.fsi.2022.04.010

Liu, D., Qin, Z., Wei, M., Kong, D., Zheng, Q., Bai, S., et al. (2022a). Genome-wide analyses of heat shock protein superfamily provide new insights on adaptation to sulfide-rich environments in *Urechis unicinctus* (Annelida, Echiura). *Int. J. Mol. Sci.* 23, 2715. doi: 10.3390/ijms23052715

Mahapatra, S., Ganguly, B., Pani, S., Saha, A., and Samanta, M. (2023). A comprehensive review on the dynamic role of toll-like receptors (TLRs) in frontier aquaculture research and as a promising avenue for fish disease management. *Int. J. Biol. Macromol.* 253, 126541. doi: 10.1016/j.ijbiomac.2023.126541

Manicassamy, S., and Pulendran, B. (2009). Modulation of adaptive immunity with Toll-like receptors. *Semin. Immunol.* 21, 185–193. doi: 10.1016/j.smim.2009.05.005

Mkaddem, S., Bens, M., and Vandewalle, A. (2010). Differential activation of Toll-like receptor-mediated apoptosis induced by hypoxia. *Oncotarget* 1, 741–750. doi: 10.18632/oncotarget.v1i8

MOA (2023). *China fishery Statistical Yearbook* (Beijing: China Agricultural Press).

Mu, Y., Ding, F., Cui, P., Ao, J., Hu, S., and Chen, X. (2010). Transcriptome and expression profiling analysis revealed changes of multiple signaling pathways involved in immunity in the large yellow croaker during *Aeromonas hydrophila* infection. *BMC Genomics* 11, 506. doi: 10.1186/1471-2164-11-506

Mu, Y., Huo, J., Guan, Y., Fan, D., Xiao, X., Wei, J., et al. (2018). An improved genome assembly for *Larimichthys crocea* reveals hepcidin gene expansion with diversified regulation and function. *Commun. Biol.* 1, 195. doi: 10.1038/s42003-018-0207-3

Napolitani, G., Rinaldi, A., Bertoni, F., Sallusto, F., and Lanzavecchia, A. (2005). Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat. Immunol.* 6, 769–776. doi: 10.1038/ni1223

Ni, L., Xu, Y., Zhao, Q., Shao, F., and Peng, Z. (2022). Genome-wide characterization of the Toll-like receptor gene family in Triplophysa rosa and expression profiles in response to *Aeromonas hydrophila* infection. *Aquaculture* 555, 738208. doi: 10.1016/j.aquaculture.2022.738208

Palti, Y. (2011). Toll-like receptors in bony fish: from genomics to function. *Dev. Comp. Immunol.* 35, 1263–1272. doi: 10.1016/j.dci.2011.03.006

Qi, D., Xia, M., Chao, Y., Zhao, Y., and Wu, R. (2017). Identification, molecular evolution of toll-like receptors in a Tibetan schizothoracine fish (*Gymnocypris eckloni*) and their expression profiles in response to acute hypoxia. *Fish. Shellfish. Immunol.* 68, 102–113. doi: 10.1016/j.fsi.2017.07.014

Rebl, A., Goldammer, T., and Seyfert, H. (2010). Toll-like receptor signaling in bony fish. *Vet. Immunol. Immunopathol.* 134, 139–150. doi: 10.1016/j.vetimm.2009.09.021

Rodriguez, M., Wiens, G., Purcell, M., and Palti, Y. (2005). Characterization of Toll-like receptor 3 gene in rainbow trout (*Oncorhynchus mykiss*). *Immunogenetics* 57, 510–519. doi: 10.1007/s00251-005-0013-1

Roesner, A., Hankeln, T., and Burmester, T. (2006). Hypoxia induces a complex response of globin expression in zebrafish (*Danio rerio*). *J. Exp. Biol.* 209, 2129–2137. doi: 10.1242/jeb.02243

Ryan, T. A. J., and O'Neill, L. A. J. (2023). An emerging role for type I interferons as critical regulators of blood coagulation. *Cells* 12, 778. doi: 10.3390/cells12050778

Sahoo, B., Basu, M., Swain, B., Maharana, J., Dikhit, M., Jayasankar, P., et al. (2012). Structural insights of rohu TLR3, its binding site analysis with fish reovirus dsRNA, poly I:C and zebrafish TRIF. *Int. J. Biol. Macromol.* 51, 531–543. doi: 10.1016/j.ijbiomac.2012.06.005

Shi, B., Zhang, Z., Xing, J., Liu, Q., Cai, J., and Zhang, Z. (2024). Perfluorooctane sulfonate causes pyroptosis and lipid metabolism disorders through ROS-mediated NLRP3 inflammasome activation in grass carp hepatocyte. *Aquat. Toxicol.* 267, 106839. doi: 10.1016/j.aquatox.2024.106839

Shun, C., Wen-Ping, J., Shi-Li, L., Jian-Bo, Z., Mei-Li, C., Xiao-Ying, H., et al. (2024). Effect of dissolved oxygen and ammonia nitrogen on *Culter alburnus*: Physiology, biochemistry, and molecular analyses. *Heliyon* 10, e27921. doi: 10.1016/j.heliyon.2024.e27921

Stridh, L., Smith, P., Naylor, A., Wang, X., and Mallard, C. (2011). Regulation of toll-like receptor 1 and -2 in neonatal mice brains after hypoxia-ischemia. *J. Neuroinflamm.* 8, 45. doi: 10.1186/1742-2094-8-45

Tran, N., Kong, T., Zhang, M., and Li, S. (2019). Pattern recognition receptors and their roles on the innate immune system of mud crab (*Scylla paramamosain*). *Dev. Comp. Immunol.* 102, 103469. doi: 10.1016/j.dci.2019.103469

Verma, S., Menon, R., and Sowdhamini, R. (2024). Structural insights into the role of deleterious mutations at the dimeric interface of Toll-like receptor interferon-β related adaptor protein. *Proteins*. doi: 10.1002/prot.26707

Vidya, M., Kumar, V., Sejian, V., Bagath, M., Krishnan, G., and Bhatta, R. (2018). Toll-like receptors: significance, ligands, signaling pathways, and functions in mammals. *Int. Rev. Immunol.* 37, 20–36. doi: 10.1080/08830185.2017.1380200

Wallach, T., Raden, M., Hinkelmann, L., Brehm, M., Rabsch, D., Weidling, H., et al. (2023). Distinct SARS-CoV-2 RNA fragments activate Toll-like receptors 7 and 8 and induce cytokine release from human macrophages and microglia. *Front. Immunol.* 13, 1066456. doi: 10.3389/fimmu.2022.1066456

Wang, K., Huang, H., Zhan, Q., Ding, H., and Li, Y. (2024). Toll-like receptors in health and disease. *MedComm. (2020).* . 5, e549. doi: 10.1002/mco2.549

Wang, X., Liu, S., Yang, Y., Fu, Q., Abebe, A., and Liu, Z. (2017). Identification of NF-kappa B related genes in channel catfish and their expression profiles in mucosal tissues after columnaris bacterial infection. *Dev. Comp. Immunol.* 70, 27–38. doi: 10.1016/j.dci.2017.01.003

Wang, B., Liu, X., Zhao, J., Cao, M., Yu, Z., Fu, Q., et al. (2022). Characterization, evolution and expression analysis of Toll-like receptor 7 (TLR7) in turbot (*Scophthalmus maximus L.*). *Fish. Shellfish. Immunol.* 125, 9–16. doi: 10.1016/j.fsi.2022.04.035

Xie, Q., Zhan, W., Shi, J., Liu, F., Niu, B., He, X., et al. (2023). Whole-genome assembly and annotation for the little yellow croaker (*Larimichthys polyactis*) provide insights into the evolution of hermaphroditism and gonochorism. *Mol. Ecol. Resour.* 23, 632–658. doi: 10.1111/1755-0998.13731

Xing, Q., Liao, H., Xun, X., Wang, J., Zhang, Z., Yang, Z., et al. (2017). Genome-wide identification, characterization and expression analyses of TLRs in Yesso scallop (Patinopecten yessoensis) provide insight into the disparity of responses to acidifying exposure in bivalves. *Fish. Shellfish. Immunol.* 68, 280–288. doi: 10.1016/j.fsi.2017.07.020

Yan, W., Qiao, Y., Qu, J., Liu, X., Zhang, Q., and Wang, X. (2021). The hsp40 Gene Family in Japanese Flounder: identification, Phylogenetic Relationships, Molecular Evolution Analysis, and Expression Patterns. *Front. Mar. Sci.* 7. doi: 10.3389/fmars.2020.596534

Yang, R., Mark, M., Gurney, A., and Godowski, P. (1999). Signaling events induced by lipopolysaccharide-activated toll-like receptor 2. *J. Immunol.* 163, 639–643. doi: 10.4049/jimmunol.163.2.639

Yao, T., Wang, R., Han, P., Liu, X., and Wang, X. (2023). Identification of olive flounder (*Paralichthys olivaceus*) toll-like receptor genes: involvement in immune response to temperature stress and *Edwardsiella tarda* infection. *Fish. Shellfish. Immunol.* 138, 108841. doi: 10.1016/j.fsi.2023.108841

Yeh, D., Liu, Y., Lo, Y., Yuh, C., Yu, G., Lo, J., et al. (2013). Toll-like receptor 9 and 21 have different ligand recognition profiles and cooperatively mediate activity of CpG-oligodeoxynucleotides in zebrafish. *Proc. Natl. Acad.* 110, 20711–20716. doi: 10.1073/pnas.1305273110

Zhang, H., Gao, S., Lercher, M., Hu, S., and Chen, W. (2012). EvolView, an online tool for visualizing, annotating and managing phylogenetic trees. *Nucleic Acids Res.* 40, W569–W572. doi: 10.1093/nar/gks576

Zhang, J., Liu, S., Rajendran, K., Sun, L., Zhang, Y., Sun, F., et al. (2013). Pathogen recognition receptors in channel catfish: III phylogeny and expression analysis of Toll-like receptors. *Dev. Comp. Immunol.* 40, 185–194. doi: 10.1016/j.dci.2013.01.009

Zhang, Y., Shen, W., Ding, J., Gao, X., Wu, X., and Zhu, J. (2022). Comparative transcriptome analysis of head kidney of *aeromonas hydrophila*-infected hypoxia-tolerant and normal large yellow croaker. *Mar. Biotechnol. (NY).* 24, 1039–1054. doi: 10.1007/s10126-022-10158-4

Zhang, X., Zhang, G., Shi, Z., Yuan, Y., Zheng, H., Lin, L., et al. (2017). Expression analysis of nine Toll-like receptors in yellow catfish (*Pelteobagrus fulvidraco*) responding to *Aeromonas hydrophila* challenge. *Fish. Shellfish. Immunol.* 63, 384–393. doi: 10.1016/j.fsi.2017.02.021

Zhang, X., Zhou, T., Chen, B., Bai, H., Bai, Y., Zhao, J., et al. (2020). Identification and expression analysis of long non-coding rna in large yellow croaker (*Larimichthys crocea*) in response to *Cryptocaryon irritans* infection. *Front. Genet.* 11, 590475. doi: 10.3389/fgene.2020.590475