Unraveling Alternative Splicing Regulations in *Seriola dumerili* Underlying Their Responses to Ambient Salinity Oscillations

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Abstract

The greater amberjack (*Seriola dumerili*) is a kind of widely distributed and economically important saltwater fish. However, its growth and development are sensitive to alterations of marine environment, for example, seawater salinity. Previous studies have unraveled the contributions of transcriptional regulations in modulating the resistance to seawater salinity fluctuations in the greater amberjack, but no attentions have been ever paid to post-transcriptional mechanisms, which also play an important role in fish growth, development and adaptation to environmental stress. In this study, we performed a comparative transcriptomic analysis to investigate the genome-wide alternative splicing (AS) dynamics in the greater amberjack under both hypo- (10 ppt) and hyper-salinity stresses. In the gills, 461 and 426 differential spliced genes (DSGs) were identified in response to 10 ppt and 40 ppt salinity, respectively. For both stress scenarios, the genes related to sodium ion transport were prone to produce a higher proportion of isoforms including more exons, suggesting an enhancement of mainly involved in. GO enrichment results showed that these genes were mainly involved in sodium ion adjustment in response to the deviation of optimal water salinity. Comparatively, pH regulation, developmental process, and positive regulation of Wnt signaling pathway were exclusively activated under 10 ppt salinity, while lipid transport and regulation of cell division were enhanced when exposed to 40 ppt saline. In the kidneys, 468 more-exon inclusive DSGs were found in response to both hypo- and hyper-salinity stress, which mainly associated with antioxidant processes, such as sulfur compound biosynthetic process and peroxisome organization. Furthermore, AS events were found to modulate different physiological processes from gene expression, suggesting AS played relatively independent role in salinity stress response in the greater amberjack. These novel findings provide us a better understanding of the post-transcriptional mechanisms underlying the resistance of the greater amberjack to ambient salinity oscillation and also provide a genetic basis for the conservation of marine organisms and biodiversity.

1 introduction

Occupying more than two-thirds of the Earth's surface, the oceans are the largest ecosystem on the

planet that hosting a huge number of organisms and play crucial roles in maintenance of the diversity and balance of life on Earth (National Geographic Society, 2017). However, in the recent few decades, global climate changes, such as global warming, glacier melting and precipitation decreasing, have substantially changed global water cycle, and altered worldwide salinity distribution among different sea areas (Durack and Wijffels 2010; Helm et al. 2010; Durack et al. 2012; Skliris et al. 2016; Li et al., 2020; Asad Ullah， 2021). One obvious phenomenon is that the fresh water is getting fresher while the saltwater becomes saltier (Durack, 2012). Till 2017, the mean salinity contrast between the low and high salinity regions was increased by more than 5% (Cheng et al., 2020). Furthermore, this imbalance has been found to be amplified by human activities (Omerspahic et al., 2022; Röthig et al., 2023). For instance, brine discharge during seawater desalination can lead to an abnormal increase of the salinity of the receiving water body of coastal marine environment (Omerspahic et al., 2022), while the enhanced urban drainage was supposed to freshen nearshore seawater (Röthig et al., 2023). Salinity fluctuation of seawater would alter the dissolved oxygen level, nutrient concentration and stratification of the oceans, thereby posting adverse impact on the respiration, metabolism, reproduction and even disease immunity of marine organisms (Lehtonen et al., 2016; Guo et al., 2020; Galkanda-Arachchige et al., 2021; Lu et al., 2022; Eric Ralls, 2023; Röthig et al., 2023). For example, the increase of water salinity was found to disrupt the normal physiological and metabolic processes of fishes, such as grass carp (Ctenopharyngodon idella) and African catfish (Clarias gariepinus), and alter their energy supply for adjusting body osmolality, thus finally inhibiting their growth and survival (Katie Smyth, 2016; Zidan et al., 2022; Liu et al., 2023). Moreover, salinity change-induced biodiversity loss of plankton, macroalgae, coral reef, seagrass may cause trophic collapse of marine ecosystem and lead to drop in fish production, which further threaten ecosystem functions and food supply of human from fishing and aquaculture (Röthig et al., 2023).

Seriola dumerili, also known as the greater amberjack, is a large saltwater bony fish of the family Carangidae. They are globally distributed in tropical and subtropical seas, including the Atlantic, Indian, Pacific and Mediterranean Oceans (Zupa et al., 2017; Araki et al., 2018). The greater amberjack is an economically important fish that is widely popular for its tasty meat, and it is also a major predator and inhabitant of artificial reefs such as coral reefs and shipwrecks, maintaining the structure and function of these ecosystems (Pereira, Maria et al, 2023). It has been widely reported that the growth and development, and electrophysiological processes in the greater amberjack were sensitive to environmental factors, such as salinity, temperature and seawater pressure (Barany 2021; Boyle et al., 2022; Navarro-Guillen et al., 2022; Peng et al., 2022; Liu et al., 2023; Ru et al., 2023). Comparative transcriptomic studies revealed that the greater amberjack has strong osmoregulatory capacity under salt stress, reflected in osmolality, muscle water content, gill and intestinal Na-K-ATPase activity and so on, (Andre Barany,Neda Gilannejad， 2021). In addition, transcriptomic analyses showed that salinity stress also affects steroid biosynthesis, lipid metabolism, ion transport proteins and immune responses in the greater amberjack (Yuhao Peng, Hongjuan Shi, 2022). Recent studies have shown that the greater amberjack get significant effects on cartilage and skeletal development, ion transport and immune response in gills, as well as pronephros development, growth regulation, ion transmembrane transport and immune response in kidneys under low (10 ppt) and high (40 ppt) salt stress (Liu, et al, 2023). However, most of the current researches only focused on the responsive regulations at transcriptional level, and no attentions have been ever paid to the role of post-transcriptional

mechanisms in modulating the resistance to seawater salinity fluctuations in the greater amberjack.

Alternative splicing (AS) is one type of post-transcriptional regulatory mechanisms in eukaryotes, and it can produce multiple isoforms with different combination of exons, thus giving rise to multiple protein products from one single gene (Tian, 2022). Therefore, AS can enrich the structural and functional diversity of proteins without any increase in gene number, and have been demonstrated to paly critical roles in regulating the growth, development and environmental adaptation of eukaryotic organisms. In zebrafish (*Danio rerio*), AS plays a key regulatory role during embryonic development (Xiao, J., et al. 2022). Antiviral activity of Japanese flounder (*Paralichthys olivaceus*) obtained by AS regulation of TRIM family genes (Guo, Yanan, et al. 2022). In Grass carp (*Ctenopharyngodon idella*), AS regulates antiviral immunity (Wan, Q., Su, J., 2015). AS regulates genes closely related to the cell cycle in the spotted sea bass (*Lateolabrax maculatus*) under hypoxic conditions (Ren, Yuhang, et al. 2022). This suggests that AS generally plays an important regulatory role in fish growth, development and adaptation to environmental stress. While the post-transcriptional regulatory mechanisms of the greater amberjack under salinity stress are not well studied, it is necessary to explore the regulatory role of AS to fully understand the physiological mechanisms of adaptation to environmental stress in the greater amberjack.

Therefore, in this study, the results of alternative splicing events under low and high salt culture conditions were obtained by analyzing the RNA-seq data of greater amberjack. And the related genes and biological process pathways were analyzed mainly for exon skipping events, in addition to predicting the premature termination codon for exon skipping events and comparing the differentially expressed genes with the differentially alternative splicing genes. The aim is to understand what kind of physiological changes occur in greater amberjack at low and high salt by the above study. This will provide some theoretical basis for the study of the conservation of marine fishes when the salinity of seawater changes due to global warming, and to understand the possible impacts of rising and falling seawater salinity caused by global climate change on this group of marine fishes, which will help us to conserve the diversity of marine organisms and maintain the stability of the marine ecosystem.

2 Materials and methods

2.1 Data preprocessing

In our previous study, Briefly, the larval fish reared in Donghai Island, Guangdong, China.They were randomly divided into 12 groups and kept in salinities of 10, 30 and 40 parts per thousand (ppt). After a period of time, some randomly selected fish from each group were dissected and gill and kidney tissues were taken for RNA seq rol group, and the remaining data from different salinities is the experimental group (Liu, 2023). Here, we re-used the transcriptome data (National Center for Biotechnology Information (NCBI) accession number: GSE220485) for alternative splicing analysis to investigate the role of post-transcriptional regulations in response to ambient salinity changes in the greater amberjack. Read quality of each data was assessed with FastQC v. 0.11.9 (Andrews, 2010). Adapter contamination and low-quality reads were cleaned up using Trim Galore v. 0.6.6 (Krueger, 2015) with a minimum quality score of 25 and length of 50. A genome index was constructed for the reference genome of the greater amberjack (NCBI

GCA_002260705.1) by the hisat2-built utility of HISAT2 program v. 2.2.1 (Kim, 2019). High-quality reads were mapped to the reference genome by hisat2. For each sample, only the uniquely aligned reads were retained for downstream analysis, and the alignment output was further assembled into full-length transcripts using StringTie v2.2.1 (Kovaka, 2019). All sets of assembled transcripts were then merged into a non-redundant set of transcripts using StringTie's merge mode. Finally, the assembled transcripts were annotated by compared against the reference annotations obtained from the NCBI RefSeq database using GFFcompare v. 0.11.2 (Pertea, 2020).

2.2 Identification of differential alternative splicing (DAS) events

rMATS v. 4.0.2 (Shen et al, 2014) was employed to detect alternative splicing events of five major types: skipped exon (SE), alternative 5′ splice site (A5SS), alternative 3′ splice site (A3SS), mutually exclusive exons (MXE), and retained intron (RI) (Mehmood, et al., 2020.). For each event, the inclusion level/percent spliced in (PSI) of a transcript element was calculated in each sample, and changes in average PSI values were compared between each treatment group (10 and 40 ppt) and the control group (30 ppt, CK) in gill and kidney, respectively, to investigate the shift in splicing patterns in response to ambient salinity alterations. For each comparison, the events with a FDR < 0.05 were considered to be significantly different between two groups, and the genes with any differential splicing event were denoted as "DSGs" for convenience. Similar to other animals, SE events are the dominant AS type the greater amberjack, thus here we first mainly focused on the functional importance of SE events. For the convenience, the genes with SE events having a PSI value under any treatment condition larger than that in CK meant that they are likely to produce a higher proportion of the transcripts containing the exon, and were denoted as "more exon-inclusive DSGs", while those with a smaller PSI value were prone to give rise to more transcripts with the exon skipped, and were denoted as "less exon-inclusive DSGs".

2.3 Functional enrichment analysis

To access the functional importance of the DSGs we identified, we first built an OrgDb database for the greater amberjack, based on the gene annotations obtained in our previous study, using the R package AnnotationForge v. 1.38.1 (Carlson, 2017). For both gill and kidney, Gene Ontology (GO) enrichment analysis was carried out for the DSGs of interest by the enrichGO function of clusterProfiler package v. 4.6.2 (Yu, 2018) . *P-value* < 0.05 was set as the criteria for statistical significance. For each comparison, the significantly enriched GO terms were then clustered according to their similarities in gene sets and visualized by a tree diagram using the treeplot function of clusterProfiler package. Furthermore, for each tissue, the more exon-inclusive DSGs were compared across different salinity treatments and the results were visualized by UpsetR package v. 1.4.0 (Conway, 2017). GO enrichment analysis was implemented for the DSGs 1) specific to 10 ppt saline treatment, 2) specific to 40 ppt saline treatment, and 3) identified under both treatments, respectively.

2.4 premature termination codons (PTCs) induced by SE events

One of the impacts of alternative splicing is introduction of premature termination codons (PTCs) to transcribed isoforms, which may cause deficiency of protein structure and function, or even

lead to nonfunctional proteins (Yeh, J, 2020). Premature termination codons (PTC) account for approximately 20% of genetic defects in human diseases and are often associated with severe phenotypes (Oren et al, 2017). Here, we first predicted putative PTCs induced by SE events. investigated the impacts of splicing-induced PTCs on Greater Amberjack gene expression. We first predicted the coding sequence (CDS) regions were first predicted for each isoform based on the annotations of the assembled transcripts. Only those with unambiguous annotations of CDS, translation initiation codon and termination codon were retained for downstream analysis. Then the coding sequences of each target isoform was extracted from the great amberjack genome, and the putative termination codon were screened in the coding regions. The first appeared termination codon before the original termination codon was considered as the PTC for the isoform. Empirically, the DSGs giving rise to a lower proportion of the isoforms with PTCs under treatment scenario than CK were supposed to produce more proteins with normal functions, thus may play a positively regulatory role in response to ambient salinity alterations.

2.5 Comparison between differential alternative splicing and gene expression

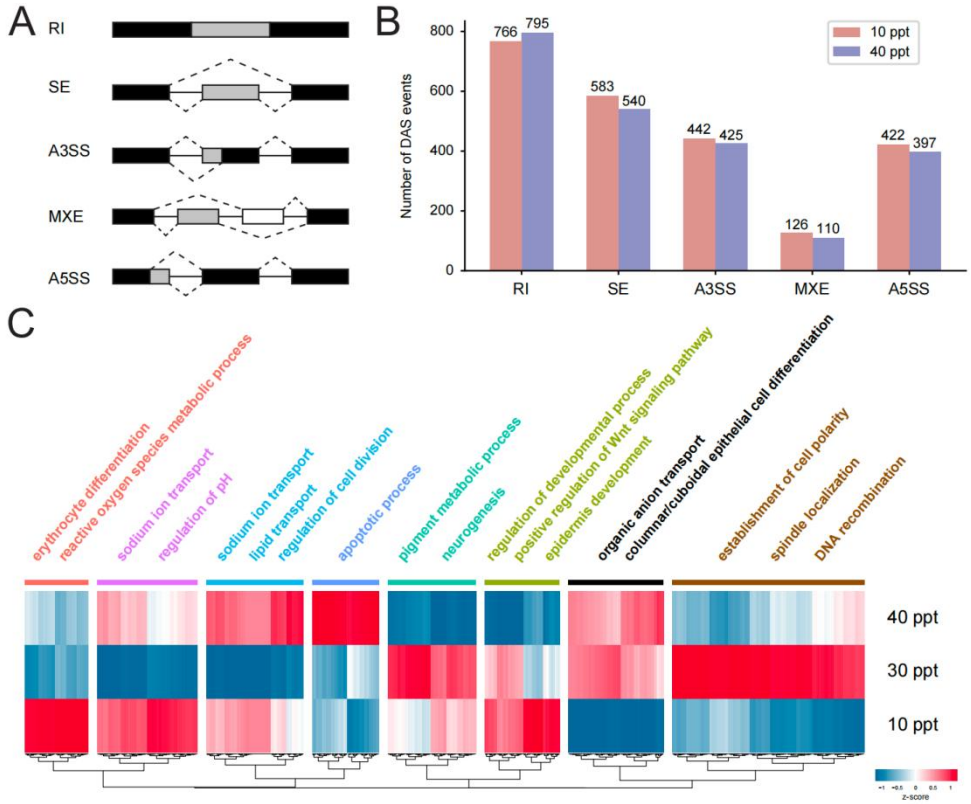
To further investigate the different roles between AS and gene expression in modulating salinity oscillation tolerance in greater amberjack, we compared the more exon-inclusive DSGs to the up-regulated differentially expressed genes (DEGs) under each treatment scenario. The DEGs were detected following the same criteria described in our previous study (Liu et al., 2023). GO enrichment analyses were then conducted for 1) the genes specifically regulated by differential AS, and 2) genes specifically regulated by differential expression.

3 Results

3.1 Alternative splicing dynamics in response to salinity alterations in gills of greater amberjack

In the gills of the greater amberjack, compared to the CK group, RI events exhibited the largest differences under both hypo- and hyper-salinity treatments, followed by SE and A3SS events (Figure 1B). Of them, the PSI of 583 SE events in 461 genes was significantly changed under 10 ppt salinity stress, where 182 genes produced transcripts with a higher inclusive proportion the representative exons, while the products of the rest 279 genes were more likely to skip the representative exons (Supplementary Figure 1). In the 40 ppt salinity group, 540 significant SE events were detected in 426 DSGs, with 187 and 239 genes being more and less exon inclusive, respectively (Supplementary Figure 1). To investigate AS dynamics across different salinity regimes, the PSI of DSGs were compared across the treatments of 10, 30 and 40 ppt saline (Figure 1C). In Cluster 1, 2 and 6, the majority of DSGs exhibited the highest inclusive level of the representative exons under 10 ppt saline than the other two conditions. GO enrichment results showed that these genes were mainly involved in erythrocyte differentiation, reactive oxygen species (ROS) metabolic process, sodium ion transport, pH regulation, regulation of developmental process, and positive regulation of Wnt signaling pathway. In contrast, the DSGs in Cluster 3 and 4 were of the highest exon inclusion in hyper-salinity treatment and these genes mainly participated in sodium ion transport, lipid transport and regulation of cell division. These results suggested that the greater amberjack activated sodium ion transport in gills to cope with deviation of normal water salinity. A large group of DSGs displayed less exon inclusion under

either hypo- or hyper-salinity than CK (Cluster 5 and 8), including those associated with pigment metabolic process, neurogenesis, cell polarity establishment, spindle localization, and DNA recombination.

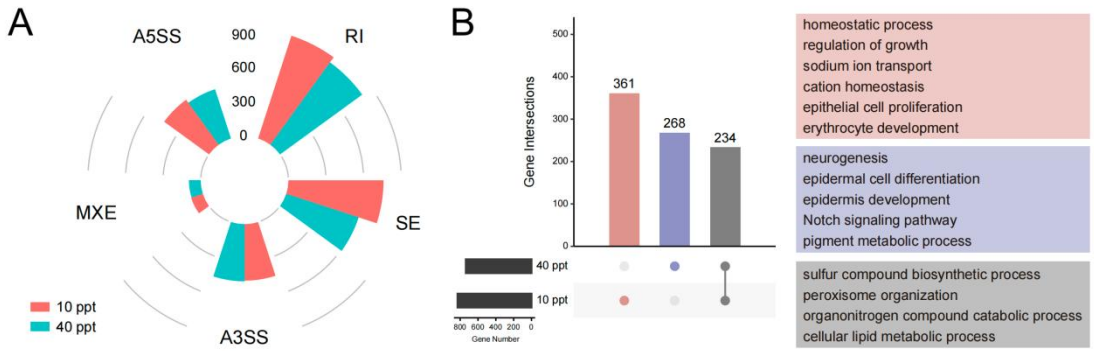


3.2 Alternative splicing dynamics in response to salinity alterations in kidneys of greater amberjack

In the kidneys, 759 significant SE events in 595 DSGs were detected under salinity stress at 10 ppt (276 more exon-inclusive DSGs and 319 less exon-inclusive DSGs), while 607 SE events of 502 DSGs were significantly changed under 40 ppt salinity stress (215 more exon-inclusive DSGs and 287 less exon-inclusive DSGs) (Figure 2A). Under 10 ppt salinity, the 276 more exon-inclusive DSGs were found to be overrepresented in the GO terms of sodium ion transmembrane transport, organonitrogen compound catabolic process, regulation of pH, erythrocyte homeostasis, response to mechanical stimulus, myeloid cell homeostasis and organophosphate metabolic process, while those with less exon inclusion were highly representative in pigment granule transport, epithelial cell differentiation involved in kidney development, chromosome organization and regulation of growth (Figure S1). In response to 40 ppt salinity stress, genes related to sulfur compound metabolism, aspartate family amino acid biosynthesis, lipid metabolism, and cellular amide metabolism process were found to include more exons than CK scenario. Conversely, genes with less exon-inclusive DSGs were enriched in regionalization, cell population proliferation, pigment metabolism and epidermis development (Figure S2).

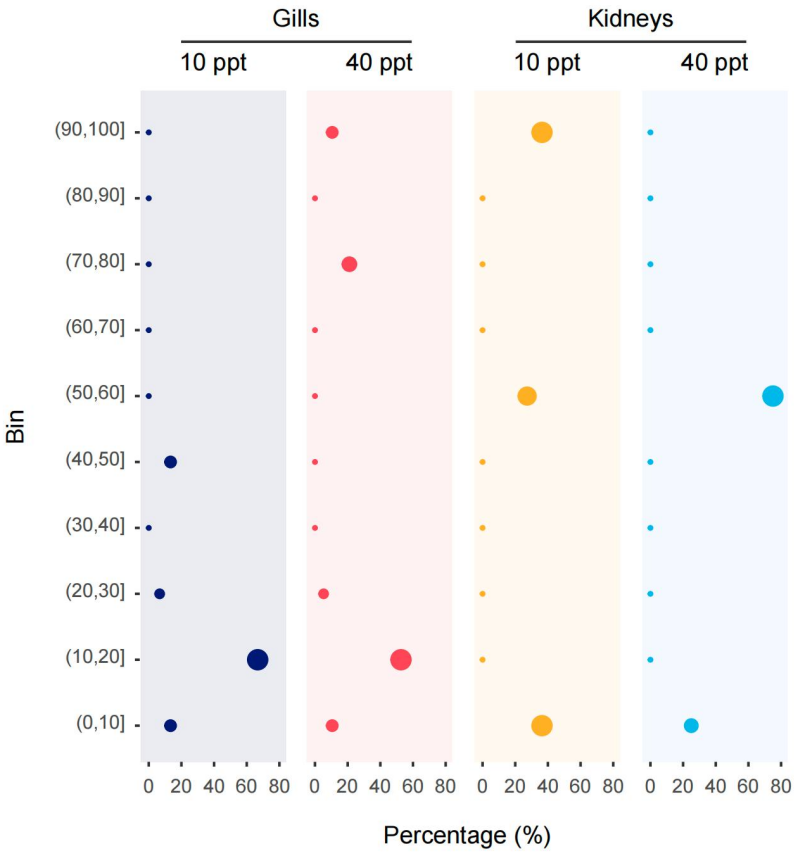
Of them, 234 DSGs were found to become more exon inclusive in response to both 10 and 40 ppt salinity stress than CK (Figure 2B). These genes were mainly associated with sulfur compound biosynthetic process, peroxisome organization, organonitrogen compound catabolic

process, and cellular lipid metabolic process. Comparatively, 361 and 268 more-exon inclusive DSGs was specifically induced under 10 and 40 ppt saline treatments, respectively (Figure 2B). Genes specifically responding to hypo-salinity stress were significantly overrepresented in the GO terms of homeostatic process, regulation of growth, sodium ion transport, cation homeostasis, epithelial cell proliferation, and erythrocyte development, while those involved in hyper-salinity responses were enriched in epidermal cell differentiation, epidermis development, Notch signaling pathway, and pigment metabolism.



3.3 Prediction of premature termination codons in the gills and kidneys of greater amberjack under hypo- and hyper-salinity

Under all the four salinity treatments, SE events were also found to affect the completeness and function of the resulting proteins by introducing PTCs (Figure 3). It is worthy to note that, in the gills, the majority of the introduced PTCs were located at the first 20% length of isoforms), that is, resulting in truncated protein products with a >80% loss in length (Figure 3). Comparatively, in the kidneys, a large proportion of PTCs occurred at the middle of the complete isoforms, indicating relatively smaller impacts on the functions of proteins.



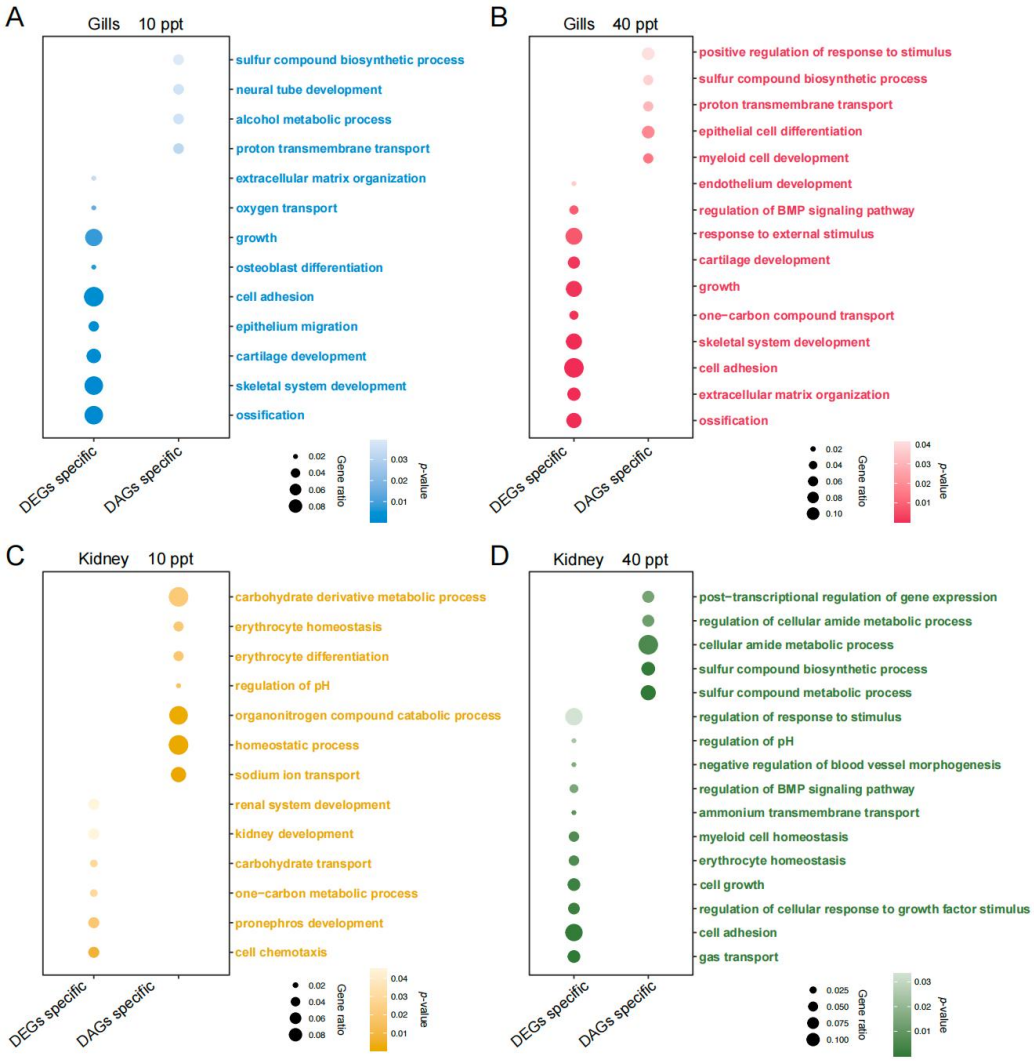
3.4 Comparative analysis between gills and kidneys of transcriptome expression and post-transcriptional regulation of greater amberjack under hypo- and hyper-salinity

We then compared the genes under AS regulations to those under differential expression regulations to investigate their roles in mitigating the adverse impacts on greater amberjack caused by salinity stress. For both gills and kidneys, the majority of genes were either modulated by differential AS or differential expression, while few genes were under the regulations of both mechanisms. More important, transcriptional and post-transcriptional regulation were found to modulate different physiological processes to enhance the tolerance of greater amberjack to water salinity oscillations.

In the gills, when treated with 10 ppt saline, DS-specific genes were mainly involved in sulfur compound biosynthesis and proton transport, while the DE-specific genes were high represented in the processes of growth, cartilage and osteoblast development, oxygen transport, and epithelium migration (Figure 4A). When exposed to 40 ppt salinity stress, the genes related to myeloid cell development, proton transport, and positive regulation of stimulus responses were mainly modulated by differential AS, while those associated with cartilage development, extracellular matrix organization, regulation of BMP signaling pathway and response to external stimulus were specifically under the regulation of different expression (Figure 4B).

In the kidneys, when exposed to low salinity environment, post-transcriptional regulatory mechanisms mainly affected the processes related to sodium ion transport, pH regulation and erythrocyte homeostasis and differentiation, and expression regulation was found to modulate the genes associated with kidney development (Figure 4C). Upon 40 ppt salinity, DSGs were significantly enriched in the GO terms of cellular amide metabolism, sulfur compound

biosynthesis and metabolism, and post-transcriptional regulation of gene expression, while DEGs were mainly related to stimulus response, ammonium transport, pH regulation, and cell growth (Figure 4D).



4 Discussion

4.1 Growth and development

Bone Morphogenetic Protein 4 (BMP4) and Growth Differentiation Factor 5 (GDF5) are closely related proteins that play a role in early cartilage patterning and differentiation (Hatakeyama et al., 2004). GDF5 can also increase the number of chondrocytes and the size of skeletal elements during late-stage skeletal development (Francis-West et al., 1999). In zebrafish, *gdf5* is expressed in the developing jaw joint, suggesting a similar role in jaw joint patterning and cartilage differentiation (Bruneau et al., 1997; Crotwell et al., 2001; Reed, 2010). The *bmp4* gene is enriched in the kidney during low salt stress, which is associated with kidney development and pronephros development. This is similar to the prominent expression of the *bmp4* gene in the zebrafish kidney, indicating its role as a primitive growth signal (Chin and Chen, 1997). Additionally, the BMP signaling pathway is also enriched in the gills and kidneys under high salt

stress. The increased expression of *bmp4* and *gdf5* plays an important role in cartilage development, osteoblast differentiation, and early kidney development in fish under unsuitable salinity conditions.

In this study, processes related to growth and development, such as skeletal system development, epithelial cell differentiation, neural tube development, and pronephric development, were activated in the gills and kidneys of the greater amberjack during changes in salinity. In the gills of the greater amberjack, bone development, and cartilage development are stimulated by low or high salt stress, indicating that increased expression of genes related to skeletal development plays an important role in maintaining normal gill structure and respiratory osmoregulation in response to environmental salinity changes. This is consistent with previous studies (Liu et al., 2023). In the kidneys, lower salinity (10 ppt) induced erythrocyte differentiation and pronephric development compared to optimal growth conditions (30 ppt). The pronephros, the first kidney formed during embryonic development in fish, plays a major role in osmoregulation (Howland, 1921; Tytler, 1988; Tytler et al., 1996; Vize et al., 1997), indicating that low salt stimulation affects normal kidney development in the greater amberjack. To maintain normal osmotic pressure, the embryonic fish specifically develops the pronephros to adapt to salinity stress.

4.2 Immune stress

Immune stress is another salt-induced biological process that we discovered in our study, which has also been mentioned in related studies on gilthead seabream (*Sparus aurata* L.) (Cuesta et al., 2005) and juvenile sablefish (*Anoplopoma fimbria*) (Kim et al., 2017). In the gills, genes related to the response to external stimuli are highly expressed due to high salt stress, primarily genes from the matrix metalloproteinase (MMP) family, such as Matrix Metallopeptidase 2 (mmp2) and Matrix Metalloproteinase 9 (mmp9). MMP family genes not only participate in normal physiological processes like embryonic development (Chen et al., 2007), reproduction (Pimentel-Acosta et al., 2023), and tissue remodeling (de Vrieze et al., 2011) by breaking down the extracellular matrix, but their immunological effects have also been clearly demonstrated in related studies on carp (*Cyprinus carpio* L.) (Chadzinska et al., 2008) and grass carp (*Ctenopharyngodon idella*) (Xu et al., 2012). The expression of *mmp2* and *mmp9* in the classical immune organs of fish (gills, head kidney, thymus, and intestine, etc.) and leukocytes indicates their important role in innate immunity during early embryonic development of teleostan. Heat Shock Protein Family B (Small) Member 7 (Hsp7) belongs to the heat shock protein family. Bartelt-Kirbach et al. (2014) found that HSPBs genes can interact to regulate stress response in organisms under stress conditions. In this study, enhanced expression of the *hspb7* gene was observed in the kidney under low salt stress, which is consistent with the significantly upregulated expression level of the *hspb7* gene in roughskin sculpin (*Trachidermus fasciatus*) under low salt stress (Kuang et al., 2020). In addition, hemopexin (HPX) not only participates in immune regulation related to hemolytic stress (Li et al., 2023), but also plays an important role in suppressing pathogen infections (Dong et al., 2023). In this study, the expression level of the *hpx* gene in the kidney was found to decrease when exposed to high salt stress, which is consistent with previous research (Xu et al., 2018), suggesting that the process of reducing hemopexin-induced destructive inflammation is downregulated under high salt conditions.

4.3 Ion homeostasis

Due to changes in seawater salinity, the ion homeostasis and osmotic pressure regulation processes of the greater amberjack also vary with the environmental salinity in order to maintain normal physiological activities and minimize the damage caused by salt stress (Liu et al., 2023). The gills serve as the main osmoregulatory and nitrogen excretory organ (Evans et al., 2005). Generally, an increase in environmental salinity lowers the excretion rate of ammonia (Uliano et al., 2010; Gracia-Lopez et al., 2006), while a decrease in salinity increases the excretion rate of ammonia (Zheng et al., 2008; Wright et al., 1995). The *sgk3* is involved in the expression of various ion channels and transport proteins related to ammonia excretion (Xu et al., 2019). In this study, the expression level of the *sgk3* in the gills of the greater amberjack exposed to high salinity conditions was reduced, while the expression level of the *sgk3* in the kidneys exposed to low salinity conditions was increased, indicating that the greater amberjack maintain osmotic balance by increasing or decreasing the expression level of the *sgk3* in response to different environmental salinity pressures. Additionally, mutations in the adaptor protein complex AP-1 subunit beta-1 (*ap1b1*) may affect the normal function of the Na/K-ATPase pump and play an important role in maintaining ion homeostasis (Grisham, 2013). For example, the expression of *ap1b1* decreases during the development process of tilapia larvae exposed to hypertonic environments (Malintha, 2022). In this study, the expression level of the *ap1b1* increased in the greater amberjack exposed to low salinity conditions, which may be related to the additional absorption of metal ions needed to reduce osmotic imbalance caused by excessive water uptake in hypoosmotic environments.

5 Conclusion

In this study, we analyzed and compared the transcriptomes and post-transcriptional regulatory mechanisms of the gills and kidneys of the greater amberjack under suitable salinity (30ppt) and unsuitable salinity (10 and 40ppt). In the gills, differentially expressed genes (DEGs) are involved in immune response activation, myeloid cell development, proton transport, and regulation of stimulus response. DEGs are enriched in cell adhesion and maintenance of extracellular matrix. Under high salt stress, *mmp2* and *mmp9* are highly expressed and play important roles in the early embryonic development process and innate immune response. In the kidneys, DSGs are enriched in cell differentiation, neural development, immune response, and substance metabolism. In addition to regulating metabolism, growth, and development, DEGs are also enriched in substance transport pathways. Under low salt conditions, *bmp4*, *hspb7*, and *sgk3* are expressed in the kidneys, mainly promoting pronephros development and maintaining osmotic pressure and immune response. Under high salt stress, the expression level of *hpx* decreases, weakening the immune response process that inhibits pathogen infection. These research findings reveal the important physiological mechanisms in the process of adapting to environmental salinity, providing theoretical guidance for understanding the survival pressure of the greater amberjack under environmental changes and the protection of future marine ecosystems.

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