Alternative splicing plays a nonredundant role in greater amberjack (*Seriola dumerili*) in the adaptation to ambient salinity fluctuations

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Abstract

Alternative splicing (AS) is an important post-transcriptional mechanism for adaptation of fish to environmental stress. Here, we performed a genome-wide investigation to explore the biological importance of AS dynamics in greater amberjack (*Seriola dumerili*), an economical marine teleost species, in response to hypo- (10 ppt) and hyper-salinity (40 ppt) stresses. The results revealed high level of differential splicing in both gills and kidney upon the exposure to undesired salinity regimes. In gills, genes involved in energy metabolism, stimulus response and epithelial cell differentiation were differentially spliced in response to the deviation of normal water salinity, while sodium ion transport, erythrocyte homeostasis and cellular amide metabolism were enhanced in kidney to combat the adverse impacts of salinity changes. More importantly, the majority of the differentially spliced genes were not differentially expressed, and AS was found to regulate different biological processes from differential gene expression, indicative of the functionally nonredundant role of AS in modulating salinity acclimation in greater amberjack. Together, our study highlights the important

contribution of post-transcriptional mechanisms to the adaptation of fish to ambient salinity fluctuations, and provides a theoretical guidance to the conservation of marine fishery resources under the increasingly extreme environmental challenges.

1 introduction

Occupying more than two-thirds of the Earth's surface, the oceans are the largest ecosystem on the planet, hosting a huge number of organisms and playing crucial roles in maintaining the diversity and balance of life on Earth. However, in the recent few decades, global climate changes, such as global warming, glacier melting, and precipitation decreasing, have substantially changed the global water cycle and altered worldwide salinity distribution among different sea areas (Durack and Wijffels, 2010; Helm et al., 2011; Durack et al., 2012; Skliris et al., 2016; Ullah et al., 2021). One noticeable trend is that the freshwater is getting fresher while the saltwater becomes saltier (Durack et al., 2012). Till 2017, the mean salinity contrast between the low and high salinity regions had 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 in salinity of the receiving water body in coastal marine environment, while the enhanced urban drainage may freshen nearshore seawater (Omerspahic et al., 2022; Röthig et al., 2023). Salinity fluctuations in seawater would alter the dissolved oxygen level, nutrient concentration, and stratification of the oceans, thereby posting an 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; Lacy et al., 2023; Röthig et al., 2023). For example, the increased water salinity was found to disrupt the normal energy allocation, and physiological and metabolic processes of fish, such as grass carp (*Ctenopharyngodon idella*) and African catfish (*Clarias gariepinus*), and finally inhibit their growth and survival (Smyth and Elliott, 2016; Zidan et al., 2022; Liu et al., 2023). Moreover, salinity change-induced biodiversity loss of plankton, macroalgae, coral reef, and seagrass may cause trophic collapse of marine ecosystem and lead to drops in fishery yield, which threaten ecosystem functions and food supply to human (Röthig et al., 2023).

Seriola dumerili, also known as 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). 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 et al., 2023). It has been widely reported that the growth, development, and physiology of greater amberjack were sensitive to the changes of water salinity, temperature, and seawater pressure (Barany et al., 2021; Navarro-Guillen et al., 2022; Peng et al., 2022; Liu et al., 2023; Ru et al., 2023). Comparative transcriptomic analyses showed that salinity alternations substantially disrupt tissue growth and development, energy metabolism, and immune responses in greater amberjack (Peng et al., 2022; Liu et al., 2023). Correspondingly, muscle water content, gill and intestinal Na-K-ATPase activity, and growth-related processes were enhanced to combat the adverse impacts (Barany et al., 2021; Liu et al., 2023). However, most of the current researches only focused on the responsive regulations at transcriptional level. No

attentions have ever been paid to the role of post-transcriptional mechanisms in modulating the resistance to seawater salinity fluctuations in greater amberjack.

Alternative splicing (AS) is one type of post-transcriptional regulatory mechanism in eukaryotes, and it can produce multiple isoforms with different combinations of exons, thus giving rise to multiple protein products from one single gene (Tian et al., 2022a). Therefore, AS can extend the diversity of structures and functions of proteins without increasing gene numbers. In fish, AS has been demonstrated to play critical roles in regulating the growth, development, and environmental adaptation of fish (Wan and Su, 2015; Tan et al., 2019; Long et al., 2020; Tian et al., 2020; Tian et al., 2022b; Xiao and Wang, 2022). The differential splicing of the genes related to spliceosome was indicated to assist the maintenance of gill homeostasis and improve the tolerance to hypoxia stress of spotted sea bass (*Lateolabrax maculatus*) (Ren et al., 2022). In another example, the expression of AS variants of *trim25* gene, which encodes an E3 ubiquitinated ligase of Tripartite Motif (TRIM) family, were up-regulated in Japanese flounder (*Paralichthys olivaceus*) in response to poly I:C treatment, indicative of their positively regulatory role of antiviral activity (Guo et al., 2022). Therefore, a comprehensive understanding of AS regulations associated with salt stress tolerance in greater amberjack allows us to fully understand the genetic basis underlying their physiological responses to fluctuating ambient salinity.

In this study, we performed a genome-wide investigation of AS dynamics in greater amberjack juveniles in response to the exposure to undesired salinity regimes. We further compared AS regulations to differential gene expression to explore their respective contributions to tolerance to water salinity fluctuations. Our findings will enrich our understanding of the molecular mechanisms underlying the adaptation to salinity stress in euryhaline fish, which provides theoretical guidance

86 for the conservation of fishery resources under the increasingly extreme global climate change.

87 **2 Materials and methods**

88 2.1 Data preprocessing and sequence alignment

89 In our previous study, we sequenced the transcriptomes for two osmoregulatory tissues, gills and
90 kidney, for greater amberjack juveniles reared under different salinity regimes: 10, 30 and 40 parts
91 per thousand (ppt) (National Center for Biotechnology Information (NCBI) accession number:
92 GSE220485) (Liu et al., 2023). Here, we re-used these RNA-seq data to investigate the role of AS
93 regulations in greater amberjack in response to ambient salinity changes. Following the data
94 preprocessing pipeline described in Liu et al. (2023), adapter contamination and low-quality reads
95 were filtered out using Trim Galore v. 0.6.6 (Krueger, 2012) with a quality score cut-off of 25 and
96 a read-length threshold of 50 bp. A genome index was constructed for the reference genome of
97 greater amberjack (NCBI GCA_002260705.1) by the hisat2-built utility of HISAT2 package v. 2.2.1
98 (Kim et al., 2019). High-quality reads were mapped to the reference genome by the hisat2 program.
99 For each sample, only the uniquely aligned reads were retained for downstream analysis. The
100 alignment output was further assembled into full-length transcripts using StringTie v2.2.1(Kovaka
101 et al., 2019). All sets of assembled transcripts were merged into a nonredundant set of transcripts
102 using StringTie's merge mode. The assembled transcripts were annotated by comparing against the
103 reference annotations obtained from the NCBI RefSeq database using GFFcompare v. 0.11.2 (Pertea
104 and Pertea, 2020).

105 2.2 Alternative splicing investigation and functional enrichment analysis

106 rMATS v. 4.0.2 (Shen et al., 2014) was employed to detect alternative splicing events of five major
107 types: skipped exon (SE), alternative 5' splice site (A5SS), alternative 3' splice site (A3SS),
108 mutually exclusive exons (MXE), and retained intron (RI) (Mehmood et al., 2020). For each event,
109 the inclusion level of an alternative fragment in each sample was measured by "percent spliced in
110 (PSI)". The changes in average PSI values were computed between the hypo- (10 ppt) or hyper-
111 salinity (40 ppt) treatment and the control group (30 ppt, CK) in gills and kidney, respectively, to
112 investigate the shift in splicing patterns in response to ambient salinity alterations. For each
113 comparison, the events with an FDR < 0.05 were considered to be significantly different between
114 the two groups, and the genes with any differential splicing event were denoted as "DSGs". Similar
115 to other animals, SE events were the dominant AS type in greater amberjack, thus here we primarily
116 focused on the functional importance of SE events. For the convenience, the genes with a larger PSI
117 value of SE event under a treatment condition than CK were going to produce a higher proportion
118 of the transcripts containing the alternative exon, and were denoted as "more exon-inclusive DSGs",
119 while those with a smaller PSI value were denoted as "less exon-inclusive DSGs", which would
120 give rise to more transcripts with the alternative exon skipped.

121 To access the functional importance of the DSGs, an OrgDb database was first built for greater
122 amberjack using the R package AnnotationForge v. 1.38.1 (Carlson and Pages, 2017), based on the
123 gene annotations obtained in our previous study (Liu et al., 2023). For both gills and kidney, Gene
124 Ontology (GO) enrichment analysis was carried out for the DSGs via the enrichGO function of
125 clusterProfiler package v. 4.6.2 (Wu et al., 2021). *P*-value < 0.05 was set as the criteria for statistical
126 significance. For each tissue, the more exon-inclusive DSGs were compared across different salinity

127 treatments, and the results were visualized by UpsetR package v. 1.4.0 (Conway et al., 2017). GO
128 enrichment analysis was implemented for the DSGs 1) specific to 10 ppt saline treatment, 2) specific
129 to 40 ppt saline treatment, and 3) identified under both treatments, respectively.

130 2.3 Premature termination codons (PTCs) induced by skipped exon events

131 One of the impacts of alternative splicing is the introduction of premature termination codons (PTCs)
132 to transcribed isoforms, which may cause deficiencies of protein structure and function or even lead
133 to nonfunctional proteins (Yeh and Hwang, 2020). PTCs account for approximately 20% of genetic
134 defects in human diseases and are often associated with severe phenotypes (Oren et al., 2017). Here,
135 we identified putative PTCs induced by SE events in greater amberjack and investigated their
136 impacts on the product proteins. The coding sequence (CDS) regions were first predicted for each
137 isoform based on the annotations of the assembled transcripts. Only those with unambiguous
138 annotations of CDS, translation initiation codon, and termination codon were retained for
139 downstream analysis. The coding sequences of each target isoform were extracted from the genome
140 of greater amberjack, and all the putative termination codons were screened out in the coding regions.
141 For the isoforms with multiple termination codons, the first appeared termination codon was
142 considered as the PTC. The impact of PTCs on the structure of product proteins was assessed
143 according to the distance between the PTC and the 5' end of transcripts, where a small distance
144 meant a large influence on the completeness of the protein structure.

145 2.4 Comparison between alternative splicing and differential gene expression

146 To further investigate the different roles between AS and gene expression in modulating salinity
147 oscillation tolerance in greater amberjack, we compared the more exon-inclusive DSGs to the up-

148 regulated differentially expressed genes (DEGs) under each treatment scenario. The DEGs were
149 detected following the same criteria described in our previous study (Liu et al., 2023). GO
150 enrichment analyses were then conducted for 1) the genes specifically regulated by differential AS
151 (DS-specific genes), and 2) genes specifically regulated by differential expression (DE-specific
152 genes).

153 **3 Results**

154 3.1 Differential splicing regulate salinity adaptation in gills of greater amberjack

155 In the gills of greater amberjack, compared to the CK group, RI events exhibited the largest
156 alterations under both hypo- and hyper-salinity treatments, followed by SE and A3SS events (Figure
157 1B). When exposed to 10 ppt saline, 583 SE events were detected in 461 genes, where the
158 transcriptional products of 182 genes displayed a higher inclusive level of the alternative exons,
159 while the rest 279 genes were prone to skip the alternative exons. GO enrichment analysis of these
160 more-exon inclusive genes revealed an enrichment of proton transmembrane transport and energy
161 metabolic processes, such as glucose and alcohol metabolism, and sulfur compound biosynthesis
162 (Figure 1C). Under 40 ppt salinity stress, 187 more exon-inclusive and 239 less exon-inclusive
163 genes were identified compared to CK, where the more exon-inclusive genes were significantly
164 overrepresented in the biological processes related to stimulus response, proton transmembrane
165 transport, cilium assembly and epithelial cell differentiation (Figure 1D).

166 These DSGs were then grouped into 8 clusters according to their changes of PSI across the
167 different salinity regimes (10, 30 and 40 ppt) (Figure 1E). In Cluster 1, 2 and 6, the majority of
168 DSGs exhibited the highest inclusive level of the alternative exons in 10 ppt saline than the other

169 two conditions. GO enrichment results showed that these genes were mainly involved in erythrocyte
170 differentiation, reactive oxygen species (ROS) metabolism, sodium ion transport, pH regulation,
171 regulation of developmental process, and positive regulation of Wnt signaling pathway. In contrast,
172 the DSGs in Cluster 3 and 4 were of the highest exon inclusion in hyper-salinity treatment and these
173 genes mainly participated in sodium ion transport, lipid transport, and regulation of cell division.
174 These results suggested that greater amberjack activated sodium ion transport in gills to cope with
175 the deviation of normal water salinity. A large group of DSGs displayed less exon inclusion under
176 either hypo- or hyper-salinity than CK (Cluster 5 and 8), including those associated with pigment
177 metabolic process, neurogenesis, cell polarity establishment, spindle localization, and DNA
178 recombination.

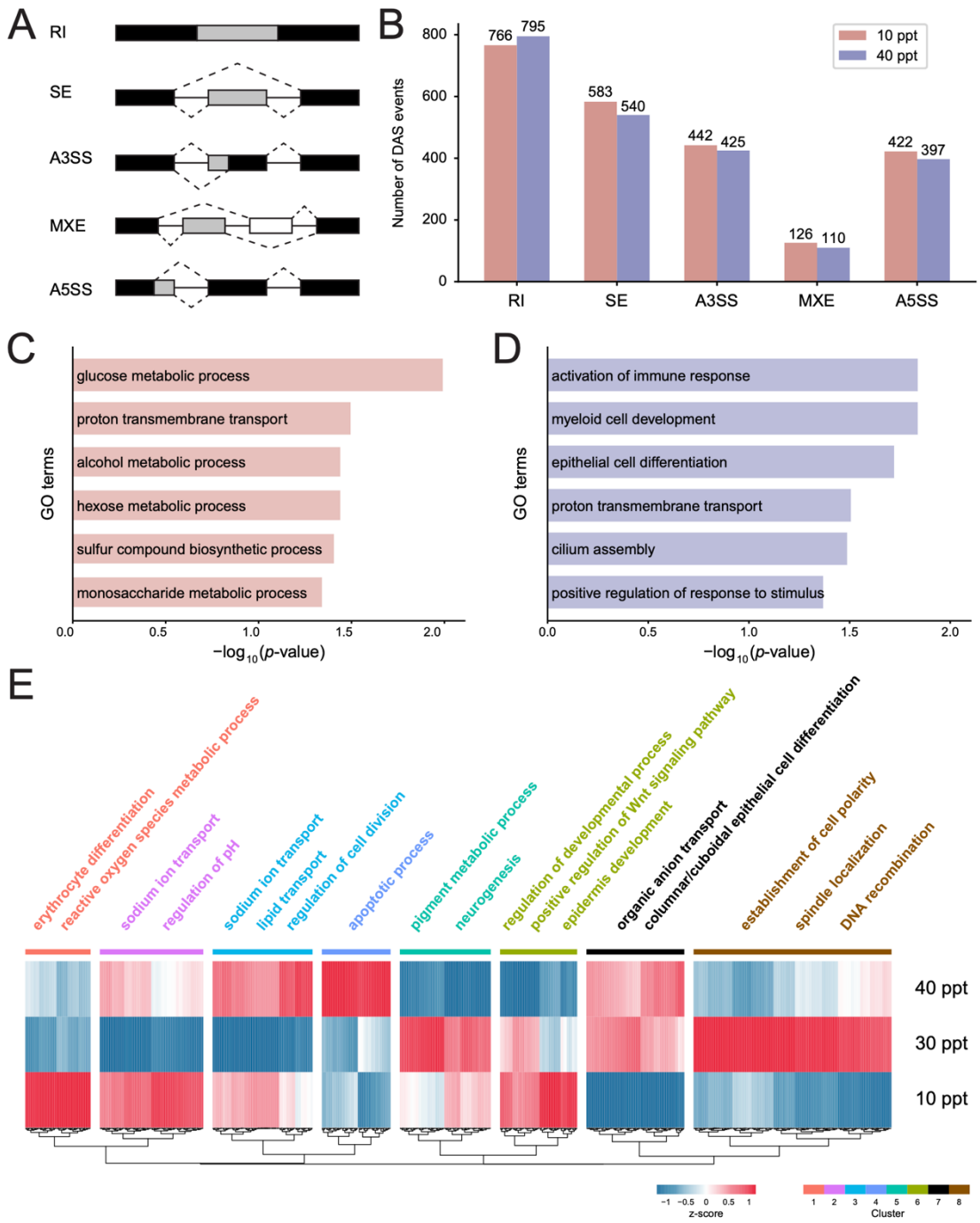

Figure 1. Alternative splicing reprogramming underlying salinity acclimation in gills of greater amberjack. (A) Schematic diagram showing five primary types of alternative splicing events we analyzed in this study. (B) Number of differential splicing events of each alternative splicing type in gills in response to hypo- (10 ppt) and hyper-salinity (40 ppt) stresses. (C) Representive Gene Ontology (GO) terms significantly enriched for the more exon-inclusive genes upon the treatment

185 of 10 ppt saline. (D) Representive GO terms significantly enriched for the more exon-inclusive
186 genes upon the treatment of 40 ppt saline. (E) Heatmap showing the percent spliced in (PSI) of the
187 alternative exon of the exon-skipping genes across different salinity regimes. Representive GO
188 terms enriched for the genes of each cluster are listed above the heatmap.
189

190 3.2 Alternative splicing responds to salinity changes in kidney of greater amberjack

191 In the kidney, 759 significant SE events in 595 DSGs were detected under salinity stress at 10 ppt
192 (Figure 2A). Of them, 276 DSGs displayed higher inclusion level of the alternative exons and these
193 genes were found to be overrepresented in the GO terms of sodium ion transmembrane transport,
194 organonitrogen compound catabolic process, regulation of pH, erythrocyte homeostasis, response
195 to mechanical stimulus, myeloid cell homeostasis, and organophosphate metabolic process (Figure
196 S1). While the 319 less exon-inclusive DSGs were highly representative in pigment granule
197 transport, epithelial cell differentiation involved in kidney development, chromosome organization,
198 and regulation of growth (Figure S1). Under 40 ppt salinity stress, 215 genes related to sulfur
199 compound metabolism, aspartate family amino acid biosynthesis, lipid metabolism, and cellular
200 amide metabolism process were found to include more exons than CK (Figure S2). Conversely, 287
201 genes were identified as less exon-inclusive DSGs and were enriched in regionalization, cell
202 population proliferation, pigment metabolism, and epidermis development (Figure S2).
203 A cross comparison showed that, the exon inclusion level of 234 genes significantly increased
204 under both 10 and 40 ppt saline treatments over CK, and these DSGs were mainly associated with
205 sulfur compound biosynthetic process, peroxisome organization, organonitrogen compound

206 catabolic process, and cellular lipid metabolic process (Figure 2B). Comparatively, 361 and 268

207 more-exon inclusive DSGs were specifically induced upon 10 and 40 ppt salinity stress, respectively

208 (Figure 2B). Genes specifically responding to hypo-salinity stress were overrepresented in the GO

209 terms of homeostatic process, regulation of growth, sodium ion transport, cation homeostasis,

210 epithelial cell proliferation, and erythrocyte development, while those involved in hyper-salinity

211 responses were enriched in epidermal cell differentiation, epidermis development, Notch signaling

212 pathway, and pigment metabolism (Figure 2B).

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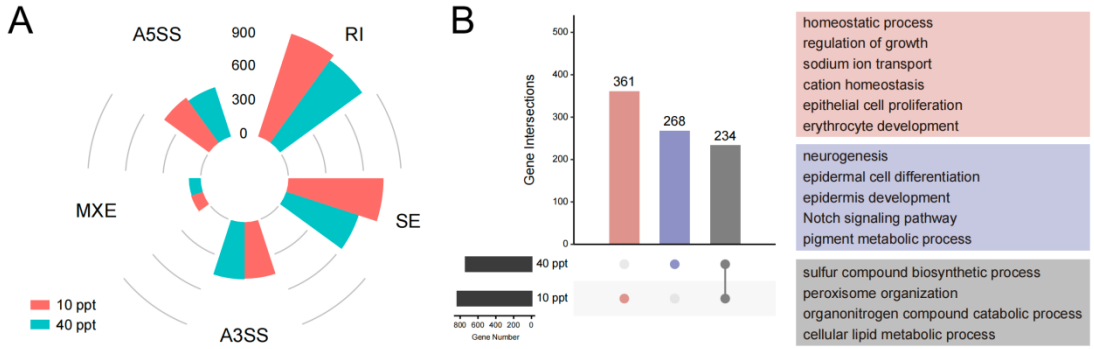

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215 Figure 2. Differential splicing in the kidney of greater amberjack in response to salinity changes.

216 (A) Number of differential splicing events of each alternative splicing type in kidney in response to

217 hypo- (10 ppt) and hyper-salinity (40 ppt) stresses. (B) Overlap of the more exon-inclusive genes

218 between 10 and 40 ppt saline treatments. The number of genes detected in each category was listed

219 in the upset intersection diagram. Representive Gene Ontology (GO) terms significantly enriched

220 for the genes of each category were listed at right, with the background color corresponding to the

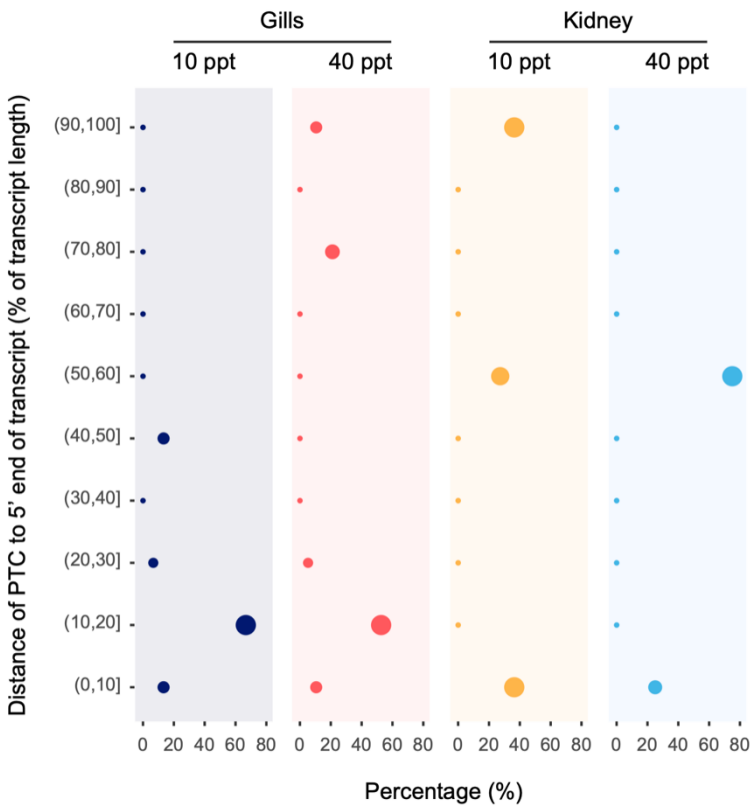
221 bars of the upset plot.

222

223 3.3 Splicing-induced premature termination codons affect completeness of product proteins

224 Alternative cassette exons can moreover introduce multiple terminations to mRNAs and finally alter
225 the coding potential of product proteins (Nikonova et al., 2020). In greater amberjack, SE events
226 were found to give rise to PTCs under all four salinity treatments, which largely affect the
227 completeness and function of the resulting proteins (Figure 3). It is worthy to note that, in gills, the
228 majority of the introduced PTCs were located at the first 20% length of isoforms), that is, resulting
229 in truncated protein products with a >80% loss in length (Figure 3). These transcripts were likely to
230 be targeted by UPF factors and degraded by nonsense-mediated mRNA decay (NMD) pathway
231 (Brogna and Wen, 2009). Comparatively, in kidney, a large proportion of PTCs occurred in the
232 middle of the complete isoforms, indicating relatively smaller impacts on the functions of proteins.

233



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235 Figure 3. Distribution of PTCs in different locations on transcripts upon hypo- (10 ppt) and hyper-

236 salinity (40 ppt) treatments in gills and kidney of greater amberjack. The location intervals of
237 transcripts are listed along the vertical axis, and dot sizes correspond to the relative proportion of
238 the PTCs in each location interval.
239

240 3.4 AS regulates different biological processes from gene expression under both hypo- and hyper-
241 salinity

242 We then compared the differentially spliced genes to those of differential expression under each
243 treatment to investigate their different roles in greater amberjack in mitigating the adverse impacts
244 of salinity stress. For both gills and kidney, the majority of genes were either modulated by
245 differential AS or differential expression, while only few genes were under the regulations of both
246 mechanisms. More importantly, gene transcription and alternative splicing were found to participate
247 in different physiological processes to enhance the tolerance of greater amberjack to water salinity
248 oscillations. In the gills, when treated with 10 ppt saline, DS-specific genes were mainly involved
249 in sulfur compound biosynthesis and proton transport, while the DE-specific genes were highly
250 represented in the GO terms of growth, cartilage and osteoblast development, oxygen transport, and
251 epithelium migration (Figure 4A). When exposed to 40 ppt salinity, the genes related to myeloid
252 cell development, proton transport, and positive regulation of stimulus responses were mainly
253 modulated by differential splicing, while those associated with cartilage development, extracellular
254 matrix organization, regulation of BMP signaling pathway and response to external stimulus were
255 differentially expressed (Figure 4B).
256 In kidney, when exposed to low salinity environment, alternative splicing mainly affected the

257 processes related to sodium ion transport, pH regulation, and erythrocyte homeostasis and
258 differentiation, while transcription regulation was found to modulate the processes associated with
259 kidney development (Figure 4C). Upon 40 ppt salinity, DSGs were significantly enriched in the GO
260 terms of cellular amide metabolism, sulfur compound biosynthesis and metabolism, and post-
261 transcriptional regulation of gene expression, while DEGs were mainly related to stimulus response,
262 ammonium transport, pH regulation, and cell growth (Figure 4D).

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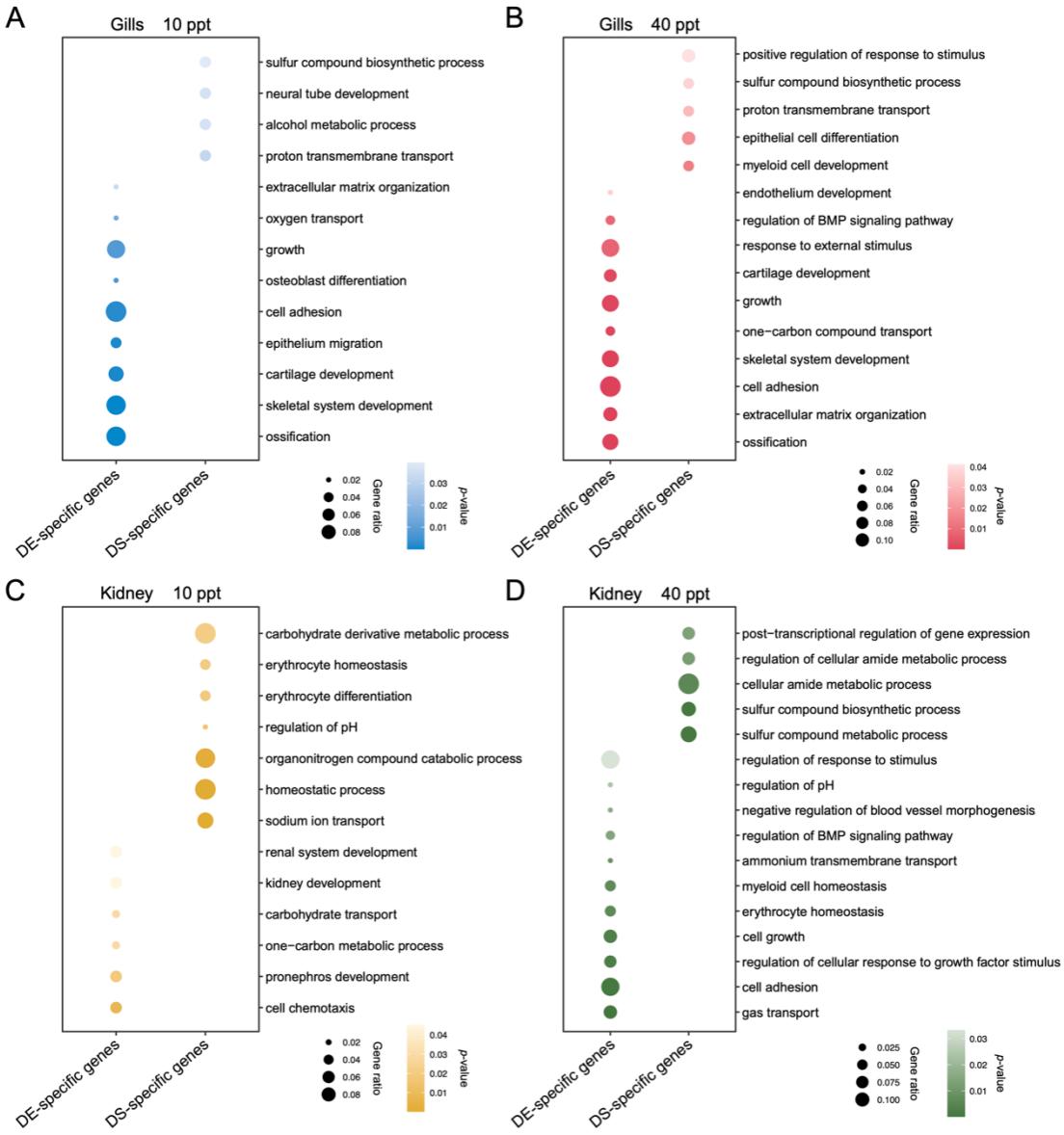

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265 Figure 4. Representive Gene Ontology (GO) terms enriched for the genes that were specifically

266 regulated by differential expression (DE-specific genes), and specifically regulated by alternative
267 splicing (DS-specific genes).
268

269 **4 Discussion**

270 Water salinity is a key environmental factor affecting the yield of aquaculture (Callaway et al., 2012).
271 In general, fish can only adapt to a limited range of water salinity changes; for instance, the optimum
272 range of greater amberjack is 29 ‰–36 ‰ (Kültz, 2015; Ru et al., 2023). Deviation from the
273 appropriate salinity range would disrupt their normal physiological and immune activities, which
274 threatens their growth and survival (Zhang et al., 2017; Cui et al., 2019). Indeed, a previous study
275 revealed a substantial up-regulation of the genes associated with organ development in both gills
276 and kidney of greater amberjack juveniles to combat the adverse impact of salinity oscillations (Liu
277 et al., 2023). In this study, we performed a genome-wide investigation of AS dynamics in greater
278 amberjack juveniles in response to the exposure to undesired salinity regimes. The results revealed
279 that both osmoregulatory organs, gills and kidney, were subjected to a high level of differential
280 splicing during acclimation to hypo- and hyper-salinity stress, and these changes were mainly
281 associated with sodium ion transport, energy metabolism, and cellular renewal and development,
282 (Figures 1 and 2; Figure S1 and S2), which highlights the important role of AS in supporting the
283 physiological reprogramming of greater amberjack to cope with short-term salinity fluctuation.
284 In gills, the genes involved in energy metabolism were significantly differentially spliced when
285 exposed to 10 ppt saline (Figure 1C). For example, one of them encodes an enzyme hexose-6-
286 phosphate dehydrogenase (H6PD) of endoplasmic reticulum. H6PD catalyzes the first two reactions

287 of the oxidative branch of the pentose phosphate pathway: the conversion from glucose-6-phosphate
288 and $NADP^+$ to 6-phosphogluconate and NADPH (Senesi et al., 2010; Ge et al., 2020). The produced
289 NADPH can reduce oxidized glutathione (GSSG) to reduced glutathione (GSH), which acts a key
290 role in scavenging ROS and maintaining cellular homeostasis, especially upon environmental
291 stresses (Senesi et al., 2010; Tomanek, 2012; Tomanek, 2015). Moreover, alcohol metabolism was
292 also enhanced in the gills. For most saltwater fish, a decrease in water salinity alters their oxygen
293 requirements in gills, thus may increase the production of lactate (Ern et al., 2014; Taugbøl et al.,
294 2022). To combat the adverse impact of lactate buildup, crucian carp and goldfish (*Carassius*,
295 Cyprinidae) can further covert lactic acid to ethanol and excrete it into ambient water via the special
296 coupling of pyruvate dehydrogenase complex (PDC) and alcohol dehydrogenase (ADH) in gills,
297 which largely improve their tolerance to low oxygen environment (Fagernes et al., 2017). However,
298 no studies yet have investigated the detailed alcohol metabolic processes in greater amberjack, and
299 future work is still required. When reared in a hyposalinity environment, the genes related to
300 stimulus response, for example, *RNF111*, were activated in gills (Figure 1D). The coding product of
301 *RNF111*, ring finger protein 111, was revealed to positively regulate DNA damage recognition in
302 nucleotide excision repair, thus alleviating stress-induced DNA damage (Poulsen et al., 2013).
303 Moreover, cilum assembly and epithelial cell differentiation were also differentially regulated by
304 AS. Previous studies have revealed that cell proliferation and differentiation of gill epithelia were
305 substantially activated in response to high salinity exposure, which can promote the osmoprotection
306 of gill cells (Chretien, 1986; Fiol et al., 2009). Moreover, the leakiness of epithelial tight junctions
307 was also increased to enhance the extrusion of sodium ions across the gill epithelium (Kültz, 2015).
308 These findings highlighted the key role of epithelial remodeling in fish gills in hypo-osmoregulation.

309 In kidney, sodium ion transport and pH regulation were enhanced to combat the decrease in
310 water salinity (Figure S1). Kidney is the main osmoregulatory organ in fish. The enhancement of
311 ion transport in kidney can increase salt excretion and maintain the ion balance and blood osmolality
312 in greater amberjack. Moreover, the splicing modes of the genes related to erythrocyte homeostasis
313 were also significantly changed. A low ambient salinity may lower the internal osmolality of fish,
314 which further alters the size and shape of red blood cells (Jensen et al., 2002). A rapid and efficient
315 regulation of blood cell volume can guarantee the oxygen transfer between red blood cells and
316 kidney. When exposed to hyper-salinity stress, cellular amide metabolic process, epidermis
317 development, Notch signaling pathway, and pigment metabolism were activated to cope with the
318 increased salinity (Figure 2B; Figure S2). The breakdown of amide/peptide bonds of proteins
319 moreover gives rise to free amino acids, which are important cellular osmolytes in euryhaline fish
320 (Aragão et al., 2010). A meta-analysis across 106 study cases showed that the cellular content of
321 free amino acids was positively correlated with the elevation of salinity, highlighting their essential
322 role in maintaining the homeostasis of osmotic pressure in fish in hyper-salinity environments
323 (Huang et al., 2023). It is noteworthy that energy metabolism, including organonitrogen compound
324 catabolism and lipid metabolism, were substantially activated by both 10 and 40 ppt saline
325 treatments (Figure 2B). For both hypo- and hyper-salinity acclimation, extra energy costs are
326 required by fish kidney for the osmoregulatory work, and the metabolism of lipids and proteins can
327 serve as alternative fuel sources to enhance the energy supply (Aragão et al., 2010; Nguyen et al.,
328 2016).

329 It is worthy to note that, in both gills and kidney, the most majority of differentially spliced
330 genes were not differentially expressed, and these two mechanisms were found to modulate different

331 biological processes (Figure 4). For example, in kidney, the genes involved in sodium ion transport
332 and pH regulation were specifically differentially spliced upon 10 ppt salinity stimulus, while those
333 associated with pronephros development were primarily under the regulation of differentially
334 expression (Figure 4C). These indicated that AS is functionally nonredundant and plays a
335 complementary role to gene transcription in regulating protein expression in greater amberjack in
336 response to ambient salinity fluctuations. It is consistent with observations in other fish species,
337 such as salmonid fish (*Salvelinus alpinus*) (Jacobs and Elmer, 2021)and spotted sea bass (Tian et al.,
338 2020). AS and gene transcription are regulated mainly by independent mechanisms. The alternative
339 procedures of pre-mRNA splicing are determined by the different recognitions and usages of
340 splicing sites of spliceosome complex (Wang et al., 2015; Liu et al., 2022). In addition, the spliced
341 mRNA products carrying PTCs would be targeted and degraded by the mRNA-surveillance
342 mechanism NMD pathway (Stamm et al., 2005; Brogna and Wen, 2009). Our study also observed
343 a large impact of PTCs on the structures and functions of the resulting proteins in both gills and
344 kidney of greater amberjack (Figure 3). These features allow AS to extend and modulate the mRNA
345 and protein repertoire of selectively constrained genes without altering their initial expressions,
346 which acts as a key mechanism in the development of phenotypic plasticity and rapid ecological
347 adaptation (Marden, 2008; Barbosa-Morais et al., 2012; Bradley et al., 2012; Jacobs and Elmer,
348 2021; Steward et al., 2022). Our current study adds further evidence for the nonnegligible role of
349 AS in regulating stress tolerance in teleost fishes.
350

351 **5 Conclusion**

352 In this study, we provide a new insight into the role of alternative splicing in regulating physiological

353 remodeling of greater amberjack to cope with the adverse impacts of fluctuated water salinity. The
354 results show that alternative splicing plays a relatively independent role in fine-tuning protein
355 expression in both gills and kidney of greater amberjacker in the early responses to salinity changes.
356 Differential splicing primarily results in structural and functional changes of the proteins involved
357 in sodium ion and proton transport, energy metabolism, and cell development and differentiation,
358 which may improve the adaptation of greater amberjacker to salinity stress. These findings unravel
359 the deeper side of the genetic basis underlying salinity acclimation in greater amberjack in response
360 to the fluctuating marine environment, and provide a genomic view for the conservation of marine
361 fishery resources.
362

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366

367 **Data availability statements**
368 Data were obtained from the National Center for Biotechnology Information (NCBI) with accession
369 number: GSE220485.
370

371 **Conflict of interst statement**
372 The authors declare that the research was conducted in the absence of any commercial or financial
373 relationships that could be construed as a potential conflict of interest.
374

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