

Original Research Articles

Tissue-specific expression responses of antioxidant, immune, and metabolic genes in juvenile yellowfin tuna (*Thunnus albacares*) under acute low-pH stress

Yitao Feng^{1,2}, Yuzhuo You^{2,3,4,5}, Xiaoyan Wang^{2,3,4,5}, Zhengyi Fu^{2,3,4a}, Zhenhua Ma^{2,3,4b}

¹ Lingshui Agricultural Investment Group Marine Aquaculture Development Co., Ltd., Lingshui 572400, China, ² Key Laboratory of Efficient Utilization and Processing of Marine Fishery Resources of Hainan Province, Sanya Tropical Fisheries Research Institute, Sanya 572018, China, ³ South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, Guangdong 510300, China, ⁴ Hainan Fisheries Innovation Research Institute, Chinese Academy of Fishery Sciences, Sanya 572024, China, ⁵ College of Fisheries and Life Sciences, Dalian Ocean University, Dalian 116023, China

Keywords: yellowfin tuna, low-pH stress, tissue-specific response, oxidative stress, immune response, energy metabolism

<https://doi.org/10.46989/001c.171979>

Israeli Journal of Aquaculture - Bamidgeh

Vol. 78, Issue 4, 2026

Acute reductions in seawater pH may disrupt acid–base regulation and physiological homeostasis in marine fish, but tissue-specific molecular responses in juvenile yellowfin tuna remain poorly understood. Juveniles of yellowfin tuna were exposed for 48 h to four seawater pH levels (8.1, 7.6, 7.1, and 6.6), with three replicate tanks per treatment. The expression of selected antioxidant-, immune-, inflammatory-, and metabolism-related genes in the liver and gill was quantified using qRT-PCR. In the gill, most examined genes were upregulated under low-pH exposure, although the magnitude and pH dependence of the responses varied among genes. In the liver, antioxidant genes and most immune-related genes were downregulated, whereas *tnf-α* was upregulated at pH 7.1. Hepatic metabolic genes also exhibited treatment-dependent responses: *mdh2* was significantly downregulated across all low-pH treatments, whereas *pck2* was significantly upregulated only at pH 7.1 and 6.6. In contrast, *dgat1b* was significantly downregulated exclusively at pH 7.1 and 6.6. These contrasting expression patterns demonstrate marked tissue specificity in the acute low-pH response of juvenile yellowfin tuna, with broader transcriptional activation in the gill and selective suppression and metabolic adjustment in the liver. The findings provide an initial molecular basis for further evaluating the physiological consequences of acute and chronic low-pH exposure in this high-metabolic-rate pelagic species.

1. INTRODUCTION

Seawater pH can be altered by natural and anthropogenic drivers, and low-pH conditions can impose substantial physiological challenges on marine organisms.¹ Seawater pH fluctuations have been recognized as important environmental stressors affecting marine ecosystems.² The reduction in seawater pH not only alters the calcium carbonate saturation state of seawater,³ but also directly interferes with critical physiological processes in marine organisms, including acid-base regulation,⁴ ion transport,⁵ respiratory function,⁶ energy metabolism, and immune defense,^{7,8} thereby affecting their growth, development, reproduction, and population dynamics.⁹ Among marine vertebrates, fish are particularly dependent on precise regulation of internal

homeostasis and may therefore exhibit complex physiological responses to low-pH stress.

Previous studies have shown that many fish possess considerable capacity to regulate acid–base balance; however, such tolerance does not necessarily indicate the absence of physiological costs.^{6,10} A number of evidence indicates that both chronic and acute low-pH stress can induce oxidative imbalance, inflammatory responses, and immune dysregulation in fish.¹¹ Particularly during the juvenile stage, when physiological regulatory systems are still undergoing development, fish exhibit heightened sensitivity to environmental changes, and the impacts of low-pH stress may be more profound and far-reaching.¹² Therefore, relying solely on adults may overlook sublethal physiological impacts of low-pH stress.

a Corresponding author. Email: zhengyifu@163.com (Zhengyi Fu),

b Corresponding author. Email: zhenhua.ma@foxmail.com (Zhenhua Ma)

Yellowfin tuna (*Thunnus albacares*), as a pelagic migratory fish, holds significant ecological and economic importance in tropical and subtropical waters.^{13,14} However, due to their distinctive migratory habits and sensitivity to changes in the marine environment, juvenile yellowfin tuna may be particularly vulnerable to environmental fluctuations due to their active metabolism and developmental requirements.¹⁵ Previous studies have demonstrated that low-pH stress can induce oxidative stress and inflammatory responses in fish by lowering blood pH and interfering with ion transport and acid-base regulation, affecting physiological processes in these tissues.^{16–18} As the primary respiratory and ion-exchange organ in fish, the gill serves as the first point of contact with environmental changes.⁵ The liver is the primary metabolic and detoxification organ, and its functional status influences energy metabolism and immune homeostasis in fish.¹⁹ In the context of global climate change, both wild populations and aquaculture systems of yellowfin tuna may face potential challenges from future changes in seawater chemistry.²⁰ However, molecular responses of tuna species to low-pH stress remain poorly understood.

The antioxidant defense system serves as a critical barrier for maintaining cellular homeostasis.²¹ Low-pH stress may disturb redox homeostasis and alter reactive oxygen species (ROS) production in fish. If the antioxidant system fails to scavenge excessive ROS in a timely manner, it will result in lipid peroxidation and protein damage.²² Among antioxidant-related genes, superoxide dismutase (*sod*), catalase (*cat*), and glutathione peroxidase (*gpx1a*) are widely used molecular markers reflecting transcriptional regulation of antioxidant defenses under environmental stress.^{23–25} In fish coping with low-pH stress, the expression levels of antioxidant genes provide molecular indicators of antioxidant-related transcriptional responses in tissues.²⁶ Because the gill is directly exposed to external water conditions, whereas the liver plays a central role in metabolism and detoxification, these tissues may exhibit distinct antioxidant responses during low-pH stress. We hypothesized that the gill would show increased expression of antioxidant-related genes under low-pH exposure, whereas the liver might exhibit a different or comparatively reduced transcriptional response.

Inflammatory and immune responses are key physiological processes that reflect immune modulation and the maintenance of immune homeostasis in organisms.²⁷ Toll-like receptor 3 (*tlr3*) is a pattern recognition receptor involved in innate immune signaling, while cytokines such as interleukin-1 β (*il-1 β*) and tumor necrosis factor α (*tnf- α*) are commonly used indicators of pro-inflammatory response.^{28,29} Interleukin-6 receptor (*il6r*) participates in cytokine-mediated signaling pathways, whereas interleukin-10 (*il10*) and transforming growth factor β (*tgf- β*) contribute to the regulation of inflammatory processes.^{30,31} We hypothesized that the gills would show increased expression of pro-inflammatory genes in response to direct environmental exposure, whereas the liver might exhibit a distinct or comparatively reduced immune-related transcriptional response. Changes in the expression

of these immune-related genes can provide insights into tissue-specific immune modulation during low-pH stress.

Energy metabolism also represents a critical component of the stress response, as fish may adjust metabolic pathways to maintain physiological homeostasis under environmental challenges.³² Malate dehydrogenase (*mdh2*) participates in the tricarboxylic acid (TCA) cycle and is associated with aerobic metabolic processes, whereas phosphoenolpyruvate carboxykinase (*pck2*) regulates gluconeogenesis and may contribute to metabolic adaptation under stress conditions.^{33,34} Diacylglycerol acyltransferase (*dgat1b*) is involved in triglyceride synthesis and lipid metabolism, and is closely linked to energy balance and cellular maintenance.³⁵ Given the central role of the gill in ion and acid-base regulation, we anticipated increased expression of metabolism-related genes in the gill under low-pH exposure. In contrast, the liver, as a major metabolic organ, was expected to exhibit a distinct and potentially selective transcriptional response in metabolism-related genes. Accordingly, the expression patterns of these metabolic genes in the liver and gills may inform tissue-specific metabolic regulation under low-pH stress.³⁶

Currently, studies on the physiological responses of fish to low-pH stress have predominantly focused on model species and coastal fishes, whereas molecular response data for economically important pelagic species, such as juvenile yellowfin tuna (*Thunnus albacares*), remain limited. To address this knowledge gap, we conducted a 48-h exposure experiment using wild juvenile yellowfin tuna collected from the South China Sea, with four pH treatments (8.1, 7.6, 7.1, and 6.6). The expression patterns of the aforementioned genes involved in antioxidative defense, inflammatory responses, and energy metabolism were examined in liver and gill tissues to test our tissue-specific prior hypotheses. By comparing transcriptional responses between these two tissues under acute low-pH stress, this study aims to elucidate the distinct physiological strategies employed by high-metabolic-rate pelagic fish in response to abrupt environmental pH fluctuations, thereby providing a theoretical foundation for assessing molecular sensitivity and guiding future investigations into long-term exposure effects.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL ANIMALS, ACCLIMATION, AND EXPERIMENTAL DESIGN

Juvenile yellowfin tuna used in this study were collected in June 2023 from the waters near Xincun Harbor in the South China Sea (18°22'31.9"N, 109°58'28.9"E), with the assistance of Sanya Tropical Fisheries Research Institute. A total of 72 healthy juveniles with intact body surfaces and active swimming behavior were selected, with a mean body length of 18.21 ± 1.09 cm and a mean body weight of 307.49 ± 49.38 g. The juveniles were first acclimated for 7 days in circular tanks (5000 L) located within the workshop of the Tropical Aquaculture Research and Development Center. During the acclimation period, the fish were fed once

daily at 8:00 a.m. to satiety with ice-preserved trash fish. Water quality parameters during both the acclimation and formal experimental periods were maintained as follows: water temperature at 28.5 ± 1 °C, dissolved oxygen > 7.5 mg/L, sodium nitrite < 0.05 mg/L, and ammonia nitrogen below the detection limit (< 0.01 mg/L). The experimental seawater used was sand-filtered natural seawater. After acclimation, the juveniles were randomly assigned to four experimental groups, each with three replicates and six fish per replicate, for a total of 72 individuals. Natural seawater at pH 8.1 was designated as the control group. Three low-pH treatment groups were established with pH values of 7.6, 7.1, and 6.6 to cover a range from moderate to severe low-pH conditions.^{37,38} The experiment lasted for 48 h, during which the fish were not fed, and the photoperiod was maintained at 12 h light:12 h dark cycle. Seawater pH was continuously monitored throughout the experimental period using a pH meter (Orion Star A211, Thermo Scientific, USA). The instrument was calibrated according to the NBS scale, and standardization was performed every 2 h using commercial buffer solutions (pH 4.01, 7.00, and 10.01). The pH of each treatment group was maintained within ± 0.1 by adding 1.0 mmol/L NaOH or 1.0 mmol/L HCl solutions.

It is worth noting that HCl/NaOH was used to adjust seawater pH instead of CO₂ bubbling, as the core objective of the experimental design was to evaluate the physiological and transcriptional effects of experimentally induced low-pH conditions on juvenile yellowfin tuna, rather than to investigate changes in the full carbonate chemistry system. Therefore, mineral acid/base adjustment provided a direct and stable means of achieving the target pH gradients, which was advantageous for isolating the biological responses associated with experimentally induced low-pH conditions.³⁹

2.2. SAMPLE COLLECTION

At the end of the 48-h exposure, all six juvenile yellowfin tuna in each tank were sampled for gene expression analysis. The mean expression value of the six fish was used as the representative value for that tank in statistical analyses, with the tank serving as the statistical unit ($n = 3$). Prior to sampling, the juveniles were euthanized with an overdose of buffered MS-222 at 250 mg/L buffered with sodium bicarbonate. After a complete loss of motor activity and cessation of opercular movement, death was confirmed, and the fish were immediately dissected.⁴⁰ All six fish in each tank were sampled at the end of the 48-h exposure, and both liver and gill tissues were collected from the same individuals for gene expression analysis. The tissues were then placed into 2 mL cryogenic vials and stored at -80°C until further use. The entire sampling procedure was performed on ice to minimize tissue degradation and exogenous contamination, thereby reducing potential interference with subsequent analytical results.

2.3. TOTAL RNA EXTRACTION AND CDNA SYNTHESIS

Total RNA was extracted from the liver and gill tissues using Trizol reagent (Lan Jie Technology Co., Ltd., Anhui,

China) according to the manufacturer's instructions. RNA concentration and purity were determined using a Nano-300 spectrophotometer (Allsheng Instruments Co., Ltd., Hangzhou, China), and RNA integrity was evaluated by agarose gel electrophoresis (DYY-6C, Beijing Liuyi Instrument Factory, Beijing, China). Only RNA samples with A260/A280 ratios between 1.8 and 2.0 and no apparent degradation were used for subsequent analyses. A total of 1 µg of total RNA was used for first-strand cDNA synthesis following the protocol of the *EasyScript*® All-in-One First-Strand cDNA Synthesis SuperMix (TransGen Biotechnology Co., Ltd., Beijing). Each 20 µL reaction contained 1 µg total RNA, 4 µL 5× *EasyScript*® All-in-One SuperMix for qPCR, 1 µL gDNA Remover, and RNase-free water, and the reaction conditions were as follows: 37 °C for 15 min, followed by 85 °C for 5 s. The synthesized cDNA was stored at -20°C for subsequent use.

2.4. GENE EXPRESSION ANALYSIS

Relative gene expression was quantified using real-time quantitative PCR (qTOWER,³ Analytik Jena GmbH, Germany) with SYBR Green fluorescent dye (Tiangen Biotech Co., Ltd., Beijing). Based on the gene sequence information of yellowfin tuna available in the NCBI database, specific primers for each target gene and the reference gene were designed using Primer Premier 5.0 software,⁴¹ and their specificity was validated by BLAST alignment against the yellowfin tuna genome. The primer sequences are listed in [Table 1](#). Each 20 µL reaction contained 10 µL of 2 × RealUniversal PreMix, 0.6 µL of each primer, 2 µL of cDNA template, and 6.8 µL of ddH₂O. The amplification program was set as follows: initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at the primer-specific temperatures listed in [Table 1](#) for 20 s, and extension at 72 °C for 30 s. Following amplification, melting curve analysis was performed to evaluate product specificity, and the results showed that all primers yielded a single melting peak, with no non-specific amplification or primer-dimer signals detected. Primer amplification efficiency was validated by the standard curve method: serially diluted cDNA templates were used to generate linear regression between Ct values and logarithmic template concentrations, and the efficiency was calculated according to the formula $E = [10^{(-1/\text{slope})} - 1] \times 100\%$. The results indicated that the amplification efficiencies of all primers ranged from 90% to 110%, with correlation coefficients (R^2) all exceeding 0.98. The stability of β -actin expression was evaluated separately in the liver and gill across the four pH treatments. Because β -actin Ct values showed low variation and no significant differences among treatments, β -actin was used as the reference gene. Each cDNA sample was analyzed in triplicate, and the mean Ct value of the three technical replicates was used for subsequent analysis. The relative mRNA expression levels of target genes were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method, with the expression level of the pH 8.1 group used as the calibrator.

Table 1. Primers used in RT-qPCR

Accession No	Primer Names	Primer Sequence (5'-3')	Amplicon size (bp)	Amplification efficiency	Annealing temperature
XM_044332395.1	<i>β-actin</i>	F: TCCCTGTATGCCTCTGGT R: TGATGTCACGCACGATTT	217	105%	54 °C
XM_044345714.1	<i>sod</i>	F: GCAGCGATAACTCAGTCT R: TATGCTAAGCCAACCTAC	127	96%	52 °C
XM_044356198.1	<i>cat</i>	F: TGACAGGCAACAACACCC R: ATCGCTAAACAGGAAGGACA	173	100%	56 °C
XM_044347776.1	<i>gpx1a</i>	F: AAGGTGGATGTGAATGGA R: GCTGTAGCGTTTGTAGGG	201	98%	54 °C
XM_044334527.1	<i>il-1β</i>	F: ACAGAACAACCTGCTGGGATA R: TCAGTGGATGATGGGAAA	176	96%	54 °C
XM_044360478.1	<i>il6r</i>	F: TTGTCACTCATTTTGGCT R: CTCTGGAGATGTTGGGGT	132	104%	52 °C
XM_044350293.1	<i>il10</i>	F: CAGCAAGATACCAACAAG R: CGACAAGAGAACCAGGAC	190	99%	54 °C
XM_044347352.1	<i>tlr3</i>	F: CAATGTGCCCAATCTTAG R: TAGCGATGTTGTTGTACTG	131	100%	50 °C
XM_044335877.1	<i>tnf-α</i>	F: AGGAGGGCTGAACTTGA R: CTGGTAGGAACTTGTGGC	193	93%	56 °C
XM_044336242.1	<i>tgf-β</i>	F: CAATGACTTTGTCTGCTAT R: GTCTCCGTGTTATCCTGT	238	92%	52 °C
XM_044358376.1	<i>mdh2</i>	F: GGTGCCCCGTTTCACTTTC R: TTCTTCTCAATGCCGTTT	143	96%	52 °C
XM_044359878.1	<i>dgat1b</i>	F: ATTCCCATCATTCAAAGC R: TCACCGTCTCAGAGTTCC	211	98%	54 °C
XM_044340148.1	<i>pck2</i>	F: AGGGAGTGAAGCGTTATG R: AGTTTACCTTGGCTGTCG	148	104%	54 °C

2.5. STATISTICAL ANALYSIS

Experimental data were organized using Excel 2010 software, and statistical analyses were performed with SPSS 25.0 software. For all detected genes (including antioxidant-, immune-, inflammatory-, and metabolism-related genes), the tank was used as the statistical unit ($n = 3$), and the mean value of the six fish sampled from each tank was used as the representative value for that tank. This procedure was applied consistently for both liver and gill tissues. Normality of the data was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. One-way analysis of variance (one-way ANOVA) followed by Duncan's multiple range test was employed to determine significant differences among treatments. Data are presented as mean $\pm$ standard deviation (Mean $\pm$ SD). A significance threshold of $p < 0.05$ was considered statistically significant. Bar graphs were generated using GraphPad Prism 9.5 software.

3. RESULTS

3.1. ANTIOXIDANT-RELATED GENE EXPRESSION IN THE LIVER AND GILL

The mRNA expression levels of antioxidant-related genes exhibited distinct patterns between the liver and gill (Fig. 1). In the liver, *sod* expression was significantly lower in the pH 7.6, 7.1, and 6.6 groups than in the pH 8.1 control group ($p < 0.01$; Fig. 1a). Hepatic *cat* expression was significantly lower at pH 7.6 and 6.6 ($p < 0.01$) and at pH 7.1 ($p < 0.05$; Fig. 1b). The expression of *gpx1a* was significantly lower at pH 7.6 and 7.1 ($p < 0.05$), whereas no significant difference was detected at pH 6.6 ($p > 0.05$; Fig. 1c).

In the gill, *sod* expression was significantly higher in all three low-pH groups than in the pH 8.1 control group ($p < 0.01$; Fig. 1A). The expression levels of *cat* and *gpx1a* were significantly higher at pH 6.6 ($p < 0.01$), whereas no significant differences were detected at pH 7.6 or 7.1 ($p > 0.05$; Fig. 1B, C).

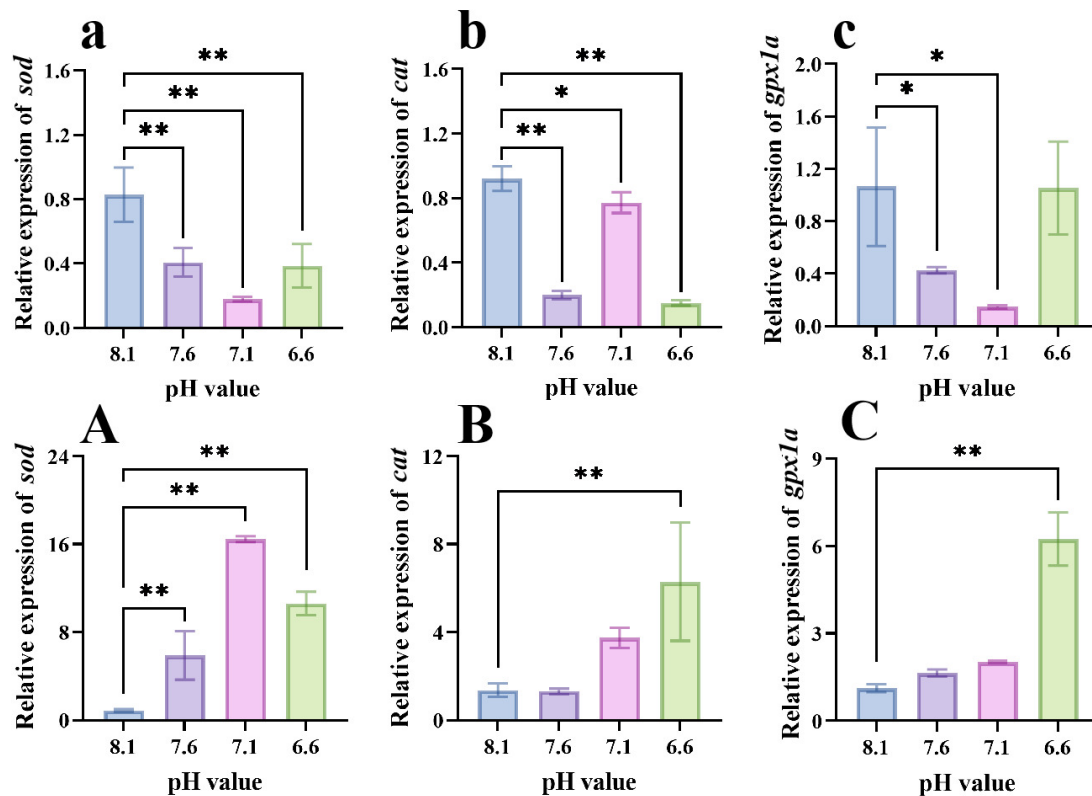


Fig. 1. Changes in antioxidant gene expression in juvenile yellowfin tuna under pH variation. (a–c) Liver, (A–C) Gill. Values are presented as mean $\pm$ SD of three replicate tanks ($n = 3$). “*” and “” indicate significant differences from the control group at $p < 0.05$ and $p < 0.01$, respectively.**

3.2. IMMUNE- AND INFLAMMATION-RELATED GENE EXPRESSION IN THE LIVER AND GILL

The mRNA expression levels of immune- and inflammation-related genes differed between the liver and gill tissues (Fig. 2). In the liver, *il-1 β* expression was significantly downregulated in the pH 7.6 and pH 6.6 groups compared with the control group ($p < 0.01$), whereas no significant difference was observed at pH 7.1 ($p > 0.05$; Fig. 2a). The expression levels of *il10*, *il6r*, and *tgf- β* were significantly decreased at pH 7.6 and pH 7.1 ($p < 0.01$), as well as at pH 6.6 ($p < 0.05$; Fig. 2b, c, f). Hepatic *tnf- α* expression was significantly upregulated at pH 7.1 ($p < 0.01$), but showed no significant changes at pH 7.6 or pH 6.6 relative to the control ($p > 0.05$; Fig. 2e). The expression of *tlr3* was significantly reduced across all low-pH treatment groups ($p < 0.01$; Fig. 2d).

In the gill, the expression levels of *il-1 β* , *il10*, and *il6r* were significantly elevated in all low-pH treatment groups compared with the control group ($p < 0.01$; Fig. 2A–C). *tlr3* expression was significantly upregulated at pH 7.1 and pH 6.6 ($p < 0.01$), as well as at pH 7.6 ($p < 0.05$), relative to the control (Fig. 2D). The expression levels of *tnf- α* and *tgf- β* were significantly higher at pH 7.1 and pH 6.6 than in the control group ($p < 0.01$), whereas no significant differences were observed at pH 7.6 ($p > 0.05$; Fig. 2E, F).

3.3. METABOLIC GENE EXPRESSION IN THE LIVER AND GILL

The transcriptional expression levels of metabolism-related genes differed between the liver and gill tissues under different pH treatments (Fig. 3). In the liver, *mdh2* expression was significantly lower in all low-pH groups than in the control group ($p < 0.01$; Fig. 3a). The expression of *dgat1b* was significantly decreased at pH 7.1 and pH 6.6 ($p < 0.01$), whereas no significant difference was detected at pH 7.6 compared with the control ($p > 0.05$; Fig. 3b). *pck2* expression was significantly increased at pH 7.1 and pH 6.6 ($p < 0.01$), but showed no significant change at pH 7.6 relative to the control ($p > 0.05$; Fig. 3c).

In the gill, the expression levels of *mdh2* and *dgat1b* were significantly upregulated in all low-pH groups ($p < 0.01$; Fig. 3A, B). *pck2* expression was significantly increased at pH 7.1 and pH 6.6 ($p < 0.01$), whereas no significant difference was detected at pH 7.6 ($p > 0.05$; Fig. 3C).

4. DISCUSSION

In this study, we characterized the transcriptional responses of antioxidant-, immune-, inflammatory-, and metabolism-related genes in the liver and gill tissues of juvenile yellowfin tuna following short-term (48 h) exposure to different pH levels (8.1, 7.6, 7.1, and 6.6). The results revealed distinct tissue-specific response patterns under

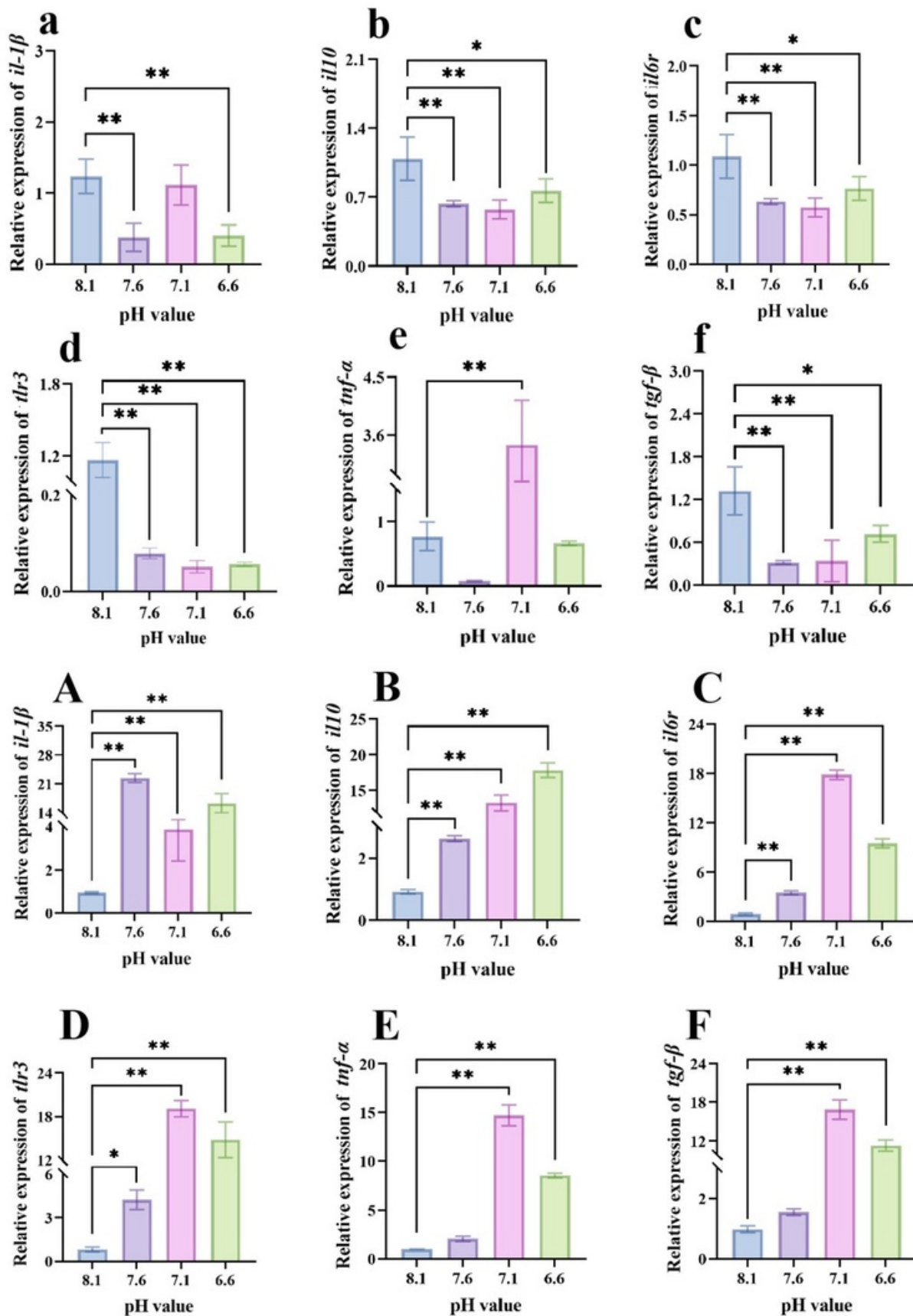


Fig. 2. Changes in inflammatory gene expression in juvenile yellowfin tuna under pH variation. (a-f) Liver, (A-F) Gill. Values are presented as mean $\pm$ SD of three replicate tanks (n = 3). "*" and "**" indicate significant differences from the control group at $p < 0.05$ and $p < 0.01$, respectively.

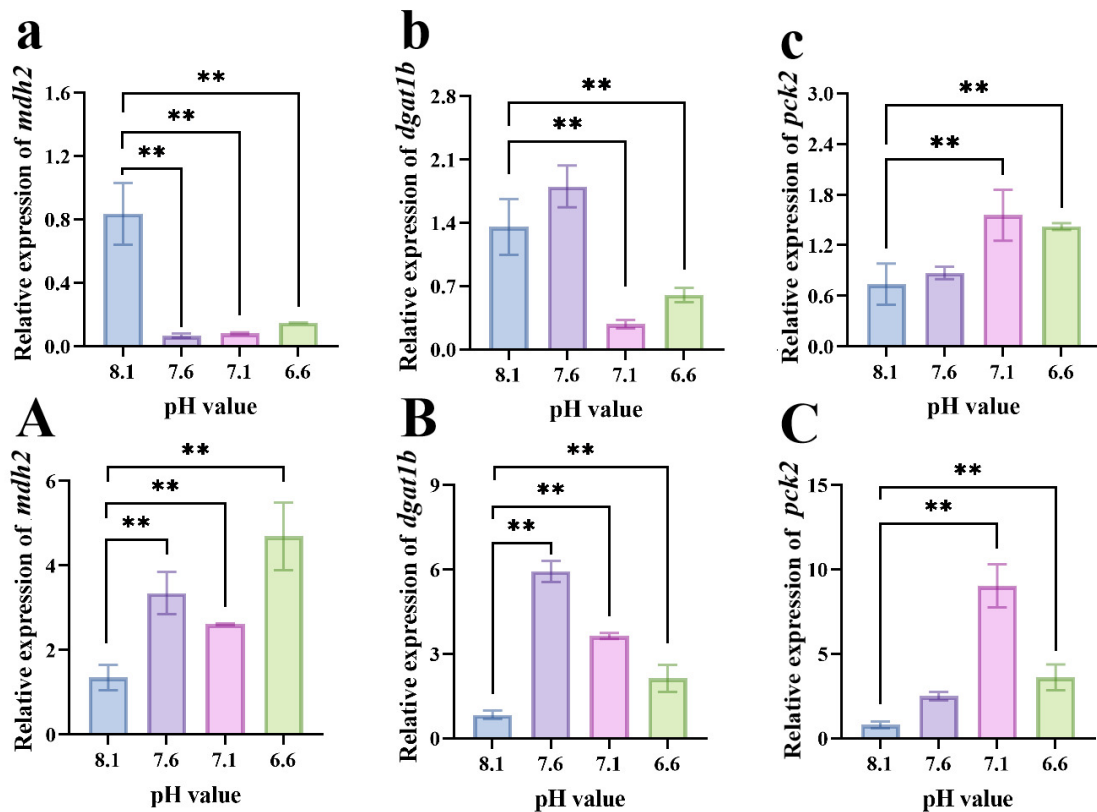


Fig. 3. Changes in metabolic gene expression in juvenile yellowfin tuna under pH variation. (a–c) Liver, (A–C) Gill. Values are presented as mean $\pm$ SD of three replicate tanks ($n = 3$). “*” and “***” indicate significant differences from the control group at $p < 0.05$ and $p < 0.01$, respectively.

acute low-pH exposure. The gill generally showed increased expression of antioxidant-, immune-, and metabolism-related genes, whereas the liver exhibited predominantly reduced expression of antioxidant and immune-related genes with selective changes in metabolic genes. These contrasting expression patterns suggest tissue-specific regulation in juvenile yellowfin tuna when responding to short-term environmental pH fluctuations.

The antioxidant defense system is an important component that maintains cellular redox balance under environmental stress. In the present study, the expression levels of *sod*, *cat*, and *gpx1a* in the gill were generally increased under low-pH exposure, with the strongest responses observed at pH 6.6. As a mucosal tissue directly exposed to surrounding seawater, the gill is likely to be the first organ sensing environmental pH fluctuations and may require rapid transcriptional regulation of antioxidant-related genes to maintain cellular homeostasis.^{5,42} Similar tissue-specific responses have been reported in large yellow croaker (*Larimichthys crocea*), where low-pH exposure resulted in pronounced transcriptional changes in gill tissues and highlighted the importance of gill-mediated regulation during environmental stress.⁴³ In contrast to the gill, hepatic expression levels of *sod*, *cat*, and *gpx1a* were significantly downregulated under low-pH treatments, with the exception of *gpx1a* at pH 6.6, which showed no significant difference compared with the control. This tissue-specific difference suggests that the liver and gill may employ dis-

tinct transcriptional responses during acute pH stress. Under environmental stress, organisms may adjust resource allocation among different physiological processes to maintain essential functions, although the specific metabolic costs and trade-offs in juvenile yellowfin tuna require further investigation.³⁶ Similar reductions in hepatic antioxidant-related responses have been reported in juvenile zebra seabream (*Diplodus cervinus*), where hepatic CAT activity decreased under acidic conditions.⁴⁴

Immune and inflammatory responses are important mechanisms for responding to environmental disturbances and maintaining physiological homeostasis. In the gill tissue, several immune- and inflammatory-related genes showed increased expression under low-pH exposure. The upregulation of the pro-inflammatory cytokines *il-1 β* and *tnf- α* suggests enhanced inflammatory-related transcriptional responses, while the concurrent increase in *il10* and *tgf- β* may indicate activation of regulatory mechanisms that modulate immune responses.⁴⁵ Proteomic studies on Atlantic halibut (*Hippoglossus hippoglossus*) also reported changes in immune-related proteins, including complement component C3 and fibrinogen, suggesting that low-pH stress can influence systemic immune responses.⁴⁶ In contrast, hepatic immune-related genes exhibited distinct expression patterns: with the exception of an increase in *tnf- α* at pH 7.1, the expression of *il10*, *il6r*, *tlr3*, and *tgf- β* was significantly downregulated across all low-pH treatments, and *il-1 β* was also significantly reduced at pH 7.6

and 6.6. These results indicate that acute low-pH stress induces alterations in hepatic immune-related transcriptional regulation. These results are comparable with a transgenerational low-pH study in European sea bass (*Dicentrarchus labrax*), where low-pH exposure affected hepatic transcripts involved in immune response, carbohydrate metabolism, and cellular homeostasis.¹⁷ Differences between acute exposure in juvenile yellowfin tuna and transgenerational exposure in European sea bass indicate that exposure duration may influence hepatic transcriptional responses to low-pH stress.

The tricarboxylic acid (TCA) cycle, gluconeogenesis, and lipid metabolism are important pathways that maintain cellular energy homeostasis.⁴⁷ In this study, changes in hepatic metabolic gene expression indicated tissue-specific transcriptional regulation of metabolic processes under acute low-pH exposure. The reduced expression of *mdh2* together with increased *pck2* expression may indicate altered transcriptional regulation of TCA cycle-related metabolism and gluconeogenesis-associated processes in the liver, rather than directly reflecting changes in metabolic flux. These transcriptional changes suggest that hepatic metabolism may undergo regulatory adjustments at the transcriptional level under acute low-pH stress.⁴⁸ In contrast, the increased expression of *mdh2*, *pck2*, and *dgat1b* in the gill suggests enhanced transcriptional regulation of multiple metabolic pathways under low-pH exposure. As an organ directly involved in ion exchange and acid-base regulation, the gill may require coordinated transcriptional responses to support physiological regulation during environmental pH fluctuations.⁴⁹ The increased expression of *mdh2* may be associated with elevated transcriptional demands related to mitochondrial processes and ion regulation, whereas increased *pck2* expression may help meet cellular metabolic demands at the transcriptional level under stress conditions.^{46,50}

Notably, several genes exhibited non-monotonic expression patterns across the tested pH levels, with expression increasing at pH 7.6 or 7.1 and subsequently decreasing at pH 6.6. This pattern suggests that transcriptional responses to acute low-pH exposure may not vary linearly with decreasing pH. One possible explanation is that moderate and severe low-pH conditions elicit different magnitudes or phases of the stress response, as stress-related energetic and regulatory processes in fishes can vary with stress intensity and physiological state.⁵¹ Similar variability in responses across pH levels has also been reported in other fishes exposed to low-pH conditions.⁵²⁻⁵⁴ However, because the present study assessed only a single 48-h endpoint and measured mRNA abundance alone, it remains unclear whether the decline observed at pH 6.6 represents reduced compensatory capacity, temporal differences in gene regulation, or other mechanisms. Studies in other marine fishes further indicate that sensitivity to severe low-pH exposure can vary substantially among species.^{54,55} Therefore, time-course experiments integrating physiological, biochemical, and protein-level measurements are needed to clarify the biological basis of these non-monotonic transcriptional responses.

It should be noted that seawater pH in this study was adjusted using HCl/NaOH rather than through CO₂ equilibration. Therefore, the observed responses primarily reflect the biological effects of low pH per se (i.e., elevated proton concentrations), rather than the full suite of carbonate chemistry changes associated with ocean acidification. Accordingly, the conclusions of this study should be interpreted strictly within the context of acute low-pH stress.⁵⁶ In addition, as the present study was conducted at a single 48 h endpoint, it is not possible to distinguish transient from sustained responses. Moreover, mRNA abundance is not equivalent to protein levels or enzymatic activities; therefore, the observed changes in gene expression should be interpreted as transcriptional-level responses and as evidence for hypothesis generation.⁵⁷ In addition, each treatment group consisted of only three replicate tanks ($n = 3$), with the average value of six fish per tank used as the representative measurement. Due to the difficulty and high cost of obtaining juvenile yellowfin tuna, the sample size was relatively small, which may have limited the statistical power to detect subtle differences. Furthermore, the Shapiro-Wilk normality test has limited ability to effectively detect deviations from normality with such a small sample size.⁵⁸ The transition from feeding during acclimation to fasting during the exposure period may constitute a nutritional confound that could influence hepatic metabolic gene expression. Future studies should integrate CO₂-driven acidification experiments, time-course sampling, protein-level analyses (e.g., Western blotting or enzyme activity assays), larger sample sizes, and extended exposure durations, as well as employ more complex statistical models (such as two-way ANOVA or regression analysis), to further disentangle the specific effects of pH from other carbonate system variables, to validate the functional consequences of these transcriptional changes, and to gain deeper insights into the non-monotonic response patterns observed in this study.

Integrating the responses of antioxidant-, immune-, and metabolism-related genes, this study highlights contrasting tissue-specific transcriptional responses of juvenile yellowfin tuna under acute low-pH exposure. The gill generally showed increased expression of genes involved in antioxidant defense, immune regulation, and metabolism, whereas the liver exhibited reduced expression of many antioxidant- and immune-related genes accompanied by selective changes in metabolic gene expression. These differences suggest that distinct tissues may regulate their transcriptional responses in accordance with their physiological roles during acute environmental stress. Such tissue-specific regulation may reflect the differential allocation of cellular resources among physiological processes under stress conditions.⁵⁶ This study provides molecular insights into the response characteristics of juvenile yellowfin tuna, an economically important pelagic fish species, under acute low-pH exposure.

5. CONCLUSIONS

By examining the expression responses of antioxidant-, immune-, inflammatory-, and metabolism-related genes in the liver and gill tissues of juvenile yellowfin tuna exposed to different pH levels (8.1, 7.6, 7.1, and 6.6), this study characterized tissue-specific molecular responses under acute low-pH stress. The gill generally exhibited increased expression of genes associated with antioxidant defense, immune regulation, and metabolism, whereas the liver showed reduced expression of several antioxidant- and immune-related genes accompanied by selective changes in metabolic gene expression. These contrasting transcriptional patterns highlight distinct regulatory responses between tissues during short-term pH stress. This study provides molecular insights into the response characteristics of juvenile yellowfin tuna, an economically important pelagic fish species, under acute low-pH exposure. Future studies integrating long-term exposure experiments with multi-omics, physiological measurements, and histopathological analyses are needed to further clarify the functional consequences of these transcriptional responses and their implications for aquaculture management.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Agriculture and Rural Affairs of the People's Republic of China Science and Technology Project Funding, the Central Public interest Scientific Institution Basal Research Fund; CAFS (2023TD58, 2027TD604, 2025XT03, 2026TS01); the 2025 Research on breeding technology of candidate species for Guangdong modern marine ranching (2025-MRB-00-001); the Hainan Province Science and Technology Special Fund (ZDYF2024XDNY247); the National Natural Science Foundation of China (32460927) and the Guangdong Province Overseas Distinguished Scholars Program (MS202500183).

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Investigation: Yitao Feng (Equal), Yuzhuo You (Equal), Xiaoyan Wang (Equal). Data curation: Yitao Feng (Equal), Yuzhuo You (Equal). Formal Analysis: Yitao Feng (Equal), Zhengyi Fu (Equal). Visualization: Yitao Feng (Equal), Zhengyi Fu (Equal). Writing – original draft: Yitao Feng (Equal), Zhengyi Fu (Equal). Methodology: Yuzhuo You (Equal), Xiaoyan Wang (Equal), Zhengyi Fu (Equal). Validation: Yuzhuo You (Equal), Xiaoyan Wang (Equal). Resources: Xiaoyan Wang (Equal), Zhenhua Ma (Equal). Conceptualization: Zhengyi Fu (Equal), Zhenhua Ma (Equal). Supervision: Zhengyi Fu (Equal), Zhenhua Ma (Equal). Project administration: Zhengyi Fu (Equal), Zhenhua Ma (Equal). Writing – review & editing: Zhengyi Fu (Equal), Zhenhua Ma (Equal). Funding acquisition: Zhenhua Ma (Equal).

ETHICAL APPROVAL

The animal study was reviewed and approved by the Animal Care and Use Committee of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The ethical code is 2020TD55, which was approved on 5 January 2020.

CONFLICTS OF INTEREST

No competing interests were disclosed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding authors.

Submitted: August 18, 2026 CDT. Accepted: September 11, 2026 CDT. Published: October 14, 2026 CDT.



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CCBY-4.0). View this license's legal deed at <http://creativecommons.org/licenses/by/4.0> and legal code at <http://creativecommons.org/licenses/by/4.0/legalcode> for more information.

REFERENCES

1. Caldeira K, Wickett ME. Anthropogenic carbon and ocean pH. *Nature*. 2003;425(6956):365-365. doi:[10.1038/425365a](https://doi.org/10.1038/425365a)
2. Doney SC, Fabry VJ, Feely RA, Kleypas JA. Ocean acidification: the other CO₂ problem. *Annual review of marine science*. 2009;1:169-192. doi:[10.1146/annurev.marine.010908.163834](https://doi.org/10.1146/annurev.marine.010908.163834)
3. Orr JC, Fabry VJ, Aumont O, et al. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*. 2005;437(7059):681-686. doi:[10.1038/nature04095](https://doi.org/10.1038/nature04095)
4. Pörtner HO, Langenbuch M, Reipschläger A. Biological impact of elevated ocean CO₂ concentrations: lessons from animal physiology and earth history. *Journal of oceanography*. 2004;60(4):705-718. doi:[10.1007/s10872-004-5763-0](https://doi.org/10.1007/s10872-004-5763-0)
5. Evans DH, Piermarini PM, Choe KP. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological reviews*. 2005;85(1):97-177. doi:[10.1152/physrev.00050.2003](https://doi.org/10.1152/physrev.00050.2003)
6. Melzner F, Göbel S, Langenbuch M, et al. Swimming performance in Atlantic Cod (*Gadus morhua*) following long-term (4–12 months) acclimation to elevated seawater PCO₂. *Aquatic toxicology*. 2009;92(1):30-37. doi:[10.1016/j.aquatox.2008.12.011](https://doi.org/10.1016/j.aquatox.2008.12.011)
7. Stumpp M, Wren J, Melzner F, Thorndyke M, Dupont S. CO₂ induced seawater acidification impacts sea urchin larval development I: elevated metabolic rates decrease scope for growth and induce developmental delay. *Comparative Biochemistry Physiology Part A: Molecular Integrative Physiology*. 2011;160(3):331-340. doi:[10.1016/j.cbpa.2011.06.022](https://doi.org/10.1016/j.cbpa.2011.06.022)
8. Wang Q, Cao R, Ning X, et al. Effects of ocean acidification on immune responses of the Pacific oyster *Crassostrea gigas*. *Fish & Shellfish Immunology*. 2016;49:24-33. doi:[10.1016/j.fsi.2015.12.025](https://doi.org/10.1016/j.fsi.2015.12.025)
9. Kroeker KJ, Kordas RL, Crim R, et al. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global change biology*. 2013;19(6):1884-1896. doi:[10.1111/gcb.12179](https://doi.org/10.1111/gcb.12179)
10. Pörtner HO. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Marine Ecology Progress Series*. 2008;373:203-217. doi:[10.3354/meps07768](https://doi.org/10.3354/meps07768)
11. Jia R, Du J, Cao L, et al. Immune, inflammatory, autophagic and DNA damage responses to long-term H₂O₂ exposure in different tissues of common carp (*Cyprinus carpio*). *Science of the Total Environment*. 2021;757:143831. doi:[10.1016/j.scitotenv.2020.143831](https://doi.org/10.1016/j.scitotenv.2020.143831)
12. Ishimatsu A, Kikkawa T, Hayashi M, Lee KS, Kita J. Effects of CO₂ on marine fish: larvae and adults. *Journal of oceanography*. 2004;60(4):731-741. doi:[10.1007/s10872-004-5765-y](https://doi.org/10.1007/s10872-004-5765-y)
13. Ramírez-Mendoza Z, Sosa-Nishizaki O, Pardo MA, et al. Mesoscale activity drives the habitat suitability of yellowfin tuna in the Gulf of Mexico. *Scientific Reports*. 2024;14(1):8256. doi:[10.1038/s41598-024-58613-7](https://doi.org/10.1038/s41598-024-58613-7)
14. Shi X, Zhang J, Wang X, et al. Reproductive biology of yellowfin tuna (*Thunnus albacares*) in tropical western and central Pacific Ocean. *Fishes*. 2022;7(4):162. doi:[10.3390/fishes7040162](https://doi.org/10.3390/fishes7040162)
15. Munday PL, Donelson JM, Dixon DL, Endo GG. Effects of ocean acidification on the early life history of a tropical marine fish. *Proceedings of the Royal Society B: Biological Sciences*. 2009;276(1671):3275. doi:[10.1098/RSPB.2009.0784](https://doi.org/10.1098/RSPB.2009.0784)
16. Michaelidis B, Spring A, Pörtner HO. Effects of long-term acclimation to environmental hypercapnia on extracellular acid-base status and metabolic capacity in Mediterranean fish *Sparus aurata*. *Marine Biology*. 2007;150(6):1417-1429. doi:[10.1007/s00227-006-0436-8](https://doi.org/10.1007/s00227-006-0436-8)
17. Auffret P, Servili A, Gonzalez AA, et al. Transgenerational exposure to ocean acidification impacts the hepatic transcriptome of European sea bass (*Dicentrarchus labrax*). *BMC genomics*. 2023;24(1):331. doi:[10.1186/s12864-023-09353-x](https://doi.org/10.1186/s12864-023-09353-x)
18. Ma Z, Zheng X, Fu Z, et al. Transcriptional analysis reveals physiological response to acute acidification stress of barramundi *Lates calcarifer* (Bloch) in coastal areas. *Fish Physiology and Biochemistry*. 2020;46(5):1729-1741. doi:[10.1007/s10695-020-00824-6](https://doi.org/10.1007/s10695-020-00824-6)
19. Bone Q, Moore R. *Biology of Fishes*. Taylor & Francis; 2008. doi:[10.1201/9781134186310](https://doi.org/10.1201/9781134186310)
20. Sumaila U, Samoilys M, Allison E, et al. *Economic Impacts of Ocean Acidification on Fisheries and Aquaculture in the Western Indian Ocean: Current Knowledge and Recommendations*. IAEA Report; 2014. doi:[10.5670/oceanog.2009.37](https://doi.org/10.5670/oceanog.2009.37)

21. Martínez-Álvarez RM, Morales AE, Sanz A. Antioxidant defenses in fish: biotic and abiotic factors. *Reviews in Fish Biology and Fisheries*. 2005;15(1):75-88. doi:[10.1007/s11160-005-7846-4](https://doi.org/10.1007/s11160-005-7846-4)
22. Mahapatra S, Maity J, Mandal S. Assessing the physiological and oxidative stress status of *Etroplus suratensis* under elevated temperature and ocean acidification. *Science of The Total Environment*. 2025;998:180294. doi:[10.1016/j.scitotenv.2025.180294](https://doi.org/10.1016/j.scitotenv.2025.180294)
23. Jomova K, Alomar SY, Alwasel SH, et al. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Archives of toxicology*. 2024;98(5):1323-1367. doi:[10.1007/s00204-024-03696-4](https://doi.org/10.1007/s00204-024-03696-4)
24. Anwar S, Alrumaihi F, Sarwar T, et al. Exploring therapeutic potential of catalase: Strategies in disease prevention and management. *Biomolecules*. 2024;14(6):697. doi:[10.3390/biom14060697](https://doi.org/10.3390/biom14060697)
25. Lee SH, Takahashi K, Hatakawa Y, Oe T. Lipid peroxidation-derived modification and its effect on the activity of glutathione peroxidase 1. *Free Radical Biology Medicine*. 2023;208:252-259. doi:[10.1016/j.freeradbiomed.2023.08.014](https://doi.org/10.1016/j.freeradbiomed.2023.08.014)
26. Gao Y, Li J, Wang W, Tian Y. Molecular and physiological responses of black rockfish (*Sebastes schlegelii*) to short-and medium-term ocean acidification. *Environmental Research*. 2025;275:121431. doi:[10.1016/j.envres.2025.121431](https://doi.org/10.1016/j.envres.2025.121431)
27. Costa RA, Hubbard PC, Manchado M, Power DM, Velez Z. Olfactory specialization in the Senegalese sole (*Solea senegalensis*): CO₂ acidified water triggers nostril-specific immune processes. *Comparative Biochemistry Physiology Part A: Molecular Integrative Physiology*. 2025;302:111820. doi:[10.1016/j.cbpa.2025.111820](https://doi.org/10.1016/j.cbpa.2025.111820)
28. Carlson KB, Nguyen C, Wcisel DJ, Yoder JA, Dornburg A. Ancient fish lineages illuminate toll-like receptor diversification in early vertebrate evolution. *Immunogenetics*. 2023;75(5):465-478. doi:[10.1007/s00251-023-01315-7](https://doi.org/10.1007/s00251-023-01315-7)
29. Pan PK, Wu TM, Tsai HY, et al. Acid external and internal environment exchange the *Oreochromis niloticus* tissue immune gene expression compared to the mouse macrophage polarization model. *Frontiers in Immunology*. 2022;13:1012078. doi:[10.3389/fimmu.2022.1012078](https://doi.org/10.3389/fimmu.2022.1012078)
30. Kaneda M, Odaka T, Suetake H, Tahara D, Miyadai T. Teleost IL-6 promotes antibody production through STAT3 signaling via IL-6R and gp130. *Developmental & Comparative Immunology*. 2012;38(2):224-231. doi:[10.1016/j.dci.2012.02.002](https://doi.org/10.1016/j.dci.2012.02.002)
31. Wei H, Yin L, Feng S, et al. Dual-parallel inhibition of IL-10 and TGF- β 1 controls LPS-induced inflammatory response via NF- κ B signaling in grass carp monocytes/macrophages. *Fish & Shellfish Immunology*. 2015;44(2):445-452. doi:[10.1016/j.fsi.2015.03.023](https://doi.org/10.1016/j.fsi.2015.03.023)
32. Ruiz-Jarabo I, Gregório SF, Alves A, Mancera JM, Fuentes J. Ocean acidification compromises energy management in *Sparus aurata* (Pisces: Teleostei). *Comparative Biochemistry Physiology Part A: Molecular Integrative Physiology*. 2021;256:110911. doi:[10.1016/j.cbpa.2021.110911](https://doi.org/10.1016/j.cbpa.2021.110911)
33. Ding W, Cao L, Cao Z, Bing X. Gill and brain transcriptomic analysis of mandarin fish (*Siniperca chuatsi*) reveals hypoxia-induced mitochondrial dysfunction and modulation of metabolism. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*. 2025;53:101367. doi:[10.1016/j.cbd.2024.101367](https://doi.org/10.1016/j.cbd.2024.101367)
34. Marandel L, Kostyniuk D, Best C, et al. Pck-ing up steam: Widening the salmonid gluconeogenic gene duplication trail. *Gene*. 2019;698:129-140. doi:[10.1016/j.gene.2019.02.079](https://doi.org/10.1016/j.gene.2019.02.079)
35. Sun J, Xu X, Huang X, et al. Nuclear factor- κ B subunit p65 is involved in lipopolysaccharide-induced lipid accumulation via regulating DGAT1b in *Ctenopharyngodon idellus* kidney cells. *Fish & Shellfish Immunology*. 2020;105:71-77. doi:[10.1016/j.fsi.2020.05.071](https://doi.org/10.1016/j.fsi.2020.05.071)
36. Stapp LS, Kreiss C, Pörtner HO, Lannig G. Differential impacts of elevated CO₂ and acidosis on the energy budget of gill and liver cells from Atlantic cod, *Gadus morhua*. *Comparative Biochemistry Physiology Part A: Molecular Integrative Physiology*. 2015;187:160-167. doi:[10.1016/j.cbpa.2015.05.009](https://doi.org/10.1016/j.cbpa.2015.05.009)
37. Ilyina T, Six KD, Segschneider J, et al. Global ocean biogeochemistry model HAMOCC: Model architecture and performance as component of the MPI-Earth system model in different CMIP5 experimental realizations. *Journal of Advances in Modeling Earth Systems*. 2013;5:287-315. doi:[10.1029/2012MS000178](https://doi.org/10.1029/2012MS000178)
38. Pörtner HO, Roberts DC, Masson-Delmotte V, et al. The ocean and cryosphere in a changing climate. *IPCC special report on the ocean cryosphere in a changing climate*. 2019;1155:10-1017.

39. Kim JH, Kim SR, Kim SK, Kang HW. Effects of pH changes on blood physiology, antioxidant responses and Ig M of juvenile olive flounder, *Paralichthys olivaceus*. *Aquaculture Reports*. 2021;21:100790. doi:[10.1016/j.aqrep.2021.100790](https://doi.org/10.1016/j.aqrep.2021.100790)
40. Neiffer DL, Stamper MA. Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. *ILAR journal*. 2009;50(4):343-360. doi:[10.1093/ilar.50.4.343](https://doi.org/10.1093/ilar.50.4.343)
41. Lalitha S. Primer premier 5. *Biotech Software Internet Report: The Computer Software Journal for Scient*. 2000;1(6):270-272. doi:[10.1089/152791600459894](https://doi.org/10.1089/152791600459894)
42. Wilson JM, Laurent P. Fish gill morphology: inside out. *Journal of experimental Zoology*. 2002;293(3):192-213. doi:[10.1002/jez.10124](https://doi.org/10.1002/jez.10124)
43. Ye T, Zhang X, Liu F, et al. Adaptive Responses of Large Yellow Croaker *Larimichthys crocea* to Ocean Acidification: Integrative Analysis of Gill and Kidney Transcriptomics and Antioxidant Enzyme Activities. *Antioxidants*. 2025;14(7):872. doi:[10.3390/antiox14070872](https://doi.org/10.3390/antiox14070872)
44. Carmona MM. *Physiological Responses to Ocean Warming and Acidification of Diplodus Cervinus*. Universidade NOVA de Lisboa (Portugal); 2019.
45. Fu Z, Yang R, Yu G, et al. Tissue comparison of transcriptional response to acute acidification stress of barramundi *Lates calcarifer* in coastal and estuarine areas. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*. 2021;38:100830. doi:[10.1016/j.cbd.2021.100830](https://doi.org/10.1016/j.cbd.2021.100830)
46. Bresolin de Souza K, Jutfelt F, Kling P, Förlin L, Sturve J. Effects of increased CO₂ on fish gill and plasma proteome. *PloS one*. 2014;9(7):e102901. doi:[10.1371/journal.pone.0102901](https://doi.org/10.1371/journal.pone.0102901)
47. Thor P, Perry D. Impact of climate change driven freshening, warming, and ocean acidification on the cellular metabolism of Atlantic Cod (*Gadus morhua*). *Scientific Reports*. 2025;15(1):37155. doi:[10.1038/s41598-025-21597-z](https://doi.org/10.1038/s41598-025-21597-z)
48. Suresh S, Welch MJ, Munday PL, Ravasi T, Schunter C. Cross-talk between tissues is critical for intergenerational acclimation to environmental change in *Acanthochromis polyacanthus*. *Communications Biology*. 2024;7(1):1531. doi:[10.1038/s42003-024-07241-y](https://doi.org/10.1038/s42003-024-07241-y)
49. Kovac A, Goss GG. Cellular mechanisms of ion and acid-base regulation in teleost gill ionocytes. *Journal of Comparative Physiology B*. 2024;194(5):645-662. doi:[10.1007/s00360-024-01560-6](https://doi.org/10.1007/s00360-024-01560-6)
50. Furukawa F, Tseng YC, Liu ST, et al. Induction of phosphoenolpyruvate carboxykinase (PEPCK) during acute acidosis and its role in acid secretion by V-ATPase-expressing ionocytes. *International Journal of Biological Sciences*. 2015;11(6):712. doi:[10.7150/IJBS.11827](https://doi.org/10.7150/IJBS.11827)
51. Hunt von Herbing I. Energetic costs of stress in developing fishes: quantifying allostasis and allostatic load. *Integrative and Comparative Biology*. 2024;64(3):1019-1033. doi:[10.1093/icb/icae094](https://doi.org/10.1093/icb/icae094)
52. Calabrese EJ. Hormesis: why it is important to toxicology and toxicologists. *Environmental Toxicology and Chemistry*. 2008;27(7):1451-1474. doi:[10.1897/07-541.1](https://doi.org/10.1897/07-541.1)
53. Val AL, Wood CM, Wilson RW, et al. Responses of an Amazonian teleost, the tambaqui (*Colossoma macropomum*), to low pH in extremely soft water. *Physiological zoology*. 1998;71(6):658-670. doi:[10.1086/515977](https://doi.org/10.1086/515977)
54. Li H, Zhang J, Ge X, Chen S, Ma Z. The effects of short-term exposure to pH reduction on the behavioral and physiological parameters of juvenile black rockfish (*Sebastes schlegelii*). *Biology*. 2023;12(6):876. doi:[10.3390/biology12060876](https://doi.org/10.3390/biology12060876)
55. Cheong J, Senoo S, Tuzan AD, et al. Effects of Acidified Seawater on Growth, Nutritional Condition, and Olfactory Sensory of the Asian Seabass (*Lates calcarifer*) Larvae. *Egyptian Journal of Aquatic Biology and Fisheries*. 2026;30(3):1015-1028. doi:[10.21608/ejabf.2026.479176.7757](https://doi.org/10.21608/ejabf.2026.479176.7757)
56. Damiani D, Moglie M, Passerini G. Effects of rapid acidification in marine seawater: Focus on Actinopterygii. *Marine Pollution Bulletin*. 2026;227:119437. doi:[10.1016/j.marpolbul.2026.119437](https://doi.org/10.1016/j.marpolbul.2026.119437)
57. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nature reviews genetics*. 2012;13(4):227-232. doi:[10.1038/nrg3185](https://doi.org/10.1038/nrg3185)
58. Ahad NA. Sensitivity of normality tests to non-normal data. *Sains Malaysiana*. 2011;40(6):637-641.