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Role of Nrf2-Keap1 signaling in the antioxidant defense response induced by low salinity in the kuruma shrimp (*Marsupenaeus japonicus*)

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Abstract
Salinity is an important environmental factor that induces oxidative stress in shrimp. This study evaluated the effects of abrupt low-salinity stress (23, 17, and 11) on histological structure, lipid peroxidation, mRNA levels and activities of antioxidant enzymes, and gene expression of Nrf2-Keap1 signaling molecules at different times (6, 12, 24, 48, and 96 h) in the gills and hepatopancreas of *Marsupenaeus japonicus*. Mild or strong increase in the levels of nuclear factor erythroid 2-related factor-2 (Nrf2) and antioxidant genes and enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) were observed after short-term exposure (6 and 12 h). After 48 and/or 96 h of exposure to low salinity, Nrf2 was significantly downregulated ($P < 0.05$), which was accompanied by downregulation of the levels of Nrf2-Keap1 pathway-related genes and enzymes such as SOD, CAT, and GPX, along with upregulation of Kelch-like-ECH-associated protein 1 (Keap1) and malondialdehyde (MDA). Pathological alterations were also observed in the gills and hepatopancreas of *M. japonicus* after 96 h of exposure to different salinities. The observed changes in antioxidant gene expression are consistent with a requirement for Nrf2 in the induction of antioxidant genes. Furthermore, there was a negative correlation between the mRNA levels of Nrf2 and Keap1, indicating that Keap1 is important for inhibition of the Nrf2 response. Negative relationships were observed between lipid peroxidation and antioxidant enzyme activities, while positive relationships were observed between activities and gene expression levels of antioxidant enzymes, suggesting the changes in molecular and enzyme activity levels may provide protection against damage from low-salinity stress. In conclusion, our data demonstrated that Nrf2-Keap1 signaling is important for modulating the gene expression levels of antioxidant enzymes. This is the first study to elucidate the effects of low-salinity stress on antioxidant responses in *M. japonicus* through the Nrf2-Keap1 pathway. The results provide insights into the mechanisms by which crustaceans resist salinity stress.

Keywords *Marsupenaeus japonicus* · Low salinity · Oxidative stress · Antioxidant enzyme · Nrf2-Keap1 signaling

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Introduction

The kuruma shrimp, *Marsupenaeus japonicus*, is a commercially important crustacean species worldwide (Tsoi et al. 2014), and its production accounts for more than 5% of global shrimp production (FAO 2020). In recent years, environmental changes and farming activities, such as heavy rain and large changes in water levels, can cause rapid declines in salinity, inducing oxidative stress in shrimp by accelerating the generation of reactive oxygen species (ROS), which lead to osmotic imbalances and metabolic disorders (Martínez-Alvarez et al. 2002; Pan et al. 2007). Therefore, it is important to investigate the antioxidant system in shrimp against low salinity-induced stress. However, there is minimal available information concerning the effects of salinity stress on the antioxidant system in *M. japonicus*.

To eliminate ROS-mediated damage, organisms have evolved a unique antioxidant defense system, which is composed of protective antioxidant enzymes and nonenzymatic radical scavengers (Storey 1996). It is reported that the levels of malondialdehyde (MDA) and antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), serve as important health indicators in marine organisms exposed to various environmental conditions (Bebianno et al. 2005; Ighodaro and Akinloye 2017). However, the underlying mechanisms of response to salinity stress at both enzymatic and molecular levels are poorly understood in shrimp. Therefore, studies regarding the activation of the antioxidant defense system are urgently needed. Numerous mammalian studies have shown that the nuclear factor erythroid 2-related factor-2 (Nrf2)-Kelch-like-ECH-associated protein 1 (Keap1) signaling plays an important role in oxidative stress by inducing the transcription of antioxidant enzymes (Matthew et al. 2015; Han et al. 2017). Wang and Zhu (2019) found that Nrf2 was activated by salinity and elevated the activity of downstream antioxidant enzymes SOD and GPX in *Coilia nasus*. Ren et al. (2020) showed that cadmium-induced cellular oxidative damage likely acts through the Nrf2 pathway in *M. japonicus*. Yun et al. (2021) demonstrated that Nrf2 is involved in the response to bacterial challenge and regulates the transcription of GPX genes in black tiger shrimp (*Penaeus monodon*). Although the contributions of Nrf2-Keap1 signaling to antioxidant functions are clear, the underlying molecular mechanism of the Nrf2-Keap1 pathway about salinity-induced oxidative stress is still relatively unexplored in crustaceans.

In this study, antioxidant responses were evaluated by histological observation, determining lipid peroxidation, and investigating the enzyme activities and expression of genes related to Nrf2-Keap1 signaling in *M. japonicus* exposure to low salinity. This investigation of Nrf2-Keap1 signaling-mediated antioxidant defense will provide new insight into the regulation of the antioxidant system in *M. japonicus* against environmental stress.

Materials and methods

Experimental animals and salinity stress

Healthy *M. japonicus* shrimp (weight 2.36 ± 0.83 g; length 5.75 ± 0.65 cm) were obtained from Guoxing Aquaculture Science and Technology Co., Ltd. (Zhanjiang,

China). Shrimp were acclimated in circular aquaculture buckets for 1 week before the salinity challenge. The tanks were continuously aerated, and water quality was monitored daily: we maintained water temperature at 26 ± 0.5 ℃, pH at 8.0, salinity at 29.5 ± 1, and the concentration of dissolved oxygen at ≥ 6 mg/L. Aquaculture water was obtained from the natural sea area and was used after sedimentation and sand filtration. Half of the tank water was renewed twice daily. *M. japonicus* shrimp were fed twice daily until 24 h prior to stress experiments. Feces and uneaten feed were removed daily using a siphon tube.

M. japonicus shrimp were randomly divided into four groups; each group had three replicates, and each replicate encompassed 30 animals ($n = 30$). The control group was set at a salinity of 29. Shrimp in the experimental group were directly transferred from natural seawater with salinity 29 (as control) to salinities of 23, 17, and 11. Low-salt water was prepared by mixing natural seawater with tap water after 24 h of aeration. Salinity was measured at 6 h intervals to maintain a constant level. After 6, 12, 24, 48, and 96 h of abrupt salinity reduction, nine shrimp (3 × 3 shrimp/replicate) per treatment and time point were randomly sampled. Gill and hepatopancreas tissues were flash-frozen in liquid nitrogen and stored at − 80 ℃ until analysis. Prior to the experiment, shrimp were fasted for 24 h to empty the gut and continuous aeration was supplied in the tank. The water was prepared ≥ 1 day before changing and aerated continuously, and 50% of the water volume was renewed daily. During the experimental period, the water conditions were identical to the acclimation conditions (26 ± 0.5 ℃; pH 8.0).

Histology of the gills and hepatopancreas

At the completion of the experiment, the gills and hepatopancreas of one shrimp from each tank at each salinity point (salinities 29, 23, 17, and 11) were dissected from the cephalothoraxes. The gills and hepatopancreas were rinsed using normal saline, then immediately fixed with 4% paraformaldehyde (Sangon Biotech, Shanghai, China) for tissue fixation; they were stored at 4 °C prior to the preparation of paraffin sections by Servicebio (Wuhan, China).

Table 1 The qRT-PCR primers used in this study

Gene	Primer	Sequence(5′-3′)
SOD	Forward	CCGAAGTGGCGCAGAAGTT
	Reverse	AAGGCGTGGATGTGGAAGC
CAT	Forward	AGGACCGTGCCATCAAAAA
	Reverse	GCAGCTAAGGCGTGAATCTG
GPX	Forward	CAGCCATCAAATCCTTCTACGA
	Reverse	GTGTTCTGCACCAGCACCAC
Nrf2	Forward	CTTGGTGGTGAGGAAGGTGTG
	Reverse	CCATTGTCTTGGGGTCGTGTA
Keap1	Forward	ATTTATCGCCACGCTTCTTG
	Reverse	ATTGGGGGTTCGCTCCTT
β-Actin	Forward	AGCCTTCCTTCCTGGGTATG
	Reverse	GGAGCGAGGGCAGTGATTT

Determination of enzyme activity

Antioxidant enzyme activities in the gills and hepatopancreas were measured by enzyme-linked immunosorbent assay kits for SOD, CAT, GPX, and MDA [Shanghai Enzyme-Linked Biotechnology Co., Ltd., catalog numbers: ml076325 (SOD), ml952441 (CAT), ml936833 (GPX), and ml022446 (MDA)], in accordance with the manufacturer's instructions.

RNA extraction, cDNA synthesis, and qRT-PCR analysis

Total RNA from the gills and hepatopancreas was extracted from samples of *M. japonicus* using the TransZol Up Plus RNA Kit (TransGen, Beijing, China). cDNA was synthesized using the EasyScript® One-step gDNA Removal and cDNA Synthesis Supermax Kit (TransGen) with an oligo dT18 primer. RNA concentrations were determined by electrophoresis; the optical densities at 260 nm and 280 nm were determined using a micro-quantitative analyzer (SimpliNano). High-quality RNA ($1.8 \leq A_{260}/A_{280} \leq 2.0$) was used for cDNA synthesis. Gene expression was detected

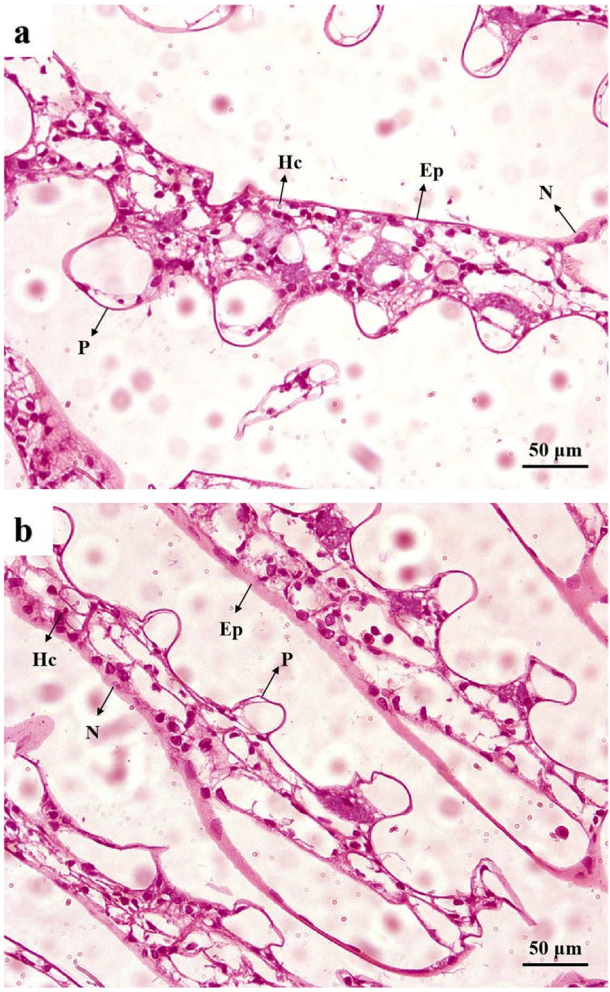
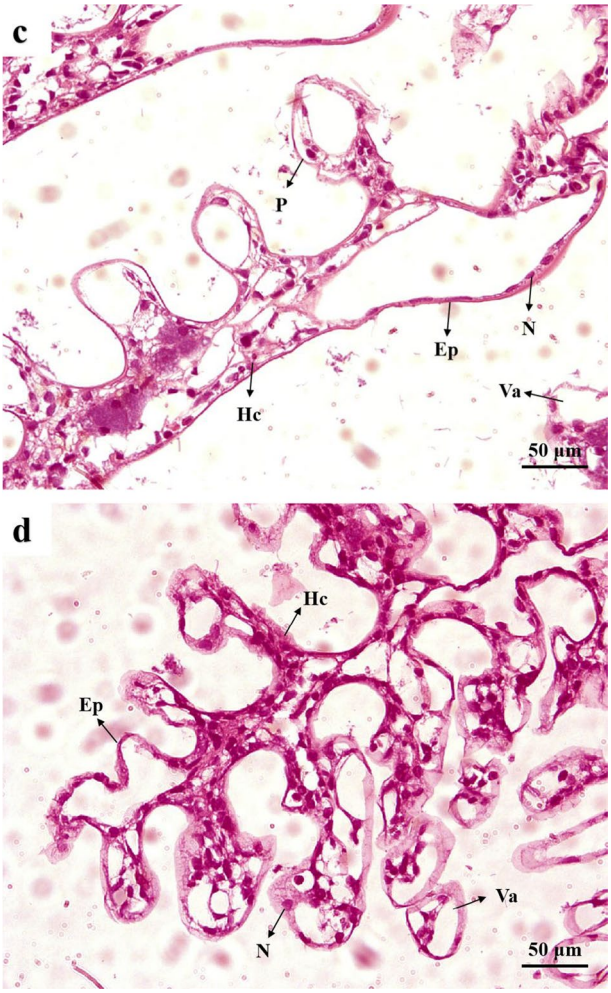


Fig. 1 The structure of gill tissues of *M. japonicus* after 96 h of exposure to different salinities. **a** Gill from the control shrimp, ×400; **b** gill of shrimp at salinity 23, ×400; **c** gill of shrimp at salinity 17, ×400; **d** gill of shrimp at salinity 11, ×400. The letters in the figure indicate (Ep) epithelial cell, (P) pillar cell, (N) epithelial nucleus, (Hc) hemocytes, and (Va) vacuole

Fig. 1 (continued)



by qRT-PCR using PerfectSTART® Green qPCR SuperMix (TransGen). The total reaction volume was 10 μL: 5 μL of 2×PerfectSTART™ Green qPCR SuperMix, 0.4 μL of forward primer, 0.4 μL of reverse primer, 3.2 μL of nuclease-free water, and 1 μL of cDNA. QRT-PCR was performed as follows: 95 ℃ for 30 s, followed by 45 cycles of 95 ℃ for 5 s, 60 ℃ for 15 s, and 72 °C for 10 s. β-actin was used as a housekeeping gene, and specific primer sequences were designed based on the coding sequences of target genes using Primer Premier 5.0 (Table 1). The β-actin gene of *M. japonicus* was selected as an internal control and each relative gene expression level is shown as the fold change in expression relative to β-actin, calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2002).

Statistical analysis

Prior to statistical analysis, all data were tested for normality using the Kolmogorov–Smirnov test. Statistical significance was determined using a one-way analysis of variance and Tukey's post hoc test for multiple comparisons. Nonparametric Spearman's

correlation analysis was used to examine relationships between parameters. Statistical analyses were performed using SPSS statistical software (version 23.0). All data were expressed as means ± standard errors of the mean. The level of significance was set at $P < 0.05$.

Results

Histological analysis

Paraffin sections of *M. japonicus* gill tissue demonstrated structural changes after 96 h of exposure to different salinities. In the control condition, gill filaments and gill fragments were complete, with epithelial cells and hemocytes neatly arranged (Fig. 1a). At salinities 23 (Fig. 1b) and 17 (Fig. 1c), the gill filaments had begun to deform and swell, narrowing of the subcuticular space was observed, and substantial disintegration of the

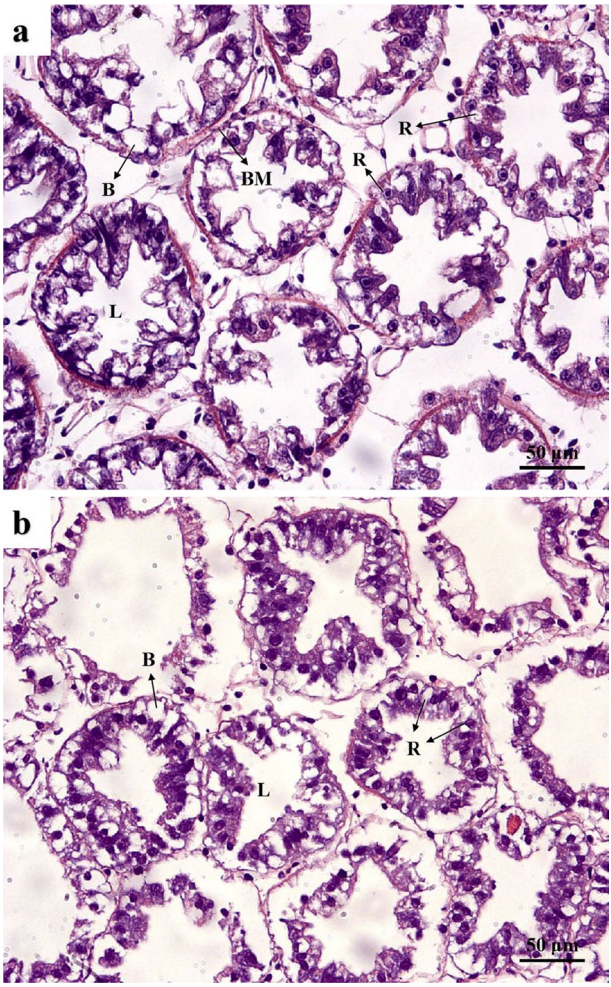
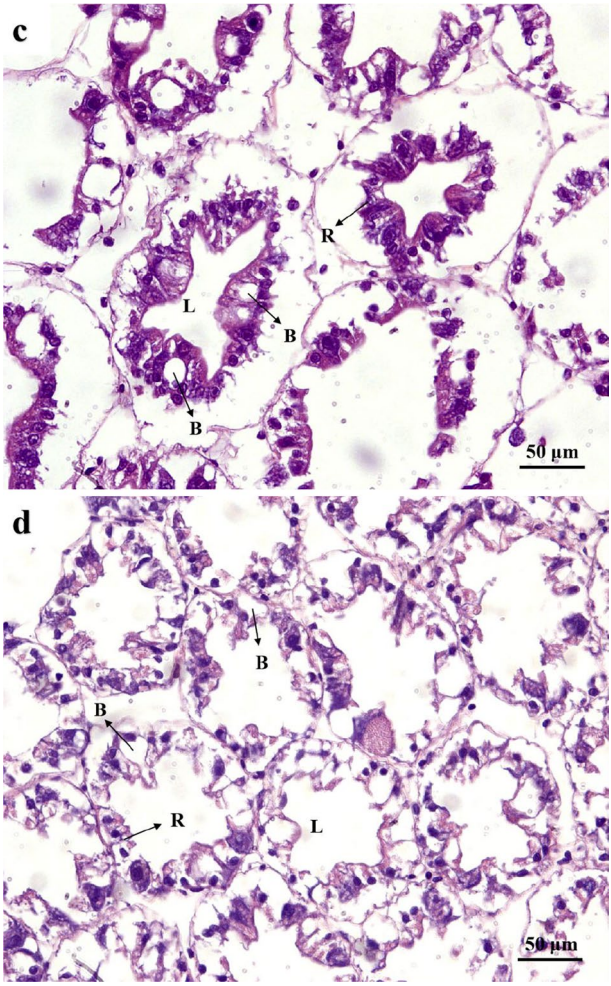


Fig. 2 The structure of hepatopancreas tissue of *M. japonicus* after 96 h of exposure to different salinities. **a** Hepatopancreas from the control shrimp, ×400; **b** hepatopancreas of shrimp at salinity 23, ×400; **c** hepatopancreas of shrimp at salinity 17, ×400; **d** hepatopancreas of shrimp at salinity 11, ×400. The letters in the figure indicate (B) B-cells, (R) R-cells, (BM) basement membranes, and (L) lumen

Fig. 2 (continued)



epithelium had occurred. At salinity 11 (Fig. 1d), we observed loose connections of the gill filaments, with numerous vacuoles in the cavity.

The hepatopancreas showed substantial pathological alterations after 96 h of exposure to different salinities. In the control condition, the wall tissue of hepatic tubules was intact, and the tubules were neatly arranged and clearly demarcated (Fig. 2a). At salinities 23 (Fig. 2b) and 17 (Fig. 2c), epithelial cells (B- and R-cells) had begun to swell, and the hepatic tubules were deformed and damaged. At salinity 11 (Fig. 2d), the arrangement of hepatic tubules was disordered, with irregular and ill-defined margins. Increasing vacuolization and necrosis of epithelial cells, as well as hemocyte infiltration in the interstitial sinus, was observed at salinity 11.

Changes in antioxidation status in the gills and hepatopancreas

Antioxidant capacity and lipid peroxidation in the gills were influenced by salinity and exposure time (Fig. 3). Compared with the control group, the levels of SOD, CAT, and

GPX significantly increased after 6 and 12 h of exposure to salinities 17 and 23 ($P<0.05$). A similar increase in the levels of CAT was observed after 6 h of exposure to salinity 11 ($P<0.05$). By the end of the experiment, the SOD, CAT, and GPX activities in shrimp exposed to salinities 17 and 23 showed no significant difference compared to the control group ($P>0.05$). There were significant decreases in SOD and GPX activities after 24 h to 96 h of exposure to salinity 11 ($P<0.05$). MDA content increased with decreasing salinity, and a substantial enhancement was observed after exposure to salinity 11 ($P<0.05$). The level of MDA in shrimp significantly decreased after 6 h of exposure to salinity 17 ($P<0.05$), but a significant increase occurred from 24 to 96 h ($P<0.05$).

The antioxidation status of the hepatopancreas was also differentially affected by salinity and exposure time (Fig. 4). SOD, CAT, and GPX activities significantly increased in shrimp after 6 h of exposure to salinities 17 and 23 ($P<0.05$) then reduced gradually and showed no significant difference compared to the control group at the end of the experiment. In contrast, SOD and GPX activities in shrimp exposed to salinity 11 showed no significant difference compared to the control group during early exposure ($P>0.05$). After 24 h of stress, SOD and GPX activities in shrimp exposed to salinity 11 were suppressed compared with activities in the control group ($P<0.05$). MDA content significantly

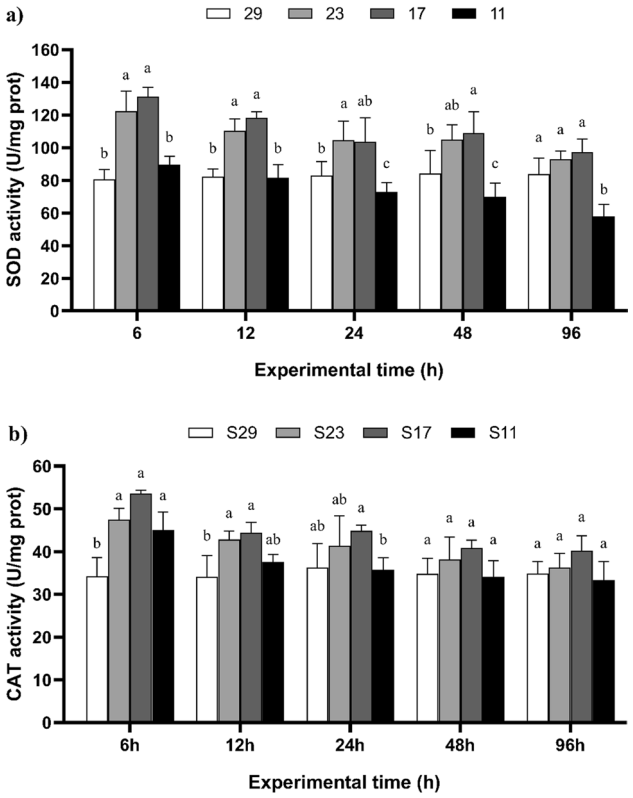


Fig. 3 Effects of different salinities on the antioxidation status in the gill of *M. japonicus*. **a** SOD; **b** CAT; **c** GPX; and **d** MDA. There was a significant difference between means with different letters at each time point ($P<0.05$)

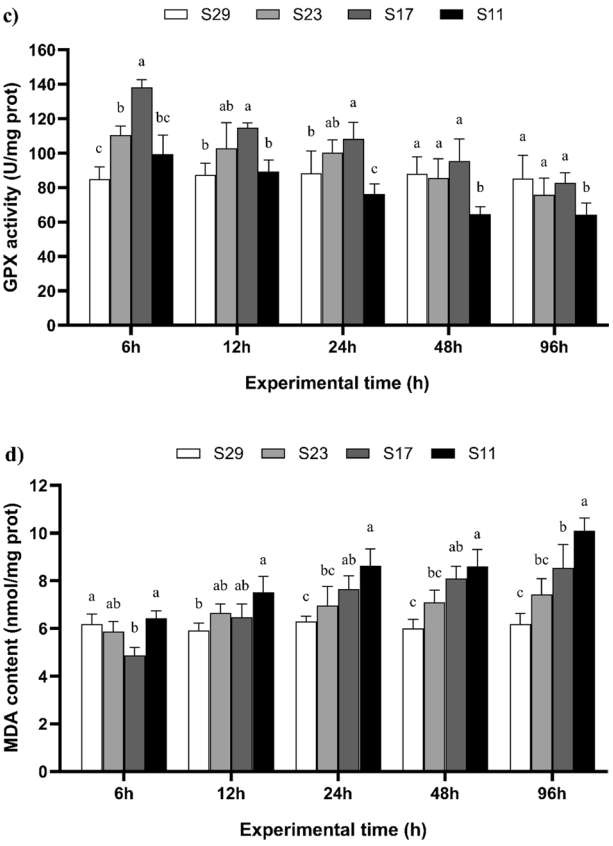


Fig. 3 (continued)

increased after 24 h of exposure to salinities 11 and 17. A similar increase was observed after 96 h of exposure to salinity 23 ($P < 0.05$).

Expression of genes involved in Nrf2-Keap1 signaling

To further characterize the mechanisms underlying the effects of low salinity on antioxidation, the mRNA levels of Nrf2, Keap1, SOD, CAT, and GPX were measured by qRT-PCR in the gills and hepatopancreas of *M. japonicus*. After 6 h of exposure, the mRNA level of SOD significantly differed between each exposure group except for salinity 11 and the control group ($P < 0.05$). Significant decreases in the mRNA level of SOD were detected after 12 h exposure to salinity 11 ($P < 0.05$) and after 48 h exposure to salinities 17 and 23 ($P < 0.05$). In the early stage of exposure, the mRNA levels of CAT were significantly greater at salinities 17 and 23 than in the control group ($P < 0.05$). The mRNA levels of CAT were significantly lower after 48 and 96 h exposure to salinities 11 and 17 ($P < 0.05$) than in the control group. After 6 h of exposure, the mRNA level of GPX was maximal in each exposure group, then gradually decreased and significantly decreased after 12 h exposure to salinity 11 ($P < 0.05$) and after 24 h exposure to salinities 17 and 23 ($P < 0.05$). After 6 h of exposure, the mRNA

level of Nrf2 was maximal in the salinity 23 group then gradually decreased and showed no significant difference compared to the control group ($P > 0.05$). After 96 h of exposure to salinities 11 and 17, the mRNA level of Nrf2 was significantly lower than in control shrimp ($P < 0.05$) (Fig. 5d). The Keap1 mRNA was visibly upregulated in shrimp exposed to salinities 11 and 17 after 24 h exposure to low salinity ($P < 0.05$). At the end of the exposure period, the Keap1 mRNA levels remained higher than the level in the control group in all three treatments (Fig. 5e).

The mRNA levels of SOD, CAT, GPX, Nrf2, and Keap1 in the hepatopancreas are shown in Fig. 6. After 6 h of exposure, the mRNA levels of SOD significantly differed between each exposure group and the control group ($P < 0.05$). After 96 h exposure to salinity 11, a significant decrease in the SOD mRNA level was detected ($P < 0.05$). The mRNA levels of CAT significantly increased after 6 and 12 h exposure to salinities 17 and 23 ($P < 0.05$), then gradually decreased and showed no significant differences compared to the control group at 48 h and thereafter ($P > 0.05$). There were no significant differences in the levels of CAT mRNA after exposure to salinity 11, although the level significantly decreased at 96 h ($P < 0.05$). The mRNA level of GPX was maximal after 6 h of exposure in each exposure group and was significantly lower after 48 h exposure to salinity 11 ($P < 0.05$) and after 96 h exposure to salinity 23 ($P < 0.05$). After 6 h of exposure, the mRNA level of Nrf2 was significantly greater in each exposure group than in the control group ($P < 0.05$) and then significantly decreased at 96 h ($P < 0.05$) (Fig. 6d). The Keap1

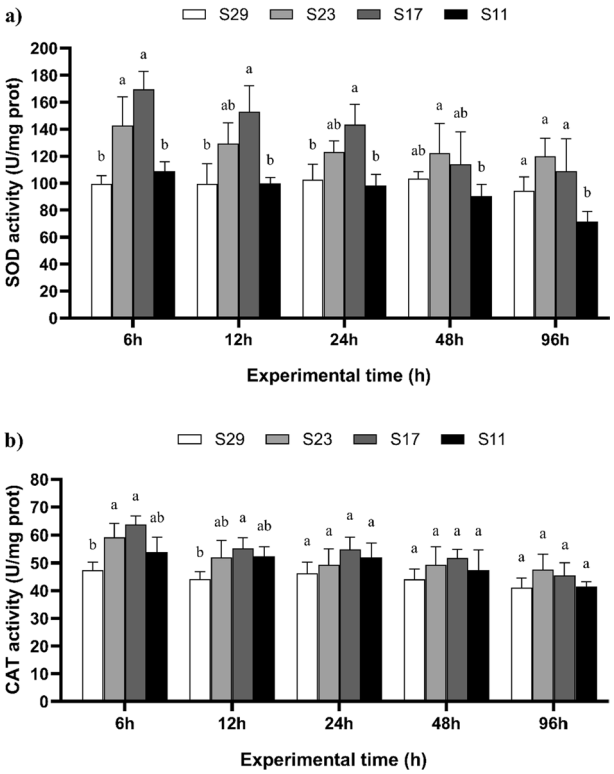
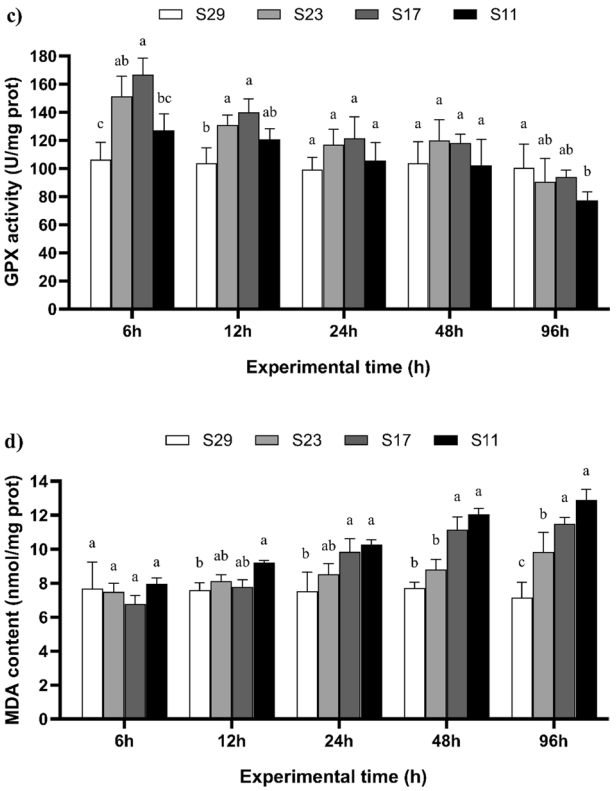


Fig. 4 Effects of different salinities on the antioxidation status in the hepatopancreas of *M. japonicus*. **a** SOD; **b** CAT; **c** GPX; and **d** MDA. There was a significant difference between means with different letters at each time point ($P < 0.05$)

Fig. 4 (continued)



mRNA level increased gradually and was significantly upregulated at the end of exposure in each exposure group ($P < 0.05$) (Fig. 6e).

Correlation analysis

MDA content was negatively correlated with SOD and GPX activities in both the gills and hepatopancreas of *M. japonicus* (Table 2). Pearson's correlation coefficients between enzymatic activities and mRNA levels of corresponding genes in the gills and hepatopancreas are shown in Table 3. Pearson's correlation coefficients between the transcription factor Nrf2 and mRNA levels of genes involved in oxidative stress in the gills and hepatopancreas are also shown in Table 3. Positive correlations between SOD activity and SOD expression, CAT activity and CAT expression, and GPX activity and GPX expression were observed. Nrf2 expression was positively correlated with the mRNA levels of SOD, CAT, and GPX and was negatively correlated with the mRNA levels of Keap1.

Discussion

Investigations of signaling molecules in the antioxidant system of shrimp under salinity stress have not received much attention thus far. The present study provides new experimental evidence of antioxidant defenses in *M. japonicus* under low-salinity stress and identifies an important role of Nrf2-Keap1 signaling in this defense response.

Salinity is an important environmental factor for aquatic organisms. The mechanism underlying salinity-induced oxidative stress is poorly understood, but it may involve changes in the activities of antioxidant enzymes (Liu et al. 2007; Li et al. 2010; Xie et al. 2014). Among these antioxidant enzymes, SOD-CAT and SOD-GPX are regarded as the first lines of defense against oxidative stress (Winston and Giulio 1991). SOD metabolizes the superoxide anion radical (O_2^-) and H^+ into O_2 and H_2O_2, which is subsequently transformed into H_2O by CAT and GPX (Halliwell 2012). Additionally, malondialdehyde (MDA), an important indicator of oxidative damage in cell membranes, can be used to evaluate the extent of peroxidation and levels of ROS in organisms (Dimitrios 2017). In the present study, a coordinated increase in the activities of SOD, CAT, and GPX was observed in the gills and hepatopancreas of *M. japonicus* after 6 h exposure to low salinity, while the MDA content remained constant or significantly decreased, suggesting a protective role for antioxidant enzymes against low salinity-induced oxidative stress during the early stage of exposure, similar to reports of fishes with acute exposure to salinity (Fei et al. 2011; Zeng et al. 2017). However, when the antioxidant system is unable to eliminate or neutralize the excess ROS, there is an increased risk of oxidative damage related to lipid peroxidation accumulation, which may reduce enzyme activity or degrade the enzymes (Lushchak 2011). In the present study, as the exposure duration increased, the SOD and GPX activities of gills and hepatopancreas were significantly diminished, while the MDA content was significantly increased. The reduction of antioxidant enzyme activity and accumulation of MDA indicated that the antioxidative defense systems of *M. japonicus* were impaired under

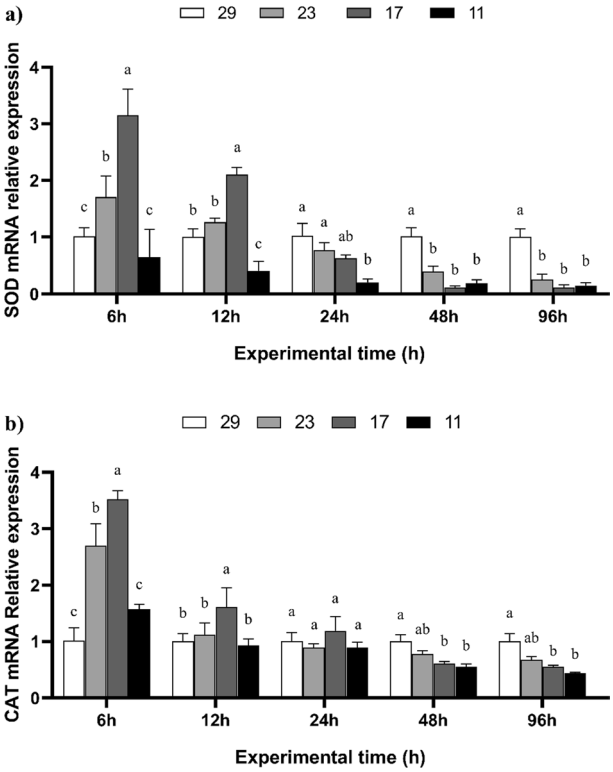
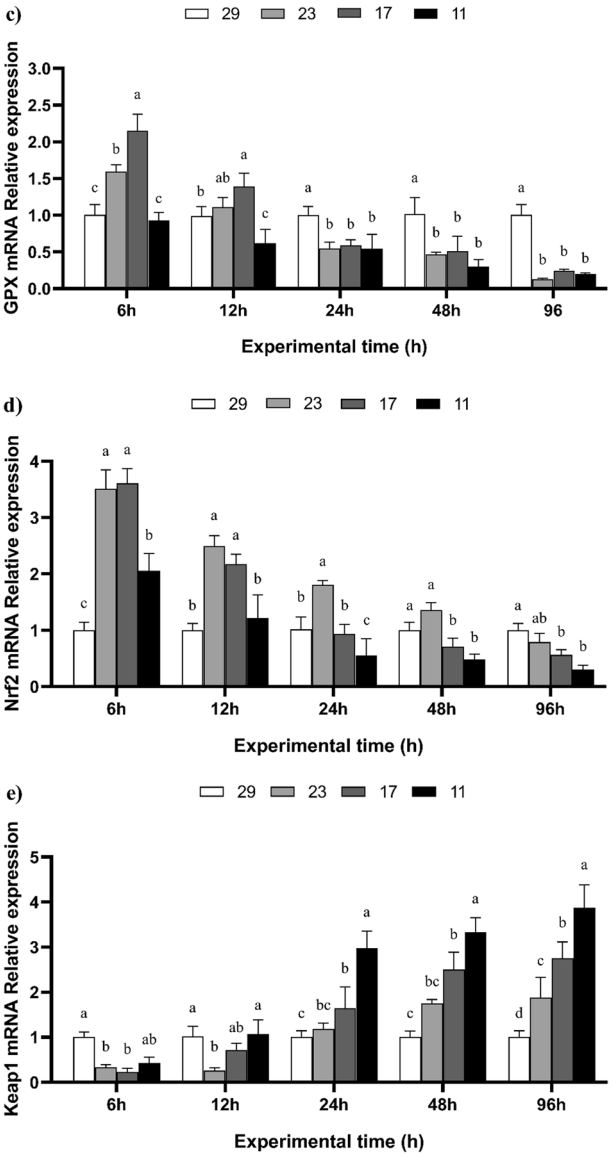


Fig. 5 The expression of genes involved in Nrf2-Keap1 signaling in the gills of *M. japonicus*. **a** SOD; **b** CAT; **c** GPX; **d** Nrf2; and **e** Keap1. There was a significant difference between means with different letters at each time point ($P < 0.05$)

Fig. 5 (continued)



low salinity-induced tissue injury. It is speculated that the increase of antioxidant enzyme activity in a short time was not sufficient to the complete protection against stress-induced cellular damage. Moreover, the results of morphological sections in our study showed that as the duration of exposure increases, the histopathological changes of the gills and hepatopancreas show signs of degeneration and necrosis, which further indicated that low-salinity stress could lead to tissues injury in *M. japonicus*.

Antioxidant gene expression is considered an accurate estimate of the antioxidant capacity for aquatic organisms, where interference of biochemical origin is not involved (Malandrakis et al. 2014). In this context, there is value in determining the origin of changes in

antioxidants under salinity stress. In this study, the mRNA levels of SOD, CAT, and GPX were upregulated during the early stage of salinity exposure, indicating that salinity stress can stimulate antioxidant capacity, and antioxidants were produced to relieve systemic oxidative stress, which were correlated with increasing antioxidant enzyme activity in *M. japonicus* in turn. However, as the exposure duration increased, the mRNA levels of SOD, CAT, and GPX were substantially reduced in the gills and hepatopancreas after 48 h exposure to salinity 11, suggesting the antioxidant capacity declined under low-salinity stress. Notably, the expression of CAT significantly decreased, while the CAT activity showed no significant difference compared with the control group during late exposure. This mismatch between mRNA level and enzyme activity may arise from posttranslational modulation of enzyme activity (Sadi et al. 2014). Additionally, the mRNA levels of antioxidant enzymes represent a snapshot of activity at a particular time, while there is a time-lag effect between transcription and translation (Nam et al. 2010).

The induction of antioxidant enzyme genes is regulated by several signaling pathways and transcription factors (Silvestre 2020). There is increasing evidence that the Nrf2-Keap1 signaling regulates the antioxidant defense system to impact oxidant homeostasis and other cellular functions (Sykiotis and Bohmann 2008). The redox-sensitive transcription factor Nrf2 has a key role in antioxidant systems and regulates the transcription of several cytoprotective and antioxidant genes through Nrf2-Keap1 signaling (Dodson et al. 2015). Under the action of oxidative stress, Nrf2 was uncoupled from Keap1 and transferred to the nucleus to activate antioxidant enzyme genes, increasing the activity of antioxidant enzymes to resist oxidative damage caused by ROS (Kaspar et al. 2009). In the

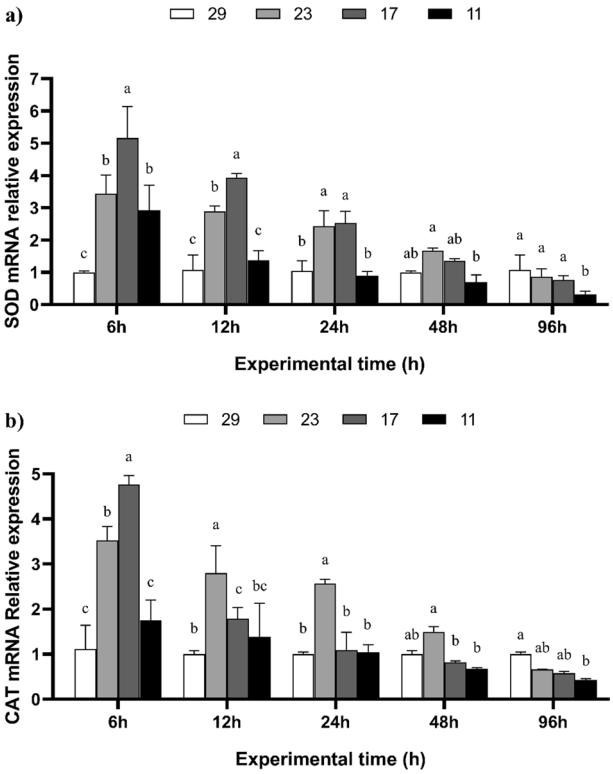
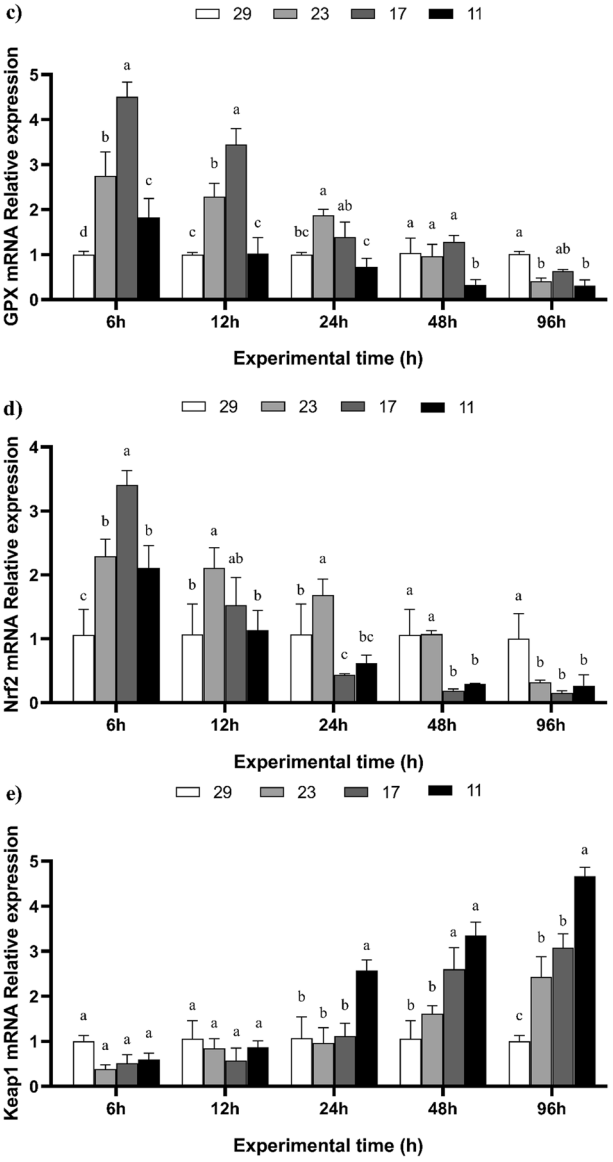


Fig. 6 The expression of genes involved in Nrf2-Keap1 signaling in the hepatopancreas of *M. japonicus*. **a** SOD; **b** CAT; **c** GPX; **d** Nrf2; and **e** Keap1. There was a significant difference between means with different letters at each time point ($P < 0.05$)

Fig. 6 (continued)



present study, increased levels of Nrf2 expression were observed in the gills and hepatopancreas of *M. japonicus* after 6 h exposure to low salinity, indicating a protective role for Nrf2. Consistent with the change in Nrf2, the mRNA levels of SOD, CAT, and GPX showed mild or strong elevation in *M. japonicus* after 6 h or 12 h exposure to low salinity. The Nrf2 expression was positively correlated with the expression levels of antioxidant genes, reflecting that Nrf2, as an upstream transcription factor in the antioxidant pathway, plays a critical role in activating antioxidant enzyme genes to stimulate the shrimp antioxidant capacity. Similar results have previously been observed in large yellow croakers (*Pseudosciaena crocea*) (Zeng et al. 2016) and *Litopenaeus vannamei* (Huang et al. 2022). However, the expression of Nrf2 was downregulated after 48 h exposure to salinities 17 and

Table 2 Pearson's correlation coefficients between MDA content and antioxidant enzymea ctivities in the gills and hepatopancreas of *M*. japonicus

			Enzymatic activities		
			SOD	CAT	GPX
Gills	MDA content	R	-0.541	-0.401	-0.679
		P	0.014	0.080	0.001
Hepatopancreas	MDA content	R	-0.459	-0.300	-0.549
		P	0.042	0.199	0.012

No significant relationship between the two variables when $P > 0.05$; positive correlation between the two variables when $R > 0$ and $P < 0.05$; negative correlation between the two variables when $R < 0$ and $P < 0.05$

23, which may be related to Nrf2 system dysfunction induced by severe and/or chronic oxidative stress. In addition, as a Nrf2 repressor, Keap1 increased during oxidative stress, which may lead to Nrf2 inactivation through the formation of a Keap1-Nrf2 complex (Liu et al. 2017; Ma et al. 2018). This phenomenon was confirmed by the upregulation of Keap1 mRNA after 24 h exposed to low salinity in our study. In this study, Keap1 was inversely correlated with the mRNA level of Nrf2. This result further supports the role of Keap1 as a negative regulator to inhibit the Nrf2 response, causing a feedback autoregulatory loop that controls the level of Nrf2, which is consistent with the hypothesis of Keap1-mediated activation of Nrf2 through a "hinge and latch model" (Tong et al. 2006). Similar results were obtained in reports on zebrafish (*D. rerio*) (Sarkar et al. 2014) and grass carp (*Ctenopharyngodon idella*) (Guo et al. 2018).

Conclusion

This study demonstrated that abrupt low-salinity stress could induce oxidative stress depending on the salinity concentration and time course. Positive correlations between gene expression and antioxidant enzyme activities were observed, suggesting that

Table 3 Pearson's correlation coefficients between enzymatic activities and mRNA levels of corresponding gene and between the transcription factor Nrf2 and mRNA levels of antioxidant genes in the gills and hepatopancreas of M. *japonicus*

			mRNA levels			Keap1
			SOD	CAT	GPX	
Gills	Enzymatic activities	R	0.652	0.760	0.805	
		P	0.002	0.000	0.000	
	Nrf2 mRNA level	R	0.828	0.896	0.833	-0.759
		P	0.000	0.000	0.000	0.000
Hepatopancreas	Enzymatic activities	R	0.895	0.737	0.916	
		P	0.000	0.000	0.000	
	Nrf2 mRNA level	R	0.848	0.932	0.861	-0.726
		P	0.000	0.000	0.000	0.000

No significant relationship between the two variables when $P > 0.05$; positive correlation between the two variables when $R > 0$ and $P < 0.05$; negative correlation between the two variables when $R < 0$ and $P < 0.05$

transcriptional regulation has an important role in defense against oxidative damage. The Nrf2-Keap1 pathway is required to modulate the activation of antioxidant genes in *M. japonicus*. Nrf2 activation resulted in increased activities of downstream antioxidant enzymes during exposure to low-salinity stress. The results of this study indicate that Nrf2-dependent gene transcription is important for resisting low salinity-induced oxidative stress and providing a theoretical basis to support the antioxidant defense function of Nrf2-Keap1 signaling in crustaceans.

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Author contribution All authors contributed to the study's conception and design. Haixin Ou contributed to the preparation of material, completion of experiments, data collection, data analysis, writing, and revising of the manuscript. Jianyong Liu contributed to the data collection, data analysis, and revising of the manuscript. The first draft was written by Haixin Ou and reviewed by all authors. The final manuscript was read and approved by all authors for publishing.

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Data availability All data in this study are available from the author. The data are not publicly available due to privacy.

Code availability Not applicable.

Declarations

Ethics approval All animal experiments were carried out in accordance with the Regulations of the Animal Ethics Committee of Guangdong Ocean University. Animal protocols were approved by the Animal Ethics Committee of Guangdong Ocean University (Zhanjiang, China).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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