



Effects of Salinity Stress on Immune Defense and Antioxidant Status in the Sea Urchin (*Strongylocentrotus intermedius*)

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SUMMARY: To explore the adaptability of *Strongylocentrotus intermedius* to different salinity stresses and its oxidative immune regulation mechanism, eight salinity concentration gradients (20‰, 22.5‰, 25‰, 27.5‰, 30‰, 32.5‰, 35‰, 37.5‰) were set based on the hemolymph lethal concentration of sea urchins for 196 hours of chronic stress. The activities of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) content, lysozyme (LZM) activity, and peroxidase (POD) activity in the coelomic fluid of sea urchins were determined at 0, 48, 96, 144, and 196 hours. The results showed that the optimal salinity range was 27.5‰ - 32.5‰. Beyond this range ($\leq 25\%$ or $\geq 35\%$), the activities of the antioxidant enzyme system (SOD, CAT, POD) were significantly inhibited ($P < 0.01$), and the accumulation peak of malondialdehyde (MDA) was triggered, and the activity of immune defense substances (LZM) weakened. Based on the results of this experiment, it is proposed that the fluctuation of salinity in aquaculture should be controlled within $\pm 2.5\%$, providing a theoretical basis for the breeding and culture of sea urchins.

KEYWORDS: Salinity stress; *Strongylocentrotus intermedius*; Immunity level

1 Introduction

Strongylocentrotus intermedius, also referred to as the Hokkaido sea urchin, is a member of the Echinidae family within the phylum Echinodermata. Presently, it primarily thrives in certain sea areas of the Yellow Sea in China and the Sea of Japan [1]. Its gonads are the sole edible part, which is highly popular among consumers because of its distinctive flavor and exceptionally high nutritional value, making it a significant variety among global economic sea urchins [2].

For the sustainable development of *Strongylocentrotus intermedius*, to optimize its artificial breeding and aquaculture techniques, and to thoroughly investigate the response mechanism of its growth and development to environmental factors is one of the key directions in current aquaculture research [3]. At present, some scholars have conducted a series of studies on the stress exerted on sea urchins by temperature, heavy metals, light, dissolved oxygen, and salinity. For example, Ma Fuheng [4] studied the impact of salinity and temperature on the feeding and survival of *Strongylocentrotus intermedius*; Li Min [5] explored the effect of Cu^{2+} on the growth, immunity, and gonad development of *Strongylocentrotus nudus*; Wang Wenpei [6] examined the effect of heat stress on the antioxidant enzyme activity and mitochondrial structure and function of *Strongylocentrotus intermedius*; and Ding Ruxin [7] looked into the effect and molecular response mechanism of hypoxic stress on *Strongylocentrotus nudus*.

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During aquaculture production, the environmental factor of salinity has a significant impact on aquatic organisms. Salinity stress triggers a cascade of physiological responses by disrupting the osmotic balance of aquatic organisms [8], and its core regulatory mechanism relies on the ion transport activity of Na⁺/K⁺-ATPase [9]. In echinoderms, this enzyme maintains the osmotic gradient between coelomic fluid and the external environment by hydrolyzing ATP to achieve transmembrane exchange of 3Na⁺ efflux/2K⁺ influx. Compared with other marine organisms, echinoderms show significant differences in salinity adaptation strategies: fish rapidly regulate osmotic pressure via chloride cells in gills [10], crustaceans respond to stress through changes in hemolymph free amino acid concentrations, and echinoderms like sea urchins maintain osmotic pressure and ion homeostasis through the dynamic balance of inorganic ions in coelomic fluid [9].

Immune enzymes are the core of the sea urchin innate immune system, including antioxidant enzymes (such as superoxide dismutase SOD, catalase CAT) and hydrolases (such as lysozyme LZM). These enzymes play important roles in eliminating pathogenic microbes in sea urchins, resisting oxidative damage, and maintaining internal environment stability [8-13]. When sea urchins are under salinity stress, there are significant changes in the expression levels and activities of their immune enzymes, and these changes are important indicators to evaluate the health status and stress resistance of sea urchins [14]. Therefore, studying the effects of salinity stress on the activity of immune enzymes in sea urchins helps to uncover their adaptation mechanisms under different salinity conditions and provides a scientific basis for optimizing aquaculture environments and improving aquaculture benefits.

Aquaculture areas in China's Yellow Sea are affected by monsoonal precipitation and runoff [15], leading to large fluctuations in sea salinity, which adversely affects the growth and survival of sea urchins. However, existing research mainly focuses on acute salinity stress (≤ 96 h), with a lack of systematic evaluation of chronic salinity fluctuations common in aquaculture. This experiment sets 8 salinity gradients to systematically explore the physiological response mechanisms of *Strongylocentrotus intermedius* under 196h of chronic salinity stress, focusing on analyzing changes in the activities of 5 immune-related enzymes (SOD, CAT, MDA, POD, LZM) in sea urchin coelomic fluid under different salinity stresses. Aimed at studying the toxic mechanism of water salinity on sea urchins, clarifying the suitable salinity range, and providing theoretical support for the healthy aquaculture and artificial breeding of *Strongylocentrotus intermedius*. Additionally, the research findings can also provide references for the protection and sustainable utilization of wild sea urchin resources, facilitating the high-quality development of China's sea urchin industry.

2 Materials and Methods

2.1 Experimental materials

The *Strongylocentrotus intermedius* used in the experiment were supplied by Liyang Sea Urchin Farm in Laizhou City. We randomly picked healthy and active 2 to 3-year-old sea urchins with a test diameter of (30 ± 5) mm. The experiment began after 48 hours of acclimation in the laboratory. The water quality parameters were: temperature (22.62 ± 1.22) °C and pH (7.6 ± 0.5) . During acclimation, oxygen was supplied and running water was maintained. After acclimation, healthy and active *Strongylocentrotus intermedius* were chosen for the experiment.

The experimental reagents included sea salt, BCA protein concentration assay kit, catalase (CAT) assay kit, total superoxide dismutase (T-SOD) test kit, peroxidase (POD) assay kit, lysozyme (LZM) test kit, and malondialdehyde (MDA) assay kit (all purchased from Nanjing

Jiancheng Technology Co., Ltd.). The experimental instruments were an optical salinometer, microplate reader, frozen high-speed centrifuge, and a -80 °C ultra-low temperature freezer.

2.2 Experimental design

In order to rationalize the salinity gradient for the chronic test, a pre-test was first performed. *Strongylocentrotus intermedius* was divided into four groups, with salinities in the breeding environment set from low to high as 15‰, 25‰, 35‰, and 45‰ respectively. Each salinity gradient had 3 replicate groups, each containing 12 sea urchins. A total of four death counts were recorded at 12h intervals. The survival rate was calculated. Regression analysis using SPSS 19.0 software was used to obtain the regression equation and data like R². The median lethal concentration at 48h under acute salinity stress was calculated, and the results were verified by significance testing.

After obtaining the median lethal concentration of *Strongylocentrotus intermedius*, the formal test was initiated. The test was conducted in the laboratory of Yantai Research Institute of China Agricultural University from October 8, 2024 to October 15, 2024. There were 8 concentration gradients set in the test: 20‰, 22.5‰, 25‰, 27.5‰, 30‰, 32.5‰, 35‰, and 37.5‰. Among them, 30‰ served as the control group. Each gradient had 3 replicates, each with 20 sea urchins. During the test, sufficient oxygen was ensured. The test lasted eight days, with five samplings carried out at 48h intervals. Meanwhile, the salinity of each group was checked every 12h, water was replenished in time to keep the salinity change of each group below 0.5‰, and dying or poorly conditioned individuals were removed in time to prevent disease spread and water pollution.

2.3 Sample collection and enzyme activity determination

When sampling at each time point, 2 sea urchins were randomly chosen from the water tanks of each experimental group. Rinse the surface of the sea urchins with sterile saline to remove sediment or any attached matter. Turn the sea urchin so its oral side is upward to expose the peristomial membrane area. Use a sterile needle to insert obliquely from the edge of the peristomial membrane, about 2-3 mm deep. Slowly draw out the coelomic fluid. After collection, transfer the fluid to a centrifuge tube and keep it on ice for no more than 15 minutes. Once all collections are done, centrifuge at 5000 r/min for 10 minutes at 4°C. Then immediately take the supernatant to measure the following parameters.

2.3.1 Determination of Superoxide Dismutase (SOD)

Determination of SOD using the TBA method: The amount of SOD that results in a 50% inhibition rate in each milliliter of the reaction solution is defined as one unit of SOD activity (U). Add the volume (ml) of the body cavity fluid to be tested to the reaction solution (mL) at a ratio of 1:26, mix thoroughly with a vortex mixer, incubate in a 37°C water bath for 40 minutes, add the chromogenic agent and mix, then let stand at room temperature for 10 minutes. Detect using a microplate reader, set the wavelength to 560 nm, zero with distilled water, and perform colorimetry.

The principle of the determination is that the superoxide anion (O₂⁻) generated by the xanthine oxidase reaction system can reduce nitroblue tetrazolium, and during the reaction, blue formazan with an absorption peak at 560 nm is produced. Since SOD can inhibit O₂⁻, the peak absorption value decreases; thus, the lighter the blue color of the final reaction solution, the higher the activity of the SOD enzyme.

The formula for calculation is:

$$\text{Total SOD activity (U/mL)} = \frac{\text{Control OD value} - \text{Measured OD value}}{\text{Control OD value}} \div 50\% \times \text{Dilution factor of reaction system} \times \text{Dilution factor before sample testing} \quad (1)$$

2.3.2 Determination of catalase (CAT)

The catalase (CAT) activity was determined using the ammonium molybdate method. The volume (ml) of the body cavity fluid to be detected was added to the reaction solution (mL) at a ratio of 1:22, immediately mixed, timed, and accurately reacted at 37°C for 1 min. The absorbance values of each tube were measured using a microplate reader at a wavelength of 405 nm with distilled water as the blank.

The principle of the determination is as follows: This determination method can rapidly terminate the reaction of CAT decomposing H₂O₂. The remaining H₂O₂ in the reaction solution can react with ammonium molybdate to finally produce a light yellow complex. The change amount is measured at 405 nm, and then the CAT activity can be calculated.

The formula for calculation is:

$$\text{CAT activity (U/mL)} = \Delta A \times 271 \div V \div T \times N \quad (2)$$

2.3.3 Determination of Lysozyme (LZM)

The content of LZM was determined using the blank control method. The volume (ml) of the body cavity fluid to be detected was added to the application bacterial solution (mL) at a ratio of 1:10, then mixed evenly. First, place it in a water bath at 37°C for 15 minutes, then immediately take it out and place it in an ice-water bath below 0°C for 3 minutes. Take out each tube and measure the absorbance at 530 nm with a microplate reader.

The principle of determination is that lysozyme can hydrolyze peptidoglycan in the bacterial cell wall, which reduces the turbidity of the bacterial solution and then increases the light transmittance of the reaction system, so as to infer the content of lysozyme.

The formula for calculation is:

$$\text{LZM content (ug/mL)} = \frac{T_{\text{determination}} - T_{\text{blank}}}{T_{\text{standard}} - T_{\text{blank}}} \times C_{\text{standard}} \times N \quad (3)$$

2.3.4 Determination of Peroxidase (POD)

Determine the activity of POD. Add the volume (ml) of the body cavity fluid to be tested to the reaction solution (mL) at a ratio of 1:39. After reacting accurately at 37°C for 30 minutes, centrifuge at 3500 revolutions per minute for 10 minutes. Take the supernatant and measure the OD value at 420nm. The principle of determination is that peroxidase (POD) can catalyze the reaction of hydrogen peroxide.

The formula for calculation is:

$$\text{POD activity (U/mL)} = \frac{A_{\text{determination}} - A_{\text{control}}}{12 \times d} \times \frac{V_{\text{total reaction}}}{V_{\text{sample}}} \div T \times N \times 100 \quad (4)$$

2.3.5 Determination of malondialdehyde (MDA)

The content of malondialdehyde (MDA) is determined using the TBA method. After sealing the centrifuge tube with a small opening, mix thoroughly, incubate in a water bath at 95°C for 40 minutes, then cool under running water. Centrifuge at 3500-4000 revolutions per minute for 10 minutes, collect the supernatant, and measure the absorbance at 532 nm with a

microplate reader.

The principle of determination is that malondialdehyde (MDA) in the degradation products of lipid peroxides can condense with thiobarbituric acid (TBA) to form a red product, which has a maximum absorption peak at 532 nm. The formula for calculation is:

$$\text{MDA content (nmol/mL)} = \frac{\text{determination OD} - \text{control OD}}{\text{standard OD} - \text{blank OD}} \times \text{standard concentration (10 nmol/ml)} \times \text{dilution factor before sample test} \quad (5)$$

2.4 Data processing and analysis

The experimental data are all presented as mean ± standard deviation (mean ± SD). One-way analysis of variance (One-Way ANOVA), multiple comparisons (Tukey), and two independent sample tests (Mann-Whitney U) were conducted using SPSS 19.0 software. A p-value less than 0.05 is considered statistically significant. Bar graphs of the experimental data were created with Prism 10.1.2.

3 Results

3.1 Changes in SOD activity in the coelomic fluid of sea urchins under chronic salinity stress

As shown in Figure 1, at the beginning of the experiment (0h), the SOD activity in the coelomic fluid of sea urchins was all kept at 74.69±0.45 U/mL, and there was no significant difference among groups (P>0.05). As the salinity stress lasted longer, the SOD activity of each experimental group first increased and then decreased. Specifically, the SOD activity of the low-salinity (20‰-27.5‰) group and the high-salinity 35‰ group reached the peak at 48h; while the peak of SOD activity in the high-salinity (30‰, 32.5‰, 37.5‰) groups appeared at 96h. Notably, at the final stages of the experiment, 144h and 192h, the SOD activity of the high-salinity (27.5‰-37.5‰) groups significantly dropped from the initial level and finally stabilized at 60 U/ml.

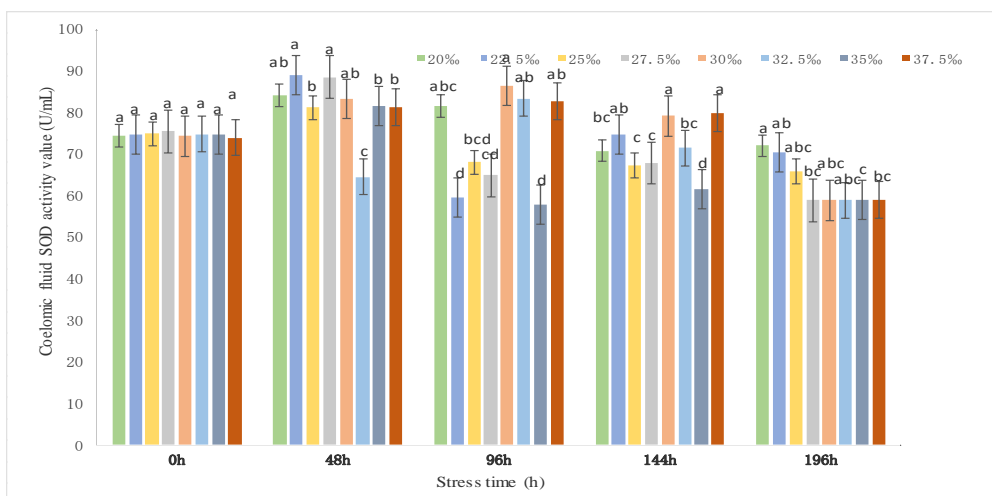


Figure 1: Trend of average SOD activity in the body cavity fluid of sea urchins

3.2 Changes in CAT activity in the coelomic fluid of sea urchins under chronic salinity stress

As shown in Figure 2, at the initial stage of the experiment (0h), the CAT activity in the coelomic fluid of sea urchins was maintained at 6.00 ± 0.30 U/mL, with no significant difference within the group ($P > 0.05$). With the extension of salinity stress time, the CAT activity in each experimental group first increased and then decreased, which was roughly the same as the change trend of SOD. Among them, the CAT activity in the 20‰ and 25‰ salinity experimental groups reached the highest at 96h, and the 22.5‰, 30‰, 32.5‰, and 35‰ salinity experimental groups reached the highest at 144h. Compared with the SOD activity level, the enzyme activity values of different salinity experimental groups fluctuated greatly. When the salinity level reached 20‰ and 37.5‰, the CAT activity was significantly different from the control group ($P < 0.05$). Compared with the control group, as the salinity difference increased, the fluctuation of CAT activity level became larger. In the high-salinity (30‰-37.5‰) experimental group, the CAT value of sea urchins fluctuated sharply, and the CAT activity level at the end of the experiment (192h) was significantly higher than the initial value ($P < 0.05$).

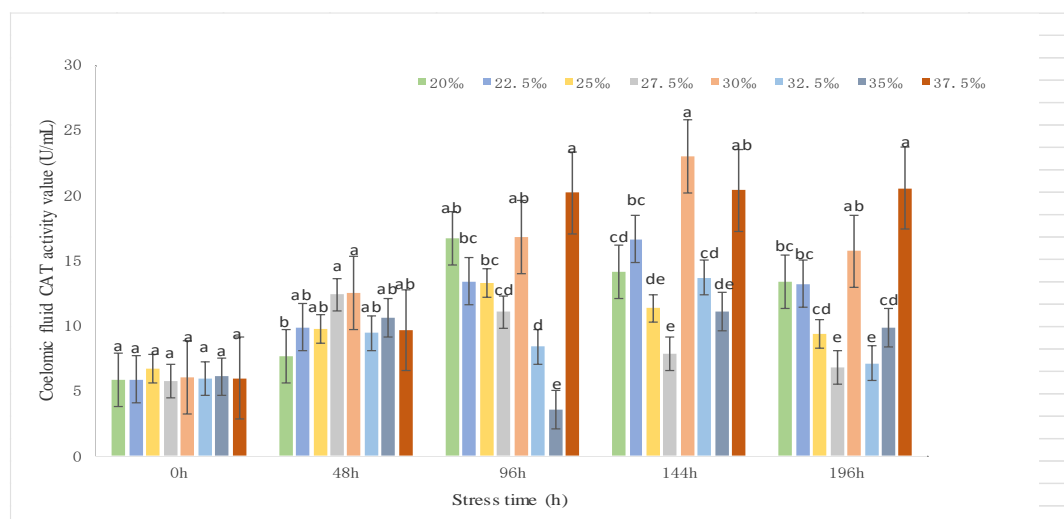


Figure 2: Trend of average CAT activity in the coelomic fluid of sea urchins

3.3 Changes in MDA content in the coelomic fluid of sea urchins under chronic salinity stress

As depicted in Figure 3, at the start of the experiment (0h), the MDA content in the sea urchins' coelomic fluid stood at 14.58 ± 0.74 nmol/mL. As the stress duration lengthened, the MDA content in each experimental group showed a clear trend: it rose initially, then declined, reaching its peak at 96h. Compared with the control group, as the salinity concentration varied more, the accumulation of MDA in the sea urchins continuously dropped. When salinity dropped to 25‰, the MDA content peaked at this point, and the accumulation level was more pronounced throughout the experimental period compared to the control group. Meanwhile, when salinity rose to 35‰, the MDA content stayed at a relatively high level, higher than that of other experimental groups. Among them, the salinity test groups of 22.5‰, 25‰, and 35‰ had a larger variation range, with the accumulated MDA content rising rapidly first and then plummeting after reaching the peak. The fluctuation of the test period for the salinity test groups of 27.5‰-32.5‰ was relatively stable.

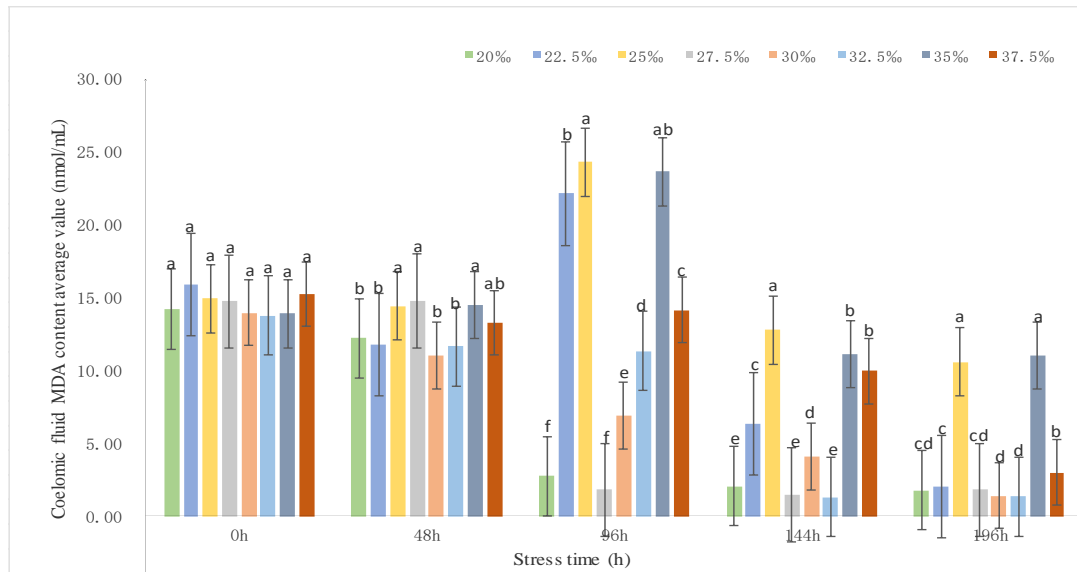


Figure 3: Trend of average MDA content in the body cavity fluid of sea urchins

3.4 Changes in POD activity in the coelomic fluid of sea urchins under chronic salinity stress

As depicted in Figure 4, at the start of the experiment (0h), the POD activity stood at approximately 60.92 ± 0.65 U/mL, with no significant intra-group differences ($P > 0.05$). As the duration of salt stress prolonged, except for the 30‰ salinity test group where POD activity remained relatively stable at around 57 U/mL, the POD activity of the other test groups first rose and then fell. The POD activity of the 22.5‰, 25‰, 27.5‰, 35‰, and 37.5‰ salinity test groups peaked at 48h. In comparison to the control group, with the increase in the range of salinity concentration variation, the POD activity fluctuated noticeably, particularly for the 22.5‰, 25‰, and 37.5‰ salinity test groups, where POD activity changed significantly with the stress duration.

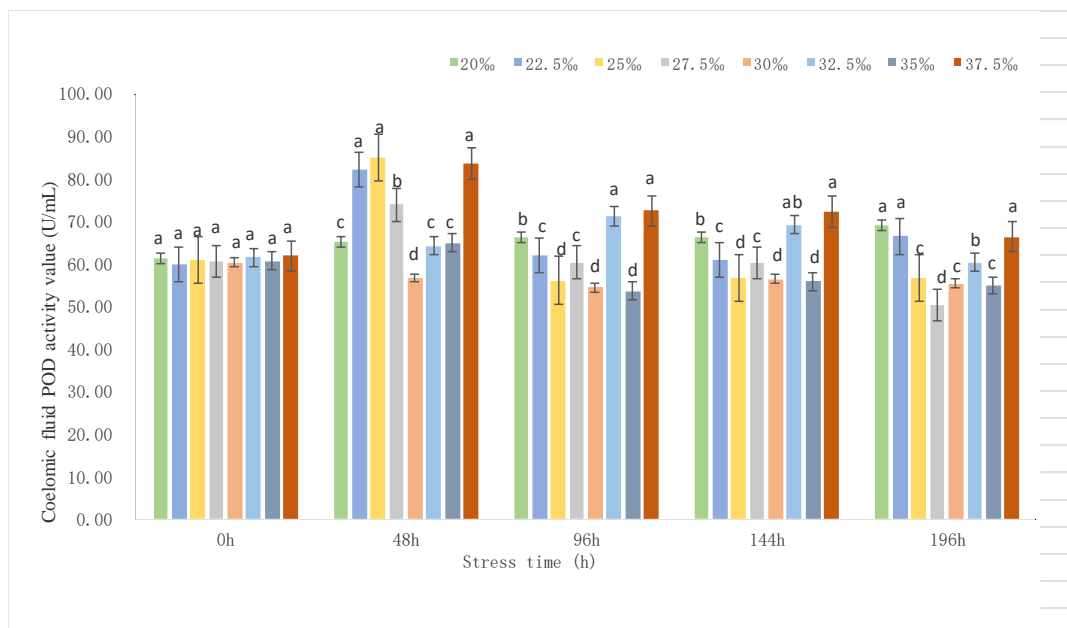


Figure 4: Trend of average POD activity in the coelomic fluid of sea urchins

3.5 Changes in LZM activity in the coelomic fluid of sea urchins under chronic salinity stress

As shown in Figure 5, at the initial stage of the experiment (0h), the activity level of LZM in the coelomic fluid of sea urchins was maintained at 2.94 ± 0.15 U/mL, and there was no significant difference within the group ($P > 0.05$). With the increase of stress time, the activity of LZM in each experimental group generally showed a trend of increasing first and then decreasing. The activity of LZM in the salinity experimental groups of 20‰, 22.5‰, and 32.5‰ reached the peak at 96h, while the activity level of the 30‰ experimental group changed slightly. Compared with the control group, with the increase of the range of salinity concentration change, the fluctuation of LZM activity increased, and the difference was significant ($P < 0.05$). At the end of the experiment (192h), the enzyme activity of LZM in 20‰, 22.5‰, 25‰, 35‰, and 37.5‰ was significantly higher than that in the control group ($P < 0.05$).

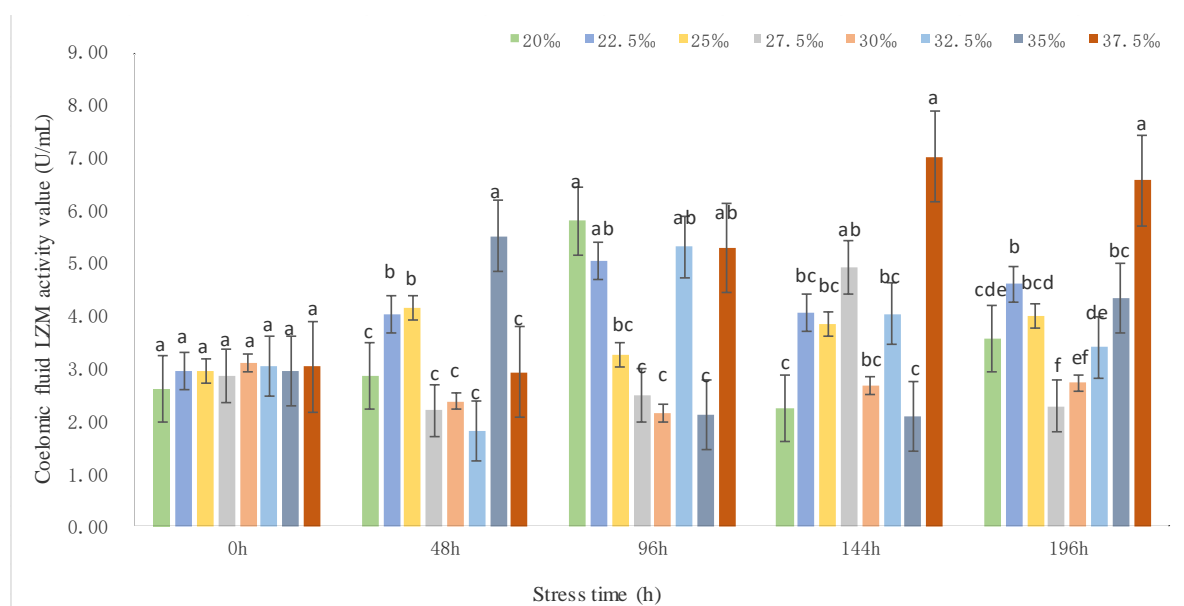


Figure 5: Trend of average LZM activity in sea urchin coelomic fluid

4 Discuss

4.1 The effect of salinity on the activities of CAT, SOD, and POD in the coelomic fluid of intermediate sea urchin

At present, a number of studies consider CAT, SOD, and POD as key markers for assessing oxidative stress triggered by exogenous compounds. In the organism's antioxidant enzyme system, SOD can catalyze the dismutation of superoxide anions to produce hydrogen peroxide and oxygen [11, 12, 16, 17], yet hydrogen peroxide is still toxic; CAT can efficiently break down hydrogen peroxide into water and oxygen, especially functioning at high concentrations [13, 18]; whereas POD can also decompose hydrogen peroxide with the help of phenolic compounds, complementing CAT's function at low concentrations. The three work in concert to jointly tackle external stress.

Salinity is a crucial environmental factor in the aquaculture of aquatic organisms, and its variations significantly impact the osmotic pressure balance and physiological functions of aquatic organisms. Salinity stress disrupts the osmotic pressure balance of aquatic organisms,

triggering a series of cascading physiological responses. The core regulatory mechanism depends on the ion transport activity of Na^+/K^+ -ATPase, indirectly causing excessive accumulation of reactive oxygen species (ROS) in aquatic organisms, leading to oxidative damage to the organism. Within the selected concentration range, the change in SOD activity shows a trend of first increasing and then decreasing, indicating that short-term salinity stress activates SOD to scavenge superoxide anions, while long-term salinity stress results in a decline in its activity. Consistent with the research findings of Sun Xuena [14, 19] et al., it is shown that SOD activity is induced when fish are subjected to mild stress and inhibited under severe stress. Especially in the high-salinity test group, the greater the difference in salinity from the control group, the more severe the damage. This is because SOD is a key metalloproteinase in organisms, and its activity relies on metal cofactors such as Cu/Zn-SOD, Mn-SOD, and Fe-SOD. In a high-salinity environment, ions like Na^+ compete with metal cofactors for binding sites, causing a reduction in enzyme activity. This is consistent with the results of He Liangyin [15, 20] et al. and Zhang Chenjie [16, 21] et al. on salinity stress in marine fish. Compared with the fluctuation ranges of CAT and POD, the overall fluctuation of SOD is smaller, possibly because this oxidation index can recover rapidly, thereby reducing damage to the antioxidant system [17, 22].

Similar to CAT, POD, and SOD, the activity levels of all groups first increase and then decrease as the stress time prolongs. This is because the increase in the substrate hydrogen peroxide leads to a short-term rise in activity. In the later stage, long-term salinity stress causes damage to the enzymes, resulting in a decline in activity. It is noteworthy that within the high salinity range, after being subjected to mild stress, the enzyme activity in the body of the CAT index goes up. However, when the salinity level is slightly elevated, the CAT activity is inhibited to some extent and then returns to the normal level. It is speculated that this may be because the *Mesocentrotus nudus* has a strong tolerance to small-amplitude salinity, gradually adapts to 32.5‰, and can eliminate oxidative stress by regulating its catalase activity to clear excess reactive oxygen free radicals in the body under high salinity. But when the salinity reaches 37.5‰, the activity is difficult to revert to the initial level, thereby causing a certain degree of damage.

In summary, the sea urchin *Strongylocentrotus intermedius* lacks a well-developed immune system. Nonspecific immunity, which depends on various coelomic oxidases, is vital for its growth. When salinity is below 25‰ or above 32.5‰, it will markedly impair the antioxidant system of *Strongylocentrotus intermedius*. The oxidative stress response thus induced may cause damage to the sea urchin's DNA, proteins, and lipids. This aligns with the findings of Cao Liang [18, 23] in the larvae of *Paralichthys olivaceus*. Moreover, appropriate salinity can enhance its antioxidant capacity [24]. During sea urchin aquaculture, it is essential to monitor the water salinity promptly and keep the salinity within the range of 27.5-32.5‰ as much as possible, so as to maintain the normal physiological functions of sea urchins and facilitate their aquaculture and breeding.

4.2 The effects of salinity and stress time on LZM activity in the coelomic fluid of intermediate sea urchin

LZM is an important innate immune defense substance in organisms, featuring broad-spectrum antibacterial properties. It can specifically hydrolyze the peptidoglycan in bacterial cell walls [19, 25], thus destroying the structure of pathogens and playing a bactericidal role. LZM can also mitigate the inflammatory response by inhibiting the chemotaxis and oxidative metabolism of neutrophils, which in turn reduces the release of inflammatory mediators and further strengthens the body's immune defense. Moreover, as the material foundation for

phagocyte bactericidal in sea urchins [21, 27], LZM can defend against external stimuli, preventing pathogenic molecules from attacking antioxidant molecules. Therefore, its activity is often an important indicator to measure the non-specific immunity of sea urchins. The level of LZM activity directly reflects how well sea urchins respond immunologically when confronted with pathogens or environmental stress.

During the stress period, there were significant differences ($P < 0.05$) in the LZM activity of coelomic fluid among sea urchins in different salinity groups. The overall trend was an initial increase followed by a decline, suggesting that as salinity stress persisted, sea urchins shifted from immune defense to adaptive adjustment. This trend implies that sea urchins may boost LZM activity to tackle environmental pressure during short-term salinity stress, but as the stress lingers, their immune system gradually adapts and regains equilibrium. Notably, 27.5‰, 30‰, and 32.5‰ were more stable than other concentrations by the end of the experiment, signifying that this salinity range is better suited for sea urchin growth and immune function maintenance.

4.3 The effect of salinity on the MDA content in the coelomic fluid of intermediate sea urchin

MDA, as a key terminal product in lipid peroxidation reactions, its content changes can effectively reflect the extent of oxidative stress and the state of cellular damage in the organism, and is a key biomarker for evaluating oxidative damage [22-24, 28-30]. When salinity stress leads to the massive accumulation of reactive oxygen species (ROS), excessive ROS will attack polyunsaturated fatty acids in the cell membrane, trigger the chain reaction of lipid peroxidation, and then decompose to generate harmful metabolites such as MDA [25, 26, 31, 32], causing damage to cellular genetic material and organismal functions. During the stress period of this experiment, the content change of MDA showed a dynamic pattern of first increasing and then decreasing, reflecting the transition process of the organism from oxidative stress to adaptive regulation. And the change of MDA content in each group at each time point showed an increase with the increase of salinity difference. The MDA content in the 20‰ and 37.5‰ salinity test groups was the highest at the initial stage of the experiment, but decreased continuously with the extension of stress, probably because the stress level was too high, damaging the oxidative stress system of sea urchins, and if not intervened in time, it may seriously affect the health status of sea urchins. The change ranges of the 22.5‰, 25‰, and 35‰ salinity test groups were relatively large, and at the end of the experiment, they were still higher than the initial level ($P < 0.05$), probably because salinity stress caused certain damage to the organism of sea urchins. The test groups with salinity of 27.5‰-32.5‰ were relatively stable during the experiment, indicating that this salinity range belongs to the adaptable range of sea urchins and can regulate themselves. The above findings are consistent with the research on salinity stress in aquatic organisms (such as *Diplodus vulgaris* and *Heteropneustes fossilis* [27, 33]), and the test results indirectly verified the above change trends of SOD, CAT, and POD indicators.

5 Conclusion

This experiment contends that under chronic stress of different salinity concentration gradients, it will have certain effects on the antioxidant defense system (SOD, CAT, POD) and the immune defense system (LZM) of *Strongylocentrotus intermedius*. During the experiment, both too high and too low salinity will significantly weaken the activities of SOD, CAT, and POD in sea urchins, weaken the activity of the immune defense substance LZM, and at the

same time, significantly increase the content of MDA in the body, causing a certain degree of damage to the body of sea urchins. When the salinity range is 27.5‰-32.5‰, the internal environment of sea urchins is relatively stable, which is the suitable salinity range for the aquaculture of sea urchins. In the process of artificial factory farming and offshore farming of *Strongylocentrotus intermedius*, it is necessary to pay timely attention to the changes in salinity in the breeding environment and reduce the large-scale fluctuations in salinity.

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Author's Profile

Xuhong Shen, an associate professor with a Ph.D. from the Yantai Research Institute of China Agricultural University, mainly focuses on the research of aquatic animal nutrition and water body ecological protection. She has participated in multiple projects including the National Natural Science Foundation of China for Young Scientists and the Natural Science Foundation of Shandong Province. She has published 5 SCI papers and over 10 core Chinese papers.

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