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Impact of Salinity Changes on the Antioxidation of Juvenile Yellowfin Tuna (*Thunnus albacares*)

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Abstract: To understand the impacts of salinity stress on the antioxidation of yellowfin tuna *Thunnus albacares*, 72 fishes (646.52 ± 66.32 g) were randomly divided into two treatments (32‰ and 29‰) and sampled at four time points (0 h, 12 h, 24 h, and 48 h). The salinity of the control group (32‰) was based on natural filtered seawater and the salinity of the stress group (29‰) was reduced by adding tap water with 24 h aeration to the natural filtered seawater. The superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) from liver, gill, and muscle tissues were used as the antioxidant indexes in this study. The results showed that the changes of SOD and GSH-Px in the gills were first not significantly different from the control group ($p > 0.05$) and finally significantly higher than the control group (SOD: 50.57%, GSH-Px: 195.95%, $p < 0.05$). SOD activity in fish liver was not significantly changed from 0 h to 48 h ($p > 0.05$), and was not significantly different between the stress group and control group ($p > 0.05$). With the increase in stress time, GSH-Px and MDA activities in the liver of juvenile yellowfin tuna increased first (GSH-Px: 113.42%, MDA: 137.45%) and then reduced (GSH-Px: −62.37%, MDA: −16.90%) to levels similar to the control group. The SOD activity in the white and red muscle of juvenile yellowfin tuna first decreased (white muscle: −27.51%, red muscle: −15.52%) and then increased (white muscle: 7.30%, red muscle: 3.70%) to the level of the control group. The activities of GSH-Px and MDA in white and red muscle increased first (white muscle GSH-Px: 81.96%, red muscle GSH-Px: 233.08%, white muscle MDA: 26.89%, red muscle MDA: 64.68%) and then decreased (white muscle GSH-Px: −48.03%, red muscle GSH-Px: −28.94%, white muscle MDA: −15.93%, red muscle MDA: −28.67%) to the level observed in the control group. The results from the present study indicate that low salinity may lead to changes in the antioxidant function of yellowfin tuna juveniles. In contrast, yellowfin tuna juveniles have strong adaptability to the salinity of 29‰. However, excessive stress may consume the body's reserves and reduce the body's resistance.

Keywords: low salinity stress; antioxidant index; superoxide dismutase; glutathione peroxidase; malondialdehyde

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1. Introduction

Thunnus albacares belongs to Perciformes, Scomberia, Scombrida, commonly known as yellowfin tuna [1]. Yellowfin tuna is an oceanic migratory fish that lives in the vast upper middle waters of tropical, subtropical, and temperate oceans. As one of the high economic value tuna species, yellowfin tuna grows quickly among the tuna species with high flesh quality [2]. It is a preferred species for offshore aquaculture [1,3,4]. Yellowfin tuna has been cultured in Mexico, Panama, and Indonesia [5]. Mexico has established a large number of yellowfin tuna culture bases, but the limited supply of wild fry has seriously

restricted the culture industry of this species [6]. The artificial cultivation of yellowfin tuna in China is still at the initial stage. The in-depth marine aquaculture technology and variety development innovation team from the Chinese Academy of Fishery Sciences has achieved indoor circulating water and offshore deep-water cage culture of yellowfin tuna in Lingshui Li Autonomous County, Hainan Province, and carried out a series of studies on aquaculture biology and disease prevention and control [7,8]. Up to now, open published biological data on yellowfin tuna farming are relatively scarce, which restricts the development of its artificial farming. Yellowfin tuna is a migratory fish in the ocean, and its migration route is closely related to salinity [1], and the physical responses of yellowfin tuna to ambient salinity changes are still unclear.

As an ecological factor, ambient salinity has a series of physiological effects on fish. As reported in previous studies, salinity is an important factor regulating fish growth, metabolism, and various physiological activities [9]. Ambient salinity can also directly affect the promotion of aquaculture [10]. An inappropriate salinity range will affect the physiology and biochemistry, immunity, growth and development, feeding, and reproduction of fish, and fish will show different adaptive states under various salinity conditions [11]. Recent studies have shown that salinity changes can cause physiological stress reactions in fish, accompanied by the production of excessive reactive oxygen free radicals, which can lead to oxidative stress reactions that damage the antioxidant defense system of fish [12,13]. Excessive oxygen free radicals produced by oxidative stress will attack biological membranes, proteins, and nucleic acids, causing oxidative damage such as cytoplasmic efflux, enzyme inactivation, and genetic replication errors [14]. Therefore, such processes will disrupt the normal physiological and behavioral activities of fish. Salinity fluctuations exist in coastal areas, with possible causes including heavy rainfall and river injection, which pose a threat to cage- and land-based culture that relies on naturally filtered seawater. The low salinity of 28.5 ‰ has been observed in the coast of Hainan, China [15], but the optimum growth salinity of yellowfin tuna is 31.2~33.3 ‰ [16]. Such temporary environmental fluctuations may pose unknown challenges to yellowfin tuna.

The antioxidant system in fish can resist oxidative damage [17]. Superoxide dismutase (SOD) is an essential antioxidant enzyme in the antioxidant system [18]. SOD is the primary substance for scavenging free radicals in organisms. It decomposes superoxide anion free radicals (O^{-2}) through disproportionation. After scavenging O^{-2}, SOD generates H_2O_2 [19]. Glutathione peroxidase (GSH-Px) is a critical non-enzymatic component [20]. As an antioxidant, GSH-Px can participate in the elimination of toxic peroxides [21]. Malondialdehyde (MDA) is a lipid peroxide, which can indirectly reflect the degree of lipid peroxidation in tissue cells [22]. In the present study, SOD, GSH-Px, and MDA were used as the antioxidant indicators to evaluate the impacts of salinity on the antioxidant system of juvenile yellowfin tuna. The purpose of this study is to understand the physiological response of yellowfin tuna to acute low salt in the process of salinity adaptation, in order to solve the change of seawater salinity reduction in a short time in subgrade culture or cage culture. The results from the present study will add biometric data to the culture of yellowfin tuna.

2. Materials and Methods

2.1. Experimental Fish and Design

The juvenile yellowfin tuna were provided by Lingshui Research Station of Tropical Fisheries Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The length and weight of the experimental fish were 28.97 ± 2.17 cm and 646.52 ± 66.32 g, respectively. A total of 72 fish were randomly distributed into six 5000 L fiberglass tanks with recirculating seawater systems for a 7-day acclimation. The fish were fed daily from 08:30 to 09:00. Fresh miscellaneous fish (4 cm × 2 cm pieces) were fed with 5–8% body weight daily by satiety. No feeding was conducted on the day before and during the experiment. During the experiment, all of the tanks were supplied with filtered seawater with a water exchange rate of 300% tank

volume per day. The ambient salinity of 32‰ was used as the control with three replicates, and 29‰ was used as the stress group with three replicates (Figure 1). The salinity of the stress group was gradually adjusted by adding tap water with 24 h aeration to the natural filtered seawater at 1‰ per 1 h. When the salinity of the stress group reached 29‰, the experiment began. The experiment lasted 48 h. The photoperiod was maintained at 14:10 h (light:dark). The salinity, water temperature, DO, and pH were monitored using an HQ40d portable multi-parameter (HQ40d18, Hach, Loveland, CO, USA), and the nitrite nitrogen and ammonia nitrogen were measured by a nitrite nitrogen testing kit (Zhecheng Biotechnology, Beijing, China). During the experimental period, the water temperature was maintained at 29.5 ± 0.5 °C, DO was >7.50 mg·L^{-1}, pH was 7.93 ± 0.12, ammonia nitrogen was <0.1 mg·L^{-1}, and nitrite nitrogen <0.05 mg·L^{-1}.

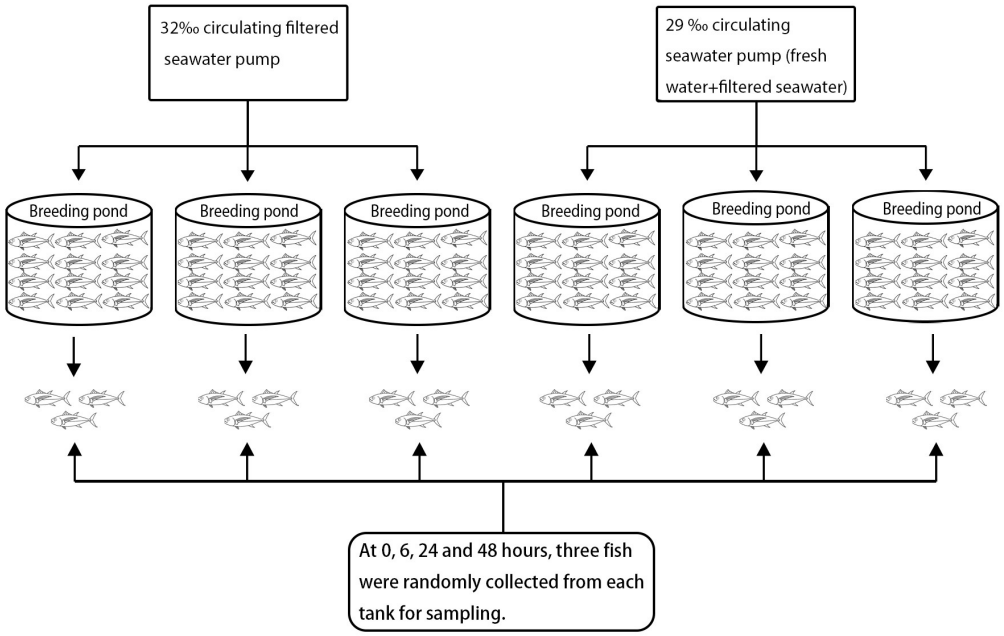

Figure 1. Experimental design of the effect of salinity change on the antioxidant capacity of juvenile yellowfin tuna (*Thunnus albacares*).

2.2. Analytical Method

At 0, 6, 24, and 48 h, three fish from each tank were randomly collected for enzyme activity measurement. The fish were anesthetized with overdose AQUI-S (100 mg L^{-1}, AQUI-S New Zealand Ltd., Lower Hutt, New Zealand) and dissected on an ice tray. The liver, gill, and muscle samples were weighed and immediately stored in liquid nitrogen. For each assay, an individual sample was partially thawed, weighed, and homogenized using a glass homogenizer on ice in 0.2 M NaCl. The suspensions were centrifuged at 2500 r·min^{-1} for 10 min at 2 °C. Then, the supernatant was incubated in the enzyme substrate and read on a spectrophotometer (Synergy H1, BioTek Instruments, Winooski, VT, USA). All of the measurements were conducted in triplicate.

The protein content was determined by the Coomassie Brilliant Blue method with bovine serum protein as the standard used the protein quantitative kit (catalog number A045-4, Nanjing, China), incubated at 37 °C for 30 min at 562 nm wavelength, and the protein concentration measured by microplate colorimetry. The SOD test kit (catalog number A001-3, Nanjing, China) was used to measure the activity of the SOD in the animal tissue samples. The activity of the SOD was determined by the xanthine oxidase method. The absorbance value was measured at the wavelength of 550 nm by colorimetry to calculate its activity. The activity unit was defined as follows: when the SOD inhibition rate reached 50% per milligram of tissue protein in 1 mL of the reaction solution, the corresponding amount of SOD was 1 SOD activity unit (U·$mgprot^{-1}$). The GSH-Px activity in tissues can

be measured with a GSH-Px determination kit (catalog number A005-1, Nanjing, China). The GSH-Px activity was expressed by the consumption rate of GSH in the enzymatic reaction, while the more stable yellow substance formed by GSH and dithiodinitrobenzoic acid can be determined by colorimetry to calculate the GSH-Px activity. Through the colorimetric method, a 1 cm optical path cuvette was used at 412 nm wavelength, the distilled water was adjusted to zero, the absorbance value was measured, and its activity was calculated. The activity unit U represents that the GSH concentration in the reaction system is reduced by 1% per milligram of protein per minute by deducting non-enzymatic reaction $\mu mol \cdot L^{-1}$. The MDA determination kit (catalog number A003-1, Nanjing, China) was used to measure the content of MDA in the animal tissues. The MDA was condensed with thiobarbituric acid to form a red substance, and MDA was determined by colorimetry at 532 nm. The method according to Gan L as well as other methods were used with slight modifications [23,24].

2.3. Statistical Analysis

The measurement of each variable was expressed as a mean of three samples. SPSS 26.0 software (SPSS, Chicago, IL, USA) was used for the statistical analysis. All figures were drawn using the Origin 2019 software (OriginLab Corporation, Northampton, MA, USA), and the data were expressed as mean ± standard deviation (mean ± SD). At the same sampling time, an independent T-test was used to compare the difference between the control and the stress groups. The mean values of the specific activities of each enzyme in the stress group between the sampling times were compared with one-way ANOVA. The significant difference was set at $p < 0.05$.

3. Results

3.1. Change of Gill State of Juvenile Yellowfin Tuna under Salinity Stress

The SOD activity in the fish gills in the stress group increased with time from 0 to 48 h and reached the maximum value at 48 h (830.86 ± 80.66 $U \cdot mgprot^{-1}$, Figure 2a). The SOD activity in the fish gills of the stress group was significantly higher than those in the control group at 24 h and 48 h ($p < 0.05$, Figure 2a). The GSH-Px activity in the stress group was not significantly different from 0 h to 24 h ($p > 0.05$), and reached the maximum value of 5.25 ± 1.01 $U \cdot mgprot^{-1}$ at 48 h ($p < 0.05$, Figure 2b). In the stress group, the MDA content in the gills of the juvenile yellowfin tuna was not significantly different from 0 h to 24 h ($p > 0.05$), and the highest value (2.87 ± 0.12 $nmol \cdot mgprot^{-1}$) was observed at 48 h (Figure 2c).

3.2. Changes in Liver Status of Juvenile Yellowfin Tuna under Salinity Stress

The SOD activity in the liver of juvenile yellowfin tuna in the stress group fluctuated slightly from 0 h to 48 h with the extension of the stress time, and no significant differences were observed between the sampling times and between the control and stress groups in the same sampling time ($p > 0.05$, Figure 3a). The GSH-Px activity in the liver of fish in the control group and the experimental group experienced a change trend of first increasing (113.42%) and then decreasing (−19.69%), and reached the maximum value at 24 h (1.50 ± 0.27 $U \cdot mgprot^{-1}$, Figure 3b). Before 24 h, the GSH-Px activity of the fish liver in the stress group was significantly higher than that observed in the control group ($p < 0.05$, Figure 3b), and the lowest GSH-Px activity in the fish liver was observed in the stress group at 48 h. The content of MDA in the liver of the fish in the stress group increased first (137.45%) and then decreased (−97.33%, Figure 3c). At 24 h, the content of MDA reached the highest value of 2.92 ± 0.23 $nmol \cdot mgprot^{-1}$. There was a significant difference between the stress group at each time point ($p < 0.05$). The MDA content in the stress group at 6 and 48 h was significantly lower than in the control group ($p < 0.05$, Figure 3c).

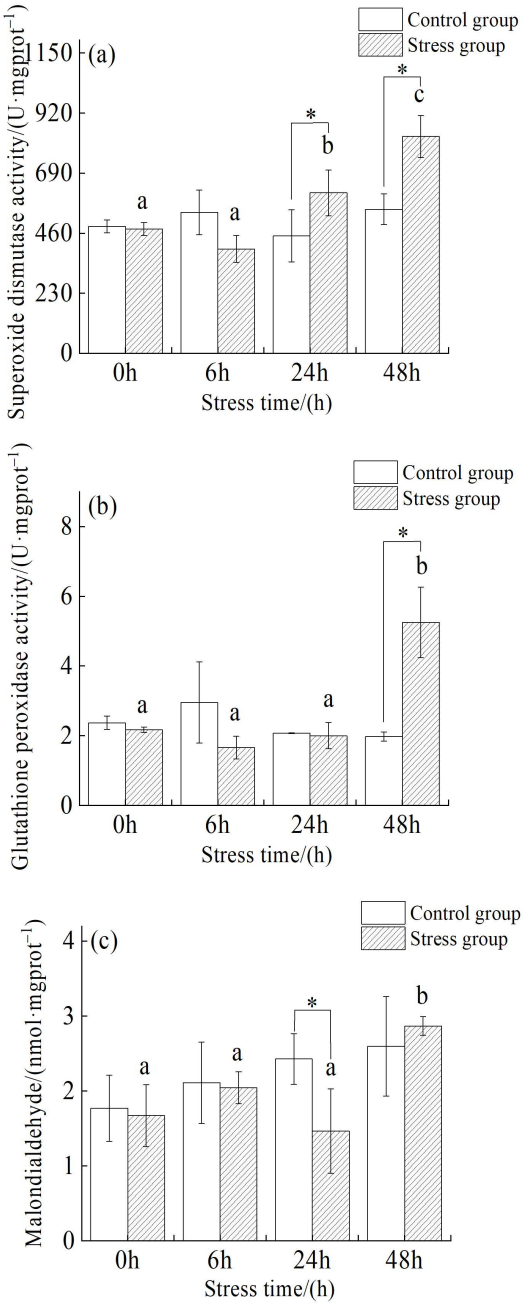

Figure 2. Effect of acute low salt stress on the antioxidation of gills of juvenile yellowfin fish (*Thunnus albacares)*, (n = 9). (**a**), Superoxide dismutase activity; (**b**), Glutathione peroxidase activity; (**c**), Malondialdehyde. Different letters indicate that there is a significant difference in the same salinity group at different time points ($p < 0.05$). Different letters indicate the difference between the experimental group and the control group over time, and * indicates the difference between the experimental group and the control group.

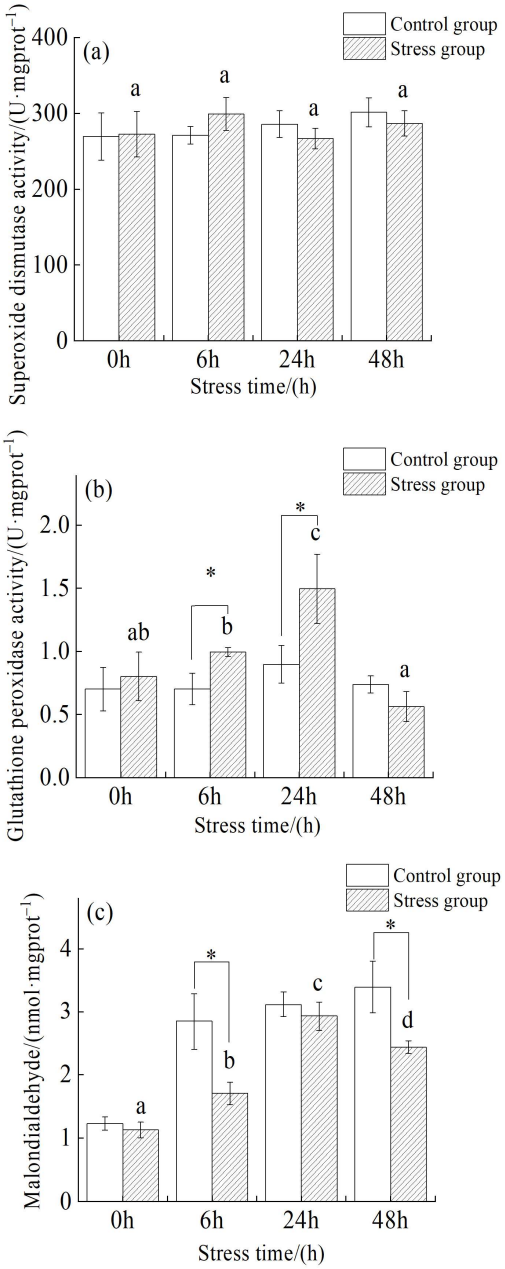

Figure 3. Effect of acute low salt stress on the antioxidation of liver of juvenile yellowfin fish (*Thunnus albacares)*, (*n* = 9). (**a**), Superoxide dismutase activity; (**b**), Glutathione peroxidase activity; (**c**), Malondialdehyde. Different letters indicate the difference between the experimental group and the control group over time, and * indicates the difference between the experimental group and the control group.

3.3. Changes in Red Muscle Status of Juvenile Yellowfin Tuna under Salinity Stress

The SOD activity in the red muscle of fish was not significantly different at 0, 24, and 48 h in the stress group ($p > 0.05$), and the lowest value of 813.13 ± 39.81 U·mgprot^{-1} was observed at 6 h ($p < 0.05$, Figure 4a). The highest GSH-Px activity (15.71 ± 1.65 U·mgprot^{-1}) in the red muscle of the fish from the stress group was observed at 24 h, and the lowest value (3.35 ± 0.10 U·mgprot^{-1}) was recorded at 48 h ($p < 0.05$, Figure 4b). In the stress group, the highest MDA value of 3.74 ± 0.36 nmol·mgprot^{-1} was observed in the red muscle of fish at 24 h ($p < 0.05$, Figure 4c), while no significant difference was observed at other sampling times ($p > 0.05$). At 6 and 24 h, the MDA value of the fish in the stress group was significantly higher than those in the control group ($p < 0.05$).

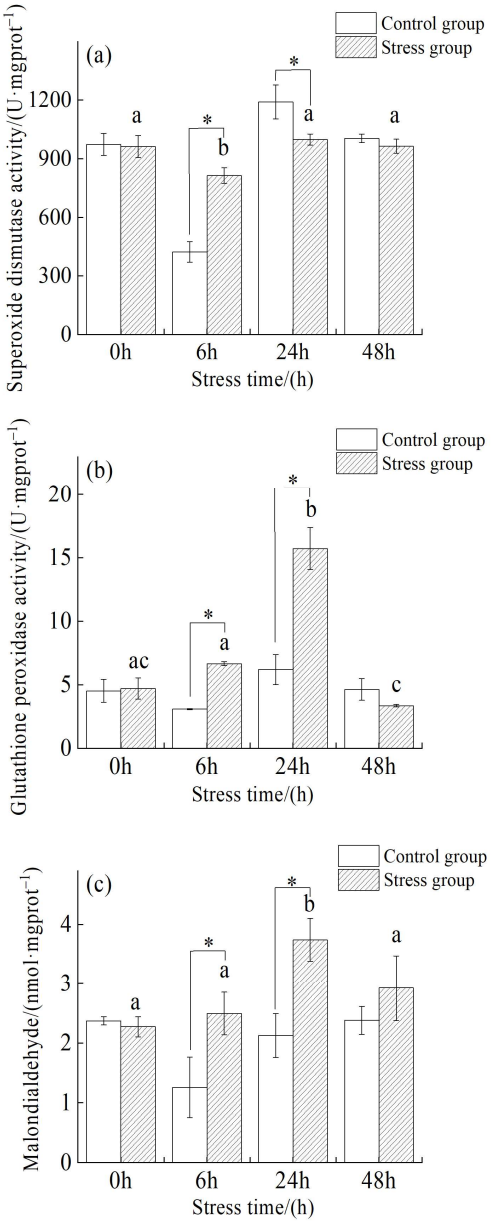

Figure 4. Effect of acute low salt stress on the antioxidation of red muscle of juvenile yellowfin fish (*Thunnus albacares*), (n = 9). (**a**), Superoxide dismutase activity; (**b**), Glutathione peroxidase activity; (**c**), Malondialdehyde. Different letters indicate the difference between the experimental group and the control group over time, and * indicates the difference between the experimental group and the control group.

3.4. Changes in White Muscle Status of Juvenile Yellowfin Tuna under Salinity Stress

The SOD activity of the white muscle of the juvenile yellowfin tuna in the stress group decreased (−28.56%) at 6 h ($p < 0.05$, Figure 5a) and gradually stabilized after 24 h ($p > 0.05$). The SOD activity in the white muscle of the fish at 6 and 48 h was significantly higher than that observed in the control group ($p < 0.05$). However, the SOD activity in the stress group was significantly lower than in the control group at 24 h ($p < 0.05$, Figure 5a). In the stress group, the highest GSH-Px activity (5.97 ± 0.14 U·mgprot^{-1}) in the white muscle of the fish was observed at 24 h, and the lowest GSH-Px activity (1.71 ± 0.16 U·mgprot^{-1}) was observed at 48 h ($p < 0.05$, Figure 5b). At 6 and 24 h, the GSH-Px activity of the fish from the stress group was significantly higher than that observed in the control group ($p < 0.05$, Figure 5b). At 48 h, the GSH-Px activity of the fish from the stress group was

significantly lower than that recorded in the control group ($p < 0.05$). The MDA value in the white muscle of the fish was not significantly different between the sampling times and treatments ($p > 0.05$, Figure 5c).

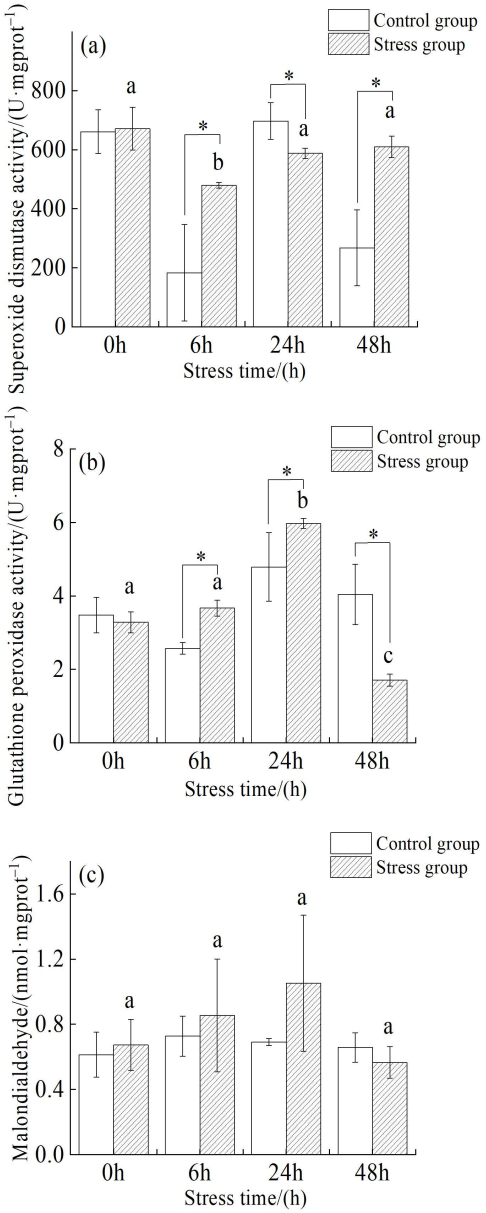

Figure 5. Effect of acute low salt stress on the antioxidation of white muscle of juvenile yellowfin fish (*Thunnus albacares*), ($n = 9$). (**a**), Superoxide dismutase activity; (**b**), Glutathione peroxidase activity; (**c**), Malondialdehyde. Different letters indicate the difference between the experimental group and the control group over time, and * indicates the difference between the experimental group and the control group.

4. Discussion

The gill is a key organ for osmoregulation in fish and plays an important role in ion regulation [25]. When salinity changes, gills not only need to carry out osmotic regulation in time, but also need to bear certain oxidative stress pressure [26]. In the present study, the change of salinity within 48 hours increased the antioxidant enzyme activity in the gills of the juvenile yellowfin tuna. The impacts of salinity on the activity of antioxidant enzymes in gills have been intensively studied. As SOD is the first part of antioxidant defense, it will encounter superoxide anion free radicals. During stress, SOD has a faster reaction speed

and higher sensitivity, which is a good indicator for monitoring the aquatic ecosystem [27]. Ghanavatinasab et al. evaluated the impact of salinity on *Sparus flavipectus* when transferring fish from 20 to 5, 12, and 34. They found no significant differences in SOD activity between the groups after 14 days of the experiment [28]. The responses of SOD activity in fish to the salinity changes are species-dependent. In black porgy *Acanthopagrus schlegeli*, when environmental salinities decreased from 35 to 10 ppt, SOD activity of fish upregulated nearly twofold [29]. Moreover, the activity of SOD in *Scapharca broughtonii* showed similar responses after exposure to low environmental salinities [30]. It is speculated that when fish are subjected to salinity stress, different tissues in the fish may undergo various levels of oxidative stress and different resistance strategies. When the salinity dropped suddenly, the SOD in the gills of the juvenile yellowfin tuna participated in scavenging free radicals. Generally speaking, the H_2O_2 produced in most animals is mainly eliminated by GSH-Px [31]. At the same time, GSH-Px can also remove lipid peroxides such as fatty acids, and maintain the normal function of the cell membrane [32]. In the present study, the GSH-Px activities in stressed fish gills reached the highest level at 24 h and were reduced to similar levels to those observed in the control group. Such changes in the GSH-Px activities of fish gills may suggest that fish may fully recover from salinity shock after a 24 h adaption. In vivo, MDA is the final product of oxidation when free radicals act on lipid peroxidation, which can cause the cross-linking and polymerization of life macromolecules such as proteins and nucleic acids and has cytotoxicity [33]. At the same time, the MDA content also shows the degree of cell plasma membrane damage [34]. When the salinity decreases, the MDA in the gills of juvenile yellowfin tuna remains stable within a certain range in the whole process of antioxidation in a low-salt environment, which also proves that the cytoplasmic membrane is not damaged.

The activity of antioxidant enzymes in different tissues of the same fish is also different. The liver is a multifunctional organ that integrates metabolism, immunity, digestion, and other functions [25]. The liver is the tissue with more oxidation reactions, so the activity of antioxidant enzymes is higher [33]. The results of this study showed that the SOD activity in the liver of juvenile yellowfin tuna had no significant impact after the sudden drop in ambient salinity, which was contrary to the research results of *Epinephelus moora* [35], *Takifugu obscurus* [36], and *Oryzias melastigma* [37]. The cultivation salinity of *Dicentrarchus labrax* decreased from 37 to 5, and the SOD activity increased significantly after 12 h of stress [38,39]. The alternations in the SOD activity were relatively low, possibly due to the low response mechanism in the liver of migratory fish in the deep sea [40]. The increased content of GSH-Px and MDA in the first 24 h may relate to the elimination of excessive reactive oxygen free radicals in the body. Afterward, the content of GSH-Px and MDA in the fish liver decreased to a level similar to the control group, possibly as a result of the reaction between glutathione peroxidase and a variety of antioxidant enzymes [41,42]. In the present study, the adaptation processes of SOD, GSH-Px, and MDA were stable after 24 h in the liver, indicating that the antioxidant system in the fish liver had adapted to the salinity reduction. In contrast, however, antioxidant changes in the gills of the yellowfin tuna were different from those of the liver. The inhalation and excretion of fish gills are closely related to the gills. In the acute low-salt environment, the yellowfin tuna is required to maintain osmotic balance, and it also bears a certain amount of oxidative stress pressure [43]. The branchial vascular system is innervated and autonomously controlled by its nerves [44]. When the antioxidation data of their own tissues are stable, the gill tissue behaves differently from the liver and muscle in response to the oxidative stress caused by the sudden change of salinity. It is speculated that different tissues are subject to different degrees of oxidative stress, and there are also differences in the resistance strategies.

The red muscle and white muscle in fish are both part of the greater lateral muscle. As the yellowfin tuna needs to rely on the gills to obtain oxygen, the red muscle and white muscle are clearly distinguished. The red muscle has a high fat content, is rich in myoglobin and has a large amount of blood, and is dark red. The yellowfin tuna has strong endurance and developed red muscle. The white muscle contains no fat and is light

white [26]. Muscle is widely distributed in the fish body and has a large proportion [26]. Determining antioxidant enzyme activity in fish muscle can also reflect the stress status of the fish. In *Acipenser schrencki*, the muscles are sensitive tissues to osmotic pressure perception and can respond positively when salinity changes [45]. A previous study has demonstrated that the SOD activity in *Rachycentron canadum* muscle increased with the decrease in salinity [46]. However, the responses of SOD activity to the salinity stress in the muscles of yellowfin tuna were different from previous studies [47]. The SOD activity in the muscle of the juvenile yellowfin tuna decreased first and then increased and tended to be stable at the end. Such responses may suggest the antioxidant system in fish muscle has fully adapted to the ambient salinity of 29‰ at 48 h.

The malondialdehyde content in the muscle increased with time, and the overall expression level of the yellowfin tuna red muscle was higher than that of the white muscle [46,47]. The higher SOD activity in the yellowfin tuna red muscle reflected the low potential mechanism in the antioxidant process of yellowfin tuna red muscle [40]. In the present study, the content of the total antioxidant capacity reached the highest value at 24 h and decreased at 48 h, which may be related to the adaptation process of muscle tissue to the salinity stress. However, there are few reports about the effect of salinity stress on antioxidant enzymes in the white and red muscles of fish, and this subject may be worthy of further investigation.

5. Conclusions

In this study, the juvenile yellowfin tuna were subjected to a 48 h acute low-salt stress experiment to evaluate the physical response of the fish. The results showed that, in terms of antioxidant levels, the juvenile yellowfin tuna could adapt to 29‰ within 48 h. Under salinity stress, the antioxidant status in the liver, gills, and muscles of the fish recovered to average levels at 48 h, indicating that the antioxidant system was able to clear the excess free radicals and oxidation intermediates and maintain the dynamic balance of the antioxidant system in the cells in this salinity changing range. This study helps to understand the physiological changes of yellowfin tuna under low salinity, and provides guidance for the artificial culture of yellowfin tuna.

Author Contributions: Conceptualization, S.Z. and G.Y.; methodology, S.Z.; software, S.Z. and Z.F.; validation, Z.F.; formal analysis, S.Z.; investigation, S.Z. and N.Z.; resources, Z.F. and L.Z.; data curation, N.Z.; writing—original draft preparation, N.Z.; writing—review and editing, Z.M. and G.Y.; visualization, N.Z. and G.Y.; supervision, Z.M. and L.Z.; project administration, Z.M.; funding acquisition, Z.M. and L.Z. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The experiment complied with the regulations and guidelines established by the Animal Care and Use Committee of the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The approval number is 2020TD55, which was approved on 5 January 2020.

Informed Consent Statement: Not applicable.

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

Conflicts of Interest: The authors declare no conflict of interest.

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