Journal of *Marine Science and Engineering*

MDPI

Article

The Effects of Acute Ammonia Nitrogen Stress on Antioxidant Ability, Phosphatases, and Related Gene Expression in the Kidney of Juvenile Yellowfin Tuna (*Thunnus albacares*)

Yongyue Sun [1,2,3,4,5], Zhengyi Fu [2,3,4,5,6] and Zhenhua Ma [2,3,4,5,6,*]

1 College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China
2 Key Laboratory of Efficient Utilization and Processing of Marine Fishery Resources of Hainan Province, Sanya Tropical Fisheries Research Institute, Sanya 572018, China
3 South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, China
4 Hainan Engineering Research Center for Deep-Sea Aquaculture and Processing, Sanya 572018, China
5 International Joint Research Center for Conservation and Application of Fishery Resources in the South China Sea, Sanya 572018, China
6 College of Science and Engineering, Flinders University, Adelaide 5001, Australia
* Correspondence: zhenhua.ma@scsfri.ac.cn

Abstract: This study investigated the effects of acute ammonia nitrogen (NH_3-N) exposure on kidney antioxidant ability and phosphatases and related gene expression in juvenile yellowfin tuna (*Thunnus albacares*). The 180 juvenile yellowfin tuna (260.39 ± 55.99 g, 22.33 ± 2.28 cm) were exposed to ammonia for 6, 24, and 36 h using natural seawater (0 mg/L) as a control and NH_3-N at 5 and 10 mg/L. The lipid peroxidation byproduct malondialdehyde (MDA) and the levels of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX), alkaline phosphatase (AKP), and acid phosphatase (ACP), were measured using the colorimetric method in the trunk kidney to determine changes in antioxidant ability and phosphatase activity of juvenile yellowfin tuna exposed to NH_3-N. Results indicated that, at 36 h, MDA, SOD, CAT, and GSH-PX levels rose in the 5 mg/L group versus the control. In the 10 mg/L group, MDA and SOD, CAT, and GSH-PX activities significantly increased after 24 and 36 h exposure compared to the control. Phosphatases play a pivotal role in the immune system. AKP activity significantly increased at 6 h, and ACP activity markedly rose at 36 h in the 5 mg/L group versus the control. Real-time fluorescence quantitative PCR was applied to detect alterations in the antioxidant genes SOD2, CAT, and glutathione peroxidase 1b (GPX1b) and immune cytokines-related genes Interleukin 10 (IL-10) and Interleukin 6 receptor (IL-6r) expression in the head kidney in juvenile tuna. Relative to the control, antioxidant gene expression in the 5 mg/L group significantly rose at 6 and 36 h, and in the 10 mg/L group, SOD2 and GPX1b were significantly elevated at 36 h. Compared to the control group, IL-10 expression in the 5 mg/L group significantly increased at 6 h, whereas IL-6r expression decreased. In the 10 mg/L group, both IL-10 and IL-6r levels were observed to be lower. Low ammonia nitrogen concentrations boost antioxidant defenses, phosphatase activities, and gene expression levels, whereas higher levels may induce suppressive effects. In yellowfin tuna juvenile farming, NH_3-N concentration significantly affects the health of the juveniles. When the NH_3-N concentration is between 5–10 mg/L, the stress duration should be limited to 24 h; if the concentration is below 5 mg/L, the stress duration can be extended to 36 h.

Keywords: NH_3-N; head kidney; trunk kidney; antioxidant enzymes; immunological enzyme; antioxidant genes; immune genes

Citation: Sun, Y.; Fu, Z.; Ma, Z. The Effects of Acute Ammonia Nitrogen Stress on Antioxidant Ability, Phosphatases, and Related Gene Expression in the Kidney of Juvenile Yellowfin Tuna (*Thunnus albacares*). *J. Mar. Sci. Eng.* **2024**, *12*, 1009. https://doi.org/10.3390/jmse12061009

Academic Editor: Nguyen Hong Nguyen

Received: 13 April 2024
Revised: 8 June 2024
Accepted: 17 June 2024
Published: 18 June 2024

1. Introduction

Ammonia nitrogen (NH_3-N) represents a prevalent stressor within the aquaculture environment, emerging as the final byproduct in the decomposition of nitrogenous wastes

from a variety of sources, such as the excrement of aquatic creatures, the breakdown of leftover feed, and the discharge from industrial and household wastewater processes [1]. In fact, there are two forms of ammonia nitrogen: ionizated (NH_4^+) and non-ionizated (NH_3), with the latter being much more toxic and more likely to enter aquatic animals, causing damage and even death to the organisms [2,3]. Elevated levels of ammonia nitrogen pose a direct threat to the liver and kidney tissues of fish, leading to conditions such as congestion, edema, hepatic coma, and, in severe cases, death [4]. Additionally, ammonia nitrogen exposure can provoke neurological responses in fish, manifesting in abnormal behaviors including reduced appetite, stunted growth, and diminished movement [5–7]. Therefore, mitigating ammonia nitrogen pollution and optimizing aquaculture conditions are crucial steps toward enhancing the efficiency of aquaculture practices.

When subjected to environmental stressors, fish respond by generating an excess of reactive oxygen species (ROS), which leads to heightened levels of malondialdehyde (MDA), a marker of oxidative stress. This escalation compromises their immune defenses and can potentially lead to mortality. In response to such oxidative threats, fish mobilize their antioxidant and immune systems for protection. The core components of the antioxidant defense mechanism include enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX), which effectively neutralize ROS, safeguarding the fish against oxidative harm [8]. Additionally, enzymes such as acid phosphatase (ACP) and alkaline phosphatase (AKP), vital elements of the lysosomal system, play crucial roles in bolstering the fish's immune response [9]. Research findings reveal that the cuttlefish (*Sepia pharaonis*) displays a decrease in the activities of SOD and CAT, coupled with an elevation in MDA levels, when subjected to ammonia nitrogen stress [10]. Similarly, Pacific white shrimp (*Litopenaeus vannamei*) exposed to ammonia stress show elevated activities of AKP and ACP, highlighting the impact of environmental stress on marine organisms' biochemical pathways [11].

In response to the stress induced by ammonia toxicity, fish can activate their antioxidant defense mechanisms through the expression of specific antioxidant genes [12]. This phenomenon has been observed in hybrid groupers (*Epinephelus fuscoguttatus*♀× *E. lanceolatus*♂), where genes responsible for SOD, CAT, and GSH-PX are significantly upregulated under ammonia-nitrogen stress [13]. Ammonia stress not only influences inflammatory cytokines in fish but also triggers inflammatory responses and tissue damage [14]. The increase in pro-inflammatory cytokines, notably Interleukin 6 (IL-6) [15], triggers such inflammatory responses. Conversely, a rise in the anti-inflammatory molecule, Interleukin 10 (IL-10), serves to mitigate these inflammatory processes [16]. Research into these cytokines across different fish species, including Atlantic halibut (*Hippoglossus hippoglossus* L.), orange-spotted grouper (*Epinephelus coioides*), Senegalese sole (*Solea senegalensis*), golden pomfret (*Trachinotus ovatus*), Atlantic cod (*Gadus morhua* L.), and large-bellied seahorse (*Hippocampus abdominalis*) [17–22], has provided insight into the nuanced interplay of enzyme activity, gene expression, and the resulting antioxidant and immune responses. This comprehensive understanding aids in deciphering the complex biological responses of fish to environmental stressors, particularly those related to ammonia exposure.

Yellowfin tuna (*Thunnus albacares*), a member of the Scombridae family within the Scombroid suborder of the order Perciformes, thrives in warm tropical and subtropical waters worldwide. Celebrated for its high protein and low fat content, yellowfin tuna is a favored selection among seafood lovers [23]. The species is abundant in the South China Sea, and advancements in aquaculture techniques, such as indoor recirculating water systems and offshore deep-water net-pan farming, have been notably successful at the Tropical Aquatic Research and Development Centre in Xincun Town, Lingshui County, Hainan Province [24]. While yellowfin tuna aquaculture is also undertaken in regions such as Mexico, Panama, and Indonesia, the industry faces challenges related to the insufficient supply of wild fry [25]. Research on yellowfin tuna has covered various aspects, including stock assessment, fisheries, reproductive biology, and genomics [26–30]. Despite these

extensive studies, the physiological response of yellowfin tuna to ammonia and nitrogen levels in artificial aquaculture environments remains largely unexplored.

The influence of ammonia nitrogen stress on the kidney health of marine fish, with a particular focus on juvenile yellowfin tuna, remains an underexplored area of research. This study seeks to examine the impact of short-term ammonia nitrogen exposure on juvenile yellowfin tuna, specifically looking at the activity of antioxidant enzymes and phosphatase, as well as antioxidant and immune-related gene expression. Our research aims to shed light on how yellowfin tuna respond to changes in ammonia nitrogen levels in their environment. The findings from this study are expected to inform improvements in aquaculture methods for yellowfin tuna, whether in recirculating water systems or cage cultures, by focusing on short-term adaptations. Through the development of well-founded and efficient management approaches, we intend to counteract the negative effects of ammonia nitrogen, thus promoting a more efficient and sustainable approach to the aquaculture of yellowfin tuna.

2. Materials and Methods

2.1. Experimental Fish and Design

Juvenile yellowfin tuna were supplied by the Sanya Tropical Fisheries Research Institute located in Lingshui County, Hainan Province, China. These juveniles had an average weight of 260.39 ± 55.99 g and measured an average length of 22.33 ± 2.28 cm. At the experiment's outset, 180 juvenile yellowfin tuna were transferred to an indoor recirculating tank and evenly distributed across nine 5000-L recirculating water tanks, ensuring 20 fish per tank for a week-long acclimation period. During this time, their diet consisted of chilled mixed fish species, and the water quality was meticulously maintained at optimal conditions for their growth: a temperature of 29 ± 1°C, ammonia nitrogen levels under 0.01 mg/L, dissolved oxygen at 7.00 ± 0.50 mg/L, a pH of 8.10, salinity at 32‰, and nitrite levels below 0.01 mg/L.

Based on prior studies [31–33], 0 mg/L (natural seawater) was used as the control group, the ammonia nitrogen concentration was set at 5 and 10 mg/L, respectively, and the experiment was set up with three replicates, where analytically pure ammonium chloride (NH_4Cl) (99.5% purity, Xilong Chemical Co., Ltd., Foshan, China) was added to seawater, dissolved, and configured into a masterbatch of 10 g/L as the ammonia nitrogen source. The relevant calculation formulas are as follows:

Supplementing ammonia nitrogen in terms of mass (mg) = The volume of water (L) × The difference in ammonia nitrogen concentration (mg/L).
The molar quantity of ammonium chloride (mol) = Supplementing ammonia nitrogen in terms of mass (mg) / The molar mass of ammonium chloride (g/mol). (The molar mass of ammonium chloride is approximately 53.49 g/mol).
The mass of ammonium chloride (g) = The molar quantity of ammonium chloride (mol) × The molar mass of ammonium chloride (g/mol).
The actual mass of ammonium chloride (g) = The mass of ammonium chloride (g)/0.995.

The fish were not fed for 24 h before and throughout the duration of the experiment. Utilizing a portable water quality detector (Wuxi Oktan Biotechnology Co. Ltd., Wuxi, China), the concentration of ammonia nitrogen is measured every two hours. Based on the results, ammonia nitrogen is calculated and replenished in a timely manner to maintain the required concentration according to the following formula. Three fish were randomly selected from each tank at 6, 24, and 36 h into the experiment for further analysis.

2.2. Sample Collection

Following the administration of eugenol anesthesia to ensure humane handling, the juvenile yellowfin tuna underwent measurement for body mass and length. Subsequently, a precise dissection was performed to extract the head and trunk kidneys, which were then meticulously placed into clearly labeled cryopreservation tubes. These samples were immediately flash-frozen in liquid nitrogen to preserve their integrity. The following day,

the samples were transferred to a −80 °C freezer, setting the stage for further detailed analyses and determinations.

2.3. Measurement of Physiological Indicators

First, 0.1 to 0.2 g of trunk kidney tissue was weighed into a 2-milliliter centrifuge tube, adhering to the reagent instructions. Then, 0.9% saline was added in a ratio of 1:9 (weight in grams to volume in milliliters). Then, 2–3 steel balls were added. The homogenate was then processed using a tissue homogenizer (Hangzhou Aosheng Instrument Co., Ltd., Hangzhou, China). Following homogenization, the mixture was centrifuged at 3500 rpm for 10 min at 4 °C using a centrifuge (Heraeus Corporation, Hanau, Germany). The supernatant was then separated from the tissue pellet so it could be aspirated and dispensed as needed. This supernatant was promptly stored at −80 °C in preparation for the measurement of antioxidant enzyme activity. The evaluation of enzyme activity was conducted utilizing the optical densities obtained with a hybrid microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) and a spectrophotometer (Mepore Instruments, Shanghai, China), ensuring accurate quantification of the enzymes.

The kit for the kidney test was purchased from the Nanjing Jiancheng Bioengineering Institute, Nanjing, China. The MDA assay kit (catalog number A003-1) was employed to quantify MDA, a byproduct of lipid peroxidation that reacts with thiobarbituric acid (TBA) to produce a red compound with a peak absorbance at 532 nm, which was then recorded. SOD activity was evaluated using a water-soluble tetrazolium salt (WST-1)-based method (Catalogue No. A001-3). CAT activity was assessed through the ammonium molybdate method, wherein the reaction between CAT and hydrogen peroxide (H_2O_2) is halted by ammonium molybdate. The remaining H_2O_2 forms a yellow complex with ammonium molybdate, the concentration of which is measured at 405 nm to determine CAT activity (Catalogue No. A007-1). GSH-PX levels were determined by measuring the consumption of reduced glutathione in the enzymatic reaction (Catalogue No. A005-1).

ACP (Catalogue No. A060-2) and AKP (Catalogue No. A059-2) activities were measured by their ability to hydrolyze disodium benzene phosphate, releasing free phenol. This phenol reacts with potassium ferricyanide in an alkaline medium and 4-aminoantipyrine to form a red quinone derivative. The intensity of the red color, indicative of enzyme activity, was then quantitatively assessed, allowing for the precise determination of ACP and AKP levels.

2.4. Expression of Antioxidant-Related and Immune-Related Genes

The head kidney was selected for the assessment of RNA gene relative expression. RNA was extracted from head kidney samples using a Trizol reagent (Lanjieke Technology Co., Ltd., Hefei, China) to isolate the total RNA. The integrity of the RNA was verified through 1% agarose gel electrophoresis (Lanjieke Technology Co., Ltd.), ensuring the RNA was not degraded. The concentration of the isolated RNA was quantified using a Nano-300 spectrophotometer (Ausheng Instrument Co., Ltd., Hangzhou, China), with the extraction deemed successful if the optical density (OD) ratios at 260 nm to 280 nm fell within the range of 1.8 to 2.0. Following RNA extraction, cDNA synthesis was carried out, as per the protocol provided by the One-Step gDNA Removal and cDNA Synthesis SuperMix kit (Quan's Gold Biotechnology Limited, Beijing, China) [34], laying the groundwork for subsequent gene expression analysis.

The gene sequences analyzed in this study of yellowfin tuna were obtained from the unpublished transcriptome data collected by our research team. Primers for the study were meticulously designed with the aid of Primer Premier 5 software (Premier Inc., Charlotte, NC, Canada), and their sequences are detailed in Table 1. These primers were then synthesized by Sangyo Bioengineering Co., Ltd. (Shanghai, China). β-actin [35] was selected as the reference gene to ensure accurate gene expression analysis.

Table 1. RT-PCR primer sequence.

Gene	Acronym		Primer sequences	Mollification Size
Superoxide dismutase 2	SOD2	F	CGGGACTTTGGTTCCTTCCA	128
	SOD2	R	GCACAAGCAGCGATACGAAG	
Catalase	CAT	F	CAGGCAACAACACCCCCA	122
	CAT	R	CCAGAAGTCCCACACCAT	
Glutathione peroxidase 1b	GPX1b	F	GACCACCAGGGATTACAC	150
	GPX1b	R	GGACGGACATACTTCAGA	
Interleukin 6 receptor	IL-6r	F	TTGTCAGTCATTTTGGCT	132
	IL-6r	R	CTCTGGAGATGTTGGGGT	
Interleukin 10	IL-10	F	CAGCAAGATACCAACAAG	190
	IL-10	R	CGACAAGAGAACCAGGAC	
β-actin	β-actin	F	CGCCCTCGTTGTTGAC	170
	β-actin	R	CCCTTTTGCTCTGTGCC	

The SYBR Green RealUniversal Colour Fluorescence Quantification Kit (Beijing Tiangen Biochemical Technology Co., Ltd., Beijing, China) was employed for cDNA quantification. The reaction mixture totaled 20 μL, comprising 10 μL of 2 × RealUniversal PreMix, 0.6 μL forward primer (10 μM concentration), 0.6ul reverse primer (10um concentration), 6.8 μL of RNase-free ddH_2O, and 2 μL of the cDNA template (the cDNA contains 1000 nanograms.) [31,32]. Real-time PCR analyses were conducted using a fluorescence quantitative PCR instrument (Langji Scientific Instruments Co., Ltd., Hangzhou, China), with the specific parameters of the reaction outlined in Table 2. Relative expression levels of the target genes were calculated employing the $2^{-\Delta\Delta Ct}$ [36] method, allowing for the precise quantification of gene expression changes.

Table 2. Fluorescence quantitative reaction procedure.

Stage	Cycle	Temperature	Times	Elements
permutability	1×	95 °C	15 min	permutability
PCR reaction	40×	95 °C	10 s	denaturation
		50–60 °C	20 s	annealing (metallurgy)
		72 °C	20–32 s	extend

2.5. Statistical Analysis

The results of the treatment and control groups are presented as mean ± standard deviation (Mean ± SD), with the difference value between the treatment and control groups presented as the treatment-control mean. Subsequently, a one-way analysis of variance (ANOVA) was conducted to evaluate the results of the treatment and control groups. Prior to conducting the ANOVA, we performed a preliminary Chi-square test to check for normal distribution and homogeneity of variances. In instances where significant differences were identified ($p < 0.05$), Duncan's multiple comparison test was employed to discern the specific differences between groups. For visual representation, the experimental data were graphically plotted using Origin 2022 software (2022 edition).

3. Results

3.1. Effect of Acute Ammonia Nitrogen Stress on the Antioxidant Ability of the Trunk Kidney of Juvenile Yellowfin Tuna

Over time, both the 5 mg/L and 10 mg/L treatment groups exhibited an upward trend in MDA concentration, with the most pronounced changes observed at the 36-h mark. Specifically, the 5 mg/L treatment group experienced an increase of 609.38 ± 70.18 nmol/mgprot, while the 10 mg/L treatment group saw an increase of 1003.97 ± 6.45 nmol/mgprot

(Figure 1a). In the 5 mg/L ammonia nitrogen treatment group, the MDA concentration in the kidney of juvenile yellowfin tuna was significantly lower than that of the control group at both the 6-h and 24-h time points (p = 0.001), yet it notably surpassed the control group's level at the 36-h mark (p = 0.001, Figure 1b). Within the 10 mg/L treatment group, the MDA concentration was significantly reduced compared to the control group at the 6-h mark (p = 0.001), but it was significantly elevated at both the 24-h and 36-h time points (p = 0.001).

The SOD activity of both the 5 mg/L and 10 mg/L treatment groups exhibited an upward trend over time, with the most significant increase observed at 36 h. Specifically, the 5 mg/L treatment group saw an increase of 13.35 ± 0.86 U/mgprot, while the 10 mg/L treatment group experienced an increase of 12.08 ± 0.72 U/mgprot (Figure 1c). In the 5 mg/L ammonia nitrogen treatment group, the SOD activity in juvenile yellowfin tuna was significantly lower than that of the control group at both the 6-h and 24-h time points (p = 0.001), but it notably surpassed the control group's level at the 36-h mark (p = 0.001, Figure 1d). Within the 10 mg/L treatment group, there was no significant difference in SOD activity compared to the control group at the 6-h mark (p = 0.425), but at both the 24-h and 36-h time points, the SOD activity in this group was significantly higher than that of the control group (p = 0.001).

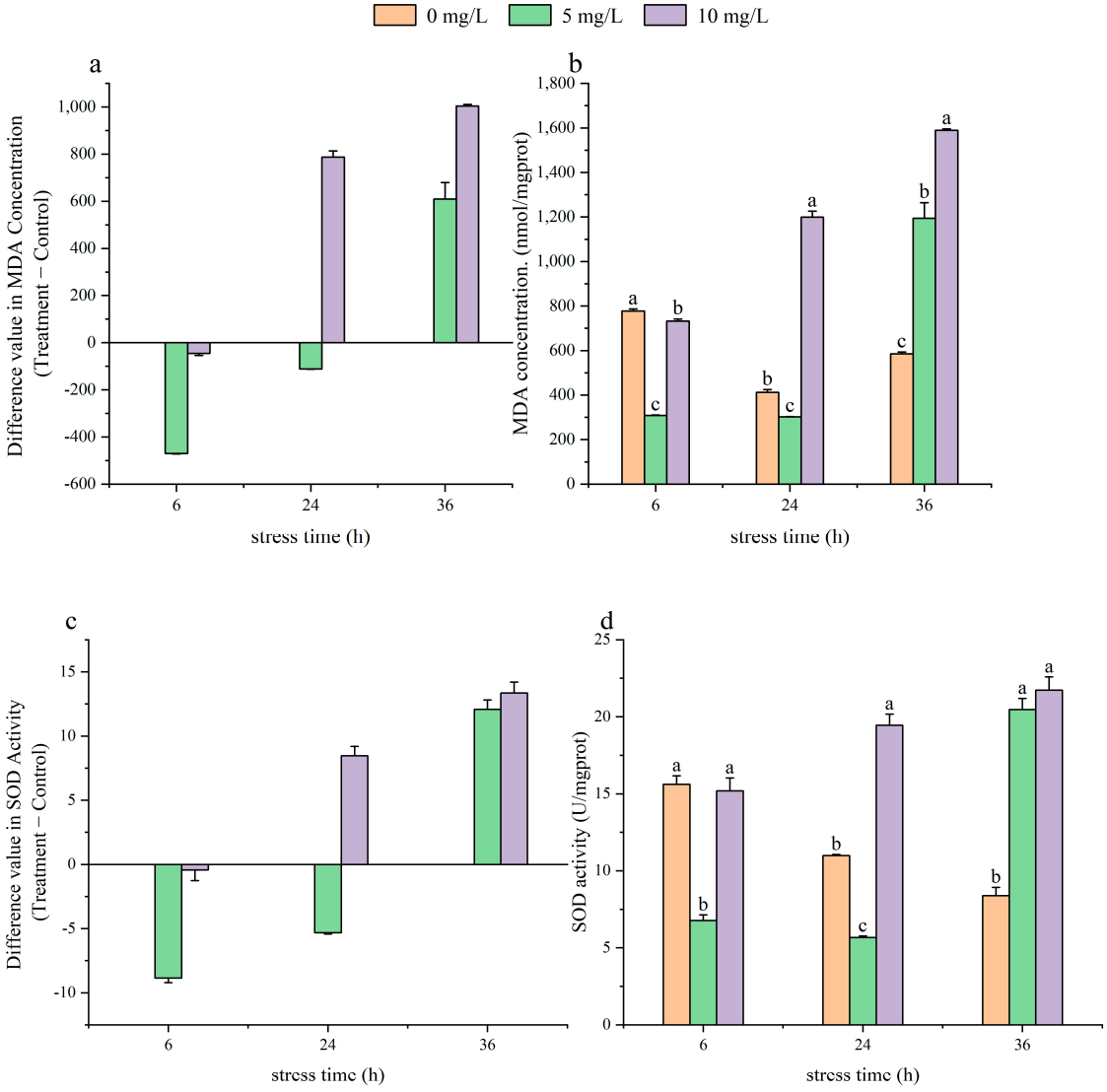

Figure 1. *Cont.*

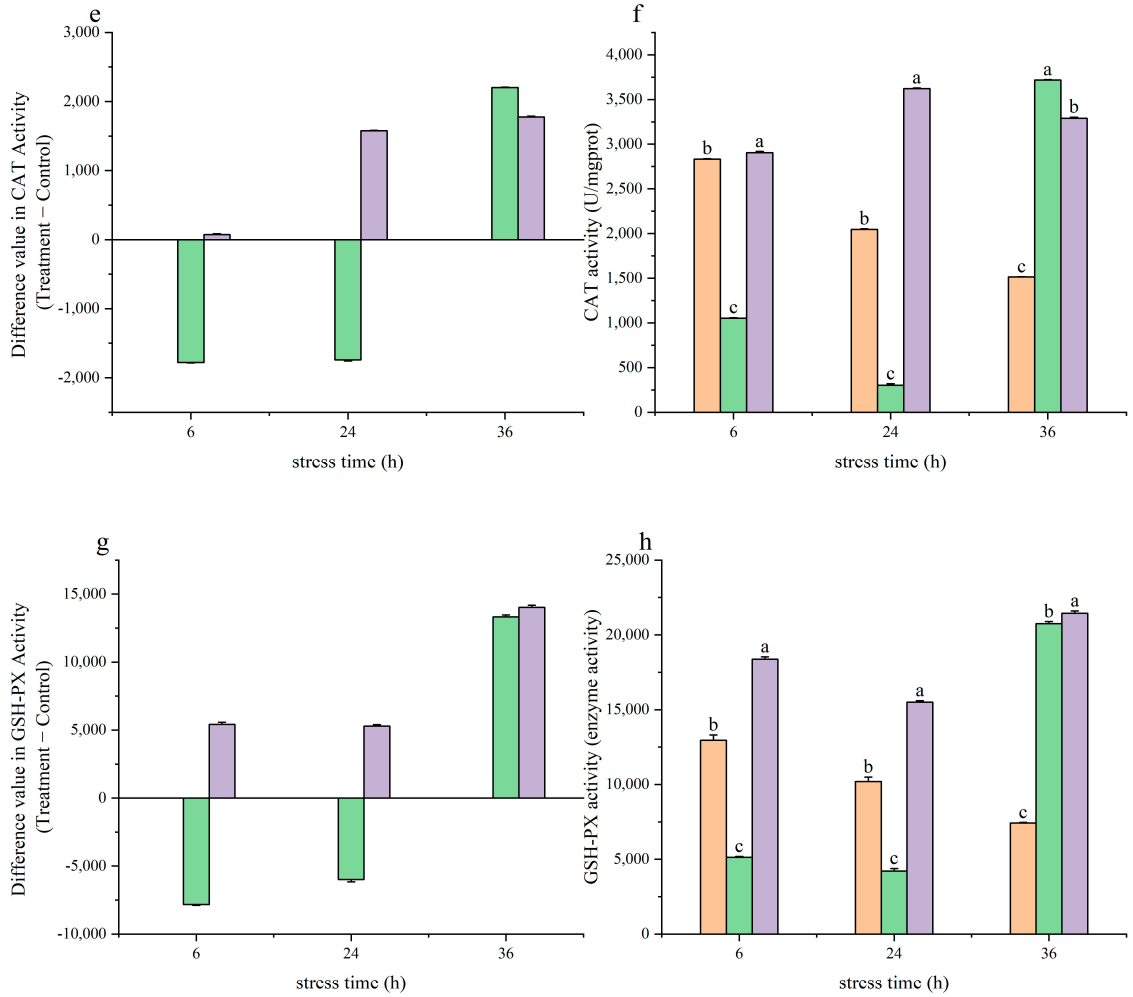

Figure 1. Effect of acute ammonia nitrogen stress on the antioxidant ability in the trunk kidney of juvenile yellowfin tuna (n = 9). (**a**) Difference value in malondialdehyde (MDA) concentration (treatment-control), (**b**) MDA concentration in treatment and control groups, (**c**) difference value in superoxide dismutase (SOD) activity (treatment-control), (**d**) SOD activity in treatment and control groups, (**e**) difference value in catalase (CAT) activity (treatment-control), (**f**) CAT activity in treatment and control groups, (**g**) difference value in glutathione peroxidase (GSH-PX) activity (treatment-control), (**h**) GSH-PX activity in treatment and control groups. The difference value = treatment value − control mean value. Different letters signify the significance of varying ammonia concentrations at the same time point ($p < 0.05$).

The CAT activity within the experimental groups displayed a consistent upward trajectory over time, with the most pronounced increase observed at the 36-h mark. Specifically, the activity rose by 2203.90 ± 4.21 U/mgprot in one group and by 11776.05 ± 11.79 U/mgprot in another (Figure 1e). In the 5 mg/L ammonia nitrogen treatment group, the CAT activity in juvenile yellowfin tuna was significantly lower than that of the control group at both the 6-h and 24-h time points (p = 0.001), but it was notably higher at the 36-h mark (p = 0.001, Figure 1f). Within the 10 mg/L treatment group, CAT activity was significantly elevated above the control group levels throughout the 6 to 36-h period (p = 0.001).

The GSH-PX activity of the 5 mg/L treatment group exhibited an upward trend over time, with the most significant increase observed at 36 h. The enzyme activity increased by 13,325.09 ± 141.68 units (Figure 1g). In the 5 mg/L ammonia nitrogen treatment group, the GSH-PX activity in juvenile yellowfin tuna was significantly lower than that of the control group at both the 6-h and 24-h time points (p = 0.001), yet it was notably higher at the 36-h mark (p = 0.001, Figure 1h). The GSH-PX activity of the 10 mg/L treatment

group showed an upward trend following a 24-h stress period, with the largest increase in activity observed at 36 h, increasing by 14,030.91 ± 150.08 enzyme activity units. Within the 10 mg/L treatment group, the GSH-PX activity was consistently elevated above the control group levels throughout the 6- to 36-h period ($p = 0.001$).

3.2. Effect of Acute Ammonia Nitrogen Stress on the Activity of Phosphatase in the Trunk Kidney of Juvenile Yellowfin Tuna

AKP activity in the 5 mg/L treatment group showed a decreasing and then increasing trend, with the greatest increase in AKP activity at 6 h, 0.063309434 ± 0.02163058 King units/gprot (Figure 2a). In the 5 mg/L ammonia nitrogen treatment group, AKP activity was significantly increased at the 6-h mark compared to the control group ($p = 0.007$), while, at the 24-h mark, it was found to be lower than the control level ($p = 0.001$). At the 36-h mark, no significant difference was observed compared to the control group ($p = 0.095$, Figure 2b). The AKP activity in the 10 mg/L treatment group exhibited a decreasing trend over time, with the largest reduction observed at 36 h, amounting to a decrease of 0.01 ± 0.01 King unit/gprot. Conversely, in the 10 mg/L treatment group, no significant differences were detected in AKP activity compared to the control group during the 6- to 36-h period ($p = 0.397$ at 6 h, $p = 0.934$ at 24 h, and $p = 0.225$ at 36 h).

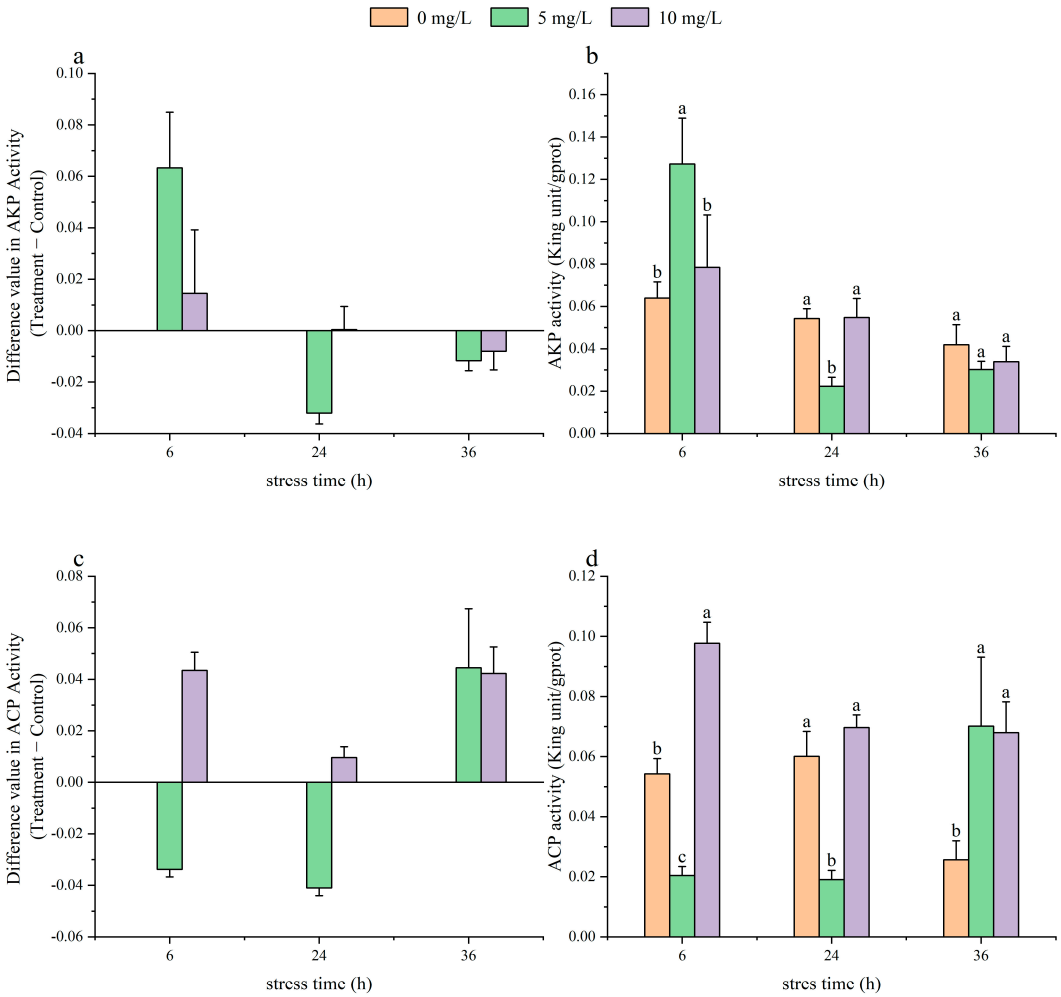

Figure 2. Effect of acute ammonia nitrogen stress on the activity of phosphatase in the trunk kidney of juvenile yellowfin tuna ($n = 9$). (**a**) Difference value in alkaline phosphatase (AKP) activity (treatment-control), (**b**) AKP activity in treatment and control groups, (**c**) difference value in acid phosphatase (ACP) activity (treatment-control), (**d**) ACP activity in treatment and control groups. The difference value = treatment value − control mean value. Different letters signify the significance of varying ammonia concentrations at the same time point ($p < 0.05$).

The ACP activity in both the 5 mg/L and 10 mg/L ammonia nitrogen treatment groups exhibited a pattern of initial decline followed by an increase over time, with the greatest decrease observed at 24 h. Specifically, the 5 mg/L group experienced a reduction of 0.041 ± 0.01 King unit/gprot, while the 10 mg/L group saw a decrease of 0.01 ± 0.01 King unit/gprot (Figure 2c). In the 5 mg/L ammonia nitrogen treatment group, ACP activity was significantly lower than that of the control group at both 6 and 24 h (p = 0.001, Figure 2d), yet it was notably higher at 36 h (p = 0.011). For the 10 mg/L treatment group, ACP activity was markedly elevated above the control levels at both 6 and 36 h (p = 0.001 at 6 h, p = 0.013 at 36 h), while, at 24 h, no significant difference was observed compared to the control group (p = 0.083).

3.3. Effects of Acute Ammonia Nitrogen Stress on Antioxidant Genes in the Head Kidney of Juvenile Yellowfin Tuna

In the 5 mg/L treatment group, the expression levels of SOD2 initially declined and subsequently demonstrated an upward trajectory over the course of the study (Figure 3a). In the 5 mg/L ammonia nitrogen treatment group, the relative expression of the SOD2 gene in juvenile yellowfin tuna was comparable to that of the control group at the 6-h mark (p = 0.201, Figure 3b), but it significantly decreased at the 24-h mark (p = 0.001) and then surpassed the control group's level at the 36-h mark (p = 0.009). In the 10 mg/L treatment group, the expression of SOD2 exhibited a consistent increase over the temporal progression. In the 10 mg/L treatment group, the SOD2 gene expression showed no significant difference from the control group at the 6-h mark (p = 0.206), was lower than the control at the 24-h mark (p = 0.004), and finally significantly exceeded the control group at the 36-h mark (p = 0.05).

The expression of the CAT gene in the 5 mg/L treatment group displayed a trend of initial decrease followed by a subsequent increase over time (Figure 3c). In the 5 mg/L ammonia nitrogen treatment group, the relative expression of the CAT gene in juvenile yellowfin tuna showed no significant difference from the control group during the 6- to 36-h period (at 6 h, p = 0.159; at 24 h, p = 0.189; at 36 h, p = 0.757; Figure 3d). In the 10 mg/L treatment group, the expression of CAT exhibited a sustained upward trend over time. Similarly, in the 10 mg/L ammonia nitrogen treatment group, the expression level of the CAT gene did not significantly differ from the control group over the same 6 to 36-h period (at 6 h, p = 0.204; at 24 h, p = 0.56; at 36 h, p = 0.936).

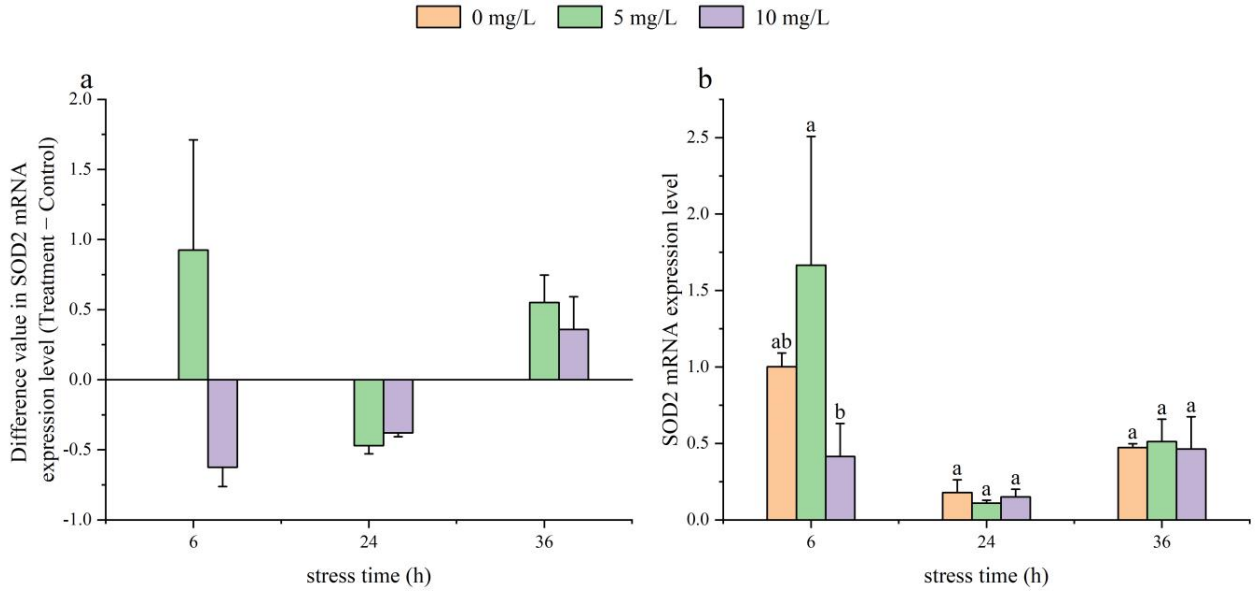

Figure 3. *Cont.*

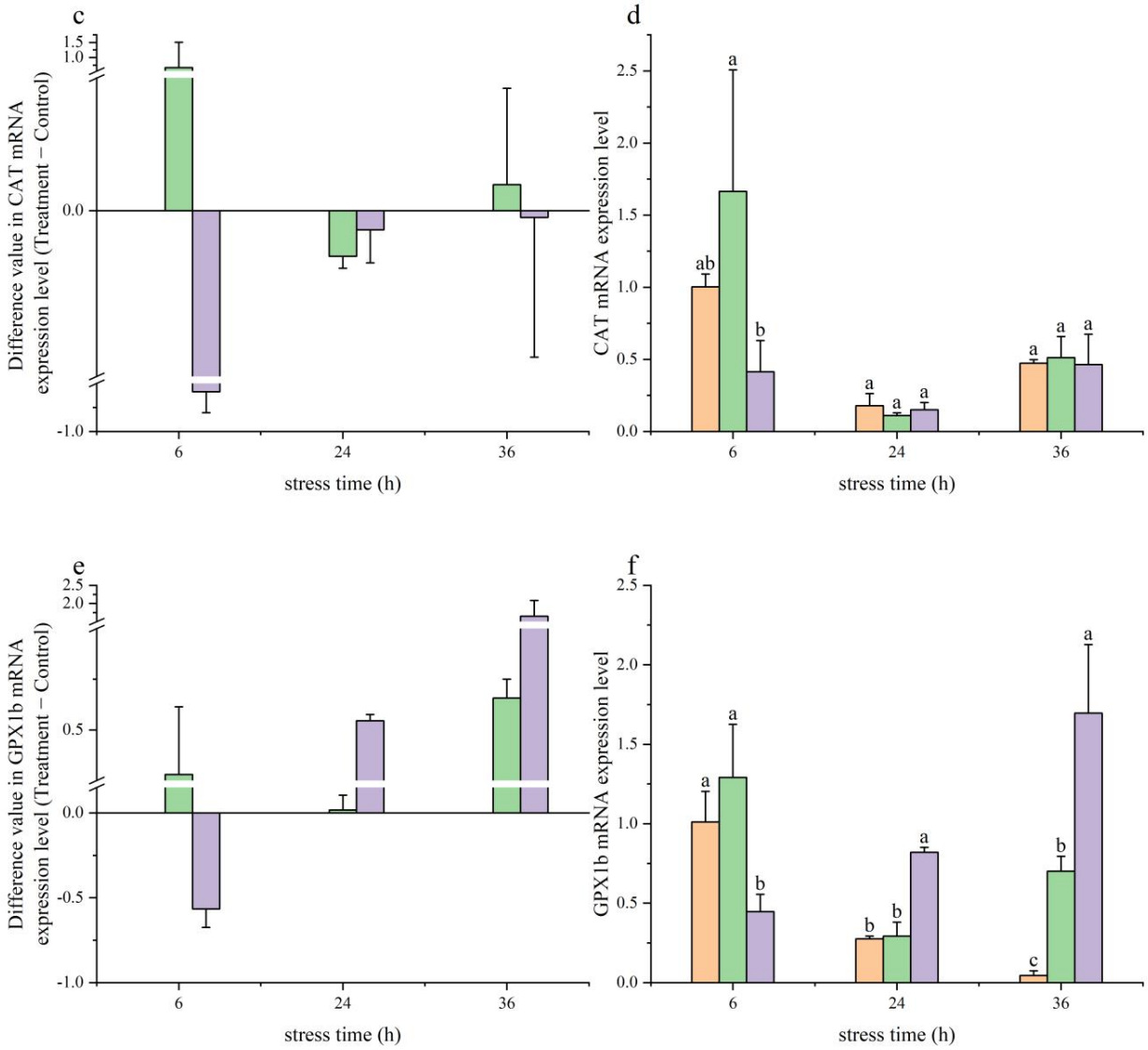

Figure 3. Effects of acute ammonia nitrogen stress on antioxidant genes in the head kidney of juvenile yellowfin tuna (n = 9). (**a**) Difference value in superoxide dismutase 2 (SOD2) mRNA expression level (treatment-control), (**b**) SOD2 mRNA expression level in treatment and control groups, (**c**) difference value in catalase (CAT) mRNA expression level (treatment-control), (**d**) CAT mRNA expression level in treatment and control groups, (**e**) difference value in glutathione peroxidase 1b (GPX1b) mRNA expression level (treatment-control), (**f**) GPX1b mRNA expression level in treatment and control groups. The difference value = treatment value − control mean value. Different letters signify the significance of varying ammonia concentrations at the same time point ($p < 0.05$).

In the 5 mg/L group, GPX1b gene expression decreased and then increased over time (Figure 3e). In the 5 mg/L treatment group, the relative expression level of the GPX1b gene in juvenile yellowfin tuna showed no significant difference from the control group at both the 6-h and 24-h time points (at 6 h, p = 0.189, at 24 h, p = 0.712, Figure 3f), but it was significantly elevated at the 36-h mark (p = 0.02). Expression of the GPX1b gene in the 10 mg/L treatment group showed an increasing trend with time. In the 10 mg/L treatment group, the expression of the GPX1b gene was significantly lower than that of the control group at the 6-h mark (p = 0.024), yet it was notably higher than the control group at both the 24-h and 36-h time points (p = 0).

3.4. Effects of Acute Ammonia Nitrogen Stress on Immune-Related Genes in the Head Kidney of Juvenile Yellowfin Tuna

IL-10 showed a trend of first decreasing and then increasing over time in the treatment group with a concentration of 5 mg/L (Figure 4a). In the 5 mg/L treatment group, the relative expression level of the IL-10 gene in juvenile yellowfin tuna was significantly higher than that of the control group at the 6-h mark (p = 0.001), but it was significantly lower at the 24-h mark (p = 0.001). By 36 h, there was no significant difference in expression levels compared to the control group (p = 0.944, Figure 4b). IL-10 demonstrated an increasing trend over time in the treatment group with a concentration of 10 mg/L. In the 10 mg/L treatment group, the IL-10 gene expression was significantly lower than the control group at both the 6-h (p = 0.002) and 24-h (p = 0.001) time points, but by 36 h, there was no significant difference from the control group (p = 0.955).

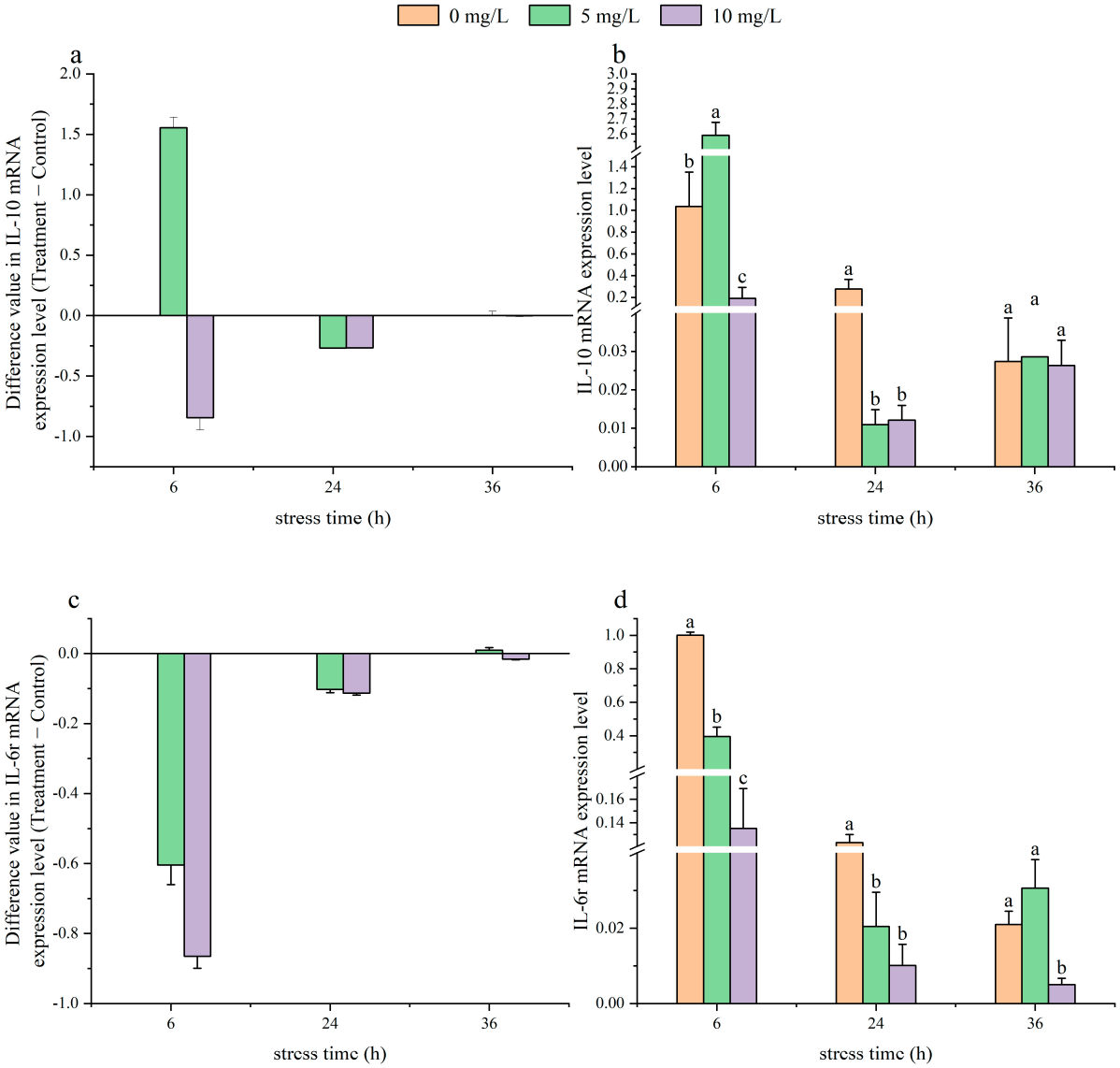

Figure 4. Effects of acute ammonia nitrogen stress on immune-related genes in the head kidney of juvenile yellowfin tuna (n = 9). (**a**) Difference value in Interleukin 10 (IL-10) mRNA expression level (treatment-control), (**b**) IL-10 mRNA expression level in treatment and control groups, (**c**) difference value in Interleukin 6 receptor (IL-6r) mRNA expression level (treatment-control), (**d**) IL-6r gene expression levels in treatment and control groups. The difference value = treatment value − control mean value. Different letters signify the significance of varying ammonia concentrations at the same time point ($p < 0.05$).

In the experimental groups treated with 5 and 10 mg/L of ammonia nitrogen, the expression of IL-6r exhibited an upward trend over time (Figure 4c). In the 5 mg/L treatment group, the expression of the IL-6r gene in juvenile yellowfin tuna was significantly lower than that of the control group at both the 6-h and 24-h time points ($p = 0.001$). By 36 h, there was no significant difference in expression levels compared to the control group ($p = 0.053$, Figure 4d). In the 10 mg/L treatment group, the IL-6r gene expression remained consistently lower than the control group from 6 h to 36 h ($p = 0.001$ at 6 and 24 h, $p = 0.007$ at 36 h).

4. Discussion

The kidneys serve as the primary detoxification and target organ for toxic substances [37]. In fish, the kidneys are situated on the ventral side, beneath the spine, and are divided into the head kidney and the trunk kidney [38]. The trunk kidney, which acts as the urinary organ, handles excretory functions [39]. Numerous studies have documented the impact of harmful environmental conditions on fish kidneys. For instance, exposure to ammonia nitrogen has been found to variably influence the activity of antioxidant enzymes in the kidneys of both amberjack (*Seriola dumerili*) and medaka (*Oryzias dancena*) [39]. Additionally, exposure to cadmium has been shown to impair both the structure and function of the kidneys in marine catfish (*Arius arius*) [39]. Ammonia nitrogen is a critical environmental factor in aquaculture, with high concentrations leading to oxidative stress in fish. This stress results in tissue damage and can severely impair fish growth, compromise their health, or even cause mortality [40]. In an experiment involving yellowfin tuna, changes were observed in the trunk kidney's MDA concentration, and the activities of SOD, GSH-PX, and CAT were monitored over time. These parameters all peaked at 36 h during a 5 mg/L ammonia nitrogen treatment. Similar trends were observed in Japanese seabass (*Lateolabrax japonicus*) [41]. The initial increase in immune response at 6 and 24 h in the 5 mg/L treatment likely facilitated the clearance of a substantial amount of ROS, reducing oxidative damage and consequently MDA production. This reduction might have led to lower activities of SOD, CAT, and GSH-PX compared to the control. As stress duration increased, the accumulation of ROS in the body heightened, causing more oxidative stress and MDA production, which in turn induced an increase in antioxidant enzyme activities due to the accumulation of H_2O_2. In bighead carp (*Hypophthalmythys*), low concentrations of ammonia stress activate its antioxidant system, leading to a significant increase in the activity of SOD and CAT. Conversely, high concentrations of ammonia stress cause damage to the antioxidant system, thereby suppressing the activity of these enzymes [42]. Conversely, in this experiment with juvenile yellowfin tuna, the 10 mg/L ammonia nitrogen treatment group exhibited significantly higher MDA concentrations and SOD, CAT, and GSH-PX activities at 6 h and 24 h compared to the 5 mg/L group. The higher concentration of ammonia nitrogen might have caused a gradual increase in ammonia accumulation in the organisms over time, leading to lipid peroxidation, a rise in MDA concentrations, and subsequent increases in SOD activity due to the production of large amounts of H_2O_2. Concurrently, CAT activity decreased while GSH-PX activity rose at 36 h, which resulted in inefficient clearance of H_2O_2, further exacerbating lipid peroxidation and increasing MDA concentrations. These processes indicate more severe damage to the organisms as the MDA concentration increases [43]. Moderate exposure to ammonia nitrogen appears to act as an adaptive challenge, stimulating the antioxidant defense mechanisms of juvenile yellowfin tuna and thereby enhancing their resistance to oxidative stress. During this process, the antioxidant capacity of juvenile yellowfin tuna was significantly improved when exposed to 5 mg/L of ammonia nitrogen for 36 h, or to 10 mg/L for 24 h. To minimize the impacts of ammonia nitrogen concentration on their antioxidant capabilities, it is crucial to strictly manage both the ammonia nitrogen levels and the duration of exposure.

ACP and AKP are critical enzymes for phosphate group transfer and transformation, playing a key role in the metabolism associated with an animal's defense mechanisms. These enzymes facilitate the absorption and transfer of substances, forming a hydrolytic

enzyme system to eliminate foreign materials and bolster the body's defense [9,44]. In juvenile barramundi (*Lates calcarifer*), ACP levels showed an increase from 24 to 72 h under ammonia nitrogen stress [45], while plasma AKP levels in Hsu's ping-pong (*Sebastes schlegelii*) increased initially and then decreased under similar stress conditions [46]. In this study, AKP activity in the 5 mg/L treatment group was elevated at 6 h, and ACP activity increased at 36 h, both compared to the control group. This may be due to the 5 mg/L ammonia nitrogen concentration enhancing oxidative stress and non-specific immunity in yellowfin tuna, leading to decreased MDA levels. The changes in AKP, related to metabolic transport, suggest increased lipid metabolism and reduced lipid oxidation. Over time, the prolonged stress leads to an excessive accumulation of ammonia nitrogen, diminishing the metabolic activities and AKP activity in juvenile yellowfin tuna, resulting in lipid oxidation. In response, antioxidant enzymes and various lysosomes, including ACP—an enzyme predominant in lysosomes involved in phagocytic processes—activate to eliminate ROS, thereby driving the increase in ACP concentration. In the context of alkalinity stress, both ACP and AKP activities first increased in low alkalinity conditions and then decreased, whereas they increased significantly under high alkalinity stress [47]. In this study, the 10 mg/L ammonia nitrogen treatment group showed significantly lower AKP activity compared to the 5 mg/L group at 6 h, while ACP activity was significantly higher in the 10 mg/L group at both 6 and 24 h. Ammonia nitrogen has a pronounced inhibitory effect on the AKP activity in juvenile yellowfin tuna. However, at lower concentrations, ammonia nitrogen exhibits a positive stimulatory effect on the ACP activity in these juvenile fish. Conversely, when ammonia nitrogen levels rise to higher concentrations, it significantly inhibits the ACP activity in the juvenile yellowfin tuna. This discovery underscores the intricate influence of ammonia nitrogen levels on the phosphatase activity in juvenile yellowfin tuna, offering a scientific basis for optimizing the aquaculture environment for these species.

The head kidney in fish is a crucial hematopoietic tissue and a site for immune cell differentiation and proliferation. In gilded snapper (*Sparus aurata* L.), researchers observed a down-regulation of some immune genes in the kidney when exposed to highly polluted marine sediments [36]. Sinha et al. reported stable SOD gene expressions but reduced CAT gene expressions in the liver of European black bass (*Dicentrarchus labrax*) under acute hypotonic stress [48]. Conversely, antioxidant genes such as SOD and CAT saw increased expression in the liver of puffer fish (*Takifugu obscurus*) subjected to high- or low-temperature stress for 1 h [49]. Ammonia stress is known to induce oxidative stress in fish, altering the expression of genes related to antioxidants [50]. In this experiment, the expression levels of antioxidant genes in the 5 mg/L ammonia-treated juvenile yellowfin tuna were not significantly higher than the control at 6 h. This pattern suggests that initial ammonia-induced oxidative stress prompted an upregulation of antioxidant genes to bolster the organism's antioxidant defenses and to efficiently remove excessive ROS, thereby maintaining metabolic balance. As the stress duration increased, the yellowfin tuna gradually adapted to the 5 mg/L ammonia concentration, but a notable increase in ammonia accumulation at 36 h led to a renewed increase in antioxidant gene expression to mitigate oxidative stress. In the 10 mg/L treatment group, the expression of GPX1b increased at 36 h compared to the control group, possibly due to the higher ammonia concentration suppressing SOD2 and CAT expressions while enhancing the immune response, leading to the overexpression of GPX1b. It is documented that the GPX1 gene is often overexpressed in many cancers, influencing tumor cell proliferation, invasion, migration, apoptosis, and immune responses, and is considered a tumor promoter [51]. The disparity between gene expression and antioxidant enzyme activity might be attributed to additional regulatory mechanisms, such as post-transcriptional and post-translational modifications, which can lead to differences between gene expression levels and their corresponding enzyme activities at the protein level [52]. Initially, low concentrations of ammonia nitrogen elevated the relative expression levels of antioxidant genes in juvenile yellowfin tuna. However, as the stress period extended, these levels began to decline. Conversely, high

concentrations of ammonia nitrogen exerted varied impacts on the relative expressions of these antioxidant genes.

IL-10, an anti-inflammatory cytokine, plays a crucial role in mitigating inflammation and autoimmune disorders by counteracting pro-inflammatory factors [53]. In contrast, IL-6r is a pro-inflammatory cytokine essential for promoting the expression of proteins linked with acute inflammation [54]. Studies have indicated that catfish (*Lophiosilurus alexandri*) exhibit significantly higher plasma IL-6r levels than the control group during 24 and 72 h of hypoxic stress [55]. Ammonia stress in fish can trigger an inflammatory response, altering the expression of associated genes [14]. In this study, within the 5 mg/L ammonia-treated group, IL-10 gene expression was up-regulated, while IL-6r gene expression was down-regulated at 6 h compared to the control group. This suggests that, early in the stress response, oxidative stress induced inflammation in juvenile yellowfin tuna, which led to an increase in IL-10 expression. This cytokine, by enhancing autoimmunity, helps prevent excessive inflammatory reactions that could damage cells and tissues. Consequently, IL-10 suppressed the expression of the pro-inflammatory cytokine IL-6r, thus exerting a regulatory effect. At 36 h, there were no significant differences in IL-10 and IL-6r levels between the ammonia nitrogen experimental group and the control group, possibly reflecting the juvenile yellowfin tuna's gradual adaptation to the ammonia nitrogen environment and the kidneys' timely regulation of genes related to the inflammatory response [56]. The interaction between IL-10 and IL-6r helped maintain a stable level that mitigated the inflammatory effects of ammonia nitrogen on the kidneys. Initially, low concentrations of ammonia nitrogen stimulated the expression of genes related to IL-10, but as time progressed, this expression was suppressed, and ammonia nitrogen exerted an inhibitory effect on IL-6r expression.

5. Conclusions

This study investigated the effects of acute ammonia nitrogen stress on the antioxidant ability and phosphatase activity in the trunk kidneys of juvenile yellowfin tuna, as well as the expression of antioxidant and immune genes in the head kidney. The results showed that ammonia exposure enhanced the antioxidant ability of trunk kidneys to varying degrees and inhibited phosphatase activity. Furthermore, ammonia nitrogen exposure reduces the expression of antioxidant and immune genes in the head kidney. These results suggest that the antioxidant system can effectively neutralize excess free radicals and oxidative intermediates, thereby maintaining the dynamic balance of the intracellular antioxidant system within the range of ammonia-nitrogen concentration changes. However, the occurrence of oxidative stress and immune-inflammatory responses in the head kidney influences the transcription levels of these genes. Based on these observations, it is recommended that ammonia nitrogen levels be strictly controlled below 10 mg/L and that the stress duration should not exceed 24 h. For ammonia nitrogen mass concentrations below 5 mg/L, the stress duration should preferably be controlled within 36 h. This study contributes to a better understanding of the physiological changes in yellowfin tuna under ammonia nitrogen stress, establishing a foundation for subsequent artificial cultivation and offering insights that can enhance the culture efficiency of yellowfin tuna.

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

Funding: This work was supported by the Hainan Major Science and Technology Project, grant number ZDKJ2021011; the Project of Sanya Yazhou Bay Science and Technology City, grant number SKJC-2022-PTDX-015; the Central Public-Interest Scientific Institution Basal Research Fund, CAFS, grant number 2023TD58, 2024XT04; the Central Public-Interest Scientific Institution Basal Research Fund South China Sea Fisheries Research Institute, CAFS, grant number 2021SD09; the Hainan Provincial Natural Science Foundation of China, grant number 323QN331; the Hainan Province Science and Technology Special Fund, grant number ZDYF2024XDNY247.

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 conflicts of interest.

References

1. Lu, J.; Yao, T.; Shi, S.; Ye, L. Effects of acute ammonia nitrogen exposure on metabolic and immunological responses in the Hong Kong oyster *Crassostrea hongkongensis*. *Ecotoxicol. Environ. Saf.* **2022**, *237*, 113518. [CrossRef] [PubMed]
2. Da Silva, F.J.R.; Lima, F.R.S.; Do Vale, D.A.; Do Carmo, M.V. High levels of total ammonia nitrogen as NH_4^+ are stressful and harmful to the growth of Nile tilapia juveniles. *Acta Scientiarum. Biol. Sci.* **2013**, *35*, 475–481.
3. Zhang, C.; Ma, J.; Qi, Q.; Xu, M.; Xu, R. Effects of ammonia exposure on anxiety behavior, oxidative stress and inflammation in guppy (*Poecilia reticulate*). *Comp. Biochem. Physiol. Part. C Toxicol. Pharmacol.* **2023**, *265*, 109539. [CrossRef] [PubMed]
4. Cong, M.; Wu, H.; Cao, T.; Ji, C.; Lv, J. Effects of ammonia nitrogen on gill mitochondria in clam *Ruditapes philippinarum*. *Environ. Toxicol. Pharmacol.* **2019**, *65*, 46–52. [CrossRef] [PubMed]
5. Rodrigues, R.V.; Schwarz, M.H.; Delbos, B.C. Acute toxicity and sublethal effects of ammonia and nitrite for juvenile cobia *Rachycentron canadum*. *Aquaculture* **2007**, *271*, 553–557. [CrossRef]
6. Xu, W.; Zhu, Z.; Ge, F.; Han, Z.; Li, J. Analysis of behavior trajectory based on deep learning in ammonia environment for fish. *Sensors* **2020**, *20*, 4425. [CrossRef] [PubMed]
7. Zhang, W.; Xia, S.; Zhu, J.; Miao, L.; Ren, M.; Lin, Y.; Ge, X.; Sun, S. Growth performance, physiological response and histology changes of juvenile blunt snout bream, *Megalobrama amblycephala* exposed to chronic ammonia. *Aquaculture* **2019**, *506*, 424–436. [CrossRef]
8. Long, S.; Dong, X.; Yan, X.; Liu, H.; Tan, B.; Zhang, S.; Chi, S.; Yang, Q.; Liu, H.; Yang, Y. The effect of oxidized fish oil on antioxidant ability, histology and transcriptome in intestine of the juvenile hybrid grouper (♀*Epinephelus fuscoguttatus* × ♂*Epinephelus lanceolatus*). *Aquac. Rep.* **2021**, *22*, 100921. [CrossRef]
9. Bao, J.; Li, X.; Xing, Y.; Feng, C.; Jiang, H. Effects of hypoxia on immune responses and carbohydrate metabolism in the Chinese mitten crab, *Eriocheir sinensis*. *Aquac. Res.* **2020**, *51*, 2735–2744. [CrossRef]
10. Chai, Y.; Peng, R.; Jiang, M.; Jiang, X.; Han, Q.; Han, Z. Effects of ammonia nitrogen stress on the blood cell immunity and liver antioxidant function of *Sepia pharaonis*. *Aquaculture* **2022**, *546*, 737417. [CrossRef]
11. Chen, S.; Yu, Y.; Gao, Y.; Yin, P.; Tian, L.; Niu, J.; Liu, Y. Exposure to acute ammonia stress influences survival, immune response and antioxidant status of pacific white shrimp (*Litopenaeus vannamei*) pretreated with diverse levels of inositol. *Fish Shellfish Immunol.* **2019**, *89*, 248–256. [CrossRef] [PubMed]
12. Abdel-Latif, H.M.; Shukry, M.; Abd-Elaziz, R.A. Clinico-pathological findings and expression of inflammatory cytokines, apoptosis, and oxidative stress-related genes draw mechanistic insights in Nile tilapia reared under ammonia-N exposure and *Aeromonas hydrophila* challenge. *Fish Shellfish Immunol.* **2022**, *127*, 1–12. [CrossRef] [PubMed]
13. Cao, J.; Mei, J.; Xie, J. Combined effects of hypoxia and ammonia-N exposure on the immune response, oxidative stress, tissue injury and apoptosis of hybrid grouper (*Epinephelus fuscoguttatus*♀× *E. lanceolatus*♂). *Environ. Sci. Pollut. Res.* **2024**, *31*, 845–856. [CrossRef] [PubMed]
14. Collet, B. Innate immune responses of salmonid fish to viral infections. *Dev. Comp. Immunol.* **2014**, *43*, 160–173. [CrossRef] [PubMed]
15. Tabaei, S.; Motallebnezhad, M.; Tabaee, S.S. Systematic review and meta-analysis of association of polymorphisms in inflammatory cytokine genes with coronary artery disease. *Inflamm. Res.* **2020**, *69*, 1001–1013. [CrossRef]
16. Yang, P.; Liu, J.; Xiao, J.; Jian, H.; Chen, H. Associations between seven common cytokine gene polymorphisms and coronary artery disease: Evidence from a meta-analysis. *Int. Arch. Allergy Immunol.* **2020**, *181*, 301–310. [CrossRef]
17. Overgard, A.; Nepstad, I.; Nerland, A.H.; Patel, S. Characterisation and expression analysis of the Atlantic halibut (*Hippoglossus hippoglossus* L.) cytokines: IL-1 beta, IL-6, IL-11, IL-12 beta and IFN gamma. *Mol. Biol. Rep.* **2012**, *39*, 2201–2213. [CrossRef] [PubMed]

18. Furne, M.; Holen, E.; Araujo, P.; Lie, K.K.; Moren, M. Cytokine gene expression and prostaglandin production in head kidney leukocytes isolated from Atlantic cod (*Gadus morhua*) added different levels of arachidonic acid and eicosapentaenoic acid. *Fish Shellfish Immunol.* **2013**, *34*, 770–777. [CrossRef]
19. Ponce, M.; Zuasti, E.; Anguís, V.; Fernández-Díaz, C. Effects of the sulfated polysaccharide ulvan from Ulva ohnoi on the modulation of the immune response in Senegalese sole (*Solea senegalensis*). *Fish Shellfish Immunol.* **2020**, *100*, 27–40. [CrossRef] [PubMed]
20. Tharuka, M.N.; Bathige, S.; Oh, M.; Lee, S.; Kim, M.; Priyathilaka, T.T.; Lee, J. Molecular characterization and expression analysis of big-belly seahorse (*Hippocampus abdominalis*) interleukin-10 and analysis of its potent anti-inflammatory properties in LPS-induced murine macrophage RAW 264.7 cells. *Gene* **2019**, *685*, 1–11. [CrossRef]
21. Peng, Y.; Cai, X.; Zhang, G.; Wang, J.; Li, Y.; Wang, Z.; Wang, B.; Xiong, X.; Wu, Z.; Jian, J. Molecular characterization and expression of interleukin-10 and interleukin-22 in golden pompano (*Trachinotus ovatus*) in response to Streptococcus agalactiae stimulus. *Fish Shellfish Immunol.* **2017**, *65*, 244–255. [CrossRef] [PubMed]
22. Chen, H.; Lin, H.; Foung, Y.; Lin, J.H. The bioactivity of teleost IL-6: IL-6 protein in orange-spotted grouper (*Epinephelus coioides*) induces Th2 cell differentiation pathway and antibody production. *Dev. Comp. Immunol.* **2012**, *38*, 285–294. [CrossRef] [PubMed]
23. Block, B.A.; Keen, J.E.; Castillo, B.; Dewar, H.; Freund, E.V.; Marcinek, D.J.; Brill, R.W.; Farwell, C. Environmental preferences of yellowfin tuna (*Thunnus albacares*) at the northern extent of its range. *Mar. Biol.* **1997**, *130*, 119–132. [CrossRef]
24. Zhang, N.; Yang, R.; Fu, Z.; Yu, G.; Ma, Z. Mechanisms of Digestive Enzyme Response to Acute Salinity Stress in Juvenile Yellowfin Tuna (*Thunnus albacares*). *Animals* **2023**, *13*, 3454. [CrossRef] [PubMed]
25. Zhou, S.; Zhang, N.; Fu, Z.; Yu, G.; Ma, Z.; Zhao, L. Impact of Salinity Changes on the Antioxidation of Juvenile Yellowfin Tuna (*Thunnus albacares*). *J. Mar. Sci. Eng.* **2023**, *11*, 132. [CrossRef]
26. Fonteneau, A. An overview of yellowfin tuna stocks, fisheries and stock status worldwide. In Proceedings of the IOTC 7th Working Party on Tropical Tunas, Phuket-Thailand, Phuket, Thailand, 18–22 July 2005.
27. Grewe, P.M.; Feutry, P.; Hill, P.L.; Gunasekera, R.M.; Schaefer, K.M.; Itano, D.G.; Fuller, D.W.; Foster, S.D.; Davies, C.R. Evidence of discrete yellowfin tuna (*Thunnus albacares*) populations demands rethink of management for this globally important resource. *Sci. Rep.* **2015**, *5*, 1–9. [CrossRef] [PubMed]
28. Andamari, R.; Hutapea, J.H.; Prisantoso, B.I. Reproduction aspects of the yellowfin tuna (*Thunnus albacares*). *J. Ilmu Teknol. Kelaut.* **2012**, *4*, 1.
29. Estess, E.E.; Klinger, D.H.; Coffey, D.M.; Gleiss, A.C.; Rowbotham, I.; Seitz, A.C.; Rodriguez, L.; Norton, A.; Block, B.; Farwell, C. Bioenergetics of captive yellowfin tuna (*Thunnus albacares*). *Aquaculture* **2017**, *468*, 71–79. [CrossRef]
30. Pecoraro, C.; Babbucci, M.; Franch, R.; Rico, C.; Papetti, C.; Chassot, E.; Bodin, N.; Cariani, A.; Bargelloni, L.; Tinti, F. The population genomics of yellowfin tuna (*Thunnus albacares*) at global geographic scale challenges current stock delineation. *Sci. Rep.* **2018**, *8*, 13890. [CrossRef]
31. He, Y.; Fu, Z.; Dai, S.; Yu, G.; Ma, Z. Dietary curcumin supplementation can enhance health and resistance to ammonia stress in the greater amberjack (*Seriola dumerili*). *Front. Mar. Sci.* **2022**, *9*, 961783. [CrossRef]
32. Wang, W.; Dai, S.; Liu, L.; Fu, Z.; Yang, R.; Yu, G.; Ma, Z.; Zong, H. Daily Rhythmicity of Muscle-Related and Rhythm Genes Expression in Mackerel Tuna (*Euthynnus affinis*). *Biology* **2023**, *12*, 1211. [CrossRef]
33. Fu, W. Comparison of Histological and Transcriptome Studies of the Gills between Juvenile and Adult Yellowfin Tuna (*Thunnus albacares*) from the South China Sea. Master's Thesis, Hainan University, Haikou, China, 2023.
34. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* **2001**, *25*, 402–408. [CrossRef]
35. Hamad, A.R. Physiological and Cellular Responses of Marine Medaka Fish (*Oryzias dancena*) to Ammonia Stress Treatment. Ph.D. Dissertation, Pukyong National University, Busan, Republic of Korea, 2018.
36. Hamed, S.B.; Guardiola, F.; Cuesta, A.; Martínez, S.; Martínez-Sánchez, M.J.; Pérez-Sirvent, C.; Esteban, M.Á. Head kidney, liver and skin histopathology and gene expression in gilthead seabream (*Sparus aurata* L.) exposed to highly polluted marine sediments from Portman Bay (Spain). *Chemosphere* **2017**, *174*, 563–571. [CrossRef]
37. Zwollo, P.; Cole, S.; Bromage, E.; Kaattari, S. B cell heterogeneity in the teleost kidney: Evidence for a maturation gradient from anterior to posterior kidney. *J. Immunol.* **2005**, *174*, 6608–6616. [CrossRef] [PubMed]
38. Hong, J.; Fu, Z.; Hu, J.; Zhou, S.; Yu, G.; Ma, Z. Dietary curcumin supplementation enhanced ammonia nitrogen stress tolerance in greater amberjack (*Seriola dumerili*): Growth, serum biochemistry and expression of stress-related genes. *J. Mar. Sci. Eng.* **2022**, *10*, 1796. [CrossRef]
39. Mani, R.; Rose, S.; Suresh, A.; Sambantham, S.; Anandan, B.; Ibrahim, M.; Meena, B. Cellular alterations and damage to the renal tissue of marine catfish *Arius arius* following Cd exposure and the possible sequestrant role of Metallothionein. *Mar. Pollut. Bull.* **2021**, *163*, 111930. [CrossRef]
40. Ching, B.; Chew, S.F.; Wong, W.P.; Ip, Y.K. Environmental ammonia exposure induces oxidative stress in gills and brain of *Boleophthalmus boddarti* (mudskipper). *Aquat. Toxicol.* **2009**, *95*, 203–212. [CrossRef] [PubMed]
41. Guo, M.; Yan, Q.; Dong, Y.; Ding, Z.; Mei, J.; Xie, J. Apoptotic Changes, Oxidative Stress and Immunomodulatory Effects in the Liver of Japanese Seabass (*Lateolabrax japonicus*) Induced by Ammonia-Nitrogen Stress during Keep-Live Transport. *Biology* **2023**, *12*, 769. [CrossRef] [PubMed]

42. Sun, H.; Lü, K.; Minter, E.J.; Chen, Y.; Yang, Z.; Montagnes, D.J. Combined effects of ammonia and microcystin on survival, growth, antioxidant responses, and lipid peroxidation of bighead carp *Hypophthalmythys nobilis* larvae. *J. Hazard. Mater.* **2012**, *221*, 213–219. [CrossRef]
43. Zhang, Z.; Liu, Q.; Cai, J.; Yang, J.; Shen, Q.; Xu, S. Chlorpyrifos exposure in common carp (*Cyprinus carpio* L.) leads to oxidative stress and immune responses. *Fish Shellfish. Immunol.* **2017**, *67*, 604–611. [CrossRef]
44. Rahman, M.F.; Siddiqui, M. Biochemical effects of vepacide (from *Azadirachta indica*) on Wistar rats during subchronic exposure. *Ecotoxicol. Environ. Saf.* **2004**, *59*, 332–339. [CrossRef] [PubMed]
45. Liu, Y.; Hu, J.; Zhou, S.; Yang, R.; Qin, J.G.; Ma, Z.; Yang, Q. Effect of acute ammonia stress on antioxidant enzymes and digestive enzymes in barramundi *Lates calcarifer* larvae. *Isr. J. Aquac.-Bamidgeh* **2018**, *70*, 70–2018. [CrossRef]
46. Gao, Y.; Gao, Y.; Wang, J.; Li, M.; Jia, Y.; Mend, Z. Effect of acute ammonia stress on the plasma biochemicalindexes of *Sebastes schlegelii*. *Mar. Sci.* **2023**, *47*, 49–59.
47. Li, M.; Wang, S.; Zhao, Z.; Luo, L.; Zhang, R.; Guo, K.; Zhang, L.; Yang, Y. Effects of alkalinity on the antioxidant capacity, nonspecific immune response and tissue structure of Chinese Mitten Crab *Eriocheir sinensis*. *Fishes* **2022**, *7*, 206. [CrossRef]
48. Sinha, A.K.; AbdElgawad, H.; Zinta, G.; Dasan, A.F.; Rasoloniriana, R.; Asard, H.; Blust, R.; De Boeck, G. Nutritional status as the key modulator of antioxidant responses induced by high environmental ammonia and salinity stress in European sea bass (*Dicentrarchus labrax*). *PLoS ONE* **2015**, *10*, e135091. [CrossRef] [PubMed]
49. Cheng, C.; Guo, Z.; Luo, S.; Wang, A. Effects of high temperature on biochemical parameters, oxidative stress, DNA damage and apoptosis of pufferfish (*Takifugu obscurus*). *Ecotoxicol. Environ. Saf.* **2018**, *150*, 190–198. [CrossRef] [PubMed]
50. Hegazi, M.M.; Attia, Z.I.; Ashour, O.A. Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure. *Aquat. Toxicol.* **2010**, *99*, 118–125. [CrossRef] [PubMed]
51. Zhao, Y.; Wang, H.; Zhou, J.; Shao, Q. Glutathione peroxidase GPX1 and its dichotomous roles in cancer. *Cancers* **2022**, *14*, 2560. [CrossRef] [PubMed]
52. Liang, X.; Wang, F.; Li, K.; Nie, X.; Fang, H. Effects of norfloxacin nicotinate on the early life stage of zebrafish (*Danio rerio*): Developmental toxicity, oxidative stress and immunotoxicity. *Fish Shellfish Immunol.* **2020**, *96*, 262–269. [CrossRef]
53. Saraiva, M.; O'Garra, A. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* **2010**, *10*, 170–181. [CrossRef]
54. Uciechowski, P.; Dempke, W. Interleukin-6: A masterplayer in the cytokine network. *Oncology* **2020**, *98*, 131–137. [CrossRef] [PubMed]
55. Baldissera, M.D.; Souza, C.F.; Boaventura, T.P.; Nakayama, C.L.; Baldisserotto, B.; Luz, R.K. Purinergic signaling as a potential target of hypoxia stress-induced impairment of the immune system in freshwater catfish *Lophiosilurus alexandri*. *Aquaculture* **2018**, *496*, 197–202. [CrossRef]
56. Li, M.; Lai, H.; Li, Q.; Gong, S.; Wang, R. Effects of dietary taurine on growth, immunity and hyperammonemia in juvenile yellow catfish *Pelteobagrus fulvidraco* fed all-plant protein diets. *Aquaculture* **2016**, *450*, 349–355. [CrossRef]