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Effects of *Ulva prolifera* Degradation on Growth Performance and Antioxidant Capacity of Japanese Flounder (*Paralichthys olivaceus*) Family

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Abstract: Massive macroalgae blooms, primarily caused by the overgrowth of *Ulva prolifera* (*U. prolifera*) in the Yellow Sea of China, pose a severe risk to both marine organisms and the aquaculture industry. This study's aim was to evaluate the impact of *U. prolifera* degradation on the growth performance and antioxidant capacity of Japanese flounder (*Paralichthys olivaceus*) and select some potential Japanese flounder families (labeled 2101–2108, established by crossbreeding) tolerating *U. prolifera* degradation conditions. Thus, a 60-day *U. prolifera* exposure experiment was conducted. The results showed that the contents of Na, K, Mg, and Fe elements in the *U. prolifera* degradation water were significantly increased. The specific growth rate and survival rate of flounder were significantly decreased under the *U. prolifera* degradation condition, while the 2101 and 2103 flounder families showed a better growth performance compared with the positive control (PC) group. Moreover, the results showed that activities of total antioxidant capacity (T-AOC), transaminases, and alkaline phosphatase (AKP) in serum were significantly decreased, while the 2103 flounder family showed higher activities. Furthermore, *U. prolifera* degradation significantly increased superoxide dismutase (SOD) activity and glutathione (GSH) content while decreasing catalase (CAT) activity and malondialdehyde (MDA) content in the liver. Specifically, SOD and CAT activities of the 2103 flounder family were higher than the 2101 flounder family and PC group. In addition, the gill SOD and CAT activities of the 2103 flounder family were significantly higher than the PC group. Similarly, the antioxidant-related gene (*sod* and *cat*) expressions were synchronously upregulated or downregulated in the liver and gills in response to *U. prolifera* degradation. These results revealed that *U. prolifera* degradation decreased the growth performance and influenced the antioxidant capacity of Japanese flounder, while the 2103 flounder family had better advantages in the *U. prolifera* degradation condition. Therefore, the 2103 flounder family could be regarded as the potential flounder family tolerating *U. prolifera* degradation. The increased Fe content in the *U. prolifera* degradation water may be one of the main causes of the physiological alterations observed in Japanese flounder.

Keywords: *Ulva prolifera*; growth performance; antioxidant capacity; family; *Paralichthys olivaceus*

Key Contribution: *U. prolifera* degradation decreased the growth performance and influenced antioxidant capacity of Japanese flounder, while the 2103 flounder family had better advantages in the *U. prolifera* degradation conditions.

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1. Introduction

In recent years, coastal areas worldwide have seen an upsurge in macroalgal blooms driven by water eutrophication and global climate change. For the past 15 years (2007–2021),

the extensive proliferation of macroalgae, primarily attributed to the excessive growth of *Ulva prolifera* (*U. Prolifera*), has consistently plagued the Yellow Sea coastline [1,2]. The proliferation of *U. prolifera* fronds has a detrimental impact on the aquaculture industry and marine fisheries, leading to substantial economic losses, estimated at 1.3 billion RMB [3]. The natural decomposition of *U. prolifera* releases substantial quantities of biogenic elements, such as carbon (C), nitrogen (N), phosphorus (P), sulfur (S), and metallic elements, such as iron (Fe). These released elements alter the chemical properties of the local seawater, resulting in hypoxia and increased metal ion content. Such changes further foster microbial growth and consequently disrupt the survival of marine organisms [4–6]. Hence, understanding the effects of *U. prolifera* blooms on marine fish is of paramount importance.

The potential Impact of *U. prolifera* degradation on biological pathways that contribute to various pathogenic infections in marine organisms has been observed [7,8]. A common characteristic observed in these diseases is an increase in oxidative stress levels [9]. Oxidative stress arises from an imbalance in the production and detoxification of reactive oxygen species (ROS) within the cells. Furthermore, the elevated ROS levels can cause irreversible damage to lipids, proteins, and nucleic acids. Specifically, lipid peroxidation leads to the formation of malondialdehyde (MDA), which is a primary contributor to cellular damage and mortality. Concurrently, antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), along with intracellular thiols, such as glutathione (GSH), play a crucial role in maintaining cellular redox balance by eliminating excess ROS [10–12]. The modulation of oxidation-related biomarker activities represents an initial response to counteract oxidative stress and can be utilized to evaluate the impact of pollution on fish [13]. However, further investigation is required to explore the effects and mechanisms of *U. prolifera* degradation on the antioxidant capacity of marine fish.

Japanese flounder (*Paralichthys olivaceus)*, a prevalent benthic economic species distributed in the Yellow Sea [14], is highly susceptible to diverse environmental pollutants, such as cadmium, arsenic, and macroalgae [15–17]. The degradation of *U. prolifera*, including metallic element Fe, was discharged into coastal seawaters, which could influence the spawning and nursery grounds of marine fish [18]. Thus, it is important to breed flounder families to tolerate *U. prolifera* degradation. This study aimed to investigate the effects of *U. prolifera* degradation on the growth performance and antioxidant capacity of Japanese flounder and select some potential Japanese flounder families with an enhanced tolerance of *U. prolifera* degradation. This study advanced the understanding of the effects of *U. prolifera* degradation on the marine fish, Japanese flounder, and promoted the sustainable development of the factory farming of aquaculture.

2. Materials and Methods

The experimental design adhered to the guidelines presented in the Regulations of the Laboratory Animal—Guideline for Ethical Review of Animal Welfare (Standardization Administration of China, 2018). Additionally, the animal care and use committee of the Yellow Sea Fisheries Research Institute thoroughly reviewed and granted approval for the experimental protocol (YSFRI-2023021). Throughout the experiment, we took great care to uphold the principles of reduction, replacement, and refinement.

2.1. Experimental Design

The normal Japanese flounder (initial average weight of 2.5 $\pm$ 0.02 g) cultured in normal seawater without *U. prolifera* degradation was considered as the negative control group (NC), while the normal Japanese flounder exposed in the *U. prolifera* degradation conditions was designated as the positive control group (PC). Eight experimental Japanese flounder families derived from the offspring of anti-vibrio breeders were generated in our lab using partial mating scheme and raised in Yellow Sea Aquatic Product Co., Ltd. (Yantai, China) [19]. The families (2101–2108) were named based on the breeding cultivation time and were established through crossbreeding by the way of artificial insemination to select and breed the better variety of Japanese flounder. Before the main experiment, a total of

150 individuals per treatment were randomly selected and separated into three replicates. Each individual was tagged with the visible implant elastomer (obtained from Qingdao starfish Instruments Co., Ltd., Qingdao, China).

The water environment of *U. prolifera* degradation was collected from Haiyang Beach (the Yellow Sea, Yantai, China) during the bloomed period. Then, the water was filtered through a quartz filtration system to remove remaining *U. prolifera* particulates and was stored in 6 tanks (d = 250 cm, h = 120 cm, volume = 6000 L) for further exposure experiments. All Japanese flounder were acclimated in normal seawater (salinity: 28–33‰, dissolved oxygen: 5.5–7.0 mg/L, water temperature: 24–28 °C, and pH: 7.5–7.7) for 7 days to adapt to the aquaculture environment. After acclimation, the selected 8 experimental Japanese flounder families and PC group were housed in a $4m^3$ opaque tank (450 fish per tank) with 2 m^3 of *U. prolifera* degradation seawater provided by water circulating for 60 days (salinity: 28–33‰, dissolved oxygen: 5.5–7.0 mg/L, water temperature: 24–28 °C, and pH: 7.30–7.45). During the trial, Japanese flounder were fed with suited commercial feed (Crude protein 52%, Crude lipid 8%; Santong Chubu Feed Co. Ltd., Weifang, China). On a daily basis, the physical condition of the fish was monitored, and the rate of mortality was documented.

2.2. Sampling Collection

At the end of the experiment, the flounder were conducted through a 24 h period of starvation followed by anesthesia using MS222 (1:10,000 diluted in water; Sigma, St. Louis, MO, USA). Subsequently, the survival rate (SR) was determined by assessing the fish count in each fiberglass tank and measuring their weight and total length. Additionally, for serum collection, blood samples were collected from six fish per treatment (enough to obtain the 80% chance of detecting a difference in a parameter) [20], which were later subjected to centrifugation. The serum was then stored at −80 °C for future biochemical analysis purposes. Moreover, nine fish from each family were randomly selected and had samples of liver and gill obtained from them to conduct antioxidant capacity and gene expression analysis. The samples were thoroughly rinsed with phosphate buffer before being stored in 1.5 mL Eppendorf tubes on ice. To preserve their integrity, the tubes were subsequently frozen using liquid nitrogen and stored at −80 °C.

2.3. Chemical Analysis

In order to evaluate the concentration of major metal ions in the experimental solutions, 100 mL of water were extracted from two different test conditions. The water samples were filtered using a mixed fiber filter membrane with a pore size of 0.45 μM. After filtration, the samples were then transferred to polyethylene bottles and stored at 4 °C. In order to ascertain the level of iron ions, the water samples were approached for the application of inductively coupled plasma mass spectrometry (ICP-MS) using the Elan DRC II instrument (Perkin Elmer, Waltham, MA, USA). The levels of Fe in the degradation water of *U. prolifera* were detected through an oxidizer reaction involving Dichloroisocyanuric acid and Trichloroisocyanuric acid.

2.4. Biochemical and Antioxidant Capacity Analysis

Serum samples were analyzed for total antioxidant capacity (T-AOC), glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), and alkaline phosphatase (AKP) using assay kits from Nanjing Jiancheng Corp., Nanjing, China. Liver and gill tissues were also assessed for malondialdehyde (MDA), catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD) levels. Before the analysis of enzyme activity, 0.1 g of frozen tissue samples were ground in a solution of 0.9% NaCl in a ratio of 1:9. Then, the homogenates were centrifugated at 4000 rpm for 15 min at 4 °C. The resulting supernatants were then transferred to a fresh centrifuge tube. These analyses were performed following the manufacturer's instructions provided by Nanjing Jiancheng Corp., Nanjing, China. The enzyme activities were finally corrected with the protein concentration of samples.

2.5. Real-Time Quantitative PCR (RT-qPCR) Analysis

To extract the total RNA, tissues were first pulverized using liquid nitrogen and then followed by the addition of Trizol reagent (Takara, Tokyo, Japan). The quality of RNA was evaluated by utilizing a 1.2% denaturing agarose gel, and the concentration of total RNA was measured using the NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The RNA underwent a DNA removal process to eliminate any potential DNA contamination, and subsequently, it was reverse transcribed into cDNA with the PrimeScript™ RT reagent Kit (Takara, Tokyo, Japan) following the manufacturer's instructions. The primers utilized in the experiment were available in Table 1. The RT-qPCR reactions were conducted three times, with a total volume of 20 µL (10 µL SYBR mix, 2 µL cDNA, 1 µL each primer, and 6 µL RNase-free H2O). The RT-qPCR program included an initial denaturation step at 95 °C for 30 s followed by 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 31 s. These reactions were performed using an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster, Waltham, MA, USA). The level of gene transcription was determined using the CT values of each selected gene based on the $2^{-\Delta\Delta CT}$ method, which was then normalized with *β-actin* [21].

Table 1. Primer information used for RT-qPCR in this study.

Gene	Sequence (5′–3′)	GenBank Accession Number
β-actin-F	GGAAATCGTGCGTGACATTAAG	XM_020109620.1
β-actin-R	CCTCTGGACAACGGAACCTCT	
sod-F	CGTTGGAGACCTGGGGAATGTG	EF681883.1
sod-R	ATCGTCAGCCTTCTCGTGGATC	
cat-F	CACGGACCAGATGAAGCAGTG	XM_020079314.1
cat-R	CCTTGGAGTAGCGGGTAATGTC	

2.6. Calculations and Statistical Analysis

Survival rate (SR%) = Nt × 100/N0

Specific growth rate (SGR, % day^{-1}) = (Ln Wt − Ln W0) × 100/t

Nt and N0 were final and initial fish number, respectively; Wt and W0 were final and initial fish weight, respectively; t was period of experimental days.

For statistical analysis, all data underwent evaluation in SPSS 23.0, a software developed by IBM in America. Various statistical tests, including independent sample t-tests or one-way analysis of variance (ANOVA), were performed, followed by Tukey's multiple-range test to determine differences between groups. Statistical significance was considered at a level of $p < 0.05$. Results were presented as means ± S.E.M (standard error of the mean).

3. Results

3.1. Metal Element Parameters of the Water Solution

First, the metal element parameters of *U. prolifera* degradation water were detected. In Table 2, the contents of Na, K, Mg, and Fe elements in the *U. prolifera* degradation water were significantly increased compared with the normal seawater ($p < 0.05$), respectively, while the Ca content showed no significant difference ($p > 0.05$). In addition, the chemical reaction test of the Fe element in the water solution was carried out. The results showed that the color of *U. prolifera* degradation water sample was turned to yellow (Figure 1).

3.2. Survival Rate and Growth Performance

In Table 3, *U. prolifera* degradation condition significantly decreased the survival rate (SR) and specific growth rate (SGR) compared with the NC group ($p < 0.05$), while SR of eight flounder families were higher than the PC group (48.0 ± 3.46%), especially 2101 (77.3 ± 9.24%) and 2103 (75.3 ± 1.15%) flounder families ($p < 0.05$). However, the SGR of eight flounder families had no significant differences compared with the PC group ($p > 0.05$).

Table 2. The content of five metal elements in two water environments.

Index	Types		
	UG	NC	*p* Value
Fe (μg/L)	7.4 ± 0.79 *	5.4 ± 0.17	0.013
Ca (mg/L)	330.3 ± 1.16	375.0 ± 29.46	0.12
K (mg/L)	439.0 ± 3.67 **	378.7 ± 18.56	0.005
Mg (mg/L)	1075.7 ± 4.04 **	970.7 ± 25.54	0.002
Na (mg/L)	9904.7 ± 65.07 **	8953.7 ± 258.72	0.000

Notes: Data are presented as means ± S.E.M. and are analyzed using independent *t*-tests. * $p < 0.05$ and ** $p < 0.01$ indicate significant differences compared with the control group. UG: *U. prolifera* degradation treatment group; NC: negative control group.

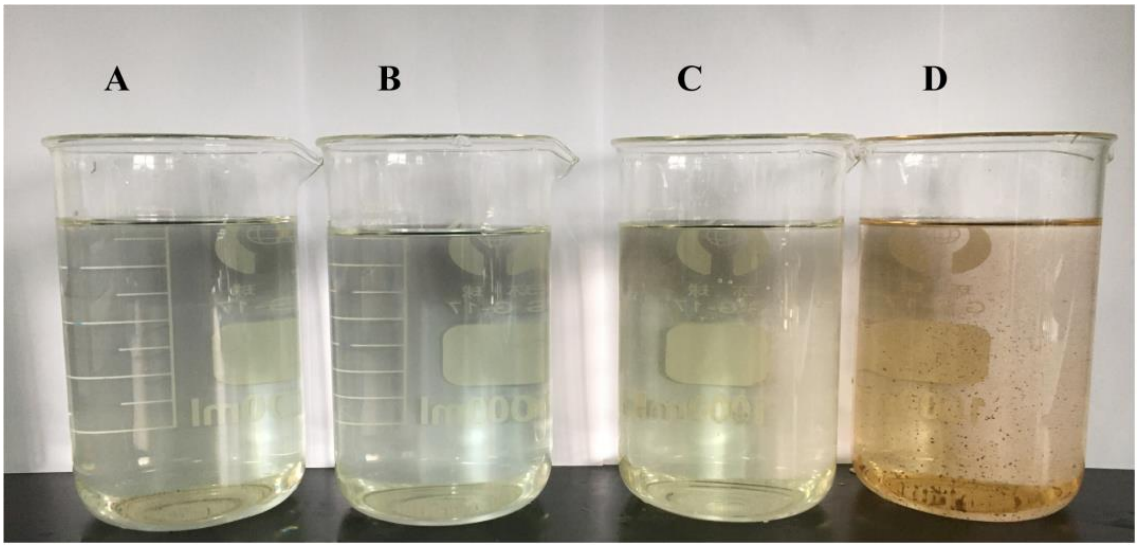

Figure 1. Chemical reaction result of Fe ion in normal seawater and *U. prolifera* degradation water environments. (**A**): normal seawater; (**B**): U. prolifera degradation water; (**C**): chemical reaction result of Fe ion in normal seawater; (**D**): chemical reaction result of Fe ion in *U. prolifera* degradation water.

Table 3. Growth performance parameters and survival rate of the Japanese flounder cultivated in different environments.

Group	IBW (g)	FBW (g)	SR (%)	SGR (%/day)
NC	2.5 ± 0.02	52.9 ± 0.87 ^{a}	88.7 ± 1.16 ^{a}	5.1 ± 0.04 ^{a}
PC	2.5 ± 0.01	40.8 ± 0.64 ^{bc}	48.0 ± 3.46 ^{c}	4.6 ± 0.01 ^{bc}
2101	2.5 ± 0.01	42.7 ± 1.83 ^{bc}	77.3 ± 9.24 ^{ab}	4.8 ± 0.07 ^{b}
2102	2.5 ± 0.01	44.6 ± 3.1 ^{b}	53.3 ± 9.87 ^{c}	4.8 ± 0.12 ^{b}
2103	2.5 ± 0.03	43.2 ± 2.29 ^{bc}	75.3 ± 1.15 ^{ab}	4.8 ± 0.04 ^{b}
2104	2.5 ± 0.01	43.6 ± 1.89 ^{b}	58.7 ± 4.16 ^{bc}	4.8 ± 0.7 ^{b}
2105	2.5 ± 0.01	42.2 ± 3.32 ^{bc}	56.0 ± 15.62 ^{bc}	4.7 ± 0.14 ^{bc}
2106	2.5 ± 0.01	39.9 ± 4.68 ^{bc}	63.3 ± 8.08 ^{bc}	4.6 ± 0.19 ^{bc}
2107	2.5 ± 0.02	36.6 ± 1.04 ^{c}	61.3 ± 10.07 ^{bc}	4.5 ± 0.04 ^{c}
2108	2.5 ± 0.02	38.3 ± 1.87 ^{bc}	52.7 ± 4.16 ^{c}	4.6 ± 0.08 ^{bc}

Notes: Data are presented as means ± S.E.M. and are analyzed using one-way ANOVA followed by Tukey's test (n = 6). Data labeled the same letters are not significantly different ($p > 0.05$). NC group: negative control group; PC group: positive control group; 2101–2108: 8 Japanese flounder families; IBW: initial body weight; FBW: final body weight; SR: survival rate; SGR: specific growth rate.

3.3. Serum Biochemical Indexes

In the present study, a significant decrease in T-AOC in the serum was observed compared with the NC group ($p < 0.05$) in *U. prolifera* degradation conditions, while the T-AOC content of 2101 and 2103 flounder families was significantly higher than the PC group

($p < 0.05$) (Figure 2A). In addition, *U. prolifera* degradation also significantly decreased GPT, GOT, and AKP activities compared with the NC group ($p < 0.05$), while these enzyme activities of 2103 flounder family were significantly higher than the PC group ($p < 0.05$) (Figure 2B–D). The 2101 flounder family had no significant difference compared with the PC group ($p > 0.05$) (Figure 2B–D).

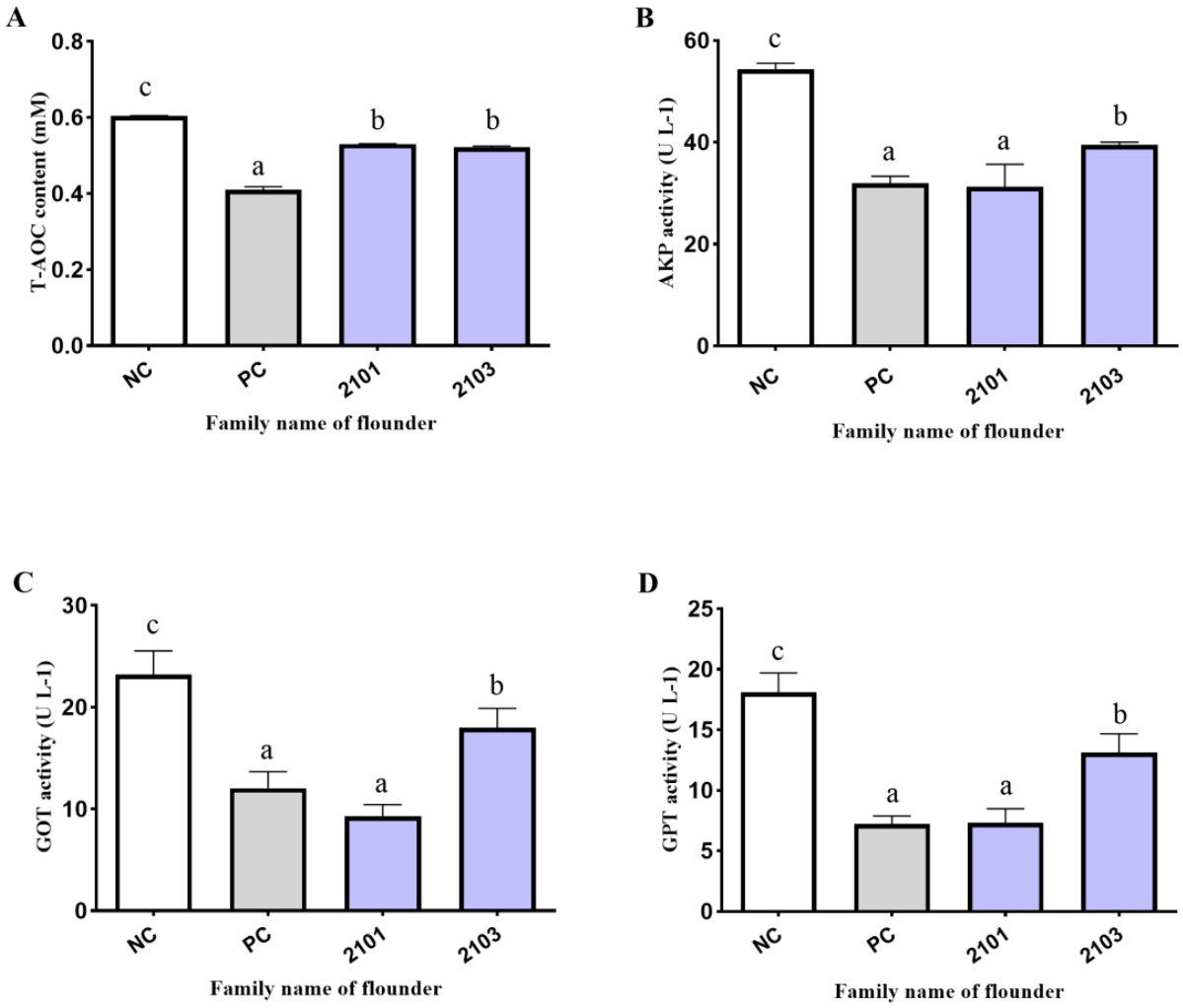

Figure 2. Effects of *U. prolifera* degradation on the serum biochemical indexes and enzyme activities ((**A**): T-AOC, (**B**): AKP, (**C**): GOT, and (**D**): GPT) of Japanese flounder. NC group: negative control group; PC group: positive control group; 2101 and 2103: 2 Japanese flounder families. Data are presented as means ± S.E.M. and are analyzed using one-way ANOVA followed by Tukey's test (n = 6). Data labeled with the same letters are not significantly different ($p > 0.05$).

3.4. Antioxidant Enzyme Activity

The effects of *U. prolifera* degradation on the antioxidant capacity in the liver and gills of Japanese flounder were detected. The results showed that the SOD activity was significantly increased in the liver compared with the NC group ($p < 0.05$) (Figure 3A). Meanwhile, the SOD activity of the 2103 flounder family was significantly higher than the 2101 flounder family and PC group in the liver ($p < 0.05$), while the SOD activity of the 2101 flounder family had no significant difference compared with PC group ($p > 0.05$) (Figure 3A). In the liver, the CAT activity was notably reduced compared to the NC group ($p < 0.05$), while the CAT activity of the 2103 flounder family was significantly higher than the 2101 flounder family and PC group ($p < 0.05$) (Figure 3B). Moreover, *U. prolifera* degradation also significantly increased liver GSH content compared with the NC group

($p < 0.05$), while the GSH content of the 2101 and 2103 flounder families had no significant difference compared with the PC group, respectively ($p > 0.05$) (Figure 3C). In addition, *U. prolifera* degradation significantly decreased the liver MDA content compared with the NC group ($p < 0.05$), and the MDA content of the 2101 and 2103 flounder families were significantly lower than the PC group ($p < 0.05$) (Figure 3D).

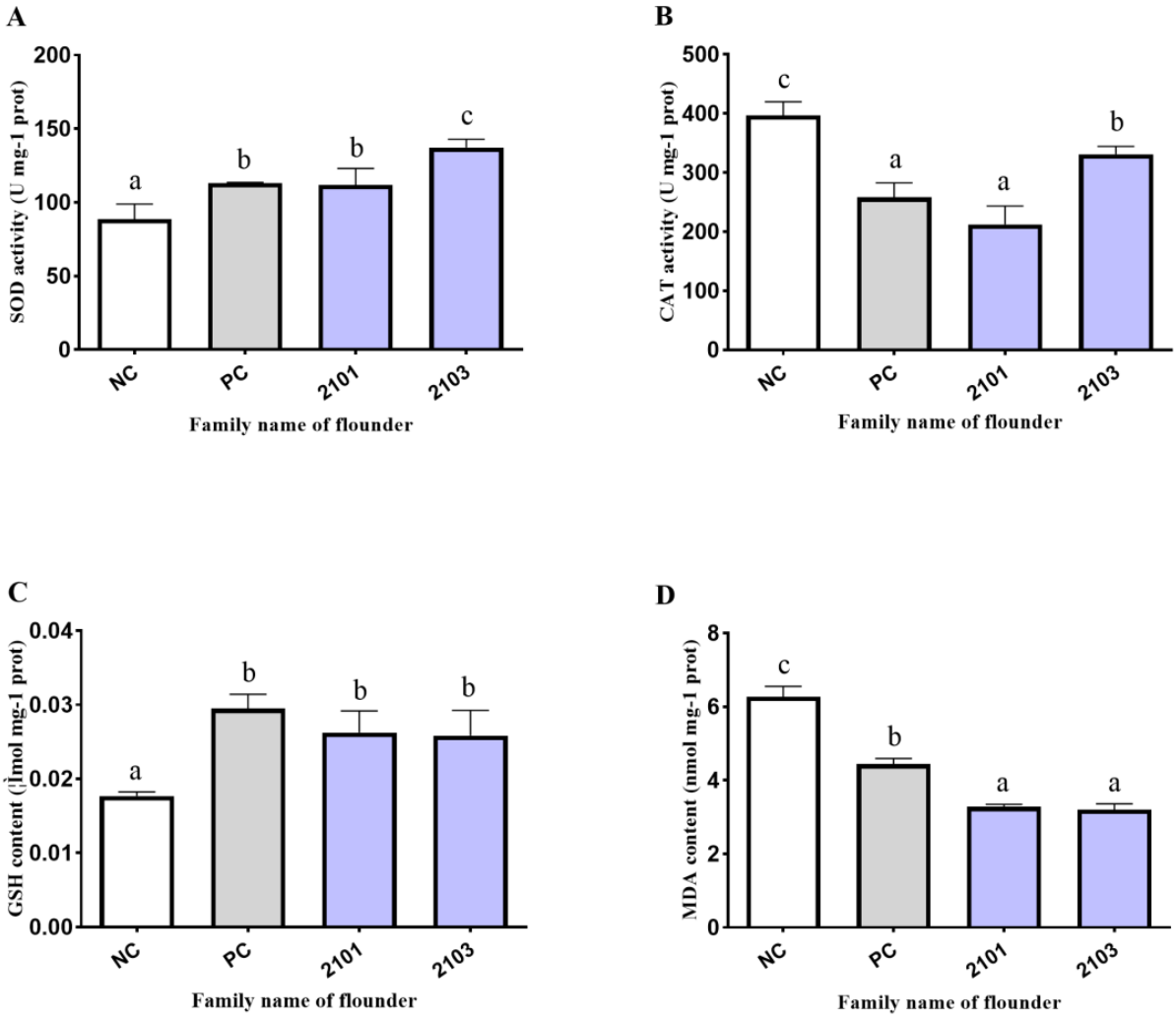

Figure 3. Effects of *U. prolifera* degradation on antioxidant capacity in liver of Japanese flounder. (**A**,**B**): enzyme activities of SOD and CAT. (**C**,**D**): contents of GSH and MDA. NC group: negative control group; PC group: positive control group; 2101 and 2103: 2 Japanese flounder families. Data are presented as means ± S.E.M. and are analyzed using one-way ANOVA followed by Tukey's test (n = 6). Data labeled the same letters are not significantly different ($p > 0.05$).

In the gills, *U. prolifera* degradation significantly increased the SOD activity compared with the NC group ($p < 0.05$), and the SOD activity of the 2103 flounder family was significantly higher than the 2101 flounder family and PC group ($p < 0.05$) (Figure 4A). In addition, *U. prolifera* degradation significantly increased the CAT activity of the 2103 flounder family in the gills, while the CAT activity of the 2101 flounder family was significantly decreased ($p < 0.05$) (Figure 4B). In addition, the GSH content of the 2103 flounder family was significantly increased in the gills compared with the NC group ($p < 0.05$) and higher than the 2101 flounder family and PC group ($p < 0.05$) (Figure 4C). The GSH content of the 2101 flounder family had no significant difference compared with the NC and PC group ($p > 0.05$) (Figure 4C). However, *U. prolifera* degradation has no significant effect on the MDA content of Japanese flounder ($p > 0.05$) (Figure 4D)

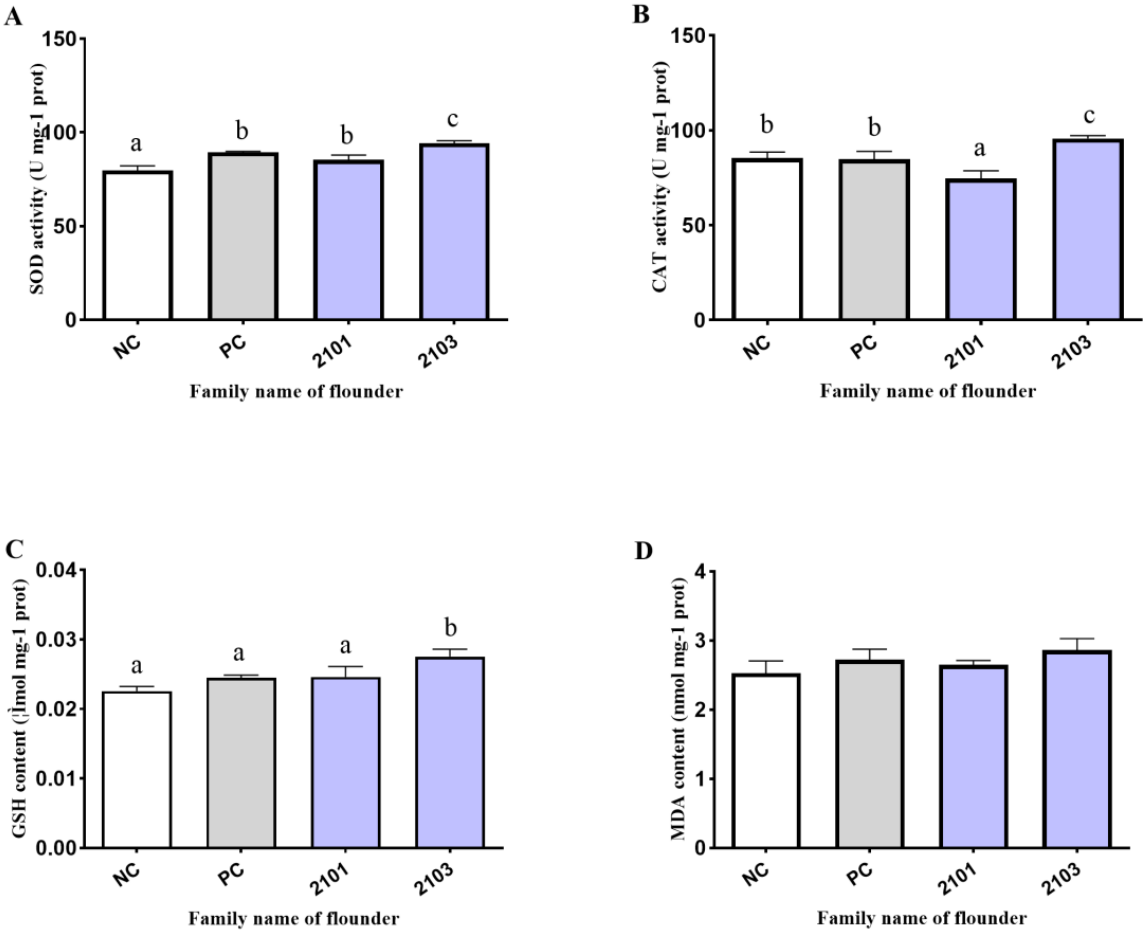

Figure 4. Effects of *U. prolifera* degradation on antioxidant capacity in gills of Japanese flounder. (**A**,**B**): enzyme activities of SOD and CAT. (**C**,**D**): contents of GSH and MDA. NC group: negative control group; PC group: positive control group; 2101 and 2103: 2 Japanese flounder families. Data are presented as means ± S.E.M. and are analyzed using one-way ANOVA followed by Tukey's test (n = 6). Data labeled the same letters and no letters are not significantly different ($p > 0.05$).

3.5. Antioxidant-Related Genes Expression

The effects of *U. prolifera* degradation on mRNA expression level of antioxidant-related genes in the liver and gills were further detected. The results showed that *U. prolifera* degradation significantly upregulated *sod* mRNA expression compared with the NC group in the liver ($p < 0.05$), and the *sod* mRNA expression of the 2101 and 2103 flounder families were significantly higher than the PC group ($p < 0.05$) (Figure 5A). In addition, *U. prolifera* degradation significantly downregulated the *cat* mRNA expression compared with the NC group in the liver ($p < 0.05$), while the *cat* mRNA expression of the 2103 flounder family was significantly higher than the 2101 flounder family and PC group ($p < 0.05$) (Figure 5B).

In the gills, *U. prolifera* degradation significantly upregulated the *sod* mRNA expression compared with the NC group ($p < 0.05$), while the *sod* mRNA expression of the 2103 flounder family was significantly higher than the 2101 flounder family and PC group ($p < 0.05$) (Figure 6A). The *sod* mRNA expression of the 2101 flounder family had no significant difference compared with the PC group ($p > 0.05$) (Figure 6A). Meanwhile, the *cat* mRNA expression of the 2103 flounder family was significantly higher than the 2101 flounder family, PC group, and NC group in the gills ($p < 0.05$) (Figure 6B), while the *cat* mRNA expression of 2101 flounder family was significantly lower than PC group and NC group in gill ($p < 0.05$) (Figure 6B).

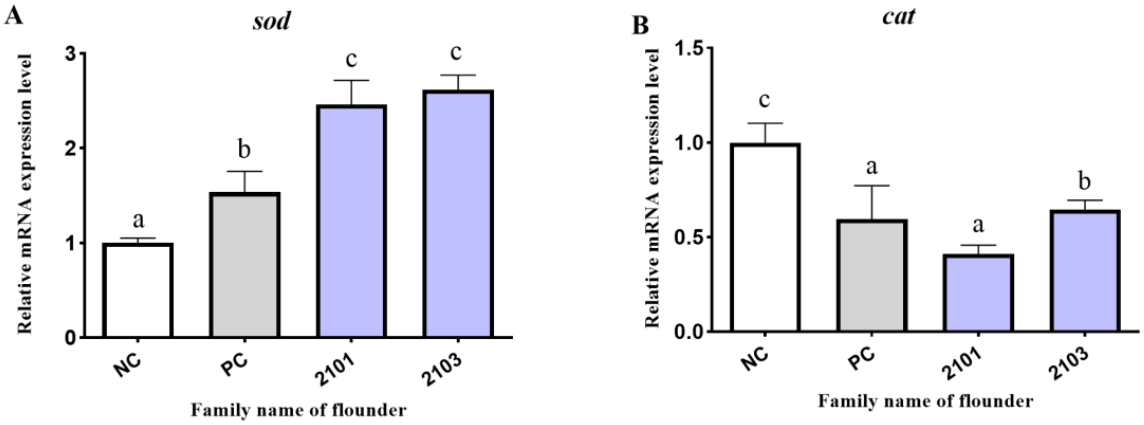

Figure 5. Effects of *U. prolifera* degradation on the mRNA expression of antioxidant-related genes ((**A**): *sod* and (**B**): *cat*) in liver of Japanese flounder. NC group: negative control group; PC group: positive control group; 2101 and 2103: 2 Japanese flounder families. Data are presented as means ± S.E.M. and are analyzed using one-way ANOVA followed by Tukey's test (n = 6). Data labeled the same letters are not significantly different ($p > 0.05$).

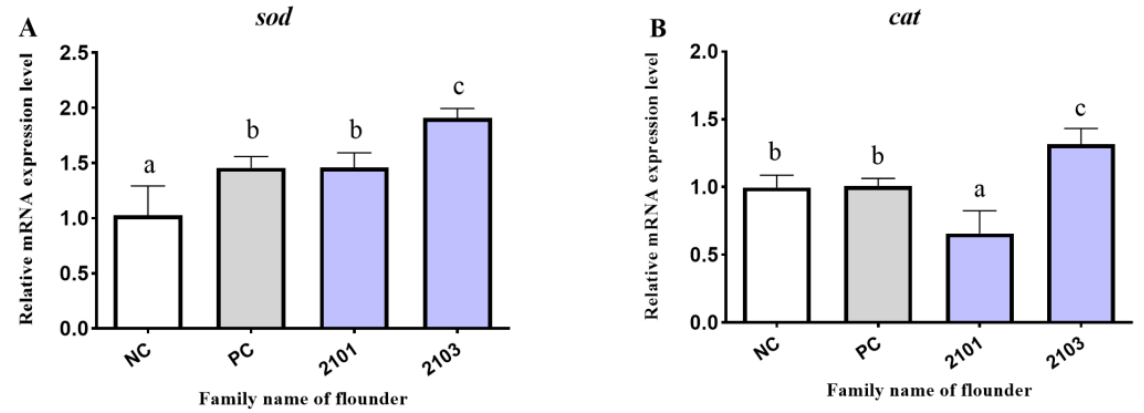

Figure 6. Effects of *U. prolifera* degradation on the mRNA expression of antioxidant-related genes ((**A**): *sod* and (**B**): *cat*) in gills of Japanese flounder. NC group: negative control group; PC group: positive control group; 2101 and 2103: 2 Japanese flounder families. Data are presented as means ± S.E.M. and are analyzed using one-way ANOVA followed by Tukey's test (n = 6). Data labeled the same letters are not significantly different ($p > 0.05$).

4. Discussion

Driven by water eutrophication and global climate change, *U. prolifera* blooms in coastal areas of the Yellow Sea of China have become increasingly common in recent years [1]. The degradation of *U. prolifera* not only disrupts marine ecosystems, but also significantly impacts aquaculture [22–24]. Therefore, it is crucially important to detect the effects of *U. prolifera* degradation on the growth performance and antioxidant capacity of Japanese flounder (*Paralichthys olivaceus*). In the present study, we first detected the element composition of the *U. prolifera* degradation water sample. The total Na, K, Mg, and Fe contents of *U. prolifera* degradation water were markedly higher than that of the normal seawater, with noticeable changes in color during chemical tests. Previous studies have shown that the total macro-, micro-, and trace-element contents of *Enteromorpha* species were higher than the values reported for some edible land vegetables and seaweeds, especially micronutrients (Fe, Zn, Mn, and Cu) [25]. However, no studies reported the metal contents of *U. prolifera* degradation water. In mammals, the possibility of excess iron promoting oxidative stress and inducing multiple disorders due to its redox reactivity has been proven [26–28]. In addition, previous studies in fish also have demonstrated that

excess iron promoted oxidative stress and mitochondrial dysfunction [29,30]. Therefore, the abnormal increase in Fe and other metal elements in the water environment and whether it affects the growth performance and antioxidant capacity of Japanese flounder need more investigation.

After determining the elements composition of *U. prolifera* degradation water, we investigated the effects of *U. prolifera* degradation on growth performance. Due to the *U. prolifera* degradation stress, the growth index and SR were significantly decreased, which was consistent with the previous findings that massive macroalgal blooms have caused the sea cucumber and shellfish death in the Yellow Sea [31]. Two flounder families (2101 and 2103) exhibited better survival and growth appearance among eight flounder families compared with the flounder of the PC group under the *U. prolifera* degradation stress, and they are considered to be the potential families for tolerance to the *U. prolifera* degradation conditions.

In addition to the growth performance, the effects of oxidative stress caused by the *U. prolifera* degradation on the antioxidant capacity of Japanese flounder were further explored. The previous study has shown that enzymatic activities of the transaminase enzymes (GOT and GPT) and alkaline phosphatase (AKP) are common indicators of hepatic damage [32]. The research demonstrated a noteworthy reduction in the activities of GOT, GPT, and AKP in the serum as a result of *U. prolifera* degradation. The findings indicated substantial damage to the liver, aligning with the cytotoxic effects observed in a rainbow trout hepatocyte analysis [33].

Additionally, SOD and CAT play a crucial role in the primary defense against oxidative stress. SOD primarily facilitates the conversion of O_2^- and H^+ into less reactive compounds, such as H_2O_2, while CAT effectively neutralizes H_2O_2 into harmless substances H_2O and O_2 [34,35]. GSH, a key non-protein thiol in cells, serves as an integral component in the cellular defense mechanism against toxic effects triggered by xenobiotics, oxyradicals, and metal cations [36]. MDA, emerges as a byproduct of lipid peroxidation, predominantly induced by excessive ROS attacking the polyunsaturated fatty acids (PUFAs) within the cellular membrane. This process can severely impair the structure and functionality of cells [37]. The current investigation revealed that *U. prolifera* degradation significantly elevated the levels of SOD and GSH while reducing CAT activity and MDA content within the liver. This suggests that *U. prolifera* degradation actively facilitates the elimination of O_2^- and prevents damage caused by oxidative stress. This effect is likely attributable to the polysaccharide found in *U. prolifera* degradation, which enhances the overall antioxidant capacity in flounder organisms. Similarly, previous studies have shown polysaccharides [38] from the *U. prolifera* degradation has a positive effect on antioxidant defense system in *Carassius auratus* [39,40]. The observed decrease in CAT activity in the liver could be attributed to iron accumulation, which generated excessive ROS through the Fenton reaction [41]. Combining the results of antioxidant enzyme activities in the serum and liver, the function and antioxidant capacity of the liver could be damaged under *U. prolifera* degradation. In addition, the 2103 flounder family displayed significantly higher activities of SOD and CAT than the 2101 and PC group in the liver, suggesting that the 2103 flounder family has greater antioxidant capacity under the *U. prolifera* degradation conditions. In the gills, the SOD and CAT activities and the GSH content of the 2103 flounder family was significantly higher than the 2101 flounder family and PC group, suggesting that the antioxidant defense system of the 2103 flounder family was influenced and higher than the 2101 flounder family in *U. prolifera* degradation conditions.

In this study, we further explored the effects of *U. prolifera* degradation on the mRNA expression of antioxidant-related genes in aquatic organisms. Our findings revealed that the degradation of *U. prolifera* had a significant impact, resulting in increased *sod* mRNA expression and decreased *cat* mRNA expression in the liver. Furthermore, in the gills of the 2103 flounder family, there was a marked rise in sod mRNA expression. These results were consistent with the changes observed in the activities of SOD and CAT in both the liver and gills. It is important to note that these findings contradict a previous study, which reported

that *Alexandrium affine* significantly affects the immunity and antioxidant capacity of red seabream gills [42]. The probable reason was that the flounder was not directly attached to the floccules of *U. prolifera*. To sum up, although the T-AOC activity of Japanese flounder was significantly decreased compared to the NC group under the *U. prolifera* degradation stress, the antioxidant ability of the liver and gills of the 2103 family was higher compared with the PC group. Therefore, the selected flounder family (2103) could be regarded as a potential flounder family tolerating *U. prolifera* degradation stress.

5. Conclusions

In conclusion, this study revealed that *U. prolifera* degradation influenced the growth performance and antioxidant capacity of Japanese flounder, while the 2103 flounder family was more resilient on the growth performance and antioxidant defense in *U. prolifera* degradation conditions. Thereby, the 2103 flounder family could be regarded as the potential family tolerating *U. prolifera* degradation stress. Furthermore, the metal element content of *U. prolifera* degradation water was detected to understand its impact on ecological environment and marine fish. The increased Fe content in the *U. prolifera* degradation water may be one of the main causes of the physiological alterations observed in Japanese flounder. Therefore, this study could advance the understanding of the effects of *U. prolifera* degradation on marine fish, specifically regarding the growth performance and antioxidant capacity, and provide scientific evidence for excellent germplasm screening.

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Data Availability Statement: Data are contained within the article.

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