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Overwinter mortality in yellow drum (*Nibea albiflora*): Insights from growth and immune responses to cold and starvation stress

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ABSTRACT

The yellow drum (*Nibea albiflora*) is an economically important maricultured fish in China, but the aquaculture of this species has recently been limited by an increase in overwinter mortalities associated with cold and starvation stress due to global climate changes. To better understand the interaction between starvation and cold-stress-driven overwinter mortality, we investigated the effects of these stresses on the growth performance, liver lesions, and immune response of yellow drum fish. The fish were subjected to different cold treatments and under starvation stress. The experiment lasted 30 days and involved four experimental groups: a fed group and a fasted group maintained at 16 °C (control), and a fed group and a fasted group subjected to cold stress at 8 °C. We found that the growth of yellow drum was severely affected by cold temperatures and starvation. Throughout the experimental period, the body weights were significantly lower in the groups subjected to starvation and cold stress than in the control group. The liver cells showed irregular shapes and disorderly arrangements in the stress groups; indicating liver lesions. The gene expressions of antioxidant enzymes (copper, zinc superoxide dismutase, manganese superoxide dismutase, iron superoxide dismutase, and catalase) in the liver were lower in the groups subjected to starvation and cold stress than in the control groups. These results were basically consistent with the enzyme activities of superoxide dismutase and catalase tested in the livers. In addition, activities of immunomodulatory enzymes (alkaline phosphatase and acid phosphatase) were also inhibited in groups subjected to stress throughout the experiment period. These findings suggested that starvation and cold stress inhibited growth, depressed liver function, and suppressed the immune system of yellow drum, which likely would lead to physiological failure and increased susceptibility to infection. The present study offers insights into the physiological and immune response of yellow drum under cold and starvation stress. These insights not only provide baseline information from which effective strategies can be established and appropriate management decisions formulated, but can also be used to improve the overwinter survival of this important fish species in China.

1. Introduction

As global climate change progresses, resulting extremely low winter temperatures have caused mass mortalities in farmed fish and have decreased fish-farm production [1]. Such overwinter mortalities have been reported for numerous fish species; mainly freshwater populations and an increasing number of marine and estuarine fishes [2]. The resulting economic losses can devastate regions and countries that are dependent on aquaculture, including China, Israel, and South America [2]. Consequently, there has been a recent and increasing shift in research focus to better understand the interactions among cold temperatures, starvation, and physiological stress that drive overwinter mortalities in fish [3,4]. The persistence of cold temperatures for long periods of time may predispose fish to “winter disease”, a syndrome that involves several factors such as generalized stress, metabolic depression, immune suppression, and opportunistic pathogen infections. Starving fish may become increasingly sensitive to cold temperatures and may face greater risks of mortality attributed to reduced physiological function [3,4]. Physiological stress is often the greatest at cold temperatures [5,6], which can preclude adequate acclimation. These causes of mortality presumably interact, and the understanding of these interactions is vital to formulate accurate predictions and take appropriate actions to avoid devastating production losses.

Environmental stresses trigger cellular and organismal responses that activate a cascade of events, which may lead to pathological conditions and even death. Previous studies have investigated several

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aspects of the physiological responses of fish to cold stress, both in natural populations and under laboratory conditions [7–11], and have provided evidence that metabolic depression and oxidative stress occur in different tissues after cold exposure. Fish livers are considered target organs for monitoring because many biological and environmental parameters can alter liver structure and metabolism: food, pollutants, toxins, parasites, and microorganisms [12]. Compared to mammals, fish have very homogeneous hepatic parenchyma and polygonal-shaped hepatocytes cells that are often weakly basophilic; therefore, they appear to be well suited as models for the analysis of interactions between natural environmental changes and hepatic morphology [12]. In the sea bream (*Sparus aurata*), one of the main pathological consequences of a limited response to cold exposure is the onset of severe hepatic steatosis, which suggests that liver dysfunction might form the basis of several winter disease symptoms [13]. In gizzard shad (*Dorosoma cepedianum*), indoor laboratory studies have demonstrated that sudden decreases in temperature induce three immediate effects: cold-induced fasting, thermal stress, and metabolic depression [14]. These authors presented evidence of the factors that were driving mortality, which included an inability of enzyme and biological membrane functions to acclimate and an inability to mobilize stored lipids, which in turn led to liver glycogen and liver tissue depletion, liver failure, and death. In most cases, overwinter mortality is probably affected by several interacting mechanisms that vary temporally and spatially throughout the winter period. However, the identification of the relative strength of these mechanisms and the clarification of their interactions remains challenging.

The yellow drum (*Nibea albiflora*) is one of the most important sciaenidae fish in commercial fisheries and a promising candidate for aquaculture in China. Cage farming of this fish has rapidly spread throughout the coastal regions of southeast China and now has the potential to reach levels of large-scale production, i.e., similar to those achieved for the large yellow croaker (*Larimichthys crocea*). The current annual production of yellow drum in Fujian and Zhejiang Province, the major production states in China, exceeds 60,000 tons [15,16]. Despite being a relatively robust species, yellow drum fish are particularly sensitive to cold and their lethal temperature is reported to be ~5 °C [17]. In water temperature conditions below 10 °C, fish become minimally active, and exhibit a reduced food intake and metabolism [15]. The fasting period is particularly severe in the major production states (Zhejiang and Fujian Province) where fish are maintained in cages over winter. The overwinter period is usually prolonged for a month, which leads to losses in body weight, a halt in production, as well as an outbreak of winter diseases that may lead to mass fish mortalities of up to 80% during the winter season. These mortalities result in significant economic losses for semi-intensive and extensive aquaculture production. A better understanding of the interaction between starvation and cold-stress-driven overwinter mortality is needed for the establishment of effective strategies and to guide management decisions to improve overwinter survival of this species. Therefore, this study aimed to investigate the growth, histological changes in the liver, and the immune response of yellow drum under cold and starvation stress.

2. Materials and methods

2.1. Experimental fish and acclimation

This study was approved by the Animal Ethics Committee of Zhejiang Ocean University and Marine Fisheries Research Institute of Zhejiang Province. All fish used in the experiments were handled according to the prescribed guidelines. The yellow drum used in this study were obtained from the hatchery at the research station of the Marine Fishery Institute of Zhejiang Province, Xishan Island, Zhou Shan, China. A total of 400 individuals (aged: 6 months, mean mass: 95.37 ± 15.30 g) were randomly selected and equally divided into 8 fiber-reinforced plastic (FRP) tanks (1000 L, 50 individuals per tank). Fish were acclimated to the tanks for two weeks prior to the commencement of the experiments. During acclimation, seawater temperature was maintained at 16.0 ± 0.2 °C using submersible heaters and an automatic temperature control (Xinlian, China). The aquarium water was exchanged over 200% per day and the filtered seawater was maintained at 16 °C by the heaters. All fish were fed a commercial pelleted diet (Tianbang, Ningbo, China) twice daily (at 8:00 and 16:00) to apparent satiation.

2.2. Experimental design and sampling

After the acclimation period, fish were then subjected to two different temperature treatments and each treatment was duplicated. Two groups of fish were maintained at 16 °C to distinguish the effect of starvation: one group was fed as previously described (hereafter referred to as the 16fed group) and the other group was fasted (not fed) throughout the experimental period (hereafter referred to as the 16unfed group). In the cold stress treatment, temperature challenges consisted of a decrease from 16 °C to 8 °C, maintenance at 8 °C for 14 days and an increase from 8 °C to 16 °C. To determine the effect of cold stress as well as the combined effect of cold and starvation stress, two groups were subjected to the previously described cold challenges, i.e., one group was fed as previously described until the fish stopped eating (hereafter referred to as the 8fed group); and the other group was fasted (hereafter referred to as the 8unfed group) throughout the experimental period. Warm- and cold-water temperature conditions were maintained by supplying tanks with two separate re-circulating systems equipped with mechanical and biological filters. The experimental design is shown in Fig. 1. To investigate the growth of the study fish, all individuals were measured at the start (n = 50 per tank) and end (n = 26 per tank) of the experiments. Fish were sampled (n = 4 per tank) on days (d) 0, 1, 8, 12, 22, 24, and 30 of the experiment. The experimental fish were anesthetized with MS-222 anesthetic (200 mg/L) before sampling, and then dissected quickly. Fresh liver tissues were frozen in liquid nitrogen and transferred to a cryogenic refrigerator (−80 °C) for storage. The liver samples were also fixed with Bouin's solution for histological analysis.

2.3. Paraffin tissue sections and observation

Fixed tissues were cut into 5 μm-thick sections using standard paraffin embedding methods and stained with hematoxylin–eosin. The samples were observed under a fluorescent microscope (Axio Imager A2; Zessis) equipped with a digital camera (Axiocam 506; Zessis).

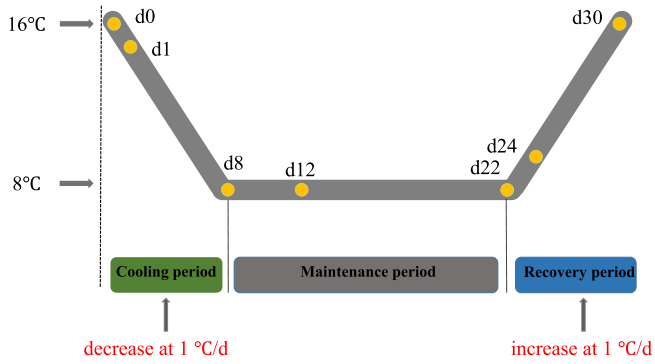

Fig. 1. Experimental schedule for cold and starvation treatments of yellow drum (*Nibea albiflora*). Temperature challenges involved a decrease from 16 °C to 8 °C at a rate of 1 °C/d, maintenance at 8 °C for 14 days, and an increase from 8 °C to 16 °C at a rate of 1 °C/d. Sampling was conducted on day (d) 0, d1, d8, d12, d22, d24, and d30 of the experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.4. Enzymatic activities

The activities of superoxide dismutase (SOD), catalase (CAT), alkaline phosphatase (AKP), and acid phosphatase (ACP) in the liver tissues were measured using reagent kits (Jiancheng Bioengineering Institute, Nanjing, China) via spectrophotometric analysis with a microplate reader. The activities of SOD and CAT were measured using reagent kits (Product Code A003-1, A007-1, respectively). The activities of AKP and ACP were measured using reagent kits (Product Code A059, A060, respectively). All procedures were performed according to the kit instructions.

2.5. Gene expression of SOD (Cu, Zn-SOD; Mn-SOD; Fe-SOD), CAT, and GPx by quantitative real-time PCR analysis (qPCR)

Total RNA was extracted from the liver samples using Trizol Reagent (RNA Extraction Kit, Invitrogen, USA) and followed by quality measurement using a 1.2% denaturing agarose gel. The concentration and quality of the total RNA were estimated by measuring the absorbance at 260 nm and agarose-gel electrophoresis, respectively. The RNA was treated with RNA-Free DNase (Takara, Japan) to remove DNA contaminants and reverse transcribed to cDNA by RevertAid First Stand cDNA Synthesis kit (Takara, Japan) following the manufacturer's instructions.

The expressions of Cu, Zn-SOD (MK780815); Mn-SOD (MK780816); Fe-SOD (MK780817); CAT (MK780818); and GPx (MK780819) were analyzed by qPCR. The primers used for the qPCRs are listed in Table 1. The qPCRs were conducted in a quantitative thermal cycler (ABI StepOnePlus, America). Amplification was performed in a total volume of 20 μL that contained 10 μL 2 × SYBR Premix Ex *Taq*II (TAKARA, Japan), 0.4 μL ROX, 0.4 μL of each primer (10 μmol/L), 6.8 μL nuclease-free water, and 2 μL of cDNA mix (100 ng/μL). The real-time RT-PCR program was as follows: denaturation at 94 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, 60 °C for 30 s, then one cycle at 95 °C for 15 s and 60 °C for 60 s. The real-time RT-PCR primer pairs for the genes and β-actin were designed by Primer Premier 5.0 and based on published nucleotide sequences. At the end of each PCR reaction, melting curve analysis of the amplification products was conducted to confirm that a single PCR product was present in these reactions. Relative gene expression levels were evaluated using the $2^{-\Delta\Delta CT}$ method [18].

2.6. Statistical analyses

All data were expressed as means ± standard errors of the mean (SEM). Two-way analysis of variance by a multiple comparison (Tukey) test was conducted to compare the significant differences among treatments using SPSS 19.0 software (SPSS, Chicago, IL, USA). *P*-values < 0.05 were considered statistically significant.

Table 1
Primers for quantitative PCR.

Primers	Sequence(5'～3')
Cu, Zn-SOD	F:TGAAAGGGGATGGGGAGG
	R:TGTGGGGATTGAAGTGAGGG
Mn-SOD	F:ATAAACCAGGTGGCTGCTTCA
	R:TCGTCACATCTCCCTTTGCTA
Fe-SOD	F:TGTCTGGATTATGGAGCCTGC
	R:GGTTCACTCTCTTGGTTTGGG
CAT	F:ACTGTGGCTGGTGAATCGG
	R:TGAAGAAAATGGGGGTGTTG
GPx	F:GCTGGAGGGACCTATTGGAG
	R:GCTTCTGAGAGAGGAGCACACG
β-actin	F:CCTCCCTGGAGAAGAGCTATGAG
	R:CGCACTTCATGATGCTGTTGTAG

3. Results

3.1. Effects of cold stress on growth parameters

During the experimental period, significant growth was observed in the 16fed group ($P < 0.05$) but no significant growth was observed in the 8fed group ($P > 0.05$) (Fig. 2). The body weight, total length, and body length were significantly decreased in the 8unfed group ($P < 0.05$); but only the body weight was significantly decreased in the 16unfed group (i.e., the total length and body length increased in the 16unfed group throughout the experimental period). The liver/body weight ratio was significantly higher in the 16fed group than those in the 16unfed and 8unfed groups ($P < 0.05$), while no significant differences were observed between the 16fed and 8fed groups ($P > 0.05$) (Fig. 2d). The food consumption in the 8fed group gradually decreased as the temperature decreased. The fish in 8fed group stopped ingesting food when the water temperature dropped to 10 °C, and they barely ingested any food until the temperature increased to 16 °C again.

The liver cells of the 16fed group were polygonal, arranged neatly and orderly, and had obvious cell boundaries, uniform cytoplasm, and distinct rounded nuclei (Fig. 3a). The cells in the 16unfed group were irregularly shaped, arranged closely and disorderly, some showed dispersion, and entire liver cells were deeply stained (Fig. 3b). The livers in the 8unfed and 8fed groups showed obvious lesions (Fig. 3c and d). The liver cells were disordered, more hepatocytes appeared vacuolized, the intercellular boundaries were cluttered, the nuclei were atrophied, and even dissolution occurred.

3.2. Effects of cold and starvation stress on gene expression in the livers of yellow drum

The expression of the antioxidant genes Cu, Zn-SOD; Mn-SOD; Fe-SOD; and CAT were analyzed among different groups. The expression levels of these genes in the 16unfed, 8unfed, and 8fed groups were significantly inhibited, and significantly lower than those in the 16fed group ($P < 0.05$) (Fig. 4). The expression levels of the Cu, Zn-SOD gene initially increased and then decreased in the 16unfed, 8unfed, and 8fed groups, while the expression levels of the Mn-SOD, Fe-SOD, and CAT genes initially decreased and then increased in the 16unfed, 8unfed, and 8fed groups throughout the experimental period. With regards to the expression of GPx, there were significant differences in various treatment groups at most sampling times. The gene expression level of GPx in the 16unfed group was significantly higher than that in the 16fed group ($P < 0.05$) (Fig. 4e). However, GPx showed a lower level of expression in the 8unfed and 8fed groups ($P < 0.05$).

3.3. Effects of cold and starvation stress on antioxidant enzymes activities in the liver of yellow drum

Two antioxidant enzymes (SOD and CAT) and two typical lysosomal enzymes (ACP and AKP) were analyzed. The activities of these enzymes showed significant changes in low temperature and starvation stress in various treatment groups ($P < 0.05$) (Fig. 5). Generally, both the SOD and CAT activities were inhibited by cold and starvation stress. Compared with the 16fed group, the activities of SOD in the 16unfed, 8unfed, and 8fed groups were lower on d1, d8, d12, and d30 ($P < 0.05$). The CAT activities in the 16unfed, 8fed, and 8unfed groups were also lower than in the 16fed group on most of the sampling days (d1, d8, d12, d22, and d30) ($P < 0.05$). With regard to the ACP and AKP, their activities differed in various treatment groups at most sampling times ($P < 0.05$). Especially at the recovery stage (d24 and d30), the activities of ACP and AKP in the stressed groups were significantly lower than those in the 16fed group ($P < 0.05$).

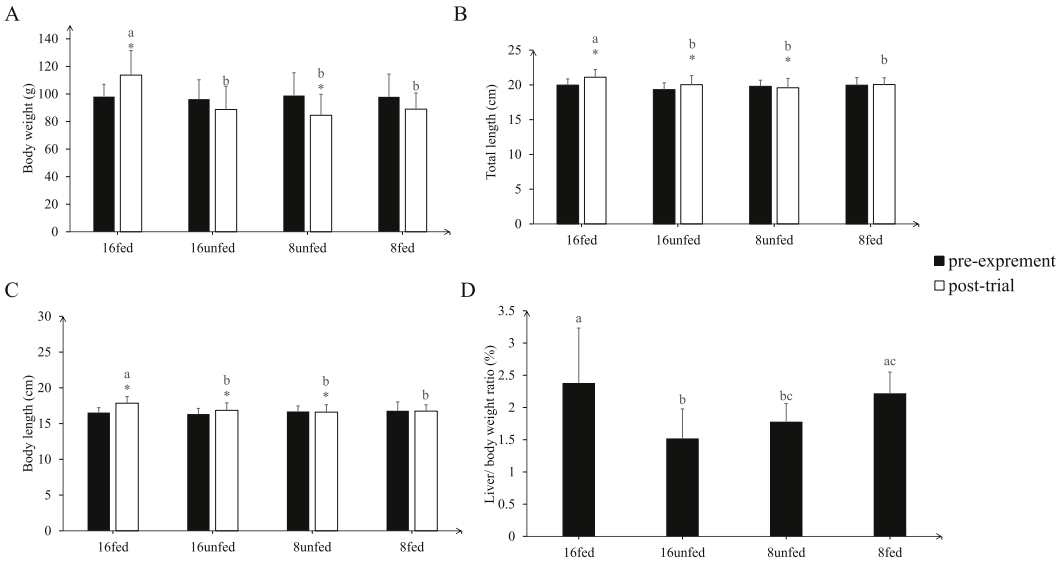

Fig. 2. Comparisons of body weight (A), total length (B), body length (C), and liver/weight ratio (D) of yellow drum (*Nibea albiflora*) in the four different groups subjected to cold and starvation stress. Two groups were maintained at 16 °C: one fed (16fed) and one fasted (16unfed) during the experimental period. The other two groups were treated with cold stress (8 °C): one fed (8fed) until the fish stopped eating, and one fasted (8unfed) during the experimental period. Data are expressed as means + SEMs. Asterisks indicate significant differences between conditions before and after the experiments. Different letters indicate significant differences between the treatment groups and the control group.

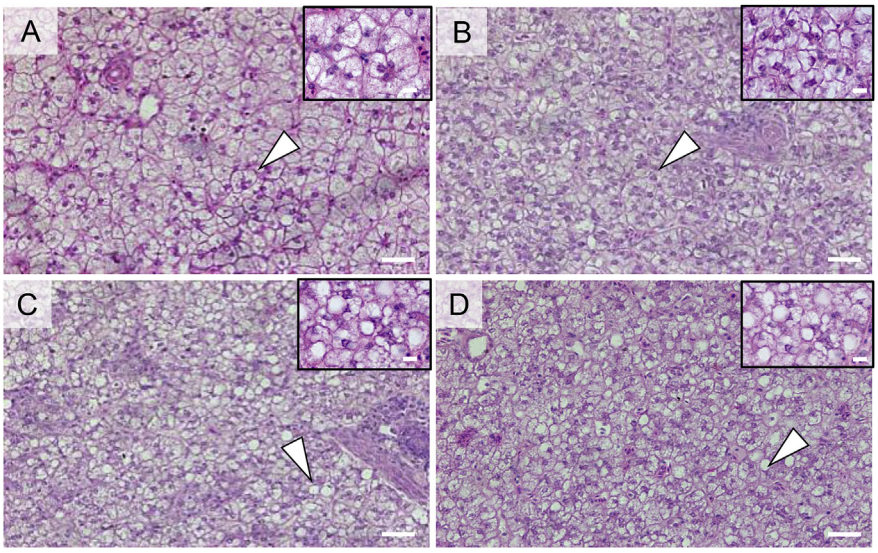

Fig. 3. The histological structure of livers in yellow drum (*Nibea albiflora*) subjected to cold and starvation stress. Histological analyses were conducted on liver tissues sampled on day 30 of the experiment, from subjects from the 16fed group (A), 16unfed group (B), 8unfed group (C), and 8fed group (D). Normal hepatocytes were observed in (A). Disordered arrangements of liver cells were observed in response to cold and starvation stress, and vacuolization, nuclear atrophy, and even dissolution were apparent in the liver cells. The magnified liver cells are shown on the right side. Scale bars = 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

Starvation and cold stress are commonly identified as the major drivers of overwinter mortalities in farmed fishes [2]. In the present study, two important factors were considered: cold and starvation stress (i.e., effects of cold stress and extended fasting on the physiological condition of yellow drum). The growth, liver lesions, and immune responses were investigated to better understand the mechanisms that drive overwinter mortality in fish. Indoor laboratory studies have demonstrated that decreases in temperature can cause cold-induced fasting, thermal stress, and metabolic depression [2–4]. We found that yellow drum body weight was significantly decreased in the groups subjected to starvation and cold stress throughout the experimental period, and thus the growth of this fish species was shown to be severely affected by such stresses. Cold-induced fasting was also observed in the 8fed group, and continued in the temperature recovery stage until the seawater temperature reached 16 °C. This observed delay in the recovery of the feeding behavior during recovery from food deprivation is consistent with the findings of Tort et al. [19]. This observed response may be an indication of a delayed recovery of the digestive and absorptive capacity after the prolonged exposure to cold stress.

The liver is a large vital organ in fish that serves a wide range of functions; including detoxification, protein synthesis, production of biochemicals necessary for digestion, and storage of glycogen from the gastrointestinal tube to regulate blood sugar balance [12]. Previous studies have demonstrated that cholesterol, triglyceride, and glucose are the first to be consumed as energy sources in the early stages of cold stress, and, thereafter, proteins and fats are metabolized and utilized in the later stages of cold stress [20]. However, fish under starvation stress decompose fat in the liver to supply energy demands [21,22], and, as a result, the liver tissue gradually shrinks and the cell volume and lipid droplets decrease. The combination of cold stress and extended fasting was likely to have intensified the lesions in the liver [23,24]. In the present study, the liver/body weight ratios were significantly lower in the stress groups than in the control group. The observed vacuolation of

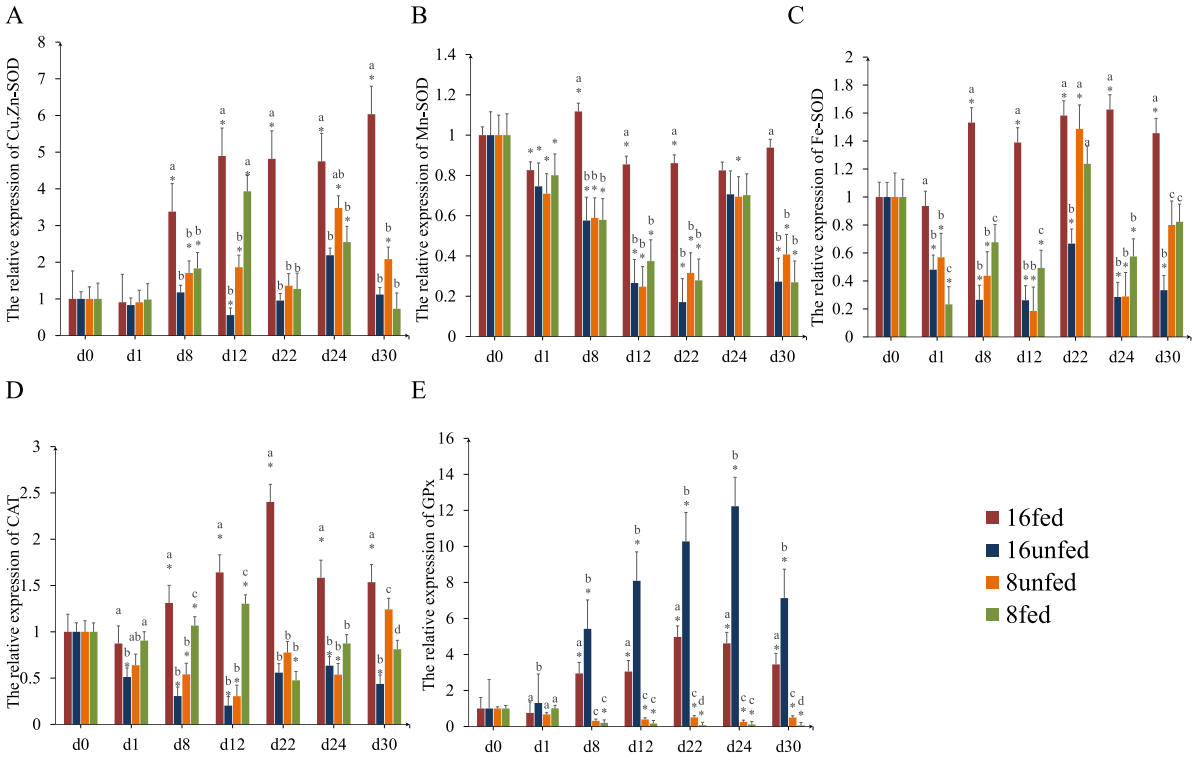

Fig. 4. Changes in gene expression in the four groups of yellow drum (*Nibea albiflora*) under starvation and cold stress. Two groups were maintained at 16 °C: one fed (16fed) and one fasted (16unfed) during the experimental period. The other two groups were treated with cold stress (8 °C): one fed (8fed) until the fish stopped eating, and one fasted (8unfed) during the experimental period. (A) Cu, Zn-SOD (copper, zinc superoxide dismutase), (B) Mn-SOD (manganese superoxide dismutase), (C) Fe-SOD (iron superoxide dismutase), (D) CAT (catalase), and (E) GPx (glutathione peroxidase). Sampling was conducted on day (d) 0, d1, d8, d12, d22, d24, and d30 of the experiment. Data are expressed as means + SEMs. Different letters indicate significant differences among treatments at the same sampling time. Asterisks indicate significant differences among data comparisons with d0. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

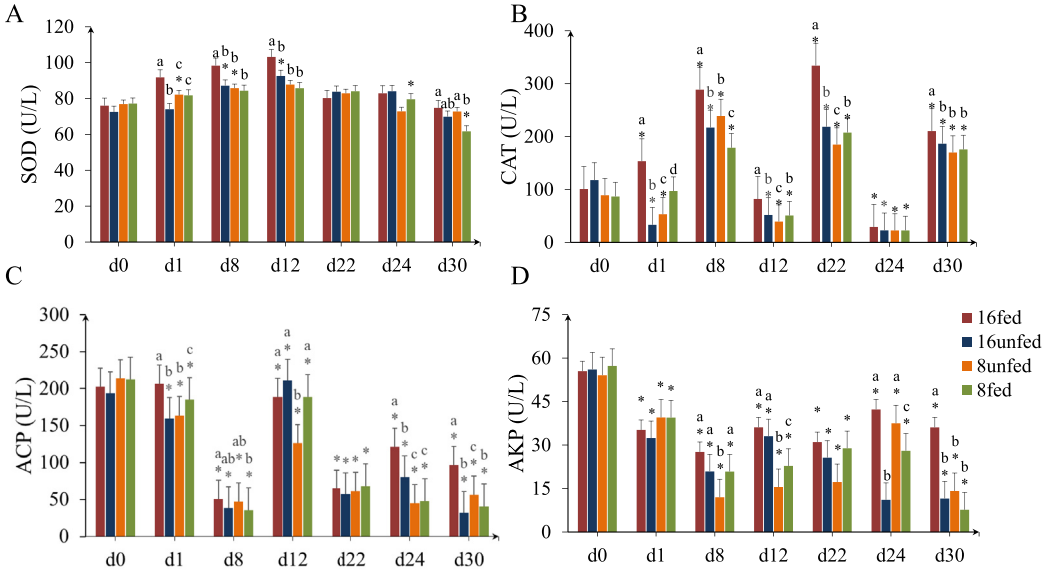

Fig. 5. Changes in enzyme activities in the four groups of yellow drum (*Nibea albiflora*) under starvation and cold stress. Two groups were maintained at 16 °C: one fed (16fed) and one fasted (16unfed) during the experimental period. The other two groups were treated with cold stress (8 °C): one fed (8 fed) until the fish stopped eating, and one fasted (8unfed) during the experimental period. (A) SOD (superoxide dismutase), (B) CAT (catalase), (C) ACP (acid phosphatase), and (D) AKP (alkaline phosphatase). Sampling was conducted on day (d) 0, d1, d8, d12, d22, d24, and d30 of the experiment. Data are expressed as means + SEMs. Data with different letters indicate significant differences among treatments at the same sampling time. Asterisks indicate significant differences among data comparisons with d0. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hepatocytes may have occurred in response to the increased oxygen consumption and the decomposition of glycogen or fat in the livers of fish under stress conditions.

Under long-term cold stress, the livers of yellow drum fish have been shown to exhibit lesions, i.e., vacuolation of hepatocytes, and some hepatocytes showed nuclear atrophy, disappearance, or even cell ablation [8]. The cellular vacuolation was also found in livers of winter flounders (*Pseudopleuronectes americanus*) exposed to chemical contaminants [25], which may be linked to hepatocarcinogenesis in these fish. Although the specific cause of the lesions was not clarified in the present study, the histopathological changes appeared to be the result of cold and starvation stress. We suspect that there would be an increased number of mortalities in the recovery period after overwintering in aquaculture production because of the lesions in the bodies of the fish exposed to such stresses. The associations between the lesions and the mortalities after overwintering should be further studied.

Cold stress can increase the production of reactive oxygen species and thus induce oxidative stress, which has harmful effects on important biological molecules, including lipids, proteins, and DNA [26–31]. Antioxidant defense systems are, therefore, likely to respond to the stress of low temperatures. As an important component of antioxidant defenses, antioxidant enzymes, such as SOD, CAT, and GPx, directly participate in the removal of reactive oxygen species and play a key role in preventing oxidative stress injury [32,33]. The responses of antioxidant enzymes under cold stress differ depending on the species, intensity, duration of the stress, etc. [34–36]. Generally, the gene expressions as well as the enzyme activities of SOD and CAT in the cold and starvation groups were inhibited during the experimental period in the present study. It was noteworthy that the SOD and CAT enzyme activities showed an increasing trend at the cooling stage and then a decreasing trend afterward in various treatments, which was not in accordance with their expression. We assumed that the oxidative stress caused by low temperature and starvation stress triggered the antioxidant defense to enhance the enzyme activities to protect the cellular machinery from the detrimental effects of the reactive oxygen species. Similar responses were also observed in other aquatic organisms, e.g., *Danio rerio* [37], *Sparus aurata* [24], and *Gasterosteus aculeatus* [36]. However, the enzyme activities of SOD and CAT decreased significantly during the cold maintenance period and the recovery period, which resulted in liver damage and was likely to exceed the self-regulation ability of the yellow drum [38].

Interestingly, we found the expression of GPx in the liver was likely to have had different responses to starvation and cold stress. Specifically, the expression of GPx in the 16unfed group was significantly higher than that of the control group, which indicated that the starvation stress resulted in a larger number of free radicals in the yellow drum that required removal via an increased expression of cell GPx mRNA [39]. However, the expression of GPx mRNA in the cold-stressed groups was significantly lower than that of the control group; indicating an inhibition of the GPx gene expression under cold stress. Collectively, these results indicated that cold stress was the main factor that drove the GPx depression.

In addition, the ACP and AKP activities were significantly affected by the cold and starvation stress, possibly due to changes in the lysosomal membrane integrity as a result of cell degranulation from prolonged cold exposure and starvation [40,41]. ACP and AKP are typical lysosomal enzymes that are responsible for preliminary immune system functions [40]. The decrease in activities of these enzymes were mainly observed in recovery stage, which implied that the immune capability was decreased in the fish subjected to cold and starvation stresses, and thus these fish were likely to have been susceptible to infection during this period. In fact, we indeed observed large-scale moralities in aquacultured yellow drum after overwintering. The associations between mortalities and immune capabilities at the recovery stage should be further clarified in future studies.

5. Conclusion

In summary, the complex etiology of overwinter mortalities in fish involve several factors, such as cold, starvation stress, metabolic depression, immune suppression, and occasional infection by opportunistic pathogens. However, damage caused by cold stress and extended fasting may enable other factors to compromise the physiology of yellow drum. In the present study, we demonstrated that cold and starvation stress resulted in growth inhibition, histopathological changes, and immune suppression in yellow drum fish. These findings offer insights into the physiological and immune responses of yellow drum under cold and starvation stress. The observed decrease in liver function may ultimately lead to physiological failure and even death. In addition, the resulting lower immune capacity in response to cold and starvation stress suggests that the fish were more susceptible to infection. The baseline information presented in this study should be used to establish effective strategies and make suitable management decisions during cold periods and recovery stages to improve the overwinter survival of yellow drum fish in China.

Author contributions

H. S., D. X., G. W., and P. T. designed the research; H. S., T. L., and R. C, performed the research; H. S and D.X wrote the paper. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.06.030.

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