Fisheries Science (2021) 87:775–784
https://doi.org/10.1007/s12562-021-01545-2

ORIGINAL ARTICLE

Biology

Acute temperature changes induce an oxidative stress response in kidney cells of grass carp *Ctenopharyngodon idellus*

Pin Ma[1,2] · Xiaoyan Yin[1,2] · Dapeng Li[1,2] · Li Li[1,2] · Rong Tang[1,2]

Received: 3 June 2020 / Accepted: 4 August 2021 / Published online: 9 September 2021

Abstract

Recent studies on the effects of temperature on the antioxidant system of fish have been conducted mostly in vivo. In vivo experimental results are influenced by many factors, and can vary widely. Hence, experiments at the cellular level can provide a new direction for scientific research. The purpose of this study was to investigate the mechanism of the antioxidant system in response to temperature changes in grass carp *Ctenopharyngodon idellus* kidney (CIK) cells. CIK cells were exposed to culture temperatures of 20 °C, 24 °C, 28 °C, 32 °C, and 36 °C for 24 h, and the results showed that heat stress significantly increased the level of reactive oxygen species (ROS), which further led to increased content of malondialdehyde (MDA) and protein carbonyl. The increase of the ratio of Rh123 fluorescence indicated a decrease in mitochondrial membrane potential ($\Delta\Psi m$), demonstrating that the changes in temperature destroyed the mitochondrial membrane of CIK cells. The acute temperature stress increased the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione reductase (GR), and improved total antioxidant capacity (TAC) and glutathione (GSH). The relative mRNA expression levels of *Cu–Zn sod*, *gpx*, and *cat* were significantly increased with the variation in temperature. In conclusion, the changes in temperature disturbed the homeostasis of the CIK cells, destroyed the mitochondrial membrane, and enhanced the activity of major antioxidant enzymes to resist oxidative stress.

Keywords *Ctenopharyngodon idellus* · Kidney cell · Reactive oxygen species · Oxidative stress · Temperature variation

Introduction

Oxidative stress is the process of generating and accumulating reactive oxygen intermediates, which results in a burst in the production of reactive oxygen species (ROS), leading to steady-state alterations in the body as the result of oxidative damage (Lesser 2006; Lushchak 2011). ROS has oxidative properties for many macromolecular substances, such as proteins, lipids, and DNA, and it can directly cause oxidative stress damage to the body (Snezhkina et al. 2019; Tanaka et al. 2006). Temperature is an important environmental factor with significant effects on organisms, and extreme temperature conditions can result in harmful effects, such as lipid peroxidation (LPO). Acute shifts in temperature can also result in failure to maintain the steady levels of ROS required for metabolism, which leads to aggravated stress levels (Lushchak 2011; Tseng et al. 2011).

Aquatic animals have evolved an efficient antioxidant defense system (ADS) to remove ROS and resist oxidative stress (Guo et al. 2018). The ADS consists of antioxidant

✉ Rong Tang
tangrong@mail.hzau.edu.cn

Pin Ma
mapin@webmail.hzau.edu.cn

Xiaoyan Yin
yitian87@qq.com

Dapeng Li
ldp@mail.hzau.edu.cn

Li Li
foreverlili@mail.hzau.edu.cn

[1] Department of Fishery Resources and Environment, College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China

[2] Hubei Provincial Engineering Laboratory for Pond Aquaculture, Wuhan 430070, China

enzymes including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), and non-enzymatic antioxidant molecules such as glutathione (GSH) (Dadras et al. 2016; Lin et al. 2018; Wilhelm Filho 1996). Hence, the activity of antioxidant enzymes and the content of non-enzymatic antioxidant molecules can reveal the oxidative status in cells (Dadras et al. 2016).

As poikilothermic animals living in water, fish will produce a stress response when the ambient temperature exceeds the optimal range. If the limit of self-regulation is exceeded, the body's immune system will be destroyed, leading to increased morbidity and mortality. Studies have shown that long-term exposure to increased temperature induces the expression of genes such as heat shock protein 70 (Hsp70) and heat shock protein 90 (Hsp90) in adult zebrafish and the phosphorylation of stress-activated protein kinases in gilthead seabream *Sparus aurata* (Feidantsis et al. 2009; Malek et al. 2004). Variation in temperature was found to affect the physiological status of both fish cells such as RTH-149 cells and organisms such as green sturgeon *Acipenser medirostris* and killifish (Babich et al. 1993; Lankford et al. 2003; Leggatt et al. 2007). It has been reported that in hepatic cells of grass carp, heat stress can increase the level of lipid peroxide and destroy antioxidant homeostasis (Cui et al. 2013). Moreover, when fish are exposed to adverse environmental conditions, for survival they will adapt to the new environment, and in that process the biosome produced accommodation; however the thermal adaption process in fish is complex. In most of the world's oceans as well as in freshwater, living organisms are suffering deadly consequences of global warming (Rossati 2017). In catfish *Heteropneustes fossilis*, for example, it was reported that when the water temperature increased, regardless of the sex of the individual catfish, the level of lipid peroxide in the respiratory apparatus increased. However, after exposure to the higher temperature, the ascorbic acid content in the air sac of the fish was decreased (Parihar and Dubey 1995). Bagnyukova et al. (2007) also noted changes to several oxidative stress markers associated with glutathione-dependent enzymes following exposure of goldfish *Carassius auratus auratus* to warm temperature stress (a change from 3 to 23 °C), which included a rapid increase in lipid peroxide levels and a 20–40% reduction in protein carbonyl content in the liver. Furthermore, the effects of temperature reduction on gene expression in adult zebrafish skeletal muscle indicated that approximately 600 genes related to the oxidative stress response were upregulated by 1.7-fold or greater (Malek et al. 2004).

As mentioned, most organisms adapt to oxidative stress by increasing antioxidant potential, as has been reported in killifish, three-spined stickleback, green sturgeon, brown trout, and goldfish (Lankford et al. 2003; Leggatt et al. 2007; Lushchak and Bagnyukova 2006; Murphy et al. 2005; Kammer et al. 2011; Hansen et al. 2006).

For further details on the process of ADS in acute temperature stress response, here the process was examined using fish cells, considering that fish cells live in a wide range of temperatures, such as grass carp *Ctenopharyngodon idellus* kidney (CIK) cells, which can live in the range of 10–40 °C. In addition, the kidney has a powerful ADS, and is a suitable model for studying temperature and oxidative stress.

Therefore, this study explored the effects of temperature on cellular antioxidant enzymes and oxidative damage indicators in CIK cells, which is a more accurate approach to understand the ADS response of the fish to temperature stress.

Materials and methods

Cell culture

CIK cells were purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, and then cultured with medium M199 (Gibco, USA), supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 mg/ml streptomycin, and grown at 28 °C in 5% CO_2. All experimental operations adhered to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Huazhong Agricultural University, Wuhan, China.

Temperature stress

After culturing at 28 °C for 2 weeks, the CIK cells (1×10^6 cells/well) were collected with 0.25% trypsin–EDTA and divided into five groups, with three replicate bottles in each group. A temperature of 28 °C was set as the control, and the incubator temperature was continuously increased or decreased relative to 28 °C, at a rate of 1 °C every 15 min. CIK cells were exposed to different temperatures (20, 24, 28, 32, 36 °C) for 24 h.

Reactive oxygen species (ROS) assay

The cells (1×10^6 cells/well) were seeded into a 25-mm^2 culture dish. After temperature stress, the cells were incubated with medium containing 2′,7′-dichlorofluorescein diacetate (DCFH-DA; 10 µmol/l) (Sigma-Aldrich, USA) at 37 °C for 20 min, then washed twice with PBS and resuspended. The levels of ROS were tested using a fluorescence microplate reader (SpectraMax i3X, Molecular Devices), at an emission wavelength of 530 nm and excitation wavelength of 488 nm.

Mitochondrial membrane potential (ΔΨm) assay

The cells (1×10^6 cells/well) were exposed to different temperatures for 24 h, then incubated with medium containing

Rh123 (1 μg/ml) (Sigma, USA) at 37 °C for 15 min, washed three times with PBS, and then resuspended. The fluorescence activity of Rh123 was tested using a fluorescence microplate reader, at an emission wavelength of 534 nm and excitation wavelength of 504 nm.

Oxidative stress indicator analysis

The activity of SOD, CAT, GPx, glutathione reductase (GR), and xanthine oxidase (XOD), the content of GSH and malondialdehyde (MDA), and the total antioxidant capacity (TAC) were measured according to the instructions of commercially available kits (Jiancheng, Nanjing, China). Protein carbonyl was measured by reaction with 2,4-dinitrophenylhydrazine (DNPH) (Lushchak et al. 2005). SOD assay was based on the method described by Beyer and Fridovich (1987). CAT activity was determined by the rate of disappearance of H_2O_2 according to Aebi (1984), with slight modifications. GPx activity was measured using GSH as a substrate to measure the conjugation of GSH and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (Drotar et al. 1985). MDA content was used as an index of LPO and was determined according to Ohkawa et al. (1979). TAC was determined using the FRAP (ferric reducing/antioxidant power) assay described by Benzie and Strain (1999). Protein content was determined by the Coomassie blue method using bovine serum albumin (BSA) as a standard.

Total RNA extraction and quantitative real-time PCR analysis

RNAiso Plus (Takara Bio, Dalian, China) was used to extract total RNA from CIK cells according to the manufacturer's instructions. The RNA samples were quantified spectrophotometrically with a NanoDrop® 2000 (Thermo Fisher Scientific), and 1 μg RNA was used to synthesize the cDNA.

The reaction mixture contained 12.5 μl SYBR Premix Ex Taq™II (Perfect Real Time) (Takara Bio, Dalian, China), 0.5 μl of each forward and reverse primer, and 9.5 μl ddH_2O. The primers used in the experiment are shown in Table 1. The reaction procedure was as follows: pre-denaturation at 95 °C for 5 min, then 35 cycles of 95 °C for 30 s, 95 °C for 5 s, 60 °C for 30 s, and 72 °C 30 s. The transcript levels of related genes were quantified with the 28 °C group as a positive control, and *eif1a* was the reference gene, the data were calculated using $2^{-\Delta\Delta C_t}$ (Livak and Schmittgen, 2001).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test; the statistical analysis was performed using SPSS 20.0 software. A value of $P < 0.05$ was considered statistically significant. All data were expressed as mean ± SD (standard deviation).

Table 1 Primers used for quantitative real-time PCR

Genes	Primer sequences (5′–3′)	Product length (bp)
Cu–Zn sod	F: TGTGACGACCCAAGTCTCCC R: TAAAGCCCAACCAGCCCC	117
gpx	F: GGCACAACAGTCAGGGATTACA R: CGGACGGACATACTTCAGAGAT	130
cat	F: CTCTACAATGCCATCTCCAACG R: CAGACCTTAGTCAAATCAAACGG	116
eif1α	F: GGGCAAGGGCTCCTTCAAAT R: GCAACAATCAGCACGGCACA	121

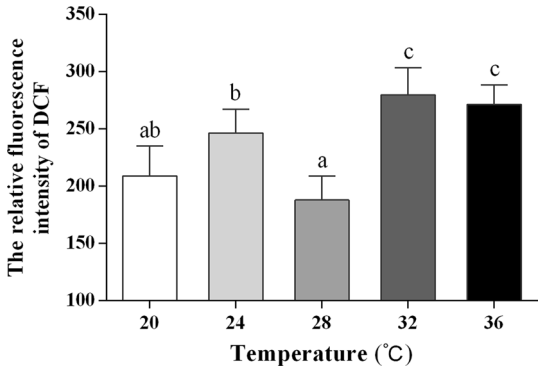

Fig. 1 The intracellular fluorescence relative intensity of dichlorofluorescein (DCF) in CIK cells after exposure to different temperatures. Significant differences between different temperature are indicated with different case letters ($P < 0.05$). All values represent the mean ± SD ($n = 9$)

Results

Reactive oxygen species (ROS)

The ROS level at 28 °C (control) was the lowest. When the temperature was reduced from 28 to 24 °C, the level of ROS increased significantly ($P < 0.05$). No significant change was observed with a decrease from 24 to 20 °C. In the heat treatment groups, the level of ROS increased significantly when the temperature increased from 28 to 32 °C ($P < 0.05$), but it showed no significant change from 32 to 36 °C. Compared with the control group, all temperature treatment groups except the 20 °C group showed significant differences in ROS levels ($P < 0.05$; Fig. 1).

Oxidative damage state indicator analysis

The ΔΨm is represented by the relative fluorescence intensity ratio of Rh123. The ΔΨm is stable in normal cells, and is decreased or lost when the membrane is damaged (Halestrap 2010). Compared with the control group, the Rh123 fluorescence ratio of cells increased significantly in the cold groups ($P < 0.05$). In the of heat treatment process, the ratio increased significantly when the temperature increased from 28 to 32 °C, while the ratio decreased significantly from 32 to 36 °C ($P < 0.05$; Fig. 2a).

The MDA content showed no significant difference between 28 and 24 °C. When the temperature decreased from 28 to 20 °C, the MDA content increased significantly ($P < 0.05$). In the heat treatment process, the MDA content increased significantly when the temperature increased from 28 to 32 °C ($P < 0.05$), but showed no difference with a temperature increase from 32 to 36 °C (Fig. 2b). The protein carbonyl content at 32 °C was 1.70 times higher than that at 28 °C ($P < 0.05$). Compared with the control group, the protein carbonyl content was significantly decreased in the 20 and 24 °C groups ($P < 0.05$; Fig. 2c).

Antioxidant relative enzyme indicator analysis

The SOD activity of CIK cells was lowest in the 28 °C group; when the temperature was increased to 32 °C, SOD activity increased significantly ($P < 0.05$). There was no significant difference when the temperature increased from 32 to 36 °C, whereas SOD activity showed a significant increase when the temperature decreased from 24 to 20 °C ($P < 0.05$; Fig. 3a).

The GPx activity of CIK cells was lowest in the 28 °C group. During the period of cold acclimatization, GPx activity showed no significant change from 28 to 24 °C, but it increased with an increase in temperature from 24 to 20 °C. However, the increase was lower than that in the warm acclimatization groups. The GPx activity of cells in the 32 °C group was 1.91 times that of the 28 °C group, and 1.24 times that in the 20 °C group ($P < 0.05$). The GPx activity in the 36 °C group was the highest (Fig. 3b).

The CAT activity of CIK cells was relatively low in the 28 and 32 °C groups, with no significant difference between them. In the heat treatment groups, when the temperature increased from 32 to 36 °C, the CAT activity

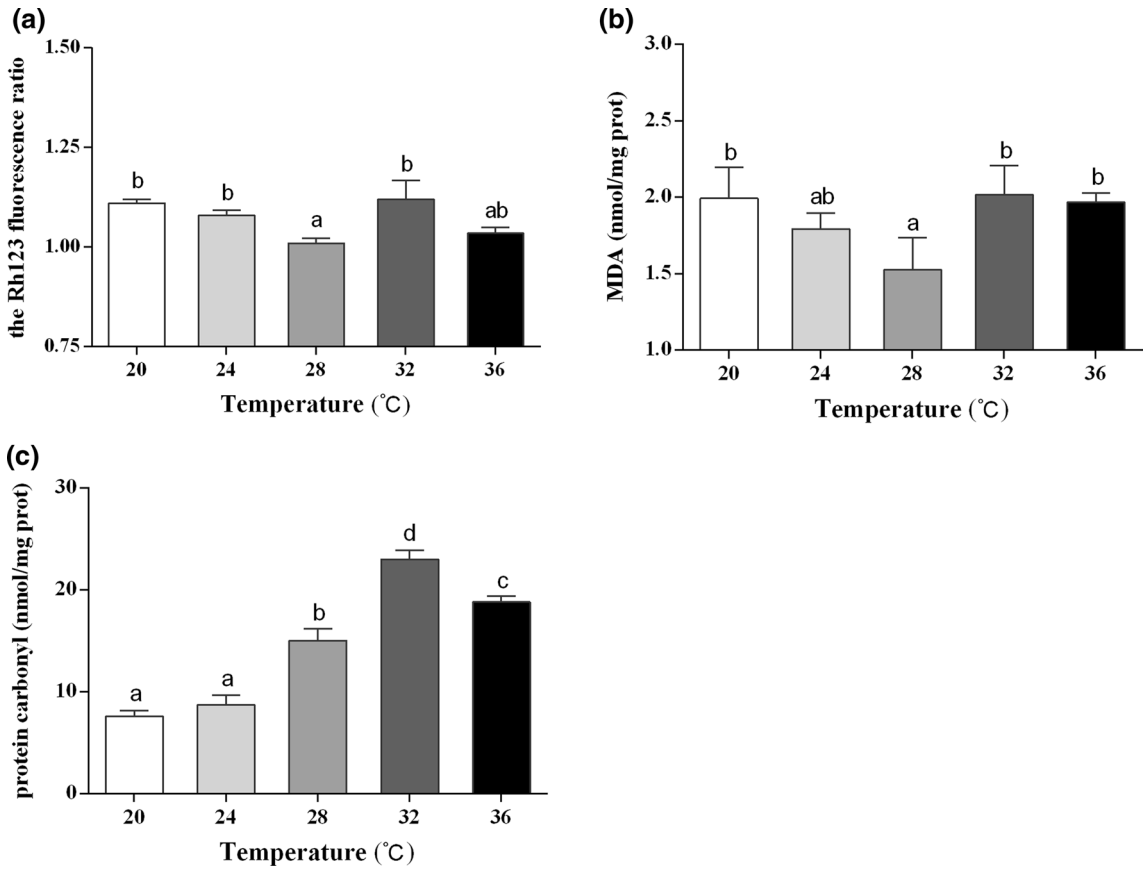

Fig. 2 The ratio of Rh123 fluorescence (**a**) and the content of MDA (**b**) and protein carbonyl (**c**) in CIK cells after exposure to different temperatures. Significant differences between temperatures are indicated with different case letters ($P < 0.05$). All values represent the mean ± SD ($n = 9$)

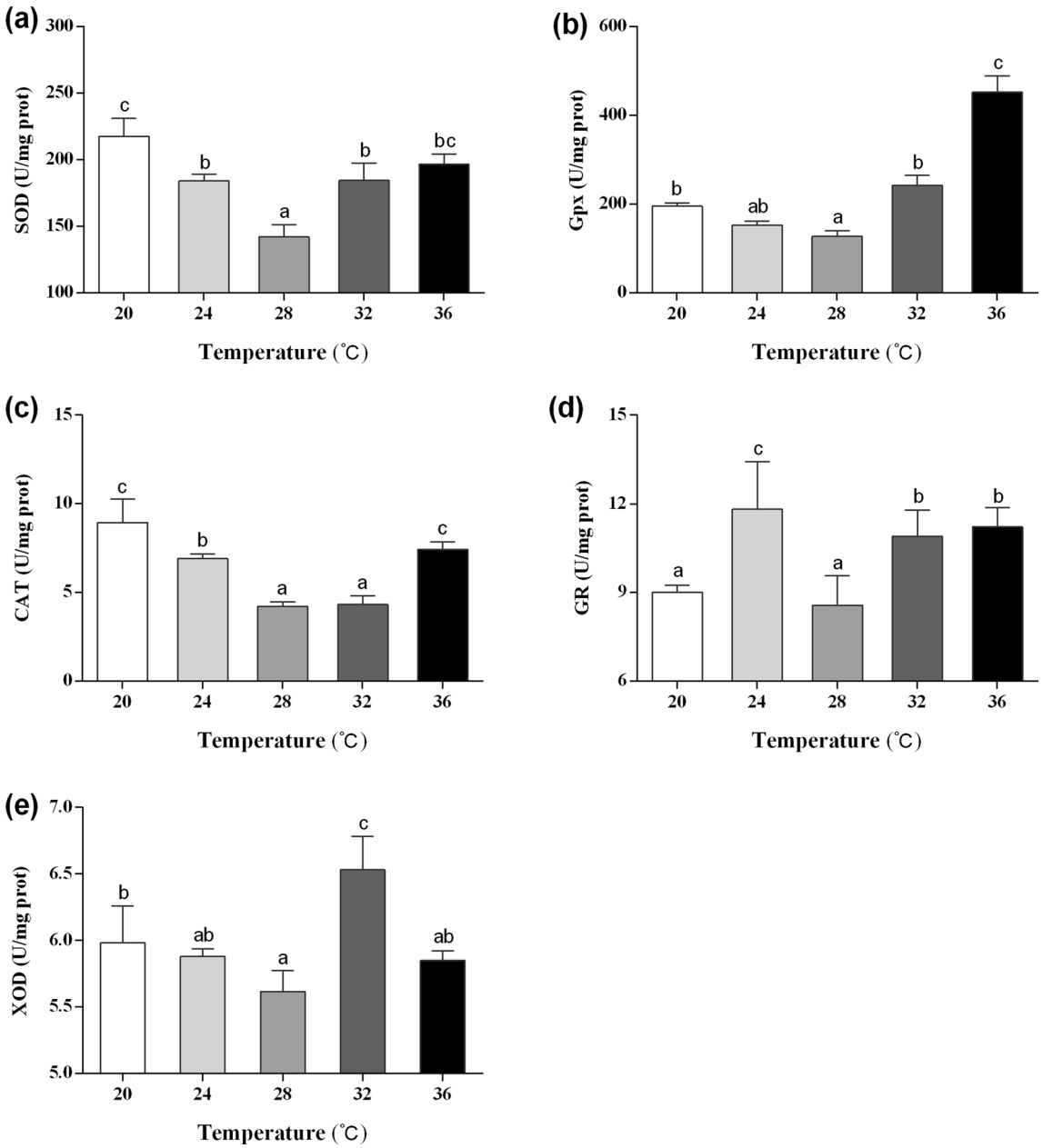

Fig. 3 The intracellular activity of SOD (**a**), GPx (**b**), CAT (**c**), GR (**d**), and XOD (**e**) in CIK cells after exposure to different temperatures. Significant differences between different temperatures are indicated with different case letters ($P < 0.05$). All values represent the mean ± SD ($n = 9$)

increased significantly ($P < 0.05$). On the other hand, CAT activity also increased significantly when the temperature decreased from 28 to 24 °C ($P < 0.05$). The CAT activity of cells incubated at 20 °C increased by 2.12 times on average compared with that in the 28 °C group ($P < 0.05$; Fig. 3c).

The GR activity increased significantly when the incubation temperature increased from 28 to 32 °C ($P < 0.05$), and showed no significant difference between the 32 and 36 °C groups. In the cool treatment groups, the GR activity increased significantly when the temperature decreased from 28 to 24 °C, while it decreased significantly when the temperature decreased from 24 to 20 °C ($P < 0.05$; Fig. 3d).

There was no significant difference in XOD activity among the 24, 28, and 36 °C groups. Compared with the control group, when the temperature decreased to 20 °C, the XOD activity of cells increased significantly ($P < 0.05$). XOD activity in the 32 °C group was the greatest, and was significantly higher than that in other groups ($P < 0.05$; Fig. 3e).

Oxidative-reductive state indicator analysis

The two indicators in Fig. 4 represent the oxidative and reductive state in the CIK cells. TAC is the total antioxidant capacity of the cells, and there were no significant differences among the 24, 28, and 32 °C groups. When the temperature was increased from 32 to 36 °C, the TAC declined ($P < 0.05$). Interestingly, when the incubation temperature decreased from 28 to 20 °C, the TAC at 20 °C was significantly higher than that in other groups ($P < 0.05$), and was 3.40 times higher than the 28 °C group (Fig. 4a).

As shown in Fig. 4b, the GSH content was lowest in the 28 °C group. When the temperature was increased to 32 °C, the GSH content increased, but there was no significant difference between the 32 and 28 °C groups. A significant increase was seen when the temperature was increased to 36 °C ($P < 0.05$). In the cold stress process, the GSH content increased. The GSH content was significantly higher in the 20 °C group than in the 24, 28, and 32 °C groups ($P < 0.05$), but there was no significant difference between the 20 and 36 °C groups.

Quantitative real-time PCR assay for target gene expression

For the transcript levels of the *cat* gene, there was no significant difference among the 20, 24, and 32 °C groups. In the cold treatment, the transcript levels of *cat* in the 20 and 24 °C groups was significantly higher than that in the control group ($P < 0.05$). In the heat treatment groups, the expression level of the *cat* gene was significantly increased when the temperature increased from 28 to 32 °C ($P < 0.05$), and continued increasing when the temperature was increased from 32 to 36 °C ($P < 0.05$; Fig. 5a).

The expression level of *gpx* in the 28 and 32 °C groups was lower than that in other groups, and there was no significant difference between the two groups. When the temperature increased from 32 to 36 °C, the expression level of *gpx* was significantly upregulated ($P < 0.05$). With cool treatment, its expression was also significantly upregulated ($P < 0.05$). There was no significant difference in the expression level of *gpx* between the 20 and 36 °C groups (Fig. 5b).

Compared with the 20, 24, 32, and 36 °C groups, the *Cu/Zn-sod* transcript level of the 28 °C group was the lowest. When the temperature increased to 32 and 36 °C, the transcript level of *Cu/Zn-sod* was upregulated, and there was no significant difference between the 32 and 36 °C groups. In the cool treatment process, the transcript level of *Cu/Zn-sod* was upregulated when the temperature decreased from 28 to 24 °C ($P < 0.05$). When the temperature decreased from 24 to 20 °C, the transcript level of *Cu/Zn-sod* was further enhanced, and was significantly higher than that in the 24 °C group ($P < 0.05$; Fig. 5c).

Discussion

In this experiment, antioxidant enzymes and oxidation indicators were investigated as research markers to demonstrate the effects of temperature stress on CIK cells (Klein et al. 2017; Van der Oost et al. 2003).

Temperature had immediate stress effects on CIK cell antioxidant status, and one of the most important effects was reflected in the elevated ROS content. ROS content was at the lowest level when CIK cells were incubated at 28 °C, and increased significantly when the incubation temperature was increased and decreased. Similar to our study, Cheng et al. (2015) found that high-temperature exposure (34 °C) increased the ROS level in the blood cells of pufferfish (*Takifugu obscurus*). ROS levels in grass carp serum increased significantly at 30 and 33 °C compared with that at 28 °C (Luo et al. 2017). High temperature improved the

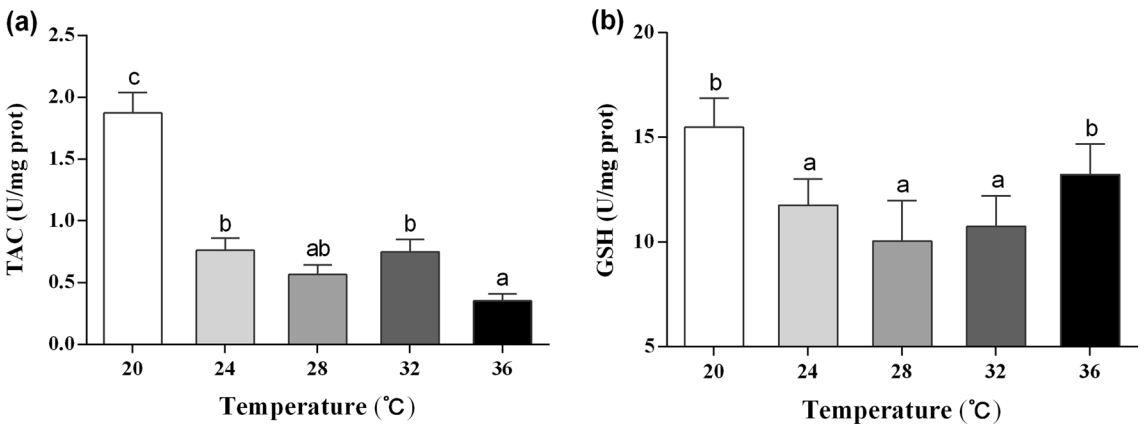

Fig. 4 The levels of TAC (**a**) and GSH (**b**) in the CIK cells after exposure to different temperatures. Significant differences between different temperatures are indicated with different case letters ($P < 0.05$). All values represent the mean ± SD ($n = 9$)

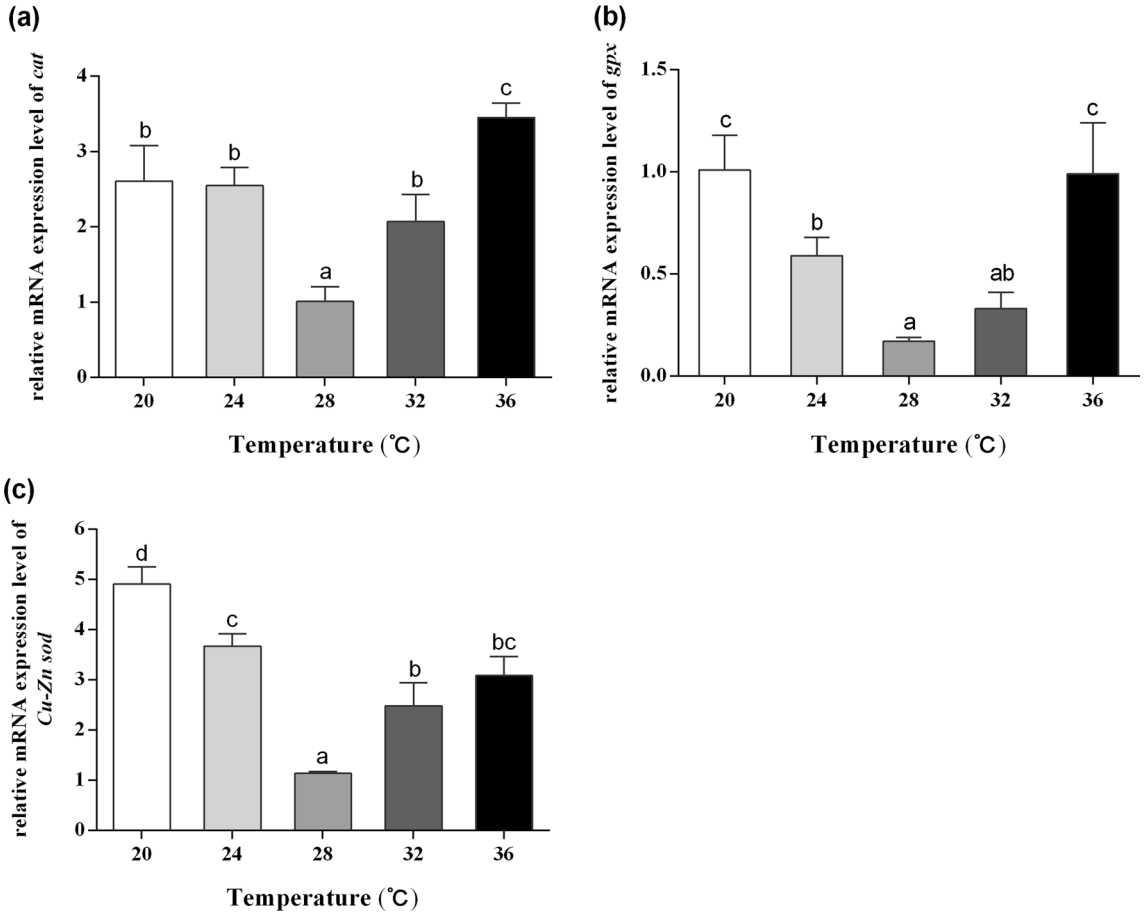

Fig. 5 The relative transcript levels of *Cu–Zn sod* (**a**), *gpx* (**b**), and *cat* (**c**) in the CIK cells after exposure to different temperatures. Significant differences between different temperatures are indicated with different case letters ($P < 0.05$). All values represent the mean ± SD ($n = 9$)

oxygen requirement and accelerated the metabolism of cells to increase the ROS level (An and Choi, 2010; Scaion et al. 2008). Xu et al. (2016) found that cold temperatures of 18 and 10 °C induced the production of ROS in a zebrafish embryo fibroblast-like cell line (ZF4 cells). Decreased temperature weakened the ADS and led to the accumulation of ROS molecules (Malek et al. 2004; Marchant et al. 2008; Niyogi et al. 2001). Acute temperature changes disturb the steady state of cells and generate excessive ROS that cannot be promptly cleared by the ADS.

The increase in the ROS level caused biological macromolecules such as lipids and proteins to be attacked (Kültz 2005). Rh123 is a cationic fluorescent dye that can penetrate the cell membrane and is an indicator of ΔΨm. At 28 °C, the Rh123 fluorescence ratio remained at the normal level, and most of the cell mitochondrial membrane remained intact. When the temperature was increased and decreased, the Rh123 fluorescence ratio increased significantly compared with that in the 28 °C group. The increased ratio of Rh123 fluorescence indicated that the mitochondria were destroyed and ΔΨm decreased (Halestrap 2010). When the temperature changed, some of the components of the mitochondrial membranes such as proteins and lipids were attacked by the elevated free radicals, leading to a loss of membrane integrity.

ROS molecules attack the lipid molecules, initiating a reaction that generates lipid peroxide, and MDA content accumulates as one of the reaction products (Kailasam et al. 2011). In the present experiment, the increased MDA content in CIK cells was associated with the elevated ROS level. Cui et al (2013) found that the MDA content in grass carp hepatic cells stressed at 32 and 34 °C was significantly higher than that in the control group, which is similar to the present result in CIK cells. Similar to in vitro experiments, the MDA content of catfish exposed to high temperature increased significantly, and cold stress increased the MDA content in the liver of juvenile rabbitfish *Siganus guttatus* (Qiang et al. 2019; Song et al. 2015; Yang et al. 2018). The variation in temperature induced lipid peroxide in fish both in vivo and in vitro.

Carbonyl protein is a product of proteins attacked by free radicals, whose content reflects the degree of protein damage

and oxidative stress status in organisms. The increase in temperature led to elevated ROS concentrations, and the carbonization rate of proteins accelerated, so the protein carbonyl content in CIK cells was significantly higher than that in the control group when the temperature increased (Hansen et al. 2006). The decrease in temperature caused a significant decrease in protein carbonyl content, and this result was not fit with ROS. In contrast to our study, the protein carbonyl level increased during cold acclimation over a period of a few weeks in three-spined stickleback *Gasterosteus aculeatus* (Kammer et al. 2011). The possible reason for this phenomenon is that the cold stress time was not long enough, and as the material itself, the stress response in fish is more complex than that in CIK cells.

It is widely known that cells have a well-developed ADS to protect against oxidative stress. SOD and CAT are vital components of the ADS, and they can indirectly reflect the body's ability to eliminate free radicals (Yang et al. 2018). SOD is a metalloenzyme that transforms superoxide anions (O^{2-}) into molecular oxygen (O_2) and H_2O_2, and the H_2O_2 that is formed is decomposed into H_2O and O_2 by CAT (Garcia et al. 2008). The present study found that SOD and CAT activity increased significantly with increasing temperature and decreasing CIK cells compared with that in the control group. Similar results were found in L8824 cells and grass carp serum, which suggests that the organisms improved the activity of antioxidant enzymes to protect against temperature stress (Cui et al. 2013; Luo et al. 2017).

The glutathione-related antioxidant system, mainly including glutathione (GSH), glutathione peroxidase (GPx), and glutathione reductase, is an important component of ADS (GR) (Li et al. 2010; Prokopenko et al. 2002). Similar to our temperature stress study, Leggatt et al. (2007) also found that increased temperature promoted GSH content in a rainbow trout *Oncorhynchus mykiss* hepatoma cell line. As an important enzyme in the GSH regeneration process, GR catalyzed oxidized GSH (GSSH) to GSH and functioned indirectly in the periods of free radical metabolism (Coelho et al. 2017). The activity of GR in CIK cells increased significantly when the incubation temperature was increased, a trend consistent with the discovery in killifish liver (Leggatt et al. 2007). GPx can use GSH or other reducing equivalents to reduce various hydroperoxides to corresponding hydroxylates (Rocha-Santos et al. 2018). The gradually increasing GPx activity with the changing temperature in CIK cells indicated that the glutathione-related antioxidant system was activated to resist oxidative stress.

TAC has been used as a comprehensive index for evaluating the functional status of cellular ADS, and reflects oxidation resistance capability in the body (Feng et al. 2012). In the present study with CIK cells, the highest level of TAC was seen in the 20 °C group and the lowest in the 36 °C group. With regard to this phenomenon, Feng et al. (2012) speculated that some constituent substances of ADS were reduced to inhibit oxidation of exogenous electrophilic groups and avoid LPO, which ultimately resulted in reduced TOC.

XOD is the main catalyzing enzyme for organs that generate free radicals when animals are under non-normal states. A previous study showed that changes in temperature affected XOD activity in juvenile Chinese sturgeon, with XOD activity highest in the 30 °C group (Feng et al. 2012). In the present study, the highest XOD activity of CIK cells appeared in the 32 °C group, which indicates that XOD was able to effectively scavenge free radicals under low-level stress to avoid the toxic effects of free radicals in the cell.

The gene expression levels of antioxidant enzymes can be used to assess cellular oxidative stress affected by temperature (Malek et al. 2004). SOD, CAT, and GPx are major antioxidant enzymes encoded by *Cu–Zn sod*, *cat*, and *gpx*. In the present study, the relative mRNA expression level of *Cu–Zn sod* increased 2.72-fold in the 36 °C group and 4.32-fold in the 20 °C group compared with the control group. Kammer et al (2011) similarly found that the transcription levels of *Cu–Zn sod* increased 1.8-fold in the liver of three-spined stickleback during cold acclimation. Moreover, the present study showed that the increased SOD activity was accompanied by increased *Cu–Zn sod* mRNA level, which both showed a trend of an increase followed by a decrease. The temperature variation upregulated the transcription levels of *gpx* and *cat* in CIK cells. In summary, genes that code for peroxisomal antioxidant enzymes were upregulated by temperature in CIK cells to resist oxidative stress (Malek et al. 2004).

In summary, changes in temperature induce oxidative stress in CIK cells, cause oxidative damage by producing ROS, and activate ADS to maintain cell stability. Temperature variation can also directly upregulate the gene expression levels of antioxidant enzymes.

Acknowledgements This research was supported by the National Natural Science Foundation (no. 31502140) and the China Agriculture Research System of MOF and MARA (CARS-45-24).

Data availability There are no linked research data sets for this submission. The data will be made available on request.

Declarations

Conflict of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

An MI, Choi CY (2010) Activity of antioxidant enzymes and physiological responses in ark shell *Scapharca broughtonii* exposed to thermal and osmotic stress: effects on hemolymph and biochemical parameters. Comp Biochem Phys B 155:34–42

Babich H, Palace MR, Stern A (1993) Oxidative stress in fish cells. In vitro studies. Arch Environ Con Tox 24:173–178

Bagnyukova TV, Lushchaka OV, Storey KB, Lushchak VI (2007) Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23 °C. J Therm Biol 32:227–234

Benzie IF, Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 299:15–27

Beyer WF Jr, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 161:559–566

Cheng CH, Yang FF, Liao SA, Miao YT, Ye CX, Wang AL, Tan JW, Chen XY (2015) High temperature induces apoptosis and oxidative stress in pufferfish (*Takifugu obscurus*) blood cells. J Therm Biol 53:172–179

Coelho ÉMP, Barbosa MC, Mito MS, Mantovanelli GC, Oliveira RS Jr, Ishii-Iwamoto EL (2017) The activity of the antioxidant defense system of the weed species *Senna obtusifolia L.* and its resistance to allelochemical stress. J Chem Ecol 43:725–738

Cui YT, Liu B, Xie J, Xu P, Tsion HM, Zhang YY (2013) The effect of hyperthermia on cell viability, oxidative damage, and heat shock protein expression in hepatic cells of grass carp *Ctenopharyngodon idellus*. J Therm Biol 38:355–361

Dadras H, Dzyuba V, Cosson J, Golpour A, Dzyuba B (2016) The in vitro effect of temperature on motility and antioxidant response of common carp *Cyprinus carpio* spermatozoa. J Therm Biol 59:64–68

Drotar A, Phelps P, Fall R (1985) Evidence for glutathione peroxidase activities in cultured plant cells. Plant Sci 42:35–40

Feidantsis K, Poertner HO, Lazou A, Kostoglou B, Michaelidis B (2009) Metabolic and molecular stress responses of the gilthead seabream *sparus aurata* during long-term exposure to increasing temperatures. Mar Biol 156(4):797–809

Feng GP, Zhuang P, Zhang LZ, Duan M, Liu JY (2012) Effects of temperature on oxidative stress biomarkers in juvenile Chinese sturgeon *Acipenser sinensis* under laboratory conditions. Adv Mater Res 343–344:497–504

Garcia SF, de Lima BC, Tie OE, Romagueira BL, Lúcia KA, Tadeu RF (2008) Antioxidant defenses and biochemical changes in pacu *Piaractus mesopotamicus* in response to single and combined copper and hypoxia exposure. Comp Biochem Physiol C Toxicol Pharmacol 147(1):43–51

Guo H, Lin W, Hou J, Wang L, Zhang D, Wu X, Li L, Li D (2018) The Protective Roles of Dietary Selenium Yeast and Tea Polyphenols on Growth Performance and Ammonia Tolerance of Juvenile Wuchang Bream *Megalobrama amblycephala*. Front Physiol 9:1371

Halestrap AP (2010) A pore way to die: the role of mitochondria in reperfusion injury and cardioprotection. Biochem Soc Trans 38:841–860

Hansen BH, Rømma S, Garmo ØA, Olsvik PA, Andersen RA (2006) Antioxidative stress proteins and their gene expression in brown trout *Salmo trutta* from three rivers with different heavy metal levels. Comp Biochem Phys C 143:263–274

Kailasam M, Kaneko G, Oo AKS, Ozaki Y, Thirunavukkarasu AR, Watabe S (2011) Effects of calorie restriction on the expression of manganese superoxide dismutase and catalase under oxidative stress conditions in the rotifer *Brachionus plicatilis*. Fish Sci 77:403–409

Kammer AR, Orczewska JI, O'Brien KM (2011) Oxidative stress is transient and tissue specific during cold acclimation of threespine stickleback. J Exp Biol 214:1248–1256

Klein RD, Borges VD, Rosa CE, Colares EP, Robaldo RB, Martinez PE, Bianchini A (2017) Effects of increasing temperature on antioxidant defense system and oxidative stress parameters in the Antarctic fish *Notothenia coriiceps* and *Notothenia rossii*. J Therm Biol 68:110–118

Kültz D (2005) Molecular and evolutionary basis of the cellular stress response. Annu Rev Physiol 67:225–257

Lankford SE, Adams TE, Cech JJ Jr (2003) Time of day and water temperature modify the physiological stress response in green sturgeon *Acipenser medirostris*. Comp Biochem Phys A 135:291–302

Leggatt RA, Brauner CJ, Schulte PM, Iwama GK (2007) Effects of acclimation and incubation temperature on the glutathione antioxidant system in killifish and RTH-149 cells. Comp Biochem Phys A 146:317–326

Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. Annu Rev Physiol 68:253–278

Li ZH, Zlabek V, Grabic R, Li P, Randak T (2010) Modulation of glutathione-related antioxidant defense system of fish chronically treated by the fungicide propiconazole. Comp Biochem Physiol C 152:392–398

Lin W, Hou J, Guo H, Li L, Wang L, Zhang D, Li D, Tang R (2018) The synergistic effects of waterborne microcystin-LR and nitrite on hepatic pathological damage, lipid peroxidation and antioxidant responses of male zebrafish. Environ Pollut 235:197–206

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408

Luo W, Xu Y, Liu XJ, Wang CF (2017) Effect of water temperature on serum content of reactive oxygen species and antioxidant defense system in grass carp *Ctenopharyngodon idella*. Freshw Fish 47(04):3–7

Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101:13–30

Lushchak VI, Bagnyukova TV (2006) Temperature increase results in oxidative stress in goldfish tissues. 1. Indices of oxidative stress. Comp Biochem Phys C 143:30–35

Lushchak VI, Bagnyukova TV, Husak VV, Luzhna LI, Lushchak OV, Storey KB (2005) Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in goldfish tissues. Int J of Biochem Cell B 37:1670–1680

Malek RL, Sajadi H, Abraham J, Grundy MA, Gerhard GS (2004) The effects of temperature reduction on gene expression and oxidative stress in skeletal muscle from adult zebrafish. Comp Biochem Phys C 138:363–373

Marchant RJ, Al-Fageeh MB, Underhill MF, Racher AJ, Smales CM (2008) Metabolic rates, growth phase, and mRNA levels influence cell-specific antibody production levels from in vitro-cultured mammalian cells at sub-physiological temperatures. Mol Biotechnol 39:69–77

Murphy BJ, Sato BG, Dalton TP, Laderoute KR (2005) The metal-responsive transcription factor-1 contributes to HIF-1 activation during hypoxic stress. Biochem Bioph Res Co 337:860–867

Niyogi S, Biswas S, Sarker S, Datta AG (2001) Seasonal variation of antioxidant and biotransformation enzymes in barnacle *Balanus balanoides* and their relation with polyaromatic hydrocarbons. Mar Environ Res 52:13–26

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358

Parihar MS, Dubey AK (1995) Lipid peroxidation and ascorbic acid status in respiratory organs of male and female freshwater catfish *Heteropneustes fossilis* exposed to temperature increase. Comp Biochem Phys C 112:309–313

Prokopenko VM, Partsalis GK, Pavlova NG, Burmistrov SO, Arutyunyan AV (2002) Glutathione-dependent system of antioxidant defense in the placenta in preterm delivery. Bull Exp Biol Med 133:442–443

Qiang J, Zhong CY, Bao JW, Liang M, Liang C, Li HX, He J, Xu P (2019) The effects of temperature and dissolved oxygen on the growth, survival and oxidative capacity of newly hatched hybrid yellow catfish larvae *Tachysurus fulvidraco*♀ ×*Pseudobagrus vachellii*♂. J Therm Biol 86:102436

Rocha-Santos C, Bastos FF, Dantas RF, Hauser-Davis RA, Rodrigues LC, Bastos VLFC, Bastos JC (2018) Glutathione peroxidase and glutathione S-transferase in blood and liver from a hypoxia tolerant fish under oxygen deprivation. Ecotoxicol Environ Saf 163:604–611

Rossati A (2017) Global Warming and Its Health Impact. Int J Occup Environ Med 8:7–20

Scaion D, Vettier A, Sébert P (2008) Pressure and temperature interactions on aerobic metabolism in migrating silver eels: results in vitro. Undersea Hyperbar M 35:27–32

Snezhkina AV, Kudryavtseva AV, Kardymon OL, Savvateeva MV, Melnikova NV, Krasnov GS, Dmitriev AA (2019) ROS generation and antioxidant defense systems in normal and malignant cells. Oxid Med Cell Longev 2019:6175804

Song ZM, Liu JY, Zhuang P, Wang Y, Zhang LZ, Hu Y, Gong P (2015) Influence of low-temperature stress on the antioxidant enzymes activities and malondialdehyde contents in liver of juvenile *Siganus guttatas*. Mar Fish 37(02):142–150

Tanaka R, Hatate H, Ito M, Nakamura T (2006) Elevation of lipid peroxide level and production of hydroxy lipids in cultured Hepa-T1 cells by oxidative stressors. Fish Sci 72:665–672

Tseng YC, Chen RD, Lucassen M, Schmidt MM, Dringen R, Abele D, Hwang PP (2011) Exploring uncoupling proteins and antioxidant mechanisms under acute cold exposure in brains of fish. PLoS ONE 6:e18180

Van der Oost R, Beyer J, Vermeulen NP (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Phar 13:57–149

Wilhelm Filho D (1996) Fish antioxidant defenses–a comparative approach. Braz J Med Biol Res 29:1735–1742

Xu QQ, Han BS, Luo JT, Yan L, Hou YW, Hu P, Zhang JF (2016) Effects of cold stress on ROS production and expression of MAPK proteins in zebrafish ZF4 cells. J Fish Sci China 23(04):771–776

Yang S, Yan T, Zhao LL, Wu H, Du ZJ, Yan TM, Xiao Q (2018) Effects of temperature on activities of antioxidant enzymes and Na^+/K^+-ATPase, and hormone levels in *Schizothorax prenanti*. J Therm Biol 72:155–160