



# Characterization and Expression Profiling of Glutathione Peroxidase 1 gene (*GPX1*) and Activity of GPX in *Onychostoma macrolepis* suffered from Thermal Stress

Haibo Yu<sup>1,\*</sup> , Chi Wang<sup>1</sup>, Wei Deng<sup>1</sup>, Guohao Liu<sup>1</sup>, Sha Liu<sup>1</sup>, Hong Ji<sup>1,\*</sup> 

<sup>1</sup>Northwest A&F University, College of Animal Science and Technology, Yangling 712100, China.

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## Corresponding Author

Tel.: +862987092937  
E-mail: yuhaiboper@nwsuaf.edu.cn  
jihong@nwsuaf.edu.cn

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## Abstract

In this study, full-length cDNA of glutathione peroxidases 1 (*GPX1*) of *Onychostoma macrolepis* was cloned by RACE, and expression of *GPX1* and activity of GPX in *O. macrolepis* suffered from heat stress were analyzed. Compared with the control group (24°C), the experimental fish were stressed for 0, 1, 3, 6, 12, 24, and 48 hours at the heated water (30°C). Liver had highest level and response speed in *GPX1* expression among various tissues after heat stress, indicated that liver was the highest sensitive tissue to heat stress. When the water was raised to the heating temperature (30°C), the GPX activity decreased in fish serum, and the consumption of GPX eliminated the increase of ROS caused by heat stress within 3h. However, after 6h and 12h stress at 30°C, GPX activity was significantly higher than that at 0h ( $P < 0.05$ ), which is due to the rapid response of GPX to heat stress. In summary, fish showed a transient stress response and was acclimated to the new temperature after 24 h according to the overall expression of the *GPX1* and the serum GPX activity, and both *GPX1* and GPX play crucial roles in this process.

## Introduction

Biotic and abiotic stresses can trigger dramatic increase of intracellular reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals and hydrogen peroxide (Fink & Scandalios, 2002; Margis et al., 2008). Aerobic organisms have developed several non-enzymatic and enzymatic systems such as glutathione peroxidases (GPX), superoxide dismutases (SOD), catalases (CAT) and ascorbate peroxidases to neutralize these compounds (Fink & Scandalios, 2002; Margis et al., 2008). Glutathione peroxidases (GPXs) are a family of antioxidant enzymes catalyzing the reduction of H<sub>2</sub>O<sub>2</sub> or organic hydroperoxides, and regulate oxidative status together with other antioxidant enzymes (Apel & Hirt, 2004). GPX1 is a vital component

of the GPXs family (Toppo et al., 2008; Flohé & Brigelius-Flohé, 2011). cDNA sequence of *GPX1* has been reported in the rainbow trout (*Oncorhynchus mykiss*) (Pacitti et al., 2013), Antarctic fish *Trematomus bernacchii* (Sattin et al., 2015) and grass carp (*Ctenopharyngodon idellus*) (Li et al., 2008).

*Onychostoma macrolepis* belongs to the Cypriniformes, Cyprinidae, Barbinae, *Onychostoma* (Yu et al., 2017). In recent years, *O. macrolepis* has been becoming a cultured species in China due to its delicious taste and high content of docosahexaenoic acid (DHA) in muscle (Yu et al., 2017). Studies have been carried out in this species, focusing on artificial breeding, genetic diversity and nutritional requirement (Chen, 2007; Zhao et al., 2011), as well as bioinformation of some genes (Yang et al., 2014; Yu et al., 2017). However, there was

no available information focusing on the gene information, molecular characterization and expression profiling of *GPX1* in *O. macrolepis* to the present.

Water temperature is an important ecological factor that affect behavior, growth, metabolism, immune response, and survival of fish (Windisch et al., 2011; Makrinos & Bowden, 2016; Dalvi et al., 2017). In nature, *O. macrolepis* commonly inhabits in mountain streams with relatively low temperature. Hence, water temperature is a key factor that affects the aquaculture of this species. *O. macrolepis* have an optimal growth temperature at 18°C-24°C (Chen, 2007), but they are usually exposed to high (over 30°C) water temperature in the summer, which can trigger their oxidative stress and poor growth performance (Yu et al., 2017). Many studies reveal that fish can modulate their antioxidant system to adapt to the temperature changes (Cheng et al., 2015; Wang et al., 2016; Madeira et al., 2016; Yu et al., 2017). *GPX1*, as one of the most important parts of antioxidant system, might be a sensitive indicator and defense barrier in response to heat stress in *O. macrolepis*. However, nothing is known about the expression profiles of *GPX1* and GPX activity changes of *O. macrolepis* after heat stress.

In this study, the full-length cDNA sequence of *GPX1* from *O. macrolepis* was identified and its molecular sequence properties were characterized including phylogenetic analysis. Expression of *GPX1* and activity of GPX in *O. macrolepis* after heat stress were also determined, aiming to provide insights into the role of *GPX1* and GPX in antioxidant defense of *O. macrolepis* suffered from heat stress.

## Materials and Methods

### Experimental Fish

The fish was purchased from an aquaculture farm in Shaanxi Province, China. Before the start of the formal experiment, the experimental fish were acclimated at 24°C for two weeks. Fish are fed three times a day at 8:00, 12:00 and 17:00, with a 5% of body wet weight (Deng et al., 2020).

### Experimental Procedures and Sample Collection

All the animal experiments and sampling procedures were approved by the Animal Care and Use Committee of Northwest A&F University and performed in accordance with animal welfare and ethics (Approval No. DKZC2019053). After acclimation, experimental fish were fasted for 24 h. One hundred forty-four fish individuals with the size of  $19.5 \pm 0.9$  g were distributed randomly into 8 groups. Each group contained three replicate tanks (45×25×30 cm) such that there were 6 fish in each tank. For heat stress, the temperature rose from 24 °C to 30 °C within 1 hour. The heat stress temperature (30 °C) was decided according to the temperature fluctuations that this fish species

encounters in its natural habitat and the results of our previous study (Yu et al., 2017). The experimental fish of the control group were sampled at 24°C. After stressed at 30°C, fish were sampled at 0, 1, 3, 6, 12, 24 and 48 hours, respectively.

Aeration was continuously provided during the experiment to maintain the dissolved oxygen (DO) higher than 5.0 mg L<sup>-1</sup>. Water temperature was adjusted and maintained using auto-regulation induction heaters. pH was within the range of 7.2 to 7.8. The parameters of the water quality were measured daily.

The experimental fish were anesthetized using tricaine methanesulfonate (MS-222, Sigma-Aldrich, USA) with the dose of 90 mg L<sup>-1</sup> before sampling. A 2.5ml hypodermic syringe was used to collect blood samples from the tail vein (3 fish per fish). After standing at 4°C for 6 hours, the serum was centrifuged (3,000xg, 10 minutes, 4°C). Thereafter, the serum samples of 3 fishes in the same tanks were pooled together. Tissues of 3 individuals were collected in each tank, including the heart, liver, kidney, spleen, gills, intestines and muscles, and pooled together at each sampling time point. The experimental samples were stocked at -80°C until being analyzed.

### Cloning of Full-length *GPX1* cDNA

Total RNA was extracted from tissue samples using TRIzol Reagent (Invitrogen, USA) according to manufacturer's protocols. Integrity of RNA was checked by denaturing on agarose gel (1%). Quantity and quality of extracted RNA was evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, United States). The 260/280 nm absorbance ratio of all samples was above 1.8-2.0. Then, according to the manufacturer's method, first-strand cDNA was synthesized using relevant reagents (TaKaRa, Japan), and a partial cDNA sequence of its template *GPX1* was amplified.

To amplify *GPX1* partial cDNA sequence, primers (Table 1) were designed according to the available genes of other fish species, including grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), zebrafish (*Danio rerio*) and Chinese rare minnow (*Gobiocypris rarus*). In order to obtain the full-length *GPX1* sequence, the first cDNA was generated to quickly amplify the 5'/3' cDNA ends according to the manufacturer's protocols of a SMARTer™ RACE cDNA Amplification Kit (Clontech, USA). The full-length *GPX1* cDNA was obtained by using 5'/3'-RACE methods with gene specific primers designed based on the obtained partial sequence of *GPX1* cDNA (Table 1). The PCR products were cloned into pMD18-T vector (Tiangen, China), and then inserted into *E. coli* strains. And positive clones were sequenced by Sangon Biotech (Shanghai, China). Finally, the partial sequence was assembled using contig Express application software.

**Table 1.** Primers used in the present study

Primer name	Primer sequence (5'-3')	Purpose
GPX1-F	GTNCTNATTGARAATGKGCCTC	Cloning
GPX1-R	AGGAACCTTYTCAAAGTTCCAGG	Cloning
GPX1-R1	TCGAAGCCATTTCAGGA	5' RACE
GPX1-R2	GACGGACTTCAGAGACAGCA	5' RACE
GPX1-F1	TGTTCTCAAGGAGAAGCTGCCTCA	3' RACE
GPX1-F2	CGACTCTGCGTCCTTGATGGTTGAT	3' RACE
GPX1-real-F	AGGCACAACAGTCAGGGATT	qRT-PCR
GPX1-real-R	CGGACTTCAGAGACAGCAGA	qRT-PCR
$\beta$ -Actin-F	TGACCCACACTGTACCCATC	qRT-PCR
$\beta$ -Actin-R	CGGACAATTTCACTCTCGGC	qRT-PCR

**Note:** Genbank No.: GPX1, KY569541;  $\beta$ -Actin, JN254630.1.

### Bioinformatics Analysis of GPX1

The ORF Finder program (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) was used to identify the ORF of GPX1. The online tool of ExPASy was used to conduct the protein analysis. The deduced GPX1 amino acids sequences of *O. macrolepis* were selected for multiple alignments with other organisms. The organisms include silver carp (*Hypophthalmichthys molitrix*) (ABU84810.1), *Acrossocheilus fasciatus* (Accession number: AIM56842.1), grass carp (*Ctenopharyngodon idella*) (ACF39780.1), crucian carp *Carassius auratus* (ABJ09418.1), zebra fish (*Danio rerio*) (NP\_001007282.2), bighead carp *Hypophthalmichthys nobilis* (ACO53608.1), Bastard halibut *Paralichthys olivaceus* (ABU49600.1), Pacific bluefin tuna *Thunnus orientalis* (BAL41419.1), mefugu (*Takifugu obscurus*) (ACR20471.1), barred knifejaw (*Oplegnathus fasciatus*) (AAU44619.1), gilthead seabream (*Sparus aurata*) (AFY97790.1), human (*Homo sapiens*) (CAA68491.1), chicken (*Gallus gallus*) (NP\_001264782.2), tropical clawed frog (*Xenopus tropicalis*) (NP\_001015740.2), chimpanzees *Pan troglodytes* (NP\_001070980.2), *Bos Taurus* (AAA16579.2), modern horses *Equus caballus* (NP\_001159951.1), *Mus musculus* (CAA27558.1), *Taeniopygia guttata* (NP\_001130041.1). The phylogenetic tree was generated by the neighbor-joining method of the MEGA 6.0 program. The protein 3D model prediction was carried out on the online tools (<http://www.expasy.org/>) using SWISS MODEL online software.

### GPX1 Expression of *O. macrolepis*

Primers used for RT-PCR were designed using the Primer Express 2.0 software (Applied Biosystems, Foster City, CA, USA) and listed in Table 1. All primers synthesized by Sangon Biotech (Shanghai, China). The housekeeping gene used for normalization of target gene was  $\beta$ -actin (GenBank: JN254630.1). RT-PCR was conducted using a CFX 96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Three replicates were performed, and each total volume was 20  $\mu$ L containing 10  $\mu$ L of 2 $\times$  SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> II (TaKaRa,

Dalian, PR China), 7.8  $\mu$ L of sterilized double-distilled water, 1  $\mu$ L of 1:10 diluted cDNA and 0.6  $\mu$ L of each forward and reverse primer. The RT-qPCR procedure including initial activation step at 95  $^{\circ}$ C for 3 min, followed by 40 cycles of 95  $^{\circ}$ C for 15 s and 60  $^{\circ}$ C for 30 s, and 5 s at 65  $^{\circ}$ C. Melt-curve analysis was performed at the end of each run. All amplification efficiencies were ranged from 96% to 103%. The comparative Ct method  $2^{-\Delta\Delta Ct}$  (Livak & Schmittgen, 2001) was used to calculate the gene expression values and normalized with  $\beta$ -actin (Genbank No. JN254630.1).

### Activity of GPX in the Serum of *O. macrolepis*

Activity of GPX was measured according to (Liu et al., 2013), using the Glutathione Peroxidase Assay Kit (Nanjing Jiancheng Bioengineering Institute, China). The activity of GPX was determined by measuring the oxidation rate of reduced glutathione (GSH) to oxidized glutathione (GSSG) form by H<sub>2</sub>O<sub>2</sub> and the catalysis of GPX. The GPX activity was expressed as the GPX U $\cdot$ mL<sup>-1</sup>.

### Statistical Analysis

The student's t test was used to analyze the difference of the control group (24 $^{\circ}$ C), compare to the experiment group at 30 $^{\circ}$ C (0h). Tukey adjustment was used to analyze the differences between groups of 30  $^{\circ}$ C for 0, 1, 3, 6, 12, 24 and 48 hours through one-way analysis of variance (ANOVA). Prior to statistical analyses, raw data were diagnosed for normality of distribution and homogeneity of variance with the Kolmogorov-Smirnov test and Levene's test, respectively (Zar, 1999). Statistical analysis and graphs were performed by Prism 5 Software (Graph Pad, La Jolla, CA). P<0.05 was considered significant for all tests.

## Results

### Identification and Bioinformatic Analyses of GPX1 in *O. macrolepis*

Full-length cDNA of GPX1 in *O. macrolepis* (Genbank accession No. KY569541) was cloned by means of RACE method. The full length of GPX1 of *O.*

*macrolepis* is 915bp, and it contains a 525bp open reading frame (ORF) and encodes 174 amino acids of putative peptide, with a theoretical pI of 5.92 and an estimated molecular weight of 16332.71 Da (Figure 1). Multiple alignment of the deduced *GPX1* amino acid sequences were conducted (Figure 2) and a phylogenetic tree was built (Figure 3). The deduced amino acid sequence of *O. macrolepis GPX1* contained a signature motif 2 (LGAPCNQF) and a conserved active site motif (WNFEKF) (Figure 2). *O. macrolepis GPX1* was grouped together with the *GPX1* of other Cypriniformes species, forming an independent clade branched from Perciformes, Pleuronectiformes and Tetraodontiformes, as well as amphibians, avian and mammalian. The predicted three-dimensional (3D) structure of *GPX1* was comparable with 5  $\alpha$ -helices and 2  $\beta$ -sheets (Figure 4).

### Expression of *GPX1* in *O. macrolepis* Suffered from Heat Stress

Tissue distribution of *GPX1* expression in *O. macrolepis* was shown in Figure 5. The expression of *GPX1* in the muscle, spleen, liver, intestine, kidney, gills and heart were tested. The highest expression level of *GPX1* was observed in the liver, and the lowest expression level of *GPX1* were observed in the gills, intestine and spleen ( $P < 0.05$ ) (Figure 5).

Expression profiles of *GPX1* of *O. macrolepis* suffered from heat stress was shown in Figure 6. After

water rose to heating temperature (30°C), expression level of *GPX1* in liver, intestine and gills significantly increased ( $P < 0.05$ ) (Figure 6), but the expression level in muscle and heart decreased significantly ( $P < 0.05$ ) (Figure 6). The response rate of *GPX1* expression to heat stress varied in different tissues. The expression level of *GPX1* reached its peak at 0 hours in the liver, 3 hours in the heart, spleen and gills, and 6 hours in the intestines and muscles after 30°C stress.

### Change in Activity of GPX in Serum of *O. macrolepis* Suffered from Heat Stress

Variation of GPX activity in serum of *O. macrolepis* subjected to heat stress was shown in Figure 7. After the water was raised to the heating temperature (30°C) for 0h, the GPX activity was found to be significantly decreased by testing the serum of *O. macrolepis* ( $P < 0.05$ ). And after the water temperature was kept at 30°C for 1 h, the GPX activity was found to be significantly decreased compared with 0 h by testing the serum of *O. macrolepis* ( $P < 0.05$ ). Studies have shown that the GPX activity in serum gradually rises within 1 hour to 6 hours, and on the contrary, it gradually decreases within 6 hours to 48 hours. The highest GPX activity in serum of *O. macrolepis* after stress at 30 °C was observed at 6 h ( $P < 0.05$ ). Activity of GPX in serum of *O. macrolepis* after stressed at 30 °C for 24 h and 48 h significantly lower than that of 0 h ( $P < 0.05$ ).

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1  aacaccactttgcgctttgaagtacgtagtaaacggcgtgttgg
46  gcatggcaggggcccgcgaagaagttttacgatctgtccgccaac
91  ttttgtcaggggacctcctaaatctttcctctctcaaaggtaaag
136 tgggtgcttattgaaaatgtggcgtctctttgaggcacaacagtc
1  M N E L H S R Y A D Q
181 gggattacacccagATGAACGAGCTCCACAGCCGCTACGCTGATC
12  G L V I L G A P C N Q F G H Q
226 AGGGGCTCGTGATCCTGGGCGCTCCCTGCAACCAGTTCGGACATC
27  E N C K N D E I L L S L K S V
271 AGGAGAACTGCAAGAACGATGAAATTCTGCTGTCTCTGAAGTCCG
42  R P G N G F E P Q F Q L L E K
316 TCCGTCCTGGAATGGCTTCGAACCACAATTCCAACCTTTGGAGA
57  L E V N G A N A H P L F M F L
361 AGCTGGAAGTGAACGGTGCGAACGCCACCCCTCTGTTTATGTTCC
72  K E K L P Q P S D D S A S L M
406 TCAAGGAGAAGCTGCCTCAGCCAGTGACGACTCTGCGTCCTTGA
87  V D P K F I I W S P V N R N D
451 TGGTTGATCCCAAATTCATCATCTGGAGTCCGGTGAACAGGAATG
102 V S W N F E K F L I G P D G E
496 ACGTCTCCTGGAACCTTTGAGAAGTTCCTCATCGGCCCGGACGGGG
117 P F K R Y S R R F L T I D I E
541 AACCGTTCAAGAGATACAGCAGAAGGTTCCCTCACCATCGACATTG
132 A D I K E L L K R T K *
586 AAGCAGATATCAAAGAGCTTCTGAAGAGGACAAAAATAAacctggc
631 atcctcctggtgttgtggccatgtgagataaacctccattccat
676 atcactttagagtggttgtttgatataggccgactgtgacagacat
721 gattaaagtgcagctgttttttagactgtttacttaaatgaagatg
766 cttcctaaaacctttttgaggggaagccttctgatgtaactgtaaat
811 gtttattataatagttgtcattttatccatgaaactgcacagctcag
856 gttttatcattcaactgttaaccaaataaaacgtaaaaaaaaaa
901 aaaaaataaaaaaaaa

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**Figure 1.** Nucleotide and deduced amino acid sequence of the *GPX1* from *O. macrolepis*. The start codon, ATG and the stop codon, TGA are circled with black boxes. GPX signature motif 2 are marked with a dotted line, and conserved active site motif is underlined.

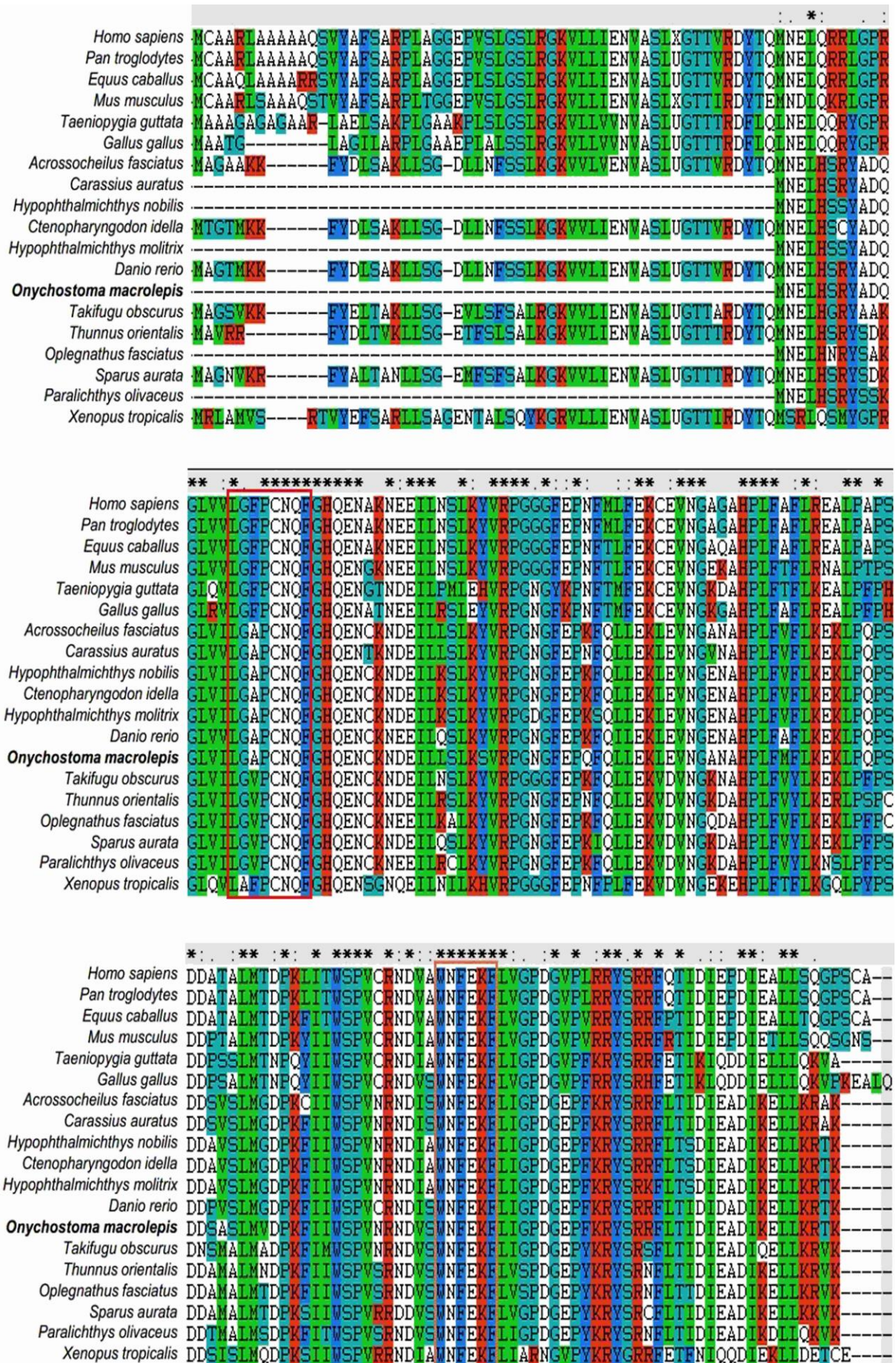


Figure 2. Multiple alignment of amino acid sequences between GPX1s from fish and other species. The GPX signature motif 2 is circled with red box and the conserved active site motif is circled with orange box.

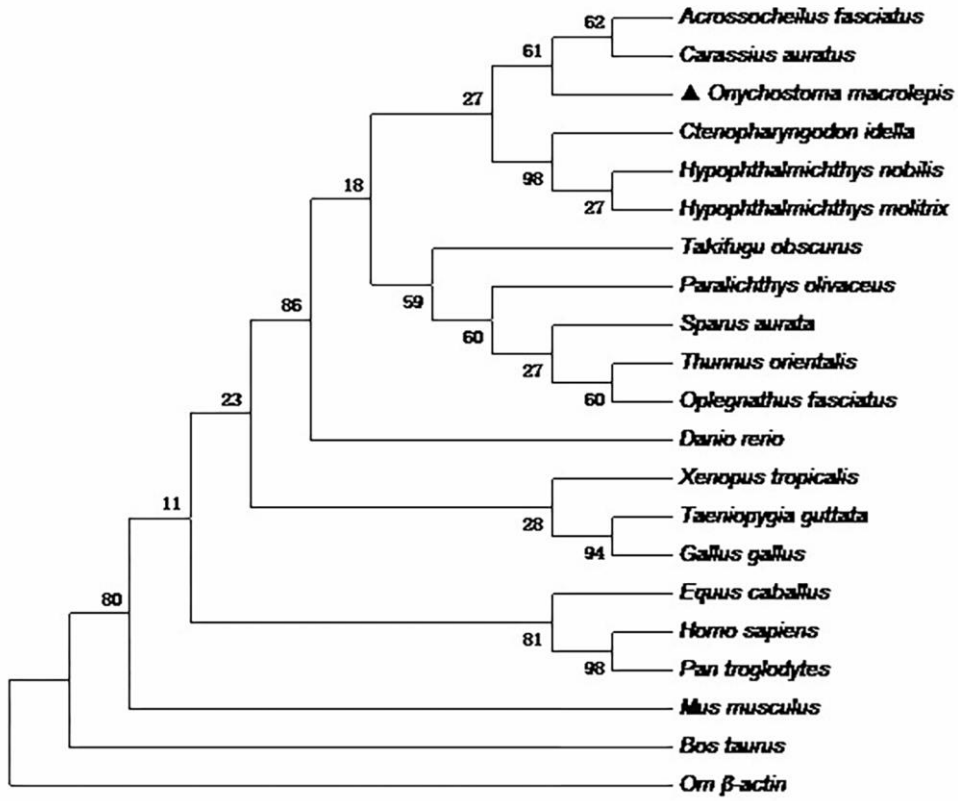


Figure 3. Phylogenetic tree of the deduced amino acid sequences of the GPX1.



Figure 4. 3D structure of the GPX1 predicted by SWISS MODEL.

## Discussion

### Identification and Bioinformatic Analyses of *GPX1* in *O. macrolepis*

In this study, full-length cDNA of glutathione peroxidases 1 (*GPX1*) of *O. macrolepis* was firstly cloned by RACE method. *GPX1* of *O. macrolepis* contains a 525bp ORF, and encodes 174 amino acids of putative peptide, with a estimated theoretical pI of 5.92 and an molecular weight of 16332.71 Da (Figure 1). The deduced amino acid sequence of *GPX1* in *O. macrolepis* contained a signature motif 2 (LGAPCNQF) and a conserved active site motif (WNFEKF), which is consistent with the sequence of Cyprinidae species, including *Acrossocheilus fasciatus*, *Carassius auratus*, *Hypophthalmichthys nobilis*, *Ctenopharyngodon Idella*, *Hypophthalmichthys molitrix* and *Danio rerio* (Pacitti et al., 2013). To further confirm the identities of *GPX1* of *O. macrolepis* and expound the phylogenetic relationship of *GPX1* between species, multiple alignment of the deduced *GPX1* amino acid sequences were conducted and a phylogenetic tree was built. *O. macrolepis GPX1* was grouped together with the *GPX1* of other Cypriniformes species, and form an independent clade branched from Perciformes, Pleuronectiformes and Tetraodontiformes, as well as avian, amphibians and mammalian. And the phylogeny of the selected organism is well reflected. The similar results were also found in other Cypriniformes species, including pengze crucian carp (*Carassius auratus* var. Pengze), grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) (Li et al., 2008; Li et al., 2013).

### Expression of *GPX1* in *O. macrolepis* in Response to Heat Stress

Previous studies in many fish species showed that liver were the primary organ that expressed antioxidant enzyme genes and antioxidant enzymes (Basha & Rani, 2003; Albano, 2006; Kim et al., 2010; Sattin et al., 2015). In this study, tissue expression profiling revealed that liver had the highest expression level of *GPX1* among the various tissues in *O. macrolepis* examined ( $P < 0.05$ ) (Figure 5). Similar result has been found that the liver of *O. macrolepis* had the highest expression level of the other antioxidant enzyme genes (such as *CAT* and *Cu/Zn SOD*) among the tissues (Yu et al., 2017). The results indicated that liver was a main place where oxidative stress occurs, and the antioxidant enzyme genes including *GPX1* play an important role in liver antioxidant defense.

Heat stress generally led to excessive generation of ROS, which can result in oxidative damage in fish if not eliminated timely (Kaur et al., 2005; Cheng et al., 2015; Madeira et al., 2016). Previous studies found that the antioxidant system can eliminate the excessive ROS and it is one of the most important defense mechanism of sturgeon (*Acipenser brevirostrum*) and turbot (*Scophthalmus maximus*) when suffering heat stress (Gradil et al., 2014; Guerreiro et al., 2014). *GPX1* is one of the stress responsive elements of the defense system against oxidative damage, and its expression could be affected by various stressors, including heat stress (Sattin et al., 2015). In this study, the key role of *GPX1* in liver intestine and gills in the body's response to heat stress was shown. This is because the expression of *GPX1* in these tissues increased significantly after exposed to the heated water (30°C) ( $P < 0.05$ ) (Figure 6).

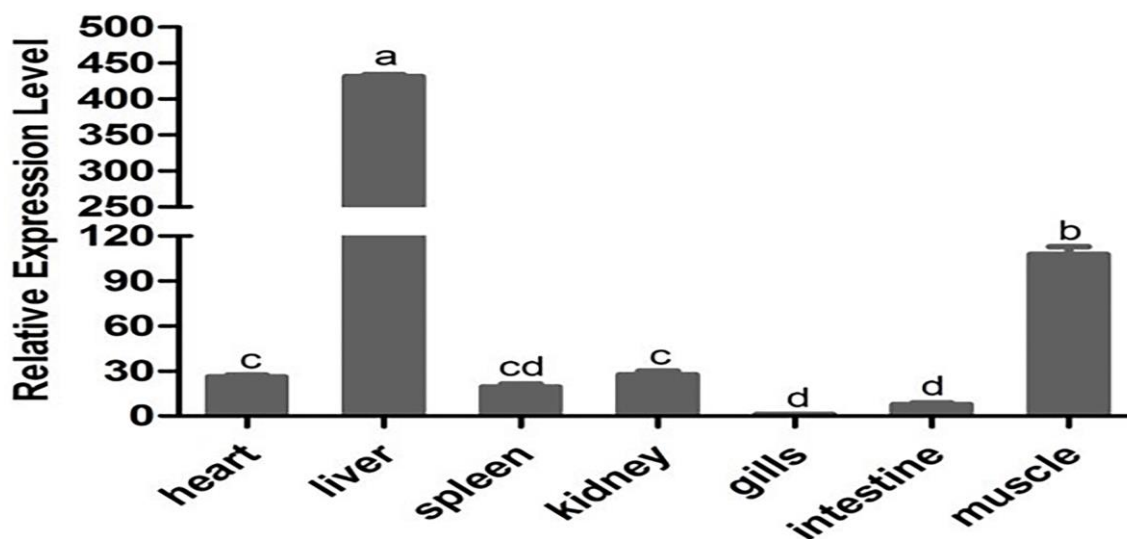
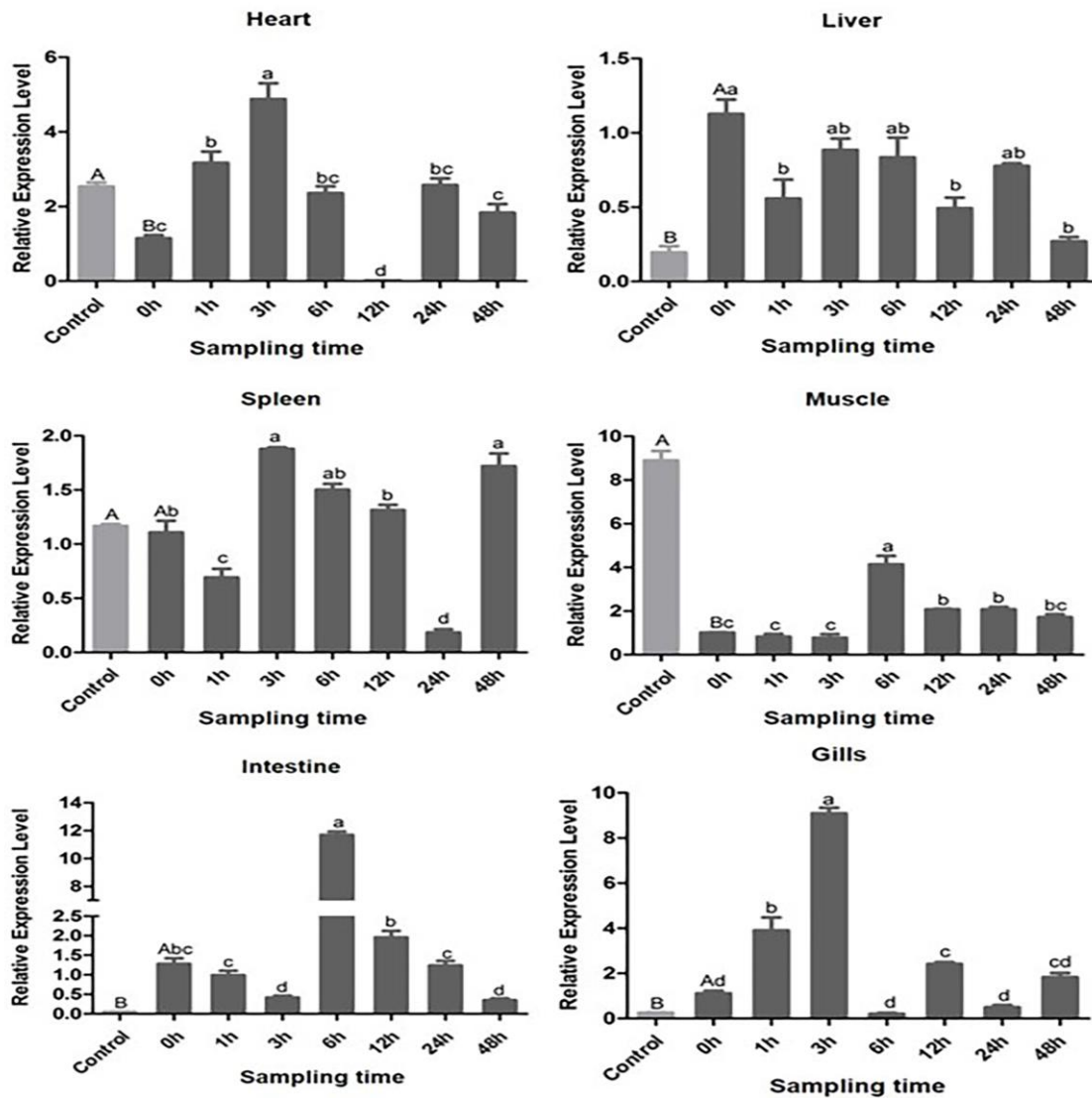
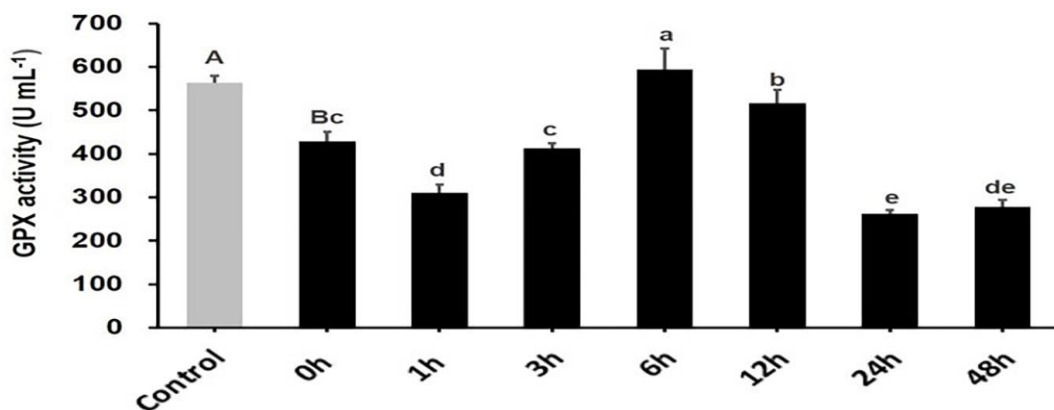


Figure 5. Tissue distribution of *GPX1* of the *O. macrolepis* cultured at 24°C. The values (n=3) were expressed as mean  $\pm$  SEM.



**Figure 6** Relative expression of the *GPX1* in different tissues of *O. macrolepis* in response to heat stress. The values (n=3) were expressed as mean  $\pm$  SEM. For different capital letter means different between control (24°C) and 0h (30°C) groups. For different lowercase letter means different among 0h, 1h, 3h, 6h, 12h, 24h and 48h groups. The gene expression level of all groups were converted to relative expression level according to the expression level in 1 h group, which mean the relative expression level in 1 h group was  $\sim$ 1. For all tests,  $P < 0.05$  was considered significant.



**Figure 7** Change in activity of GPX in the serum *O. macrolepis* in response to heat stress. The values (n=3) were expressed as mean  $\pm$  SD. For different capital letter means different between control (24°C) and 0h groups. For different lowercase letter means different among 0h, 1h, 3h, 6h, 12h, 24h and 48h groups. For all tests,  $P < 0.05$  was considered significant.



Similar result was also found in pufferfish (*Takifugu obscurus*) (Cheng et al., 2015) in which high water temperature significantly induced mRNA expression of antioxidant enzyme genes including *GPX*.

In addition, the quick response in expression of *GPX1* is important in antioxidant defense processes of fish suffer from heat stress. In this study, the speed of response to heat stress varied in different tissues in terms of *GPX1* expression. The expression level of *GPX1* reached its peak at 0 hours in the liver, 3 hours in the heart, spleen and gills, and 6 hours in the intestines and muscles after 30°C stress. Liver had the highest response speed of *GPX1* expression to heat stress among the tissues examined, indicating that liver was the highly sensitive tissue in response to heat stress. The above results showed that the liver had the highest level and response speed of *GPX1* expression. This is reasonable as liver is a key organ of ROS generation (Albano, 2006) so that high stock and quick generation of antioxidant is required to protect this organ against lipid peroxidation induced by heat stress.

Interestingly, our previous studies have shown that the mRNA level of Cu/Zn-SOD reaches its peak in most tissues of *O. macrolepis* after 24 hours of stress at 30°C (Yu et al., 2017). However, in this study, expression of *GPX1* can respond quickly to heat stress at 30 °C within 6 h, though the response speed varied in different tissues. These results indicated that expression response of *GPX1* to heat stress was more prompt compared to *Cu/Zn-SOD* in *O. macrolepis*. Up-regulation of *GPX1* might strengthen the antioxidant defense of *O. macrolepis* suffered from heat stress at the early stages (within 6 h).

#### Change in Activity of GPX in Serum of *O. macrolepis* Suffered from Heat Stress

Many previous study selected activity of antioxidant enzyme in serum as a indicator to evaluate the antioxidant status of fish (Jiang et al., 2009; Tang et al., 2016; Yu et al., 2017). Therefore, in this serum study of *O. macrolepis*, the activity of GPX in heat stress was determined. When temperature rose to the 30 °C (0 h), it indicated that the GPX activity decreased significantly from the results of the serum study from  $563.6 \pm 16.3$  U mL<sup>-1</sup> to  $429.0 \pm 22.3$  U mL<sup>-1</sup> (P<0.05), and kept decreasing within 3 h after stressed at 30 °C. Decrease of GPX activity at early stages (within 3 h) of heat stress might be due to consumption of GPX to eliminate the overproduced ROS caused by heat stress. Similar result was also found by Kaur et al. (2011) in which GPX activity decreased significantly in liver, kidney and gills of *Channa punctata* Bloch with 3 h heat stress (32 °C) compared to control group (20 °C).

After stressed at 30 °C, *GPX1* transcription was rapidly activated within 6 h to synthesize new GPX to eliminate excessive ROS. But, increase of GPX activity in the serum was observed from 6 h to 12 h after stressed at 30 °C. This unsynchronized changes in gene

expression and enzyme activity might be due to lag of translation to transcription, and many previous studies have found that there was a time window between transcription and translation (Amado et al., 2011; Kong et al., 2017; Yu et al., 2017). In addition, some enzyme or proteins are also subjected to post-transcriptional regulation, which might lead to the lack of similarity between mRNA levels and enzyme activity (Hansen et al., 2007). With the continuous transcription and translation, the levels of GPX were recovered after stressed at 30 °C from 6 h to 12 h, indicating an enhanced antioxidant capacity of *O. macrolepis* to the short-term heat stress. Interestingly, compared to the 0 h, it was found that the activity of GPX decreased significantly by testing the serum after stressed at 30 °C for 24 h and 48 h (P<0.05), which may because of the adaptation of *O. macrolepis* to the elevated temperature.

In conclusion, a 915 bp *GPX1* cDNA was cloned from *O. macrolepis*, which encodes a protein consisting of 174 amino acids. The liver had the highest level and response speed in *GPX1* expression among various tissues in response to heat stress, indicated that liver was the highest sensitive tissue in response to heat stress, and the *GPX1* plays a crucial role in defense of oxidative stress resulted from heat stress in liver. GPX activity in the serum declined after exposed to the heated water at 30 °C (0 h), and kept decreasing within 3 h, which may be due to the over consumption of GPX for eliminating the increased ROS caused by heat stress. However, compared with 0 h, GPX activity increased significantly after 6 h and 12 h at the heated water, due to its rapid response to heat stress. It appeared that the fish showed a transient stress response and was acclimated to the new temperature after 24 h according to the overall expression of the *GPX1* and the serum GPX activity, and both *GPX1* and GPX play crucial roles in this process. However, further study on the expression response of *GPX1* and GPX to a longer term heat stress also needs to be addressed in the future.

#### Ethical Statement

Not applicable.

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#### Author Contribution

**HY:** Methodology; Investigation; Formal analysis, Roles/Writing - original draft, Funding acquisition; Project administration; Supervision. **CW:** Writing -

review & editing, Investigation. **WD:** Formal analysis; Investigation. **GL:** Investigation. **SL:** Investigation. **HJ:** Methodology; Project administration; Supervision.

### Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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