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# Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23 °C

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**Abstract**

The effects of a rapid transfer from a low (3 °C) to a warm (23 °C) temperature on oxidative stress markers and antioxidant defenses were studied in the brain, liver and kidney of the goldfish, *Carassius auratus*. Cold-acclimated fish were acutely moved to 23 °C and sampled after 1, 6, 12, 24, 48 or 120 h of warm temperature exposure. Lipid peroxide levels increased quickly during the first few hours at 23 °C, but thiobarbituric acid-reactive substances changed little. Protein carbonyl content was reduced by 20–40% in the liver over the entire experimental course, but increased transiently in the kidney. The content of high-molecular mass thiols decreased by two-thirds in the brain and was affected slightly in other organs. By contrast, total low-molecular mass thiols (e.g. glutathione and others) increased transiently. Activities of the primary antioxidant enzymes—superoxide dismutase and catalase—were generally unaffected in goldfish organs, whereas glutathione-dependent enzymes were elevated in the brain and kidney after 24–48 h at 23 °C. Glutathione peroxidase increased by 1.5–2.3-fold and glutathione-*S*-transferase by 1.7-fold. Hence, a short-term exposure to warm temperature disturbed several oxidative stress markers, but only slightly affected the activities of antioxidant enzymes. However, comparison of the current data for cold-acclimated winter fish with the same parameters in summer fish suggests that longer exposure to high ambient temperature requires the enhancement of activities of glutathione-dependent enzymes for maintaining the steady-state levels lipid peroxidation and protein oxidation in goldfish tissues.


*Keywords:* Goldfish; Temperature adaptation; Oxidative stress; Antioxidant enzymes; Lipid peroxidation

## 1. Introduction

Ectotherms, including fish, are consistently subjected to varying ambient temperature and must cope with its fluctuations. The metabolic rate of ectothermic animals and, hence, oxygen consumption is proportional to environmental temperature (Hochachka and Somero, 1984). Furthermore, the rate of reactive oxygen species (ROS) generation, including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($^{\cdot}OH$) is related to oxygen consumption (Halliwell and Gutteridge, 1989). Therefore, it can be expected that the intensification of respiration at higher temperatures would result in enhanced ROS production and, therefore, that adaptive responses by antioxidant defenses would be expected for ectotherms when shifting from low to high ambient temperatures.

Seasonal variations in free radical metabolism of ectotherms may mainly reflect the influence of environmental temperature changes over the year. This issue has been studied in a variety of ectotherms (Viarengo et al., 1991; Power and Sheehan, 1996; Wilhelm Filho et al., 2001; Bagnyukova et al., 2003; Ramos-Vasconcelos and Hermes-Lima, 2003; Ramos-Vasconcelos et al., 2005) but fish have not received much attention (Wilhelm Filho et al., 2001). Another way to examine antioxidant responses to temperature changes is to use experimental induction of temperature shock, that is short-term transfer to significantly higher or lower temperature. This action can disturb antioxidant potential and cause oxidative stress (Parihar and Dubey, 1995; Heise et al., 2006; Lushchak and Bagnyukova, 2006a, b).

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Eurythermal species are of special interest owing to their ability to survive a wide range of temperatures in nature. One of such species, the goldfish, *Carassius auratus* L., is highly tolerant of a wide spectrum of natural environmental factors including both oxygen (anoxia, hyperoxia) and temperature extremes (van den Thillart et al., 1983; Lushchak et al., 2001, 2005; Ford and Beitinger, 2005; Lushchak and Bagnyukova, 2006a, b). Well-developed antioxidant defenses of goldfish may contribute to its ability to adapt to widely varying environmental conditions. This species possesses high constitutive activities of antioxidant enzymes and glutathione levels, which allow it to cope with a broad range of stressful factors. Moreover, goldfish show so-called "preparation for oxidative stress" by increasing their antioxidant potential under anoxic conditions when ROS production is low in anticipation of a large increase in ROS output when oxygen is reintroduced (Lushchak et al., 2001). These peculiarities allow goldfish to be a useful model to study adaptations of antioxidant systems to a variety of natural conditions.

Previously, we have shown that acute temperature changes from an average summer temperature (21 °C) to extremely high temperature (35 °C) led to oxidative stress development and responses by antioxidant enzymes in goldfish tissues (Lushchak and Bagnyukova, 2006a, b). The present study was aimed to determine whether oxidative stress develops and antioxidant potential of goldfish tissues increases when fish are transferred from a low temperature (3 °C) to a higher temperature (23 °C) over the course of 5 days. Products of oxidative damage to proteins (protein carbonyls (CP)) and lipids (lipid peroxides (LOOH) and thiobarbituric acid-reactive substances (TBARS)) as well as changes in the activities of six antioxidant enzymes were quantified in the brain, liver and kidney of goldfish. In addition, we assessed levels of both low-molecular mass thiols (L-SH), which represent the sum of various soluble thiol-containing metabolites such as glutathione, cysteine and others, and high-molecular mass thiol (H-SH) groups, which are the sum of thiol groups in acid-precipitable proteins.

## 2. Materials and methods

### 2.1. Reagents

*N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), $NADP^+$, NADPH and Tris–HCl were purchased from Reanal (Hungary). Butylated hydroxytoluene (BHT), 1-chloro-2, 4-dinitrobenzene (CDNB), cumene hydroperoxide, 2,4-dinitrophenylhydrazine (DNPH), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), ethylenediamine-tetraacetic acid (EDTA), glucose-6-phosphate (G6P), reduced and oxidized glutathione (GSH, GSSG), guanidine-HCl, phenylmethylsulfonyl fluoride (PMSF), thiobarbituric acid (TBA), sodium azide, xylenol orange and yeast glutathione reductase (GR) were from Sigma-Aldrich Co. (USA). All other reagents were of analytical grade.

### 2.2. Experimental design

Goldfish (*C. auratus*) of both sexes weighing 29–59 g of 2–3-year age were purchased in March at a local fish market. They were previously caught in natural ponds and held in cold water. Fish were kept in 1000 l tanks with dechlorinated tap water without feeding. The temperature of the water was maintained at $3 \pm 1$ °C with a natural light–dark cycle. Fish were held in these conditions for at least 3 weeks before experimentation.

For experimentation, fish were carefully transferred into 100 l aquaria aerated with bubblers at a water temperature of $23 \pm 1$ °C; 6–7 fish were placed in each aquarium. After 1, 6, 12, 24, 48 or 120 h exposure to a higher temperature, fish were killed by trans-spinal dissection and the brain, liver and kidneys were quickly removed, frozen in liquid nitrogen and stored for several days at −20 °C. Cold-adapted fish that were hold at 3 °C were used as the control group. The levels of oxidative stress indices were evaluated within the first 2–4 days after the sampling.

### 2.3. Indices of oxidative stress

Tissue samples were homogenized (1:10 w/v) using a Potter–Elvehjem glass homogenizer in 50 mM potassium phosphate buffer, pH 7.0, containing 0.5 mM EDTA; a few crystals of PMSF were added prior to homogenization to inhibit proteases. A 250 μl aliquot of this homogenate was then mixed with 0.5 ml of 10% (final concentration) trichloroacetic acid and centrifuged for 5 min at 13,000*g*. CP levels were measured in the resulting pellets, and TBARS were assayed in the supernatants using a spectrophotometer SF-46 (USSR). Carbonyl groups of proteins were measured by reaction with DNPH as described earlier (Lushchak et al., 2005). The amount of CP was evaluated spectrophotometrically at 370 nm using a molar extinction coefficient of $22 \times 10^3$ M/cm (Lenz et al., 1989). The values are expressed as nanomoles of CP per protein milligram in the guanidine chloride solution. Levels of low-molecular mass products of lipid peroxidation, including malondialdehyde, were assessed spectrophotometrically by the TBARS assay as described by Lushchak et al. (2005). A molar extinction coefficient of $156 \times 10^3$ M/cm at 535 nm was used for calculation of TBARS concentration (Rice-Evans et al., 1991). The values are expressed as nanomoles of TBARS per gram wet mass (gwm). LOOH were assayed by reaction with xylenol orange (Hermes-Lima et al., 1995) as described by Lushchak et al. (2005). LOOH content is expressed as nanomoles of cumene hydroperoxide equivalents per gwm.

Content of L-SH and H-SH were measured with DTNB as described previously (Lushchak and Bagnyukova, 2006a). Total content of free thiols, both L-SH and H-SH, was measured in supernatants prepared as for enzyme assays (see below). L-SH content was assessed in the supernatants after precipitation of proteins with trichloroacetic acid. The H-SH content was calculated by

subtracting the L-SH concentration from the total thiol level. The thiol levels are expressed as micromoles of SH-groups per gwm.

### 2.4. Antioxidant enzyme activities

Tissue homogenates were prepared as for the TBARS/CP assay and centrifuged at 4 °C for 15 min at 15,000*g* in a K-24 centrifuge (Germany). Supernatants were removed and enzyme activities at 21 °C were measured using a spectrophotometer Specol-221 (Germany). Activities of total superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione-*S*-transferase (GST), GR and glucose-6-phosphate dehydrogenase (G6PDH) were measured as described previously (Lushchak et al., 2005). One unit of SOD activity is defined as the amount of enzyme that inhibits the quercetin oxidation reaction by 50% of maximal inhibition. One unit of catalase, GPx, GST, GR and G6PDH activity is defined as the amount of enzyme consuming 1 μmol of substrate or generating 1 μmol of product per minute at 21 °C. All enzyme activities are expressed per protein milligram.

### 2.5. Protein measurements and statistics

Protein concentration was measured by the Bradford method with Coomassie Brilliant Blue G-250 (Bradford, 1976) using bovine serum albumin as a standard. Data are presented as means ± SEM, with 5–7 independent determinations on tissues from different animals. Statistical analysis was performed using analysis of variance (ANOVA) followed by a Student–Newman–Keuls test.

## 3. Results

### 3.1. Oxidative stress markers

Fig. 1 shows the levels of oxidative stress markers in three organs of cold-acclimated goldfish at 3 °C and after acute transfer of fish to 23 °C for 1, 6, 12, 24, 48, or 120 h. LOOH are one of the initial products of free radical attack on lipids. LOOH content was similar in the three organs (the brain, liver and kidney) of winter-acclimated goldfish that were assessed (Fig. 1A). Transfer to the higher temperature (23 °C) resulted in an immediate strong increase in LOOH levels within 1 h; LOOH content rose by 3-fold in the brain, 3.3-fold in the liver and 5.3-fold in the kidney. Brain and liver levels remained high up to the end of the 120 h (5 days) exposure, whereas kidney levels of LOOH quickly decreased by 6 h at 23 °C and were similar to initial values over the rest of the experimental course.

End products of lipid peroxidation were assessed as TBARS. Their levels in the brain, liver and kidney of cold-acclimated fish were 7.70 ± 0.84, 7.83 ± 0.36 and 17.6 ± 0.8 nmol/gwm, respectively. Experimental exposure to the higher temperature had no effect on this parameter in either the brain or kidney (data not shown). Exposure for 1 h at 23 °C doubled TBARS content in the liver. However, this increase was transient, and levels returned to near control values by 6 h exposure and did not change significantly at any other time point (data not shown).

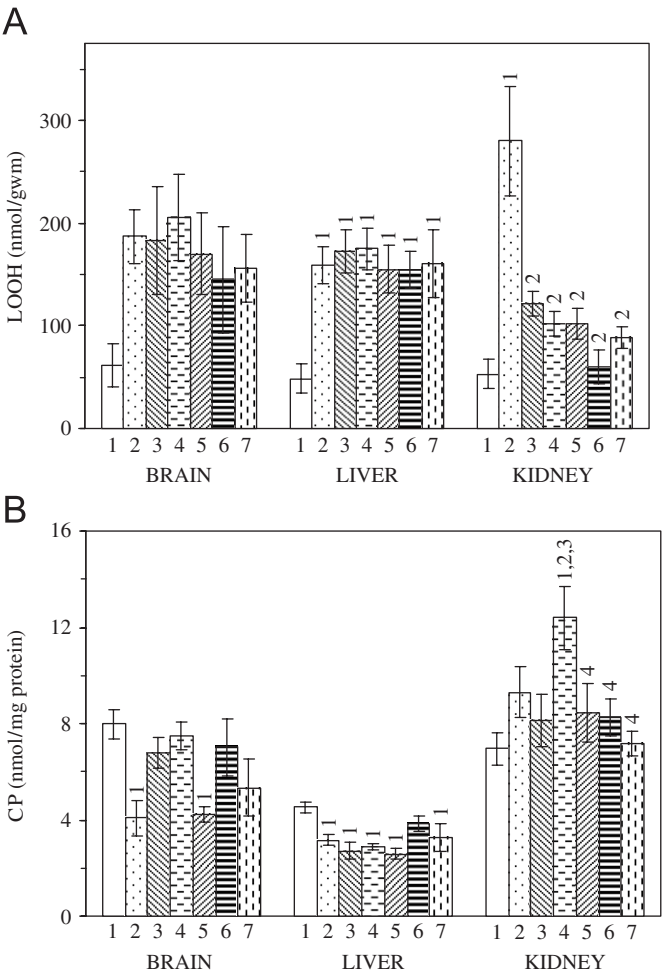


Fig. 1. Effect of acute transfer from 3 to 23 °C on the levels of lipid peroxides (A) and protein carbonyls (B) in the brain, liver and kidney of goldfish, *Carassius auratus*. The bars show 3 °C cold-acclimated animals (bar 1) and fish transferred to 23 °C for 1, 6, 12, 24, 48 and 120 h values (bars 2, 3, 4, 5, 6 and 7, respectively). Data are means ± SEM, $n = 5$–6 independent determinations on samples from different fish. Superscripts indicate significant difference from indicated groups, $P < 0.05$.

CP content in the liver decreased significantly by 30% after 1 h at 23 °C and remained low over the rest of experimental course (Fig. 1B). A similar tendency was seen in the brain with significantly reduced CP content (by 50%) within 1 h but variable levels were seen subsequently. Oppositely, kidney CP levels rose transiently by 1.8-fold after 12 h at 23 °C, but with longer exposures were not significantly different from the values at 3 °C (Fig. 1B).

The content of L-SH in tissues varied with small transient alterations. Brain L-SH levels were largely unaffected by exposure to high temperature except for a

strong decrease by 65% after 48 h; however, levels were restored to control values after 120 h (Fig. 2A). Both the liver and kidney showed a strong increase in L-SH content that peaked after 24 h at 23 °C. In the liver the increase was 1.9-fold higher than the initial value at 3 °C and in the kidney the increase was 2.4-fold; the kidney also showed a smaller elevation of L-SH levels after 6 h at 23 °C. With longer exposures at 23 °C, L-SH levels returned to initial value in both tissues.

Transfer to 23 °C substantially affected H-SH content in the brain; total H-SH dropped by two-thirds within the first hour and after 6 h reached a minimum value of only 18% of the value at 3 °C. Levels remained low over the remainder of the 23 °C exposure (Fig. 2B). Liver H-SH content was near initial values or slightly reduced at most time points and decreased by about 41% after 120 h exposure. Oppositely, kidney levels of this parameter increased after longer exposures at 23 °C with values after 24 and 48 h that were about 1.5-fold higher than the initial values in 3 °C acclimated fish.

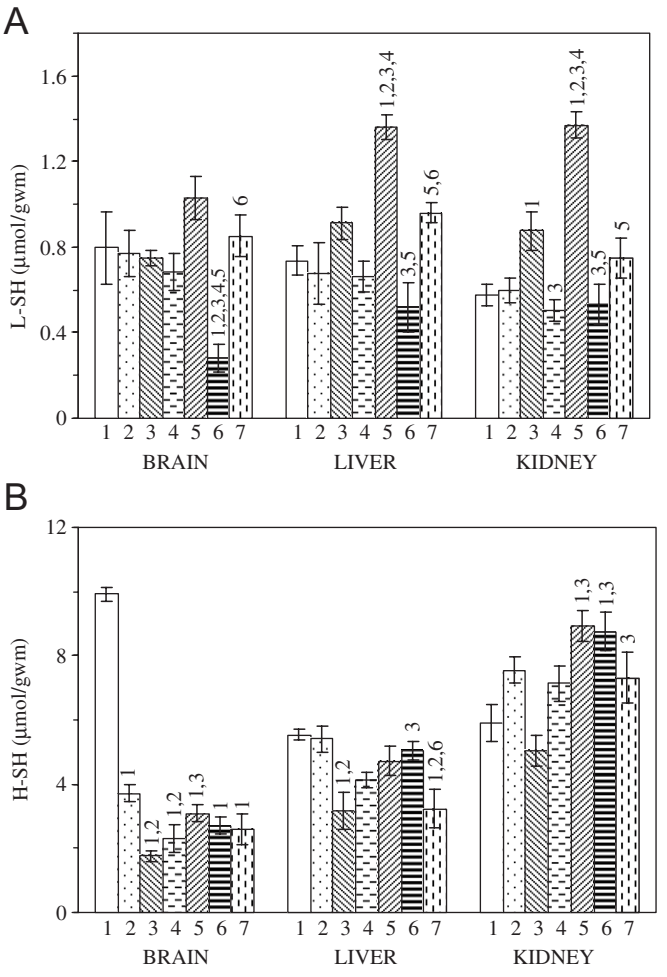


Fig. 2. Effect of acute transfer from 3 to 23 °C on the levels of soluble thiol metabolites, assessed as low-molecular mass thiols (A), and thiol groups in proteins, assessed as high-molecular mass thiols (B). Other information as in Fig. 1.

### 3.2. Antioxidant enzyme activities

SOD activities in the brain, liver and kidney of winter-acclimated (3 °C) fish were 47.4±4.1, 116±4 and 77.7±13.3 U/mg protein, respectively. Experimental exposure to 23 °C did not affect activity in any of the tissues (data not shown). Catalase activities in the tissues were only slightly modified by exposure to higher temperature (Table 1). Catalase activity in the brain was elevated by 23–28% over the intermediate times (6, 12 and 24 h) after transfer to 23 °C but after longer times was reduced again. Catalase in the liver showed a decreasing trend over time, which was significant after 48 h when activity dropped to just 55% of the initial value. In the kidney, catalase activity was largely unchanged over time.

GPx activities in the brain and kidney increased by ~1.5- and ~2.3-fold, respectively, by 24–48 h after transfer to 23 °C, but activity in both tissues was reversed to control values after 120 h (Fig. 3A). An increase in liver GPx activity occurred earlier, after 6 h, but thereafter returned to the initial level.

GST activities rose by 1.7-fold in the brain after 48 h of exposure at warm temperature, but fell again to initial values by 120 h (Fig. 3B). A similar pattern of change was seen in the kidney, but significant elevation of GST activity was registered earlier, after 12 h exposure; high GST was also found at 48 h but was reduced to control levels by 120 h. Liver GST activity was unaffected by experimental conditions.

GR activities in cold-acclimated fish were 9.00±0.92, 10.4±1.4 and 5.51±0.51 mU/mg protein in the brain, liver and kidney, respectively, and showed little change under the experimental conditions. The kidney showed a 1.5-fold increase in GR activity after 6 h at 23 °C and activity remained elevated thereafter (data not shown). No significant changes in brain or liver GR were found.

G6PDH activities were unchanged in the brain and elevated in the liver and kidney within the first hours of exposure; liver G6PDH rose quickly within 1 h to ~1.6-fold higher than control, whereas kidney G6PDH reached 1.5-fold higher than control after 12 h (Table 2).

Table 1
The activities of catalase (U/mg protein) in different tissues of goldfish after acute transfer from 3 to 23 °C

| Experimental group | Brain | Liver | Kidney |
|---|---|---|---|
| 1—Control, 3 °C | 8.12±0.72 | 106±16 | 19.9±0.5 |
| Time at 23 °C: | | | |
| 2—1 h | 8.18±0.34 | 129±16 | 23.6±2.2 |
| 3—6 h | 10.4±0.8 | 92.3±13.0 | $18.3\pm0.9^{2}$ |
| 4—12 h | $10.3\pm0.6^{2}$ | 85.4±10.1 | 21.8±1.1 |
| 5—24 h | $9.99\pm0.58^{2}$ | 73.2±5.6 | $18.5\pm1.2^{2}$ |
| 6—48 h | $6.26\pm0.56^{3-5}$ | $58.8\pm15.0^{2}$ | $16.6\pm1.0^{2}$ |
| 7—120 h | $7.29\pm0.52^{3-5}$ | 74.8±9.0 | $16.9\pm0.9^{2}$ |

Data are means±SEM, $n = 5$–7 independent determinations on samples from different fish. Superscript numerals indicate significance difference ($P<0.05$) from the indicated groups.

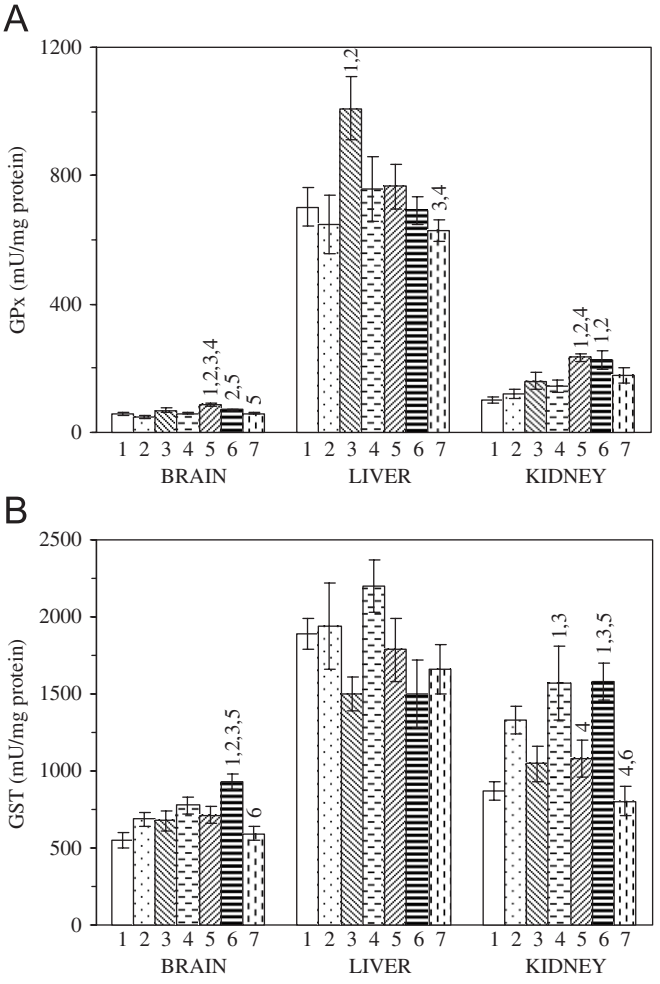


Fig. 3. Effect of acute transfer from 3 to 23 °C on the activities of glutathione peroxidase (A) and glutathione-*S*-transferase (B). Other information as in Fig. 1.

Table 2
The activities of glucose-6-phosphate dehydrogenase (mU/mg protein) in different tissues of goldfish after acute transfer from 3 to 23 °C

| Experimental group | Brain | Liver | Kidney |
|---|---|---|---|
| 1—Control, 3 °C | 8.14±0.67 | 17.1±2.3 | 14.4±0.9 |
| Time at 23 °C: | | | |
| 2—1 h | 8.05±0.55 | 27.0±3.7[1] | 19.4±1.8 |
| 3—6 h | 7.86±0.54 | 18.3±3.2 | 15.3±1.7 |
| 4—12 h | 8.65±0.79 | 19.3±1.0 | 22.6±2.3[3] |
| 5—24 h | 8.51±0.45 | 19.5±1.1[2] | 19.6±1.9 |
| 6—48 h | 7.92±0.51 | 17.2±1.6 | 17.6±0.9 |
| 7—120 h | 8.01±0.23 | 15.8±1.8[2] | 20.5±1.3 |

Data are means±SEM, $n = 5$–7 independent determinations on samples from different fish. Superscript numerals indicate significance difference ($P<0.05$) from the indicated groups.

## 4. Discussion

Temperature change crucially affects many aspects of metabolism, and the effects often depend on gradients and directions of alterations. An increase in ambient temperature usually causes oxidative stress in ectotherms (Parihar and Dubey, 1995; Bagnyukova et al., 2003; Heise et al., 2006), correlated with enhanced respiration, oxygen consumption and metabolic rate as temperature increases (Watters and Smith, 1973; Sollid et al., 2005). A shift to lower temperatures may also result in oxidative stress and may require enhanced antioxidant defenses. It appears that lipids are a specific target for ROS action under such conditions. The maintenance of membrane fluidity at low temperature requires a higher degree of fatty acid unsaturation and polyunsaturated fatty acids (PUFAs) accumulate (Rady et al., 1990; Abele and Puntarulo, 2004). PUFAs are prime targets for free radical attack and hence the shift to a cold temperature may require increased defenses against lipid peroxidation.

In this study, we found that lipid peroxidation was disturbed by a sharp increase in temperature from 3 to 23 °C. Signs of oxidative stress development were seen within 1 h of exposure to the higher temperature. Lipid peroxidation products increased in most tissues although their levels were rapidly reduced again in the kidney. Lipid peroxidation is a very sensitive marker of oxidative damage to lipids. LOOH and TBARS content in goldfish tissues rose quickly within the first hours of oxidative stress induced by hyperoxia (Lushchak et al., 2005) or heat shock (Lushchak and Bagnyukova, 2006a, b). Lipid peroxidation products are not only a marker of oxidative damage to lipids; they are also possibly involved in triggering the up-regulation of antioxidant enzymes (Lushchak and Bagnyukova, 2006c). As a result, an enhanced antioxidant response suppresses further lipid peroxidation. The experimental conditions used here constituted a "mild" stress, affecting lipid peroxidation (an intermediate product) transiently but with no substantial effect on TBARS, a final end product of peroxidation. In another carp species, the common carp, *Cyprinus carpio*, 7-day acclimation to warmer temperature was enough to decrease the percentage of PUFAs and produce a corresponding increase in the saturated fatty acid content (Rady et al., 1990). We can conclude that a similar rapid modification of lipid composition might occur within the experimental period examined by us and, hence, be weakly disturbed by high temperature.

Unexpected results were obtained for CP changes during exposure to the higher temperature. Kidney data fit the conventional concept of development of oxidative stress and the metabolic response to it (Halliwell and Gutteridge, 1989). More surprisingly, the brain and especially the liver showed lowered CP levels when moved to the higher temperature as compared to cold acclimation. It is known that multicatalytic protease degrades oxidatively modified proteins (Starke et al., 1987) and oxidative stress activates this enzyme. It is possible then that, under the experimental conditions used, the degradation of oxidized proteins exceeded (in the liver) or counterbalanced (in the brain and kidney) the possible intensification of protein oxidation at the elevated temperature.

Transfer to the warmer temperature also affected the thiol content of goldfish organs. Elevated levels of L-SH in the liver and kidney suggest a possible increase in the synthesis of glutathione, which is the main low-molecular mass antioxidant (Halliwell and Gutteridge, 1989). It seems that a short-term increase was enough to adjust the antioxidant potential to deal with the enhanced ROS production at high temperature because after longer times at 23 °C L-SH levels were reduced. The content of protein thiols varied in a tissue-specific manner. Firstly, a decrease in brain and liver H-SH content could be a result of direct oxidation of such thiols at elevated temperatures in the absence of physiological changes in this parameter. Secondly, in the kidney and partially in the liver by the end of experimental exposure, protein disulfides might be reduced by activated antioxidant defenses. Thirdly, high temperature might change the composition of proteins with enhanced synthesis of proteins (possibly, with different cysteine content) needed in the more active physiological state at the higher environmental temperature.

The disturbance of free radical processes at varying temperatures affects antioxidant defenses. Thus, long-term acclimation of zebrafish to a lower temperature (a decrease from 28 to 18 °C) resulted in elevated activities of a set of antioxidant enzyme genes, including GPx, Cu,Zn-SOD, Mn-SOD, GST and others (Malek et al., 2004). On the other hand, an increase in ambient temperature also raises antioxidant enzyme activities (Abele and Puntarulo, 2004). This increase may occur at transcriptional, translational or posttranslational levels. Changes in antioxidant enzyme activities can occur rather quickly. In the rat brain, both cold and heat stress increased Cu,Zn-SOD and Mn-SOD activities within 1–3 h (Djordjević et al., 2004). Similar fast enhancement of antioxidant defenses was found during arousal after estivation in the land snail *Helix aspersa* (Ramos-Vasconcelos et al., 2005) and during recovery from winter hibernation in frogs *Rana ridibunda* (Bagnyukova et al., 2003).

In our study, some antioxidant enzymes showed an "acute stress" response to the temperature change, increasing their activities over the first hours of 23 °C exposure. This was the case for liver GPx and G6PDH although the rise was transient. In some cases, antioxidant enzyme activities increased after longer times at 23 °C—kidney and brain GPx and GST, and kidney GR showed this pattern. This might be directed to establishing long-term adaptation to high temperature. Notably, glutathione-dependent enzymes, but not the primary antioxidant enzymes, SOD and catalase, showed the most marked response to the transition from low to high temperature. These enzymes, especially GPx and GST, also seem to play an important role in antioxidant defenses of goldfish subjected to other kinds of stress (Bagnyukova et al., 2005a, b; Lushchak et al., 2005).

The data presented here document only the first 5 days of exposure to higher ("summer") temperature. In order to compare oxidative stress indices and antioxidant enzyme activities of goldfish fully acclimated in the long term to cold (winter) and high (summer) temperatures, we compared the data for cold-acclimated goldfish in winter (this study) with the equivalent indices for summer fish sampled in July and reported in Lushchak and Bagnyukova (2006a, b). The data, presented in Table 3, show that the levels of oxidized proteins and lipid peroxidation products of intact goldfish tend to be higher in the summer reflecting more intensive oxidative processes in this warm season related to active physiological conditions such as feeding, etc. Enhanced levels of these oxidative stress markers are probably a common feature of ectotherms at warmer temperatures; for example, CP levels in the liver of eelpout (*Zoarces viviparus*) were more than 3-fold higher in summer compared to autumn fish (Heise et al., 2006). Summer oxidative stress in goldfish is dealt with by a 3-fold higher content of liver L-SH, presumably due to enhanced synthesis of glutathione, which plays a crucial role in detoxification of xenobiotics taken in with food.

Table 3
Oxidative stress indices and antioxidant enzyme activities in goldfish tissues in winter and summer

| Parameters | Brain | | Liver | | Kidney | |
|---|---|---|---|---|---|---|
| | Winter | Summer | Winter | Summer | Winter | Summer |
| CP, nmol/mg protein | 7.98±0.62 | 9.41±2.22 | 4.53±0.22 | 6.94±1.78 | 6.95±0.68 | 12.8±1.60* |
| LOOH, nmol/gwm | 61.3±21.1 | 110±27 | 84.9±25.5 | 182±47 | 52.7±14.2 | 136±67 |
| TBARS, nmol/gwm | 7.70±0.84 | 11.1±2.8 | 7.83±0.36 | 30.0±5.1* | 17.6±0.8 | 21.9±5.1 |
| L-SH, μmol/gwm | 0.80±0.17 | 0.72±0.06 | 0.74±0.07 | 2.21±0.50* | 0.57±0.05 | 0.56±0.25 |
| H-SH, μmol/gwm | 9.93±0.21 | 1.94±0.29* | 5.53±0.17 | 4.77±1.59 | 5.91±0.58 | 5.69±0.25 |
| SOD, U/mg protein | 47.4±4.1 | 47.2±7.8 | 116±4 | 77.8±13.8* | 77.7±13.3 | 51.8±17.1 |
| Catalase, U/mg protein | 8.12±0.72 | 3.16±0.27* | 106±16 | 144±27 | 19.9±0.5 | 23.2±4.0 |
| GPx, mU/mg protein | 57.6±4.7 | 123±12* | 703±60 | 4460±570* | 101±9 | 1030±140* |
| GST, mU/mg protein | 550±50 | 1230±120* | 1890±99 | 2420±280* | 871±61 | 1740±290* |
| GR, mU/mg protein | 9.00±0.92 | 22.5±1.5* | 10.4±1.4 | 28.4±3.5* | 5.51±0.51 | 46.0±6.3* |
| G6PDH, mU/mg protein | 8.14±0.67 | 17.4±0.9* | 17.1±2.3 | 46.9±8.7* | 14.4±0.9 | 95.2±9.6* |

Data for winter fish are the 3 °C cold-acclimated values; data for summer fish are from Lushchak and Bagnyukova (2006a, b). Data are means±SEM, $n = 5$–7 independent determinations on samples from different fish. *Significantly different from winter values, $P < 0.05$.

Interestingly, protein thiol levels in the brain of summer goldfish are much lower than in winter fish and this coincides with the effect of high-temperature exposure on brain H-SH reported in the present study. Among enzymes examined, the glutathione-dependent and associated enzymes, GPx, GST, GR and G6PDH, seem to be more important in coping with seasonal oxidative stress than are the primary antioxidant enzymes, SOD and catalase. Thus, activities of the first four enzymes were 2–10-fold higher in the summer in all tissues, whereas SOD and catalase activities do not differ much between two seasons or decrease in some organs of summer fish. Catalase is located in peroxisomes, whereas GPx is a cytosolic enzyme; hence, intracellular localization of these hydrogen-catabolizing enzymes may be responsible for different responses of them to oxidative stress. SOD activity could be high enough for dismutating superoxide anion; moreover, lowered SOD in the liver of summer goldfish might be compensated for by increased levels of hepatic glutathione, which can scavenge ROS itself.

Generally, the transfer from the low-temperature (3 °C) characteristic of winter to the higher temperature (23 °C) characteristic of summer water temperatures acutely disturbed lipid peroxidation in goldfish tissues. The quick increase in lipid peroxide levels might serve as a signal for enhancement of overall antioxidant potential, and notably antioxidant enzyme activities generally showed more protracted increases after 1–2 days at 23 °C, a length of time that could be consistent with a longer term enhancement of gene expression and protein synthesis. Goldfish tissues possess high constitutive activities of antioxidant enzymes (Lushchak et al., 2005; Lushchak and Bagnyukova, 2006b), which might be enough to cope with short-term disturbance in free radical processes. However, long seasonal changes of ambient temperature require extensive reorganization of cellular metabolism, including antioxidant defenses. This results in a marked increase in the activities of glutathione-dependent enzymes and decreased protein thiol content at least in the brain. In nature, a gradual increase in some antioxidant enzyme activities during the transition from cold winter conditions to warmer summer conditions would reflect the needs of an organism to cope with elevated oxidative stress that accompanies enhanced oxygen consumption and metabolic rate at higher environmental temperatures.

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