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Seasonal changes in antioxidant defence system of liver and gills of *Salmo trutta caspius, Salmo trutta labrax* and *Salmo trutta macrostigma*

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Seasonal changes in antioxidant enzyme activities (superoxide dismutase, SOD, EC 1.15.1.1; catalase, CAT, EC 1.11.1.16; glutathione peroxidase, GPx, EC 1.11.1.9; glutathione reductase, GR, EC 1.6.4.2; glucose-6-phosphate dehydrogenase, G6PD, EC 1.1.1.49 and glutathione *S*-transferase, GST, EC 1.5.1.18) and lipid peroxidation (LPO) levels of livers and gills of female Caspian trout *Salmo trutta caspius*, Black Sea trout *Salmo trutta labrax* and mountain trout *Salmo trutta macrostigma* were investigated. SOD, CAT, GPx, G6PD and GST activities were higher in liver compared to gills of all sub-species; concomitantly, the GR activity was also higher in the livers of *S. t. caspius* and *S. t. labrax*, but the reverse was seen in *S. t. macrostigma*. LPO levels were higher in the gills compared to the liver of all sub-species. There was no general trend in the seasonal changes in the gill antioxidant enzyme (AE) activities or LPO levels. Higher AE activities, however, were found in the liver of each sub-species during autumn, and this coincided with an increase in the gonado-somatic index. © 2009 The Authors
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Key words: antioxidant enzymes; gonado-somatic index; oxidative stress; reactive oxygen species; *Salmo trutta* sp.; seasonal variations.

INTRODUCTION

Free radicals and other reactive oxygen species (ROS) are generated by all aerobic cells (Winston & Di Giulio, 1991), and if the cell is unable to neutralize ROS activity then acute damage to the most major macromolecules such as DNA, proteins and lipids occurs (Berger, 2005). To minimize the negative effects of ROS, fishes like other vertebrates possess an antioxidant defence system (ADS), which utilizes enzymatic and non-enzymatic mechanism (Jos *et al.*, 2005). Key enzymatic players in ADS are superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.16), glutathione peroxidase (GPx, EC 1.11.1.9), glutathione reductase (GR, EC 1.6.4.2), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) and glutathione *S*-transferase (GST, EC 1.5.1.18) (Halliwell & Gutteridge, 1999; Bayir, 2005).

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ROS reacts with polyunsaturated fatty acids (PUFA) to induce the release of toxic and reactive aldehyde metabolites such as malondialdehyde (MDA), one of the end products of lipid peroxidation (LPO) (Yeh *et al.*, 2005). LPO leads to the loss of cellular functions through the inactivation of membrane enzymes. Increased ROS modulates the lipid composition thus altering the phospholipid fatty acid profile of the cell membrane. Because PUFAs are the major targets of ROS, they are vulnerable and sensitive to metabolic conditions associated with oxidative stress (Vijayavel *et al.*, 2007).

Increased antioxidant defence (AD) in some fish species has been related to pollutants or chemical exposure (Lemaire *et al.*, 1996; Vaglio & Landriscina, 1999; Lopes *et al.*, 2001; Wilhelm Filho *et al.*, 2001; Li *et al.*, 2003) and can be induced by other stress factors such as hyperoxia (Ritola *et al.*, 2002) and hypoxia or anoxia (Lushchak *et al.*, 2001). Moreover, it is also known that in aquatic ecosystems, seasonal and daily changes in dissolved oxygen and temperature are environmental variables that are likely to influence oxidative processes (Parihar *et al.*, 1997; Cunha Bastos *et al.*, 2007).

Brown trout *Salmo trutta* L. are native to North America, Europe, west and north Asia (Geldiay & Balik, 1996). In Turkey, the sub-species of *S. trutta*: Caspian trout *Salmo trutta caspius* Kessler, Black Sea trout *Salmo trutta labrax* Pallas and mountain trout *Salmo trutta macrostigma* Dumeril occur naturally in the streams of the Aras (Tortonese, 1955; Aras, 1974), the Coruh (Kuru, 1971; Aras, 1974) and the Karasu Basins (Kuru, 1975), respectively. Degradation of spawning habitats and river damming are endangering *S. trutta* populations (Alp *et al.*, 2005), and there is a need for good management practices for the conservation of stocks in the East Anatolian Region of Turkey. To fulfil this task, it is necessary to achieve a sound knowledge of the biology of these sub-species (Gortázar *et al.*, 2007), and the current study forms part of a wider project investigating the biology of *S. trutta* in the Turkey and more specifically and aims to assess the seasonal changes in the ADS of the liver and the gills, as well as the seasonal gonado-somatic index (I_G) of *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma*.

MATERIALS AND METHODS

FISH SAMPLING

Female fishes were collected quarterly between summer 2006 and autumn 2007 (14–16 July, October, February and April); *S. t. caspius* (96–117 g) were captured from the Hamzanlar Creek (Aras Basin), Tekman, Erzurum, Turkey (39°27′37″ N; 41°15′39″ E), *S. t. labrax* (86–124 g) were captured from Yagli Creek (Coruh Basin), Ispir, Erzurum, Turkey (40°21′55″ N; 41°4′22″ E) and *S. t. macrostigma* specimens (111–123 g) were captured from Yesildere Creek (Karasu Basin), Dumlu, Erzurum, Turkey (40°7′31″ N; 41°25′18″ E) by cast-nets in spring and summer and by electrofishing in autumn and winter. Five fish of each of the three sub-species were killed with a sharp blow on the head. The fishes were weighed (M, g) and gonads removed and weighed (M_G, g). The livers and gills were excised and frozen immediately in liquid nitrogen. Samples were transferred to the laboratory and stored at −84° C until analyses. The I_G of fishes was determined from $I_G = 100\ M_G\ M^{-1}$. Water temperature and dissolved oxygen values were measured at the sites with a WTW360i digital analyser (WTW; www.wtw.com).

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DETERMINATION OF ENZYME ACTIVITIES AND LIPID PEROXIDATION LEVELS

Liver and gills of the female trout were washed in 0·9% NaCl, dried with filter paper and *c*. 1 g sample was weighed. Samples were homogenized in 10 vol (w/v) 1·15% KCl for 5 min. The homogenates were centrifuged at 11 312 *g* for 30 min at +4° C, and the resulting supernatants were used for analysis.

The following enzyme activities were measured by using a double-beam spectrophotometer (Thermo Evolution 100; Accelonix Ltd; www.accelonix.co.uk).

SOD activity was assayed by inhibition of xantine–xantine oxidase generated O_2^- reduction of nitroblue tetrazolium (NBT) (Sun *et al.*, 1988). NBT was reduced to blue formazon by O_2^- which has a strong absorbance at 560 nm. One unit was defined as the amount of protein producing 50% inhibition of reaction. The reaction mixture contained 1 M Tris–HCl, 5 mM ethylenediaminetetraacetic acid (EDTA) buffer (pH 8·0), 150 μM NBT, 400 mM Na_2CO_3, 1 g l^{-1} bovine serum albumin, 3 mM l^{-1} xantine, 0·833 U ml^{-1} xantine oxidase, 0·8 mM $CuCl_2$ and tissue homogenate (30 μl).

GPx activity was determined by following the rate of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation at 340 nm by the coupled reaction with GR (Beutler, 1975, 1984). The assay system contained 1 M Tris–HCl, 5 mM EDTA buffer (pH 8·0), 0·1 M reduced glutathione (GSH), 10 U ml^{-1} GR, 2 mM NADPH, 7 mM t-buthylhydroperoxide, distillated water and tissue homogenate (10 μ1).

CAT activity was measured according to the method of Beutler (1984) after the decomposition of H_2O_2 at 230 nm ($\varepsilon = 0{\cdot}071$ mM^{-1} cm^{-1}). The assay mixture consisted of 0·05 M phosphate buffer (pH 7·0), 0·019 M H_2O_2 and tissue homogenate (10 μ1).

GR activity was determined as described by Beutler (1984) by measuring the oxidation of NADPH at 340 nm using the extinction coefficient 6·22 $mM^{-1}cm^{-1}$. The reaction mixture contained 1 M Tris–HCl, 5 mM EDTA buffer (pH 8·0), 0·033 M oxidized glutathione (GSSG), 2 mM NADPH, distillated water and tissue homogenate (10 μ1).

G6PD activity was assayed as described by the method of Beutler (1984). The activity assay was done by monitoring the increase in absorption at 340 nm ($\varepsilon = 6{\cdot}22$ mM^{-1} cm^{-1}) due to the reduction of 2 mM $NADP^+$. The assay system contained 1 M Tris–HCl, 5 mM EDTA buffer (pH 8·0), 0·1 M $MgCl_2$, 2 mM $NADP^+$, 6 mM G6P, distillated water and tissue homogenate (50 μ1).

GST activity was evaluated with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate in a final reaction mixture containing 0·11 M phosphate buffer (pH 6·5), 30 mM CDNB, 0·1 M GSH, distillated water and tissue homogenate (50 μ1) (Habig *et al.*, 1974). The activity rate was measured as change in optical density at 340 nm ($\varepsilon = 9{\cdot}6$ mM^{-1} cm^{-1}).

The level of LPO was determined by assessment of MDA as total thiobarbituric acid (TBARS)-reactive products by method of Uchiyama & Mihara (1978). The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard. Tissue homogenates were mixed with phosphoric acid (1% v/v) and thiobarbituric acid (TBA) (% 0·6 w/v); the mixture was mixed strongly and incubated in a boiling water-bath for 45 min. After cooling the tubes, n-butanol was added and mixed, and the precipitates were removed by centrifugation at 1756 *g* for 10 min. Finally, the MDA concentration in the supernatant fraction was determined spectrophotometrically at 532 nm.

All processes were carried out in the ice or at +4° C. Specific enzyme activities were defined as enzyme unit (EU) per mg protein, and MDA concentrations were expressed as nmol per mg protein. All chemicals were obtained from Sigma-Aldrich (www.sigmaaldrich.com).

PROTEIN CONTENT OF SAMPLES

The protein contents of samples were determined by Bradford (1976) method using bovine serum albumin as a standard.

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DATA ANALYSES

The statistical analyses were performed with SPSS version 10.0 for Windows (SPSS, www.spss.com). Data are presented as mean ± S.D. Data were analysed by one-way ANOVA, and significant differences determined using the Duncan's multiple range *post hoc* test.

RESULTS

There were wide changes in season temperatures (Table I) with the maximum at Hamzanlar Creek (16·5° C) and lowest at Yesildere Creek (0·4° C). Dissolved oxygen levels were relatively stable with the highest being in Yagli Creek (10·12 mg l^{-1}; in the winter) and lowest at Hamzanlar Creek (8·11 mg l^{-1}; in the summer) (Table I).

The highest I_G values were determined in the autumn for all sub-species, and they dramatically decreased in the winter. This suggest that reproduction in all three sub-species occurs between the second half of autumn and first half of winter (Fig. 1).

ANTIOXIDANT ENZYME ACTIVITIES AND LIPID PEROXIDATION LEVELS

Generally, the activities of SOD, GPx, CAT, G6PD, GR and GST were higher in liver compared to gills of all sub-species. The exception being in the *S. t. labrax* were GR activity was higher in the gill compared to the than liver. In contrast, the LPO levels of gills were higher than those of liver (Figs 2–7).

Antioxidant enzyme activities and lipid peroxidation levels of liver

SOD, CAT and GPx activities of liver showed similar behaviour, *i.e.* significantly higher activities were recorded during the autumn (prespawning period) [Figs 2(a)–(c), 3(a)–(c) and 4(a)–(c)].

GR activities of liver showed important seasonal variations, with the highest activities measured in the spring for the *S. t. caspius*, 1·27 ± 0·13 EU mg protein^{-1} and *S. t. labrax*, 1·46 ± 0·20 EU mg protein^{-1}, and in the autumn for the *S. t. macrostigma*, 5·11 ± 0·29 EU mg protein^{-1}. The lowest activities were assayed in the summer for the *S. t. caspius*, 0·33 ± 0·05 EU mg protein^{-1},

TABLE I. Water temperature and dissolved oxygen values during the study

	Temperature (° C)			Dissolved oxygen (mg l^{-1})		
	HC	YC	YAC	HC	YC	YAC
Summer	16·5	10·8	14·8	8·11	8·70	8·35
Autumn	4·3	3·8	4·8	9·87	9·58	10·04
Winter	0·8	0·4	0·5	10·03	9·69	10·12
Spring	10·6	7·9	11·4	8·90	9·03	8·42

HC, Hamzanlar Creek; YC, Yesildere Creek; YAC, Yagli Creek.

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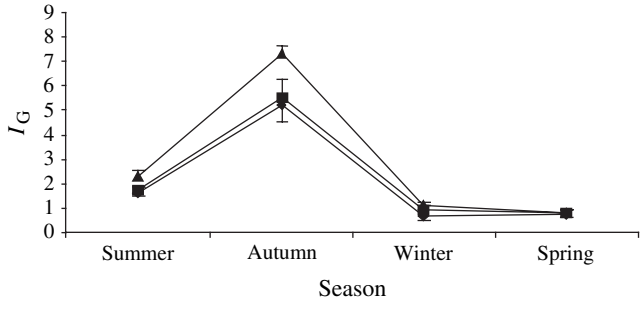


FIG. 1. Mean + S.D. gonado-somatic index (I_G) values of female *Salmo trutta caspius* (■), *Salmo trutta labrax* (▲) and *Salmo trutta macrostigma* (◆) during an annual cycle.

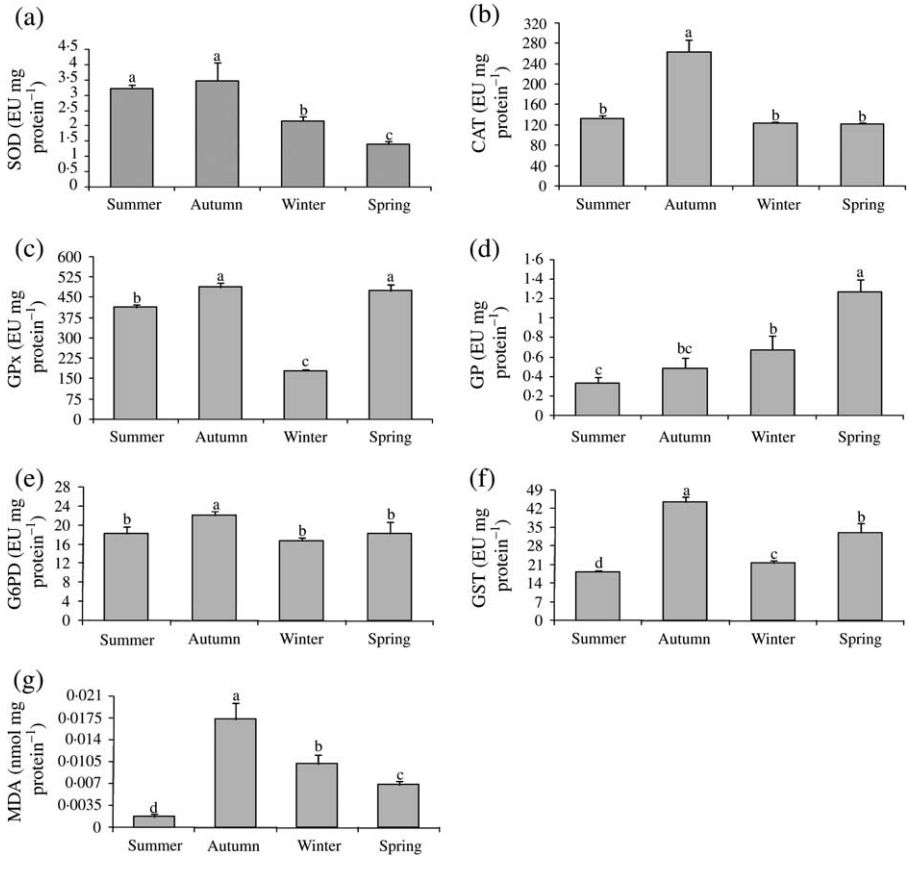


FIG. 2. Seasonal changes in antioxidant defence system of liver of *Salmo trutta caspius*: (a) superoxide dismutase (SOD) activity, (b) catalase (CAT) activity, (c) glutathione peroxidase (GPx) activity, (d) glutathione reductase (GR) activity, (e) glucose-6-phosphate dehydrogenase (G6PD) activity, (f) glutathione *S*-transferase (GST) activity and (g) lipid perioxidation (LPO) levels as malondialdehyde (MDA). Values are means ± S.D. ($n = 5$). Data were analysed by one-way ANOVA. Means were compared by Duncan's multiple range tests. Means with different lower case letters are significantly different ($P < 0{\cdot}05$). EU, enzyme unit.

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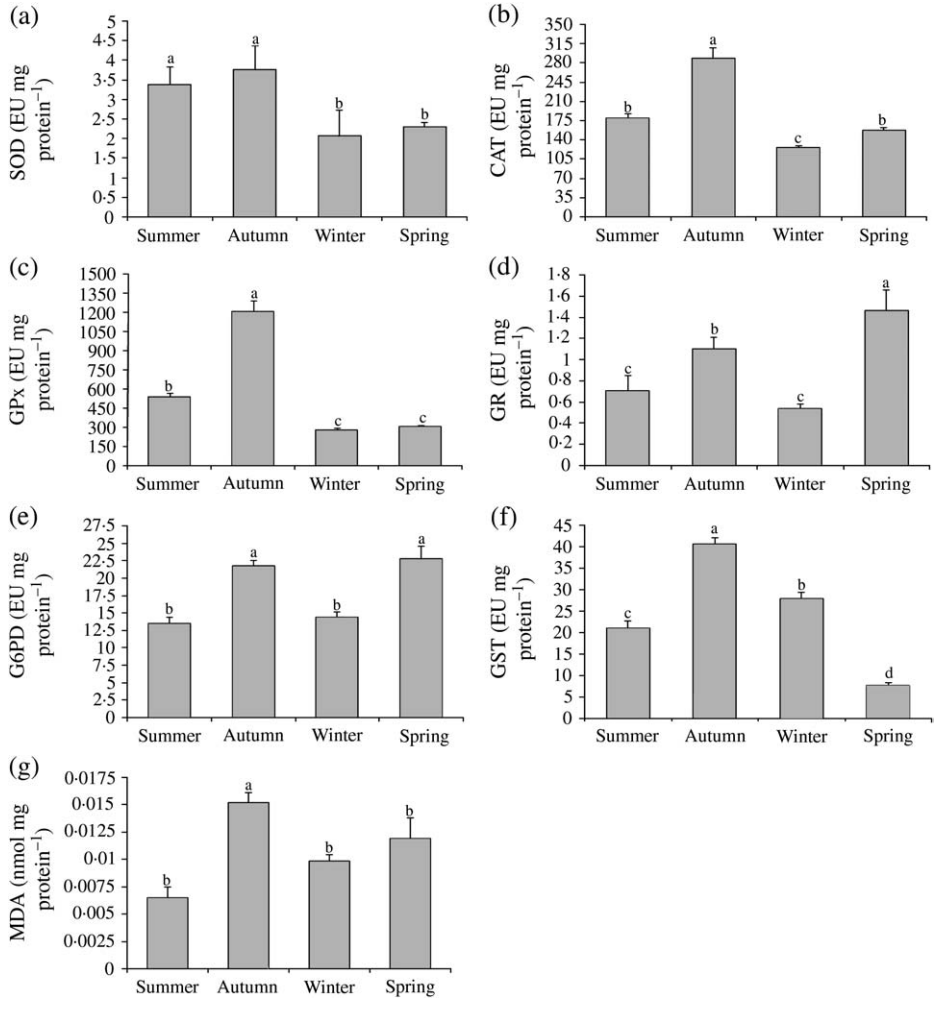


FIG. 3. Seasonal changes in antioxidant defence system of liver of *Salmo trutta labrax*: (a) superoxide dismutase (SOD) activity, (b) catalase (CAT) activity, (c) glutathione peroxidase (GPx) activity, (d) glutathione reductase (GR) activity, (e) glucose-6-phosphate dehydrogenase (G6PD) activity, (f) glutathione *S*-transferase (GST) activity and (g) lipid perioxidation (LPO) levels as malondialdehyde (MDA). Values are means ± S.D. ($n = 5$). Data were analysed by one-way ANOVA. Means were compared by Duncan's multiple range tests. Means with different lower case letters are significantly different ($P < 0{\cdot}05$). EU, enzyme unit.

and *S. t. macrostigma*, 0·66 ± 0·04 EU mg protein^{-1}, and in the winter (post-spawning period) for the *S. t. labrax*, 0·54 ± 0·04 EU mg protein^{-1} [Figs 2(d), 3(d) and 4(d)].

The highest levels of liver G6PD activities of the *S. t. labrax* and *S. t. macrostigma* were determined in the spring; 22·81 ± 1·93 EU mg protein^{-1} and 23·62 ± 0·96 mg protein^{-1}, respectively, and autumn for the *S. t. caspius*, 21·98 ± 1·02 EU mg protein^{-1} [Figs 2(e), 3(e) and 4(e)]. The lowest activities were found in the winter for the *S. t. caspius*, 16·62 ± 0·76 EU mg protein^{-1}, and in the

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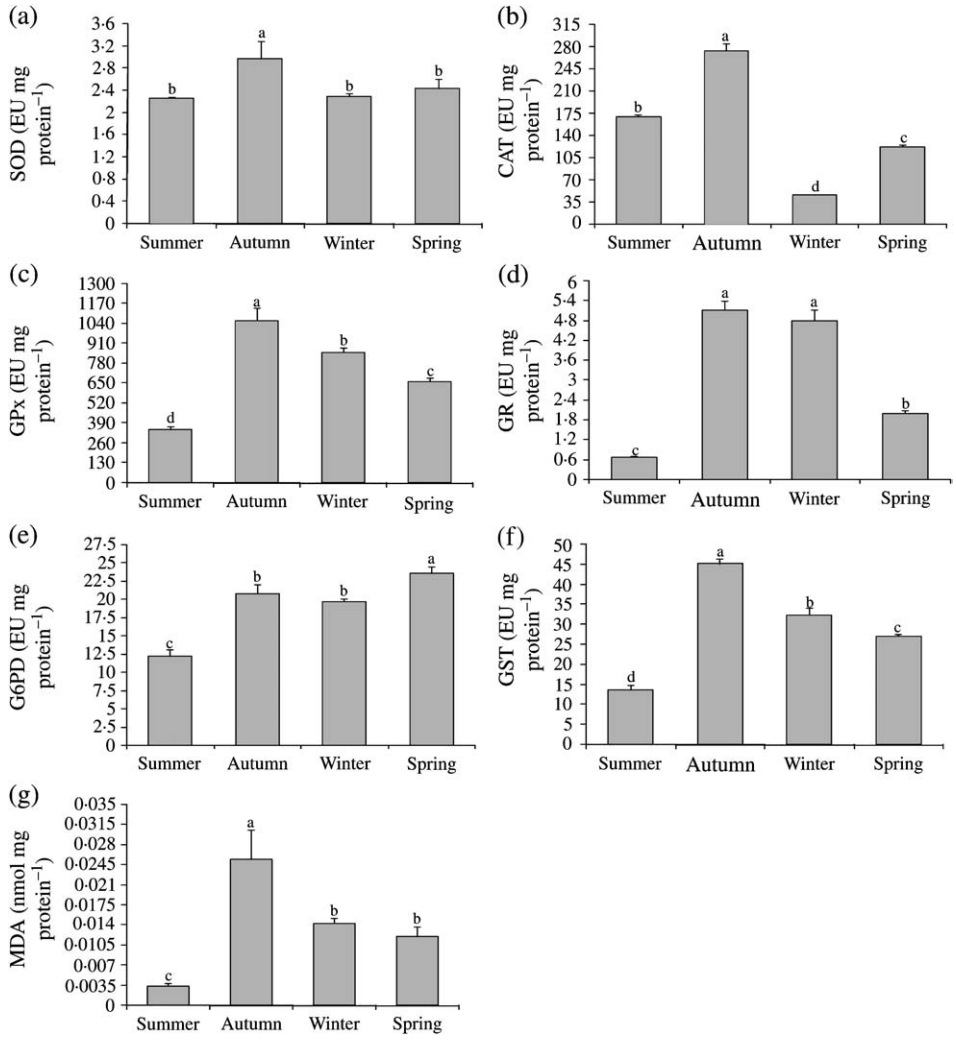


FIG. 4. Seasonal changes in antioxidant defence system of liver of *Salmo trutta macrostigma*: (a) superoxide dismutase (SOD) activity, (b) catalase (CAT) activity, (c) glutathione peroxidase (GPx) activity, (d) glutathione reductase (GR) activity, (e) glucose-6-phosphate dehydrogenase (G6PD) activity, (f) glutathione *S*-transferase (GST) activity and (g) lipid perioxidation (LPO) levels as malondialdehyde (MDA). Values are means ± S.D. ($n = 5$). Data were analysed by one-way ANOVA. Means were compared by Duncan's multiple range tests. Means with different lower case letters are significantly different ($P < 0{\cdot}05$). EU, enzyme unit.

summer for the *S. t. labrax* and *S. t. macrostigma*, 13·46 ± 1·01 and 12·23 ± 0·86 EU mg protein^{-1}, respectively [Figs 2(e), 3(e) and 4(e)].

The highest liver GST activities were measured in the autumn: *S. t. caspius*, 44·51 ± 2·01 EU mg protein^{-1}, *S. t. labrax*, 40·53 ± 1·79 EU mg protein^{-1} and *S. t. macrostigma*, 45·26 ± 1·24 EU mg protein^{-1}. The lowest activities were found in the summer for the *S. t. caspius*, 18·05 ± 0·94 EU mg protein^{-1} and *S. t. macrostigma*, 13·65 ± 1·25 EU mg protein^{-1}, and in the spring for the *S. t. labrax*, 7·62 ± 0·82 EU mg protein^{-1} [Figs 2(f), 3(f) and 4(f)].

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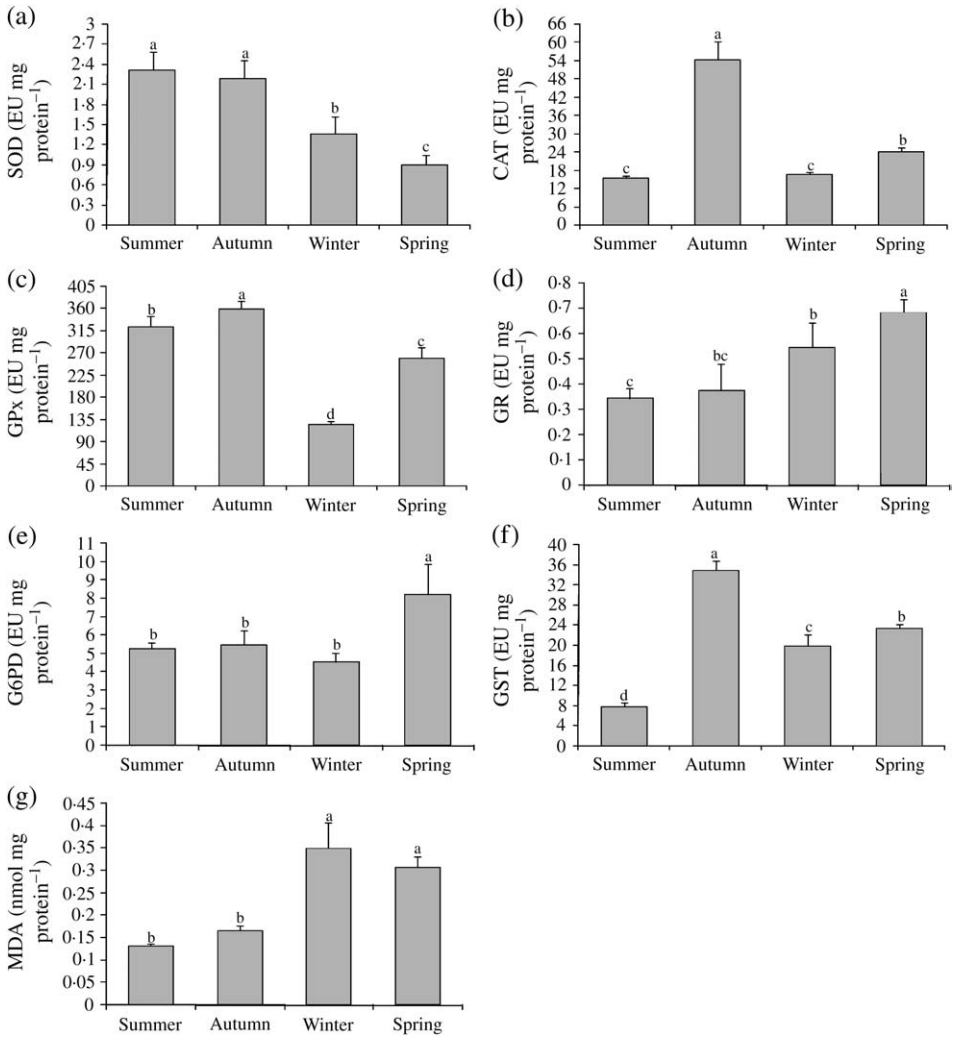


FIG. 5. Seasonal changes in antioxidant defence system of gill of *Salmo trutta caspius*: (a) superoxide dismutase (SOD) activity, (b) catalase (CAT) activity, (c) glutathione peroxidase (GPx) activity, (d) glutathione reductase (GR) activity, (e) glucose-6-phosphate dehydrogenase (G6PD) activity, (f) glutathione *S*-transferase (GST) activity and (g) lipid perioxidation (LPO) levels as malondialdehyde (MDA). Values are means ± S.D. ($n = 5$). Data were analysed by one-way ANOVA. Means were compared by Duncan's multiple range tests. Means with different lower case letters are significantly different ($P < 0{\cdot}05$). EU, enzyme unit.

LPO values of liver of three sub-species showed similar changes during the study, with significantly higher levels in the autumn and decreases in the summer [Figs 2(g), 3(g) and 4(g)].

Antioxidant enzyme activities and lipid peroxidation levels of gills

The highest gill SOD activities were measured in the summer for the *S. t. caspius*, 2·32 ± 0·26 EU mg protein^{-1} and *S. t. labrax*, 2·48 ± 0·31 EU mg

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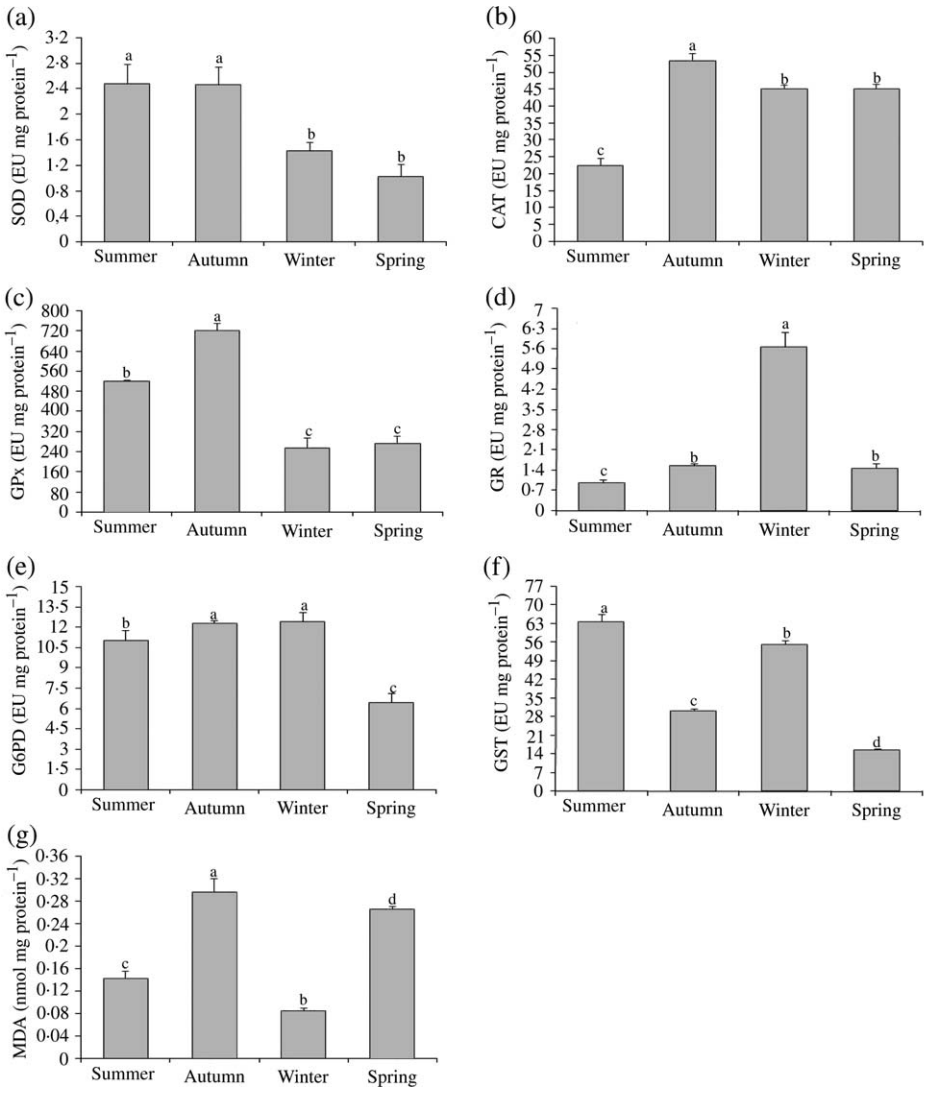


FIG. 6. Seasonal changes in antioxidant defence system of gill of *Salmo trutta labrax*: (a) superoxide dismutase (SOD) activity, (b) catalase (CAT) activity, (c) glutathione peroxidase (GPx) activity, (d) glutathione reductase (GR) activity, (e) glucose-6-phosphate dehydrogenase (G6PD) activity, (f) glutathione *S*-transferase (GST) activity and (g) lipid perioxidation (LPO) levels as malondialdehyde (MDA). Values are means ± S.D. ($n = 5$). Data were analysed by one-way ANOVA. Means were compared by Duncan's multiple range tests. Means with different lower case letters are significantly different ($P < 0{\cdot}05$). EU, enzyme unit.

protein^{-1}, and in the winter for the *S. t. macrostigma*, 3·84 ± 0·12 EU mg protein^{-1}. The lowest levels where measured in the spring for all sub-species. In addition, the maximum gill CAT and GPx activities were recorded in the autumn for all fish, but the lowest activities were measured in different seasons for each sub-species [Figs 5(a)–(c), 6(a)–(c) and 7(a)–(c)].

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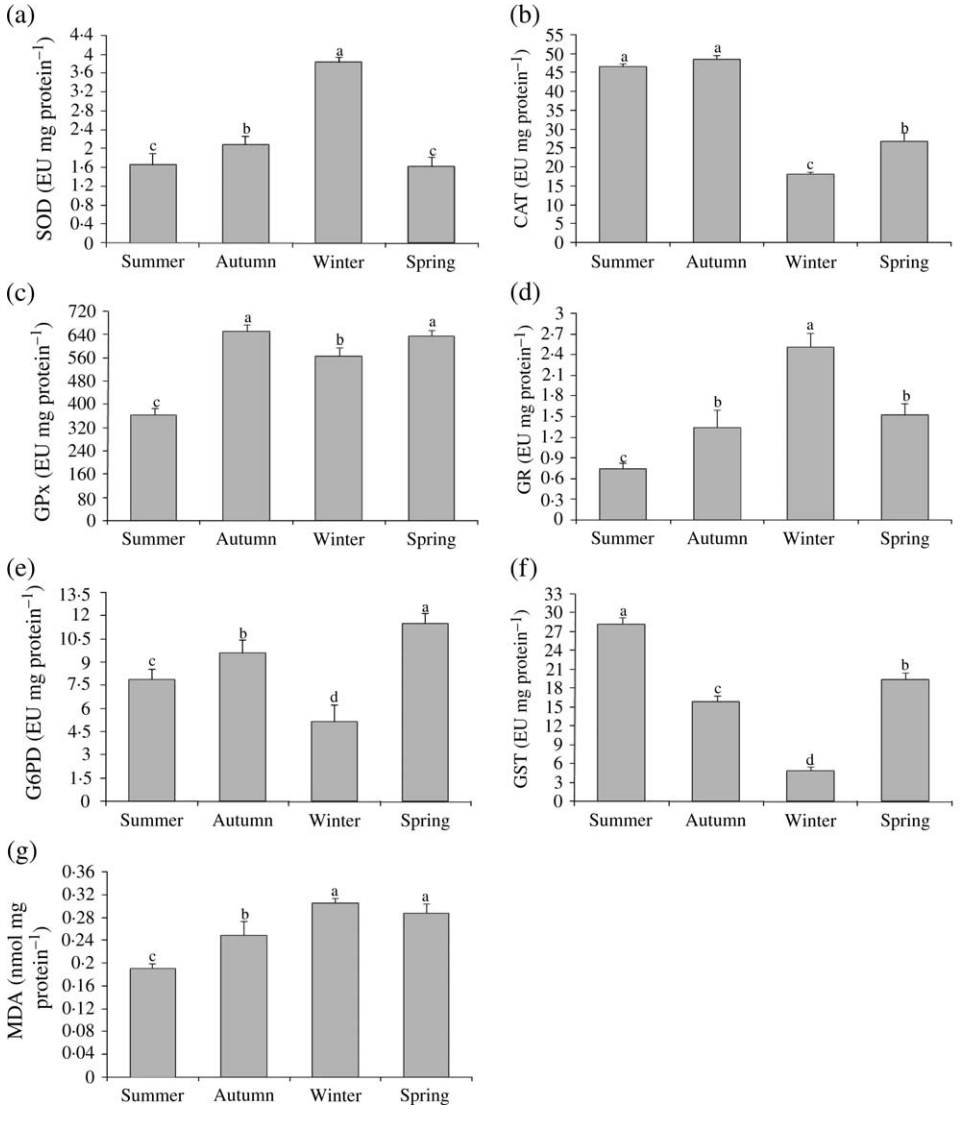


FIG. 7. Seasonal changes in antioxidant defence system of gill of *Salmo trutta macrostigma*: (a) superoxide dismutase (SOD) activity, (b) catalase (CAT) activity, (c) glutathione peroxidase (GPx) activity, (d) glutathione reductase (GR) activity, (e) glucose-6-phosphate dehydrogenase (G6PD) activity, (f) glutathione *S*-transferase (GST) activity and (g) lipid perioxidation (LPO) levels as malondialdehyde (MDA). Values are means ± S.D. ($n = 5$). Data were analysed by one-way ANOVA. Means were compared by Duncan's multiple range tests. Means with different lower case letters are significantly different ($P < 0{\cdot}05$). EU, enzyme unit.

The minimum gill GR activities were recorded in the summer for all sub-species, with the maximum activities were found in the winter for the *S. t. labrax*, 5·66 ± 0·54 EU mg protein^{-1} and *S. t. macrostigma*, 2·50 ± 0·20 EU mg protein^{-1}, and in the spring for the *S. t. caspius*, 0·68 ± 0·05 EU mg protein^{-1} [Figs 5(d), 6(d) and 7(d)].

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The highest G6PD activities of gills were recorded in the winter for the *S. t. labrax*, 12·42 ± 0·65 EU mg protein^{-1} and in the spring for the *S. t. caspius* and *S. t. macrostigma*, 8·19 ± 1·71 EU mg protein^{-1} and 11·48 ± 0·70 EU mg protein^{-1}, respectively. Although the lowest activities were found in the winter for the *S. t. caspius*, 4·55 ± 0·51 EU mg protein^{-1} and *S. t. macrostigma*, 5·12 ± 1·11 EU mg protein^{-1}, it was determined in the spring for the *S. t. labrax*, 6·46 ± 0·63 EU mg protein^{-1}.

The maximum gill GST activities for the *S. t. labrax* and *S. t. macrostigma* were assayed in the summer, 63·54 ± 3·23 and 28·14 ± 1·22 EU mg protein^{-1}, respectively, and autumn for the *S. t. caspius*, 34·74 ± 2·15 EU mg protein^{-1}. The minimum GST activities were also found in the different seasons [Figs 5(f), 6(f) and 7(f)].

The LPO levels in the gill of *S. t. labrax* exhibited the highest value in the autumn, 0·30 ± 0·027 nmol mg protein^{-1}, and lowest in the winter, 0·085 ± 0·005 nmol mg protein^{-1}. The *S. t. caspius* and *S. t. macrostigma* gill LPO levels also displayed significant increases during the winter, 0·35 ± 0·06 and 0·31 ± 0·007 nmol mg protein^{-1}, respectively, and decreases during the summer, *S. t. caspius*, 0·13 ± 0·006 nmol mg protein^{-1} and *S. t. macrostigma*, 0·19 ± 0·007 nmol mg protein^{-1} [Figs 5(g), 6(g) and 7(g)].

DISCUSSION

SOD, CAT AND GPX ENZYME ACTIVITIES OF LIVERS AND GILLS

SOD, CAT and GPx are three main antioxidant enzymes (Wilhelm Filho, 1996). SOD is an oxidoreductase and it provides the first defence against oxidative stress. SOD greatly accelerates the conversion of O_2^- to water and H_2O_2. The efficacy of SOD relies on its co-operation with CAT and GPx (Parihar *et al.*, 1997; Pandey *et al.*, 2003). Gabryelak *et al.* (1983) and Palace & Klaverkamp (1993) suggested that antioxidant defence enhancement in fishes during the spring and summer compared to colder winter months. But, Ronisz *et al.* (1999) reported no correlation between activities of GPx and CAT and water temperature in eelpout *Zoarces viviparous* (L). In the present study, an increase in the main liver antioxidant enzyme (AE) activities did not relate to increased temperature but was found to occur in the autumn for all sub-species. This suggests that the fishes are experiencing oxidative stress at this period. Autumn corresponds to the season with the highest I_G recorded and suggesting that this is the period prior to spawning (Fig. 1). In addition, this is the season when there is a rapid temperature drop, a decrease in daylight and increase in rainfall, all factors that may cause stress in the fishes, and potentially alter the status of the ADS.

GR, G6PD AND GST ENZYME ACTIVITIES AND LIPID PEROXIDATION LEVELS OF LIVERS

GR has an important role in cellular antioxidant protection. Because it converts the GSSG to its reduced form GSH, one of the main soluble antioxidants

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acting both as direct scavengers of oxyradicals and as cofactor of several AEs (Regoli *et al.*, 2002). G6PD catalyzes the first reaction of the pentose phosphate pathway, and the main physiological function of pentose phosphate pathway is to produce NADPH (used in lipid biosynthesis and in the protection of the cell from oxidative stress) and 5-phosphoribosyl pyrophosphate (used in RNA synthesis). Therefore, G6PD is the first and key enzyme of the pentose phosphate pathway. NADPH is necessary as a coenzyme of GR and essential for CAT activity (Slenzka *et al.*, 1994; Ciardiello *et al.*, 1997). Hence, the high liver GR activities of the *S. t. caspius* and *S. t. labrax* in the spring are probably due to the high liver G6PD activity that elevates NADPH production. Theoretically temperature and food availability induce oxygen consumption and cellular oxyradical generation, which are compensated by increasing antioxidant defence (Manduzio *et al.*, 2004), thus it is difficult to explain why the lowest G6PD and GR activities of livers of the *S. t. labrax* and *S. t. macrostigma* were found in the summer [Figs 3(e) and 4(e)]. Many other factors, however, could influence metabolism and AE activities, *e.g.* mode of life and feeding habits (Ronisz *et al.*, 1999) that were not assessed in the present study.

The conjugation of electrophilic compounds with GSH is catalyzed by GST. Apart from their essential functions in intracellular transport and the biosynthesis of leukotrienes and prostaglandins, a critical role for GSTs is defence against oxidative damage and peroxidative products of DNA and lipids (George, 1994). Saez *et al.* (1990) described that GPx and GST work in concert to counteract pro-oxidant processes. This result is in accordance with the present results, because the highest liver GPx and GST activities for all fishes were also observed in the autumn [Figs 2(c), 3(c) and 4(c)], with a decline in liver GST activities during the spring and summer [Figs 2(f), 3(f) and 4(f)].

A typical reaction during oxidative stress is peroxidative damage to PUFAs, and the measurement of LPO levels is an indicator of oxidative damage (Pandey *et al.*, 2001). Therefore, the high LPO levels of *Salmo trutta* species in the autumn probably indicates increased ROS activity.

GR, G6PD AND GST ENZYME ACTIVITIES AND LIPID PEROXIDATION LEVELS OF GILLS

The gill is more readily exposed to changeable water variables than the liver, and thus the increase in gill GST activities for the *S. t. labrax* and *S. t. macrostigma* in the summer may be related to higher water temperatures and lower dissolved oxygen. The low GR and G6PD activities of gills in the summer, however, suggests that the drop in dissolved oxygen content (from *c.* 10–8 mg l^{-1}) did not cause an oxidative stress, and the gill LPO levels showed seasonal fluctuations, within each sub-species. It is difficult to explain why there were such differences in the ADS of the gills between seasons and sub-species. But, developmental and seasonal metabolic cycles reflect the complex interactions between exogenous factors such as food availability, temperature and growth and endogenous factors such as reproductive activities (Gabbott, 1983), which may influence the results.

In conclusion, the activities of SOD, CAT, GPx, G6PD and GST were higher in livers than gills, whereas the reverse was found for GR in the *S. t.*

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macrostigma and for LPO in all fishes. Liver and erythrocytes usually show higher AE activities, which could be due to high rates of free radical generation in these tissues (Martínez-Álvarez *et al.*, 2005). The GR difference may indicate an important role for the glutathione system in gill epithelial cells as a front line of antioxidant defence (Livingstone, 2001; Ritola *et al.*, 2002), and probably the gill is more sensitive than liver against ROS. The ADS of *S. t. caspius*, *S. t. labrax* and *S. t. macrostigma* appear to be affected by many endogenous and exogenous factors, and this study provides reference values throughout the seasons of ADS status for these economically valuable and endangered sub-species. But, it is of interest to note that the increase in liver AE activity occurred during the season where the fishes exhibit the highest I_G and is the period just prior to spawning.

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