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ORIGINAL RESEARCH

Annual variations in hepatic antioxidant defenses and lipid peroxidation in a temperate fish, common bream *Abramis brama* (L.)

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Abstract Alternation of physiological states during the annual cycle (pre-spawning mobilization, spawning energy loss, post-spawning accumulation of energy reserve, declined metabolic activity during the wintering) occurring in the wide temperature range requires high plasticity of fish defense systems. The article presents a 2-year study of hepatic antioxidant defenses and lipid peroxidation in the temperate cyprinid fish, common bream *Abramis brama* (L.), in relation to basic annual rhythms, such as photoperiodic and temperature cycling, reproduction and feeding activity. The objective of the study was to evaluate the plasticity of antioxidant defenses in bream under normal conditions throughout the annual cycle, by measuring hepatic antioxidants (superoxide dismutase, catalase, glutathione-*S*-transferase activities and reduced glutathione concentrations) and products of lipid peroxidation (thiobarbituric acid reactive substances concentrations). The results suggested that hepatic levels of antioxidants in bream varied dramatically during the annual cycle allowing bream to develop necessary adaptation and implement reproduction program. The lowest values of all studied antioxidants were witnessed in the middle of winter. In early spring, antioxidant enzyme activities increased rapidly reaching their maxima in late spring and summer. Toward the end of autumn, enzyme activities were declining toward the levels observed before the ice breakup. Spring–summer up-regulation of antioxidant defenses along with increased lipid peroxidation indicated bream to be experiencing mild oxidative stress.

Keywords Antioxidants · Lipid peroxidation · Oxidative stress · Seasonal fluctuations

Introduction

Dynamic equilibrium between an organism and its environment is maintained by the effective regulation of metabolic processes in response to the external factors. In the temperate-boreal climatic zone, environmental conditions change dramatically through the year demanding a large adaptive potential from fish. Alternation of physiological states during the annual cycle (pre-spawning mobilization, spawning energy loss, post-spawning accumulation of energy reserve, declined metabolic activity during the wintering) occurring in the wide temperature range requires high plasticity of fish defense systems.

Energetic life support in aerobic organisms is maintained by the complex combination of processes, in which the key role is played by the oxidative reactions involving molecular oxygen as the main oxidizing

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agent or electron acceptor. Due to electrons escaping the mitochondrial electron-transport chain to molecular oxygen, reactive oxygen species (superoxide anion radical, hydrogen peroxide, hydroxyl radical, and hydroxyl anion) are produced. The amount of electrons escaping the electron-transport chain depends on the physiological state of the organism and oxygen availability. Living organisms possess a multilevel and complicated antioxidant defense system operating to prevent the formation of reactive oxygen species (and reactive oxygen species modified molecules) and to eliminate them, or minimize their negative effects (Winston and Di Giulio 1991; Menshchikova et al. 2006; Lushchak 2014, 2016).

Primary antioxidant enzymes use reactive oxygen species as substrates, thus performing direct detoxification. Superoxide dismutase (SOD, EC 1.15.1.1) converts superoxide anion radical to hydrogen peroxide and molecular oxygen. Catalase (CAT, EC 1.11.1.6) destroys hydrogen peroxide to molecular oxygen and water. Secondary antioxidant enzymes assist the primary ones. For instance, glutathione-*S*-transferase (GST, EC 2.5.1.18) protects cells by reducing hydroperoxyl groups of oxidized phospholipids directly in the membranes. A no less important component of the antioxidant defense is a non-enzymatic antioxidant—reduced glutathione (GSH), which can be used as a cofactor by primary and secondary antioxidant enzymes, such as glutathione-dependent peroxidase (EC 1.11.1.9) and GST, respectively. GSH is involved in reduction and conjugation reactions and can directly neutralize many reactive oxygen species and stabilize cell membranes (Hayes et al. 2005; Menshchikova et al. 2006; Di Giulio and Meyer 2008; Forman et al. 2009; Lushchak 2014).

Normally, antioxidant defenses effectively maintain very low steady-state levels of reactive oxygen species, protecting cells from the oxidative damage. However, under certain circumstances, an imbalance between production and elimination of reactive oxygen species can occur, causing oxidative stress (Lushchak 2014; Sies 2015) which has specific physiological consequences including lipid peroxidation (Cajaraville et al. 2003; Di Giulio and Meyer 2008). Changes in water temperature, oxygen deficiency as well as reoxygenation may affect steady-state levels of reactive oxygen species and antioxidant defenses in fish (Martínez-Álvarez et al. 2005). It is also known that release of sexual products during spawning leads to tissue degradation coupled with oxidation processes (Shulman and Love 1999), therefore, spawning could induce natural oxidative stress (Soldatov et al. 2007).

Useful information on the state of the organism could be obtained by accessing antioxidant enzyme activities (Sies 2015). The objective of our study was to evaluate the plasticity of antioxidant defenses in the temperate cyprinid fish, the common bream (*Abramis brama* L.), under normal conditions throughout the annual cycle, by measuring hepatic antioxidants (SOD, CAT, GST activities, and GSH concentrations) and products of lipid peroxidation (concentrations of thiobarbituric acid reactive substances, TBARS).

Materials and methods

Fish and sampling

Common bream is a bottom dwelling fish. It inhabits slow-flowing waters and forms shoals. The bream is a typical benthivore, feeding on macrozoobenthos including oligochaetes, chironomid larvae, and bivalves. Most actively, it feeds during the summer time. The bream spawns when the water temperature reaches 12–16 °C (in the study area, it usually happens in May). Under normal weather conditions, spawning is of short duration (Nikolsky 1978; Yakovlev et al. 2001).

Bream was collected during 2 years, January 2008–December 2009. In February, November, December 2008, January–April and November 2009 sampling could not been conducted due to legal reasons. Mature fish was captured using a seine in the mouth area of the Sutka River (58°1.18′N 38°15.976′E, Upper Volga basin). Based on hydrobiological and hydrochemical parameters of the water, the river is characterized as mesotrophic water object. Concentrations of the main ions (ppm) in the sampling area varied in the following ranges 18.1–76.1 (Ca^{2+}), 5.3–23.3 (Mg^{2+}), 2.4–13.1 (Na^{+}), 1.1–3.0 (K^{+}), 1.8–12.4 (Cl^{-}), 2.9–23.4 (SO_4^{2-}), and 73.8–344.4 (HCO_3^{-}); pH values varied from 7.40 to 8.09 (Tsvetkov et al. 2015). Sampling site may be considered as a reference site as it is free of industrial discharges, surrounded by forests and paludified lands.

Table 1 Length, weight, sex, and age of the studied bream, *Abramis brama*

Sampling date	*n* (♂, ♀)	Standard length (cm)	Total weight (g)	Eviscerated weight (g)	Age
16 Jan 2008	9 (3, 6)	34.7 ± 0.6 cd	833 ± 40 c	742 ± 34 c	7–10
1 Apr 2008	16 (7, 9)	24.6 ± 0.5 a	218 ± 15 a	199 ± 14 a	4–7
24 Apr 2008	11 (3, 8)	35.0 ± 0.5 cd	861 ± 39 c	741 ± 23 c	7–10
5 May 2008	11 (9, 2)	38.4 ± 0.6 fg	825 ± 34 c	738 ± 30 c	9–12
19 June 2008	10 (3, 7)	40.5 ± 0.8 g	1053 ± 67 d	937 ± 62 d	11–15
3 July 2008	10 (4, 6)	36.8 ± 0.7 def	794 ± 47 c	694 ± 40 c	8–10
7 Aug 2008	10 (4, 6)	36.0 ± 1.1 cde	887 ± 76 c	775 ± 65 c	6–11
25 Sep 2008	10 (5, 5)	30.1 ± 1.8 b	279 ± 12 a	251 ± 11 a	4–10
30 Oct 2008	10 (4, 6)	23.2 ± 0.3 a	245 ± 5 a	226 ± 5 a	3–4
20 May 2009	10 (5, 5)	34.9 ± 0.5 cd	847 ± 37 c	740 ± 34 c	7–10
25 June 2009	10 (2, 8)	38.4 ± 0.8 fg	1077 ± 79 d	939 ± 75 d	8–12
16 July 2009	10 (4, 6)	36.9 ± 0.6 def	1072 ± 51 d	938 ± 43 d	9–12
13 Aug 2009	10 (5, 5)	30.2 ± 0.4 b	478 ± 37 b	399 ± 15 b	5–8
24 Sep 2009	10 (4, 6)	29.2 ± 1.2 b	504 ± 52 b	454 ± 43 b	4–8
16 Oct 2009	10 (7, 3)	37.2 ± 0.3 ef	1112 ± 28 d	969 ± 17 d	9–10
30 Dec 2009	10 (4, 6)	33.9 ± 0.7 c	890 ± 62 c	786 ± 51 c	7–9

Standard length, total weight, eviscerated weight values are given as mean ± SEM. Age values are given as min–max. The differences in length and weight among the sampling events were examined by Kruskal–Wallis test with Mann–Whitney *U* post hoc test; means with same letters do not have significant differences

After fish was sacrificed by cervical transection, the abdominal cavity was opened on ice, and the liver was excised. Each liver sample was immediately frozen at 20 °C until analyzed. A total of 167 bream were analyzed. Sampling dates, number of individuals analyzed, fish size, age and sex are presented in Table 1.

The duration of daylight at each sampling date and additional dates to build a photoperiod curve was calculated using the day length calculator (http://www.solartopo.com). Water temperature was measured at depth 0.5 m at each sampling event.

Biochemical assays

Each liver sample was homogenized in ice-cold potassium phosphate buffer pH 7.5 at a 1:5 ratio (weight/volume) for enzyme activity assays (1 g tissue in 5 ml buffer) and a 1:1 ratio for GSH assay (1 g tissue in 1 ml buffer) using an Ultra-Turrax T10 Basic homogenizer (IKA). Homogenates were centrifuged at 12,000*g* for 15 min at 4 °C. After centrifugation, lipid phase was removed, and supernatants were collected. Biochemical assays were carried out using a Lambda 25 spectrophotometer (PerkinElmer). Each sample was measured in duplicate. Protein contents were measured using the method of Bradford (1976) with bovine serum albumin as a standard.

SOD activity was measured by following the inhibition of nitroblue tetrazolium reduction in reaction with phenazine methosulfate and NADH under basic conditions (Spitz and Oberley 1989). Measurement was performed in 100 mM potassium phosphate buffer pH 7.8 at 20 °C. The reaction mixture contained 0.33 mM EDTA, 0.6 mM phenazine methosulfate, 1.35 mM nitroblue tetrazolium, 78 mM NADH, and 0.3 ml sample aliquot. SOD activity determination was performed at 540 nm and reported as the concentration of nitroformazan produced per mg protein in the sample per minute of reaction time (μM/mg protein/min).

CAT activity was measured by following the rate of H_2O_2 decomposition, which can be determined directly by the decrease in absorbance at 240 nm, using a molar extinction coefficient of 22.2×10^3 M^{-1} cm^{-1}. Additional dilution in the potassium phosphate buffer at a 1:9 ratio was applied to the liver supernatant. An aliquot of the resultant solution was added to a quartz cuvette with a reactive mixture of 100 mM potassium phosphate buffer pH 7.0 and 1.2 mM H_2O_2. Change of absorbance per minute was measured and reported as μM/mg protein/min (Aebi 1984).

GST activity was determined by the method of Habig et al. (1974) by monitoring the conjugation of GSH with 1-chloro-2,4-dinitrobenzene used as a substrate. The reaction mixture was composed of 100 mM potassium phosphate buffer pH 6.5, 100 mM GSH, 50 mM 1-chloro-2,4-dinitrobenzene, and 0.1 ml of the sample aliquot. Absorbance was measured at 340 nm, 20 °C. The increase in absorbance was recorded for 3 min. Enzyme activity was measured using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1} and reported as the concentration of 1-GS-2,4-dinitrobenzene produced per mg protein in the sample per minute of reaction time (μM/mg protein/min).

GSH concentrations were detected in reaction with 5,5′-dithiobis(2-nitrobenzoic acid). Trichloroacetic acid (25%) was added to homogenate aliquot, mixed thoroughly, and placed on ice for 1 h. The resulting precipitate was separated by centrifugation at 5000 *g* for 10 min at 4 °C. Each sample cuvette contained 100 mM potassium phosphate buffer pH 8.0, 0.6 mM 5,5′-dithiobis(2-nitrobenzoic acid), and 0.5 ml supernatant fraction. The colored complex was registered at 412 nm. GSH content was calculated using a molar extinction coefficient of 13.6×10^3 M^{-1} cm^{-1} and reported as nM/mg protein (Moron et al. 1979).

TBARS concentrations were assayed by color reaction with 2-thiobarbituric acid (Buege and Aust 1978). Trichloroacetic acid solution at 30%, 0.5 M HCl solution, and 0.75% 2-thiobarbituric acid solution were successively added to tissue homogenate. The mixture was stirred vigorously and heated for 15 min in a boiling water bath. After cooling, samples were separated by centrifugation. Ethanol solution of ionol was added to supernatant (final concentration 5 mM) in order to prevent lipid peroxidation in the sample. Samples were measured at 532 nm. A molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} was used for calculations. TBARS concentration was reported as nM/mg protein.

Statistical analysis

The data are presented as the means with the standard errors (mean ± SEM). The statistical analyses were performed using Statistica software. The data were assessed for normality using the Shapiro–Wilk test. As the test results suggested that the data did not come from a normal distribution, nonparametric methods were used. The differences in the levels of antioxidants and TBARS, length and weight of the bream among the sampling points were examined by Kruskal–Wallis test with Mann–Whitney *U* post hoc test. The strength of the association between the studied variables was estimated using the Spearman rank correlation coefficient (*R*). The graphs were constructed using Microsoft Office Excel software.

Results

No significant influence of the fish size on the antioxidant activities and TBARS levels was detected. Weak relationships were registered between the standard length and SOD ($R = 0.38$, $p < 0.05$), CAT ($R = 0.31$, $p < 0.05$), and TBARS ($R = 0.24$, $p < 0.05$).

Annual variations of SOD activity are shown in Fig. 1. The lowest SOD activities were found in winter and fall with the minimum registered in January 2008 (12.76 ± 1.35 μM/mg protein/min). In spring, by the time

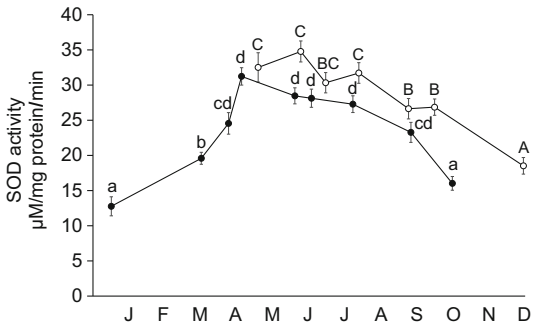

Fig. 1 Superoxide dismutase (SOD) activity (μM/mg protein/min) in the liver of bream in the years 2008 (*filled circle*) and 2009 (*open circle*). Data are presented as mean ± SEM. Means with *different letters* are significantly different (Kruskal–Wallis test with Mann–Whitney *U* post hoc test, *lower case* the year 2008, *upper case* the year 2009)

of spawning, a fast significant increase in activity was observed. The greatest value was registered in May 2008 (31.23 ± 1.23 μM/mg protein/min), and then a slow decrease in activity was observed. In 2009, higher SOD levels were witnessed. Two small peaks were registered in summer with the maximum in June (34.77 ± 1.49 μM/mg protein/min).

CAT activity increased rapidly in spring (Fig. 2) reaching its maximum of 50.19 ± 1.97 μM/mg protein/min by May 2008 (spawning period). In 2009, the greatest value was registered in May as well (52.43 ± 1.69 μM/mg protein/min). Both years, since June, CAT activities were decreasing to a lower winter level (24.41 ± 1.41 μM/mg protein/min, December).

Similar to CAT and SOD activities, GST level increased significantly in spring by the time of spawning (Fig. 3). Slight decreases in GST activity followed by new increases were observed in summer months. The greatest GST values were registered in August of each year (3.26 ± 0.12 and 3.13 ± 0.1 μM/mg protein/min). In 2008, the period of high enzyme activity was more prolonged, GST level stayed high from June to the late September, while the next September GST activity was significantly lower as compared with the values registered in August (Mann–Whitney test, $p < 0.05$).

GSH pattern differed from those witnessed for SOD, CAT, and GST activities (Fig. 4). Spring values were significantly greater as compared with winter level, being relatively small on a whole year scale though. Pronounced peaks were observed in August of each year (11.21 ± 0.84 and 8.12 ± 0.33 nM/mg protein). Late autumn and winter levels were equal to those registered in spring and early summer.

TBARS content did not peak through the annual cycle (Fig. 5), though May–August levels were significantly greater than those seen in April, October, and December ($p < 0.05$). TBARS level started to rise during spawning and continued to elevate slowly reaching the annual maximum in August of each year (1.83 ± 0.1 and 1.79 ± 0.09 nM/mg protein).

Moderate relationships were observed between SOD and CAT ($R = 0.61$, $p < 0.05$), SOD and GST ($R = 0.55$, $p < 0.05$). Weaker associations were evident between SOD and TBARS ($R = 0.43$, $p < 0.05$), CAT and GST ($R = 0.43$, $p < 0.05$). Other relationships were much weaker or non-significant.

Water temperature values registered at the sampling dates are given in Fig. 6, showing an increase from April (after the breakup) to July. The photoperiod curve for the sampling site is also presented in Fig. 6.

Statistically significant correlations (Spearman coefficient, $p < 0.05$) were observed between all studied parameters and photoperiod (R_p) and water temperature (R_t), except for GSH. The strength of association varied from moderate to strong. Correlation coefficient values were as follows SOD $R_p = 0.83$, $R_t = 0.78$; CAT $R_p = 0.82$, $R_t = 0.6$; GST $R_p = 0.59$, $R_t = 0.7$; TBARS $R_p = 0.77$, $R_t = 0.88$.

Discussion

It is well established by numerous studies that seasonal rhythms govern various vital functions of fish (Bulow et al. 1981; Eliassen and Vahl 1982; Zapata et al. 1992; Gozdowska et al. 2006; Prihartato et al. 2015; etc.). Along with the alternation of seasons, temperate fish experience changes in mean temperature, amounts of

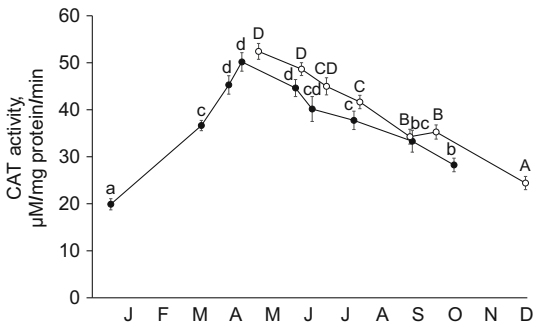

Fig. 2 Catalase (CAT) activity (μM/mg protein/min) in the liver of bream in the years 2008 (*filled circle*) and 2009 (*open circle*). Data are presented as mean ± SEM. Means with *different letters* are significantly different (Kruskal–Wallis test with Mann–Whitney *U* post hoc test, *lower case* the year 2008, *upper case* the year 2009)

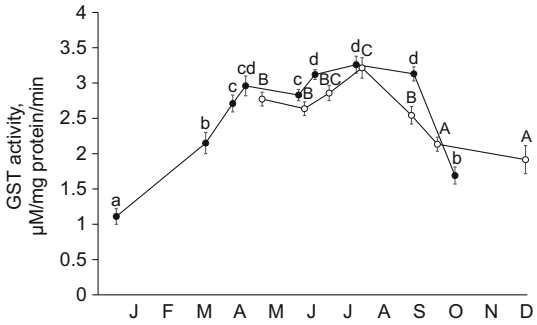

Fig. 3 Glutathione-*S*-transferase (GST) activity (μM/mg protein/min) in the liver of bream in the years 2008 (*filled circle*) and 2009 (*open circle*). Data are presented as mean ± SEM. Means with *different letters* are significantly different (Kruskal–Wallis test with Mann–Whitney *U* post hoc test, *lower case* the year 2008, *upper case* the year 2009)

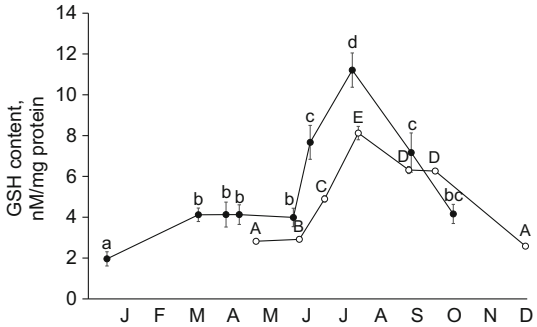

Fig. 4 Reduced glutathione (GSH) concentrations (nM/mg protein) in the liver of bream in the years 2008 (*filled circle*) and 2009 (*open circle*). Data are presented as mean ± SEM. Means with *different letters* are significantly different (Kruskal–Wallis test with Mann–Whitney *U* post hoc test, *lower case* the year 2008, *upper case* the year 2009)

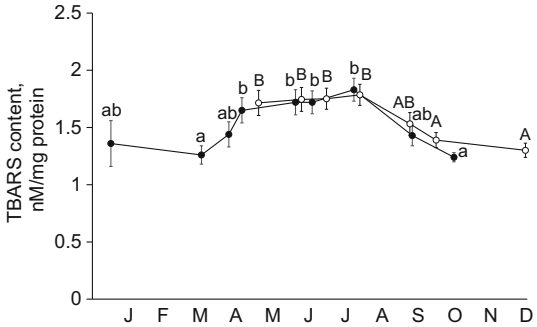

Fig. 5 Thiobarbituric acid reactive substances (TBARS) concentrations (nM/mg protein) in the liver of bream in the years 2008 (*filled circle*) and 2009 (*open circle*). Data are presented as mean ± SEM. Means with *different letters* are significantly different (Kruskal–Wallis test with Mann–Whitney *U* post hoc test, *lower case* the year 2008, *upper case* the year 2009)

oxygen, food availability large enough to present organisms with a physiological challenge (Clarke 1993). Yet, these fluctuations have a constant regime for which certain functional adaptations exist.

The lowest values of all studied antioxidants were seen in the middle of winter. This fact reflects a general decline in fish metabolic activity during the wintering period (Shulman and Love 1999).

Enzyme activities and GSH level increased in early spring before the ice breakup and water temperature elevation. At that, the TBARS level decreased as compared with January level. In the obvious absence of temperature compensation on the enzymatic activities, one might consider a cueing role of photoperiod. Temperate fish's fundamental metabolic processes are driven to maintain seasonality in reproduction; photoperiod is an important synchronizing factor inducing changes in trends and rates of metabolism (Shulman

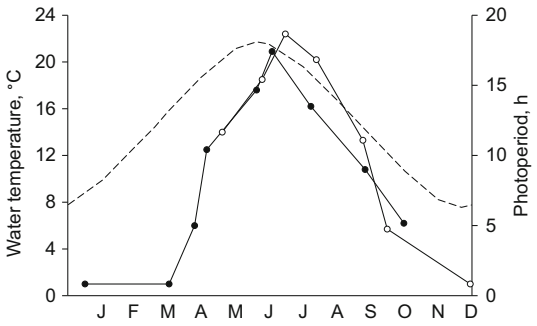

Fig. 6 Photoperiod, h (*dashed line*) and water temperature courses, °C, in the years 2008 (*filled circle*) and 2009 (*open circle*)

and Love 1999; Wang et al. 2010). The second possible explanation of the observed elevation of the antioxidant levels might be the fish's response to hypoxia termed "preparation to oxidative stress" (Hermes-Lima et al. 1998). This response is considered as an evolutionary adaptation playing a protective role during oxygen deficient conditions, which usually occur by the end of ice-covered period. Hypoxia activates antioxidant defences, allowing fish to cope with oxidative stress that might arise from tissue reoxygenation (Martínez-Álvarez et al. 2005). The possibility of such post-breakup oxidative stress is also illustrated by the fact that the bream inhabiting the studied area experiences spring (post-breakup) increase of plasma concentrations of triiodothyronine, known to enhance tissue oxygen uptake (Bolotovskiy and Levin 2015).

By the end of April, when the water temperature started to grow, SOD, CAT, and GST activities were at their rapid rise, while the GSH level was still relatively low and equal to that registered before the ice breakup. TBARS concentrations heightened before the beginning of spawning. At the period from May to August, increased TBARS concentrations along with elevated levels of the studied antioxidants indicated a state of oxidative stress. According to the intensity-based classification of oxidative stress proposed by Lushchak (2014), a low-intensity/mild oxidative stress is that is witnessed here. The spawning may be considered as a physiological state causing naturally induced oxidative stress in aquatic organisms (Soldatov et al. 2007). Thus, Soldatov et al. (2007) observed increased glutathione peroxidase and CAT activities along with elevated TBARS level in bivalve mollusk *Mytilus galloprovincialis* Lam, during the spawning period.

In the present study, the signs of oxidative stress were registered prior to spawning and throughout the summer. Endogenous compounds, both involved in gametogenesis and resorption of unspawned gametes, may be considered as effectors inducing oxidative stress. For instance, metabolism of steroid hormones involves hepatic cytochrome P450-dependent enzymes (Whyte et al. 2000). In the cytochrome P450 metabolic pathways, reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radical can be generated, evoking certain responses (Menshchikova et al. 2006).

It was evident that SOD and CAT activities had a tendency to decline after spawning. Apart from these enzymes, maximum of GST activity was observed in August. Although there were no direct associations between the enzyme activities and water temperature, SOD, CAT, and GST were likely to be under relatively strong temperature impact. Enhanced temperature can be considered as a natural pro-oxidant factor during summer months (Gorbi et al. 2005). High ambient temperature can disturb metabolic processes causing elevation of steady-state concentrations of reactive oxygen species, which required adjustments in the antioxidant defense. Each year, while SOD and CAT levels were decreasing after a spring rise, GST activity increased to its new high. At that, the TBARS levels, although relatively high, were almost even throughout the summer indicating elevated but steady level of oxidative modification.

An important antioxidant action of GSH is that of being a substrate to antioxidant enzymes such as glutathione-dependent peroxidase and GST. Thus, the highest levels of GSH were timed to the GST activity maxima. However, in a whole year scale, their fluctuations were not in complete agreement. No changes of the GSH concentrations were found during the spawning period when GST activity was at significant rise. Summer GSH peaks appear to be not only GST-related. As the spawning is accomplished, the bream leaves the nearshore zones and moves to the deep in search for areas rich with benthos (Poddubny 1971). Active feeding governed by the need for energy reserve accumulation that will cover vital functions later when food consumption is ceased, stimulates intensive digestion. A sharp summer increase of the GSH levels might be

related to the intensification of feeding and digestion. The uptake of amino acids formed in the result of proteolysis is controlled by passive or active mechanisms functioning via diffusion or requiring cellular energy (Forrest et al. 2011; Hediger et al. 2013). Presumably, GSH may act as an amino acid transporter in the γ-glutamyl cycle (Orlowski and Meister 1970; Meister 1974). According to the initial γ-glutamyl cycle hypothesis, a series of catalytic events involves the synthesis and degradation of GSH, and the coupled uptake and release of free amino acids. GSH is known to play multiple roles in animal metabolism (Wu 2013). Therefore, the up-regulation of GSH synthesis witnessed in summer may be associated with not only antioxidant functions of GSH but also its non-antioxidant contributions to metabolic and regulation processes as well.

As observed in 2008 and 2009, spring–summer up-regulation of the studied antioxidants along with relatively intensified but steady lipid peroxidation indicate bream's adaptation to periodically occurring natural oxidative stress. Such high plasticity of antioxidant defenses provides restraining of lipid peroxidation and thus surviving under oxidative stress and realization of reproduction program.

Toward the end of autumn, after abrupt decline of water temperature before the ice-covered period, the bream ceases feeding (Poddubny 1971). At this time, fish metabolism is adjusted to a stable state preparing for the wintering period (Shulman and Love 1999). In the present study, in autumn, enzyme activities along with TBARS concentrations were approaching the levels observed prior to the ice breakup.

As shown in this study, hepatic levels of antioxidants in bream vary dramatically during the annual cycle. Spring–summer levels significantly exceed those registered in autumn and winter. Driven by endogenous and exogenous factors, the greatest levels of antioxidants are timed to the periods of spawning and active feeding. In spring prior to spawning and further to the end of summer, bream seems to be experiencing mild oxidative stress.

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