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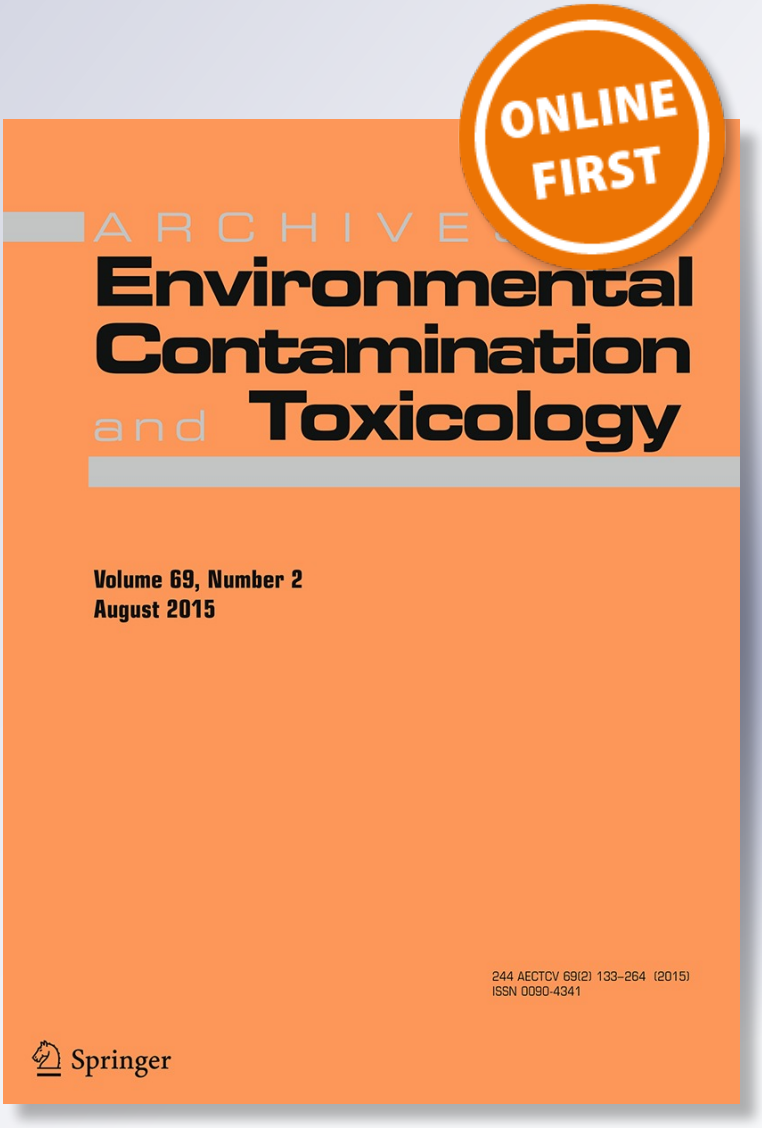

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Factors Affecting Antioxidant Response in Fish from a Long-term Mercury-Contaminated Reservoir

M. Sevcikova[1] · H. Modra[1] · J. Blahova[1] · R. Dobsikova[1] · J. Kalina[2] · O. Zitka[3] · R. Kizek[3] · Z. Svobodova[1]

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Abstract The objective of this work was to evaluate antioxidant defence and oxidative damage in organs (liver, gills, kidney, and brain) of five fish species (*Aspius aspius, Esox lucius, Sander lucioperca, Abramis brama, Rutilus rutilus*) from the long-term mercury-contaminated Skalka Reservoir in the Czech Republic. Special emphasis was placed on a comprehensive assessment of the factors that may affect the antioxidant response to mercury in fish. Antioxidant enzymes (glutathione reductase, glutathione peroxidase, and glutathione-S-transferase) did not significantly respond to mercury contamination. Levels of the analysed enzymes and oxidative damage to lipids were predominantly determined by a separate organ factor or species factor, or by the combination of both ($p < 0.001$). Levels of total glutathione and the reduced/oxidized glutathione ratio were influenced by mercury contamination in combination with their specific organ distribution ($p < 0.001$). Our results suggest that species and type of organ alone or in combination are more important factors than chronic exposure to mercury contamination with respect to effects on antioxidant defence in fish under field conditions. Our findings suggest that the main antioxidant defensive mechanism in fish from the studied long-term mercury contaminated site was the inter-tissue distribution of glutathione.

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Mercury is a metal whose toxicity to humans and wildlife has been intensively studied since mercury poisoning in Minamata Bay (Japan) was revealed in 1956 (Harada 1995). The naturally ubiquitous presence of mercury in the environment is connected with volcanic activity, the weathering of rocks, water movements, and biological processes. The increasing mercury pollution of the aquatic environment is heavily associated with various human activities (WHO 1976). The tendency of fish to accumulate mercury relatively to their position in the food chain and to their feeding habits was demonstrated in many studies (Rincon Leon et al. 1993; Dusek et al. 2005). Mercury, particularly methylmercury, has high affinity to lipids, which allows it to move easily across cell membranes and alter cell metabolism (Bebianno et al. 2007). Methylmercury is readily absorbed across the gills and gut of fish and is mainly accumulated in fish muscle, liver, and kidney (Downs et al. 1998). The harmful effects of chronic mercury exposure on fish include neurological and behavioural disorders, such as direct brain lesions and reduced swimming activity (Berntssen et al. 2003), the inhibition of growth and gonadal development (Friedmann et al. 1996; Hammerschmidt et al. 2002), the induction of oxidative stress (Berntssen et al. 2003; Larose et al. 2008; Monteiro et al. 2013), and direct tissue damage (liver, kidney, gills), leading to the impairment of basic metabolic pathways (Wester and Canton 1992; Liao et al. 2007).

Although the exact mechanisms of mercury toxicity in fish have not been completely explored, recent studies suggest that its biochemical mode of action includes the generation of radical oxygen species (ROS) leading to lipid

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peroxidation, oxidative DNA damage, and the oxidation of proteins, with cell death via apoptosis and necrosis as a consequence-in general, the state termed oxidative stress (Kelly et al. 1998; Ercal et al. 2001).

One of the most important mechanisms behind mercury-induced oxidative damage is the depletion of reduced glutathione (GSH) (Stohs and Bagchi 1995). Glutathione is a tripeptide (γ-L-glutamyl-L-cysteinyl-glycine), which participates as a free-radical scavenger in several nonenzymatic antioxidant reactions and is involved in cellular defence against the toxic action of metal cations. Glutathione acts as an antioxidant via two mechanisms: it can act nonenzymatically by directly scavenging ROS and also as a cofactor of antioxidant enzymes (Mason and Jenkins 1995). The overproduction of ROS by mercury can lead to oxidation of the reduced glutathione form (GSH) to the oxidized form (GSSG) (Meister and Anderson 1983). The ratio of GSH to GSSG is considered as an indicator of the intracellular redox state (Srikanth et al. 2013).

Important components of enzymatic antioxidant defence connected with GSH are glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) (Lushchak 2011). Glutathione peroxidase is a selenium-dependent enzyme that reduces a variety of peroxides to their corresponding alcohols. Glutathione is employed as a cofactor in these reactions. Glutathione reductase is a crucial enzyme for maintaining the GSH/GSSG ratio catalysing the transformation of GSSG to GSH, with the simultaneous oxidation of NADPH (nicotinamide adenine dinucleotidephosphatehydrate) to $NADP^+$ (nicotinamide adenine dinucleotide phosphate) (Van der Oost et al. 2003). The conjugation of electrophilic compounds with GSH is catalysed by GST. Apart from the essential functions of GST in intracellular transport and the detoxifying mechanism, a critical role is played by GST in the defence against oxidative damage and the peroxidative products of DNA and lipids (Leaver and George 1998).

Traditionally, studies dealing with fish antioxidant response to mercury have focused mainly on the liver, the main target organ for toxic substances (Elia et al. 2003; Larose et al. 2008). Gills and kidney are the next most frequently studied organs (Navarro et al. 2009; Monteiro et al. 2013), because both play an important role in osmoregulation function in fish. In addition, gills are directly involved in metal uptake due to their permanent direct contact with the aquatic environment (Wendelaar Bonga and Lock 2008), and the kidney was proven to be the most susceptible organ to the pro-oxidative action of mercury (Mieiro et al. 2014). Mercury neurotoxicity has been described in fish, but the role of the oxidative stress mechanism in these neurodegenerative processes in fish is less clear than in mammalian models (Mieiro et al. 2011c). Therefore, taking into account the specific roles of organs in metal metabolism and toxicity, the liver, kidney, gills, and brain were chosen for the present study.

Components of antioxidant defence, both enzymatic and nonenzymatic, are useful tools for assessing the effects of a large number of pollutants in the aquatic environment (Van der Oost et al. 2003). These biomarkers were used in several field studies involving mercury, dealing separately with different fish species (Larose et al. 2008; Mieiro et al. 2011a), differences between organs (Bebianno et al. 2007; Navarro et al. 2009; Mieiro et al. 2011b), and various levels of mercury contamination (Guilherme et al. 2008; Mieiro et al. 2010) in connection with antioxidant defence; however, the overall effect of long-term mercury contamination covering several factors at once has rarely been described.

Therefore, the main objective of this work was to assess the overall effect of long-term mercury contamination on different fish species by evaluating various compartments of antioxidant defence and also oxidative damage in different organs under field conditions. Special emphasis was placed on a comprehensive assessment of the factors that may affect a fish's antioxidant response to mercury.

Materials and Methods

Sampling Sites

The study was performed at the Skalka and Zelivka Reservoirs. The Skalka Reservoir is located in western Bohemia on the River Ohre near the border with Germany (Kruzikova et al. 2011). Contamination of the Skalka Reservoir by effluent containing mercury from a chemical factory in Marktredwitz (Germany) was revealed in 1974 and from that moment monitoring was initiated (Svobodova and Hejtmanek 1976). Between 1974 and 1996, the range of total mercury in muscle was 0.14–3.4 mg kg^{-1} wet weight depending of fish species (Marsalek et al. 2005). In 2004, the concentration of total mercury was up to 0.215 μg l^{-1} in water and up to 27.27 mg kg^{-1} in sediments (Soukup 2004). A study performed in 2011 by Sevcikova et al. (2013) confirmed high mercury levels in fish tissues. The mean values of mercury contents in muscle and liver of predatory fish varied from 0.26 to 0.73 and from 0.19 to 0.75 mg kg^{-1} wet weight, respectively. Methylmercury constituted the major part of the total mercury concentration in all muscle and liver samples. Other metals (cadmium, copper, lead, nickel, zinc, and arsenic) in low concentrations also were detected in fish tissues (Sevcikova et al. 2013).

The Zelivka Reservoir is situated in central Bohemia, 4 km above the confluence of the River Sazava. The reservoir was gradually filled from 1970 to 1974. A short

time after filling, a high level of mercury was detected in fish tissue, although no source of mercury pollution was discovered (Svobodova et al. 1988). During the monitoring of mercury contents in fish from 1974 to 2011, a significant decrease in mercury content was observed (Kruzikova et al. 2011). Nowadays, the Zelivka Reservoir is used as a source of drinking water; therefore, regular monitoring of the water quality is undertaken by a public research institute (T.G. Masaryk's Water Research Institute). According to data collected from 2003 to 2011, the measured concentrations of various pollutants (metals, pesticides, tensides, polycyclicaromatichydrocarbons, etc.) were very low (Masaryk 2011). The levels of mercury in water did not exceed 0.2 μg l^{-1} during this period. Currently, the reservoir is considered to be uncontaminated with respect to mercury; thus, it was used as a control site in the present study.

Fish Collection

Sampling was performed by electrofishing at the end of April 2011. The physicochemical parameters of the water at the sampling sites were as follows: temperature 8.5 °C, pH 7.2, conductivity 294 S cm^{-1} for the Skalka Reservoir; temperature 7.7 °C, pH 7.6, conductivity 272 S cm^{-1} for the Zelivka Reservoir. Twenty-five fish were sampled from the Skalka Reservoir and 35 from the Zelivka Reservoir (Table 1). Three carnivorous (asp, pike, and pikeperch) and two benthophagous (bream, roach) species were selected for this study to cover different feeding habits and diet spectra. Fish were weighed and scales were collected for age determination. Samples of liver, gill, caudal kidney, and brain were taken and frozen in liquid nitrogen, transported to the laboratory, and stored at −85 °C for later analysis. The same samples of liver and muscle were also used for total mercury and methylmercury determination; data published by Sevcikova et al. (2013). Concentrations of total mercury in liver are recorded in Table 1.

The sampling procedure was in compliance with national legislation (Act No. 246/1992 Coll., on the Protection of Animals Against Cruelty, as amended, and Decree No. 419/2012 Coll., on the Protection, Breeding, and Use of Experimental Animals, as amended).

Determination of Detoxifying Enzyme and Oxidative Stress Parameters

Tissue samples (liver, gill, caudal kidney, and brain) were homogenized in 50 mM potassium phosphate buffer with 1 mM EDTA (pH 7.4) and centrifuged at 11,200 × *g* for 20 min at 4 °C. The supernatant was pipetted into separated Eppendorf tubes and kept at −85 °C for later analyses. It was used for the determination of GR, GPx, and GST activity, and protein concentration. Noncentrifuged homogenate (stored at −85 °C) was used to estimate lipid peroxidation. The protein concentration was quantified by means of Bicinchoninic Acid Protein Assay Kit (Sigma–Aldrich, St. Louis, MO) using bovine serum albumin as a standard (Smith et al. 1985).

The total catalytic concentration of GST was determined by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione at 340 nm (Habig et al. 1974). The specific activity was expressed as the nmol of the formed product (the conjugate of CDNB with GSH) per min per mg of protein.

The catalytic concentration of GR was determined spectrophotometrically by measuring NADPH oxidation at 340 nm (Carlberg and Mannervik 1975). The catalytic concentration of GPx was calculated from the rate of NADPH oxidation in the reaction with GR at 340 nm

Table 1 Biometric data [body weight (g), age (year)] and total mercury concentration in liver (mg kg^{-1} wet weight) of fish from both sampling sites

Species	Site	*n*	Body weight (g)	Age (range of years)	Total mercury in liver (mg kg^{-1} wet weight)
Asp (*Aspius aspius*)	Skalka Reservoir	5	1727 ± 279	4–7	0.67 ± 0.18
	Zelivka Reservoir	9	1288 ± 1155	2–8	0.06 ± 0.04
Pike (*Esox lucius*)	Skalka Reservoir	5	1797 ± 727	3–5	0.19 ± 0.04
	Zelivka Reservoir	7	824 ± 574	2–5	0.05 ± 0.01
Pikeperch (*Sander lucioperca*)	Skalka Reservoir	5	1054 ± 899	2–7	0.75 ± 0.30
	Zelivka Reservoir	4	2055 ± 1104	3–7	0.04 ± 0.02
Bream (*Abramis brama*)	Skalka Reservoir	5	670 ± 157	3–7	0.22 ± 0.15
	Zelivka Reservoir	9	653 ± 126	4–7	0.06 ± 0.03
Roach (*Rutilus rutilus*)	Skalka Reservoir	5	193 ± 63	4–5	0.11 ± 0.05
	Zelivka Reservoir	6	389 ± 444	2–7	0.02 ± 0.01

Data published by Sevcikova et al. (2013). Values presented as mean ± SD

Table 2 Numbers of samples used for statistical analysis defined by the factors of site, species, and organ

Site	Species/organ	Brain	Gills	Liver	Kidney	Total
Skalka Reservoir	Asp (*Aspius aspius*)	5	5	5	5	20
	Bream (*Abramis brama*)	5	5	5	5	20
	Pike (*Esox lucius*)	5	5	5	5	20
	Roach (*Rutilus rutilus*)	5	5	5	5	20
	Pikeperch (*Sander lucioperca*)	5	5	5	3	18
Zelivka Reservoir	Asp (*Aspius aspius*)	8	9	9	7	33
	Bream (*Abramis brama*)	9	9	9	8	35
	Pike (*Esox lucius*)	6	7	7	7	27
	Roach (*Rutilus rutilus*)	6	6	6	4	22
	Pikeperch (*Sander lucioperca*)	4	4	4	4	16
Total		58	60	60	53	231

(Flohe and Gunzler 1984). The specific activities of GR and GPx were expressed as the nmol of NADPH consumption per min per mg of protein.

Lipid peroxidation was determined using the TBARS (thiobarbituric acid reactive substances) method at 535 nm with a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Lushchak et al. 2005). The concentration was expressed as nmol of TBARS per gram of wet weight tissue. All spectrophotometric methods were performed using a Varioskan Flash Spectral Scanning Multimode Reader (Thermo Scientific).

Levels of GSH and GSSG in liver, gill, and caudal kidney samples were measured by high-performance liquid chromatography with electrochemical detection (HPLC-ED). The system consisted of two solvent delivery pumps operating in the range of 0.001–9.999 ml min^{-1} (Model 582 ESA Inc., Chelmsford, MA), a Zorbax eclipse AAA C18 (150 × 4.6; 3.5 µm particle size; Agilent Technologies, USA), and a CoulArray electrochemical detector (Model 5600A, ESA, USA). The sample (20 µl) was injected using an autosampler (Model 542, ESA, USA). Supernatants from the centrifuged homogenates (15,000 × *g*, 20 min, 4 °C) were used for the measurements. Total GSH was calculated as the sum of GSH and GSSG, and expressed as the µmol per g of wet weight.

Statistical Analysis

The analysis was performed for a total of 240 tissue samples taken from four different organs (60 samples from each organ), five fish species, and two sampling sites. Numbers of samples within groups differed significantly (Table 2). The whole dataset divided into four groups along with the three independent factors of site, species, and organ yielded 40 groups for analysis. This structure allowed the use of generalized estimating equations (GEE) to estimate the effects of mixed factor combinations on selected parameters and their statistical significance. The GEE method was used to statistically analyse the activities of GST, GPx, and GR, and the levels of lipid peroxidation (LPO). Because the activities of GPx in liver and GST in gills of pike were markedly different than the others, additional analyses excluding these data also were performed. When there were no measurements for any of the 40 groups, the GEE method was unable to be used; therefore, the generalized linear models method (GLM) was employed to statistically analyse the levels of total GSH and the GSH/GSSG ratio. Bonferroni correction was used to eliminate false-positive results.

Results

The results of statistical analysis are summarized in Table 3. The factor *species* significantly differed in the activity of GST and the level of LPO. Furthermore, the results revealed the significant ($p < 0.001$) influence of the factor *organ* in the activities of GST and GPx, the level of LPO, and the GSH/GSSG ratio. All analysed enzymes ($p < 0.001$) and also the level of LPO ($p < 0.001$) and the GSH/GSSG ratio ($p < 0.016$) were significantly influenced by the factor combination *species* + *organ*. The additional analysis excluding data of GPx activity in liver and GST activity in gills of pike revealed still significant ($p < 0.003$) influence of the factor *organ* and also the factor combination *species* + *organ*. Surprisingly, the factor combination *site* + *organ* were significant ($p < 0.001$) only in total GSH content and the GSH/GSSG ratio. All of these significant differences were confirmed by Bonferroni correction (data not shown) tightening the significance level to reduce a risk of multiple comparison. Although some other factor combinations appear to be significant due to the use of the GEE or GLM methods, their significance was not confirmed by Bonferroni correction (data not shown);

Table 3 Estimation of mixed-factor effects on glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), and lipid peroxidation (LPO) using the GEE method and on total glutathione (Total GSH) and the reduced/oxidized glutathione ratio (GSH/GSSG) using the GLM method

Method Parameter	GEE GST	GEE GPx	GEE GR	GEE LPO	GLM GSH/GSSG	GLM Total GSH
Factor combination	*p* value					
Species	<0.001**	0.128	0.115	<0.001**	0.220	0.380
Site	0.590	0.282	0.039*	0.699	0.193	0.029*
Organ	<0.001**	<0.001**	0.193	<0.001**	<0.001**	0.050*
Species + site	0.660	0.568	0.143	0.431	0.073	0.079
Species + organ	<0.001**	<0.001**	<0.001**	<0.001**	<0.016**	0.227
Site + organ	0.320	0.216	0.028*	0.006*	<0.001**	<0.001**
Species + organ + site	0.320	0.015*	0.022*	0.002*	0.482	0.077

* Results with decreased significance by Bonferroni correction

** Results with high significance of the factor combination

therefore, they are considered to be nonsignificant. The real measured values of all analysed parameters are shown in Table 4.

Discussion

Field studies are useful for assessing the potential effects of pollutants on aquatic biota in realistic conditions, but attention should be paid to the possible misinterpretation of results. A wide range of pollutants, such as metals, pesticides, pharmaceuticals, and oil products, are able to induce oxidative damage (Lushchak 2011). The interaction of these pollutants can result in both protective and deleterious synergistic effects (Livingstone 2003). In our study, mercury was the main pollutant in the studied (Skalka) reservoir, which has been documented in many studies and confirmed by means of long-term monitoring (Svobodova and Hejtmanek 1976; Marsalek et al. 2005; Kruzikova et al. 2011). Therefore, we consider mercury to be the major possible promoter of oxidative stress in the studied reservoir.

The variable sensitivity of different fish species to toxic substances is a frequent finding of laboratory and field studies dealing with oxidative stress indices (Larose et al. 2008; Eyckmans et al. 2011; Miller and Hontela 2011). As for antioxidative stress response, it covers a variable induction of both enzymatic and nonenzymatic compartments of antioxidant defence. However, antioxidant defence also can be influenced by many other factors apart from aquatic pollution, such as, for example, feeding habits and metabolic characteristics (Hellou et al. 2012). Fundamental differences in physiological and biochemical processes have been documented even between closely related fish species (Krogdahl et al. 2004; Miller et al. 2009). These differences alone may contribute to a variety of antioxidant responses among different fish species independent of the influence of aquatic pollutants.

A variety of biochemical processes was described in a comparative study focused on the activities of superoxide dismutase, catalase, and glutathione peroxidase in two herbivorous and three omnivorous fish species (Radi et al. 1985). The authors revealed lower enzymatic activity in herbivorous species compared to those with omnivorous feeding-behaviour. A higher trophic position of fish species in the food chain represents a greater risk of the accumulation of various toxic substances in their feed (Farrington 1991). Higher basal antioxidant defence may be an evolutionary mechanism of adapting to constant pressure from the environment. This corresponds with the higher GST activity in pike determined in our study, which can be explained by the top food chain position of this predatory species relative to the other chosen fishes. The significant influence of species on oxidative defence also was reported by Miller et al. (2009) in salmonids from selenium-impacted streams. The species factor was evaluated to be significant in several compartments of antioxidative defence (GSH, GPx, vitamin E, and vitamin A), but not in the level of LPO (Miller et al. 2009).

An organ-specific response to oxidative stress stimuli was described in fish as a consequence of exposure to various metals in the aquatic environment (Campana et al. 2003; Ahmad et al. 2006; Hansen et al. 2006). The involvement of individual antioxidant enzymes and nonenzymatic antioxidants depends on the particular organ's role in fish metabolism and the detoxification process.

Studies with a multiorgan approach usually revealed varied results with respect to oxidative stress indices, pointing to the fact that the type of organ plays an important role in the oxidative stress mechanism pertaining to mercury in fish (Berntssen et al. 2003; Navarro et al. 2009; Monteiro et al. 2013). Our results that the organ factor separately and significantly influenced the majority of the studied oxidative stress indices are not so surprising, and on a more general level, are consistent with the

Table 4 Oxidative stress parameters in liver, kidney, gills, and brain [glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), lipid peroxidation (LPO), the reduced/oxidized glutathione ratio (GSH/GSSG), and total GSH (Total GSH)] of different fish species from the Skalka and Zelivka Reservoirs

Tissue/species	Site	*n*	GST	GPx	GR	LPO	GSH/GSSG	Total GSH
Liver								
Asp	Skalka Reservoir	5	21.0 ± 4.9	536.1 ± 199.2	7.9 ± 4.7	10.3 ± 1.8	12.5 ± 3.8	1.0 ± 0.4
	Zelivka Reservoir	9	16.8 ± 4.2	584.6 ± 263.5	7.9 ± 4.5	19.8 ± 13.3	8.3 ± 3.5	2.3 ± 1.6
Pike	Skalka Reservoir	5	72.6 ± 12.7	2494.3 ± 960.1	12.5 ± 1.9	6.9 ± 3.3	11.4 ± 4.8	0.6 ± 0.2
	Zelivka Reservoir	7	54.5 ± 24.3	1126.9 ± 510.6	14.2 ± 6.4	4.6 ± 0.5	9.2 ± 4.0	2.4 ± 1.1
Pikeperch	Skalka Reservoir	5	29.8 ± 3.4	507.3 ± 156.2	9.9 ± 4.3	4.6 ± 1.0	5.1 ± 2.4	1.0 ± 0.3
	Zelivka Reservoir	4	29.5 ± 3.4	558.8 ± 90.4	7.9 ± 1.0	4.9 ± 1.8	8.0 ± 2.0	3.0 ± 1.6
Bream	Skalka Reservoir	5	31.8 ± 3.0	432.1 ± 98.9	7.0 ± 0.4	23.9 ± 15.2	10.6 ± 6.3	0.5 ± 0.4
	Zelivka Reservoir	9	36.8 ± 11.5	417.6 ± 105.1	10.0 ± 4.6	10.3 ± 2.5	6.7 ± 4.1	1.6 ± 0.7
Roach	Skalka Reservoir	5	39.1 ± 17.0	380.1 ± 127.5	14.6 ± 5.6	18.2 ± 9.5	8.9 ± 5.0	0.9 ± 0.4
	Zelivka Reservoir	6	30.6 ± 13.0	622.5 ± 204.9	10.4 ± 1.6	7.8 ± 3.1	6.2 ± 2.7	1.4 ± 0.6
Kidney								
Asp	Skalka Reservoir	5	27.3 ± 21.2	65.5 ± 33.6	9.0 ± 2.9	58.2 ± 24.5	13.5 ± 4.2	2.1 ± 0.9
	Zelivka Reservoir	9	18.2 ± 2.4	40.1 ± 11.3	7.9 ± 1.5	60.0 ± 20.3	6.9 ± 4.2	0.7 ± 0.4
Pike	Skalka Reservoir	5	23.8 ± 22.1	104.9 ± 48.5	6.8 ± 4.3	47.6 ± 30.8	7.3 ± 3.9	1.6 ± 0.5
	Zelivka Reservoir	7	37.8 ± 18.3	137.5 ± 19.9	10.9 ± 4.0	42.1 ± 33.9	5.2 ± 1.7	0.9 ± 0.6
Pikeperch	Skalka Reservoir	5	22.6 ± 7.9	73.1 ± 5.6	12.8 ± 4.0	11.5 ± 6.0	7.7 ± 1.2	2.2 ± 0.6
	Zelivka Reservoir	4	36.2 ± 4.8	44.1 ± 18.0	9.1 ± 2.1	21.1 ± 7.7	5.5 ± 0.2	0.7 ± 0.4
Bream	Skalka Reservoir	5	27.5 ± 5.2	117.9 ± 7.8	9.8 ± 0.5	75.0 ± 13.8	7.8 ± 2.1	1.7 ± 0.5
	Zelivka Reservoir	9	33.9 ± 4.2	107.9 ± 16.1	9.2 ± 1.5	51.0 ± 23.1	4.9 ± 2.3	0.4 ± 0.2
Roach	Skalka Reservoir	5	28.1 ± 4.8	68.5 ± 23.9	12.6 ± 1.3	50.5 ± 37.0	10.0 ± 3.9	2.4 ± 0.6
	Zelivka Reservoir	6	21.9 ± 2.2	39.0 ± 17.9	9.4 ± 2.2	82.7 ± 62.8	5.5 ± 1.5	0.4 ± 0.1
Gills								
Asp	Skalka Reservoir	5	24.5 ± 9.8	46.4 ± 20.7	9.2 ± 2.7	66.9 ± 21.5	7.6 ± 4.2	1.6 ± 0.5
	Zelivka Reservoir	9	29.2 ± 22.7	63.6 ± 18.2	8.6 ± 4.1	97.8 ± 23.7	8.7 ± 4.9	1.6 ± 0.6
Pike	Skalka Reservoir	5	367.3 ± 53.6	42.5 ± 11.5	9.7 ± 1.8	62.8 ± 11.3	12.3 ± 5.1	0.8 ± 0.2
	Zelivka Reservoir	7	330.3 ± 77.1	52.2 ± 11.3	11.7 ± 2.2	65.4 ± 26.3	11.4 ± 2.7	1.8 ± 1.0
Pikeperch	Skalka Reservoir	5	39.0 ± 11.4	63.8 ± 32.2	6.6 ± 2.7	19.7 ± 9.0	6.1 ± 3.5	1.9 ± 0.9
	ZelivkaReservoir	4	38.0 ± 11.4	67.6 ± 32.2	11.3 ± 0.7	40.7 ± 29.5	12.6 ± 2.9	1.5 ± 1.2
Bream	Skalka Reservoir	5	74.8 ± 14.8	152.7 ± 52.4	9.2 ± 3.3	16.6 ± 4.5	6.9 ± 4.2	0.7 ± 0.5
	Zelivka Reservoir	9	80.8 ± 20.0	116.8 ± 40.0	13.5 ± 4.2	34.2 ± 14.7	11.8 ± 4.7	2.4 ± 0.5
Roach	Skalka Reservoir	5	53.3 ± 15.4	69.6 ± 29.8	4.6 ± 2.2	64.7 ± 27.4	10.1 ± 3.8	1.2 ± 0.4
	Zelivka Reservoir	6	32.5 ± 13.4	46.1 ± 27.7	7.9 ± 4.1	72.0 ± 29.9	15.4 ± 0.1	2.1 ± 0.7
Brain								
Asp	Skalka Reservoir	5	47.9 ± 5.9	46.6 ± 3.4	14.4 ± 2.8	30.7 ± 5.0	n/a	n/a
	Zelivka Reservoir	9	46.9 ± 5.7	43.3 ± 7.9	12.8 ± 4.1	31.4 ± 17.4		
Pike	Skalka Reservoir	5	49.4 ± 30.8	24.6 ± 5.8	5.7 ± 1.7	23.9 ± 6.7	n/a	n/a
	Zelivka Reservoir	7	40.2 ± 11.5	35.0 ± 13.5	16.4 ± 7.4	10.1 ± 0.7		
Pikeperch	Skalka Reservoir	5	57.5 ± 10.7	47.5 ± 10.4	6.9 ± 3.1	11.8 ± 2.7	n/a	n/a
	Zelivka Reservoir	4	60.9 ± 9.1	44.8 ± 10.3	10.6 ± 4.7	12.6 ± 2.2		
Bream	Skalka Reservoir	5	53.9 ± 11.1	56.1 ± 10.9	6.8 ± 1.9	16.8 ± 5.8	n/a	n/a
	Zelivka Reservoir	9	57.8 ± 9.1	62.5 ± 16.0	9.5 ± 6.2	15.6 ± 5.1		
Roach	Skalka Reservoir	5	60.8 ± 10.0	52.8 ± 14.9	8.8 ± 2.6	28.0 ± 10.6	n/a	n/a
	Zelivka Reservoir	6	59.0 ± 16.1	57.6 ± 17.2	13.0 ± 5.2	16.2 ± 8.4		

Values presented as mean ± SD. GST (nmol min^{-1} mg of $protein^{-1}$); GPx (nmol NADPH min^{-1} mg of $protein^{-1}$); GR (nmol NADPH min^{-1} mg of $protein^{-1}$); LPO (nmol of TBARS g^{-1} of wet weight); Total GSH (μmol g^{-1} of wet weight)

n/a non analysed

above mentioned studies. Similar conclusions also were reached in a multiorgan study of mercury in fish under field conditions, in which significant differences in GST, GR, and GPx activities, and the content of total GSH were found between liver, gills, and kidney in *Liza aurata* sampled from a reference (unpolluted) site (Mieiro et al. 2011b).

In the present study, the measured GPx activity in the liver was clearly higher than in the other analysed organs. Therefore, GPx activity seems to be a more important component of enzymatic antioxidant defence in the liver compared with other fish organs. Conversely, low levels of LPO were found in the liver, probably as a result of effective antioxidant defence. These results are consistent with the fact that the liver is considered to be metabolically the most active organ in antioxidant defence, playing also an important role in redox metabolism (Huang et al. 2012).

Interesting results were obtained when taking into account the combination of site and organ factors. In this case, only the level of total GSH and the GSH/GSSG ratio were affected. In mammals, GSH synthesis was confirmed mainly in the liver and also in skeletal muscle, from both of which glutathione can be exported to other organs (kidney, lung, and intestine) (Kretzschmar 1996). Unlike the well-known and documented regulation of the GSH metabolism in liver and other tissues in mammals, there is a lack of detailed information about tissue specific GSH biosynthesis and the products of GSH degradation in fish (Hellou et al. 2012).

The assessment of total GSH content is frequently used in the evaluation of the oxidative impact of mercury on fish. However, under field conditions, it has a wide range of responses, both induction and depletion being recorded. For instance, there were no differences among lakes with different levels of methylmercury contamination in liver of walleye (*Sander vitreus*); in contrast, however, GSH levels tended to increase nonsignificantly with liver methylmercury concentration in perch (*Perca flavescens*) (Larose et al. 2008). Conversely, total GSH levels demonstrated a significantly increasing trend together with increasing mercury contamination and mercury concentration in liver, kidney, and gills of wild European bass (*Dicentrarchus labrax*). The highest levels of total GSH were found in gills, followed by kidney, and then by liver at a noncontaminated reference site as well as in mercury-contaminated coastal lagoons (Mieiro et al. 2014).

A more meaningful value is attributed to the GSH/GSSG ratio (Van der Oost et al. 2003), which unfortunately is not commonly determined in either field or laboratory studies on fish exposed to mercury. In the present study, the GSH/GSSG ratio was found to be positive in favour of GSH, even in liver samples from the mercury-polluted reservoir. Moreover, when comparing fish from the mercury-polluted reservoir with those from the unpolluted site, a higher ratio was found in the majority of fish from the mercury-polluted reservoir.

With regard to the real values of total GSH, a different distribution between organs depending on site is apparent. While fish from the mercury-polluted reservoir exhibited the highest levels of GSH in kidney and the lowest in liver, in those from the unpolluted reservoir the values were found to be reversed. The diminished pool of total GSH in liver could be due to the primary inhibition of GSH biosynthesis or to its transport to other organs-in our case, to the kidneys-via plasma or bile (Kretzschmar 1996). This specific distribution of total GSH between the organs may be considered one of the adaptive mechanisms that fish have developed in response to the chronic effects of mercury.

Overall, the impact of mercury contamination on the components of antioxidant defence in fish was not clearly demonstrated in the present study, with the exception of that on levels of glutathione.

Conclusions

Our results suggest that fish species and type of organ, alone or in combination, have a greater influence on antioxidant defence than chronic exposure to mercury contamination under field conditions. However, comparing various antioxidant parameters in fish between contaminated and unpolluted reservoirs confirmed the key role of glutathione in mercury detoxification. In the light of the findings from the long-term mercury-contaminated reservoir investigated in this study, it appears that the distribution of glutathione between organs in fish under such conditions could be regarded as an adaptive protective mechanism.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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