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OXIDATIVE STRESS AND ENDOCRINE ENDPOINTS IN WHITE SUCKER (*CATOSTOMUS COMMERSONI*) FROM A RIVER IMPACTED BY AGRICULTURAL CHEMICALS

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Abstract—The effects of agricultural chemicals on cortisol secretion, antioxidants, and lipid peroxidation were investigated in hepatic and adrenal tissue of white sucker (*Catostomus commersoni*) from a river (Yamaska) that drains an agricultural region in Québec (Canada). Plasma cholinesterase (ChE) activity, used as a biomarker of exposure to pesticides, was elevated in fish from the reference site compared to fish from the contaminated sites. Plasma concentrations of cortisol and thyroid hormones (T_3 and T_4) were higher in fish from the reference site compared to contaminated sites; reduced glutathione (GSH) levels, catalase (CAT), and glutathione peroxidase (GPx) activities were higher and lipid peroxidation (LPO) was lower. Levels of antioxidants (CAT, Gpx, and GSH) were higher (10–90%) and LPO levels were lower (50%) in the liver than in the adrenal tissue. The present in situ study provided evidence that antioxidants, lipid peroxidation, and plasma hormones were altered in fish sampled in areas impacted by agricultural chemicals. Endocrine-disrupting effects were associated with oxidative stress. The results suggest that antioxidants and lipid peroxidation could be used as markers of contaminant exposure in fish.

Keywords—Oxidative stress   Agricultural chemicals   Agricultural pesticides   Agricultural fish   Agricultural endocrine disruption

INTRODUCTION

Oxidative stress is defined as a disruption of the prooxidant–antioxidant balance in favor of the former, leading to potential damage [1]. It is a result of one of three factors: An increase in reactive oxygen species (ROS), an impairment of antioxidant defense systems, or an insufficient capacity to repair oxidative damage. Damage induced by ROS includes alterations of cellular macromolecules such as membrane lipids, DNA, and/or proteins. The damage may alter cell function through changes in intracellular calcium or intracellular pH, and eventually can lead to cell death [2,3].

Specially adapted systems normally counteract the damaging effects of ROS either by repairing the oxidative damage or by directly scavenging oxygen radicals. Antioxidant systems include antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) and free radical scavengers (e.g., vitamins C and E, glutathione) that remove ROS, thereby protecting organisms from oxidative stress. Superoxide dismutase metabolizes superoxide anion (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2); the H_2O_2 is detoxified into H_2O and O_2 by peroxysomal catalase (CAT). Hydroperoxides and H_2O_2 also are detoxified in the cytosolic and mitochondrial compartments by peroxidases. The main peroxidase is glutathione peroxidase (GPx), which uses reduced glutathione (GSH) as an electron donor and generates oxidized glutathione. Glutathione reductase (GR) regenerates GSH in the glutathione redox cycle. Although several antioxidant enzymes have been characterized in fish [4], the role of the antioxidant systems in maintaining the physiological and endocrine function in fish following exposure to pesticides and other agricultural chemicals in the field have not been investigated yet.

Studies with aquatic vertebrates and invertebrates have shown that antioxidant systems are sensitive indicators of exposure to various xenobiotics [4–6]. Several studies reported the capacity of pesticides such as endrin, lindane, and hexachlorocyclohexane to induce oxidative stress in different organs of mammals [7–9]. In fish, modulation of antioxidant systems in liver by endosulfan, and the modulatory effect of preexposure to copper on the endosulfan-induced oxidative stress in vivo have been reported [10]. Recent in vitro studies [11,12] provided evidence that endosulfan induces oxidative stress in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*).

Several in vivo and in vitro studies demonstrated that some environmental pollutants act as endocrine-disrupting chemicals in fish [13–16], amphibians [17], and birds [18], and that one of the endocrine targets of xenobiotics is the hypothalamo-pituitary-adrenal axis [19]. Although the endocrine-disrupting and cytotoxic potential of pesticides such as *o,p′*-DDD (1-[2-chlorophenyl]-1-[4-chlorophenyl]-2,2-dichloroethane), mancozeb, diazinon, atrazine, or endosulfan have been assessed in teleost and amphibian adrenal cells in vitro [16,20–22], only recently has oxidative stress been identified as a mechanism mediating the adrenal toxicity of endosulfan in rainbow trout [11,12].

However, it is not known whether chronic field exposures

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to a mixture of agricultural chemicals, including pesticides, induce oxidative stress and impair adrenal function in teleosts. Moreover, the relative importance of the various antioxidant systems in maintaining the integrity of the endocrine function in fish has not been assessed. Thus, the objectives of the present study were to investigate the effects of agricultural chemicals (agrichemicals) on cortisol secretion and antioxidant parameters, to validate the endocrine dysfunction and antioxidant systems as markers of chronic exposure to agrichemicals, and to test the hypothesis that endocrine-disrupting effects of chronic exposure to agrichemicals are mediated by oxidative stress. Cortisol and thyroid hormone plasma levels, as well as lipid peroxidation (LPO), antioxidant activities (CAT, GPx), and GSH levels in the liver and the adrenal tissue were evaluated in white sucker (*Catostomus commersoni*) from the Yamaska, a river impacted by agricultural activities in Québec (Canada). Hepatic glycogen levels, Na/K-ATPase (adenosine triphosphatase) activity in the gill, plasma cholinesterase activity and histopathology of the thyroid tissue also were used to assess the physiological status of the fish.

MATERIALS AND METHODS

Chemicals

Reduced α-nicotinamide adenine dinucleotide phosphate, reduced GSH, glutathione reductase, cumene hydroperoxide, metaphosphoric acid, 1-chloro-2,4-dinitrobenzene, bovine serum albumin, assay kit for cholinesterase (ChE), amyloglucosidase, ammonium molybdate, ouabain, and sucrose were obtained from Sigma (St. Louis, MO, USA). Radioimmunoassay kits for cortisol, T_3 (triiodothyronine), T_4 (thyroxine), GSH, and LPO assay kits were obtained from Oxis Research Medicorp (Montreal, PQ, Canada). The 3-aminobenzoic acid ethyl ester for anesthesia was purchased from ICN Pharmaceuticals (Orangeburg, NY, USA). Hydrogen peroxide (H_2O_2) was obtained from Fisher Scientific (Nepean, ON, Canada). Glycogen standard and glucose oxidase–phenazone/phenol were obtained from Boehringer Mannheim (Laval, PQ, Canada).

Fish sampling

White sucker (*C. commersoni*) were sampled in June 2001 and 2002 in the Yamaska River (QC, Canada) that drains an agricultural area situated in the southern part of Québec (45°18′N,73°16″W). Concentrations of pesticides in ambient water and limnological characteristics at the sampling sites are summarized in Table 1. Sites A, B, and C (Fig. 1), situated in the area of intensive crop agriculture (corn and soya) represent the contaminated sites and a site in Green Bay, Lake Memphremagog (QC, Canada), situated in a green space (mixed forest) within the same watershed, was used as the reference site. White sucker was sampled because it is an abundant species at each of the four sites. Fish were captured with gill nets (25-m long, 2-m high, 2.5–15-cm mesh) between 15 and 20 h each day within a period of two weeks in June. To minimize the variability of the hormonal and biochemical responses of fish sampled at different sites, the sampling procedure was standardized. The gill nets were set for periods of 30 min; fish were anesthetized immediately with 3-aminobenzoic acid ethyl ester when removed from the nets and a blood sample was taken from the caudal vasculature. The bled fish then were placed on ice and transported to the laboratory within 90 min where the liver, adrenal tissue, and gills were dissected, frozen, and stored in liquid nitrogen (−80°C) before analysis. The thyroid was stored in Bouin's solution for histological analysis. Organ and body mass and length were recorded to evaluate the condition factor ([weight (g)]/[length (cm)]^3 × 100), the hepatosomatic index (hepatosomatic index = [liver weight (g)]/[total weight (g)] × 100), and the gonadosomatic index (gonadosomatic index = [gonad weight (g)]/[total weight (g)] × 100).

Table 1. Water concentrations of pesticides and limnological characteristics at sites situated in an area of intensive agriculture in Quebec, Canada

		Sites[a]	
Pesticides[b] (μg/L)	Reference	A	C
Deethyl-atrazine	<0.04	0.06	0.04
Simazine	<0.01	0.01	<0.01
Atrazine	<0.02	0.32	0.27
Metolachlor	<0.01	0.33	0.48
Dimethenamide	<0.02	0.07	0.04
Dicamba	<0.03	0.25	0.2
Mecoprop	<0.01	0.04	0.03
2,4-D	<0.02	<0.02	0.05
Diazinon	<0.03	<0.03	<0.03
Carbaryl	<0.03	<0.03	<0.03
Parathion	<0.03	<0.16	<0.03
Limnological characteristics			
Total N (mg/L)	0.22	1.01	1.85
Total P (μg/L)	16.2	100	195
Conductivity (μS; 25°C)	130–158	165	400
pH	6.4–8.4	7.8	9.1

[a] Sites were identified in the *Materials and Methods* section, *Fish sampling* section and in Figure 1 (A and C are contaminated sites).
[b] Pesticides (triazines, organophosphates, and aryloxyacides) were separated and identified by gas chromatography and mass spectrometer using quality controls for each sample. Each measure corresponds to the average of two replicates.

Plasma analyses

Heparinized blood was centrifuged at 5,600 *g* for 6 min and plasma was frozen at −20°C before cortisol, triiodothyronine (T_3), and thyroxine (T_4) radioimmunoassays. The characteristics of the assays were described previously [23].

Plasma ChE activity was measured using a Sigma diag-

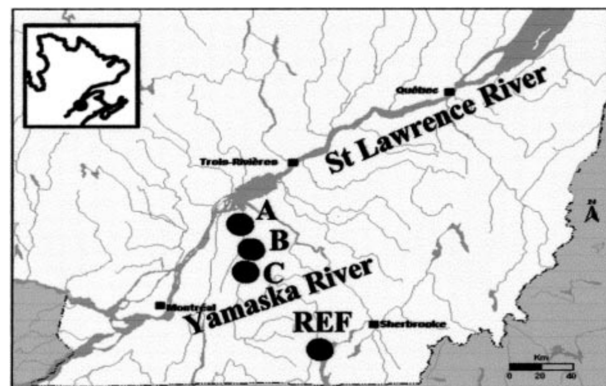

Fig. 1. Sampling sites in the Yamaska River (45°18′N,73°16″W) that drains an area of intensive agriculture in Quebec (Canada). Sites A, B, and C are the contaminated sites; Green Bay, Lake Memphremagog, is the reference site (REF). Inset in the upper left of the map represents Quebec; the dark circle (●) indicates the area where sampling was done.

nostics procedure with acetylcholine as the substrate. A blank was prepared by inactivation at 60°C for 10 min to compensate for background absorbance contributed by the sample. Absorbances of both blank and test were read at 420 nm and the difference was used to estimate the ChE level. One unit of ChE was defined as the amount of ChE that will liberate 1 μmol of acetic acid in 30 min at 25°C.

Tissue enzyme assays

The activities of antioxidant enzymes were measured in the liver and adrenal tissues under earlier established conditions [11,12] that were optimal for enzyme activity analysis. The tissues were homogenized (Brinkmann Homogenizer, Mississauga, ON, Canada) in 4 ml of 0.01 M phosphate buffer (pH = 7.4) and the homogenates were centrifuged at 2,000 *g* (IEC Micromax centrifuge, Fisher Scientific) for 10 min. Supernatants then were removed and centrifuged at 14,000 *g* for 10 min. Protein concentrations in the supernatants were measured by the method of Bradford [24] using bovine serum albumin as a standard.

The activity of CAT was estimated by measuring the decrease in absorbance at 240 nm ($\varepsilon = 0.04$/mM/cm; Ultrospec 2000 Pharmacia Biotech, Fisher Scientific) resulting from H_2O_2 consumption [25]. One unit of CAT activity was defined as 1 μmol of H_2O_2 consumed/min/mg of protein.

The activity of GPx was estimated by the decrease in absorbance at 340 nm ($\varepsilon = 6.22$/mM/cm) due to NADPH oxidation. The activity was assayed in a coupled enzyme system where oxidized glutathione is reduced to GSH from excess glutathione reductase [26]. One unit of GPx activity was defined as 1 nmol of NADPH oxidized/min/mg of protein.

Glutathione-*S*-transferase (GST) activity was determined at 340 nm using 1-chloro-2,4-dinitrobenzene as substrate ($\varepsilon = 9.6$/mM/cm) [27]. Several isoenzymes of GST exist with distinct but overlapping substrate specificities, however, 1-chloro-2,4-dinitrobenzene conjugates much more rapidly than other substrates [28]. One unit of GST activity was defined as 1 μmol of GSH consumed/min/mg of protein.

The activity of Na/K-ATPase was measured in homogenized gills with an assay period of 30 min at 30°C [29]. One unit of Na/K-ATPase activity was defined as 1 μmol of PO_4 formed/h/mg of protein.

Reduced glutathione levels

The GSH levels were measured with the GSH-400 method (Oxis Research, Medicorp) involving a chemical reaction that proceeds in two steps: The first is the formation of substitution products (thioethers) between 4-chloro-1-methyl-7-trifluoromethyl-quinolinium (reagent) and all mercaptans that are present in the sample. The second is a β-elimination reaction that specifically transforms the substitution product (thioethers) obtained with GSH into a chromophoric thione that has an absorbance maximum at 400 nm. The apparent molar extinction coefficient (ε) was determined using a GSH 0.5 mmol/L in 5% metaphosphoric acid solution as standard.

Lipid peroxides levels determination

Lipid hydroperoxides (LOOH) levels were measured with the LPO-560 method (Oxis Research, Medicorp). This method involves a direct colorimetric measurement of LOOH. The assay is based on the oxidation of ferrous ions to ferric ions by hydroperoxides and the binding of ferric ions to an indicator dye (xylenol orange), which forms a stable, colored complex that absorbs strongly at 560 nm. To eliminate interference in LOOH measurement by H_2O_2, the samples were pretreated with CAT. A sample blank also was assayed to account for non-LOOH absorbance at 560 nm.

Glycogen levels

Liver glycogen was measured with a method described previously [30,31], except that digestion at 100°C was replaced by sonication and incubation at room temperature for 60 min.

Histology

Standard histological procedures were used to prepare tissues for histomorphometry [29]. The thyroid region was removed and fixed in Bouin's solution, then rinsed with water, dehydrated in an alcohol series, and embedded in paraffin. Sections (6-μm thick) were stained by Trichrome Masson and observed by light microscope (Panasync computer linked to a color Panasonic digital 5000 camera, Future Shop, Montreal, QC, Canada) using the WinTV 2000 Program (Hauppauge Computer Works, Hauppauge, NY, USA). We measured the heights of epithelial cells in six thyroid follicles in each fish.

Pesticides analyses

Pesticide concentrations in water samples were measured by the Centre d'Expertise en Analyse Environnementale du Québec (Sainte Foy, QC, Canada). For organophosphorous pesticides, 150 ml of water was extracted with conditioned solid-phase extraction cartridge C18 (JT Baker, Phillipsburg, NJ, USA) and concentrated 1,000-fold before analysis by gas chromatography/mass spectrometry [32]. For herbicides, 250 ml of water was extracted on conditioned C18 (1 g), concentrated 100-fold and methylated with diazomethane before analysis by gas chromatography/mass spectrometry [33]. A quality control was made for each sample with extraction and injection standard (respectively, propoxur, atrazine-d_5, iprodione, and terbutryne for triazines and organophosphates; dicamba-d_3, 2,4-D-d_3, and 1,3,5-tribromobenzene for phenoxyacids).

Statistics

Statistical differences between parameters in fish from different sites were analyzed using either Student's and Tukey-Kramer tests or one-way analysis of variance with a confidence range of $p < 0.05$ with JUMPIN software model (Jump Statistics, Faerie Glen, Pretoria, South Africa).

RESULTS

Pesticide concentrations and ChE activity

Pesticide concentrations measured in the water column and limnological characteristics at the sampling sites are presented in Table 1. A gradient was evident, with the highest levels of pesticides, total phosphorus, and nitrogen detected in sites A and C, suggesting that these two sites are impacted by agricultural activities. No pesticides as well as low N and P were detected in Green Bay situated in the same watershed, so this site was used as the reference site. Atrazine, metolachlore, and dicamba were relatively abundant; only traces of simazine, mecoprop, and 2,4-D were found. Diazinon, carbaryl, and parathion were undetectable. The concentrations of waterborne pesticides in the Yamaska River also are measured in monitoring programs by the Ministere of the Environment of Québec [34]. These Ministere of the Environment of Québec data collected for several years show a maximal peak of pesticide concentrations in water at the beginning of June.

Table 2. Plasma cholinesterase (ChE) activity and liver glycogen concentrations (mean ± standard error of mean) in white sucker (*Catostomus commersoni*) sampled at sites situated in an area of intensive agriculture in Quebec, Canada

	Sites[a]		
	Reference	A	C
Plasma ChE activity (U/ml)[b]	120 ± 14.3*	73 ± 12.6	71 ± 20
Liver glycogen (mg/g)	9.8 ± 6.0*	30.5 ± 7.2	40.9 ± 10

[a] Sites are identified in the *Materials and Methods* section, *Fish sampling* section, and in Figure 1 (A and C are contaminated sites).
[b] Cholinesterase activity is expressed in U/ml with one unit of ChE defined as the amount of ChE that will liberate 1 μmol of acetic acid in 30 min. The asterisk (*) indicates significant difference from sites A and C (Student's and Tukey-Kramer test, α = 0.05). The number of fish was 9 for reference site, and 4 and 7 for sites A and C, respectively.

Plasma ChE activity was significantly higher in fish from the reference site (Green Bay) compared to contaminated sites (Table 2), but no significant differences were observed between fish from contaminated sites A and C.

Morphological characteristics

In June 2001 and June 2002, sexually immature fish of both sexes were captured and no significant differences were observed in the length (average 41.55 ± 5.3) or weight (average 796.40 ± 331.7) of fish from the four sites. Moreover, no differences between sites were observed in the condition factor (average 1.04 ± 0.09), the hepatosomatic index (average 1.7 ± 0.5) or the gonadosomatic index (average 0.87 ± 0.5).

Cortisol, T_3, and T_4 levels

Plasma cortisol, T_3, and T_4 in white sucker exposed to agrichemicals in the Yamaska River are shown in Figure 2. Post-stress plasma cortisol levels in fish sampled under standardized stress protocols were significantly higher in fish from the reference site compared to contaminated sites A, B, and C (Fig. 2A). The plasma cortisol levels of reference fish were around 100% of the maximal levels reported in this species [13]. No significant differences were observed between contaminated sites. Plasma T_3 and T_4 levels also were significantly higher at the reference site than at the contaminated sites (Fig. 2B). No significant differences in plasma T_3 and T_4 were observed between contaminated sites.

Antioxidants and LPO levels

Activities of CAT and GPx in the liver and the adrenal tissue of white sucker from the Yamaska River are shown in Figure 3. The activity of CAT (Fig. 3A) was significantly higher in both tissues in fish from the reference site compared to contaminated sites and no significant differences were observed between the three contaminated sites. The activity of CAT was 7.5 times higher in the liver compared to the adrenal. The same pattern was observed for GPx activity (Fig. 3B), with GPx activity being 1.3 times higher in the liver compared to the adrenal in contaminated sites, and 2.2 times higher at reference site.

No significant differences in GST activity were observed between any sites in both tissues. However, hepatic GST activities (average 60 ± 20 U, units expressed as μmol GSH consumed/min/mg protein) were 2.5 times higher than GST activity in the adrenal (average 24 ± 8.0 U).

The levels of GSH and LPO in the liver and the adrenal tissue are shown in Figure 4. The GSH levels in both tissues were significantly higher in fish from the reference site than in fish from the contaminated sites (Fig. 4A). Moreover, GSH levels were 1.6 times higher in the liver compared to GSH in the adrenal tissue. The levels of LPO were significantly higher in fish from contaminated sites than in fish from the reference site in both tissues (Fig. 4B). Adrenal levels were 1.9 times higher than hepatic levels.

Activity of Na/K-ATPase and hepatic glycogen

No significant differences between sites were observed for the gill Na/K-ATPase activity (mean activity 0.33–0.38 ± 0.12 U). Hepatic glycogen levels were significantly higher in fish from contaminated sites A and C than in fish from the reference site (Table 2).

Histopathology

Epithelial cells in thyroid follicles were significantly thicker in fish from the reference site (4.29 ± 0.91 μm) compared to

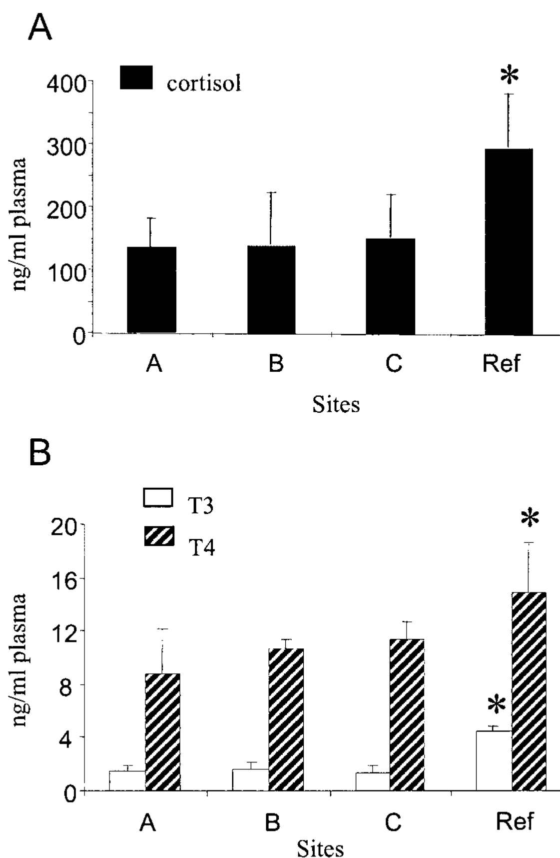

Fig. 2. (**A**) Concentrations of plasma cortisol and (**B**) plasma triiodothyronine (T_3) and thyroxine (T_4); mean ± standard error of mean in white sucker exposed to agrichemicals. Asterisk (*) indicates significant differences between sites (Student's and Tukey-Kramer test, α = 0.05). The number of fish was 18, 12, 12, and 9 for sites A–C and reference, respectively.

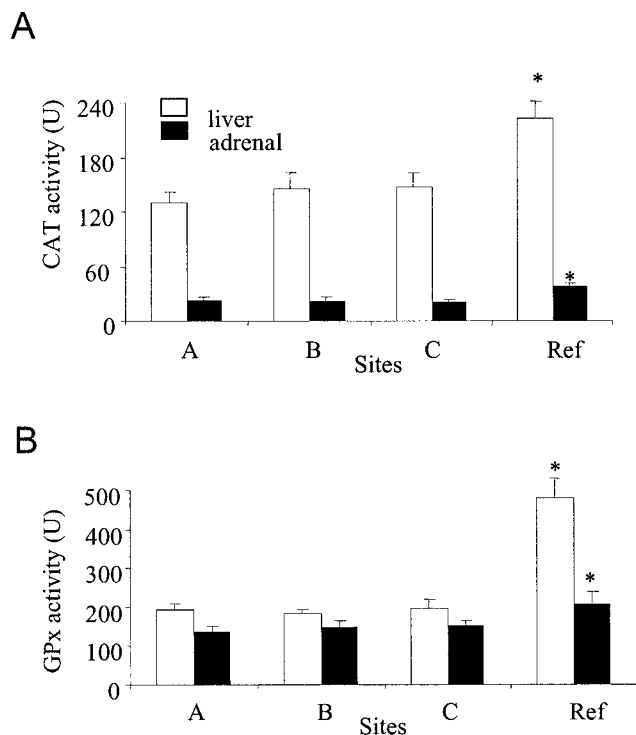

Fig. 3. (**A**) Catalase (CAT) and (**B**) glutathione peroxidase (GPx) activities (mean ± standard error of mean, expressed in units of activity [U], with one unit of CAT activity defined as 1 μmol of H_2O_2 consumed/min/mg of protein and one unit of GPx activity as 1 nmol of reduced nicotine adenine diphosphate oxidized/min/mg of protein) in the liver and the adrenal gland of white sucker exposed to agrichemicals. Asterisk (*) indicates significant differences between sites (Student's and Tukey-Kramer test, α = 0.05). The number of fish was 18, 12, 12, and 9, respectively.

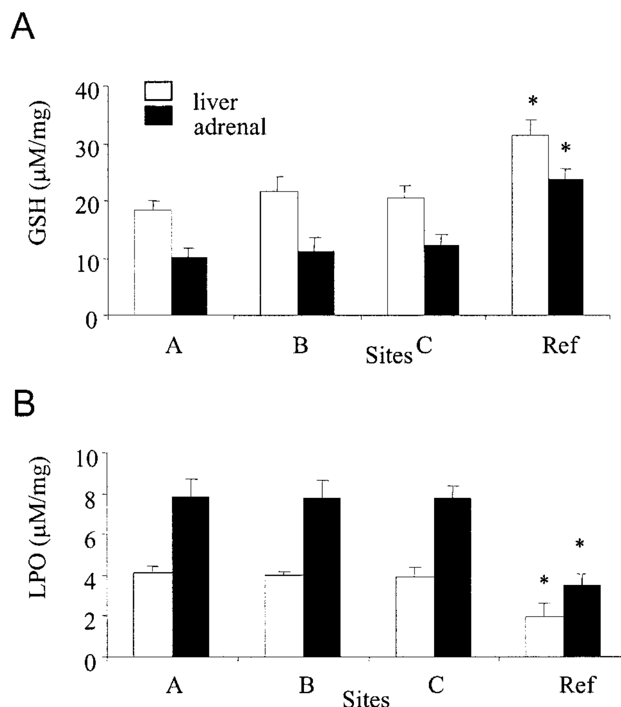

Fig. 4. (**A**) Reduced glutathione (GSH) and (**B**) lipid peroxidation (LPO) levels (mean ± standard error of mean, expressed as μM/mg of protein) in the liver and the adrenal gland of white sucker exposed to agrichemicals. Asterisk (*) indicates significant differences between sites (Student's and Tukey-Kramer test, α = 0.05). The number of fish was 18, 12, 12, and 9, respectively.

fish from contaminated sites (3.26 ± 0.44 μm), as shown in Figure 5. Moreover, follicles in the thyroid of fish from the reference site seemed to be more abundant than in fish from contaminated sites.

DISCUSSION

Our study provided evidence that antioxidants (CAT, Gpx, and GSH), lipid peroxidation and plasma cortisol, and thyroid hormone levels were altered in fish sampled in areas impacted by agrichemicals. Endocrine-disrupting effects were associated with oxidative stress. Moreover, evidence that tissues differ with respect to defense capacity against pollutant-induced oxidative stress has been provided.

Oxidative stress is a result of an increase in reactive oxygen species or an impairment of antioxidant defense systems. Antioxidants may be induced or inhibited by exposure to environmental pollutants, but inhibition of antioxidants impairs the capacity to prevent ROS formation and cell damage. Several studies provide evidence that antioxidants may be used as biomarkers of exposure to environmental pollutants [4,35,36]. Moreover, recent studies report that various pesticides can induce oxidative stress in different tissues [7,8,37]. Endosulfan, an organochlorine pesticide, recently was identified in vitro as a chemical that reduced cortisol secretion by teleost adrenocortical cells, affected the activity of enzymes involved in oxidative stress, and increased levels of lipid hydroperoxides [11,12].

The present field study in an agricultural region was designed to test the link between cortisol impairment and oxidative stress induced by chronic exposure to a mixture of agrichemicals. The activities of CAT and GPx, GSH levels, and plasma cortisol, T_3, and T_4 in white sucker from the Yamaska River were significantly higher at the reference site compared to sites contaminated by agrichemicals. These results suggest, for the first time, that endocrine-disrupting effects of agrichemicals, including pesticides, may be mediated by oxidative stress. Antioxidants thus may be useful for assessing the environmental impact of pollutants in the field by measuring exposure and providing a reliable indication of toxic effects such as an endocrine dysfunction. Moreover, these results complete previous work in our laboratory reporting an inhibition of cortisol secretion after in vitro exposure to pesticides [16,20,21]. Lipid hydroperoxide levels also appeared as potential markers of chronic exposure to agrichemicals. In contrast, no significant differences were observed for GST activity, one of the several enzymatic systems (phase I or II biotransformation enzymes) often activated during exposure to contaminants.

Little is known about the nature and function of GST in fish. In mammals, GST greatly enhances its own activity by sulfhydryl-reactive metabolites and reduced oxygen species during oxidative stress [38]. Increase in the activity of GST has been used as a marker of exposure to organochlorine contaminants [28]. In the present study, GST activity may not differ between sites because GSH levels, essential to GST activity, are depleted at contaminated sites. Indeed, GST participates in pollutant detoxification by adding a GSH-group to xenobiotics or their metabolites, so they become more water-soluble and, thus, excreted more easily [38].

Gill Na/K-ATPase activity did not differ significantly among sites in our study, suggesting that this parameter might

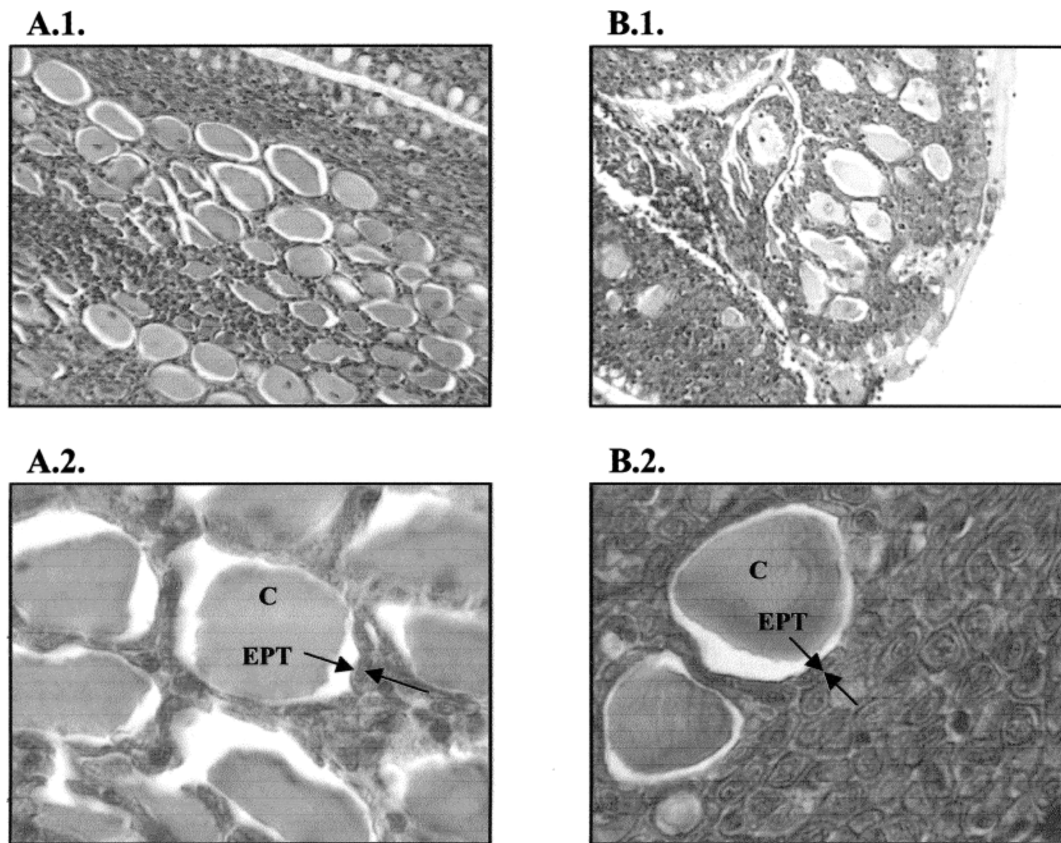

Fig. 5. Histomorphometric analysis of the thyroid of white sucker exposed to agrichemicals. (**A1**) thyroid follicles in fish from reference site, magnified ×400; (**A2**) reference site, ×1,000; (**B1**) thyroid follicles in fish from a contaminated site, ×400; (**B2**) contaminated site, ×1,000). C = Colloid; EPT = Epithelium. The number of follicles analyzed was at least six by fish and the number of fish was six for contaminated and eight for reference site.

not be a good biomarker of chronic exposure to agrichemicals, even though it is an excellent biomarker of exposure to metals [29]. Hepatic glycogen levels in June were significantly higher in fish from contaminated sites compared to reference site, similar to yellow perch (*Perca flavescens*) from metal-contaminated lakes [31]. Evidence for a thyroid impairment also was observed because the epithelial cells in thyroid follicles were significantly higher in fish from the reference site compared to contaminated sites, as has been observed in fish exposed to metals [29].

Chronic exposure to agrichemicals may affect endocrine and metabolic functions as well as the activity of enzymes that are involved in oxidative stress. Moreover, this study suggests that endocrine dysfunction may be associated with oxidative stress. A previous in vitro study also identified CAT, Gpx, and GSH as essential antioxidants in maintaining the function and integrity of rainbow trout (*Oncorhynchus mykiss*) adrenocortical cells and suggested that the glutathione redox cycle is more efficient than CAT in protecting adrenocortical cells against oxidative stress induced by endosulfan [12]. This study also provided new data about tissue differences in response to pollutant-induced oxidative stress. Differences in CAT and GPx activities, GSH, and lipid peroxidation levels were observed between the adrenal tissue and liver. Hepatic GSH levels, CAT, and GPx activities were higher compared to the adrenal, though lipid peroxidation was lower. The tissue differences in catalase and GPx, two components of the defense system against H_2O_2, along with differences in GSH, an essential cofactor of several enzymes and also a direct scavenger of oxyradicals [39,40], suggest that liver may be less vulnerable to oxidative stress than the adrenal tissue. The deficiency of the antioxidative systems and alterations in the activity of enzymes involved in oxidative stress likely affects the capacity of cells to defend themselves and respond to oxidative stress. Lipid peroxidation, a well-recognized mechanism of cellular injury [1] used as an indicator of oxidative stress in cells and tissues, was indeed higher in the adrenal than the liver. However, in both tissues, chronic exposure to agrichemicals was associated to an impairment of antioxidative enzymes and increased lipid peroxidation, potentially compromising adrenal and hepatic cell function and leading to reductions of hormone levels and glycogen. Indeed, if oxidative species are not eliminated rapidly, loss of physiological integrity of the cell may impair its functional capacity or reduce its survival.

CONCLUSION

In conclusion, this in situ study provided evidence that antioxidants and plasma cortisol, T_3, and T_4 were altered in fish residing in areas experiencing agricultural impacts. Moreover, the endocrine-disrupting effects were associated with oxidative stress, and differences in the capacity of tissues to protect themselves from oxidative stress were shown. Additional investigations are needed to assess the effects of chronic exposure to agrichemicals and pesticides on the physiological status, including intermediary metabolism in fish. The measure of pesticide levels in tissues may be relevant to identify the specific pesticides that potentially bioaccumulate and induce lipid peroxidation. Laboratory experiments using controlled in

vivo exposures to selected pesticides are in progress to test the causal relationship between exposure to a specific pesticide, induction of oxidative stress, and endocrine-disrupting effects.

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