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HORMONAL, MORPHOLOGICAL, AND PHYSIOLOGICAL RESPONSES OF YELLOW PERCH (*Perca flavescens*) TO CHRONIC ENVIRONMENTAL METAL EXPOSURES

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The effects of a chronic environmental exposure to metals on the hormonal, physiological, and reproductive status were assessed in yellow perch (Perca flavescens) sampled in six lakes situated along a contamination gradient of Cd, Zn, Cu, Pb, and Ni in the mining region of Rouyn-Noranda, Québec. Fish were captured in the summer and fall, and sampled before or after a confinement of one hour. Metal concentrations in the kidneys and the interrenal tissues (homologous to mammalian adrenals) were measured to compare tissue-specific metal accumulation. An exposure-related decrease of condition factor, gonadosomatic index (GSI), branchial Na^+/K^+-ATPase activity, plasma thyroxine (T4), triiodothyronine (T3), and 17β-estradiol and an impaired capacity to enhance cortisol levels after confinement were observed. Fish from the metal-contaminated lakes possessed gonads at less mature stages and exhibited structural alterations of their gills, interrenal cells, and thyroid follicle epithelium. A comparison of the morphological, biochemical, and physiological endpoints measured in the present study revealed that plasma concentrations of hormones and parameters of gill function were the most affected by metal contamination. The results of this study indicate that lifelong exposures to sublethal concentrations of metals alter the physiological functions of fish and delay reproduction.

In fish, metals accumulate in liver, gills, kidneys, and gonads (Harrison & Klaverkamp, 1989) as well as the interrenal tissue (homologous to mammalian adrenals) where corticosteroid hormones are synthesized (Laflamme et al., 2000).

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Metals have a high reactivity toward macromolecules and their O, S, and N groups, and the potential to perturb multiple processes within the organism. The present study was designed to characterize the hormonal, physiological, and morphological responses to chronic field exposures to metals in yellow perch, an indigenous teleost fish species, and to compare the sensitivity of various biomarkers of effects.

Adverse effects of metals on interrenal steroidogenesis in teleost fish have been documented. Even though acute exposures (hours to days) to metals elevate plasma cortisol levels in salmonids (Fu et al., 1990; Hontela et al., 1996), chronic field exposures were associated with attenuated cortisol stress responses (Lockart et al., 1972; Norris et al., 1999; Laflamme et al., 2000). Mammalian studies suggested that adrenal dysfunction can be produced by direct effects of toxicants on the adrenal or by secondary effects produced by toxicant-induced anomalies in other physiological systems (Harvey, 1996). Although recent in vitro studies provided evidence for direct action of Cd, Hg, or Zn on adrenal (interrenal) steroidogenesis and a dose-dependent inhibition of cortisol synthesis in rainbow trout (Leblond & Hontela, 1999), adverse effects in other systems, contributing directly or indirectly to the interrenal dysfunction in vivo, cannot be excluded at present.

Since functional interactions occur between the adrenal and thyroid axes, and corticosteroids often act in synergism with thyroid hormones, one of the objectives of the present study was to investigate the effects of chronic environmental exposure to metals on the thyroid function in yellow perch. Both corticosteroids and thyroid hormones produce metabolic effects, and in teleosts these hormones also play an important role in osmoregulation (Laurent & Perry, 1990; Vijayan et al., 1997). Cortisol and thyroid hormones stimulate gill Na^+/K^+-ATPase activity, an enzyme controlling fluxes of electrolytes across the gill epithelium (Shrimpton & McCormick, 1998). Several studies demonstrated a decrease in gill Na^+/K^+-ATPase in fish exposed to cadmium and chromium (Lemaire-Gony & Mayer-Gostan, 1994; Thaker et al., 1996; Lionetto et al., 2000); however, an increase after an exposure to Cu has been reported as well (McGeer et al., 2000). Thyroid function and plasma levels of thyroxine (T4), the pro-hormone, and triiodothyronine (T3), the biologically active hormone (Brown et al., 1998), are affected by metals. Laboratory exposure to Cd lowered plasma thyroid hormones levels in fish (Hontela et al., 1996; Gupta et al., 1997) and in mammals (Gupta & Kar, 1997). Zhou et al. (1999) reported that chronic exposures to heavy metals and organic pollutants such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and dichlorodiphenyltrichloroethane (DDTs) were associated to decreased plasma T4 levels, and increased follicle epithelial cell height and size of thyroid follicles.

It is well established that the mammalian reproductive system and the synthesis of sex steroids are targeted by metals (Laskey & Phelps, 1991). The effects of xenobiotics on reproduction and sex steroid hormones in teleost fish have been documented in systems contaminated by pulp and paper effluents

(Van Der Kraak et al., 1992); however, studies concerning the effects of metals on teleost reproduction are scarce. Cadmium was shown to decrease sperm motility in several teleost species (Kime, 1999). In tilapia (*Oreochromis niloticus*), Cd, Zn, and Pb affected the activity of the hypophyseal-gonadal axis and produced a decrease of sperm volume and degeneration of oocytes (Mousa & Mousa, 1999).

The objectives of the present environmental study were to evaluate the major endocrine systems (adrenal, reproductive, and thyroid) and aspects of gill physiology in yellow perch (*Perca flavescens*) chronically exposed to metals in lakes situated along a contamination gradient of Cd, Zn, Cu, Pb, and Ni in a mining region to determine if (1) sublethal exposure to metals impairs multiple physiological systems, (2) various biomarkers exhibit different sensitivity to metals, and (3) the hormonal endpoints are highly sensitive to metals.

MATERIALS AND METHODS

Study Sites and Fish Species

Yellow perch (*Perca flavescens*) were sampled in six lakes of the mining area of Rouyn-Noranda, located in northwestern Québec (48°00′N, 79°00′W), in September 1998 and June 1999. The sampling was always completed within 4 wk. Fish were sampled in Lake Opasatica and Lake Dasserat, two reference lakes, situated upwind from the smelter. The two most contaminated lakes were Lake Osisko and Lake Dufault, situated near and downwind from the smelter. Lake Bousquet and Lake Du Moulin, located about 20 km downwind from the smelter, were the two intermediate lakes. Lakes have been contaminated by Cd, Zn, and Cu by the atmospheric emissions from the smelting complex (Laflamme et al., 2000). The water temperature of the lakes was 18 ± 2 °C in the fall and 20 ± 2 °C in the summer, pH ranged from 7.1 to 7.9. For more details of the limnological characteristics, see Brodeur et al. (1997), Laflamme et al. (2000), and Sherwood et al. (2000).

Yellow perch were used in this study, because it is an abundant and relatively sedentary species (Aalto & Newsome, 1990). Sampling procedure and age structure distribution of perch from the lakes sampled in this study have been described previously in Levesque et al. (2002) and Sherwood et al. (2000). Briefly, adult fish ranging in length from 130 to 200 mm, 4 yr and older (Sherwood et al., 2000), were captured by fishing rods, gill nets, or seine, and then transferred to a floating enclosure and divided into 2 groups. The day after capture, fish were anesthetized and sampled without confinement (control fish) or after a confinement of 1 h (confined group, 10 fish in a 20-L container). In both sampling periods (summer and fall), body mass and length were recorded to evaluate the condition factor ([weight (g)]/[length (cm)]^3 × 100. Plasma was kept in liquid nitrogen and transferred to −80 °C on return to the university for further biochemical analyses. In the fall sampling, gonads were

weighed to calculate the gonadosomatic index ([gonad weight (g)]/[total weight (g)×100]) and conserved in Bouin's solution for histology. In summer, gills, head, kidney, and the thyroid were fixed for histology, and a sample of gills were kept in liquid nitrogen.

Analyses of Metals

Concentrations of Cd, Cu, Zn, Pb, and Ni were measured by inductively coupled plasma atomic emission spectrophotometry (ICP-AES) in the kidneys and interrenals where corticosteroidogenic cells are located, using tissue homogenates corresponding to 100 mg dry weight. Tissues from two to four fish were pooled when the tissues were too small. One microgram of metals per gram dry weight corresponds to aproximatively 0.29 µg metals per gram wet weight.

Hormone Assays

Plasma cortisol, T3, and T4 were assayed with commercial radioimmunoassay kits (ICN Biochemicals). The characteristics of the assays were described previously (Hontela et al., 1995). Plasma testosterone and 17β-estradiol were measured by radioimmunoassay (RIA), as described in Van Der Kraak et al. (1984, 1990).

Na^+/K^+-ATPase Activity in the Gills

ATPase activity was evaluated according to the methods of Holliday (1985) and Morgan et al. (1997). Briefly, samples of homogenates (about 50 mg of branchial filaments in a buffer containing sucrose, ethylenediamine tetraacetic acid [EDTA], imidazole-HCl) were added to 200 µl of buffer A (NaCl 167 m*M*, KCl 50 m*M*, imidazole-HCl 33 m*M*, pH 7.2, with NaOH 1 *M*), or buffer B (NaCl 237 m*M*, ouabain 1.67 m*M*, imidazole-HCl 33 m*M*, pH 7.2, with NaOH 1 *M*). The Na^+/K^+-ATPase activity was obtained by determining the difference in inorganic phosphate production between the reactions in the two different media. The assay period was 30 min at 30 °C. Enzyme activity was expressed as micromoles of inorganic phosphate per hour per milligram of protein.

Histomorphometric Analyses

Standard histological procedures were used for the preparation of tissues for histomorphometric analyses. Gonads, head kidney, thyroid region, and gills (secondary lamellae) were removed and fixed in Bouin's solution for 48 h, then rinsed in water, dehydrated in an alcohol series, and embedded in paraffin. Sections (6 µm) were stained by Trichrome Masson and observed by light microscope (Quadra 840AV MacIntosh computer linked to a color camera) using the NIH Image program.

For the gills, six randomly chosen fields were examined from each prepared section, with four measurements performed within each field. The four morphometric measurements were: (1) interlamellar distance (ILD), defined as

the distance between the outer epithelial surface of two adjacent secondary lamellae, (2) blood-water diffusion distance (DD), the distance between the outer epithelial surface and the nearest blood space on any given lamellae, (3) lamella thickness (LT), and (4) height of epithelial padding between two lamellae (H). Measurements of ILD, DD, and LT were made approximately midway between the distal and proximal ends of the secondary lamellae, and measurements of H were made on the primary lamellae.

For ovaries, four stages of oocytes were identified, as described in Chan and Chua (1980): (1) perinucleolus stage, including oocytes in the early nucleolus stage (diameters, d=20–150 μm) and oocytes in late perinucleolus stage (d=110–160 μm); (2) vesicle stage (d=150–400 μm), oocytes that contain yolk vesicle in the periphery of the cytoplasm; (3) primary and secondary yolk stage (d=350–900 μm), oocytes that contain yolk globules in the inner part of the cytoplasm; and (4) atretic follicle stage, oocytes with hypertrophied granulosa cells or empty follicules. The number of oocytes in each stage was counted in six sections of each gonad. The average number of cells in 0.25 mm^2 of the gonad was used in the calculation of the number of cells in 1 mm^2.

The development stage of the testes was assessed using the classification presented by Lokman and Young (1998) as follows: stage I, stem-cell proliferation (only type A and early type B spermatogonia present); stage II, early spermatogenesis (spermatogonial proliferation, late type B spermatogonia present), and stage III, midspermatogenesis (all stages of sperm development present).

For thyroid follicles, 10 follicles randomly selected from each fish and the heights of 4 epithelial cells in each follicle were measured.

In the interrenal tissue, nuclear diameter was determined as described in Hontela (1997), for 10 nuclei of 10 adrenocortical cells randomly selected from 5 sections (50 nuclei in total).

Statistical Analyses

Differences between groups were tested by either t-test or one-way analysis of variance (ANOVA) followed by a Tukey-Kramer test. A statistical significance level of α=.05 was used for all tests. Except for the sex steroid and GSI data, males and females were grouped for analyses, since no sex differences were detected within each site (t-test). Data were transformed to obtain normality and homoscedasticity, if necessary.

RESULTS

Exposure of the Fish

Kidney concentrations of Cd, Pb, and Zn (Table 1 and Figure 1) in fish sampled in two consecutive years followed a significant contamination gradient from the reference lakes (Lake Opasatica and Lake Dasserat), to intermediate lakes (Lake Bousquet and Lake Du Moulin) and highly contaminated lakes (Lake Osisko and Lake Dufault). There were no significant differences in kidney

TABLE 1. Concentrations of Cu, Zn, Pb, and Ni (means ± SE) in Kidney and Interrenal Tissues of Adult Yellow Perch (*Perca flavescens*) Collected in Six Lakes of the Rouyn-Noranda Mining Region

			Kidney					Interrenal tissue			
Lakes	Year	*n*	Cu (µg/g)	Zn (µg/g)	Pb (µg/g)	Ni (µg/g)	*n*	Cu (µg/g)	Zn (µg/g)	Pb (µg/g)	Ni (µg/g)
Reference lakes											
DA	1998	26	11.6 ± 0.3^{a}	697.2 ± 41.9^{b}							
	1999	23	9.7 ± 0.4^{a}	486.8 ± 37.7^{a}	4.8 ± 0.5^{a}	$2.9 \pm 0.3^{a,b}$	18	3.7 ± 0.21^{a}	199.2 ± 29.4^{b}	4.2 ± 0.6^{a}	2.7 ± 0.4^{b}
OP	1998	19	9.7 ± 0.4^{a}	673.3 ± 24.5^{b}							
	1999	19	9.3 ± 0.4^{a}	459.9 ± 29.8^{a}	3.8 ± 0.4^{a}	2.4 ± 0.3^{a}	18	3.1 ± 0.07^{a}	133.2 ± 3.5^{a}	3.2 ± 0.3^{a}	1.9 ± 0.2^{a}
Intermediate lakes											
BO	1998	15	11.21 ± 0.4^{a}	$561.9 \pm 29.9^{a,b}$			18	3.1 ± 0.13^{a}	117.9 ± 4.4^{a}	2.7 ± 0.2^{a}	1.6 ± 0.1^{a}
	1999	16	6.61 ± 0.4^{a}	395.7 ± 19.4^{a}	$5.79 \pm 0.4^{a,b}$	$3.31 \pm 0.4^{a,b}$					
DM	1998	19	12.17 ± 0.6^{a}	1108.2 ± 56.4^{c}			14	6.1 ± 0.46^{c}	$156.6 \pm 6.8^{a,b}$	$5.5 \pm 0.8^{a,b}$	$3.4 \pm 0.5^{b,c}$
	1999	13	9.21 ± 0.3^{a}	757.9 ± 33.3^{b}	7.20 ± 1.3^{b}	4.49 ± 0.8^{c}					
Highly contaminated lakes											
OS	1998	28	17.38 ± 1.1^{b}	1565.2 ± 67.5^{d}			18	6.23 ± 0.43^{c}	$232.8 \pm 16.4^{b,c}$	7.67 ± 1.4^{b}	1.98 ± 0.1^{a}
	1999	22	13.59 ± 0.9^{a}	1647.4 ± 122.6^{d}	7.31 ± 0.8^{b}	$3.74 \pm 0.4^{b,c}$					
DT	1998	15	$15.84 \pm 1.5^{a,b}$	1862.2 ± 116.2^{d}			20	5.17 ± 0.30^{b}	266.6 ± 18.7^{c}	10.97 ± 2.2^{c}	4.25 ± 0.4^{c}

Note. Means followed by letters that are different indicate significantly different values ($p < .05$), ANOVA followed by Tukey-Kramer test). OP, Lake Opasatica; DA, Lake Dasserat; BO, Lake Bousquet; DM, Lake Du Moulin; OS, Lake Osisko; DT, Lake Dufault; *n* indicates number of samples (individual fish or pooled samples).

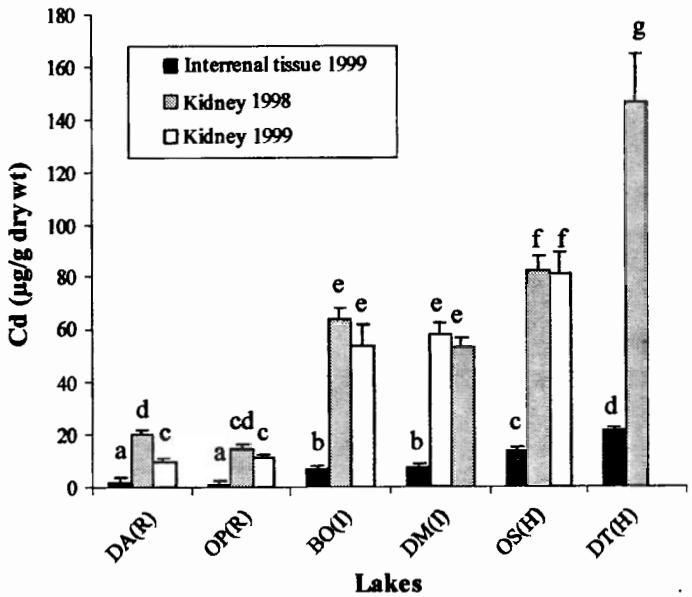

FIGURE 1. Concentrations of Cd (means ± SE) in interrenal tissue and kidney of adult yellow perch collected in six lakes of the Rouyn-Noranda mining area. Lakes were ranked as reference (R), intermediate (I), or highly contaminated (H) according to the concentration of metals accumulated in the tissues (Table 1) (see Table 1 for the names of the lakes); $n = 20$ (tissues from individual fish or pooled samples from 2–4 fish). Letters that are different indicate significantly different means ($p < .05$, ANOVA followed by Tukey-Kramer test).

Cd concentrations between the 2 years of sampling (Figure 1). Kidney Cu concentrations did not exhibit a gradient but they were higher in fish from Lake Osisko in 1999 and in fish from Lake Dufault in 1998 (Table 1). Metal concentrations (Cd, Pb, Zn, Cu) in the interrenal tissue also followed a significant contamination gradient similar to kidney; however, the concentrations were lower than in the kidney. Concentrations of Ni in 1999 did not follow the contamination gradient, in either the interrenal tissue or the kidney.

Morphological Characteristics of the Fish

Fish from the two most contaminated lakes had a markedly lower condition factor (ratio weight to length) than fish from the reference lakes in both summer and fall (Table 2).

Gill Na^+/K^+-ATPase and Gill Morphology

The activity of gill Na^+/K^+-ATPase of fish from reference lakes was significantly higher than in fish from the most contaminated lake (Lake Dufault) (Figure 2A). Moreover, histomorphometric analyses of the gills revealed significant morphological differences between fish from the reference lake and the contaminated lake (Figure 2B). Fish from the contaminated lake had a greater blood water diffusion distance (DD), lamellae thickness (LT), and height of

TABLE 2. Morphological Characteristics (Mean ± SE) of Adult Yellow Perch (*Perca flavescens*) Collected in Six Lakes of the Rouyn-Noranda Mining Region

	Summer				Fall			
Lakes	*n*	Length (mm)	weight (g)	Condition factor	*n*	Length (mm)	Weight (g)	Condition factor
OP	39	186 ± 4^{a}	74.8 ± 4.7^{b}	1.08 ± 0.02^{b}	38	190 ± 3^{b}	76.8 ± 4.6^{b}	1.08 ± 0.001^{b}
DA	41	188 ± 4^{a}	92.1 ± 4.6^{a}	1.22 ± 0.02^{a}	32	209 ± 3^{a}	111.9 ± 6.9^{a}	1.18 ± 0.02^{a}
BO	35	$178 \pm 4^{a,b}$	75.8 ± 4.9^{b}	1.25 ± 0.02^{a}	23	185 ± 4^{b}	78.1 ± 5.5^{b}	1.18 ± 0.02^{a}
DM	30	154 ± 4^{b}	$37.3 \pm 5.4^{c,d}$	0.96 ± 0.02^{c}	20	164 ± 4^{c}	41.9 ± 3.7^{c}	0.90 ± 0.01^{c}
OS	61	$177 \pm 3^{a,b}$	48.2 ± 3.8^{c}	0.88 ± 0.02^{d}	44	194 ± 2^{b}	$65.4 \pm 2.7^{b,c}$	0.88 ± 0.01^{c}
DT	41	148.3 ± 3^{b}	28.8 ± 4.6^{d}	0.86 ± 0.02^{d}	61	130 ± 10^{d}	19.2 ± 0.3^{d}	0.87 ± 0.01^{c}

Note. Means followed by the same letter are not significantly different ($p < .05$, ANOVA followed by Tukey-Kramer test); comparisons of means were made within the same season. Condition factor = ([body weight]/[body length]^3) × 100. See Table 1 for names of the lakes.

epithelial padding (H) than fish from the reference lakes (Figure 2C). There were no differences between lakes in the interlamellar distance (ILD). Deformities in gill lamellae, such as club-shaped lamellae (CSL) and hypertrophy of epithelial cells, were also observed in gills of fish from the contaminated lake (Figure 2B).

Plasma Cortisol and Morphology of the Adrenocortical Cells

Plasma cortisol was determined in a control group and a group subjected to a confinement test (Figure 3A). The increase in plasma cortisol levels in response to confinement was lower in yellow perch from the most contaminated lake (Lake Osisko), compared with fish from the reference lakes, which exhibited a significant increase in cortisol levels. Moreover, cortisol levels in fish not subjected to the confinement were lower in fish from the contaminated lakes than fish from the reference and intermediate lake. Nuclear diameter of steroidogenic cells in the interrenal tissue of fish from a contaminated lake was smaller than in fish from a reference lake (Figure 3, B and C).

Plasma Thyroid Hormones and Morphology of Thyroid Follicles

Plasma levels of T4 and T3 of control fish and fish subjected to confinement were lower in fish from the most contaminated lake than in fish from reference lake (Figure 4A) in the summer. There were no differences between lakes and treatment in the fall, with average concentrations of 18.8 ± 2.2 ng/ml for T4 and 2.4 ± 0.43 ng/ml for T3 (data not shown). Epithelial cell height of thyroid follicles was lower in fish from the contaminated lake than in fish from reference lake sampled in the summer.

Plasma Sex Steroids, GSI, and Morphology of Ovaries

Fish from the most contaminated lakes had a lower GSI in the fall (recrudescing females) than fish from reference lakes (Figure 5A). Similar differences

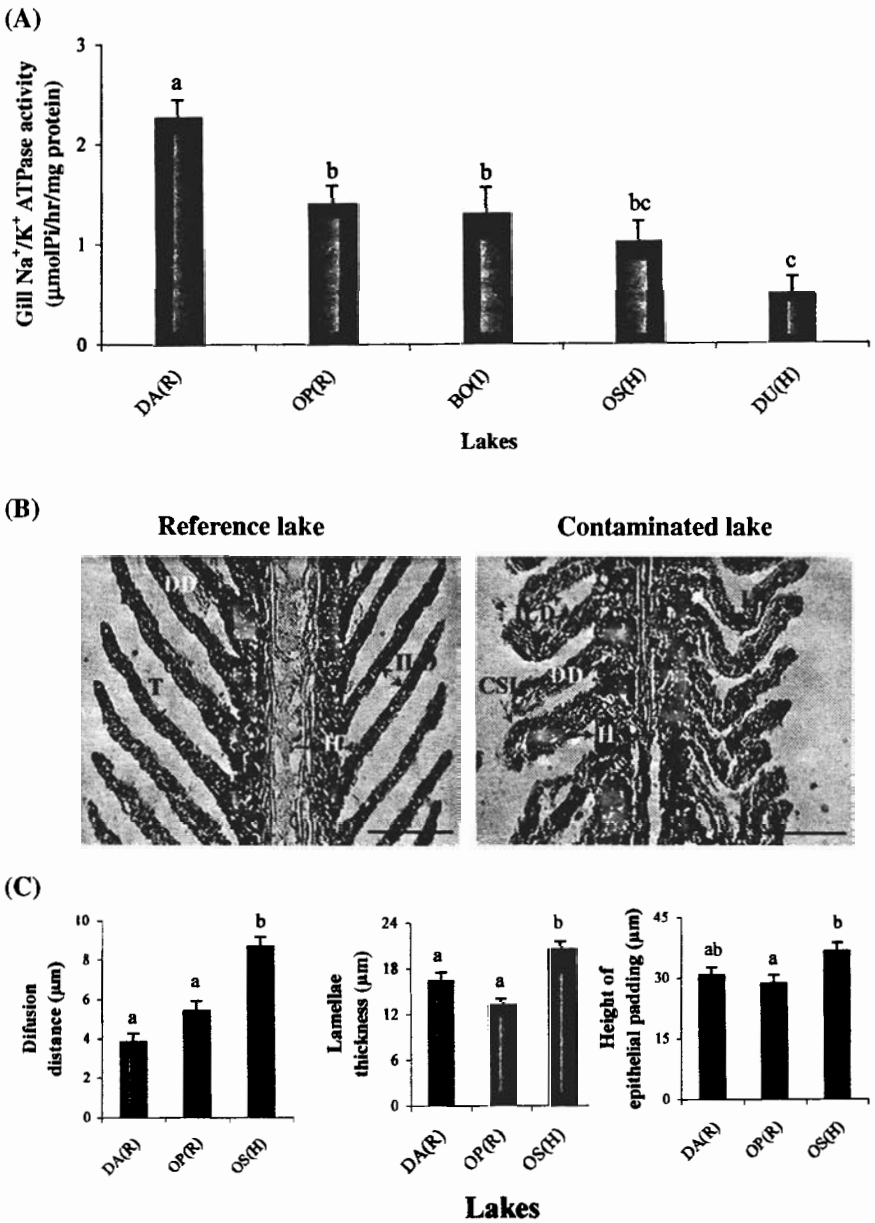

FIGURE 2. (A) Activity of Na^+/K^+-ATPase (means ± SE) in gills of yellow perch sampled in lakes of the mining region of Rouyn-Noranda. Letters that are different indicate significantly different means ($p < .05$, ANOVA followed by Tukey-Kramer test); $n = 12$ (see Table 1 for names of the lakes). (B) Morphology of gills from fish sampled in a reference lake (Lake Dasserat) and a contaminated lake (Lake Osisko), ×400. DD, blood-water diffusion distance; LT, lamellae thickness; H, height of epithelial padding; ILD, interlamellae distance; CSL, club-shaped lamellae; black bar measures 50 µm. (C) Histomorphometric analyses of DD, LT, and H in gills from fish sampled in a reference lake and a contaminated lake ($p < .05$, t-test, $n = 10$).

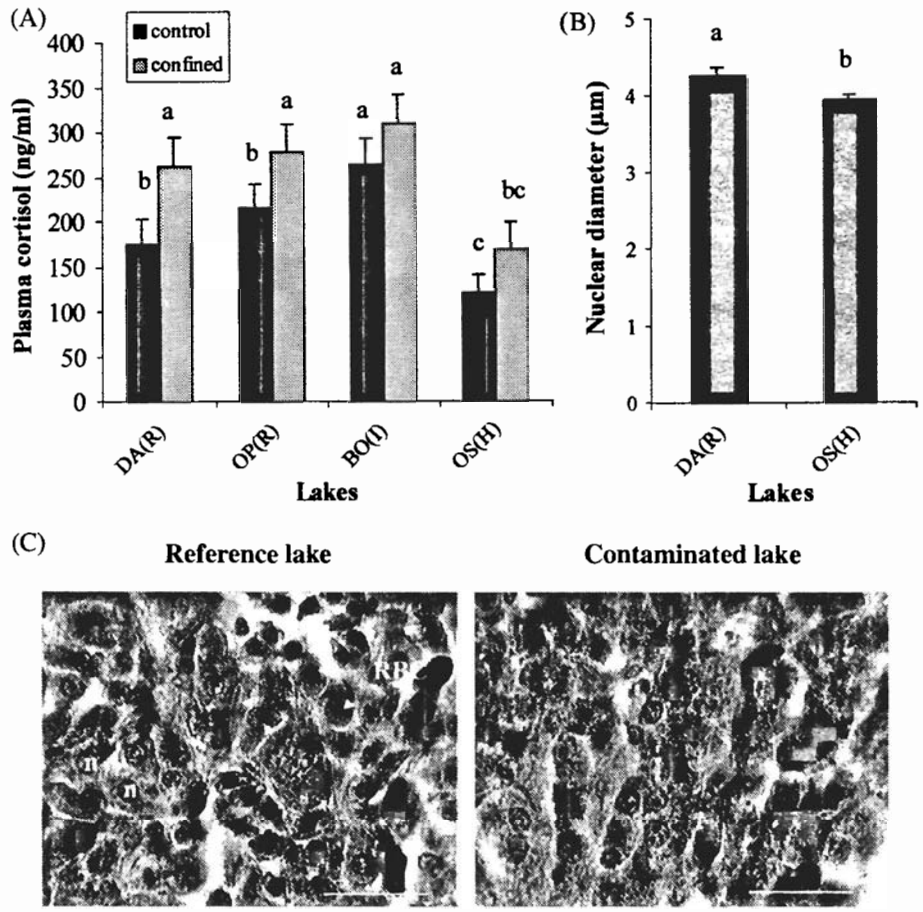

FIGURE 3. (A) Plasma cortisol concentrations (mean ± SE) in adult yellow perch from lakes of the Rouyn-Noranda mining region. Fish were sampled before (controls) or after confinement. Different letters indicate means that are significantly different ($p < .05$, ANOVA followed by Tukey-Kramer test); $n = 15$ (see legend of Table 1 for names of the lakes). (B) Histomorphometric analyses of interrenal tissue of fish from a reference lake and a contaminated lake, ×1000: n, nucleus; RBC, red blood cells; arrows indicate the nucleus thickness and white bar measures 50 µm. (C) Morphology of interrenal tissue in fish from a reference lake and a contaminated lake ($p < .05$, t-test, $n = 10$).

were also detected in plasma 17β-estradiol levels, except for Lake Dufault, where only three fish were assayed. Plasma testosterone levels in the fall and in the summer and 17β-estradiol in the summer (postspawning females) did not follow the contamination gradient (Table 3). Histological examination of ovaries from females sampled in the fall revealed that fish from the contaminated lake had less developed gonads (Figure 5C), with more oocytes in immature stages (perinucleolus and vesicle stages) than fish from the reference lakes. Fish sampled in the summer, following spawning, were sexually regressed and had no measurable gonadal tissue in all the lakes.

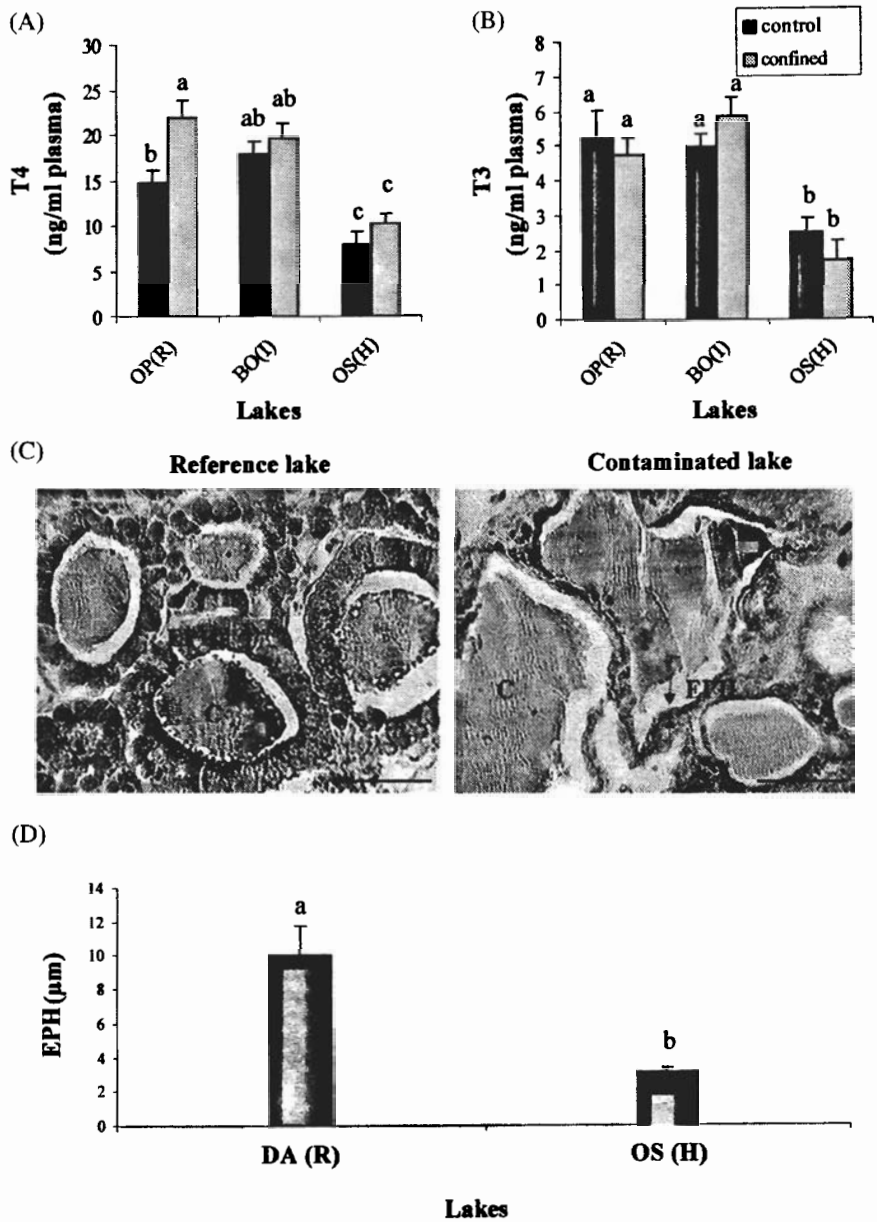

FIGURE 4. (A, B) Plasma thyroid hormone concentrations (mean ± SE) in adult yellow perch from lakes of the Rouyn-Noranda mining region. Fish were sampled before (controls) and after confinement, in the summer. Different letters show means that are significantly different ($p < .05$, ANOVA followed by Tukey-Kramer test); $n = 15$ (see legend of Table 1 for names of the lakes). (C) Morphology of thyroid follicles of fish from a reference lake and a contaminated lake, ×400. EPH, epithelial cell height; C, colloids; black bar measures 50 µm. (D) Histomorphometric analyses of thyroid follicles of fish from a reference lake and a contaminated lake ($p < .05$, t-test, $n = 10$).

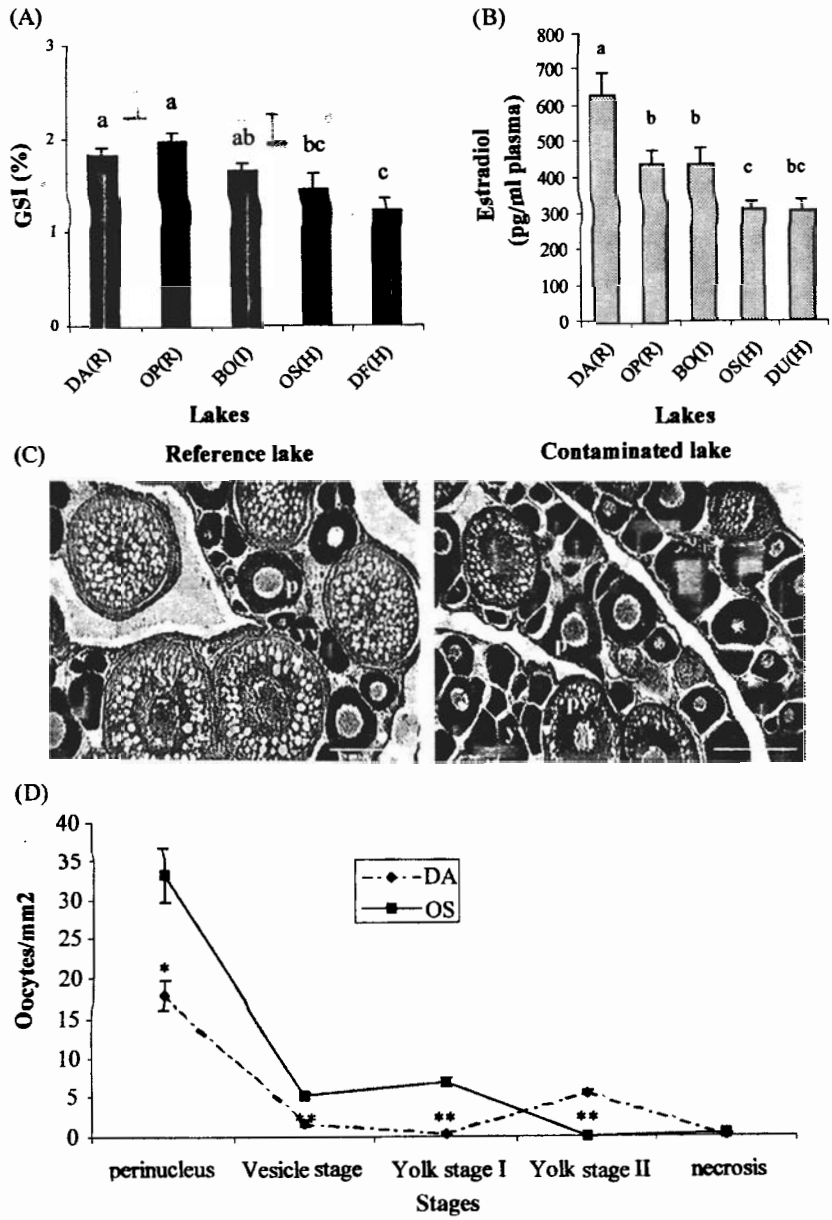

FIGURE 5. (A) Gonadosomatic index and (B) plasma estradiol concentrations (mean ± SE) in female adult yellow perch sampled in fall in lakes of the Rouyn-Noranda mining region. Different letters indicate means that are significantly different ($p < .05$, ANOVA followed by Tukey-Kramer test); $n = 15$. (C) Morphology of ovaries of fish from a reference lake and a contaminated lake, ×100; n, nucleus; p, oocytes in perinucleolus stage; yv, yolk vesicle; py, oocyte in 1° yolk stage; sy, oocytes in 2° yolk stage; white bar indicates 50 µm. (D) Histomorphometric analyses of ovaries of fish from a reference lake and a contaminated lake ($p < .05$, t-test, $n = 10$).

TABLE 3. Plasma Sex Steroids (Mean ± SE) of Adult Yellow Perch (*Perca flavescens*) Collected in Lakes of the Rouyn-Noranda Mining Region

	Fall testosterone (pg/ml)				Summer				
					Estradiol (pg/ml)		Testosterone (pg/ml)		
Lakes	*n*	Female	*n*	Male	*n*	Female	Female	*n*	Male
DA	10	2831.00 ± 237.9^{a}	7	3200.86 ± 264.7^{a}	6	588.16 ± 60.6^{a}	$184.67 \pm 28.5^{a,b}$	12	241.00 ± 24.5^{a}
OP	15	1044.73 ± 75.4^{c}	3	$1824.67 \pm 821.2^{a,b}$	13	422.23 ± 37.8^{b}	$155.00 \pm 15.0^{b,c}$		na
BO	7	$578.43 \pm 43.9^{c,d}$	8	1862.00 ± 554.6^{b}	10	577.00 ± 92.5^{a}	239.80 ± 28.1^{a}	5	251.25 ± 68.1^{a}
OS	20	2015.55 ± 105.1^{b}	3	$2771.00 \pm 160.9^{a,b}$	14	328.00 ± 53.7^{b}	102.14 ± 6.9^{c}	4	104.00 ± 7.0^{b}
DT	3	2808.33 ± 418.5^{a}	6	$2821.71 \pm 492.2^{a,b}$	13	736.92 ± 105.6^{a}	$150.42 \pm 40.3^{b,c}$	4	$130.00 \pm 26.0^{a,b}$

Note. Means followed by the same letter are not significantly different ($p < .05$, ANOVA followed by Tukey-Kramer test); comparisons of means were made within the same season. See Table 1 for names of the lakes. na, not available.

Plasma Sex Steroids, GSI, and Morphology of Testes in Yellow Perch

Fish from the most contaminated lakes had lower GSI than fish from reference lakes (Figure 6A). Plasma testosterone did not follow the contamination gradient (Table 3). Histological analyses of the testes in the fall showed that fish from contaminated lake had more immature gonads than fish from reference lake (Figure 6B), according to the classification of Chan and Chua (1980). In all fish sampled, lobular organization, previously defined by Kristoffersson and Pekkarinen (1975), was evident. In fish from contaminated lakes, testes were in stage II, spermatogonia were predominant, and the lumen was absent, whereas testes of fish from the reference lakes were in stage III, spermatids and sperm-

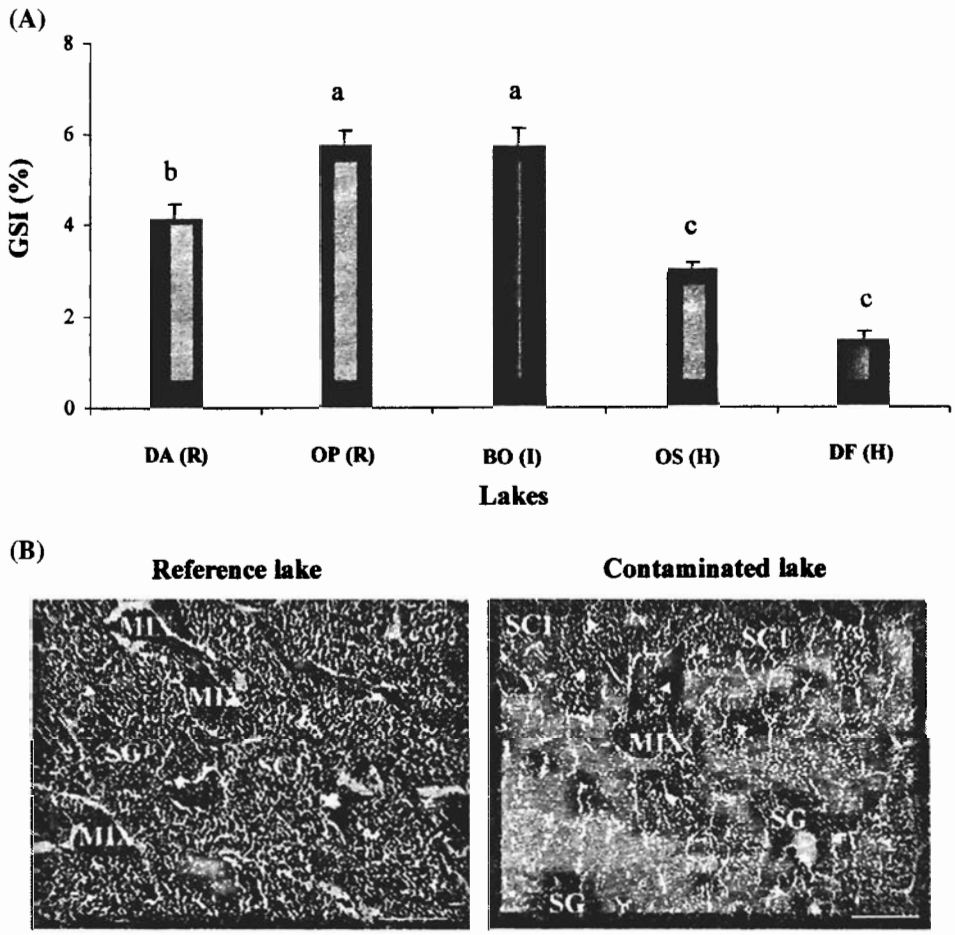

FIGURE 6. (A) Gonadosomatic index (mean ± SE) in male adult yellow perch sampled in fall from lakes of the Rouyn-Noranda mining region. Different letters show means that are significantly different ($p < .01$, ANOVA followed by Tukey-Kramer test); $n = 8$, except for Lake Dufault where $n = 3$. (B) Morphology of testis of fish from a reference lake and a contaminated lake. SG, spermatogonia; SC1, spermatocytes in stage 1; MIX, mixture of spermatocytes, spermatids, and spermatozoides. ×400. White bar indicates 50 µm.

atozoa were predominant, and some sperm was present in the lumen of the tubule (Figure 6B).

DISCUSSION

This study is a large-scale field investigation of the effects of lifelong exposures to metals on the hormonal, physiological, and reproductive status of yellow perch sampled in lakes situated in a mining region. Tissue burdens of Cd, Zn, and Pb in the kidney and in the interrenal tissue were used to characterize the gradient of exposure and to rank the fish populations as reference, intermediate, and highly contaminated. Although the contamination gradient was evident in both tissues, the concentrations of metals in the kidney were 3–10 times higher than in the interrenal tissue. Tissue-specific metal burdens were reported previously (Harrison & Klaverkamp, 1989; McGeer et al., 2000), with kidney as the major site of Cd accumulation. Concentrations of Cu in the kidney did not follow a gradient, as predicted by studies that suggested that Cu is an essential metal and its levels are regulated by the organism (Andes et al., 2000). In contrast, interrenal Cu levels did follow a gradient in the present study, as has been reported by Laflamme et al. (2000), for the same fish species sampled in the same lakes. The capacity of the interrenal tissue to accumulate Cu, as suggested by the concentration gradient present in the interrenal tissue, requires further investigations.

The increased tissue burdens of metals were linked to a lower condition factor, indicative of a growth impairment. Previous studies in this region also reported a lower condition factor (Laflamme et al., 2000; Levesque et al., 2002) and reduced growth efficiency, the capacity to convert food into biomass, in perch from the most contaminated lakes (Sherwood et al., 2000; Sherwood, Pazzia et al., 2002). Moreover, fish chronically exposed to metals exhibit abnormal seasonal cycling of liver glycogen and triglycerides reserves (Levesque et al., 2002), and the food sources may be depleted in the contaminated lakes as well (Sherwood, Kovecses et al., 2002). Although it is difficult at the present time to differentiate between direct effects of metals on various cellular and systemic targets, and indirect effects of metals through modified prey basis and nutritional status, the present study provides substantial evidence that fish sampled in this study are subjected to metal-induced environmental stress and that their growth is impaired.

Along with condition factor, an indicator of growth, biochemical, hormonal, and morphological responses were characterized in the present study. While gill morphology in fish from the reference lake was quantitatively similar to that described for healthy salmonids by Skidmore (1970), morphological anomalies characterized by thickened lamellae were diagnosed in gills of fish from contaminated lakes. In addition, the activity of gill Na^+/K^+-ATPase, a key enzyme in osmoregulation and acid base balance, decreased with the contamination gradient in an exposure dependent pattern. Previous studies investigating the effects of metals on Na^+/K^+-ATPase are not in agreement. McGeer et al. (2000) detected an increase in the activity in gills of rainbow trout exposed to Cu and

no differences in gills exposed to Cd. In contrast, Lionetto et al. (2000), similar to our results, reported a decrease in the activity of Na^+/K^+-ATPase in branchial and intestinal cells of eel (*Anguilla anguilla*) exposed to Cd. The mechanisms through which Cd influences the activity of Na^+/K^+-ATPase are not known at present. Cd may affect the formation of the phosphorylated enzyme, as it does for Ca^{2+}/K^+-ATPase (Lemaire-Gony & Mayer-Gostan, 1994); altered cortisol and thyroid hormones levels may also be involved. Shrimpton and McCormick (1998) demonstrated that both cortisol and thyroid hormones increase the activity of Na^+/K^+-ATPase.

The present study provided evidence that chronic exposure to metals impairs the cortisol stress response to confinement, in agreement with previous studies (Brodeur et al., 1997; Laflamme et al., 2000) characterizing the impact of a chronic exposure to metals on the hypothalamo-pituitary-interrenal axis. An impaired capacity of the interrenal tissue from metal-contaminated fish to secrete cortisol in response to an ACTH stimulation was also reported in vitro (Laflamme et al., 2000). Histomorphometric analyses of adrenocortical cells demonstrated in the present study that the nuclei were smaller in fish from the contaminated lakes, in agreement with Hontela (1997). In contrast, Norris et al. (1997) reported an increase in the diameter of interrenal cells nuclei, associated with overstimulation in brown trout (*Salmo trutta*) exposed to Cd and Zn. The cellular atrophy of the interrenal tissue in fish from our study is consistent with the lower cortisol secretion in perch from contaminated compared to reference lakes.

Plasma thyroid hormone levels and epithelial height of thyroid follicles were assessed as indicators of thyroid function in the present study, since cortisol and thyroid hormones act in synergy in numerous physiological processes. Epithelial cell height is a reliable indicator of the activity of thyroid follicle (Yamano et al., 1991). In our study, epithelial cell height of thyroid follicles was lower in fish from contaminated lake than fish from reference lakes, similarly to Scherer et al. (1997) in lake trout exposed 6 and 9 mo to 5 µg Cd/L. Fish chronically exposed to metals had decreased plasma T3 and T4 levels in the summer (postspawning season) compared to fish from reference lakes. There were no differences in the fall period of gonadal recrudescence, suggesting that thyroid function may be less sensitive to metals at that period. It is important to note that seasonal differences in plasma levels of thyroid hormones in the reference lakes were similar to the differences reported in other teleosts such as Atlantic salmon (*Salmo salar*) (Morin & Dodson, 1989), sea bass (*Dicentrarchus labrax*), and sea bream (*Sparus aurata*) (Cerdà-Reverter et al., 1996).

Previous laboratory studies with rainbow trout reported an increase in plasma T4 after acute and subchronic (30 d) exposure to Cd but no differences in T3 levels (Hontela et al., 1996), and a decrease of T4 levels after longer exposure periods in rainbow trout and yellow perch (Hontela et al., 1995, 1996). The plasma levels of T3, the biologically active hormone (Brown et al., 1998), may be maintained at the control levels, while T4 is converted to T3. In the present field study and in the study of Gupta et al. (1997), T3 levels were also lower in fish from contaminated lakes, suggesting that the levels of

T4 were too low to maintain T3 levels, possibly due to an inhibition in synthesis and release of T4 at the follicular level (Gupta et al., 1997). Cadmium may also have an inhibitory effect on the activity of extrathyroidal 5'-monodeiodinase, implicated in the conversion of T4 to T3 in peripheral tissue.

Cortisol and thyroid hormones exert, among their numerous actions, effects on the reproductive axis (McKenzie et al., 1989; Pankhurst & Van Der Kraak, 2000). Male and female fish from the contaminated lakes had lower GSI and their gonads were in a less mature stage compared to fish from reference lakes. However, the effects of metals on plasma levels of reproductive hormones were not consistent, since only females exhibited lower plasma estradiol levels in contaminated lakes, and no effects could be seen on testosterone levels in males and females. A delayed reproduction was reported by Brown et al. (1994) in rainbow trout exposed to 9.3 and 29.1 µg Cd/L; however, sex steroids were not measured. The lower estradiol levels in female fish from contaminated lakes may be linked to the increase in metallothionein (Laflamme et al., 2000), which could limit the availability of Zn for synthesis of sex steroid hormones (Kime, 1999). Other mechanisms could be involved as well, since Cd is a well-known reproductive toxicant in mammals. Hew et al. (1993) reported an adverse effect of Cd on rat spermination 24 h after an injection of 1 mg/kg. A decrease in sperm count and percentage of motile sperm was detected in mice exposed to Pb (Wadi & Ahmad, 1999). A decrease in hCG-stimulated and db-cAMP-stimulated testosterone production by rat Leydig cells exposed in vitro to Cd^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} was demonstrated by Laskey and Phelps (1991). The mechanisms through which metals may compromise synthesis of sex steroid hormones in fish remain to be elucidated, as do the interactions between the thyroid and reproductive axes in fish chronically subjected to metals in their environment.

Our study with yellow perch demonstrated that a lifelong exposure to metals has sublethal effects on the interrenal, thyroid, and reproductive axes, the major endocrine systems interacting through complex physiological processes. The various endpoints measured in the present study were affected differently by chronic exposure to metals (Table 4). Although kidney was the most important tissue where metals accumulated, compared to liver and the interrenal tissue, the most important difference between reference and contaminated lake was observed for Cd concentrations in the liver (increase by 762% from reference). The present study indicated that gill parameters are sensitive indicators of metal impact on the health status of fish since the activity of Na^+/K^+-ATPase and blood water diffusion distance (DD) were variables that changed the most following metal exposure (80% and 124%, respectively). Plasma levels of cortisol, thyroid hormones, and sex steroids, depending on the season of sampling, were also sensitive to metals (decrease of 30–53% from reference). GSI and condition factor exhibited a smaller change (decrease of 20–27% and 20%, respectively) but remain important biomarkers of metal effects since they are easily measured and are indicators of reproductive competence and growth, two responses related to fitness.

TABLE 4. Comparison of the Change in Hormonal, Morphological, and Physiological Responses of Yellow Perch (*Perca flavescens*) From a Reference and a Metal-Contaminated Lake of the Rouyn-Noranda Mining Region

Tissue	Biomarker	Units	Change[a]	Magnitude of change	Ranking
Gill	Gill Na^+/K^+-ATPase	µmol Pi/h/mg protein	–	80%	5
	DD	µm	+	124%	4
	LT	µm	+	24%	13
Interrenal	Plasma cortisol	ng/ml	–	32%	11
	Adrenocortical cell nuclei diameter	µm	–	8%	15
	Cadmium burden	µg/g	+	548%	3
Thyroid	Plasma T4	ng/ml	–	53%	8
	Plasma T3	ng/ml	–	48%	10
	Thyroid follicle epithelial cell	µm	+	69%	6
Gonad	GSI females	%	–	20%	14
	GSI males	%	–	27%	12
	Plasma estradiol	pg/ml	–	50%	9
Whole body	Condition factor	%	–	20%	14
Kidney	Cadmium[b]	µg/g	+	611%	2
Liver	Cadmium[c]	µg/g	+	762%	1
	Glycogen[d]	mg glycogen/g liver	–	58%	7

[a] The comparison is made between the response in fish from a contaminated lake (Lake Osisko) and a reference lake (Lake Dasserat), except for thyroid hormones, where the reference lake used was Lake Opasatica.

[b] Kidney cadmium concentrations are from 1999 samples.

[c] Liver cadmium concentrations are from Laflamme et al. (2000).

[d] Liver glycogen data are from Levesque et al. (2002).

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