Interrenal metallothionein and cortisol secretion in relation to Cd, Cu, and Zn exposure in yellow perch, *Perca flavescens*, from Abitibi lakes

J.-S. Laflamme, Y. Couillard, P.G.C. Campbell, and A. Hontela

Abstract: The concentrations of Zn, Cu, and Cd in the interrenal tissue and liver of yellow perch, *Perca flavescens*, sampled in six lakes in the mining region of Abitibi (Quebec) revealed a gradient of contamination: reference lakes, intermediate-contaminated lakes, and highly contaminated lakes. Interrenal and hepatic metallothionein concentrations followed a similar gradient, and there was a strong relationship between metallothionein and total metal (Zn + Cu + Cd) content in the liver and Cu + Cd content in the interrenals. Following a standardized confinement stress test, plasma cortisol and glucose were significantly higher in fish from reference lakes compared with the two most contaminated lakes. No differences in plasma chloride were observed. The secretory response of the interrenal tissue to in vitro stimulation by adrenocorticotropic hormone and dibutyryl cyclic AMP was significantly lower in fish from a contaminated lake compared with fish from a reference lake. Condition factor was significantly lower in yellow perch from the most contaminated lakes. This study demonstrated that a chronic field exposure to base metals increases hepatic and interrenal tissue metallothionein concentrations and disrupts the physiological capacity of yellow perch to generate the normal hormonal stress response.

Résumé : Les concentrations du Zn, Cu et Cd dans le tissu interrénal et le foie de la perchaude, *Perca flavescens*, échantillonnée dans six lacs de la région minière de l'Abitibi (Québec) ont démontré un gradient de contamination : lacs référence, lacs à contamination moyenne et lacs à contamination forte. Les concentrations de la métallothioneine interrénale et hépatique ont suivi un gradient similaire, avec une corrélation entre métallothioneine et les métaux totaux (Zn + Cu + Cd) dans le foie et le Cu + CD dans les interrénales. Suite à un test de stress standardisé, le cortisol et glucose plasmatique étaient plus élevés chez les poissons des lacs référence, comparé aux lacs le plus contaminés, tandis que le chlorure plasmatique n'était pas différent. La réponse secrétrice du tissu interrénal à une stimulation in vitro par l'hormone adrénocorticotropique and le dibutyryl AMP cyclique, ainsi que le facteur de condition, était plus faibles chez les poissons des lacs contaminés comparé aux lacs référence. L'étude a démontré que l'exposition chronique sur le terrain à des métaux augmente les concentrations de la métallothioneine et perturbe la capacité physiologique normale de générer la réponse hormonale au stress chez la perchaude.

Introduction

The hypothalamo–pituitary–interrenal axis of teleost fish is activated by abiotic and biotic stressors (Barton and Iwama 1991; Hontela 1997) that promote the release of hypothalamic corticotropin-releasing hormone and pituitary adrenocorticotropic hormone (ACTH) to stimulate the synthesis and release of cortisol by the interrenal tissue located in the pronephros (Wendelaar Bonga 1997). Cortisol has effects on intermediary metabolism (Van Der Boon et al. 1991), osmoregulation (Laurent and Perry 1990), the reproductive system (Pankhurst and Van Der Kraak 2000), and the immune system (Pickering 1989). The stress response in fish, characterized by elevated plasma cortisol, favours, together with other hormones, recovery of homeostasis and its maintenance (Barton and Iwama 1991; Wendelaar Bonga 1997).

Numerous laboratory studies have reported increased plasma cortisol levels following acute exposure of fish to environmental pollutants, including metals (reviewed in Hontela 1997). In contrast, studies of chronic exposure effects on cortisol levels and the interrenal function are scarce. Chronic (180 days) laboratory exposure to Hg reduced cortisol levels in walking catfish, *Clarias batrachus* (Kirubagaran and Joy 1991), and juvenile walleye, *Stizostedion vitreum* (Friedmann et al. 1996). Lower cortisol response following a stress has been documented in northern pike, *Esox lucius*, and yellow perch, *Perca flavescens*, sampled at sites contaminated by metals (Lockhart et al. 1972; Hontela et al. 1992; Brodeur et al. 1997*a*). Recent in vitro studies in our laboratory demonstrated that short-term (60 min) exposure of interrenal cells to Cd, Hg, or Zn impairs cortisol se-

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cretion at doses that are not cytotoxic (Leblond and Hontela 1999). However, the mechanisms through which chronic environmental exposures to base metals disrupt cortisol secretion in fish have not been elucidated thus far.

In the present field study, we investigated some of the cause–effect relationships between contamination by metals and interrenal dysfunction in yellow perch by sampling fish in lakes representing a gradient of metal concentrations. The study was designed to determine if dose–response relationships can be established between the in situ exposure to metals, the tissue load (burden), and effects on the physiological and endocrine status of the fish. Metal concentrations were measured in the liver, an important site of metal accumulation, in order to rank selected sites according to hepatic contamination of the indigenous yellow perch. For the first time in teleost fish, metals were also measured in the interrenal tissue to test the link between exposure and metal levels in this endocrine tissue. To assess the capacity of the interrenal tissue to resist metal toxicity, metallothionein (MT), a detoxification protein, was measured in both the liver and the interrenals. MT is a low molecular mass ubiquitous protein, rich in cysteine, and its synthesis is mainly induced by metals. It plays a key role in the detoxification of base metals and the regulation of essential metals such as Cu and Zn (Roesijadi 1992). To test the link between tissue load of metals and effect on the endocrine status, the capacity of the interrenal tissue to generate the normal cortisol stress response was assessed: first in vivo by measuring plasma cortisol in yellow perch subjected to a 1-h confinement stress and then in vitro by measuring cortisol output in response to ACTH and, for the first time in a wild fish, to dibutyryl cyclic AMP (dbcAMP), a second messenger mediating the effect of ACTH (Patiño et al. 1986). Because cortisol has a role in intermediary metabolism, the link between tissue load and effects was also tested by measuring the condition factor of all the fish. Thus, the objectives of this study were to (*i*) characterize the exposure of yellow perch from lakes in a mining region, (*ii*) test the link between base metal and MT levels in liver and interrenal tissue, and (*iii*) test the link between exposure, the capacity to generate the normal cortisol response, and effects on growth (condition).

Materials and methods

Study sites and fish species

Fish were sampled in six lakes (Lakes Opasatica, Dasserat, Vaudray, Bousquet, Dufualt, and Osisko) of the mining area of Rouyn-Noranda, located in northwestern Quebec, Canada (48°00′N, 79°00′W), in June 1997. Lakes in the area are affected by acid and metal contamination from current metal mining operations, abandoned mine sites, and atmospheric deposition from a nearby smelter (Couillard et al. 1993). Previous studies (Couillard et al. 1993; Tessier et al. 1993; Brodeur et al. 1997*a*) provided data on metal concentrations in water, sediments, and yellow perch livers and enabled us to make a preliminary ranking of the lakes based on contamination: Lakes Opasatica and Dasserat as reference sites, Lakes Vaudray and Bousquet as intermediate-contaminated sites, and Lakes Osisko and Dufault as the most highly contaminated lakes. Table 1 shows concentrations in water and sediments from a more recent sampling. For a detailed map of the area and limnological characteristics of the lakes, see Couillard et al. (1993) and Brodeur et al. (1997*a*).

Yellow perch was used for this study because it is a relatively sedentary fish (Aalto and Newsome 1990) that reflects the contamination of the site where it is sampled, and it is an abundant species in the six sampled lakes. Males and females were sampled; fish were sexually immature (spawning occurs in May). The fish were collected in the postspawning period to minimize variation of the cortisol response due to differences in gonadal status (Pottinger et al. 1995).

Experimental treatments

Fish were captured by rod, seine, or gill net, since capture method does not influence plasma cortisol in fish kept in in situ enclosures following capture and blood sampled the next day (Brodeur et al. 1997*b*). Fish were placed into floating enclosures (0.5 m wide × 1 m long × 1 m deep) made of net (10 fish per enclosure) for at least 16 h so they could partially recover from the stress of capture. They were not fed during this period. Previous studies have shown that a 15- to 24-h rest period in enclosures postcapture facilitates recovery from the initial stress of capture, as indicated by significantly lower plasma cortisol levels compared with values immediately postcapture (Brodeur et al. 1997*a*, 1997*b*; Girard et al. 1998).

Standardized in vivo stress test

The day after capture, fish were taken from the enclosures and placed into 20-L containers by groups of 10 for 1 h. The 1-h confinement stress used in the present study following the period of recovery significantly elevated cortisol levels in comparison with fish that were not confined. However, the stress was not too severe, since morbidity or mortality of yellow perch was not observed during the confinement period. The confinement stress test always began at 10:00, and each additional group was put into different containers 15 min later. The fish were stressed during the same period of the day to avoid diel effects on cortisol secretion (Audet and Claireaux 1992). After 1 h of confinement stress, fish were anaesthetized by adding 150 mg tricaine methanesulfonate (MS 222)·L^{-1} to the container. Yellow perch were bled from the caudal vasculature with a 1-mL heparinized syringe. Another group of 12 fish·$site^{-1}$·day^{-1} were removed from the enclosures and at 10:00 were anaesthetized directly in the MS 222 solution: these fish are referred to as the "unstressed group." A maximum of 40 fish·day^{-1} were sampled, and about 10 min was necessary to bleed each group of 10 fish. Bleeding of all the fish was completed within 1 h. Fish and syringes were put on ice in a cooler and transported to a field laboratory; sites were located 20–45 min from the laboratory. The time between the end of blood sampling at the site and the beginning of dissection in the laboratory was kept the same (45 min) for all the lakes.

At the field laboratory, blood was centrifuged (5 min at 10 000 × *g*) and plasma was frozen at –20°C for later analyses. Body mass and length of fish were recorded for the calculation of condition factor (weight (grams)/(length (centimetres))^3 × 100). Liver and interrenal tissue were dissected for metals and MT analyses and immediately frozen and kept in liquid nitrogen to avoid oxidation. Upon return to the university facilities (1 month later), tissue samples were transfered to a –80°C freezer and maintained in oxygen-free sealed bags. Hepatosomatic index was calculated as liver weight (grams)/body weight (grams) × 100.

In vitro ACTH and dbcAMP challenge

The functional integrity of the interrenal tissue was tested in vitro in yellow perch from a reference lake (Lake Opasatica) and a contaminated lake (Lake Osisko). The day after capture, a group of 12 fish·$site^{-1}$·day^{-1} were removed from the enclosures and at 10:00

Table 1. Trace metal concentrations (mean ± SE) in water, sediments, and yellow perch sampled in previous studies in the Rouyn-Noranda region.

	Free metal ion in the dissolved phase (nmol·L^{-1})				Metals in oxic sediments (nmol·g dry weight^{-1})					Metals in yellow perch liver (nmol·g wet weight^{-1})[b]			
Lake	Year	*N*	[Zn^{2+}]	[Cu^{2+}]	[Cd^{2+}]	Year	*N*	Extract {Zn}	Extract {Cu}	Total {Cd}	Year	*N*	Zn	Cu	Cd
Opasatica	1998	8	19.3±3.0	3.4±0.7	0.12±0.06	1997	4	105±20	144±25	5.1±0.7	1996	20	612±21	153±25	28±7
	1989				0.36[a]	1989				3.76[a]					
Dasserat											1996	20	474±35	121±19	17±2
Bousquet	1998	6	39.0±2.0	2.9±0.4	0.40±0.01	1997	3	204±44	143±9	10.0±0.1					
	1989				2.32[a]	1989				30.1[a]					
Vaudray	1998	6	35.0±1.0	8.9±4.6	0.46±0.02	1997	1	320	238	12					
						1989				38.3[a]					
Osisko	1997	1	20	2.2	0.47	1997	1	68 500	105 000	590	1996	20	765±31	425±44	125±7
Dufault	1998	4	250±20	9.6±0.8	1.65±0.14	1997	1	18 000	19 400	200	1996	20	749±90	299±36	205±16

Note: Free metal ion concentrations were directly measured in aqueous media using dialysers installed at 10 cm above the sediments and an ion-exchange technique; see Fortin and Campbell (1998) for details on the ion-exchange technique. [M^{2+}] were estimated from sediment–water sorptive equilibria; see Tessier et al. (1993) for details. {M} were extracted for 6 h at 96°C with 0.04 M $NH_2OH{\cdot}HCl$ in 25% (v/v) HOAc and the residue was extracted at 85°C for 5 h with 30% H_2O_2 adjusted to pH 2 and then at room temperature with 3.2 M NH_4Oac in 20% (v/v) HNO_3.

[a]Data from Tessier et al. (1993).

[b]Data from Brodeur et al. (1997*a*).

were anaesthesized directly in MS 222 solution ("no-stress groups"). Fish were bled and transported to the field laboratory.

The microplate method has been described elsewhere (Benguira and Hontela 2000). In summary, each head kidney was dissected, cut into small fragments (1 mm^3), and divided into three equal parts. Each part was put into a microplate well containing 1 mL of nutritive medium (MEM). After 2 h of incubation necessary to reach the basal rate of cortisol secretion, the microplate was centrifuged and the supernatant removed and each of the three wells received a different treatment: stimulation by 1 mL of MEM containing 2 IU of porcine ACTH (Sigma Chemical Co., St. Louis, Mo.), stimulation by 1 mL of MEM containing 4 mmol of dbcAMP (Sigma Chemical Co.), both optimal doses determined in a pilot study, and incubation in 1 mL of MEM only. After 3 h of incubation, each well was centrifuged and the supernatant frozen for later analyses of cortisol by radioimmunoassay.

Analyses of metals and MT

Metals and MT levels were measured in the liver and the interrenal tissue from fish that were subjected to the in vivo stress test. Concentrations of Cd, Cu, and Zn in both tissues were measured by flame atomic absorption spectrometry using tissue homogenates corresponding to 100 mg dry weight. Cytosolic MT concentrations were measured by a Hg saturation assay adapted from Dutton et al. (1993) and Couillard et al. (1993). Fresh bovine hemoglobin was used as the exogenous protein for removal of excess non-MT-bound Hg. Homogenates of both tissues were prepared from combined samples from at least three randomly chosen fish from the same lake to obtain sufficient dry weight for both analyses.

The summation of the metals in each combined sample ([Zn + Cu + Cd]), in order to establish a relationship with MT concentrations, was calculated as

$$(1)\qquad [Zn + Cu + Cd] = [Zn]/K_{Zn}AM_{Zn} + [Cu]/K_{Cu}AM_{Cu} + [Cd]/K_{Cd}AM_{Cd}$$

where [Zn], [Cu], and [Cd] are the concentrations of the metals in the combined samples (micrograms per gram dry weight), K is the specific capacity of each metal to bind to MT by metal thiolate linkages (seven equivalents for bivalent metals (Zn and Cd) and 12 equivalents for univalent metals (Cu), as reported by Kägi and Schäffer (1988), and AM is the atomic mass of each metal. To calculate Cu and Cd summation ([Cu + Cd]) in combined samples of the interrenal tissue, the same calculation was done as eq. 1, except that [Zn] was not added.

Analyses of blood parameters

Concentrations of cortisol in the plasma and the incubation medium from the microplates were measured with a radioimmunoassay kit (ICN Biomedicals Canada Ltd., Montreal, Que.). Plasma chloride and glucose levels were determined using a colorimetric reagent method (Sigma Chemical Co.).

Statistical analyses

For all the tests, we used a statistical significance level of $\alpha = 0.05$. Since no difference between sexes was detected within each site (t test, $P < 0.05$), the two sexes were grouped for analysis. Differences amoung groups (except for in vitro results) were tested using one-way analysis of variance (ANOVA, SAS 6.12 program). The Tukey–Kramer test was used to differentiate different means. Data were transformed, when necessary, to obtain normality and homoscedasticity. Relationships between concentrations of metals and MT were evaluated with Pearson's correlation coefficient. Data were ln transformed to obtain homogeneity and normality of the residue values. Bonferroni's correction of the statistical signifi-

cance level was used. Analysis of covariance (ANCOVA) was used for the in vitro method because final cortisol secretion correlated with basal cortisol secretion before stimulation (Pearson's test, $r = 0.74$, $P < 0.001$). Final cortisol secretion was used as the dependent variable, basal cortisol secretion as the covariate, and lake and treatment as the independent variables. Data were ln transformed to obtain homogeneity. The model used explained 86.9% of the variability.

Results

Characteristics of sampled yellow perch

Yellow perch from Lake Opasatica were larger (greater length and weigth) and those from Lake Dufault were smaller compared with fish from all the other lakes (Table 2), which were not different from each other in length and weight. Condition factor was greater in yellow perch from reference (Lakes Opasatica and Dasserat) and intermediate-contamination lakes (Lakes Bousquet and Vaudray) than in those from high-contamination lakes (Lakes Osisko and Dufault) (Table 2). The hepatosomatic indices of fish collected in Lakes Dasserat and Vaudray were higher than in the other lakes, all of which were similar (except Lake Dufault < Lake Opasatica).

Metals and MT concentrations

The levels of Zn, Cu, and Cd were higher in pooled samples of liver and interrenal tissue of yellow perch from the most contaminated lakes (Lakes Osisko and Dufault) compared with the reference and intermediate-contamination lakes (Table 3). Liver and interrenal concentrations of Cd were higher in intermediate-contamination lakes (Vaudray and Bousquet) than in reference lakes (Lakes Opasatica and Dasserat). Concentrations of Cu and Cd were consistenly higher in the liver, compared with the interrenal tissue, in yellow perch within the same lake.

Tissue MT concentrations in pooled samples of liver or interrenal tissue of yellow perch increased progressively from the least to the most contaminated lakes: fish from the two reference sites (Lakes Opasatica and Dasserat) had the lowest MT concentrations, and fish from the two most contaminated lakes (Lakes Osisko and Dufault) had the highest levels (Fig. 1). Similarly to the concentrations of metals, MT levels were higher in liver compared with the interrenal tissue in yellow perch within the same lake. MT concentrations correlated well with the concentrations of each metal (Zn, Cu, and Cd) in both tissues (Table 3). In the liver, MT levels highly correlated in a linear way with the sum of the metals, [Zn + Cu +Zn] (Fig. 2*a*). In the interrenal tissue, MT concentrations highly correlated in a linear way with the sum of Cu and Cd, [Cu + Cd] (Fig. 2*c*), but the relationship between MT levels and the summation of the three metals seemed to reach a plateau (Fig. 2*b*).

Cortisol response

Following a standardized stress test, plasma cortisol levels were generally higher in sampled adult yellow perch from reference lakes (Lakes Opasatica and Dasserat) and an intermediate lake (Lake Vaudray) compared with the most contaminated lakes (Lakes Osisko and Dufault) (Fig. 3). Fish from Lake Bousquet (intermediate) had higher plasma cortisol levels than fish from Lake Dufault but not fish from Lake Osisko. In vitro cortisol secretion by the interrenal tissue in response to 2 IU of porcine ACTH or 4 mmol of dbcAMP stimulation was higher in interrenal tissue from the reference lake (Lake Opasatica) compared with Lake Osisko, the highly contaminated lake (Fig. 4). Within each lake, no difference in the cortisol secretion was observed between the ACTH and dbcAMP stimulated responses. No difference was detected for the unstimulated interrenal tissue secretion between the two lakes (Fig. 4).

Blood parameters before and after a standardized stress test

Plasma chloride, glucose, and cortisol levels were compared (Table 4; Fig. 3) between a yellow perch group subjected to a standardized confinement stress test and a group sampled without the stress test from three lakes: Lake Opasatica (reference), Lake Vaudray (intermediate contamination), and Lake Osisko (high contamination). Plasma cortisol levels were higher in the group after the standardized stress test compared with the unstressed group for each lake (means from unstressed fish ranged between 82.9 and 118.9 $ng \cdot mL^{-1}$ and were not significantly different between the three lakes; data not shown). Plasma chloride levels within each type of stress status group were not different between the lakes. Stressed yellow perch from Lakes Opasatica and Osisko, but not Lake Vaudray, had lower plasma chloride levels compared with unstressed fish from the same lake. Stressed fish from the highly contaminated lake had lower glucose levels than stressed fish from the less contaminated lake. Plasma glucose levels of the stressed fish were higher in Lakes Opasatica and Vaudray compared with those of the unstressed fish from the same lake. No difference in plasma glucose levels between stressed and unstressed yellow perch groups from the highly contaminated lake (Lake Osisko) was observed. (Table 4).

Discussion

Exposure of yellow perch was characterized by measuring metals in the liver and the interrenal tissue. Concentrations of Zn, Cu, and Cd in the liver of wild yellow perch sampled in the present study increased progressively from the least to the most contaminated lake: the six lakes sampled thus provide an excellent in situ system for investigating the effects of metal contamination on the physiology of resident yellow perch. Levels of Cu and Cd in the liver of yellow perch from our reference lakes were similar to those reported for wild European perch, *Perca fluviatilis*, from reference sites by Olsson and Haux (1986). Concentrations of Cd in the liver of yellow perch from the two most contaminated lakes (Lakes Osisko and Dufault) ranged from 41 to 61 $\mu g \cdot g$ dry $weight^{-1}$. These values were higher than mean hepatic Cd levels from the most contaminated sites in various in situ studies with either European perch (6–31 $\mu g \cdot g$ liver dry $weight^{-1}$; Olsson and Haux 1986; Hogstrand et al. 1991) or salmonids (2–19 $\mu g \cdot g$ liver dry $weight^{-1}$; Roch et al. 1982; Farag et al. 1995).

This was the first study to characterize metal concentrations in the interrenal tissue of wild fish. Similar to Zn, Cu, and Cd levels in the liver, concentrations of metals in the

Table 2. Characteristics (mean ± SE) of adult yellow perch collected in six lakes of the Rouyn-Noranda mining region.

	N	Length (cm)	Weight (g)	Hepatosomatic index (%)	Condition factor
Reference lakes					
Opasatica	31	21.1±0.6 *a*	100.1±7.8 *a*	1.20±0.05 *b*	1.13±0.02 *ab*
Dasserat	63	17.6±3.3 *b*	67.1±3.9 *b*	1.44±0.02 *a*	1.15±0.01 *a*
Intermediate-contamination lakes					
Bousquet	32	17.7±0.5 *b*	70.0±6.1 *b*	1.07±0.02 *bc*	1.16±0.02 *a*
Vaudray	32	17.4±0.4 *b*	58.3±3.4 *b*	1.51±0.03 *a*	1.08±0.01 *b*
High-contamination lakes					
Osisko	38	20.2±0.2 *b*	73.8±2.7 *b*	1.12±0.04 *bc*	0.88±0.01 *c*
Dufault	47	14.0±0.2 *c*	25.7±1.5 *c*	1.06±0.03 *c*	0.90±0.01 *c*

Note: Means followed by the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$).

Table 3. Concentrations of Zn, Cu, and Cd (mean ± SE, μg·g dry weight^{-1}) and correlations (Pearson's *r*) between concentrations of MT (nmol metal binding sites·g dry weight^{-1}) and metal (M) in pooled samples of liver or interrenal tissue of adult yellow perch collected in six lakes of the Rouyn-Noranda mining area.

	Liver				Interrenal tissue			
	N	[Zn]	[Cu]	[Cd]	*N*	[Zn]	[Cu]	[Cd]
Reference lakes								
Opasatica	8	92.4±3.6 *a*	10.4±1.8 *a*	2.9±0.4 *a*	8	104.6±2.3 *a*	2.3±0.2 *a*	0.9±0.1 *a*
Dasserat	7	98.6±4.2 *ab*	10.8±0.9 *a*	5.3±0.6 *b*	8	118.8±2.6 *b*	2.9±0.3 *a*	1.7±0.2 *b*
Intermediate-contamination lakes								
Bousquet	7	106.5±5.4 *ab*	20.4±4.5 *b*	20.3±2.9 *c*	8	96.08±1.2 *a*	2.2±0.1 *a*	3.8±0.2 *c*
Vaudray	8	108.9±1.6 *b*	12.9±0.7 *ab*	25.1±1.7 *c*	8	127.6±4.7 *b*	2.7±0.2 *a*	5.7±0.7 *cd*
High-contamination lakes								
Osisko	8	177.2±9.0 *c*	246.5±29.8 *c*	45.7±3.2 *d*	8	153.6±6.6 *c*	6.5±1.0 *b*	8.0±0.5 *de*
Dufault	8	151.1±3.7 *c*	148.5±11.1 *c*	61.3±5.3 *d*	4	227±10.3 *d*	6.4±1.5 *b*	12.6±0.4 *e*
Correlation between [MT] and [M]	46	0.93**	0.95**	0.91**	36	0.82**	0.77**	0.95**

Note: Means followed by the same letter are not significantly different; comparison between lakes only (Tukey–Kramer test, $P < 0.01$). **$P < 0.001$ (Pearson's test).

interrenal tissue also increased along the gradient of water and sediment contamination. The levels of Zn in the interrenal tissue were similar to concentrations in the liver, possibly reflecting basal homeostatically regulated physiological concentrations. However, Cu and Cd levels were much greater in the liver than in the interrenal tissue: in yellow perch from the most contaminated lakes, Cu levels in liver were 25- to 35-fold higher than the interrenal tissue levels. Studies in salmonids have shown higher Cd concentrations in the kidney compared with the liver after chronic exposure to Cd in the laboratory (Harrison and Klaverkamp 1989) and in the field (Farag et al. 1995). Preliminary analyses of kidney metal concentrations suggested that the tissue distribution was similar in wild yellow perch (H. Levesque, Département des Sciences Biologiques, Université du Québec à Montréal, personal communication). Thus, it could be expected that Cd levels in the interrenal tissue, which is located in the head kidney, would be greater than liver concentrations. However, our data clearly showed that Cd accumulates more in the liver of yellow perch than in the head kidney (around a fivefold difference in the most contaminated lakes).

The metal exposure of the yellow perch was further characterized by measures of MT. MT has been proposed in various studies as a biomarker of exposure to base metals. In Canada, the use of MT to monitor the effects of the mining industry is under technical evaluation (Couillard and St-Cyr 1997). Our data showed a significant difference in MT levels between reference, intermediate-contaminated, and highly contaminated lakes in the liver and in the interrenal tissue. Liver MT levels of yellow perch from the contaminated lakes were 10-fold higher than those of yellow perch from the reference lakes; interrenal MT levels were about threefold higher. This was the first study to measure MT levels in the interrenal tissue of wild fish and to demonstrate its increase related to metal levels. However, to prove unequivocally that MT is synthesized de novo in the interrenal tissue, it would be necessary to detect MT mRNA in the interrenal tissue and characterize the structure of the interrenal MT.

There is some evidence in the literature that MT levels correlate with metal (Zn, Cu, or Cd) levels in the liver of salmonids and European perch exposed in the laboratory or in the field (Hogstrand et al. 1991; Marr et al. 1995; Dallinger et al. 1997). In our study, MT levels in the liver and the interrenal tissue correlated strongly with the levels of Cd, Zn, or Cu. Since all three metals increased gradually in both tissues of yellow perch from the six lakes, no one metal could be identified as a putative inducer of MT synthesis. It is interesting that MT in the interrenal tissue strongly correlated in a linear way with Cu and Cd summation, but with the addition of Zn to the correlation, MT pro-

Fig. 1. MT concentrations (mean ± SE) in pooled samples of (*a*) liver and (*b*) interrenal tissue of adult yellow perch collected from six lakes of the Rouyn-Noranda mining region. Means followed by the same letter are not significantly different (Tukey–Kramer test, $P < 0.01$). For each lake, $N = 8$ pooled samples. OP, Lake Opasatica; DS, Lake Dasserat; BO, Lake Bousquet; VA, Lake Vaudray; OS, Lake Osisko; DT, Lake Dufault. Lakes were ranked as reference (R), intermediate contaminated (I), or highly contaminated (H) according to the concentrations of metals accumulated in tissues.

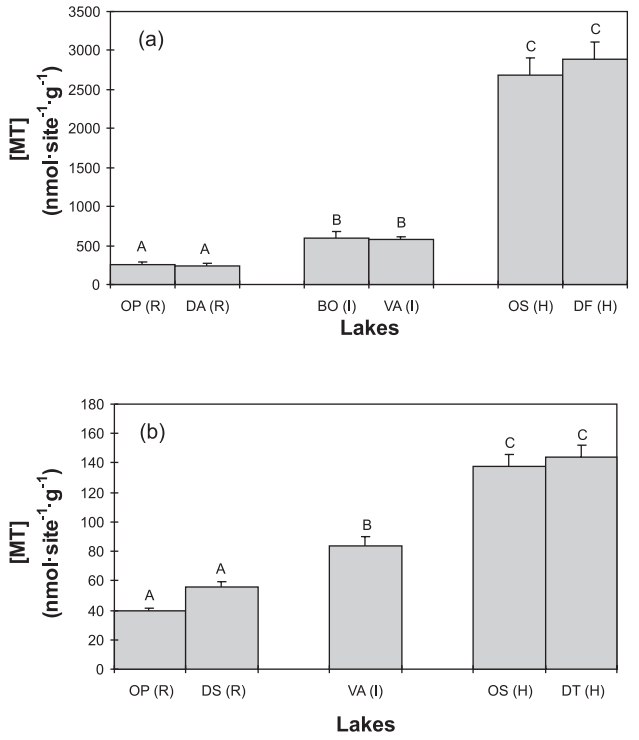
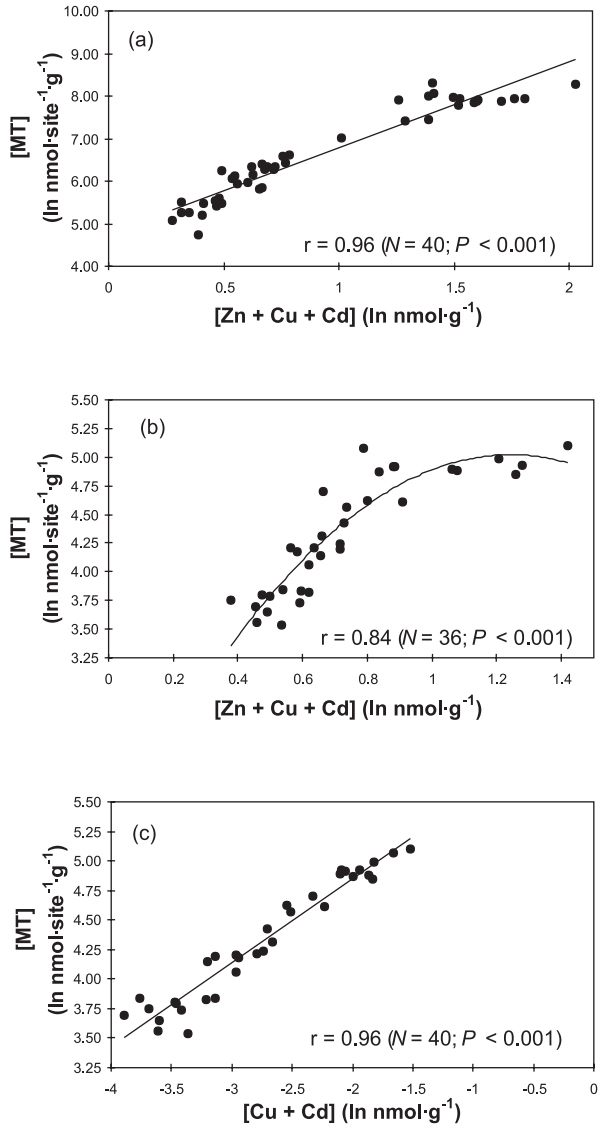


Fig. 2. Relationship (Pearson's *r*) between MT concentrations (ln nmol metal binding sites·g dry weight^{-1}) and summation of metals (ln nmol·g dry weight^{-1}) in combined samples of (*a*) liver and (*b* and *c*) interrenal tissue in adult yellow perch collected from six lakes of the Rouyn-Noranda mining region. OP, Lake Opasatica; DS, Lake Dasserat; BO, Lake Bousquet; VA, Lake Vaudray; OS, Lake Osisko; DT, Lake Dufault.



duction reached a plateau. This might indicate a Zn spillover onto other cytosolic ligands, but further investigations of cytotoxicity and subcellular partitioning of the metals would be needed to support this hypothesis.

Once the metal exposure and the MT response had been quantified in the liver, an important site of metal accumulation in the fish (Heath 1995), and in the interrenal tissue, a component of the hypothalamo–pituitary–interrenal axis, the link between contamination and physiological effects could be investigated. The physiological capacity to respond to a stressor is a fundamental endocrine response (Hontela 1997; Wendelaar Bonga 1997), and the results of our in vivo and in vitro functional tests of the interrenal tissue indicated an impairment of the cortisol stress response in adult yellow perch from the highly contaminated lakes. Following the standardized confinement stress test, yellow perch from the two most contaminated lakes had lower plasma cortisol levels than those from the reference lakes. Three previous studies in metal-contaminated sites also showed lower cortisol response either postcapture (Lockhart et al. 1972; Brodeur et al. 1997*a*) or after a 1-h confinement (Norris et al. 1999). If we compare the pattern of plasma cortisol levels in confinement-stressed yellow perch and MT levels, in relation to contamination, MT appears to be more sensitive as an indicator of metal exposure, since a significant difference is detected between each degree of contamination, while a difference in the capacity to generate the cortisol stress response is observed only between reference and the most contaminated lakes. This finding conforms to previous models of sensitivity at different levels of biological organization (Adams 1990), since the endocrine stress response represents a higher level of response than induction of MT, a biomarker of exposure. The in vivo evidence for an interrenal dysfunction in yellow perch chronically exposed to high ambient metal concentrations in the field was further supported by the in vitro experiment. Cortisol secretion of the interrenal tissue stimulated by optimal concentrations of ACTH or dbcAMP, an agonist of the second messenger cAMP, was

Table 4. Plasma chloride and glucose (means ± SE (*N*)) in an unstressed group and a stressed group (subjected to a standardized stress test) of adult yellow perch collected from three lakes of the Rouyn-Noranda mining area.

	Chloride ($mg \cdot mL^{-1}$)		Glucose ($mg \cdot mL^{-1}$)	
Lake	No stress	Stress	No stress	Stress
Opasatica (reference)	141±4 (22)	126±4 (29)*	0.14±0.02 (22)	0.26±0.02 (31)*
Vaudray (intermediate)	146±16 (11)	129±8 (29)	0.33±0.03 (11)*	0.44±0.02 (31)*#
Osisko (high)	149±6 (21)	116±2 (31)*	0.18±0.03 (21)	0.18±0.01 (38)#

Note: Lakes were ranked as reference, intermediate contaminated, or highly contaminated according to the concentrations of metals accumulated in tissues. *Significantly different from the no-stress group (*t* test, $P < 0.05$); #significantly different from the other two lakes (*t* test, $P < 0.05$).

Fig. 3. Plasma cortisol levels (mean ± SE) after a standardized stress test in adult yellow perch collected from six lakes of the Rouyn-Noranda mining region. Means followed by the same letter are not significantly different (Tukey–Kramer test, $P < 0.05$). Numbers of fish sampled in each lake are indicated inside the bars. Lakes were ranked as reference (R), intermediate contaminated (I), or highly contaminated (H) according to the concentrations of metals accumulated in tissues.

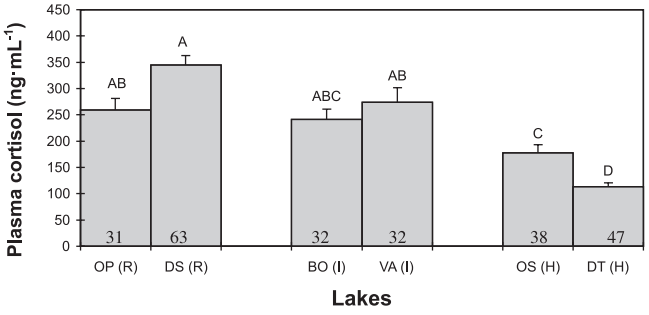
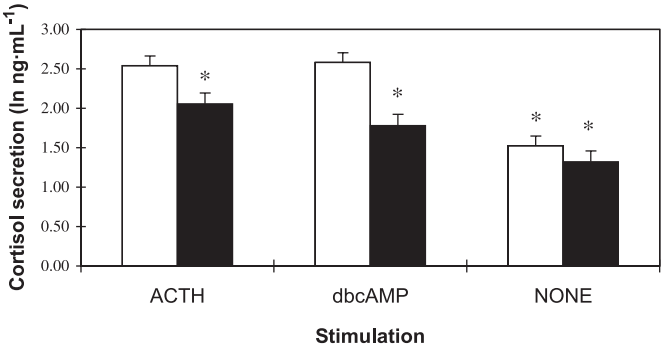


Fig. 4. In vitro cortisol secretion (least squares mean ± SE for the mean value of basal cortisol secretion before stimulation) of the interrenal tissue of adult yellow perch from a reference lake (Lake Opasatica, open bars, $N = 22$) and a contaminated lake (Lake Osisko, solid bars, $N = 17$) stimulated by 2 IU of porcine ACTH, 4 mmol of dbcAMP, or not stimulated. *Significantly different from the stimulated interrenal tissue of the reference lake (ANCOVA test, $P < 0.05$).



also lower in yellow perch from the most contaminated lake. It is the capacity to respond to stimulation that was different between interrenals from the reference and contaminated lakes; the basal cortisol secretion was the same. The interest in comparing cortisol output following ACTH, which interacts with a membrane receptor, or dbcAMP, which diffuses directly into the cells, bypassing the membrane receptor, was to determine if cortisol secretion in fish exposed to metals is affected at the membrane receptor or postmembrane steps. Indeed, since cortisol secretion in response to ACTH was impaired in interrenals from fish sampled in Lake Osisko, the most contaminated lake, and stimulation with dbcAMP could not restore the secretory response, we can postulate that metals disrupt cortisol biosynthesis at multiple steps, including postmembrane steps. Similar results were obtained in a laboratory study using rainbow trout, *Oncorhynchus mykiss*, interrenal cell suspensions exposed in vitro to Cd and Zn (Leblond and Hontela 1999).

Even though further studies are needed to elucidate the precise mechanisms through which chronic exposure to metals alters the normal capacity to generate the hormonal stress response, the present study provided new data linking field exposure to metals to an endocrine dysfunction. The physiological consequences of this dysfunction were also investigated in the present study. Lower plasma chloride levels have been reported in wild fish from metal-contaminated sites or in fish exposed to metals in laboratory experiment (Larsson et al. 1985). Cortisol promotes proliferation and differentiation of chloride cells in the gills, increasing chloride body influxes but not plasma chloride levels (Laurent and Perry 1990; Madsen 1990). Despite differences in cortisol status and in tissue metal burdens between fish from different lakes in our study, difference in chloride levels were not detected. Stressed fish did, however, have lower plasma chloride levels compared with unstressed fish from the same lake, probably reflecting osmoregulatory disturbances due to the 1-h confinement stress.

A rise in plasma glucose is another expected response to stress (Barton and Iwama 1991). In our study, stressed fish from the reference and intermediate lakes were able to raise their plasma glucose levels compared with unstressed fish from the same lake, but this capacity was significantly inhibited in yellow perch from the most contaminated lake. The mechanisms underlying this metabolic dysfunction are under investigation in our laboratory; disruption of corticosteroid or catecholamine synthesis, abnormal gluconeogenesis, and (or) exhaustion of the energy reserves are considered. Fish from the two most contaminated lakes also had a lower condition factor, an indication of decreased energy reserves (Goede and Barton 1990). Many biotic and abiotic variables, other than xenobiotics, influence the growth and condition factor of fish. However, there is some evidence suggesting a significant metabolic cost associated with resisting chemical contaminants (Heath 1995). A recent study by Sherwood et

al. (2000) provided evidence that yellow perch from the two most contaminated lakes (Lakes Osisko and Dufault) have lower growth efficiency (capacity to convert consumed food into body mass) compared with yellow perch from the reference lakes. Our data on lower condition of the same yellow perch populations gave further support to the evidence for growth impairment in yellow perch subjected to chronic environmental exposures to metals.

This was the first study to characterize metals and MT levels in the interrenal tissue of wild fish and to demonstrate that metals increase in the interrenal tissue in relation to exposure, that MT is present in this tissue, and that its levels are related to tissue metal concentrations. Clear differences in MT levels between reference, intermediate-contaminated, and highly contaminated lakes were observed in the liver and the interrenal tissues of yellow perch. There was also an exposure-related impairment of the cortisol response in vivo and of the capacity to respond to ACTH and dbcAMP in vitro. In addition, fish from the most contaminated lakes exhibited a metabolic impairment characterized by a lower capacity to increase plasma glucose in response to a confinement stress and a lower condition factor.

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