Disruption of the hypothalamo-pituitary-interrenal axis in 1+ yellow perch (*Perca flavescens*) chronically exposed to metals in the environment

A. Gravel, P.G.C. Campbell, and A. Hontela

Abstract: Although it has been reported that adult yellow perch (*Perca flavescens*) chronically exposed to metals in the environment exhibit endocrine impairment characterized by blunted cortisol secretion, little is known about the vulnerability of early life stages. Young-of-the-year (YOY) and 1+ yellow perch were captured, subjected to a standardized stress test or adrenocorticotropic-hormone stimulation in lakes situated along a contamination gradient of Cd, Cu, and Zn in the mining region of Abitibi, Quebec. For the first time, whole-body cortisol concentrations were measured. The 1+ fish with elevated whole-body Cd, Cu, and Zn concentrations had an impaired capacity to respond to an acute stress challenge. Although YOY perch had similar whole-body Cd concentrations to 1+ perch, no effects on physiological status were detected in relation to body burdens of metals. Metal contamination did not affect whole-body thyroid-hormone concentrations, condition factor, or hepatosomatic index in 1+ or YOY perch. These results indicate that effects of Cd, Cu, and Zn on the functional integrity of the hypothalamo-pituitary-interrenal axis in yellow perch are detectable after only 1 year of environmental exposure.

Résumé : Bien qu'on ait signalé que les perchaudes adultes exposées de façon chronique aux métaux dans leur environnement subissent une déficience endocrinienne, caractérisée par une sécrétion inadéquate de cortisol, on connaît mal la vulnérabilité des jeunes stades. Nous avons capturé des jeunes de l'année (YOY) et d'âge 1+ de la perchaude (*Perca flavescens*) et les avons soumis à une épreuve de stress standardisée ou à une stimulation à l'ACTH (l'hormone adrénocorticotrope) dans des lacs situés le long d'un gradient de contamination au Cd, au Cu et au Zn dans la région minière de l'Abitibi, Québec. Nous avons mesuré pour la première fois les concentrations corporelles globales de cortisol. Les poissons (1+) ayant des concentrations corporelles élevées de Cd, de Cu et de Zn ont une capacité réduite de réagir à une épreuve aiguë de stress. Bien que les perchaudes YOY aient des concentrations corporelles de Cd semblables à celles des perchaudes 1+, nous n'avons pas mesuré d'effet sur l'état physiologique en fonction des charges corporelles de métaux. La contamination par les métaux chez les perchaudes YOY et 1+ n'affecte pas les concentrations corporelles d'hormone thyroïdienne, ni le coefficient de condition, ni l'indice hépatosomatique (HSI). Ces résultats indiquent que les effets d'expositions au Cd, au Cu et au Zn dans le milieu sur l'intégrité fonctionnelle de l'axe hypothalamo-hypophyso-interrénal (HPI) ne sont décelables chez la perchaude qu'après une année d'exposition.

[Traduit par la Rédaction]

Introduction

Metals are ubiquitous pollutants detected in the aquatic environment, where they can accumulate at different trophic levels (Per-Arne et al. 1997; Mason et al. 2000). In fish, metals are absorbed by the digestive tract and gills (Verbost et al. 1989) and can accumulate in different organs such as kidney, liver, and interrenal tissue (Laflamme et al. 2000; Lévesque et al. 2002, 2003). Although some metals (Zn, Cu, Co, Fe, Mn, Mg, Mo, Se, Ni) are essential for normal physiological processes, Cd, Hg, Pb, and As (nonessential metals) do not have a known biological function in animals. In excess concentrations, all metals have toxic effects, ranging from histological lesions (Forlin et al. 1986; Lévesque et al. 2003) to disruption of metabolic and endocrine functions (Lévesque et al. 2002, 2003; Campbell et al. 2003).

Impairment of the hypothalamo-pituitary-interrenal (HPI) axis, characterized by lower plasma cortisol levels in response to acute stress or adrenocorticotropic-hormone (ACTH) stimulation in vivo has been reported in adults of

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several species of fish sampled from metal-contaminated sites (Lockhart et al. 1972; Hontela et al. 1995; Lévesque et al. 2003). Since cortisol plays an important role in osmoregulation (Laurent and Perry 1990), immune defence (Wendelaar Bonga 1997), reproduction (Pankhurst and Van Der Kraak 2000), and metabolism (Vijayan and Moon 1992; Vijayan et al. 1997; Mommsen et al. 1999), it is important to understand the mechanisms and physiological consequences of pollutant-induced disruption of the HPI axis.

Cortisol interacts with other hormones such as thyroid hormones and growth hormone through permissive actions (Young and Lin 1988; De Jesus et al. 1990). It has been shown that cortisol-impaired adult yellow perch (*Perca flavescens*) from metal-contaminated lakes had lower plasma triiodothyronine (T_3) and thyroxine (T_4) levels (Lévesque et al. 2003), a lower condition factor (Laflamme et al. 2000; Sherwood et al. 2000; Eastwood and Couture 2002), and reduced growth efficiency (Sherwood et al. 2002), i.e., the capacity to convert consumed food to body mass.

Cortisol and T_4 have synergistic effects during larval development of fish, promoting growth and larval differentiation and modulating metabolism (Plisetskaya et al. 1983; De Jesus et al. 1990). Although eggs, larvae, and early juveniles are the stages in the life cycle of a fish that are the most vulnerable to pollutants, and also the most vulnerable in populations according to some authors (McKim 1977; von Westernhagen 1988; Sorensen 1991), few studies have investigated the effects of metals on the metabolic and endocrine functions of young fish exposed in the field. Moreover, the minimum duration of field exposure to metals that is necessary to cause physiological anomalies has not so far been determined.

The objectives of the present study were, therefore, to assess the physiological status and metal concentrations in young-of-the-year (YOY) and 1+ yellow perch (*Perca flavescens*) in metal-contaminated lakes situated along a contamination gradient in a mining region. To evaluate the functional integrity of the HPI axis in young fish sampled in the field and too small to blood-sample, cortisol and thyroid-hormone levels following capture of YOY or following a confinement-stress test or an ACTH injection for 1+ fish, and concentrations of metals accumulated in the fish, were measured in the whole body.

Materials and methods

Study sites

Fish were sampled in six lakes in the mining area of Rouyn-Noranda, northwestern Quebec, Canada (48°00′N, 79°00′W), in June of 1999, 2000, and 2001. Lakes in the area are affected primarily by their proximity to the Horne smelter and atmospheric deposition brought by dominant winds, and also by mining operations and abandoned mine sites in the area. Previous studies (Laflamme et al. 2000; Perceval et al. 2002; Lévesque et al. 2003) provided data on metal concentrations in water, sediments, and yellow perch tissues that enabled us to make a preliminary ranking of the lakes based on contamination levels (Fig. 1). Lakes Osisko and Dufault, subjected to point-source and atmospheric metal inputs, are classified as high-contamination lakes; Lakes Vaudray and Bousquet, affected by metals via atmospheric transport, are classified as intermediate-contamination lakes; and Lakes Dasserat and Opasatica, upwind from the smelter, are classified as reference lakes. Limnological characteristics of the lakes are detailed in Sherwood et al. (2002) and Perceval et al. (2002).

Capture of the fish

The yellow perch was chosen for this study because of its relative sedentariness (Aalto and Newsome 1990), tissue metal levels that reflect the contamination of the sites where it is sampled, and its abundance in North American lakes. Age 1+ yellow perch (average body mass 2.5 g) were captured by seine or small gill net. They were placed in floating enclosures (1 m × 1 m × 0.5 m depth) made of nylon net subdivided into four individual compartments (15 fish per compartment) near shore for at least 16 h to allow recovery from the stress of capture, as demonstrated previously (Brodeur et al. 1997; Girard et al. 1998). YOY perch (body mass 0.006–0.12 g) were captured at night with a mysis net and immediately frozen at –20 °C.

Stress test and ACTH-challenge test for 1+ fish

The day after capture, 1+ fish were removed from the enclosures and subjected to a standardized confinement and net stress test. Each group of 15 fish was held in a small aquarium net and taken in and out of the water for 90 s. Fish were then placed back in the compartment for 15, 30, 45, 60, or 120 min and euthanized with 150 $mg \cdot L^{-1}$ tricaine methanesulfonate (MS 222). One group of 15 fish was removed from the compartment and euthanized directly in the MS 222 solution, without the confinement-stress test. These fish are referred to as the basal group.

The last two groups of fish that did not undergo the net stress were subjected to the ACTH-challenge test (Girard et al. 1998) modified for use with young fish. Following light anaesthesia in MS 222, one group (ACTH group) received an intraperitoneal injection of 4 IU ACTH·100 g body $mass^{-1} \cdot 100$ µL saline (0.7% NaCl)$^{-1}$ and the control group (saline group) received an intraperitoneal injection of 100 µL saline·100 g body $mass^{-1}$. After injection, fish were put back in their compartments to recover from the anaesthesia, which occurred within a few minutes. These two groups were euthanized in MS 222 after 120 min.

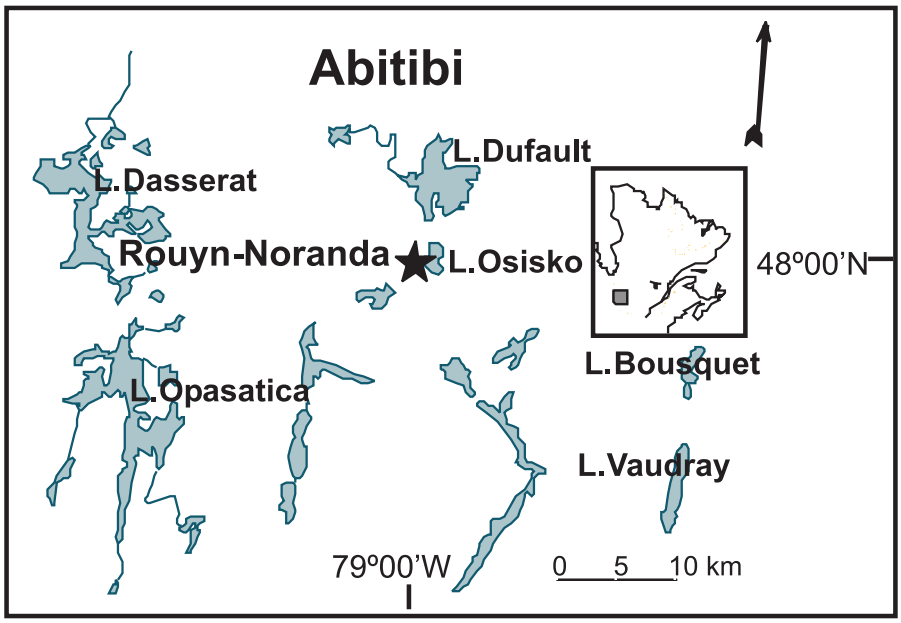
All fish were treated during the same period of the day to avoid diel effects on hormone secretion (Audet and Claireaux 1992), and after treatment they were frozen whole at –20 °C for later analysis in the laboratory.

Sample preparation

In the laboratory, body mass and length of thawed fish were recorded for calculating the condition factor, $(g \cdot cm^{-3}) \times 100$. Livers of 1+ fish were dissected out for glycogen analysis and calculation of the hepatosomatic index (HSI), $(g \cdot g^{-1}) \times 100$, and opercular bones were removed for age determination. Identification of YOY was confirmed in the laboratory using identification keys (Norden 1961; Auer 1982).

Because of the small body mass of these fish and difficulty with blood sampling in the field, an alternative method of hormone assessment was required. The whole body of each fish (without the liver in 1+ perch) was homogenized in three volumes (mL) of phosphate buffer (0.168 g KH_2PO_4

Fig. 1. Map showing the study area with the six lakes sampled in the Rouyn-Noranda region in Quebec, Canada, and the location (★) of the Horne smelter. The reference lakes are Dasserat (DS) and Opasatica (OP); the intermediate-contamination lakes are Bousquet (BO) and Vaudray (VD); and the high-contamination lakes are Osisko (OS) and Dufault (DF). The direction of dominant winds is west to east.



and 1.26 g Na_2HPO_4 in 1 L of deionized water), 0.01 mol·L^{-1} at pH 7.5. Homogenates of YOY perch were made up from pools of fish to obtain a final weight of about 2.0 g. One aliquot of the homogenate was used for metal analysis by inductively coupled plasma atomic emission spectrophotometry (ICP-AES), and a second aliquot of the homogenate was centrifuged (13 000*g*) three times for 10 min each time and the supernatant was frozen (–20 °C) for hormone analyses.

Biochemical analyses

Total body cortisol, T_4, and T_3 were assayed with a radioimmunoassay (ICN Biochemicals No. 07-221102; ICN No. 06B263676; ICN No. 06B254221) according to the manufacturer's protocol. The characteristics of the assays were described previously (Hontela et al. 1995). Liver glycogen was measured by a method described previously (Lévesque et al. 2002).

Statistical analysis

Differences among groups were tested using one-way analysis of variance followed by a Tukey–Kramer test to compare more than two groups, or a Student's *t* test. Data were transformed when necessary to obtain normality. A statistical significance level of $\alpha = 0.05$ was used. Relationships between plasma cortisol and total body cortisol levels in the homogenates were evaluated with Pearson's correlation coefficient.

Results

Whole-body metal concentrations

Whole-body concentrations of Cd in YOY (Fig. 2*a*) and 1+ yellow perch (Fig. 2*b*) increased along a contamination gradient among lakes. Differences in total body concentrations of Ni or Pb in YOY and 1+ fish either did not vary significantly among lakes or did not follow a consistent gradient, while Zn and Cu concentrations were higher in fish sampled in Osisko and Dufault, the most contaminated lakes (Table 1).

Stress response

The stress test and the use of whole-body cortisol levels were initially validated in a pilot field study with 1+ yellow perch (Fig. 3*a*) from a reference site near Montreal (Île Perrot) and in a laboratory study with 1+ rainbow trout (*Oncorhynchus mykiss*). A significant ($P < 0.006$) linear correlation ($r^2 = 0.38$) was established between plasma and body cortisol concentrations in the laboratory test with rainbow trout (data not shown). The pilot field study with 1+ yellow perch (Fig. 3*a*) demonstrated that body cortisol concentrations increased significantly with time following a net-stress test lasting 90 s and reached a maximum value (4× the resting basal value) after 45 min, maintained for up to 2 h. Homogenates of whole fish were either extracted as described in De Jesus et al. (1991) or simply centrifuged prior to radioimmunoassay. The pattern of the stress response was similar with both assay methods but whole-body cortisol concentrations after extraction were about 25% of the concentrations in homogenates that were only centrifuged. Based on the results from the pilot study, the maximal time allocated for hormone secretion post stressor exposure was 120 min and the assay method chosen for hormone analysis of the whole body of small fish for the present project was homogenization without extraction.

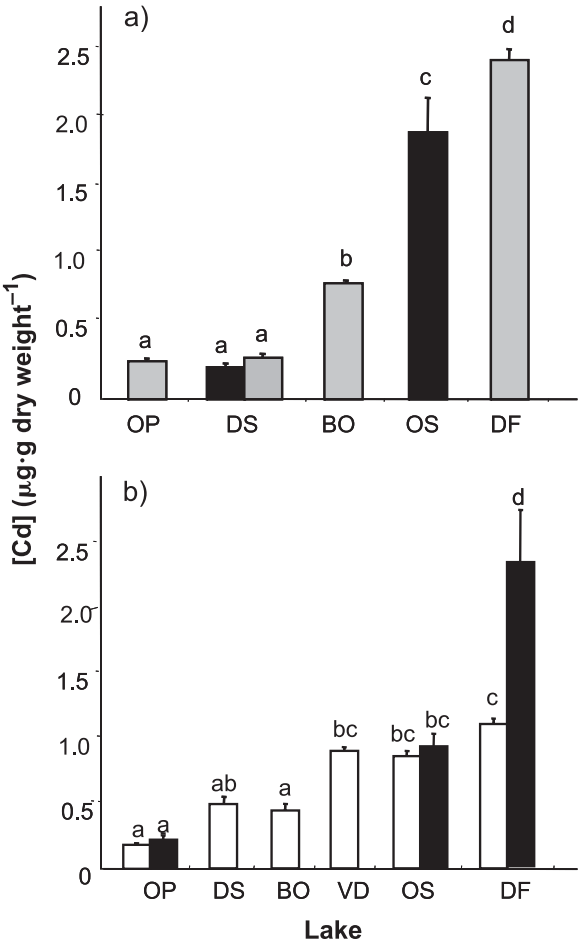
The sampling was repeated in a reference lake in the Rouyn-Noranda region (Fig. 3*b*). Whole-body cortisol concentrations in 1+ yellow perch increased significantly 30 min following a net and confinement stress test in a reference lake, and the maximal value (twice the resting value)

Table 1. Whole-body concentrations of Cu, Zn, Pb, and Ni (mean ± standard error of the mean; $\mu g \cdot g^{-1}$ dry weight) in young-of-the-year (YOY) and 1^+ yellow perch, *Perca flavescens*, in six lakes in the Rouyn-Noranda region.

	YOY yellow perch						1+ yellow perch					
Lake	Year	*n*	[Cu]	[Zn]	[Pb]	[Ni]	Year	*n*	[Cu]	[Zn]	[Pb]	[Ni]
OP	2001	8	7.6±0.3c	97.6±0.8a	1.25±0.04a	2.0±0.2a	1999	22	5.8±0.5b	153.7±4.9b	1.4±0.1de	18.1±3.4cd
							2000	10	5.7±0.3bc	132.0±4.7ab	0.6±0.02ab	20.0±2.3d
DS	2000	6	5.5±0.3ab	126.7±1.2b	1.07±0.02a	24.0±3.6a	1999	15	5.1±0.3ab	144.4±9.1b	0.7±0.02b	53.6±26.7d
	2001	13	5.4±0.3a	145.0±2.7c	1.05±0.04a	2.2±0.2a						
BO	2001	16	6.6±0.3b	119.3±0.9b	1.01±0.03a	5.1±0.6b	1999	21	4.8±0.3ab	124.9±4.7ab	1.1±0.03d	14.6±3.7cd
VD							1999	14	5.2±0.4ab	145.5±2.9ab	0.8±0.04bcd	12.7±5.9bc
OS	2000	6	14.1±0.5e	142.1±1.0c	1.10±0.04a	29.5±5.2c	1999	15	3.8±0.2a	114.9±2.2a	0.6±0.1a	10.1±1.0cd
							2000	10	4.2±0.4ab	124.4±3.2ab	0.7±0.03ab	0.4±0.07a
DF	2001	12	11.8±0.5d	149.7±1.4c	1.94±0.22b	3.2±0.5ab	1999	23	4.5±0.4ab	175.8±6.5c	1.6±0.1ef	4.2±1.2b
							2000	10	9.3±1.9c	176.7±13.0c	2.4±0.4f	13.5±2.8cd

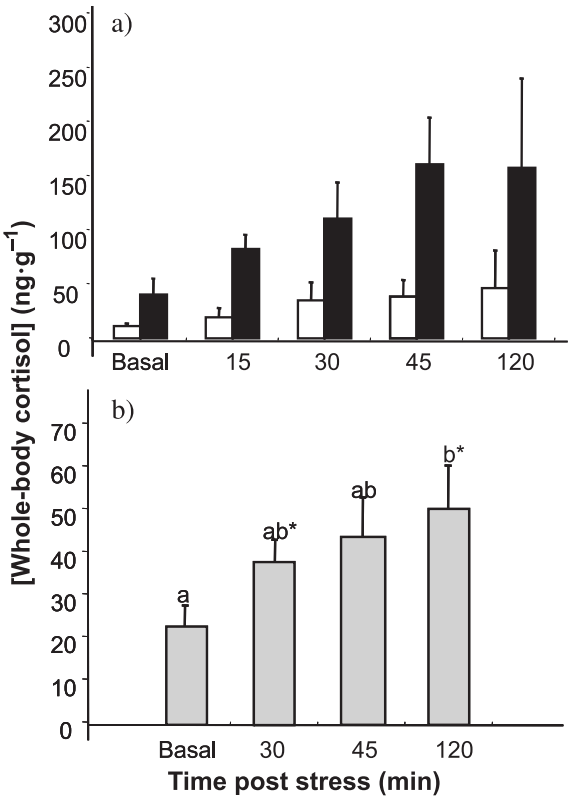
Note: Values followed by a different letter are significantly different (Tukey–Kramer test, $P < 0.05$) from those for the other lakes; *n* is the number of 1+ fish or the number of pools of YOY. Opasatica (OP) and Dasserat (DS) are reference lakes, Bousquet (BO) and Vaudray (VD) are intermediate-contamination lakes, and Osisko (OS) and Dufault (DF) are high-contamination lakes.

Fig. 2. Whole-body Cd concentrations (mean ± standard error of the mean) in young-of-the-year (YOY) (*a*) and 1+ yellow perch, *Perca flavescens* (*b*), from metal-contaminated lakes sampled in 1999 (open bars), 2000 (solid bars), or 2001 (shaded bars) in the Rouyn-Noranda region. Opasatica (OP) and Dasserat (DS) were the reference lakes, Bousquet (BO) and Vaudray (VD) were intermediate-contamination lakes, and Osisko (OS) and Dufault (DF) were the most contaminated lakes. The samples consisted of 6–16 pools of YOY and 10–23 individual 1+ fish. A different letter above the bar indicates a significant difference (Tukey–Kramer test, $P < 0.05$) from the other lakes.



was reached 2 h post stressor exposure. Cortisol concentrations attained in fish from the reference site in Île Perrot (not situated in a mining region) were significantly higher than those in fish from the reference lake in the Rouyn-Noranda mining region. Whole-body cortisol concentrations in the two Rouyn-Noranda reference lakes (Opasatica and Dasserat) increased significantly above basal levels in response to the net stress at 30 min, increasing still further after 120 min in fish from Lake Opasatica, the cleanest lake (Fig. 4). Although cortisol levels after exposure to the stress test were elevated in fish from the intermediate-contamination lakes (Bousquet and Vaudray), no detectable increase in body cortisol concentrations following the stress test was detected in the most contaminated fish, those from Lake Dufault.

Fig. 3. Validation of the standardized stress test and the whole-body cortisol assay. Whole-body cortisol concentrations (mean ± standard error of the mean) before (basal) and after a stress test in 1+ yellow perch from a reference lake in a pilot study (*a*) at Île Perrot and (*b*) in the Rouyn-Noranda region. Cortisol was measured in the homogenate after extraction (open bars) or centrifugation only (solid and shaded bars). Number of fish sampled was 9–10 each for Île Perrot and 12–15 in the Rouyn-Noranda region. A different letter above the bar indicates a significant difference (Tukey–Kramer test, $P < 0.05$) from the other groups. An asterisk indicates a significant difference (Student's *t* test, $P < 0.05$) from the no-stressor group (basal).



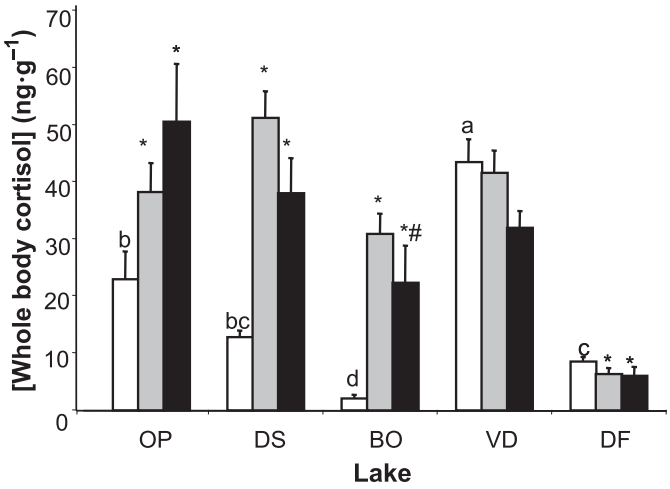
An in-vivo injection of ACTH increased whole-body cortisol concentrations in 1+ fish from a reference lake (Opasatica) or intermediate-contamination lake (Vaudray) but not in fish from the most contaminated lake (Dufault) sampled in 1999 (Fig. 5*a*). A similar trend was also observed in 2000 (Fig. 5*b*). The cortisol levels reached after injection were higher in 1999 (first study with ACTH in young fish) than in 2000 (second ACTH study), when the handling of the fish was better standardized.

Whole-body concentrations of cortisol in YOY and thyroid hormones in YOY and 1+ perch (Table 2) varied among lakes, independently of the metal-contamination gradient.

Condition factor and HSI

Condition factor and HSI values in YOY and 1+ fish varied among lakes independently of the contamination gradient (data not shown). Mean values for each group are given in Table 2.

Fig. 4. Whole-body cortisol concentrations (mean ± standard error of the mean) in 1+ yellow perch from metal-contaminated lakes in the Rouyn-Noranda region, sampled without a stress test (open bars), 30 min after a stress test (shaded bars), or 120 min after a stress test (solid bars). The number of fish sampled was 10–15 each time. A different letter above the bar indicates a significant difference (Tukey–Kramer test, $P < 0.05$) from the other lakes. An asterisk indicates a significant difference (Student's *t* test, $P < 0.05$) from the basal level; # denotes a significant difference (Student's *t* test, $P < 0.05$) from the value after 30 min.



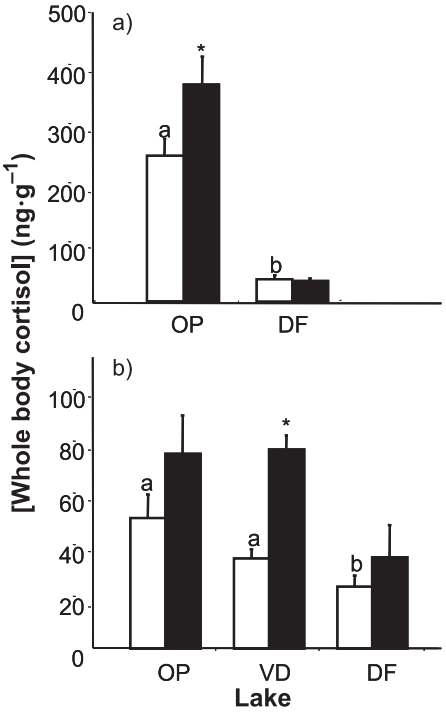
Glycogen

Hepatic glycogen reserves before stress (basal) in 1+ yellow perch varied among lakes, independently of contamination levels (Table 3). However, 120 min following the stress test, the decrease of glycogen concentration in the liver was significant in fish from the two reference lakes (Opasatica and Dasserat) but not in intermediate- (Bousquet and Vaudray) or high-contamination (Osisko and Dufault) lakes.

Discussion

Characterization of exposure is an integral part of all ecotoxicological studies and, given the lack of field data on young fish, it was particularly important in evaluating the sensitivity of young perch to metals. A gradient in whole-body Cd concentrations — consistent with the gradient of Cd concentrations within the lakes from which the fish were sampled (Laflamme et al. 2000; Perceval et al. 2002) — was evident in 1+ and YOY fish. Metal concentrations in the whole-body homogenates were similar from one year to another, except for those of 1+ perch from the most contaminated lake (Dufault), which had, for unknown reasons, a more than twofold increase of Cd in 2000 compared with 1999. The pattern of Cd accumulation in the body of young fish followed the same pattern as that reported for liver, kidney, and interrenal tissue of adult yellow perch from the same study sites (Brodeur et al. 1997; Laflamme et al. 2000; Lévesque et al. 2003). Metal concentrations in the whole-body homogenates (without liver) of young perch were, however, a small fraction of the concentrations in tissues of adult fish. Other studies have shown that Cd, Cu, and Zn accumulate in internal organs, with Cd tending to accumulate

Fig. 5. Whole-body cortisol concentrations (mean ± standard error of the mean) after an intraperitoneal injection of saline (open bars) or adrenocorticotropic hormone (ACTH; solid bars) in 1+ yellow perch sampled from metal-contaminated lakes in the Rouyn-Noranda region in (*a*) 1999 and (*b*) 2000. The number of fish sampled (each bar) was 9–15. A different letter above the bar indicates a significant difference (Tukey–Kramer test, $P < 0.05$) from the other lakes. An asterisk indicates a significant difference (Student's *t* test, $P < 0.05$) from the saline-injected fish in the same lake.



in kidney and Cu in liver (McGeer et al. 2000; Lévesque et al. 2003). Since liver was not included in the homogenates of 1+ perch, the gradient of whole-body Cd concentrations could be attributed, in large part, to accumulation in the kidney. Metal accumulation increases with time of exposure (McGeer et al. 2000), and in the present study, higher concentrations of metals were expected after 1 year of exposure (1+ perch) than after a few weeks (YOY). However, Cd and also Cu concentrations expressed per gram of body mass of YOY from the two highly contaminated lakes tended to be higher than those of 1+ perch. The higher whole-body concentrations of metals in the YOY than in the 1+ perch, both chronically exposed to nonlethal metal concentrations in the present study, could be related to a change in the accumulation rate of Cd with age and in ambient metal concentrations, as was seen before in a study on early life stages of *Cyprinus carpio* exposed to Cd (Suresh et al. 1993). Our results with young perch could also be explained by the way metal accumulation is expressed, either as a body burden (μg·fish^{-1}) or as a concentration (μg·g^{-1}). The hypothesis, proposed by others (Spehar 1976; Arnac and Lassus 1985), that fish grow at a faster rate than they accumulate contaminants is another possible explanation for the lower Cd accumulation in 1+ than in YOY perch homogenates.

Similar to metal concentrations in adult perch kidney (Lévesque et al. 2002), Cu, Zn, Ni, and Pb concentrations in young perch did not follow the contamination gradient among lakes, in contrast to Cd concentrations. However, 1+ and YOY fish from Lake Dufault were, in general, the most contaminated according to whole-body metal concentrations, despite the fact that livers were removed from the 1+ perch for glycogen analyses and that the liver is an important organ for accumulation of some metals, particularly Cu (Laflamme et al. 2000; McGeer et al. 2000). Unlike Cd, a nonessential metal, Zn and Cu are trace metals required for the metabolic activity of fish, and their intake and elimination are regulated (Arnac and Lassus 1985; Collvin 1985). The ability of fish to reestablish homeostasis rapidly after exposure to Cu (Laurén and McDonald 1987*a*, 1987*b*) and Zn (Alsop et al. 1999) was demonstrated in laboratory studies. Exposure to higher concentrations or for a longer period would likely saturate these mechanisms of regulation of Cu and Zn and eventually lead to the tissue accumulation patterns reflecting the gradient of ambient contamination that were observed with adult fish exposed for several years (Lévesque et al. 2002). The pattern of response to Cd, however, is suggestive of a process that is not metabolically regulated. McGeer et al. (2000) suggested that Cd detoxification would be more passive, involving storage, particularly in the kidney, rather than active regulation and elimination, resulting in continuous metal accumulation over an extended time course.

To test the link between exposure of young life stages of perch and the capacity to generate the normal stress response, changes in cortisol levels in response to an acute stressor were measured. In adult fish, plasma cortisol concentrations increase with time, following exposure to diverse types of acute stressors (Barton and Iwama 1991). To characterize the stress response in young fish, too small to blood-sample in the field, it was necessary to validate the use of measured whole-body cortisol levels. Changes in whole-body cortisol levels of fish larvae following extraction of whole-body homogenates using diethylether were reported previously (Feist et al. 1990; De Jesus et al. 1991; Barry et al. 1995). However, in view of the important loss of corticosteroid hormones caused by the extraction, as shown in the pilot study, and the technical difficulties of working with ether, we chose homogenization without extraction for the present project. A significant positive linear correlation was demonstrated between plasma cortisol and whole-body cortisol concentrations, providing further support for the use of a whole-body homogenate as an alternative to plasma. In the pilot study the standardized confinement-stress test led to a normal and detectable stress response characterized by a significant increase in cortisol concentration with time in 1+ perch from reference sites. Whole-body cortisol concentrations in 1+ perch from reference lakes in Abitibi also increased with time following confinement, compared with basal levels. It should be noted that the increase in cortisol levels in fish from the reference site in the mining region was less than that in fish from the reference site near Montreal, providing further evidence for metal-induced impairment of the cortisol stress response. Although Lakes Opasatica and Dasserat were used as reference lakes in the Rouyn-Noranda region, metal concentrations in these lakes

Table 2. Gross morphology and hormone levels (mean ± standard error of the mean) for young-of-the-year (YOY) and 1+ yellow perch sampled in six lakes in the Rouyn-Noranda region.

	YOY yellow perch	1+ yellow perch
Body length (cm)	1.8±0.08 (n = 34)	6.6±0.04 (n = 300)
Body mass (g)	0.07±0.04 (n = 34)	2.49±0.06 (n = 300)
Condition factor (%)	1.19±0.07 (n = 34)	0.84±0.01 (n = 300)
Hepatosomatic index (%)	nd[a]	1.52±0.09 (n = 300)
[T_3] ($ng \cdot g^{-1}$)	1.39±0.06[b] (n = 48)	0.32±0.03 (n = 84)
[T_4] ($ng \cdot g^{-1}$)	9.6±1.2[b] (n = 48)	3.0±0.2 (n = 84)
[Cortisol] ($ng \cdot g^{-1}$)	10.50±2.51[b] (n = 48)	—[c]

[a]Not determined.
[b]Hormone concentrations in YOY are expressed in nanograms per gram of body mass of pooled fish sampled after capture.
[c]See data in the Stress response section of Results.

were higher than in reference lakes near Montreal (Hontela et al. 1995).

Whole-body cortisol concentrations demonstrated, for the first time, that 1+ yellow perch from metal-contaminated lakes have a reduced capacity to increase their cortisol secretion following an acute stress test or ACTH stimulation. In contrast, YOY perch were not impaired, despite similar or even higher whole-body Cd concentrations. Further studies are required to confirm this finding, since eggs and larvae are normally considered the most sensitive life stages (Spehar 1976; McKim 1977; Sorensen 1991). However, a certain period of time may be required before the endocrine impairment becomes detectable.

Although impairment of the HPI axis in 1+ perch exposed to metals was revealed, no other physiological anomalies linked to metal exposure were detected. Thyroid-hormone levels in young perch, measured in whole-body homogenates, were not affected by 1 year of exposure to metals even though thyroid-hormone and cortisol levels often follow a similar pattern because of paracrine regulation (Young and Lin 1988), and adult perch from the same metal-contaminated sites had lower plasma T_3 and T_4 levels than perch from the reference lakes (Lévesque et al. 2003). Our data suggest that an impaired cortisol stress response and impaired thyroid function do not necessarily occur together.

Condition factor and HSI were measured to assess whether metal exposure and HPI impairment led to altered somatic growth. Again, similar to plasma thyroid-hormone levels, although the condition factor of adult perch in the metal-contaminated lakes sampled in this study was lower (Sherwood et al. 2000), no differences in condition were detected in YOY or 1+ perch. There was no effect of metal exposure on HSI in young or adult fish (Laflamme et al. 2000; Sherwood et al. 2000; Lévesque et al. 2002), suggesting that HSI is not sensitive to pollutant-induced stress.

Liver glycogen reserves were assessed to evaluate whether the metal-induced disruption of the HPI axis had an effect on the metabolic status of fish. In the two reference lakes, there was a significant decrease of glycogen stores in the liver following the stress challenge. These findings are consistent with the mobilization of glycogen and the increase in plasma glucose that coincides with an elevation in plasma cortisol concentrations after exposure to a stressor in rainbow trout and adult yellow perch (Haux and Larsson 1984; Vijayan and Moon 1992; Lévesque et al. 2002). Acute laboratory exposures to Cd also led to a decrease in liver glycogen accompanied by increased plasma glucose (Haux and Larsson 1984; Lowe-Jinde and Niimi 1984; Pratap and Wendelaar Bonga 1990). In the present study, stress-related glycogen mobilization was significant in the reference lakes, while in the intermediate- and high-contamination lakes, only a trend toward lower liver glycogen levels after exposure to the stress test was observed. This trend toward reduced mobilization of glycogen could be the first sign of an alteration in metabolic functions of young perch exposed to Cd. Field studies on adult perch have shown that fish from contaminated sites maintained liver glycogen stores after exposure to the stress test but plasma glucose was depleted (Hontela 1998; Lévesque et al. 2002). Moreover, the seasonal cycling of glycogen reserves was altered in metal-contaminated adult perch (Lévesque et al. 2002). Surprisingly, the highest liver glycogen levels were recorded in young perch from the most contaminated lake. Whether this reflects a seriously impaired ability to mobilize energy reserves in these fish remains to be demonstrated.

Table 3. Liver glycogen concentrations (mean ± standard error of the mean) before and after a confinement-stress test of 1+ yellow perch sampled in six lakes in the Rouyn-Noranda region.

	Glycogen concentration ($mg \cdot g^{-1}$)	
Lake	Before stress (basal)	After stress
Opasatica	5.09±0.26ab	3.36±0.22*
Dasserat	10.30±1.74bc	4.13±1.66*
Bousquet	6.91±1.20ab	4.85±0.49
Vaudray	7.56±1.35ab	4.99±0.54
Osisko	3.60±0.60a	2.61±0.36
Dufault	13.83±2.12c	9.65±1.24

Note: The number of fish sampled in each group was 14. Values followed by a different letter are significantly different (Tukey–Kramer test, $P < 0.05$).
*Significantly different from basal levels in fish sampled in the same lake (Student's t test, $P < 0.05$).

Our study demonstrated that effects of environmental exposure to metals on the HPI axis in yellow perch are detectable after only 1 year of exposure. This chronic exposure caused an increase in Cd body burdens of YOY and 1+ yellow perch. Cd accumulation was linked to an impaired stress

response characterized by an inability to increase whole-body cortisol concentrations and reduced mobilization of glycogen reserves in response to an acute stressor. Our study characterized for the first time the physiological status of young life stages of yellow perch exposed to metals in the field. The disruption of cortisol secretion seems to be one of the first physiological alterations detectable in young fish exposed to metals in the environment. The stress response of 1+ fish could be used as an early biomarker of toxicity in fish chronically exposed to contaminants.

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