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Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field

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Abstract

The effects of heavy metals on growth, intermediary metabolism and enzyme activities were investigated in yellow perch (*Perca flavescens*), sampled in summer and fall from lakes situated along a contamination gradient of Cd, Zn and Cu in the mining region of Rouyn–Noranda, Québec. An exposure-dependent decrease in condition factor was observed in both seasons. Liver glycogen and triglyceride reserves were higher in summer than in fall in fish from the reference lake, while the seasonal pattern was different in fish from the contaminated lakes. Plasma free fatty acids (FFA) levels were also influenced by season and contamination. Activities of malic enzyme (ME) and glucose 6-phosphate dehydrogenase (G6PDH) in the liver were higher in the summer than in the fall in reference lakes whereas no seasonal variations were detected in fish from contaminated lakes. Activities of pyruvate kinase (PyK), aspartate transaminase (AST), phosphoenolpyruvate carboxykinase (PEPCK) and malate dehydrogenase (MDH), were higher in fish from contaminated lakes in fall but not in summer. Chronic exposure of yellow perch to sublethal levels of heavy metals impairs growth and alters the seasonal cycling of liver glycogen and triglycerides as well as the activities of metabolic enzymes.

Keywords: Fish; Cadmium; Metals; Intermediary metabolism; Lipids; Carbohydrates; Enzymes; Field; Yellow perch

1. Introduction

The impact of pollutants on growth, survival and fertility of fish is an important concern, yet few studies have investigated the effects of life-long exposures to metals on intermediary metabolism and growth (Hontela, 1997). Fish subjected to metals such as Cd, Cu or Zn, have a reduced condition factor (Kearns and Atchinson, 1979; Munkittrick and Dixon, 1988; Laflamme et al., 2000), and a reduced growth efficiency (Sherwood et al., 2000), the capacity to convert con-

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sumed food into body mass. Since reduced growth can be mediated through direct effects of metals on physiological functions of the fish or through effects on the food web (Sherwood et al., 2002), it is often difficult to establish direct cause–effect relationships between contamination and reduced growth in the field. Previous studies have shown that chronic exposure to metals blunted the normal cortisol stress response (Brodeur et al., 1997; Laflamme et al., 2000) and perturbed carbohydrate metabolism in perch (Sjöbeck et al., 1984). Lowered muscle and visceral lipid stores in white sucker (*Catostomus comersioni*) chronically exposed to Cu and Zn (Munkittrick and Dixon, 1988) were also reported. Exposure up to 30 weeks to Cd in the laboratory decreased growth as well as liver glycogen reserves in rainbow trout (Haux and Larsson, 1984; Ricard et al., 1998) and tilapia (Pratap and Wendelaar Bonga, 1990), and increased the activity of aspartate transaminase (AST) and alanine transaminase (ALT), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) in *Channa punctatus* (Sastry et al., 1997). However, the effects of chronic field exposures to metals on intermediary metabolism of indigenous fish and the seasonal variation of these effects have not been determined thus far.

The present large-scale field study investigated carbohydrate and lipid metabolism, and liver enzymes involved in gluconeogenesis (ALT, AST and PEPCK), glycolysis (LDH and PyK) and lipid metabolism (triglyceride lipase (TGL), ME and G6PDH) in yellow perch (*Perca flavescens)* sampled in spring (post-spawning) and fall (gonadal recrudescence) from five lakes representing a gradient of metal contamination.

2. Material and methods

2.1. Description of the sites and fish species

Yellow perch were sampled in six lakes of the Abitibi mining region of Rouyn–Noranda, located in western Quebec (48°00′N, 79°00′W) in fall 1998 and summer 1999. A large smelter has operated in Rouyn since 1927 and many lakes in the surrounding area are contaminated by Cd, Zn and Cu from atmospheric emissions from the smelting complex and from runoff affected by mining residues, resulting in a metal contamination gradient (Laflamme et al., 2000).

The two most contaminated lakes, Lake Dufault and Lake Osisko, are situated near Rouyn–Noranda, while the intermediate lake, Lake Bousquet is situated 20 km east downwind of the smelter. The two reference lakes, Lake Opasatica and Lake Dasserat, are located upwind of the smelter. To more easily identify these lakes within the manuscript, they will be numbered from the reference sites to the most contaminated as L1 to L5. The water temperature of the lakes was 18 ± 2 °C in the fall and 20 ± 2 °C in the summer. For detailed limnological characteristics, see Brodeur et al. (1997), Laflamme et al. (2000), Sherwood et al. (2000). Ambient water metal concentrations are presented in Table 1.

Yellow perch was used for this study, because, it is a relatively sedentary fish (Aalto and Newsome, 1990) that reflects the contamination of the sites where it is sampled (Girard et al. 1998). Moreover, it is an abundant species in the five lakes sampled.

2.2. Capture of the fish

In the two sampling seasons, fish of both sexes were captured by fishing rod, seine (30 m long, 2 m high at the extremities, 4 m high at the center, 1.5 cm mesh), or gill net (25 m long, 2 m high, 2.5 cm to 15 cm mesh), between 1600 and 2000 h in the five lakes within a period of 3 weeks in September and in June. The sampling procedure is described in detail in Laflamme et al. (2000). Briefly, captured fish were transferred to a large holding tank in the boat, then transferred in groups of ten to floating enclosures (0.5 m width × 1 m long × 1 m deep) overnight (at least 16 h) to reduce the effects of capture stress and to standardize the handling procedures. The day after capture, one group of fish was immediately anesthetized with MS222 (control group), whereas, fish from the other group were taken from the enclosure and placed into a 20 l container for 1 h of confinement (confinement group) before anesthesia with MS222. Anesthetized fish

were bled from the caudal vessels using a heparinized syringe, then transported on ice to a field laboratory located 20–45 min from the field site. The time between the end of the blood sampling at the site and the beginning of dissection in the laboratory was kept the same (45 min) for all the lakes. Blood was centrifuged (5 min, 13000 rpm, Micro Centaur, Sanyo, VWR Scientific) and plasma was frozen at −20 °C. Body mass, length, and organ mass were recorded to evaluate the condition factor ([weight (g)]/[length (cm)]^3 × 100), the hepatosomatic index (HSI) ([liver weight (g)]/[total weight (g)] × 100) and the gonadosomatic index (GSI) ([gonad weight (g)]/[total weight (g)] × 100). Liver and plasma were held in liquid nitrogen in the field and were stored at −80 °C until analysis.

2.3. Laboratory analyses

2.3.1. Analyses of metals

Concentration of Cd, Cu and Zn in the kidney were measured by inductively coupled plasma atomic emission spectrophotometry (ICP-AES) using tissue homogenates corresponding to 100 mg of dry weight, as described previously by Laflamme et al. (2000) for perch liver and head kidney samples. When the tissue was too small, samples (from four or fewer fish) were pooled. One μg of metals per dry weight corresponds to approximately 0.29 μg of metals per wet weight (Ricard et al., 1998).

2.3.2. Plasma analyses

Plasma cortisol was measured by radioimmunoassay (ICN Biomedicals, no. 07221106). Plasma glucose and free fatty acids (FFA) were determined with a calorimetric method (GOD-PAP, Boehringer–Mannheim Diagnostica, number 166391 and Sigma, number RO-1383-175, respectively).

2.3.3. Tissue analyses

Liver glycogen was measured using the enzymatic method described by Foster and Moon (1986), Bleau et al. (1996), except digestion at 100 °C was replaced by sonication. Total lipids in the liver were extracted according to the method of Folch et al. (1957), Fournier and Weber (1994). After evaporation under nitrogen, lipids were resuspended in dimethylsulfoxide (DMSO), and the triglycerides were measured by spectrophotometry at 540 nm (Sigma kit, number 337-A). Triglyceride standards (Lipid-Lin-Trol L2648) and blank controls were used with each series of samples to validate the extraction processes.

Enzyme activities were measured under saturating substrate conditions according to standard procedures of Moon and Mommsen (1987), Mommsen et al. (1985). Briefly, liver pieces (0.2 g)

Table 1
Ambient water metal concentrations (mean ± S.E.) in lakes from the Rouyn–Noranda mining region

Lake	Dissolved metal levels measured by in situ dialysis (nmol l^{-1})*			N	Metal in oxic sediments (nmol g per dry weight)		
	[Cd]	[Cu]	[Zn]		Total {Cd}	Extract {Cu}	Extract {Zn}
L1[a]	0.53 ± 0.26	69 ± 14	168 ± 82		n.a.	n.a.	n.a.
L2	0.076 ± 0.045	64 ± 16	6.5 ± 2.8	4	5.1 ± 0.7	144 ± 25	105 ± 20
L3	0.553 ± 0.064	62.1 ± 12.3	403.8 ± 6.10	3	10.0 ± 0.1	143 ± 9	204 ± 44
L4	n.a.	n.a.	n.a.	1	590	105 000	68 500
L5	2.7 ± 0.3	127 ± 19	433 ± 88	1	200	19 400	18 000

*Free metal ion concentrations were directly measured in aqueous media using dialysers installed at 10 cm above the sediments and an ion-exchange technique (Fortin, unpublished data; see Fortin and Campbell, 1998 for details on the ion-exchange technique). Means followed by the same letter are not significantly different ($P < 0.05$, Tukey–Kramer); comparisons of means were made within the same season. n.a, not available.

[a] Names of lakes are given in Section 2.1 and Section 3.1. Numbers of samples analyzed are between two and three.

were homogenized (1:4, W/V) using sonication in a buffer consisting of 0.5 M glycerol, 20 mM Na2HPO4, 0.5 mM EDTA and 5 mM ß-mercaptoethanol, 0.2% BSA, in the presence of a crystal of phenylmethylsulfonyl fluoride (PMSF), a protease inhibitor, and 1 μl aprotonin (10 mg ml^{-1}). The homogenate was centrifuged for 12 min at 12000 rpm at 4 °C (Micro Centaur, Sanyo, VWR Scientific) and the supernatant was used for enzyme assays. Reaction rates were assayed spectrophotometrically at 340 nm following the appearance or disappearance of NADH or NADPH. Kinetic assays were run at 20 °C.

Assay conditions used were as follows, imidazol 50 mM, pH 7.8, was used as buffer:

- LDH (E.C.1.1.1.27): NADH (0.15 mM), pyruvate (5 mM).
- MDH (E.C.1.1.1.37): NADH (0.15 mM), oxaloacetate (0.5 mM).
- G6PDH (E.C.1.1.1.49): $MgCl_2$ (10 mM), NADP (0.5 mM), glucose 6-phosphate (10 mM).
- ME (E.C. 1.1.1.40): $MgCl_2$ (10 mM), NADP (0.5 mM), malate (1 mM).
- PyK (E.C.2.7.1.40): KCl (25 mM), $MgCl_2$ (10 mM), NADH (0.15 mM), ADP (5 mM), PEP (5 mM), excess PK-free LDH.
- PEPCK (E.C.4.1.1.32): $MnCl_2$ (1.75 mM), 2 deoxyGDP (dGDP) (0.4 mM), $NaHCO_3$ (20 mM), glycerol-free MDH (10 U ml^{-1}), PEP (5 mM), NADH (0.15 mM).
- AST (E.C.2.6.1.1.): α-ketoglutarate (13 mM), aspartate (40mM), NADH (0.15 mM), pyridoxal phosphate (2.5 mM).
- ALT (E.C.2.6.1.2): α-ketoglutarate (13 mM), alanine (500 mM), NADH (0.15 mM), pyridoxal phosphate (2.5 mM).

The activity of liver TGL was measured using the hydrolysis of ^{14}C-triolein to [^{14}C] oleic acid, according to the method of Sheridan and Allen (1984), Harmon et al. (1991). Briefly, liver pieces were homogenized in buffer (1:10 V/W, 0.25 M sucrose, 25 mM Tris, 1 mM EGTA, pH 7.4), containing a crystal of PMSF added before sonication. The homogenate was centrifuged for 20 min at 38000 g at 4 °C. The supernatant was used to measure the activity of TGL. The radioactive substrate was purified using an Amberlite ion-exchange resin according to Khoo and Steinberg (1974). The reaction was stopped after 30 min with a solution of chloroform:methanol:benzene (1:2.4:2) with 0.1 μM of oleic acid added. Radioactive ^{14}C-oleic acid released from ^{14}C-triolein was counted using a Packard 2500 TR liquid scintillation analyzer.

2.4. Statistical analyses

For all tests, a statistical significance level of 0.05 ($\alpha = 0.05$) was used using the computer program JUMP IN. Since no differences between sexes were detected within each site (t-test), except for GSI values, the two sexes were grouped for analysis. Differences among groups were tested using one-way analysis of variance (ANOVA). The Tukey–Kramer test was used to differentiate means. Data were transformed when necessary to obtain normality and homoscedasticity.

3. Results

3.1. Exposure of the fish

The ambient metal concentrations measured in the water column and the sediments are presented in Table 1. A gradient was detected, with the lowest levels measured in L. Opasatica and the highest in L. Dufault. Kidney concentrations of Cd and Zn also followed a significant ($P < 0.05$) contamination gradient from the most contaminated lakes (Osisko and Dufault), to intermediate (Bousquet) and the least contaminated lakes used as reference (Opasatica and Dasserat) (Fig. 1A and B). Kidney concentrations of Zn were about ten times higher than Cd, concentrations of Cu were similar among lakes. Lakes are coded as L1 to L5, with L1 and L2 representing the most pristine lakes (Opasatica, Dasserat), L3 intermediate (Bousquet), and L4 (Osisko) and L5 (Dufault) the two most contaminated lakes.

3.2. Morphological characteristics

Fish from the most contaminated lakes (L4, L5) had significantly lower ($P < 0.05$) length,

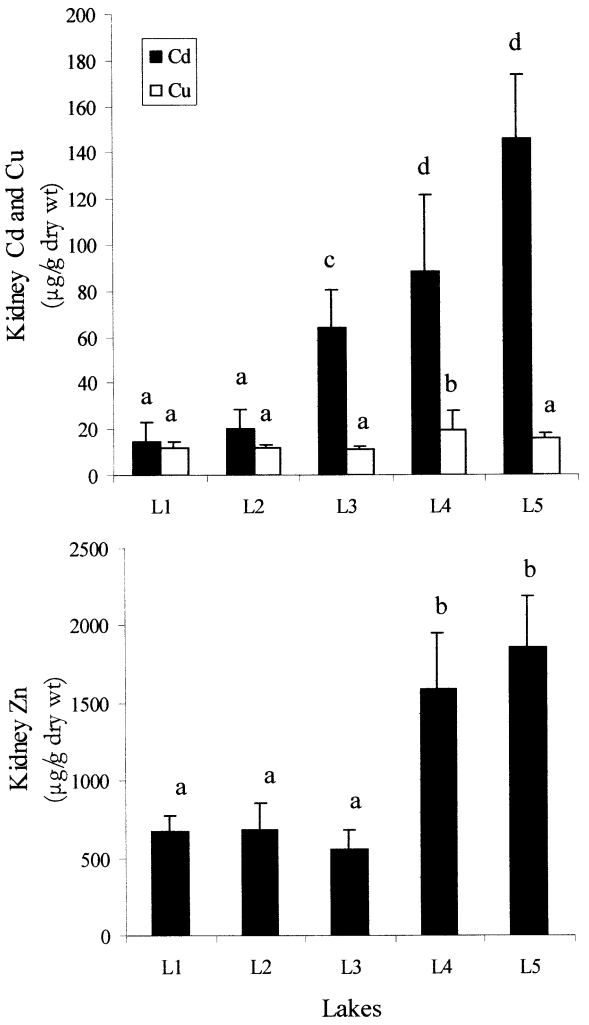

Fig. 1. Concentrations of Cd, Cu (A) and Zn (B) (mean ± S.E., μg g^{-1} dry weight), in pooled samples of kidney from adult yellow perch collected in five lakes of the Rouyn–Noranda mining area. Letters that are different indicate significantly different values ($P < 0.01$, Tukey–Kramer test). For each lake, $N = 20$ samples or pooled samples. Lakes are coded as L1 to L5, (see Section 2), with L1 and L2 representing the most pristine lakes and L5 the most contaminated lake, according to the concentrations of metals in the water (Table 1) and in fish kidneys.

weight and condition factor than fish from the reference lakes (L1, L2) in both summer and fall (Table 2). The HSI was not different between lakes in the fall and although differences between lakes were detected in the summer, they did not follow the contamination gradient. In the fall, fish were in gonadal recrudescence, and the GSI in females and males decreased with the contamination gradient. In summer, the GSI could not be determined, because, the sampling was done just after the spawning season.

3.3. Glycogen and triglyceride reserves

Liver glycogen and triglyceride levels, measured as indicators of energetic reserves, were significantly ($P < 0.05$) higher in the summer compared with fall in fish from the reference (L1) lake (Fig. 2A and B). However, this distinct seasonal increase in liver reserves was not observed in the contaminated lakes (L4 and L5). There were no differences between lakes and seasons in liver protein content (data not shown).

3.4. Metabolic enzymes

3.4.1. NADPH production

The production of liver NADPH (nicotinamide adenine dinucleotide phosphate), implicated in lipid synthesis, was estimated by measuring the activity of two enzymes, glucose-6-phosphate dehydrogenase (G6PDH), an enzyme of the pentose phosphate pathway, and ME. The activities of G6PDH (Fig. 3A) and Me (Fig. 3B) were lower ($P < 0.05$) in fish from the contaminated lakes L4 and L5, as compared with intermediate (L3) and reference (L1) lakes in the summer, indicating that less NADPH was produced. There were no differences among lakes in the fall and the activities were low. The seasonal increase in activity of both enzymes observed in the reference lake was not detected in the more contaminated lakes.

3.4.2. Lipid metabolism

Lipolysis was assessed by measuring TGL activity (Fig. 3C). Liver TGL activity was not different among lakes in summer, but in the fall, TGL activity was higher ($P < 0.05$) in fish from the most contaminated lake (L5), compared with the other lakes.

3.4.3. Gluconeogenic and amino acid metabolic enzymes

Gluconeogenesis, the ability to produce glucose

from non-carbohydrate substrates, was evaluated by estimating the activity of liver phosphoenolpyruvate carboxykinase (PEPCK), alanine transaminase (ALT), AST and MDH (Table 4). The activities of PEPCK, ALT and MDH were significantly higher ($P < 0.05$) in fish from the most contaminated lake (L5) in the fall, whereas there were no differences in the summer. No differences were found among lakes and between seasons for AST.

3.4.4. Glycolytic enzymes

Glycolytic activity was estimated by liver PyK and lactate dehydrogenase (LDH) (Table 4). The activity of PyK was higher ($P < 0.05$) in fish from the most contaminated lake (L5) compared with fish from the reference and intermediate lakes in the fall. No differences were found among lakes or between seasons for liver LDH, an indicator of anaerobic metabolism processes in the tissue.

3.5. Plasma FFA, cortisol, and glucose

Plasma FFA levels (Table 3) were lower in the summer compared with fall in fish from the reference (L1) and intermediate (L3) lakes. The seasonal pattern of plasma FFA was reversed in contaminated lakes (L4 and L5).

Plasma cortisol and glucose were determined in fish subjected to a standardized 1 h confinement test and in unconfined controls (Table 3). The increase in plasma cortisol and glucose levels in response to confinement was significant only in fish from the reference lakes (L1 and L2).

4. Discussion

This large-scale field study was designed to test the hypothesis that chronic exposures to metals impair growth of teleost fish through a perturbation of intermediary metabolism and the activity of metabolic enzymes. The exposure of yellow perch was characterized by measuring metals in the kidney of fish from five lakes situated along a contamination gradient in a mining region. Concentrations of Cd and Zn increased in a steep gradient up to about 150 μg g^{-1} dry weight (45 μg g^{-1} wet weight) for Cd and 2000 μg g^{-1} dry weight (590 μg g^{-1} wet weight) for Zn, in the most contaminated lake. Substantially lower kidney burdens were reported in other field studies

Table 2
Morphological characteristics (mean ± S.E.) of adult yellow perch collected in lakes of the Rouyn–Noranda mining region

Lake	Length (cm)[a]	Weight (g)[a]	HIS (%)[a]	Condition factor[a]	GSI (%) males	GSI (%) females
Fall						
L1[b]	208.6 ± 3.8b	111.9 ± 6.9b	1.22 ± 0.01a	1.18 ± 0.02b	4.244 ± 0.34a	1.668 ± 0.12ab
L2	190.1 ± 3.4a	76.8 ± 4.6a	1.20 ± 0.07a	1.08 ± 0.001a	5.65 ± 0.05a	1.972 ± 0.08a
L3	184.7 ± 4.5b	78.1 ± 5.5a	0.89 ± 0.04b	1.18 ± 0.02b	5.61 ± 0.39a	1.659 ± 0.18ab
L4	193.8 ± 2.8b	65.4 ± 2.7ac	0.91 ± 0.04b	0.88 ± 0.01c	1.36 ± 0.61c	1.458 ± 0.04b
L5	130.4 ± 0.9d	19.2 ± 0.3d	1.25 ± 0.02a	0.87 ± 0.01c	2.963 ± 0.22d	1.236 ± 0.12d
Summer						
L1	188.4 ± 3.8a	92.1 ± 4.6b	1.60 ± 0.04b	1.22 ± 0.02b	n.a	n.a
L2	186.1 ± 3.8a	74.8 ± 4.7a	1.23 ± 0.04ac	1.08 ± 0.02a	n.a	n.a
L3	178.2 ± 4.1a	75.8 ± 4.9a	1.17 ± 0.04a	1.25 ± 0.02b	n.a	n.a
L4	177.1 ± 3.1ab	48.2 ± 3.8c	1.238 ± 0.03ac	0.88 ± 0.02d	n.a	n.a
L5	148.3 ± 3.8b	28.9 ± 4.6d	1.146 ± 0.04c	0.86 ± 0.02d	n.a	n.a

Note: Means followed by the same letter are not significantly different ($P < 0.05$, Tukey–Kramer); comparisons of means were made within the same season. n.a., not available.

[a] Numbers of fish sampled are greater than 20 for all the lakes. Condition factor, HSI, and GSI are defined in Section 2.2, arrows indicate the contamination gradient.

[b] Names of lakes are given in Section 2.1 and Section 3.1.

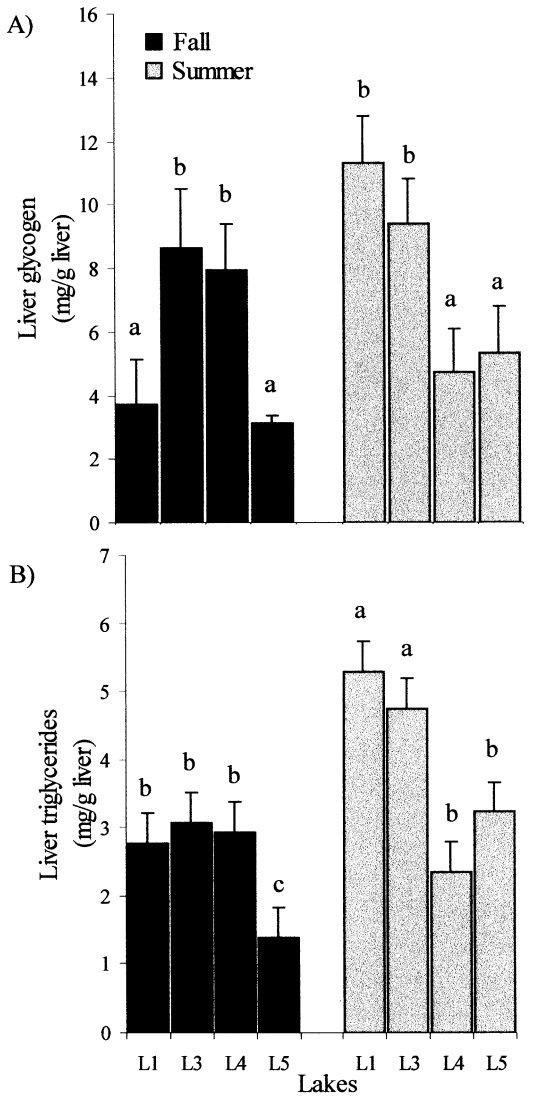

Fig. 2. Seasonal variation in hepatic glycogen (A) and hepatic triglycerides (B) concentrations (mean ± S.E.) in adult yellow perch from lakes of the Rouyn–Noranda mining region. Different letters show means that are significantly different ($P < 0.05$, Tukey–Kramer). For each lake, $N = 15$. See legend of Fig. 1 for details.

with fish; 15 μg g^{-1} wet weight for Cd and 195 μg g^{-1} wet weight for Zn in brown trout (*Salmo trutta*) by Olsvik et al. (2000), and 8 μg g^{-1} dry weight for Cd by Farag et al. (1995). Concentrations of Cd and Zn measured in the kidney in the present study were three times higher than those reported by Laflamme et al. (2000) for liver in perch from the same lakes. Higher metal accumulation in kidney than in liver has been shown in other studies with fish (Harrison and Klaverkamp, 1989; Olsvik et al. 2000). There was no evidence for a gradient in Cu concentrations in the kidney among fish from different lakes, and the levels were similar to concentrations reported previously for liver (Laflamme et al., 2000). Collvin (1985) suggested that perch (*Perca fluviatilis*) actively regulate Cu concentrations in the gills and in the liver by detoxification and excretory processes.

A metal exposure-related decrease in condition factor was detected in the present study, similar to the growth impairment reported by Laflamme et al. (2000), Sherwood et al. (2000) for this same species from these lakes. A decrease in gonad size was also evident in fish from the contaminated lakes. Other size-related endpoints, including HSI, varied among lakes but did not follow the contamination gradient. The metabolic status, specifically liver reserves of glycogen and triglycerides, and enzyme activities of growth-impaired fish from lakes situated along a contamination gradient were investigated in the present study. Fish use glycogen for immediate energy requirements during acute stress (Lowe-Jinde and Niimi, 1984; Vijayan and Moon, 1992), and they maintain their liver glycogen reserves by mobilizing other energy stores such as lipids and proteins (Sheridan and Mommsen, 1991). In the present study, in the summer, the lower liver glycogen content in fish from the most contaminated lakes was associated with the inability to increase plasma glucose, and also cortisol, following confinement. Fish with the highest tissue burdens of metals might not have the capacity to increase their plasma glucose concentrations following an acute stress, because of low liver glycogen reserves. The summer sampling was carried out just after the spawning season, an energetically demanding process further exhausting the liver glycogen reserves. Despite the differences observed in plasma glucose and liver glycogen of fish from the contaminated lakes compared with reference fish, no differences among lakes in the summer could be detected in PyK and LDH (glycolytic enzymes) or PEPCK,

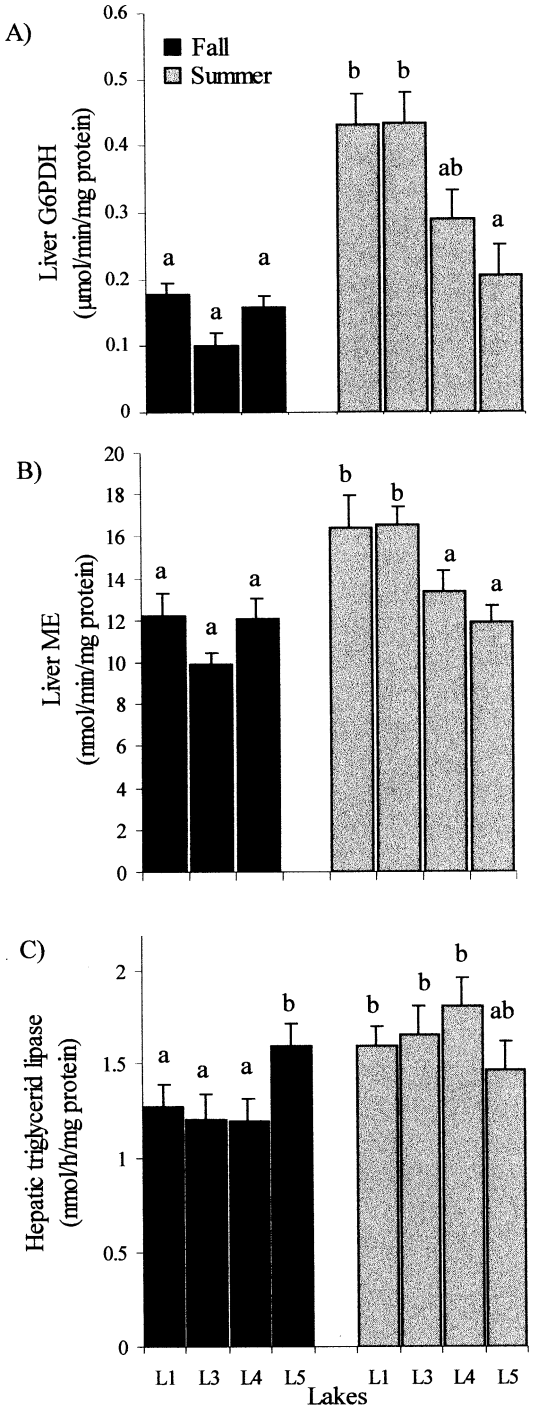

Fig. 3.

AST, ALT and MDH (gluconeogenic and transaminase enzymes).

In contrast to the pattern in the summer, liver glycogen reserves in the fall were higher in fish from the contaminated and intermediate lakes, but lower for the most contaminated lake, compared with the reference lake. Our data show that fish from the reference lake exhibit a distinct seasonal cycle of liver glycogen reserves, with low levels in early summer (post-spawning period) and high levels in the fall (period of gonadal recrudescence). Fish from contaminated lakes, in contrast, exhibit a very different pattern; low levels and no seasonal cycling (L5), or a shifted cycle with higher glycogen reserves in the fall instead of summer (L4). This difference in the glycogen reserve-cycling, and probably in related metabolic parameters, may be linked through a disrupted capacity to use energy, to the lowest condition factor and to the delayed gonadal recrudescence diagnosed by low GSI (Table 1) in fish from contaminated lakes.

Along with differences in plasma glucose and liver glycogen reserves, the activities of gluconeogenic (PEPCK, MDH and AST) and glycolytic (PyK) enzymes were increased in liver of fish from the contaminated lakes during the fall. Furthermore, the higher activities of PyK and AST, both in the summer and fall, in fish with the highest tissue burdens of Cd and Zn, provide additional evidence for higher metabolic demands in fish chronically impacted by metals. Fish from the reference lake exhibited a seasonal cycling in the activity of AST and PyK, with low levels in the summer, compared with high levels in the fall, whereas fish from the most contaminated lake (L5) exhibit high levels and no seasonal cycling. An increase in the activity of hepatic PEPCK and PyK, as well as a decrease in liver glycogen, have been reported in fish subjected to acute stress caused by factors other than metals (hypoxia,

Fig. 3. Seasonal variation in hepatic G6PDH (A), ME (B) and TGL (C) activities (mean ± S.E.) in adult yellow perch from lakes of the Rouyn–Noranda mining region. Different letters show lakes that are significantly different ($P < 0.05$, Tukey–Kramer). For each lake $N = 14$. See legend of Fig. 1 for details.

Table 3
Plasma cortisol, glucose and FFA levels (mean ± S.E.) in yellow perch collected in lakes of the Rouyn–Noranda

	Fall	Summer				
	FFA (mM)	FFA (mM)	Glucose (mg ml^{-1})		Cortisol (ng ml^{-1})	
	Control	Control	Control	Confined	Control	Confined
L1[a]	n.a	n.a	1.99 ± 0.16	2.53 ± 0.17*	214.1 ± 28.1	278.0 ± 33.1*
L2	1.26 + 0.15	0.83 ± 0.14	2.18 ± 0.27	2.96 ± 0.19*	175.5 ± 28.9	261.2 ± 30.1*
L3	1.27 + 0.22	0.83 ± 0.15	1.42 ± 0.14	1.62 ± 0.15	263.8 ± 29.7	309.9 ± 31.2
L4	0.76 + 0.08[b]	1.88 ± 0.39[b]	2.52 ± 0.20	2.34 ± 0.23	199.4 ± 19.9	175.8 ± 32.1
L5	0.65 + 0.11[b]	0.78 ± 0.25	1.96 ± 0.22	1.72 ± 0.20	n.a	n.a

Note: *, Significantly different from the control group of the same lake (t-test, $P < 0.05$).
[a] Names of lakes are given in Section 2.1 and Section 3.1.
[b] Significantly different from the reference lakes of the same treatment (t-test, $P < 0.05$). n.a., not available; $N = 15$ for each groups; control, fish sampled without confinement; confined, fish sampled following a 1 h confinement.

Wright et al., 1989; confinement, Vijayan et al., 1997). Our results suggest that fish challenged by environmental pollution may have a higher turnover of glucose and more glucose may be produced from non-carbohydrate substrates and used.

Further evidence for increased energetic costs and altered intermediary metabolism in fish from polluted environments was provided by estimating lipid metabolism. Fish sampled in the fall from the two most contaminated lakes had low liver triglycerides (and also glycogen) reserves and the seasonal build-up of reserves observed in the reference lake, was not evident. The amount of liver protein was not different (data not shown) among lakes or between seasons. Whether fish rely on muscle protein instead and whether this underlies, at least in part, the smaller size of fish from the most contaminated lakes, remains to be investigated.

The activity of three enzymes involved in lipid metabolism, glucose 6-phosphate dehydrogenase

Table 4
Enzyme activities (mean ± S.E.) in the liver of adult yellow perch, collected in lakes of the Rouyn–Noranda mining region in the fall and summer

Lake	Enzymes					
	AST	ALT	PEPCK	LDH	PyK	MDH
Fall						
L1[a]	0.335 ± 0.03a	0.387 ± 0.04a	5.956 ± 0.75a	0.117 ± 0.02a	35.68 ± 5.10a	1.59 ± 0.24a
L4	0.364 ± 0.03a	0.415 ± 0.04a	5.024 ± 1.21a	0.055 ± 0.02b	33.19 ± 3.63a	2.58 ± 0.28ab
L5	0.557 ± 0.03b	0.449 ± 0.04ab	9.700 ± 1.15b	0.170 ± 0.02a	73.23 ± 7.88b	3.04 ± 0.27b
Summer						
L1	0.493 ± 0.03b	0.413 ± 0.03a	n.a	0.140 ± 0.02a	62.11 ± 6.55b	1.86 ± 0.22a
L3	0.529 ± 0.04b	0.516 ± 0.04b	6.110 ± 0.99a	0.060 ± 0.02b	46.63 ± 6.76a	1.75 ± 0.23a
L4	0.409 ± 0.03ab	0.442 ± 0.03ab	n.a	0.125 ± 0.02a	65.04 ± 6.35b	2.11 ± 0.21a
L5	0.604 ± 0.04b	0.588 ± 0.05c	5.95 ± 1.15a	0.146 ± 0.02a	72.76 ± 7.56b	2.14 ± 0.23a

Note: Means followed by the same letter are not significantly different ($P < 0.05$, Tukey–Kramer). n.a., not analyzed. Activities of AST, ALT, LDH and MDH are expressed in μmol min^{-1} mg per protein; activity of PEPCK and PyK are expressed in nmol min^{-1} mg per protein. $N = 15$. Fish were not subjected to the confinement stress test.
[a] Names of lakes are given in Section 2.1.

(G6PDH), ME and TGL, were measured, for the first time in an indigenous fish species exposed to metals in the field. Both ME and G6PDH use NADP as coenzyme, generating NADPH, which is used for lipid synthesis. In fish sampled in the summer in the contaminated lakes, the activities of ME and G6PDH were lower than in fish from the reference lakes, indicating that less NADPH was produced. This could significantly compromise lipogenesis. There were no differences in the activity of these two enzymes among lakes in the fall. Importantly, the activity of G6PDH and ME in fish from the reference lakes exhibited a significant seasonal variation, being higher in the summer than in the fall, whereas activities remained low during both seasons in fish from the contaminated lakes. Concentrations of liver TG lipase, an enzyme that hydrolyses triglyceride reserves, was enhanced and liver triglyceride reserves were significantly lower in the fall in fish from the most contaminated lake. Plasma FFA levels were also lower in fish from the two most contaminated lakes, suggesting a significantly enhanced utilization of lipid reserves and FFA in fish challenged by chronic exposure to metals. Since the triglyceride reserves were higher in the summer compared with the fall in the reference lake, it appears that fish from the contaminated lakes may be unable to increase their lipid reserves in the summer whereas the fish from reference lakes can do so. This metabolic alteration may, as has been postulated for glycogen reserves, contribute to the delayed gonadal recrudescence and lower condition factor observed in fish from contaminated lakes and possibly jeopardize winter survival.

The present study provides, for the first time in an indigenous fish species, evidence that chronic exposure to sublethal levels of Cd, Zn and Cu in the environment can disturb the normal processes of intermediary metabolism and energy cycling. Our study suggests that the energetic costs of detoxification processes have an impact on fish growth through alterations of intermediary metabolism. Yellow perch from contaminated lakes could not respond normally to acute confinement by increasing their plasma cortisol and glucose levels, and their capacity to build up and then use the glycogen and triglyceride reserves was altered, compared with fish from the reference lakes. Our study also clearly demonstrated that seasonal variation in metabolic processes must be considered when interpreting the effects of environmental contamination on fish physiology.

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