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Differentiating Between Direct (Physiological) and Food-Chain Mediated (Bioenergetic) Effects on Fish in Metal-Impacted Lakes

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ABSTRACT

The objectives of this field project were to test relationships between the physiological and population status of indigenous fish and (a) ecological factors (habitat quality, food resources), (b) toxicological factors (ambient and tissue metal concentrations), and (c) metal detoxification factors (metallothionein induction and subcellular metal partitioning). The sentinel species, yellow perch (YP: *Perca flavescens*), was collected from lakes with contrasting metal levels located on the Canadian Precambrian Shield, downwind and downstream from metal smelters. In lakes at the high end of our exposure gradient, metals (Cu, Ni, and especially Cd) accumulate in YP to concentrations well above background tissue values; increases in tissue Zn concentrations were much more modest, despite the existence of a very marked gradient in ambient [Zn]. Metal accumulation in YP is accompanied by metallothionein induction, but all evidence to date suggests that metal detoxification by metallothionein is incomplete. Indeed, direct effects of metal toxicity are detected at multiple levels of biological organization, from effects at the cellular level, to effects in organs and tissues, to individuals and populations, in a pattern linked to accumulated metal concentrations (*i.e.*, along the contamination gradient). In addition to direct or physiological effects, we also documented indirect, food-web-mediated effects of metals on YP in the most contaminated lakes. The

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most common indication of such indirect effects on YP is severely stunted growth coupled with a high degree of zooplankton dependence throughout their life.

Key Words: Yellow perch, metals, bioaccumulation, detoxification, endocrine, food web.

INTRODUCTION

Attempts to characterize the impacts of metals on aquatic ecosystems have usually involved laboratory experiments under defined conditions (toxicity tests) and, to a lesser extent, field observations on impacted indigenous populations. Traditional ecological risk assessment (ERA) approaches have largely relied on the former approach, comparing a laboratory-derived "predicted no effect concentration" (PNEC) with the "predicted environmental concentration" (PEC). PNEC values have been derived from single species laboratory toxicity tests, the results of which are used to develop a distribution of species sensitivities to a particular metal; it is then assumed that this distribution (derived from laboratory test species) represents the distribution of species sensitivities in a generic and diverse aquatic community – see Brix *et al.* (2001) for a recent example of this approach, as applied to copper. Based on statistical analysis of this distribution, PNEC values are then chosen such that a certain proportion of the aquatic species, usually 95%, will be protected.

This laboratory-based approach is predicated on the assumption that metals are affecting the target species of interest *directly*, either via waterborne or diet-borne metal exposure.[1] It is also possible that metal effects on a consumer organism may be *indirect*, that is, they may be mediated via the wood web. A classic example of this type of ecotoxicological effect was observed in the whole lake acidification experiments carried out in the Experimental Lakes Area, Ontario, Canada (Schindler 1988). The top predator in these lakes, the lake trout *(Salvelinus namaycush)*, was more or less insensitive to acidification *per se*, but its major prey items were progressively eliminated in the lakes as the pH declined, and the lake trout exhibited severe physiological and reproductive effects that could be linked to malnutrition. Such food-web mediated effects are not accounted for in the aquatic toxicity testing approach.

In recognition of the shortcomings of the laboratory approach, a limited number of workers have ventured into the field to collect indigenous aquatic organisms from metal-contaminated waters, and to compare these organisms with specimens from reference environments. Comparisons have been made at the physiological, population, and community levels, and differences attributed to the presence of metals in the contaminated systems. Both lakes and rivers have been examined with this approach, usually in areas that have been grossly impacted by mining activities, for example, Manitouwadge Lakes, Ontario, Canada — see Munkittrick and Dixon 1988; Munkittrick *et al.* 1991; Clark Fork River, Montana, USA — see Cain *et al.* 1992; Cain and Luoma 1998). However, almost without exception these studies have concentrated on the biological differences among sites and have neglected to define

[1] In most jurisdictions PNEC values are in fact expressed in terms of dissolved metal, *i.e.*, there is no explicit consideration of metal speciation in water column, nor of the possible contribution of diet-borne metal to metal toxicity – see Niyogi and Wood (2003).

the metal exposure regime ([M^{z+}], pH, hardness, dissolved/particulate metal concentrations, *etc.*) to which the indigenous organisms have been exposed.

To link these two approaches, one needs a common measure of metal exposure in laboratory and field settings. The determination of metal concentrations or burdens in tissues (or whole organisms) has been suggested as a means of achieving this linkage (Luoma 1996, Borgmann *et al.* 2001a;). In the present project we have explored this approach with indigenous fish, and expanded the concept of metal "body burden" to take into account the speciation of the metal within the organism, that is, the organism's ability to detoxify the metal. The project has been designed to look for metal-induced effects on a sentinel species, yellow perch (*Perca flavescens*), in lakes located along an existing metal gradient, downwind and downstream from past/current metal smelters. We are seeking evidence both for direct physiological effects, as defined above, and for food-web-mediated bioenergetic effects.

In the case of **direct** effects, a key hypothesis to be tested is that there exists a mechanistic link between the intracellular speciation of the metals and the manifestation of deleterious effects at the organism (physiology, endocrine and metabolic status, growth, reproductive status) and population (abundance, production, reproductive fitness) levels. Specifically, we sought to demonstrate the following linkages: chronic metal exposure → metallothionein induction → perturbed intracellular metal partitioning → endocrine/physiological impairment → diminished growth efficiency → reduced survival, altered population age structure and population dynamics. In the case of **food-web mediated** effects, we investigated the following sequence: chronic metal exposure → reduced food abundance of certain dietary components → increased energetic costs of feeding → reduced growth efficiency and ultimately stunting. Thus we looked for evidence of "energetic bottlenecks" imposed by the absence of key prey components that are necessary for normal diet shifts and growth to occur.

MATERIALS AND METHODS/DESCRIPTION OF EXPERIMENTAL APPROACH

Summary

Our main study area was centered around Rouyn-Noranda (Abitibi, NW Quebec), a region for which we had already collected geochemical data relating to the degree of metal exposure in more than 20 lakes. Additional complementary sampling was carried out on lakes in the Sudbury, Ontario, region. The project involved a study of lakes with contrasting metal levels (especially Cd, Ni, Zn) but similar trophic status (Perceval *et al.* 2002). The lakes, all of which possess resident fish populations, have been characterized (1) from a *limnological* perspective, to evaluate the habitat quality for fish and the benthic invertebrate populations; (2) at the *geochemical* level, to measure the ambient exposure of the fish to metals; and (3) in a *biochemical* context, to determine the degree to which accumulated metals have been successfully detoxified in the indigenous fish (liver tissue). For each lake, we have studied the response of the indigenous fish to the metal gradient at three different levels: cellular/biochemical (tissue metal levels, subcellular metal partitioning), physiological (endocrine and metabolic status; reproductive status) and ecological (growth, bioenergetics, age structure, food webs). Appropriate statistical

techniques were used to treat the results within each data type (limnological, geochemical, biochemical, biological), and to explore the links among the different hierarchical levels.

Sentinel Species Selection

We chose yellow perch (*Perca flavescens*) as the sentinel organism for our studies, based on the following selection criteria: ubiquity; abundance; relative immobility; ease of sampling; metal tolerance; metal bioaccumulation capacity; dynamics of metal accumulation; capacity to synthesize metallothionein; available physiological and behavioral data; ecological role. Abundant and widely distributed across North America, this species does not travel over long distances and therefore its metal body burden tends to represent local sources (Aalto and Newsome 1990; Hontela *et al.* 1995).

Metals Studied

The suite of metals (Cd, Cu, Zn, Ni, Pb) corresponds to the Metals in the Environment Research Network (MITE-RN <www.mite-rn.org>) core analytes, and all five are high profile metals under the Canadian Environmental Protection Act (CEPA). Known natural and anthropogenic sources of Cd, Cu, and Zn exist in the main study area (Rouyn-Noranda, QC) and in earlier work we demonstrated the existence of clear metal concentration gradients for these metals in lakes from this region (Couillard *et al.* 1993; Wang *et al.* 1999; Perceval *et al.* 2002); at the biochemical level these three metals are known to induce metallothionein (MT) biosynthesis in the laboratory and to bind to MT in the cytosol. Comparable spatial gradients for Cu and Ni have been documented in the Sudbury area (Borgmann *et al.* 2001a,b; Eastwood and Couture 2002). Lead concentration gradients have been documented downwind from the Rouyn-Noranda smelter (*e.g.,* in lake sediments), but concentrations in biological tissues were frequently below our analytical detection limits.

Site Selection and Characterization

In choosing our study lakes we considered three main factors: the need to cover a spatial metal gradient; the accessibility of the lakes and their proximity to regional laboratories; the availability of relevant biological and geochemical data. Recent geochemical and limnological data were available for 20+ lakes in the Abitibi area, located both upwind and downwind from the Horne smelter in Rouyn-Noranda, and fish data were available from 12 of these lakes. A subset of these 12 lakes, of similar trophic status but distributed along the metal concentration gradient, was considered for organism- and population-level studies. In selecting lakes of similar trophic status we sought to minimize differences in fish habitat quality (including estimates of available prey). The final group of lakes retained for intensive study included some pristine lakes, with low ambient metal levels, to serve as controls. Very recently we have extended our study to lakes in the Sudbury, Ontario region that have been investigated by other MITE-RN researchers (Borgmann *et al.* 2001a,b; Eastwood and Couture 2002).

To evaluate the *habitat quality* and *trophic status* of the initially selected 20+ lakes, we required time-series data on key limnological variables: nutrients (total P, inor-

ganic N), dissolved organic carbon, chlorophyll-*a*, and water chemistry (O_2, Ca, pH), as well as ambient metal levels (*e.g.*, Couillard *et al.* 1993; Croteau *et al.* 1998; Wang *et al.* 1999; Perceval 2002). To compare the quantity and quality of the food resources available to the fish populations, we collected planktonic and benthic invertebrates from each lake and examined the stomach contents of captured fish.

Fish Measurements

To determine the biochemical and physiological status of the indigenous fish in each lake, young-of-the-year (YOY), 1^+ and adult yellow perch of similar age (age 4+, N=20) were collected in the early summer. Adult specimens were also collected in the middle phase of the reproductive cycle (late Summer). Fish in gonadal recrudescence were sampled so that reproductive effects could be detected as well as effects on growth and physiology. These specimens were used for the following determinations:

a. *Contamination indices*: accumulated Cd, Cu, Ni, Pb and Zn in three tissue groups (gills, liver and kidney, including the pronephros where cortisol is secreted); liver cytosolic metals — total concentrations + subcellular partitioning (size-separation HPLC and differential centrifugation); liver cytosolic metallothionein. The analytical methods for determining these various indices of contamination are described in Wang *et al.* (1999) and Wallace *et al.* (1998: differential centrifugation). These measurements revealed how the contamination indices vary along the environmental metal gradient, and enabled us to monitor for perturbations in sub-cellular metal partitioning in feral fish.

b. *Physiological indices*: In fish captured in lakes located along the metal gradient, blood, liver and kidney samples were collected and analyzed for hormones (cortisol, triiodothyronine T3, thyroxine T4) and various biochemical parameters (glucose, glycogen, lipids). The functional integrity of the interrenal organs secreting cortisol was assessed *in vitro* using the adrenocorticotropic hormone (ACTH) challenge test (Laflamme *et al.* 2000) and a histopathological examination of vital organs such as gills, liver and kidney was performed (Levesque *et al.* 2003). In addition, reproductive status was estimated by gonad size (gonado-somatic indices or GSI, gonad weight as % of body weight). These measures, obtained on adult specimens of similar age (4+ age class, N=20) from the studied lakes, enabled us to evaluate the endocrine and metabolic status of the indigenous fish.

c. *Ecological parameters*: Growth rates and population dynamics of the indigenous fish populations were determined from age-size and abundance curves. Quantitative seine hauls (Pierce *et al.* 1990, 1994) and experimental gill samples (n=10) from each lake were used to assess the proportion of different age classes of YP in each population. Measurements done on each fish included: age, sex identification, length, and weight. Fish ages were determined using scales, otolithes and opercular bones; condition factor was calculated as weight (grams)/(length (cm))^{3}x100. Measurements of ^{137}Cs levels in yellow perch

and in their gut contents, together with our existing data on ^{137}Cs dynamics in this species, were used to evaluate their bioenergetic budget (feeding rate, growth rate, growth efficiency). ^{137}Cs is a globally dispersed radioisotope that accumulates in fish tissues through trophic transfer. This radioisotope has a very long biological half-life, and the changes that take place in the body burden throughout life (across age classes) can be used to calculate the feeding rates from a whole body mass-balance model (Rowan and Rasmussen 1996; Tucker and Rasmussen 1999). In addition to age-specific body burdens, this model requires data on [^{137}Cs] in prey (diet), and laboratory-derived data on assimilation and elimination rates of the contaminant. Using this method (see Sherwood *et al.* 2000 for details) we were able to compare specific growth rates of fish to specific consumption rates, and thus compare growth efficiency among our different study populations.

Statistical Analyses

In the analysis and interpretation phase of this project, we dealt with various types of data: limnological (habitat quality); geochemical (ambient metal levels); cellular (effectiveness of detoxification at the biochemical level); whole organism (physiology, endocrine and metabolic status, growth, reproductive status); and population (growth curves, bioenergetics, food webs, age-class structure). Statistical techniques were used to treat the results within each data type, and to explore the anticipated links among the different hierarchical levels.

RESULTS

Using a variety of end-points (tissue metal concentrations; subcellular metal partitioning; endocrine physiology; reproductive status; population status), we assessed inter-lake variability in the responses of indigenous yellow perch (YP) collected from lakes situated along a marked metal concentration gradient. In the following section we summarize the main conclusions of our work to date. Some of the results have already been published in the peer-reviewed literature, and they are appropriately cited; some are currently in review but are nevertheless included in the bibliography; unpublished results are cited in the text only.

Metal Exposure Gradient

Total dissolved and calculated free metal concentrations in the study lakes varied markedly among lakes (Table 1). Metal "gradients", defined as the ratios of the highest observed metal concentration/lowest metal concentrations, are given in the last two lines of Table 1. For example, in the lakes sampled in 2000 (all in the Rouyn-Noranda area) the maximum/minimum ratios decreased in the order $[Zn]_d$ (~100) > $[Cd]_d$ (~70) >> $[Cu]_d$ (8). Gradients for the free metal ions were even greater, but in this case the order was reversed: $[Cu^{2+}]$ (4700) >> $[Zn^{2+}]$ (400) » $[Cd^{2+}]$ (350). The pronounced gradient for free copper stems from the extremely low estimates of free $[Cu^{2+}]$ in the reference lakes, reflecting its important complexation with dissolved organic matter.

Table 1. Water quality parameters for the studied lakes. [a,b]

Lake	Year	$[H^+]$ (nM)	[Cd][c] (nM)	[Cu][c] (nM)	[Zn][c] (nM)	[Ni][c] (nM)	[DOC][d] (mg/L)	$[Cd^{2+}]$ [e] (nM)	$[Cu^{2+}]$ [e] (nM)	$[Zn^{2+}]^e$ (nM)	$[Ni^{2+}]^e$ (nM)	$[Ca^{2+}]^e$ (µM)
Dufault (DU)	2000	32	7.7	250	2430	-	5.5	3.7	0.14	1150	-	590
	2001	35	6.7	180	1100	10	5.3	2.3	0.024	370	4.8	310
Osisko (OS)	2000	630	2.4	120	470	-	3.5	1.4	0.63	300	-	950
	2001	0.3	1.6	140	110	20	3.1	0.22	<0.001	0.9	2.8	420
Bousquet (BO)	2000	250	1.1	56	160	-	14.7	0.21	0.012	45	-	150
Vaudray (VA)	2000	110	0.81	54	110	-	9.0	0.16	0.006	29	-	130
	2001	63	0.50	42	60	10	8.8	0.03	0.001	5.2	2	53
Héva (HE)	2000	790	0.54	35	56	-	9.4	0.16	0.029	23	-	93
Dasserat (DA)	2000	32	0.18	32	57	-	9.5	0.02	<0.001	7.6	-	290
Ollier (OL)	2000	13	0.12	46	23	-	6.3	0.02	0.001	4.3	-	520
Opasatica (OP)	2000	6	0.11	44	31	-	7.7	0.01	<0.001	2.8	-	340
	2001	18	0.30	33	20	10	7.7	0.02	<0.001	1.4	2.7	160
Hannah (HA)	2001	95	2.4	370	50	2500	3.7	1.0	0.43	24	1600	200
Wavy (WA)	2001	9770	1.5	110	120	900	2.8	1.2	11	100	750	34
Raft (RA)	2001	210	1.7	130	160	1820	2.6	0.87	0.092	79	1280	72
Laurentian (LA)	2001	430	0.6	140	30	840	4.6	0.25	0.066	12	510	87
max/min	2000	-	130	70	8	100	-	4	350	$4.6 \cdot 10^3$	400	-	10
max/min	2001	-	$3.4 \cdot 10^4$	23	11	59	300	3	117	$1.1 \cdot 10^5$	420	860	12

[a]Water samples were collected using duplicate *in situ* diffusion samplers. Three replicate samples (3.5 mL) were removed from each sampler for the determination of trace metal (Cd, Cu, Zn, Al, Fe) and major cation (Ca, Mg, Na and K) concentrations; two replicate samples (~1.5 mL) were removed from each sampler for major anions (SO_4^{2-}, NO_3^-, Cl^-). Average concentrations were calculated for each sampler resulting in 2 measurements for each lake. Pb has been omitted from the table because its concentrations in many water and tissue samples were below our analytical detection limits. [b]For comparison purposes, the Canadian Water Quality Guidelines for these metals are as follows: Cd = 0.03→0.19 nM (for hardness = 10→60 mg $CaCO_3$/L); Cu = 31 nM; Ni = 425 nM; Zn = 460 nM. [c]Total dissolved metal concentration (*in situ* dialysis). [d]Total dissolved organic carbon (*in situ* dialysis). [e] The free Ca^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+} concentrations were estimated with the Windermere Humic Aqueous Model (WHAM 6.0.1; NERC 2001). Input data included the pH, the total dissolved metal concentrations (including Fe and Al), and concentrations of inorganic ligands and organic carbon.

Metal Bioaccumulation

In juvenile and adult YP, Cd levels increased in all body parts except the carcass along the metal concentration gradient, with higher concentrations in the kidney and liver than the other organs. The relative Cd burdens in different organs changed along the gradient: the liver's contribution to the total body burden became more important in the more contaminated lakes, whereas the contribution from the carcass decreased (Giguère *et al.* 2001; 2003a; Gravel *et al.* 2003; Levesque *et al.* 2002; 2003). Increases in tissue Cu and Zn concentrations were much more modest than in the case of Cd, suggesting some degree of homeostatic control for these two essential metals (especially true for Zn, even though ambient dissolved Zn levels vary enormously in the Rouyn-Noranda lakes — Giguère *et al.* 2003a). Tissue Pb concentrations were frequently below our analytical detection limits.

In pilot-scale transplant experiments, where juvenile YP (1^+) were moved from a relatively clean lake (Opasatica) to a metal-contaminated one (Dufault), marked accumulation of Cd (but not of Cu or Zn) occurred over the first 30 d; this accumulation was most marked in the gastrointestinal tract, suggesting that food is an important source of Cd to juvenile YP in the wild (Kraemer *et al.* 2002).

Subcellular Metal Partitioning

Juvenile YP (1^+) were collected from 8 lakes (Table 1, 2001 sampling) for this part of the study. Liver was chosen for metal subcellular analysis since our earlier results on metal distributions among whole organs had shown that the relative contribution from this organ to the total Cd body burden increased in the more contaminated

lakes. In addition, the liver accumulated the highest Cu concentrations and exhibited the greatest inter-lake variation in Cd concentrations. Kidney tissue was also considered, but its small mass relative to liver tissue mitigated in favor of the liver. The fractionation protocol was designed to distinguish among sub-cellular pools associated with metal sequestration (*e.g.*, metallothionein (HSP) and granules) and those linked to cellular toxicity (mitochondria, nuclei and heat-denatured cytosolic proteins other than metallothionein (HDP)). The method used did not allow us to differentiate between lysosomes and endoplasmic reticulum (fragmented ER = microsomes). If metal concentrations detected in this fraction are mostly attributable to an accumulation in the lysosomal fraction, then they would be indicative of metal elimination; on the other hand, if they were due to an accumulation in the endoplasmic reticulum, they could be indicative of toxicity.

Trends in subcellular partitioning are best illustrated by the results for Cd, an non-essential metal (Figure 1). Most of the hepatic Cd burden was present in the cytosol, in the metallothionein-like fraction (corresponding to our HSP fraction — see Figure 1C). As shown in Figure 1A, tissue metallothionein (MT) concentrations increased along the exposure gradient (largely in response to the Cd exposure gradient — Laflamme *et al.* 2000; Giguère *et al.* 2003b). However this induction in MT was *in*sufficient to protect the other subcellular fractions, that is, metal concentrations in fractions corresponding to potential targets of metal toxicity (mitochondria, nuclei, heat-denatured cytosolic proteins) also increased along the spatial gradient (Figures 1A and 1B).

Physiological Effects

Physiological responses of organisms to contaminants are integrators of subcellular and cellular processes, and may be indicative of the overall fitness of the individual organism (Cappuzzo 1988; Hontela 1997). Adult and 1^+ perch collected from the more contaminated lakes exhibited endocrine impairment but YOY did not (Gravel *et al.* 2003). This impairment was characterized by an attenuated cortisol stress response *in vivo* (Figure 2A) and a lower secretory capacity in response to ACTH *in vitro* (Laflamme *et al.* 2000; Levesque *et al.* 2002). Moreover, lower levels of the thyroid hormones T3 and T4, key hormones for regulation of intermediary metabolism and osmoregulation, were measured in adult YP from contaminated lakes but not in 1^+ fish (Levesque *et al.* 2003; Gravel *et al.* 2003), suggesting that an exposure of at least 1 year is necessary to induce this specific endocrine impairment.

In addition to the endocrine response, anomalies in intermediary metabolism and use of energy reserves were observed in adult YP from contaminated lakes (Figure 2B). Seasonal cycles of build-up of liver energy reserves (glycogen, triglycerides) were altered and enzymatic activities mediating lipid, carbohydrate and protein metabolism were perturbed (Levesque *et al.* 2002). Results for the 1^+ YP suggested an impaired capacity to mobilize liver glycogen reserves. Overall, the physiological effects tended to increase in the sequence YOY < 1^+ < adult perch.

Histopathological alterations were observed in organs of fish from contaminated lakes, specifically in gills (club-shaped secondary gill lamellae, increase in diffusion distance), thyroid tissue (deformed thyroid follicles and thinner follicular epithe-

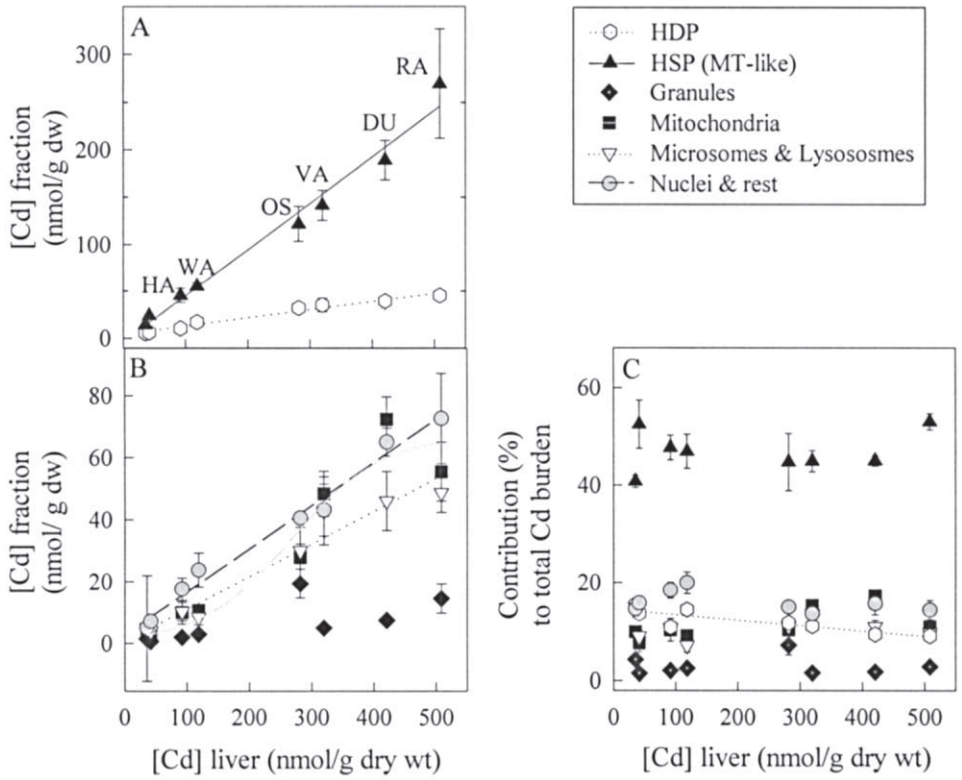

Figure 1. Relationships between liver metal concentrations and (A) Cd concentrations in two protein fractions of the hepatic cytosol (HDP = heat-denatured proteins; HSP = heat-stable proteins), (B) Cd concentrations in various particulate fractions, and (C) the Cd contribution of each subcellular fraction to the total Cd burden. Each point represents data for fish collected in a single lake (mean ± SD; N=3). Sampled lakes are shown in Table 1 (Year = 2001). Note that as the total concentration of Cd in the liver increases, so too do Cd concentrations in all the subcellular fractions (except the granules).

lium), interrenal tissue (smaller nuclei of steroidogenic cells), and gonads (delayed gonadal maturation) in adult YP (Figure 2C: Levesque *et al.* 2003).

Ecological Effects

Sherwood *et al.* (2000) showed that yellow perch in two highly contaminated lakes, Osisko and Dufault, grew more slowly, less efficiently relative to ration, and reached a smaller maximum size than did fish from two reference lakes, Opasatica and Dasserat. In addition, the fish from Osisko and Dufault did not undergo the normal sequence of diet shifts (zooplankton to littoral macroinvertebrates to littoral fish) that yellow perch display in reference lakes (Sherwood *et al.* 2002a). Instead, they continued to utilize smaller prey throughout their lives, although in both contaminated lakes some piscivory was evident in the larger fish. Stunting was linked

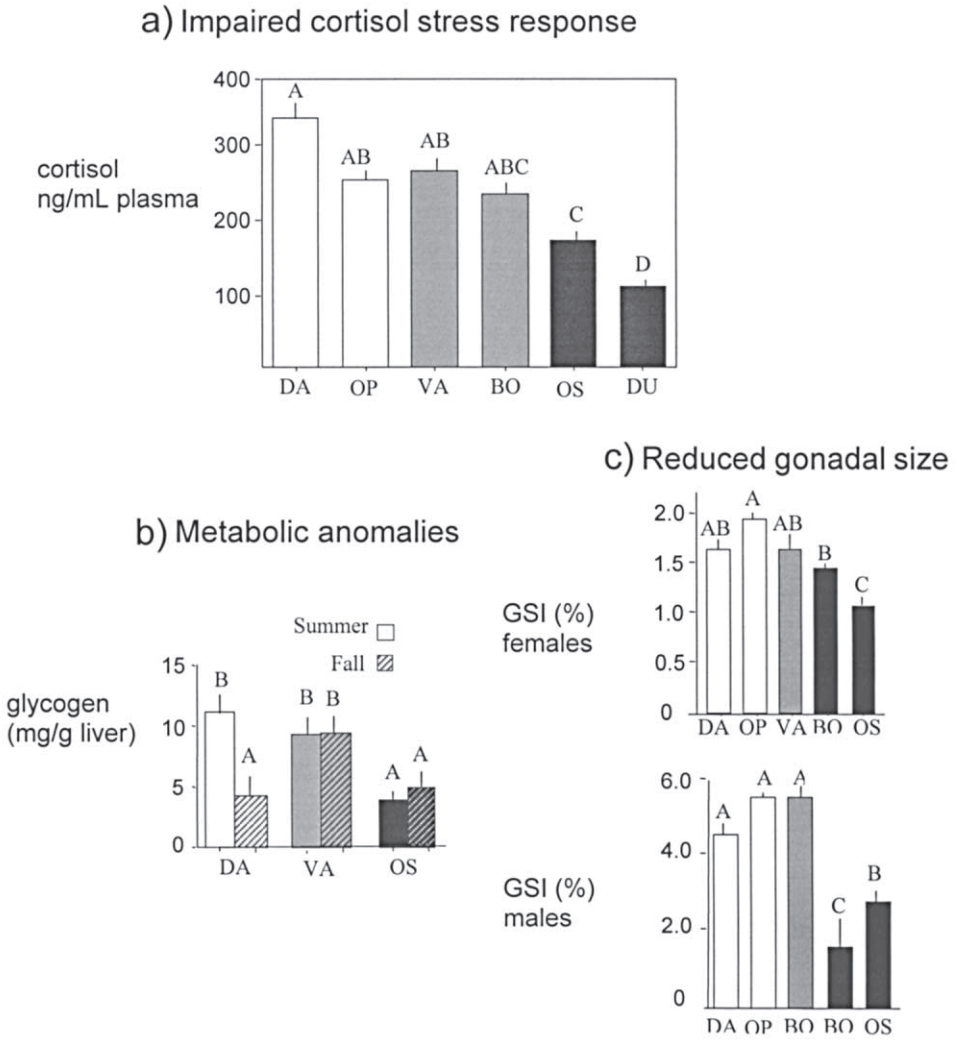

Figure 2. **Yellow perch from metal-contaminated lakes exhibit (a) endocrine anomalies such as a reduced capacity to elevate plasma cortisol levels in response to a standardized confinement stress, (b) metabolic anomalies such as differences in seasonal cycling of liver glycogen reserves, and (c) reduced gonadal size (GSI, gonadosomatic index).** Fish were sampled from reference lakes, Opasatica and Dasserat (white bars), intermediate Bousquet and Vaudray (gray) and highly contaminated lakes Osisko and Dufault (black). Letters that are different indicate significantly different means ($P<0.01$, Tukey-Kramer test). (Modified from Laflamme *et al.* 2000; Levesque *et al.* 2002, 2003.)

to the failure to complete diet shifts to larger-sized prey, and in both lakes this was associated with an impoverished littoral benthic community (Sherwood *et al.* 2000; Kovecses 2002). In reference lakes the littoral benthos constituted a diverse community of macro-invertebrates which serve as a bridge of intermediate-sized prey that allow fish to select gradually larger and larger prey as they grow; in these lakes YP make the shift from zooplankton to littoral macrobenthos during their second year of growth (12 to 15 g), and then begin to include a significant amount of fish in their diet during their third to fifth year of growth (25 to 50 g).

Kovecses (2002) recently extended the original population study to include lakes Bousquet and Vaudray, two lakes with intermediate levels of metal exposure. In these lakes, she observed no clear-cut evidence of food web disturbance leading to bioenergetic bottlenecks and impaired diet development, as seen in the more highly contaminated lakes. Littoral macro-invertebrate taxa such as amphipods, Ephemeroptera and Odonata were abundant in lakes Vaudray and Bousquet, within the same range as the reference lakes, and these prey items made up the majority of the perch diet in these lakes.

In addition to these indirect effects on YP energetics, acting through food web bottlenecks, Sherwood *et al.* (2000) also reported data on yellow perch age structures that indicate that the younger age classes of yellow perch in the most metal-contaminated lakes experience reduced survivorship, perhaps as a result of direct toxic effects of metal exposure. Yellow perch populations are usually dominated by younger age classes. In reference lakes, fish of age 2^+ (the youngest age class that we could consistently sample) made up on average 40% of the total catch, with the percentage declining gradually with age. Thus, annual survivorship appears to be approximately 75% or better over the age interval 2 to 9 yr. Conversely, in the metal-contaminated lakes, age 2^+ perch made up only about 10% of the total catch, with the remainder of the catch being distributed throughout the remaining age classes. Thus survivorship appears to be very low in the first two years of life, but becomes essentially normal after age 3. The age structure of yellow perch in intermediate lakes was between the two previously outlined extremes, with age 2^+ fish making up approximately 20% of the total catch, and slightly higher representation in the 3^+ and 4^+ age categories. In contrast to metal effects that contribute to reduced survivorship in fish of age 3^+ and less, the food web bottlenecks that lead to impaired growth (stunting) are most clearly manifested in adult-age (4^+) fish, which supports the idea that they are acting through a different mechanism.

DISCUSSION

Direct Effects

Our studies provide unambiguous evidence that metals (Cu, Ni, and especially Cd) accumulate to higher levels in yellow perch in lakes at the high end of our exposure gradient than in fish from the control lakes; for Cd our preliminary results suggest that this accumulation is largely diet-borne. The liver and the kidney are the organs where this accumulation is most noticeable (*i.e.*, the organs where the internal metal concentrations are highest, and thus where toxicological effects are most likely). Note that both the kidney and the interrenal tissue accumulate metals; to our knowledge this is the first field demonstration that an endocrine tissue such

as the interrenal tissue (the head kidney, which secretes corticosteroid hormones) accumulates metals along an environmental metal gradient.

Metal accumulation in YP is accompanied by induction of metallothionein, in what might be construed as a detoxification response, *but* all our evidence to date suggests that metal detoxification by metallothionein is *incomplete*. For example, even in the moderately contaminated lakes, some of the Cd is found bound to potentially sensitive subcellular fractions other than metallothionein. There is no indication of a threshold level below which all the incoming Cd is sequestered by metallothionein.

As pointed out by Mason and Jenkins (1995) in their comprehensive review, our current models of metal detoxification mechanisms have been derived almost entirely from exposure experiments where test organisms are exposed to a range of metal concentrations (*e.g.*, experiments analogous to those run by Brown and Parsons (1978) or by Jenkins and Mason (1988)). Under such conditions, at high external metal concentrations, one might well expect the metal detoxification mechanism(s) to be overwhelmed by the influx of metal (*e.g.*, Cd). Metallothionein synthesis would cease and the Cd-MT levels would reach a plateau. Indeed, just such results were reported by Baudrimont *et al.* (1999) for a field experiment in which bivalves (*Corbicula flumineae*) were transplanted from an uncontaminated site to a grossly contaminated site on the River Lot in southeastern France — the plateau in MT concentrations preceded by several weeks the complete mortality of the transplanted molluscs. However, the present study does *not* correspond to a time-course experiment. We have instead sampled indigenous animals that are chronically exposed to high but sublethal metal concentrations. Under such conditions, the measured subcellular metal distribution represents a steady-state situation, where the incoming metal is detoxified sufficiently to allow the organisms to survive indefinitely, but where there may well be a metabolic or physiological penalty. The observation of nonessential metals such as Cd in potentially sensitive subcellular fractions (heat-denatured cytosolic proteins; microsomes; nuclei + residue fraction) may be taken as evidence of incomplete detoxification.

Given this evidence of incomplete Cd detoxification, one might reasonably expect to find evidence of toxicity at the cellular and physiological levels. Indeed, metal toxicity is detected at multiple levels of biological organization, from effects at the cell level, to effects in organs and tissues, to individuals and populations, in a pattern linked to metal concentrations (*i.e.*, along the contamination gradient). Morphological characteristics of several organs (gills, interrenal tissue, thyroid, gonads) are altered in metal concentration-dependent patterns. In parallel with some of these morphological changes, physiological impairment is also observed. A decreased capacity to secrete cortisol and thyroid hormones T4 and T3, key hormones for regulation of intermediary metabolism and osmoregulation, is detected in adult fish. Although cortisol impairment is evident in 1^{+} YP, the capacity to secrete cortisol seems normal in YOY perch from contaminated lakes, suggesting that an exposure of at least 1 year is necessary to induce this specific endocrine impairment.

To return to the question posed in the Introduction, regarding the evidence that metals have a direct effect on indigenous yellow perch in the Rouyn-Noranda and Sudbury lakes, we would argue that observed changes in endocrine and physiological status are very likely direct effects. The diagnosis of cortisol impairment observed

in 1^+ perch, life stages that feed on plankton in all the lakes (both reference and metal-contaminated), provides strong evidence that cortisol impairment is caused by direct effects of metals, either waterborne or diet-borne, rather than by effects mediated through changes in food webs. The effects on organ morphology are also clearly direct effects of metals — there are no reports in the literature to indicate that differences in food (web or quality) can cause histopathological changes in organ morphology. The metabolic effects are however more difficult to interpret since quantity of food could influence intermediary metabolism (and consequently energy reserves and enzyme activities).

Indirect Effects

In our most contaminated lakes (Osisko and Dufault), the evidence for indirect, food-web-mediated effects of metals on yellow perch is compelling (Figure 3). In reference lakes, YP make the shift from zooplankton to littoral macrobenthos during their second year of growth (12 to 15 g), and then begin to include a significant amount of fish in their diet during their third to fifth year of growth (25 to 50 g). Fish in lakes Osisko and Dufault did not undergo this normal sequence of diet shifts. Instead they continued to utilize smaller prey throughout their lives, although in both lakes some piscivory was evident in the larger fish. We hypothesize that this failure to complete diet shifts to larger-sized prey, associated with the impoverished littoral benthic community in these contaminated systems, leads to a bioenergetic bottleneck and stunted growth. Sherwood *et al.* (2002b), using measurements of lactate dehydrogenase in muscle tissue, showed that immediately upon shifting to larger prey sizes (*e.g.*, from zooplankton to macro-invertebrates, or from macro-invertebrates to fish), energetic costs decreased. This observation is consistent with the higher growth efficiency reported by Sherwood *et al.* (2000) for fish populations that successfully carried out diet shifts as they grew (Figure 3B); these latter *in situ* estimates of growth efficiency were obtained with a ^{137}Cs radio-tracer technique.

The indirect, food-web-mediated effects on YP perch diet development and growth, demonstrated in our lakes, are not unique to metal-contaminated environments; very similar patterns can be seen in eutrophic lakes, and also in lakes that have been acidified. In Saginaw Bay, MI, which has gradually become eutrophied over the last 40 years, yellow perch have been greatly impacted (Schaeffer *et al.* 2000). When *Hexagenia*, a large benthic macro-invertebrate, was the most abundant prey species, perch grew rapidly to large sizes (several hundred g, 25 to 30 cm). Following eutrophication, *Hexagenia* and other large macro-invertebrates disappeared, and perch now feed mainly on small chironomid larvae, grow very slowly and stunt at a maximum size of 15 to 18 cm. Similar changes took place in eutrophic basins of Lake Erie (Hayward and McGraf 1987). In a study of YP populations in eastern Quebec, Boisclair and Rasmussen (1996) found that growth and consumption rates were negatively affected by eutrophication. Growth, consumption, and activity rates decreased with the increase of the percent contribution of small prey to the invertebrate community. In small Danish lakes, Jeppesen *et al.* (2000) reported that eutrophication leads to decreases in the abundance of piscivorous fish relative to planktivores. Changes in YP growth and feeding patterns are important

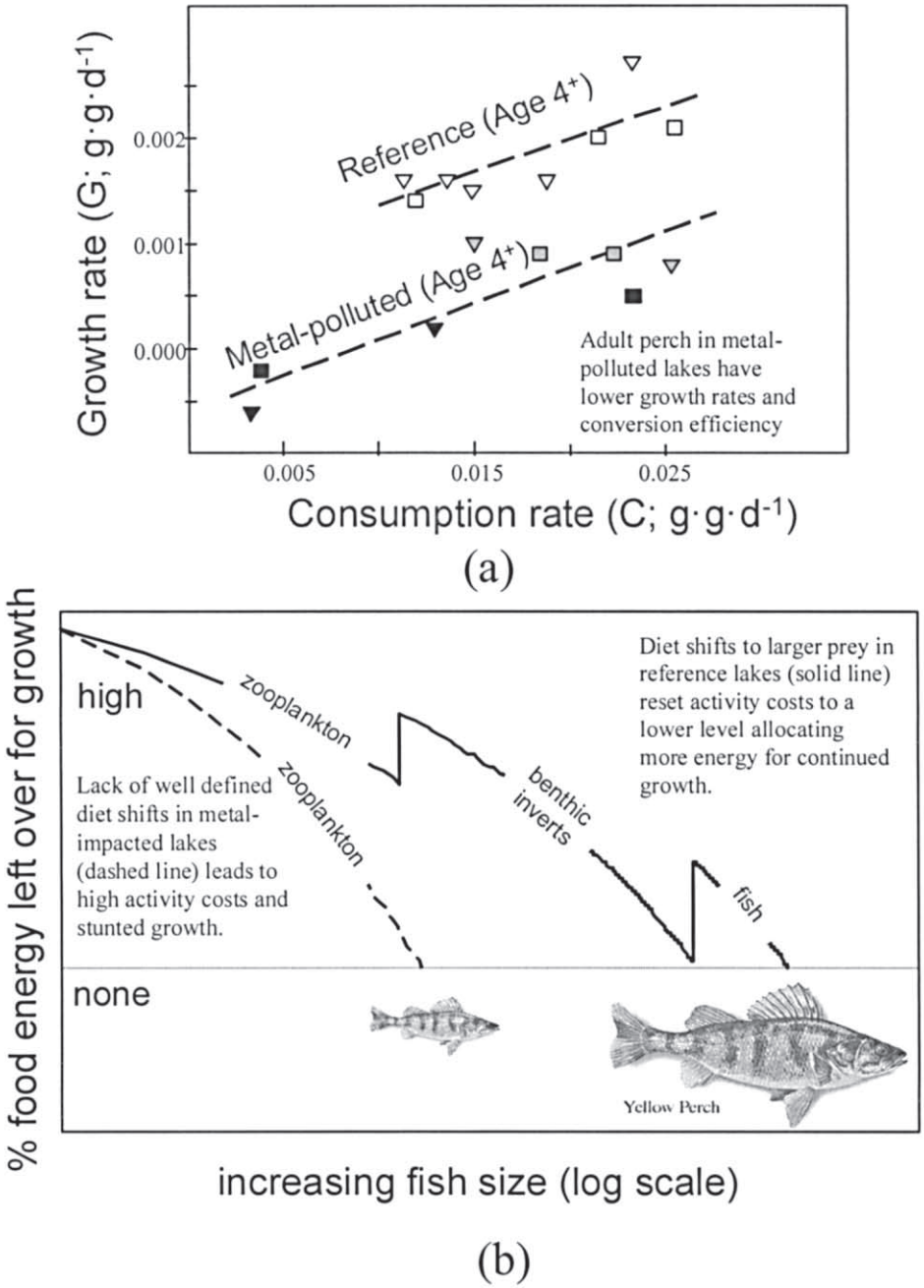

Figure 3. **(a) Relationship between specific daily growth rate and specific daily consumption rate in female (squares) and male (triangles) 4+ yellow perch from reference and metal-contaminated lakes.** Clear symbols denote reference lakes, dark symbols denote metal-contaminated lakes, and grey symbols denote intermediate

Figure 3 (continued)

lakes. (Modified from Sherwood *et al.* 2000.) Specific growth rate (y-axis) and specific consumption rate (x-axis) are both measured in g fresh biomass per g body fresh body weight per day ($g \cdot g \cdot d^{-1}$) and the scales denote a relative linear comparison. (Modified from Sherwood *et al.* 2000). **(a) Schematic outline comparing bioenergetic budgets for yellow perch in metal-contaminated lakes, where well-defined diet shifts do not occur, to perch in reference lakes where well-defined diet shifts occur allowing fish to select larger prey as they grow.** Growth efficiency declines as fish become larger since more individual prey items must be consumed to maintain the same specific consumption rate (daily ration). This ultimately reduces growth to zero (stunting), unless shifts to larger prey can be made. Larger prey allow the fish to obtain a normal daily ration while consuming far fewer individual prey, and thus growth efficiency increases sharply at each diet shift. (Modified from Sherwood *et al.* 2002a.)

in this respect since as lakes become richer, perch become progressively less abundant. In eutrophic lakes they rarely grow large enough to be piscivorous, and as planktivores, they lose out in competition with cyprinids. As lakes become richer and phytoplankton biomass increases, transparency decreases leading to reductions in the productivity and biomass of benthic and epiphytic algae and in aquatic macrophyte standing stock and extent of coverage (Vadeboncoeur *et al.* 2001; Vander Zanden and Vadeboncoeur 2002). These littoral production sources play an important role in providing food, habitat, and refuge for littoral macrophytic invertebrates, such as amphipods, which play an important role in the diet of yellow perch.

Although much less studied, a very similar scenario occurs in lakes experiencing acidification (Ryan and Harvey 1980). As lakes become mildly acidified (pH 5 to 6), YP often become very numerous, since they are among the most acid-tolerant fish species. In such lakes, in spite of the scarcity of competing species and an abundant supply of zooplankton, the yellow perch rarely exceed 15 cm in length, and feed mainly on zooplankton and tiny chironomid larvae, which is the benthic invertebrate taxon most resistant to acidification (Schindler *et al.* 1990). In such systems the acidification has severe impacts on the macrophyte and macro-invertebrate communities, in particular on large invertebrate species important to the dietary development of perch, such as amphipods and burrowing mayflies (Lonergan and Rasmussen 1996). While acidified lakes often also suffer high metal loadings, the lakes in Killarney Park, Ontario, are of special interest since on account of their extremely low alkalinity levels they became strongly acidified in spite of being far from smelters and exposed to very low metal loadings (Keller and Yan 1998). Although these lakes experienced significant species losses of zooplankton, benthos and fish, they are recovering rapidly following reduction in smelter emissions. The pH has returned to preacidification levels, as inferred from diatom microfossils in sediment cores, and the zooplankton communities are nearly fully recovered in abundance and species richness (Snucins *et al.* 2001). Whereas many of these lakes still contain few fish species, most support large populations of stunted yellow perch that remain largely dependent on zooplankton for most of their lives. Littoral benthic commu-

nities in these lakes are largely dominated by tiny chironomid larvae. Larger taxa such as amphipods and burrowing mayflies, which are important for good growth and diet development in yellow perch, are usually very scarce or absent in these lakes despite the lakes apparent chemical recovery.

In summary, the invertebrate species that are most vital to growth and diet development of perch are among the most sensitive to a variety of contaminants, and are among the slowest to recover. Perch themselves tolerate a wide range of environmental conditions and are among the most widely distributed fish across the northern hemisphere. Thus, the "stunted perch" scenario that results from pollutant impacts on large macro-invertebrates is a widespread and common occurrence.

Relative Importance of Direct and Indirect Effects

Assessing the relative importance of the direct and indirect effects that we have described for yellow perch is difficult: mechanisms are incompletely known, there may be as yet undiscovered direct/indirect effects, and finally, endpoints such as growth (biomass production) will be influenced by a combination of these types of effects that could only be "teased apart" with elaborate experimental manipulations in the laboratory and field. However, based on the mechanisms that are known, it is possible to distinguish: (a) endpoints that we can interpret as direct physiological/biochemical effects of metal exposure, (b) endpoints that are primarily a result of reduced energy input resulting from food-web impoverishment, and (c) endpoints that could plausibly be a result of direct or indirect effects, or both acting together.

a. *Direct effects*: In the first 2 years of life yellow perch in all of our study lakes, regardless of metal exposure status, feed on zooplankton, and whereas metal exposure may have reduced the abundance of certain species in the zooplankton community, the overall availability of this resource to fish appeared adequate. Thus effects on juvenile fish, such as cortisol impairment (Gravel *et al.* 2003), reduced condition (Giguère *et al.* 2003), elevated muscle LDH levels in young fish, and reduced survivorship of young age classes are probably direct effects of metal exposure. Effects on adult fish for which no plausible links to reduced energy intake are known should also be interpreted as direct effects on metal exposure. These would include reductions in Na^+/K^+ ATP-ase activity, gill histopathology, reduced sex steroid production, delayed gonadal recrudescence (Levesque *et al.* 2003), and cortisol impairment (Levesque *et al.* 2002; Hontela and Lacroix 2003).

b. *Indirect effects acting through food web impoverishment*: The reduced availability of larger prey in lakes with high metal exposure leads to a sharp reduction in energy allocation to growth in adult perch, and results in stunting and a high degree of zooplanktivory at all ages (Sherwood *et al.* 2002a,b). The fact that such stunting effects are not evident early in life, when many of the direct effects are already evident, supports the interpretation that the stunting is primarily a result of the inability to switch to large prey as fish grow larger. However, it should be noted that direct physiological effects such as cortisol impairment, gill histopathology, and reduced membrane bound ATP-ase ac-

tivity, would also be expected to have some energetic costs, and thus probably exacerbate growth reductions in adult fish. These effects could also have an impact on survivorship, a parameter that is difficult to estimate accurately without capturing inordinate numbers of fish. It should however be noted that "old" perch (8+ and older) were not uncommon even in the most contaminated lakes that we studied.

c. *Effects for which the mechanism (direct vs. indirect) is not clear*. Effects that are first observed in juvenile yellow perch (and are thus attributable to direct effects — see above), but which persist in adult perch, such as reduced condition index, reduced GSI, reductions in thyroid hormones, and changes in liver metabolic enzymes and energy stores (Levesque *et al.* 2002, 2003), are likely to be modulated by the energy budg*et al*terations brought about by food web impoverishment. Such effects could plausibly lead to impaired spawning and recruitment to early age classes, and thus contribute to the under-representation of young age classes in the perch populations in metal-exposed lakes (Sherwood *et al.* 2000).

Finally, it should be noted that there may be indirect effects acting through other mechanisms that we have not yet studied. For example, reduced recruitment or survivorship of young perch could lead to compensatory increases in growth and condition in young fish that survive due to reduced intra-(or inter-) specific competition. "Positive" indirect effects of this kind were reported by Ryan and Harvey (1980) for moderately acidified lakes, where yellow perch growth rates in the first 2 years of life were generally higher than in reference lakes.

CONCLUSIONS

Given that our project is still ongoing, this summary is probably best viewed as an interim progress report. Nevertheless, a number of ERA-relevant observations should be mentioned.

- Our preliminary work indicates that food is an important source of Cd (and other metals?) to yellow perch. If this observation is confirmed, it should trigger consideration of the question whether PNECs expressed in terms of *dissolved* metal will be under- or overprotective.

- Metal "spillover theory" does not seem to apply in the case of chronic/life-long metal exposures (cf. our evidence for incomplete metal detoxification even in moderately contaminated lakes, and the apparent absence of a threshold exposure concentration below which the incoming metals are completely detoxified).

- Consistent with this diagnosis of incomplete metal detoxification, multiple responses (biochemical, physiological, morphological, demographic) can be detected in fish from metal-contaminated lakes. It is however difficult, in a multifactorial environment, to establish unambiguous mechanistic functional linkages among the various responses (*i.e.*, an observed response may be mediated by more than one biochemical or physiological pathway).

- Effects of metals on yellow perch can occur as a result of direct toxic effects of water- or foodborne metals on fish physiology, or they can occur indirectly through effects of metals on prey organisms at the base of the food chain.

- The most common indication of such indirect food-web-mediated effects on yellow perch is severely stunted growth coupled with a high degree of zooplankton dependence throughout life (> 8 years).

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REFERENCES

Aalto SK and Newsome GE. 1990. Additional evidence supporting demic behaviour of a yellow perch (*Perca flavescens*) population. Can J Fish Aquat Sci 47:1959-62

Baudrimont M, Andrès S, Metivaud J, *et al.* 1999. Field transplantation of the freshwater bivalve *Corbicula fluminea* along a polymetallic contamination gradient (River Lot, France): II. Metallothionein response to metal exposure. Environ Toxicol Chem 18:2472-7

Boisclair D and Rasmussen JB. 1996. Empirical analysis of the influence of environmental variables associated with lake eutrophication on perch growth, consumption, and activity rates. Ann Zool Fenn 33:507-15

Borgmann U, Norwood WP, Reynoldson TB, *et al.* 2001a. Identifying cause in sediment assessments: bioavailability and the Sediment Quality Triad. Can J Fish Aquat Sci 58:950-60

Borgmann U, Reynoldson TB, Rosa F, *et al.* 2001b. Final Report of the Effects of Atmospheric Deposition of Metals from the Sudbury Smelters on Aquatic Ecosystems. NWRI Report 01-023. Environment Canada, National Water Research Institute, Burlington, ON, Canada

Brix KV, DeForest DK, and Adams WJ. 2001. Assessing acute and chronic copper risks to freshwater aquatic life using species sensitivity distributions for different taxonomic groups. Environ Toxicol Chem 20:1846-56

Brown DA and Parsons TR. 1978. Relationship between cytoplasmic distribution of mercury and toxic effects to zooplankton and chum salmon (*Oncorhynchus keta*) exposed to mercury in a controlled ecosystem. J Fish Res Board Can 35:880-4

Cain DJ and Luoma SN. 1998. Metal exposures to native populations of the caddisfly *Hydropsyche* (Trichoptera: Hydropsychidae) determined from cytosolic and whole body metal concentrations. Hydrobiologia 136:103-17

Cain DJ, Luoma SN, Carter JL, *et al.* 1992. Aquatic insects as bioindicators of trace element contamination in cobble-bottom rivers and streams. Can J Fish Aquat Sci 49:2141-54

Cappuzzo JM. 1988. Physiological effects of a pollutant gradient - summary. Mar Ecol Prog Ser 46:147-8

Couillard Y, Campbell PGC, and Tessier A. 1993. Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. Limnol Oceanogr 38:299-313

Croteau M-N, Hare L, and Tessier A. 1998. Refining and testing a trace metal biomonitor (*Chaoborus*) in highly acidic lakes. Environ Sci Technol 32:1348-53

Eastwood S and Couture P. 2002. Seasonal variations in condition and liver metal concentrations of yellow perch (*Perca flavescens*) from a metal contaminated environment. Aquat Toxicol 58:43-56

Giguère A, Campbell PGC, Jeanniard Du Dot T, *et al.* 2001. Metal partitioning at the organ and subcellular levels in indigenous yellow perch. 22nd Annual Meeting, Society of Environmental Toxicology and Chemistry, Baltimore, MD, USA, 12-16 November 2001

Giguère A, Campbell PGC, Hare L, *et al.* 2003a. Influence of lake chemistry and fish age on Cd, Cu and Zn concentrations in various organs of indigenous yellow perch. Can J Fish Aquat Sci (*in prep*)

Giguère A, Campbell PGC, Jeanniard Du Dot T, *et al.* 2003b. Metal partitioning at the organ and subcellular levels in indigenous yellow perch. Environ Toxicol Chem (*in prep*)

Gravel A, Campbell PGC, and Hontela A. 2003. Disruption of the hypothalamo-pituitary-interrenal axis in 1^+ yellow perch from metal contaminated lakes. Can J Fish Aquat Sci (*in prep*)

Hayward RS and Margraf FJ 1987. Eutrophication effects on prey size and food available to yellow perch in Lake Erie. Trans Amer Fisheries Soc 116:210-23

Hontela A. 1997. Endocrine and physiological responses of fish to xenobiotics: Role of glucocorticosteroid hormones. Rev Toxicol 1:1-46

Hontela A, Dumont P, Duclos D, *et al.* 1995. Endocrine and metabolic dysfunction in yellow perch *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. Environ Toxicol Chem 14:725-31

Hontela A and Lacroix A. 2003. Heavy metals. In: Carr J and Norris DO (eds), Endocrine Disruptors: Biological Basis for Health Effects in Wildlife and Humans, Chapter 17, Oxford University Press, Oxford, UK (*in press*)

Jenkins KD and Mason AZ. 1988. Relationships between subcellular distributions of cadmium and perturbations in reproduction in the polychaete *Neanthes arenaceodentata*. Aquat Toxicol 12:229-44

Jeppesen E, Jensen JP, Sondergaard M, *et al.* 2000. Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. Freshwater Biol 45:201-18

Keller W and Yan ND. 1998. Biological recovery from lake acidification: Zooplankton communities as a model of patterns and processes. Restoration Ecol 6:364-75

Kovecses J. 2002. Impacts of Heavy Metals on Lake Food Webs: Changes to the Littoral Benthic Invertebrate Communities and the Consequences for Yellow Perch (*Perca flavescens*). MSc Thesis, Dept Biol, McGill University, Montreal, QC, Canada

Kraemer LD, Campbell PGC, and Hare L. 2002. A field study examining the uptake of metals in yellow perch (*Perca flavescens*). 23rd Annual Meeting, Society of Environmental Toxicology and Chemistry, Salt Lake City, UT, USA, 16-20 November 2002

Laflamme J-S, Couillard Y, Campbell PGC, *et al.* 2000. Interrenal metallothionein and cortisol secretion in relation to Cd, Cu, and Zn exposure in yellow perch, *Perca flavescens*, from Abitibi lakes. Can J Fish Aquat Sci 57:1692-700

Levesque HM, Moon TW, Campbell PGC, *et al.* 2002. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. Aquat Toxicol 60:257-67

Levesque HM, Dorval J, Van Der Kraak GJ, *et al.* 2003. Hormonal, morphological and physiological responses of yellow perch (*Perca flavescens*) to chronic environmental metal exposures. J Toxicol Environ Health 66:87-106

Lonergan SP and Rasmussen JB. 1996. A multi-taxonomic indicator of acidification: Isolating the effects of pH from other water-chemistry variables. Can J Fish Aquat Sci 53:1778-87

Luoma SN. 1996. The developing framework of marine ecotoxicology: Pollutants as a variable in marine ecosystems? J Exp Mar Biol Ecol 200:29-55

Mason AZ and Jenkins KD. 1995. Metal detoxification in aquatic organisms. In: Tessier A and Turner D (eds), Metal Speciation and Bioavailability in Aquatic Systems, pp 479-608. John Wiley & Sons, Chichester, UK

Munkittrick KR and Dixon DG. 1988. Growth, fecundity, and energy stores of white sucker (*Catostomus commersoni*) from lakes containing elevated levels of copper and zinc. Can J Fish Aquat Sci 45:1355-65

Munkittrick KR, Miller PA, Barton DR, *et al.* 1991. Altered performance of white sucker populations in the Manitouwadge chain of lakes is associated with changes in benthic macroinvertebrate communities as a result of copper and zinc contamination. Ecotoxicol Environ Safety 21:318-26

NERC (Natural Environment Research Council). 2001. The Windermere Humic Aqueous Model. Version 6.0.1, Equilibrium Chemical Speciation for Waters. Centre for Ecology and Hydrology, Ambleside, Cumbria, UK

Niyogi S and Wood CM. 2003. The effects of chronic waterborne and dietary exposure on gill-metal binding: Implications for the Biotic Ligand Model. Human Ecol Risk Assess 9 [*this issue*]

Perceval O, Pinel-Alloul B, Méthot G, *et al.* 2002. Cadmium accumulation and metallothionein synthesis in freshwater bivalves (*Pyganodon grandis*): relative influence of the metal exposure gradient versus limnological variability. Environ Pollut 118:5-17

Pierce CL, Rasmussen JB, and Leggett W.C. 1990. Sampling littoral fish with a seine: corrections for variable capture efficiency. Can J Fish Aquat Sci 47:1004-10

Pierce CL, Rasmussen JB, and Leggett WC. 1994. Abundance, variation, and structure of littoral fish communities in southern Quebec Lakes. Can J Fish Aquat Sci 51:1128-38

Rowan DJ and Rasmussen JB. 1996. Measuring the bioenergetic cost of fish activity *in situ* using a globally dispersed radiotracer ^{137}Cs. Can J Fish Aquat Sci 53:734-45

Ryan PM and Harvey HH. 1980. Growth responses of yellow perch (*Perca flavescens* Mitchill) to lake acidification in the La Cloche mountains lakes of Ontario. Env Biol Fishes 5:97-108

Schaeffer JS, Diana JS, and Haas RC. 2000. Effects of long-term changes in the benthic community on yellow perch in Saginaw Bay, Lake Huron. J Great Lakes Res 26: 340-51

Schindler DW. 1988. Effects of acid rain on freshwater ecosystems. Science 239:149-57

Schindler DW, Frost TM, Mills KK, *et al.* 1990. Comparisons between experimentally-acidified and atmospherically-acidified lakes during stress and recovery. Proc Roy Soc Edinburgh Sect B - Biol Sci 97:193-226

Sherwood GD, Rasmussen JB, Rowan DJ, *et al.* 2000. Bioenergetic costs of heavy metal exposure in yellow perch (*Perca flavescens*): *in situ* estimates with a radiotracer (^{137}Cs) technique. Can J Fish Aquat Sci 57:441-50

Sherwood GD, Kovecses J, Hontela A, *et al.* 2002a. Simplified food webs lead to energetic bottlenecks in polluted lakes. Can J Fish Aquat Sci 59:1-5

Sherwood GD, Pazzia I, Moeser A, *et al.* 2002b. Shifting gears: enzymatic evidence for the energetic advantage of switching diet in wild-living fish. Can J Fish Aquat Sci 59:229-41

Snucins E, Gunn J, Keller B, *et al.* 2001. Effects of regional reductions in sulphur deposition on the chemical and biological recovery of lakes within Killarney Park, Ontario, Canada. Environ Monit Assess 67:179-94

Tucker S and Rasmussen JB. 1999. Using radiocesium (^{137}Cs) to measure and compare bioenergetic budgets of juvenile Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) in the field. Can J Fish Aquat Sci 56:875-87

Vadeboncoeur Y, Lodge DM, and Carpenter SR. 2001.Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. Ecology 82:1065-77

Vander Zanden MJ and Vadeboncoeur Y. 2002. Fishes as integrators of benthic and pelagic food webs in lakes. Ecology 83:2152-61

Wallace WG, Lopez GR, and Levinton JS. 1998. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar Ecol Prog Ser 172:225-37

Wang D, Couillard Y, Campbell PGC, *et al.* 1999. Changes in subcellular metal partitioning in the gills of freshwater bivalves (*Pyganodon grandis*) living along an environmental cadmium gradient. Can J Fish Aquat Sci 56:774-84