

## Direct and Indirect (Foodweb Mediated) Effects of Metal Exposure on the Growth of Yellow Perch (*Perca flavescens*): Implications for Ecological Risk Assessment

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### ABSTRACT

Yellow perch (YP) are metal tolerant fish that form large populations in many metal-impacted regions across Canada. While they are able to survive and reproduce successfully in environments with water and sediment metal concentrations that are toxic to many invertebrates, perch experience a suite of direct and indirect impacts. YP were studied in a series of Canadian Precambrian Shield lakes near Sudbury, Ontario, along a gradient of metal exposure downwind from metal smelters. In lakes at the high end of our exposure gradient, concentrations of metals (Cu, Ni, and Cd) in YP liver and kidney were well above levels seen in fish from reference lakes. Direct effects linked to metal-exposure were observed, ranging from effects at the cellular level, to effects in organs and tissues, individuals, and populations. In addition to direct or physiological effects, we also documented indirect, foodweb-mediated effects of metals on YP in the most contaminated lakes resulting from the elimination of metal-sensitive large benthic invertebrates and their replacement by small metal-tolerant taxa. The most common indication of such indirect effects on YP is severely stunted growth coupled with a high degree of zooplankton dependence throughout their life. Such indirect effects have important implications for ecological risk assessment (ERA) because they indicate that higher trophic levels may be functionally altered even though the functional groups that they depend on are present and abundant. Although the functional groups important to yellow perch

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can be abundant in metal-impacted lakes, their benthic communities are impoverished and this is strongly reflected in their size structure upon which energy transfer to higher trophic levels depends. Thus *indirect* (foodweb-mediated) effects can be important in situations where invertebrate size structure is impacted in such a way as to reduce the efficiency of energy transfer to higher trophic levels, and therefore measures should be taken to protect and/or restore large sensitive benthic species.

**Key Words:** metals, fish, yellow perch, direct effects, indirect effects, foodweb, cortisol, energetics.

## INTRODUCTION

### Combining Laboratory and Field Studies to Detect Ecosystem-Level Metal Impacts

Attempts to characterize the effects of metals on aquatic biota usually rely on laboratory toxicity tests performed on individual species. Their aim is to evaluate key toxic endpoints (both lethal and sublethal) and to determine the “predicted no-effect concentration” (PNEC) of the metal in water, sediment or tissues, for a prescribed set of test species that span a broad range of sensitivities (Borgmann *et al.* 2001; Milani *et al.* 2003). In applying guidelines determined in this way, it is assumed that in a field situation a high proportion of the species can be protected. This approach assumes that the statistical distribution of sensitivities among the test species reflects that found in the natural system (see Brix *et al.* 2001) and also, that metals are affecting the target species *directly*, either via water-borne or diet borne metal exposure.

Species interact within ecosystems and can be assigned to different functional groups (*e.g.*, herbivores, benthivores, or piscivores); each functional group contains species that are considered to be more or less similar in terms of their energetic role (functional redundancy, Huston 1994). PNEC values can be chosen so as to protect ecosystem function (*i.e.*, at least a portion of each functional group is preserved), although elements of functional group structure, for example, the number and type of species present, may be compromised. An indirect effect is an effect on a species resulting secondarily from a direct effect on another species, for example its prey. If significant *indirect* effects are detected—even though all functional groups are present (although some taxa may be missing), then it could be argued that the presence of all functional groups is not sufficient to preserve ecosystem integrity, and that changes in the structure (or composition) of functional groups can have important consequences. Thus indirect (foodweb mediated) effects should be important in situations where functional group composition is affected by direct metal impacts in such a way as to affect energy transfer within the foodweb.

An alternative to the laboratory approach to setting PNEC for ecosystems involves direct comparison of ecosystem parameters between impacted and reference systems, in order to determine levels of exposure in water or sediment that can be considered acceptable. Such field studies are capable of detecting indirect effects on fish resulting from effects at lower trophic levels, as well as direct sublethal effects on the fish. In a whole lake acidification experiment, Schindler (1988) described bioenergetic and reproductive effects on the top predator, the lake trout (*Salvelinus namaycush*), a relatively acid-tolerant species, resulting from the elimination of its major

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prey species by direct effects of low pH. Several highly impacted mining areas have been studied using this approach (Munkittrick and Dixon 1988; Munkittrick *et al.* 1991; Cain *et al.* 1992; Cain and Luoma 1998), but these studies have often concentrated on site-specific biological comparisons at the expense of general applicability. Although this field-based approach is more capable of detecting shifts in foodweb dynamics resulting indirectly from metal-impoverished functional groups than a purely lab-based approach, field studies have sometimes failed to characterize the exposure regime in sufficient detail (*e.g.*, metal concentrations and speciation in water and sediment, pH, hardness, alkalinity, DOC) to allow direct comparison with laboratory studies and to generalize results for use in risk assessment (Campbell *et al.* 2003).

### The Yellow Perch: A Sentinel for Direct and Indirect Sublethal Effects of Metals

Historically, lakes in the Sudbury, Ontario region were subjected to very high levels of acid and metal loading from industry emissions, which resulted in the elimination of many common species of fish and invertebrates (Keller 1992). One of the species that has proved most resistant to this impact, by virtue of high acid and metal tolerance in gills and other tissues (Niyogi *et al.* 2004; Lacroix and Hontela 2004), is the yellow perch (*Perca flavescens*, YP), which remains the sole abundant species in many lakes that formerly held diverse communities composed of salmonids, percids, and a variety of centrarchids and cyprinids (Gunn *et al.* 1995; Gunn & Mills 1998). The geographic range of YP is broad, and in addition, YP are relatively sedentary, therefore metal body burdens reflect local sources (Aalto and Newsome 1990; Hontela *et al.* 1995; Miller 2003).

#### *Direct effects of metal exposure on yellow perch*

YP that survive in a metal-impacted environment can still be adversely affected throughout life by sublethal toxic effects acting directly on a variety of biochemical, cellular, and systemic processes. The defense mechanisms that YP rely on to resist and survive metal toxicity are poorly understood. Many trace metals induce synthesis of metallothionein (MT) (Laflamme *et al.* 2000; Giguère *et al.* 2005, 2006), which sequesters metals and likely enhances survival. YP have been shown in laboratory studies to be less sensitive to metals than rainbow trout; for example the LC50 for Cd is ~400 times higher for YP than rainbow trout (Niyogi *et al.* 2004), and the viability, functional integrity, and membrane transport characteristics of cortisol-secreting head kidney cells are more resistant to Cd than those of rainbow trout (Lacroix and Hontela 2004; Raynal *et al.* 2005). Although YP survive in metal-impacted lakes, adverse sublethal effects on gill morphology and function (Levesque *et al.* 2003) and on a variety of endocrine and physiological functions have been demonstrated (Brodeur *et al.* 1997; Hontela 1997; Laflamme *et al.* 2000; Rajotte and Couture 2002; Levesque *et al.* 2003). These cellular and biochemical alterations involve homeostatic challenges that, when considered together, are likely energetically costly.

#### *Indirect (foodweb mediated) effects of metal exposure on yellow perch*

Many of the prey species that YP feed on are highly sensitive to metal impacts and their absence can reduce the size these fish attain, despite the presence of all functional groups that YP require (Sherwood *et al.* 2000, 2002b; Kovacs *et al.* 2005).

Young YP feed on zooplankton during their first year, and shift to progressively larger prey (first zoobenthos, then fish) as they grow (Boisclair and Leggett 1989a). These diet shifts reflect the presence of a diverse size range of benthic organisms in most lakes and therefore reflect the functional integrity of the foodweb (Figure 1a). Although zooplankton community impoverishment occurs in metal-impacted lakes, mainly due to impacts on *Daphnia* spp (Yan *et al.* 1996), the most impoverished functional group in metal-impacted lakes is the zoobenthos, and the effect is greatest on the larger taxa such as *Hexagenia*, amphipods and macrocrustaceans (Gunn and Mills 1998; Kovacs *et al.* 2005; Iles and Rasmussen 2005). The indirect reduction of YP growth resulting from the absence of large zoobenthic taxa in metal-impacted lakes is likely due to a reduction in the efficiency of energy transfer (Sherwood *et al.* 2002a,b) because the ability of fish to feed and grow on small prey is progressively reduced as fish grow (Persson 1987) and diet shifts to larger prey are necessary to maintain high growth efficiency (Pazzia *et al.* 2002). Thus the zoobenthic impoverishment, which we observed in metal-impacted lakes of the Rouyn/Noranda region, limits the opportunities for YP to switch to larger prey, and ultimately the size they attain (Figure 1b). Thus the trophic ecology of YP can indirectly reflect the integrity of the lake foodweb.

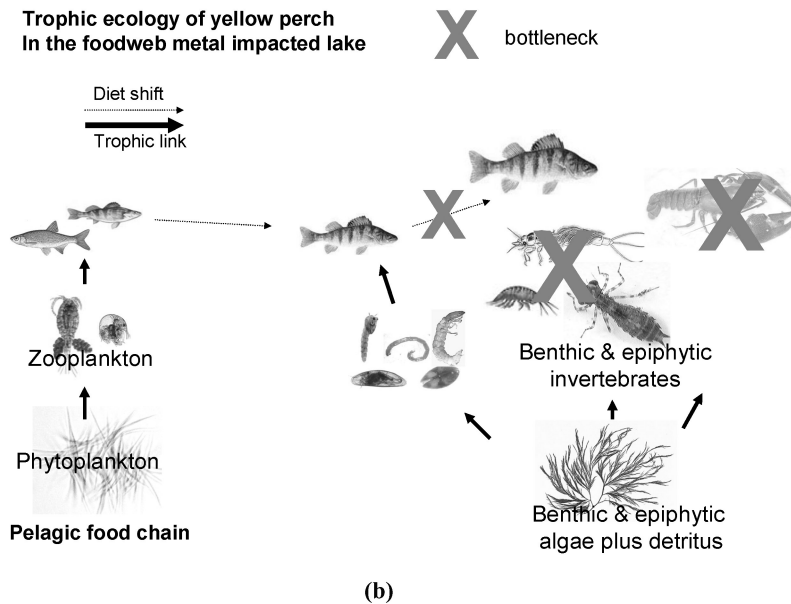
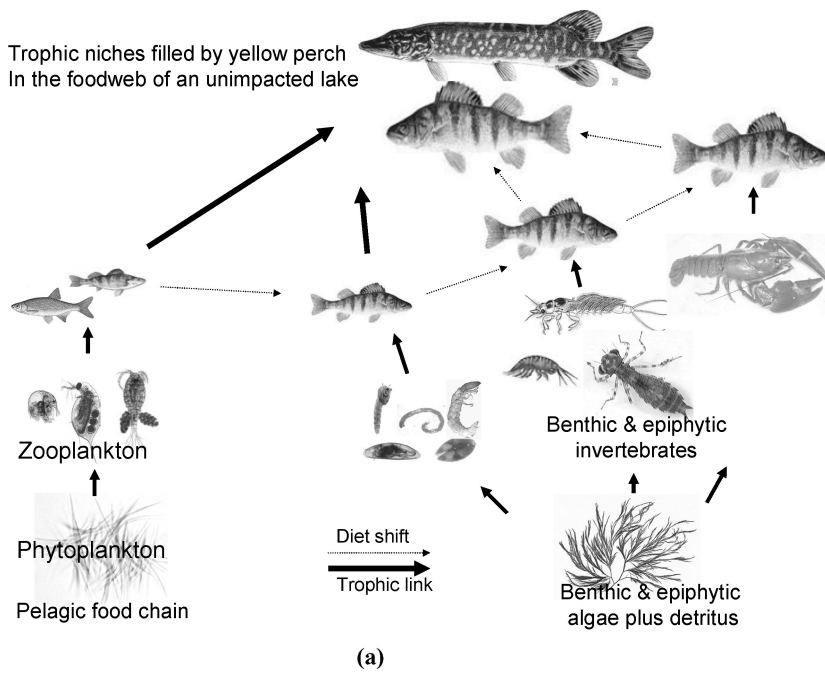
Impoverishment of zoobenthos (and other functional groups as well) is very striking in lakes of the Sudbury area where acid and metal-rich smelter emissions have impacted lakes for many years (Keller *et al.* 1999). Borgmann *et al.* (2001), using the Sediment Quality Triad, showed that benthic community impoverishment in the Sudbury lakes could be explained as a direct result of the toxicity of the metal-enriched surficial sediments to sensitive benthic species. The Sediment Quality Triad (Chapman 1996, 2000) combines sediment chemical analysis, *in situ* analysis of benthic communities, and bioassays of sediment toxicity measuring both survival and growth of key benthic taxa such as *Hexagenia*, *Hyaella*, *Chironomus*, and *Tubifex*. Borgmann *et al.* (2001) linked the Triad to metal bioaccumulation by *Hyaella* (see Chapman *et al.* 1997) and showed that the metal most often exceeding toxic limits in the Sudbury lakes was nickel (Borgmann *et al.* 2001). Thus, metal-related impoverishment of the benthic community in lakes of the Sudbury region and in other metal-impacted regions is well established, and is understood to be a direct result of metal burdens in water and sediment.

## Hypotheses

Our model of the trophic shifts of YP as a sentinel for indirect foodweb effects of contaminants was developed on the basis of studies in the Rouyn/Noranda region where copper smelter emissions have simplified the zoobenthic communities in the lakes east of Noranda (Sherwood *et al.* 2000, 2002a; Campbell *et al.* 2003; Kovacs *et al.* 2005). In order to establish the generality of this model, and to examine the relationship between direct and indirect effects of metals, we conducted a foodweb and physiological field study on a series of lakes reflecting a gradient of metal impacts near Sudbury, Ontario, Canada.

The key hypothesis to be tested regarding **direct effects** was that there exists a mechanistic link between metal concentrations in fish tissues and the manifestation of deleterious physiological effects at the organism and population levels. We

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**Figure 1.** Comparison of the trophic niche of yellow perch within the foodweb of (a) an unimpacted lake with a diverse size range of benthic prey, and (b) an impacted lake where large benthic prey have been lost. Based on Sherwood *et al.* (2002a) and Kovacs *et al.* (2005).

hypothesized that we would observe an exposure-dependent impairment of the cortisol stress response similar to the pattern observed in our Rouyn/Noranda studies, even though the metal loading profile differed between the two regions.

For indirect (**foodweb mediated**) effects, we investigated the following linkage: chronic metal exposure → reduced food abundance of key dietary components → increased energetic costs of feeding → reduced growth/condition 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. We hypothesized that:

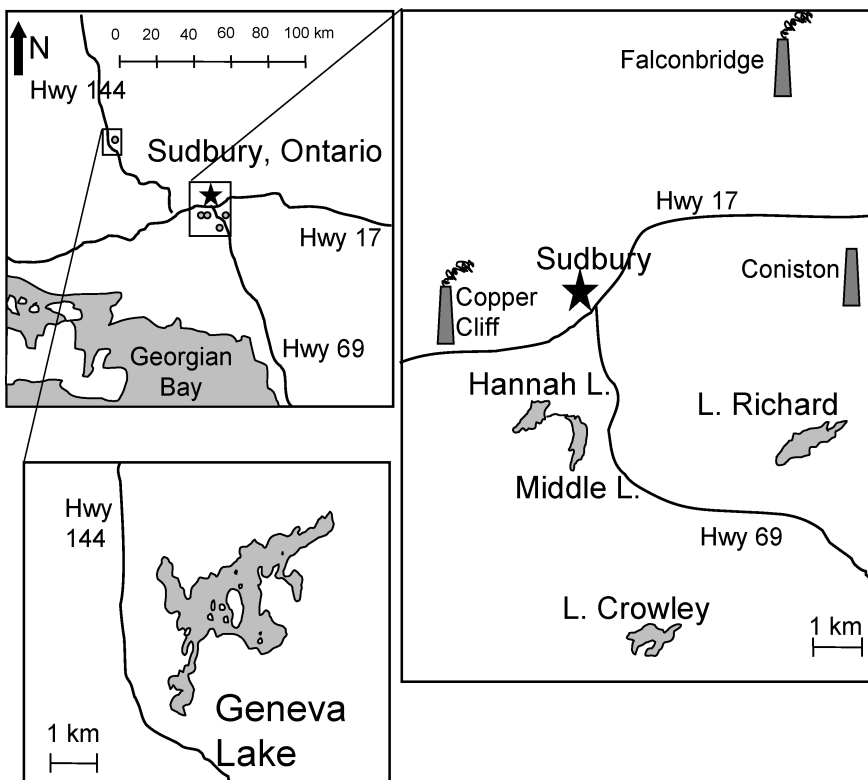
1. Diversity and size of the zoobenthic community would be reduced in contaminated lakes, and that adult YP would exhibit a higher degree of reliance on small prey in metal-impacted lakes than in the reference lake.
2. YP populations would exhibit stunted growth and reduced condition at age 2+ and older in contaminated lakes where diet shifts do not occur, and more rapid growth in reference lakes, where the benthic community is conducive to diet shifting.
3. Activity costs would be lowered (down regulation of the glycolytic pathway as reflected by LDH activity in white muscle) following well-defined diet shifts (planktivory to benthivory at age 2+, and benthivory to piscivory at age 3+) in the reference lake but not in metal impacted lakes.

We expected that both direct and indirect effects related to metal exposure would affect the metabolic budget of the YP and be difficult to partition. However, we hypothesized that energetic bottlenecks resulting from benthic community impoverishment would mainly affect fish 2+ and older, and our study thus centers mainly on this benthivorous stage of their life-history. Analysis of the zooplanktivorous stage is limited to a comparison of size and condition of 1+ YP among lakes, along with a comparison of the size of zooplankton consumed across the gradient of lakes.

### The Study Area

Lakes in the Sudbury region (latitude 46°37', longitude 80°48", Figure 2) have been subjected to high levels of metal loading from industry emissions. The metal contamination profile for Sudbury area lakes is different from that of Noranda lakes where our previous studies were conducted (Campbell *et al.* 2003), and the difference reflects the ore bodies mined in the two regions. Thus [Ni] is ~10 times [Cu] in Sudbury lakes, whereas, the opposite is true in Noranda lakes; [Cu] in water is roughly similar in the most contaminated lakes of the two regions. Although [Cd] << [Cu] in both regions, it is somewhat more important in the Noranda region (Cd:Cu ~ 1:30) than in the Sudbury region (Cd:Cu ~ 1:100). Despite improved emission controls in recent years that have led to declines in concentrations of metals such as nickel, copper, and cadmium in lake sediments (Keller 1992), the metal concentrations in the lakes (Table 1) still remain high enough to be toxic to aquatic communities. Borgmann *et al.* (2001) compared present day Ni and Cu concentrations in lakes near Sudbury that were directly impacted by smelter fallout (surficial sediments >10-fold elevated), to lakes located 10–15 km downwind (surficial sediments 2–3 fold elevated), and to unimpacted reference lakes northwest of Sudbury. In their work,

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**Figure 2.** Map showing the relationships between the study lakes, the municipality of Sudbury ON, and the Coniston, Copper Cliff, and Falconbridge smelters.

sediment toxicity to benthic invertebrates was measured with a battery of growth and survival bioassays with *Hyaella azteca*, *Hexagenia limbata*, and *Chironomus riparius*. The structure of the benthic community in lakes of the Sudbury area reflected their toxicity. *Hyaella*, as well as other amphipods, were rare except in the reference lakes; *Hexagenia* were also scarce, and *Pisidium* and other mollusks were absent from the most contaminated lakes. Estimates of 96-h LC25 and LC50 values determined for *Hyaella*, *Hexagenia*, *Chironomus*, and *Tubifex* by Milani *et al.* (2003) were all below the range of sediment Ni, Cu, and sometimes Cd concentrations observed in the highly contaminated lakes, and for *Hyaella* and *Hexagenia*, below the levels measured in the medium range lakes as well.

Comparing the LC25 and LC50 values for the four benthic taxa to the range of sediment concentrations for Ni found in Sudbury lakes, Milani *et al.* (2003) indicated that sediment metal levels in all but the reference lakes were highly toxic to *Hyaella*, most of those in the highly contaminated lakes were highly toxic to *Hexagenia*, marginally toxic to *Chironomus*, and relatively benign to *Tubifex*. Although the laboratory sediment toxicity tests used to obtain the LC50 and LC25 estimates in these studies involve spiking sediments with metal salts that may potentially be more bioavailable than metals found in lake sediments, it should be noted that the

**Table 1.** Metal concentrations measured in water, surficial sediment, and perch tissues from Lakes of the Sudbury region.

	Highly contaminated		Intermediate Crowley <sup>††</sup>	Richard <sup>††</sup>	Reference Geneva <sup>†</sup>
	Hannah <sup>††</sup>	Middle <sup>††</sup>			
<b>Water</b>					
Cd ( $\mu\text{g/L}$ ) <sup>†</sup>	0.5	0.3	0.2	0.2	<0.03
Cu ( $\mu\text{g/L}$ )	22	24	11	8	1.27
Ni ( $\mu\text{g/L}$ )	111	114	55	57	1.9
pH	7.3	6.9	6.3	7.3	6.6
Alkalinity (mg/L CaCO <sub>3</sub> )	16.9	11.7	2.1	29.9	8.2
DOC mg/L	3.6	3.6	3.3	4.1	7.2
Ca (mg/L)	10.6	11.0	2.1	17.4	3.0
Area (ha)**	27.7	28.1	43.5	83.6	356
Mean depth (m)**	4	2	6.4	3.9	6.3
Max depth (m)	8.5	15	16	9.5	25.3
Distance from Sudbury (km)	6	6	12	9	53
<b>Surficial Sediment<sup>§</sup></b>					
Cd ( $\mu\text{g/g ww}$ )	0.69	0.93	0.14	0.16	0.16
Cu ( $\mu\text{g/g ww}$ )	629	555	61	87	4.9
Ni ( $\mu\text{g/g ww}$ )	558	641	55	142	9.9
<b>Perch livers (<math>\pm\text{SEM, n}</math>)*</b>					
Cd ( $\mu\text{g/g dw}$ )	15 $\pm$ 0.8, 30	19 $\pm$ 1.1, 23	29.3 $\pm$ 2.2, 37	9.6 $\pm$ 0.7, 27	2.0 $\pm$ 0.7, 26
Cu ( $\mu\text{g/g dw}$ )	672 $\pm$ 113, 30	272 $\pm$ 50, 23	69 $\pm$ 7.1, 37	33.9 $\pm$ 4.0, 27	13.7 $\pm$ 0.5, 26
Ni ( $\mu\text{g/g dw}$ )	5.6 $\pm$ 0.8, 30	3.6 $\pm$ 0.8, 23	1.9 $\pm$ 0.6, 37	3.9 $\pm$ 0.7, 27	0.14 $\pm$ 0.6, 26
<b>Perch kidney (<math>\pm\text{SEM, n}</math>)*</b>					
Cd ( $\mu\text{g/g dw}$ )	18.2 $\pm$ 1.5, 30	42.3 $\pm$ 3.4, 23	36.5 $\pm$ 5.2, 37	15.3 $\pm$ 1.7, 27	3.5 $\pm$ 0.6, 26
Cu ( $\mu\text{g/g dw}$ )	112 $\pm$ 41.9, 30	16.5 $\pm$ 1.4, 23	13.2 $\pm$ 1.7, 37	14.7 $\pm$ 1.7, 27	9.6 $\pm$ 0.5, 26
Ni ( $\mu\text{g/g dw}$ )	71.6 $\pm$ 16.3, 30	51.0 $\pm$ 25, 23	17.4 $\pm$ 1.7, 37	14.5 $\pm$ 2.1, 27	8.7 $\pm$ 6.5, 26

\*Gagnon and Hontela, unpublished data, <sup>†</sup>Couture, Laurentian University, pers comm.,

<sup>††</sup>Keller *et al.* (2004). \*\*Carbone *et al.* (1998), <sup>§</sup>J. M. Gunn unpublished data.

sediment bioassays carried out by Borgmann *et al.* (2001) on unspiked lake sediments also support the conclusion that sediments in Sudbury area lakes are often toxic to *Hexagenia* and *Hyalella*.

Our study examined five lakes from the Sudbury region. Although the set of lakes we studied overlapped only partially with the set studied by Borgmann *et al.* (2001), they spanned the same range of metal concentrations in water and sediment (Table 1). Because the precise make-up of the metal loadings in the Sudbury region is different from those impacting the Rouyn/Noranda lakes and YP are abundant in the Sudbury lakes, the Sudbury region provided an excellent system to test the general applicability of our previous findings regarding direct and indirect effects.

#### *Metal exposure regime of the Sudbury lakes*

We studied a series of Precambrian Shield lakes near Sudbury along a gradient of metal exposure at different distances from the smelters (Table 1; Figure 2).



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None of the lakes have significant fisheries and all except Crowley Lake (46° 23' N, 80° 58' W) have some shoreline houses, but no significant development. All sampling took place during June 2002. Hannah (46° 26' N, 81° 02' W) and Middle (46° 26' N, 81° 02' W) Lakes are located in the same catchment, 6 km south of one of Sudbury's largest smelters. Both lakes were highly acidified and metal-contaminated by the 1950s, but have been recovering since they were limed in the 1970s. This intervention raised the pH to circumneutral levels and lowered the metal concentrations in the water column. Nevertheless, sediment metal concentrations remain high (Table 1), so these lakes are categorized as "highly contaminated" in the present study. YP in both lakes comprise >90% of fish biomass, with small populations of brown bullhead (*Ameiurus nebulosus*) and Iowa darters (*Etheostoma exile*). Small populations of golden shiners (*Notemigonus crysoleucas*) and northern redbelly dace (*Phoxinus eos*) are also present in Middle Lake (Iles and Rasmussen 2005).

Richard (46° 26' N, 80° 55' W) and Crowley Lakes are categorized as "intermediately contaminated" lakes based on metal concentrations in water and sediments (Table 1). The Crowley Lake fish community is similar to that of the most contaminated lakes (Iles and Rasmussen 2005) in that it is dominated by YP with a small population of Iowa darters. The Richard Lake fish community is more diverse, with additional populations of largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), northern pike (*Esox lucius*), pumpkinseed (*Lepomis gibbosus*), white suckers (*Catostomus commersonii*), golden shiners (*Notemigonus*), and central mudminnows (*Umbra limi*).

Geneva Lake (46° 46' N, 81° 33' W), located north of Sudbury upwind from industry emissions, was never acidified or heavily contaminated with metals, and served as a reference lake for this study. It is considerably larger than all the other lakes; however, only the southern bay was sampled, which is of comparable size to the rest of the lakes and is only connected to the main part of the lake by a narrow channel. In addition to YP, Geneva Lake has a very diverse fish community (Iles and Rasmussen 2005) including: walleye (*Sander vitreus*), largemouth bass, smallmouth bass, northern pike, brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), pumpkinseeds, white suckers, brown bullhead, burbot (*Lota lota*), darters, and golden shiners.

## METHODS

### Fish Measurements

To determine the biochemical and physiological status of the indigenous fish in each lake, YP (age 1+ to 4+) were collected in the early summer (June 2002). These specimens were used for the following determinations.

### Contamination indices

Cd, Cu, and Ni concentrations were measured in liver and kidney. These measurements revealed how the contamination indices vary within the Sudbury region and enabled us to test the link between exposure and effects on fish.

### *Physiological indices*

Blood samples were collected and analyzed for hormones (cortisol). The functional integrity of the interrenal organs secreting cortisol was assessed *in vitro* using the adrenocorticotrophic hormone (ACTH) challenge test (Laflamme *et al.* 2000). These measures, obtained on adult specimens of similar age (2+ to 4+ age class,  $n = 20$ ) from the studied lakes, enabled us to evaluate the endocrine status of the indigenous fish.

### *Ecological parameters*

*Fish collection.* Each lake was sampled over 1 day in the last week of June 2002, over a month after YP had finished spawning. Fish were caught in gill nets, 1.5 cm mesh minnow nets and by angling. No attempt was made to sample young-of-the-year. Nets were set during the afternoon, and were checked for fish every 30–45 min. Approximately 120 YP were obtained from each lake, from as wide a range of body sizes as possible. Fish were sacrificed after capture, immediately placed on ice, and were later frozen at  $-20^{\circ}\text{C}$  for subsequent diet and enzyme analysis. Both weight (nearest 0.1 g) and total length (nearest mm) were measured. Fish were aged using opercular bones. Most of the YP  $>3+$  years of age were aged, but only about half of the 1+ and 2+ group were directly aged. The remaining fish were assigned to age groups based on interpolation of length together with the length-frequency distribution cohort they belonged to. Fulton's condition factor (FCF), an indicator of energetic performance, was calculated as:

$$\text{FCF} = 100 * \text{Weight (g)} / \text{Length (cm)}^3 \quad (1)$$

*Invertebrate collection.* From 5–9 zoobenthic samples were taken from the study lakes at the same time and location as the fish. Samples were taken at a range of depths in the littoral zone in a variety of microhabitats. Kicknet samples ( $0.2 \text{ m}^2$ ) were taken at 0.5 m depth, and Ekman grabs ( $0.025 \text{ m}^2$ ) were taken at 1 and 2 m depths. Samples were stored for a few hours on ice prior to being frozen at  $-20^{\circ}\text{C}$ . Zoobenthic samples were sorted using a dissecting microscope and invertebrates were identified to order, counted, measured, and dried at  $55^{\circ}\text{C}$  for 48 h to obtain a dry weight measurement and to estimate the abundance. The Shannon–Weiner Index of Diversity ( $H'$ ; Magurran 1988) was used to compare average invertebrate diversity of benthic samples between lakes:

$$H' = \sum p_i \ln p_i \quad (2)$$

where  $p_i$  is  $n_i/N$ ,  $n_i$  is the number of individuals in the  $i$ th order and  $N$  is the total number of individuals in the sample. The benthic invertebrates were assigned to size classes based on individual dry weight. The limits defining the eleven size classes were the same as those chosen by Boisclair and Leggett (1989b).

*Perch diet composition.* YP diet was determined from stomach contents of 45 fish per lake. Individual prey items were identified, counted, measured, and dried at  $55^{\circ}\text{C}$  for 48 h to obtain a dry weight estimate of each order. The % occurrence of each prey taxon (percentage of fish containing the prey taxon) and the % weight of each prey taxon (percentage of total stomach contents dry weight that prey taxon accounted

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for) were determined for each age class for each lake. Those prey taxa with both a high per cent occurrence (a common prey) and a high percent weight (a profitable prey) were deemed to be the most important prey for that age class in that lake.

*Lactate dehydrogenase analysis.* Lactate dehydrogenase (LDH) activity was measured for the same fish used for stomach analysis and chosen to encompass the range of YP sizes present in each lake. Sample preparation and analysis methods closely followed the protocol described by kit specifications (LDH-optimized, Sigma Diagnostics) and the slight modifications of Sherwood *et al.* (2002a). All samples were run in duplicate and the average coefficient of variation for all LDH assays was <5%.

*Statistical analyses.* Mean YP, prey, and invertebrate weights were compared using one-way ANOVA after checking for normality and uniform variance, followed by Tukey–Kramer multiple comparison tests ( $p = .05$ ). The significance of diet shifts for resetting YP LDH activity was determined using ANCOVA with body weight as the continuous variable and diet categorized with two dummy variables defining the shifts from planktivory to benthivory and from benthivory to piscivory (see Sherwood *et al.* 2002a for details). ANCOVA models were compared with simple linear models with  $F$ -ratio tests to determine if the inclusion of diet categories accounted for a significant proportion of the variation in the data. Diet categories were assigned based on stomach contents. YP with empty stomachs were not included in the ANCOVA analyses.

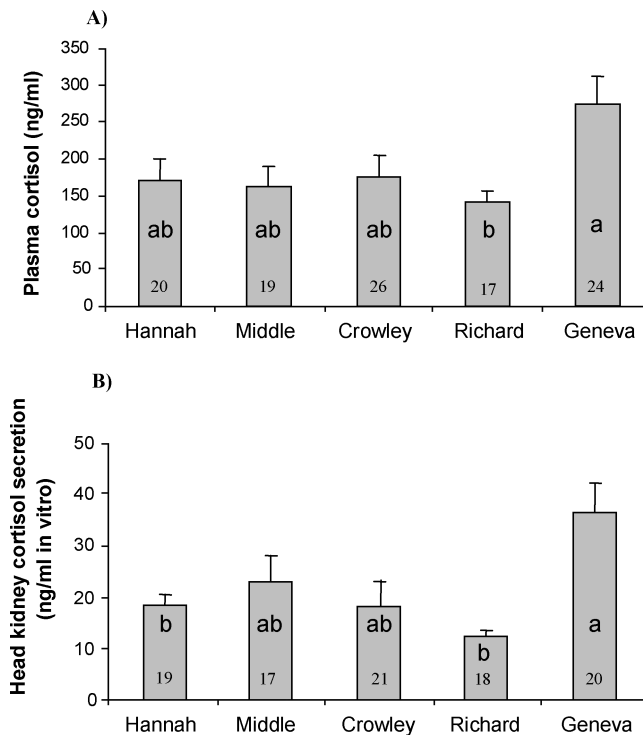
## RESULTS AND DISCUSSION

### Analysis of Metal Concentrations in Fish Tissues

Metal concentrations in YP livers generally reflected levels in water and sediment. Of the three metals analyzed, liver Cu exhibited the best relationship to the exposure regime. Levels were highest in Hannah and Middle lakes, intermediate in Crowley and Richard lakes, and much lower in Geneva than in any of the four impacted lakes (Table 1). For Cd and Ni, levels in all four impacted lakes were higher than in Geneva, but concentrations were no higher in the highly contaminated lakes than in the intermediate lakes. Although Ni concentrations in water and sediment were higher than those of Cu, tissue concentrations of Ni were lower. Metal concentrations in kidney tissue did not reflect sediment and water levels as well as liver concentrations. Although kidney concentrations of all three metals, Cd, Cu, and Ni, were higher in the exposed lakes than in the reference lake, with the exception of high Cu levels in Hannah Lake, the concentrations observed in the highly contaminated lakes were in the same range as those seen in the intermediate lakes. Thus the metal that best reflects the exposure gradient in fish livers in the Sudbury lakes is Cu, whereas in the Rouyn/Noranda lakes the best relationship to exposure was seen in liver Cd concentrations (Laflamme *et al.* 2000).

### Impairment of the Cortisol Stress Response—Direct Effect

We tested the relationship between metal exposure and the capacity to secrete cortisol (an endocrine indicator of physiological fitness. Two measures were carried out using 2–4+ YP; plasma cortisol, which is a measure of the capacity to respond to



**Figure 3.** Cortisol stress response in yellow perch sampled in Sudbury lakes. A) Plasma cortisol  $F_{4,110} = 3.1$ ,  $p = .01$ ; B) Maximal cortisol secretion by head kidney stimulated by ACTH *in vitro*.  $F_{4,97} = 5.68$ ,  $p = .0004$ .

the capture and confinement challenge (Figure 3a), and *in vitro* cortisol secretion by isolated head kidneys stimulated with an optimal dose of ACTH (Figure 3b), a potent secretagogue for cortisol secretion. Fish from Geneva lake (reference) responded to the stress of capture and confinement by mounting a strong cortisol stress response, with levels within the normal range for unimpacted YP (Laflamme *et al.* 2000; Levesque *et al.* 2003). Fish from the four impacted lakes, although captured, handled, and confined in the same way, mounted a much weaker response, with plasma cortisol levels on the order of half of those exhibited by the Geneva Lake fish. Although the differences were large, they were only marginally significant due to the large variability within each lake (Figure 3), and only Richard Lake was significantly lower than the reference lake ( $F_{4,112} = 3.1$ ,  $p = .01$ ). In the *in vitro* ACTH challenge test, the responses exhibited by the head kidneys isolated from Geneva Lake fish were high, and within the normal range seen previously in unimpacted YP (Laflamme *et al.* 2000). The head kidney tissues from metal-impacted fish had a lower capacity to respond to the ACTH challenge. In this *in vitro* test the variance was not as great as that seen for plasma cortisol levels, and therefore the level of significance was greater for this comparison (Figure 3b); Geneva Lake was significantly different from Middle, Hannah, and Richard lakes ( $F_{4,97} = 5.68$ ,  $p < .0005$ ). Interestingly, the fish from strongly impacted lakes (Middle and Hannah) had very

## Direct and Indirect Effects of Metals on Yellow Perch

similar levels of both plasma cortisol and *in vitro* cortisol production to those of fish from intermediate lakes. In fact, fish from Richard Lake had the most impaired cortisol stress response, both in terms of plasma cortisol and *in vitro* production, and this matches the pattern seen for growth of 1+ YP.

### Analysis of the Benthic Invertebrate Community and the Perch Diet

Our analysis of the zoobenthic communities in Sudbury lakes support the findings of Borgmann *et al.* (2001) using the Sediment Triad toxicity assessment, and the 96-h benthic invertebrate LC25 and LC50 estimates of Milani *et al.* (2003). Many of the large invertebrates that play a key role in the diet of YP were absent or rare in metal-contaminated lakes. Thus crayfish, *Hexagenia*, amphipods, large odonates, and gastropods were rare or absent in the metal-impacted lakes, and replaced by small, metal-tolerant taxa such as chironomid larvae and trichopteran larvae. The last two taxa made up nearly the entire benthic community in Hannah, Middle, and Crowley lakes (Iles and Rasmussen 2005). These differences are well reflected in the size distribution of the zoobenthic community, which mirrors the exposure gradient (Table 2). The mean size of the benthic animals (total biomass/number present) as well as the 50 percentile of the biomass size spectrum (see Rasmussen 1993) were both much lower in the metal-impacted lakes. The two largest size classes (30–49 mg) and (>50 mg) (mainly *Hexagenia* and large crustaceans) were found only in Geneva Lake and the top four size classes were absent from Hannah and Middle lakes.

The youngest age class of YP that we sampled (1+) were beginning to include zoobenthic prey in their diet, but still fed mainly on zooplankton. 1+ YP consumed the largest prey in Geneva and Richard lakes and the smallest prey in Crowley Lake, whether benthic prey were included, or only zooplankton considered (Table 2). The size of benthic prey consumed by the 2+ and 3+ YP, the ages where shifts to larger benthic prey generally take place, also reflects the absence of large benthic prey in the contaminated lakes (Table 2). For 2+ YP, the size of the benthic prey was smaller in all of the impacted lakes than in Geneva, but the highly contaminated and intermediate lakes were not different; however, for the 3+ YP, the size of the prey closely reflected the contamination gradient (Table 2).

In Geneva Lake, 2+ and 3+ YP shifted their diet to larger invertebrates in a manner consistent with the pattern outlined in Figure 1a (Table 3). Although the 2–4+ fish in the impacted lakes did adopt a benthivorous diet, the benthic invertebrates consumed were small, and not much larger than the zooplankton consumed by 1+ fish. This pattern was consistent with the pattern outlined in Figure 1b (Table 3). Whereas this pattern persisted throughout life in the strongly impacted lakes, the fish in intermediate lakes eventually became piscivorous by age 4+ and thus size at age 4+ was significantly larger in Richard and Crowley lakes than in Hannah and Middle lakes.

Thus the feeding opportunities for benthivorous YP in the metal-impacted lakes support the pattern illustrated in Figure 1, and also our first hypothesis related to indirect/foodweb mediated effects that was derived from our previous work on Rouyn/Noranda lakes (Sherwood 2002a, Kovacs *et al.* 2005). The overall diversity

**Table 2.** Comparison of benthic community parameters and perch growth and condition<sup>a</sup> among lakes in the Sudbury region.

	Highly contaminated		Intermediate		Reference		<i>p</i>
	Hannah	Middle	Crowley	Richard	Geneva	F	
Size of benthos (total biomass/#) mg dw <sup>*</sup>	0.27b	0.04 a	0.05 a	0.17a,b	0.91 c	F <sub>4,28</sub> = 24	<.0001
%50 of benthic biomass distribution (mg dw)	0.75	0.03	0.05	3	19		
H' diversity of benthic community	0.35a	0.54a,b	0.1 a	0.84 b	0.88 b	F <sub>4,217</sub> = 86	<.0001
Prey size (mg dw)							
(1+ perch) (all prey)	0.053 a,b	0.041a,b	0.021 a	0.122 b	0.131 b	F <sub>4,55</sub> = 4.1	<.006
(1+ perch) (zoopl)	0.030 a,b	0.019 a	0.015 a	0.054 b	0.055 b	F <sub>4,42</sub> = 7.2	<.0002
(2+ perch) (benthos)	0.08 a	2.5 a,b	2.9 b	2.5 a,b	9 c	F <sub>4,85</sub> = 3.4	<.01
(3+ perch) (benthos)	9 a,b	2.2 a	8.6 a,b	25 b,c	66.7 c	F <sub>4,55</sub> = 4.1	<.01
Perch weight g fw							
1+	3.7 a/a	5.1 a/b	4.6 a/b	3.1 a/a	6.9 a/c	F <sub>4,274</sub> = 70	<.0001
2+	9.5 b/a	9.4 b/a	9.6 a/a	9.7 b/a	20.4 b/b	F <sub>4,217</sub> = 17.1	<.0001
3+	12.9 c/a	15.9 c/a,b	22.6 b/a,b	28.7 c/b	60.6 c/c	F <sub>4,120</sub> = 72	<.0001
4+	13.0 c/a	21.5 d/a	44.7 b/b	42.4 d/b	—	F <sub>3,49</sub> = 59	<.0001
	F <sub>3,171</sub> = 495	F <sub>3,100</sub> = 182	F <sub>3,161</sub> = 108	F <sub>3,114</sub> = 181	F <sub>2,123</sub> = 179		
	<i>p</i> < .0001	<i>p</i> < .0001	<i>p</i> < .0001	<i>p</i> < .0001	<i>p</i> < .0001		
Perch length cm							
1+	6.9 a/b	8.0 a/c	8.0 a/c	6.5 a/a	8.4 a/d	F <sub>4,274</sub> = 89	<.0001
2+	10.1 b/a	9.8 b/a	10.1 b/a	9.5 b/a	12.0 b/b	F <sub>4,217</sub> = 36	<.0001
3+	10.8 b/a	12.4 c/a,b	13.8 c/b	13.9 c/b	17.0 c/c	F <sub>4,120</sub> = 82	<.0001
4+	11.1 b/a	14.1 d/b	17.1 d/d	15.9 d/c	—	F <sub>3,49</sub> = 82	<.0001
	F <sub>3,171</sub> = 445	F <sub>3,100</sub> = 141	F <sub>3,161</sub> = 272	F <sub>3,114</sub> = 113	F <sub>2,123</sub> = 380		
	<i>p</i> < .0001	<i>p</i> < .0001	<i>p</i> < .0001	<i>p</i> < .0001	<i>p</i> < .0001		

(Continued on next page)

**Table 2.** Comparison of benthic community parameters and perch growth and condition<sup>a</sup> among lakes in the Sudbury region. (*Continued*)

	Highly contaminated			Intermediate Crowley	Richard	Reference Geneva	F	<i>p</i>
	Hannah	Middle						
Perch condition								
1+	1.09 a/a	1.01 a/b	0.90 a/c	1.10 a/a	1.12 a/a	$F_{4,274} = 70$	<.0001	
2+	0.92 b/a,b	0.98 a/b	0.89 a/a	1.11 a/c	1.15 a,b/c	$F_{4,217} = 54$	<.0001	
3+	0.94 b/b	0.84 b/a	0.84 a/a	1.02 a/b	1.18 b/c	$F_{4,120} = 55$	<.0001	
4+	0.96 b/b	0.77 b/a	0.89 a/b	1.04 a/c	—	$F_{3,49} = 16$	<0.0001	
	$F_{4,171} = 26.5$	$F_{3,97} = 29$	$F_{3,161} = 1.2$	$F_{3,114} = 1.2$	$F_{2,123} = 5$			
	<i>p</i> < .0001	<i>p</i> < .0001	<i>p</i> = .4	<i>p</i> = .3	<i>p</i> < .01			

<sup>a</sup>Modified from Iles and Rasmussen (2005). \*dw indicates dry weight and fw indicates fresh weight.

**Table 3.** Summary of diet shifts of yellow perch in lakes of the Sudbury region.<sup>a</sup>

Lake	Age class	Prey taxon	% occurrence	% dry wt	n
Geneva	1+	Zooplankton	83.3	85.9	6
	2+	Ephemeroptera	38.5	43.6	13
	3+	Malacostraca	35.3	81.2	17
Crowley	1+	Zooplankton	100	90.7	7
	2+	Diptera	77.8	9.4	11
		Odonata	22	82	
	3-4+	Odonata	57.1	67.8	7
Richard	1+	Zoopl/Diptera	100	100	6
	2+	Diptera/Amphipoda	100	45	12
		Odonata	33.3	31.7	
	3-5+	Fish	60	80	7
Middle	1+	Zooplankton	83.3	86.4	18
	2+	Trichoptera/Odonata	100	45	7
	3-4+	Trichoptera	71.4	22.9	5
Hannah	1+	Zooplankton	54.5	53.8	11
	2+	Diptera	63.6	18.3	3
	3+	Diptera/Trichoptera	100	20	10
	4-5+	Diptera/Trichoptera	100	55	7

<sup>a</sup>Modified from Iles and Rasmussen (2005).

of benthic taxa was also generally lower in the metal-impacted lakes, but Richard Lake had diversity on par with that seen in Geneva Lake. Similar results showing the sensitivity of large benthic taxa to metal contamination have been obtained in lakes (Johnson *et al.* 1992; Gower *et al.* 1994), streams (Clements *et al.* 2000) and estuaries (Gaston *et al.* 1998) from many different parts of the world.

### Analysis of Perch Growth and Condition

Age 1+ YP were smallest in Richard (3.1 g) and Hannah lakes (3.8 g), larger in Crowley (4.9 g) and Middle lakes (5.1g), and much larger (6.9 g) in the reference lake (Geneva). The same pattern was seen in lengths. For condition, Crowley (0.90) was the lowest, followed by Middle (1.01) and Hannah (1.07), and the highest condition in 1+ YP was seen in Richard (1.10) and Geneva (1.12) lakes, which were not significantly different (Table 2). At this age YP fed mainly on zooplankton in all the lakes and were only beginning to include benthic prey in their diets. Thus the pattern of significantly better growth and condition in the reference lake is already established after one year of life prior to the shift to benthivory, where effects of benthic impoverishment begin to appear. The comparison among lakes in size of 1+ YP did not reflect the comparison in size of prey consumed (Table 2) in that Richard Lake YP consumed large prey but had the lowest 1+ size of all the lakes.

In the reference lake, YP nearly tripled their weight in their second year of life, reaching an average weight of 20.4 g at 2+. All of the impacted lakes had similar sized YP (9.4-9.7 g) at age 2+, which were all significantly smaller than the Geneva Lake fish (Table 2). Comparison of fish lengths yielded the same pattern. In terms



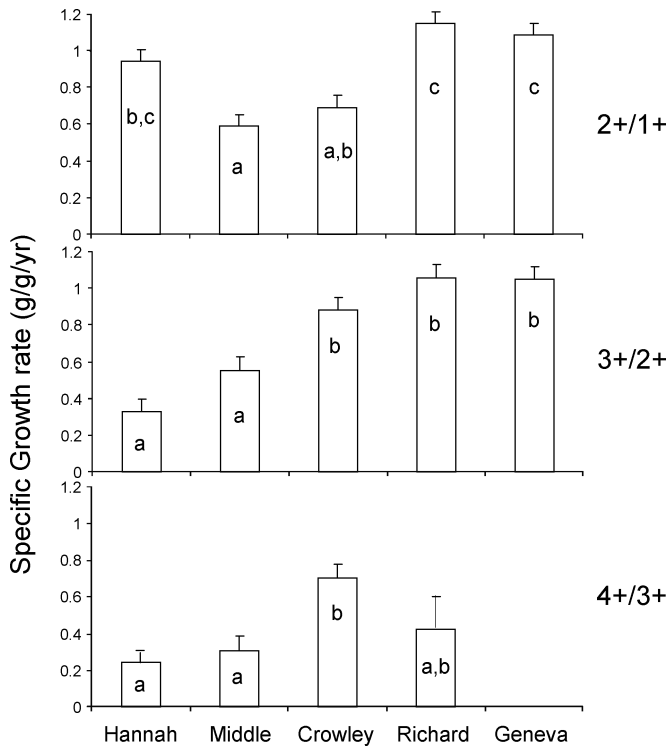
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of condition, Crowley Lake fish were lowest at 0.89, followed by Hannah Lake fish at 0.92, and Middle Lake fish (0.98) where condition remained at the 1+ levels, and then Richard Lake at 1.11. Condition was the highest in Geneva Lake (1.15), which was the only lake where condition increased with age. Whereas at age 1+ Geneva fish were 66% larger than the average size of 1+ YP in all of the other lakes, by age 2+ they were more than twice the size of similar aged fish in metal-impacted lakes.

By age 3+, Geneva Lake YP had again tripled their weight to 60.4 g. In Richard Lake they also tripled their weight to 28.7 g, and in Crowley Lake they more than doubled to 22.6 g. In the two most impacted lakes weight increased by only around 50% to 12.9 g in Hannah Lake and 15.9 g in Middle Lake. Condition factor again increased in Geneva Lake to 1.15, decreased slightly in Richard (1.04) and Crowley lakes (0.84), dropped substantially in Middle Lake to 0.84, and stayed constant at 0.94 in Hannah Lake. Thus Geneva Lake was the only lake where YP increased in condition factor throughout, whereas in the intermediate lakes slight decreases occurred, and in the strongly impacted lakes significant decreases in condition factor occurred with age. Although no 4+ YP were caught in Geneva Lake, the patterns among the remaining lakes seen at 3+ were replicated at 4+. That is, YP from the moderately impacted lakes exhibited substantial growth from 3+ to 4+, whereas those from the strongly impacted lakes did not.

The among-lake comparisons in YP size for age 2+ and older (Table 2) represent cumulative differences. In order to partition the effects among year classes, we calculated specific growth rates (SGR) such that  $W_t = W_{t-1}e^{SGR}$ . Thus the weight obtained by a 2+ fish would be evaluated relative to that of a 1+ fish from the same lake and the SGR estimated by  $\ln W_t - \ln W_{t-1}$ . The comparisons of SGR for 2+/1+, 3+/2+ and 4+/3+ are shown in Figure 4. This comparison shows that 2+ and 3+ fish from Richard Lake had SGR values as high as those from Geneva Lake, even though they were not nearly as large. Although SGR comparisons show that 2+ and 3+ YP from the highly impacted lakes (Hannah and Middle) grew more slowly during this phase of their lives than those from the reference lake, those from Richard Lake did not. Although 2+ YP from Crowley Lake did grow more slowly than 2+ YP from Geneva Lake, the difference between the two lakes was not significant at 3+. Thus partitioning growth rate by year classes in this way shows that much of the size difference, between Geneva Lake YP and those from the intermediate lakes for 2+ and 3+ age groups, was actually a result of the larger size that Geneva Lake fish had already achieved by age 1+, prior to their shift to benthivory. However, even after the shift to benthivory, growth rates in Geneva Lake fish continued to be higher than those seen in the highly impacted lakes. This difference was especially significant for the 3+ fish (Figure 4).

These patterns support hypothesis 2, and also our previous findings. Sherwood *et al.* (2000) showed that YP in highly contaminated lakes grew more slowly, less efficiently relative to ration, and reached a smaller maximum size than did fish from two reference lakes. In addition, the fish from the contaminated lakes did not undergo the normal sequence of diet shifts (zooplankton to littoral macroinvertebrates to littoral fish) that YP 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 to the failure to complete diet shifts to larger-sized prey, and in both lakes this was associated



**Figure 4.** Comparison of specific growth rate estimates ( $\ln W_t - \ln W_{t-1}$ ), for 2+/1+, 3+/2+, and 4+/3+ age increments. Error bars represent pooled SE estimates obtained from  $(SE_{\ln W_t}^2 + SE_{\ln W_{t-1}}^2)^{0.5}$ . Statistical comparisons within each age series are based on Bonferoni corrected *t*-tests ( $p < .05$ ). Bars sharing the same letter are not significantly different. Geneva has no bar in the 4+/3+ series since no 4+ fish were caught in this lake.

with an impoverished littoral benthic community (Sherwood *et al.* 2000; Kovacs *et al.* 2005). In reference lakes the littoral benthos constitutes 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).

### Age Structure of Yellow Perch Populations

In addition to these indirect effects on YP energetics, acting through foodweb bottlenecks, Sherwood *et al.* (2000) also reported data on YP age structures indicating that the younger age classes of YP in metal contaminated Noranda lakes experience reduced survivorship, perhaps a result of direct toxic effects of metal exposure (Campbell *et al.* 2003). In reference lakes, 1+ fish made up on average 40% of the population, whereas in the most impacted lakes and intermediate lakes

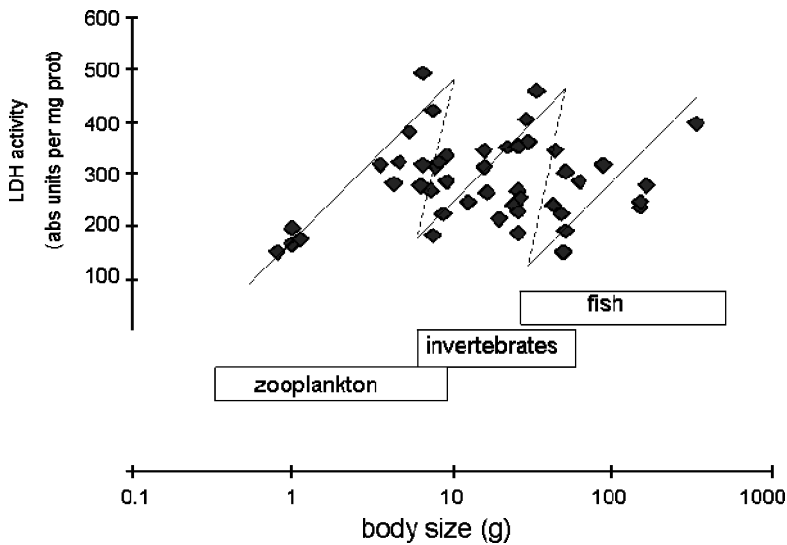
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this proportion dropped to 10% and 20%, respectively. No such pattern was evident in our Sudbury lakes data. In Geneva Lake, 1+ fish made up 32% of the catch, while this age group made up 28% and 33%, respectively, in Richard and Crowley lakes, and 54% and 56%, respectively, in Middle and Hannah lakes. Thus survivorship of young YP through their first season seems to be excellent in these lakes, and the proportion of the population in the 1+ age class tended to be higher in the highly contaminated lakes. The absence of 4+ and older fish in Geneva Lake might indicate poor survivorship, and this could be a result of the rich predator community in that lake. However, another possibility is that older fish moved from the bay where we sampled and became piscivorous in some other portion of this rather large lake.

### Diet Shifts and the Activity of the Glycolytic Pathway (Muscle LDH Activity)

Fish such as YP will utilize anaerobic pathways while feeding, if feeding activity involves activity bursts that exceed the aerobic threshold repeated often enough that lactate is not cleared within the available recovery time (Childress and Somero 1990; Sherwood *et al.* 2002a). The activity of glycolytic (anaerobic) enzymes (including LDH) in fish muscle does not follow the same metabolic size-scaling paradigm that governs activities of aerobic enzymes (Sullivan and Somero 1980; Childress and Somero 1990). As opposed to negative allometry confined to a narrow range of scaling coefficients (typical values for aerobic enzymes centre around  $-0.25$ ), glycolytic enzymes possess highly variable and mostly positive size dependence. The fact that glycolytic enzymes usually scale positively to body size may be related to increasing anaerobic power requirements in larger fish that may experience higher drag forces when generating short swimming bursts (Sullivan and Somero 1980). The large variability among species in glycolytic scaling coefficients has been interpreted to reflect differences in power requirement associated with different locomotory and feeding types of fish (Childress and Somero 1990).

There is also considerable variability in glycolytic scaling within species, and down regulation of the white muscle glycolytic pathway (as reflected by muscle LDH activity) following shifts to larger sized prey has been shown in many species of fish including YP (Sherwood *et al.* 2002a) and walleye (Kaufman *et al.* 2006). Although individual feeding bursts directed toward larger prey likely exceed the aerobic threshold more than bursts directed at smaller prey, the much greater time interval between bursts (recovery time) will allow lactate to be cleared by aerobic pathways, and thus permit glycolytic pathways to be down-regulated (LDH activity levels reset to a lower level). Down regulation of the glycolytic pathway leads to reductions in metabolic costs, and increases in growth rate, condition factors, and the maximum size attained (Sherwood *et al.* 2002a). This downshift, or resetting of LDH to a lower level, was attributed to a lower need for glycolytic potential because of the sharp decline in the number of burst swimming prey attacks required once prey size becomes much larger. By increasing prey size, the number of prey items required decreases, allowing more time to recycle lactate between active bursts, and therefore glycolytic potential is downregulated because of lower anaerobic demands (Sherwood *et al.* 2002b). This explained why higher growth efficiency was observed in lakes where prey size was larger and diet shifts occurred, and why the absence of large prey led to



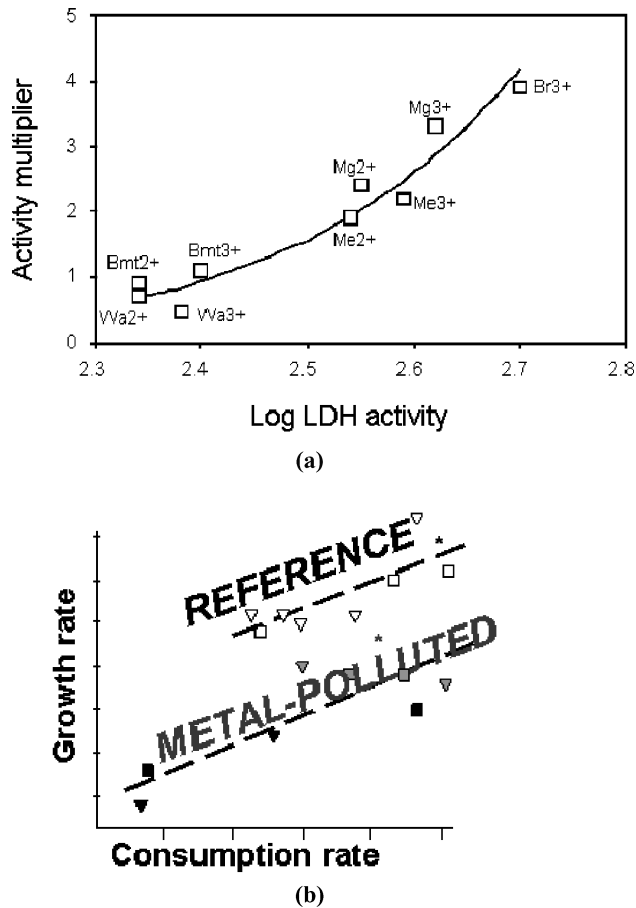
**Figure 5.** ANCOVA regression fitted to diet shifts of yellow perch from an unimpacted lake, Lac Hertel, QC. The best fit model is  $LDH = 2.33 + 295 \log W - 227c - 488d$ ,  $R^2 = 0.27$ ,  $F = 5.5$ ,  $N = 38$ .  $c$  represents a dummy variable 1 = benthivore, 0 = non-benthivore,  $d$  represents a dummy variable 1 = piscivore, 0 = non-piscivore.

energetic bottlenecks, stunted growth, and lower condition in metal-impacted lakes (Sherwood *et al.* 2002a; Kaufman *et al.* 2006).

Statistically, such “downshifts” were demonstrated by fitting ANCOVA models to LDH activity *versus* body size plots (covariate), with diet type coded categorically (Figure 5) leading to a regression with a sawtoothed pattern. When the ANCOVA model explained significantly more variance than the simple linear regression, the LDH downshift associated with the diet shift was considered statistically significant. Evidence for a statistically significant downshift was associated with a diet shift from *Daphnia* to benthos (a diverse benthic diet dominated by *Hexagenia*) in Geneva Lake 2+ YP ( $F$ -ratio test,  $F_{11,28} = 2.88$ ,  $p < .05$ ; see Iles and Rasmussen 2005 for details), but no significant downshift was associated with the shift from *Hexagenia* to crayfish (3+ YP). Although diet shifts did sometimes occur in the metal-impacted lakes (*e.g.*, a shift from zooplankton to small Diptera and Trichoptera in Hannah and Middle lakes, Table 3), they did not involve significant downshifts in LDH activity, and LDH generally increased with body size (*i.e.*, the ANCOVA model did not explain more variance than the simple allometric regression).

These results support hypothesis 3, which was based on patterns observed in our previous studies. In reference lakes, YP shifted from zooplankton to littoral macrobenthos during their second year of growth (12 to 15 g), and then began to include a significant amount of fish in their diet during their third to fifth year of growth (25 to 50 g). Fish in the most contaminated lakes 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. In the

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**Figure 6.** (a) The relationship between white muscle LDH activity in 2+ and 3+ yellow perch and the activity multipliers calculated from bioenergetic budgets. Me = Lake Memphremagog, Quebec, Mg = Lake Magog, Quebec, Wa = Lake Waterloo, Bmt = Lake Bromont, Br = Lake Brome. Modified from Sherwood *et al.* (2002a). (b) Specific growth rate *vs.* daily ration measured with using a tissue  $^{137}\text{Cs}$  mass balance model, for metal-impacted *vs.* reference lakes in Rouyn/Noranda. Modified from Sherwood *et al.* (2000).

reference lakes the shifts to larger prey (zooplankton to benthos, followed by benthos to fish) resulted in significant downshifts of white muscle LDH, from which we infer that energetic costs decreased. This inference is supported by a strong correlation between LDH activity and the activity multipliers (total metabolic costs divided by basal metabolism) (Figure 6a) calculated from bioenergetic budgets (see Boisclair and Rasmussen 1996; Sherwood *et al.* 2002b). This observation is also consistent with the higher growth efficiency reported by Sherwood *et al.* (2000) for fish populations that successfully carried out diet shifts as they grew (Figures 3b and 6b); these latter *in situ* estimates of growth efficiency were obtained with a  $^{137}\text{Cs}$  radio-tracer technique.

Such downshifts did not occur in metal-impacted lakes where prey size tended to remain small throughout life and stunted growth was observed.

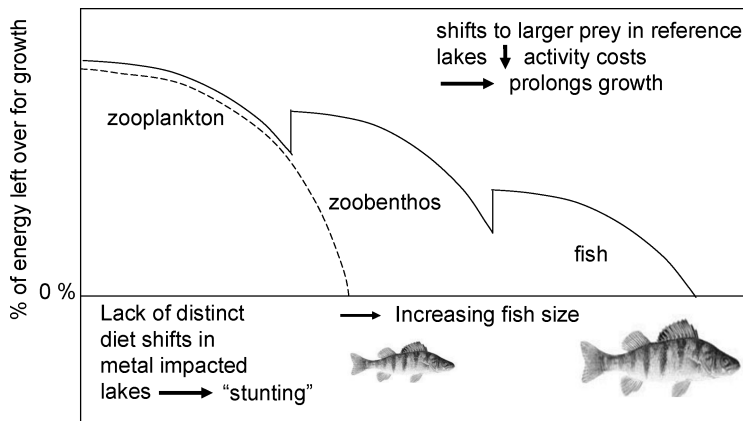
### **Energy Available for Growth Depends on Activity Costs**

Stunting in YP populations is a well-documented phenomenon that has often been attributed to food shortage because of low zoobenthic production (Persson 1987; Diehl 1993; Heath and Roff 1996) or high YP densities (Hansson 1985). However, there is evidence that energy expenditure for active metabolism is also an important contributor to stunting in YP populations. It has been argued that activity costs are higher when fish are required to attack many, small prey (requiring upregulation of anaerobic pathways) than when they are able to obtain their daily ration by capturing a few, large prey (downregulated anaerobic pathway), and that high activity costs limit the amount of energy available for growth (Krohn and Boisclair 1994; Sherwood *et al.* 2002a; Rennie *et al.* 2005; Kaufman *et al.* 2006). This hypothesis is supported by the fact that YP growth rates are not well related to the quantity of food consumed (Boisclair and Leggett 1989a; Sherwood *et al.* 2000; Rennie *et al.* 2005), and also by the fact that YP have higher growth rates following diet shifts (Boisclair and Leggett 1989b; Sherwood *et al.* 2002a) despite the overall lower abundance of larger invertebrates (Rasmussen 1993). Moreover, growth rates of YP decrease as the proportion of small prey items in the diet increases (Diehl 1993; Boisclair and Rasmussen 1996; Lott *et al.* 1996; Hjelm *et al.* 2000). Furthermore, Sherwood *et al.* (2000) showed that YP from reference and contaminated lakes in Rouyn-Noranda did not differ in rates of prey consumption, but did differ in food conversion efficiencies. Thus, fast growing and stunted YP populations may differ as much or more in their metabolic costs, as in their rate of food consumption. Ideally, YP will maximize their growth efficiency by switching their diet to optimal-sized prey as they grow, and thus maintain high growth rates. Under this scenario, if a fish outgrows its prey, its growth rate will slow down until it is large enough to exploit the next size class of prey, at which time growth should return to normal. This period of slow growth constitutes what has been termed a growth "bottleneck" (Heath and Roff 1996; Hjelm *et al.* 2000; Sherwood *et al.* 2002a,b).

Stunted YP populations result from a gap in the prey size structure that is so large that growth ceases, and the fish never become large enough to efficiently utilize the next size class of prey. Metal contamination, by eliminating large metal sensitive prey taxa, creates gaps in the prey size structure, producing the patterns in YP growth as described earlier (Figure 7). Of the Sudbury lakes YP populations, only the one from the reference lake (Geneva) showed a significant resetting of LDH activity following the shift to benthivory. Piscivory was low in Geneva Lake, despite the fact that YP were larger than some of the piscivorous YP from the intermediate lakes. The presence of large invertebrate prey in Geneva Lake would increase the optimal size for switching to piscivory. It has previously been noted that piscivory is not necessary for high YP growth rates where there is an invertebrate prey base of good size (Lott *et al.* 1996).

YP in the intermediate lakes experienced reduced growth throughout the size range typically associated with benthivory. We suggest that the absence of large-bodied invertebrates, such as crayfish and mayfly larvae, is the cause of the reduced growth. In the intermediate lakes, YP appear to eventually make it through the slow growing benthivorous stage to become piscivorous. In the most contaminated

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**Figure 7.** Graphical model illustrating how downshifting of activity costs allows fish to maintain high growth efficiency and offset the tendency for stunting. Fish that are unable to make diet shifts that scale back costs ultimately dissipate their entire energy budget on activity costs required to obtain food. Modified from Sherwood *et al.* (2002a).

lakes, where YP were extremely stunted, there was an absence or low abundance of intermediately sized prey in addition to the absence of large invertebrates. YP in these systems quickly outgrow any energetic benefits of switching to small benthic prey, which are not much larger than zooplankton, as evidenced by their slow growth and lack of a significant lowering of activity costs. A slight resetting of LDH activity would likely not be detectable, given the variation in the LDH data (Figure 5).

### The “Stunted Perch” Scenario Is Not Unique to Metal-Impacted Lakes

These patterns of indirect, foodweb mediated effects on YP trophic ecology, bioenergetics, growth, and condition are not restricted to metal-contaminated lakes. In Saginaw Bay, MI, USA, which has gradually become eutrophied over the last 40 years, YP have been greatly impacted (Schaeffer *et al.* 2000). When *Hexagenia*, a large benthic macro-invertebrate, was the most abundant prey species, YP grew rapidly to large sizes (several hundred g, 25 to 30 cm). Following eutrophication, *Hexagenia* and other large macro-invertebrates disappeared, and YP now feed mainly on small chironomid larvae. The enriched conditions led to increased zooplankton and chironomid production and improved feeding and growth for 0+ and 1+ YP. However, the increased sediment oxygen demand and low sediment Eh associated with eutrophication led to the loss of large burrowing mayflies (Rasmussen 1988), which were key elements in the diet of older age classes. Eutrophication also leads to increased turbidity and light extinction causing a retraction of the littoral zone to the shallow margin of the lake, which tends to reduce the overall abundance of the littoral zoobenthic community. Decreased abundance of large benthic invertebrates caused stunting of older perch at a maximum size of 15–18 cm (Schaeffer *et al.* 2000).

Hayward and Margraf (1987) and Tyson and Knight (2001) also observed low growth rates among YP inhabiting the eutrophic western basin of Lake Erie, coinciding with loss of burrowing mayflies, and a heavier reliance on small prey. In a study

of YP populations in eastern Quebec, Boisclair and Rasmussen (1996) found that growth and consumption rates were negatively correlated with lake trophic status. Growth, consumption, and activity rates decreased with the increase of the percent contribution of small prey to the invertebrate community. The lakes that supported the highest growth rates of 2+ and 3+ YP were those where large taxa were abundant (Rasmussen 1993). Changes in YP growth and feeding patterns are important in this respect since as lakes become richer, YP 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 and burrowing mayflies, which play an important role in the diet of YP.

A very similar, although much less studied, scenario occurs in lakes experiencing acidification (Ryan and Harvey 1980; Gunn and Keller 1990). As lakes become mildly acidified (pH 5 to 6), YP often become very numerous, because 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 small zooplankton, the YP rarely exceed 15 cm in length, and feed mainly on zooplankton and tiny chironomid larvae, 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 YP, such as amphipods and burrowing mayflies (Lonergan and Rasmussen 1996).

Although acidified lakes often also suffer high metal loadings, the lakes in Killarney Park, Ontario, are of special interest because they have extremely low alkalinity and became strongly acidified in spite of being far from smelters and exposed to relatively low metal loadings (Keller and Yan 1998; Keller *et al.* 2003). Although these lakes experienced significant species losses of zooplankton, benthos, and fish, pH in many of the lakes is now above 6, and some recovery has been noted in zooplankton community structure following reduction in SO<sub>2</sub> emissions from smelters (Sprules 1975; Holt and Yan 2003). However, zoobenthic communities have experienced limited recovery, with many ephemeroptera and amphipods still absent from many lakes (Snucins 2003). The littoral benthic communities in these lakes became dominated by tiny chironomid larvae (Harvey and McArdle 1986) and the larger taxa such as amphipods and burrowing mayflies, which are important for good growth and diet development in YP, are still 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 YP are among the most sensitive to a variety of contaminants, and are among the slowest to recover. YP 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, which results as an indirect effect of pollutant impacts on large macro-invertebrates, is not unique to metal-impacted systems. Moreover, its generality provides further support for the idea that the



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size-structure of the benthic functional group is important to ecosystem integrity because of its effect on energy transfer efficiency to benthivorous fish and as a “stepping stone” to piscivory.

### Restoration of Metal Damaged Aquatic Communities: Management Options

Considerable effort has been invested into restoring acid- and metal-damaged lakes in the Sudbury region, which involves both re-establishment of large sensitive invertebrate species and the rehabilitation of littoral habitat, which in unimpacted lakes is the site of maximum diversity and productivity for benthic algae, aquatic macrophytes, and zoobenthos. Lake restoration in the Sudbury region, and rehabilitation of littoral habitats in particular, is linked to the reduction of smelter emissions. This reduction has been successful, leading to reduced metal loadings and increased pH (>6) in lakes throughout the region (Keller *et al.* 2007). Large-scale deforestation and soil damage also occurred in this region (Gorham and Gordon 1960) and greatly impacted lakes through a combination of severely episodic runoff, high metal loadings from the landscape (Nriagu *et al.* 1998), and major reductions in organic matter inputs (Dudka *et al.* 1995) essential for metal detoxification and chemical buffering. The littoral margins of lakes were particularly vulnerable to these impacts because of their close proximity to the damaged terrestrial landscape.

Reforestation efforts throughout the watershed have involved soil liming, fertilization, seeding of grasses and other cover species, and tree planting (Gunn 1996; Gunn *et al.* 2001; Winterhalder 1996). Reforestation has increased the input of organic detritus, leading to increased sediment organic matter concentrations (Matson 2007), which tend to reduce both the concentrations and toxicity of sediment metals and help rebuild both aquatic macrophyte and invertebrate communities. In addition, reforestation increases the input of coarse woody debris that, together with aquatic macrophytes, contributes to littoral habitat structure providing both food and shelter for fish communities (Gunn *et al.* 1995). Because lake and landscape processes are closely linked, management efforts to speed up the normally slow process of reforestation and soil formation help to promote biological recovery both directly, by reducing concentrations and toxicity of metals and thus facilitating survival and growth of sensitive species, and indirectly, by rebuilding habitat to re-establish normal foodweb interactions, including diet shifts of key species such as YP.

### Relative Importance of Direct and Indirect Effects of Metals on Yellow Perch

Assessing the relative importance of the direct and indirect effects that we have described for YP 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 foodweb impoverishment; and (c) endpoints that could plausibly be a result of direct or indirect effects, or both acting together.

### **Direct effects**

Direct effects of metals are those that involve interactions between metals taken up through water or diet and biochemical targets within the organism, and cannot plausibly be interpreted as effects of reduced energy intake, or other ecological interactions. YP in metal impacted lakes exhibit increased concentrations of metallothionein, a metal binding and detoxifying protein, in their livers and kidneys (Brodeur *et al.* 1997; Laflamme *et al.* 2000; Giguère *et al.* 2005). Impaired mitochondrial function (liver and muscle citrate synthase) causing decreased aerobic swimming capacity has been reported in YP from metal impacted lakes near Sudbury (Rajotte and Couture 2002; Couture and Kumar 2003). Both young and mature YP in metal contaminated lakes also exhibit cortisol impairment, wherein toxic metal species disrupt the synthesis of cortisol, a steroid hormone that is the effector of the physiological stress response through actions on gill, liver, intestines, and muscle. We demonstrated this impairment both in the field, by reduced plasma cortisol response to capture and handling stress, or by testing *in vitro* the cortisol secretion capacity of head kidneys isolated from field exposed fish (Laflamme *et al.* 2000 and this study). We interpret these impairment measures as indications of chronic, sublethal contaminant stress resulting from direct effects of metals acting on secretory pathways of the head kidney (Gravel *et al.* 2005).

Evidence for this direct mechanism has been provided through laboratory studies on the signaling pathways leading to cortisol synthesis in head kidney cells isolated from hatchery reared uncontaminated rainbow trout, and exposed to metals in primary cell cultures. In this way, the steps disrupted by specific metals within the cortisol signaling pathway were identified (Lacroix and Hontela 2004; Raynal *et al.* 2005). This type of endocrine disruption has been associated with a broad range of adverse effects such as reductions in gill Na<sup>+</sup>/K<sup>+</sup> ATP-ase activity, gill histopathology, reduced sex steroid production, and delayed gonadal recrudescence (Levesque *et al.* 2003; Levesque *et al.* 2002; Hontela and Lacroix 2006)); however, the causal links to cortisol impairment remain unclear. It is likely also that cortisol impairment has energetic consequences; however, these have yet to be identified, and would in all likelihood be difficult to isolate from indirect effects acting through the metabolic budget. 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” YP (8+ and older) were not uncommon even in the most contaminated lakes that we studied in Rouyn/Noranda and the Sudbury regions.

### **Indirect effects acting through foodweb impoverishment**

The reduced availability of larger prey in lakes with high metal exposure leads to a sharp reduction in energy allocation to growth in YP 2+ and older, and results in stunting and strong dependence on zooplankton and/or small benthic prey at all ages (Sherwood *et al.* 2002a, b; Kovacs *et al.* 2005). The same pattern was seen in the highly contaminated Sudbury lakes, Hannah and Middle, and to a lesser extent in the moderately contaminated Crowley Lake. Although growth differences between reference and metal-impacted fish are evident early in life, while fish from all lakes still feed predominantly on zooplankton, the differences become much

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more pronounced and growth becomes severely stunted in YP from metal-impacted lakes at the age where fish in reference lakes switch to large prey and continue to grow to large sizes.

### Effects for which the mechanism (direct vs. indirect) is not clear

A variety of effects of metals on YP physiology have been published, where it is possible that both direct effects and indirect effects resulting from the metabolic budget may be involved. Examples are gonadosomatic index (GSI) reductions, reductions in thyroid hormones, and changes in liver metabolic enzymes and energy stores (Levesque *et al.* 2002, 2003), reduction in condition index, reduced RNA/DNA ratio, and effects on anaerobic capacity and swimming performance (Eastwood and Couture 2002; Audet and Couture 2003; Taylor *et al.* 2004).

Giguère *et al.* (2004) reported reduced condition factors in 1+ YP from metal contaminated lakes and interpreted this as a direct effect of metal toxicity, on the assumption that community impoverishment effects acting through the feeding regime were unlikely to be manifested on the early juvenile zooplanktivorous YP. Metal impacted lakes near Sudbury have, however, been shown to have impoverished zooplankton communities that are deficient in large species (*e.g.*, daphniids) and often dominated by copepods and small cladocera (*e.g.*, bosminids) (Yan *et al.* 1996, 2004). Although we were not able to link size and condition of 1+ YP in our lakes to the size of zooplankton in the diet, impoverishment effects resulting from metal effects on zooplankton size structure cannot be ruled out, without detailed study. Moreover, in a recent study by Lippert *et al.* (2007) on lakes in the Sudbury area where piscivorous fish have recently colonized, it was shown that in the first few years following invasion by bass and walleye, the abundance of YP was greatly reduced and the size and condition of age 1+ YP, was significantly reduced. This result might indicate that the small size of 1+ Richard Lake YP seen in our study may in fact be a consequence of this lake having been recently colonized by pike and smallmouth bass.

It should be noted that there may be indirect effects acting through other mechanisms that we have not mentioned. For example, reduced recruitment or survivorship of young YP 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 YP growth rates in the first 2 years of life were generally higher than in reference lakes. Predation by reintroduced predators (Gunn *et al.* 1990) or by cannibals within some perch populations (Persson *et al.* 2003) can also significantly affect population abundance, growth, and body condition.

### ERA IMPLICATIONS

1. In order to preserve ecosystem integrity/benthic size-structure, environmental standards must be sufficient to protect large metal-sensitive invertebrates, for which PNEC values are amongst the lowest in the aquatic ecosystem. Impoverishment of the benthic community through direct metal impacts on large sensitive benthic taxa leads to stunted growth of YP and impairment of trophic shifts that

are important to the integrity of whole foodweb. Thus, preserving size-structure within functional groups (*e.g.*, zoobenthos) can be vital to maintaining ecosystem integrity.

2. Watershed management strategies involving emission reduction, revegetation and soil remediation have been shown to promote recovery of littoral communities and would be expected to help shift the trophic structure in impacted lakes from that shown in Figure 1b in the direction of that shown in Figure 1a.
3. Environmental standards or management practices that protect sensitive benthic species or promote their recovery, would likely also have similar enhancing effects on other functional groups, whose impoverishment might also be contributing to the stunted perch scenario, and thus promote the functional integrity of the overall ecosystem.

## CONCLUSIONS

- YP, because they survive well in metal-impacted systems, can serve as a suitable sentinel “model” with which to examine both direct and indirect sublethal effects of metal exposure.
- Effects of metals on YP can occur as a result of direct toxic effects of water- or food-borne metals on fish physiology (see Campbell *et al.* (2008) for a discussion of the incomplete detoxification of metals in YP from the Rouyn-Noranda and Sudbury lakes).
- Direct effects on YP include: impairment of the cortisol stress response, reduced gill Na<sup>+</sup>/K<sup>+</sup> ATP-ase activity and gill histopathology, and buildup of metallothionein in liver and other body tissues. Direct effects of metals resulting from toxic exposures in sediment and water lead to significant shifts in the structure of the benthic community, with many of the larger more sensitive taxa such as burrowing mayflies, amphipods, and crayfish and mollusks being rare or absent.
- Effects of metals on YP can also occur indirectly through effects of metals on prey organisms at the base of the food chain. The most common indication of such indirect foodweb mediated effects on YP is severely stunted growth, which is linked to energetic bottlenecks arising from impoverished benthic communities preventing normal diet development, and leading to a high degree of dependence on zooplankton and small insects throughout life (>8 years).
- The arrival of recolonizing predators (*e.g.*, bass, walleye) can further intensify the energetic bottleneck in high metal contaminated lakes by forcing YP to confine their feeding to littoral refuge areas where metal sensitive benthic taxa are scarce.
- The significant indirect effects that metals exert on the growth and trophic shifts of YP resulting from direct effects on large metal-sensitive benthic taxa, demonstrate that preserving size-structure within functional groups (*e.g.*, zoobenthos) can be vital to maintaining ecosystem integrity. This has significant consequences for ecological risk assessment in that PNEC values must be set at levels low enough to protect large metal-sensitive taxa (Borgmann *et al.* 2001; Milani *et al.* 2003). Furthermore, policy decisions, management strategies, and land-use decisions aimed at protecting the integrity of zoobenthic communities and/or

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promoting their recovery will likely also promote similar effects on other functional groups (*e.g.*, zooplankton and piscivorous predators) and thus promote functional integrity of the whole ecosystem.

- The suite of direct and indirect effects seen in lakes impacted by Sudbury smelters was similar to that reported previously in lakes of the Rouyn/Noranda regions although the metals involved are considerably different.

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