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Live Fast and Die Young: Metal Effects on Condition and Physiology of Wild Yellow Perch from along Two Metal Contamination Gradients

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ABSTRACT

This review summarizes some of the main findings of our work with the Metals in the Environment Research Network examining seasonal and regional effects on metal accumulation, growth, condition, and physiology in wild yellow perch (*Perca flavescens*) from 10 lakes comprising two metal contamination gradients in the industrial regions of Sudbury, Ontario and Rouyn-Noranda, Québec, Canada. The specific objectives of this review are: (1) to propose threshold tissue metal concentrations to discriminate between fish from contaminated and reference sites; (2) to identify factors that can influence metal accumulation and fish condition; and (3) to define an experimental approach for measuring metal effects in wild yellow perch. Using tissue thresholds appeared useful not only for discriminating fish from clean or contaminated environments, but also provided a simple approach to examine metabolic consequences of tissue metal accumulation. Overall, fish from Sudbury grew faster, expressed higher aerobic capacities, and died younger, but also appeared better at limiting accumulation of some metals than Rouyn-Noranda fish. The condition of the latter fish was clearly more affected by metals than Sudbury fish. Finally, our dataset allows us to propose that yellow perch are highly suitable for ecological risk assessment studies of metal effects in wild fish, but that fish size, season, and region must be considered in sampling design and that several reference sites must be studied for meaningful conclusions to be reached.

Key Words: wild yellow perch (*Perca flavescens*), seasonal and regional variation, tissue metal concentration thresholds, metabolic enzyme activity, longevity, fish condition.

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INTRODUCTION

The field-based research reviewed here was conducted on yellow perch (*Perca flavescens*), a species of fish that has not received the attention it deserves in ecological risk assessment (ERA) of metal contamination in North American freshwater systems. Yellow perch is a freshwater-only percid distributed widely across North America. In Canada, it occurs naturally in every province except Newfoundland. It is found as far north as the Great Slave Lake and its distribution extends south to Ohio and Illinois. Its occurrence in most of the areas in Canada where mining and smelting activities take place makes it one of the most relevant fish to examine in metals ERA. The preferred habitat of yellow perch is lakes and ponds of all sizes, as well as rivers and creeks, but it avoids areas where significant currents are present. Therefore, this species is a less appropriate choice when impact sites of mining effluent release are situated in small creeks, but an ideal choice in lakes contaminated by these creeks. Yellow perch feed in shallow, vegetated areas. The young are planktivorous, but will rapidly incorporate increasing amounts of benthic invertebrates and eventually small fish as they grow. Therefore, dietary metal uptake, effects and risk could vary depending on fish size. Yellow perch reproduction takes place in early spring around the time of ice breakup, which in Sudbury and Rouyn-Noranda occurs in April (unpublished data). This is an important advantage over other species of small fish such as minnows, which typically breed during summer, because reproduction and associated changes in energy allocation and behavior could bias the responses of fish to metal contamination, making it inappropriate to carry out sampling around the reproductive period.

Contrary to other fish species co-existing in cleaner lakes, the yellow perch is commonly the only species still present in the most metal-contaminated lakes. For example, in the mid 1990s, yellow perch was the only species present in Hannah Lake and a dominant species in Whitson Lake, the two most contaminated Sudbury lakes included in this review (unpublished data). These lakes are rapidly recovering and today, brown bullhead (*Ameiurus nebulosus*), pumpkinseed (*Lepomis gibbosus*), and various minnows can be captured in Hannah Lake, while Whitson Lake also shelters a growing population of northern pike (*Esox lucius*) and walleye (*Sander vitreus*; unpublished data). Yellow perch is also a dominant species in metal-contaminated lakes of Rouyn-Noranda (Sherwood *et al.* 2000).

There is extensive literature, a review of which is beyond the scope of this article, indicating that natural abiotic (temperature, environmental chemistry) and biotic factors (reproduction, competition, predation, parasitism, food web productivity) influence condition and contaminant uptake in fish. However, our knowledge of factors influencing metal accumulation and condition in wild yellow perch is more limited, and warrants a brief review.

Several studies have reported that yellow perch tissue metal concentrations are influenced by environmental contamination (Brodeur *et al.* 1997; Laflamme *et al.* 2000; Levesque *et al.* 2002; Campbell *et al.* 2003; Couture and Rajotte 2003; Pyle *et al.* 2005; Couture *et al.* 2008a). A number of reports also indicate that metal-contaminated wild yellow perch suffer a range of metabolic and energetic impairments, including an impaired cortisol stress response (Hontela *et al.* 1996; Brodeur *et al.* 1997; Laflamme *et al.* 2000; Lacroix and Hontela 2004), bioenergetics (Sherwood *et al.*

2000; Sherwood *et al.* 2002), intermediary metabolism (Levesque *et al.* 2002), aerobic metabolism (Rajotte and Couture 2002; Audet and Couture 2003; Couture and Kumar 2003), and even basic morphometric condition indicators (Laflamme *et al.* 2000; Levesque *et al.* 2002; Pyle *et al.* 2005; Couture *et al.* 2008b). Although this substantial literature leaves little doubt that metals affect the condition of wild yellow perch, natural variations in fish condition likely confound interpretation of most indicators of yellow perch health. Limited sample size, small number of study sites, and single sampling events also limit application of these studies. Three studies have reported seasonal variations within a year in yellow perch tissue metal concentrations and condition. Two of them (Eastwood and Couture 2002; Kraemer *et al.* 2006b) examined seasonal variations in four or five lakes within a single region, and only examined the condition factor as an indicator of yellow perch health. The third (Audet and Couture 2003) focused on only two lakes from the Sudbury area but also examined variations in metabolic capacities. These combined studies indicate that both tissue metal concentrations and condition vary seasonally in yellow perch. Yet in order to better understand the implications for ERA of seasonal variations in condition and tissue metal concentrations, a larger study was required.

Here we review work that our group has conducted on tissue metal accumulation patterns and effects on both morphometric (growth, condition factor) and physiological (tissue protein contents and metabolic enzyme activities) condition in yellow perch from two of the most metal contaminated gradients of lakes in Canada (Sudbury (S), Ontario and Rouyn-Noranda (RN), Québec). We designed a large study in which 120 yellow perch of all sizes available from each of 5 lakes in each region (James (S1), Geneva (S2), Crowley (S3), Whitson (S4) and Hannah (S5) in Sudbury and Opasatica (RN1), Ollier (RN2), Bousquet (RN3), Osisko (RN4) and Dufault (RN5) in Rouyn-Noranda; see Couture *et al.* 2008a for information on these lakes including location, water quality and metal contamination levels) were sampled once in late spring and once in late summer. Morphometric indicators were recorded for all fish, and a subset of each age class was further analyzed for tissue metal concentrations and indicators of metabolic capacities. The resulting database is the largest of its kind, with morphometric parameters (length, mass, age, condition factor) available for 2400 fish. Liver and kidney enzyme activities and metal concentrations (the latter also measured in gut contents) are available for 400 of these fish of all age classes. The dataset generated is available through the MITE website (http://www.mithern.org/ mite_rn/research/Results-Details.asp?MetaDataID = 6). Details of this research are published separately (Pyle *et al.* 2008; Couture *et al.* 2008a; Couture *et al.* 2008b).

The general objective of this research was to improve the ecological relevance of ERA by studying a fish species (yellow perch) that is not commonly considered under the current ERA paradigm but is widely distributed throughout North America and is known to inhabit many of the metal-contaminated environments around northern industrial regions. The specific objectives of this review are: (1) to propose thresholds for identifying above-normal accumulation of metals in yellow perch tissues; (2) to identify the factors that influence yellow perch metal contamination and condition; and (3) to define the experimental approach for fairly measuring metal effects in wild yellow perch.

TISSUE METAL ACCUMULATION AS AN INDICATOR OF EXPOSURE

Thresholds of Reference Values

Our studies to date have focused on five metals (Cd, Cu, Ni, Se, and Zn) in the mining and smelting areas of Sudbury and Rouyn-Noranda. Because these metals all occur naturally, their presence can theoretically be detected in the tissues of all yellow perch, regardless of environmental contamination. Even though we have noted and reported important seasonal variations in yellow perch tissue metal concentrations (reviewed later), the ranges within which they fluctuate in clean lakes have upper thresholds efficiently separating them from values in contaminated fish for Cd and Cu, but not for Ni. This approach could not be used for Se for which water concentrations were not determined in several lakes (below analytical detection limits), and was irrelevant for Zn, which is strongly regulated and weakly affected by environmental contamination (Couture *et al.* 2008a). To determine these upper thresholds of normal variation, all available data from clean sites should be used. In this review, the tissue metal-concentration thresholds that we proposed were exceeded by 10% of the reference fish in 5 to 7 lakes depending on the metal (in 200 to 300 fish) in 2 seasons (spring and summer). The value of 10% was selected as high enough to capture the natural variability in clean fish (whereas 20% could not), while avoiding the inclusion of uncommonly high values found on occasion even in clean fish (which would be included using 5%). It should be emphasized that the value of 10% proposed here, although generating threshold concentrations in agreement with published literature, was based on our dataset and must be validated empirically in other datasets. Tissue metal concentrations measured in fish from contaminated lakes (exceeding the Ontario Provincial Water Quality Objectives [OPWQO], see Table 1) were then compared to the thresholds determined in clean fish. In cases where the majority of contaminated fish exceeded the 10% threshold in clean fish, we propose that (1) fish from these lakes are at a risk of toxicity; and (2) the use of thresholds for a specific metal and tissue could be useful for ERA. From an ERA perspective, measuring tissue metal concentrations in exposed fish and comparing them to threshold values has the advantage of providing a more direct measure of exposure and risk of toxicity than measuring contaminant concentrations in the different media from which the contaminant may be obtained (water, fish food items).

Below, we present evidence from empirical measurements in support of our suggestion that exceeding these thresholds may be linked to toxic effects in wild yellow perch. However, direct experimental evidence will be required to validate our hypothesis and, therefore, our thesis is limited to the reasonable suggestion that exceeding these tissue metal thresholds represents a risk of toxicity. Also, although metal speciation in water and food, as well as water chemistry in the case of aqueous metal uptake and subcellular partitioning for food-borne metals, are known to influence accumulation in fish tissues, the influence of these factors is complex and not fully understood. In the system studied, because inter-lake variations in water chemistry and their potential influence on differential tissue metal accumulation among fish populations remained small (Couture *et al.* 2008a), we have chosen to ignore these factors. Nonetheless, we cannot exclude that some of the regional differences

Table 1. Upper thresholds of kidney and liver Cd, Cu, and Ni concentrations (μg/g dry weight) exceeded by 10% of yellow perch in clean lakes from Sudbury and Rouyn-Noranda in spring and fall combined. Lakes were classified as clean when aqueous concentrations of Cd, Cu or Ni were below the Ontario Provincial Water Quality Objective (Ontario Ministry of Environment and Energy (OMEE) 1994) of 0.1, 5, and 25 μg/L, respectively.

Metal	Clean lakes	Upper 10% threshold in clean fish	% above threshold in pooled contaminated fish	% above above threshold per lake
Kidney				
Cd	RN1-2-3; S1-2	19.9 (211)	68.3% (186)	RN4: 32.5% (40) RN5: 92.2% (51) S3: 67.5% (40) S4: 90.9% (22) S5: 60.6% (33)
Cu	RN1-2-3; S1-2	20.2 (202)	31.2% (167)	RN4: 74.2% (31) RN5: 27.5% (51) S3: 16.7% (36) S4: 35.0% (20) S5: 6.9% (29)
Ni	RN1-2-3-4-5; S1-2	14.4 (259)	17.9% (84)	S3: 18.9% (37) S4: 29.4% (17) S5: 10.0% (30)
Liver				
Cd	RN1-2-3; S1-2	11.0 (207)	72.6% (175)	RN4: 53.3% (45) RN5: 100% (51) S3: 55.2% (29) S4: 57.9% (19) S5: 68.4% (31)
Cu	RN1-2-3; S1-2	38.8 (207)	72% (164)	RN4: 75.6% (45) RN5: 78.4% (51) S3: 45.5% (22) S4: 68.4% (19) S5: 77.8% (27)
Ni	RN1-2-3-4-5; S1-2	12.1 (296)	8.3% (72)	S3: 0% (21) S4: 9.1% (22) S5: 13.8% (29)

Refer to Couture *et al.*(2007a) for water metal concentrations in each lake. The proportion (in %) of fish from contaminated lakes with tissue concentrations above the threshold is indicated for all contaminated fish pooled, and for fish from each contaminated lake (sample size in parentheses). Contaminated lakes were those not considered as clean (metal-specific) among Sudbury (S1 to S5) and Rouyn-Noranda (RN1 to RN5) lakes.

in metal accumulation, which we attribute to selection, may be partly explained by abiotic factors such as water chemistry.

Tissue Cd concentrations in all samples examined in Couture *et al.* (2008a) ranged from 0.3 to 178 μg/g dw ($n = 397$) and from 0.1 to 77 μg/g dw ($n = 382$) in kidney and liver, respectively (data not shown). The thresholds proposed for Cd of 19.9 μg/g dw in kidney and 11.0 μg/g dw in liver (Table 1) appear highly useful at separating clean from Cd-contaminated fish, with 10% of reference fish above the thresholds, and about 70% of contaminated fish from both regions combined above the thresholds, for both liver and kidney. These results support conclusions in Couture *et al.* (2008a) that fish from both regions are incapable of regulating Cd. Finally, the proportion of fish from contaminated lakes above the threshold Cd concentration was higher in the most Cd-contaminated lakes (RN5 and S4) compared to lakes classified as Cd-contaminated but where aqueous Cd concentrations were intermediate between the most Cd-contaminated lakes and reference lakes. We propose that the proportion of fish with liver and kidney Cd concentrations above thresholds could be used as an indicator of risk for Cd toxicity at the population level. Although a threshold for discriminating between clean and Cd-contaminated yellow perch has never been proposed for kidney, for liver the value of 11 μg/g dw proposed here is in strong agreement with a value of 10 μg/g dw proposed earlier (Couture and Rajotte 2003).

Tissue Cu concentrations in all samples examined in Couture *et al.* (2008a) ranged from 0.2 to 171 μg/g dw and from 0.1 to 1078 μg/g dw in kidney and liver, respectively (data not shown). Although the liver Cu threshold value of 38.8 μg/g dw proposed here efficiently discriminates clean from Cu-contaminated fish with 72% of the latter yielding liver Cu concentrations above this threshold, the threshold for kidney above which 10% of fish from Cu-clean lakes are found would not allow discriminating clean from contaminated fish, as only about 30% of the latter expressed values above the threshold of 20.2 μg/g dw. This suggests that kidney Cu is more strongly regulated than liver Cu, and highlights the role of liver for Cu storage as well as the essential and tightly-regulated nature of this metal. As for Cd, a higher proportion of fish were above thresholds in the most Cu-contaminated lakes (RN5, S4, and S5) but in liver only. Therefore, we propose that the proportion of fish with liver Cu concentrations above thresholds could be used as an indicator of risk for Cu toxicity at the population level. Finally, the value of 38.8 μg/g dw proposed for liver is about 20% lower than the value of 50 proposed earlier (Couture and Rajotte 2003) and supported by other research (Kraemer *et al.* 2006b).

Tissue Ni concentrations in all samples examined in Couture *et al.* (2008a) ranged from lower than 0.1 to 81 or 89 μg/g dw in kidney and liver, respectively (data not shown). In contrast to Cd and Cu, the thresholds of both liver and kidney Ni concentrations above which 10% of fish from Ni-clean environments fell was not useful in discriminating between fish from high vs. low aqueous Ni concentrations, because only 8 to 18% of fish from Ni-rich environments were above these threshold values (Table 1). This odd result is due to exceptionally high tissue Ni concentrations in fish from RN in the spring, even though all RN lakes were classified as low-Ni (Couture *et al.* 2008a). As discussed in Couture *et al.* (2008a), it appears that Sudbury yellow perch can better regulate their tissue Ni concentrations than RN fish. Therefore, also in contrast with Cd and Cu, even though a threshold of tissue

Ni concentrations could be proposed for S yellow perch, it could not be applied to RN fish in the spring, as fish from these Ni-clean environments would exceed the threshold. As for Cd and Cu, ongoing investigations are attempting to establish whether fish tissue Ni concentrations that exceed these tissue metal accumulation thresholds will result in toxicity. Some of the evidence in support of this hypothesis is reviewed in the following sections.

SEASONAL VARIATIONS IN TISSUE METAL CONCENTRATIONS AND IMPLICATIONS FOR ERA

Several studies have reported that yellow perch tissue metal concentrations vary seasonally, in contaminated lakes (Eastwood and Couture 2002; Audet and Couture 2003; Kraemer *et al.* 2006b) but also sometimes in clean lakes (data examined later from Couture *et al.* 2008a). Here we provide evidence with implications for ERA of how ignoring these seasonal variations could falsely lead to conclusions that fish from contaminated lakes do not differ in tissue metal concentrations compared to clean fish.

In the first example, lake RN2, with an aqueous Cd concentration of 0.03 μg/L (Couture *et al.* 2008a), is considered clean with respect to Cd contamination, whereas RN4 is considered contaminated (Table 1). In spring, kidney Cd concentrations were higher in fish from RN4 compared to fish from RN2 (Figure 1). However, if sampling was carried out in both lakes in summer, or if RN2 was sampled in the spring and RN4 in summer, we would conclude that kidney Cd concentrations did not differ between these fish, and therefore that RN4 fish do not face a higher risk of Cd toxicity than clean fish. Thus, based on a statistical comparison of kidney Cd concentrations, a higher risk for RN4 fish could only be detected in spring. Using the 10% upper threshold approach described above of 19.9 μg/g dw for kidney Cd (Table 1) and applying it to concentrations measured in RN2 and RN4 fish, 32 to 33% of RN4 fish, but only 4 to 8% of RN2 fish fell above the threshold,

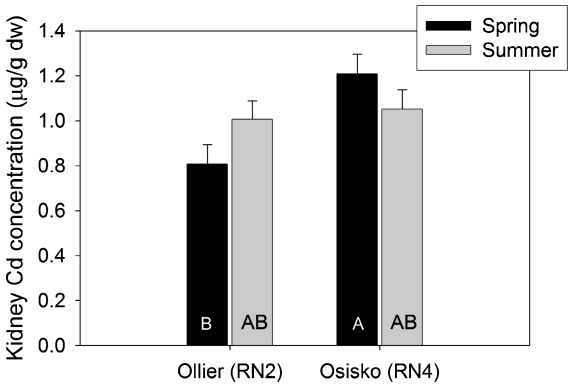

Figure 1. Mean log kidney Cd concentrations (+SEM) in spring and summer in a Cd-clean lake (RN2) and in a Cd-contaminated lake (RN4). Bars sharing the same letters are not significantly different from one another (two-way ANOVA; $p > .05$).

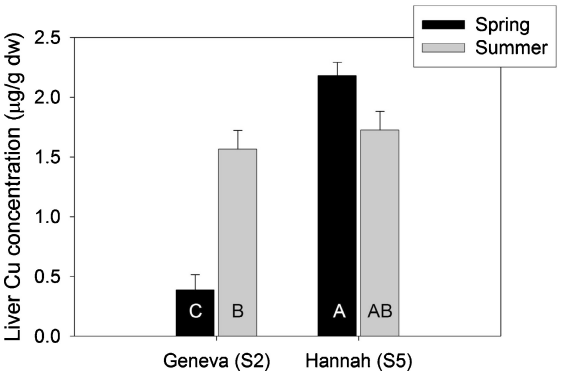

Figure 2. Mean log liver Cu concentrations (+SEM) in spring and summer in a Cu-clean lake (S2) and in a Cu-contaminated lake (S5). Bars sharing the same letters are not significantly different from one another (two-way ANOVA; $p > .05$).

in spring and summer, respectively. Combining these approaches of sampling two seasons and using concentration thresholds for comparison, we can conclude that yellow perch from RN4 face a higher risk of Cd toxicity compared to reference fish in both seasons, but that differences between clean and contaminated fish are more important in spring. Given that only a third of fish from RN4 exceeded the threshold of kidney Cd at any time, we could also propose that the risk of Cd toxicity is moderate.

Similarly, we compared liver Cu concentrations in fish from one Cu-clean lake (S2) and one contaminated lake (S5) (Figure 2). Liver Cu was higher in S5 compared to S2, but only in spring, because of a major increase in liver Cu in S2 fish in summer. Interestingly, while 0% of S2 fish were above the liver Cu threshold reported in Table 1 in the spring, in summer 44% of fish from this clean lake exceeded the threshold. In comparison, there was 83% and 67% of S5 fish above the threshold in spring and summer, respectively. Combined, these analyses suggest that S5 fish constantly face a high risk of Cu toxicity as the majority of them sustain liver Cu concentrations higher than 90% of values in clean fish, but that nearly half of S2 fish, although living in a lake low in aqueous Cu, may also have to face the metabolic consequences of excess liver Cu in summer.

Although only two concrete examples have been provided, comparing fish from other clean lakes with fish from other contaminated lakes in our database, or performing these comparisons with the other metals (Ni, Se and Zn) for which yellow perch showed seasonal variations in tissue concentrations (Couture *et al.* 2008a), would similarly support that sampling fish from all study lakes within a narrow time window and more than once a year is required for determining whether tissue metal accumulation represents a risk to fish. Because seasonal variations in tissue metal concentrations are inconsistent among metals and lakes and between the two regions studied, no recommendation of a better season for maximizing differences between fish from clean and contaminated lakes can be made.

REGIONAL DIFFERENCES IN TISSUE METAL ACCUMULATION AND IMPLICATIONS FOR ERA

Yellow perch from different regions sometimes differ widely in their accumulation of metals from environments having the same degree of metal contamination. Several studies have shown that, regardless of region (Sudbury or Rouyn-Noranda), yellow perch cannot regulate tissue Cd. As a result, yellow perch from both regions demonstrate similar patterns of tissue Cd accumulation when exposed to either dietary or waterborne sources (Giguere *et al.* 2004; Kraemer *et al.* 2006a; Couture *et al.* 2008a). Consequently, when studying Cd contamination, the distance between reference and contaminated lakes may not be as important as for Ni, because S fish may have evolved superior Ni-regulatory capacities than RN fish. Although a broad statistical approach has already described this general phenomenon (Couture *et al.* 2008a), this case study allows us a better understanding of the implications for ERA.

We compared mean liver and kidney Ni concentrations in the spring in fish from two lakes (RN3 and S1) with similarly low aqueous (1.2 *vs.* 0.9 μg/L, or 1.3-fold higher in RN3) and dietary (6.7 *vs.* 3.9 μg/g dw, or 1.7-fold higher in RN3) Ni concentrations. Liver Ni concentration was 11.1-fold higher in RN3 compared to S1 fish (15.6 *vs.* 1.4 μg/g dw), and kidney Ni concentration was 7.9-fold higher in RN3 compared to S1 fish (15.6 *vs.* 1.4 μg/g dw). Comparing fish tissue concentrations in spring in RN4 vs. S2 lakes, both with similarly low aqueous and dietary Ni exposure for yellow perch, also suggested much greater Ni-accumulation in RN fish (1.9-fold and 10.5-fold in liver and kidney, respectively). Some of the tissue Ni concentrations measured in RN fish were in the same range as the highest values recorded in S fish. Comparisons in high-Ni lakes from both regions could not be performed due to the absence of such lakes in RN, but we can only speculate on the tissue Ni concentrations that would be reached if RN fish were subjected to the high dietary and aqueous Ni exposure faced by S fish. We could not apply the threshold approach to a pooled sample of fish from both regions owing to the high tissue Ni concentrations in low-Ni lakes in RN (see earlier). Therefore, it is clear, at least for Ni, that tissue concentrations do not reflect risk based on aqueous and dietary exposure in RN fish. However, tissue-Ni accumulation patterns in S fish more predictably reflected environmental concentrations (data reported in Couture *et al.* 2008a).

We have discussed elsewhere the regional differences in metal accumulation between yellow perch from Sudbury and Rouyn-Noranda.(Couture *et al.* 2008a) and proposed that selective pressures may have allowed S fish to evolve better capacities for regulating Ni, Cu, and perhaps also Se compared to RN fish. Therefore, when studying metal exposure and accumulation patterns in wild fish, it is best to select reference sites that are situated in reasonably close proximity to metal-contaminated sites to allow for a reasonable assumption of relatively close genetic similarity among comparative populations, when it cannot be tested. Testing differences in tissue metal accumulation in genetically distant fish may be meaningless from an ERA perspective.

EFFECTS OF SIZE AND AGE ON METAL ACCUMULATION

There is no controversy, and an abundant literature, on the bioaccumulation of several organic contaminants over time in fish. Among metals, the organic form of Hg (CH_3Hg) is well known to accumulate in older fish, including yellow perch (Ion *et al.* 1997). However, evidence of accumulation of inorganic forms of metals remains anecdotal and the literature contains limited, and sometimes contradictory, evidence. Literature on metal accumulation in fish in general and specifically in yellow perch has been reviewed elsewhere (Sorensen 1991; Couture *et al.* 2008a). The overwhelming evidence from these reviews is that inorganic metals do not generally accumulate in fish over time, except for anecdotal reports including two studies indicating that Cd accumulates with size in yellow perch from RN4 (Giguère *et al.* 2004) and other RN lakes (Couture *et al.* 2008a). However, in spite of an absence of a general pattern of increasing or decreasing tissue metal concentrations with size in yellow perch, in each individual lake and depending on the season, tissue metal concentrations (Cd, Cu, Ni, Se, and Zn) are sometimes correlated with size, either positively or negatively (Couture *et al.* 2008a). Yellow perch tissue metal concentrations have been shown by several studies (briefly reviewed in the Introduction) to be strongly influenced by environmental contamination but not consistently by size, implying that tissue metal concentrations are largely the reflection of both recent (because they vary seasonally) accumulation from aqueous and dietary sources and depuration (Kraemer *et al.* 2005). From an ERA perspective, if size or age did not affect fish tissue metal concentrations, fish size could be ignored in the sampling design and data interpretation. On the other hand, if accumulation patterns with size were consistent, models could be used to correct the effects of size on tissue metal concentrations. However, because neither of these scenarios appears to reflect the reality in the field, careful size selection must be considered in metals ERA studies with yellow perch.

MORPHOMETRIC AND PHYSIOLOGICAL CONDITION

Morphometric Fish Condition

Fish condition is commonly assessed by environmental scientists who wish to evaluate the general "well-being" of fish in a specific population. Condition is typically estimated as a ratio of the actual weight of a fish against an expected weight estimated from a double-log plot of fish weight and length from fish sampled from a population of interest (for a detailed discussion about traditional fish condition metrics, see Pyle *et al.* 2008). This kind of condition metric, which we shall refer to as "morphometric condition" hereafter, is thought to reflect recent feeding activities in sampled fishes. Fish assessed to be of high condition are heavy relative to their length, which corresponds to increased energy storage (*i.e.*, fat deposition) from abundant food resources relative to physiological energetic requirements. In contrast, low-condition fish deposit less fat because of reduced food availability and (or) increased physiological demand for energetic resources, which may be the case in metal-contaminated systems where fish allocate significant energetic resources toward metal detoxification (Smith *et al.* 2001). Therefore, fish morphometric condition is often used

in ERAs to provide a rough approximation of the relationship between available resources (*i.e.*, food availability and possibly food quality) and a fish's energetic demand.

From the aforementioned, it seems intuitive that fish inhabiting metal-contaminated environments are likely to be of lower morphometric condition than those inhabiting clean environments. Fish allocate energetic resources to detoxify metals taken up through food or water, and the presence of relatively high environmental metal concentrations may reduce food availability if concentrations are high enough to induce toxicity in food organisms (Sherwood *et al.* 2000). However, published studies investigating morphometric condition in wild yellow perch populations have led to contradictory conclusions in the literature.

Several studies have concluded that fish inhabiting metal-contaminated lakes are of lower morphometric condition than those inhabiting reference lakes (Leis and Fox 1994; Laflamme *et al.* 2000; Lohner *et al.* 2001; Levesque *et al.* 2002; Rajotte and Couture 2002; Levesque *et al.* 2003; Bervoets and Blust 2003; Couture and Rajotte 2003). In contrast, other studies have reported higher morphometric condition in fish inhabiting metal-contaminated lakes relative to reference lakes (Farkas *et al.* 2003; Pyle *et al.* 2005). Therefore, based on the published literature, it is difficult to make any general statements about the relationship between metal contamination and morphometric condition in fish.

To illustrate this, we calculated relative condition (K_n) in wild yellow perch from 10 lakes comprising 2 5-lake metal-contamination gradients. Data reported here are from Pyle *et al.* (2008). Relative condition is a slope-corrected condition factor, where slopes are derived from a double-log plot of fish weight (W in g) versus fish length (L in mm) using the formula, $K_n = W/L^s \times 10$, where s is the slope of the double-log plot. Slopes were calculated for each individual lake (regression details are in Table 2), and K_n for each lake by season is reported in Figure 3.

In each metal-contamination gradient, we sampled two reference lakes, two metal-contaminated lakes, and one intermediate lake. Had we restricted our analysis to fewer lakes, we would not have been able to draw any meaningful conclusions with respect to the relationship between metal-contamination and fish condition. For example, if we had selected S1 as our only reference lake to compare K_n against fish from the two most metal-contaminated lakes in the Sudbury region, S4 and S5 lakes, we may have concluded that fish from the contaminated lakes yielded lower condition than those from a reference lake (see Table 2 for statistical details). However, we could have just as appropriately selected S2 as our reference lake, and the conclusions we could draw would be vastly different: that is, fish from S5 were of significantly higher condition than those from S2, and fish from S4 showed no significant difference in K_n relative to those from S2 (Table 2). A similar effect can be observed in Rouyn-Noranda lakes. For example, if we had selected RN1 as a reference lake, we would have concluded that there was no significant difference in K_n between fish sampled from the reference lake and those from the most contaminated lakes RN4 and RN5. The only significant difference in K_n between reference and contaminated fish is with fish from RN3, the intermediate lake, which had significantly lower condition than those from RN1 (Table 2). However, if we were to use RN2 as the reference lake, our conclusions would have been different. There was no significant difference in K_n between fish sampled from RN2 and one of the

Table 2. Results of log-log weight (g)-length (mm) analysis of covariance on wild yellow perch sampled from 10 lakes along two metal-contamination gradients in Rouyn-Noranda, Quebec and Sudbury, Ontario ($n = 2449$) to determine if slopes (b; scaling coefficients, which are used in the calculation of K_n) varied by lake ($F_{1,9} = 257\,964$, $p < .0001$) or intra- (Rouyn-Noranda: $F_{1,4} = 154\,204$, $p < .0001$; Sudbury: $F_{1,4} = 111\,820$, $p < .0001$) or inter- ($F_{1,1} = 237\,157$, $p < .0001$) regionally.

Region	Lake	Code	Intercept (a)	Slope (b)	K_n
Rouyn-Noranda	Opasatica	RN1	−5.24	3.13	1.09 ± 0.36 (240)^{bc}
	Ollier	RN2	−5.31	3.18	1.15 ± 0.35 (236)^{b}
	Bousquet	RN3	−5.43	3.25	0.86 ± 0.49 (169)^{d}
	Dufault	RN4	−5.11	3.05	1.00 ± 0.55 (240)^{c}
	Osisko	RN5	−5.45	3.21	1.08 ± 0.39 (240)^{bc}
Sudbury	James	S1	−4.86	2.91	1.34 ± 0.27 (257)^{a}
	Geneva	S2	−5.16	3.07	0.87 ± 0.35 (257)^{d}
	Crowley	S3	−4.95	2.96	1.09 ± 0.32 (278)^{bc}
	Whitson	S4	−5.05	3.00	0.80 ± 0.42 (254)^{d}
	Hannah	S5	−5.32	3.15	1.00 ± 0.30 (278)^{c}

Mean $K_n \pm$ SD (n) is provided for fish sampled from each lake. Lakes sharing the same alphabetical superscript in the K_n column are not significantly different from one another (Tukey-Kramer HSD; $p > .05$). All slopes (except Whitson; $p = .92$) are significantly different from 3 ($p < .02$).

most contaminated Rouyn-Noranda lakes, RN4. However, RN2 fish had significantly higher condition than those from RN3 and RN5 (Table 2).

Establishing seasonal trends in fish condition as a function of metal contamination is equally problematic (see Figure 3). In Rouyn-Noranda, fish from one of the reference lakes (RN1) showed significantly higher condition in the spring relative to the summer ($t = 2.9$, $d.f. = 219.1$, $p = .004$), whereas there was no seasonal difference in the other reference lake (RN2; $t = -1.1$, $d.f. = 234$, $p = .28$). No seasonal differences were observed in any other Rouyn-Noranda lake ($p > .05$). This situation is different than what was observed in Sudbury-area lakes. Both reference lakes in the Sudbury region (S1 and S2) yielded fish having higher condition in the summer than in the spring (S1: $t = -7.3$, $d.f. = 231.2$, $p < .0001$; S2: $t = -2.5$, $d.f. = 202.6$, $p = .01$), a phenomenon also observed in one of the most contaminated Sudbury-area lakes, S5 ($t = -14.1$, $d.f. = 221.9$, $p < .0001$).

Another issue that must be considered when comparing fish from different populations is the statistical assumptions inherent in the estimation of commonly used fish condition metrics. Fulton's condition factor (K_F) assumes that the slope of the log-log fish weight-length plot is 3, because K_F is estimated as weight/length^3. Of the 10 lakes that we sampled, only 1 (S4) yielded a slope that was not significantly different from 3 (Table 2). In every other lake, the slope was either significantly higher or lower than 3, violating a major assumption of the K_F model. But even when slopes are used in the calculation of fish condition, such as the slope-corrected K_n,

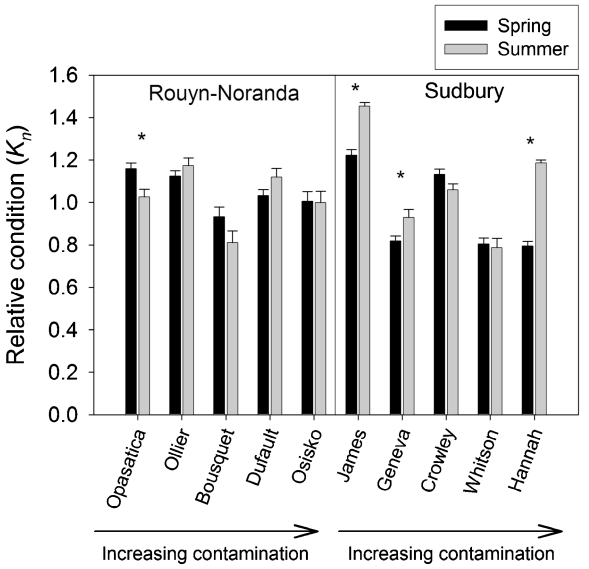

Figure 3. Effect of season on yellow perch relative condition (K_n) in each study lake. Bars represent means + SEM ($n = 75$–148); asterisks (*) indicate a significant difference between spring and summer (t-test; $p \leq .05$).

which replaces 3 in the calculation with a scaling coefficient (*i.e.*, the slope of the double-log plot), the model still assumes homogeneity of slopes among comparative populations (Cone 1989). Again, our analysis revealed that slopes varied among our comparative populations (Table 2), violating this statistical assumption.

These results indicate that common fish condition metrics, such as the Fulton's condition factor (K_F) or the slope-corrected relative condition factor (K_n, which we used here for illustrative purposes), can only provide a rough approximation of fish "well-being" at a particular place and time. Condition factor is an integrative metric that can be influenced by a number of abiotic (temperature, environmental conditions such as water quality or contamination, *etc.*) and biotic (predation pressure, competition, food availability, population genetics, *etc.*) factors. Therefore, it is difficult to draw any meaningful conclusions about fish condition if the full range of variability among comparative populations is not considered. This analysis resulted in similar confusing, and sometimes contradictory, conclusions as those reported in the literature. Consequently, a better approach to assessing fish condition is to sample a large number of fish representing the full size/age range in each population using more than one reference site, and optimally, over more than one season. Condition can be estimated using a least-squares approach, originally proposed by Le Cren (1951) and later modified by Patterson (1992). This is the approach we adopted for assessing the influence of environmental conditions on fish condition, which we reported in Pyle *et al.* (2008).

In Pyle *et al.* (2008), we speculated that fish from Rouyn-Noranda and Sudbury probably had significant genetic differences based largely around differences in observed growth patterns (*e.g.*, see Figure 1 in Pyle *et al.* 2008). Those growth pattern

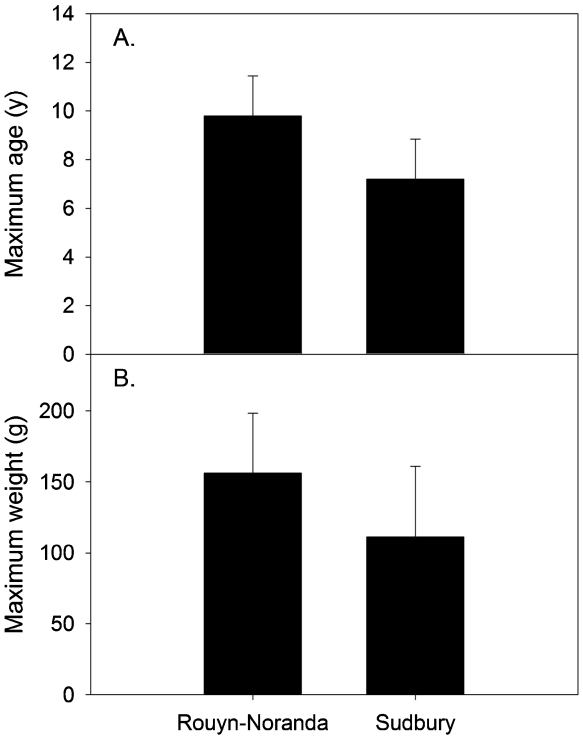

Figure 4. Mean maximum age (A) and weight (B) (±SD; $n = 5$) in wild yellow perch collected from 10 lakes forming two 5-lake contamination gradients in Rouyn-Noranda, QC, and Sudbury, ON. Statistical comparisons were not attempted given that fish from each region were sampled in different years.

differences indicated that Rouyn-Noranda fish grew relatively quickly while they were young, but slowed as they approached maximum age (approx. 11 y). In Sudbury-area lakes, however, fish started out growing slowly, but then increased their growth rate until eventually slowing as they approached maximum age (approx. 9 y). At approximately 4 y of age (*i.e.*, when both groups of fish demonstrated their fastest growth rates) Sudbury-area fish grew significantly faster (1.8 g/y) than Rouyn-Noranda fish (1.7 g/y; instantaneous slopes compared using ANCOVA, $F_{1,1817} = 9282$, $p < .0001$). The average maximum age of fish collected from the five lakes comprising the Rouyn-Noranda gradient was 9.8 y (range: 7–11 y), whereas in Sudbury it was 7.2 y (range: 5–9 y) (Figure 4). Similarly, maximum fish weight was 41% greater in Rouyn-Noranda than in Sudbury (Figure 4). Using the least squares approach to estimate fish condition mentioned earlier, we also found that fish from Rouyn-Noranda were generally of higher condition than those from Sudbury (Pyle *et al.* 2008).

Therefore, Rouyn-Noranda fish were of higher condition, lived longer, grew to larger sizes, but grew slower (and demonstrated a different long-term growth pattern) than fish from Sudbury, regardless of environmental contamination in any specific lake. Together, these results suggest that fish from Sudbury and

Rouyn-Noranda probably derived from a different genetic heritage. Mitochondrial DNA haplotype analysis, as has been done on Ontario lake trout (*Salvelinus namaycush*) populations (Wilson and Hebert 1996 1998), would provide important insights on these population differences, which have important implications to ERA, because several of the measures it considers such as growth and metal handling capacities may have strong genetic influences.

Metal Effects on Metabolic Enzyme Activities and Longevity

To evaluate the physiological condition of the wild yellow perch we sampled from each of the 10 lakes comprising the two metal-contamination gradients, we measured total protein concentration, lactate dehydrogenase (LDH) (an indicator of anaerobic capacities), citrate synthase (CS), and cytochrome C oxidase (CCO) activities (indicators of aerobic capacities) in muscle and liver tissues. We reported regional and seasonal effects in Couture *et al.* (2008b). Here, we wanted to determine the extent to which total protein concentration or tissue enzyme activities varied on the basis of whether or not tissue metal concentrations were above or below metal accumulation thresholds reported in Table 1. In each region, we compared total protein concentration and tissue enzyme activities (in muscle and liver) between fish having tissue metal concentrations (in kidney or liver) above or below the metal accumulation thresholds. Results of this analysis are reported in Tables 3 (muscle enzymes) and (liver enzymes).

Exceeding tissue metal thresholds did not affect liver or muscle protein concentrations in Sudbury fish (Tables 3 and 4). In RN fish, however, whereas excess Cu (either in liver or kidney) negatively affected muscle protein concentrations, in contrast, exceeding tissue thresholds of Cd, Cu, or Ni yielded higher liver protein concentrations. Because elevated muscle protein concentration is an indicator of muscle energy reserves (Lambert and Dutil 1997), this finding highlights a regional difference in metal tolerance, with energy reserves of RN but not S fish being negatively affected by Cu. Our data support that yellow perch are a metal-tolerant species, but that metal exposure exerts a metabolic cost that varies according to the region of origin of the fish. Specifically, the tissue metal threshold approach followed here indicates that RN fish exceeding these thresholds increase their liver protein concentration, presumably in an effort to combat metal intoxication (for example, by increasing their production of metallothioneins, Campbell *et al.* 2005), thus (at least when the Cu thresholds are exceeded) decreasing energy available for muscle growth. Implications for ERA are that tissue protein concentrations are useful indicators of metal stress in RN, but not in S, fish.

Evidence from the literature of metal effects on muscle and liver LDH activity is contradictory and complicated by a number of factors, including a lack of allometric corrections when comparing different-sized fish (Levesque *et al.* 2002), or studies limited to two lakes (Audet and Couture 2003). Two studies conducted in the Sudbury area have examined in more detail the relationships between tissue anaerobic capacities and metal contamination in yellow perch. In the first, Rajotte and Couture (2002) concluded that metal contamination did not affect yellow perch anaerobic capacities, whereas the second suggested that Cd and Cu contamination

Table 3. Muscle protein concentration ($\log_{10}$ mg/g wet weight) and enzyme activity ($\log_{10}$ IU*/g wet weight) in wild yellow perch from two metal-contamination gradients in Sudbury, ON, and Rouyn-Noranda, QC, which have tissue (kidney or liver) metal concentrations (Cd, Cu, or Ni) either above or below tissue metal thresholds reported in Table 1.

			Protein			LDH			CS			CCO		
			Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	n
Kidney metals														
Sudbury	Cd	Above	2.296	0.122	68	2.515	0.228	68	0.442	0.209	68	**0.943**$^{+}$	0.157	68
		Below	2.264	0.145	88	2.557	0.176	88	0.420	0.181	88	**0.886**	0.165	88
	Cu	Above	2.270	0.121	16	**2.669**$^{+}$	0.234	16	**0.177**$^{-}$	0.220	16	0.871	0.181	16
		Below	2.281	0.141	129	**2.516**	0.192	129	**0.468**	0.170	129	0.910	0.165	129
	Ni	Above	2.325	0.069	13	2.595	0.213	13	0.447	0.168	13	0.967	0.148	13
		Below	2.280	0.142	128	2.529	0.202	128	0.450	0.188	128	0.902	0.172	128
Rouyn-Noranda	Cd	Above	2.287	0.117	81	**2.036**$^{+}$	0.210	81	0.312	0.207	81	0.713	0.130	81
		Below	2.289	0.104	157	**1.879**	0.213	157	0.344	0.179	157	0.712	0.140	157
	Cu	Above	**2.261**$^{-}$	0.087	56	1.919	0.285	56	**0.376**$^{+}$	0.174	56	**0.745**$^{+}$	0.140	56
		Below	**2.308**	0.109	163	1.944	0.207	163	**0.307**	0.196	163	**0.699**	0.134	163
	Ni	Above	2.325	0.074	26	**1.859**$^{-}$	0.235	26	**0.458**$^{+}$	0.174	26	0.740	0.119	26
		Below	2.296	0.112	172	**1.952**	0.220	172	**0.296**	0.179	172	0.701	0.138	172
Liver metals														
Sudbury	Cd	Above	2.285	0.125	51	2.565	0.231	51	0.415	0.210	51	**0.942**$^{+}$	0.169	51
		Below	2.265	0.154	84	2.532	0.182	84	0.433	0.188	84	**0.885**	0.158	84
	Cu	Above	2.282	0.111	50	2.566	0.250	50	**0.346**$^{-}$	0.220	50	0.913	0.158	50
		Below	2.246	0.162	74	2.532	0.170	74	**0.457**	0.166	74	0.883	0.165	74
	Ni	Above	2.244	0.087	8	2.497	0.140	8	**0.525**$^{+}$	0.069	8	0.946	0.141	8
		Below	2.260	0.143	123	2.539	0.208	123	**0.410**	0.199	123	0.898	0.168	123
Rouyn-Noranda	Cd	Above	2.288	0.108	94	**1.992**$^{+}$	0.254	94	0.338	0.203	94	**0.735**$^{+}$	0.127	94
		Below	2.289	0.108	143	**1.894**	0.195	143	0.327	0.178	143	**0.701**	0.139	143
	Cu	Above	**2.270**$^{-}$	0.115	85	**1.982**$^{+}$	0.245	85	0.357	0.195	85	**0.738**$^{+}$	0.147	85
		Below	**2.300**	0.102	152	**1.905**	0.210	152	0.317	0.183	152	**0.701**	0.127	152
	Ni	Above	2.296	0.072	26	**1.703**$^{-}$	0.202	26	**0.539**$^{+}$	0.123	26	**0.841**$^{+}$	0.087	26
		Below	2.292	0.111	203	**1.961**	0.215	203	**0.301**	0.180	203	**0.696**	0.133	203

Significant differences (*t*-test; $p < .05$) between fish that are above or below specific tissue metal thresholds are indicated in **bold**; superscripts indicate whether fish above threshold values have higher (+) or lower (–) muscle enzyme activities relative to those below threshold. *1 IU = 1 μmol substrate converted to product per minute.

Table 4. Liver protein concentration ($\log_{10}$ mg/g wet weight) and enzyme activity ($\log_{10}$ IU*/g wet weight) in wild yellow perch from two metal-contamination gradients in Sudbury, ON, and Rouyn-Noranda, QC, which have liver metal concentrations (Cd, Cu, or Ni) either above or below liver metal thresholds reported in Table 1.

			Protein			LDH			CS			CCO		
			Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	n
Sudbury	Cd	Above	2.203	0.151	42	0.725	0.163	41	0.300	0.218	41	**1.560**$^{-}$	0.309	37
		Below	2.159	0.194	79	0.697	0.241	78	0.302	0.203	78	**1.747**	0.237	63
	Cu	Above	2.196	0.143	43	0.739	0.231	42	0.290	0.232	42	1.652	0.288	41
		Below	2.148	0.222	69	0.665	0.248	68	0.275	0.243	68	1.724	0.263	52
	Ni	Above	2.061	0.112	5	0.550	0.244	5	0.189	0.267	5	1.535	0.255	5
		Below	2.162	0.194	111	0.693	0.249	109	0.283	0.234	109	1.696	0.272	92
Rouyn-Noranda	Cd	Above	**2.031**$^{+}$	0.226	93	**0.562**$^{+}$	0.210	71	**0.299**$^{+}$	0.127	79	**1.635**$^{-}$	0.081	94
		Below	**1.901**	0.245	98	**0.487**	0.242	98	**0.207**	0.186	96	**1.665**	0.109	98
	Cu	Above	**2.036**$^{+}$	0.218	83	0.508	0.188	64	**0.283**$^{+}$	0.138	71	**1.672**$^{+}$	0.089	84
		Below	**1.909**	0.250	108	0.526	0.254	105	**0.224**	0.183	104	**1.633**	0.101	108
	Ni	Above	**2.095**$^{+}$	0.118	26	**0.442**$^{-}$	0.124	18	**0.341**$^{+}$	0.112	26	**1.612**$^{-}$	0.111	26
		Below	**1.934**	0.253	157	**0.534**	0.240	143	**0.233**	0.175	141	**1.652**	0.093	158

Significant differences (*t*-test; $p < .05$) between fish that are above or below specific tissue metal thresholds are indicated in **bold**; superscripts indicate whether fish above threshold values have higher (+) or lower (–) liver enzyme activities relative to those below threshold.*1 IU = 1 μmol substrate converted to product per minute.

was associated with increased liver and muscle LDH activities (Couture and Kumar 2003). The threshold approach used here in fish from two metal gradients provides a more complete perspective. In general, exceeding tissue metal thresholds did not affect muscle and liver LDH activities in S yellow perch, with the minor exception that excess kidney Cu was associated with higher liver and muscle LDH activities, in overall agreement with Rajotte and Couture (2002) and Couture and Kumar (2003). In contrast, RN fish exceeding tissue Cd and Cu concentration thresholds generally expressed higher liver and muscle LDH activities, but exceeding Ni thresholds led to lower liver and muscle LDH activity. Muscle LDH activity in yellow perch has been used as an indicator of activity cost (Sherwood *et al.* 2002; Kaufman *et al.* 2006). Our data indicate that, at least for RN fish, metal contamination must be considered when LDH activity is used as an indicator of locomotion in contaminated yellow perch.

There is strong evidence, reviewed elsewhere (Couture *et al.* 2008b), of aerobic impairment in metal-contaminated yellow perch. The tissue metal threshold approach provides an opportunity to generalize our understanding of how metal contamination affects tissue aerobic capacities in yellow perch. Using this approach, there was no evidence that metals negatively affected muscle aerobic capacities in S fish, except for CS activity that was lower in Cu-contaminated fish (Table 3), as previously reported (Rajotte and Couture 2002; Audet and Couture 2003; Couture and Kumar 2003). Exceeding tissue metal thresholds was more generally associated with increased muscle enzyme activities, especially in RN fish, which expressed higher muscle CS activities when kidney Cu or Ni or liver Ni concentrations exceeded thresholds. Sudbury fish exceeding the liver Ni threshold also exhibited higher muscle CS activity. Exceeding tissue thresholds of Cd in fish from both regions, and Cu and Ni in RN fish, was also associated with higher muscle CCO activity. Therefore, although the reduction in aerobic capacities of Cu-contaminated S fish reported in earlier studies using CS as an indicator is strongly demonstrated here using the threshold approach, by examining other metals and adding CCO as an additional indicator of aerobic capacities, we can conclude that aerobic capacities are generally increased in yellow perch muscle fibers, especially in RN fish. Aerobic capacities of whole fish, measured in S fish using swim performance tests (Rajotte and Couture 2002) or oxygen consumption rate at rest and post-exercise (Couture and Kumar 2003) are instead lower in metal-contaminated fish. If this is also the case for contaminated RN fish, then their increased tissue aerobic capacities could reflect metabolic costs of metals for repair and detoxification or direct mitochondrial damage. In turn, this metal-induced increase in aerobic enzyme capacities could be involved in the reduced longevity of these fish (see later). It is important to note that, while the threshold approach used here suggests higher CCO activities in fish contaminated with all three metals examined, straight correlations between tissue metals and CCO activity (data not shown) indicated inconsistent trends. This is likely because factors other than metals also affect tissue aerobic capacities. Whereas aerobic capacities are primarily affected by metals in fish above contamination thresholds, variations induced by other natural factors in cleaner fish weaken general correlations between tissue metal concentrations and CCO activity. The influence of ecological variables on yellow perch tissue metabolic capacities (locomotory activity and predation) has been briefly reviewed elsewhere (Couture *et al.* 2008b).

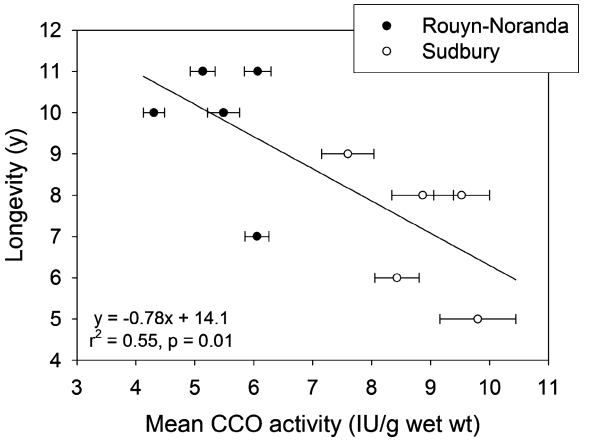

Figure 5. Relationship between muscle cytochrome C oxidase (CCO) activity and wild yellow perch longevity in 10 lakes comprising two metal-contamination gradients.

Although exceeding liver thresholds of all three metals examined led to increased liver CS activity and excess liver Cu enhanced liver CCO activity in RN fish (Table 4), in contrast to muscle, liver Cd and Ni contamination led to decreased liver CCO activities. Regional differences were much stronger for liver enzymes (Table 4) than for muscle enzymes (Table 3), because in S fish only one liver enzyme (CCO) was affected by one metal (Cd). Decreased CCO activities in the liver of Cd and Ni contaminated RN fish is an indication of uncompensated toxicity, again supporting a lower metal tolerance of RN compared to S fish.

Muscle CCO activity may have an influence on longevity. Increasing mean muscle CCO activities led to decreasing maximum age (as a surrogate for longevity) in wild yellow perch from each of the 10 lakes sampled, regardless of region Figure 5). Based on the significant equation of the linear relationship between longevity and muscle CCO activity, increasing muscle CCO from 4 to 11 IU/g (ww; *i.e.*, the approximate range measured in this study representing a 64% increase) leads to a 55% reduction in longevity. In support of a metabolic cost for enhanced mitochondrial enzyme activity, increasing muscle CCO activity was also related to decreasing K_m in all RN fish (see Figure 6 for an example in RN3; however, the effect was significant in all RN lakes and the slopes were not statistically different from each other) and near significant in S5 ($p = .06$). We have speculated that S fish have probably adapted better metal-regulatory processes to help cope with chronic metal exposure (Couture *et al.* 2008). It appears from the results reported earlier that RN fish are more susceptible to the negative influence of metal contamination on muscle CCO activity, probably because of their relatively inferior ability to regulate uptake and accumulation of dietary Cu and Ni relative to S fish.

Combining this evidence, we propose that exposure of yellow perch to elevated concentrations of metals over their lifetime leads to increases in mitochondrial respiration, which vary depending on evolved capacities for metal tolerance. Increased aerobic respiration in turn reduces longevity. In addition, our study indicates that adapting to metal tolerance or a faster growth rate in S fish also exerts a metabolic

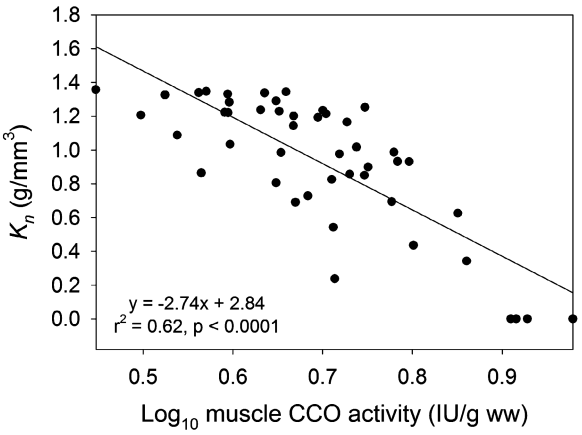

Figure 6. Relationship between muscle CCO activity and K_n in wild yellow perch sampled from RN3 (Lac Bousquet) ($n = 47$).

cost. Indeed, as reviewed later, S fish grew faster, but died younger than RN fish as a whole. Consistent with this interpretation, muscle CCO and CS activities were also significantly higher in S compared to RN fish (data not shown), supporting that metal tolerance, longevity, and aerobic capacities may be linked. Although overall longevity was higher in RN fish, it was more negatively affected by metal contamination than in S fish. Metals have been reported in rats to damage mitochondrial membranes and increase their permeability (Garcia *et al.* 2000; Belyaeva and Korotkov 2003; Belyaeva *et al.* 2004). In addition, metallothionein, which is induced by cellular metal accumulation in several species including yellow perch (Giguère *et al.* 2005), has also been reported to increase inner membrane permeability of rat mitochondria (Simpkins *et al.* 1998). We do not know whether mitochondrial permeability is increased in metal-contaminated yellow perch, and if this would lead to compensatory increases in the activity of mitochondrial enzymes such as CS and CCO. However, a study of oxidative stress in RN yellow perch does not allow supporting that metal-contaminated fish are under significant oxidative stress (Giguère *et al.* 2005) although this hypothesis cannot be ruled out based on only one study in one region (RN) and one season (late spring) and that used a narrow set of indicators of metal stress (malondialdehyde and glutathione). Therefore, the relationships reported here between metal contamination, increased mitochondrial enzyme activity and longevity remain to be fully elucidated.

GENERAL CONCLUSIONS

Results from this study reveal that yellow perch are suitable for studying metal effects in wild populations that have experienced life-long metal exposures. However, in order to get at the complex relationships between metal accumulation and fish condition and metabolic capacities, a proper sampling design is required. The sampling design must be established with a focused research question in mind. If one is interested in understanding the relationship between metal accumulation

and metabolic effects or fish condition, then fish size must be considered in the design. Fish from comparative populations must be of the same size, or allometric corrections must be made prior to interpreting results. Seasonal variations in condition and metabolic capacities also necessitate that fish from comparative populations must be sampled within a narrow temporal window in order to minimize significant seasonal effects, and sampling repeated at least once in another season of the same year to capture seasonal variability in the parameters under investigation. In either case, the most important consideration is choosing adequate reference populations. Our results clearly demonstrate that sampling a single reference site is not suitable for drawing conclusions on metal accumulation patterns, fish growth and condition, or metabolic capacities. Suitable reference sites should be in close proximity to contaminated sites to maximize the probability that comparative populations have a similar genetic constituency, and minimize any significant regional effects. Moreover, because of myriad competing influences of non-target variables (*e.g.*, temperature, water chemistry, competition, predation) any attempt to maximize reference-population variability among target variables by sampling more than one reference site will ultimately improve ERA predictions.

Similarly, sampling wild fish populations for a single indicator of metal effects provides little information. To understand the complex, yet subtle, influences of metal contamination on growth patterns or metabolic activities, study designs should consider more than one indicator variable. Fish growth is inextricably linked to fish physiology. Therefore, in any given population, when one variable is insensitive to metal contamination there is a greater likelihood of observing a subtle effect when more than one indicator variable is considered. Multivariate statistical analyses are encouraged for this sort of a study to consider all of the sources of variability (*e.g.*, region, season, lake, fish size, several indicator variables), and their interrelationships, simultaneously.

Finally, this is one of the largest studies of its kind examining the complex relationships among sampling season and region, metal accumulation, fish growth and condition, and metabolic capacities on wild yellow perch. We have provided tissue metal accumulation thresholds for Cd and Cu, and have demonstrated how tissue metabolic capacities are affected in fish that exceed those thresholds. Although metal-contaminated yellow perch do not appear impaired in their early growth, and even demonstrate higher growth rates than reference fish, their longevity is reduced. From this analysis, it appears that fish inhabiting metal-contaminated environments grow fast and die young, perhaps as a result of oxidative damage from up-regulated aerobic metabolic processes.

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