

Seasonal and Regional Variations of Metal Contamination and Condition Indicators in Yellow Perch (*Perca flavescens*) along Two Polymetallic Gradients. I. Factors Influencing Tissue Metal Concentrations

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ABSTRACT

This study examined relationships among water, sediment, diet, and fish tissue metal (Cd, Cu, Ni, Se, and Zn) concentrations in yellow perch from metal gradients in two regions (Sudbury (S), Ontario, and Rouyn-Noranda (RN), Québec, Canada) in two seasons (spring and summer). The objectives of this study were (1) to examine the influences of aqueous and dietary metal contamination on yellow perch liver and kidney metal accumulation; (2) to compare the seasonal and regional variations in gut content and tissue metal concentrations along the two gradients studied; and (3) to investigate the potential of metals for tissue accumulation under conditions of life-long chronic exposure. Our results suggest a greater aqueous than dietary influence on tissue metal concentrations for all metals examined except Cd, where the opposite was observed. Metals did not accumulate in older fish, except for Cd that accumulated with age in RN, but not S, fish. Regional, but also metal-specific differences in metal handling capacities are proposed. Fish from neither region appeared capable of regulating tissue Cd concentrations, but fish from both regions regulated Zn tightly. Sudbury fish appeared better at regulating tissue Cu, Ni, and perhaps also Se concentrations than RN fish, suggesting acclimation or selection for metal tolerance. There were several significant seasonal effects on tissue metal concentrations. However, close examination of the dataset does not allow proposing the presence of a season-linked mechanism explaining these variations, precluding

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a modeling approach and implying that repeat sampling within and among years is required for proper ecological risk assessment.

Key Words: metals, yellow perch, dietary and aqueous influences, seasonality, regional variations.

INTRODUCTION

A large proportion of research on metal effects in fish has been carried out with rainbow trout (*Oncorhynchus mykiss*) or fathead minnows (*Pimephales promelas*) exposed to single metals at relatively high aqueous concentrations under tightly controlled laboratory conditions. Moreover, the exposure route is often assumed to be exclusively waterborne. In contrast, our previous observations clearly indicate that in metal-contaminated lakes around the Sudbury and Rouyn-Noranda areas, yellow perch (*Perca flavescens*) is a dominant species, although other species are also present. Wild yellow perch inhabiting metal-contaminated lakes are exposed to metals from both waterborne and dietary sources (Audet and Couture 2003; Giguère *et al.* 2004). Consequently, it has been difficult to extrapolate effects observed under laboratory conditions back to fish populations inhabiting natural metal-contaminated environments.

To address these shortcomings, we examined metal accumulation and effects in wild yellow perch along two gradients of increasing metal contamination. Yellow perch are ubiquitous across northeastern North America, and are found in freshwater ecosystems ranging in size from the St. Lawrence River and Great Lakes to small streams, rivers, and lakes. As it grows from a small schooling forage fish to a predator that can reach 500 g or more in larger water bodies, yellow perch feed successively on zooplankton, benthic invertebrates, and small fish (Lott *et al.* 1996). Because yellow perch have a high tolerance to metals, they are often the only fish species present in highly metal-contaminated lakes (Couture and Rajotte 2003).

The two metal contamination gradients investigated in this study were located in Sudbury, Ontario, and Rouyn-Noranda, Québec, Canada. Intensive hard rock metal mining and ore smelting has been taking place in the Sudbury region since the late 19th century, resulting in widespread acidification and metal contamination in 7000 lakes over a 17,000 km² industrial “zone of impact” (Keller *et al.* 1992). Although aggressive reclamation efforts have resulted in partial recovery of ecological damage in these lakes (Keller *et al.* 1992, 1999), elevated metal concentrations will likely persist long into the future (Nriagu *et al.* 1998). Cadmium (Cd), copper (Cu), nickel (Ni), and zinc (Zn) are of particular concern. A recent study in Sudbury-area lakes confirmed that metal concentrations remain elevated above background concentrations (i.e., concentrations measured in nearby lakes never affected by mining activities) (Pyle *et al.* 2005). Lakes surrounding the industrial region of Rouyn-Noranda in northwestern Québec have been affected by mining and smelting operations since about 1927 (Couillard *et al.* 1993). Although the extent of industrial contamination is considerably smaller in the Rouyn-Noranda region than in Sudbury, recent studies have demonstrated elevated concentrations of Cd, Cu, and Zn in contaminated lakes relative to background concentrations (Couillard *et al.* 1993; Brodeur *et al.* 1997; Laflamme *et al.* 2000; Sherwood *et al.* 2000; Levesque *et al.* 2002).

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For this research, we collected wild yellow perch from two qualitatively different but long-established metal-contamination gradients in order to conduct three studies, the first examining contamination gradients in lakes and fish (this study), and the second and third investigating metal effects on yellow perch morphometric (Pyle *et al.* 2008) and metabolic (Couture *et al.* 2008) condition indicators, respectively. Several studies have examined these questions at smaller sampling scales, and results are often contradictory. The large sampling and analytical efforts undertaken in this research were designed to provide, in combination with a review of published studies, a better understanding of metal accumulation and effects in wild fish. The general objective of this research is to provide data to improve the relevance of ecological risk assessment (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 objectives of the first study (this article) were: (i) to examine the influences of aqueous and dietary metal contamination on yellow perch liver and kidney metal accumulation; (ii) to compare seasonal and regional variations in gut content and tissue metal concentrations along the two gradients studied; and (iii) to investigate the potential of metals for tissue accumulation under conditions of life-long chronic exposure. The second study (Pyle *et al.* 2008) examines how metals, seasons, and regions affect yellow perch morphometric condition and growth. The last study (Couture *et al.* 2008) investigates the influences of metal contamination, seasons and regions on tissue metabolic capacities.

MATERIALS AND METHODS

Study Lakes

We studied five lakes in the Sudbury area, northeastern Ontario, and five lakes in the Rouyn-Noranda area, northwestern Québec, forming two geographically and geologically distinct polymetallic gradients (Figures 1 and 2). Lakes were selected based on aqueous and sediment metal concentration data from our own previous research for Sudbury and data from other researchers for Rouyn-Noranda (PGC Campbell, INRS-ETE, pers comm). Sudbury area lakes, sampled in 2002, consisted of two contaminated (Hannah (S5) and Whitson (S4)), one intermediate (Crowley (S3)), and two reference lakes (Geneva (S2) and James (S1)). The location of each lake and their relation to Sudbury smelting operations is shown in Figure 1. Rouyn-Noranda area lakes, sampled in 2003, consisted of two reference lakes (Opasatica (RN1) and Ollier (RN2)), one intermediate (Bousquet (RN3)), and two contaminated lakes (Osisko (RN4) and Dufault (RN5)). The location of each lake and their relation to Rouyn-Noranda is shown in Figure 2.

Yellow Perch Sampling and Processing

Yellow perch were sampled from each lake in two seasons. Spring sampling was carried out after spawning, which in the lakes studied occurred in April. In Sudbury (2002), spring sampling was carried out between May 29 and June 16, whereas summer sampling took place between August 17 and September 5. In Rouyn-Noranda

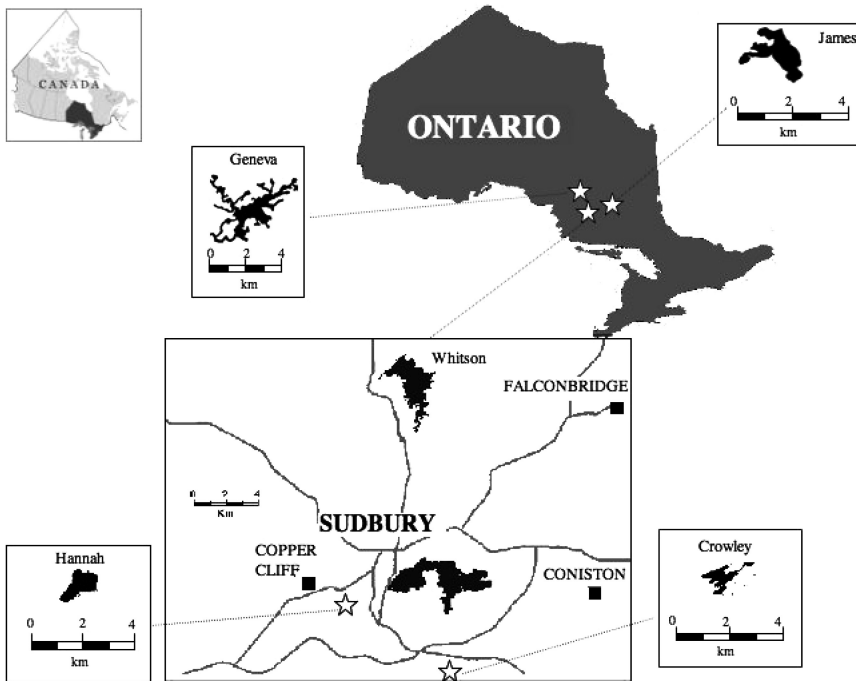


Figure 1. Location of the five Sudbury (S) study lakes (James (S1), Geneva (S2), Crowley (S3), Whitson (S4), and Hannah (S5)) in relation to the two active smelters, Coniston and Falconbridge.

(2003), spring sampling was carried out between May 27 and June 18, whereas summer sampling took place between August 7 and 21. A minimum of 120 yellow perch were sampled from each lake and for each season when possible and returned alive to the laboratory in 40 L Rubbermaid containers filled with lake water and aerated with portable aerator units. Fish were transported back to the laboratory within two hours of capture. This method yielded very low mortality and only live fish were used in the analyses. In order to capture the full size range available, several fishing techniques were used and fishing was carried out on two consecutive days in each lake regardless of fishing success on the first day. Minnow traps were baited with Styrofoam chips, placed in the littoral zone of each study lake and checked daily. Fish were also captured using seine netting and angling also in the littoral zone, and were selected to represent the greatest available range of sizes. Upon arrival at the laboratory, fish were sacrificed by concussion and immediately dissected. Fork length, total weight, and liver weight were recorded for each fish. A sample of white muscle collected above the lateral line and below the dorsal fin as well as half of each liver were removed and stored in liquid nitrogen for tissue enzymatic analysis carried out as another part of this study, while kidneys, gut contents, and half of each liver were stored at -20°C for metal analysis. Gut contents collected included everything found in gut, and were not screened prior to metal analysis. Therefore gut contents may have included zooplankton, benthic invertebrates, terrestrial insects, fish, and

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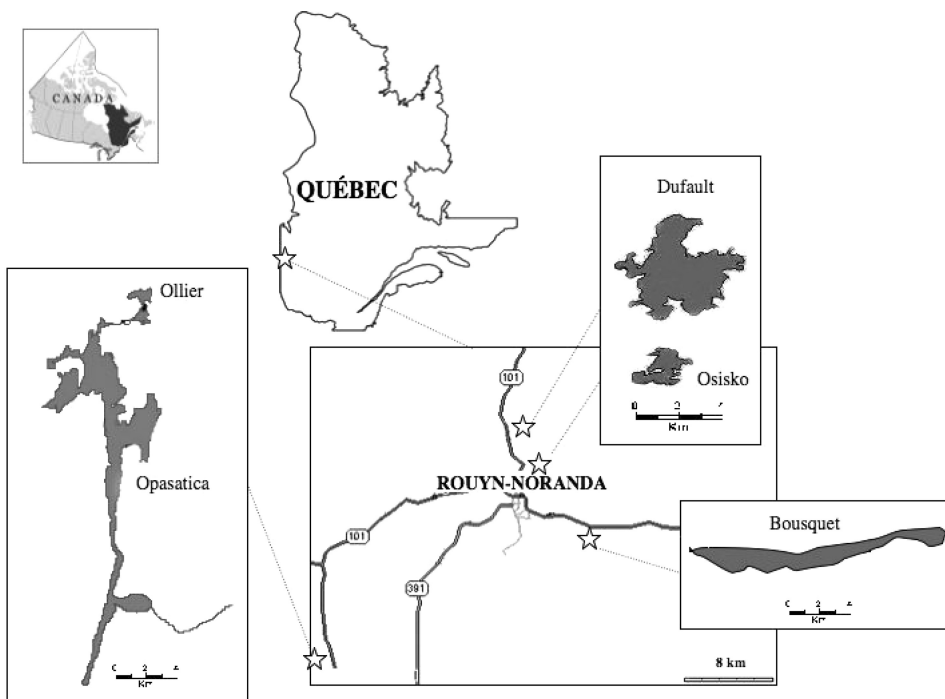


Figure 2. Location of the five Rouyn-Noranda (RN) study lakes (Opasatica (RN1), Ollier (RN2), Bousquet (RN3), Osisko (RN4) and Dufault (RN5)).

sediments contained in the benthic invertebrates. Gut contents were also partly and unevenly digested between samples and therefore, metal concentrations in gut contents were likely often different from concentrations that would be measured in live prey. In addition, some of the metals present in the gut may not come from the prey, but rather from biliary excretion. Metal concentrations measured in gut contents may also not be totally bioavailable, depending on how the metals were stored in the prey. A factor that could influence the trophic transfer of metals is the proportion of metals stored in insoluble fractions, such as metal-rich granules, relative to fractions that may be more bioavailable (*i.e.*, proteins, organelles) which vary among invertebrates (Wallace and Luoma 2003). Therefore, although the term dietary metals was used on occasion in this article to refer to gut content metal concentrations, neither term should be taken as indicating that metal concentrations measured in these samples were either an exact reflection of concentrations in prey or totally available for dietary uptake. Opercular bones were kept for ageing by annular ring count (refer to Pyle *et al.* 2008 for details on the aging method).

Fish Tissue Preparation for ICP-OES (Optical Emission Spectroscopy) and Metal Analysis

Samples of yellow perch liver, kidney, and gut contents for metal analysis were digested as described by Pyle *et al.* (2005). Fish tissue samples were analyzed for metal concentrations by ICP-OES at the Elliot Lake Field Research Station (Elliot

Lake, Ontario), and followed their Quality Assurance and Quality Control (QA/QC) procedures.

Water and Sediment Sampling and Analysis

In order to allow for comparisons with previous research in these geographical areas, water and sediment collection and analysis methods differed in each region. In Sudbury, the method followed previous research in these lakes by researchers at Laurentian University (Sudbury, Ontario), whereas in Rouyn-Noranda lakes we used protocols developed by researchers at the Institut National de la Recherche Scientifique, centre Eau, Terre et Environnement (Université du Québec) for those lakes. In Sudbury, water samples were taken from each study lake at 3 depths (1, 3, and 5 m) on the day of fish capture for each sampling event (season) from the most successful fish collection sites using a Van Dorn bottle. Because no depth- or season-related trends were observed, mean values are presented in this study. Containers were rinsed with lake water three times, filled to capacity (*i.e.*, no head space), and placed on ice for transport back to the laboratory. Sediment samples were collected from the same locations as water samples using an Eckman grab, which collects approximately the top 5 cm of sediment. Sediments were mixed, one sample bottle was filled with the mixed sediment and returned to the laboratory on ice. Both water and sediment samples were refrigerated upon return to the laboratory. Water samples (150 mL) for metal analysis were acidified to a pH of 2 by addition of 1 N trace metal grade nitric acid. Water temperature and pH (using a ThermoOrion portable pH meter, Fisher Scientific) were recorded on site during water sampling. Sudbury water and sediment samples for metal analysis were prepared as previously described (Pyle *et al.* 2005). Metal analyses were carried out by Testmark Laboratories Ltd. and followed their QA/QC procedures. Dissolved organic carbon (DOC) was determined spectrophotometrically (Varian Cary 100 UV/Vis spectrophotometer) according to the method of Beauclerc and Gunn (2001).

In Rouyn-Noranda, water samples were collected at the time of fish sampling by *in situ* dialysis. The dialysis sampler consisted of eight adjacent 4-mL cells separated from the lake water by a polysulfone membrane (0.2 μm) (Pall Gelman Sciences, HT-200, Ville St-Laurent, QC). Samplers were fixed to a plastic rod approximately 30 cm above the sediment. After an equilibration time of 3 d with the surrounding waters, the samplers were recovered, sealed, and sent on ice to the INRS-ETE in Québec City within 24 h of retrieval. Ambient pH was measured at a depth of 0.5 m, near the dialysis samplers. Sub-samples of water were removed from the samplers for analysis of major cations (Ca, Mg), total dissolved trace metals (Cd, Cu, Ni, and Zn) and dissolved organic carbon. These constituents were measured at the INRS-ETE as follows: metals and major cations by ICP-AES (Varian Vista AX) and organic carbon by a total organic carbon analyzer (Schimazu, TOC-5000A). Sediments of Rouyn-Noranda lakes were collected as for Sudbury lakes, but were analyzed at the INRS-ETE by ICP-AES. All Rouyn-Noranda water quality and sediment metal measurements followed INRS-ETE QA/QC procedures. For both Sudbury and Rouyn-Noranda water samples, water hardness was calculated as:

$$\text{Hardness (mg/L)} = ([\text{Ca}] \times 2.497) + ([\text{Mg}] \times 4.118)$$

where [Ca] and [Mg] are the aqueous concentrations of Ca and Mg, in mg/L.

Calculations and Statistics

Relationships between water or sediment metal concentrations and concentrations of the same metals in gut contents (GC), liver, and kidney, as well as relationships between fish age and GC, liver or kidney metal concentrations were examined using linear regression on log values. Regional (Sudbury and Rouyn-Noranda) and seasonal (spring and summer) effects on tissue (kidney, liver, and gut contents) metal (Cd, Cu, Ni, Se, and Zn) concentrations were analyzed using a multivariate analysis of variance (MANOVA). All data were \log_{10} -transformed in order to improve parametric assumptions. Effects were considered significant when $p \leq .05$. In cases where the statistical interaction (region \times season) was significant, subsequent analyses focused on the interaction rather than the main effects regardless of whether or not the main effects were significant (Zar 1999). Significant effects identified by the MANOVA (using a transformed Wilk's Λ to yield an approximated F statistic) were subsequently analyzed using ANOVA followed by a Tukey's Honestly Significant Difference (HSD) test using least squares means corrected for the multivariate model. A canonical correlation analysis (CCA) is a purely descriptive analysis (not an inferential analysis, such as ANOVA), which is used to visualize complex relationships among several variables. Points on the ordination (*i.e.*, the graphical representation of the data) are centroids calculated as an optimal linear combination of all variables in the analysis. Points that plot close to one another are most similar with respect to all of the metal concentrations used to generate the ordination. Those that plot further away from each other are more dissimilar. A CCA was conducted as part of the MANOVA, which allowed for the characterization of complex relationships among tissue metal concentrations and seasonal and regional effects. Mean scores from the CCA were used to establish relationships (via Pearson correlation analysis) with water quality data, including pH, hardness, dissolved organic carbon (DOC), and dissolved metals (except dissolved Se, which was below detection in most lake water samples). All analyses were performed using the statistical software package JMP 5.1.

RESULTS

Metal Concentrations in Water and Sediments along the Rouyn-Noranda (RN) and Sudbury (S) Gradients

Water pH was circumneutral (pH range 6.6–7.8, median pH 7.3), with RN lakes having slightly higher pH than S lakes (Table 1). Water hardness was typically low in all lakes and approximately the same in both gradients, ranging from 10 to 100 mg/L as CaCO_3 . Dissolved organic carbon (DOC) varied by less than 4-fold among study lakes, ranging from 3 to 11 mg/L. Metal contamination gradients in either water or sediment were unrelated to water pH, DOC, or hardness.

Lake rankings (Figures 1 and 2) were used to characterize each contamination gradient based on water Cd, Cu, Ni, and Zn concentrations (Table 1). The first two ranks represented clean (reference) lakes, the third was classified as intermediate, and ranks 4 and 5 lakes could be considered as similarly contaminated. Water Cd concentrations increased along the RN gradient, and were also higher in lakes S3,

Table 1. Water quality parameters and sediment metal concentrations in the 10 study lakes.

| Lake | Water | | | | | | | Sediment | | | | |
|------|-------|----------|-------|------|------|-------|------|----------|-------|------|------|------|
| | pH | Hardness | DOC | Cd | Cu | Ni | Zn | Cd | Cu | Ni | Se | Zn |
| DL | — | — | — | 0.01 | 0.5 | 0.2 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| RN1 | 7.66 | 50.8 | 6.95 | 0.01 | 3.0 | <0.2 | 0.3 | 1.62 | 46 | 40 | <0.5 | 110 |
| RN2 | 7.68 | 49.6 | 5.90 | 0.03 | 4.2 | <0.2 | 4.8 | 0.70 | 28 | 19 | <0.5 | 86 |
| RN3 | 6.91 | 14.5 | 11.12 | 0.06 | 3.3 | 1.2 | 3.3 | 0.18 | 9 | 12 | <0.5 | 20 |
| RN4 | 7.81 | 84.8 | 3.00 | 0.16 | 5.7 | 3.5 | 13.3 | 96.42 | 12111 | 104 | 46.4 | 9263 |
| RN5 | 7.65 | 56.5 | 4.85 | 0.62 | 14.3 | <0.2 | 66.4 | 26.85 | 1965 | 30 | 8.9 | 2728 |
| S1 | 6.78 | 98.5 | 11.23 | 0.10 | 1.6 | 0.9 | 13.9 | 2.34 | 23 | 15 | 31.8 | 221 |
| S2 | 6.55 | 11.0 | 7.21 | 0.02 | 2.0 | 4.5 | 7.8 | 1.60 | 49 | 99 | 22.6 | 138 |
| S3 | 7.16 | 9.8 | 4.24 | 0.17 | 15.6 | 73.2 | 16.3 | 1.92 | 568 | 550 | 24.6 | 71 |
| S4 | 7.36 | 25.3 | 6.62 | 0.52 | 20.7 | 149.1 | 22.8 | 3.83 | 973 | 1384 | 32.0 | 193 |
| S5 | 7.03 | 42.7 | 4.88 | 0.45 | 24.4 | 174.8 | 13.6 | 2.67 | 1051 | 1093 | 26.4 | 124 |

Hardness and DOC are expressed in mg/L; metal concentrations are in $\mu\text{g/L}$ (water) or $\mu\text{g/g}$ dry weight (sediments). $n = 1$ to 6. DL = Detection Limit. Water Se was omitted from the analysis because it was only detected in S4 and S5 (1.0 and 2.6 $\mu\text{g/L}$, respectively; DL = 1 $\mu\text{g/L}$).

S4, and S5 compared to lakes S1 and S2. Waterborne Cu concentrations varied from 2 to 24 $\mu\text{g/L}$, and generally increased with increasing rank. Lakes S3 to S5 had the highest aqueous Cu concentrations. Waterborne Ni was detected in only two of the five lakes of the RN gradient (RN3 and RN4), and in all of the lakes in the S gradient. Nickel concentration tended to increase with increasing rank, and ranged from 1 to 175 $\mu\text{g/L}$. Waterborne Zn increased with increasing rank, except for RN3, S2, and S5, and ranged from 0.3 to 66 $\mu\text{g/L}$. Although S lakes generally demonstrated slightly higher waterborne Zn concentrations than RN lakes, the maximum water Zn concentration was measured in RN5.

Sediment metal concentrations generally tended to increase with increasing rank (Table 1). Lakes RN4 and RN5 had higher sediment Cd, Cu, and Zn than any other lake. Lakes S3-S5 demonstrated the highest sediment Ni concentrations, which were approximately five to ten times higher than the highest sediment Ni concentrations recorded in RN lakes. Sediment Se concentrations were higher in S lakes than in RN lakes, except for RN4, which had the highest sediment Se concentration among all ten study lakes.

Influence of Water and Sediment Metal Contamination on Gut Content Metal Concentrations

Using pooled mean values from RN and S lakes, water, but not sediment, Cd concentration correlated with GC Cd concentration (Table 2). Water and sediment Cu and Ni concentrations were correlated with GC concentrations of the same metals. Sediment but not water Zn concentration was correlated to GC Zn concentration. There was no relationship between GC Se concentration and sediment Se concentration.

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Table 2. Coefficients of determination (r^2 ; p is given in parentheses) describing linear relationships between mean metal concentrations in gut contents (GC) and water and sediment metal concentrations.

| | GC Cd | GC Cu | GC Ni | GC Se | GC Zn |
|----------|-------------|---------------|---------------|-------|--------------|
| Water | 0.41 (0.04) | 0.53 (0.02) | 0.95 (0.0002) | NC | NS |
| Sediment | NS | 0.91 (0.0001) | 0.74 (0.001) | NS | 0.59 (0.009) |

All data were log-transformed (log/log). $n = 10$ in all cases except for GC versus water Ni ($n = 7$); NS, not significant ($p > .05$); NC, not calculated, because of missing water Se values. All significant relationships were positive.

Influence of Diet and Water Metal Contamination on Liver and Kidney Metal Concentrations

Using individual data, both liver and kidney Cd were strongly correlated to GC Cd ($n = 305$ – 349 , Table 3). Gut content Cu was also strongly related to liver Cu. As indicated by lower correlation coefficients, a weaker influence of GC metal content was observed for kidney Cu, as well as liver and kidney Ni. Gut contents Se and Zn exhibited weak relationships, if any, with liver and kidney concentrations of these metals.

Relationships between log-transformed mean values of GC metal concentrations for all fish pooled within each lake were also compared to log-transformed mean liver and kidney concentrations of the same metals ($n = 10$) and reflected the relative strengths of the relationships using individual points (Table 3). Mean liver and kidney Cd concentrations were strongly positively correlated with GC Cd ($r^2 = 0.89$, $p < .0001$ and $r^2 = 0.74$, $p = .002$, respectively). Liver and kidney Cu concentrations were also positively correlated with GC Cu ($r^2 = 0.62$, $p = .007$ and $r^2 = 0.65$, $p = .005$, respectively). Neither GC Ni, Se, or Zn correlated with tissue concentrations of the same metals.

Correlations of tissue versus water metal concentrations were examined using mean values for each lake ($n = 10$). Liver and kidney Cd concentrations were strongly positively correlated with water Cd ($r^2 = 0.56$, $p = .009$ and $r^2 = 0.63$, $p < .006$, respectively). Liver but not kidney Cu concentration was also correlated with water Cu ($r^2 = 0.68$, $p = .003$). Neither water Ni, nor Zn correlated with tissue concentrations of the same metals.

Table 3. Coefficients of determination (r^2 ; p is given in parentheses) describing linear relationships between metal concentrations in gut contents (GC) and those of liver and kidney.

| | GC Cd | GC Cu | GC Ni | GC Se | GC Zn |
|--------|---------------|---------------|---------------|-------------|--------------|
| Liver | 0.34 (0.0001) | 0.30 (0.0001) | 0.09 (0.0001) | 0.01 (0.05) | 0.03 (0.005) |
| Kidney | 0.35 (0.0001) | 0.12 (0.0001) | 0.14 (0.0001) | NS | 0.03 (0.005) |

All data were log-transformed (log/log). $n = 305$ – 349 ; NS, not significant ($p > .05$). All significant relationships were positive.

Concentrations of all metals examined within individual tissues were positively correlated with each other (data not shown). However, in general tissue concentrations of Ni and Se were not as strongly correlated with other metals as Cd, Cu, and Zn together. Kidney Cd was correlated with liver Cd concentration in all fish pooled. This was also the case for Cu, Ni, and Se but not for Zn. The differences in tissue Zn concentrations between liver and kidney were more important than observed for other metals but the concentrations showed little variability compared to other metals, and were not correlated between tissues.

Relationships between Gut Contents Metal Concentrations and Age

Because yellow perch feed on plankton as juveniles, and switch to benthos and in some cases to fish as they grow, we examined whether the diet of yellow perch changed in metal concentration in older fish. Changes in dietary (GC) metal concentration associated with aging were examined in fish from individual lakes for each season (Table 4). Dietary Cd concentrations decreased systematically in older fish from all RN lakes examined, clean or contaminated during summer, but only in RN1 and RN4 fish in spring. This phenomenon was not observed in Sudbury,

Table 4. Significant linear regressions between age and log gut content metal concentrations for each metal, lake and season.

| Response variable | Lake | Intercept | Slope | r ² | <i>p</i> | <i>n</i> |
|-------------------|-------|-----------|--------|----------------|----------|----------|
| Gut content Cd | | | | | | |
| Spring | RN1 | 1.119 | -0.113 | 0.222 | .027 | 22 |
| | RN4 | 1.705 | -0.224 | 0.538 | <.001 | 19 |
| Summer | S2 | 0.248 | -0.215 | 0.219 | .028 | 22 |
| | RN1 | 0.669 | -0.132 | 0.402 | .003 | 20 |
| | RN2 | 0.711 | -0.133 | 0.440 | <.001 | 24 |
| | RN3 | 0.660 | -0.075 | 0.259 | .009 | 25 |
| | RN4 | 0.987 | -0.044 | 0.237 | .035 | 19 |
| RN5 | 1.566 | -0.103 | 0.337 | .002 | 26 | |
| Gut content Cu | | | | | | |
| Spring | S1 | -0.883 | 0.336 | 0.511 | <.001 | 28 |
| | S2 | -1.651 | 0.469 | 0.544 | .006 | 12 |
| Summer | RN2 | 1.713 | -0.106 | 0.312 | .005 | 24 |
| Gut content Se | | | | | | |
| Spring | RN2 | 1.219 | -0.039 | 0.217 | .025 | 23 |
| | S3 | 1.732 | -0.077 | 0.315 | .001 | 30 |
| | S5 | 0.067 | 0.202 | 0.527 | <.001 | 18 |
| Summer | RN1 | 1.382 | -0.070 | 0.301 | .010 | 21 |
| Gut content Zn | | | | | | |
| Spring | RN2 | 2.056 | 0.056 | 0.186 | .040 | 23 |
| | RN4 | 2.939 | -0.085 | 0.204 | .046 | 20 |
| | S1 | 1.709 | 0.101 | 0.283 | .006 | 23 |
| | S4 | 2.436 | -0.192 | 0.235 | .049 | 17 |
| Summer | RN3 | 2.378 | -0.050 | 0.405 | <.001 | 25 |

Only significant (*p* < .05) relationships are presented. Data from S1 to S5 were excluded from the analysis in summer due to low sample size (*n* ≤ 12).

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with the exception of S2 in spring and S5 in summer. Dietary Cu generally did not change with fish size. However, while dietary Cu decreased in older RN2 fish during summer, there were significant increases in dietary Cu with age in S1 and S2 fish in spring only. Dietary Ni was unrelated to age in all spring-captured fish and in all RN fish. Finally, dietary Se and Zn concentrations often decreased with age in fish from both regions, but on occasion the opposite trend was observed (S5 in spring for Se; RN2, and S1 in spring and S5 in summer for Zn).

Interestingly, if a significant relationship was observed between age and gut content Se or Zn concentration in one season, then no trend was observed during the other season. Overall, season clearly affected the relationships between age and diet metal contamination, because decreasing diet contamination in older fish was much more commonly observed in summer than in spring. These relationships also differed between regions. Specifically, a decrease of Cd in the diet of larger fish was much more common in RN than in S fish, although similar decreases of dietary Ni were only observed in some S fish. Finally, we examined whether age-related changes in diet metal concentrations were more frequent in highly contaminated lakes compared to clean lakes. Surprisingly, age-related changes in dietary metal content occurred just as frequently in clean lakes (Ranks 1 and 2; 14 significant relationships) than in metal-contaminated lakes (Ranks 4 and 5; 11 significant relationships).

Liver and Kidney Metal Accumulation with Age

The relationships between the duration of chronic exposure (age, in years) and tissue metal concentrations were examined in each region and season and are reported in Tables 5 (liver) and 6 (kidney). Significant relationships between age and tissue metal concentrations were just as common in liver as in kidney, but in general, relationships were stronger in kidney. Although in several instances a significant relationship in a tissue was not significant in the other, in only three cases there were opposite trends between tissues (Cd in spring in RN4 fish; Se in summer in RN5 fish; Zn in spring in RN1 fish). There was also good agreement between seasons for these relationships, with only one disagreement for liver Cd in RN4 fish, which decreased with age in spring but increased in summer. Low sample size for tissue metal analysis in Sudbury fish during summer prevented us from examining relationships with age in many of these fish. In spring, the majority (76%) of relationships between age and tissue metal concentrations were found in RN fish.

Overall, liver and kidney Cd tended to increase with age whereas Cu, Ni, Se and Zn more typically decreased as fish aged (Tables 5 and 6). However, there were important regional differences: Significant relationships between tissue metal concentrations and age were more typical of RN than S fish. Seasons also affected the strength (and sometimes the direction) of these relationships. Specifically, Cd accumulation with age was observed in both liver and kidney of all RN fish in at least one season (except for liver in RN4 where a decrease was reported in the spring), regardless of the level of metal contamination. In contrast, a similar increase of tissue Cd with age was not observed in any Sudbury fish. No consistent trend of hepatic Cu accumulation with age was observed in fish from any region. Kidney Cu concentration however decreased with age in fish from almost all RN lakes in

Table 5. Significant linear regressions between age and log liver metal concentrations for each metal, lake, and season.

| Response variable | Lake | Intercept | Slope | r ² | <i>p</i> | <i>n</i> |
|-------------------|------|-----------|--------|----------------|----------|----------|
| Liver Cd | | | | | | |
| Spring | RN1 | 0.415 | 0.038 | 0.226 | .022 | 23 |
| | RN2 | 0.307 | 0.051 | 0.273 | .013 | 22 |
| Summer | RN4 | 1.262 | -0.046 | 0.221 | .027 | 22 |
| | RN1 | 0.034 | 0.098 | 0.242 | .020 | 22 |
| | RN2 | 0.338 | 0.053 | 0.238 | .012 | 26 |
| | RN3 | 0.734 | 0.075 | 0.469 | <.001 | 25 |
| | RN4 | 0.921 | 0.023 | 0.214 | .026 | 23 |
| | RN5 | 1.334 | 0.039 | 0.180 | .028 | 27 |
| Liver Cu | | | | | | |
| Spring | RN1 | 1.561 | -0.052 | 0.376 | .002 | 23 |
| | RN3 | 1.481 | -0.065 | 0.484 | <.001 | 22 |
| | S5 | 1.374 | 0.238 | 0.534 | <.001 | 18 |
| Summer | RN2 | 0.669 | 0.126 | 0.323 | .002 | 26 |
| Liver Ni | | | | | | |
| Spring | RN1 | 0.508 | -0.179 | 0.571 | <.001 | 23 |
| | RN2 | 0.030 | -0.139 | 0.425 | .003 | 18 |
| | RN3 | 1.722 | -0.257 | 0.478 | <.001 | 21 |
| | RN4 | 1.669 | -0.318 | 0.711 | <.001 | 22 |
| | RN5 | 1.503 | -0.292 | 0.412 | <.001 | 24 |
| | S1 | 0.664 | -0.253 | 0.696 | <.001 | 32 |
| | S2 | 1.825 | -0.457 | 0.600 | .001 | 14 |
| Summer | RN2 | 0.524 | -0.310 | 0.899 | <.001 | 26 |
| | RN3 | 0.048 | -0.208 | 0.730 | <.001 | 21 |
| | RN5 | 0.232 | -0.165 | 0.356 | .002 | 25 |
| Liver Se | | | | | | |
| Spring | RN3 | 1.461 | -0.058 | 0.606 | <.001 | 22 |
| | RN4 | 1.632 | -0.053 | 0.486 | <.001 | 22 |
| | RN5 | 1.517 | -0.033 | 0.343 | .003 | 24 |
| Summer | RN1 | 0.893 | 0.074 | 0.195 | .024 | 26 |
| | RN2 | 1.288 | -0.061 | 0.377 | <.001 | 26 |
| | RN3 | 1.435 | -0.107 | 0.614 | <.001 | 25 |
| | RN5 | 1.341 | -0.031 | 0.241 | .009 | 27 |
| Liver Zn | | | | | | |
| Spring | RN1 | 1.971 | 0.022 | 0.223 | .023 | 23 |
| | RN2 | 2.105 | -0.047 | 0.255 | .023 | 20 |
| | RN3 | 2.540 | -0.056 | 0.384 | .002 | 22 |
| | RN4 | 2.759 | -0.098 | 0.473 | <.001 | 22 |
| Summer | RN5 | 2.311 | -0.026 | 0.271 | .005 | 27 |

Data from S1 to S5 were excluded from the analysis in summer due to low sample size (*n* ≤ 12).

Only significant (*p* < .05) relationships are presented.

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Table 6. Significant linear regressions between age and log kidney metal concentrations for each metal, lake and season.

| Response variable | Lake | Intercept | Slope | r ² | p | n |
|-------------------|------|-----------|--------|----------------|-------|----|
| Kidney Cd | | | | | | |
| Spring | RN1 | 0.373 | 0.067 | 0.373 | .004 | 20 |
| | RN2 | 0.279 | 0.150 | 0.723 | <.001 | 23 |
| | RN3 | 1.024 | 0.057 | 0.384 | .002 | 22 |
| | RN4 | 1.020 | 0.055 | 0.532 | <.001 | 22 |
| | S2 | 1.218 | -0.137 | 0.241 | .024 | 21 |
| Summer | RN1 | 0.307 | 0.099 | 0.220 | .028 | 22 |
| | RN3 | 0.856 | 0.106 | 0.650 | <.001 | 24 |
| | RN5 | 1.392 | 0.098 | 0.426 | <.001 | 27 |
| Kidney Cu | | | | | | |
| Spring | RN1 | 1.363 | -0.072 | 0.792 | <.001 | 23 |
| | RN2 | 0.424 | -0.141 | 0.451 | <.001 | 23 |
| | RN3 | 1.504 | -0.061 | 0.785 | <.001 | 22 |
| | RN4 | 1.878 | -0.101 | 0.780 | <.001 | 18 |
| | S4 | 1.040 | -0.261 | 0.463 | .015 | 12 |
| Summer | RN1 | 1.088 | -0.053 | 0.314 | .003 | 26 |
| Kidney Ni | | | | | | |
| Spring | RN1 | 1.393 | -0.122 | 0.802 | <.001 | 16 |
| | RN2 | 1.472 | -0.120 | 0.302 | .007 | 23 |
| | RN5 | 0.911 | -0.174 | 0.231 | .017 | 24 |
| | S1 | 0.234 | -0.108 | 0.337 | .001 | 29 |
| | S5 | -0.015 | 0.135 | 0.247 | .019 | 22 |
| Summer | RN1 | 0.450 | -0.094 | 0.189 | .043 | 22 |
| | RN3 | 0.283 | -0.111 | 0.182 | .048 | 22 |
| Kidney Se | | | | | | |
| Spring | RN1 | 1.511 | -0.081 | 0.343 | .003 | 23 |
| | RN2 | 1.243 | -0.045 | 0.292 | .008 | 23 |
| | RN4 | 1.512 | -0.052 | 0.607 | <.001 | 22 |
| | S5 | 0.556 | 0.097 | 0.211 | .032 | 22 |
| Summer | RN5 | 0.779 | 0.106 | 0.841 | <.001 | 23 |
| Kidney Zn | | | | | | |
| Spring | RN1 | 3.231 | -0.058 | 0.523 | <.001 | 23 |
| | S1 | 2.248 | 0.067 | 0.229 | .009 | 29 |
| | S5 | 2.142 | 0.154 | 0.668 | <.001 | 19 |
| Summer | RN2 | 3.028 | -0.034 | 0.336 | .002 | 26 |
| | RN5 | 3.264 | -0.046 | 0.411 | <.001 | 27 |

Only significant ($p < .05$) relationships are presented. Data from S1 to S5 were excluded from the analysis in summer due to low sample size ($n \leq 12$).

the spring. Liver Ni concentration decreased strongly and consistently with age in the spring in all fish examined from RN and in S1 and S2 fish, where the lowest aqueous and sediment Ni concentrations were measured. In contrast, in S5, where environmental Ni was highest, kidney Ni concentration increased in the spring with age. By summer, most of these relationships had disappeared or weakened. For Se, there was a general trend of decrease with age in RN, but not in S fish, although

in three instances tissue Se concentration increased with age (RN1 in summer for liver, and RN5 in summer and S5 in spring for kidney). There was no evidence of Zn accumulation with age in any fish examined, except in liver for RN1 and in kidney for S1 and S5, in the spring. Instead, liver and to a lesser extent kidney Zn decreased in larger fish, but mostly in RN fish and in the spring.

Regional and Seasonal Influences on Gut Content (GC) and Tissue Metal Concentrations

A MANOVA analysis was conducted in order to simplify the interpretation of seasonal and regional influences on GC and tissue metal concentrations in this large and complex dataset. Mean values and univariate statistics for each of the variables measured and included in the model (GC, liver, and kidney concentrations of Cd, Cu, Ni, Se, and Zn for each season and lake) are available from the authors on request. Complex relationships among region, season, and tissue metal concentration are depicted in the ordination displayed in Figure 3. In this ordination, circles represent 95% confidence intervals around centroids (points) for regional and seasonal effects, and vectors represent the magnitude (represented by vector length) and direction of canonical correlations of tissue metals with the two displayed axes. Objects that plot near to one another are more similar with respect to an optimal linear combination of tissue metal concentrations than those that plot further away. Regional effects on tissue metal concentrations appear to separate along Axis 1. Fish from Rouyn-Noranda lakes plot high on Axis 1 relative to those from Sudbury. Seasonal effects appear to discriminate along Axis 2, with fish caught in the spring plotting at higher magnitudes on Axis 2 than those caught in the summer. In general, seasonal and regional effects on tissue metal concentrations were less variable in Rouyn-Noranda fish than in Sudbury fish.

The strongest vector in Figure 3 was for dietary Cd, which showed a high degree of positive relationship with Axis 1. Dietary Se and Zn also correlated positively with Axis 1 (with a smaller degree of association with Axis 2), which is in the direction of most RN objects. In contrast, dietary Cu and Ni vectors were negatively associated with Axis 1 and radiated in the direction of most S objects.

To help resolve the identity of the ordination axes of Figure 3, Pearson correlation coefficients (r) were calculated between mean ordination scores for each axis in each lake and water (and sediment) quality variables. Axis 1 was significantly and positively correlated with pH ($r = 0.71$; $p = .02$). Axis 2 was significantly and positively correlated with dissolved Cd ($r = 0.79$, $p = .006$), Cu ($r = .68$, $p = .03$), and Zn ($r = 0.64$, $p = .04$). No water quality variables were negatively associated with either of the ordination axes, and no sediment quality variables were significantly correlated with either of the ordination axes ($p > .05$). Eigenvector (λ) loadings for each tissue metal used in establishing the CCA were also used to establish the identity of the two ordination axes (Table 7). Dietary Cd ($\lambda = 0.122$) and Ni ($\lambda = -0.070$) had the highest and lowest loadings on Axis 1, respectively, whereas liver Se ($\lambda = 0.075$) and kidney Cu ($\lambda = -0.114$) had the highest and lowest loadings on Axis 2, respectively. Taken together, Axis 1 represents increasing pH and dietary Cd and decreasing dietary Ni, whereas Axis 2 represents increasing liver Se and dissolved metal concentrations, especially Cd, Cu, and Zn, and decreasing kidney Cu.

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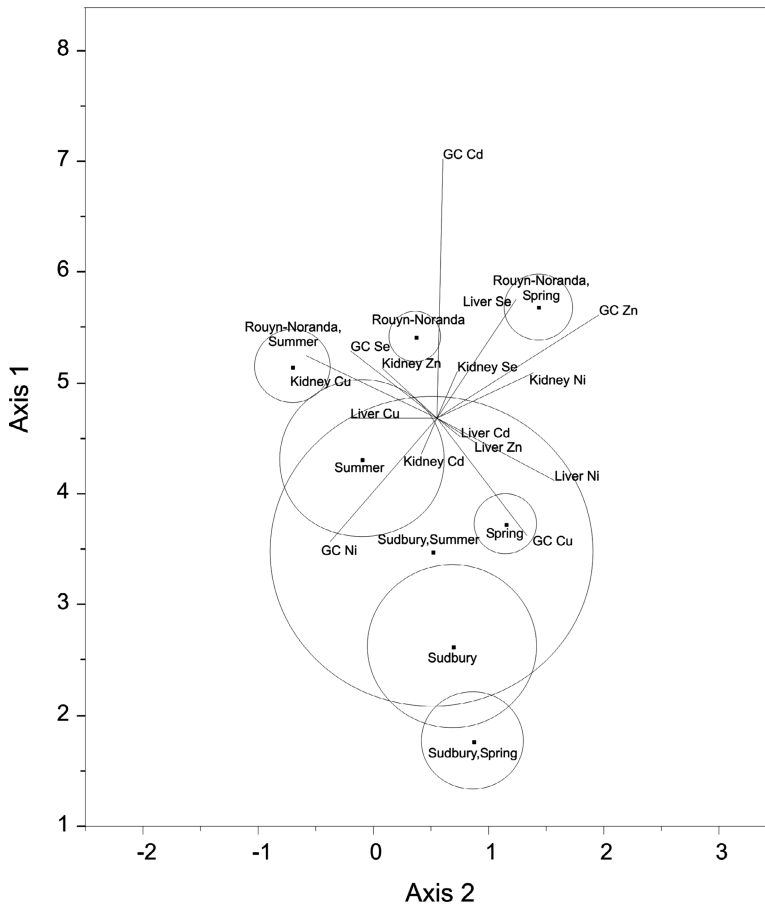


Figure 3. Canonical correlation ordination depicting the complex relationships among tissue (kidney, gut contents [GC], and liver) metal (Cd, Cu, Ni, Se, and Zn) concentrations in wild yellow perch sampled over two seasons (spring and summer) from two regions (Rouyn-Noranda and Sudbury). Objects plotting close to one another are most similar based on an optimal linear combination of tissue metal concentrations than those that plot further away from each other. Circles represent 95% confidence intervals around centroids (points) associated with seasonal or regional effects. Vectors of tissue metals radiate from a grand mean in the direction of maximum canonical correlation; vector length corresponds with the magnitude of correlation.

The whole MANOVA model indicated that significant effects on tissue metal concentrations were present (Wilk's $\Lambda = 0.14$, $p < .0001$), including significant regional ($F_{15,185} = 7.52$, $p < .0001$) and seasonal ($F_{15,185} = 2.71$, $p = .0009$) effects, as well as region-by-season interactions ($F_{15,185} = 2.88$, $p = 0.0004$). Subsequent two-way ANOVAs, summarized in Table 8, were conducted for metals in each tissue.

Table 7. Eigenvector loadings of tissue metal concentrations for the first two canonical axes from the ordination presented in Figure 3. Highest and lowest magnitude eigenvectors for each axis are depicted in boldface type.

| Variable | Axis 1 | Axis 2 |
|-----------------|---------------|---------------|
| Kidney Cd | -0.028 | -0.012 |
| Kidney Cu | 0.057 | -0.114 |
| Kidney Ni | 0.035 | 0.072 |
| Kidney Se | 0.047 | 0.020 |
| Kidney Zn | 0.052 | -0.055 |
| Gut contents Cd | 0.122 | 0.003 |
| Gut contents Cu | -0.057 | 0.042 |
| Gut contents Ni | -0.070 | -0.058 |
| Gut contents Se | 0.043 | -0.054 |
| Gut contents Zn | 0.039 | 0.059 |
| Liver Cd | -0.015 | 0.018 |
| Liver Cu | 0.000 | -0.054 |
| Liver Ni | -0.040 | 0.072 |
| Liver Se | 0.116 | 0.075 |
| Liver Zn | -0.037 | 0.055 |

Dietary Cd concentrations were significantly higher in RN fish during the spring than in the summer (Figure 4a). However, the lowest dietary Cd concentrations were in fish caught during the spring in S lakes, which were significantly lower than dietary Cd concentrations measured in S fish during the summer (Figure 4a). Dietary Cu concentrations were significantly higher in RN fish caught in the spring relative to S fish caught in the spring (Figure 4b). Dietary Ni concentrations were highest in fish caught from S lakes during the summer, relative to any other fish (Figure 4c). Dietary Se concentrations were significantly lower in fish caught from S lakes during the spring than any other group (Figure 4d). The highest dietary Zn concentrations were measured in fish collected during the spring in RN lakes, which were significantly higher than in fish collected in the summer in the same region or in spring in S fish (Figure 4e). In fish from S lakes, dietary Zn concentrations did not vary seasonally.

Kidney Cd was the only tissue metal that demonstrated no significant seasonal or regional effect or significant region-by-season interaction ($p > .05$; Table 8). Both kidney Se and Zn varied by region but not by season, and yielded no significant interaction (Table 8). In both cases, kidney Se and Zn were significantly higher in fish collected from RN lakes than in those from S lakes (Figure 5). Every other tissue metal examined yielded a significant region-by-season interaction (Table 8).

Kidney Cu concentrations in fish collected from S lakes in the spring were approximately five times lower than values in fish from Rouyn-Noranda (in either season) or in S fish collected in the summer (Figure 6a). Kidney Ni was approximately four times higher in fish collected from RN lakes in the spring than in fish collected in the summer or in Sudbury-area lakes (Figure 6b).

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Table 8. Statistical summary of main effects of region and season and the interaction between region and season on wild yellow perch metal concentrations in kidney, gut contents, and liver.

| Tissue | Metal | Region | | Season | | Region × season | |
|--------------|-------|--------|----------|----------|----------|-----------------|----------|
| | | F* | <i>p</i> | <i>F</i> | <i>p</i> | <i>F</i> | <i>p</i> |
| Kidney | Cd | 3.55 | .06 | 0.53 | .47 | 1.79 | .18 |
| | Cu | 13.97 | .0002 | 9.84 | .002 | 6.65 | .01 |
| | Ni | 0.10 | .75 | 0.40 | .53 | 9.05 | .003 |
| | Se | 12.42 | .0005 | 0.0002 | .99 | 2.51 | .11 |
| | Zn | 6.04 | .01 | 1.14 | .29 | 0.63 | .43 |
| Gut contents | Cd | 9.49 | .002 | 4.19 | .04 | 18.69 | <.0001 |
| | Cu | 0.001 | .97 | 2.95 | .09 | 10.27 | .002 |
| | Ni | 3.61 | .06 | 7.11 | .008 | 9.19 | .003 |
| | Se | 4.49 | .04 | 6.98 | .009 | 5.07 | .03 |
| | Zn | 6.51 | .01 | 0.18 | .67 | 5.02 | .03 |
| Liver | Cd | 1.34 | .25 | 4.34 | .04 | 9.91 | .002 |
| | Cu | 0.75 | .39 | 5.43 | .02 | 5.60 | .02 |
| | Ni | 1.08 | .30 | 0.17 | .68 | 13.49 | .0003 |
| | Se | 13.55 | .0003 | 3.35 | .07 | 13.53 | .0003 |
| | Zn | 0.01 | .91 | 0.17 | .68 | 5.64 | .02 |

Statistical output was generated from two-way comparisons on log₁₀-transformed data using a MANOVA platform. Effects were considered significant when *p* < .05. *All *F* values reported in this table are transformed Wilk's Λ and have been corrected for the entire MANOVA model. Numerator and denominator degrees of freedom were 1 and 199, respectively.

Liver Cd and Cu concentrations were significantly lower in fish collected in the spring from S lakes relative to fish collected in the other region or season (Figures 7a, b). In RN lakes, fish collected in the spring had significantly higher liver Ni and Zn concentrations than those collected in the summer and than those collected from S lakes in the spring (Figures 7c, e). Liver Se concentrations showed opposite trends in each of the two regions; fish in RN lakes had significantly higher liver Se concentrations in the spring than in the summer, whereas fish from S lakes had significantly higher liver Se concentration in the summer than in the spring (Figure 7d).

DISCUSSION

Although the two gradients studied were geographically distinct, with nearly 500 km separating them, there were many similarities in the physical characteristics of lakes in each region. Both areas are lightly populated by humans and largely forested except for the zones of maximal impact from mining and smelting activities where high concentrations of metals released to the environment have severely contaminated terrestrial and aquatic environments. Variations in water quality aside from metal concentrations (pH, hardness, DOC) remained small, providing

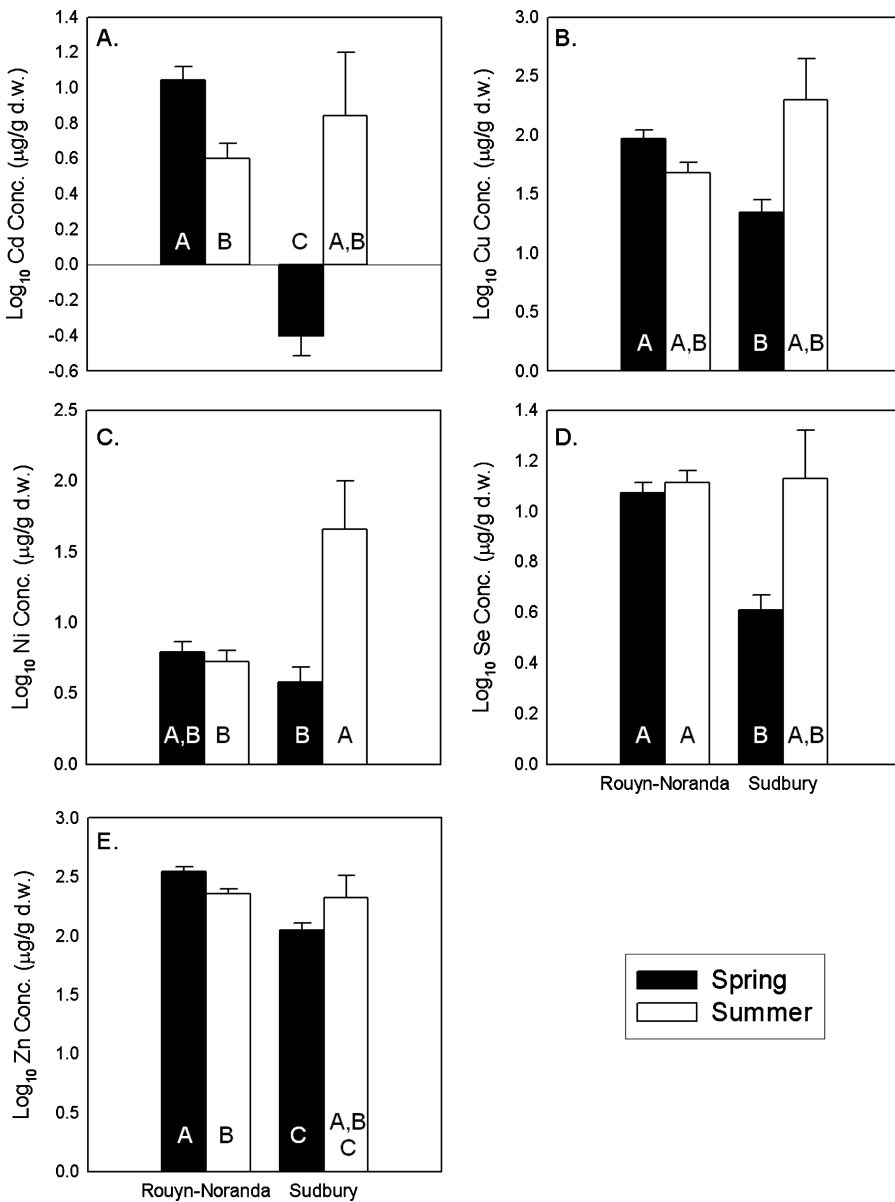


Figure 4. Significant (Table 8) region × season interactions for (a) Cd, (b) Cu, (c) Ni, (d) Se, and (e) Zn concentrations in gut contents of wild yellow perch. Bars represent least squares means (+SEM; least square means are means corrected for all shared variability associated with the full MANOVA model used to establish significant differences) using log₁₀-transformed data. Bars sharing the same letter designation are not significantly different from one another ($p > .05$).

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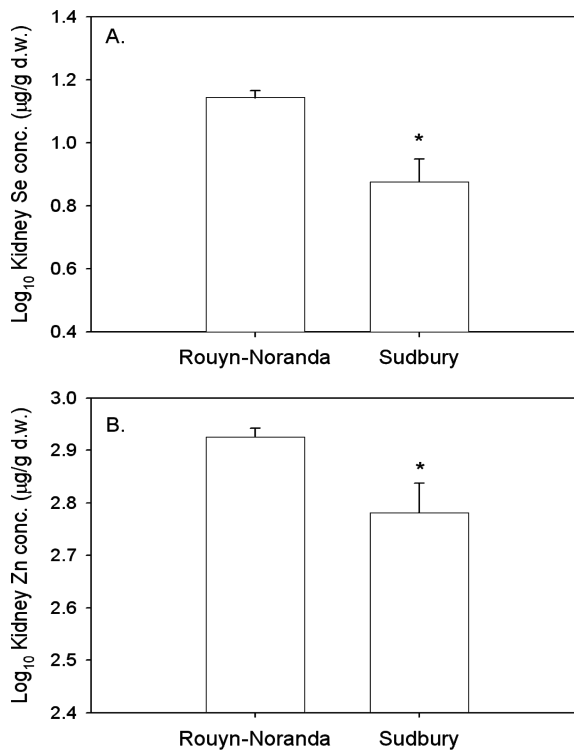


Figure 5. Significant ($p < .05$, Table 8) regional effects on wild yellow perch kidney (a) Se and (b) Zn concentrations. Bars represent least squares means (+SEM) using \log_{10} -transformed data. Asterisks indicate significant differences ($p \leq .05$).

a suitable comparative system both within and between the two five-lake gradients. Water and sediment metal concentrations, however, increased drastically but differently along both gradients. Specifically, contaminated RN lakes were characterized by elevated concentrations of Cd, Cu, and Zn in water and sediment, whereas contaminated Sudbury lakes were characterized by elevated concentrations of Cd, Cu, and Ni in water, and Cu and Ni in sediments. Although there was no evidence of Ni contamination in RN lakes, the highest sediment Cu concentration, measured in RN4, was around 10 times higher than the 4-week lowest effect concentration of 1118 $\mu\text{g/g dw}$ reported for *Hyalella azteca* (Borgmann and Norwood 1997). In the most contaminated Sudbury lakes (S4 and S5), aqueous Ni concentrations exceeded the 48-h LC50 value of 81 $\mu\text{g/L}$ reported for *Ceriodaphnia dubia* in water having hardness values close to those reported for these lakes (Keithly *et al.* 2004).

Given the wide range of yellow perch dietary preferences, the variability in contamination within a particular food type and seasonal variations (e.g., benthic invertebrates, Hare *et al.* 2001), instead of measuring metal concentrations in a range of potential food items that yellow perch may or may not eat, we analyzed metal contamination in the diet that fish themselves sampled. The contamination of these food items was strongly correlated to both water and sediment contamination for Cu and

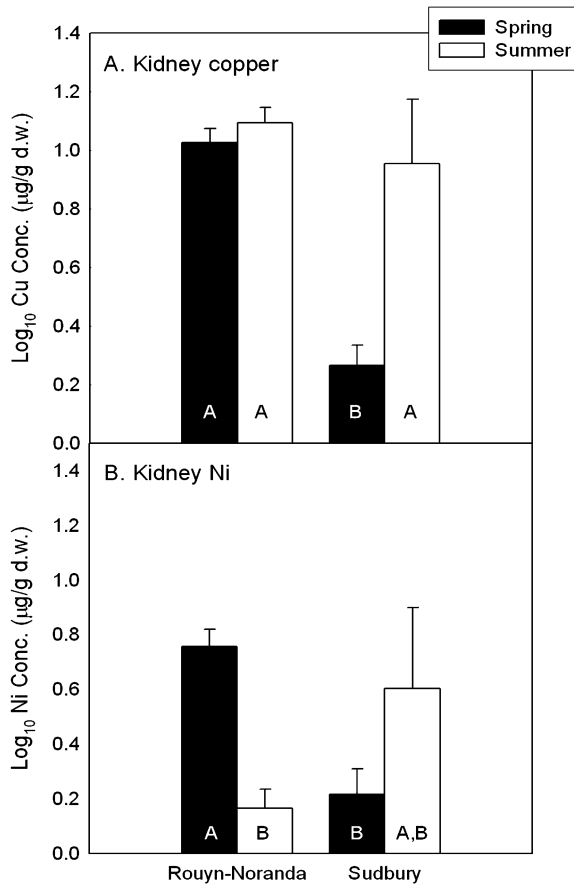


Figure 6. Significant (Table 8) region × season interactions for (a) Cu and (b) Ni concentrations in kidneys of wild yellow perch. Format as for Figure 4.

Ni, but only water influenced dietary Cd concentrations, whereas sediment, but not water, Zn was positively correlated to dietary Zn contamination. Although sediment Se was not correlated to Se in prey items, missing aqueous Se concentrations (below detection limits) preclude any conclusions regarding the influence of environmental Se on contamination of food items. Overall, for the four metals for which sufficient data were available (Cd, Cu, Ni, and Zn), environmental contamination clearly led to elevated concentrations of these elements in fish food, implying that fish living in these environments are necessarily exposed to both dietary and aqueous metal contamination. Because we did not sort fish diet by prey type (zooplankton, invertebrates, and fish) and data for fish of all sizes were pooled for the statistical analysis, we cannot interpret the influences of water and sediment on metal accumulation in specific prey items. Interpretation of metal concentrations in gut contents must also consider that these samples may have contained negligible amounts of sediment (from visual observation during dissection), and that their contamination would reflect daily and seasonal variations in invertebrate abundance and availability. Several

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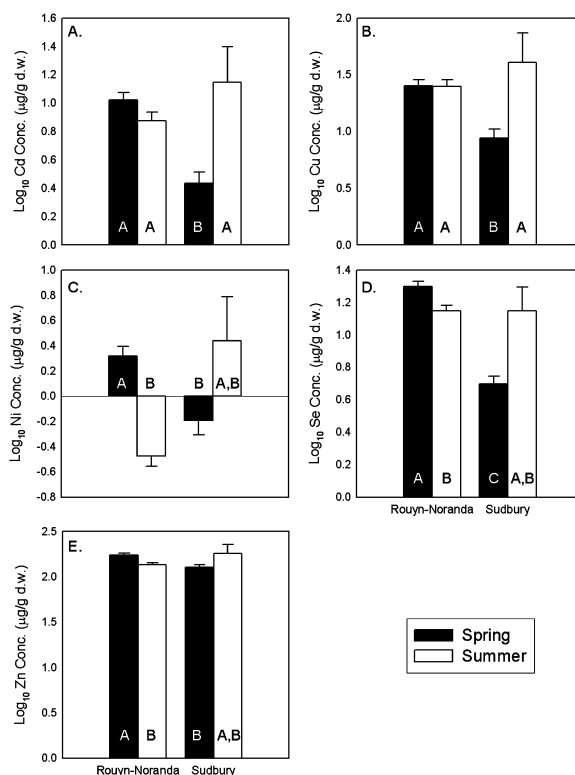


Figure 7. Significant (Table 8) region \times season interactions for (a) Cd, (b) Cu, (c) Ni, (d) Se, and (e) Zn concentrations in livers of wild yellow perch. Format as for Figure 4.

authors have already reported on biotic and abiotic influences on metal bioaccumulation by zooplankton and benthic invertebrates (Hare and Tessier 1996; Borgmann and Norwood 1997; Warren *et al.* 1998).

Our approach did not discriminate relative influences of aqueous versus dietary metal sources on metal accumulation in fish tissue. These influences may only be determined using radiotracers or transplant experiments in enclosures allowing for inclusion or exclusion of contaminated food (Kraemer *et al.* 2006b). In our study, although GC metal concentrations were measured for each fish and could be compared to tissue metal concentrations of the same fish, contamination of the gut content sampled by an individual fish on the day of capture may not accurately represent its average dietary metal exposure. Therefore, the use of mean values to examine the relationships between GC and tissue metal concentrations may be more realistic. Because water contamination was the same for all fish in a given lake, the relationships between water and tissue metal concentrations could only be examined using mean values.

Dietary Cd had a stronger influence than aqueous Cd on both liver and kidney Cd concentrations, relative to other metals examined where aqueous exposures had a greater influence. This result, which is not surprising given that there are no known

branchial or renal regulatory mechanisms for this non-essential metal, is in good agreement with an earlier report (Audet and Couture 2003). In a recent caging experiment with juvenile yellow perch from Rouyn-Noranda (Kraemer *et al.* 2006b), both aqueous and dietary sources contributed significantly to liver Cd uptake, but only aqueous Cd appeared to influence kidney Cd uptake. Kraemer *et al.* (2006b) suggested that the lack of influence of dietary Cd on kidney Cd concentrations they reported, which contrasts with the results of the present study, reflected that liver, but not kidney, receives blood directly from the digestive system. Therefore, the 30-day exposure in the caging experiment may not have been long enough to allow for dietary Cd to influence kidney Cd concentrations.

In contrast, Zn, which is strongly regulated, showed much weaker relationships between diet and tissue concentrations in this study, which supports similar observations by Kraemer *et al.* (2005) who reported that yellow perch liver Zn concentrations did not vary more than 1.5-fold in a 100-fold aqueous Zn gradient. In our study, dietary Se was also weakly correlated to tissue Se concentration, contrasting sharply with a recent review describing the dominant importance of diet as a source of Se in fish and other aquatic organisms (Hamilton 2004). This discrepancy could reflect limitations in our correlative approach. Because aqueous Se concentrations are not reported here, further speculation on the discrepancies between this study and current literature on the relative influence of dietary Se on tissue accumulation would be inappropriate. However, our study justifies further investigations in areas such as Sudbury or Rouyn-Noranda where elevated environmental concentrations of several metals could interfere with dietary Se uptake. The strong relationship between dietary and liver Cu supports recent suggestions that yellow perch tissue Cu concentrations are loosely regulated (Couture and Rajotte 2003; Kraemer *et al.* 2005) and are influenced by ambient Cu concentrations (Giguère *et al.* 2004). Although Ni essentiality has not been firmly established in aquatic organisms, Ni appears essential and is physiologically regulated in several species of bacteria, plants, birds, and mammals, but not in humans (Eisler 1998). To our knowledge, there is no direct evidence of homeostatic control mechanisms for Ni in aquatic organisms, although recent work suggests that dietary Ni uptake may be controlled in lake whitefish (*Coregonus clupeaformis*) (Ptashynski and Klaverkamp 2002). Muysen *et al.* (2004) have suggested that Ni may be essential and regulated in aquatic fauna. Our data indicate that dietary and aqueous Ni exerted a measurable but modest influence on liver and kidney Ni concentrations. Since the aqueous and dietary Ni gradients were much more important than for other metals (more than 100-fold between the lowest and highest concentrations for water) and tissue Ni gradients remained smaller (10-fold in liver and 20-fold in kidney, data not shown), our data add to the indirect evidence supporting the existence of homeostatic control mechanisms for Ni.

Current literature presents contradictory evidence regarding the influence of diet on tissue metal concentrations. In the Rouyn-Noranda region, preliminary model simulations suggest that the relative influence of dietary Cd on liver Cd concentration decreased with increasing aqueous Cd (Lisa Kraemer, INRS-ETE, pers comm). In a study of metal transfer between invertebrates and yellow perch along a river contaminated by gold mine tailings (Draves and Fox 1997), Cd, Cu, and Zn in fish diet, but not in water, were reflected in whole juvenile yellow perch. Similar conclusions were reached by Dallinger and Kautzky (1985) in a field study of rainbow

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trout for the same metals as well as Cr, Mn, and Pb. A strong dietary influence on Cd concentrations of all tissues examined (liver, gill, and muscle) was also reported in stickleback, *Gasterosteus aculeatus* (Bervoets *et al.* 2001). These authors also reported a dietary influence on liver Cu concentrations, in agreement with our findings, but not for other tissues. In contrast, Miller *et al.* (1992) reported no correlation between diet and tissue Cu or Zn concentrations in white sucker, and suggested that water may be a better predictor of tissue metal contamination than diet. Similarly, Köck *et al.* (1996) found no correlation between Cd in food items and in Arctic char (*Salvelinus alpinus*) from an alpine lake. They proposed that increases in metabolic rate during summer led to increasing uptake rates of contaminants, which had a greater influence on tissue Cd concentrations than Cd contents in food items.

Yellow perch shift their diet as they grow (Lott *et al.* 1996; Sherwood *et al.* 2000). Juvenile yellow perch feed on large zooplankton, whereas older fish increase their reliance on benthic invertebrates. In lakes where yellow perch can reach a large size, they will eventually start to feed on small fish. In this context, we examined whether these gradual shifts in dietary preferences were accompanied by corresponding changes in dietary metal exposure within each lake population and season. Giguère *et al.* (2004) examined this question and reported no relationship between age and Cd or Cu concentrations in gut content of yellow perch, but their study was limited to one metal-contaminated lake (Osisko, RN4 in our study). Although our results agree with theirs for Cu, our study indicated a decrease of dietary Cd in RN4 fish with age in both seasons examined. Given the gradual nature of dietary shifts in yellow perch, the general lack of abrupt changes in dietary metal concentration in our dataset was expected. Overall, neither dietary Cu, Se nor Zn consistently varied with fish age, suggesting that concentrations of these metals in food items are similar regardless of the specific diet type (i.e., zooplankton, benthos, or fish). In contrast, the concentration of Cd decreased in the diet of older fish from RN as well as in S5 fish. In those lakes where environmental Cd was elevated (RN5 and S5), our data indicated that larger food items (assuming larger yellow perch consumed larger prey) contained lower Cd concentrations, implying a reduced risk of dietary-induced toxicity for older fish. However, we do not know why dietary Cd also decreased in larger fish from cleaner RN lakes. Similarly, the decreased dietary Ni in older fish from lake S5 would suggest lower Ni concentrations in larger food items and imply a reduced risk of adverse effects, but the same observation in S1 and S2 fish, where environmental Ni was low, cannot be explained at this stage.

The age-related changes in dietary metal exposure were inconsistent between the two seasons examined, except for Cd in fish diet from lakes RN1 and RN4, which consistently decreased in both seasons. Likely, dietary items, including benthic invertebrates but perhaps also small fish, vary their body metal concentration and burden during the course of the growth season, either due to growth dilution or changes in metabolism which may modify metal uptake and excretion rates. The allometric decrease in specific growth rate and metabolic rate and therefore in food consumption may also reduce dietary metal uptake in larger fish. Overall, dietary metal concentrations often decreased, but never increased, in larger fish, supporting that larger yellow perch face a reduced risk of dietary metal poisoning, assuming that tissue metal concentrations are directly correlated to risk and that the relationships between these two variables do not vary with age.

Our data revealed noteworthy regional differences in tissue Cd accumulation with age. Although very few increases in tissue Cd with age were reported in any Sudbury fish, tissue Cd accumulation was common in older RN yellow perch even in those from clean sites, suggesting regional differences in metal handling capacities and strategies. In an early study, McFarlane and Franzin (1980) also reported increased liver Cd concentrations in large northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) from both clean and contaminated sites. Similarly, Arctic char also showed increasing liver Cd concentration with increasing age in an Austrian lake (Köck *et al.* 1996). In the only other study examining the influence of age on yellow perch tissue metal concentrations, Giguère *et al.* (2004) sampled 81 fish from RN4 in June 2000 (comparable to our spring sampling), and reported that both liver and kidney Cd concentration increased with age. In contrast, when we examined yellow perch from RN4 in both spring and summer 2003, we reported that liver Cd increased with age in summer but decreased in spring, whereas kidney Cd did not vary with age in summer but increased in spring. Combining these studies, our data indicate that patterns of age effects on yellow perch tissue Cd accumulation cannot be generalized even within a single lake because they vary both seasonally and annually. Clearly then, no single mechanism is involved in the accumulation of Cd in aging fish.

The patterns of tissue metal accumulation as a function of age for the other metals examined differed from the patterns observed for Cd. Although yellow perch from both regions appeared capable of preventing liver from accumulating Cu over the years, decreases in liver Cu accumulation were uncommon. In all lakes where environmental Ni concentrations were low (RN lakes, S1 and S2), tissue Ni decreased with age in the spring, but not in Ni-contaminated lakes (S3-S5). Possibly due to rapid growth and consequent metal dilution in younger fish, many of the age-related decreases in tissue Ni that were observed in the spring had disappeared in summer. Even though tissue Se and Zn varied with size in some lakes and seasons, patterns of variation with age were inconsistent. Overall, there was no evidence that any of the metals examined increased in concentration with age, except for Cd in many RN, but not S, fish. The latter finding implies an increased risk of Cd toxicity in older RN but not S fish, even when comparing lakes where environmental Cd exposure is similarly high (RN5 vs. S4 and S5). In contrast to Cd, the systematic decrease in tissue Ni concentration with age in RN, but not S, fish, indicates a decreased risk of Ni toxicity in older RN, but not S, fish.

In order to evaluate the single and combined effects of region and season on tissue metal accumulation, a multivariate statistical approach (MANOVA) was conducted. The first two axes generated by the CCA as part of the MANOVA model allowed for the separation of seasonal and regional effects on gut contents and tissue metal concentrations, indicated in Figure 3 by non-overlapping circles (where circles represent 95% confidence intervals). Along Axis 1, dietary metals contributed to regional differences, with RN fish associated with higher dietary Cd, Se and Zn values, whereas elevated dietary Cu and Ni were more typically associated with S fish. However, seasons and regions interacted, such that regional differences in dietary metal concentrations were not always apparent in both seasons. In general, RN fish exhibited decreased dietary metal contamination in summer compared to spring,

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whereas no significant seasonal variations were observed in S fish, except for dietary Ni, which increased sharply during summer.

Although kidney Cd was not influenced by region or season, liver Cd concentrations were lower in S fish in spring than in summer, and lower than measured in RN fish, where seasonal variations were not observed. In agreement with these observations for RN fish, Kraemer *et al.* (2006a) reported in the same year as this study (2003) that liver Cd concentrations decreased between May and July in RN5, but increased again in August to reach levels similar to those measured in May. Supporting our observation of seasonal variations in liver Cd in S fish, Köck *et al.* (1996) also observed higher tissue Cd concentrations during summer compared to spring in Arctic char and proposed that temperature, rather than water Cd concentration or diet contamination, was the major factor contributing to tissue Cd, through enhanced uptake associated with increased metabolic rate. Our study does not support this hypothesis, because RN yellow perch would also increase their tissue Cd concentrations during summer. Finally, in a recent study (Audet and Couture 2003), we reported no seasonal variations in tissue Cd concentrations of wild yellow perch from Whitson Lake (S4). Therefore, seasonal variations in liver Cd concentrations observed at the regional scale may not always be observed in individual lakes within a region.

As observed for liver Cd, increased tissue Cu concentrations in summer relative to spring in S fish only was also observed in both liver and kidney, whereas no seasonal variations were detected in RN fish tissue Cu concentrations, which were on average as high as those measured during summer in S fish. Overall, because Cu contamination in both gradients was similar, the lower tissue Cu concentrations in S fish in the spring compared to values in RN fish suggest regional differences in Cu handling and regulation strategies, which would be superior in S fish. However, although we did not observe any major regional differences in seasonal variations of biotic or abiotic factors that could lead to the regional differences observed for tissue metal accumulation, the influence of factors not considered in this study cannot be ruled out.

Seasonal variations of tissue Ni were only observed in RN fish, where environmental Ni concentrations were lowest. The high tissue Ni values in spring RN fish compared to summer, and compared to S fish in spring, were therefore surprising. Given the very low dietary and aqueous Ni contamination in all RN lakes (with the only exception of summer where gut content Ni concentration was elevated in some RN5 fish; data not shown), the sources of Ni leading to such high liver and kidney Ni concentrations in RN fish in the spring (higher than in yellow perch from comparably Ni-clean or even Ni-contaminated lakes from the Sudbury gradient; data not shown) remain unidentified and warrant further investigations. As for Cu, our data support the hypothesis of an evolved capacity for tissue Ni regulation in S, but not RN, fish.

Compared to Cd, Cu, and Ni, different patterns of seasonal and regional variations were apparent for tissue Se and Zn concentrations. In the case of kidney, these were affected by region but not by season, with higher values in RN fish, reflecting environmental and dietary contamination. However, seasonal variations were observed in liver. Zinc concentrations in liver were higher in the spring compared

to summer for RN fish, but did not vary seasonally in S fish. Although seasonal variations in tissue concentrations of other metals observed in one region were not always present in the other, liver Se was the only tissue metal that showed opposite regional trends of seasonal variations, with decreasing concentrations between spring and summer in RN fish, but increasing in S fish. Although RN fish clearly accumulated more tissue Se than environmental (sediment) contamination would suggest, in this case the higher dietary Se input in RN than in S fish, which was positively correlated with tissue Se in liver, may explain regional differences in tissue Se accumulation.

Seasonal variations in tissue metal concentrations within the same fish populations can be due to a range of factors, including shifts in diet items, temperature- or activity-driven modifications in metabolic rate (that would influence both aqueous and dietary metal uptake as well as elimination rates), recent growth that may lead to a decrease (i.e., growth dilution, Thomann 1989) or an increase (if more contamination is ingested via the food, *e.g.*, Farkas *et al.* 2002). Overall, it is clear from our dataset that no generalizations can be made to explain seasonal variations in tissue metal concentrations, or that many competing mechanisms are at work, and therefore that the only way to properly evaluate the risk of toxicity associated with higher tissue metal concentrations in wild fish is to repeat the measurements at regular intervals within and among years.

Because climate is similar in both regions examined here, regional differences in tissue metal concentrations may largely reflect genetic differences associated with either growth or metal handling capacities. Fish from both regions similarly increased their tissue Cd concentrations in proportion to aqueous and dietary Cd, suggesting that Cd handling capacities were similarly low in fish from both regions. In contrast, fish from both regions appeared capable of regulating tissue Zn concentrations, explaining why regional and even seasonal differences in tissue Zn concentrations, although sometimes statistically significant, remained small compared to variations of other metals. Finally, for Ni, Cu, and perhaps Se but not for Cd or Zn, this study suggests that S fish, which have historically been exposed to higher concentrations of these metals, may have evolved metabolic strategies to better regulate and maintain lower tissue values.

Results from this study should serve to improve current ERA predictions for wild yellow perch inhabiting metal-contaminated lakes. By sampling a large number of fish of all ages and sizes from two distinct metal-contamination gradients over two distinct seasons, we have demonstrated that metal-accumulation patterns must be interpreted under an appropriate context. That context must take into account the specific metal (regulated or not), metal source (water or diet), age or size of the fish (which should be consistent among comparative populations), sampling season, and sampling region. Single sampling events with the objective of characterizing metal accumulation in any given lake provide only a brief and incomplete snapshot of the complex patterns that emerge over a fish's lifetime, seasons, and regions. Moreover, careful consideration must be given to the selection of an appropriate reference population because of potential genetic, environmental, and ecological differences inherent among disparate populations. Studies that consider all of these confounding influences with respect to tissue metal accumulation in wild

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yellow perch populations will result in improved ERAs, which will ultimately improve decision-making in environmental management and regulatory activities.

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