

**Effects of Environmental Metal Contamination on the Condition
of Northeastern Ontario Yellow Perch (*Perca flavescens*)**

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**A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology**

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ABSTRACT

Yellow perch (*Perca flavescens*) from 7 northeastern Ontario lakes were examined to assess the impact of varying levels of Cu, Ni, Al, and Zn on their condition. Indicators of condition including the relative condition factor (K_n), hepatosomatic index (HSI), liver protein and water contents, and scaling coefficients were used to determine if fish were physiologically stressed by the presence of elevated environmental metal concentrations.

Nickel and aluminum did not appreciably bioaccumulate in these fish and showed no relationship to fish condition. However, liver Cu and Zn concentrations were negatively associated with various indicators of condition. Liver Cu concentrations were negatively associated with HSI ($r = -0.3072$; $p < 0.005$) and scaling coefficients ($r = -0.78$; $p < 0.05$). Liver Zn concentrations were negatively correlated with K_n ($r = -0.3003$; $p < 0.01$) and scaling coefficients ($r = -0.90$; $p < 0.05$) and positively correlated with liver protein contents ($r = 0.2003$; $p < 0.05$). Since liver Cu and Zn concentrations covaried ($r = 0.49$; $p < 0.001$), the extent to which either metal impacted yellow perch condition is unknown.

This study revealed negative physiological effects in fish subjected to elevated environmental metal concentrations. Further research is needed to determine the extent to which trace metals affect (direct physiological impairment, variations in diet, competition, and/or genetic variations) fish condition.

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1.0 Introduction

The sulphide mineral deposits in Sudbury, Ontario, contains about 50% of the world's nickel supply, large amounts of iron and copper, and lesser quantities of twelve other metals. Smelting emissions in the Sudbury area have released vast quantities of SO₂ and substantially increased the concentration of certain trace metals within local soils, vegetation, and lakes (Semkin and Kramer, 1976; Freedman and Hutchinson, 1980; Hazelett et al., 1984). Many lakes near Sudbury have exhibited both depressed pH and elevated trace metal concentrations (Scheider et al., 1975; Dillon et al., 1977; Keller et al., 1992).

As a result of reduced sulphur emissions and to a lesser extent because of Sudbury's reclamation projects (i.e., liming), there were increases in pH and acid-neutralizing capacity in many lakes surveyed in the Sudbury area during the period 1981-1989 (Keller et al., 1992). The biota in many damaged lakes have made a substantial natural recovery in response to reduced emissions (Gunn and Keller, 1990), although this response appears to be more prevalent in acidified lakes that were farther from Sudbury and less metal contaminated (Keller and Gunn, 1995a, 1995b). However, the physiological status of fish populations in these "recovering lakes" have not been studied in detail following the increased pH and lowered trace metal concentrations that have been observed during the past few decades.

Four trace metals were chosen for examination in this study (Cu, Ni, Al, and Zn). Copper, Zn, and Ni were chosen because their levels in water (Nriagu et al., 1998) and soils (Winterhalder, 1996) near Sudbury far exceed those found in

undisturbed sites. There has been little change shown by water Cu and Ni concentrations between 1978/1979 and 1993/1994 in spite of the massive emission control and ecosystem rehabilitation efforts (Nriagu et al., 1998). In fact, it is believed that many of Sudbury's catchment areas have become saturated with Cu and Ni, and with Zn and Pb to a lesser degree. It is estimated that the mobilization of metals from saturated soils can sustain the high concentrations of Cu and Ni in many lakes for the next 1000 years (Nriagu et al., 1998). Within the Sudbury region, concentrations of Cu and Ni in many lakes exceed suggested safe values (Provincial Water Quality Objectives-PWQO) for the protection of fish and other aquatic life. Although the concentration of Al has generally decreased in lake waters as the acid leaching of watersheds was reduced and the pH of acidic lakes increased (Keller et al., 1992), Al (along with the other metals examined in this study) could detrimentally impact local fish populations where concentrations exceed current PWQO (MOEE, 1994). High trace metal concentrations may continue to impede the recovery of many Sudbury area lakes and their aquatic inhabitants for decades or even centuries to come (Nriagu et al., 1998).

In aquatic ecosystems, fish population characteristics integrate and reflect the effects of the numerous biotic and abiotic factors that interact in an ecosystem (Larkin, 1978; Adams and McLean, 1985). Although anthropogenic stresses such as metal contaminants may exert adverse effects, fish are typically subjected to numerous stressors including unfavorable or fluctuating temperature, low dissolved oxygen concentrations, and limited food availability. These factors, individually or together, can impose considerable stress on

physiological systems of fish and impair their health or condition (Wedemeyer et al. 1990).

Since the early 1950's, fish populations throughout the region began to diminish (Uutala and Smol, 1995). It was believed that this decline was a result of the combination of the increased environmental burden of metals and acids in Northeastern Ontario waters (O.W.R.C., 1970). The increased environmental burdens of trace metals in Sudbury area lakes have been potentially stressful to local fish populations (Bradley and Morris, 1986; Marr et al., 1995). However, the significance of increased metal burdens in wild fish populations have not been fully assessed.

Yellow perch (*Perca flavescens*) were chosen for examination in this study because they are relatively tolerant to pollution (Hontela et al., 1992; Ion et al., 1997) and are present in many Sudbury area lakes where few other species can survive. Preliminary studies (Eastwood, 1997) have identified the yellow perch as a useful bioindicator species because they bioaccumulate trace metals quite readily under natural environmental conditions.

Trace metals mainly accumulate in the livers of fish and to a lesser extent in other tissues such as the muscle, gills, kidney, and various parts of the brain (Bradley and Morris, 1986; Boeck et al., 1997; Baker et al., 1998). The liver is therefore an ideal bioindicator organ for studying the impact of trace metals on the condition of fish. This study measured the concentration of Cu, Ni, Al, and Zn in the livers of yellow perch from 7 lakes with varying levels of trace metals to determine if metals were bioaccumulated from their respective environments. Increased metal content of fish tissues can be an informative sublethal response,

indicating increased metal availability and potential metal stress (Bradley and Morris, 1986).

Body condition reflects the success of an individual or a population of fish in finding and storing energy under prevailing environmental conditions (Dutil et al., 1995). Field (Adams and McLean, 1985; Farag et al., 1995) and laboratory studies (Wilson et al., 1993; Baker et al., 1998) have both shown that fish exhibited lowered stored energy in the form of tissue growth (i.e., weight and length parameters) in response to high trace metal exposure.

Several indicators of condition were used in my study including the relative condition factor (K_n), the hepatosomatic index (HSI), liver protein and water contents, and scaling coefficients. The relative condition factor (K_n) measures the 'girth' of fish. During periods when fish have high-energy intake, the growth of tissues and the storage of energy (protein, lipids, glycogen etc...) in muscle and liver can cause an individual to have greater-than-usual weight at a particular length. This storage can be estimated by measuring K_n . Although considerable debate exists over the legitimacy of using condition factors in fish assessments (Bolger and Connolly, 1989; Liao et al., 1995), the relative condition factor has been shown to be a useful indicator of fish condition when certain assumptions have been met (Le Cren, 1951). The relative condition factor is a function of the slope of the weight-length relationship and is only useful for comparisons within a particular sample or between samples with a similar slope (Le Cren, 1951). This assumption was met in the present study where the slope (3.31) of the weight-length relationship for the entire fish sample was used to calculate K_n . This slope

is similar to the slope used to develop the standard weight equation for yellow perch (Willis et al., 1991).

Tissue protein and water contents were also used as indicators of yellow perch condition. The liver is the main site for the synthesis of metallothionein (MT). Metallothionein is necessary for the sequestration of elevated levels of trace metals (George et al., 1996). Metallothionein can be induced by and bind metals such as Cu, Cd, Hg, Ag, and Zn (Kägi and Kojima, 1987). In fact, the capacity of MT to bind to metals in the liver has been proposed as a useful approach for biochemical monitoring for metal pollution in aquatic environments (Bayne, 1979; Bayne et al., 1980). A higher liver protein content in response to elevated trace metal concentrations could be indicative of elevated MT synthesis and help explain lowered physiological condition in wild fish populations. Higher liver water content is also indicative of lowered energy content and hence, lowered condition in fish (Dutil et al., 1995). This study will examine relationships between liver water and protein contents and liver metal concentrations to determine if fish were physiologically impaired by elevated environmental metal exposure.

The HSI (the ratio of liver to body weight) is a useful indicator of condition in assessing the general condition of fish captured in the field (Goede and Barton, 1990). Fish accumulate energy in the liver as lipids (fat content) and glycogen. During periods of high-energy intake, the relative size of their livers should correlate with their nutritional state and growth rates (Delahunty and De Vlaming, 1980; Bolger and Connolly, 1989; Dehn, 1992; and Dutil et al., 1995). Decreases in HSI are frequently seen in fish under stress (Lee et al., 1983). In

fish exposed to sublethal trace metal concentrations, physiological stress can impose an energy drain on fish and result in a loss of energy stores such as liver glycogen and lipids.

Data on the weight and length of fish are easily obtained and can be accurately measured under controlled laboratory conditions. Scaling coefficients are a useful measure of the weight-length relationship and illustrate the growth pattern that exists in all fish populations. The scaling coefficient is the exponent (n) of the weight-length relationship ($W = aL^n$), and usually ranges between 2.5 and 4. Fish grow isometrically when their scaling coefficient is 3. This means they will maintain the same shape as they grow. A fish following a growth pattern with a scaling coefficient less than 3 will become leaner as it grows in length, an indication of lower condition.

The productivity of lakes and streams has long been known to influence fish condition. Productivity refers to complex interactions between living substances in communities and the environment (Reid, 1961). Three common qualitative measures of ecosystem productivity as a community attribute include: standing crop, rate of removal of material, and rate of production. Standing crop is the instantaneous measure of the quantity of organisms or biomass within a particular ecosystem. Rate of removal pertains to the yield of an ecosystem per unit time whereas the rate of production pertains to the amount of organic substance synthesized in a prescribed space per unit of time (Reid, 1961). The relationships between a chemical indicator of productivity and fish condition will be examined to assess the potential influence of lake productivity (food availability) on fish condition. Alkalinity was used as the chemical indicator of

productivity in this study because it has been shown to be correlated with lake productivity (Moyle, 1956; Turner, 1960).

The primary objective of this study was to determine if yellow perch exposed to elevated environmental metal concentrations demonstrated lowered condition. Liver metal concentrations were used as the principal measure of environmental metal exposure. To this end, condition and liver metal concentrations were compared among fish samples from 7 lakes varying in aqueous metal concentrations. The second objective was to examine the relationships between liver metal concentrations and alkalinity (estimator of productivity), and indicators of condition within and among fish samples. I hypothesize that fish exposed to elevated environmental metal concentrations will exhibit higher tissue metal concentrations and lower condition.

2.0 Methods

2.1 Lake Characteristics

Seven northeastern Ontario lakes were selected for this study. Hannah, Ramsey, Whitson, and Vermilion lakes were located in the Sudbury region within 20 km of Sudbury's smelters. The additional 3 lakes, Michiwakenda, Larder, and Round lakes, are located 60-95 km from the smelters. Figure 1 illustrates the location of the lakes. Physical characteristics of the lakes are indicated in Table 1.

2.2 Water Chemistry Collection and Analysis

Water quality data were collected from July 28 to August 12 of 1998. Water samples were collected approximately 1 meter below the water's surface and were filtered with a Nalgene hand-operated vacuum pump on site through a 0.45 µm membrane filter (to remove bound non-available trace metals) and filled into 50-ml prewashed Histoplex containers. Each Histoplex container was rinsed twice with double distilled water (DDW) and once with trace metal grade (TMG) nitric acid (Fisher-Scientific). Each sample was diluted 1:20 with TMG nitric acid (Fisher Scientific) and stored in a refrigerator at 4°C for 1 to 2 days. Copper, Ni, and Al were analyzed using a graphite furnace (Perkin-Elmer 5000 with attached HGA 500 programmer, AS40 autosampler, and Zeeman background correction) atomic absorption (AA) spectrophotometer. Zinc was measured by flame (Perkin-Elmer 5000) AA spectrophotometry. Five blanks and 5 certified reference material (lobster hepatopancreas – Tort1) samples were used to correct for

Figure 1. Location of the 7 Northeastern Ontario study lakes. The Copper Cliff, Coniston, and Falconbridge smelters are indicated.

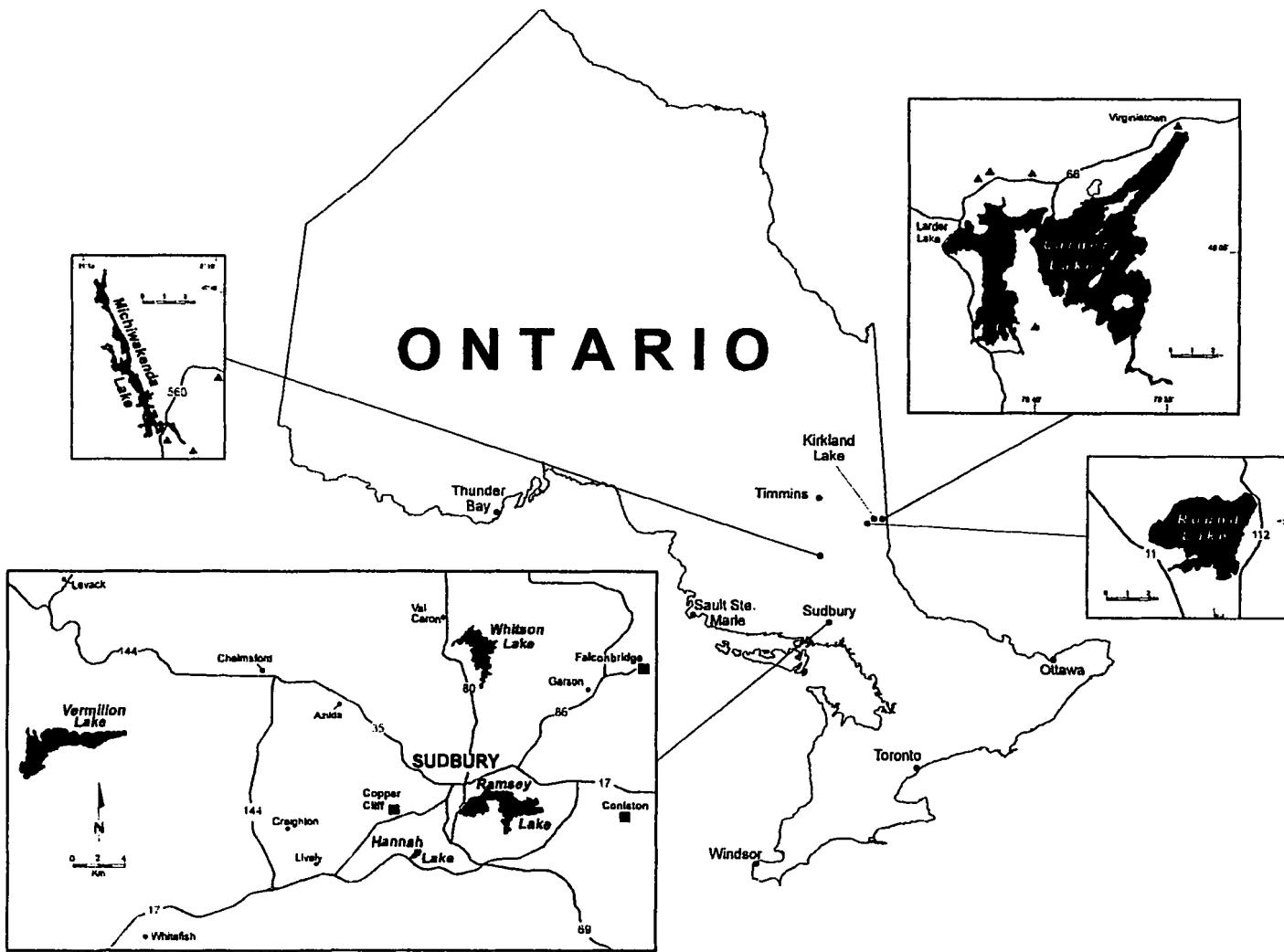


Table 1. Physical characteristics and location of the 7 study lakes.

| | Sudbury Area Lakes | | | | Distant Comparative Lakes | | |
|---|--------------------|----------|--------|-----------|---------------------------|--------|--------------|
| | Hannah | Whitson | Ramsey | Vermilion | Michiwakenda | Larder | Round |
| Longitude | 81°34' | 80°52' | 80°57' | 81°24' | 80°14' | 79°40' | 80°02' |
| Latitude | 46°12' | 46°35' | 46°29' | 46°31' | 47°36' | 48°05' | 48°01' |
| Township | Broder | Blezzard | Broder | Fairbank | Churchill | Hearst | Otto/Marquis |
| Surface Area (ha) | 27.2 | 472.3 | 795.2 | 1124.5 | 302.2 | 3703.7 | 1213 |
| Distance From Nearest Sudbury Smelter (km) | 5 | 8 | 6 | 18 | 60 | 95 | 85 |

background contamination and monitor analytical accuracy and recovery rates. See Section 2.6 for more detailed methodology. Additional water samples were also collected and submitted to the Cooperative Freshwater Ecology Unit (COOP) and the Ontario Ministry of the Environment. Water chemistry parameters analyzed by the COOP include, pH, alkalinity, and conductivity, whereas dissolved organic carbon analysis was completed by the MOEE.

2.3 Fish Sampling

Fish were sampled using multi mesh gillnets between early September and mid October, 1997. Gill nets were randomly set (using a random stratified sampling technique) (George Morgan, Laurentian University, Sudbury, Ontario, pers. comm.) in each lake. Gill net mesh size increased from 25 to 152 mm and each panel (8 in total) was approximately 7.6 by 1.8 meters in size. To avoid water depth-related bias in sampling, half the nets were placed with the small mesh towards shore whereas the other half were placed with the large mesh starting at the shoreline. Sampling was carried out for 1 to 5 days in each lake with up to 4-gill nets until at least 20 fish were obtained. Because of the cost of metal analysis, the maximum sample size was set at 20. However, because of time constraints in capturing fish, sample sizes were less than the maximum in Round (18), Vermilion (14), Michiwakenda (9), and Larder (8) lakes. When more than 20 fish were available, the most representative range in length from each lake sample was selected. To obtain a representative range from each lake sample, all fish were lined up on the bench from smallest to largest (based on fork length) and 20 fish that represented the full size range were selected. Fish

sampled from Larder and Round lakes were captured in the same manner and during the same period by personnel from the COOP, and were stored in labeled plastic bags in a freezer at -20°C until dissection.

2.4 Fish Dissection and Tissue Preservation

Immediately after recovery, fish were killed with a blow to the head and covered with ice until they were returned to the laboratory. Ice was used to minimize any tissue decay and to maintain a moist environment during transport. Fork body length (\pm 0.1 cm), body weight (\pm 0.01 g), carcass weight (\pm 0.01 g), and the wet weight of the livers (\pm 0.01 g) were recorded. Each liver was cut into 2 separate portions, for determination of metal and protein contents. Each liver sample was placed into a pre-weighed 10-ml Teflon container and re-weighed to obtain the wet liver weight. The liver samples were placed in 1 ml labeled prewashed Eppendorf plastic containers (Eppendorf containers were washed in the same manner as the Histoplex containers mentioned previously) and were stored at -20°C.

2.5 Fish Ageing

Scale samples were taken medially from below the dorsal fin and the opercular bone was removed and cleaned. These two calcified structures were later dried and aged (Labman Ageing, Gogama). Scales were used as the primary structure for age determination following the recommendations of Niewinski and Ferreri (1999).

2.6 Determination of Liver Water and Trace Metal Contents

To determine liver water content, liver samples were placed in 10 ml Teflon vials (Cole-Parmer) and weighed before and after dehydration at 60°C for 24 hrs. Preliminary studies have shown that this temperature and duration were adequate to completely dry liver samples of the size range used in this study (~ 20-80 mg wet liver). Each dry sample was digested in 5 ml of TMG nitric acid in a microwave oven at maximum output for 3 intervals of 30 seconds. The Teflon vial caps were sealed tightly to prevent sample evaporation during the microwave procedure. The samples were allowed to cool between intervals to prevent excess pressure within the vials. When 5 ml of nitric acid was insufficient to completely digest the dried liver sample, an additional 2 ml of TMG nitric acid was added and the samples were re-digested according to the previous microwave protocol. Certified reference material (lobster hepatopancreas – Tort1) was used to monitor analytical accuracy and recovery rates. Recoveries ranged from 96.3 ± 4.5 , 93.7 ± 4.5 , 95.3 ± 1.5 , and 94.0 ± 5.3 for Cu, Ni, Al, and Zn concentrations respectively in all samples examined ($n = 107$). Five blanks and each metal standard were measured after every 10th sample to ensure analytical accuracy of our measurements. The detection limit was also determined and is defined as the concentration of the element that will produce a signal twice the value of the baseline noise (Andre Bourrie, pers. Comm., 1999). The baseline noise refers to the variability in the blank absorbance readings. Blank absorbance readings and detection limit values for Cu, Ni, Al, and Zn are

reported in Appendix 1. Tissue metal concentrations were measured using the same equipment outlined in Section 2.2.

Copper, Ni, Al, and Zn standards were diluted in double distilled water. From these standards, various concentrations were prepared to assess the linearity of the absorbance in the concentration range. Since the relationships for each range of metal concentrations and their respective range of absorbencies were linear (Appendix 2), only the highest concentration of each metal along with a set of blanks were used to calibrate the spectrophotometer prior to determining the metal concentrations in each series of fish liver samples. Liver Zn concentrations were determined against a 500 ppb standard whereas liver Cu, Ni, and Al concentrations were determined using a 100 ppb standard. The absorbance readings obtained for tissue sample analyses were converted to metal concentrations using the following equation:

$$[\text{Metal}] = \frac{(\text{Sample Abs} - \text{Blank Abs})(\text{Std } [\]) (\text{Dilution}) (\text{N.A. Volume}) (1000)}{(\text{Std Abs}) (\text{Sample Wt})}$$

where: [Metal] = metal concentration of liver sample in $\mu\text{g}\cdot\text{g}^{-1}$ wet liver (ppm)

Sample Abs = atomic absorption reading of the sample

Blank Abs = average atomic absorption reading of the procedural blanks

Std [] = concentration in $\text{ng} \cdot \text{ml}^{-1}$ (ppb)

Dilution = dilution factor

N.A. Volume = Total volume of TMG nitric acid (ml) used to digest sample

Std Abs = atomic absorption reading from standard concentration

Sample Wt = wet weight of sample in grams (g)

Dilutions were used to ensure that the sample absorbencies were within the absorbance range used to assess the linearity of the concentration range used in this study (i.e., Zn = 0-500 ppb; Cu, Ni, and Al = 0-100 ppb). Five blanks were created using the same microwave protocol as for the liver samples. These were used to correct for contamination and background values of each examined metal.

2.7 Protein Analysis

Frozen liver samples were thawed 6-8 weeks after dissection. Wet liver samples were diluted 1:10 with homogenization buffer and homogenized at an intermediate speed at three intervals of 20 seconds with a Ultra-turrax homogenizer. Samples were measured in triplicate using a Varian Cary 100 UV-VIS spectrophotometer. Detailed methodology can be found in Appendix 3.

2.8 Indicators of Condition - Calculations

2.8.1 Scaling Coefficient

The scaling coefficient for the entire fish sample ($n=107$) was obtained from the weight-at-length relationship shown in Appendix 4. The scaling coefficients for each lake sample are shown in Appendices 5a-g. The scaling coefficient is the exponent of the power equation describing each weight-at-length relationship examined. The power trendline calculates the least squares fit through points by using the following equation:

$$y = cx^b$$

b = slope = scaling coefficient
y = y-axis point
x = x-axis point
c = constant

2.8.2 Relative Condition Factor (K_n)

The relative condition factor was calculated using the equation:

$$K_n = \text{body weight}/\text{length}^m * 1000 \quad m = \text{scaling coefficient (3.31)}$$

2.8.3 Hepatosomatic Index

The hepatosomatic index or liver index was calculated using the following equation:

$$\text{HSI} = \frac{\text{wet liver weight (g)} * 100}{\text{total body weight (g)}}$$

2.9 Statistical Evaluation of Data

Analysis of variance (ANOVA) was used to detect significant differences between mean liver metal concentrations and/or burdens (Table 4) and mean indicators of condition (Table 5) among the 7 yellow perch samples. The relative condition factor was normally distributed within each lake sample. No other lake sample variables followed a normal distribution and were therefore log-transformed. A Tukey-Kramer post-hoc test was used in conjunction with an

ANOVA to distinguish which groups differed with each other. When a non-parametric distribution was found or if the assumption of homogeneity of variance was violated in an ANOVA, a non-parametric Kruskal-Wallis analysis of variance test was used in conjunction with a non-parametric multiple comparison test for equal and unequal sample sizes (Sokal and Rohlf, 1981).

Regression and Pearson correlation analyses were used to examine the associations between water metal concentrations, liver metal concentrations, and indicators of condition. Neither liver metal concentrations nor any indicators of condition followed a normal distribution when all yellow perch were pooled. Therefore, all regression and Pearson correlation analyses including that of our pooled yellow perch sample were log-transformed to obtain normally distributed data.

Since liver Cu and Zn concentrations were found to significantly covary (Figure 10), partial correlation analyses were used to determine associations between these metals and various measures of yellow perch condition. A partial correlation coefficient measures the correlation between any pair of variables when other specified variables have been held constant (Sokal and Rohlf, 1981).

An analysis of covariance was used to detect significant differences between scaling coefficients.

2.10 Allometric Corrections

Allometric scaling refers to the structural and functional consequences of changes in size and may explain the proportional change of one characteristic to

another, usually body size (Cates and Gittleman, 1997). The relationship between liver metal concentrations or the various indicators of condition with fish wet body weight is a case of allometric scaling. Liver Cu burden, water content, and K_n were positively correlated with body weight. Allometric corrections were applied to the aforementioned variables to accurately interpret any relationships between liver metal content and liver water content and K_n . Liver Cu burden, K_n , and liver water content were scaled (log/log plot) according to the average body weight of all yellow perch sampled ($n=107$, mean body weight = 55 g). Allometric scaling was accomplished with the use of the following equation:

$$\text{Scaled variable} = \left[\frac{\text{average body weight (g)}}{\text{body weight}} \right]^{\text{slope}} * \text{variable value}$$

Where:

scaled variable = variable that has been corrected for size

average body weight = average body weight of pooled yellow perch sample

body weight = body weight of yellow perch

variable value = variable to be corrected for size

slope = slope of the regression between body weight and the variable to be corrected for size (each variable were log-transformed)

3.0 Results and Discussion

3.1 Lake Water Quality

Sudbury lakes contained elevated levels of aqueous Cu, Ni, Al, and Zn in relation to Michiwakenda and Round lakes (Table 2). Larder lake also exhibited aqueous Cu and Ni concentrations that exceeded PWQO. Larder lake is a contaminated lake due to the presence of tailings from a nearby gold mine. This is consistent with other studies documenting increased trace metals in aquatic ecosystems proximal to point sources of contamination such as mining industries (Bradley and Morris, 1986; Nriagu et al., 1998). Water Zn and Al concentrations from all lakes were lower than PWQO (Table 2) whereas Vermilion lake had a water Al concentration ($72.2 \mu\text{g}\cdot\text{L}^{-1}$) that was approaching the PWQO of $75 \mu\text{g}\cdot\text{L}^{-1}$. However, water Cu concentrations from 6 of the 7 lakes exceeded the PWQO of $5 \mu\text{g}\cdot\text{L}^{-1}$; Round Lake was the only exception with a Cu concentration of $4.5 \mu\text{g}\cdot\text{L}^{-1}$. Aqueous Ni concentrations were above PWQO in Hannah, Whitson, Ramsey, and Larder lakes. Further discussion of lake characteristics will be presented in Section 3.8.

3.2 Fish Sample Description

Size-age characteristics are reported in Table 3. Michiwakenda, Larder, and Round lakes fish consistently demonstrated higher body weight and fork length values than the 4 Sudbury area lake samples. Hannah and Whitson lakes fish exhibited the smallest weight and length measurements among the 7

Table 2. Chemical characteristics of the 7 study lakes during late summer, 1998. Provincial Water Quality Objectives (PWQO) ($\mu\text{g L}^{-1}$) (MOEE, 1994) are listed in bold lettering.

| Lake | Date | Cu ($\mu\text{g L}^{-1}$) * | Ni ($\mu\text{g L}^{-1}$) * | Al ($\mu\text{g L}^{-1}$) * | Zn ($\mu\text{g L}^{-1}$) * | pH | Alkalinity mg L^{-1} ** | Conductivity μScm^{-1} ** | DOC mg L^{-1} ** |
|--------------|---------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|------|-------------------------------------|---|------------------------------|
| Hannah | 28/7/98 | 21.3 | 108.5 | 24.1 | 14.8 | 7.56 | 15.76 | 123 | 3.2 |
| Whitson | 30/7/98 | 27.6 | 81.7 | 10.0 | 10.1 | 7.05 | 5.63 | 144 | 3.8 |
| Ramsey | 28/7/98 | 8.2 | 170.0 | n.d. | 8.4 | 7.86 | 29.65 | 335 | 5.7 |
| Vermilion | 31/7/98 | 11.2 | 18.8 | 72.2 | 6.3 | 7.62 | 20.56 | 123 | 4.1 |
| Michiwakenda | 3/8/98 | 5.1 | 7.1 | n.d. | n.d. | 7.92 | 25.27 | 71 | 8.4 |
| Larder | 12/8/98 | 10.0 | 41.0 | n.d. | n.d. | 8.25 | 35.79 | 136 | 5.5 |
| Round | 12/8/98 | 4.5 | 9.8 | n.d. | n.d. | 8.41 | 43.77 | 150 | 7.8 |
| PWQO | | 5 | 25 | 75 | 20 | | | | |

n.d. = not detectable

* Shawn Eastwood, 1998.

** MOEE, 1998

Table 3. Size-age characteristics (Mean \pm S.E.) of yellow perch (*Perca flavescens*) samples taken from 7 Northeastern Ontario lakes. Sample size is indicated in parentheses.

| | Hannah (18) | Whitson (20) | Ramsey (20) | Vermilion (14) | Michiwakenda (9) | Larder (8) | Round (18) |
|-------------------------|------------------|------------------|------------------|-------------------|-------------------|-------------------|-------------------|
| Body Weight (g) | 32.04 \pm 4.25 | 13.49 \pm 0.37 | 54.53 \pm 7.25 | 58.63 \pm 14.41 | 89.35 \pm 23.49 | 72.63 \pm 17.43 | 72.90 \pm 11.84 |
| Fork Length (cm) | 13.80 \pm 0.58 | 10.85 \pm 0.13 | 15.83 \pm 0.74 | 15.10 \pm 1.06 | 17.29 \pm 1.56 | 16.61 \pm 0.97 | 16.86 \pm 0.74 |
| Age (yrs) | 2.74 \pm 0.10 | 2.00 \pm 0.19 | 2.90 \pm 0.20 | 2.93 \pm 0.27 | 3.22 \pm 0.55 | 2.75 \pm 0.31 | 2.56 \pm 0.18 |

samples examined. Mean fish age ranged from 2.00 ± 0.19 to 3.22 ± 0.55 for Whitson and Michiwakenda lakes respectively. Seventy-four percent of all captured yellow perch were either year class 2^+ or 3^+ . The remaining 26% of fish were year class 1^+ (9.0%), 4^+ (14.7%), 5^+ (2%) and 6^+ (0.1%) (Appendix 6).

3.3 Copper Bioaccumulation

Table 4 shows mean liver Cu concentrations and burdens from each lake. Liver Cu concentrations ranged from 5.58 ± 0.89 to 17.51 ± 4.67 (mean \pm S.E.) $\mu\text{g}\cdot\text{g}^{-1}$ wet weight for Round and Whitson lakes yellow perch respectively. Mean liver Cu concentrations from Hannah and Whitson lakes yellow perch were significantly higher than those from Vermilion and Round lakes (ANOVA) ($F_{(6,106)} = 5.78$; $p<0.0001$), however, no other significant differences were observed. The lack of significance between mean liver Cu contents among the 7 lake samples may suggest that Cu is under homeostatic control. Copper is an essential metal necessary for many metabolic processes and is therefore under strict homeostatic control (Chapman et al., 1996).

Miller et al. (1992) obtained similar results in a study where they examined tissue metal concentrations in white suckers from 2 reference lakes, 2 intermediate contaminated lakes, and 4 contaminated lakes in Northern Ontario. Liver Cu concentrations in their study ranged from 46.0 ± 9.0 to $98.0 \pm 11.0 \mu\text{g}\cdot\text{g}^{-1}$ dry for the least and most contaminated lakes respectively. In comparison, our mean liver Cu concentrations (converted to $\mu\text{g}\cdot\text{g}^{-1}$ dry wt) ranged from 23.90 ± 3.66 to 78.83 ± 21.30 for Round and Whitson lakes yellow perch respectively. Bradley and Morris (1986), Stripp et al. (1990), and Pourang (1995) have all

Table 4. Mean (S.E.) liver metal concentrations and burdens for 7 Northeastern Ontario yellow perch populations. Sample size is indicated in parentheses. Among lakes, mean values with different letters are significantly different from each other.

| | Cu | Ni | Al | Zn | Cu | Ni | Al | Zn |
|---------------------------|--|------------------|------------------|-------------------|--|------------------|-------------------|--------------------|
| | <u>Mean liver metal concentration ($\mu\text{g} \cdot \text{g}^{-1}$ wet)</u> | | | | <u>Liver burden (Total μg)</u> | | | |
| Hannah (n=18) | 14.05 ± 1.48 b | 4.10 ± 2.96 a | 2.64 ± 0.87 a | 28.84 ± 1.87 a | 5.19 ± 1.06 ab | 1.26 ± 0.17 b | 1.17 ± 0.42 ab | 11.22 ± 2.22 a |
| Whitson (n=20) | 17.51 ± 4.67 b | 2.52 ± 0.77 a | 1.83 ± 0.90 a | 35.87 ± 3.17 a | 3.06 ± 0.68 a | 0.55 ± 0.19 a | 0.29 ± 0.11 a | 6.72 ± 0.72 a |
| Ramsey (n=20) | 8.67 ± 0.76 ab | n.d. | n.d. | 22.55 ± 1.96 a | 8.03 ± 1.08 b | n.d. | n.d. | 24.80 ± 5.22 a |
| Vermilion (n=14) | 6.07 ± 1.71 a | n.d. | 2.09 ± 0.34 a | 25.79 ± 1.81 a | 6.26 ± 3.39 ab | n.d. | 1.52 ± 0.46 ab | 18.45 ± 5.02 a |
| Michiwakenda (n=9) | 12.85 ± 2.90 ab | n.d. | 2.89 ± 0.47 a | 26.90 ± 2.92 a | 18.35 ± 7.58 b | n.d. | 3.59 ± 1.13 b | 35.72 ± 13.17 a |
| Larder (n=8) | 6.94 ± 1.29 ab | n.d. | 2.29 ± 0.95 a | n.d. | 5.27 ± 3.11 ab | n.d. | 0.66 ± 0.26 ab | n.d. |
| Round (n=18) | 5.58 ± 0.89 a | n.d. | 1.12 ± 0.46 a | n.d. | 4.00 ± 1.00 ab | n.d. | 0.55 ± 0.25 a | n.d. |

n.d. = not detectable

demonstrated similar liver Cu concentrations in yellow perch and other carnivorous and detritivorous fish from contaminated and non-contaminated sites throughout North America.

Many field (Ellenberger et al., 1994; Swales et al., 1998) and laboratory (Anderson and Spear, 1980; Collvin, 1984; Boeck et al., 1997; Baker et al., 1998) studies have demonstrated the deleterious effects of Cu on fish condition. Since Sudbury area fish are subjected to elevated Cu levels (Table 2), this metal is a prime candidate for examination of its influence on fish condition.

Regression analysis (Figure 2a,b) showed that liver Cu concentration was not significantly associated with fork length ($p>0.05$) or total weight ($p>0.05$) in the entire fish sample ($n=107$). This demonstrates that no allometric scaling of liver Cu concentrations existed in the overall yellow perch sample ($n=107$). Six of the 7 yellow perch samples exhibited similar results (Table 5). The Vermilion Lake sample was the only exception (Figure 3a,b). Log liver Cu concentrations in fish from Vermilion lake were positively correlated with log fork length and log body weight ($R^2 = 0.43$; $F_{(1,12)} = 8.21$; $p<0.05$ and $R^2 = 0.44$; $F_{(1,12)} = 8.62$; $p<0.05$, respectively). Lack of correlation between tissue Cu concentrations and fish length and weight have also been reported by several other researchers studying the impact of metals in fish (Cross et al., 1973; Pourang, 1995). In contrast, Koli et al. (1978) and Linde et al. (1996) found liver Cu concentrations to be positively associated with fish length and age respectively, as was observed in Vermilion lake fish (Figure 3a,b). No reports of negative relationships between tissue Cu content and fish size were found in the literature. This suggests that a

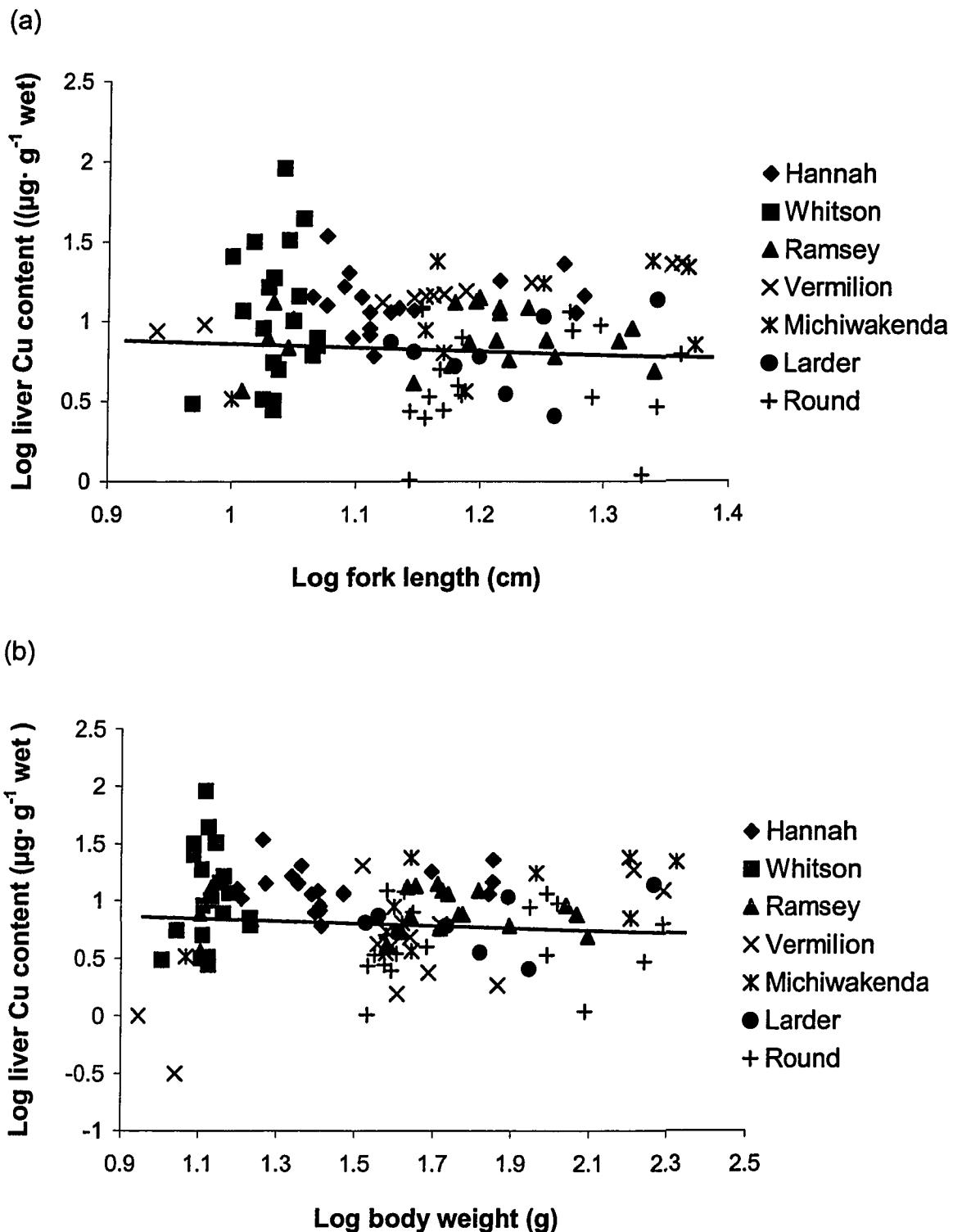


Figure 2. Relationship between log liver Cu concentrations ($\mu\text{g} \cdot \text{g}^{-1}$ wet) and (a) log fork length (cm) ($p>0.05$; $n=107$) and (b) log body weight (g) ($p>0.05$; $n=107$) in yellow perch sampled from 7 lakes in Northeastern Ontario during the fall of 1997.

Table 5. Regression coefficient and F ratio values for the relationships between log liver Cu content and log body weight (g) and log fork length (cm) in yellow perch populations in the 7 lakes examined in the fall of 1997. Significant associations are indicated by asterisks ($p<0.05$). Sample size is indicated in parentheses.

| Lake | log Cu content vs log body weight | log Cu content vs log fork length |
|--------------------|-----------------------------------|-----------------------------------|
| Hannah (n=18) | $F = 0.26$ $R^2 = 0.01$ | $F = 0.41$ $R^2 = 0.02$ |
| Whitson (n=20) | $F = 0.104$ $R^2 = 0.006$ | $F = 0.37$ $R^2 = 0.02$ |
| Ramsey (n=20) | $F = 0.09$ $R^2 = 0.005$ | $F = 0.13$ $R^2 = 0.007$ |
| Vermilion (n=14) | $F = 8.62 *$ $R^2 = 0.44$ | $F = 8.21 *$ $R^2 = 0.43$ |
| Michiwakenda (n=9) | $F = 5.09$ $R^2 = 0.42$ | $F = 3.37$ $R^2 = 0.32$ |
| Larder (n=8) | $F = 0.49$ $R^2 = 0.075$ | $F = 0.39$ $R^2 = 0.061$ |
| Round (n=18) | $F = 0.153$ $R^2 = 0.009$ | $F = 0.029$ $R^2 = 0.002$ |

* $p<0.05$

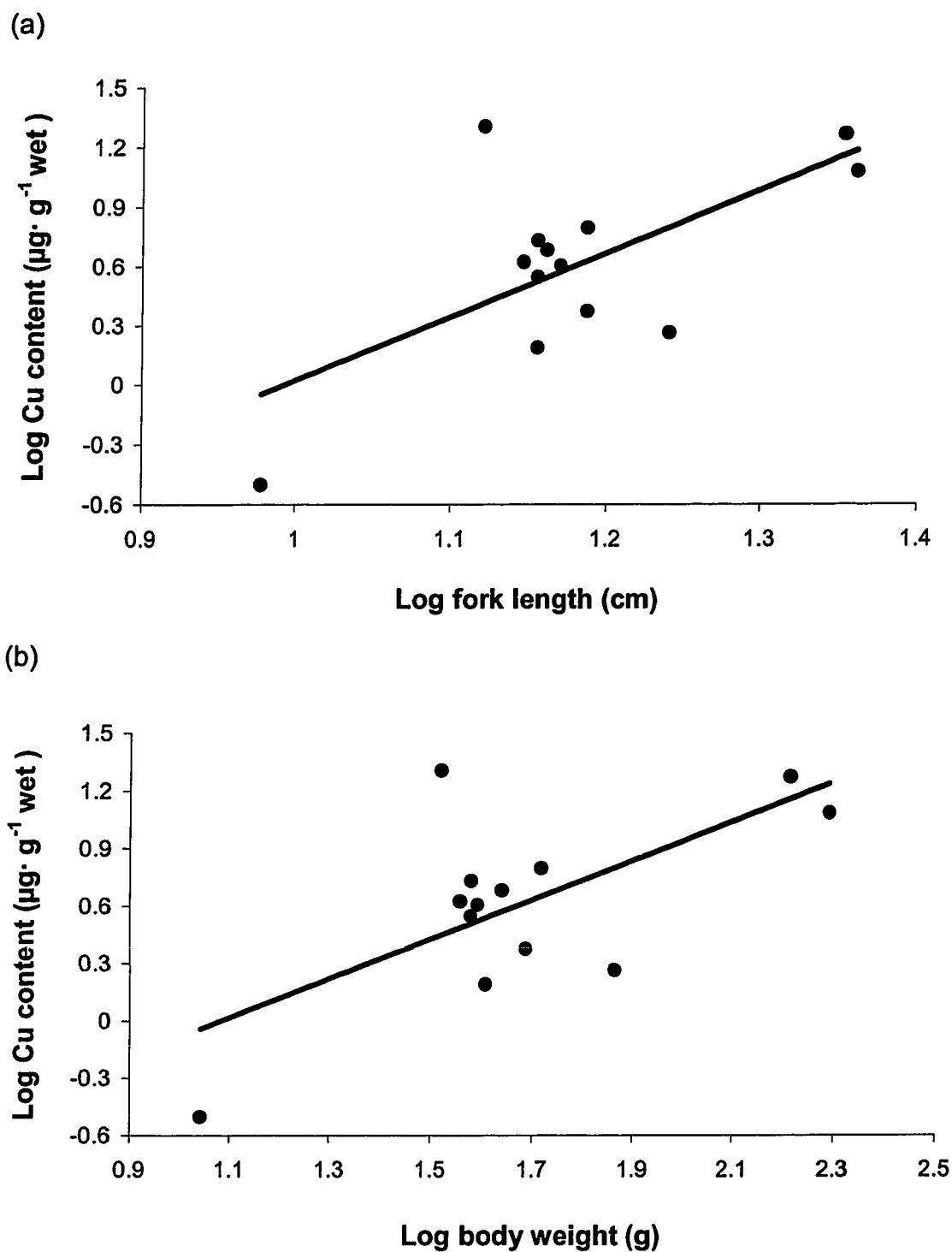


Figure 3. Relationship between log liver Cu contents ($\mu\text{g} \cdot \text{g}^{-1}$ wet) and (a) log fork length (cm) ($F_{(1,12)} = 8.62$; $p < 0.05$) and (b) log body weight (g) ($F_{(1,12)} = 8.21$; $p < 0.05$), in yellow perch sampled from Vermilion lake during the fall of 1997.

growth related 'dilution effect' among the 7 examined yellow perch populations does not exist under natural environmental conditions. Because this study will examine possible relationships between fish condition and liver metal contents (Section 3.7), it was essential to establish whether or not liver metal concentrations varied systematically with fish size. If a dilution effect (decreasing metal concentrations in larger fish due to a higher relative accumulation of anabolic products) is natural in yellow perch, we could wrongly interpret a negative association between various indicators of condition (most of which have been calculated using weight and/or length values) and liver metal concentrations. This would lead to the false interpretation that high liver metal concentrations are responsible for negatively influencing condition.

Regression analyses demonstrated a significant positive correlation between liver Cu burden and fish body weight ($R^2 = 0.39$; $F_{(1,106)} = 63.70$; $p < 0.01$) as displayed in Figure 4. This figure illustrates that yellow perch accumulate Cu as they become larger with no change in concentration (see above). As these fish become larger, their livers increase in size and in Cu burden in a proportional fashion. Although Hannah and Whitson lakes mean liver Cu concentrations were not significantly higher than the remaining 5 lake samples, they did contain the highest Cu levels overall. These fish were the smallest in both fork length and body weight (Table 3), yet exhibited higher liver Cu burdens for their size. Fish from our lesser contaminated lakes (Vermilion, Ramsey, Larder, Round, and Michiwakenda) demonstrated liver Cu burdens that were distributed evenly along the burden/body weight trendline. The higher Cu burden values (in relation to

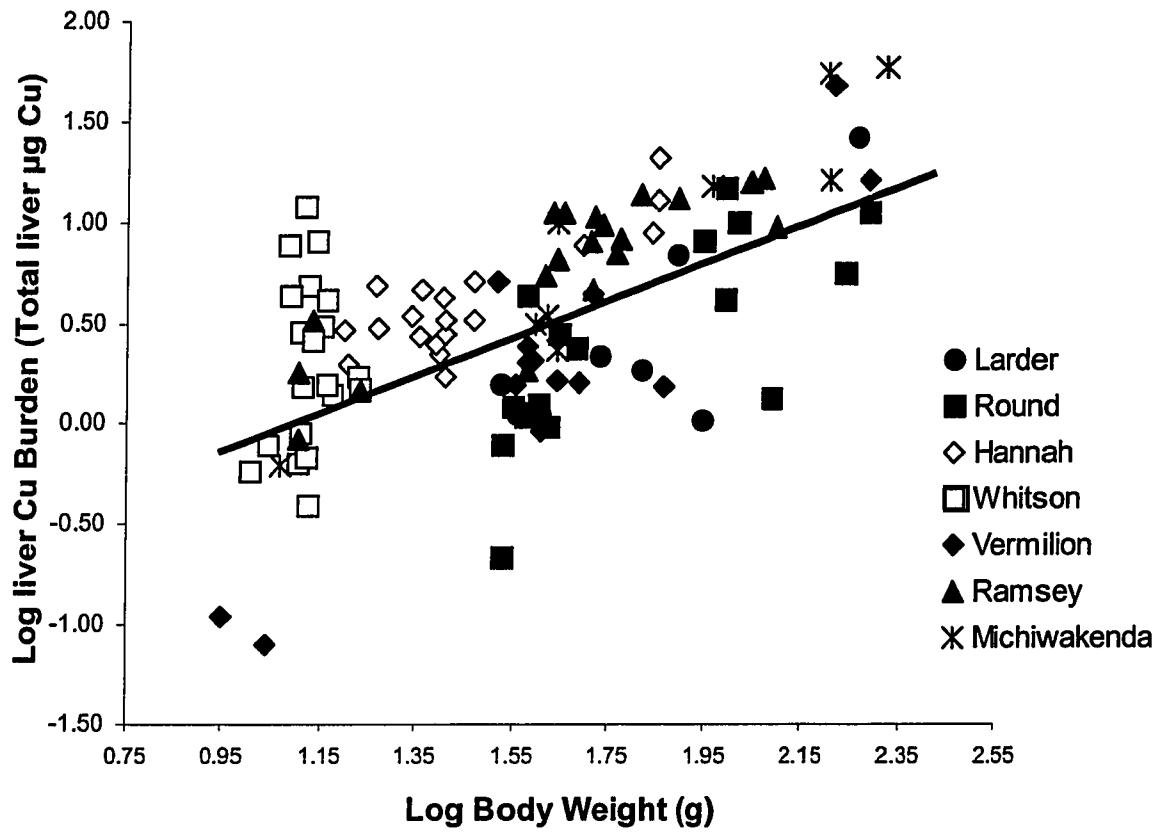


Figure 4. Relationship between log body weight (g) and log liver Cu burden (Total liver µg Cu) ($R^2 = 0.39$; $F_{(1,106)} = 63.70$; $p < 0.0001$).

size as seen in Figure 4) seen in Hannah and Whitson lake fish account for the higher liver Cu concentrations reported in Table 4. Although metal burden allows for the examination of total metal load within a particular tissue, it does not consider size differences between and within each lake liver sample. Liver metal concentrations compensate for differences in liver size, making this measurement more appropriate in the examination of fish condition with varying levels of trace metal concentrations. In addition, toxicological effects at the cellular level would be expected to be directly dose or concentration dependent.

Figure 5 illustrates the relationship between water Cu concentrations (Table 2) and mean liver Cu concentrations (Table 4) in the 7 lakes examined in this study. Pearson correlation analysis showed that water Cu concentrations were positively associated with mean liver Cu concentrations ($r = 0.76$; $p < 0.05$; $n = 7$). Bioconcentration (the contribution to bioaccumulation that results from aqueous exposure and is taken up by the gills) is one of the many factors that can contribute to the bioaccumulation of trace metals in fish under natural (Miller et al., 1992) and laboratory (Anderson and Spear, 1980; Collvin, 1984) conditions. These results suggest that yellow perch subject to higher water Cu concentrations bioaccumulate more liver Cu, regardless of their size. If Hannah and Whitson Lakes were removed from this relationship it would no longer be significant at the 5% probability level. Yellow perch from our other 5 study lakes are probably within their homeostatic control capacities with regards to tissue Cu loads. This suggests that the bioaccumulation of Cu may only occur at higher water Cu concentrations. Other important factors that influence the

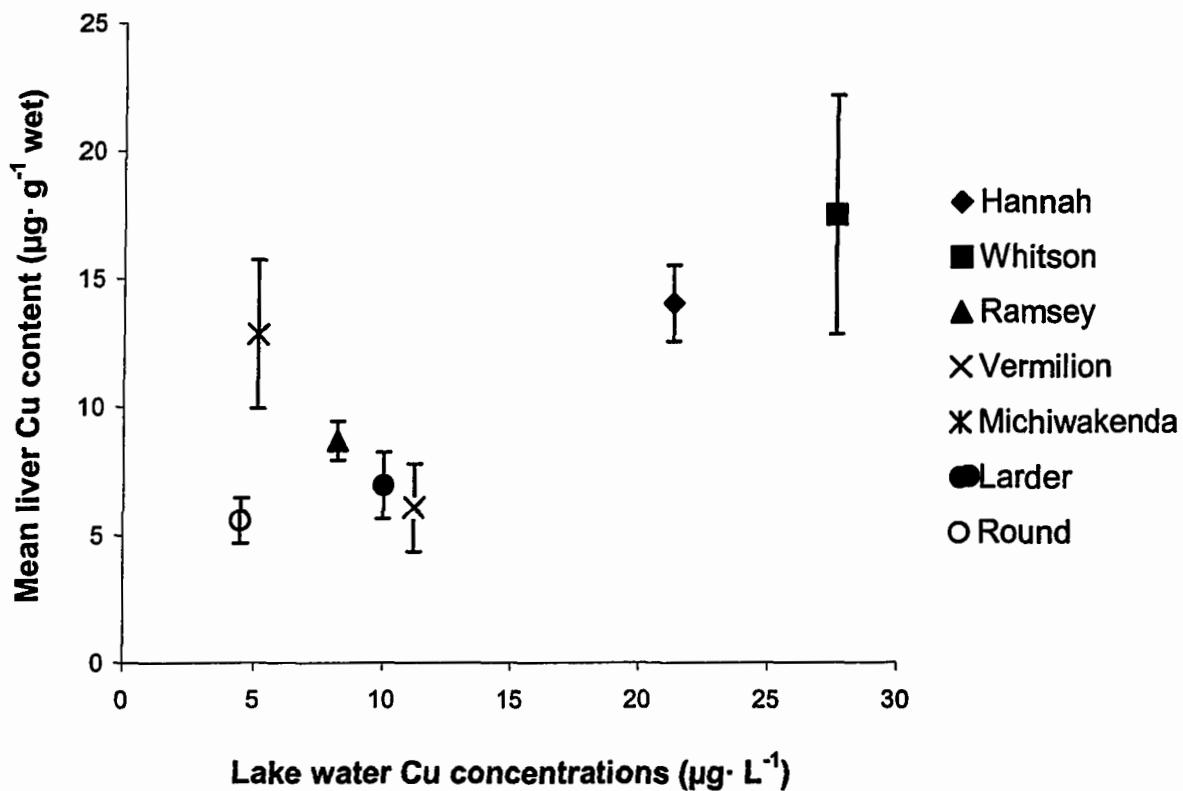


Figure 5. Relationship between lake water Cu concentration (1998) ($\mu\text{g}\cdot\text{L}^{-1}$) and mean liver Cu concentrations (1997) ($\mu\text{g}\cdot\text{g}^{-1}$ wet) in yellow perch from 7 Northeastern Ontario lakes. $r = 0.76$; $p < 0.05$; $n = 7$.

bioaccumulation of trace metals by fish are, feeding behavior, growth rate, temperature, hardness, salinity, dissolved organic carbon, age, and pH (Mance, 1987). The extent to which these factors affect the bioaccumulation of Cu in the fish in this study are unknown. Although water Cu concentrations are not entirely responsible for the bioaccumulation of trace metals in aquatic organisms, they are one of the most important parameters (Ellenberger, et al., 1994; Pourang, 1995; Chapman et al., 1996; Boeck et al., 1997), a second being diet (Hammar et al., 1993; Chapman et al., 1996).

3.4 Nickel, Aluminum, and Zinc Bioaccumulation

In my study, liver Ni levels were below detection limits ($2.25 \mu\text{g} \cdot \text{g}^{-1}$ wet), with the exception of Hannah and Whitson lakes fish (Table 4). In these fish, Ni concentrations were approaching detection limits. Ni may not bioaccumulate within the livers of fish in their natural habitat. Nickel is believed to be accumulated preferentially by kidney tissue (N.R.C.C., 1981b). Bradley and Morris (1986) also found similar results. In their study, yellow perch liver Ni levels were below detection limits for 8 of 10 lakes in the Sudbury region. In lakes that did contain detectable levels, liver Ni concentrations ranged between 2.2 and 2.9 $\mu\text{g} \cdot \text{g}^{-1}$ dry, considerably less than liver Cu and Zn concentrations (5-185 and 80-170 $\mu\text{g} \cdot \text{g}^{-1}$ dry, respectively) which were also examined. Mean liver Ni concentrations (converted to $\mu\text{g} \cdot \text{g}^{-1}$ dry) from my study ranged from 9.3 ± 1.85 to 20.5 ± 14.8 for Whitson and Hannah lakes yellow perch respectively. Although these values were somewhat higher than those found in the Bradley and Morris

study (1986), our mean liver Ni levels were also considerably lower than liver Cu and Zn concentrations (30-88 and 113-180 $\mu\text{g}\cdot\text{g}^{-1}$ dry, respectively).

Mean liver Al concentrations ranged from 1.12 ± 0.46 to $2.89 \pm 0.47 \mu\text{g}\cdot\text{g}^{-1}$ wet in yellow perch from Round and Michiwakenda lakes respectively. These values are lower than I found in Laurentian, Clear, Hannah, and Falconbridge lakes during the fall of 1996 (Appendix 7) and lower than those observed by Yeardley et al. (1998) and Heiny and Tate (1997) from non-contaminated sites throughout the United States. Water Al levels were not detectable among 4 of the 7 study lakes (Table 2). The 3 Sudbury area lakes that did exhibit measurable Al concentrations were Hannah, Whitson, and Vermilion lakes. Mean liver Al concentrations were also very low (approaching non-detectable concentrations) in my 7 lake samples (Ramsey lake fish exhibited non-detectable liver Al concentrations) (Table 4) and showed no relationship to smelter proximity and fish condition. However, short-lived acidification events (pulses) associated with snowmelt and intense rain storms can severely stress fish populations (Dillon et al., 1984; Harvey and Whelpdale, 1986). Adverse effects of acid pulses on fishes are not only attributable to increased concentrations of hydrogen ions but also to the mobilization of metals such as aluminum, manganese, zinc, lead, and copper (Baker, 1982). Although the results of my study suggests that Al is not a problem contaminant in these circumneutral lakes, the influence of Al on fish condition during such events (acid pulses) has not been determined in this study.

Liver Zn concentrations ranged from 22.6 ± 2.0 to $35.9 \pm 3.2 \mu\text{g}\cdot\text{g}^{-1}$ in all lakes except Larder and Round lakes where Zn levels were below detection

limits. No statistical differences were detected among the lakes with Zn present. These values are similar to those obtained in the livers of yellow perch for Sudbury lakes studied by Bradley and Morris (1986). Similar results have been found in the threespine stickleback and the Sacramento sucker (Saiki et al., 1995), white sucker (*Catostomus commersoni*) (Miller et al., 1992), and various other species of fish in both contaminated and non-contaminated lakes in the lower (Schmitt and Brumbaugh, 1990) and Northeastern (Yeardley et al., 1998) United States. Although liver Zn concentrations resembled levels normally found in fish not influenced by anthropogenic sources, it has been shown that fresh water adapted fish can become acclimatized to elevated Zn levels without any detectable increase of Zn accumulation in the gills or liver (Hogstrand and Wood, 1995). Zinc is likely under homeostatic control resulting in the narrow Zn range observed in the fish examined in this study.

Figure 6 shows the relationship between log liver Zn concentrations and log fork length (cm) and log body weight (g) for the entire fish sample (n=81) (Larder and Round lake fish were not included due to non-detectable Zn concentrations). These results are similar to those found in section 3.3. (Cu Bioaccumulation). Liver Zn concentrations showed no allometric scaling with fish size. Within-lake associations (Table 6) demonstrated similar results with the exception of Ramsey lake. Log liver Zn concentrations were positively associated with log fork length and log body weight in Ramsey lake fish ($R^2 = 0.57$; $F_{(1,19)} = 24.0$; $p < 0.01$; and $R^2 = 0.52$; $F_{(1,19)} = 19.41$; $p < 0.01$, respectively). These relationships (Table 6) were necessary to discount a growth related 'dilution

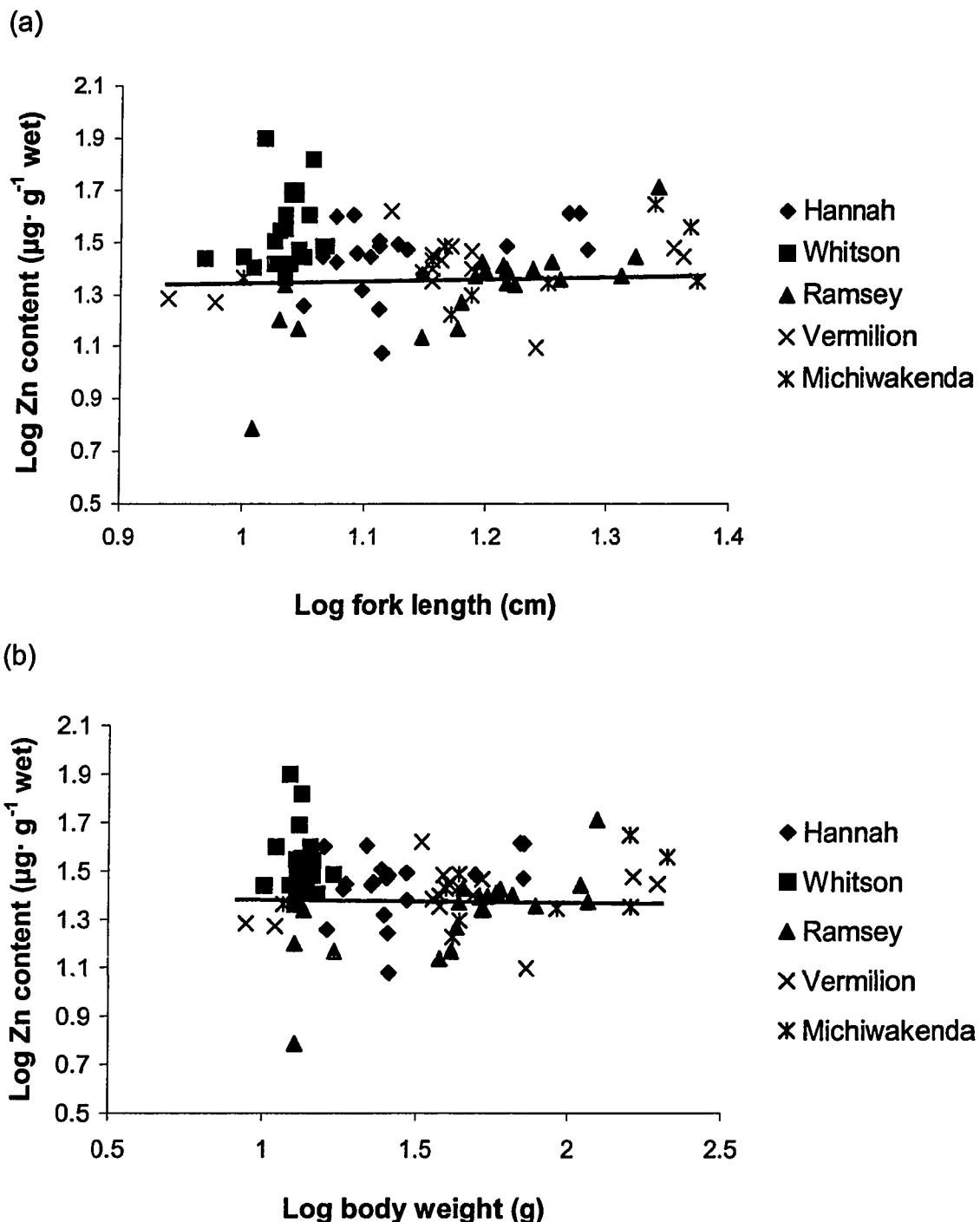


Figure 6. Relationship between log liver Zn concentrations ($\mu\text{g} \cdot \text{g}^{-1}$ wet) and log fork length (cm) ($p>0.05$; $n=81$) and (b) log body weight (g) ($p>0.05$; $n=81$) in yellow perch sampled from 5 lakes in Northeastern Ontario during the fall of 1997.

Table 6. Regression coefficient and F ratio values for the relationships between log liver Zn content and log body weight (g) and log fork length (cm) in yellow perch populations from 5 lakes examined in the fall of 1997. Significant associations are indicated by asterisks ($p<0.05$). Sample size is indicated in parentheses.

| Lake | log Zn content vs log body weight | log Zn content vs log fork length |
|--------------------|-----------------------------------|-----------------------------------|
| Hannah (n=18) | $F = 0.197$ $R^2 = 0.10$ | $F = 2.71$ $R^2 = 0.14$ |
| Whitson (n=20) | $F = 0.33$ $R^2 = 0.018$ | $F = 0.39$ $R^2 = 0.021$ |
| Ramsey (n=20) | $F = 19.41$ * | $F = 24.01$ * |
| | $R^2 = 0.52$ | $R^2 = 0.57$ |
| Vermilion (n=14) | $F = 0.73$ $R^2 = 0.06$ | $F = 0.68$ $R^2 = 0.054$ |
| Michiwakenda (n=9) | $F = 2.18$ $R^2 = 0.24$ | $F = 1.65$ $R^2 = 0.19$ |
| Larder (n=8) | Insufficient data | Insufficient data |
| Round (n=18) | Insufficient data | Insufficient data |

* $p<0.05$

'effect' in the fish samples, which may have lead to a false interpretation of lowered fish condition in response to higher liver Zn concentrations.

3.5 Comparison of Condition Indicators Between Lake Samples

The means of K_n , HSI, and liver water and protein contents for the 7 lake samples are reported in Table 7. Significant differences between indicators of condition were determined by a K-W analysis of variance test with a non-parametric multiple comparison test for equal and unequal sample sizes. Hannah and Whitson lakes fish exhibited K_n values that were significantly lower than the Vermilion and Larder lake yellow perch, however, they were not significantly different from the other 3 lake samples. Fish with a high K_n are heavier for their length compared to fish with a low K_n . The relative condition factor reflects large changes in energy reserves (lipid, protein, glycogen, and total energy) occurring over extended periods of time (Chellappa et al., 1995), and is therefore an indirect measure of growth in fish. The lower mean body weight and fork length values (Table 3) for Hannah and Whitson lakes fish samples in conjunction with their lower K_n support lower growth. Hannah and Whitson lakes are two of the most contaminated lakes in this study based on liver metal residues (Table 4) and water metal concentrations (Table 2). The other 5 lakes exhibit a wide range of metal levels of which Round lake is the least contaminated. The lower K_n and weight and length values in Hannah and Whitson lakes fish samples are indicative of fish whose energy reserves had been utilized for physiological processes other than growth. The physiological changes permitting metal

Table 7. Comparison of condition indicators for yellow perch captured from our 7 study lakes in the fall of 1997. Sample size is indicated in parentheses. Sample means \pm 1 S.E. are indicated. Within columns, mean values with different letters are significantly different from each other ($p < 0.05$) based on the K-W oneway analysis of variance test with a non-parametric multiple comparison test for equal and unequal sample sizes.

| Lakes | Kn | HSI | mg H ₂ O g ⁻¹ wet liver | mg PRT g ⁻¹ |
|---------------------------|-----------------|-----------------|---|------------------------|
| | | | (scaled to body weight) | wet liver |
| Hannah (n=18) | 5.04 \pm 0.12 | 1.13 \pm 0.05 | 732 \pm 7.5 | 127 \pm 9.0 |
| | a | ac | a | a |
| Whitson (n=20) | 5.08 \pm 0.16 | 1.42 \pm 0.08 | 780 \pm 4.8 | 141 \pm 5.0 |
| | a | bc | b | a |
| Ramsey (n=20) | 5.20 \pm 0.11 | 1.75 \pm 0.09 | 770 \pm 5.1 | 134 \pm 11.3 |
| | ab | b | b | a |
| Vermilion (n=14) | 6.00 \pm 0.12 | 1.42 \pm 0.19 | 793 \pm 6.1 | 121 \pm 4.6 |
| | c | bcd | b | a |
| Michiwakenda (n=9) | 5.78 \pm 0.21 | 1.26 \pm 0.09 | 779 \pm 7.6 | 129 \pm 7.8 |
| | abc | ab | b | a |
| Larder (n=8) | 5.92 \pm 0.20 | 0.68 \pm 0.08 | 768 \pm 8.1 | 124 \pm 4.2 |
| | bc | a | b | a |
| Round (n=18) | 5.56 \pm 0.11 | 0.94 \pm 0.06 | 769 \pm 5.4 | 124 \pm 2.8 |
| | abc | ab | b | a |

detoxification and homeostasis cost energy and reduced growth caused by exposure to elevated trace metal concentrations has been attributed to metabolic demands associated with metal detoxification and excretion processes (Hogstrand et al., 1995). Other studies examining the effects of Cu and Zn on brown (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) condition have demonstrated similar results (Marr et al., 1995; Linde et al., 1996).

The hepatosomatic indices that are reported in Table 7 showed no apparent pattern in relation to the level of contamination seen in the 7 study lakes (Table 4). Although the HSI is considered by some authors to be a reliable indicator of the energy status of fish (Wootton et al., 1978; Campbell and Love, 1978), it is known to vary spatially and seasonally with climate changes. As such, the HSI is not an appropriate indicator of condition for comparative purposes between fish populations (this will be further discussed in the following section).

Although the mean liver water content from Hannah Lake yellow perch was significantly lower than for the other 6 lake samples, all 7 mean water contents were quite similar (Table 7) and portrayed a range of values comparable to those found in the three-spined stickleback (*Gasterosteus aculeatus*) (Chellappa et al., 1995). No differences were found between mean liver protein contents among the 7 lake samples (Table 7). Yellow perch living in the wild appear to have an ability to maintain a stable liver composition. These parameters are likely under careful homeostatic control, which would explain the narrow range in mean water and protein contents observed.

Scaling coefficients for our 7 yellow perch samples are illustrated in Figure 7. In this study, scaling coefficients were used to comparatively illustrate lower physiological condition in relation to varying levels of trace metal exposure and liver metal residues. Hannah and Whitson lake fish were the only two lake samples that demonstrated scaling coefficients that were less than 3. The Hannah and Whitson lake fish scaling coefficients were significantly lower than the other 5 lake samples. Furthermore, the scaling coefficient from the Whitson lake yellow perch sample was significantly lower than the Hannah lake sample. The scaling coefficients from the remaining 5 lake samples are similar to those found in a study by Willis and Guy (1991). In this study, weight and length data for 78 natural populations of yellow perch in 20 states and 6 Canadian provinces were used to develop a standard weight equation. The scaling coefficient that best described these range of lakes was 3.23. This is similar to the scaling coefficient obtained for the entire fish sample in my study (3.31). Furthermore, the scaling coefficients from Hannah and Whitson lakes were very low (lower 5% range) in comparison to those from this study. Yellow perch from these two lakes do not appear to grow in the same manner as other yellow perch populations throughout North America. Elevated trace metals may be responsible for this lowered condition and will be discussed in Section 3.7.

3.6 Relationship Between Metals and Condition in Pooled Yellow Perch

Of the 4 metals examined in this study, only liver Cu concentrations were found in elevated concentrations that were similar to those found in contaminated

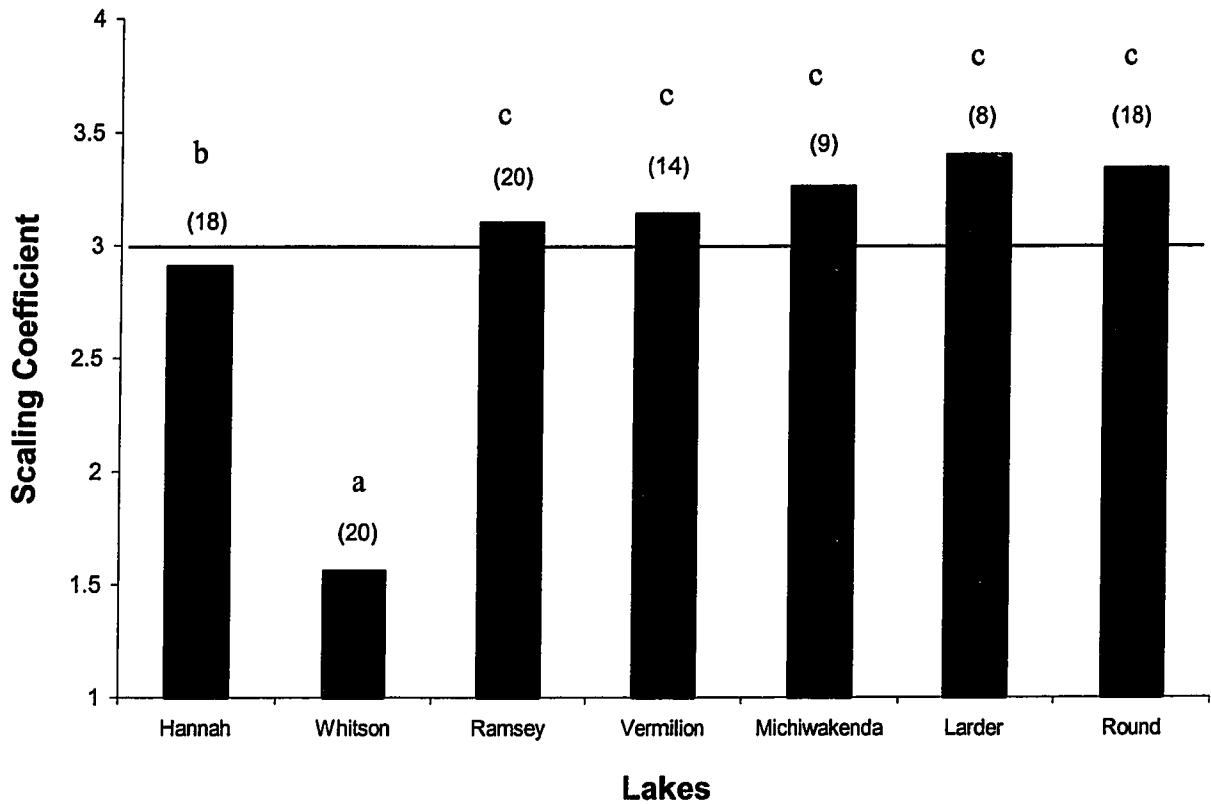


Figure 7. Scaling coefficients for yellow perch in the seven lakes investigated during the fall of 1997. Sample size is indicated in parentheses. See methods for the derivation of these values. The line intersecting at coefficient = 3 reflects conditions in fish that do not change their shape as they grow (isometric growth). Yellow perch with a scaling coefficient below this line are fish that become leaner as they grow in length.

fish from other ecotoxicological studies (Miller et al., 1992; Ellenberger et al., 1994). Unlike liver Cu concentrations, Ni and Al did not appreciably accumulate in the livers of these fish (Table 4), nor did they correlate with any indicators of condition examined in this study. Consequently, all further discussion will be focused on liver Cu and Zn concentrations and their relationships with the condition of yellow perch.

Initially, no relationships involving liver Zn concentration and our indicators of condition were found. However, because liver Cu concentration was positively correlated ($r = 0.49$; $p < 0.001$) with liver Zn concentration (Figure 8), it was necessary to remove any shared variation among these two variables (remove the masking effect of Cu on Zn) to further examine relationships between liver Zn concentrations and condition. Following partial correlation analyses (effects of Cu held constant), liver Zn concentration was found to be negatively associated with K_n and positively correlated with liver protein content (Table 8). Liver Zn content was also negatively correlated with K_n in Ramsey Lake fish, but no other relationships between liver Zn and other indicators of condition were found in the other lake samples.

Zinc is an important essential trace metal, necessary for the function of various enzymes, including various metalloenzymes. Metallothioneins (MT) (metal detoxifying proteins) can be induced by the presence of elevated Zn to bind and detoxify it (Kägi and Kojima, 1987; Stegeman et al., 1992; George et al., 1996). Elevated levels of Zn have been shown to induce metallothionein production in the liver (Udom and Brady, 1980; Cousins, 1985). The positive

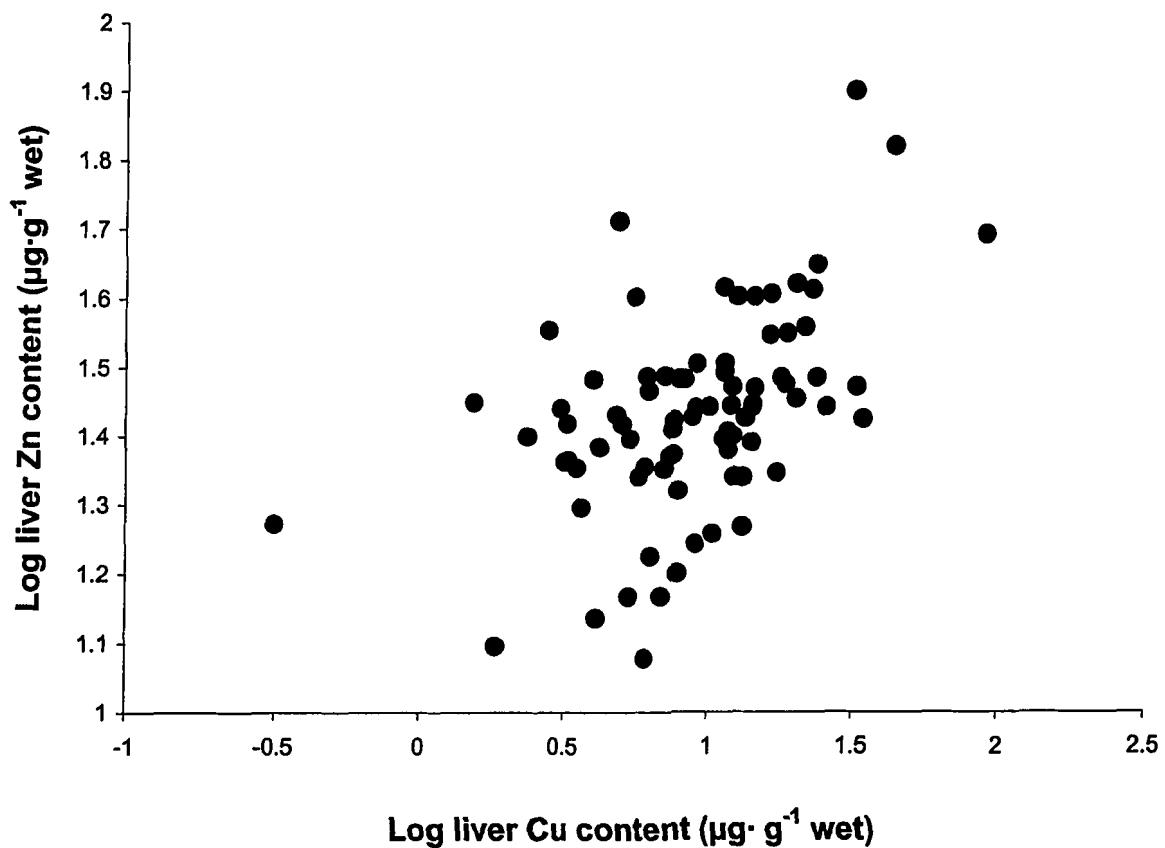


Figure 8. Relationship between log liver Cu and log liver Zn concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ wet) ($r = 0.49$; $p < 0.001$; $n = 81$) in yellow perch captured from 5 Northeastern Ontario lakes in the fall of 1997. Larder and Round lakes fish were excluded from this relationship due to non-detectable levels of Zn (Table 4).

Table 8. Partial correlation coefficients for liver Cu (statistically controlling for Zn) and Zn (statistically controlling for Cu) contents with Kn, HSI, and liver protein (mg Prt g⁻¹ wet liver) contents in pooled yellow perch and yellow perch captured from Ramsey, Vermilion and Michiwakenda lakes in the fall of 1997. No significant relationships were found in other lakes. Sample size is indicated in parentheses.

| | | K _n | HSI | Protein |
|-------------------------------|----|----------------------|----------------------|--------------------|
| POOLED YELLOW PERCH (n=72) | Cu | n.s. | r = -0.31 p<0.005 | n.s. |
| | Zn | r = -0.30 p<0.01 | n.s. | r = 0.20 p<0.05 |
| RAMSEY (n = 17) | Cu | n.s. | n.s. | n.s. |
| | Zn | r = -0.61 P<0.005 | n.s. | n.s. |
| VERMILION (n = 9) | Cu | n.s. | r = -0.64 p<0.05 | n.s. |
| | Zn | n.s. | n.s. | n.s. |
| MICHIWAKENDA (n = 6) | Cu | n.s. | r = -0.69 p<0.05 | n.s. |
| | Zn | n.s. | n.s. | n.s. |

n.s. = not significant

relationship between liver Zn concentrations and liver protein contents may suggest that an increased synthesis of metallothionein proteins has occurred to sequester Zn in fish exposed to sublethal Zn concentrations. In 1999, Laflamme showed that liver concentrations were higher in yellow perch from metal-polluted lakes in Abitibi. Sherwood et al. (2000) suggested that the synthesis of MT might directly or indirectly involve a significant metabolic expenditure which could lead to the energetic impairment of yellow perch in metal-polluted environments.

The uptake of essential metals like Zn is a natural process required by organisms for metabolism and growth (Chapman et al., 1996). The concentration range of essential metals can vary substantially between organisms, but optimal concentration ranges are often narrow and frequently under careful homeostatic control (Leland and Kuwabara, 1985). Essential metals that exceed this range must be actively excreted, compartmentalized in cells or tissues, or metabolically immobilized, otherwise, toxic effects will occur (Chapman et al., 1996). The major organ for the sequestration of elevated trace metals in fish is the liver (George et al., 1996). The aforementioned processes that deal with excess metals in fish, consume important energy reserves necessary for the growth of tissues (Marr et al., 1995). This energy expenditure can be seen in the relationship between liver Zn and K_n (Table 8). The relative condition factor is an indirect indicator of energy expenditure in fish as it measures the 'girth' of fish and hence, tissue growth or lack thereof. Furthermore, liver protein content was negatively correlated with K_n (Figure 9). This relationship further supports the contention that yellow perch exposed to higher liver Zn levels were deleteriously influenced (exhibited a lower

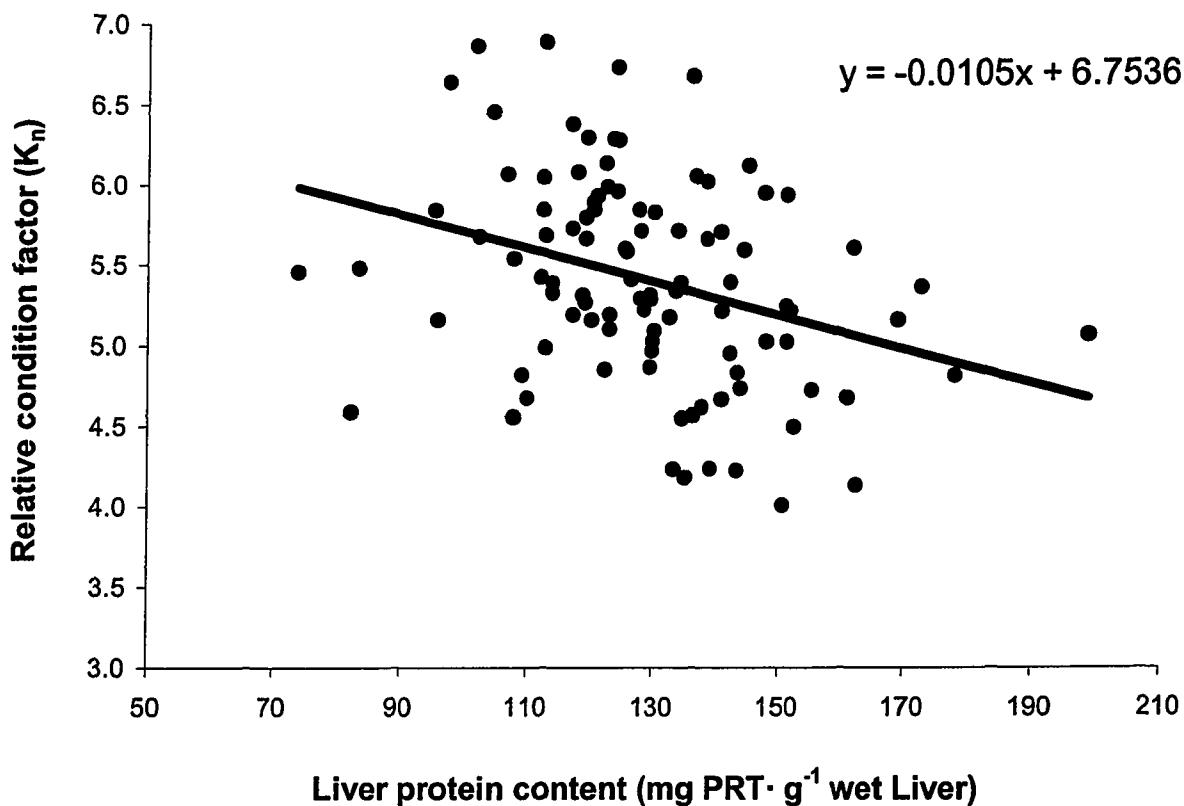


Figure 9. Relationship between liver protein content (mg PRT · g⁻¹ wet liver) and the relative condition factor in yellow perch captured from 7 Northeastern Ontario lakes in the fall of 1997. r = -0.33; p<0.001; n = 98. Some fish were not included in this relationship due to unavailable protein data.

K_n due to increased long-term energy expenditures involving increased liver protein synthesis either through increased metallothionein production or, possibly through higher protein turnover due to tissue damage by elevated Zn levels (Gwozdinski, 1992).

Partial correlation analyses were also performed on Cu (effects of Zn held constant) with our indicators of condition. The results from these analyses are reported in Table 8. Liver Cu concentration was only associated with one of the 4 (K_n , HSI, and liver protein and H₂O contents) measures of condition after partial correlation analyses were completed. Liver Cu concentrations were negatively correlated with HSI in the pooled sample as well as the Vermilion and Michiwakenda lake samples. Although these results suggest lowered yellow perch condition in response to elevated Cu exposure, I have come to the conclusion that the HSI was not an appropriate indicator of condition in assessing fish condition in the wild. This conclusion was based on two factors. First, elevated environmental metal exposure is known to increase the production of metallothioneins (a sequestering protein) in the liver. Even though transitory changes in liver size are largely due to variations in glycogen and fat content (Heath, 1984), an increase in liver protein content (elevated metallothionein concentrations) and a decrease in glycogen and/or fat content in response to increased trace metal exposure would cause difficulties in the interpretation of HSI measurements. To appropriately interpret the HSI, one must measure a variety of parameters that include: liver water, protein, glycogen, carbohydrate, and lipid contents as well as metallothionein and various metalloenzyme

concentrations in the liver. Although these parameters would help interpret changes in HSI with respect to varying levels of environmental metal concentrations, obtaining this information would defeat the original purpose of the HSI, which is to provide a quick, general assessment of fish condition. Second, the hepatosomatic index can vary seasonally and spatially (Dutil et al., 1995) which makes it difficult to obtain an unbiased sample. The HSI may not be an appropriate indicator of condition due to constant changes between and within study lakes over time.

3.7 Relationship Between the Scaling Coefficient and Liver Cu and Zn Concentrations

Figure 10 illustrates negative correlations between mean liver Cu and Zn concentrations and scaling coefficients. These relationships suggest that growth impairment has occurred in fish that have bioaccumulated Cu and Zn from their respective habitats. The scaling coefficient is a measure of the growth pattern in fish. Fish that exhibit a scaling coefficient of less than 3 will become leaner as they grow in length (ie., Hannah and Whitson lake fish). To add further support to this relationship, length, weight, and water metal data were obtained from Crowley, Camp, Linton, and Middle lakes in the summer of 1992 in a study by Wright (1995). These results demonstrate a significant negative association between water Cu and Zn concentrations and the scaling coefficients of yellow perch (Figure 11).

In addition, Brodeur et al. (1997) demonstrated that yellow perch from metal-polluted lakes were chronically stressed and exhibited an impaired cortisol

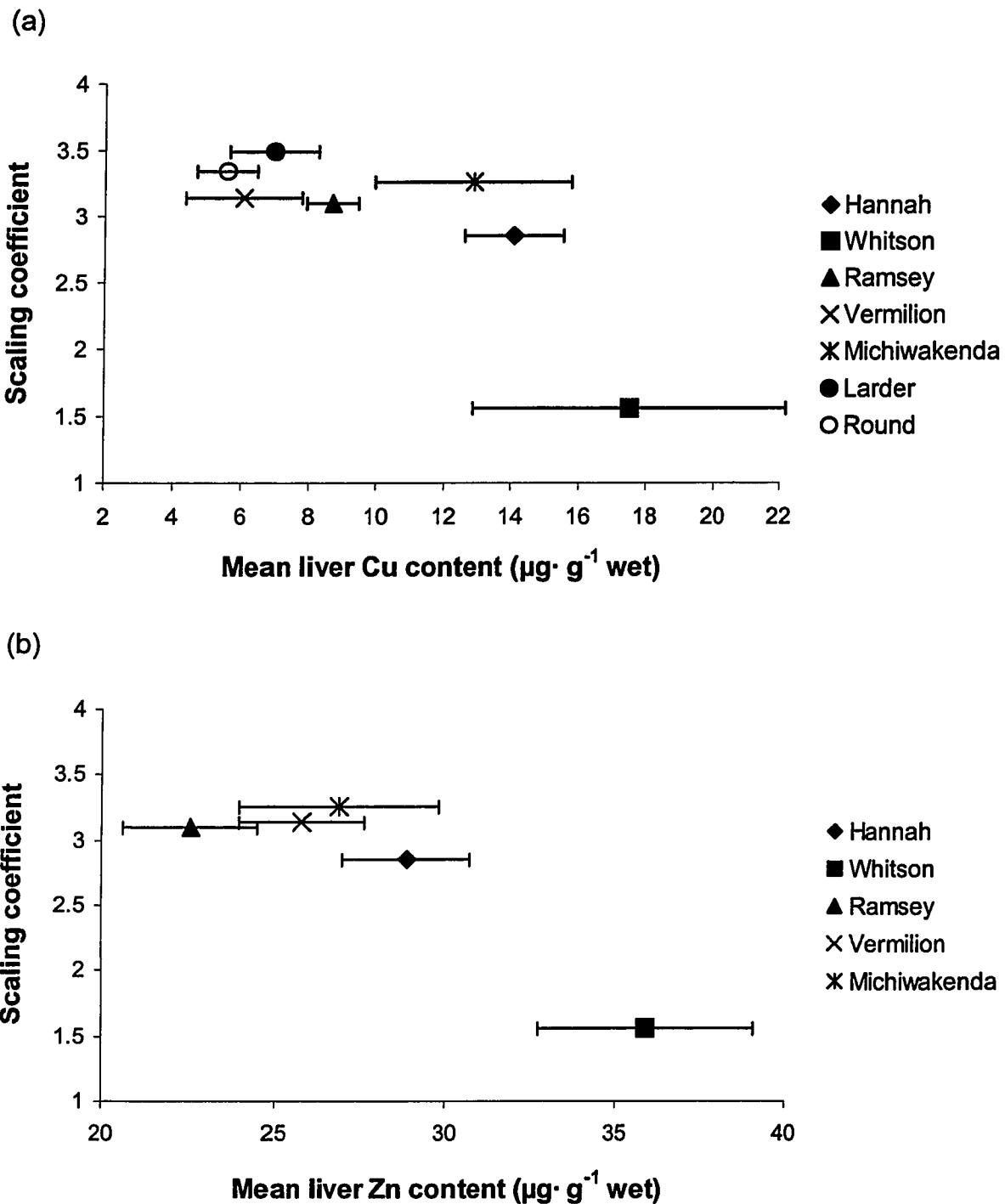


Figure 10. Relationship between scaling coefficients and (a) mean liver Cu contents ($\mu\text{g} \cdot \text{g}^{-1}$ wet) ($r = -0.80$; $p < 0.05$; $n = 7$) and (b) mean liver Zn contents ($\mu\text{g} \cdot \text{g}^{-1}$ wet) ($r = -0.91$; $p < 0.05$; $n = 5$) for yellow perch examined from Northeastern Ontario lakes during the fall of 1997.

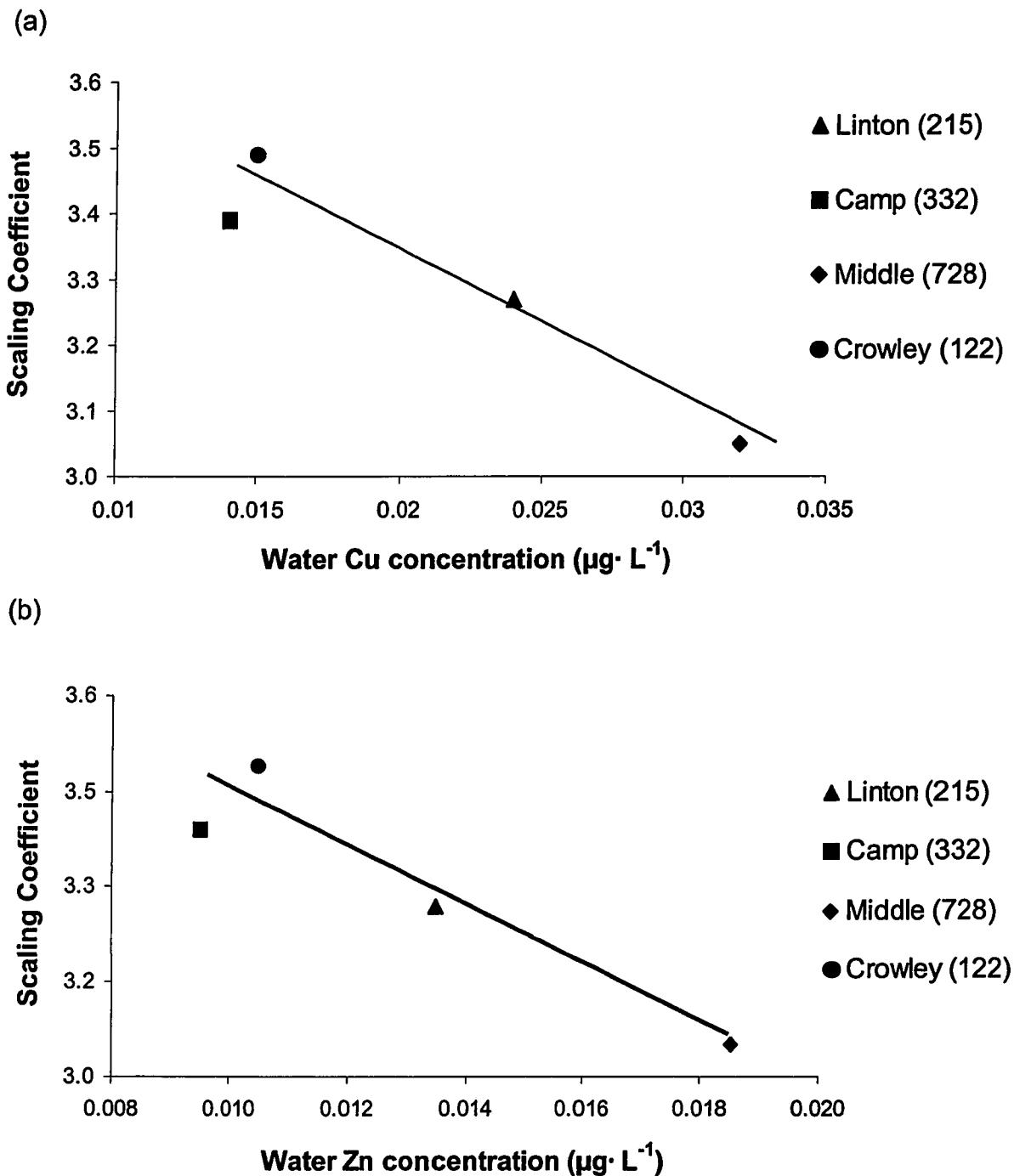


Figure 11. Relationship between scaling coefficients and (a) water Cu concentrations ($r = -0.96$; $p < 0.05$; $n = 4$) and (b) water Zn concentrations ($r = -0.95$; $p < 0.05$; $n = 4$) for yellow perch sampled from 4 Sudbury area lakes during the summer of 1992. The number of fish used to calculate the scaling coefficients are indicated in parentheses (data acquired from Wright (1995)).

stress response, a general bioindicator of chronic contaminant stress in fish. In 2000, Sherwood et al. compared the bioenergetic performance (growth rate, consumption rate, and conversion efficiency) of yellow perch from lakes of varying degrees of trace metal pollution in Quebec. Their results demonstrated a significant bioenergetic cost associated with living in an environment subject to chronic sublethal exposure to trace metal pollution (Cu, Zn, and Cd). Their study is similar to my own in that it was conducted primarily in the mining region of Abitibi (Northwestern Quebec) and includes 5 lakes along a metal contamination gradient.

According to the relationships in Table 8, liver Zn concentrations were also negatively associated with K_n after the influence of Cu was removed. Furthermore, mean liver Zn concentrations were more significantly associated with scaling coefficients even though this relationship was based on a lower sample size (n=5 for Zn and n=7 for Cu). Larder and Round lakes data were excluded from the relationships between mean liver Zn contents and scaling coefficients because Zn was undetectable in the livers of these fish. Nonetheless, Larder and Round lakes fish demonstrated the highest scaling coefficient values among the 7 lake samples examined in this study. Hence, if we could have measured the low Zn levels in the livers of these fish, the addition of these two lakes in Figure 10b would likely increase the significance of these associations. Because of the low sample size involved in the formation of these relationships, multivariate analyses were not appropriate in determining the extent to which

either of these metals influenced the scaling coefficient (growth pattern) of these yellow perch.

The growth of fish can be used as a sensitive and reliable endpoint in chronic toxicological investigations (Boeck et al., 1997). Sublethal levels of a wide variety of toxicants have been shown to slow the growth of fish (Woltering, 1984). The results from this study do not indicate the extent to which Cu and/or Zn negatively influence the growth patterns described by scaling coefficients. Furthermore, the effect of these metals examined in this study could either be direct (physiologically impairing yellow perch condition) or indirect (i.e., affecting food prey abundance). Lowered condition in fish can be influenced by numerous environmental stressors including fluctuating temperatures, low dissolved oxygen concentrations, and limited food availability (Adams et al., 1993). However, fish that have been exposed to sublethal trace metal concentrations have also demonstrated increased metabolic expenditure for detoxification and maintenance of normal body functions (Marr et al., 1995). In a laboratory study in 1998, Baker et al. determined that depressed growth rates in the grey mullet (*Chelon labrosus*) were a result of increased metabolic costs associated with Cu exposure. In elevated water Cu exposure, these costs included the synthesis of hepatic MT, increased Cu deposition, and elevated lipid peroxidation.

3.8 Relationship Between Water Chemistry and Liver Metal Concentrations and Condition

The associations between water chemistry characteristics (pH, alkalinity, conductivity, and DOC) and water and liver metal concentrations were examined

to assess the potential bioaccumulation and bioavailability of trace metals in the yellow perch examined from this study. No significant associations were found. However, a larger sample of lakes would likely increase the likelihood of significant correlations between these variables.

3.9 Productivity and Fish Condition

Several water quality parameters have been used as indicators of the productivity of aquatic ecosystems. One predictor, alkalinity, was measured in this study and is reported in Table 2. Moyle (1956) and Turner (1960) have both related increased alkalinity to elevated fish production. However, recent reports supporting the use of alkalinity as a chemical indicator of lake productivity have not been found. A significant positive association between alkalinity and scaling coefficients was found and is shown in Figure 12. This relationship suggests that elevated ecosystem productivity positively influenced fish condition. This is not an uncommon event, as food availability is essential for growth, reproduction and fish survival. Numerous studies have demonstrated increased abundance, growth, and overall condition of fish in response to high ecosystem productivity (Mills and Forney, 1981; Abbey and Mackay, 1991; Cobb and Watzin, 1998). Round lake in particular is a highly productive lake resulting from a sewage spill in 1995 (Ferguson, 1997). This led to increased nutrification with a potential increase in zooplankton and phytoplankton production (Kerr et al., 1997). The increased productivity in Round lake is clearly seen in the condition of its yellow perch. In fact, Round lake fish displayed higher condition than 3 of the 4 Sudbury

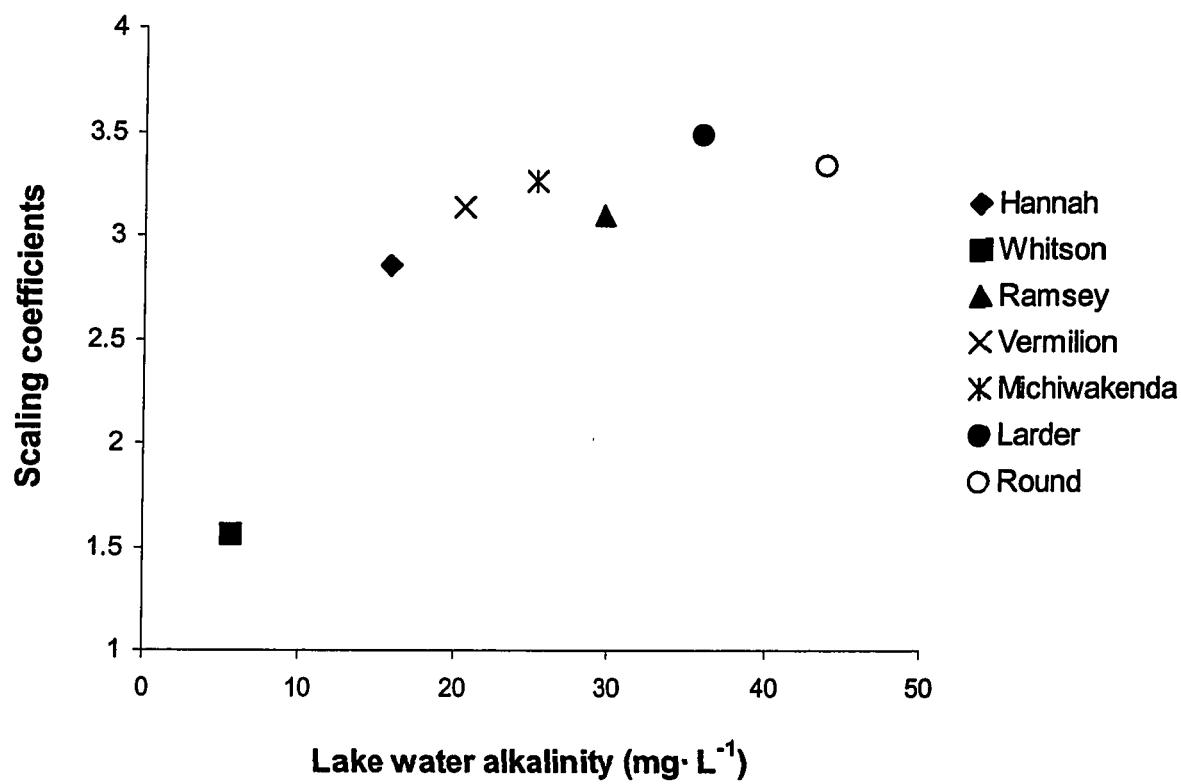


Figure 12. Relationship between lake water alkalinity ($\text{mg} \cdot \text{L}^{-1}$) and the scaling coefficients ($r = 0.82$; $p < 0.05$; $n = 7$) from our yellow perch samples examined from 7 Northeastern Ontario lakes during the fall of 1997.

area fish populations (Table 3, Table 5 and Figure 12). The only exception was in Vermilion lake where fish demonstrated a higher K_n .

However, alkalinity has also been shown to influence the bioavailability and uptake of trace metals by organisms in aquatic systems (Welsh et al., 1993; Playle et al., 1993; Horne and Dunson, 1995a). Alkalinity, among other water quality parameters (i.e., DOC, pH), has been shown to inhibit the uptake of contaminants by aquatic organisms (Welsh et al., 1993; Horne and Dunson, 1995a; Horne and Dunson, 1995b). The low tissue metal concentrations (Table 4) shown by Larder Lake fish is likely due to the low bioavailability of metals resulting from elevated pH, alkalinity and DOC. Larder lake is a contaminated lake due to the presence of tailings from an active gold mine located on the Northeast arm of the lake (Ferguson, 1997). The presence of these tailings resulted in increased ion and contaminant concentrations and a drastic decrease in abundance of benthic organisms and primary production (Kerr et al., 1997). Although productivity (as predicted by alkalinity) is likely influencing fish condition (Figure 12), pH and DOC may also be influencing metal availability by inhibiting their uptake in aquatic systems where trace metal concentrations are elevated (i.e., Larder Lake). Alkalinity is known to affect the bioavailability of trace metals in aquatic systems by reducing the amount of free cations through the formation of metal carbonates and other complexes available for uptake by aquatic organisms. The particular role of alkalinity (whether it is an estimator of productivity or whether it decreases metal bioavailability) on fish condition in this study is unknown.

The relationships between total phosphorus (a more reliable measure of productivity) and alkalinity with scaling coefficients were also examined from 4 additional Sudbury area lakes (Data acquired from Beth Wright's thesis, 1995). These relationships were not significant at the 5% probability level (Appendix 8). This lack of significance may be a result of the narrow range of productivity (Figure 11) characterized by these Sudbury area lakes. If in my study, the 3 Northern lakes with the highest alkalinity values (Michiwakenda, Larder, and Round) were removed from Figure 10a and b, the negative trend between scaling coefficients and liver Cu and Zn concentrations would remain. Although productivity likely contributes to the variability in condition examined from this study (Figure 12), fish condition may only be positively influenced in lakes with high productivity where trace metal concentrations are low (or high yet not available for uptake). On the other hand, elevated trace metal concentrations (Cu and Zn) appears to deleteriously affect fish in lakes with lower ecosystem productivity (i.e., Sudbury area lakes). As a result, high productivity and elevated environmental metal concentrations may be influencing fish condition simultaneously in the wide range of lakes examined in this study.

4.0 Conclusions

The results presented in this thesis suggest that elevated water and liver metal concentrations (Cu and Zn) are associated with lower yellow perch condition. This was supported by other unpublished data and is in agreement with other reports from the literature.

Yellow perch that were exposed to higher environmental trace metal concentrations (Hannah and Whitson lakes) exhibited elevated tissue Cu and Zn concentrations. This supports my first hypothesis that fish exposed to elevated environmental metal exposure would demonstrate elevated tissue metal concentrations.

Liver Cu and Zn concentrations were both negatively associated with various indicators of fish condition. Scaling coefficients were negatively associated with liver Cu and Zn concentrations in my 7 study lakes (1997) and with aqueous Cu and Zn concentrations in 4 additional Sudbury area lakes examined (Wright, 1995). Although the scaling coefficient (by itself) has never been used in fish ecotoxicological studies, I propose that it can be a precise and reliable measure of population condition, only requiring a measure of weight and length from each fish sampled within a population and a proper sampling technique. The cost involved in obtaining the scaling coefficients of numerous populations is low compared to other physiological methods (i.e., enzyme, metallothionein, RNA/DNA analyses) used to assess the condition of fish.

Following partial correlation analyses, it was determined that Cu was masking the relationship of Zn with liver protein content and the relative condition

factor (K_n). Elevated Zn exposures to fish have been shown to induce metallothionein (MT) synthesis in the liver. In my study, Zn was positively correlated with liver protein content and negatively associated with K_n . Furthermore, K_n was negatively associated with liver protein content suggesting that increased protein synthesis in response to elevated Zn concentrations may be one of many factors responsible for the lowered condition of yellow perch exposed to increased environmental Zn concentrations. Elevated protein synthesis (i.e., hepatic MT) is an important physiological process necessary for the sequestration and excretion of excess metals. The continuous synthesis of protein in response to elevated environmental Zn concentrations involves energy expenditure thus reducing the scope for growth. This explanation is supported by the observed reduction in the indicators of condition such as the weight and length data, K_n , and the scaling coefficients in response to elevated aqueous and tissue Cu and Zn exposure. These results support my second hypothesis, that yellow perch would demonstrate lower condition in response to elevated environmental metal exposure and liver metal concentrations.

Farag et al. (1995) observed that the most complete link available in the published literature between metal exposure and effects on growth was metallothionein concentrations and tissue metal residues. They stated that the health status of a fish population could be compromised if tissue metal concentrations alone were high. They also suggested that if elevated tissue metal concentrations were associated with an indication of physiological impairment in the same fish, it may be concluded that the health status of a fish

population could be impaired and that further associations among tissue metal accumulations, oxidative stress, and growth reduction would add more weight to a determination of fish health impairment. This study presents similar evidence of an impairment of physiological condition due to high environmental metal exposure (particularly Cu and Zn) in Northeastern Ontario yellow perch populations. However, this effect appears to be focused on those lakes in the high range of metal concentrations (i.e., Hannah and Whitson lakes) rather than in the intermediate and/or low range of trace metal concentrations. Furthermore, it is still unknown how these metals affect the condition of yellow perch (directly or indirectly) in these ecosystems.

Lowered food prey abundance has also been shown to negatively impact fish populations (Cobb and Watzin, 1998) and may be partly responsible for the lowered condition observed in these yellow perch. The relationship between alkalinity (indicator of lake productivity) and scaling coefficients suggests that productivity plays a role in the condition of fish examined in this study.

5.0 Recommendations for Future Research

Although the liver has proven to be a reliable bioindicator organ for studying the potential adverse effects of trace metals on fish, I recommend the use of other organs such as the kidney and gills in the determination of certain trace metals. Nickel for instance, has been shown to preferentially bioaccumulate in the kidney (N.R.C.C., 1981b) whereas many morphometric and physiological studies indicate that low pH and elevated aluminum primarily cause damage at the gills (Mueller et al., 1991). Therefore, kidney (for Ni) and gill lamellae (rinsed with double distilled water - to remove unbound Al) would provide better bioindicator organs for studying the effects of Ni and Al on fish condition.

Future studies should sample a larger number of fish to make statistical comparisons and associations more defendable. Because some of the sample sizes in this study were below 10, it is debatable whether or not this number of fish can accurately represent an entire fish population. A sample of 50 fish per lake would be more adequate in a physiological study such as this. However, costs associated with metal analyses are quite high and may require that a smaller sample be taken.

Differences in morphometric and physiological parameters between sexes may be partly responsible for explaining some of the variation in fish condition. As such, further studies should investigate the effects of trace metal concentrations on each gender individually to assess whether or not a difference in condition is attributable to sex differences.

Future research is needed in determining the extent to which trace metals affect fish condition. Additional studies should examine direct measures of lake productivity (i.e., standing crop, rate of removal, and rate of production) in relation to measures of fish condition. This information will help identify the extent to which productivity is influencing fish condition.

Lastly, the determination of MT would be recommended in future studies as an indication of physiological impairment in fish when exposed to sublethal trace metal concentrations.

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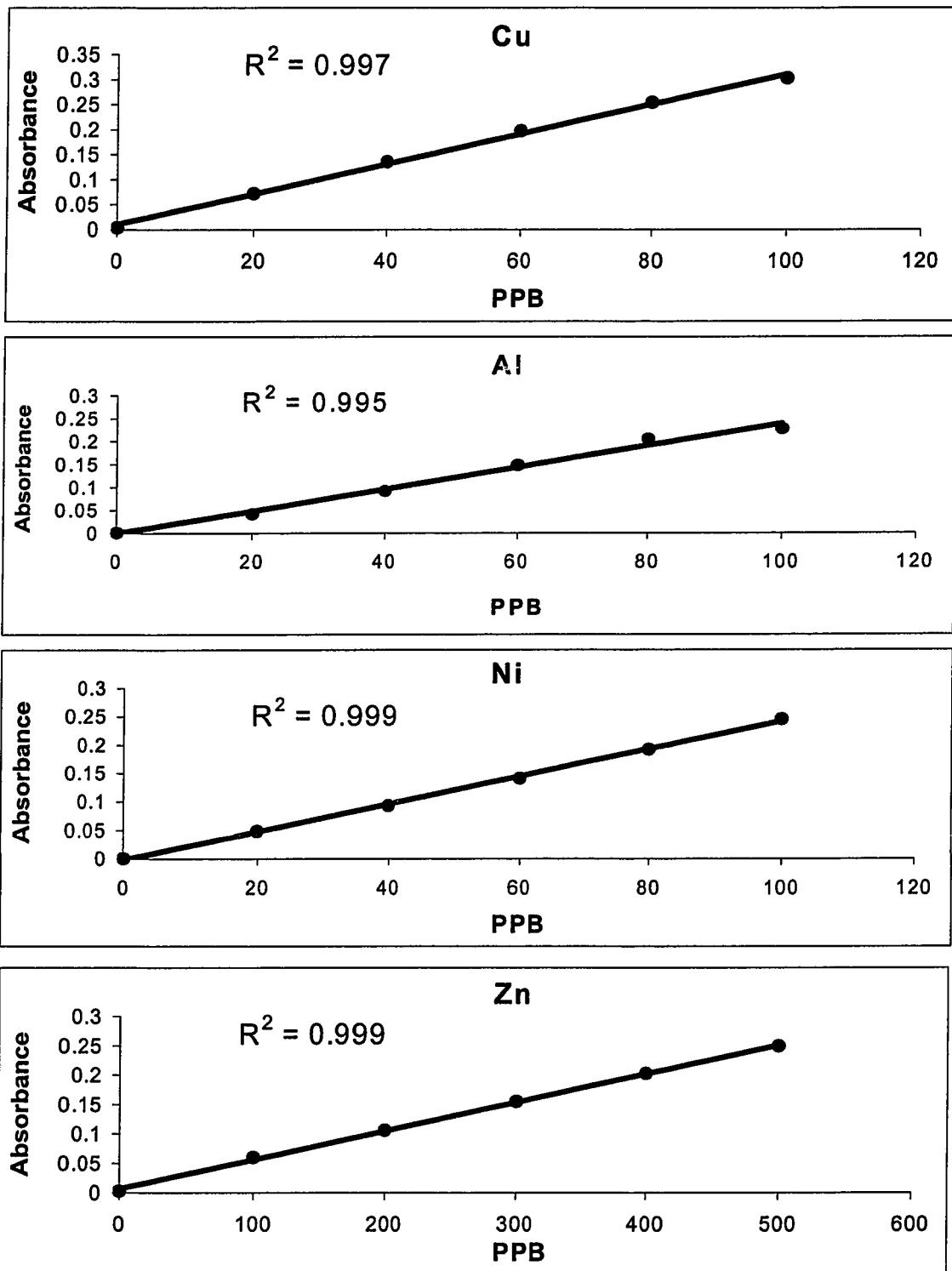
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Appendix 1. Blank absorbance readings and detection limits for Cu, Ni, Al, and Zn.

| Sample Blanks (n = 107) | Absorbency Reading | | | |
|--|--------------------|----------------|----------------|----------------|
| | Cu | Ni | Al | Zn |
| Blank(1) | 0.000 | 0.002 | 0.002 | 0.008 |
| Blank(2) | 0.000 | 0.001 | 0.003 | 0.009 |
| Blank(3) | 0.001 | 0.001 | 0.003 | 0.005 |
| Blank(4) | 0.000 | 0.000 | 0.002 | 0.008 |
| Blank(5) | 0.001 | 0.002 | 0.003 | 0.007 |
| Baseline Noise x 2 | 0.001 0.002 | 0.002 0.004 | 0.001 0.002 | 0.004 0.008 |
| Detection Limit (μgg^{-1} wet) | 2.90 | 2.25 | 0.79 | 1.80 |

Detection limit values were obtained by substituting the new baseline value (baseline noise x 2) into the standard curve equation for each respective metal. Standard curves are illustrated in Appendix 2.

Appendix 2. Standard curves for Cu, Ni, Al, and Zn standards.



Appendix 3 Protein analysis protocol modified from Lowry's protein assay (1951).

LOWRY'S PROTEIN ASSAY

Reagents

| | | |
|-------------------------------|---------------------------------------|------------------------------|
| <u>Solution A</u> | Na ₂ CO ₃ | 2% w/v in 0.1N NaOH |
| <u>Solution B₁</u> | Na-K tartrate-4H ₂ O | 2% w/v |
| <u>Solution B₂</u> | CuSO ₄ · 5H ₂ O | 1% w/v |
| <u>Standard BSA</u> | 1 mg · mL ⁻¹ | double distilled water (DDW) |

MODIFIED METHOD FROM LOWRY

Mix fresh before use:

Reagent 1: 1 part of B₁ + 1 part of B₂ + 48 parts A

Reagent 2: 1 part Folin-Ciocalteu's phenol reagent + 1 part DDW

- To a 1.5 mL cuvette, add 50 µL DDW to sample containing 0-40 µg protein
- Complete volume to 200 µL with 0.1 N NaOH.
- Prepare a standard curve containing 0-10-20-30-40 µg BSA and first complete volume to 50 µL with homogenization buffer, then to 200 µL with 0.1 N NaOH.
- Add 1000 µL Reagent 1.
- Leave 10 minutes at room temperature.
- Add 100 µL Reagent 2 and mix.
- Leave 30 minutes at room temperature.
- Read absorbance at 690 nm.

Appendix 3 (continued)

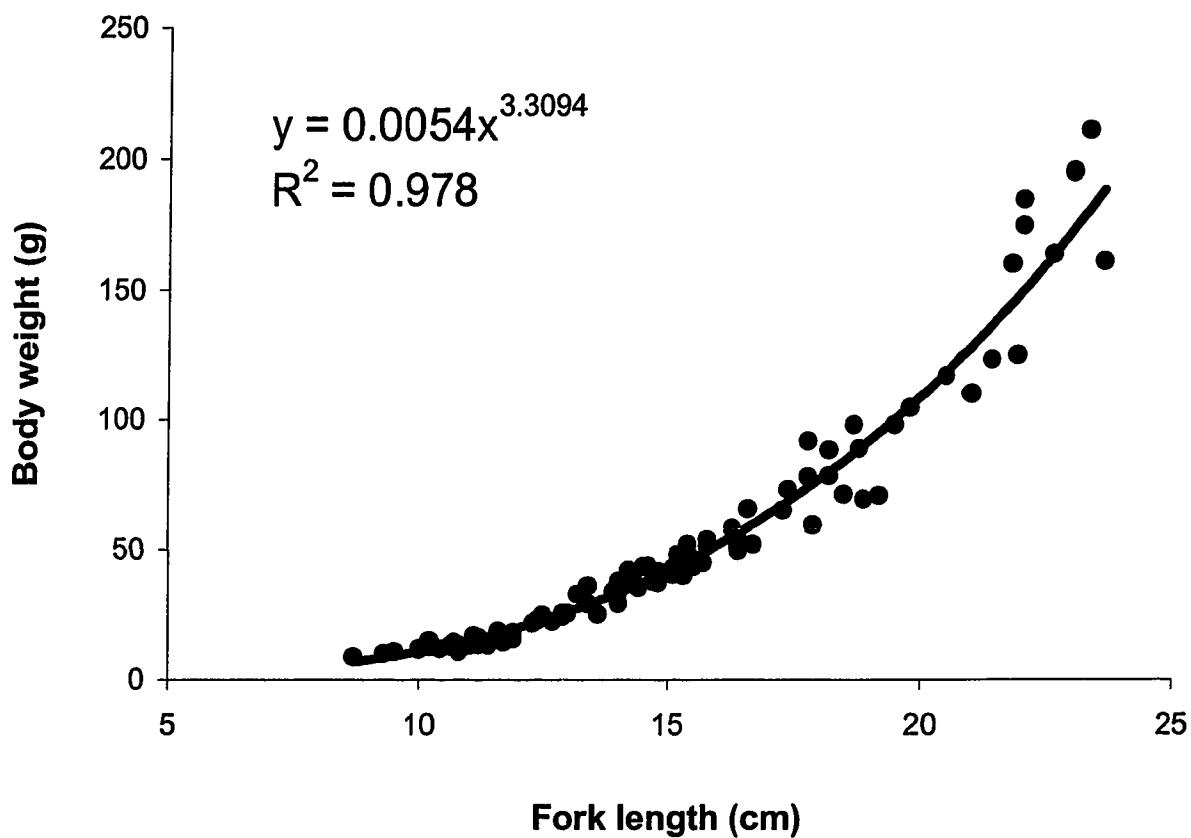
HOMOGENIZATION BUFFER (pH = 7.5)
(Stable for 1 week at 4°C)

| Ingredients | [mM] | M.W. | g/500 mL |
|--|--------|--------|----------|
| Imidazole 0.2033 | 50 | 68.08 | 1.7020 |
| MgCl ₂ ·6H ₂ O 0.6806 | 2 | 203.30 | |
| Na-EDTA 0.1537 | 5 | 272.24 | |
| Glutathione (reduced) 0.1537 | 1 | 307.30 | |
| Triton X-100 | 0.1% | | 0.5 mL |

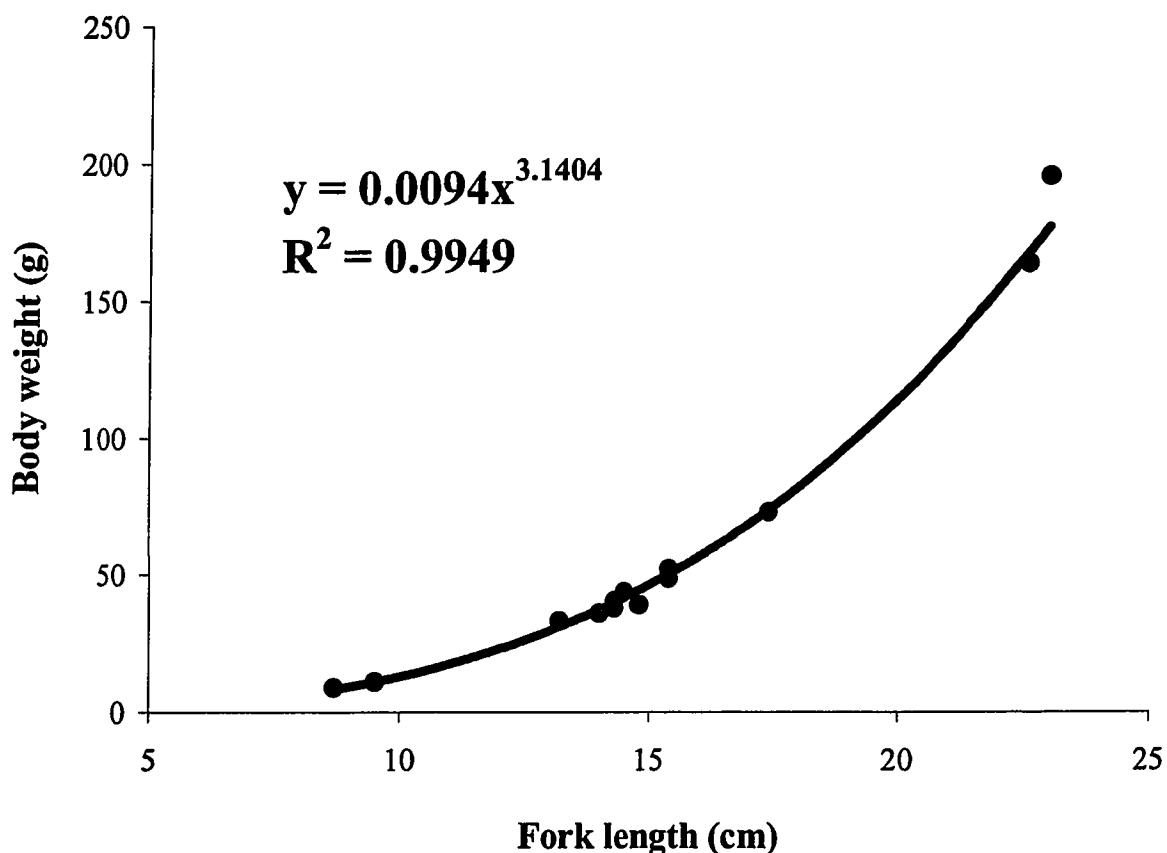
METHOD:

1-2 g diluted 1:10 with homogenization buffer with Ultra-turrax at intermediate speed, 3 * 20 seconds with 20 second pauses, keeping sample on ice at all times.

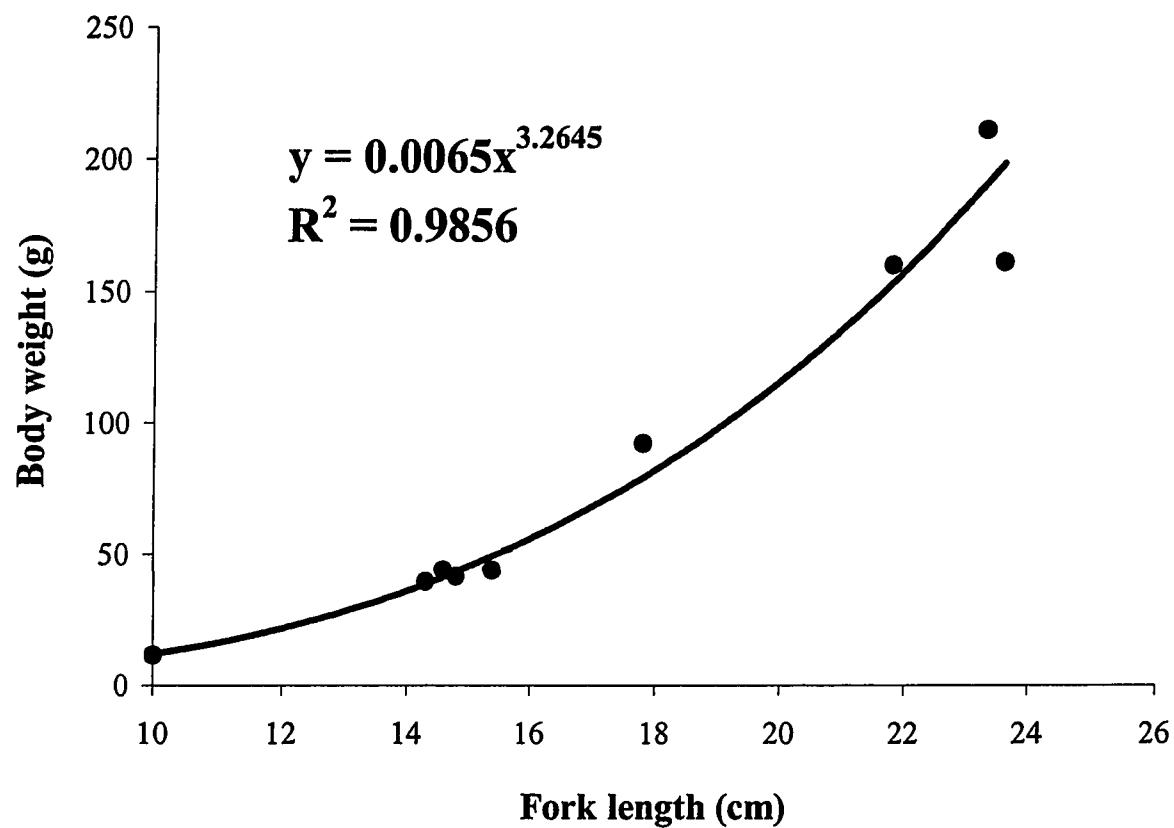
Appendix 4. Weight-at-length relationship (power) demonstrating the scaling coefficient for all yellow perch examined from 7 Northeastern Ontario lakes in the fall of 1997; $p < 0.0001$, $n = 107$.



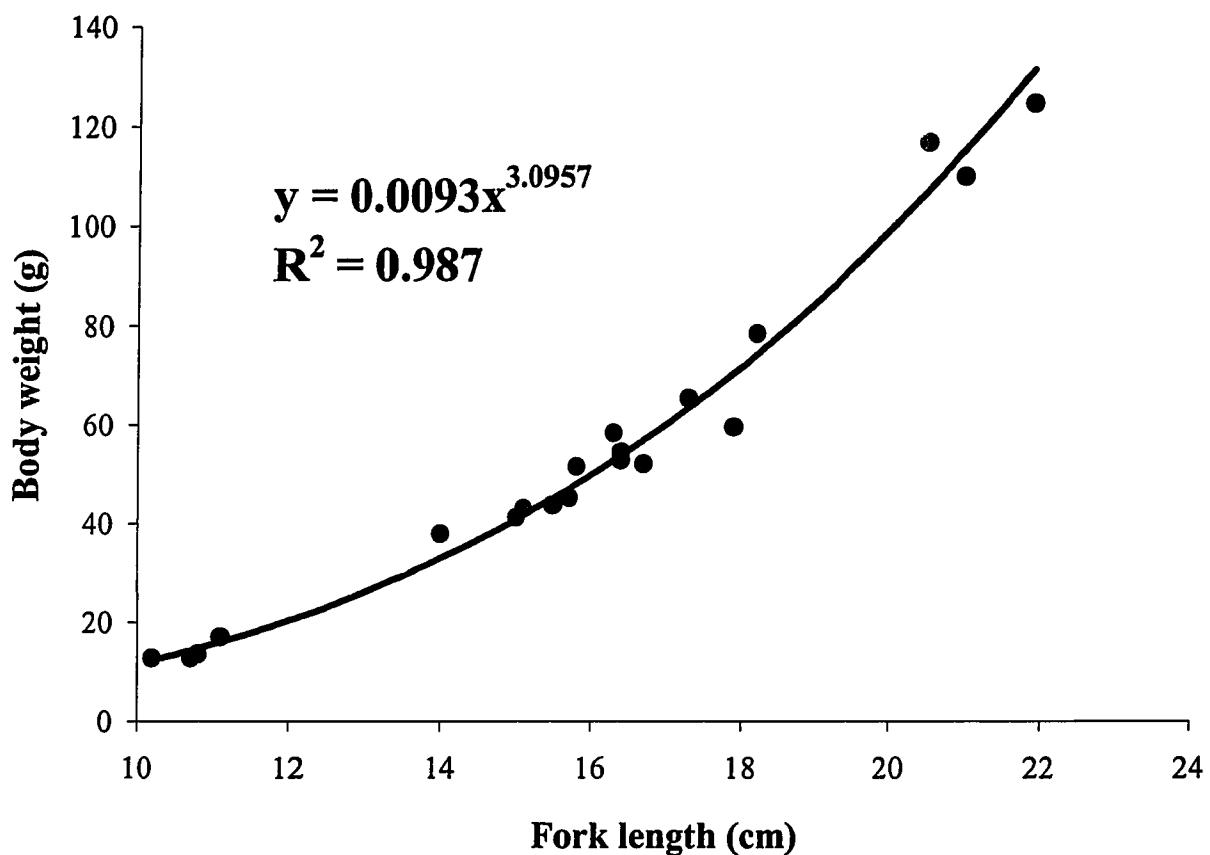
Appendix 5a. Weight-at-length relationship (power) for yellow perch captured from Vermilion lake during the fall of 1997; $p<0.0001$, $n = 14$.



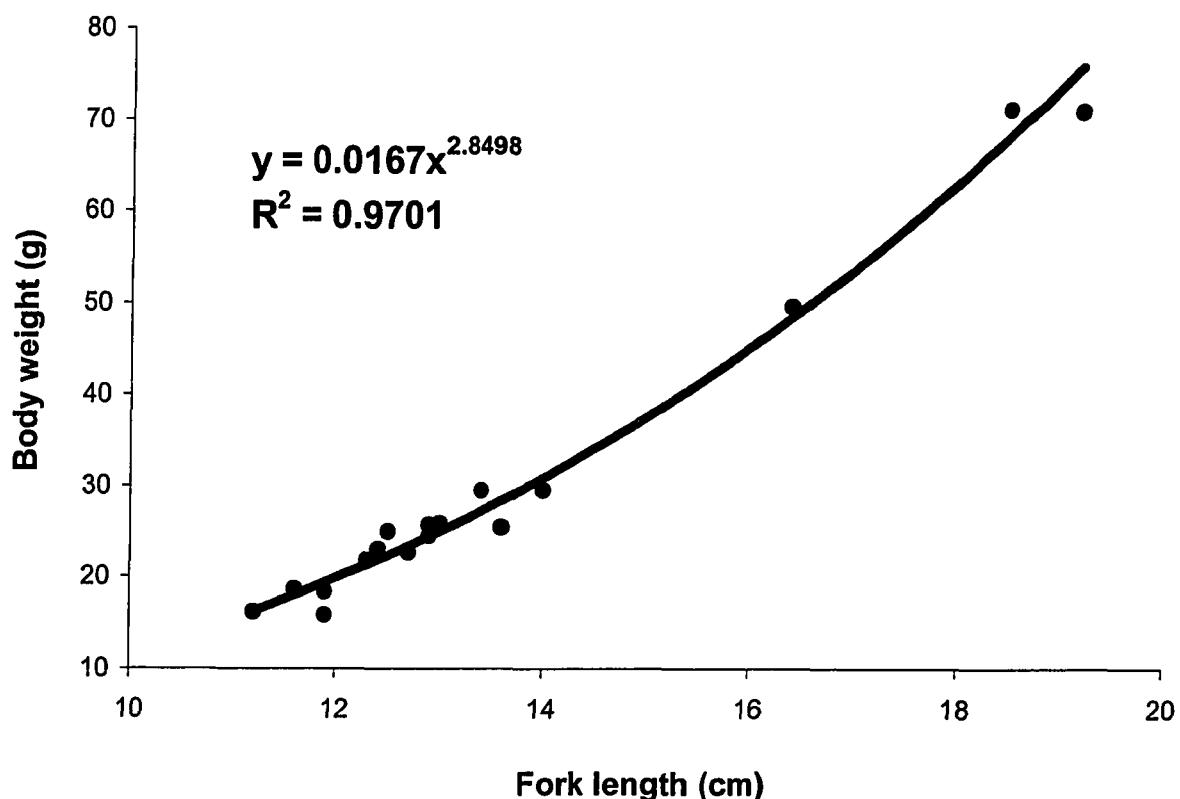
Appendix 5b. Weight-at-length relationship (power) for yellow perch captured from Michiwakenda lake during the fall of 1997; $p<0.0001$, $n = 9$.



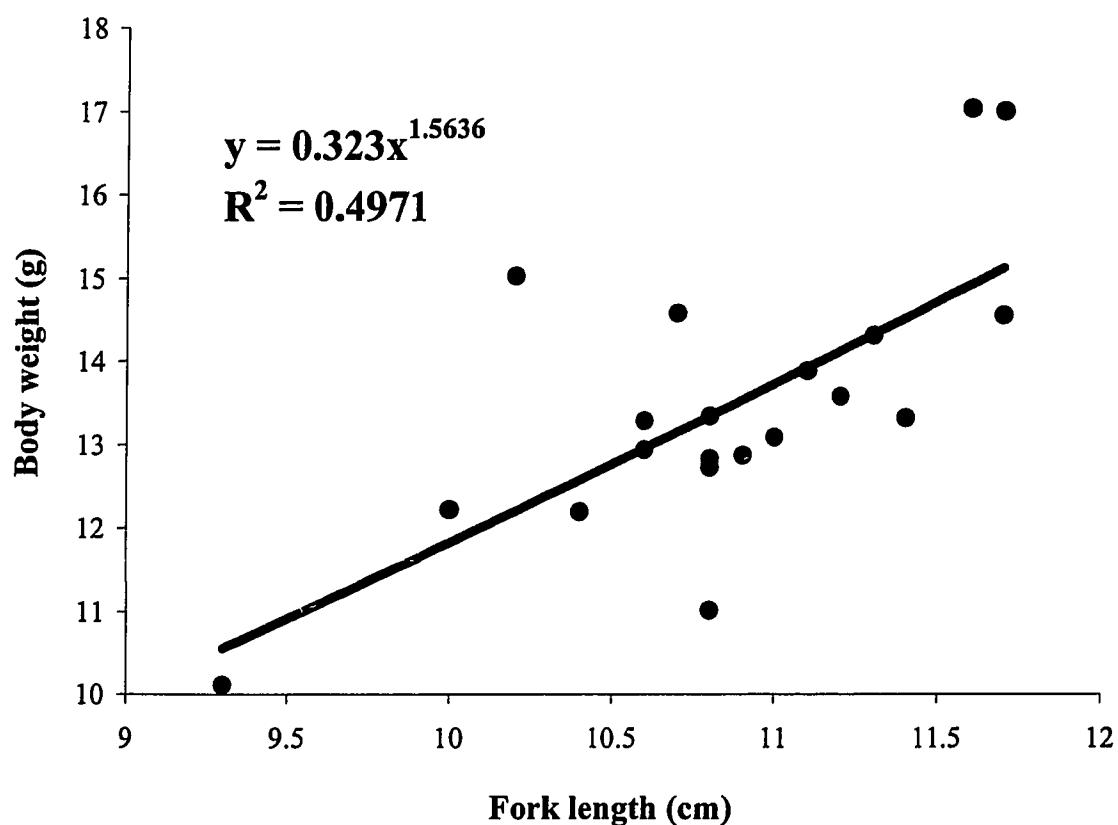
Appendix 5c. Weight-at-length relationship (power) for yellow perch captured from Ramsey lake during the fall of 1997; $p<0.0001$, $n = 20$.



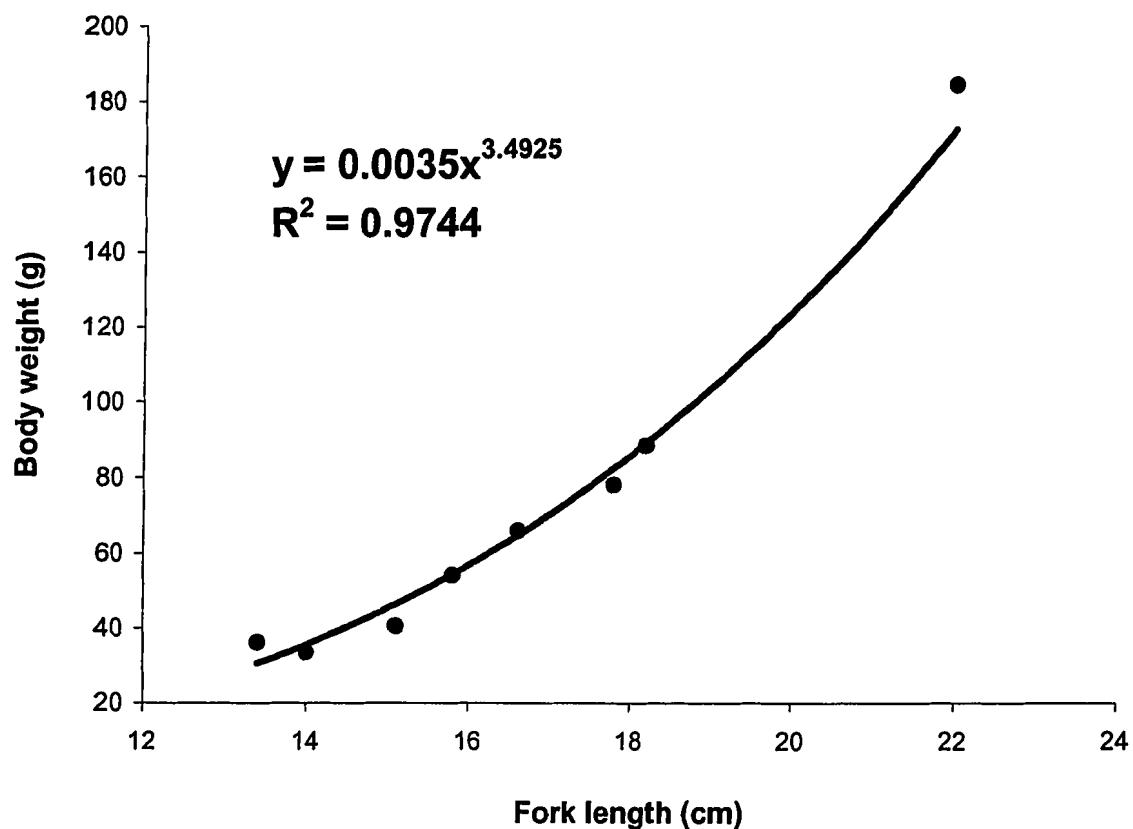
Appendix 5d. Weight-at-length relationship (power) for yellow perch captured from Hannah lake during the fall of 1997; $p<0.0001$, $n = 18$.



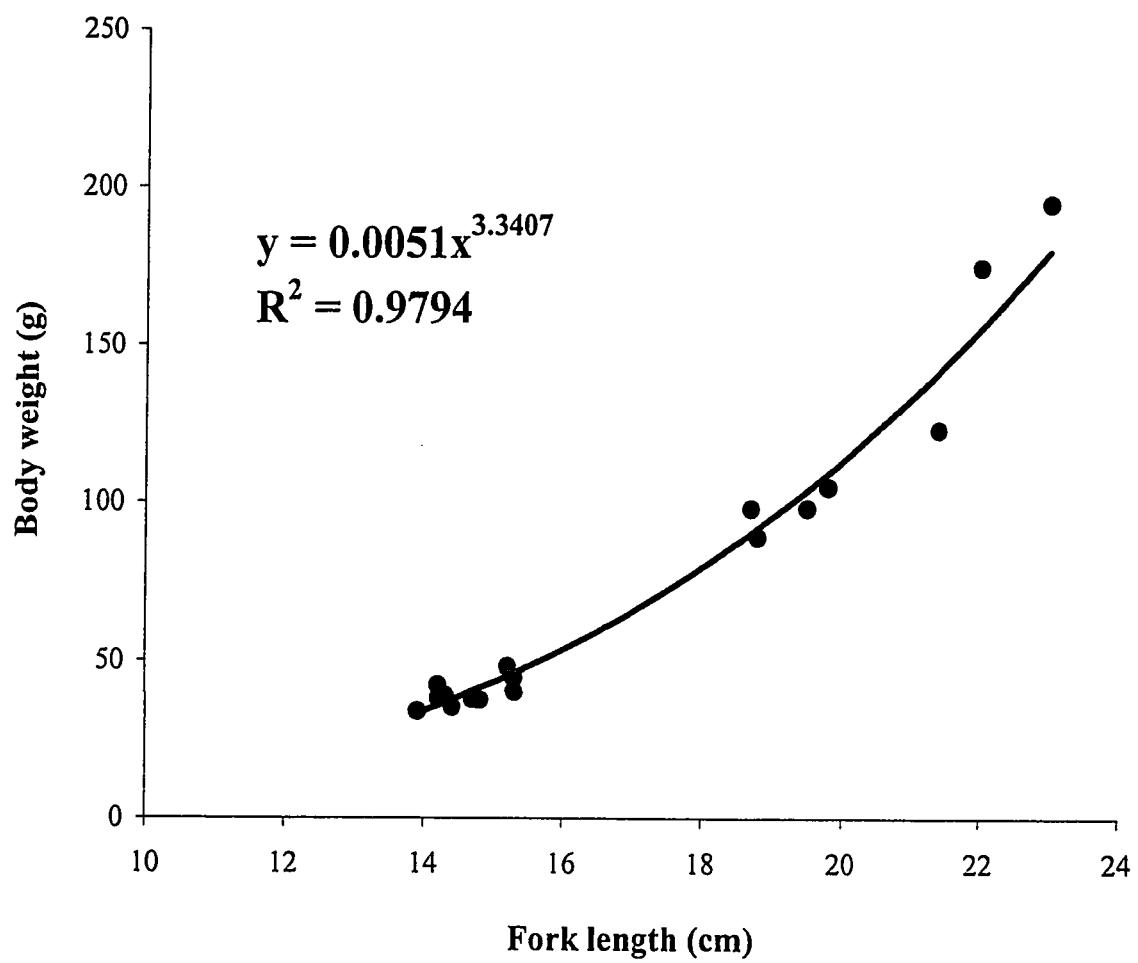
Appendix 5e. Weight-at-length relationship (power) for yellow perch captured from Whitson lake during the fall of 1997; $p>0.05$, $n = 20$.



Appendix 5f. Weight-at-length relationship (power) for yellow perch captured from Larder lake during the fall of 1997; $p<0.001$, $n = 8$.



Appendix 5g. Weight-at-length relationship (power) for yellow perch captured from Round lake during the fall of 1997; $p<0.0001$, $n=18$.



Appendix 6. Age (yrs) categories of yellow perch showing sample size of each age class for individual lakes and for all lakes combined. Total percentage of each age class is also indicated.

| Lake | Age - 1 | Age - 2 | Age - 3 | Age - 4 | Age - 5 | Age - 6 |
|-----------------------------------|---------|---------|---------|---------|---------|---------|
| Hannah (n = 18) | 1 | 5 | 12 | 0 | 0 | 0 |
| Whitson (n = 20) | 6 | 9 | 4 | 1 | 0 | 0 |
| Ramsey (n = 20) | 1 | 6 | 7 | 6 | 0 | 0 |
| Vermilion (n = 14) | 1 | 3 | 7 | 2 | 1 | 0 |
| Michiwakenda (n = 9) | 1 | 3 | 1 | 2 | 1 | 1 |
| Larder (n = 8) | 0 | 4 | 2 | 2 | 0 | 0 |
| Round (n = 18) | 0 | 11 | 4 | 3 | 0 | 0 |
| All yellow perch (n = 107) | 10 | 41 | 37 | 16 | 2 | 1 |
| Percent (%) | 9.1 | 38.0 | 36.0 | 14.7 | 1.8 | 0.1 |

Appendix 7. Mean (S.E.) liver metal concentrations ($\mu\text{g g}^{-1}$ dry) in yellow perch examined from 4 Sudbury area lakes in the summer of 1996 (Eastwood, 1997).

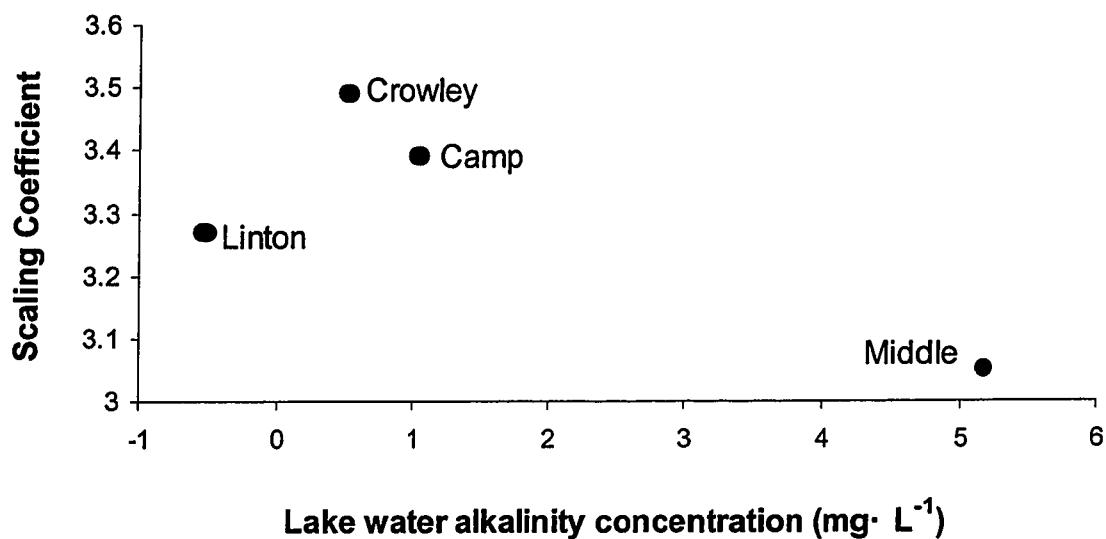
| Lake | Cu | Ni | Al | Zn |
|----------------|-----------------|-----------------|-----------------|------------------|
| Laurentian | 182 \pm 47.5 | 6.24 \pm 2.09 | 25.9 \pm 10.5 | 267.7 \pm 50.9 |
| Clear | 30.9 \pm 5.6 | 5.1 \pm 1.5 | 39.2 \pm 13.5 | 121.8 \pm 42.9 |
| Hannah | 678.8 \pm 101 | 4.32 \pm 1.68 | 64.4 \pm 10.3 | 157.5 \pm 38.3 |
| Falconbridge * | 31.3 \pm 7.6 | 12.73 \pm 9.7 | 33.5 \pm 11.4 | 64.8 \pm 8.0 |

Note: Approximate conversion from dry to wet weight concentration is to divide by 5

* Falconbridge wetland treatment pond

Appendix 8. Relationships between yellow perch scaling coefficients and (a) water alkalinity ($p>0.05$) and (b) total phosphorus ($p>0.05$) from 4 Sudbury area lakes during the summer of 1992 (Wright, 1995).

(a)



(b)

