METAL CONCENTRATION AND CALCIFICATION OF BONE OF WHITE SUCKER (CATOSTOMUS COMMERSONI) IN RELATION TO LAKE pH

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ABSTRACT. The concentrations of 17 elements were determined in the bone of white suckers (Catostomus commersoni) netted from 5 acid (pH range 4.8 to 5.8) and 2 circumneutral (pH=6.2 and 6.3) lakes in south-central Ontario. The bone Ca:P dry weight ratios were similar (2.0:1) for all fish populations except those of George Lake (pH=4.8) which showed a significantly lower Ca:P ratio (1.9:1, $P < 0.05$). Magnesium was also lower in the bone of these fish and in fish from 2 other acid lakes. Only bone Ba and S concentrations in the 7 fish populations correlated significantly to lake pH (R=-0.9 and R=-0.8, respectively, $P < 0.05$). Bone Mn concentrations correlated to dissolved lake Mn concentrations (R=0.8, $P < 0.05$), and was 7 fold greater in the bone of fish from George Lake and 2 fold greater in King Lake (pH=5.0) fish, vs fish from the 2 circumneutral lakes. Bone Zn was significantly greater in white sucker from George Lake, and tended to be higher in this species from King Lake, compared to all other fish populations. Bone concentrations of Fe, Cu, Ni and Al showed no apparent trends among the 7 fish populations. Cd, Co, Cr, Mo, V and Be were not detected. The occurrence of a reduced Ca:P ratio coincident with the highest concentrations of Mn, Zn and Ba in the bone of fish from the most acidified environment suggests that increased metal concentrations which occur in surface waters coincident with lake acidification may affect bone calcification.

1. INTRODUCTION

The mechanisms by which acidification affect fishes in lakes of north-eastern North America are complex. One possible mechanism is that acid stress interferes with the normal Ca dynamics, resulting in bone deformities (Beamish et al., 1975) and bone demineralization (Fraser and Harvey, 1982). However, recent field (Mills, 1984) and experimental (Rogers, 1984) evidence has indicated that conditions of chronic acid exposure alone are not sufficient to interfere with the normal Ca dynamics of fish.

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Coincident with lake acidification are increases in metal concentrations such as Cd and Zn (Dickson, 1975; Schindler et al., 1980). These metals can interfere with the Ca metabolism of bony tissues causing a reduction of bone Ca and P concentrations and a reduced Ca to P ratio coincident with increased bone metal concentrations (Muramoto, 1981; Sauer and Watabe, 1984), as well as inducing bone deformities (Bengtsson et al., 1985). It is possible therefore that increased metal concentrations in acid lakes may play a role in altering the Ca dynamics of fish. This may be apparent as a correlation between reduced bone Ca and P concentrations and a reduced Ca to P ratio and increased bone metal concentrations in the bone of white sucker from acid lakes. The objective of this study was to use a multielemental analysis of the bone of white sucker captured from acid and non-acid lakes to determine if such a relationship exists. A strong correlation may be indicative of a possible role of trace metals in acid waters interfering with the normal calcification of fish bone.

2. STUDY AREA AND METHODS

Seven lakes were chosen based on location, previous knowledge of the existing fish species and known water chemistry (Bendell-Young and Harvey, 1986). A detailed outline of the study area (location and lake morphology). Additional methods and water chemistry have been published (Bendell-Young and Harvey, 1986). Water and sediment chemistry for the 7 lakes are presented in Table I. The following elements were included in the analysis of the fish bone; Ca, P, Mg, S, Mn, Zn, Ba, Fe, Cu, Al, Ni, Cd, Co, Cr, Mo, V and Be. The 17 elements grouped as follows: macroelements; Ca, P, S and Mg; trace elements; Mn, Zn, Fe, Cu, Al, Ni and Ba and (post-analysis) non-detectable trace elements; Cd, Co, Cr, Mo, V, and Be. Accuracy of the bone analysis was measured by the inclusion of a National Bureau of Standards (NBS) bovine standard spiked with a 1,000 $\mu g.L^{-1}$ Ca standard to simulate the bone matrix. Experimentally determined values for Ca, P, S, Mn, Zn, Fe and Cu were within 10% of the NBS certified values. Determined values for Mg were within 15% of the certified values. Ba, Ni and Al were not detected in the NBS standard. Limits of detection for these 3 elements plus Cd, Co, Cr, V, Be and Mo were 0.005, 0.033, 0.027, 0.006, 0.007, 0.005, 0.003, 0.006 and 0.01 $\mu g.L^{-1}$, respectively.

Fish mean weight, fork length, bone elemental concentrations and the Ca to P dry weight ratios for the 7 white sucker populations are presented in Table II. Since only trace amounts of Ni were detected in the fish bone, ranges are presented. Elemental concentrations did not differ between sexes for the 7 fish populations (Students t-test; $P > 0.05$) and were not dependent on fish weight or fork length (Pearson's product correlation coefficient; $P > 0.05$). Since within-lake variability was not dependent on either fish sex or size, comparisons among the 7 fish populations were made. Homogeneity of group variances for the elemental concentrations in white sucker bone were verified through Levene's test prior to an one-way analysis of variance between elemental means. Where significant differences occurred,

Duncan's multiple comparisons were applied to indicate where they occurred. Pearson's product correlation coefficients were calculated to determine relationships between mean bone metal concentrations and
1) water chemistry parameters, 2) sediment chemistry parameters, and
3) mean bone elemental concentrations. Significant correlations are presented in Table III.

TABLE I

	WATER			SEDIMENT ± 1 SD						
Lake	Al	Zn	Fe	Mn	Al	Fe	Zn	Ni	Cu	Ba[a]
		$\mu g.L^{-1}$			$mg.g^{-1}$			$\mu g.g^{-1}$		
George	180	30	50	3.3 ±0.7	27 ±4	48 ±22	256 ±102	113 ±16	106 ±24	830 ± 50
King	90	10	280	0.3 ±0.1	12 ±2	16 ± 2	123 ± 21	22 ± 7	23 ±11	634 ±180
McDonald	120	62	250	0.3 ±0.1	7 ±1	6 ± 2	97 ± 32	11 ± 3	15 ± 6	681 ±149
Crosson	50	10	430	0.3 ±0.1	14 ±2	26 ± 4	141 ± 63	16 ± 4	31 ± 8	319 ± 67
Chub	105	NA[b]	430	0.3 ±0.06	12 ±2	22 ± 8	142 ± 30	17 ± 4	28 ± 6	781 ±241
Red Chalk	15	10	180	2.3 ±1.5	15 ±5	45 ±26	210 ± 37	16 ± 1	64 ±40	393 ± 47
Harp	50	NA	130	3.2 ±1.0	15 ±5	40 ±19	165 ± 73	18 ± 6	33 ±20	1191 ±251

a: Ba sediment concentrations are "total" metal as compared to the "acid extractable" concentrations of the other metals.
b: Not available.

3. RESULTS AND DISCUSSION

3.1. Elemental concentrations in the bone of white suckers

Of the 11 elements that were detected in the fish vertebrae only Ba and S correlated to lake pH ($R=-0.9$ and $R=-0.8$, respectively; $P < 0.05$) (Table III). Barium is a large, highly electropositive cation and behaves differently than the transition metals such as Zn and Mn. For example, Schindler et al. (1980) reported uptake by seston and the rate of removal from the water column following acidification was the greatest for the transition metals and the least for the highly electropositive metals such as Ba. Similarly, Jackson et al. (1980) noted metal ions of large ionic radius, such as Ba, showed a greater affinity for the aqueous phase than did the smaller cations and attributed the observed effect to the low charge/radius ratio of Ba and the associated weak physical adsorption of this ion in an aqueous environment. These authors further suggested that under some circumstances Ba may be preferentially released from lake sediments to the interstitial water and overlying water column. This difference in the behavior of Ba as compared to the transition metals may explain in part the strong correlation that was found between lake pH and bone Ba concentrations in the 7 fish populations (Figure 1).

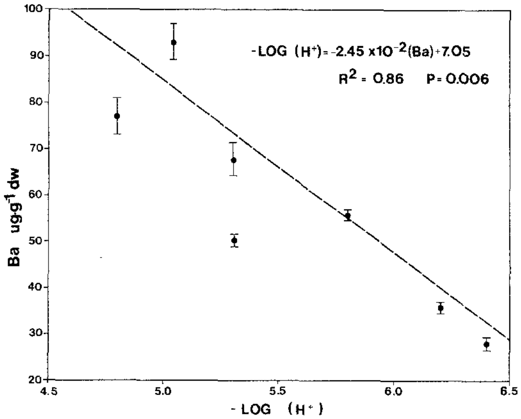

Figure 1. The regression of mean bone Ba concentration (± 1 SE) on lake pH for the 7 white sucker populations.

Bone S concentration was significantly greater in fish from 4 of 5 acid lakes as compared to fish from the 2 circumneutral lakes (Table II) and also correlated to lake pH (Table III). Neither the accumulation in nor toxicity of sulphate to fishes has received much study. Limited research has indicated that fish will accumulate S in skeletal tissue when exposed to increased water concentrations (Phillips et al., 1959). However, the sulphate anion passes through biological membranes with difficulty and must be taken up by a selected process with an expenditure of energy (Phillips et al., 1959).

Manganese was elevated 7 fold in the bone of white sucker from the most acid lake and 2 fold in the bone of fish from acid King Lake compared to white sucker from the remaining acid and circumneutral lakes (Table II). However, whereas white sucker bone Mn concentrations were strongly correlated to water Mn ($R=0.9$, $P < 0.05$), they were not related to lake pH (Table III). Similarly, Fraser and Harvey (1982) and Moreau et al. (1983) have noted that no simple relationship exists between lake pH and bone Mn concentrations. Recently, Bendell-Young and Harvey (1986) have shown that dissolved water and lake sediment concentrations of Mn are mediated strongly by the redox cycling of this metal as well as being influenced by changes in lake acidity. Bone concentrations of Mn in white sucker probably are correlated better with dissolved Mn

TABLE II

Lake	Wt	FL	Ca	P	Mg	S	Mn	Zn	Fe	Al	Cu	Ni	Ca:P
	g	cm		mg.g^{-1}					μg.g^{-1}				
George (16)	555 ±123	33.5 ±3.2	208^{c} ±22	109^{bc} ±13	2.8^{d} ±0.5	4.9^{b} ±0.3	675^{a} ±100	138^{a} ±25	6^{ab} ±5	15^{b} ± 8	2.3 ±1	(0.0- 6.6)	1.9^{b} ±0.08
King (19)	651 ±125	34.4 ±2.6	242^{ab} ±29	122^{a} ±12	3.9^{a} ±0.4	4.9^{b} ±0.4	210^{b} ± 62	90^{bc} ±11	8^{ab} ±5	17^{ab} ± 6	8.9 ±1	(0.0- 4.2)	1.98^{ab} ±0.07
McDonald (19)	209 ± 80	25.5 ±4.0	218^{c} ±26	106^{c} ±13	3.3^{cd} ±0.7	4.6^{a} ±0.8	62^{d} ±15	80^{c} ±15	8^{ab} ±5	22^{a} ±17	NA*	ND	2.05^{a} ±0.07
Crosson (7)	665 ±421	32.7 ±6.3	233^{bc} ±25	117^{c} ±13	3.2^{cd} ±0.3	4.9^{b} ±0.4	101^{c} ± 58	87^{c} ±19	9^{a} ±7	12^{ab} ± 7	6.4 ±2	ND	2.02^{ab} ±0.1
Chub (19)	669 ±115	35.1 ±1.3	243^{ab} ±33	123^{a} ±14	3.7^{a} ±0.3	5.0^{b} ±0.3	123^{c} ± 15	83^{c} ±13	7^{ab} ±7	22^{c} ±11	9.4 ±1	(0.0- 2.3)	1.96^{ab} ±0.08
Red Chalk (18)	1044 ±271	39.6 ±4.1	249^{a} ±29	123^{a} ±14	3.6^{ab} ±0.4	4.6^{a} ±0.2	147^{c} ± 65	83^{c} ±15	11^{a} ±7	15^{a} ± 9	9.6 ±1	(0.0- 1.4)	2.02^{a} ±0.06
Harp (34)	557 ±176	34.7 ±4.0	227^{b} ±16	111^{b} ±10	3.4^{bc} ±0.4	4.3^{a} ±0.3	118^{c} ± 33	64^{d} ±10	3^{b} ±3	8^{a} ± 4	NA	ND	2.05^{a} ±0.09

NA: Not available
ND: Not detected

TABLE III

II

	pH	ALK	COND	DOC	Mn	Al	Zn	Fe	Al	Fe	Ni	Cu	Ba	Zn	Mn	Ba	Mn	Zn	Fe	Al	Cu	Ni	Ca	P	Mg	S	Ca:P
	WATER								SEDIMENT							BONE											
pH	1																										
ALK	.8	1																									
COND	-	.76	1																								
DOC	-	-	-	1																							
Mn	-.73	-	-	-	1																						
Al	-.75	-	-	-	.8	1																					
Zn	-	-	-	-	-	-	1																				
Fe	-	-	-	-	-	-	-	1																			
Al	-	-	-	-.8	-	-	-	-	1																		
Fe	-	-	-	-.9	-	-	-	-	.8	1																	
Ni	-	-	-	-	.8	.73	-	-	.9	-	1																
Cu	-	-	-	-.9	-	-	-	-	.9	.8	.9	1															
Ba	-	-	.8	-	-	-	-	-	-	-	-	-	1														
Zn	-	-	-	-.9	-	-	-	-	.9	.9	-	.9	-	1													
Mn	.8	.9	-	-	-	-	-	-	-	-	-	-	-	-	1												
Ba	-.9	-.8	-	-	.75	-	-	-	-	-	-	-	-	-	-.8	1											
Mn	-	-	-	-	.9	-	-	-	.9	-	.9	.9	-	.8	-	-	1										
Zn	-	-	-	-	.9	-	-	-	.8	-	.9	.8	-	-	-	-	.9	1									
Fe	-	-	-.8	-	-	-	-	-	-	-	-	-	-.9	-	-	-	-	-	1								
Al	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1							
Cu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1						
Ni	-	-	-	-	.9	-	-	-	-	-	.8	-	-	-	-	-	.9	.8	-	-	-	1					
Ca	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.8	-	1				
P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.9	-	.9	1			
Mg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.9	.8	1		
S	-	-.8	-	-	-	-	-	-	-	-	-	-	-	-	-.8	-	-	-	-	-	-	-	-	-	-	1	
Ca:P	-	-	-	-	-.8	-	-	-	-	-	-.8	-	-	-	-	-	-.8	-.9	-	-	-	-.9	-	-	-	-	1

concentrations, where the latter are the result of both acid leaching and the geochemical cycling of this metal than with increased water concentrations resulting from pH depression alone.

Bone Zn concentration was significantly higher in white sucker from the most acid lake and tended to be elevated in this species from King Lake (Table II). Bone Zn concentrations were not correlated to either lake pH, or sediment concentrations (Table III). Since concentrations of dissolved Zn in 5 of the 7 study lakes were below detection, it was not possible to correlate lake Zn concentrations to white sucker bone Zn concentrations. Moreau *et al*. (1983) have reported the elevation of Zn in the opercula of brook trout (*Salvelinus fontinalis*) from 3 acidified as compared to 3 non-acidified lakes. Greater bone Zn concentrations in white sucker from lakes George and King are probably attributable to the increased mobilization and greater solubility of this metal in acid waters.

Although Fe, Al and Cu were detected in fish bone, there were no apparent trends in concentrations of these elements among the 7 fish populations studied (Table II). Bone Ni was only detected in trace amounts. Rather than the absence of significant differences being indicative of the availability of these 4 metals in acid and non-acid systems, it is more likely that the bone is not the best site for metal accumulation. For example, the liver has been shown to be the site of Cu storage and accumulation in the white sucker (Bendell-Young and Harvey, 1985) and gill accumulation of Al is indicative of its toxicity to fish (Schofield, 1976).

3.2. Elemental bone concentrations in relation to bone calcification

The mineralized portion of bone is composed of calcium triphosphate, which has a theoretical Ca:P weight ratio of 2.16:1 (Glimcher, 1959). Experimentally, this ratio has been shown to equal 2.11 in the scales of the mummichog (*Fundulus heteroclitus*) (Sauer and Watabe, 1984), and 2.05 in the vertebrae of carp (*Cyprinus carpio*) (Muramoto, 1981). Exposure of the mummichog to sublethal levels of Zn and the carp to sublethal levels of Cd resulted in bone demineralization and an overall reduction of the Ca:P ratio to 1.65 and 1.71 respectively, concurrent with an increase in bone metal concentrations.

In our study, fish from acid George Lake which contained the greatest bone metal concentrations of Mn, Zn and Ba also contained a significantly lower Ca:P ratio as compared to the 2 circumneutral fish populations (Table II). Bone Mg was also low in this population and tended to be low in the fish from 2 other acid lakes as compared to concentrations found in fish captured from 2 circumneutral lakes (Table III). It is possible that Zn, Mn and Ba are directly replacing Ca^{2+} in the hydroxapatite matrix, impairing bone calcification. However, direct ion replacement can only account for 7% of the noted reduction of bone Ca^{2+} in George Lake white suckers.

Rather than direct replacement of the metal ions in the bone matrix, one or a combination of these metals may be inhibiting the mineralization of the bone. For example, in addition to Cd and Zn inducing bone deformities *in vivo*, Mn and Zn have been shown to interfere with bone mineralization *in vitro* (Gluggenheim and Gaster, 1973). As well, even though it is a larger ion, Ba^{2+} can compete with Ca^{2+} for large anions (i.e. SO_4^{2-} and $ROSO_3^-$) because of favorable solvation factors. The complexes formed with such anions have low solubility, and in this way Ba may interefere with metabolic processes involving Ca (Williams, 1976).

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