Size- and age-specific patterns of trace metal concentrations in freshwater clams from an acid-sensitive and a circumneutral lake

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We collected freshwater clams (*Elliptio complanata*) from an acid-sensitive and a circumneutral lake in south central Ontario and compared tissue metal concentrations. Clams from the acid-sensitive lake had higher concentrations of Cu and Cd and lower concentrations of Zn and Mn than clams from the circumneutral lake. Tissue concentrations did not reflect metal levels in the water. Competition may be occurring between metals for binding substrate in clam tissue. Clam size and (or) age successfully predicted tissue metal concentrations, but in a metal-specific and tissue-specific manner. Clam biomonitoring studies should therefore control for size and age variability. Lake buffering capability was not very important in influencing size- and age-specific patterns of tissue metal concentrations. However, this conclusion is based solely on data from two lakes.

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Nous avons récolté des moules d'eau douce (*Elliptio complanata*) dans un lac peu tamponné et un lac presque neutre du centre sud de l'Ontario et avons évalué les concentrations de métaux dans leurs tissus. Les moules du lac acide avaient des concentrations de Cu et de Cd plus élevées et des concentrations de Zn et de Mn plus faibles que les moules du lac neutre. Les concentrations dans les tissus ne reflétaient pas les concentrations des métaux contenues dans l'eau. Il semble y avoir compétition entre les métaux pour se lier aux substrats dans les tissus. La taille des moules ou leur âge, ou la combinaison des deux facteurs, permettent de prédire assez bien les concentrations de métaux dans les tissus, mais d'une façon spécifique à chaque métal et spécifique à chaque tissu. Il est donc essentiel de contrôler la variabilité de la taille et celle de l'âge dans ce genre d'études. La capacité-tampon du lac ne semble avoir que peu d'influence sur les variations des concentrations tissulaires de métaux en fonction de la taille et en fonction de l'âge. Cependant, cette conclusion n'est basée que sur les données obtenues en deux lacs.

[Traduit par la revue]

Introduction

Freshwater unionid clams are used to biologically monitor variation in environmental levels of trace metals through *in situ* (Forester 1980; Imlay 1982; Great Lakes Institute 1984) and transplantation (Krauss *et al.* 1981; Great Lakes Institute 1985) studies. Much of the variability in trace metal tissue concentrations in marine clams has been attributed to variability in clam size or age (Hugget *et al.* 1973; Boyden 1974, 1977; Ayling 1974; Watling and Watling 1976; Bryan and Uysal 1978; Romeril 1979; Cossa *et al.* 1980; Strong and Luoma 1981; Popham and D'Auria 1983; Szefer and Szefer 1985; Cain and Luoma 1986). However, it is uncertain whether freshwater clams exhibit similar patterns. In this study we examine size- and age-specific patterns of trace metal concentrations in the freshwater clam *Elliptio complanata* (Unionidae).

The bioavailability of many trace metals is enhanced by increasing environmental acidity (Tri-Academy Committee on Acid Deposition 1985; Campbell and Stokes 1985; Stokes *et al.* 1985; Kelso *et al.* 1986). In acid-stressed, poorly buffered waters, bioaccumulation of trace metals has been reported in algae (Stokes *et al.* 1985), fish (Scheider *et al.* 1979; Harvey and Frazer 1982; Kelso and Gunn 1984), and clams (Forester 1980; Graney *et al.* 1984). We collected *E. complanata* from an acid-sensitive and a circumneutral lake to compare differences in metal concentrations in clam tissue between the two lakes with differences in size- and age-specific trace metal patterns.

Methods

We collected 50 *E. complanata*, including as wide a size range as possible (lengths, 45.9 to 84.9 mm), using SCUBA from a low exposure (low water turbulence) site in each of two small lakes during the 1st week of August 1985. Beech Lake (45°05′ N, 78°42′ W) and Tock Lake (45°16′ N, 78°53′ W) are located in south central Ontario on the Precambian Shield (Fig. 1). Although morphologically similar, the lakes differ greatly in total inflection point alkalinities (Tock and Beech lakes, 22 and 238 μequiv·L^{-1}, respectively; Ontario Ministry of Environment data, 1980–1983). Total concentrations of trace metal in water from the two lakes are very similar, although Beech Lake has slightly higher Mn levels (Table 1). Shell length, height, and width (as defined in Hinch *et al.* 1986) were measured to the nearest 0.01 mm using Mitutoyo 500-110 digimatic calipers. Live clams were brought back to the laboratory and were kept alive for 1 week in their original lake water in which time they purged the contents of their digestive system. Clams were then frozen alive in their shells and kept frozen (−20°C) until they were needed for analysis. Clam soft parts were removed from the shell while still frozen using a clean stainless steel scalpel and were thoroughly rinsed with distilled water. The gills were dissected from the remaining visceral mass (body) and analyzed for metals separately because they often contain larger concentrations of metals (Hemelraad *et al.* 1986). Gill water (i.e., water contained in gill tubes) was included in analyses. Tissues were oven-dried at 80°C in preweighed, acid-washed Coors evaporating dishes, and then digested in concentrated ultrapure nitric acid for 18 h. Solutions were heated at temperatures just below boiling until near dryness. After cooling, 1 *N* ultrapure nitric acid was added to the solutions, which were then filtered through acid-washed ashless No. 40 filter paper. The filtrate was analysed for levels of Cu, Cd, Zn, and Mn using flame spectrophotometry on a Perkin-Elmer model 5000 atomic absorption spectrophotometer. Background correction was used. Blanks were run at the beginning of each analysis session to ensure that extraneous metals were not entering the samples. Standard additions of randomly chosen samples demonstrated that there were no spectral interferences or signal suppression during the analyses.

Dry shells were coated with epoxy resin and a radial cut was made on all left valves from the umbo to the farthest point on the shell ventral margin using a Buehler Isomet low-speed saw with a diamond wafering blade. The cut shell edge was polished with fine-grain diamond grit paper and was affixed to a frosted microscope slide using epoxy resin. Once dried, most of the shell was cut off the slide using the Isomet saw, leaving a thin section of shell, approximately 0.5 mm thick, on the

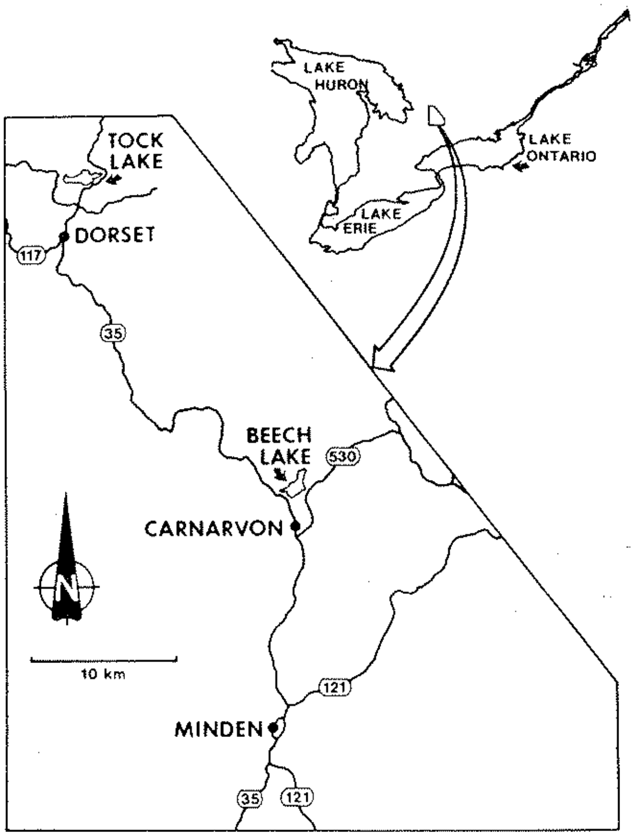


FIG. 1. Location of study areas in Haliburton County, south central Ontario.

slide. Clams were aged under a light microscope by counting the continuous thick, dark bands in a thin section inside the shell. These dark bands represent cessation of a year's growth (Isely 1914; Negus 1966; Haukioja and Hakala 1978; Strayer *et al.* 1981; McCuaig and Green 1983) and were easily distinguishable from noncontinuous thin bands, which are probably formed in response to environmental disturbances. Ghent *et al.* (1978) used a mark–recapture technique to verify growth ring annularity in *Anodonta grandis* and Imlay (1982) concluded from his literature review that aging unionids by counting dark bands within the shells was a very reliable method. In the present study *E. complanata* were independently aged by two people. Results from both were in agreement ($P < 0.05$, $r^2 = 0.70$). Clam ages ranged from 2 to 14 years.

One-way analysis of variance (ANOVA) was employed (using log-transformed trace metal concentrations) to compare trace metal concentrations in gill and body tissue between the two lakes. To assess the interrelationships between clam metal concentrations, canonical variates analyses were also performed on gill and body trace metal data. Analysis of covariance (ANCOVA) was used to compare the relationships between clam age and length and clam metal concentration in the two lakes. Clam length was used as measure of size since it was highly correlated with clam height and width and since it exhibited the greatest range out of these variables.

Results

Trace metal concentrations in both gill and body tissues differed between Beech Lake and Tock Lake clams (Table 2). Tissues of Tock Lake clams had higher concentrations of Cu and Cd and lower concentrations of Zn and Mn than those of Beech Lake clams ($P < 0.05$ in all cases). A canonical variates analysis of trace metal variability in gills defined an axis from Tock Lake to Beech Lake of increasing Cu and Cd concentrations relative to those of Zn and Mn. A similar analysis of trace metal variability in body tissues derived an axis from Beech Lake to Tock Lake of increasing Mn concentrations relative to those of Cu and Cd (Table 3). Both models accounted for significant ($P < 0.0001$) proportions of the total variability of trace metals in gill ($r^2 = 0.51$) and body ($r^2 = 0.62$) tissues.

Tock Lake clams were slightly larger (means, 65.2 vs. 61.8 mm; $P < 0.05$) and older (means, 6.4 vs. 4.6 years; $P < 0.05$) than Beech Lake clams. However, there was no relationship between size and age for these populations, possibly because of the great variability in *E. complanata* growth rates among individuals and the lack of extremely young individuals in the samples from both lakes. Size and age were therefore used as independent covariates in each analysis.

Each ANCOVA was successful in predicting tissue trace metal

TABLE 1. Mean concentration (SE) of metals ($mg \cdot L^{-1}$) in Tock Lake and Beech Lake

	Tock Lake		Beech Lake
Mn	0.011 (0.001)	*	0.014 (0.001)
Cd	0.0003[a]		0.0003[a]
Cu	0.001[a]		0.001[a]
Zn	0.008 (0.005)		0.005 (0.002)

NOTE: Samples were collected just above the clam populations in the water column. Water samples (three at each site) were taken at the time of clam collection. Metal analysis was performed by the Ontario Ministry of the Environment.
*Significant difference between lakes ($P = 0.05$).
[a]At or near minimum detectable limits.

TABLE 2. Mean metal concentrations (SE) ($\mu g \cdot g^{-1}$ based on dry weight) in gill and body tissues of *E. complanata* from Beech Lake and Tock Lake

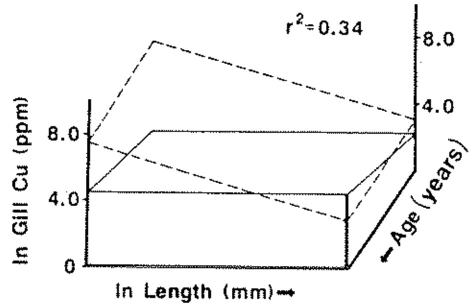
	Beech Lake ($n = 50$)	Tock Lake ($n = 50$)
Gills		
Cu	13.28(0.80)	18.51(1.11)
Cd	10.10(0.61)	12.78(0.64)
Zn	371.32(15.38)	273.81(10.30)
Mn*	140.80(5.70)	104.90(4.63)
Body		
Cu	7.29(0.26)	10.29(0.98)
Cd	10.98(0.82)	14.51(0.65)
Zn	155.50(6.21)	134.17(8.93)
Mn*	42.20(2.77)	23.50(1.70)

*Mean Mn concentrations have been divided by 100.

TABLE 3. Standardized canonical coefficients for each variable and the correlation of each variable with the canonical score from the canonical variates analyses of gill metal variation and body metal variation

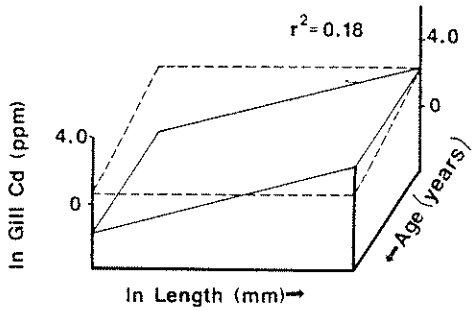
	Gills		Body	
	Standardized coefficient	Structure coefficient	Standardized coefficient	Structure coefficient
Cu	0.771	0.625	0.318	0.428
Cd	0.587	0.417	1.140	0.457
Zn	−1.009	−0.643	0.069	−0.385
Mn	−0.075	−0.590	−1.490	−0.657

a



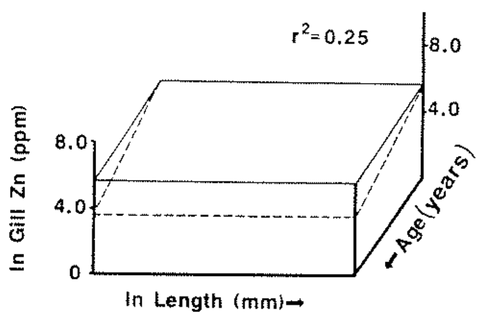
Source of variation	P
ln length	0.106
age	0.001
lake	<0.001
ln length x age	0.521
ln length x lake	0.250
age x lake	0.462
ln length x age x lake	0.014

b



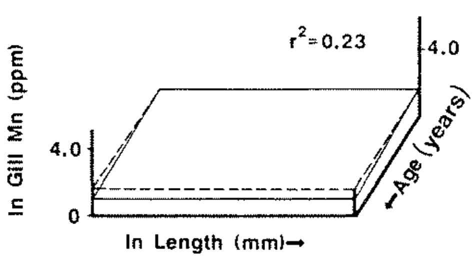
Source of variation	P
ln length	0.002
age	0.443
lake	0.021
ln length x age	0.457
ln length x lake	0.737
age x lake	0.170
ln length x age x lake	0.456

c



Source of variation	P
ln length	0.759
age	0.003
lake	<0.001
ln length x age	0.642
ln length x lake	0.378
age x lake	0.278
ln length x age x lake	0.403

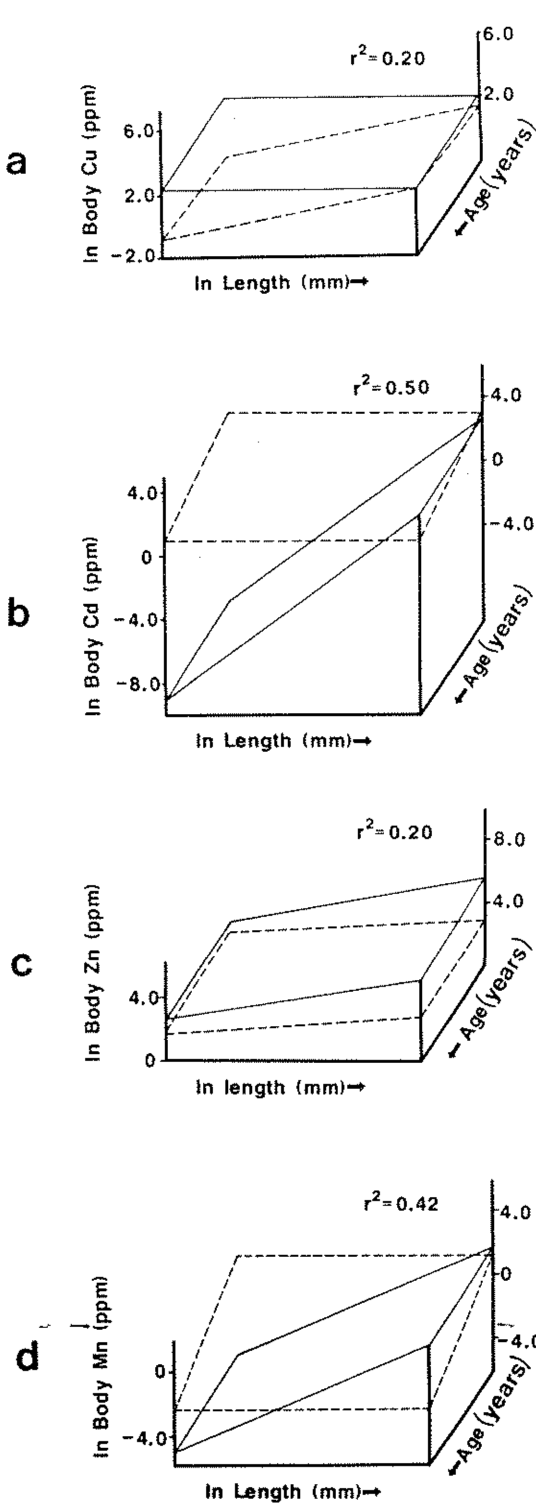
d



Source of variation	P
ln length	0.923
age	<0.001
lake	<0.001
ln length x age	0.717
ln length x lake	0.634
age x lake	0.303
ln length x age x lake	0.692

FIG. 2. Gill metal concentrations versus ln length and age by lake (———, Beech Lake; - - - -, Tock Lake). Planes were derived from ANCOVA-predicted values. Source of variation and probability levels from each ANCOVA are included. Negative concentrations may be present because original metal concentrations were log-transformed.

variability and in each case the lake was a significant contributor to the model. Clam age was a good predictor of gill Zn and Mn concentrations (Figs. 2*c* and 2*d*) while gill Cd was better predicted by clam size (Fig. 2*b*). Young, small clams had proportionately higher gill Cu concentrations in Tock Lake than young, small clams in Beech Lake relative to old, large clams from both lakes (Fig. 2*a*). Clam age and size were useful in predicting Zn concentrations in body tissues (Fig. 3*c*) while clam age was primarily responsible for modelling body tissue Mn concentrations (Fig. 3*d*). Big, old Tock Lake clams had greater body tissue Cu concentrations than small, young Tock Lake clams (Fig. 3*a*). Young Tock Lake clams had relatively



a

Source of variation	P
ln length	0.049
age	0.073
lake	0.004
ln length x age	0.043
ln length x lake	0.532
age x lake	0.885
ln length x age x lake	0.241

b

Source of variation	P
ln length	<0.001
age	0.300
lake	<0.001
ln length x lake	0.015
ln length x age	0.033
age x lake	0.602
ln length x age x lake	0.887

c

Source of variation	P
ln length	0.035
age	<0.001
lake	0.005
ln length x age	0.739
ln length x lake	0.598
age x lake	0.952
ln length x age x lake	0.727

d

Source of variation	P
ln length	0.119
age	<0.001
lake	<0.001
ln length x age	0.911
ln length x lake	0.549
age x lake	0.261
ln length x age x lake	0.406

FIG. 3. Body metal concentrations versus ln length and age by lake (———, Beech Lake; - - - -, Tock Lake). Planes were derived from ANCOVA-predicted values. Source of variation and probability levels from each ANCOVA are included. Negative concentrations may be present because original metal concentrations were log-transformed.

high body tissue Cd concentrations at a given length while large Beech Lake clams showed a similar response at a given age (Fig. 3*b*).

Discussion

The trace metals examined in this study are known to increase in geochemical mobility (i.e., leave soils and sediments in an aqueous form) with increasing acidification (Tri-Academy Committee on Acid Deposition 1985) and are generally found in greater concentrations in nonbuffered than in buffered waters in Ontario (Forester 1980). Tock Lake is acid sensitive and has low buffering capabilities while Beech Lake is circumneutral and buffers acidic input. However, water metal levels were similar between the lakes. Although Beech Lake had slightly higher Mn levels in the water than Tock Lake, and Beech Lake clams had higher Mn concentrations than Tock Lake clams, clam metal

TABLE 4. Relationships between Cu, Cd, Zn, and Mn concentrations and size and age (inferred from size) in marine molluscs: metal concentrations increase with size (+), decrease with size (−), or are not related to size (o)

	Trace metal	Metal–size relationship	Reference
Mercenaria mercenaria	Cu	+	Romeril 1979
	Zn	+	Romeril 1979
	Mn, Zn	−	Boyden 1977
Mytilus edulis	Cu, Cd, Zn	o	Brix and Lyngby 1985
	Cd, Mn, Zn	+	Szefer and Szefer 1985
	Cu	−	Szefer and Szefer 1985
	Cu	−	Popham and D'Auria 1983
	Cd	+	Ritz *et al.* 1982
	Zn	o	Ritz *et al.* 1982
	Cu	−	Ritz *et al.* 1982
	Cu, Cd, Zn, Mn	−	Cossa *et al.* 1980
	Cd, Zn	+	Harris *et al.* 1979
	Mn, Cu	−	Harris *et al.* 1979
	Cd	o	Boyden 1977
Ostrea edulis	Mn	+	Boyden 1977
	Zn, Cu, Cd	o	Boyden 1977
Patella vulgata	Cd	+	Boyden 1977
	Zn	−	Boyden 1977

concentrations did not reflect lake metal levels. In the more alkaline environment (Beech Lake) clams had higher Mn/Cu, Mn/Cd, Zn/Cu, and Zn/Cd ratios. These results are similar to those of Servos *et al.* (1987), who examined bioaccumulation of metals by *E. complanata* over a 2-month period during acidic snowmelt. In their study, Zn concentrations increased relative to Cd concentrations in gill and body tissues in a less acidic environment (pH 6.2 to 6.8) as compared with more acidic environments (pH 4.5 to 5.5). Mechanisms that control this type of phenonmenon are not fully understood.

Marine clams are known for their ability to selectively accumulate trace metals (Romeril 1979), although mechanisms that are responsible for accumulation, storage, and elimination of metals may differ depending on the metal (Robinson and Ryan 1986). There can be competition between metals for a limited supply of binding substrate (ligand) within mollusc tissue (Mason and Simkiss 1983). Mason and Simkiss suggest that the competitive nature of metals for ligands can be altered depending on environmental metal levels. In other words, one or a few particular metals could alter the competitive nature of many metals for ligands. Competition between trace metals for binding substrate in *E. complanata* has been reported by Tessier *et al.* (1984) who proposed that iron oxyhydroxides and manganese oxides, which originate in the sediments, compete with Cu and Zn for binding substrate within the clam tissue. They suggested that Fe and Mn play a protective role as they may be the principal factors controlling the dissolved trace metal concentrations to which the clam is exposed. Mn may play a similar role in this study. Where Mn levels were relatively low (Tock Lake), Cu and Cd concentrations were greater in the clams. Where Mn levels were relatively high (Beech Lake), Cu and Cd concentrations were lower in the clams. Patterns of clam Zn concentrations cannot be explained in this manner. Metal concentrations in sediment fractions from the lakes may have been more useful than water metal concentrations for predicting metal concentrations in clam tissues.

Gill and body tissue metal concentrations exhibited similar patterns of variability within each lake, although concentrations in the gills were usually twice those in body tissue. Unionid gill tissues are often found to have the greatest concentration of metals when organs are separately analysed (Tessier *et al.* 1984); V.-Balough and Salanki 1984; Hemelraad *et al.* 1986). However, gill metal concentrations may not reflect what is actually in the gill tissue. The gills are covered in mucus which aids in trapping food. Indigestible material accumulates on the gills as pseudofeces (mucous balls) and is eventually eliminated (Morton 1983). It is quite likely, therefore, that both the mucus and pseudofeces, which are not easily washed off, represent an additional source of metals. Thus concentrations of metals in the gill tissue may be overestimated. During the clams' reproductive season, glochidia raised in the gills may also contribute to gill metal levels. However, none of the clams in the present study appeared gravid and it is known that *E. complanata* generally spawn 4 to 6 weeks earlier than our sampling period (Matteson 1948). For these reasons the current practice in large-scale biomonitoring studies of including gills in whole body analyses should be reassessed. Gill inclusion may be inappropriate if gills increase body tissue trace metal variability. Body tissues probably reflect longer-term environmental metal levels than gills; therefore exclusion of gills from whole body analyses would also allow more accurate comparisons in long-term transplantation studies.

Relationships between metal concentration and clam size and age varied among metals. This type of variability is common in marine molluscs where size-specific metal concentrations vary among and within metals, among and within species, and among studies (Table 4). There are many factors that could enhance variability in size- and age-specific patterns of trace metals in clams. For example, feeding habits may influence the amount of particulate metals that are ingested (Smock 1983); reproductive state and seasonal variation in growth rate (Strong and Luoma 1981) could alter metal flux by altering metabolic processes (Cain and Luoma 1986) which are responsible for filtering rates (Cossa *et al.* 1980). It is also possible that clam growth could dilute tissue concentrations despite environmental levels if tissue is added faster than metals are accumulated (Strong and Luoma 1981). Physiological explanations for the observed size- and age-specific patterns are not within the scope of this paper. However, it is clear that investigators who wish to compare *E. complanata* metal concentrations should standardize clam size and age. This would especially apply to clam biomonitoring studies where comparisons are made among sites and years.

In only two of eight cases (gill Cu and body Cd) did lake interact with clam size and (or) age in modelling tissue trace metal variability. Buffering capability, which is the primary difference between the two lakes, is therefore not very important in influencing size- and age-specific variability in trace metal concentrations. Popham and D'Auria (1983) came to similar conclusions about *Mytilus edulis*, which were sampled along a pollution gradient. They determined that size- and age-specific metal uptake was independent of population location. However, with only two lakes in our study, caution should be used when our results are extrapolated to larger-scale systems.

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