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Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient

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Abstract

Surficial (oxic) sediments, overlying water, and specimens of the freshwater bivalve *Anodonta grandis* were collected by divers at 11 littoral lacustrine sites located along a geochemical gradient of pH, [Cd], [Cu], and [Zn] in an area influenced by mining and smelting activities. The molluscs were dissected (gills; hepatopancreas; remaining tissues) and analyzed for Cd, Cu, Zn, and metallothioneins (MT). Tissue concentrations of MT in *A. grandis* varied between 100 and 440 nmol metal binding sites (g dry wt)$^{-1}$. Metallothionein levels in the gills, the remaining tissues, and the whole organism were significantly correlated ($P < 0.01$) with tissue Cd concentrations. In contrast, correlations between [MT] and tissue levels of Cu or Zn were weak or nonexistent. The intersite variations in MT concentrations were best related, not to total dissolved metal concentrations at the time of sampling, nor to extractable metal concentrations in the sediments, but to the free Cd^{2+} concentration at the sediment–water interface, as estimated from sediment-water sorptive equilibria. These observations suggest that Cd^{2+} activity is the key environmental factor to which metallothionein levels in *A. grandis* are responding in the studied lakes. The mollusc condition indices (C.I. = 1,000 × total dry wt of flesh of the animals/total intervalval volume) deteriorated as Cd concentrations in the tissues increased.

Freshwater and marine molluscs are recognized for their capacity to concentrate metals from their environment (Phillips 1980). Their tolerance of the resulting metal burdens has been attributed to the existence of an effective detoxification mechanism involving the trapping of the incoming metals by specific ligands present in the cytosol (Viarengo 1989). Possible candidates for this role include metallothioneins (MT), low-molecular-weight, cysteine-rich metal-binding proteins with high affinity for group IB and IIB metal ions. Stability constants measured in vitro for metal ions such as Cd^{2+}, Cu^{2+}, Hg^{2+}, and Zn^{2+} are usually several orders of magnitude higher than those of the comparable metal complexes with other cellular ligands.

Given its ubiquity in the animal kingdom and its strong affinity for "soft" metal cations, MT is well positioned to play a metal detoxification role (Stegeman et al. 1992). Consistent with such a role is the observation of enhanced metal tolerance associated with the induction of metallothionein. Thus, prior exposure of the marine mussel *Mytilus edulis* in the laboratory to Cu, Cd, or Zn at concentrations sufficient to induce metallothioneinlike proteins conferred increased tolerance to inorganic Hg (Roesijadi and Fellingham 1987). Similar results have been reported for freshwater fish (Klaverkamp and Duncan 1987; NRCC 1985).

However, most of the available evidence supporting the idea that aquatic organisms synthesize metallothionein as a defense against toxic metals derives from laboratory trials where exposure conditions can differ greatly from those encountered in natural environments. In particular, metal concentrations shown to induce MT synthesis in laboratory experiments are often orders of magnitude higher than those found in even the most contaminated aquatic systems where the studied organisms are found. Other putative functions have been proposed for metallothionein: as a

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Zn- and Cu-transfer protein, as a free-radical scavenger, as a stress protein (response to heat stress, starvation, bacterial infection, etc.), and as a cysteine reservoir (Engel and Brouwer 1987; Bremner and Beattie 1990; Hidalgo et al. 1991). The diversity of roles attributed to MT and the perceived artificiality of the laboratory experiments have cast doubt on the primary involvement of MT in metal detoxification in aquatic animals living in their natural environments (Cosson et al. 1991).

An important criterion of an effective detoxification system is that its activity increase as a function of toxicant concentration (Simkiss et al. 1982). In the present study, we have examined the hypothesis that metallothionein concentrations in the freshwater mollusc *Anodonta grandis* respond in a dose-dependent manner to increasing Cd bioavailability in the environment. A companion study (Tessier et al. 1993) examined the partitioning of Cd between the bottom sediments and the overlying water, gave details of the geochemical model used to estimate the environmental $[Cd^{2+}]$ gradient, and explored relationships between $[Cd^{2+}]$ and Cd tissue concentrations in *A. grandis*.

Study area

A preliminary survey in the mining area of Rouyn-Noranda in northwestern Québec indicated the presence of both the required spatial gradient of metal contamination and an appreciable bivalve population along this gradient. Major mining and smelting operations have been carried out in the region since 1927. According to data available for 1977, annual atmospheric emissions from the smelting complex included 485,000 t of SO_2, 75 t of Cd, 34 t of Cu, 1,540 t of Pb, 610 t of Zn, and 16 t of Hg. Lesser emissions of Bi, Co, Fe, Ni, Sb, Se, and Te were also reported (BEST 1979*a*). Air and water pollution controls have recently been introduced, but surficial sediments in the surrounding lakes still reflect their history of contamination.

Eleven lakes, representing a wide range of Cd, Cu, and Zn contamination, were chosen along the spatial gradient identified in the preliminary survey. Sediment, overlying water, and bivalves were collected from 12–25 June 1989 at a single littoral site in each lake (Fig. 1). The principal source of metal contamination for these lakes is thought to be atmospheric deposition from smelter emissions, since no mining operations or mine residues are evident in the immediate lake catchments. During the weather conditions normally prevailing in the region, 9 of the 11 sites are downwind from the smelting activities in Rouyn-Noranda (Fig. 1).

All the lakes studied lie at elevations of <300 m on glaciolacustrine deposits (rich in clay and silt) left by the postglacial lake Barlow-Ojibway. Higher elevations in the region are usually dominated by Precambrian igneous and metamorphic rocks [surficial geology map No. 1639A (Geol. Surv. Can.; Energy, Mines and Resources Canada)].

Methods

Sediment sampling and analyses—At each sampling site, SCUBA-equipped divers collected three or four sediment cores within the area of bivalve collection. The cores were extruded on shore and samples of the uppermost 0.5 cm, i.e. from the oxidized layer, were pooled, placed in acid-cleaned polypropylene centrifuge bottles, covered with lake water, transported to the laboratory at ~4°C, and stored at −20°C until analyzed (within 1 month). Before analysis, the samples were thawed, centrifuged at 13,700 × *g* for 30 min, and the supernatants discarded. After removal of any visible large fragments with polypropylene tweezers (e.g. pebbles, shell fragments, roots), an amount of wet sediment corresponding to ~1 g dry wt was extracted for 6 h at 96°C with 0.04 M $NH_2OH \cdot HCl$ in 25% (vol/vol) acetic acid. This single extraction should release nondetrital metals associated with carbonates and amorphous Fe- and Mn-oxyhydroxides or adsorbed to particulate organic matter (NRCC 1988). A second set of sediment samples, collected in a similar manner at the same sites as part of the companion study (Tessier et al. 1993), was used to determine the geochemical variables needed to estimate the partitioning of Cd in the surficial sediments and $[Cd^{2+}]$ at the sediment–water interface (i.e. organic C, amorphous {Fe-ox} and total Cd, $\{Cd\}_T$). Note that $\{\cdot\}$ and $[\cdot]$ refer here and elsewhere in the text to concentrations of solid and dissolved species respectively.

Concentrations of Fe, Cd, Cu, and Zn in the

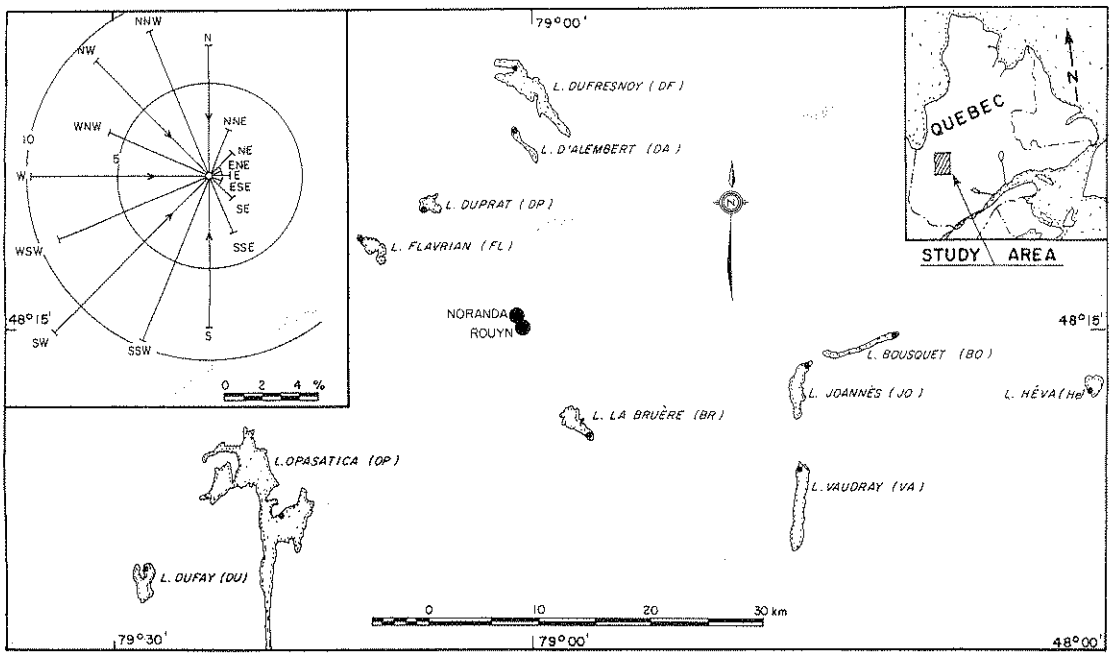

Fig. 1. Locations of sampling stations. Average wind directions at the Rouyn-Noranda smelting complex are also shown.

sediment extracts were measured by flame atomic absorption spectrophotometry (AAS) (Varian Spectra AA-20). The Hg content of the sediments was determined, after acid digestion (HNO_3) and reduction ($SnCl_2$) steps, by cold-vapor AAS (Varian AA VGA 76). Sediment organic C concentrations, {C-org}, were determined by combustion (Carlo-Erba CNS analyzer, model NA 1500) after removal of inorganic C by acidification with 0.05 M H_2SO_4 (15 min, 100 ml per g sediment dry wt).

Water sampling and analyses—Water samples for determination of total dissolved metals ($[Cd]_d$, $[Cu]_d$, and $[Zn]_d$) were collected on one occasion at each littoral sampling site. For all the lakes except Joannès and La Bruère, divers collected samples from ~10 cm above the sediments in clean Teflon bottles. Each sample was filtered in the field through a prewashed polycarbonate membrane (0.4 μm, Nuclepore). The first 4-ml portion of the filtrate was discarded and a subsequent 2-ml volume was transferred to a clean Teflon bottle and acidified with 200 μl of ultrapure HNO_3 (J. T. Baker Chemical Co., Ultrex grade, 0.1 N). For Joannès and La Bruère, dissolved metal concentrations were obtained by in situ dialysis in summer 1987 and 1988 respectively (Tessier et al. 1993). Metal concentrations were measured by flameless AAS (Varian Spectra AA-30 equipped with a GTA-96 graphite tube atomizer).

Values obtained for a certified reference water sample (SLRS-1, Riverine water reference material, NRCC) were within ±7% of the certified values for Cu and Zn; the Cd concentration in the reference sample was too close to our analytical detection limit to allow meaningful comparison. Deionized water blanks that had been taken into the field and carried through the filtration and preservation procedures showed low but nonnegligible contamination (Cd: 0.2 nM; Cu: 4.6 nM; Zn: 10 nM). In addition, refiltration of a filtered sample (Lake Héva) through a second polycarbonate filter had no effect on the measured trace metal concentrations, suggesting that adsorptive losses during the filtration step itself were unimportant.

Special care was taken to avoid sample contamination, especially by Zn. All the plasticware was soaked for 24 h in 5% HNO_3, then in 7% HCl, and finally rinsed thoroughly with deionized water. The membrane filters were

mounted in their holders under a laminar flow hood in the laboratory. These assemblies were placed in low-density polyethylene bags and used in the field only once. Contact time of the sample with the ambient air was kept to a minimum.

Water samples for determination of pH, alkalinity, Ca, and dissolved organic C (DOC) were collected at ~10 cm above the sediments in acid-cleaned polyethylene bottles washed with the lake water at the time of collection. Samples for pH measurement were collected with the same procedure on several other occasions during the summer in order to obtain time-averaged pH values, mean pH = $-\log_{10}(\Sigma[H^+]/N)$. Ca was determined by flame AAS with the addition of a La-Cs ionic suppressor (2,000 mg liter^{-1}). DOC was measured by persulfate-UV oxidation followed by conductimetric determination on a Technicon autoanalyzer of the CO_2 released. Alkalinity was evaluated by Gran titration.

Bivalve collection and analysis—At each site, divers collected 20 actively filtering specimens of *A. grandis* of uniform size (nominal length, ~8 cm; actual means ranged from 7.3 to 8.5 cm). Sampling was done in June to avoid possible complications due to the reproductive cycle; none of the animals were gravid. The sampling areas at each individual site ranged from 300 to 1,500 m^2 (approx radius, 10–22 m). The bivalves were dissected within 12 h of collection into three tissue groups: gills, hepatopancreas, and remaining tissues (mantle, foot, visceral mass, labial palps, kidneys, heart, muscles—hereafter referred to as the "body"). Tissues from five animals were pooled (yielding four replicate samples per site), sealed in polyethylene bags filled with nitrogen, and stored at −40°C until the homogenization step, carried out 2 weeks to 5 months later. The gut contents of the animals were removed during the dissection step by flushing the digestive tracts with deionized water to minimize the influence of ingested particulate material on estimates of pollutant body burdens (Hare et al. 1989).

Partially thawed tissues were homogenized with a tissue grinder (Brinkman Kinematica CH-6010) in an equal weight of ice-cold 0.9% NaCl solution (for the hepatopancreas, 4× weights were used). Homogenization was performed in a glove bag filled with nitrogen (Atmosbag, Aldrich Chem. Co.) to minimize oxidation of MT during this step, and the homogenized tissues were kept on ice. A subsample (3 ml) was centrifuged at 30,000 × *g* for 30 min at 4°C and the supernatant analyzed the same day for metallothionein by the Hg-saturation method described below. Additional subsamples of the whole homogenate were retained for determinations of Cd, Cu, and Zn concentrations and to determine the {dry wt : wet wt} ratio. The following condition index was calculated for each replicate (Davenport and Chen 1987):

$$\frac{\text{total dry wt of flesh (g)}}{\text{total intervalval volume (ml)}} \times 1{,}000. \quad (1)$$

This index is widely used and involves measurements that are easily performed.

Metallothionein concentrations in the various tissues were measured with a mercury-saturation assay, in which samples were incubated with excess ^{203}Hg in the presence of 10% trichloroacetic acid (Fig. 2). The method was shown by Dutton et al. (1993) to be simple, specific, and rapid; they demonstrated successful displacement of Cu (96% release), Zn, and Cd by Hg in trials with rainbow trout (*Oncorhynchus mykiss*) hepatic MT, known to have a high Cu content. The characteristic overestimation of MT observed in the original method of Piotrowski et al. (1973) was overcome by Dutton et al. in the modified assay by adding an exogenous protein (egg albumin) to scavenge Hg bound to cytosolic ligands other than MT.

The Hg-saturation assay we used was adapted slightly from that of Dutton et al. (1993). Fresh rat hemoglobin was substituted for egg albumin as the exogenous protein; it formed firmer pellets on centrifugation, which facilitated withdrawal of the supernatant for γ-counting. Sample dilution series recommended by Dutton et al. to detect nonspecific metal binding (at the end of the assay) were used in preliminary assays with various mollusc tissues. Because nonspecific binding was unimportant, serial dilution was not performed on a routine basis. With the adapted assay, recovery of an internal standard of commercially available rabbit liver MT (Sigma

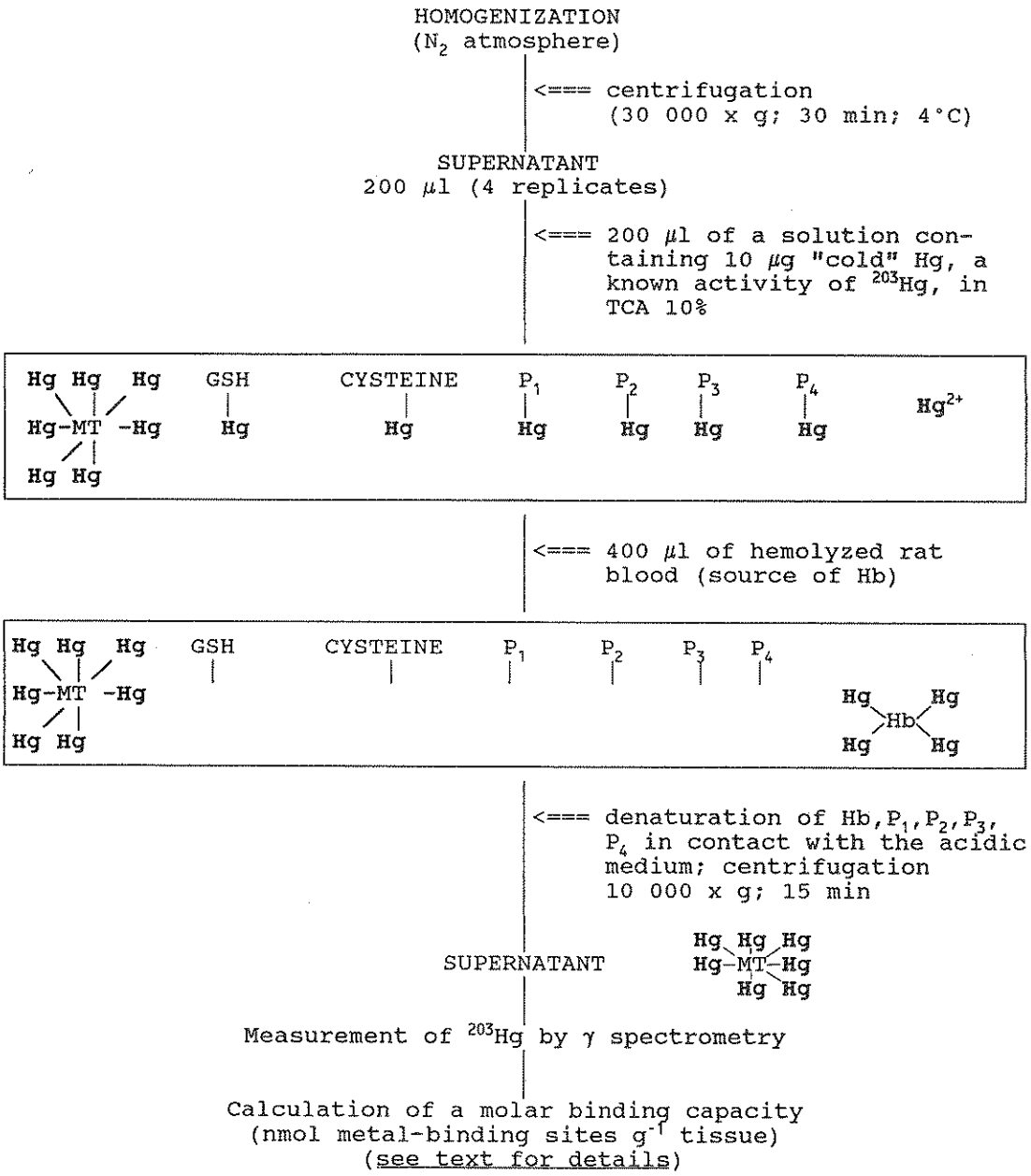

Fig. 2. Flow diagram for the ^{203}Hg-saturation assay for measuring metallothioneins. Abbreviations: GSH—glutathione; P_1, P_2, P_3, P_4—supernatant labile proteins and enzymes; Hb—hemoglobin; TCA—trichloroacetic acid.

Co.), added to a mollusc tissue homogenate preparation before ^{203}Hg addition, averaged 96±1% (N = 4). In addition, MT concentrations determined in tissues that had been stored for 15 or 60 d at −40°C under a nitrogen atmosphere were indistinguishable from those determined at t = 0 on fresh samples of the same tissues; we conclude that the tissue preservation procedure was appropriate.

Metallothionein concentrations are expressed as nmoles metal binding sites per gram of dry tissue weight (based on measured Hg-

binding capacities). Concentrations in the whole organism, [MT(org)] were calculated as

$$[\mathrm{MT(org)}] = \frac{\Sigma[\mathrm{MT(tissue)}]_i W_i}{\Sigma W_i} \qquad (2)$$

where $[\mathrm{MT(tissue)}]_i$ is the metallothionein concentration and W_i the dry weight of the ith tissue. Precision of the mean (SE/mean) for MT concentrations, as estimated from the four replicates at each site, ranged from 4 to 20% (avg 13%), 2 to 39% (avg 17%), 3 to 23% (avg 11%), and 3 to 16% (avg 9%) for the gills, hepatopancreas, body, and whole organism, respectively.

For trace metal analyses, an amount of tissue homogenate corresponding to 100 μg dry wt was pipetted into a 30-ml Teflon bomb (Parr Instr. Co.) and preheated for 2 h in an oven heated at 70°C; ultrapure concentrated nitric acid (3 ml; BDH Chemicals, Aristar grade) was then added and the digestion carried out in a microwave oven (700 W, ≤2 min) up to a pressure of 6,900 kPa. Cooled, digested samples were diluted with deionized water up to a volume of 25 ml and Cd, Cu, and Zn concentrations were determined by plasma atomic emission spectrometry (Thermo Jarrell Ash, Atom Scan 25; sequential reading). Samples of similar weight of a certified reference material (U.S. Natl. Inst. Std. Technol.; Oyster tissue, SRM No. 1566) were digested during every other analytical run; measured trace metal concentrations in the standard varied little over time (C.V. 1–12%, $N = 6$) and were within the certified ranges for each element. Digestion blanks were also run and no contamination was detected.

Metal concentrations in the whole organism, [M(org)], were calculated in the same way as [MT(org)] (Eq. 2). Precision of the mean (SE/mean), as obtained from the four replicates at each site, was similar for all tissues: ≃16% for Cd, ≃12% for Cu, and ≃8% for Zn.

All labware was soaked in 15% nitric acid for 24 h and rinsed repeatedly in deionized water before use, in order to minimize trace element contamination. High purity water for analytical purposes (>17 Mohms cm^{-1}) was obtained from a commercial system by means of mixed-bed ion exchange, charcoal adsorption, and filtration (0.2 μm) steps.

Statistical analyses—Relationships between tissue concentrations (MT; metals) and various environmental variables were initially examined in bivariate scatter diagrams and then tested by calculating Pearson correlation coefficients (r) and associated probabilities. The assumption of normality required for use of the Pearson r-value was generally met with the nontransformed data.

Results and discussion

Contamination gradient—The sampling area exhibits an extensive spatial contamination gradient, as can be judged from Tables 1 and 2. Concentration ranges are greatest for Cd^{2+} and for Cu and Zn extracted from surficial oxic sediments with a reducing solution, $NH_2OH \cdot HCl/HOAc$ (max : min values ≃ 25). H^+-ion activities, Gran alkalinity values, and dissolved Zn also show appreciable interlake variability, whereas the remaining variables (Ca, DOC, $[Cd]_d$, $[Cu]_d$) are somewhat less variable. As anticipated, the highest sediment metal concentrations are found in lakes downwind from the smelting complex (D'Alembert, Duprat, Flavrian, Joannès, La Bruère, and Vaudray—*see Fig. 1*). The chosen lakes do not appear to be highly contaminated by Hg (also a potential inducer of MT biosynthesis); Hg concentrations in the surficial sediments fall within the range of sediment background levels for this region: 0.10–0.45 nmol Hg (g dry wt)$^{-1}$ (as determined in pre-1900 strata from lake sediment cores, BEST 1979*b*—Table 2).

Metal concentrations in the tissues of *A. grandis* also vary along the contamination gradient (Table 3), though to a lesser extent than do the metal concentrations in the sediments. Interlake variability in the tissue metal concentrations is greater for Cd than for Cu or Zn; for a given metal, ranges are wider for the gills than for the other tissues. The relative constancy of the Zn levels in the tissues of *A. grandis,* despite the presence of an appreciable Zn gradient (Tables 1 and 2), may reflect its status as an essential trace element and the existence of some form of homeostatic control over its tissue concentrations. Langston and Zhou (1986) observed that body concentrations of Cu and Zn in the gastropod *Littorina littorea* did not respond to fluctuations in environmental Cu and Zn contamination along the coasts of Great Britain.

The tissue concentrations of Cu and Zn reported in Table 3 are not unusual for freshwater lamellibranch molluscs (cf. Manly and

Table 1. Surface areas of the 11 lakes studied and geochemical data for water samples collected close to the sediment–water interface at each sampling site. Mean pH was estimated from a summer series (June–October).

Lake, area in ha	Ca (10^{-4} M)	Gran alk. (μeq liter^{-1})	DOC (mg C liter^{-1})	Mean pH	Dissolved M (nM)		
					Cd	Cu	Zn
Bousquet, 176	1.05	147	9.7	6.34	2.8	63	205
D'Alembert, 109	1.19	173	6.5	7.14	2.1	101	108
Dufay, 435	0.85	529	7.2	6.51	2.1	56	53
Dufresnoy, 1,158	1.66	328	10.9	7.17	1.0	75	49
Duprat, 218	1.88	326	7.7	7.10	1.2	145	162
Flavrian, 308	1.58	108	6.4	7.39	2.7	74	245
Héva, 228	0.59	47	7.1	6.18	2.2	38	142
Joannès, 430	1.70	287	—	7.22	1.1*	51*	42*
La Bruère, 388	3.44	298	—	7.33	0.8*	113*	15*
Opasatica, 5,128	1.65	292	6.5	7.39	0.8	56	40
Vaudray, 746	0.90	93	6.5	6.56	1.2	53	174
Max : min	5.8	11	1.7	16†	3.5	3.8	16

* Measurements with pore-water peepers.
† Ratio calculated for [H$^+$], not pH.

George 1977: 340–1,630 nmol Cu g^{-1}, 6,170–27,800 nmol Zn g^{-1}), but the Cd levels are considerably higher than those found in indigenous populations from uncontaminated systems. For example, in their study Manly and George reported values from 4 to 52 nmol Cd g^{-1}, well below our range of 140–1,150 nmol g^{-1}. In our companion study values as low as 25–70 nmol Cd g^{-1} were found for molluscs in lakes unaffected by mining activities (Tessier et al. 1993). Malley et al. (1989) determined a whole-body Cd concentration of $\simeq$36 nmol (g dry wt)$^{-1}$ for specimens of *A. grandis* from a pristine Precambrian Shield lake in western Ontario (0.01 $\pm$ 0.001 nM dissolved Cd). Similarly, Segar et al. (1971) reported concentrations of 11, 47, and 1,835 nmol (g dry wt)$^{-1}$ for Cd, Cu, and Zn for specimens of the genus *Anodonta* collected in a lake with very low metal contamination in Ireland.

Relationship between metallothionein and tissue metal levels—Earlier work in our laboratory (Legrand et al. 1987) demonstrated the presence of a metallothioneinlike protein in the gills and the hepatopancreas of *A. grandis* specimens that had previously been exposed to artificially high concentrations of Cd in the laboratory. This Cd-, Cu-, and Zn-binding protein was subsequently detected in specimens collected from lakes in the Rouyn-Noranda mining area. The molecular weight of this metal-binding protein ($\simeq$10 kDaltons) as

Table 2. Geochemical data for the surficial sediments from the 11 lakes studied. Estimation of free Cd^{2+} concentrations from sediment–water sorptive equilibria is described in the text.

Lake	[M] extracted with $NH_2OH \cdot HCl$ (nmol g^{-1})		Total metal (nmol g^{-1})		Sorbents (μmol g^{-1})		Calculated [Cd^{2+}] (nmol liter^{-1})
	{Cu}	{Zn}	$\{Hg\}_T$	$\{Cd\}_T$	{Fe-ox}*	{OM}†	
Bousquet	28	1,300	0.32	33	207	3,415	1.77
D'Alembert	102	2,700	0.26	93	119	6,530	0.47
Dufay	25	430	0.12	9.1	88	1,175	0.88
Dufresnoy	67	800	0.31	18	126	3,250	0.17
Duprat	114	4,280	0.15	66	86	5,120	0.46
Flavrian	70	2,100	0.15	28	123	4,200	0.12
Héva	25	750	0.16	10	122	1,840	1.43
Joannès	112	2,260	—	62	120	4,675	0.32
La Bruère	547	1,600	—	36	73	1,095	0.68
Opasatica	54	150	0.10	4.4	41	260	0.28
Vaudray	88	1,200	0.10	38	129	1,885	2.22
Max : min	22	28	3.3	21	5	25	18

* Amorphous Fe oxyhydroxides extracted from the sediment with $NH_2OH \cdot HCl$.
† Organic matter expressed as μmol organic C.

Table 3. Mean metallothionein, MT [nmol metal binding sites (g dry wt)$^{-1}$], and metal concentrations [nmol M (g dry wt)$^{-1}$] in tissues of *Anodonta grandis* collected from the 11 lakes studied. C.V. given in the methods section ($N = 4$).

Lake	Gills				Hepatopancreas				Body				Whole organism			
	MT	Cd	Cu	Zn	MT	Cd	Cu	Zn	MT	Cd	Cu	Zn	MT	Cd	Cu	Zn
Bousquet	195	1,390	1,470	9,060	358	430	445	1,630	365	610	260	2,380	287	710	630	3,980
D'Alembert	238	2,130	3,540	9,070	319	365	590	1,460	269	420	300	1,820	270	710	900	3,020
Dufay	220	770	710	4,460	232	420	330	1,480	244	340	150	1,500	237	450	300	2,180
Dufresnoy	154	340	870	4,100	242	180	320	1,330	173	90	130	1,270	183	140	280	1,710
Duprat	217	720	6,180	11,890	258	70	990	1,730	253	90	300	1,580	249	160	1,070	2,830
Flavrian	129	490	1,070	5,110	232	200	470	1,380	198	140	270	1,260	192	200	410	1,830
Héva	262	1,170	820	4,040	310	580	390	1,710	359	420	130	1,490	330	620	330	2,130
Joannès	199	2,050	2,500	6,400	387	380	370	1,430	421	660	390	2,170	339	1,150	1,160	3,650
La Bruère	156	740	5,370	12,320	307	240	630	1,340	259	240	390	1,600	240	350	1,520	3,970
Opasatica	100	260	860	5,520	212	170	370	1,390	178	130	230	1,790	163	170	400	2,670
Vaudray	408	2,400	2,540	5,860	292	540	370	1,460	436	480	150	1,450	414	840	610	2,260
Highest value / lowest value	4.1	9.1	8.7	3.1	1.8	7.9	3.1	1.3	2.5	7.3	3.0	1.9	2.5	8.0	5.5	2.3

well as its chromatographic behavior, UV absorbance spectrum, stability after heat and acid treatment, polarographic behavior, and sulfhydryl content (ratio of 0.3 -SH group per amino acid residue) are all consistent with its designation as a metallothionein (metallothionein class 1 of Fowler et al. 1987).

As in the case of tissue metal levels, variations in metallothionein concentrations along the contamination gradient are also greater for the gills than for the hepatopancreas or body (Table 3). For the gills or body considered separately, or for the reconstituted whole organism, MT levels are significantly correlated with the Cd concentrations in the corresponding tissues (Fig. 3, Table 4). In contrast, no such correlations are observed between [MT] and tissue or whole organism levels of Cu or Zn. Multiple regression analyses between MT and Cd, Cu, and Zn concentrations in the gills, the body, or the whole organism confirm that only [Cd] is a significant predictor of MT in our study area ($P < 0.05$; results not shown). These observations do not however rule out the possible binding of metals other than Cd (e.g. Cu and Zn) to MT in the freshwater mollusc. Indeed, MT concentrations in the body and the hepatopancreas of specimens from lakes Opasatica, Flavrian, Dufresnoy, and Duprat are more than sufficient to bind all tissue [Cd] (Table 3), suggesting that complexes other than Cd–MT are present (we assume similar stoichiometries for Cd and Hg binding to MT).

The correlation between [MT] and tissue levels of Cd is consistent with the reported differences in potency of Cd, Cu, and Zn in inducing MT biosynthesis in laboratory experiments. For example, Roesijadi et al. (1988) exposed specimens of the marine mussel *M. edulis* to Cd, Cu, or Zn for 28 d and measured MT concentrations of 20 μg g^{-1} for 89 nM Cd, 3.7 μg g^{-1} for 79 nM Cu, and 1.3 μg g^{-1} for 153 nM Zn. They considered Zn to be ineffective as an inducer of these proteins—the increase of MT over the baseline level (0.5 μg MT g^{-1}) being minimal. From experiments with mice and rats reported in the literature, Jones et al. (1988) showed that the relative ability of metals to induce metallothionein synthesis is inversely correlated with their softness parameter, σ_p. A soft electron acceptor is characterized by a high polarizability of its outer electronic shell and a tendency to form sta-

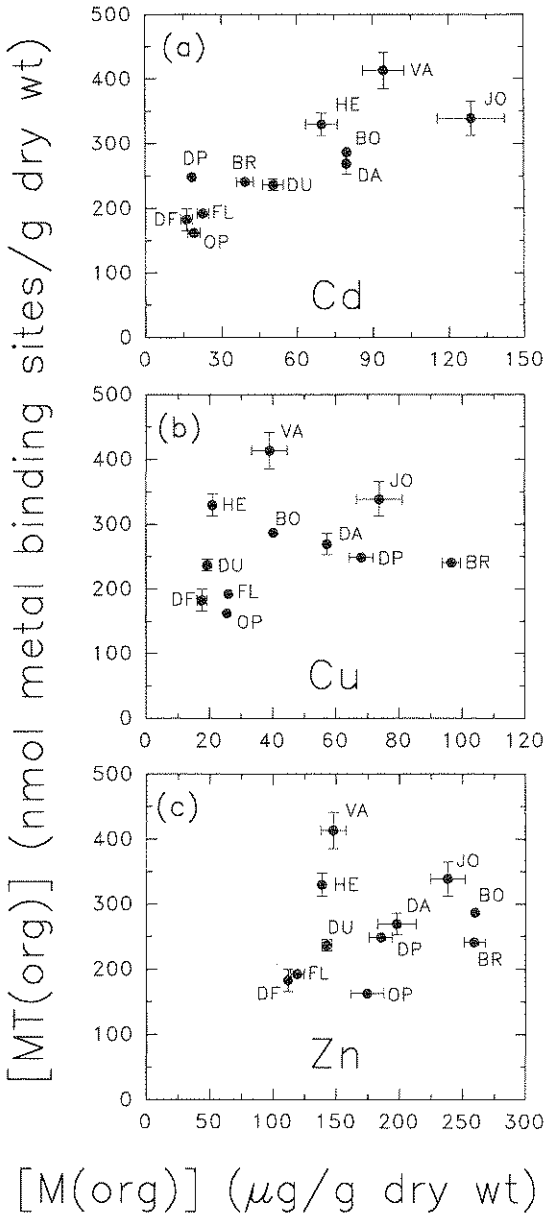

Fig. 3. Scatter diagrams of metallothionein concentrations vs. concentrations of Cd, Cu, and Zn in *Anodonta grandis* (mean ± SE; $N = 4$). Pearson correlation coefficients between whole organism [MT] and whole organism [Cd], [Cu], and [Zn] are respectively 0.83 ($N = 11$; $P < 0.01$), 0.22 ($N = 11$; $P > 0.05$), and 0.21 ($N = 11$; $P > 0.05$). For correspondence between symbols and individual lakes, *see Fig. 1.*

Table 4. Correlations (Pearson's r) between mean metallothionein concentrations in *Anodonta grandis* and mean tissue metal concentrations for specimens collected in lakes of the Rouyn-Noranda mining area. Asterisks: **—$P < 0.01$; ***—$P < 0.001$. ($N = 11$.)

	Correlation between [MT] and [M] for each tissue		
	[Cd]	[Cu]	[Zn]
Gills	0.77**	0.12	−0.06
Hepatopancreas	0.51	−0.01	0.24
Body	0.87***	0.08	0.47
Whole organism	0.83**	0.22	0.21

ble bonds with soft ligands, e.g. those containing free thiol groups, RS^-. Lower values of σ_p correspond to softer ions; σ_p values for Cd^{2+}, Cu^{2+}, and Zn^{2+} are 0.081, 0.104, and 0.115 (Ahrland 1968).

Relationship between metallothionein concentrations and the contamination gradient—Having shown that variations in metallothionein levels in *A. grandis* are correlated with changes in tissue Cd (but not tissue Cu or Zn), we now test the obvious corollary that metallothionein concentrations should vary in a predictable manner in response to changes in Cd bioavailability along the contamination gradient. (Note that no relationships were found between [MT] and dissolved or NH_2OH-extractable Cu or Zn; values given in Tables 1 and 2.)

In a parallel field study, carried out along a more extensive metal contamination gradient (Tessier et al. 1993; $N = 19$ sites, of which 10 were common to the present study), we found that variations in Cd levels in the soft tissues of *A. grandis* were related to dissolved Cd^{2+} ion concentrations, $[Cd^{2+}]$, at the sediment–water interface, as computed from lake water pH and sediment–water sorption equilibria. For example, correlations (Pearson's r) between $[Cd^{2+}]$ and Cd concentrations in the gills, the hepatopancreas, and the whole organism were 0.78, 0.88, and 0.91 ($N = 19$; $P < 0.001$). In contrast, neither total sediment Cd nor extractable Cd proved to be good predictors of tissue Cd. With these observations as a starting point, we can explore potential relationships between [MT(tissue)] and [Cd] in water.

Benthic invertebrates are exposed to both dissolved and particulate trace metals and can in principle accumulate metals directly from the ambient water and from ingested particles

(Luoma 1983; NRCC 1988). For *A. grandis* (and for another filter-feeding bivalve, *Elliptio complanata*), circumstantial evidence exists in support of the hypothesis that water is the main vector for Cd uptake in our study area. In these species, the gills and mantle are flushed with large amounts of water and have large surface areas. Calculations taking into account estimated daily water filtration rates and daily sediment ingestion rates (Tessier et al. unpubl. data) indicate that the potential contribution of the ambient water to Cd uptake is more important than that of the ingested sediment. Consistent with this suggestion is the observation that large quantities of Cd are associated with the gills and mantle of the two bivalves. Despite their relatively small contribution to the total weight of the organism (35–40%), these organs account for ~75% of the Cd body burdens in *A. grandis* in the studied lakes. This high contribution of the gills and mantle to the Cd body burden is particularly evident in the contaminated lakes examined in our study—somewhat lower contributions (35–50%) were noted for lakes in southern Québec uninfluenced by mining activities (Tessier et al. 1993).

Given the correlation between tissue Cd and computed dissolved $[Cd^{2+}]$ at the sediment–water interface, and the correlation between tissue Cd and tissue MT, one might expect metallothionein levels to vary as a function of $[Cd^{2+}]$ in the overlying water (Jenkins and Sanders 1986) or, if it is assumed that complexation of Cd is negligible in these dilute freshwaters (*see* Tessier et al. 1993), as a function of $[Cd]_d$. Such is not the case, however; dissolved Cd concentrations, determined as single values on water sampled at the time of the bivalve collections, are not good predictors of MT levels in the bivalves (Fig. 4, Table 5).

Transplant experiments with *A. grandis* indicate that tissue metal levels change very slowly over time and thus reflect long-term changes in ambient pollutant concentrations ($t_{1/2}$ for Cd >0.9 yr: Tessier et al. unpubl. results). The same experiments also indicate that appreciable metal accumulation occurs only during the ice-free season. Because dissolved Cd may vary appreciably over the course of the ice-free period in lakes on the Precambrian Shield as a result of changes in water chemistry (notably pH) and phytoplankton biomass (e.g.

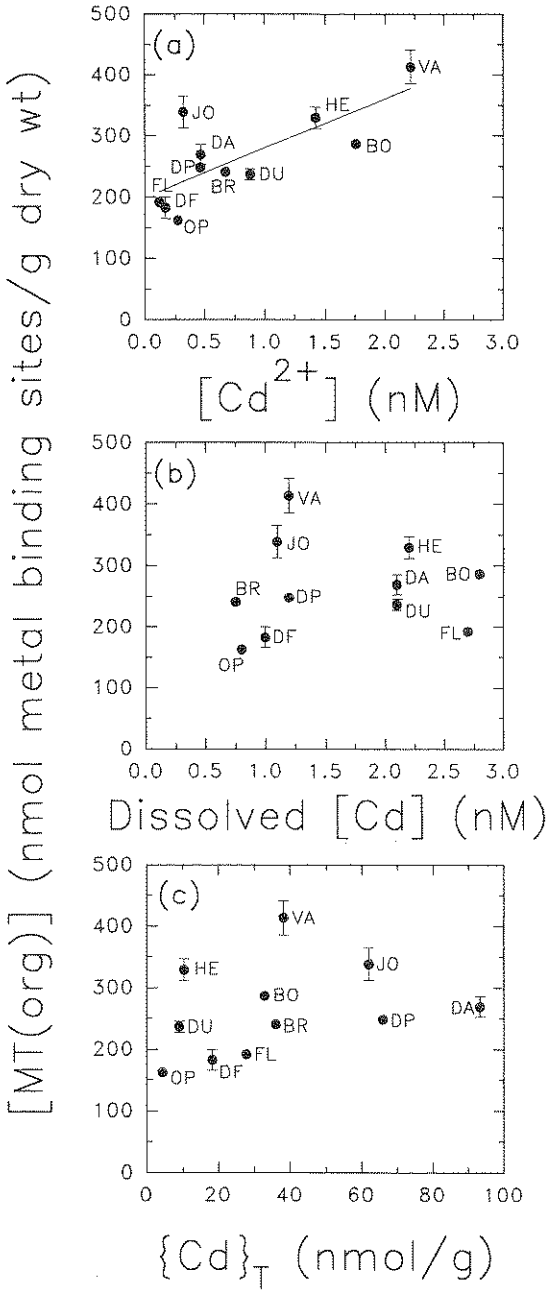

Fig. 4. Scatter diagrams of metallothionein concentrations in *Anodonta grandis* (mean ± SE; $N = 4$). [a.] Whole organism MT vs. $[Cd^{2+}]$, as calculated with Eq. 4 ($r = 0.75$, $N = 11$; $P < 0.01$). [b.] Whole organism MT vs. $[Cd]_d$ ($r = 0.05$, $N = 11$; $P > 0.05$). [c.] Whole organism MT vs. $\{Cd\}_T$ ($r = 0.24$, $N = 11$; $P > 0.05$). For correspondence between symbols and individual lakes, *see Fig. 1.*

Yan et al. 1990), the apparent independence of [MT] and $[Cd]_d$ in our study may simply reflect the paucity of $[Cd]_d$ values and their inability to represent the temporal variability of dissolved Cd. Inadvertent contamination of the water samples may also have contributed to the apparent independence of [MT] and $[Cd]_d$.

To obtain an integrated estimate of the Cd exposure conditions and to circumvent the difficulty of actually measuring free metal ion concentrations along the contamination gradient, we have derived an estimate of $[Cd^{2+}]$ based on the sorptive partitioning of Cd between water and the surficial sediments.

In a parallel field study of the partitioning of Cd in oxic littoral sediments from 35 lakes (including 10 of the 11 lakes chosen for our study), Tessier et al. (1993) showed that lake-to-lake variations in Cd partitioning could be satisfactorily explained by Cd sorption on two sediment components: amorphous Fe oxyhydroxides and organic matter. Surface complexation concepts were used to describe this sorption (Eq. 3):

$$\equiv S\text{–}OH_p + Cd^{2+} \overset{{}^*K_a}{\longleftrightarrow} \equiv S\text{–}OCd + p\,H^+; \quad (3)$$

charges on the solid species are omitted for simplicity, $\equiv S$ are sorbent sites (either amorphous Fe–ox or sediment organic matter, OM), ${}^*K_a$ is an apparent overall equilibrium constant, and p is the average apparent number of protons released per Cd ion sorbed.

According to this partitioning model, the development of which is discussed in detail by Tessier et al. (1993), $[Cd^{2+}]$ can be calculated from

$$[Cd^{2+}] = \frac{\{Cd\}_T[H^+]^{x+y}}{(N_{Fe} \times {}^*K_{Fe\text{–}Cd}\{Fe\text{–}ox\}[H^+]^y + N_{OM} \times {}^*K_{OM\text{–}Cd}\{OM\}[H^+]^x)} \quad (4)$$

where {Fe–ox} is the concentration of amorphous Fe oxyhydroxides (μmol g^{-1}), {OM} the concentration of organic C in the surficial sediments (μmol C g^{-1}), $\{Cd\}_T$ the total Cd concentration in the sediments (nmol g^{-1}), x and y the apparent average numbers of protons released per Cd^{2+} ion adsorbed on Fe oxyhydroxides and organic matter, N_{Fe} the number of moles of sorption sites on Fe oxyhydroxides per mole Fe–ox, N_{OM} the number of moles of sorption sites on sediment organic matter per mole organic C, and ${}^*K_{Fe\text{–}Cd}$ and ${}^*K_{OM\text{–}Cd}$ the apparent overall equilibrium constants for the sorption of Cd on Fe oxyhydroxides and organic matter. The values of the geochemical constants x (0.82), y (0.97), $N_{Fe} \times {}^*K_{Fe\text{–}Cd}$ ($10^{-1.30}$), and $N_{OM} \times {}^*K_{OM\text{–}Cd}$ ($10^{-2.45}$) were determined experimentally from the field geochemical data (Tessier et al. 1993). Given the slow dynamics of Cd exchange between *A. grandis* and its environment, the estimates of Cd^{2+} were based on mean pH values in bottom waters, estimated from a summer series (June–October) in each lake, rather than on the instantaneous pH values measured at the time of bivalve collection. The values of $[Cd^{2+}]$ calculated for each lake are presented in Table 2.

Table 5. Correlations (Pearson's r) between mean metallothionein concentrations (MT) in *Anodonta grandis* and environmental Cd concentrations in lakes of the Rouyn-Noranda mining area. Asterisks: *—$P < 0.05$; **—$P < 0.01$. ($N = 11$.)

	MT concentrations			
Predictor	Gills	Hepato-pancreas	Body	Whole organism
$[Cd^{2+}]$†	0.79**	0.34	0.72*	0.75**
$[Cd]_d$	0.05	0.14	0.10	0.06
$\{Cd\}_T$	0.24	0.51	0.27	0.31

† Calculated with Eq. 4.

The estimates of Cd^{2+} activity at the sediment–water interface vary 18-fold along the contamination gradient and prove to be good predictors of tissue metallothionein concentrations. Highly significant positive relationships are found between MT concentrations (in the gills, the body, or the whole organism) and the Cd^{2+} concentrations (Fig. 4; Table 5). This result supports our initial hypothesis, namely that MT biosynthesis can be induced at contaminant levels typical of those encountered in polluted sediments and that tissue concentrations of these proteins respond in a dose-dependent manner as a function of metal bioavailability (in this case Cd) in the natural environment.

Relatively few studies have dealt with relationships between metallothionein concentrations in indigenous organisms and environmental metal levels (Roch and McCarter 1984; Deniseger et al. 1990; Hogstrand and Haux 1990; Klaverkamp et al. 1991). In these studies, all of which have focused on fish, the degree of contamination has generally been as-

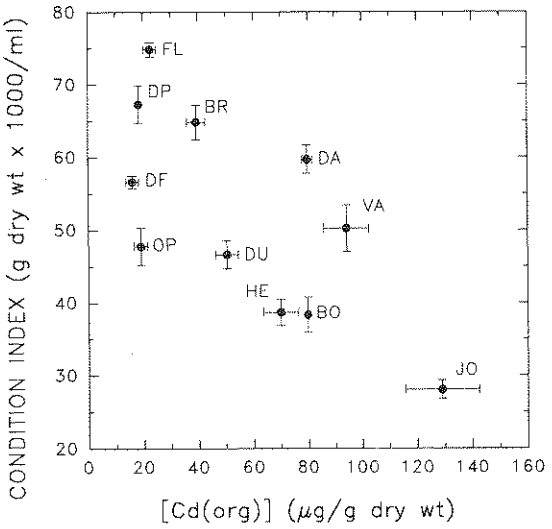

Fig. 5. Scatter diagram of the mollusc condition indices as a function of the concentration of Cd in *Anodonta grandis* (whole organism; mean ± SE; $N = 4$). For correspondence between symbols and individual lakes, *see Fig. 1.*

sessed by measuring either dissolved metal concentrations (Roch and McCarter 1984; Deniseger et al. 1990) or total sediment metal levels (Klaverkamp et al. 1991); neither measure is necessarily a good estimation of metal bioavailability. To our knowledge, our study is the first demonstration that metallothionein levels in field populations of freshwater invertebrates vary along a metal contamination gradient in a consistent manner, i.e. as a function of metal exposure as defined by the free-metal-ion concentration, $[Cd^{2+}]$. The generality of the relationship must, however, be verified for a broader array of surface waters with varied physicochemical conditions, for other benthic organisms, and with other trace metals.

As a cautionary note, it should be emphasized that while the present results are consistent with the free-ion model of metal toxicity (Morel 1983), they do not constitute an unambiguous field validation of the model. Equilibrium calculations indicate that along our contamination gradient virtually all the dissolved inorganic Cd should be present as the free Cd^{2+} ion (as mentioned above). If complexation of Cd by dissolved organic matter is also unimportant, or remains constant from lake to lake (*see* Tessier et al. 1993 for a discussion of this point), the observed relation between tissue MT and $[Cd^{2+}]$ is mathematically indistinguishable from a relation between [MT] and $[Cd]_d$.

Effects of metal accumulation—We examined relationships between the accumulation of Cd, Cu, and Zn by the bivalves and their general health. Average condition indices for *A. grandis* (C.I.) range from a low of ~25 g ml^{-1} (Lake Joannès) to a high of ~75 g ml^{-1} (Lake Flavrian) along the contamination gradient. The condition indices are negatively correlated with Cd concentrations in the reconstituted whole organism (Fig. 5; $r = -0.70$); correlations between C.I. and [Cu] ($r = 0.13$) or [Zn] ($r = -0.28$) in the whole organism are not significant.

A condition index should reflect variations in the intensity of anabolic activity and in particular tissue growth; a low value of a given index is indicative of a period of stress (Lucas and Beninger 1985). The condition index of freshwater molluscs would be expected to be influenced by various factors, notably the trophic status of the studied lakes and the concentration and nutritional quality of the suspended food particles. Seasonal cycles of condition index may differ among the bivalve populations studied. One might also expect some variation in tolerance to toxic agents among different populations of a mollusc species, whether on a phenotypic or genetic basis (Klerks and Weis 1987). Given this inherent lack of specificity, the significant negative relationship between C.I. and whole organism Cd (Fig. 5) is noteworthy and implies that lake-to-lake variability in the condition indices of the molluscs is at least partly explained by their Cd tissue burdens.

Brown and Parsons (1978) suggested that if the rate of metal influx exceeded the net rate of metallothionein biosynthesis, the incoming excess metal would tend to bind nonspecifically to other intracellular ligands (notably the enzyme pool). They further postulated that this phenomenon would correspond to the onset of detectable adverse effects. Although this hypothesis could not be formally tested in our study, we did attempt to evaluate spillover indirectly by comparing condition indices of *A. grandis* with a variable reflecting the potential saturation of tissue metallothionein, i.e. the ratio of tissue Cd concentration to cytosol MT concentration; the implicit assumption is that

higher ratios correspond to less-effective Cd detoxification. Low condition indices are indeed generally associated with high ratios of [Cd(organism)]:[MT(organism)]. For example, specimens with poor condition indices from lakes Joannès, Bousquet, and Héva (C.I. < 40 in Fig. 5) display average [Cd]:[MT] ratios of 3.30, 2.47, and 1.88. The correlation between C.I. and the [Cd(organism)]:[MT(organism)] ratio is significant ($r = -0.69$, $P < 0.05$), but it does not represent a statistical improvement on the simple correlation between C.I. and [Cd(organism)] ($r = -0.70$, $P < 0.05$).

This absence of improvement is perhaps not surprising, given the subcellular distribution of Cd and MT in mollusc tissues. As indicated above, we calculated the concentration ratios of Cd in whole tissues to MT in the tissue cytosol; comparison of [Cd(cytosol)] and [MT(cytosol)] might well have yielded a better indication of the effectiveness of Cd detoxification (e.g. Hamilton et al. 1987). Cytosolic metal represents an important bioactive pool that should respond rapidly to metal exposure, whereas whole organ concentrations may include large pools of bound metals minimally involved in metal metabolism (Johannson et al. 1986). For example, in gills of specimens of *A. grandis* from lakes at either end of the contamination gradient (Opasatica and Vaudray), Cd in the cytosol represented only 5–8% and 2–4% of total gill Cd (Y. Couillard unpubl. results).

The difference between metallothionein levels in the cytosol and whole-tissue Cd concentrations, which is particularly marked for the gills (Table 3), indicates that metallothionein is not the sole molecule involved in Cd binding and detoxification. For example, molluscs from Lake Flavrian appear to be the healthiest based on their condition index, yet there is almost 4 times more Cd than available MT binding capacity in their gill tissue ([Cd(gill)]:[MT(gill)] = 0.26). Clearly gill Cd must be bound in forms other than Cd–MT. This observation is not without precedent—in marine invertebrates, in contrast to mammals or fish, MT frequently does not constitute the major intracellular sink for metals in target tissues (Stegeman et al. 1992). Metallothionein may still play an important role in Cd detoxification in the gills, e.g. as the cytosolic ligand that initially complexes the incoming metal and acts to transfer it to other storage sites (lysosomes/granules or concretions). Such a function has indeed been suggested for marine invertebrates (Viarengo 1989).

Metallothionein and the biochemical indicator concept—The biochemical indicator concept is based on the principle that all biological effects of toxic chemicals begin with the interaction of the toxic chemical with receptor sites in a living organism. The assumption is made that effects at the ecosystem level are preceded by reactions in individual organisms at the molecular level and that concentrations of the contaminant needed to initiate these reactions are lower than those required to provoke a life-threatening situation for the target organism or a perceptible degradation of the ecosystem. The detection and quantification of these chemical reactions could then be developed as an early, sensitive, and specific indicator of environmental stress (NRCC 1985).

For metals, much of the attention in the area of biochemical indicators has focused on metal-binding proteins (Roesijadi 1981; NRCC 1985; Engel and Roesijadi 1987; Stegeman et al. 1992); in particular, the metallothionein content of an organism or a tissue has been proposed as an indicator of prior metal exposure. The results of our study, in particular the increase in MT along a contamination gradient in a dose-dependent manner, support this contention. Several issues remain to be resolved, however, before variations in MT levels in indigenous populations can be interpreted unambiguously. For example, the dynamics of metallothionein synthesis and degradation in freshwater invertebrates are virtually unknown, as are the effects of seasonal factors (e.g. reproductive cycle) on MT levels. These variables should be evaluated in the natural environment under realistic exposure conditions.

References

AHRLAND, S. 1968. Thermodynamics of complex formation between hard and soft acceptors and donors. Struct. Bonding **5**: 118–149.

BEST. 1979*a*. Détermination de la quantité des substances toxiques rejetées dans l'environnement de la région de Rouyn-Noranda. Gouv. Quebec BEST Rapp. T-6.

———. 1979*b*. Etude écologique de la région de Rouyn-Noranda. Gouv. Quebec BEST Rapp. E-17.

BREMNER, I., AND J. H. BEATTIE. 1990. Metallothionein and the trace minerals. Annu. Rev. Nutr. **10:** 63–83.

BROWN, D. A., AND T. R. PARSONS. 1978. Relationship between cytoplasmic distribution of mercury and toxic effects to zooplankton and chum salmon (*Oncorhynchus keta*) exposed to mercury in a controlled ecosystem. J. Fish. Res. Bd. Can. **35:** 880–884.

COSSON, R. P., C. AMIARD-TRIQUET, AND J. C. AMIARD. 1991. Metallothioneins and detoxication. Is the use of detoxication protein for MTs a language abuse? Water Air Soil Pollut. **57–58:** 555–567.

DAVENPORT, J., AND X. CHEN. 1987. A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.). J. Molluscan Stud. **53:** 293–297.

DENISEGER, J., L. J. ERICKSON, A. AUSTIN, M. ROCH, AND M. J. R. CLARK. 1990. The effects of decreasing heavy metal concentrations on the biota of Buttle Lake, Vancouver Island, British Columbia. Water Res. 24: 403–416.

DUTTON, M. D., M. STEPHENSON, AND J. F. KLAVERKAMP. 1993. A modified mercury displacement assay for measuring metallothionein in fish. Environ. Toxicol. Chem. In press.

ENGEL, D. W., AND M. BROUWER. 1987. Metal regulation and molting in the blue crab, *Callinectes sapidus*: Metallothionein function in metal metabolism. Biol. Bull. **173:** 239–251.

———, AND G. ROESIJADI. 1987. Metallothioneins: A monitoring tool, p. 421–438. *In* W. B. Vernberg et al. [eds.], Pollutign physiology of estuarine organisms. Univ. South Carolina.

FOWLER, B. A., C. E. HILDEBRAND, Y. KOJIMA, AND M. WEBB. 1987. Nomenclature of metallothionein, p. 19–22. *In* Metallothionein 2. Experientia Suppl. 52. Birkhäuser.

HAMILTON, S. J., P. M. MEHRLE, AND J. R. JONES. 1987. Evaluation of metallothionein measurement as a biological indicator of stress from cadmium in brook trout. Trans. Am. Fish. Soc. **116:** 551–560.

HARE, L., P. G. C. CAMPBELL, A. TESSIER, AND N. BELZILE. 1989. Gut sediments in a burrowing mayfly (Ephemeroptera, *Hexagenia limbata*): Their contribution to animal trace element burdens, their removal, and the efficacy of a correction for their presence. Can. J. Fish. Aquat. Sci. **46:** 451–456.

HIDALGO, J., J. S. GARVEY, AND A. ARMARIO. 1991. On the metallothionein, glutathione and cysteine relationship in rat liver. J. Pharm. Exp. Theor. **255:** 554–564.

HOGSTRAND, C., AND C. HAUX. 1990. Metallothionein as an indicator of heavy metal exposure in two subtropical fish species. J. Exp. Mar. Biol. Ecol. **138:** 69–84.

JENKINS, K. D., AND B. M. SANDERS. 1986. Relationships between free cadmium ion activity in seawater, cadmium accumulation and subcellular distribution, and growth in polychaetes. EHP Environ. Health Perspect. **65:** 205–210.

JOHANNSON, C., D. J. CAIN, AND S. N. LUOMA. 1986. Variability in the fractionation of Cu, Ag, and Zn among cytosolic proteins in the bivalve *Macoma balthica*. Mar. Ecol. Prog. Ser. **28:** 87–97.

JONES, M. M., M. J. MEREDITH, M. L. DODSON, R. J. TOPPING, AND E. BARALT. 1988. Metallothionein synthesis and its induction mechanism: Correlation with metal ion electronic configurations and softness parameters. Inorg. Chim. Acta **153:** 87–92.

KLAVERKAMP, J. F., AND D. A. DUNCAN. 1987. Acclimation to cadmium toxicity by white suckers: Cadmium binding capacity and metal distribution in gill and liver cytosol. Environ. Toxicol. Chem. **6:** 275–289.

———, M. DUTTON, H. S. MAJEWSKI, R. V. HUNT, AND L. J. WESSON. 1991. Evaluating the effectiveness of metal pollution controls in a smelter by using metallothionein and biochemical responses in fish, p. 33–64. *In* M. C. Newman and A. W. McIntosh [eds.], Metal ecotoxicology: Concepts and applications. Lewis.

KLERKS, P. L., AND J. S. WEIS. 1987. Genetic adaptation to heavy metals in aquatic organisms: A review. Environ. Pollut. **45:** 173–205.

LANGSTON, W. J., AND M. ZHOU. 1986. Evaluation of the significance of metal-binding proteins in the gastropod *Littorina littorea*. Mar. Biol. **92:** 505–515.

LEGRAND, C., D. HUIZENGA, R. SCHENCK, A. TESSIER, AND P. G. C. CAMPBELL. 1987. Cadmium-, copper-, and zinc-binding proteins in various tissues of the freshwater pelecypod *Anodonta grandis* collected from a mining area [Abstract], p. 711. *In* Metallothionein 2. Experientia Suppl. 52. Birkhäuser.

LUCAS, A., AND P. G. BENINGER. 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture **44:** 187–200.

LUOMA, S. N. 1983. Bioavailability of trace metals to aquatic organisms—a review. Sci. Total Environ. **28:** 1–22.

MALLEY, D. F., P. S. S. CHANG, AND R. H. HESSLEIN. 1989. Whole lake addition of cadmium-109: Radiotracer accumulation in the mussel population in the first season. Sci. Total Environ. **87/88:** 397–417.

MANLY, R., AND W. O. GEORGE. 1977. The occurrence of some heavy metals in populations of the freshwater mussel *Anodonta anatina* (L.) from the River Thames. Environ. Pollut. **14:** 139–154.

MOREL, F. M. M. 1983. Principles of aquatic chemistry. Wiley.

NRCC. 1985. The use of biochemical indicators in the assessment of ecosystem health—their development and validation. Natl. Res. Counc. Can. NRCC Rep. 24371.

———. 1988. Biologically available metals in sediments. Natl. Res. Counc. Can. NRCC Rep. 27694.

PHILLIPS, D. J. H. 1980. Quantitative aquatic biological indicators. Appl. Sci.

PIOTROWSKI, J. K., W. BOLANOWSKA, AND A. SAPOTA. 1973. Evaluation of metallothionein content in animal tissues. Acta Biochim. Polon. **20:** 207–215.

ROCH, M., AND J. A. MCCARTER. 1984. Hepatic metallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*)—2. Held in a series of contaminated lakes. Comp. Biochem. Physiol. **77C:** 77–82.

ROESIJADI, G. 1981. The significance of low molecular weight metallothionein-like proteins in marine invertebrates—current status. Mar. Environ. Res. **4:** 167–179.

———, AND G. W. FELLINGHAM. 1987. Influence of Cu, Cd, and Zn preexposure on Hg toxicity in the mussel *Mytilus edulis*. Can. J. Fish. Aquat. Sci. **44:** 680–684.

———, M. E. UNGER, AND J. E. MORRIS. 1988. Immunochemical quantification of metallothioneins of a marine mollusc. Can. J. Fish. Aquat. Sci. **45:** 1257–1263.

SEGAR, D. A., J. D. COLLINS, AND J. P. RILEY. 1971. The distribution of the major and some minor elements in marine animals. Part 2. Molluscs. J. Mar. Biol. Assoc. U.K. **51:** 131–136.

SIMKISS, K., M. TAYLOR, AND A. Z. MASON. 1982. Metal detoxification and bioaccumulation in molluscs. Mar. Biol. Lett. **3:** 187–201.

STEGEMAN, J. J., AND OTHERS. 1992. Molecular responses to environmental contamination: Enzyme and protein systems as indicators of chemical exposure and effect, p. 235–335. *In* R. A. Huggett et al. [eds.], Biomarkers—biochemical, physiological and histological markers of anthropogenic stress. Lewis.

TESSIER, A., Y. COUILLARD, P. G. C. CAMPBELL, AND J. C. AUCLAIR. 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. Limnol. Oceanogr. **38:** 1–17.

VIARENGO, A. 1989. Heavy metals in marine invertebrates: Mechanisms of regulation and toxicity at the cellular level. CRC Crit. Rev. Aquat. Sci. **1:** 295–317.

YAN, N. D., G. L. MACKIE, AND P. J. DILLON. 1990. Controls on cadmium in *Holopedium gibberum* (Crustacea, Cladocera) in Canadian Shield lakes. Environ. Toxicol. Chem. **9:** 895–908.

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