RICHARD J. NEVES

Coullard et al 1995

With my compliments

Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. I. Temporal changes in metallothionein and metal (Cd, Cu, and Zn) concentrations in soft tissues

Y. Couillard, P.G.C. Campbell, A. Tessier, J. Pellerin-Massicotte, and J.C. Auclair

Abstract: To test the response of the freshwater bivalve *Pyganodon grandis* (formerly *Anodonta grandis*) to increased metal exposure in the field, we transferred specimens (8 cm length; 4–6 years old) from a less to a more contaminated lake in the mining area of Rouyn-Noranda, in northwestern Québec. The transplanted bivalves were maintained in open enclosures placed in the bottom sediments of the contaminated lake. Up to 16 individuals were removed from pairs of enclosures at times $t = 0$ (June 1990), 5, 14, 30, 60, 90, and 400 d; tissue concentrations of metallothionein (MT) and metals were monitored over time. Measurements on control molluscs enclosed in their lake of origin showed that enclosure per se had no apparent effect on tissue [MT] or tissue metal levels, but did decrease shell growth. Metallothionein levels in specimens transplanted to the more contaminated lake showed a slow but steady increase with time; in contrast, MT levels in the control populations showed only modest seasonal fluctuations. The increase in MT over time in the transplanted bivalves was closely correlated with a similar slow increase in soft tissue [Cd]. We conclude that MT in the freshwater bivalve *P. grandis* is a promising biochemical indicator of metal exposure.

Résumé : Pour évaluer la réponse du bivalve *Pyganodon grandis* (anciennement *Anodonta grandis*) à une brusque augmentation de métaux en milieu naturel, nous avons transplanté des spécimens de ce bivalve (longueur 8 cm; 4–6 ans) d'un lac relativement peu contaminé vers un lac très contaminé dans la région minière de Rouyn-Noranda, dans le nord-ouest du Québec. Les bivalves transplantés et témoins (gardés dans leur lac d'origine) furent confinés dans des enclos enfoncés dans les sédiments littoraux. Jusqu'à 16 individus furent retirés de chaque série d'enclos aux temps 0 (juin 1990), 5, 14, 30, 60, 90 et 400 jours; les concentrations de métallothionéine (MT) et de métaux furent suivis dans le temps. Le confinement des mollusques n'affecta pas les niveaux tissulaires de MT ou de métaux, mais provoqua un ralentissement de la croissance en longueur de la coquille. Les bivalves transplantés vers le lac le plus contaminé montrèrent un accroissement lent de MT dans le temps; cet accroissement était corrélé avec une augmentation tout aussi lente des concentrations tissulaires de Cd. Les variations saisonnières de [MT] dans les bivalves témoins étaient modestes en comparaison avec la hausse régulière en [MT] notée chez les bivalves transplantés. La métallothionéine dans le bivalve d'eau douce *P. grandis* s'avère prometteur comme indicateur biochimique d'exposition aux métaux.

Received November 29, 1993. Accepted October 24, 1994.
J12181

Y. Couillard. The Great Lakes Institute for Environmental Research, University of Windsor, 304 Sunset Street, Windsor, ON N9B 3P4, Canada.
P.G.C. Campbell[1], **A. Tessier, and J.C. Auclair.** INRS-Eau, Université du Québec, C.P. 7500, Ste-Foy, QC G1V 4C7, Canada.
J. Pellerin-Massicotte. Département d'Océanographie, Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada.

[1] Author to whom all correspondence should be addressed.

Introduction

Traditionally, attempts to assess the impacts of contaminants on aquatic ecosystems have involved laboratory experiments performed under defined conditions (toxicity tests) and, to a lesser extent, field observations on impacted indigenous populations. To date these approaches have met with only limited success (NRCC 1985; Cairns 1992). Extrapolation of laboratory-derived toxicological data to the field is fraught with difficulties, as is the unambiguous interpretation of field observations, e.g., changes in relative abundance, species diversity, community structure, and biomass. An alternative and complementary approach involves the use of biochemical indicators to monitor the response of individual organisms to toxic chemicals.

According to the biochemical indicator concept (NRCC 1985), behavioral effects at the organism level and those operating at the population, community, and ecosystem levels are preceded by biochemical reactions in individual organisms. Concentrations of a contaminant needed to initiate these reactions are assumed to be lower than those required to provoke a life-threatening situation for the target organism or perceptible degradation of the ecosystem. The detection and quantification of these biochemical reactions could then be developed as an early, sensitive, and specific indicator of environmental stress (NRCC 1985). The following criteria for such indicators have recently been proposed (Stegeman et al. 1992; Haux and Förlin 1989).

(1) The indicator should present an early warning capacity, i.e., the biochemical response should be predictive of effects at higher levels of biological organization (population, community) and should precede them.
(2) It should be specific to a particular contaminant or a class of contaminants.
(3) It should respond in a concentration-dependent manner to changes in ambient levels of the contaminant.
(4) Endogenous and exogenous factors that affect the indicator should be known, so that sources of uncontrolled variation can be minimized.
(5) The indicator should be related to the health or fitness status of the organism.

For metals, much of the attention regarding biochemical indicators has focused on metal-binding proteins. Metallothioneins (MT) are low molecular weight, cysteine-rich metal-binding proteins that are ubiquitous in the animal kingdom and show high affinity for Group IB and IIB metal ions. Studies involving aquatic animals have suggested a central role for these molecules in the regulation of the essential metals Zn and Cu; in the detoxification of these metals, when present in excess, and of the nonessential metals Cd and Hg; and in the acquisition of metal tolerance for populations living in metal-contaminated environments (Roesijadi 1992). The vast majority of studies supporting this model have, however, been performed in the laboratory at artificially high metal concentrations and for short exposure times. Extrapolation of this model of MT involvement in metal metabolism to field conditions is accordingly tenuous. More has to be determined about the physiological and toxicological functions of MT in organisms in their natural environment (Roesijadi 1992; Stegeman et al. 1992; Luoma and Carter 1991; Viarengo 1989) before definitive conclusions can be drawn as to the usefulness of MT as a biochemical indicator.

Earlier work in our laboratory demonstrated the presence of a MT-like protein in the gills and the digestive gland of specimens of the freshwater bivalve *Pyganodon grandis* (formerly *Anodonta grandis*) that had previously been exposed to artificially high concentrations of Cd in the laboratory. This metal-binding protein was subsequently detected in specimens collected from lakes in a mining area (Couillard et al. 1993; Legrand et al. 1987). In the present paper we evaluate the potential of MT in *P. grandis* as a biochemical indicator of metal exposure. Specimens of this bivalve were transplanted from a relatively unpolluted lake to a lake contaminated by Cd and Zn. Our objectives were to assess the seasonal variability in MT levels of specimens kept in their source lake (criterion 4), and to compare this variability with that caused by an abrupt increase in ambient metal levels (criterion 3). Provided relatively stable conditions prevail over time in the contaminated lake, MT and metal concentrations in transplanted mussels would be expected eventually to reach those in the indigenous bivalves. A companion paper (Couillard et al. 1995) extends this evaluation of MT and examines its conformity to criterion 1 (early warning capacity) and criterion 5 (links to organism fitness).

Materials and methods

Study area

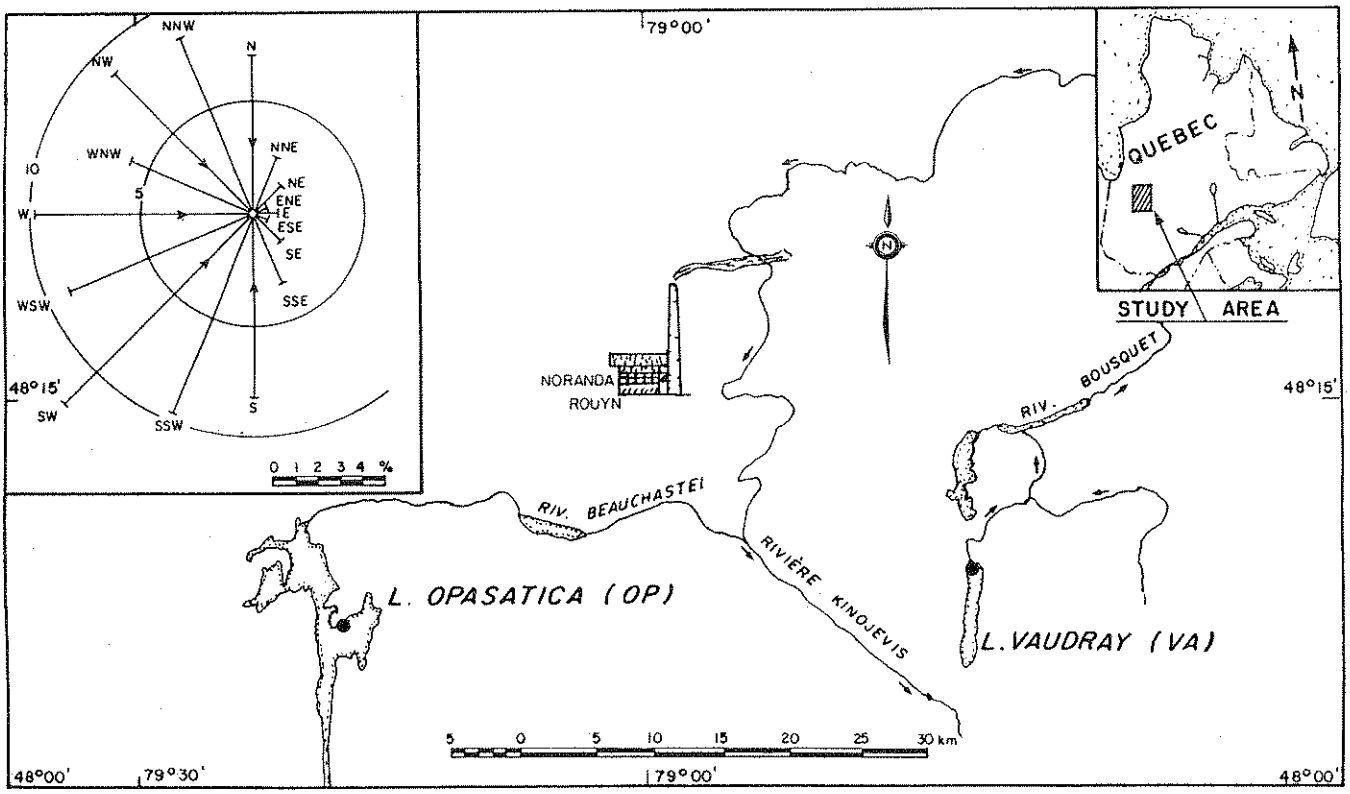
Since 1927, major mining and smelting operations have been carried out in the Rouyn-Noranda region in northwestern Québec. According to data available for 1977, annual atmospheric emissions from the smelting complex included 485 000 tonnes (t) SO_2, 75 t Cd, 34 t Cu, 1540 t Pb, 610 t Zn, and 16 t Hg. Lesser emissions of Bi, Co, Fe, Ni, Sb, Se, and Te were also reported (BEST 1979*a*). Sediment cores obtained in 1982 indicated that surficial sediments in lakes located within 10 km of the smelters were severely contaminated with Cu, Zn, and Pb; sediment enrichment factors ($[M]_{0-7\ cm}/[M]_{background}$, where M = metal) ranged from 118 for Zn to 166 for Cu (Arafat 1985). Air and water pollution controls have recently been introduced, but surficial sediments in lakes surrounding the smelter still reflect their history of contamination.

In a study carried out in the region in 1989, we demonstrated that MT concentrations in indigenous populations of *P. grandis* were significantly correlated with the free Cd^{2+} concentration at the sediment–water interface, as estimated from sediment–water sorptive equilibria (Couillard et al. 1993). As the present experiment was designed to monitor changes resulting from increased metal exposure as a function of time, we selected two lakes from the 11 lakes sampled in the former study (lakes Opasatica and Vaudray; Fig. 1). These two lakes were of similar trophic status but of widely different levels of Cd and Zn contamination; metallothionein concentrations in the bivalves also differed markedly between the two lakes (Table 1). The rationale for choosing these lakes is discussed below.

Experimental design

Specimens of the benthic bivalve *P. grandis* of a nominal length ≈8 cm were transplanted from Lake Opasatica (low

Fig. 1. Location of the study sites, indicated by solid circles. Average wind directions at the Rouyn-Noranda smelting complex are shown in the left-hand inset.



metal contamination) to Lake Vaudray (high metal contamination) (N = 112; mean length = 80.1 ± 4.3 mm (mean ± SD); herein referred to as T-VA). Indigenous mussels from both Lake Opasatica (referred to as C-OP: N = 64; length = 81.3 ± 4.5 mm) and Lake Vaudray (referred to as C-VA: N = 112; length = 82.6 ± 3.9 mm) served as control groups. None of the animals were found to be gravid in a preliminary sampling carried out 2 weeks before the transplantation experiment. All groups, including the controls, were maintained within enclosures (plastic borders, 10 cm high, arranged in circles of 60 cm diameter) inserted into littoral sediments (water depth ≈5 m). The enclosures, 24 in Lake Vaudray and 6 in Lake Opasatica, were arranged in pairs along a rope anchored on the lake bottom. Each enclosure contained eight molluscs; the resulting density of 8 animals/0.28 m^2 was comparable with that of the indigenous populations. The animals transferred to Lake Vaudray (T-VA) and the Lake Vaudray controls (C-VA) were sacrificed according to the sequence 0, 5, 14, 30, 60, 90, and 400 d (t = 0: 20 June 1990; two enclosures sampled and thus 2 × 8 = 16 molluscs collected on each date, unless mortality had occurred). The C-OP control group was sampled at times 0, 5, 90, and 400 d. In coding the various groups, we have used the following conventions: C = control; T = transferred; OP = Lake Opasatica; VA = Lake Vaudray; IND = indigenous, free living. The number of days of exposure completes the code, e.g., T-VA-90 d designates molluscs transferred to Lake Vaudray and collected after 90 d exposure.

To follow the growth of individual organisms, we identified specimens destined to be collected at 400 d by engraving a number with a sharp nail on the umbone region of the shell. Similarly, to determine whether marking the bivalves and maintaining them within the enclosure had an influence on metallothionein and metal concentrations, and on condition indices, we collected indigenous bivalves on day 400 outside the enclosures in each lake (samples referred to as OP-IND and VA-IND) and compared them to marked specimens from groups C-OP-400 d and C-VA-400 d. For the groups C-OP-400 d and C-VA-400 d, we also evaluated the possibility of enclosure and marking effects on shell growth 90 d after initiating the experiment; this evaluation will be discussed later.

Bivalve ageing

To compare the ecological characteristics of the contaminated and control sites, we assessed the growth rates of their indigenous bivalve populations. Two weeks before the transplantation experiment, we selected 21 and 29 specimens, respectively, from lakes Opasatica and Vaudray; specimens were chosen to represent the range of size classes present. The external annual growth rings were then counted independently by two evaluators. Differences between the independent age estimates were infrequent (5 differences among 50 determinations). Shell lengths as well as interannular lengths were measured to the nearest 0.1 mm using digital calipers. Shell length is defined as the longest dimension of the shell, measured with a caliper without compressing the outer shell margin. Interannular length is the widest dimension within a given annual ring, i.e., along the axis of greatest shell length. The age of each of the

Table 1. Surface areas, geochemical and biological data for the two sites chosen for the transplantation experiment.

	Geochemical data			Biological data	
	Lake Opasatica	Lake Vaudray		Lake Opasatica	Lake Vaudray
			Whole organism		
Area (ha)	5128	746	[MT] (nmol M sites·g^{-1})	154±10	596±16
[Ca] (10^{-4} M)	1.65	0.9	[Cd] (nmol·g^{-1})	178±6	1560±60
Gran alkalinity (meq·L^{-1})	292	93	[Cu] (nmol·g^{-1})	680±43	1260±60
DOC (mg C·L^{-1})	6.5	6.5	[Zn] (nmol·g^{-1})	3130±150	4880±280
Mean pH[a]	7.39	6.56			
Dissolved [M]			Gills		
[Cd] (nM)[b]	0.8	1.2	[MT] (nmol M sites·g^{-1})	109±14	411±20
[Cu] (nM)[b]	56	53	[Cd] (nmol·g)	279±19	2560±150
[Zn] (nM)[b]	40	174	[Cu] (nmol·g)	1435±55	3060±200
Calculated [Cd^{2+}] (nM)[c]	0.28	2.2	[Zn] (nmol·g)	6660±260	10 140±830
[M] in oxic sediments			Organism characteristics		
Extract. [Cu] (nmol·g^{-1})[d]	54	88	Condition index	0.110±0.003	0.103±0.009[e]
Extract. [Zn] (nmol·g^{-1})[d]	150	1200			
Total [Cd] (nmol·g^{-1})	4.4	38	Growth rate	Similar[f]	
Total [Hg] (nmol·g^{-1})	0.10	0.10			

Note: Geochemical data are from Couillard et al. (1993). Biological data were determined on specimens collected outside the enclosures at the end of the experiment (OP-IND and VA-IND; $N = 3$ replicates; each replicate comprises four animals; mean ± SE). Metal (M) and MT concentrations in sediments and biological tissues are expressed on a dry weight basis.

[a]Mean pH estimated from summer time series (June to October); mean pH = $-\log_{10}(\Sigma[H^+]/N)$.

[b]Single measure at 10 cm above the sediments (June 1989).

[c][Cd^{2+}] estimated from sediment/water sorptive equilibria; see Tessier et al. (1993) for details.

[d][M] extracted for 6 h at 96°C with 0.04 M NH_2OH·HCl in 25% (vol/vol) HOAc.

[e]A condition index based on soft tissue dry wt. standardized by intervalval volume gave similar values for the two sites. See Figure 5 in Couillard et al. (1993).

[f]See Figure 2*a*.

specimens making up the groups C-OP-400 d and C-VA-400 d was also determined.

Comparison of ecological characteristics of the two sites depended on accurate age and interannular length determinations. In a few cases, shell erosion made it difficult to distinguish the first annulus. In the determinations of age–growth relationships for the indigenous bivalves, we avoided trying to discriminate among crowded rings on the shell edges of older specimens. Such concerns were raised by Metcalfe-Smith and Green (1992) with reference to the use of external rings of unionids for ageing. The appearance, once a year, of a new external growth ring for populations of bivalves from northern climates has been generally accepted, based on mark–recapture studies (reported by Metcalfe-Smith and Green 1992; *P. grandis*, Ghent et al. 1978). However, in a recent experiment of this type, Downing et al. (1992) observed that external annuli on several shells of *P. grandis* from a lake in Minnesota were formed less frequently than annually. The generality of this observation cannot be established at this time.

Bivalve analyses

The bivalves were dissected within 4 h of collection into four tissue groups: gills, mantle, digestive gland, and remaining tissues (foot, visceral mass, labial palps, kidneys, heart and muscle, which are hereafter referred to as the remainder). To minimize the influence of ingested particulate material on the estimation of metal concentrations in the remainder (Hare et al. 1989), the animals' gut contents were removed during the dissection by flushing the digestive tracts with deionized water. Tissues from four animals were pooled yielding four replicates per treatment and per date when no mortality had occurred. Tissues were put in two polyethylene bags: the inner bag was vacuum sealed, and the outer was filled with nitrogen. They were stored at −40°C until homogenization, carried out 1–5 months later.

Partially thawed tissues were homogenized with a tissue grinder (Brinkman Kinematica CH-6010) in ice-cold 25 mM Tris solution adjusted to pH 7.2. Weights of added buffer solution varied for the different tissues: digestive gland (4:1 wt:wt ratio of buffer:tissue), mantle (3:1), gills (2:1), or remainder (1:1). Homogenization was performed in a glove bag filled with nitrogen (Atmosbag, Aldrich Chemical 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 was analyzed the same day for MT. Additional subsamples of the homogenate were retained for determinations of Ca, Cd, Cu, and Zn concentrations. To determine the dry weight (wt.) to wet wt. ratio, an additional

subsample of the tissue homogenate was weighed before and after drying at 70°C for 24 h; dry wt. to wet wt. ratios were corrected for the contribution of the added buffer. The following condition index (CI) was calculated for each of the replicates (Lucas and Beninger 1985):

$$[1] \quad CI = \frac{\text{total flesh dry weight (g)}}{\text{total shell weight (g)}}$$

Metallothionein

Metallothionein concentrations in the various tissues were measured with a mercury saturation assay adapted slightly from Dutton et al. (1993) and described in detail in Couillard et al. (1993). The recoveries of MT standards during the assay (MT from rabbit liver, Sigma Co.; stoichiometry of 7 mole metal·mole^{-1} MT) were in the ranges of 87–114, 85–110, 85–114, and 87–113% for the gills, mantle, digestive gland, and remainder, respectively.

Metallothionein concentrations are expressed as nanomoles metal binding sites per gram of dry tissue wt. (based on measured Hg binding capacities). Concentrations in the whole organism, [MT(org)], were calculated as follows:

$$[2] \quad [MT(org)] = \frac{\sum([MT(tissue)]_i W_i)}{\sum W_i}$$

where $[MT\ (tissue)]_i$ and W_i are the metallothionein concentration and the dry wt. of the ith tissue, respectively.

Tissue metal analyses

An amount of tissue homogenate corresponding to 100 mg dry wt. was pipetted into a 30-mL Teflon bomb (Parr Instrument Co.) and preheated for 2 h in an oven 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 watts, ≤2 min) up to a pressure of 6900 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. National Institute of Standards and Technology, NIST; oyster tissue, SRM No. 1566) were digested during every other analytical run; measured trace metal concentrations in the standard varied little over time (coefficients of variation 3–17%, $N = 14$) and were within the certified ranges for each element. Low to negligible contamination was detected in digestion blanks (Cd, 0.03 ± 0.03 nmol·g^{-1} dry wt.; Cu, 5 ± 2.1 nmol·g^{-1} dry wt; Zn, 7.6 ± 5.1 nmol·g^{-1} dry wt.; Ca, 89 ± 25 nmol·g^{-1} dry wt.; $N = 12$; calculated for a nominal tissue weight of 100 mg).

Metal concentrations in the whole organism, [M(org)], were calculated as described above for metallothionein (Eq. 2).

Labware

To minimize trace element contamination, all labware was soaked in 15% nitric acid for 24 h and rinsed repeatedly in deionized water prior to use. High purity water for analytical purposes (>17 Mohms/cm) was obtained from a commercial system with mixed-bed ion exchange, charcoal adsorption, and filtration (0.2 μm) steps.

Statistical analyses

One-way analyses of variance were applied to test for significant differences in the temporal profiles of MT, metals, and other measured variables for each treatment series: T-VA, C-OP, and C-VA. Differences among successive group means (T-VA-i d, T-VA-j d, etc.) were examined using a posteriori Scheffe contrasts. Because the groups in each treatment had unequal sample sizes (owing to mortality), a priori orthogonal contrasts could not be used. Increases in MT or metal concentrations in the transplanted bivalves were considered significant when the T-VA replicates were significantly different from the C-OP replicates collected after the same exposure time (Student t-test for differences between two means) and were significantly different from the T-VA replicates collected at $t = 0$ d. Relationships between MT and tissue metal concentrations were initially examined in bivariate scatterplots and tested by calculating Pearson correlation coefficients (r). Partial correlation analyses were used to detect spurious relationships between MT and tissue metal concentrations, notably those caused by changes in organism dry weights. Shell growth rates of the bivalve populations from the two lakes studied were determined using bivariate plots of the length delimited by an annulus in year $n + 1$ versus length for year n, the so-called Walford plots (McCuaig and Green 1983). The similarity between the two linear regressions was tested by an analysis of covariance (ANCOVA). The assumptions required for use of these parametric methods were generally met with the raw (nontransformed) data.

Results and discussion

Comparison of the two transplantation sites

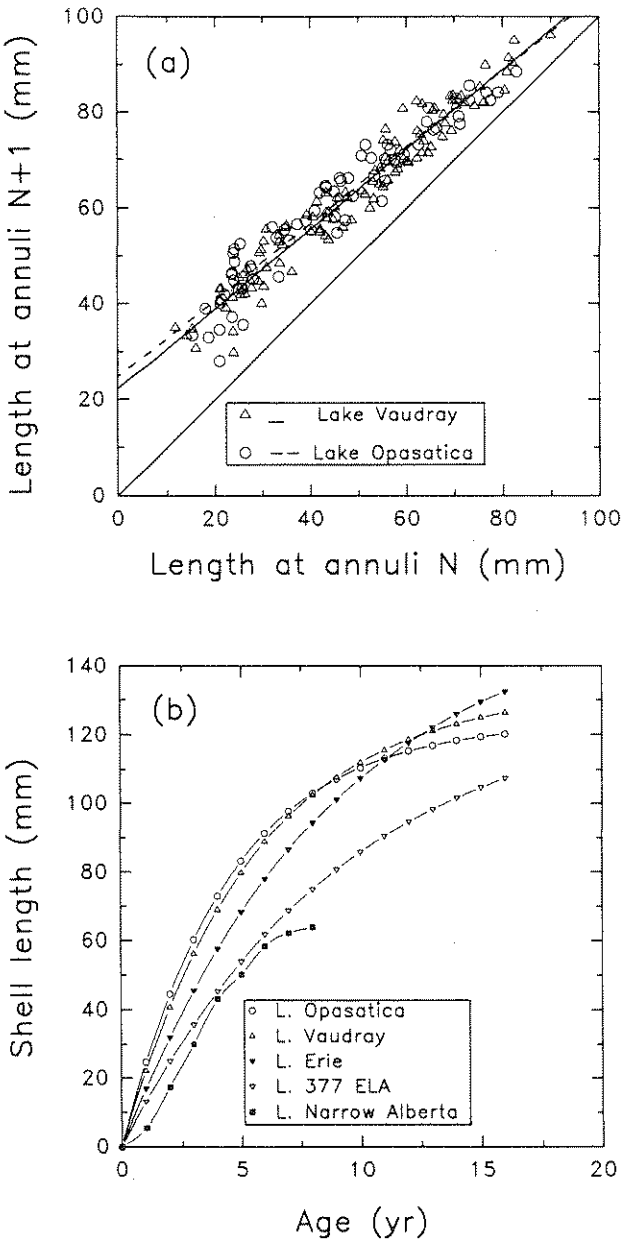
Lake Opasatica is situated upwind from the Rouyn-Noranda smelting complex whereas Lake Vaudray is located downwind (Fig. 1). Both lakes lie at elevations of less than 300 m on glaciolacustrine deposits left by the postglacial Lake Barlow-Ojibway (Surficial geology map No. 1639A, Geological Survey of Canada; Energy, Mines and Resources Canada 1987). Superimposed on this geological similarity, the chosen sites exhibit the desired differences in the levels of contamination of their biotic and abiotic components, as shown in Table 1. The trace metal concentration differences are greatest for Cd and Zn in the surficial oxic sediments, and for estimated dissolved Cd^{2+} (eight- to nine-fold differences). H^+ ion activities, Gran alkalinity values and dissolved Zn also show appreciable differences between the two sites, whereas the remaining variables, notably [Ca], [DOC], dissolved [Cu], and sediment [Cu], are of similar magnitude. The two sites are apparently not contaminated by Hg, also a potential inducer of MT biosynthesis. Mercury concentrations in the surficial sediments fall within the range of sediment background levels for the region, 0.10–0.45 nmol Hg·g^{-1} dry wt., as determined in pre-1900 strata from lake sediment cores (BEST 1979*b*).

Metallothionein and Cd concentrations in the tissues of *P. grandis* are considerably higher in indigenous specimens from Lake Vaudray than in those from Lake Opasatica; tissue Cu and Zn levels also differ between the two sites but not to the same extent as the Cd concentrations (Table 1). Whereas the tissue concentrations of the essential elements

(Cu and Zn) are not unusual for freshwater lamellibranch molluscs (cf. Manly and George 1977: 340–1630 nmol Cu·g^{-1} dry wt., 6170 – 27 800 nmol Zn·g^{-1} dry wt.; whole body concentrations), MT and Cd levels are much higher than those reported for indigenous populations from uncontaminated systems. For example, for specimens of *P. grandis* living in a pristine Precambrian Shield lake in northwestern Ontario, Canada (0.01 ± 0.001 nM dissolved Cd), Malley et al. (1989) reported a mean whole body concentration of ≈36 nmol Cd·g^{-1} dry wt. and a gill MT concentration of ≈9 nmol metal binding sites·g^{-1} wet wt. (estimated from Malley et al. 1993, assuming a molecular weight of 10 kDa for MT and a stoichiometric ratio of 7 mole Hg·mole^{1} MT). On a wet wt. basis, gill MT concentrations for the relatively uncontaminated bivalve population of Lake Opasatica were somewhat higher (16 ± 2.6 nmol metal binding sites·g^{-1} wet wt.).

To minimize the influence of factors other than metal contamination on the biochemical and physiological responses considered, we selected sites likely to share similar ecological characteristics. It is known that environmental conditions and, in particular, quality and availability of food are well integrated by the condition index (Lucas and Beninger 1985) and shell growth rate (McCuaig and Green 1983) of bivalve populations. Comparison of these characteristics for the indigenous mollusc populations suggested that the two sites were indeed of similar trophic status (Table 1 and Fig. 2a). For example, the length delimited by an annulus for year $n + 1$ was regressed against the length for year n for samples of mussels collected at the sites in lakes Opasatica and Vaudray 2 weeks before the beginning of the experiment. The regression coefficients and the Y-intercepts are not significantly different (ANCOVA: $F_{slope}(1, 174) = 1.13$; $F_{position}(1, 175) = 2.48$; $P > 0.05$). The regressions in Fig. 2*a* were used to derive the Von Bertalanffy shell growth curves (McCuaig and Green 1983; Fig. 2*b*). Visual comparison of these shell growth curves again suggests that the growth rates for the bivalves are similar in lakes Opasatica and Vaudray. In addition, these bivalves apparently grow faster and to larger sizes than bivalve populations at similar latitudes in Canada, e.g., in Lake 377, Experimental Lakes Area, Ontario (Malley et al. 1989) and at a 1-m depth in Lake Narrow, Alberta (Hanson et al. 1988). However, they are apparently surpassed in asymptotic length by *P. grandis* from Lake Erie (McCuaig and Green 1983). If shell growth rate is to be assessed for the purposes of the transplantation experiment, then water turbulence must be taken into consideration because of its effect on the allometric growth of the shell (Green et al. 1989). Because both sites were located at similar depths and close to the lee shore (Fig. 1), it seems reasonable to assume that the bivalves experienced similar water turbulence regimes. We conclude that the two transplantation sites are ecologically similar.

Fig. 2. (*a*) Walford plots of lengths at consecutive annual rings for the bivalve *P. grandis* from lakes Opasatica ($N = 21$) and Vaudray ($N = 29$). The specimens were collected 2 weeks before the transplantation experiment. The solid line originating from 0,0 is the line of zero growth. (*b*) Von Bertalanffy growth curves for populations of *P. grandis* from some lakes in Canada. Data source: lakes Opasatica and Vaudray, this study; Lake Erie, McCuaig and Green (1983); Lake 377 ELA, Malley et al. (1989); Lake Narrow, Hanson et al. (1988).



Evaluation of an enclosure and marking effect on the bivalves used in the transplantation experiment (control groups C-OP and C-VA)

Before discussing the effects of the changed metal exposure regime on tissue MT and tissue metal concentrations, we should consider whether these latter parameters were affected by the experimental design (i.e., by enclosing the molluscs and restricting their movement). For enclosed specimens in Lake Opasatica (treatment C-OP), comparisons between marked mussels in the enclosures of the group C-OP-400 d and indigenous mussels collected outside the enclosures at the end of the experiment (OP-IND) did not reveal any corral or marking effect on condition index (Fig. 3), on MT, nor

Fig. 3. Variations over time of condition indices of transplanted and control bivalves. T-VA = transplanted bivalves; C-OP and C-VA = specimens put in enclosures in their source lakes Opasatica and Vaudray, respectively; OP-IND and VA-IND = specimens collected outside the enclosures in lakes Opasatica and Vaudray, respectively.

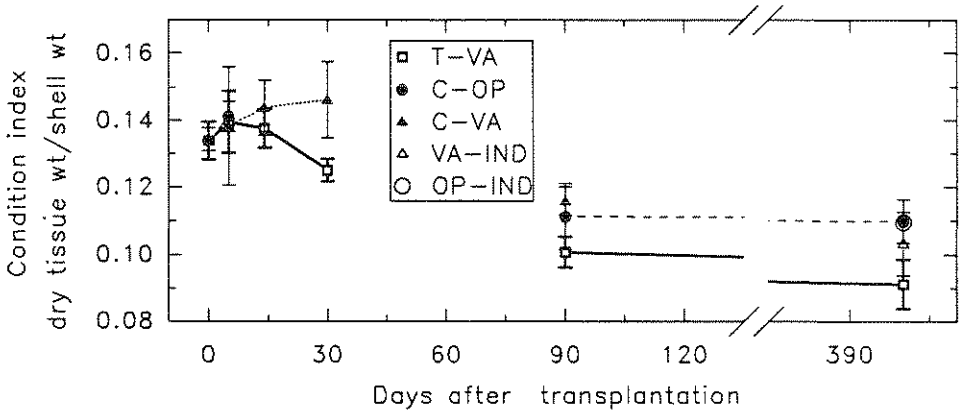


Table 2. Comparisons between marked bivalves kept in enclosures (group C-OP-400 d) and their congeners collected outside the enclosures at the end of the experiment (OP-IND).

Group identification	Enclosure and marking effect on			
	[MT]	[Cd]	[Cu]	[Zn]
Gills				
C-OP-400 d	No	No	No	No
Mantle				
C-OP-400 d	Yes ($P \approx 0.02$)	No	No	No
Digestive gland				
C-OP-400 d	No	No	No	Yes ($P \approx 0.04$)
Remainder				
C-OP-400 d	No	No	No	No
Whole organism				
C-OP-400 d	No	No	No	No

Note: Replicate sample sizes: groups C-OP-400 d and OP-IND, $N = 3$.

on tissue metal concentrations (with the exception of [MT(mantle)] and [Zn(digestive gland)]: Table 2). The marking effect concerns only the enclosed specimens collected at 400 d; specimens destined to be sacrificed earlier in the experiment were not marked as individual growth measurements were not planned for these animals.

Organism dry weights decreased over time in all treatments, C-OP, C-VA, and T-VA, affecting the mollusc condition indices (Fig. 3). A similar decrease also occurred in the indigenous free populations in the two lakes (Fig. 3; compare C-VA-0 with VA-IND, C-OP-0 with OP-IND). Cain and Luoma (1990) also observed a constant decrease for 2 consecutive years in soft tissue weight in indigenous specimens of the marine bivalve *Macoma balthica* in San Francisco Bay. Yearly profiles (with a high sampling frequency) for several consecutive years would be needed to evaluate interannual variations in condition index and to relate these to year-to-year variations in such factors as the abundance and nutritional quality of the suspended particulate matter.

We were unable to perform similar comparisons between the marked mussels in the C-VA-400 d group and the indigenous mussels collected in Lake Vaudray at the end of the experiment (VA-IND). Mortality was much higher for the group C-VA-400 d than for the other groups (Table 3); numbers of live bivalves among the 16 marked individuals after 90 and 400 d were, respectively, 7 and 1. In comparison, at the end of the experiment we recovered 14 live individuals from the original 16 transplanted mussels (T-VA-400 d), and 9 of the 16 control mussels in Lake Opasatica (C-OP-400 d). We suspect that the mortality in the C-VA-400 d group was precipitated by marking the molluscs, because we inadvertently pierced several of the rather thin shells during the engraving step. Fortunately, this marking artifact only affected the C-VA-400 d group, as C-VA specimens destined to be collected at 0, 5, 14, 30, 60, and 90 d were not marked.

We also evaluated the enclosure and marking effect on shell growth by temporarily removing the molluscs in the C-OP-400 d and C-VA-400 d enclosures after 90 d, i.e., towards the end of the first growing season. These individual molluscs were collected by divers, measured, and returned to their enclosures. We compared the shell increments of the marked specimens from groups C-OP-400 d and C-VA-400 d with the expected shell increments for the same individuals, if they had been left unmarked outside the enclosures, for the same period of time. The expected shell increments were assessed by using the Von Bertalanffy shell growth equation of each of the bivalve populations from lakes Opasatica and Vaudray (Fig. 2*b*). We assumed that the period of active growth averages 120 d per year in the Rouyn-Noranda area, based on the average number of days between the spring and fall turnovers (F. Girard, Ministère du Loisir, de la Chasse et de la Pêche du Québec, Direction régionale de l'Abitibi-Témiscamingue, 180 boulevard Rideau, suite 180, Rouyn-Noranda, QC, J9X 1N9, personal communication). For the first 90 days of the experiment, the specimens would reach 75% of their annual growth increments. Specimens from the group C-OP-400 d grew on average 1.74 ± 0.26 mm (mean ± SE) as opposed to the expected increment of 5.30 ± 0.20 mm

Table 3. Fate of bivalves during the transplantation experiment: C-OP and C-VA correspond to specimens kept in enclosures in their source lakes, Opasatica and Vaudray, respectively; the T-VA group corresponds to the bivalves transferred from Lake Opasatica to Lake Vaudray. Initially (day 0) 16 individuals were present in each set of two enclosures.

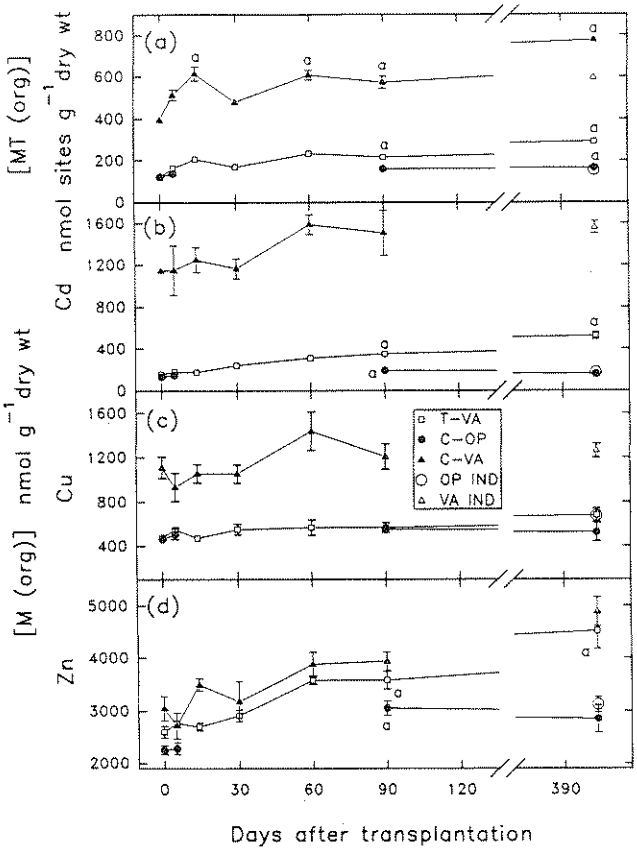
	Missing individuals		
Day of collection	C-OP	T-VA	C-VA
0	0	0	0
5	1	0	0
14	—[a]	0	0
30	—[a]	0	0
60	—[a]	3	0
90	6[b]	5[b]	2[b]
400	6[c]	2[c]	15[c]

[a]No collections were planned for these days.

[b]On this occasion (17 September 1990), the water temperature was ≈10°C. It is possible that some individuals had already burrowed into the sediments for the winter.

[c]These enclosures were also inspected on day 90: missing specimens for the C-OP group = four, including two live specimens found close by but outside the enclosures; missing specimens for the T-VA group = zero; missing specimens for the C-VA group = nine dead (empty shells found in the enclosure).

Fig. 4. Variations of MT, Cd, Cu, and Zn concentrations over time in whole transplanted and control bivalves (note different scales). Each point is the mean ± SE of the replicate samples. Lowercase letters indicate, for a group in a given treatment, means that are significantly different from the mean at 0 d. For the transplanted mussels (T-VA), lowercase letters indicate means that are significantly different from both the C-OP replicates collected on the same dates and the T-VA replicates at 0 d ($P < 0.05$). The high MT concentration for the group C-VA-400 d is discounted because it was obtained for a single individual that exhibited anomalously high Cd and Cu concentrations in the mantle and in the remainder. Only one specimen of the C-VA-400 d group survived to the end of the experiment.



calculated for molluscs if they had been left unmanipulated in their natural environment. Likewise, specimens of the group C-VA-400 d grew on average 0.99 ± 0.46 mm as opposed to the expected increment of 4.38 ± 0.14 mm. Thus, for each of the two groups, a strong enclosure and marking effect on shell growth was detected ($P < 0.001$). Similar conclusions were reached for a subsequent manipulation carried out during summer 1992, in which specimens were marked more gently by attaching plastic labels to the posterior margin of the valves with underwater glue (Y. Couillard, unpublished data).

Hanson et al. (1988) restricted specimens of *P. grandis* to one depth zone, by the use of enclosures, in Lake Narrow, Alberta, from June 1985 to August 1986. Mussels reared at 7 or 5 m grew more slowly than mussels reared at 1 and 3 m. Differences in growth were strongly correlated with the water temperature measured for the different depths. Given these results, a possible explanation for the apparent enclosure and marking effect on shell growth observed with our experimental design would be that bivalves in their natural environment grow faster than bivalves kept in enclosures because they are able to migrate to microenvironments more favourable to growth such as a particular substrate type, warmer waters, etc. At our two sites, bivalves need to migrate less than 100 m to move from 5 m, the depth at which the enclosures were placed, to 1 m. A shortage of food in the enclosures does not seem to be a plausible explanation for the enclosure and marking effects on shell growth because, as mentioned earlier, condition indices were similar for the enclosed molluscs and for the free-living indigenous specimens (Fig. 3).

Temporal changes in metallothionein and metal concentrations during the transplantation experiment

Figure 4 shows temporal profiles for MT and metal concentrations in whole organisms for all three treatments, i.e., the transplanted bivalves plus the control bivalves at each site. The main statistical features concerning the temporal changes of [MT] and [M] for the individual body parts are summarized in Tables 4 and 5.

Table 4. Statistical analysis of metal (Cd, Zn) bioaccumulation and metallothionein (MT) induction in transferred bivalves.

Body part	[MT] (Day No.)	[Cd] (Day No.)	[Zn] (Day No.)
Gills	90*, 400**	90*, 400**	N.S.
Mantle	400**	90**, 400**	400*
Digestive gland	90*, 400**	90**, 400**	90*, 400**
Remainder	400**	90**, 400**	90*, 400**
Reconstituted organism	90*, 400**	90**, 400**	90*, 400*

Note: Collection days are indicated for which the mean metallothionein or mean tissue metal concentrations of the T-VA replicates were significantly different from (*i*) the C-OP replicates collected at the same occasions and (*ii*) the T-VA replicates at 0 d. (*, $P < 0.05$; **, $P < 0.01$). Explanation of the sample code: T-VA = specimens transferred from the less contaminated Lake Opasatica (OP) to the more contaminated Lake Vaudray (VA); C-OP and C-VA = specimens kept in enclosures in their source lakes OP and VA, respectively.

Metallothionein

At each site, seasonal variations in organism MT levels were insignificant ($P > 0.05$) relative to the marked differences between MT concentrations in the bivalves from the two sites (C-VA vs. C-OP; VA-IND vs. OP-IND). In effect, only moderate seasonal variations in [MT(org)] were observed for control mussels from lakes Vaudray and Opasatica (C-VA and C-OP; Fig. 4*a*); for Lake Vaudray, the ratio of the highest mean value obtained (14 d) to the lowest mean value (0 d) was 1.55[2] whereas for Lake Opasatica the ratio was 1.34. Differences between C-OP and C-VA mean MT concentrations at 0, 5, 90, and 400 d were always highly significant ($P < 0.001$). Similar statistical conclusions hold for the body parts.

Specimens transplanted from Lake Opasatica to Lake Vaudray (T-VA) showed a slow but steady increase in metallothionein concentrations with time. The ratio of the mean highest MT level (400 d) to the mean lowest MT level (0 d) reached 2.45 (Fig. 4*a*). Ignoring any possible enclosure effect on MT kinetics, and assuming a linear increase in MT concentrations with time all year long, we calculate that it would have taken two more years for the transplanted molluscs to reach the MT levels found in indigenous bivalves from Lake Vaudray. Because marked bivalves kept in enclosures grew more slowly than their congeners outside the enclosures, we cannot exclude the possibility that the experimental design may also have affected the dynamics of metal accumulation and perhaps MT biosynthesis.

Significant increases in [MT] in the gills, digestive gland, and in the whole organism were first noted at 90 d and persisted until the end of the experiment (Fig. 4*a*; Table 4). In contrast, increases in [MT] in the mantle and in the remainder were not detectable in the transplanted bivalves before 400 d (Table 4). Malley et al. (1993) analyzed specimens of *P. grandis* from a lake where epilimnetic mean total Cd concentrations were raised purposely from 0.01 to 0.66 nM. They observed that the gills, mantle, visceral mass, foot, and kidney all produced MT in response to the increased metal exposure. Our results are consistent with these findings.

[2] The high MT concentration observed at 400 d is discounted because it was obtained for a single animal that exhibited unusually high Cu and Cd concentrations in the mantle and in the remainder. MT and metal concentrations in this organism were excluded from the statistical analyses.

Table 5. Correlations (Pearson's *r*) between metallothionein (nmol metal binding sites·g^{-1} dry wt.) and metal concentrations (nmol·g^{-1} dry wt.) in the tissue replicates of *P. grandis* for the different treatments of the transplantation experiment.

Treatment	Metal		
	Cd	Cu	Zn
Gill MT			
T-VA	0.76***	−0.04	0.66***
C-OP	−0.44	−0.51	0.04
C-VA	0.27	0.21	0.31
Mantle MT			
T-VA	0.70***	0.41*	0.64***
	*0.64***	*0.36**	*0.58***
C-OP	0.59*	0.19	0.50
C-VA	0.49*	0.19	0.22
Digestive gland MT			
T-VA	0.56**	−0.24	0.57**
	*0.48***		*0.42**
C-OP	0.57*	−0.18	0.20
	0.40		
C-VA	0.15	−0.45*	0.30
Remainder MT			
T-VA	0.75***	0.12	0.78***
	*0.75***		*0.78***
C-OP	0.73**	0.36	0.72**
C-VA	0.71***	0.59**	0.70***
	*0.53***	*0.32*	*0.44**
Reconstituted organism MT			
T-VA	0.82***	0.58**	0.86***
	*0.77***		*0.82***
C-OP	0.59*	0.16	0.60*
C-VA	0.61**	0.39	0.46*
			0.37

Note: Partial correlations between [MT] and [M], holding the tissue dry weight constant, are indicated (*italic underlined*) where significant relationships between tissue dry weights and [MT] and [M] have been detected (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Cadmium

Mean whole body Cd, [Cd(org)], for control bivalves from lakes Vaudray and Opasatica (C-VA and C-OP) did not vary markedly during the transplantation experiment (Fig. 4*b*). Ratios of the mean highest [Cd(org)] to the mean lowest [Cd(org)] for the C-VA and C-OP groups were both equal to 1.4. In contrast, mean [Cd(org)] increased 3.3-fold from 0 to 400 d for the transplanted bivalves (T-VA). After 400 d, the mean [Cd(org)] in the T-VA replicate sample (525 nmol·g^{-1} dry wt.) was still only about one third of

the whole organism Cd level in the indigenous Lake Vaudray bivalves. Statistically significant increases in [Cd] for all body parts were first observed for the 90 d sample and persisted until the end of the experiment (Fig. 4*b*; Table 4).

Copper

As might have been anticipated from the similarity in the ambient dissolved [Cu] at the two sites (Table 1), [Cu] in transplanted bivalves (T-VA) did not significantly increase relative to the control bivalves in Lake Opasatica (Fig. 4*c*). However, Cu levels in the control or indigenous bivalves from Lake Vaudray (C-VA or VA-IND) were about twice those of control or indigenous specimens from Lake Opasatica (C-OP or OP-IND). The difference in whole-body concentrations was attributable to anomalously high gill [Cu] in specimens originating from Lake Vaudray (overall means for gill tissue: C-VA: ≈225 $\mu g \cdot g^{-1}$ dry wt.; C-OP: ≈80 $\mu g \cdot g^{-1}$ dry wt.); Cu levels in the other body parts were similar for C-VA and C-OP bivalves. The higher Cu concentrations in the Lake Vaudray molluscs may be related to differences in the water chemistry of the two lakes; Lake Vaudray has lower Ca concentrations, lower alkalinity, and lower pH than Lake Opasatica (Table 1).

Zinc

Zinc concentrations in the bivalves differed less than did dissolved and sediment-bound [Zn] at the two sites (Table 1). Overall means for [Zn(org)] in the transplantation experiment were 235 and 171 $\mu g \cdot g^{-1}$ dry wt., respectively, for the C-VA and C-OP bivalves. No statistical difference existed between the two groups at t = 0, 14, or 90 d ($P > 0.05$); a statistically significant difference was, however, noted between Zn concentrations in the groups OP-IND and VA-IND at 400 d (Fig. 4*d*). In marked contrast to the slow Cd dynamics described earlier, Zn concentrations in all body parts of the transplanted bivalves (T-VA) rapidly attained values similar to those in indigenous bivalves from Lake Vaudray (Table 4).

These observations are consistent with the status of Zn as an essential trace element and the existence of some form of regulation of Zn availability for Zn-dependent functions. Similar conclusions were reached by Langston and Zhou (1986), who observed that Zn concentrations in the gastropod *Littorina littorea* did not respond to fluctuations in environmental Zn contamination along the coasts of Great Britain.

Relationships between metallothionein and tissue metal concentrations

Transplanted bivalves

Relationships between MT and tissue concentrations of Cd, Cu, or Zn were examined for bivalves transplanted from Lake Opasatica to Lake Vaudray (T-VA; Fig. 5, Table 5). For the body parts considered separately, or in combination (reconstituted whole organism), [MT] were significantly correlated with [Cd] in the corresponding body parts (e.g., compare points labelled 0, 5, 30, 60, 90, and 400 in Fig. 5*a*). Whereas [MT] and [Cd(org)] increased, organism dry weights decreased during the course of the

Fig. 5. (*a*) and (*b*): Scatterplots of metallothionein concentrations (X_2) versus Cd concentrations (X_1) or organism dry weights (X_3) of transplanted bivalves. Each number corresponds to the day of sampling of the replicate samples. (*c*) Scatterplot of the partial correlation between metallothionein (X_2) and Cd concentrations (X_1) of the transplanted bivalves, holding organism dry weight constant (X_3). Solid circles are the organism individual replicates. Lines indicate lack of departure of an observation from its estimation by X_3.

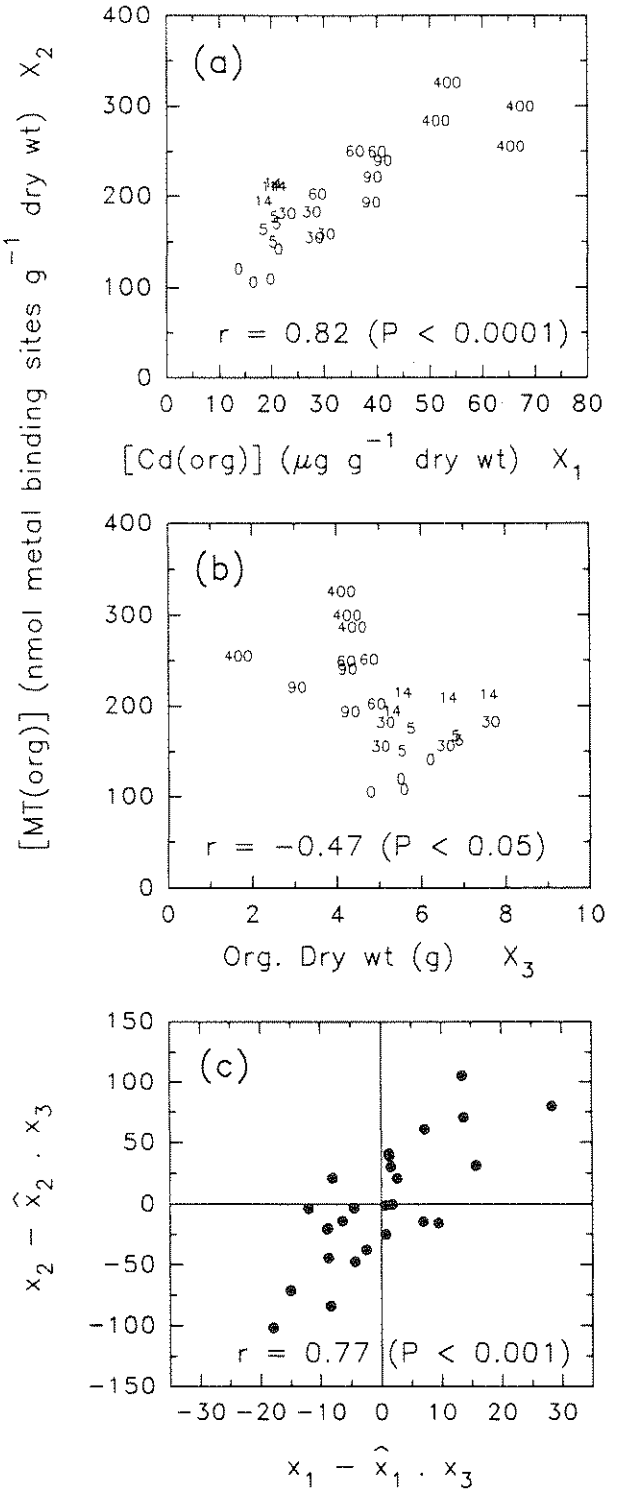
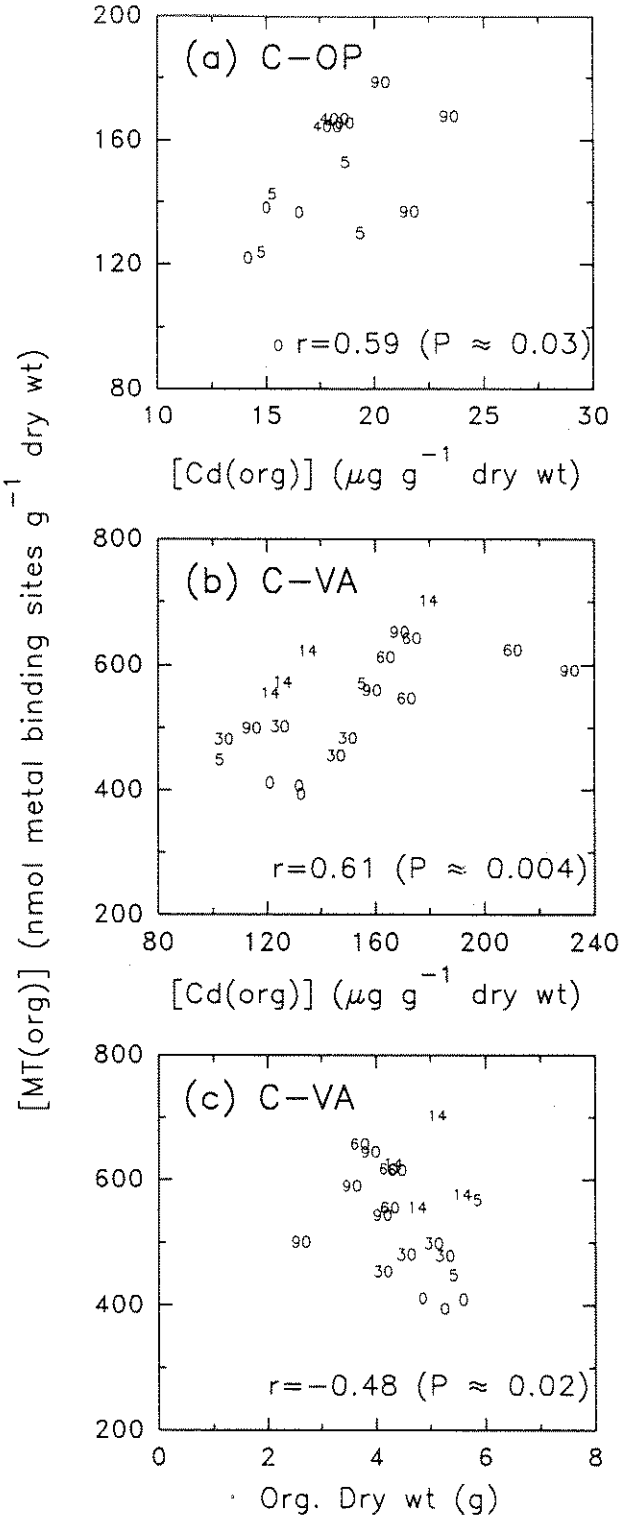


Fig. 6. (*a*) and (*b*): Scatterplots of metallothionein versus cadmium concentrations of control bivalves from lakes Opasatica (C-OP) and Vaudray (C-VA), respectively. (*c*) Scatterplot of metallothionein concentrations versus replicate dry weights of control bivalves from Lake Vaudray. Each number indicates the day of collection of the replicate samples.



experiment (Fig. 5*b*). We initially suspected that the relationship between [MT] and [Cd] might be spurious, caused by the decreases in organism weight. However, partial correlation analysis, holding organism dry weight constant, showed that the correlation between [MT(org)] and [Cd(org)] within the T-VA group remained significant (Fig. 5*c*).

For all body parts except the gills, and for the reconstituted whole organism, we also found significant correlations between [MT] and [Zn] in the corresponding body parts, holding dry weight constant (Table 5, T-VA). This result is in apparent contrast to our earlier study of the spatial variability of MT (Couillard et al. 1993). In the earlier study we examined MT and Cd, Cu, and Zn levels in specimens of *P. grandis* from 11 lakes of the Rouyn-Noranda area (including the two lakes of the present study). MT concentrations in the gills, in the remaining tissues (whole organism minus gills and digestive gland) and in the whole organism were significantly correlated ($P < 0.01$) with tissue [Cd]. In contrast, correlations between [MT] and tissue levels of Zn were either weak or nonexistent. In addition, in a laboratory exposure experiment Roesijadi et al. (1988) found Zn to be ineffective as an inducer of these proteins in the marine mussel *Mytilus edulis* (in contrast to Cd and Cu). We hypothesize that the relation between [MT] and [Zn] in the present transplantation experiment is an artifact and suggest that if the experiment had been prolonged, organism [Cd] and [MT] would have continued to increase whereas organism [Zn] would have remained more or less constant as it had already reached levels found in indigenous bivalves from Lake Vaudray. As a result the relationships between organism [Zn] and [MT] would weaken over a longer time frame.

We thus suggest that MT concentrations in the transplanted bivalves (T-VA) increased in the course of the transplantation as a result of organism weight losses and, to a greater extent, as a result of induction caused by the bioaccumulation of Cd (Fig. 5). The response time of MT levels in transplanted *P. grandis* to a change in metal exposure was less than 90 d and increased levels of MT persisted thereafter until the end of the experiment. This dose–response relationship for Cd is also observed spatially along a contamination gradient. In an earlier study (Couillard et al. 1993) we noted a strong correlation between MT concentrations in *P. grandis* and Cd^{2+} concentrations at the sediment–water interface. No such correlations were observed with Cd, Cu, or Zn in other abiotic compartments. Thus, the results of these two studies suggest that MT satisfies criterion 3 mentioned in the Introduction, i.e., a concentration-dependent response.

Control bivalves

Within the groups of control mussels, C-OP and C-VA, MT concentrations were correlated with tissue Cd and Zn levels. However, these correlations due to seasonal variations were weaker than for transplanted mussels (T-VA), and were not systematically observed in gills, mantle, and digestive gland (Table 5). In Lake Vaudray, changes in dry weights had a significant effect on the seasonal variations of [MT], [Cd], and [Zn] in the whole organism and remainder (C-VA control bivalves; Table 5,

Fig. 6*c*). This was not the case with control bivalves from Lake Opasatica. Note that, because of the covariations with dry weight, correlations between remainder [MT] and [Cu], and between organism [MT] and [Zn], are spurious (group C-VA; Table 5).

Strong seasonal variations in metal concentrations have been reported for marine bivalves (Cain and Luoma 1990; Boyden and Phillips 1981). These variations were largely related to changes in soft tissue weight, which in turn were dependent (Boyden and Phillips 1981) or independent (Cain and Luoma 1990) of the gametogenesis–spawning cycle. Fowler et al. (1986) have shown that production of Cd-binding protein (at present defined as CdBP) in the oyster *Crassostrea virginica* was related to water temperature and to the reproductive cycle on the east coast of United States. Production of CdBP was at a minimum in winter and increased to a maximum during gonadal development and spawning from June to August. In addition, Cd associated with the protein was displaced by Cu during the late prespawning and spawning periods. Similarly, we observed that [MT(org)] of C-OP and C-VA bivalves tended to be high in specimens carrying eggs or immature glochidia in gill marsupia, i.e., 60 d (20 August 1990), 90 d (17 September 1990), and 400 d (24 July 1991) after transplantation (Fig. 6). Monitoring of indigenous mollusc populations for several consecutive years might help to determine how annual growth and gametogenesis–spawning cycles affect tissue MT and metal concentrations, as well as the metal competition for MT.

If MT is to be used as a biomarker or prior metal exposure and metal-induced stress, intrasite seasonal variations in its concentrations should be less important than intersite differences. The relatively modest seasonal variations in the MT levels of control bivalves compared with the same variations in transplanted bivalves are, therefore, reassuring. Temporal variations in [MT] in populations of *P. grandis* from other environments with more complex hydrologic and geochemical processes might, however, be more appreciable. In addition, longer continuous records of this kind are needed if we are to be able to account for the effects of endogenous and exogenous factors on MT levels in indigenous populations (criterion 4).

Concluding remarks

Most of the evidence supporting the notion that aquatic organisms synthesize MT as a defence against toxic metals has been derived from laboratory experiments. However, the metal exposure conditions shown to induce MT synthesis in these laboratory experiments are often environmentally unrealistic: short exposure times and metal concentrations orders of magnitude higher than those found in even the most contaminated aquatic systems where the studied organisms are found. The present habitat-swap experiment provides convincing field-based evidence for MT induction in a representative freshwater invertebrate, the bivalve *P. grandis*, in response to an abrupt but realistic increase in metal exposure (Cd, Zn); in our study area, Cd is the key metal to which metallothionein levels in *P. grandis* respond. The dynamics of the in situ response were, however, rather slow; after 400 d Cd and MT levels in specimens transplanted to the more contaminated site were only about one third as high as those in the indigenous molluscs present in the contaminated lake. Given the observed effect of enclosure on bivalve growth, we cannot exclude the possibility that the experimental design may also have affected the dynamics of Cd accumulation and perhaps MT biosynthesis. It is, however, reassuring that the in situ response of tissue Zn levels was rapid.

The slow increase of MT concentrations in the transplanted bivalves does not necessarily mean that MT induction/synthesis is slow. Overall changes in MT concentrations in *P. grandis* integrate both biosynthetic and degradative processes. Induction of MT synthesis might well have been rapid in the T-VA replicates, but owing to the inevitable biological variability of the controls, this phenomenon could not be demonstrated statistically before day 90. To comprehend MT biosynthesis and degradation processes fully in such an in situ experiment would require studies at the molecular level.

The present field transplantation experiment was designed to evaluate the potential of MT in *P. grandis* as a biochemical indicator of metal exposure and metal-induced stress. To evaluate this potential we can consider the data with respect to the five criteria proposed for such indicators by Haux and Förlin (1989) and by Stegeman et al. (1992) (see Introduction). The temporal correlation of MT and metal (Cd) concentrations in the transplanted bivalves suggests that MT conforms to criteria 2 and 3 for such indicators, namely that the indicator should respond in a concentration-dependent manner to changes in ambient levels of a particular contaminant or class of contaminants. Moreover, the relatively modest seasonal variations of the MT levels in the control bivalves suggest that characteristics related to the basic biology/physiology of *P. grandis*, e.g., dietary regime, growth and development, and reproduction, are less important than changes in metal bioavailability as sources of variation in [MT]. This is a particularly important requirement if MT is to satisfy criterion 4: factors, endogenous as well as exogenous, affecting the indicator should be known so that sources of uncontrolled variation can be minimized.

To complete this evaluation of MT as a biochemical indicator, we should consider links between the MT status of the organism and its general health, as well as relationships between MT responses and possible effects at higher levels of biological organization. These questions, and the compliance of MT with respect to criteria 1 and 5, are examined in Couillard et al. (1995).

Acknowledgements

This research was supported by the Natural Sciences and Engineering Research Council of Canada, the World Wildlife Fund, and by the Fonds de Développement académique du Réseau de l'Université du Québec (FODAR). Y.C. received a NSERC postgraduate scholarship. The assistance of R. Rodrigue, F. Fournier, P. Goupil, C. Mathieu, A. Vachon, and S. Bélanger in the field and M. Geoffroy-Bordeleau, B. Veilleux, and S. Saint-Pierre in the laboratory is acknowledged. Special recognition is extended to J.F. Klaverkamp and M. Dutton for their help in teaching

the details of the ^{203}Hg-saturation assay for metallothionein. The sediment Hg analyses were performed by the Laboratoire d'Écologie Fondamentale et Écotoxicologie of the Université de Bordeaux, France. Helpful comments on draft versions of this paper were furnished by D.W. Engel, A.Z. Mason, and two anonymous reviewers.

References

Arafat, N.M. 1985. The impact of mining and smelting on trace metal distribution in lake sediments around Rouyn-Noranda, Québec. Water Pollut. Res. J. Can. **20**: 1–8.

BEST. 1979*a*. Détermination de la quantité des substances toxiques rejetées dans l'environnement de la région de Rouyn-Noranda. Bureau d'étude sur les substances toxiques, Gouvernement du Québec, Québec. Rapp. T-6.

BEST. 1979*b*. Étude écologique de la région de Rouyn-Noranda. Bureau d'étude sur les substances toxiques, Gouvernement du Québec, Québec. Rapp. E-17.

Boyden, C.R., and Phillips, D.J.H. 1981. Seasonal variation and inherent variability of trace elements in oysters and their implications for indicator studies. Mar. Ecol. Prog. Ser. **5**: 29–40.

Cain, D.J., and Luoma, S.N. 1990. Influence of seasonal growth, age, and environmental exposure on Cu and Ag in a bivalve indicator, *Macoma balthica*, in San Francisco Bay. Mar. Ecol. Prog. Ser. **60**: 45–55.

Cairns, J. 1992. The threshold problem in ecotoxicology. Ecotoxicology, **1**: 3–16.

Couillard, Y., Campbell, P.G.C., and Tessier, A. 1993. Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. Limnol. Oceanogr. **38**: 299–313.

Couillard, Y., Campbell, P.G.C., Pellerin-Massicotte, J., and Auclair, J.C. 1995. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. II. Metallothionein response to Cd and Zn exposure, evidence for cytotoxicity, and links to effects at higher levels of biological organization. Can. J. Fish. Aquat. Sci. **52**: 703–715.

Downing, W.L., Shostell, J., and Downing, J.A. 1992. Non-annual external annuli in the freshwater mussels *Anodonta grandis grandis* and *Lampsilis radiata siliquoidea*. Freshwater Biol. **28**: 309–317.

Dutton, M.D., Stephenson, M., and Klaverkamp, J.F. 1993. A mercury saturation assay for measuring metallothionein in fish. Environ. Toxicol. Chem. **12**: 1193–1202.

Fowler, B.A., Engel, D.W., and Brouwer, M. 1986. Purification and characterization studies of cadmium-binding proteins from the American oyster *Crassostrea virginica*. Environ. Health Perspect. **65**: 63–69.

Ghent, A.W., Singer, R., and Johnson-Singer, L. 1978. Depth distributions determined with SCUBA, and associated studies of the freshwater clams *Elliptio complanata* and *Anodonta grandis* in Lake Bernard, Ontario. Can. J. Zool. **56**: 1654–1663.

Green, R.H., Bailey, R.C., Hinch, S.G., Metcalfe, J.L., and Young, V.H. 1989. Use of freshwater mussels (Bivalvia: Unionidae) to monitor the nearshore environment of lakes. J. Great Lakes Res. **15**: 635–644.

Hanson, J.M., Mackay, W.C., and Prepas, E.E. 1988. The effects of water depth and density on the growth of a unionid clam. Freshwater Biol. **19**: 345–355.

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

Haux, C., and Förlin, L. 1989. Nine selected assays for health status in natural fish populations. *In* Chemicals in the aquatic environment: advanced hazard assessment. *Edited by* L. Lander. Springer-Verlag, Berlin. pp. 197–215.

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

Legrand, C., Huizenga, D., Schenck, R., Tessier, A., and Campbell, P.G.C. 1987. Cadmium-, copper-, and zinc-binding proteins in various tissues of the freshwater pelecypod *Anodonta grandis* collected from a mining area. *In* Metallothionein II. *Edited by* J.H.R. Kägi and Y. Kojima. Birkhäuser-Verlag, Experientia **52**(Suppl.): 711. (Abstr.).

Lucas, A., and Beninger, P.G. 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture, **44**: 187–200.

Luoma, S.N., and Carter, J.L. 1991. Effects of trace metals on aquatic benthos. *In* Metal ecotoxicology—concepts and applications. *Edited by* M.C. Newman and A.W. McIntosh. Lewis Publishers, Chelsea, Mich. pp. 261–300.

Malley, D.F., Chang, P.S.S., and Hesslein, R.H. 1989. Whole lake addition of cadmium-109: radiotracer accumulation in the mussel population in the first season. Sci. Total Environ. **87/88**: 397–417.

Malley, D.F., Klaverkamp, J.F., Brown, S.B., and Chang, P.S.S. 1993. Increase in metallothionein in freshwater mussels *Anodonta grandis grandis* exposed to cadmium in the laboratory and the field. Water Pollut. Res. J. Can. **28**: 253–273.

Manly, R., and George, W.O. 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.

McCuaig, J.M., and Green, R.H. 1983. Unionid growth curves derived from annual rings: a baseline model for Long Point Bay, Lake Erie. Can. J. Fish. Aquat. Sci. **40**: 436–442.

Metcalfe-Smith, J.L., and Green, R.H. 1992. Ageing studies on three species of freshwater mussels from a metal-polluted watershed in Nova Scotia, Canada. Can. J. Zool. **70**: 1284–1291.

NRCC. 1985. The use of biochemical indicators in the assessment of ecosystem health—their development and validation. Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada, Ottawa, Ont. NRCC Rep. 24371.

Roesijadi, G. 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat. Toxicol. **22**: 81–114.

Roesijadi, G., Unger, M.E., and Morris, J.E. 1988. Immunochemical quantification of metallothioneins of a marine mollusc. Can. J. Fish. Aquat. Sci. **45**: 1257–1263.

Stegeman, J.J., Brouwer, M., Di Giulio, R.T., Förlin, L., Fowler, B.A., Sanders, B.M., and Van Veld, P.A. 1992. Enzyme and protein synthesis as indicators of contaminant exposure and effect. *In* Biomarkers—biochemical, physiological, and histological markers of anthropogenic stress. *Edited by* R.J. Hugget, R.A. Kimerle, P.M. Mehrle, Jr., and H.L. Bergman. Lewis Publishers, Chelsea, Mich. pp. 235–335.

Tessier, A., Couillard, Y., Campbell, P.G.C., and Auclair, J.C. 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.