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EFFECTS OF INDUSTRIAL EFFLUENTS ON POPULATIONS OF THE FRESHWATER MUSSEL *LAMPSILIS RADIATA* IN THE ST.LAWRENCE RIVER

by

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Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario
August 1995

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ABSTRACT

The objective of this study was to assess the effects of industrial discharges, contaminated primarily with trace metals, on populations of *Lampsilis radiata* (Bivalvia. Unionidae) in the upper St. Lawrence River. Effects on shell growth were examined by (a) comparing growth curves derived from analyses of external annual rings of clams sampled from sites located upstream and downstream of four localized sources of discharges, and (b) measuring shell growth for one year from clams that were transplanted into contaminated and uncontaminated sites. Allozyme frequencies in clams from above and below discharges were determined for glucose-phosphate isomerase (GPI) and phosphoglucomutase (PGM), two enzyme systems reported to be sensitive to selection by polluted conditions. Field collected clams and sediment were analysed for trace metal content to correlate soft tissue burdens with degree of environmental contamination. Toxicity tests with glochidia larvae were performed with zinc to test for differential resistance based on the source (upstream versus downstream of a zinc - enriched discharge), and the GPI and PGM genotypes of the mother clams.

Minor depressions were detected in shell growth patterns of clams from downstream of discharges relative to clams from upstream in three out of four discharge areas. However, no shell growth depression was observed in clams transplanted into contaminated sites. Shell growth of transplanted clams was strongly affected by their collection source, suggesting that genetic factors or irreversible physiological compensation is more important in controlling shell growth that environmental contaminants. There was no evidence for differential selection of allozymes in clams from upstream and downstream of discharges. Metal burdens were elevated in clam tissue only in those from the most polluted site. Although glochidia of clams from polluted and unpolluted sites exhibited significant among - clam variability in resistance to zinc toxicity, differences were not related to source (site) or genotype of mother clams.

These results suggest that populations of *L. radiata* in the upper St. Lawrence River are either resilient to effects of industrial discharges, or that gene flow between sites is sufficiently high to offset selective pressure by contaminants.

ACKNOWLEDGEMENTS

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1. GENERAL INTRODUCTION

1.1. Assessing Ecological Impairment

As running waters throughout the world are increasingly degraded, and recognition of their ecological importance and societal value rises, their preservation and restoration becomes imperative. For these actions to be effective, some measure of ecosystem integrity or "health" (Nriagu 1989; Karr 1993) is required to determine the degree to which environmental conditions are disturbed and to evaluate the effectiveness of remedial efforts.

Ecosystems comprise a complex array of structural and functional components, whose states and interactions define system integrity. Ecosystem disturbance can be assessed in terms of physical, chemical and biological variables. Physical and chemical attributes (such as water flow regime and contaminant concentrations in sediments) are easier to assess than the comparatively more complex and variable biological ones (such as population abundance or mean fitness). However, it is in terms of biotic components (and their interactions with the rest of the environment) that ecosystem "damage" is commonly recognized (Beanlands and Duinker 1983; McIntyre 1984).

Assessing biological damage is a difficult task, ideally involving the identification of important and relevant components followed by a comprehensive study of their structure and function (Beanlands and Duinker 1983). Lacking sufficient resources, technical capability or theoretical understanding for such a study, researchers often concentrate on a restricted set of components that are indicative of the general health of the ecosystem (Cairns and Pratt 1993). Thus, the monitoring of "indicator species" has found wide application in the assessment of aquatic pollution (Phillips 1980; Hellawell 1986).

Benthic macroinvertebrates offer many advantages in biological monitoring and, in fact, are the group of organisms most commonly used to assess water quality (Rosenberg and Resh 1993). Bivalves appear particularly suitable. Phillips (1977) and Green et al. (1985) discuss the criteria of good biological indicators of environmental quality, and why bivalves satisfy many of the requirements. Their main points are that bivalves (a) continually filter water at

relatively stationary positions for several years or decades, (b) are responsive (ie. show changes in some measureable parameters) to various pollutants at subcellular, cellular, individual and populational levels of organization, (c) are often abundant and easily sampled in polluted areas, and (d) are comparatively well studied taxonomically, physiologically and genetically. Marine bivalves, especially the blue mussel (*Mytilus edulis*), have been used extensively for monitoring pollutants such as trace metals and organic xenobiotic compounds (Phillips 1977; Bayne et al. 1979; Cunningham 1979; Bayne 1989). Studies have examined contaminant accumulation, enzyme induction, histopathology, effects on energy reserves, growth and reproduction, and community characteristics such as species abundance and distribution. Effects of environmental disturbance in freshwaters have been assessed using unionid, corbiculid and dreissenid bivalves (Foster and Bates 1978; Imlay 1982; Green et al. 1989; Elder and Collins 1991; Johnson et al. 1993; Metcalfe-Smith 1994).

1.2. Study Objectives

The purpose of this study was to assess effects of industrial effluents on populations of unionid bivalves in the upper St. Lawrence River. As a river of great regional socio-economic importance, it has been subjected to a wide range of industrial, municiple and recreational uses (St. Lawrence River Remedial Action Team [SLRRAPT] 1992). In addition to non-point sources of contaminants (e.g. agricultural and urban runoff, leachate from landfills, atmospheric deposition and upstream loadings from tributaries and the Great Lakes), numerous installations situated on the river's shorelines discharge effluents containing trace metals, xenobiotic organic compounds, and raw sewage (Environnement Canada 1985; SLRRAPT 1992). The degree to which the St. Lawrence River is now polluted has become a public concern (Picard 1989), and governments at the provincial, state and federal levels have developed remedial action plans (Metcalfe-Smith 1994).

Lampsilis radiata radiata (Gmelin), hereafter refered to as *L. radiata*, is one of the two dominant unionids in the upper St. Lawrence River. It is found throughout the St. Lawrence River (Clarke 1981), even in sites immediately downstream of some of the largest dischargers

of contaminants. Based on the above criteria of Phillips (1977) and Green et al. (1985), the physiological and ecological characteristics of the species suggest it has great potential as a an indicator of biological effects of industrial discharges. I therefcre examined several physiological and ecological aspects of *L. radiata* - shell growth patterns, population genetic structure, soft tissue contaminant burdens, and larval survivorship in contaminant treatments - for relationships to contaminant fields in several areas of the St. Lawrence River.

1.3. Structure of the Thesis

The main body of the thesis is divided into seven sections. This general introduction (Sec. 1) identifies the overall rationale and purpose of the research, describes the study area, introduces the study organism, outlines the mechanisms by which industrial effluents can affect populations of bivalves, and states some general expectations about what information can be obtained from the results. The next four sections deal with the different methods used to assess effects of industrial effluents on clams. Sections 2 and 3 are about the effects of such effluents on shell growth. They describe an observational study of differences in growth patterns between populations upstream and downstream of contaminant discharges (Sec. 2), and transplantation experiments with clams from contaminated and uncontaminanted sites (Sec. 3). Description and discussion of the methods used to reconstruct shell growth patterns are given in Appendix B. In Sec. 4, allozyme genotypes of two loci (*Gpi*, *Pgm*) are determined for the same clams collected for the observational shell growth study. Frequencies of alleles and genotypes are analysed for differences between groups from upstream and downstream of outfalls. Interrelationships among sediment contaminant levels, clam soft tissue contaminant burdens, shell growth patterns and allozyme genotypes are examined for groups of clams in Sec. 5. The toxicity of zinc, an important contaminant in the Cornwall area, is tested with glochidia larvae, the life stage likely the most sensitive to pollution, in Sec. 6 Maternal source (habitat) and allozyme genotype are considered as additional factors in the toxicity response. Section 7 summarizes the main findings and states the overall conclusions from the study.

The clams that were studied from downstream of the industrial outfalls lived in some of the most contaminated regions of the St. Lawrence River. The fact that clams survive in substantial numbers in these habitats raises several questions. Do they suffer from sublethal toxicological effects? Has there been selection for genotypes resistant to pollution? Are they actually exposed to discharged contaminants? The components of this study were designed to answer these specific questions, and to determine if unionid bivalves such as *L. radiata* are suitable as an indicator organism (i.e., a species whose biological response at the biochemical, physiological or autecological level is informative of specific environmental conditions) of effects of industrial effluents.

1.4. Study Areas

Situated along the upper two-thirds St. Lawrence River, between its source at Lake Ontario and the city of Sorel at the junction of the Richilieu River, are over 50 industrial installations that presently or have historically ($\leq$ 1990) discharged effluents (Environnement Canada 1985; SLRRAPT 1992). The majority of these outfalls are located adjacent to the cities of Cornwall, Montreal and Sorel (Fig. 1.1). Studies were designed to assess the effects of effluents from four areas of discharges - (a) the north channel of the river at Cornwall, (b) the south channel of the river at Cornwall, (c) the Montreal area, and (d) the Sorel area. Although effluents affecting these areas originate from a variety of industries, trace metals, iron and polychlorinated biphenyls (PCBs) are the main contaminants (Environnement Canada 1985; SLRRAPT 1992).

1.4.1. Sources of contaminants The Cornwall, N. Channel area received wastewater from three major sources: a submerged diffuser shared by a kraft pulp mill (Domtar Fine Papers) and an inorganic and organic chemicals manufacturer (ICI(formerly CIL)/Cornwall Chemicals), a diffuser from an organic chemicals manufacturer (Courtaulds Fibres and Courtaulds Films [closed 1989]), and the outfall from the City of Cornwall sewage treatment plant (SLRRAPT 1992). Effluent from the the kraft pulp mill, which is the second highest

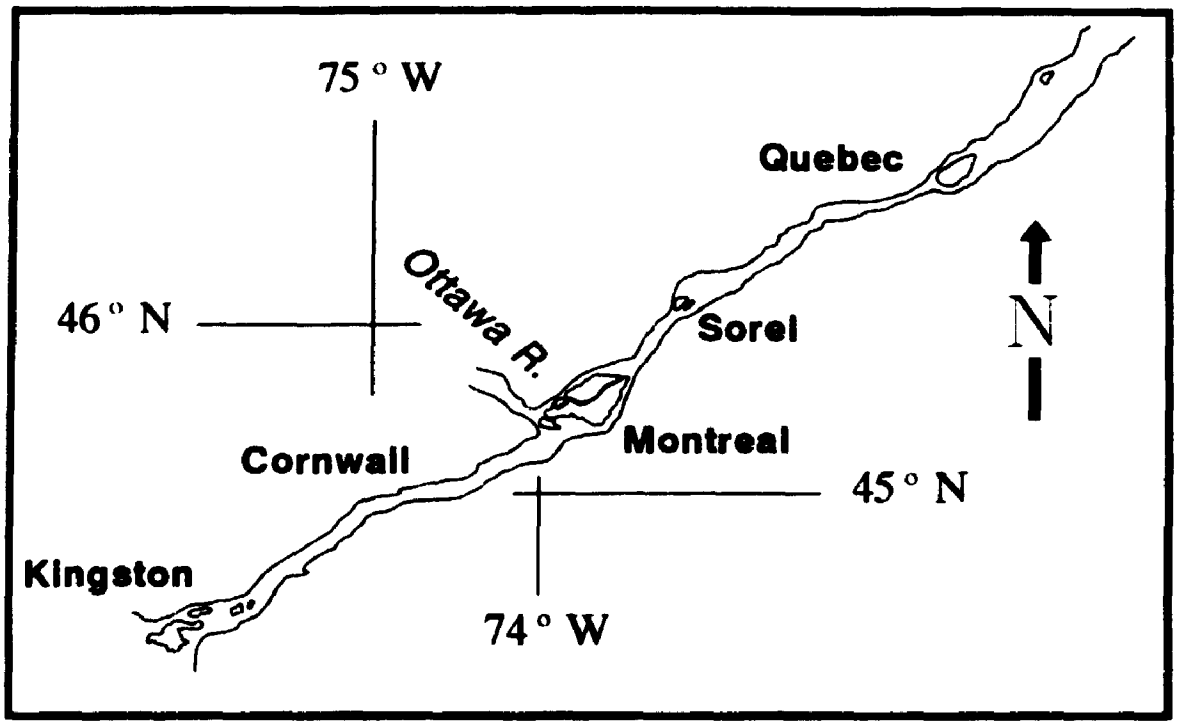

Fig. 1.1. Locations of study areas in the St. Lawrence River.

in volume among 27 other such plants in Ontario (Ontario Ministry of the Environment [OME] 1991), had relatively high 5-day BOD (biochemical oxygen demand) and total suspended solids, and previous to 1980, mercury (Kauss et al. 1988). Effluent from the inorganic and organic chemicals manufacturer also contained elevated amounts of mercury in the past (Kauss et al. 1988), but has recently been in compliance with federal chlor-alkali regulation (SLRRAPT 1992). The organic chemicals manufacturer has released amounts of zinc, mercury and lead substantially in excess of provincial water quality objectives (OME 1992).

The Cornwall, S. Channel area has been influenced by three aluminum smelters -two located on the adjacent shoreline (Reynolds Metals, General Motors Central Foundry) and the third (ALCOA) 10 km up the Grasse River in Massena, NY. These installations were reported to be the largest source of PCBs to the St. Lawrence River west of the Quebec border (SLRRAPT 1992). The Grass River itself is also a potential source of contaminants (Kauss et al. 1988). The U.S. Environmental Protection Agency recently targeted this section of the river for "Superfund" remediation (SLRRAPT 1992).

The main stem of the St. Lawrence River in the Montreal area has been subject to the discharges from at least two dozen industries, urban runoff from a large city, and the inflow of the Ottawa River (Environnement Canada 1985). A wide range of metal contaminants were reported in effluents from these sources.

Sorel, and the adjacent town of Tracy, are the homes of several smelters (QIT - Fer et Titane Inc., Rio Algom Ltée., Acieries Slater Ltée.) and a pigment manufacturer (Tioxide Canada Inc.). These industries have released enormous amounts of metals - at one time accounting for 97% of the Pb, 80% of the Ni, 72% of the Cr, 67% of the Fe, 55% of the Zn, and 34% of the Cu loads to the St. Lawrence River between Cornwall and Sorel (Environnement Canada 1985). The Richilieu River, which enters the St. Lawrence R. at Sorel, also contributes loads of Cu and Zn greatly in excess of those from the local sources (Environnement Canada 1985).

1.4.2. Study sites The general study design for assessing the effects of industrial effluents involved sampling clam beds immediately upstream and downstream of each of the above four industrial areas. Eight primary sites (nos. 2 -5, and 8 - 11) of four upstream/downstream pairs, and five secondary sites (nos. 1, 6, 7, 13, 15) were selected for study (Fig.1.2, Table 1.1). Based on the locations of outfalls (Environnement Canada 1985; SLRRAPT 1992), effluent flow distributions and dilution measurements (Nettleton 1988), and surveys of contaminant distributions in sediments (Kauss et al. 1988; Anderson 1990; Richman 1994; this study, App. A), sites 2, 4, 6, 7, 8, 11 and 15 are considered chemically impacted and sites 1, 3, 5, 9, 11 and 13 comparatively unimpacted by local industrial disharges.

1.4.3. Observed contaminants field: dissolved substances Water quality of the St. Lawrence River is strongly influenced by that of Lake Ontario. At Cornwall, 95% of the flow in the St. Lawrence R. is estimated to be from the Great Lakes (SLRRAPT 1992). Based on multivariate analyses of 19 water quality variables (physical characteristics, major ions, nutrients, metals) for 77 stations between Cornwall and Quebec City, Désilets and Langlois (1989) identified two major water masses - one from Lake Ontario and another from the rivers that drain from the Precambrian Shield (including the Ottawa and others entering on the north shore). Mixing of these waters is poor until well downstream of Sorel, with the softer Shield water confined to the northern side of the St. Lawrence River. Sites in the present study should share much of the same water mass, except in localized, nearshore areas immediately below inflows from tributaries and discharges from riparian industries (Kauss et al. 1988; Désilets and Langlois 1989).

Data from 25 stations between Cornwall and Sorel (M. Charette, Water Quality Branch, Environment Canada, pers. comm.; App. A) support this. Specific conductance, a coarse measure of the concentration of dissolved ions, is about 300 µS/cm, except for stations near the Ottawa River inflow or the north shoreline. Calcium concentration and alkalinity, two factors important to bivalve shell growth (Green 1972; Burky 1983), show similar trends. For most stations Ca concentration was about 35 mg/L, and alkalinity averaged 80-90 mg

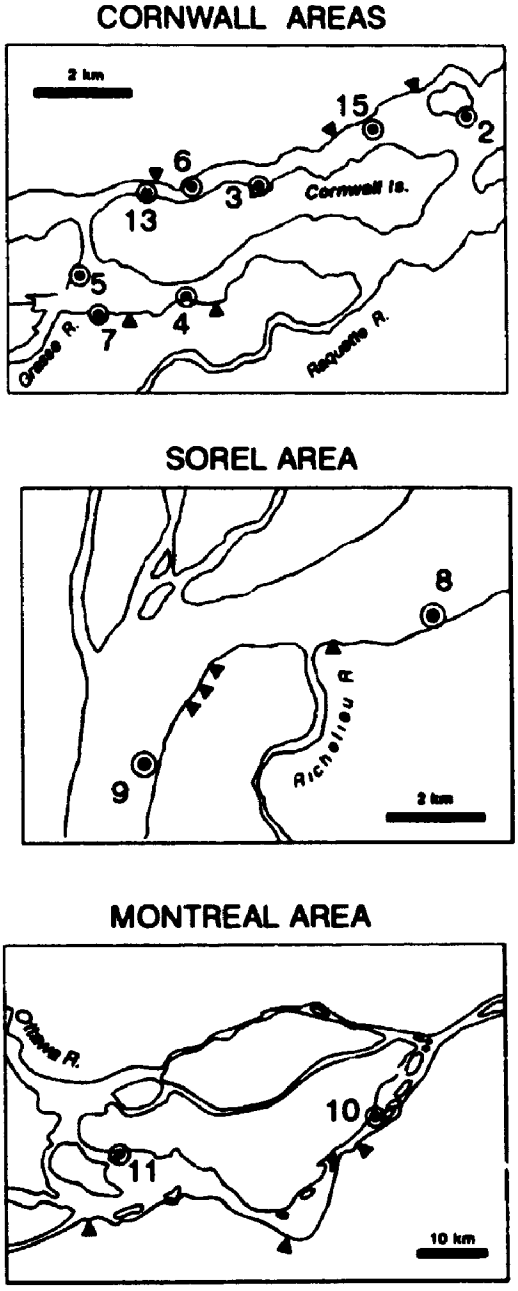

Fig. 1.2. Study sites in the St. Lawrence River. Outfalls are indicated by ▼. The Grasse R. also receives effluent 10 km upstream from its mouth. Based on river flow (which is from left to right) and movements of discharge plumes, sites 3, 5, 9, 11 and 13 are "above" discharges, and sites 2, 4, 6, 7, 8, 10 and 15 are below. (Site 1, at the source of the St. Lawrence R., is not shown.)

Table 1.1. Locations of study sites in the St. Lawrence River.

Site	Latitude (N)	Longitude (W)
1	44° 12' 24"	76° 14' 18"
2	45° 01' 22"	74° 39' 50"
3	45° 00' 34"	74° 43' 04"
4	44° 59' 18"	74° 44' 18"
5	44° 59' 34"	74° 46' 01"
6	45° 00' 36"	74° 44' 05"
7	44° 59' 09"	74° 45' 35"
8[a]	46° 03' 10" (1989)	73° 05' 21" (1989)
	46° 03' 02" (1990)	73° 05' 40" (1990)
9	46° 01' 37"	73° 09' 42"
10	45° 35' 10"	73° 29' 45"
11	45° 24' 32"	73° 53' 40"
13	45° 00' 27"	74° 44' 48"
15	45° 01' 15"	74° 41' 09"

[a] Dredging immediately upstream of Site 8 in 1990 resulted in the heavy deposition of sediment on the clam bed sampled in 1989. Collections in 1990 were therefore conducted several 100 m upstream, above the dredging.

$CaCO_3/L$. Turbidity, which may be correlated to the supply of food available to filter feeders such as unionids, shows less variability among sites than within sites.

Dissolved metals show little importance as factors discriminating sites. Concentrations were frequently near or below detection limits, or not different among sites in the mainstream sections of the St. Lawrence River (App. A).

1.4.4. Observed contaminants field: suspended particulates Studies in the Cornwall area in 1979 (Kauss et al. 1988) and 1988 (Anderson and Biberhofer 1991) suggest that contaminant - bearing particulates in industrial effluents are deposited within several kilometres downstream. Kauss et al. (1988) reported higher concentrations of Cr, Hg, Pb, Zn and PCBs in particulates near site 15 (Fig. 1.2), just below the Courtaulds outfall, compared to a location 1 km downstream of site 2. Effluents from Courtaulds sampled in 1988 contained substantial amount of Hg, Zn, Cu and Pb, but there was very little difference in the concentrations of these or any other of the metals analysed (Al, Ar, Cd, Co, Cr, Fe, Ni, Se) in particulates from 3 km above site 13 and 4 km downstream of site 2 (Anderson and Biberhofer 1991). Furthermore, Anderson (1990) linked elevated sediment concentrations of Hg, Pb and Zn in the Cornwall area with the same inorganic contaminants in the effluents of local sources.

1.4.5. Observed contaminants field: sediments Three surveys in the Cornwall study areas in 1979 (Kauss et al. 1988), 1985 (Anderson 1990) and 1991 (Richman 1994), in addition to samples collected in 1989 and 1990 for the prese:. study (App. A), indicate substantial contamination of sediments in sites downstream of sources of industrial effluents. In the Cornwall, N. Channel area, the major contaminants are Hg, Zn, and Pb, especially near sites 15 and, to a lesser degree, site 2 (Fig. 1.2). Methyl mercury, the most bioavailable form of mercury (Luoma 1983), was also elevated in sediments immediately downstream of Hg sources, and was highest near site 6 (0.012 µg/g vs 0.001 µg/g upstream of the discharges; Richman 1994). Concentrations of total metals in sediments from stations resampled for the

surveys in 1979, 1985 and 1991 generally show no significant decreases through time (Anderson 1990; Richman 1994).

Sediments from downstream of discharges in the Cornwall, S. Channel area are primarily polluted with PCBs. Samples from stations along the southern shoreline, including ones within several hundred metres of sites 4 and 7 (Fig. 1.2), contain levels of total PCBs 1 - 2 orders of magnitude greater that samples from the N. Channel and upstream of local sources (1000 - 14000 ng/g vs < 100 ng/g; Kauss et al. 1988; Anderson 1990). This condition has existed since at least 1975 (Metcalfe and Charleton 1990). Site 7 also showed substantially elevated concentrations of Cd, Cu, Mn, Ni, Se and Zn (Fig. A.3, A.4; App. A).

Characterizations of sites downstream of the Montreal (site 10) and Sorel (site 8) areas are based solely on samples from the present study (App. A). Below Montreal, sediment concentrations of total Hg, Zn, Cu, Fe and As are higher than those measured upstream of discharges (Fig.A.3), and all except As exceed Provincial Sediment Quality Guidelines (Persaud et al. 1992). Extractable concentrations of metals, generally considered a better measure of the bioavailable fraction than total concentrations (Tessier and Campbell 1987; Luoma 1989), show only Zn and Mn as elevated (Fig. A.4). Sediments from downstream of the Sorel industries are heavily polluted by both total and extractable concentrations of Cu, Co, Ni, Cr, Pb, Fe and Zn (Figs. A.3, A.4).

Table 1.2 lists the important contaminants identified in the study sites downstream of the industrial discharges. Based on sediments sampled for the present study, all the primary sites below outfalls (except site 4) appear chemically impacted by metals. Combined metal concentrations (in units of mmol/kg) for the eight primary study sites (Figs. A.5 and A.6), show higher overall levels in sites 2, 8 and 10 relative to their paired upstream sites. An ordination of sites by PCA (App. A), further supports this conclusion.

Table 1.2. Major contaminants in sediments of study sites (Fig. 1 2) downstream of industrial outfalls in the St. Lawrence River.

SITE	AREA	CONTAMINANTS[a]	REFERENCES[b]
2	Cornwall, N. Channel	Hg, Zn, Pb	2, 3, 4
4	Cornwall, S. Channel	PCB	1, 2, 4
6	Cornwall, N. Channel	Hg	2, 3
7	Cornwall, S. Channel	PCB, Cu, Ni, Cd, Zn, Mn, Se	1, 2, 4
8	Sorel	Cr, Fe, Ni, Co, Cu, Pb, Zn	4
10	Montreal	Hg, Zn, Mn, Cu	4
15	Cornwall, N. Channel	Zn, Hg, Pb	2, 3

[a] in approximate order of importance, which among metals is based on the percent concentration exceeds Provincial Sediment Quality Guidelines (Persaud et al. 1992)

[b] 1. Kauss et al. (1988) 2. Anderson (1990) 3. Richman (1994) 4. this study (App. A)

1.5. Study Organism

Lampsilis radiata radiata (Gmelin), also known as the eastern lampmussel, is a common unionid bivalve found in the lower St. Lawrence River and Atlantic Slope drainages from southern Quebec and eastern Ontario to South Carolina (Burch 1975; Clarke 1981). In the upper St. Lawrence River, there is evidence of some intergradation with the closely related *L. radiata siliquoidea* (Barnes) from the Interior Basin (Clarke 1981). (Kat (1986) regards this latter taxon as a distinct species, *L. siliquoidea*, with which *L. radiata* can hybridize.) Clarke and Berg (1959) described an intergradation in the St. Lawrence River drainage, with the influence of *siliquoidea* highest in Lake Ontario and western New York and lowest in Lake Champlain and the St. Lawrence River near Cornwall. According to Clarke (1981), pure *L. r. siliquoidea* are rare below Lake Erie, and intergrades with *L. r. radiata* can be refered to as *Lampsilis radiata* (*sensu lato*). Based on shell morphology, clams[1] collected for the present study generally resembled *L. r. radiata*, but in recognition of the potential inclusion of intergrades, clams will be refered to as *L. radiata*.

Lampsilis radiata, of the tribe Lampsilini in the subfamily Ambleminae (Davis and Fuller 1981), is eurytopic and one of the most abundant of unionids in its range (Clarke and Berg 1959; Strayer 1987). Like all unionids, a salient feature of its life history is a mode of reproduction involving the obligate parasitism by its larvae on a fish. This characteristic has important consequences for the organism's dispersal (Kat 1984) and possibly its ecotoxicological responses to pollutants (Huebner and Pynnönen 1992). The larvae, or glochidia, develop from fertilized eggs in modified chambers (marsupia) of the gills in females. When mature, the bivalved glochidia are discharged through special holes at the bases of the marsupia and released from the clam to be taken up by a host. The breeding season for *L. radiata* begins in late summer and lasts almost a year (Clarke 1981). In the Lampsilini, edges of the mantle are modified to resemble a small fish which, coupled with a particular wriggling

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[1] Although unionids are traditionally and, perhaps, in the strict sense more correctly referred to as "mussels" and not "clams", the latter will be used here to avoid confusion with the recently exotic zebra mussel (*Dreissena polymorpha*).

behavior, serve to attract a host (Kat 1984) Although the host fishes for *L. r. radiata* are not known (Clarke 1981), it is likely that they include some of the 5 - 10 species used by *L. r. siliquoidea* (Clarke and Berg 1959). Glochidia clamp on to the host, usually at the gills, and encyst for several weeks. During this period the adult organ systems develope. Juvenile clams then erupt from gill tissue, and drop from the host (Coker et al. 1922). On suitable substrate, juveniles burrow into the bottom and continue development to adults.

Adult clams are sedentary, and feed by filtering organic, particulate matter from the water column. Sexual maturity is attained after several years (Coker et al. 1922). Adult survivorship is high and lifespans can be as long as several decades (Burky 1983), which in addition to low survivorship past the juvenile stage, accounts for the dominance in natural populations of large, adult individuals (McMahon 1991).

Substrate type, water chemistry (especially hardness and oxygen concentration) and food supply are important natural factors that affect distribution, growth and abundance of unionids (Fuller 1974; Burky 1983). These factors are not, however, independent of each other. Maximum abundance of clams occurs in fast currents and at depths less than 10 m (Burky 1983). Fuller (1974) remarks that the major biotic relationship of clams (excluding humans) involves their parasitism of fishes. Unlike marine bivalves, unionids show few adaptations in shell structure and behavior that suggest that predators have been of significant evolutionary consequence (Vermeij and Dudley 1985).

1.6. Effects of Metal and PCB Contaminants on Bivalves

Exposure to biologically available contaminants can produce toxicological effects throughout the organizational hierarchy of organisms, from the subcellular and cellular levels, through organs and individuals, and up to populations and communities (Haux and Förlin 1988). As outlined below, initial responses include uptake and accumulation in tissues. If cellular detoxification and depuration systems are inadequate, altered structure and deficient function of important macromolecules such as enzymes and nucleic acids can occur, resulting in impairment of dependent physiological processes. Reduced survival can occur if

impairment is severe, and reproductive output may be lower under sublethal impairment. Resistance to the effects of toxicants incurs some degree of energetic costs that would otherwise be available for production (Calow 1991).

1.6.1. Metals The bioavailability of metal contaminants to aquatic organisms is affected by numerous geochemical and biological factors (reviewed by Luoma 1983; Campbell and Tessier 1989). Although these factors can interact in complex and incompletely understood ways (Luoma 1989), several generalizations relevant to the present study are possible. It is accepted that a substantial fraction of most metals entering aquatic systems are partitioned into colloidal and particulate phases and eventually transported to sediments (Yeats and Bewer 1982; Batley 1983; Moore and Ramamoorthy 1984; McIntosh 1991; Warren and Zimmerman 1993). Filter-feeders such as unionids are thus likely to be exposed to metals downstream of outfalls polluted with metals. Bioaccumulation of metals from contaminanted freshwater environments has been widely observed in bivalves (Havlik and Marking 1987; Elder and Collins 1991), indicating that metals are bioavailable to some degree (Bayne 1989; Luoma and Carter 1991).

Uptake of a metal is affected by a variety of factors related to the form of the metal and physiology of the organism (Luoma 1983; Elder and Collins 1991; Roesijadi and Robinson 1994). In bivalves, the gills, foot, mantle and digestive tract have been implicated as sites of uptake (Simkiss and Mason 1983). Metals are generally taken up more efficiently from solution than from particle-bound forms (Luoma 1983; Roesijadi and Robinson 1994). However, ingestion is often the most important source of metals to organisms that feed on particles because the concentrations of most metals are orders of magnitude greater in sediments than in solution (Simkiss and Mason 1983; Luoma 1983, 1989).

Regulation and toxicity of metals in aquatic organisms has been recently reviewed by Simkiss and Mason (1983), George and Viarengo (1985), Luoma and Carter (1991), and Roesijadi and Robinson (1994). Protection against toxic levels of metals within cells appears to involve uptake from the cytosol, sequestration in an inactive form, and excretion. Specific mechanisms of the metabolism of metals, many of which are essential to enzymatic processes,

are not completely understood. However, it has been observed that in a variety of taxa these processes involve specific metal binding proteins (metallothioneins, which are inducible by high metal concentrations, ferritin, and possibly transferrin), subcellular inclusions such as granules, lysosomes and other vesicles. Excretion, where observed, possibly occurs through renal or digestive pathways, at a slower rate than metal binding and storage. Certain granules appear to accumulate without being eliminated. Metal influxes that overwhelm cellular regulation processes can result in biochemical toxicity by binding and impairing the function of cytosolic enzymes. Damage to lysosomes has also been observed, resulting in leakage of hydrolytic enzymes and consequent damage to cell structure and function and, because of the role played by lysosomes in metal sequestration, a reduction in the rate of detoxification.

Because metal detoxification requires increased protein synthesis for the induction of metal-binding proteins, replacement of degraded enzymes, and repair of damaged cellular structures, less energy is available for growth and the production of gametes (Bayne 1989). In bivalves, specific physiological and whole organism processes that show sensitivity to metal contamination include feeding rate, respiration, nitrogen excretion (indicative of protein use), gamete and embryological development, "scope for growth" (sensu Bayne et al. 1985) and tissue growth (reviewed in Leland and Kuwabara 1985; Luoma and Carter 1991)

Population - level effects of metal contaminants on bivalves, such as reduced abundance, are not well documented. Difficulties in establishing causal relationships between multiple, interacting environmental factors and population factors (e.g. abundance and density) may partially account for this. Responses of populations are often more subtle, involving reduced fecundity and differential survival related to age, size, or genetic factors, which can alter population age structure, or select for pollutant-tolerant genotypes (Luoma and Carter 1991; Mulvey and Diamond 1991). Furthermore, physiological effects, such as reduced growth and reproductive output may not necessarily result in decreased population size. In organisms where larval mortality is naturally high (e.g. bivalves), density-dependent regulation of recruitment may make pollutant-related effects negligible (Underwood and Peterson 1988).

1.o.2. PCBs Although the reactivity and transfer of polychlorinated biphenyls in aquatic environments differ among congeners, in general PCBs are highly mobile and persistent (Eisenreich et al. 1983). Owing to their hydrophobicity, many PCBs (especially higher chlorinated congeners) tend to adsorb onto particles and settle to sediments, which can serve as reservoirs for further exchanges among other compartments, including biota (Eadie et al. 1983; Thomann and Mueller 1983, Allan 1989). PCBs are very soluble in lipids and have been widely observed to bioconcentrate in food webs (Safe 1984; Gilbertson et al. 1991), including unionid clams in the Great Lakes and St. Lawrence River (Metcalfe and Charlton 1990).

Potential toxicological effects of PCBs on animals have been reviewed by Safe (1984). Following uptake, PCBs can induce the cytochrome P-450, ethoxyresorufin *O*-deethylase (EROD), or benzo[*a*]pyrene hydroxylase systems, whose synthesis is, as for metal binding-proteins, energetically expensive (Bayne 1989). Failure to detoxify certain isomers and congeners can result in damaging interactions with proteins, DNA and RNA. Observed effects (in mammals, birds, and some fishes) include hepatic toxicity, immunotoxicity, reproductive impairment, genotoxicity and epigenetic effects (Safe 1984).

2. SHELL GROWTH PATTERNS IN NATURAL POPULATIONS FROM UPSTREAM AND DOWNSTREAM OF INDUSTRIAL OUTFALLS

2.1. Introduction

Growth of shells in many marine and freshwater bivalves is strongly affected by environmental conditions, such as temperature, water chemistry, substrate type, turbulence or current velocity, food supply, turbidity, and anthropogenic contaminants (Hallam 1965; Seed 1980; Tevesz and Carter 1980; Bayne et al. 1985). The relationship of shell structure and growth rate to environmental conditions has been applied by paleontologists in reconstructing past environments (Rhoads and Lutz 1980). Growth is also considered a relevant character by ecotoxicologists in assessing the health of an individual. Studies of physiological responses to pollutants often involve the balance within an organism of energy gained from food and energy lost to maintenance processes (Bayne 1989). Growth and reproduction, both of which bear heavily on fitness, are possible only when the energy balance is positive. Furthermore, because growth results from numerous biochemical activities, it integrates toxicological responses from lower levels of biological organization.

Studies of intra-specific variability in shell form of freshwater clams related to environmental factors suggest some degree of phenotypic plasticity in response to these factors (Green 1972; Green et al. 1989). The influence of natural conditions on the shell morphology of unionaceans has been reviewed by Eager (1978) and Tevesz and Carter (1980), and examined more recently by Kat (1982; substrate), Mackie and Flippance (1983; water chemistry), Hinch et al. (1986; substrate), Bailey and Green (1988; current velocity, substrate), Hinch and Bailey (1988; current velocity), and Hinch et al. (1989; water chemistry, substrate). In general, thicker and larger shells are associated with hard, fast flowing, warm and productive waters. Shell obesity or width at a given length appears negatively correlated with substrate particle size. These relationships are not consistent though, especially among lakes and rivers. Often correlations exist among factors, such as current velocity and substrate particle size or food supply. In addition, the relationship between environmental

factors and shell structure may not be monotonic. For example, moderately fast currents likely supply more food particles than slower currents, promoting larger, faster growing individuals. With further increase in current, however, the energetic cost of maintaining position can result in smaller, "stunted" clams (Bailey 1987).

Rate of growth is also influenced by the same environmental factors affecting shell form. The effect of temperature on shell growth is well known for unionids in temperate regions. After accounting for effects of habitat type, clams in warm waters grow faster than clams in cold waters (Tevesz and Carter 1980). Regarding other conditions, those which decrease energy intake or increase body maintenance costs leave less energy available for growth and reproduction (Bayne 1989). Thus, clams in poorly-productive, calcium-deficient, low-water flow environments tend to grow slower than those from highly-productive, calcium-rich, high-flow environments.

Environmental pollutants have long been recognized as stresses to freshwater bivalves (Fuller 1974; Havlik and Marking 1989). Metal contaminants can retard growth through several routes: reduced energy intake and assimilation, due to valve closure to avoid exposure to toxicants or enzyme impairment; increased metabolic maintenance costs, due to metal detoxification and repair of damaged tissues; and slower tissue production, due to impairment of required biochemical pathways (see Sec. 1.6.1). Although observed in marine systems (Luoma and Carter 1991), depression of bivalve shell growth due to metal pollution has not been as widely examined in freshwater systems, especially for unionids (Elder and Collins 1991).

The pattern of mineral deposition in bivalve shells results from interacting environmental and physiological factors (Lutz and Rhoads 1980). Both externally and internally, bivalve shells commonly show discontinuities in structure due to growth interruption, corresponding to both regular periods related to lunar and solar cycles, and episodic events such as predator attacks, storms, pollution and spawning (Lutz and Rhoads 1980). In many species of unionids from temperate regions, several kinds of growth rings or lines have been described. Workers as early as Isley (1914), Coker et al. (1922), and Chamberlain (1931) recognized two types: dark, macroscopic rings ("annuli") visible on the exterior and believed due to

winter growth cessation; and lighter, microscopic rings ("pseudoannuli") less regularly visible and considered due to shorter-term growth disturbances. These structures, as well as ultrastructural rings, are also visible in cross sections of shells as darkened lines, and are apparently caused by altered arrangement and composition of $CaCO_3$ (calcite, aragonite) and protein (conchiolin) in response to environmentally induced valve movements (Lutz and Rhoads 1980; Day 1984).

There has been some debate about the annularity of the macroscopic growth lines. Whereas the periodicity of growth lines in marine bivalves has been extensively investigated and established (Lutz and Rhoads 1980), evidence of annularity of freshwater shell growth rings (Isley 1914; Coker et al. 1922; Negus 1966; Podemski 1992) is more limited. Imlay (1982), Tevesz and Carter (1980) and McCuaig and Green (1983) accept that, in at least some species, true annual growth rings can be identified, especially when cross-checked with internal lines and verified by field mark-recapture studies. Downing et al. (1992), on the other hand, observed that the number of annuli formed per year in two species of marked and recaptured clams was variable and often less than one, especially in larger individuals. They caution against the use of data from annuli whose annularity has not been verified.

For many bivalve taxa, shell growth is fitted well by the von Bertalanffy growth model

$$(1) \qquad L_t = L_\infty (1 - e^{-kt})$$

where L = size, t = time, L_∞ = maximum size and k = instantaneous growth rate at $t = 0$ (Seed 1980). This asymptotic growth model describes an increase in size over time whose rate is proportional to the degree L_t is less than L_∞. Growth of this pattern has been previously observed in *Lampsilis* from several locations: *L. teres* from the lower Mississippi River drainage (Chamberlain 1931); *L. radiata siliquoidea* from the upper Mississippi River (Chamberlain 1931); *L. radiata* from the St. Lawrence River (Magnin and Stanczykowska 1971); *L. r. siliquoidea* from Lake Erie (McCuaig and Green 1983; Bailey 1987); *L. r. radiata* from Lake Champlain (Day 1984); and *L. radiata* from Lake Ontario (Podemski 1992). McCuaig and Green (1983) applied the "Walford Plot" method to describe patterns

fitted by this model. The method involves the use of consecutive annual rings and the linear regression equation

(2) $$L_{t+1} = a + bL_t$$

where L_{t+1} and L_t are the lengths of two rings for years t+1 and t. The intercept, a, represents length after the first year, and the slope, b, the fraction of the total growth remaining after the first year. The two Walford Plot parameters are related to the von Bertalanffy parameters by

(3) $$L_\infty = a/(1 - b) \quad \text{and}$$

(4) $$k = -\ln b$$

McCuaig and Green (1983) and Green et al. (1989) proposed using unionid shell growth rate (as described by the above parameters) as a sensitive indicator of environmental conditions. Beds of *L. radiata* are found throughout the St. Lawrence River (Clarke 1981), including below several areas discharging industrial effluents in sites heavily contaminanted with metals and PCBs. The purpose of this study was to estimate mean rates of shell growth for populations of clams living above and below of four areas of contaminant discharge, and to determine if clams downstream of the outfalls show depression in their rate of growth relative to those upstream.

2.2. Methods

2.2.1. Sampling design and collection of clams *Lampsilis radiata* were collected in June 1989 from clam beds located upstream and downstream of four areas receiving point source discharges in the St. Lawrence River: sites 2 and 3 in the north channel of the river at Cornwall; sites 4 and 5 in the south channel of the river at Cornwall; sites 8 and 9 at Sorel; and sites 10 and 11 at Montreal (Fig. 1.2). Historical information on the effluents discharged

from these sources and measurements of contaminant concentrations in sediments (Sec. 1.4 ; App. A) suggest that clams in the downstream sites (2,4,8,10) have been exposed to substantially higher levels of trace metals or PCBs (Table 1.2) than clams in the upstream sites (3,5,9,11).

From each of the eight paired collection sites, at least 50 clams of all available sizes were collected from depths of 2 - 9 m by divers or oyster tongs. Within several hours, the clams were shucked, and the shells labelled. Both valves were later cleaned of residual soft tissue, and as much externally attached algae and sediment as possible without scraping off periostracum, and dried.

2.2.2. Identification and measurement of growth rings In contrast to some other unionids, the external, macroscopically visible growth lines on the shells of *L. radiata* are generally distinct and easily identified (McCuaig and Green 1983; Day 1984; Podemski 1992). This was true for the present study, except for the earliest one or two rings (which were usually eroded) and for the oldest rings in some large clams (which were often closely spaced and weakly defined). These external growth rings were assumed to mark winter growth interruptions because internal growth lines, which were examined in cross-sections prepared from 15 shells (App. B), corresponded well to external rings in both position and number. Although field verification of growth ring annularity was not attempted in the St. Lawrence River, Podemski (1992) demonstrated yearly ring deposition in *L. radiata* marked with oxytetracycline in the Bay of Quinte, Lake Ontario, which is within 100 km from the outflow of the St. Lawrence River.

Clams were aged using rings from both valves. The valve with the least amount of erosion was examined wet, which improved ring visibility. The other valve was examined dry. Shells were examined using reflected and transmitted light from an incandescent lamp. The dark, often raised rings on the periostracum were counted from the umbo to the shell edge. In preliminary examinations of shells, the first growth ring, when visible, tended to be 10 - 20 mm in length, which agreed with estimates from other studies of the length at one year for *L. radiata* (McCuaig and Green 1983; Day 1984). Therefore, for shells with extensive erosion

in the umbo region, rings larger than 20 mm were assumed to be the second year's growth ring. Possible bias and annual ring misidentification were checked by thin-sectioning (App. B) approximately 15 shells and comparing age estimates with those obtained by external ring counts. Agreement was usually within 1-2 years. Measurement error was estimated to range from ±0 to ±3 rings, depending on the age and degree of umbo erosion of the individual.

At the same time as aging, maximum lengths (Fig. B.1) of as many as 10 external rings were measured from the left valve (or right if left valve extensively eroded) of each clam using digital or dial calipers (precision = 0.01 mm). All measurements were made by the same individual. Independent remeasurements of clams indicated measurement error to be ≈0.1 mm.

2.2.3. Growth pattern determination and analysis Separate growth patterns were derived for each of 421 clams which had at least three measureable ring pairs (App. B). Initially, Walford Plot regressions (eq. 2) were fitted to ring lengths using the Geometric Mean method, as recommended by McArdle (1988) for data of this type. This method is preferred over the Ordinary Least Squares method when error in observations of the independent (X) variable is approximately equal to error in observations of the dependent (Y) variation. Walford Plot parameter estimates a and b were used to estimate von Bertalanffy parameters L_∞ and k (eqs. 3 and 4). Lastly, with equation 1, growth curves were derived for ages 0 - 12 years.

Shell growth patterns in the eight groups were analyzed by one-way multivariate analysis of variance (MANOVA), which tested for differences among sites based on Walford Plot estimates of intercept a and slope b. Observations were weighted by the degrees of freedom of the regression from which a and b were estimated. Following the MANOVA, canonical discriminant analysis (CDA) was performed on the same data to determine which groups of clams were different and, in particular, whether clams from downstream of industrial dis- ges showed different growth patterns from those of clams from upstream of discharges. Two uncorrelated canonical variates (CVs) were derived that maximized discrimination among the eight groups. Group differences in growth pattern were assessed

for each CV using one-way ANOVA and Fisher's LSD multiple comparison of means. Significance levels for comparisons were adjusted using the Bonferroni inequality (Jones 1984) to limit experiment-wise type I error rate to ≤ 0.05.

2.3. Results

Between one-half to two-thirds of the external rings on the shells of *L. radiata* were measured for length. Estimates of Walford Plot regression parameters (a and b) and von Bertalanffy growth model parameters (L_∞ and k) are summarized for each of the eight study sites in Table 2.1. Out of the 421 shells to which growth models were fitted, 13 were excluded from statistical analyses either because b was estimated to be > 1, implying an ever increasing growth rate, or L_∞ was estimated as > 200 mm, which greatly exceeds maximum observed sizes of *L. radiata* (Clarke 1981).

Growth patterns of clams differed among sites. Based on the MANOVA (Table 2.2), mean a and b varied significantly among sites ($P < 0.001$). In terms of the two CVs (Table 2.3), upstream and downstream groups within each of the four study areas were distinct (Bonferroni adjusted $P < 0.04$; Fig. 2.1). This is also clear in bivariate plots of Walford Plot intercept against slope (Fig. 2.2). Comparisons between upstream and downstream sites within each pair show higher mean slope for the two downstream sites in the Cornwall area. No difference in slope between upstream and downstream populations at Sorel was detected, but for the Montreal area the slope was higher in the upstream population than in the downstream population. Mean intercept of Walford Plots for the downstream Cornwall - north channel population was lower than that for the upstream site, indicating less growth in the first year. Differences in mean intercept between sites in the Cornwall - south channel and Sorel areas were not significant. At Montreal the upstream population had a much lower mean intercept than the downstream population.

Derived growth curves based on von Bertalanffy models are shown in Fig. 2.3. Maximum asymptotic lengths (L_∞) were lower in the downstream than in the upstream clams at Sorel; not different between upstream and downstream sites at Cornwall, south channel; and higher in the downstream than in upstream populations at Cornwall - north channel and Montreal

Table 2.1. Summaries of Walford Plot regression and von Bertalanffy equation parameters estimated by analyses of *Lampsilis radiata* shells from eight sites in the St. Lawrence River.

	a	*b*	L_∞	*k*
		Site 2 (n = 47)		
mean	22.8	0.747	97.3	0.299
SD	5.53	0.0929	24.4	0.126
Q1	19.3	0.684	83.1	0.214
median	22.6	0.745	90.7	0.294
Q3	27.0	0.808	101.2	0.380
minimum	7.9	0.547	64.4	0.071
maximum	34.9	0.931	189.0	0.603
		Site 3 (n = 56)		
mean	27.0	0.668	83.1	0.412
SD	5.59	0.0841	10.9	0.128
Q1	23.1	0.590	75.4	0.305
median	26.0	0.676	81.2	0.392
Q3	32.2	0.737	89.7	0.527
minimum	17.3	0.496	64.8	0.194
maximum	38.6	0.824	120.6	0.702
		Site 4 (n = 56)		
mean	26.0	0.712	91.7	0.345
SD	5.35	0.0674	10.6	0.0953
Q1	23.1	0.657	86.3	0.293
median	25.3	0.720	90.9	0.329
Q3	28.8	0.746	97.2	0.420
minimum	15.3	0.574	71.2	0.135
maximum	40.4	0.873	129.0	0.556
		Site 5 (n = 56)		
mean	28.8	0.669	88.3	0.408
SD	4.61	0.0697	9.75	0.106
Q1	26.0	0.616	80.7	0.324
median	28.0	0.676	88.1	0.392
Q3	30.8	0.723	93.2	0.485
minimum	20.0	0.532	69.6	0.205
maximum	40.4	0.816	119.6	0.632

Table 2.1. continued

	a	b	L_∞	k
		Site 8 (n = 54)		
mean	25.5	0.703	89.1	0.359
SD	5.18	0.0827	14.9	0.120
Q1	20.8	0.656	77.0	0.280
median	26.4	0.698	88.3	0.359
Q3	28.4	0.756	96.9	0.422
minimum	13.5	0.487	65.3	0.103
maximum	37.8	0.902	138.2	0.721
		Site 9 (n = 35)		
mean	26.7	0.728	105.0	0.325
SD	4.53	0.0860	26.7	0.119
Q1	22.1	0.658	83.0	0.243
median	28.4	0.737	100.5	0.305
Q3	29.9	0.784	114.7	0.419
minimum	16.8	0.561	72.1	0.108
maximum	34.1	0.898	187.1	0.579
		Site 10 (n = 56)		
mean	25.2	0.685	92.9	0.387
SD	5.20	0.0892	16.2	0.133
Q1	21.8	0.633	74.8	0.314
median	25.7	0.676	79.5	0.392
Q3	28.4	0.731	86.0	0.457
minimum	12.3	0.434	59.2	0.100
maximum	37.0	0.905	166.0	0.836
		Site 11 (n = 48)		
mean	12.1	0.828	74.7	0.198
SD	4.18	0.0807	19.7	0.105
Q1	8.7	0.792	65.5	0.139
median	11.6	0.828	69.6	0.189
Q3	14.6	0.870	78.5	0.234
minimum	4.9	0.537	44.8	0.045
maximum	24.2	0.956	155.3	0.622

Table 2 2 Effect of clam bed site on shell growth patterns of *Lampsilis radiata*.

ANOVA

Source	df	SS	MS	*F*	*P*
Dependent variable: Walford Plot regression intercept (*a*)					
Site	7	44803	6401	66.68	<0 001
Error	400	38395	96		
Dependent variable: Walford Plot regression slope (*b*)					
Site	7	4.4863	0.6409	28.27	<0.001
Error	400	9 0681	0.0227		

MANOVA for Site

Criterion	Test Statistic	*F*	df (num., den.)	*P*
Wilk's Lambda	0.32674	42.7	14, 798	<0.001
Lawley-Hotelling Trace	0.82910	52.0	14, 796	<0.001
Pillai's Trace	0.74888	34.2	14, 800	<0.001

Table 2.3. Canonical discriminant analysis of growth pattern parameters (a and b estimates from Walford Plot regressions). Canonical variate (CV) scores are summarized for each ot the eight study sites. Following, for each CV, are given the eigenvalue, the squared canonical correlation (R^2), and the results of the ANOVAs testing for site differences.

	Canonical Variate 1		Canonical Variate 2	
	mean	SD	mean	SD
Site 2	-1.59	5.53	0.0157	0.0426
Site 3	2.61	5.59	-0.0171	0.0408
Site 4	1.64	5.35	0.0161	0.0283
Site 5	4.39	4.61	0.0033	0.0339
Site 8	1.13	5.18	0.0020	0.0444
Site 9	2.27	4.54	0.0392	0.0521
Site 10	0.75	5.20	-0.0209	0.0436
Site 11	-12.32	4.18	-0.0245	0.0394
eigenvalue	0.902		0.229	
R^2	0.474		0 187	
F (df = 7, 400)	51.6		13.1	
P	<0.001		<0 001	

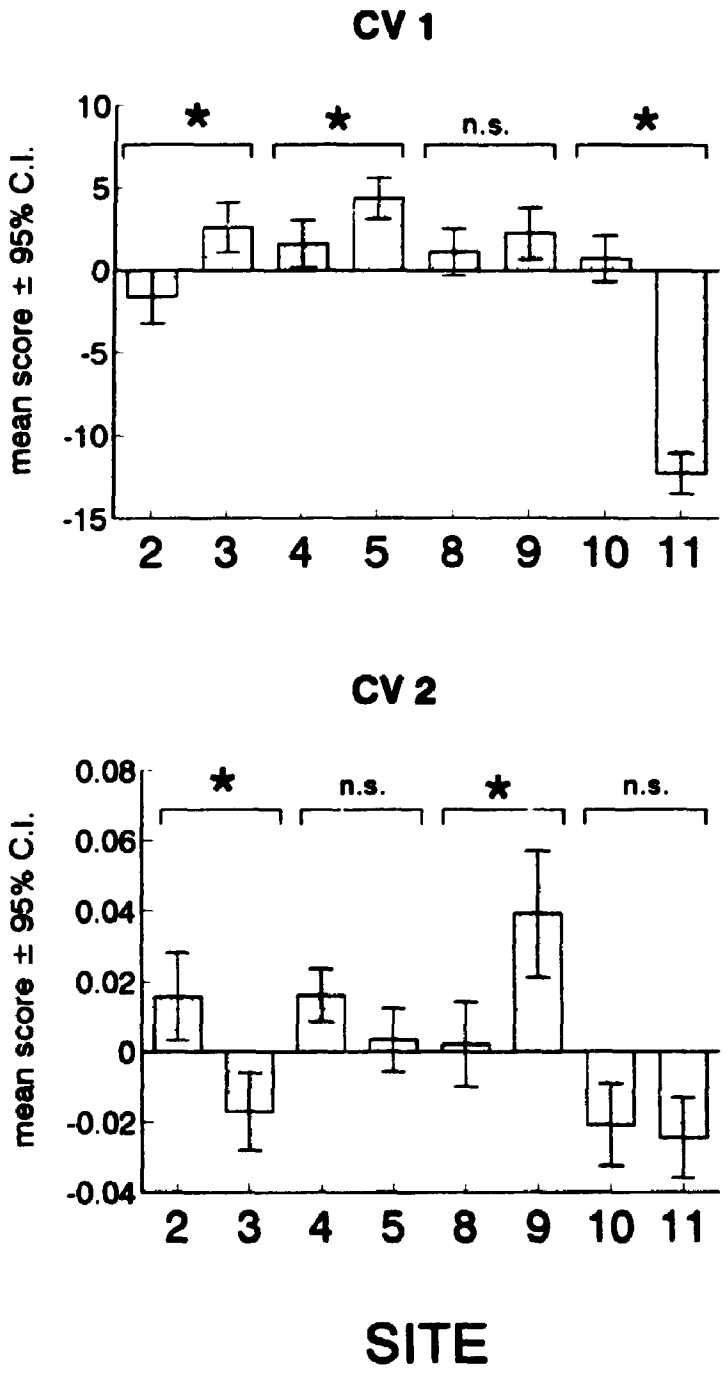


Fig. S01. Differences in shell growth patterns of *Lampsilis radiata* between sites in the St. Lawrence River. Scores of canonical variates (CV1, CV2) are from CDA on estimated intercepts and slopes from Walford Plot regressions of annual shell lengths. Comparisons between upstream (3,5,9,11) and downstream (2,4,8,10) groups from four pairs of sites were made for each CV. * = significantly different at experiment-wise $\alpha < 0.04$. n.s. = not significantly different.

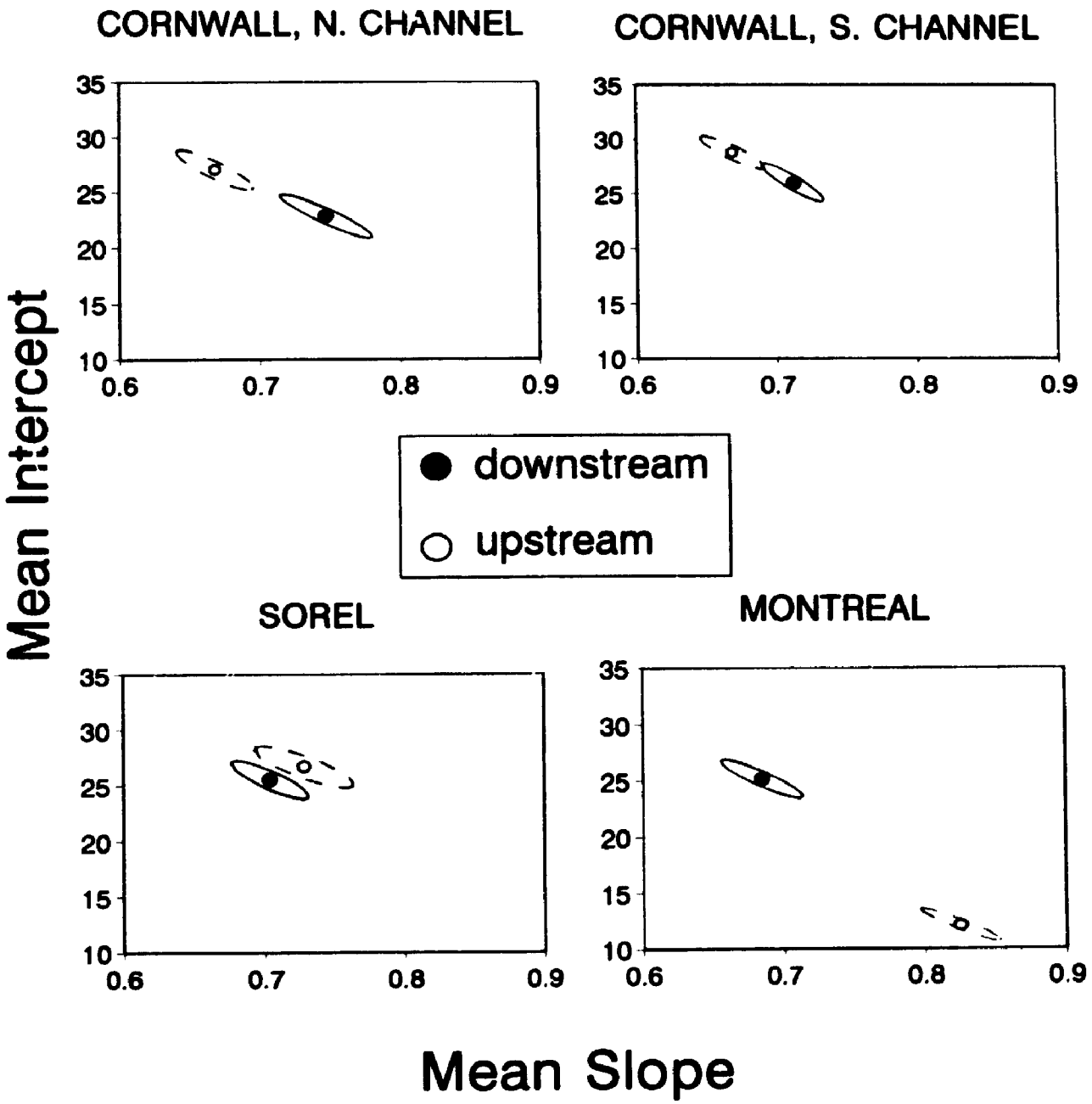


Fig. 2.2. Differences in growth patterns of *Lampsilis radiata* from upstream and downstream of four sources of industrial discharges in the St. Lawrence River. Mean intercept and slope of Walford Plot regressions from individual clams are shown with 95% confidence ellipses for each of the eight group means.

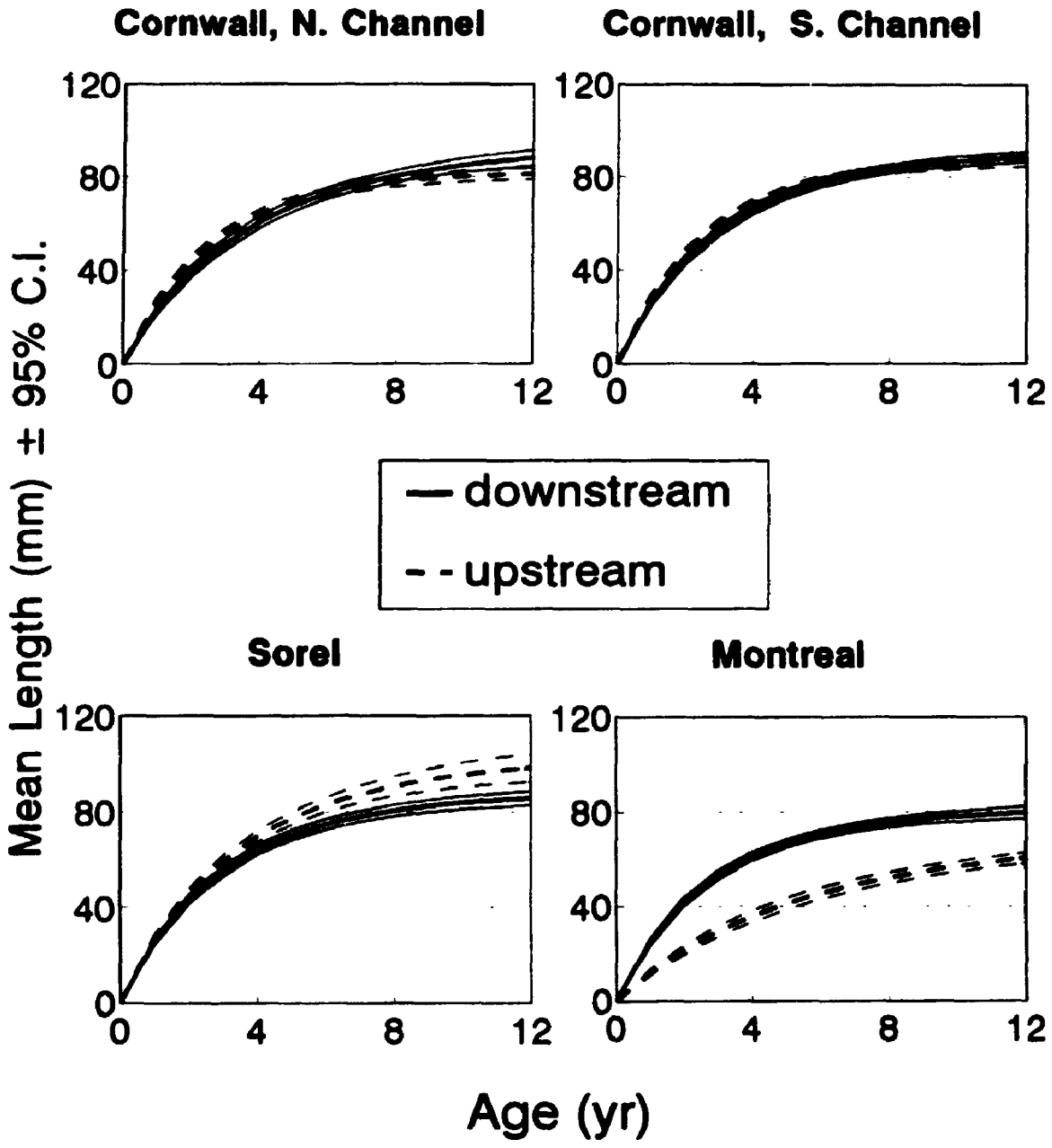


Fig. 2.3. Fitted von Bertalanffy growth curves for eight groups of *Lampsilis radiata* from the St. Lawrence River. Sites were sampled upstream and downstream of four sources of industrial discharges (Fig. 1.2).

2.4. Discussion

2.4.1. Depression of shell growth In three out of the four site pairs, some aspect of shell growth was depressed in the downstream clams relative to those from upstream. Lower Walford Plot regression intercept (a), as in clams from site 2, indicates slower growth early in life. Higher Walford Plot regression slope (b), as in sites 2 and 4, implies a lower initial instantaneous growth rate (von Bertalanffy model parameter k) from eq. 4. Low a and high b values correlate to low CV1 scores (Fig. 2.1), and correspond to positions in the lower right of the plots in Fig. 2.2. Where growth in the early years in the site below the outfall did not differ from that in the site above the outfall, as in site 8 (Fig. 2.2), maximum length (L_∞) was lower below the outfall (Fig. 2.3). In contrast to the other three pairs of sites, clams from the downstream site in the Montreal area showed substantially faster shell growth relative to clams from above discharges in the region (Fig. 2.2, 2.3).

2.4.2. Factors affecting growth patterns Except for the Montreal area (discussed below), upstream and downstream sites do not differ greatly from each other in natural conditions that are known to affect clam growth, such as water chemistry (specific conductance, alkalinity, dissolved calcium concentration), or sediment particle size distribution (App. A). Thus, variability in growth patterns among these six groups is difficult to explain by natural factors. Sediments in sites below industrial discharges are however, polluted by trace metals or PCBs (Sec. 1.4.5.). Excessive amounts of such contaminants in tissues can impair a variety of biochemical and physiological processes including shell growth in bivalves (Sec 1.6). Therefore, if degree of sediment contamination is indicative of contaminant bioavailability, differences in shell growth patterns between upstream and downstream sites possibly are due to toxicity of trace metals or PCBs.

Although Imlay (1982) recommended the use of annual shell growth to monitor metal pollution in freshwaters, few studies appear to have examined effects of metal pollutants on

shell growth of freshwater clams (reviewed by Havlik and Marking 1987; Elder and Collins 1991; and Johnson et al. 1993). Belanger et al. (1986) found reduced shell growth in *Corbicula* sp. exposed to zinc (0.05 - 1.0 mg/L) for 30 days in artificial streams. The lowest observed effect (0.05 mg Zn/L) was reportedly equal to the level of zinc considered protective of life by the USEPA. Effects of copper on shell growth by *Corbicula fluminea*, in both artificial streams and in the Clinch River below an industrial discharge were studied by Belanger et al. (1990). Growth was depressed both in adults and, especially, in juveniles exposed to copper. These responses to experimental treatments agreed with the relationship observed between densities of natural populations and copper concentrations.

Clams from the upstream site (11) in the Montreal area showed a markedly lower rate of growth and estimated age-specific length compared to those from the downstream site. Located near the inflow of the Ottawa River, alkalinity, dissolved calcium concentration and specific conductance near site 11 were all roughly less than a third of the values for the other seven sites which are all in the mainstream of the St. Lawrence River (Fig. A.1, A.2: note stations 9002 and 9008). Slower growth and smaller size in these clams compared to those from the mainstream of the St. Lawrence River, are probably due to the influence of the softer water of the Ottawa River, an effect which was also observed by Magnin and Stanczykowska (1971).

2.4.3. Ecological significance of growth depression Differences in shell growth patterns between upstream and downstream groups in each of the four study areas were statistically significant, but these results may have little ecological significance. The statistical power to detect differences in growth patterns was high, as evidenced by the results for the Cornwall - south channel groups. Despite having nearly overlapping derived growth curves (Fig. 2.3), these groups were different at a Bonferroni adjusted $\alpha < 0.04$ (Fig. 2.1, 2.2). Differences of only a few millimetres between groups in mean age-specific lengths or maximum asymptotic size may suggest differences in environmental conditions or genetic factors related to growth, but may not necessarily correlate with differential mean fitness. Fitness of a unionid bivalve can be affected by its growth rate in several ways. Rapid growth in the first few years lessens

the time the clam spends in the juvenile stage, whose surviorship is low relative to that of the adult stage (Coker et al. 1922; MacMahon 1991). Earlier maturation also allows earlier reproduction and thus greater potential lifetime fecundity. Fecundity in bivalves is also generally proportional to body size (Brousseau 1977; Bayne et al. 1983; Sprung 1983; Bayne et al. 1985). Thus faster growing and larger clams should be selectively favored over slower growing and smaller individuals.

Direct measurement of fitness was beyond the scope of this study. However, if survival or fecundity are strongly related to shell size, clams from downstream of discharges in the Sorel area may suffer reduced fitness. Unionids require several years to reach maturity (2 - 8 yr according to Coker et al. 1922; at least 6 yr according to McMahon 1991). The minimum lengths of reproducing *L. radiata* sampled from the St. Lawrence River study sites ranged from 60 to 70 mm, corresponding to ages of 3 - 6 yr. This agrees with observations of Podemski (1992) on gravid *L. radiata* from the Bay of Quinte, Lake Ontario, which were as small as 60 - 70 mm (4 - 8 yr old). In the Sorel pair of groups, mean shell length of the downstream clams diverged from that of the upstream clams by age four (Fig. 2.3). Mean lengths thus differed throughout the reproductive lives of the clams, possibly resulting in lower fecundity in the downstream than in the upstream population.

Depressions in growth attributed to pollutants should also be evaluated relative to variability due to non-anthropogenic stresses. Among all groups, the smallest and slowest growing clams were from site 11 - upstream of the Montreal area discharges but within the inflowing Ottawa River water mass. The depression in growth here, likely due to low alkalinity and low calcium concentration, easily exceeds any of those in clams from the polluted sites. Hinch et al. (1986) and Green et al. (1989) also observed variability in growth pattern due to natural environmental heterogeneity at levels exceeding the differences observed here between upstream and downstream groups (excluding sites 10 and 11) In addition, variability in shell growth pattern among study areas is of a similar magnitude to the detected pollutant effects. Thus, the levels of industrial discharges and sediment contamination observed in the study sites, though high by some standards (Sec. 1.4), are not the most important factors affecting shell growth in *L. radiata*.

3. EFFECTS ON SHELL GROWTH OF SOURCE AND DESTINATION OF CLAMS TRANSPLANTED INTO CONTAMINATED AND UNCONTAMINATED SITES

3.1. Introduction

Analyses of shell growth patterns of *L. radiata* sampled upstream and downstream of industrial discharges at four areas in the St. Lawrence River indicated significant variation among sites in growth rate and maximum length of clams (Sec. 2). In three of the four pairs of sites, clams from downstream of the discharges exhibited growth patterns that were depressed relative to those of clams from immediately upstream of the discharges. Sites below outfalls are exposed to levels of trace metals and PCBs that are substantially higher than levels in the sites above outfalls. In the absence of major environmental heterogeneity among sites in other factors known to affect shell growth, such as water chemistry and substrate particle size, these results suggest that shell growth patterns may be related to habitat contamination.

Although growth of bivalve shells is strongly influenced by environmental conditions (Seed 1980; Tevesz and Carter 1980; Bayne 1989), genetic factors can also be important (Singh and Zouros 1978; Berger 1983; Zouros et al. 1988; Koehn and Bayne 1989; Skibinski and Roderick 1989). Clams in each of the study sites could belong to populations with different inherent rates of growth that are coincident with the observed differences in contaminant levels. To assess the relative importance of genetic and environmental factors in explaining differences in growth patterns among sites, two field experiments were conducted in which a year's shell growth was measured for clams collected from common sources and transplanted into polluted and unpolluted sites. Variability between transplantation sites in growth of clams from the same source should be due to environmental conditions, whereas variability in growth within transplantation sites between clams from different sources should be attributed to genetic factors.

3.2. Methods

3.2.1. Study sites and experimental designs The two experiments involved three polluted and two unpolluted sites located in two areas in the upper St. Lawrence River, in the north channel of the river at Cornwall, and in the Sorel area (Fig. 1.2). For the Cornwall experiment, *L. radiata* were collected from site 13, upstream of a submerged diffuser shared by a kraft pulp mill and a chemical manufacturer, and transferred to (a) site 6, several hundred metres downstream from the diffuser; (b) site 3, across the river from site 6 but outside the discharge plume; and (c) back into site 13 (Fig. 3.1A). Sediments at site 6 are heavily contaminated with mercury. Concentrations of total mercury immediately downstream of site 6 averaged 3.26 μg/g in 1991 (Richman 1994). In comparison, mercury levels in sites 3 and 13 were 0.07 and 0.03 μg/g, respectively, in 1990 (J. L. Smith, pers. comm.). Methyl mercury levels were also elevated near site 6 - 0.012 μg/g vs 0.001 μg/g in sites upstream of the outfall (Richman 1994). Unlike all other sites sampled in the St. Lawrence River, few live unionids were found at site 6 prior to transplantation.

The purpose of the Sorel experiment was to examine the effect of source, as well as destination on shell growth. In the Sorel experiment clams were reciprocally transplanted between site 8, below the discharges of three smelters and a pigment manufacturer, and site 9, upstream of the discharges (Fig. 3.1B). Sediments in site 8 are heavily contaminated with Cu, Co, Ni, Cr, Pb, Fe and Zn (App. A).

3.2.2. Cornwall experiment Over 300 *L. radiata* were collected by divers from 2 -3 m depth in site 13 on 20-21 June 1990. During handling, clams were held in tubs of cooled, aerated river water. One hundred clams were randomly assigned to each of the three transfer groups, and then marked with coded plastic tags glued to the right valve with a wet surface repair epoxy putty. Maximum shell lengths were measured with dial calipers to the nearest 0.01 mm. Mean length of these shells was 82.4 mm, with SD = 7.7. On 22 June 1990, clams were returned to the river with no mortality during handling. Clams were released onto

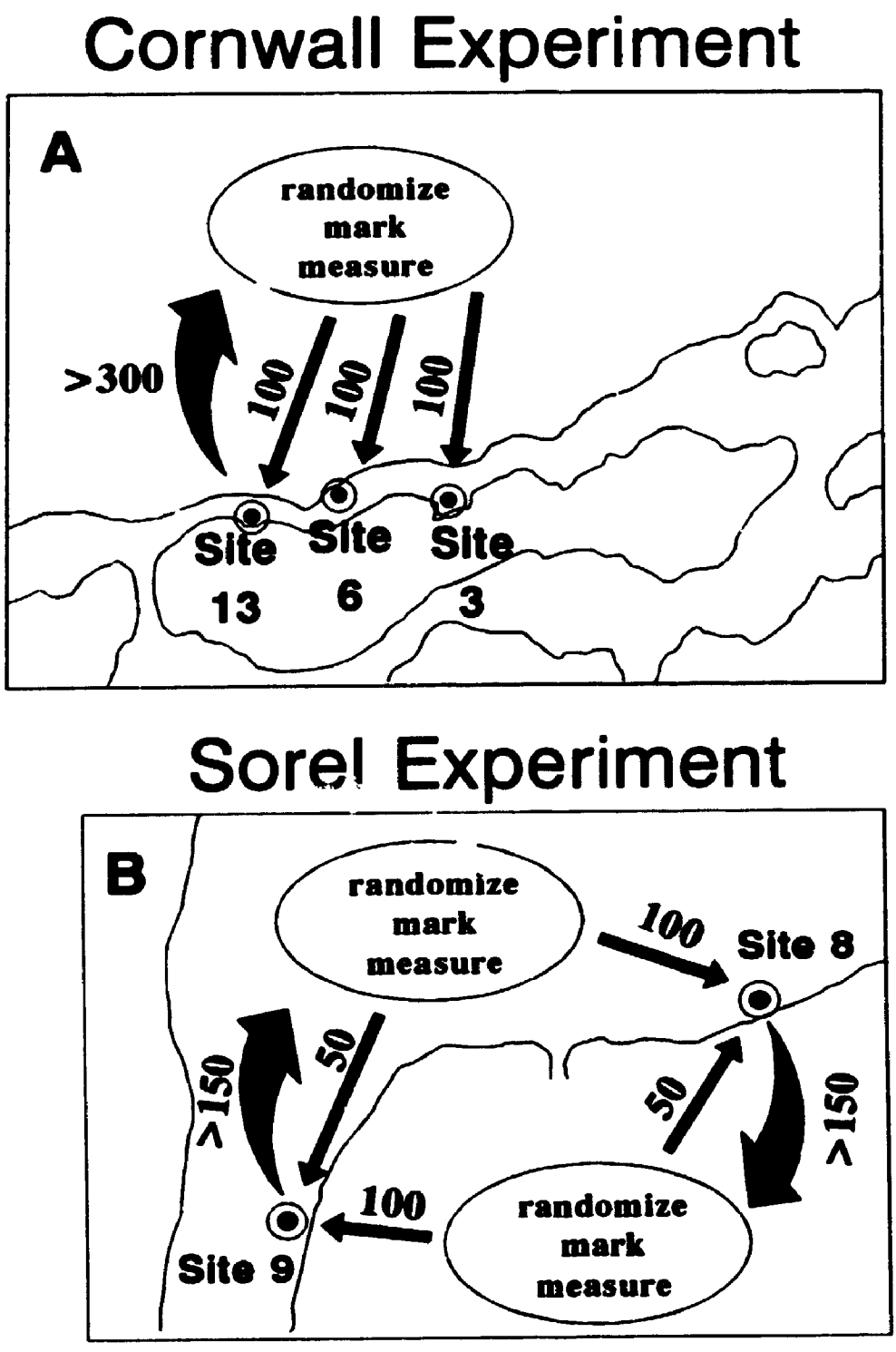


Fig. 3.1. Designs of transplantation experiments with *Lampsilis radiata* in two areas of the St. Lawrence River. See text for details.

flagged, 2-3 m^2 - areas of river bed at 2 - 4 m depth, on substrate shared with other clams (except for the virtually uninhabited site 6).

During 23-28 May 1991, transplant sites of clams released in 1990 were relocated. At each site, an area 10 m in radius was systematically searched for tagged clams. Surviving tagged individuals were recaptured and remeasured for maximum lengths.

Differences in shell growth among sites were assessed by analysis of covariance (ANCOVA). The effect of the transplant destination (DESTIN) was tested on shell length in 1991 (L_{91}) adjusted for length in 1990 (L_{90}), because smaller, younger clams grow faster than larger, older ones. This is equivalent to an ANCOVA on "length gain" (L_{91} - L_{90}), but with fewer statistical assumptions. Because the DESTIN*L_{90} interaction term was not significant ($P = 0.111$), the model was reduced to:

$$L_{91} = \mu + \text{DESTIN} + bL_{90} + e .$$

3.2.3. Sorel experiment Concurrent with the Cornwall experiment, over 150 *L. radiata* were collected from depths of 2 - 4 m at each of sites 8 and 9 in the Sorel area on 26 June 1990. Clams were maintained in cooled, aerated river water prior to transplantation. From each site, 100 randomly chosen clams were assigned to the opposite site and 50 clams were replaced at their source. Shells were marked and measured for length in the same manner as for the Cornwall experiment. Mean length ± SD of shells in 1990 was 83.8 ± 10.1. On 27 June 1990, clams were released onto flagged river bed in their assigned destinations. These positions were relocated and searched over 10 m - radius areas during 30 May - 02 Jun 1991. Recovered live clams were remeasured for maximum length.

Variation in shell growth among transplant groups was assessed by an ANCOVA similar to the Cornwall experiment, except that the effect of collection source (SOURCE) on length in 1991 (adjusted for length in 1990) was tested in addition to transplant destination. Again, a full model with all interaction terms was initially used after which nonsignificant ($P > 0.274$) interaction terms were removed, leaving the reduced model :

$$L_{91} = \mu + SOURCE + DESTIN + bL_{90} + SOURCE*L_{90} + e .$$

3.3. Results

3.3.1. Cornwall experiment Of the 100 tagged clams placed into each of the three study sites, recapture numbers were 80, 73 and 52 for sites 3, 6 and 13, respectively. These numbers included some dead individuals: 6, 10 and 2 for sites 3, 6 and 13, respectively. Analysis of the live recaptures indicated that growth in shell length, as determined by L_{91} adjusted for L_{90}, did not differ among transplant groups (Table 3.1). Mean length in 1991 of clams transferred to site 6, the most highly contaminated with mercury, was lower than the mean lengths of clams from sites 3 and 13, but the differences were not significant (Fig. 3.2).

3.3.2. Sorel experiment The recapture rate of tagged shells from site 8 was 52% (78 live, 0 dead), and from site 9, 70% (98 live, 6 dead). Results from the ANCOVA on 1991 lengths showed strong effects of clam source ($P < 0.001$) and destination ($P = 0.015$) on growth (Table 3.1). Clams that came from upstream of the discharges (site 9) grew more than those from downstream (site 8), regardless of where they were transplanted (Fig. 3.3A). But among clams from the same source, those put into the downstream site grew more than those put into the upstream site (Fig. 3.3B). The transplanted group collected from upstream and released downstream of the discharges showed the greatest growth (Table 3.2).

The SOURCE*L_{90} interaction was also significant ($P < 0.001$), indicating that the relationships between L_{90} and L_{91} differed between clams from site 8 and those from site 9. In Fig. 3.4, these relationships are shown in terms of predicted gain in length vs. L_{90} for clarity. A year's growth by clams from site 9 was on average greater than that by clams from site 8, except for individuals longer than roughly 90 mm. For shells >90 mm in length, (over 90% of the estimated maximum lengths for *L. radiata* from sites 8 and 9; Table 2.1), annual growth is expected to be near zero (Fig. 2.3). Some individuals even showed degrowth of shells.

Table 3 1 Variation in shell growth of *L. radiata* among transfer groups: ANCOVAs using reduced models with L_{91} (length in 1991) as the dependent variable. Factors are (a) site to which clams were transplanted (DESTIN), and (b) site from which clams were collected (SOURCE). Length in 1990 (L_{90}) was the covariate.

Source	df	Type III SS	MS	*F*	*P*
		Cornwall Experiment			
DESTIN	2	2.0	1.0	2.53	0 082
L_{90}	1	10964.6	10964.6	28200	<0.001
Error	183	71 2	0 4		
		Sorel Experiment			
SOURCE	1	21.8	21.8	17.97	<0.001
DESTIN	1	7.4	7.4	6.07	0 015
L_{90}	1	10506.5	10506.5	8652.78	<0 001
SOURCE*L_{90}	1	15.4	15.4	12.67	<0.001
Error	171	207.6	1.2		

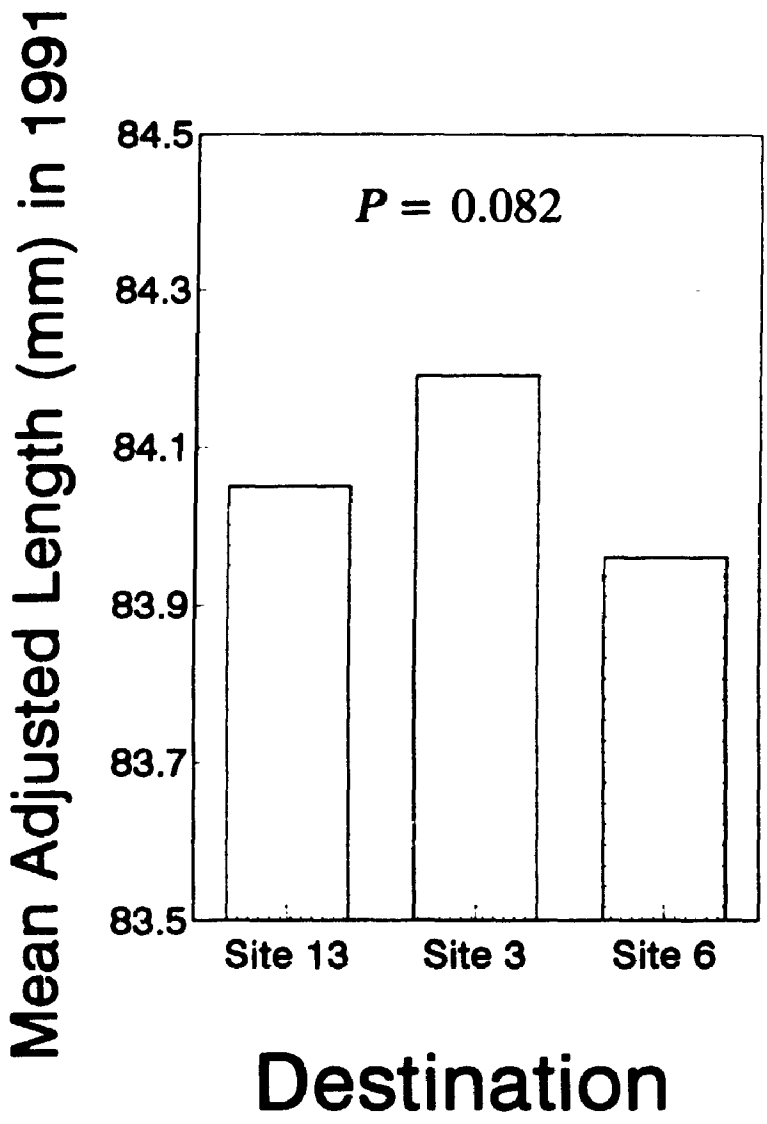


Fig. 3.2. Mean adjusted lengths of shells from Cornwall transplantations. Differences among sites were not significant (P = 0.082).

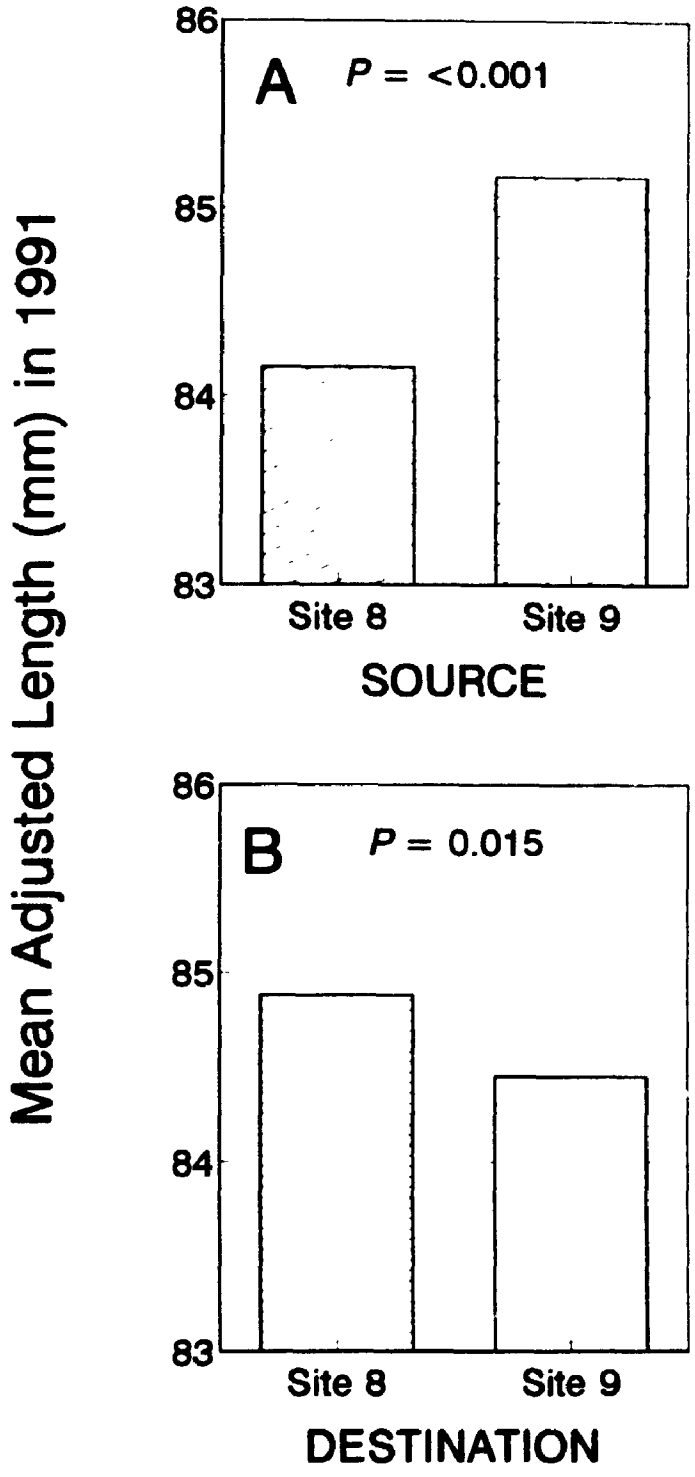


Fig. 3.3. Effects of transplantation factors on shell growth in the Sorel sites. P - values are for significance of differences between groups.

Table 3 2. Gain in shell lengths (mm) of transplanted *L. radiata* in the Sorel experiment. Values for each of the four transplant groups are mean ± SE of predicted differences in lengths between 1991 and 1990 from a reduced model ANCOVA using "L_{91} - L_{90}" as the dependent variable in place of L_{91} in the analysis described in the text.

	Source of Clams	
Destination of Clams	Upstream of Discharges (site 9)	Downstream of Disharges (site 8)
Upstream (site 9)	1.83 ± 0.17	0.32 ± 0.04
Downstream (site 8)	2.25 ± 0.14	0.66 ± 0.05

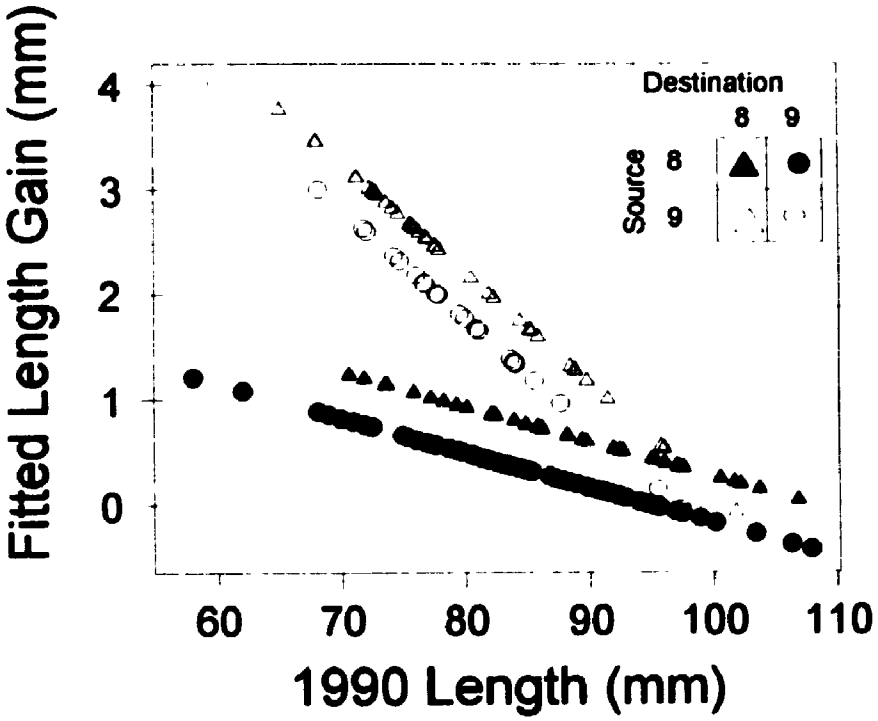


Fig. 3.4. Effects of source, destination and 1990 length on shell growth of transplanted clams in Sorel area sites. Site 8 is downstream and site 9 is upstream of discharges.

3.4. Discussion

In both the Cornwall and the Sorel experiments, over a year clams that were transplanted into metal-contaminated sites showed no depression of shell growth relative to clams put into comparatively uncontaminated sites. In fact, in the Sorel experiment shell growth was better in the polluted site than in the unpolluted site for clams collected from the same source. These results are surprising given that metal concentrations in the sediments of sites 6 and 8 are well above the "severe effects level" of Ontario's Provincial Sediment Quality Guidelines (Persaud et al. 1992).

Clams from upstream of the Sorel area discharges grew more in a year than those from downstream, which is in agreement with the analyses of growth patterns in Sec. 2 (Fig. 2.3). This effect of source was clearly greater than the effect of destination on shell growth (Fig. 3.3, 3.4), suggesting that a genetic factor, or possibly an irreversible physiological adaption (Hinch et al. 1986; Bayne 1987; Peterson and Beal 1989), influences growth rate. Furthermore, slower growing clams from site 8 did not exhibit enhanced growth after transfer to the less contaminated site 9, as expected if the stress of trace metal resistance restricts shell growth.

Similar results were obtained from reciprocal transplant experiments by Hinch et al. (1986), on *L. radiata* from two sites in Lake Erie differing in substrate type, and by Hinch and Green (1989), on *Elliptio complanata* from three southern Ontario lakes differing in alkalinity. In both cases, the effect of clam source on shell growth dominated that of destination. Peterson and Beal (1989) found that source had the greatest effect of several factors on shell growth in field experiments with the estuarine clam *Mercenaria mercenaria*, and that this effect was likely to have a genetic rather than a physiological basis. Growth of *Mytilus edulis* was also found to depend on collection source (Dickie et al. 1984; Mallet et al. 1987).

Several circumstances constrain the conclusions to be drawn from the results. Loss of marked clams, although not high compared to other studies with *L. radiata* in lakes (Hinch et al. 1986; Downing et al. 1992; Podemski 1992), were not negligible and somewhat uneven

among transplant groups. Unrecovered clams could have burrowed into the sediment, dispersed from the search area, or died and been buried or swept away by the current, possibly in response to environmental conditions. Emigration and/or mortality should account for most of the loss of marked clams, because it is unlikely that many clams would have been endobenthic at the time of sampling. Amyot and Downing (1991) reported that in a population of *Elliptio complanata* in a Quebec lake, no clams in late June, and only approximately 10% in early August were endobenthic. It is difficult to determine however, if the loss of marked clams is due more to emigration or to mortality Recapture rates in the two experiments were not consistent with respect to degree of site contamination. In the Cornwall experiment, most dead shells were recovered in the contaminated site, but the poorest recapture rate was in the uncontaminated, source site. At Sorel, the reverse occurred: all dead shells were found in the upstream site, where the recovery rate was lowest

The clams used in the experiments, though representative of the beds from which they were taken, were generally over 70 mm in length. Because annual growth at these sizes is only a fraction of that in younger clams, the potential response to environmental conditions was low. Measured gains in length were commonly less than 2 mm; some individuals even lost length, a condition also observed by Downing et al. (1992). Resistance to environmental stress is likely higher in older than in younger age groups (Cherr et al. 1990; Yeager et al. 1994; Warren et al. 1995). Thus, while the conclusions from these experiments are relevant to most individual encountered in clams beds, inference concerning younger clams should be guarded.

In summary, it is evident that shell growth of transplanted *L. radiata* is not inhibited in the sites contaminated with trace metals. Clams from upstream of the Sorel area discharges grew faster than those from downstream. Growth rates observed here appear to be constrained by genetic factors, or possibly by irreversible physiologic acclimatization. Selection may have favored slower growing clams in contaminated sites as a trade-off against greater resistance to trace metal toxicity. Perhaps enhanced growth shown by clams from the upstream site (9) in the downstream site (8) may be explained by the existence of conditions at site 8 more favorable to growth over the short term than those at site 9.

4. VARIATION IN ALLOZYME GENOTYPE FREQUENCIES AMONG NATURAL CLAM POPULATIONS UPSTREAM AND DOWNSTREAM OF INDUSTRIAL DISCHARGES

4.1. Introduction

Aquatic invertebrates are affected by pollutants at levels of biological organization ranging from subcellular to community. At the population level, a strong lethal effect decreases total abundance. With less severe effects, individuals (and their offspring) resistant to contaminant toxicity may replace those suffering reduced survival or reproductive output. If there is a genetic component underlying variation in resistance, pollutants can act as selective force, altering the genetic structure of populations before eliminating them. Populations in contaminated and uncontaminated environments should differ in the frequencies of genotypes that confer differential fitness under contaminant exposure (Nevo et al. 1983; Bayne 1987).

Electrophoretic analysis of allelic variants of enzymatic proteins (allozymes) has been an important tool for the study of population genetic structure for several decades (Richardson et al. 1986). Pollutant-related variation in allozyme genotype frequencies has been reported in field observations of marine shrimp and gastropods (Nevo et al. 1984; Nevo 1993), barnacles (Patarnello et al. 1991; Montero et al. 1994), freshwater insects (Benton and Guttman 1990) and freshwater fishes (Gillespie and Guttman 1989; Heagler et al. 1993). These studies have all involved trace metal contaminants and one or both of the enzymes glucose-phosphate isomerase (GPI) and phosphoglucomutase (PGM). GPI and PGM are commonly polymorphic and easily detected in animal taxa (Richardson et al. 1986), and elevated concentrations of certain metals inhibit the function of PGM (Milstein 1961) and GPI (Montero et al. 1994). Frequencies of GPI and PGM allozymes have also exhibited variations in field studies related to oil contaminantion, thermal pollution, and degree of eutrophication (reviewed in Hummel and Patarnello 1994). For these reasons, the analysis of PGM and GPI genotypes has been suggested as a means of detecting disturbances to populations due to environmental pollution.

The purpose of this study was to (a) determine if allozyme genetic markers could be analyzed in populations of the freshwater bivalve *Lampsilis radiata* from industrialized areas of the St. Lawrence River, and (b) assess if industrial discharges affect the genetic structure of these populations with respect to these markers. In preliminary electrophoretic screening of several allozyme systems, GPI and PGM alone were both resolvable and polymorphic. The presence of beds of reproducing *L. radiata* in several sites severely chemically impacted by effluents suggests that adaptation to the contaminants may have occurred. Variation in GPI and PGM allozyme frequencies has been associated with variation in fitness, under a variety of polluted conditions in laboratory experiments (reviewed by Hummel and Patarnello 1994). Thus, frequencies of GPI and PGM allozymes are expected to differ between groups of clams from sites upstream and downstream of discharges.

4.2. Methods

4.2.1. Study sites Eight clam beds located downstream and upstream of four industrialized areas in the St. Lawrence River (sites 2 and 3 in the north channel of the river at Cornwall; sites 4 and 5 in the south channel of the river at Cornwall; sites 8 and 9 at Sorel; and sites 10 and 11 at Montreal; Fig. 1.2) were sampled to examine allozyme frequencies. Concentrations of contaminants in effluents and in sediments indicate substantial contaminantion by trace metals or PCBs in the sites downstream of outfalls (Sec. 1.4; Table 1.2).

4.2.2. Collection and handling of samples Approximately 50 *Lampsilis radiata* were collected from each of eight clam beds (one upstream and one downstream of the four study areas) from depths of 2 - 9 m using oyster tongs or SCUBA during June 1989. Within several hours, clams were shucked. Shells were labelled, aged (App. 2) and retained for growth pattern analysis (Sec. 2). Several grams of foot tissue were dissected, snap-frozen in vials with liquid nitrogen, and stored initially on dry ice and later in an ultra-cold (-80°C) freezer.

4.2.3. Electrophoresis Allozyme electrophoresis was performed on all samples using the general procedures of Richardson et al. (1986). To prepare samples for for electrophoresis, approximately 0.2 cm^3 of frozen or freshly thawed foot tissue was placed in a centrifuge tube with 0.50 mL of homogenizing solution (Table 4.1), and ground with glass beads and a rod while immersed in an ice bath. Samples were spun in a centrifuge at 1800 rpm for 3 - 4 min., and five or more 10-µL subsamples of the supernatent were refrozen in liquid nitrogen and stored at -80°C.

Electrophoresis was conducted on cellulose acetate gel plates. The usual running buffer was 0.015 M Tris-EDTA-borate-$MgCl_2$ at pH 7.8 - 8.0. For cases in which allozyme band separation or resolution was insufficient, additional runs with 0.025 Tris-glycine at pH 8.2 - 8.5 as buffer were performed. Homogenates were transferred to Titan III Plates with a Super Z-12 Application System (Helena Laboratories, Beaumont, Texas). Plates were run two per buffer tank, with constant 200 V direct current, usually for 60 minutes. Temperatures of buffers were maintained at < 5°C by ice baths. Following each run, one plate was stained for GPI (E.C. Number 5.3.1.9) and the other plate for PGM (E.C. Number 2.7.5.1) using the agar overlay method of Hebert (1986) and solutions (Table 4.1) modified from Richardson et al. (1986). Positions of bands were recorded immediately after appearance by either photocopying the plate or tracing band outlines on an overlying transparency. Line-up gels were run to confirm apparent mobility similarities across plates and between buffers. Repeated electrophoresis of the earliest run samples after all others had been run indicated no change in allozyme activity or migration.

4.2.4. Analysis Interpretation of banding patterns followed the procedures of Richardson et al. (1986). Different allozymes (phenotypes) as detected by banding patterns were assumed to result from allelic substitutions at the locus coding for the enzyme. For each enzyme system, the fastest migrating allozyme was labelled "A", the next fastest "B", and the slowest "C". An individual designated GPI-BC, for example, showed allozyme bands corresponding to the product of the heterozygote Gpi^{bc}. Allelic and genotypic frequencies of *Gpi* and *Pgm* were then estimated for each of the eight clam groups (= putative populations) using

Table 4.1. Recipes for solutions used in electrophoresis.

Homogenization Solution

0.303 g Tris
0.093 g EDTA (di-sodium salt)
250 mL water (deionized, distilled)

Adjust to pH 6.8 with conc. HCl

10 mg NADP
18 µL ß-mercaptoethanol

Staining Solution of GPI (agar overlay method)

1.5 mL 0.276 M Tris-HCl pH 8.0
0.2 mL 0.16 M Fructose-6-PO_4
1.0 mL 5.0 mM NADP
0.75 mL 0.4 M $MgCl_2$
0.25 mL 14.5 mM Methyl thiazolyl blue (MTT)
0.25 mL 6.5 mM Phenazine methosulphate (PMS)
4.4 I.U. Glucose-6-phosphate dehydrogenase
2 mL agar (70°C)

Staining Solution of PGM (agar overlay method)

1.5 mL 0.276 M Tris-HCl pH 8.0
0.25 mL 0.32 M Glucose-1-PO_4 (with $\geq$ 1% glucose-1,6-diphosphate)
1.5 mL 5.0 mM NADP
0.3 mL 0.4 M $MgCl_2$
0.25 mL 14.5 mM MTT
0.25 mL 6.5 mM PMS
4.4 I.U. Glucose-6-phosphate dehydrogenase
2 mL agar (70°C)

BIOSYS-1 (Swofford and Selander 1981). Linkage disequilibrium between the loci was assessed by Chi-square homogeneity test on the genotype distributions (Richardson et al. 1986).

Within- and among - group variation was assessed for both enzymes. Based on allelic frequencies, genotype frequencies in a population are predicted by the Hardy-Weinberg Principle if, among other assumptions, there is no differential selection among genotypes (Crow 1986). Deviations of observed from expected frequencies within groups were tested by Chi-square analysis.

Selection by industrial effluents (resulting in differential survival among *Gpi* and *Pgm* genotypes) should be evident by comparing allozyme frequencies in groups from downstream of outfalls with those in groups from upstream. Differences in genotype frequencies among groups were tested for significance by chi-square contigency analysis.

Genetic differentiation of the eight groups was assessed by calculating Wright's F - statistics (Crow 1986) using BIOSYS-1. F_{ST}, the fixation index, is a measure of population subdivision in terms of the amount of allelic heterozygosity within groups (subpopulations) relative to that expected for the whole population (groups pooled). In other words, it is a measure of the reduction in heterozygosity due to population subdivision. The stronger the subpopulation structure, possibly due to low levels of genetic mixing among groups or locally variable selection regimes, the higher the F_{ST} value.

4.3. Results

Both GPI and PGM were polymorphic in all groups of clams: 3 alleles with 5 genotypes for GPI, and 3 alleles with 4 genotypes for PGM (Tables 4.2 and 4.3). There appears to be no linkage between the two loci ($X^2 = 9.085$, d.f.= 12, $P = 0.70$). Staining for PGM often showed activity in a second zone further from the sample origin, indicating a second detectable locus. However, these bands were not always apparent and sufficiently intense to be scored. Results reported here are thus from the *Pgm-2* locus.

Table 4.2. Allele frequencies in groups of *Lampsilis radiata* collected from St. Lawrence River sites in 1989.

		GPI			PGM		
SITE	N	A	B	C	A	B	C
2	51	.020	.833	.147	.020	.902	078
3	44	.034	.886	.080	.011	.864	125
4	48	.042	.854	.104	.021	.906	073
5	50	.020	.850	.130	.020	.880	100
8	49	.031	.847	.122	031	857	112
9	50	.020	.860	.120	.020	.910	.070
10	50	.030	.870	.100	.010	.870	.120
11	45	.011	.856	.133	.022	.778	200

Table 4.3. Genotype frequencies in groups of *Lampsilis radiata* collected from St. Lawrence River sites in 1989.

		GPI					PGM			
SITE	N	AB	AC	BB	BC	CC	AB	BB	BC	CC
2	51	.020	.020	.745	.157	.059	.039	.804	.157	0
3	44	.068	0	.773	159	0	.023	.750	.205	.023
4	48	.063	.021	.750	'46	.021	.042	.833	.104	.021
5	50	.020	.020	.740	.200	.020	.040	.78	.160	.020
8	49	.061	0	.694	.245	0	.061	.714	.225	0
9	50	.020	.020	.760	.180	.020	.040	.840	.100	.020
10	50	.060	0	.740	.200	0	.020	.780	.160	.040
11	45	0	.022	.756	.200	.022	.044	.600	.311	.044

4.3.1. GPI Allelic frequencies of GPI were similar in all groups, with GPI-B at proportions >0.83, GPI-C at 0.08 - 0 15, and GPI-A at <0.05 (Fig. 4.1). Genotype frequencies (Fig. 4.2) were also similar among groups and predominated by GPI-BB (0 69 - 0.77) and GPI-BC (0.15 - 0 24). These frequencies showed no significant departures from Hardy-Weinberg equilibrium values in any of the groups (Table 4.4). Genotype frequencies were not different between upstream and downstream groups from any of the four pairs of sites (P = 0.30 - 0.82) In fact, genotype frequencies were the same in all eight sites (X^2 = 18 470, d f.= 28, P = 0.91). In the 4 downstream sites, where selective pressure due to pollutants should have been greatest, genotype frequecies (pooled among sites) were homogeneous in age classes 2 - 7 yr, 8 - 11 yr, and 12 - 22 yr (Fig. 4.3). This suggests genotype frequencies have not changed over the last 10 - 20 years.

4.3.2. PGM The results for PGM were similar to those for GPI. The PGM-B allele was the most common in all groups, at proportions of 0.78 - 0.91 (Fig. 4.4). PGM-C and PGM-A occurred at levels of 0.07 - 0.20 and 0.01 - 0.03, respectively. The most important genotype proportions (Fig. 4.5) were consistently for PGM-BB (0.60 - 0.84) and PGM-BC (0 10 - 0.31). Frequencies of genotypes in all groups were the same as those expected from the Hardy-Weinberg Principle (Table 4.4). There were no differences in genotype frequencies either between upstream/downstream group pairs (P = 0.25 - 0.88), or among all groups (X^2 = 17.059, d.f. = 21, P = 0.71). Among age classes, the distribution of PGM genotypes appeared stable (Fig. 4.3).

4.3.3. Population subdivision Based on *Gpi* and *Pgm-2* allelic frequencies, very little genetic differentiation exists among groups. F_{ST} values for *Gpi* and *Pgm-2* were 0 003 and 0.014, respectively. For both loci, F_{ST} was 0.008 (Table 4.5).

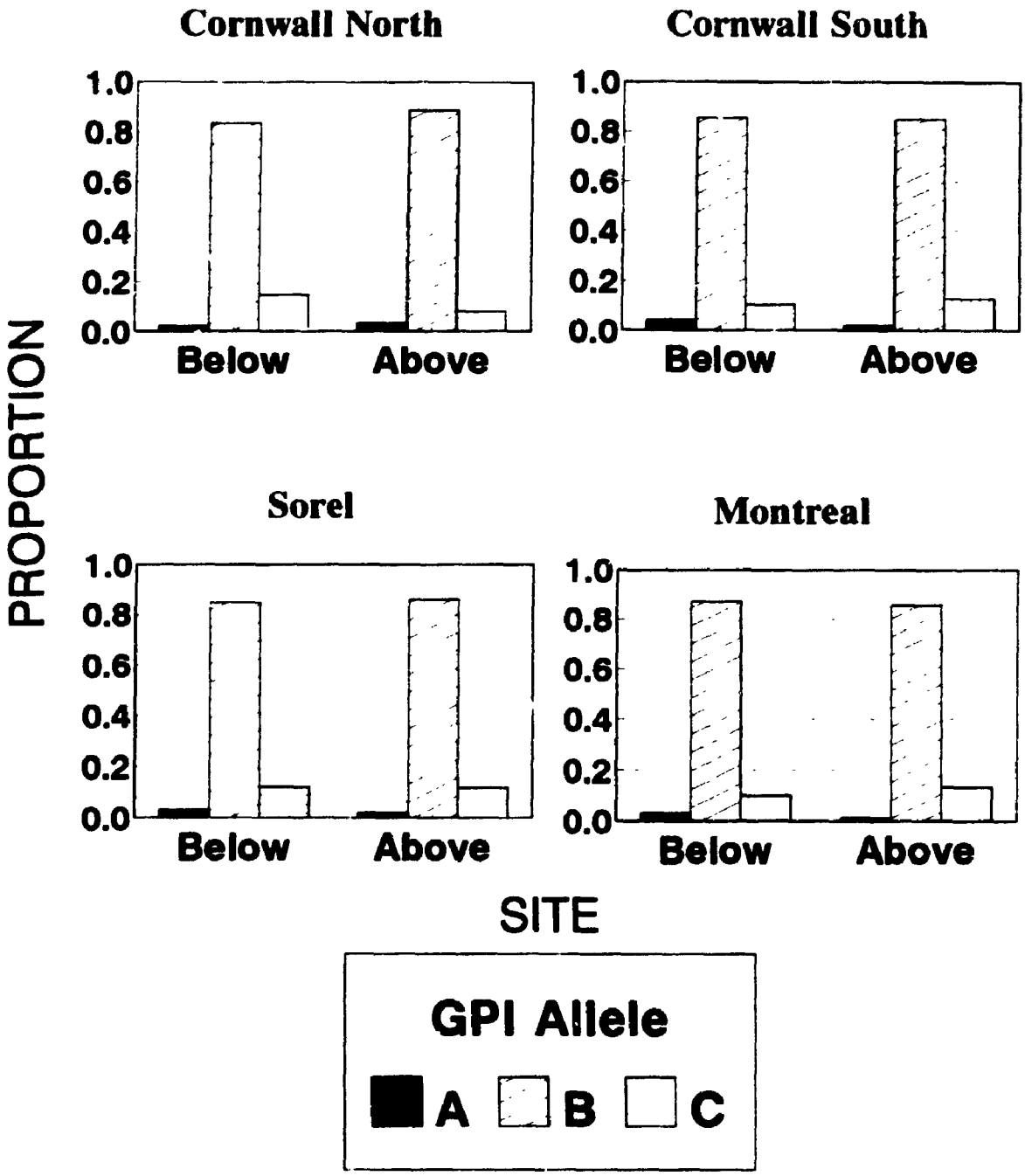


Fig. 4.1. Allelic frequencies of GPI allozymes in *Lampsilis radiata* sampled above and below industrial discharges in four areas of the St. Lawrence River.

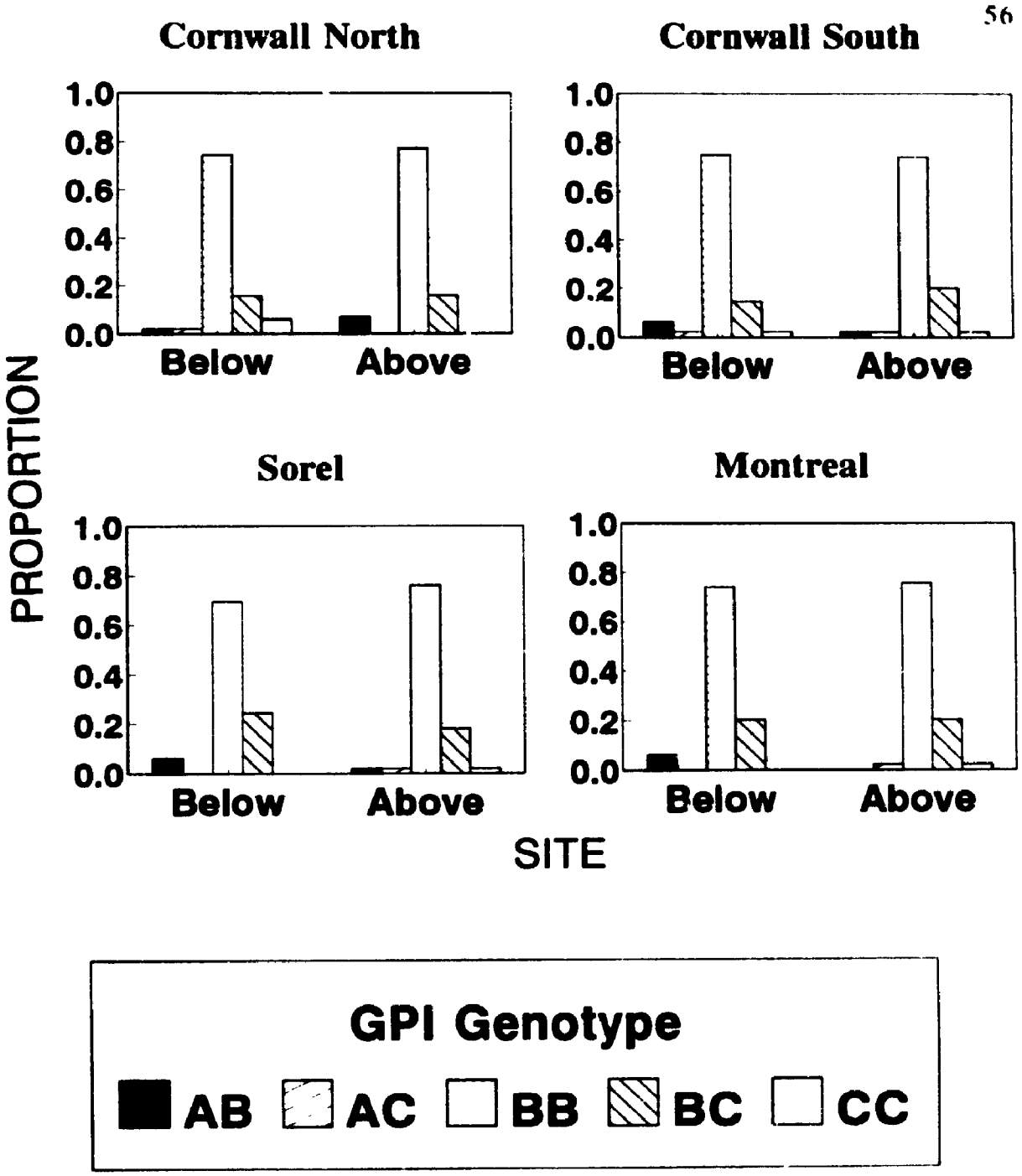


Fig. 4.2. Genotype frequencies of GPI allozymes in *Lampsilis radiata* sampled above and below industrial discharges in four areas of the St. Lawrence River.

Table 4.4. Tests for deviation of genotype frequencies from Hardy-Weinberg equilibrium (rare genotypes pooled, d.f. = 1).

GPI

POPULATION	CHI-SQUARE	P
Cornwall N, below	6.784	0.009
Cornwall N, above	0.723	0.395
Cornwall S, below	1.287	0.257
Cornwall S, above	0.942	0.332
Sorel, below	1 600	0.206
Sorel, above	1 435	0.231
Montreal, below	1 116	0.291
Montreal, above	1.638	0.201

PGM

POPULATION	CHI-SQUARE	P
Cornwall N, below	0.603	0.438
Cornwall N, above	0.054	0.816
Cornwall S, below	0.965	0.326
Cornwall S, above	0.141	0.708
Sorel, below	1.361	0.243
Sorel, above	1.056	0.304
Montreal, below	2.086	0.149
Montreal, above	0.037	0.848

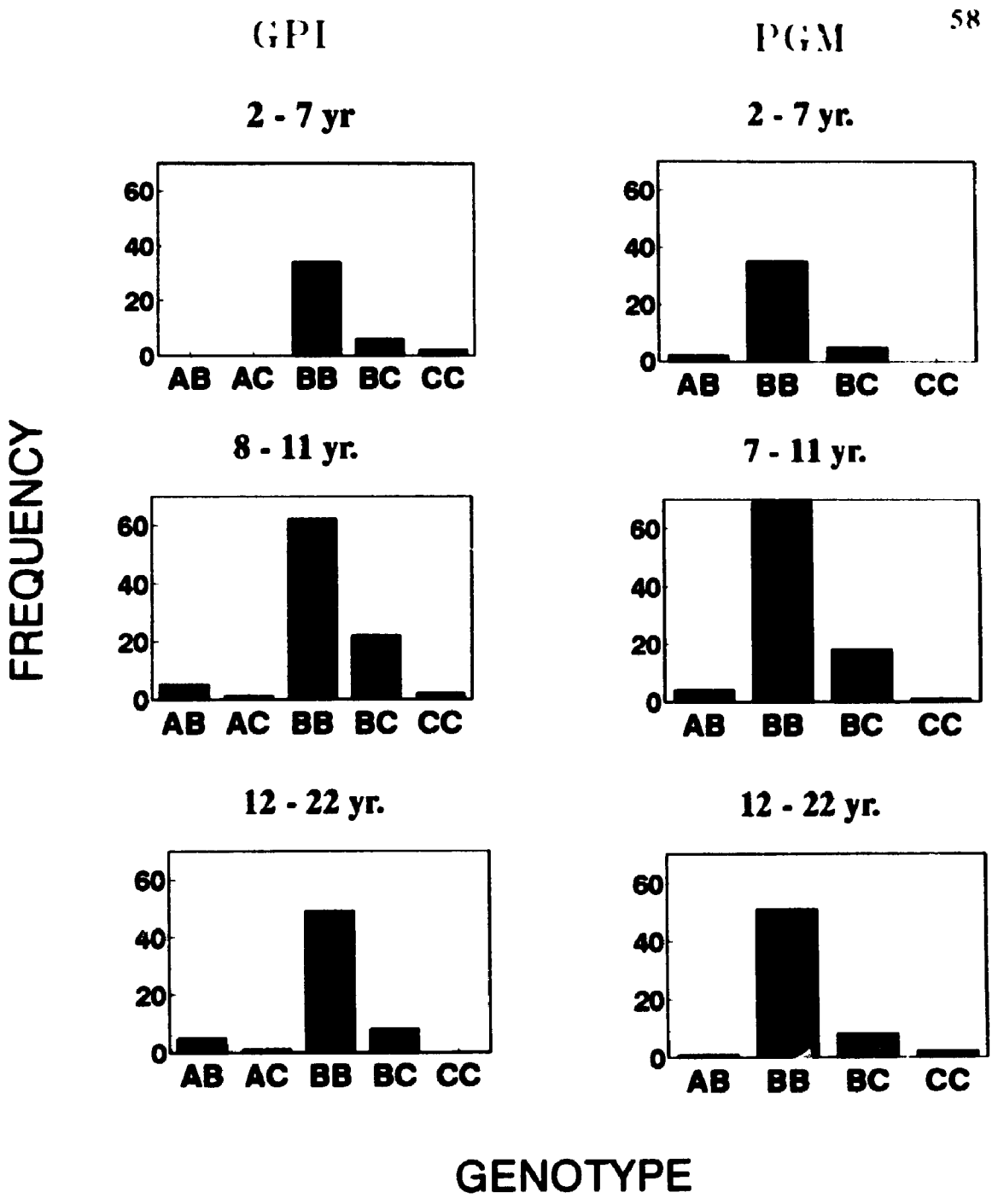


Fig. 4.3. Frequencies of allozyme genotypes of GPI and PGM partitioned by age class and pooled across sites (2,4,8,10) downstream of outfalls.

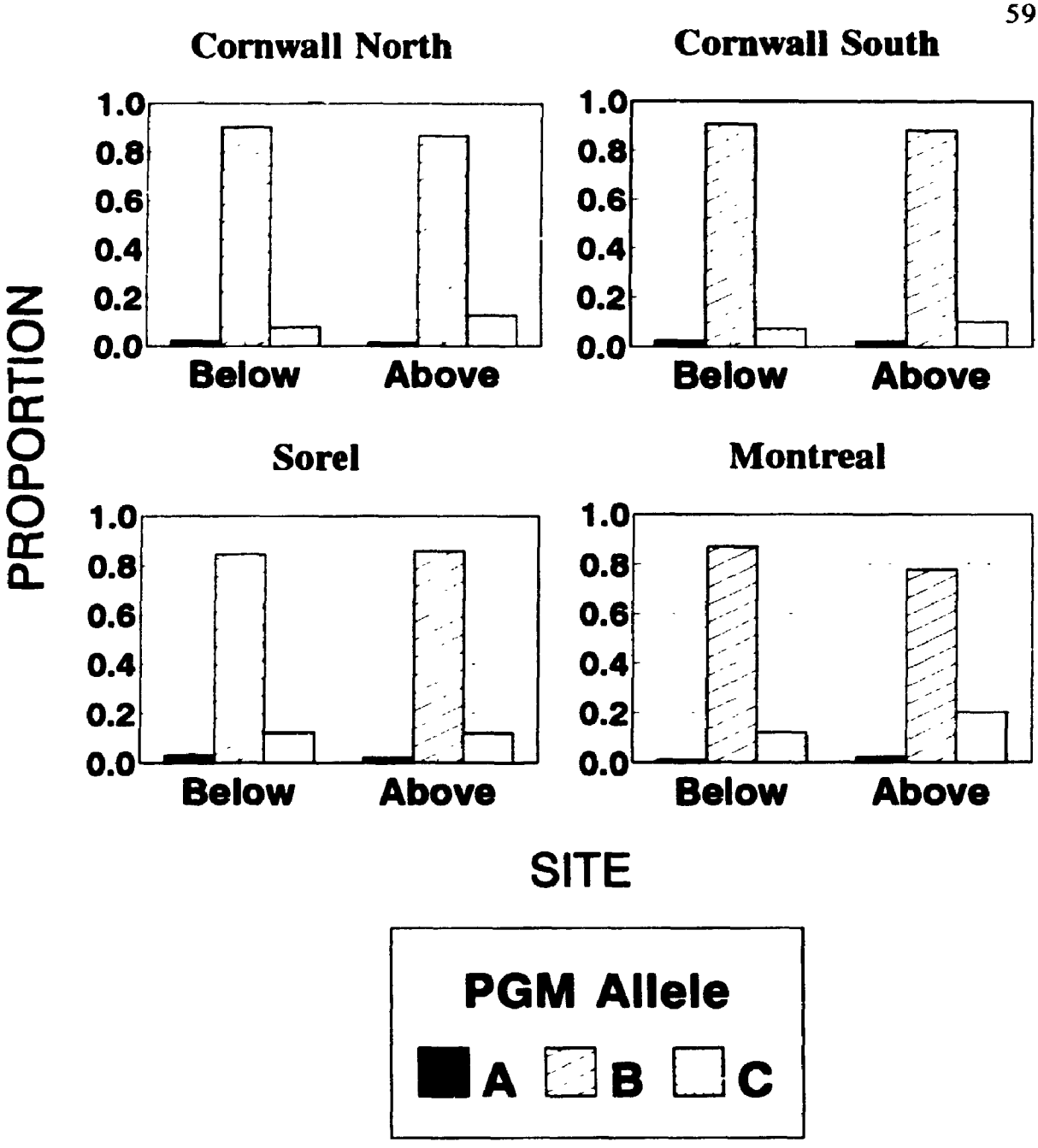


Fig. 4.4. Allelic frequencies of PGM allozymes in *Lampsilis radiata* sampled above and below industrial discharges in four areas of the St. Lawrence River.

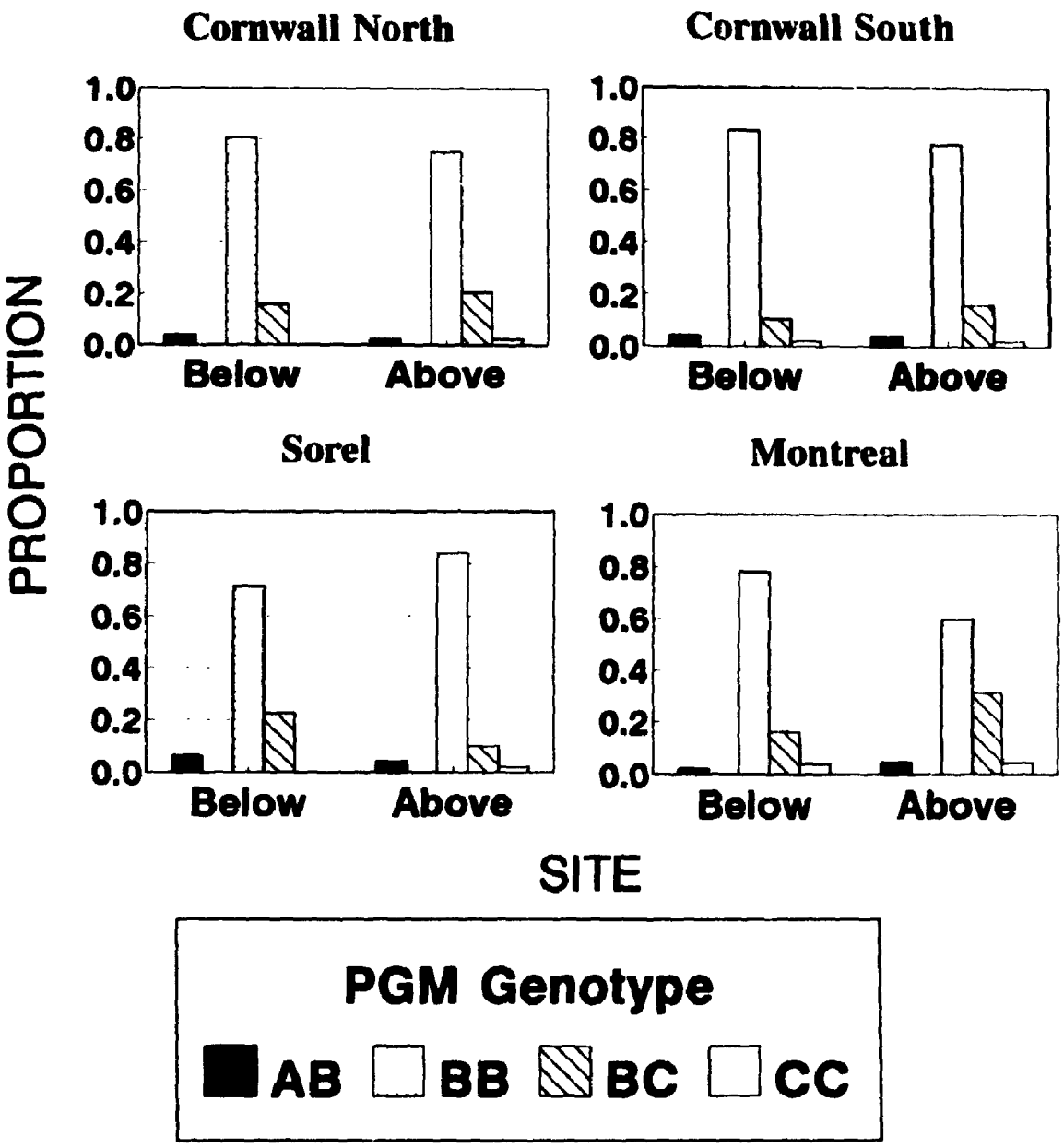


Fig. 4.5. Genotype frequencies of PGM allozymes in *Lampsilis radiata* sampled above and below industrial discharges in four areas of the St. Lawrence River.

Table 4.5 Summary of F-statistics for groups of *L. radiata* from the St. Lawrence River, 1989.

LOCUS	F_{IS}	F_{IT}	F_{ST}
GPI	.051	.054	.003
PGM	.043	.056	.014
Mean	.047	.055	.008

4.4. Discussion

Populations of the unionid *L. radiata* situated in the St. Lawrence River study areas were expected to experience a microevolutionary scenario involving several features. Panmixis among clam beds is likely, due to the obligate parasitization by their larvae of a variety fish species (Kat 1984) able to range throughout the river. Following settlement from the host, the generally sedentary lifestyle of juveniles and adults would result in little migration among study clam beds (which are separated by at least 2 km). In the sites downstream of industrial outfalls, survival of *Gpi* and *Pgm* genotypes resistant to contaminant toxicity should be favored over genotypes that suffer impairment. Selection for specific genotypes due to pollution has been cited to explain heterogeneous allozyme frequencies across contaminant gradients in several field studies, involving (a) PGM in the shrimp *Palaemon elegans* and GPI in the gastropod *Monodonta turbinata* and mercury pollution in the Mediterranean Sea (Nevo et al. 1984), (b) PGM and MDH in the fish *Campostoma anomalum* and discharges of a uranium processing plant to a river (Gillespie and Guttman 1989), (c) GPI in the fish *Gambusia holbrooki* and mercury pollution in a river (Heagler et al. 1993), and (d) GPI and PGM in the barnacle *Balanus amphitrite* in a chemically polluted channel of the Mediterranean Sea (Montero et al. 1994).

The observed genetic structure based on GPI and PGM allozyme freqencies of the 8 clam groups provided no evidence for any contaminant-mediated selection in habitats downstream of the industrial outfalls. The allozyme frequencies, which were nearly identical among groups and all in agreement with Hardy-Weinberg equilibrium values, are consistent with a single panmictic population and a uniformly weak selection regime across the study sites. Homogeneity of allozyme frequencies among age groups suggests little temporal variability in selection pressure. Bailey (1987) also found little divergence in GPI and PGM allozyme frequencies among *L. radiata* in the lower Great Lakes.

This apparent "lack of response" by GPI and PGM, two of the most often cited enzyme systems affected by contaminants (Hummel and Paternello 1994), in clam beds downstream of some of the largest point source dischargers of metal-containing pollution in Quebec and

Ontario (Environnement Canada 1985; SLRRAPT 1992) is surprising. Three conditions that could account for these results are (a) inadequate power in the sampling design to detect differences in allozyme frequencies, (b) exposure of clams to contaminants at levels below toxicity, and (c) lack of differential resistance of allozymes to toxicity. These will be evaluated below.

The difference in allele frequencies between groups that can be determined as significant using a chi-square test depends on the sample size (n), frequency of the allele (p), Type I error rate (α), and Type II error rate (β) (Richardson et al. 1986). For both GPI and PGM, the frequencies for the A, B and C alleles were approximately 0.8, <0.2 and <0.05, respectively, and sample sizes ranged from 44 to 51. With $\alpha = 0.05$ and $\beta = 0.2$, the detectable significant difference should be about 0.2 (Richardson et al. 1986). In other words, allele frequencies in paired upstream and downstream sites that differed by ≥ 0.2, should have been determined as significant 80% of the time. The fact that significant differences were found in none of the four site pairs tested further reduces the likelihood of having accepted a null hypothesis that is not true.

In contrast to other studies comparing allozyme frequencies in metal-contaminanted and reference sites, the differences in genotype frequencies found in the present study are low. The greatest difference in genotype frequencies between upstream and downstream paired clam groups observed was 0.065 for GPI, and 0.18 for PGM. With the exception of Heagler et al. (1993), who found *Gpi-2*^{38} in mosquito fish from a mercury polluted canal at a frequency 0.028 lower than that in an unpolluted river, differences in genotype frequencies reported as significant range from 0.1 (*Pgm*^{MS} in shrimp and *Pgi*^{MM} in gastropods involving marine mercury pollution; Nevo et al. 1984), to 0.37 (*Pgm*^{bb} in central stonerollers from a uranium and technetium polluted stream; Gillespie and Guttman 1989).

The degree to which aquatic organisms are exposed to environmental contaminants depends on a variety of geochemical and biological factors and processes whose actions and interactions are not well understood (Luoma and Carter 1991). Although contaminant availability to *L. radiata* was not directly addressed in this study, some generalizations can be made. Elevated concentrations of metals in sites downstream of outfalls relative to those

upstream indicate past deposition of contaminant-bearing particles (Sec. 1.4). An important fraction of contaminants in freshwaters are associated with particulate matter (Batley 1983, McIntosh 1991; Warren and Zimmerman 1993), and thus potentially available to filter feeding bivalves. Ingestion is an important route by which metals are taken up in bivalves (Luoma 1983; Simkiss and Mason 1983). Therefore, it is difficult to suggest that the clam beds downstream of outfalls could not have been exposed to contaminants.

Laboratory experiments on a variety of marine and freshwater invertebrates and fishes have demonstrated differential tolerance (measured by survivorship, time-to-death) among allozymes of *Gpi* and *Pgm* to elevated metal treatments (Mulvey and Diamond 1991; Hummel and Paternello 1994). Several studies have also determined that some genotypes showing resistance in laboratory experiments are also found at disproportionately high frequencies in natural populations exposed to metal pollution (Nevo et al. 1984; Gillespie and Guttman 1989; Heagler et al. 1993; Montero et al. 1994). These studies provide strong evidence that *Gpi* and *Pgm* (or ≥. least other loci to which they are linked) are subject to selection by environmental contaminants. However, in comparing results among studies, consistent trends are not apparent (Hummel and Paternello 1994). The allozymes observed in *L. radiata* may differ structurally from those observed in other studies, and may exhibit differences in fitness. It also is possible that observed genotypes are equally resistant to St. Lawrence River discharges.

In conclusion, the homogeneity of allozyme distributions among sites results suggests that selection by the contaminant field (or by any factor) is insufficient to counter gene flow among clam beds through the settlement of juveniles. Based on the GPI and PGM enzyme systems, industrial effluents do not affect the genetic structure of populations of *L. radiata* in the upper St. Lawrence River.

5. INTERRELATIONSHIPS AMONG SHELL GROWTH PATTERNS, ALLOZYME GENOTYPES, SEDIMENT CONTAMINATION AND SOFT TISSUE CONTAMINANT BURDENS

5.1. Introduction

Beds of *Lampsilis radiata* are found in all of the St. Lawrence River study sites described in Sections 1 - 4 (although substantially reduced in site 6), including those downstream of discharges heavily contaminated with metals. The general physiological "health" of clams sampled from below discharges, as assessed by analyses of shell growth patterns, does not appear severely impaired. Statistically significant but minor depressions in growth were detected in some of these groups relative to clams sampled from sites immediately above discharges (Sec. 2). However, mature clams transplanted from locations upstream of outfalls into more polluted sites downstream show no growth depression, and clams transferred from polluted to less polluted sites showed no growth acceleration (Sec. 3). These results challenge the premise that these clams take up metals and, that once taken up, the metals cause deleterious effects.

Sediments in the sites below outfalls are contaminated by a variety of metals (except site 4), in some cases heavily (Sec. 1.4.5.). A large fraction of most metals entering freshwater partitions into particulate phases and, eventually, into sediments (Yeats and Bewer 1982; Batley 1983; Moore and Ramamoorthy 1984; McIntosh 1991; Warren and Zimmerman 1993). Sedentary, long-lived, filter-feeders such as unionids are thus likely to be exposed to discharged metals. Assimilation of metals, however, is difficult to assess. Trace metal bioavailability depends on numerous factors related to both the physico-chemical environment and the biological system involved (Luoma 1983; Tessier et al. 1984; Campbell and Tessier 1989). Quantifying the relationship between uptake and environmental contaminant levels has proven difficult (Luoma 1989).

To obtain an initial evaluation of metal bioavailability in *L. radiata* in several of the study sites, two questions were addressed: (a) Are soft tissue metal concentrations influenced by

sediment metal concentrations?, and (b) Do sediment and/or tissue metal levels account for variation in shell growth patterns among sites?

Among - clam variation in shell growth pattern was also examined for association with specific GPI and PGM genotypes. Growth rates in bivalves are known to be partially controlled by genetic factors, likely involving many loci (Berger 1983) However, if specific genotypes confer physiological advantages in metal - contaminated environments, as has been suggested for GPI and PGM in other studies (Hummel and Patarnello 1994), dependent processes such as growth patterns should differ among genotypes.

5.2. Methods

5.2.1. Clam metal burdens and sediment metal contamination In June 1989, sediment was sampled from each of sites 1 - 5 and 7 - 11 (Fig. 1.2) and analyzed for extractable Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn; total As, Hg, and Se; and organic content (Sec. A.2). At the same time, 9 - 10 *L. radiata* between 60 and 100 mm in length were collected by divers or oyster tongs from each site. Clams were rinsed with river water, wiped dry, wrapped in aluminum foil and plastic bags and frozen on dry ice without allowing depuration. (Because clams remained closed, foil and soft tissue were not in contact.) After return from the field, clams were thawed and shucked. For each site, the tissues of 5 specimens were pooled into a composite sample for analysis; the remaining 4 or 5 were analyzed individually. Composite samples were homogenized in a stainless steel blender, and subsampled. Individual and composited tissues were then freeze dried, and ground to a homogeneous powder with stainless steel blades. Sample digestion and analyses for the above metals (except Pb, for which there were interference problems) were performed by the National Laboratory for Environmental Testing (NLET, Environment Canada, Burlington, Ontario) using standard methods, including quality assurance and quality control procedures (NLET 1994). All elements except As, Se and Hg were analyzed by atomic absorption spectroscopy (AAS). Inductively coupled plasma - atomic emission spectroscopy (ICP-AES) was used for As and Se. Mercury was analyzed by flameless AAS.

Sediment and clam tissue metal concentrations were statistically analyzed by both univariate and multivariate methods. Pearson correlations were calculated between sediment and composited tissue metal concentration for each metal (excluding Co, which was largely below detection in tissues, and the toxicologically less important Al, Fe and Mn). Tessier and Campbell (1987) noted that the prediction of metal levels in benthic organisms is often improved when extracted metal concentrations in sediment are normalized for iron and organic content of sediments. Therefore, sediment metal concentrations were each regressed against Fe level and organic content (as percent loss on ignition). Correlations between the residuals from these regressions and clam metal levels were then calculated.

Metal burdens in clams and concentrations in sediments were also compared by collapsing the number of variables in each data set using principal components analysis (PCA), and plotting the main components against each other. The metals-in-sediment PCA is described in Sec. A.2. For the metals-in-clams PCA, the individual-clam data for 11 metals (Co was mostly below detection and therefore omitted) from sites 2, 3, 8, 9, 10 and 11 were used. A covariance based PCA was performed on ln - transformed concentrations. The first two components (PCs) from this analysis were then plotted against PC1 from the sediment PCA.

5.2.2. Shell growth and metal levels in clams and sediment Concurrent with the collections of sediments and clams for metals analyses, an additional 52 - 56 clams were sampled from each of sites 2 - 5 and 8 - 11. Shell growth patterns were described by fitting von Bertalanffy growth models to annual external ring lengths using the Walford Plot method (Sec. 2). Means of estimated growth parameters for clams from each site were then compared to clam metal burdens and sediment metal levels. Correlations were calculated between site means of Walford Plot regression intercept a and slope b (Table 2.1), with (i) composited metal burdens from clams (used as estimates of site mean burdens) and (ii) metal concentrations in sediments (from Sec. 5.2.1.). To detect multivariate relations, mean growth parameters were plotted against PC1 and PC2 from the metals-in-clams PCA, and PC1 from the metals-in-sediments PCA.

5.2.3. Shell growth and allozyme genotypes Variation in shell growth among previously collected individuals from metal - contaminated sites was examined to test for relationships to individual GPI and PGM genotypes. Between 42 and 50 clams from each of sites 2, 8 and 10 were analyzed for both shell growth (in Sec. 2) and allozyme genotype for GPI and PGM (in Sec. 4). A multivariate analysis of variance (MANOVA) was performed with Walford Plot growth parameters a and b as response variables, and site (sites 2, 8, 10), GPI genotype (AB, AC, BB, BC, CC) and PGM genotype (AB, BB, BC, CC) as predictors Responses were weighted by the degrees of freedom of the regressions from which a and b were estimated.

5.3. Results

5.3.1. Metal burdens in clams and metal contamination in sediment Based on single metal correlations between clam tissue concentrations and sediment concentrations (Table 5.1), few of the metals appear bioaccumulated (ie. taken up in proportion to environmental concentrations) in spite of the fact that all correlations were positive. Highest correlations between tissue and unadjusted sediment metal levels were for Ni and Cr (P <0.01), followed by Zn and Fe (P <0.05). Concentrations in clams and sediments from site 8 (the most heavily polluted), strongly influenced the values obtained. Normalizing sediment metal levels for Fe and organic content improved correlations for five of the nine metals, although for Se the sign of the correlation was reversed and the significance levels for Ni and Cr decreased

As estimates of mean metal burdens for each group of clams, the composite samples appear reliable. Agreement between composite samples and mean values from individually analysed clams from each site was good for Al, Cd, Cr, Fe, Mn, Ni and Zn (correlations between the estimates ranged from 0.806 to 0.999). Similarly, the trend among sites in sediment metal levels for 1989 matched that for 1990 samples (App. A).

Results from the PCA on tissue metal concentrations (Fig 5.1) showed that clams from site 8 are distinct from all others along the first axis, mainly due to comparatively high levels of Cr and Fe, and low levels of Cd. Scores from the second axis further separate the sites,

Table 5.1 Correlations between concentrations of metals in composited soft tissues of *L. radiata* and concentrations of metals in sediments (unadjusted and adjusted for Fe levels and organic content) N = 10 sites.

Metal	Correlation[a]	
	Sediment [M] unadjusted	Sediment [M] adjusted
As	0.442	0.662
Cd	0.254	0.313
Cr	0.809	0.741
Cu	0.518	0.273
Hg	0.345	0.561
Ni	0.872	0.753
Se	-0.161	0.197
Zn	0.687	0.708

[a] Critical values for $r = 0.602$ (α=0.05) and 0.765 (α=0.01)

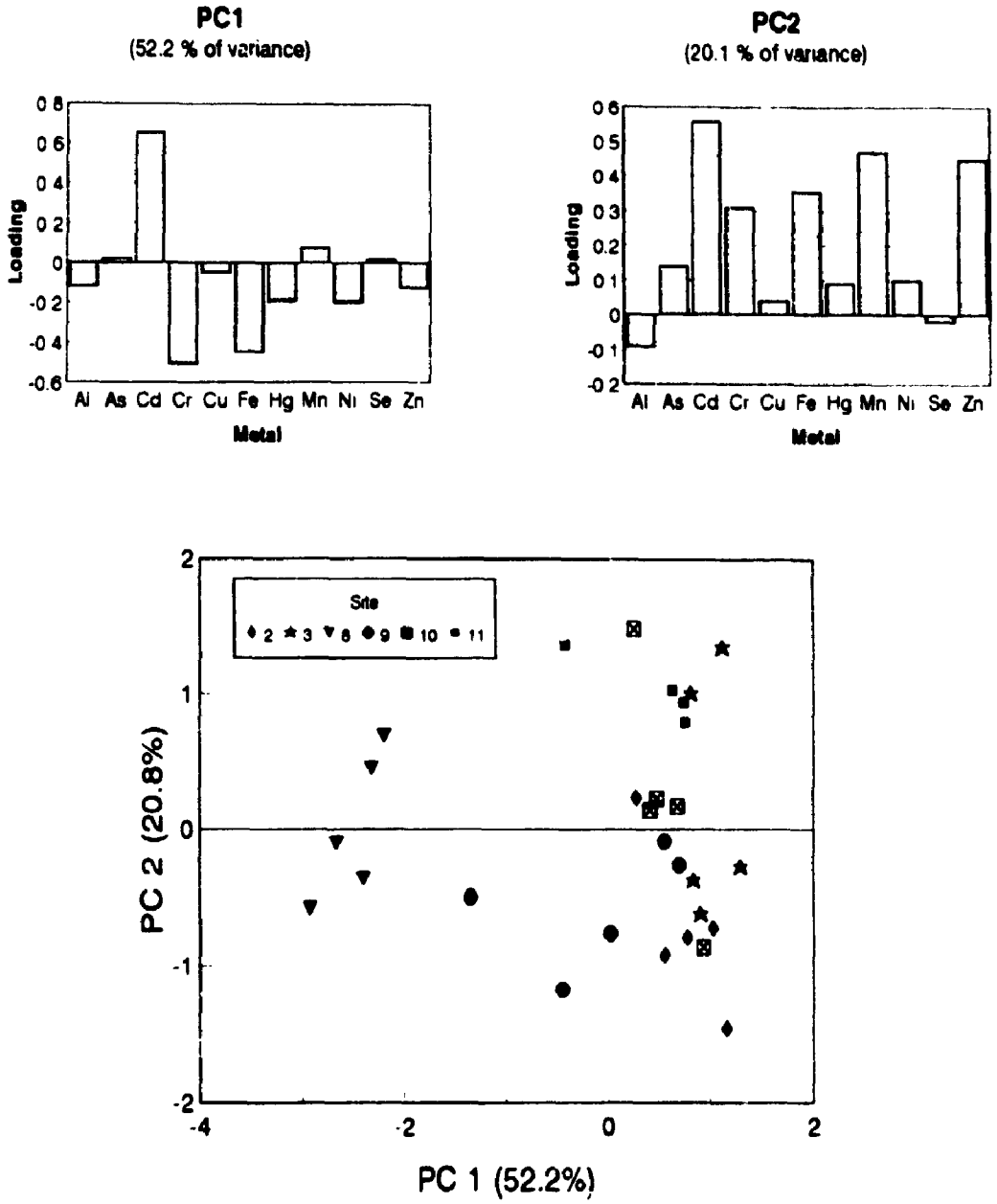


Fig. 5.1. Component loadings and plot of scores for PC1 and PC2 from ordination of soft tissue metal concentrations in individual clams from six sites in the St. Lawrence River.

but indicate that clams from downstream of industrial discharges (sites 2 and 10) tend to have lower metal burdens than clams from their corresponding upstream sites (sites 3 and 11). A lack of a clear positive relationship between clam burdens and sediment contamination is also evident in Fig. 5.2, in which PC1 scores from the metals-in-clams PCA are plotted against PC1 scores from the sediment ordination. Metal levels in clam tissue are elevated only in those from the most polluted site. No trend is evident in the relationship between metals-in-clam PC2 and metals-in-sediment PC1.

5.3.2. Shell growth and metal levels in clams and sediment Shell growth patterns, as measured by intercept and slope from Walford Plot (WP) regressions of annual shell lengths, bear virtually no relationships to single metal levels in either clam soft tissues or sediments (Table 5.2). Correlations between sediment Cd concentration and intercept ($P < 0.01$) and slope ($P < 0.01$), and clam Mn level and intercept ($P < 0.01$), were the only significant relationships out of 50 calculations. It is not unlikely that these correlations occurred by chance. Multivariate comparisons suggest a similar conclusion. No trends are evident in plots of intercept and slopes against PC1 and PC2 for metals-in-clams (Fig. 5.3), nor are there in plots of intercept and slope against the metals-in-sediments PC1 (Fig. 5.4).

5.3.3. Shell growth and allozyme genotypes After accounting for effects of site, variation in shell growth pattern among individual clams from sites contaminated by metals was not related to differences in allozyme genotypes (P ranged from 0.493 to 0.984 for effects of GPI and PGM in the MANOVA; Table 5.3). Fitted shell growth parameters from the MANOVA show no meaningful differences among any allozyme genotypes (Fig. 5.5).

5.4. Discussion

Levels of trace metal contaminants in soft tissues of *L. radiata* were not proportional to metal levels in sediments. Only in the most polluted site was there evidence for increased tissue burdens. These results suggest that in most of the study sites metals are either not bioavailable, or if they are taken up, they are regulated. Although measurements of metal

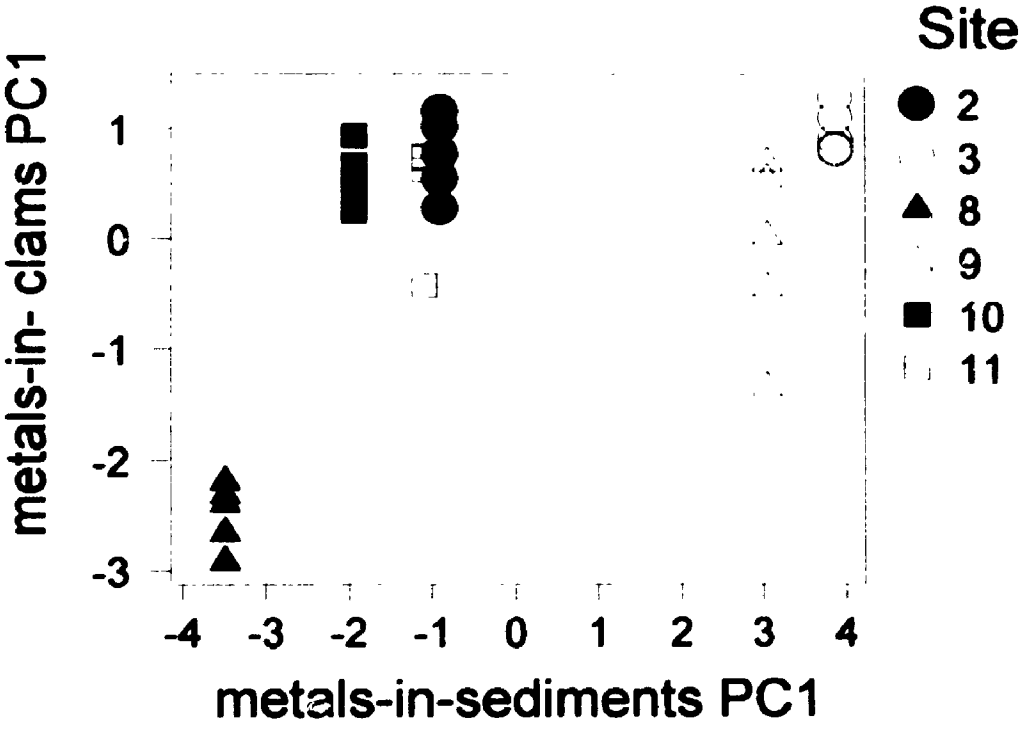


Fig. 5.2. Relationship between metal burdens in clams and metal levels in sediments. Scores are from separate PCAs on metal concentrations in clams and sediments. Solid symbols denote sites downstream of outfalls.

Table 5.2. Correlations between metal concentrations (in composited soft tissues of *L. radiata* and in sediments) and shell growth patterns (mean Walford Plot regression intercept and slope). N = 8 sites.

	Correlation[a]	
Metal	WP Intercept	WP Slope
Clams		
Al	-0.385	0.545
As	-0.427	0.133
Cd	-0.229	-0.061
Cr	-0.089	0.092
Cu	-0.442	0.339
Fe	-0.126	0.095
Hg	-0.616	0.459
Mn	-0.804	0.533
Ni	0.230	-0.260
Se	0.442	-0.460
Zn	-0.542	0.344
Sediment		
Al	-0.537	0.420
As	-0.059	-0.148
Cd	-0.911	0.761
Co	-0.152	0.060
Cr	0.063	-0.074
Cu	-0.035	-0.037
Fe	-0.002	-0.114
Hg	-0.250	0.152
Mn	-0.199	-0.011
Ni	0.007	-0.051
Pb	-0.192	0.122
Se	-0.281	0.245
Zn	-0.292	0.193

[a] Critical values for $r = 0.666$ (α=0.05) and 0.798 (α=0.01)

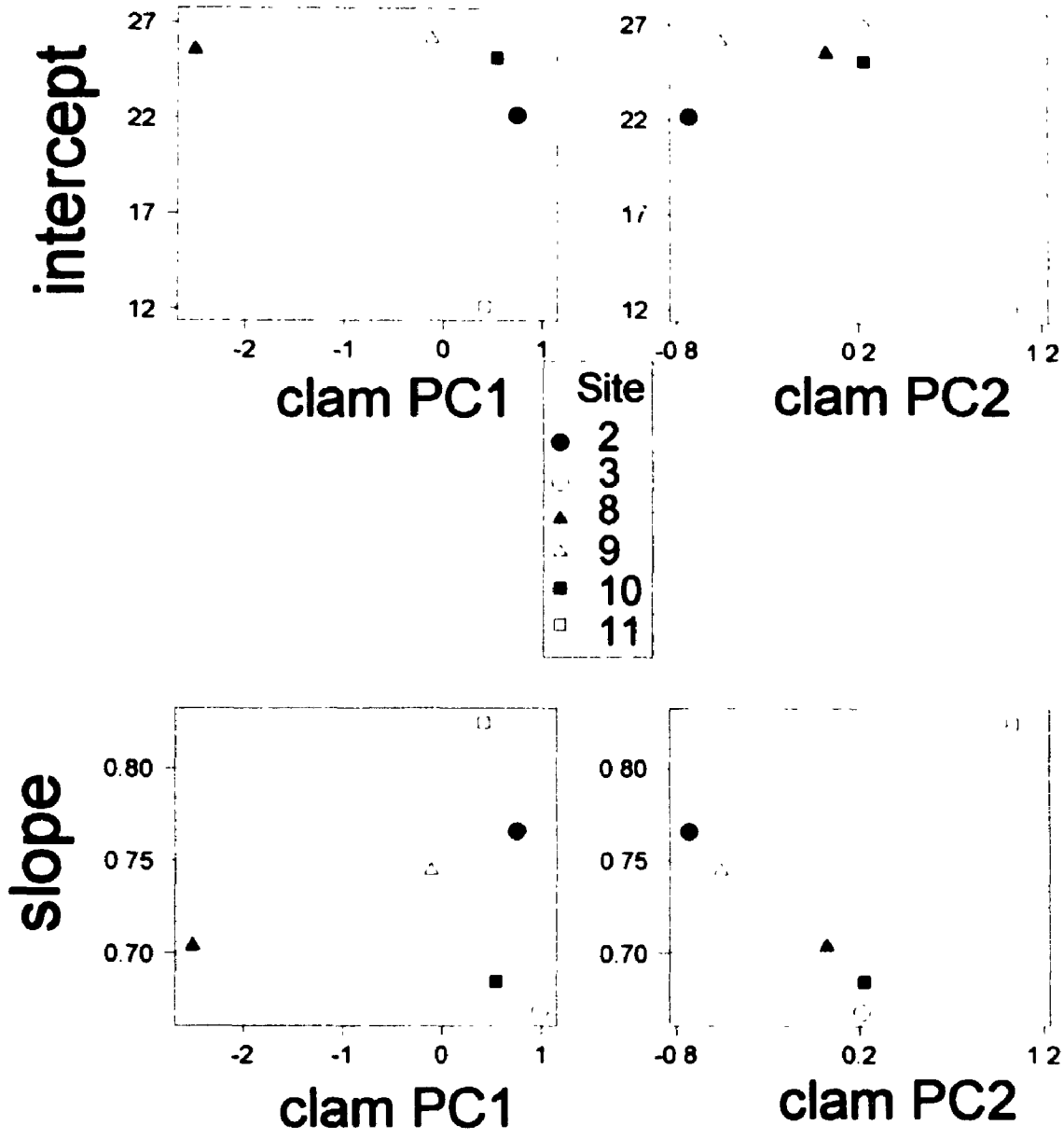


Fig. 5.3. Relationships between shell growth pattern and clam metal burdens. Mean estimates of Walford Plot regression intercept and slope for 6 sites are plotted against mean scores of main components from the metals-in-clams PCA.

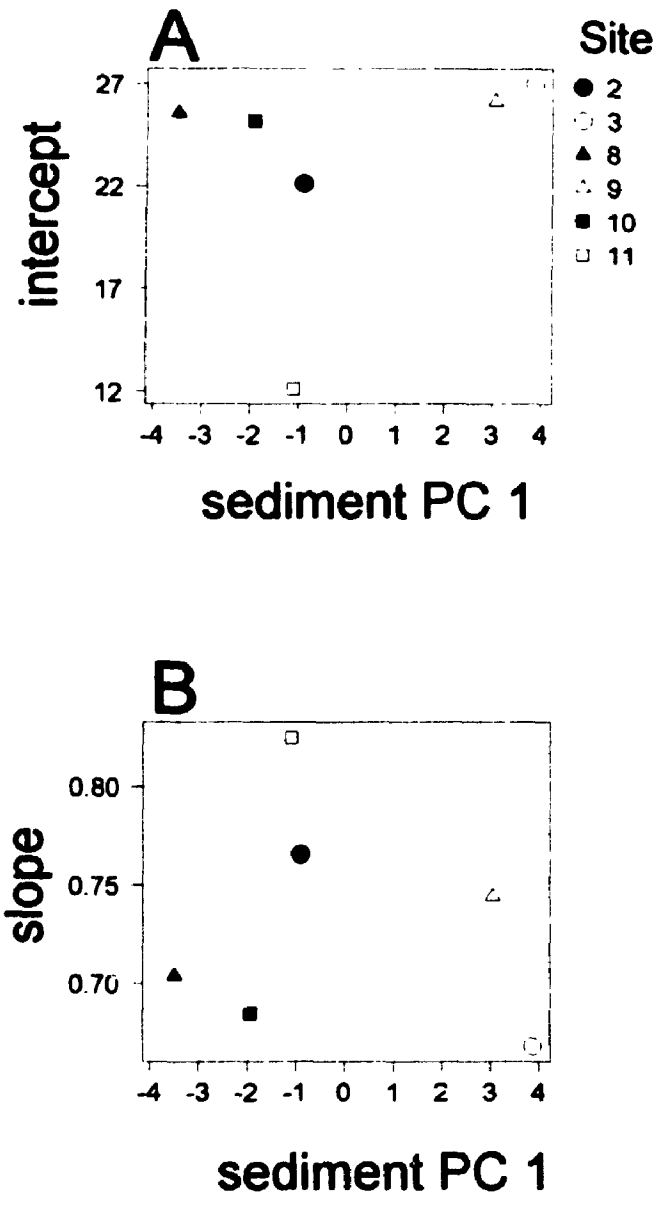


Fig. 5.4. Relationships between shell growth pattern and metal levels in sediments. Mean estimates of Walford Plot regressions are plotted against first component scores from the metals-in-sediments PCA.

Table 5.3. Effects of GPI and PGM genotypes on shell growth patterns of *L. radiata* from metal - contaminated sites 2, 8 and 11.

ANOVA

Source	df	SS	MS	*F*	*P*
Dependent variable: Walford Plot regression intercept (*a*)					
Site	2	968.2	484.1	4.47	0.013
GPI	4	342.9	85.7	0.14	0.532
PGM	3	46.5	15.5	0.14	0.934
Error	129	13961.9	108.2		
Dependent variable: Walford Plot regression slope (*b*)					
Site	2	0.3618	0.1809	6.69	0.002
GPI	7	0.0925	0.0231	0.85	0.493
PGM	3	0.0044	0.0015	0.05	0.984
Error	129	3.4898	0.0271		

MANOVA for GPI

Criterion	Test Statistic	*F*	df (num., den.)	*P*
Wilk's Lambda	0.96843	0.517	4, 256	0.843
Lawley-Hotelling Trace	0.03245	0.515	4, 254	0.845
Pillai's Trace	0.03172	0.520	4, 258	0.841

MANOVA for PGM

Criterion	Test Statistic	*F*	df (num., den.)	*P*
Wilk's Lambda	0.97616	0.518	4, 256	0.795
Lawley-Hotelling Trace	0.02440	0.516	4, 254	0.796
Pillai's Trace	0.02386	0.519	4, 258	0.794

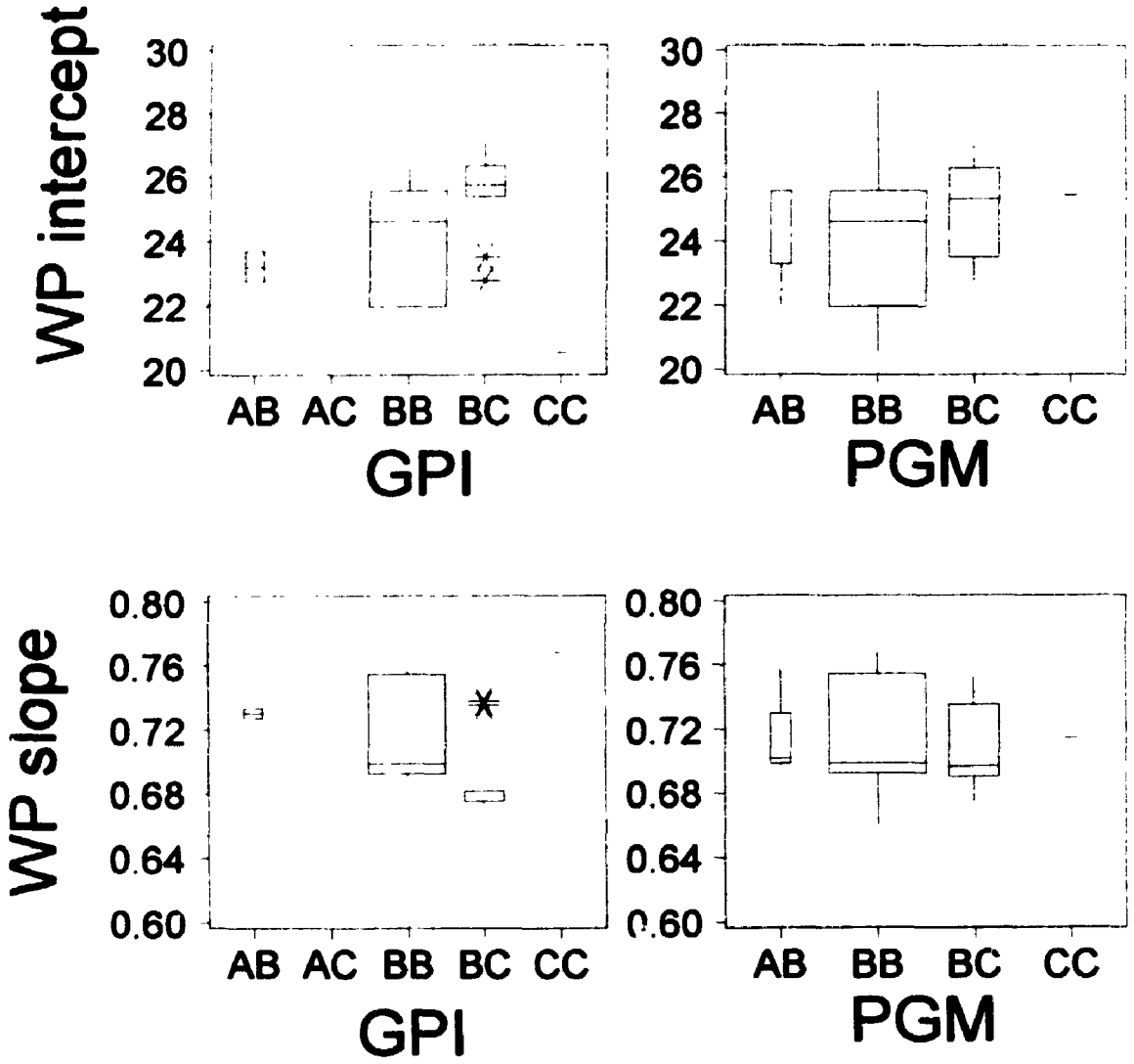


Fig. 5 5. Influence of allozyme genotypes on shell growth patterns (intercept and slope for Walford Plot regressions). Data are fitted values from MANOVA and are adjusted for the effect of site. Boxes show medians and inter-quartile ranges, whiskers extend to adjacent values, and asterisks indicate outliers. Boxes are proportional to sample size.

uptake rates were not made, if clams do regulate internal metal levels, it should be at an energetic cost and leave less energy available for other processes such as growth (Bayne 1989). Shell growth rates though, were poorly related to metal levels in the sediments.

Growth patterns were also not related to tissue metal concentrations when compared across six sites. However, if site 11 is neglected, which may be warrented because the unusually slower shell growth shown by its clams appears due to alkalinity and dissolved calcium levels (Sec. 2), an inversely proportional relationship with WP regression slope and PC2 from the clam burden PCA is apparent (Fig. 5.3). The slope parameter is the fraction of growth to maximum length remaining after the first year. For clams that grow the same amount in the first year (i.e., WP intercepts equal), a lower slope results in a smaller maximum size. Thus for sites 3, 8, 9 and 10, higher levels of metals in tissues (i.e., greater PC2 scores) correspond with smaller maximum lengths. This interpretation is tenuous because of the small number of sites and because both slope values and PC2 scores are site means, but suggests an area of further research.

In several studies involving marine and freshwater invertebrates and fishes, individuals with specific genotypes of GPI and PGM exhibited superior fitness in polluted environments based on their higher frequencies of occurence in contaminated versus uncontaminated sites (Nevo et al. 1984; Gillespie and Guttman 1989; Benton and Guttman 1990; Patarnello et al. 1991; Heagler et al. 1993; Nevo 1993; Montero et al. 1994). In addition, differential survival and physiological impairment in laboratory metal toxicity experiments were observed among GPI and PGM genotypes (Nevo et al. 1984; Chagnon and Guttman 1989; Gillespie and Guttman 1989; Diamond et al. 1989; Benton and Guttman 1990, 1992; Kramer et al. 1992; Heagler et al. 1993; Montero et al. 1994). No such phenomena are evident in *L. radiata* from the metal - contaminated study sites. The lack of differences in shell growth rates among individuals of different GPI and PGM genotypes is evidence against these allozymes being important in mediating affects of metal contaminants on *L. radiata*.

6. TOXICITY OF ZINC TO GLOCHIDIA LARVAE AND THE INFLUENCE OF MATERNAL HABITAT AND ALLOZYME GENOTYPE

6.1. Introduction

The presence of clam beds in heavily contaminated sites in the St. Lawrence River suggests the possible development of trace metal tolerance (i.e., the ability to live indefinitely in conditions eventually lethal to nontolerant individuals). Responses to environmental stress can be genetically based (Koehn and Bayne 1989; Kramer et al. 1992; Soares et al. 1992), leading to the evolution of tolerant populations. In studies comparing the toxicological responses of organisms from different field populations, often (but not always) those from metal - contaminated sites show greater tolerance of the contaminants in laboratory tests than those from unpolluted sites (Mulvey and Diamond 1991).

Evidence for metal - mediated selection on populations has been obtained from examinations of allozyme genotype variation among organisms in polluted and unpolluted sites (Sec. 4). Two allozyme systems in particular, glucose-phosphate isomerase (GPI) and phosphoglucomutase (PGM), have exhibited distributions of alleles and genotypes related to the degree of field contamination (Mulvey and Diamond 1991; Hummel and Patarnello 1994). In some cases individuals with the allozyme predominant in the polluted sites were more tolerant of metals in laboratory exposures than individuals of different allozymes. Specific variants of GPI or PGI, or some other allozyme whose locus is linked to that of GPI or PGM, confered differential fitness under stressful metal conditions.

The purpose of this experiment was to determine if *L. radiata* differs in its toxicological response to an important metal contaminant, zinc, based on (a) the source of the clams (a polluted and an unpolluted site), and (b) their GPI and PGM genotypes. The collection sites are upstream and downstream of a major discharger of zinc - contaminated effluents in the St. Lawrence River. If metals such as zinc are important selective forces in natural populations, clams from downstream of this discharge should be more resistant (i.e., able to survive longer) than upstream clams to zinc treatments in toxicity tests. Differential resistance

may be shown among different allozymes of GPI or PGM if these enzymes are prone to selection as has been demonstrated in other taxa and habitats

Laboratory assessments of the toxicity of contaminants using adult clams are difficult and of questionable relevance to field environments. Adult clams can shut their valves for several days, and so avoid exposure to short-term doses of contaminants (Doherty et al. 1987). This phenomenon, coupled with a relatively slow growth rate makes adults generally less sensitive to contaminants than immature clams (Elder and Collins 1991). Immature stages of bivalves are therefore frequently used to study effects of contaminants (Harrison et al. 1984; McKim (1984); Cherr et al. 1990; Keller and Zam 1991; Jacobson et al. 1993; Warren et al 1995). Recently, Huebner and Pynnönen (1992) exposed the glochidia larvae of two species of *Anodonta* to low pH and several metals in acute toxicity tests. Glochidia larvae were therefore used here to measure the response of *L. radiata* to dissolved zinc. As in other unionids, these larvae are obligate parasites on fish. After developing from fertilized eggs in the marsupium (modified parts of the gills of females), glochidia are expelled into the water column and sediments to be taken up by their host (Sec. 1.5). If taken up within several days, the glochidia can encyst into the host's tissue, develop to the juvenile stage, and drop back to the sediment. Thus, in the period between leaving the mother and encysting in the host, glochidia are exposed to potential contaminants (Silverman et al. 1987; Huebner and Pynnönen 1992). This period - lasting several days at most (Mackie 1984) - and possibly during the juvenile stage, are the times when effects due to contaminant stress should be greatest.

6.2. Methods

6.2.1. Collection and maintenance of clams On 13 September 1993, >50 *L. radiata* were collected from each of study sites 13 and 15 in the north channel of the St. Lawrence River at Cornwall (Fig. 1.2). Site 15 is several hundred metres downstream of the discharge from an organic chemical manufacturer that was highly enriched with zinc, mercury and lead (OME 1992). Sediment samples from site 15 contained 143.3, 0.98 and 30.8 mg/kg dry weight of

total Zn, Hg and Pb, respectively in 1991 (Richman 1994). For Zn and Hg, these concentrations were several times higher in sediments from a site within 100 m of the discharge. Site 13 is several kilometres upstream of this discharge. For comparison, sediments sampled from there in 1990 for the present study had concentrations of total Zn, Hg and Pb equal to 49.4, 0.03 and 3.54 mg/kg dry weight, respectively.

Following collection and return from the field, clams were placed into two aerated aquaria (74 and 88 L) containing tap water (hardness ≈ 100 mg $CaCO_3$/L, conductivity = 280 µS) and fine gravel (5 - 10 cm depth). Within a few days, gravid female clams began to display their modified mantle flaps in the manner characteristic of the Lampsilini, which serves to attract potential fish hosts (Sec. 1.5). No non-experimental mortality occurred.

6.2.2. Experimental design The main purpose of the experiment was to assess whether glochidia differ in their toxicological response to dissolved zinc based on (a) the mother clam from which they came, (b) the site in which their mother was collected, and (c) the allozyme genotype of their mother. This was achieved by measuring viability (see below) of 3 groups of glochidia from each of 30 clams (15 per site) at 4 nominal concentrations of zinc (0, 1, 5, 25 mg/L) after 24 and 48 hr. The overall design is a nested factorial experiment (Fig. 6.1). Glochidia groups, which were tested in individual vials, are nested within clam, which in turn is nested within site. Because of limitations in setting up treatments, collecting glochidia, and measuring viability, the experiment was conducted in 6 trials, which complicated the data analyses (described below). Genetic influences on resistance to zinc were examined by comparing viability of glochidia in treatments to genotypes of GPI and PGM obtained from electrophoretic analyses of foot tissue from the mother clams.

6.2.3. Exposure of glochidia to treatments Glochidia were exposed to 20 mL of static test solutions in 23 mL - glass vials at room temperature. Treatments were prepared using reagent grade $ZnCl_2$ (in a stock solution of distilled, deionized water acidified to pH 2) and water filtered (1.2 µm pore) and mixed from both aquaria that contained clams. For each trial, three serial dilutions of 25, 5 and 1 mg/L zinc solutions were made up in nine 400 mL -

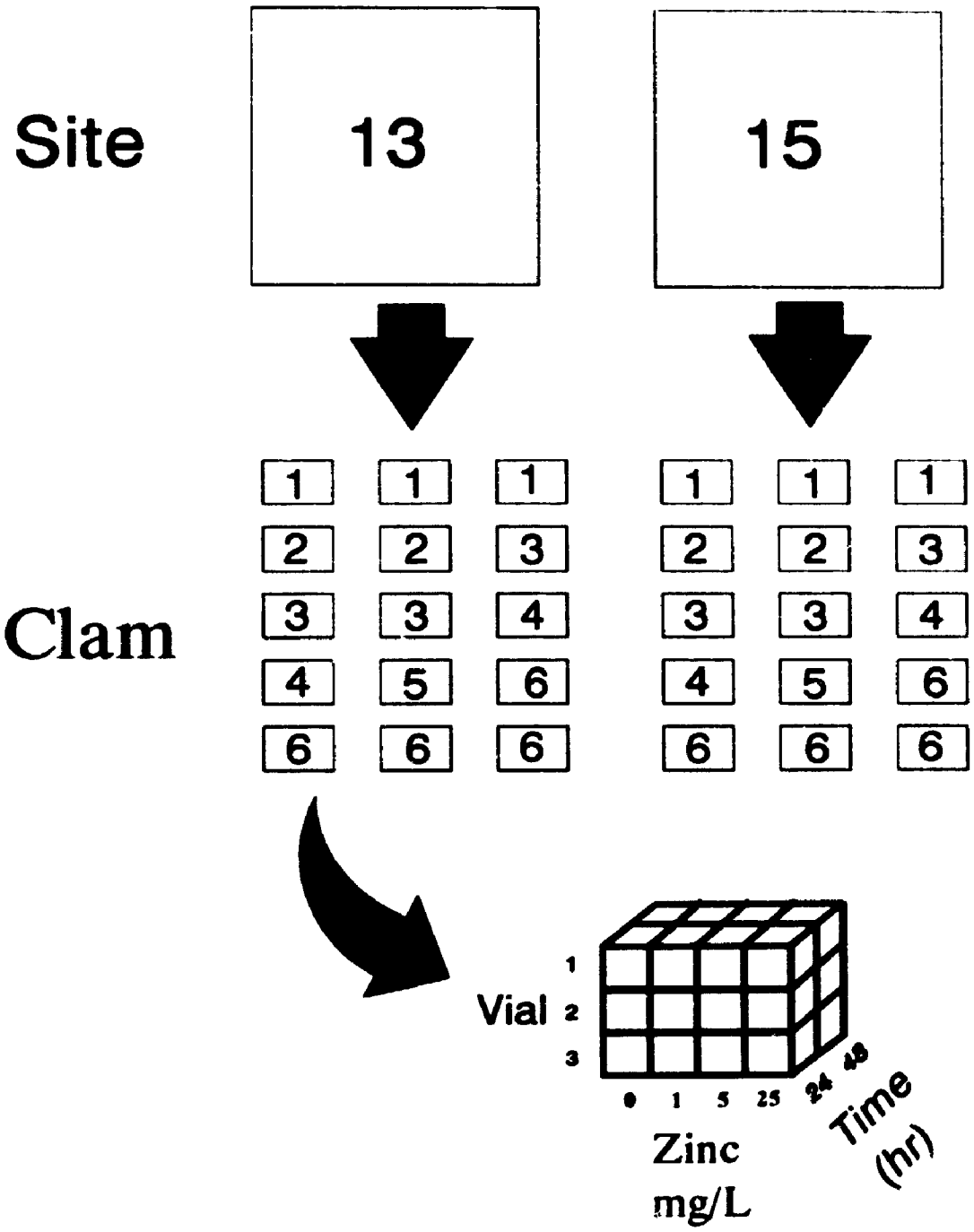


Fig. 6.1. Overall experimental design of the zinc toxicity experiment. Clams used in common trials (1 - 6) are indicated with the same number.

beakers, which together with four control solutions were then added to vials systematically arrayed such that glochidia from each of the clams tested were exposed to each of the twelve test solutions. Conductivity, monitored periodically, ranged from 280 - 410 µS Minimum pH, in the highest Zn treatment, was > 7.0.

After toxicologic measurements in each trial, water was sampled from twelve vials, one for each prepared test solution, and preserved with nitric acid for analysis of Zn concentration. Samples were analysed by flame-absorbance atomic absorption spectroscopy calibrated with certified zinc standard solution. The lower detection limit was 0.06 mg/L Concentrations of Zn in vials were slightly lower than targetted, but agreement among trials and treatment replicates was good (Fig. 6.2).

Glochidia were dissected from clams immediately before placement into treatment vials Marsupial gills were removed from active gravid female clams, cut open and stripped of glochidial packets. These were then dispersed in 100 mL of filtered aquarium water and rinsed three times by decanting off water after glochidia settled. Several hundred glochidia were transferred by pipette to each of 12 test vials (4 Zn treatment x 3 replicates) for each clam.

6.2.4. Assessment of viability Viability of glochidia in experimental treatments was assessed by testing their ability to close their valves in response to a noxious stimulus, 2 5 M KCl, using the methods of Huebner and Pynnönen (1992). Glochidia unable to close their valves cannot attach to a fish host and complete their development into juvenile clams Response to a negative stimulus was used as a substitue for a positive stimulus such as fish plasma or tactile arousal, which indicated similar degrees of viability *Anodonta* (Lefevre and Curtis 1910; Huebner and Pynnönen 1992).

Using Pasteur pipettes, samples of glochidia (in approx. 1 mL of solution) were transferred from treatment vials to 2 mL - plastic wells. Numbers averaged 63 individuals, and ranged from 21 to 151. Under a dissecting microscope, the numbers of glochidia open and closed in each well were counted. A drop (≈ 0 04 mL) of 2.5 M KCl was then applied to each well, and the numbers open and closed recounted. The difference between the

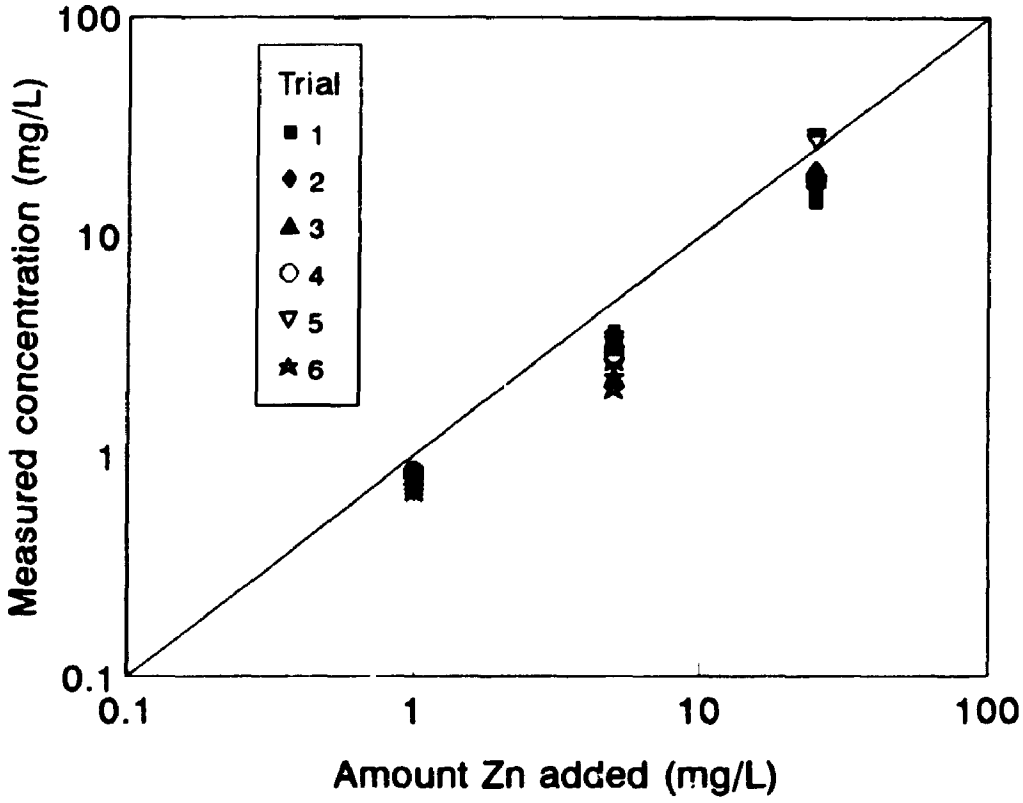


Fig. 6.2. Concentrations of zinc in experimental vials. Samples were collected 2 - 3 days after addition of zinc and analysed by AAS. Concentrations in all control treatment vials were below detection limit (0.06 mg/L).

numbers closed before and after KCl application was the number capable of responding to a stimulus, and thus viable. Individuals either closed before or open after KCl addition were considered either moribund or dead.

Before the transfer of freshly collected glochidia in the rinsing beaker to the vials containing test solutions, the proportion of individuals viable was estimated from each of three samples. For all clams, the proportion viable was 95 - 100% Viability of a sample of glochidia from each experimental vial was assessed 24 and 48 hr after introduction to the treatments. Samples were discarded after counting glochidia.

Tests on glochidia from the 30 experimental clams were conducted in 6 trials on separate dates. Equal numbers of clams from sites 13 and 15 were used in each trial.

6.2.5. Genetic procedures Immediately after dissection of gills for glochidia, several grams of foot tissue was cut from each mother clam and frozen and stored in cryovials at -80°C These samples were later homogenized and analysed by allozyme electrophoresis for GPI and PGM using the methods described in Sec. 4.2.3 and 4.2.4.

6.2.6. Statistical analyses Counts of the number of viable individuals in samples of glochidia were converted to proportions of the total number counted. To improve homogeneity of variance among groups and normality of the distribution for statistical analyses, these values were transformed into logit units by.

$$\text{logit } V = \ln(V/(1 - V))$$

where V = proportion viable. (For V = 0 and 1, 0.01 and 0.99 were substituted)

Nested factorial analysis of variance (ANOVA) models were used to test for the effects of the following factors: trial, site, clam (nested within site), zinc treatment and time at which viability was assessed. Response variability among groups of glochidia in vials (nested with clams and sites) represented experimental error. Initially, a full model that included all factors was computed, but due to unequal allocation of clams among trials a single analysis on the

whole data set was difficult. Therefore, ANOVAs were performed for each trial using the model

LOGIT V = SITE|CLAM(SITE)|ZINC|TIME VIAL(SITE CLAM)

where LOGIT V = logit transformed proportion viable, SITE = source of parent clams (site 13, site 15), CLAM = parent clams of glochidia (n = 1 - 4, depending on trial), ZINC = Zn treatments (0, 1, 5, 25 mg added/L), TIME = time of viability assessment (24, 48 hr), and VIAL = groups of glochidia from the same parent subjected to the same treatment (n = 3). The response variable was weighted by the number of glochidia counted for each viability measurement. Factors CLAM(SITE) and VIAL(SITE CLAM) were considered as random effects. Mean squares were calculated with sequential (Type I) sums of squares. Error terms used for F - tests (given in Table 6.1) were generally not the error mean square. In trial 5, only one clam per site was tested, which left SITE and CLAM(SITE) confounded. For the ANOVA, these terms were combined into a single factor and the results interpreted with recognition of this problem.

The effect of trial on viability was examined by comparing fitted values for LOGIT V from each of the separate ANOVAs. A one-way ANOVA was performed on the pooled data, with trial as a the single factor. Variance in the fitted values due to trial was not significant (P = 0.221), so the data were then pooled across trials and re-analysed using the same ANOVA model to increase treatment replication and improve the power of tests.

Differences in viability related to allozyme genotypes were assessed by comparing fitted responses from the ANOVA models among GPI and PGM genotypes. Lack of sufficient balance across factor combinations in the numbers of genotypes precluded statistical tests, so comparisons were made graphically.

Table 6.1. Analyses of viability of glochidia of *L. radiata*: ANOVAs on data separated by experimental trials. The response variable is logit - transformed proportion viable

No.	Source	df	MS	Error term	*F*	*P*
			Trial 1			
1	Site [S]	1	1134	2	4.13	0 112
2	Clam(Site) [C(S)]	4	274.7	12	2 58	0 091
3	Zinc treatment [Z]	3	14712	7	145 72	<0 001
4	Time [T]	1	3405 7	8	73.90	0 001
5	S * Z	3	185.1	7	1 83	0.195
6	S * T	1	235.5	8	5 11	0.087
7	Z * C(S)	12	101.0	12	0.95	0 535
8	T * C(S)	4	46.1	12	0.43	0.782
9	Z * T	3	577.3	11	12.1	0 001
10	S * Z * T	3	42.6	11	0.89	0 473
11	Z * T * C(S)	12	47.7	12	0.45	0.910
12	Vial(Site Clam)	12	106.3	13	1.95	0 039
13	Error	84	54.5			
			Trial 2			
1	Site	1	873.9	2	2 25	0 273
2	Clam(Site)	2	389.2	12	12.89	0.003
3	Zinc treatment	3	11753.3	7	233.91	<0.001
4	Time	1	849.5	8	40.14	0.024
5	S * Z	3	211.5	7	4.21	0 064
6	S * T	1	70.6	8	3.34	0 209
7	Z * C(S)	6	50.3	12	1.66	0 247
8	T * C(S)	2	21.2	12	0.70	0.524
9	Z * T	3	461.1	11	12.91	0.005
10	S * Z * T	3	4.2	11	0.12	0.947
11	Z * T * C(S)	6	35.7	12	1.18	0.402
12	Vial(Site Clam)	8	241.6	13	1.39	0.223
13	Error	56	21.8			

Table 6.1 continued.

No.	Source	df	MS	Error term	*F*	*P*
			Trial 3			
1	Site	1	977.3	2	1.90	0.240
2	Clam(Site)	4	514.6	12	17.88	<0.001
3	Zinc treatment	3	16918.0	7	224.81	<0.001
4	Time	1	1058.0	8	10.48	0.032
5	S * Z	3	73.7	7	0.98	0.435
6	S * T	1	29.2	8	0.29	0.619
7	Z * C(S)	12	75.3	12	2.61	0.055
8	T * C(S)	4	101.0	12	3.51	0.041
9	Z * T	3	431.4	11	8.56	0.003
10	S * Z * T	3	52.1	11	1.03	0.412
11	Z * T * C(S)	12	50.4	12	1.75	0.173
12	Vial(Site Clam)	12	28.8	13	1.32	0.225
13	Error	84	21.9			
			Trial 4			
1	Site	1	0.4	2	<0.01	0.987
2	Clam(Site)	2	1352.2	12	29.95	<0.001
3	Zinc treatment	3	12794.5	7	78.84	<0.001
4	Time	1	1673.4	8	25.22	0.037
5	S * Z	3	58.2	7	0.36	0.786
6	S * T	1	0.8	8	0.01	0.923
7	Z * C(S)	6	162.3	12	3.59	0.050
8	T * C(S)	2	66.4	12	1.47	0.286
9	Z * T	3	375.3	11	48.45	<0.001
10	S * Z * T	3	79.0	11	10.20	0.009
11	Z * T * C(S)	6	7.8	12	0.17	0.977
12	Vial(Site Clam)	8	45.1	13	1.62	0.142
13	Error	54	27.9			

Table 6.1 continued .

No.	Source	df	MS	Error term	*F*	*P*
			Trial 5			
2	Clam(Site)	1	487.6	12	11 58	0 027
3	Zinc treatment	3	6478	7	58 07	0 004
4	Time	1	1389	8	26.35	0 122
7	Z * C(S)	3	111 6	12	2 65	0.185
8	T * C(S)	1	52.7	12	1.25	0.326
9	Z * T	3	178.8	11	1.69	0 338
11	Z * T * C(S)	3	105.7	12	2.51	0 198
12	Vial(Site Clam)	4	42.2	13	0.85	0.503
13	Error	28	49.4			
			Trial 6			
1	Site	1	430.3	2	0.56	0 484
2	Clam(Site)	6	775 1	12	17.04	<0 001
3	Zinc treatment	3	34019.7	7	158.63	<0.001
4	Time	1	2053.1	8	46.18	<0.001
5	S * Z	3	48.4	7	0.23	0.877
6	S * T	1	23.5	8	0.53	0 494
7	Z * C(S)	18	214.5	12	4.71	0.002
8	T * C(S)	6	44.5	12	0.98	0.472
9	Z * T	3	531.8	11	16 77	<0 001
10	S * Z * T	3	21.1	11	0.66	0.585
11	Z * T * C(S)	18	31.7	12	0.70	0.771
12	Vial(Site Clam)	16	45.5	13	2.06	0.015
13	Error	112	22.1			

6.3. Results

Separate analyses of glochidia viability in each trial gave generally consistent levels of significance for the effects tested (Table 6.1). Poorest agreement between analyses was for the ZINC*CLAM(SITE) interaction, which was highly significant ($P = 0.002$) in trial 6, but borderline or nonsignificant ($P \geq 0.05$) in the other trials. Results from the ANOVA of the combined data (Table 6.2) were congruent with those from the separate ANOVAs and provide a description of the overall effects shown in the toxicity trials.

The greatest effect by far on glochidia viability was due to zinc treatment ($P < 0.001$, Fig. 6.3A). Viability was approximately 90% in control, close to 0% in the highest zinc treatment, and monotonically decreasing between the two. The difference in viability at 24 and 48 hr represented the second greatest effect ($P < 0.001$, Fig. 6.3B). Not surprisingly, viability was lower after 48 hr than after only 24 hr. The source from which parent clams were collected had no effect on the viability of glochidia ($P = 0.519$ from pooled ANOVA, and ranged from 0.112 to 0.987 from separate ANOVAs). This was due to significant variability observed among clams ($P < 0.001$ from pooled ANOVA, Fig. 6.3C). Some clams thus showed greater resistance to zinc than others, but these individuals were not more prevalent in the groups from site 13 than from the group from site 15.

Two second order interactions were also significant. Overall, variability among clams in viability of glochidia was not constant across zinc treatments ($P < 0.001$ for ZINC*CLAM(SITE), Table 6.2). The response of clams was more heterogeneous in the 1 and 5 mg Zn/L treatments, than in the control and 25 mg Zn/L ones (Fig. 6.4A). Similarly, mean resistance differed more between 24 and 48 hr in intermediate concentrations of zinc than in the control and 25 mg Zn/L conditions (ZINC*TIME $P < 0.001$, Fig. 6.4B).

Homozygotes for the most common alleles of both GPI and PGM predominated in the clams from both sites 13 and 15 (Table 6.3). When back - transformed fitted viability was averaged for all zinc treatment - genotype combinations, there was little indication of differential resistance among maternal genotypes. For GPI, glochidia from the clam having the GPI-CC homozygote showed higher median viability than that of genotypes with the

Table 6.2. Analyses of viability of glochidia of *L. radiata*. ANOVA on data pooled across experimental trials. The response variable is logit - transformed proportion viable

No.	Source	df	MS	Error term	*F*	*P*
1	Site [S]	1	273.4	2	0 43	0.519
2	Clam(Site) [C(S)]	28	640.7	12	12.32	<0 001
3	Zinc treatment [Z]	3	94096.1	7	468 98	<0 001
4	Time [T]	1	9542.2	8	124.57	<0.001
5	S * Z	3	85.0	7	0 42	0.736
6	S * T	1	128.2	8	1.67	0.206
7	Z * C(S)	84	200.6	12	3.86	<0.001
8	T * C(S)	28	76.6	12	1.47	0 105
9	Z * T	3	2293.1	11	52.5	<0.001
10	S * Z * T	3	14.4	11	0.33	0.804
11	Z * T * C(S)	84	43.7	12	0.84	0.772
12	Vial(Site Clam)	60	52.0	13	1.67	0.002
13	Error	418	31.1			

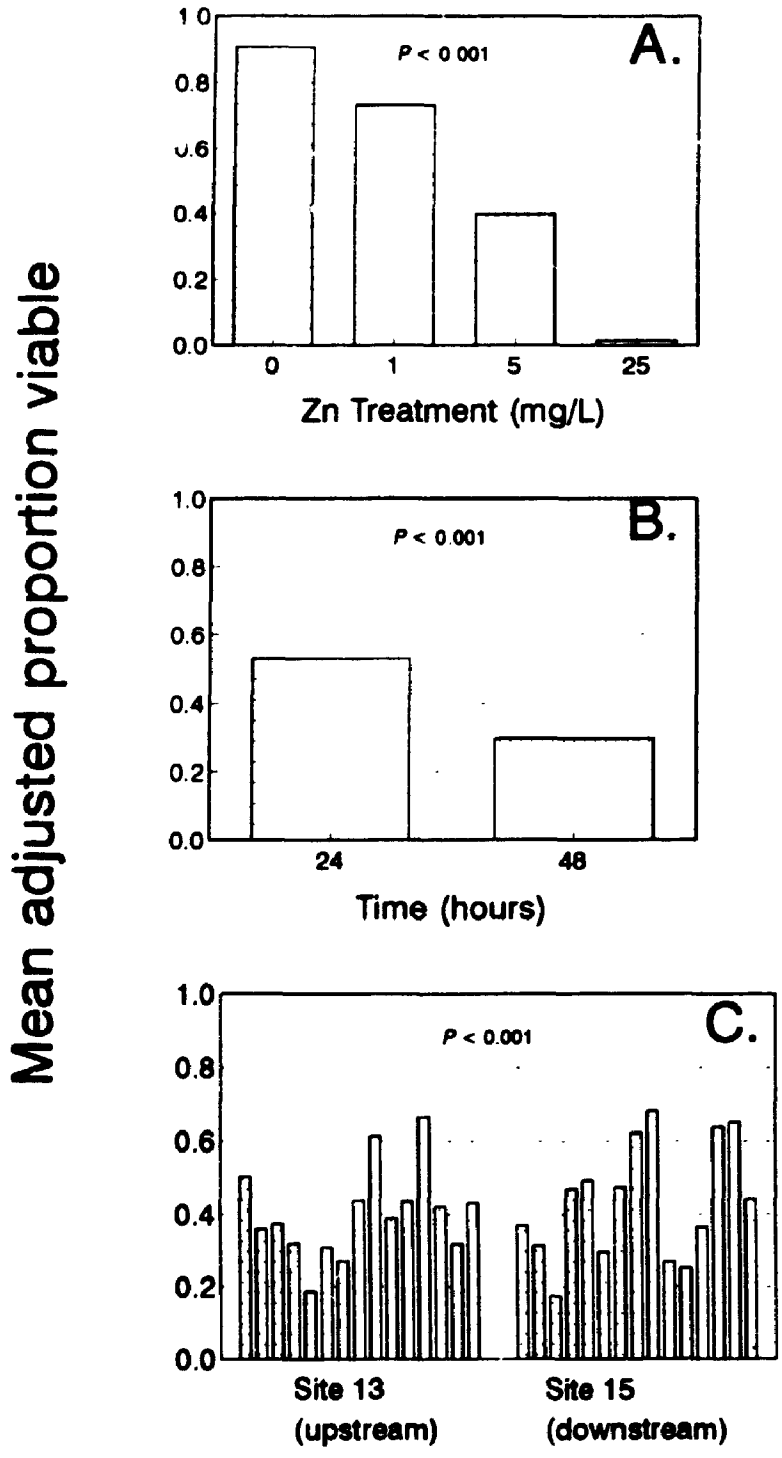


Fig. 6.3. Viability of glochidia in experimental treatments: main effects for (A) zinc treatment, (B) time in treatment, and (C) clam. P -values indicate significance of effect from ANOVA of pooled data.

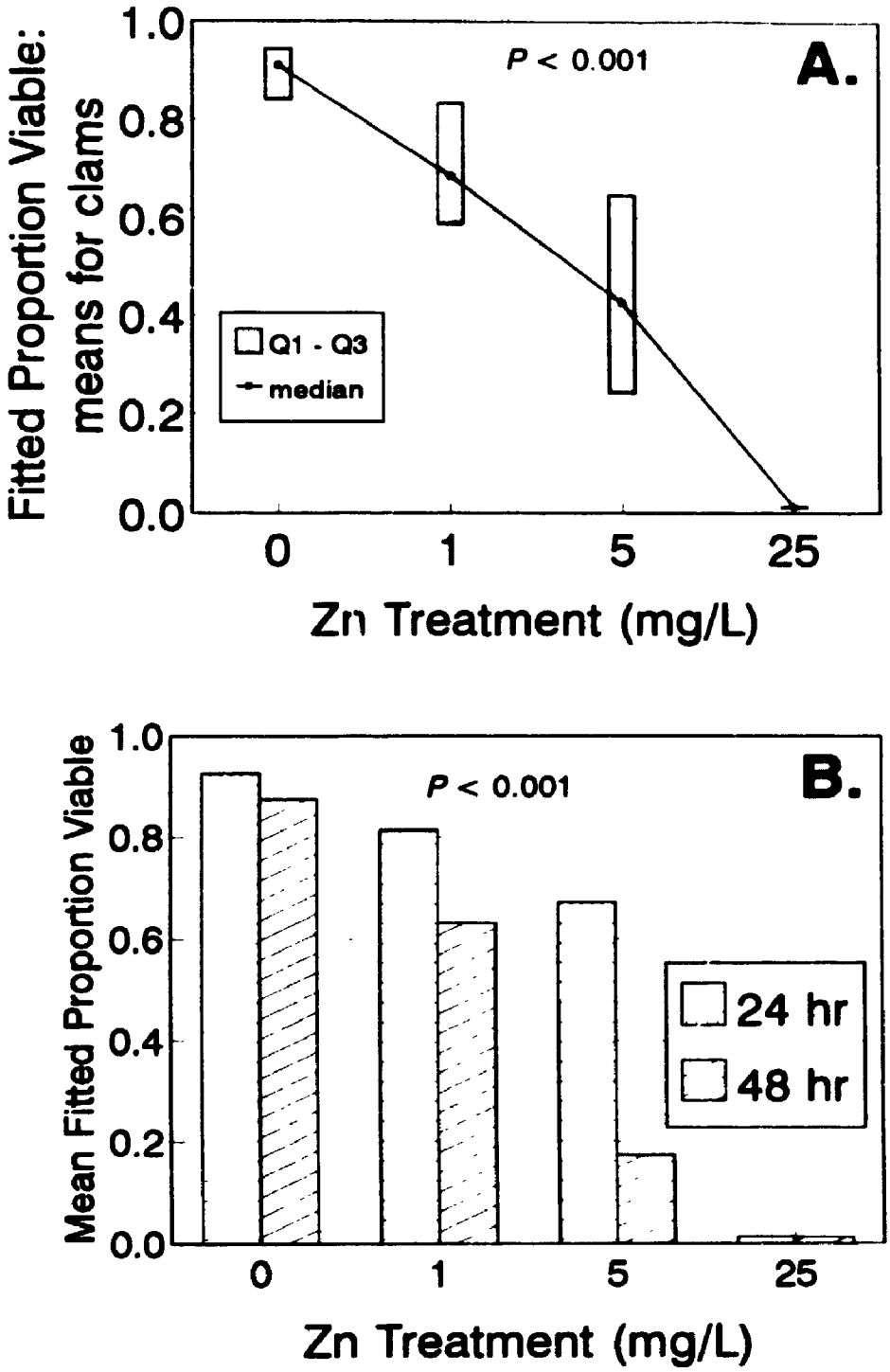


Fig. 6.4. Viability of glochidia in experimental treatments: interactions for (A) ZINC × CLAM(SITE) and (B) ZINC × TIME. Shown in A are the inter-quartile ranges of the 30 clam means for each zinc treatment.

Table 6.3 Genotype frequencies of GPI and PGM for gravid *Lampsilis radiata* used in the zinc toxicity trials.

	GPI				PGM	
Site	AB	BB	BC	CC	BB	BC
13	0	14	1	0	11	4
15	1	12	1	1	11	4

GPI-B allele in three of the zinc treatments (Fig. 6 5) Additional data are required to determine the significance of these differences. For PGM, mean viability was approximately equal for the two genotypes in all treatments (Fig. 6 6).

6.4. Discussion

6.4.1. Effect of zinc Viability of glochidia in the experimental treatments was strongly related to zinc concentrations. Although EC_{50}s (effective concentrations that reduce viability to 50%) were not formally determined, interpolations from Fig. 6.4B suggest approximate values of 5 - 7 mg Zn/L for 24 hr and 1 - 2 mg/L for 48 hr. Inability of glochidia to close in response to stimulation likely has the same effect on fitness as death. Thus EC_{50}s can be considered equivalent to LC_{50}s. Glochidia of *L. radiata* glochidia were less sensitive to zinc than other immature unionids. Huebner and Pynnönen (1992) estimated 24- and 48-hr EC_{50}s for glochidia of *Anodonta cygnea* to be 0.1979 and 0.0691 mg Zn/L. Juvenile *A. imbecilis* displayed 48-hr LC_{50}s of 0.355 - 0 588 mg Zn/L, depending on water hardness (Keller and Zam 1991). In contrast, Millington and Walker (1983) reported a 14-d LC_{50} for adult *Velesunio ambiguus* as 66 mg Zn/L, and Rodgers et al. (1980) determined 96-hr LC_{50} for *Corbicula* sp. to be 6.04 mg Zn/L in a static system.

Although field measurements of dissolved zinc concentrations were not made in this study, observations by Tessier et al. (1989) from the oxic sediments of 40 lakes exposed to metals from atmospheric deposition and mining activities suggest that the experimental treatments were not unrealistic. In lakes of pH > 7, up to 2.6 mg Zn/L was measured in the overlying 5 cm of water; concentrations of Zn in interstitial waters were even higher Sediment Zn levels ranged from 0.13 - 436 µg/g. The authors also noted that in natural waters Zn is not strongly complexed with ligands (i.e., Zn^{2+} is the primary species in the dissolved phase). Thus, glochidia settled on and in the sediments near site 15 could be exposed to conditions similar to those in the toxicity treatments.

6.4.2. Variability in resistance to zinc Substantial variability in resistance to zinc was observed among glochidia. In addition to the effect of assessment time, much of this was

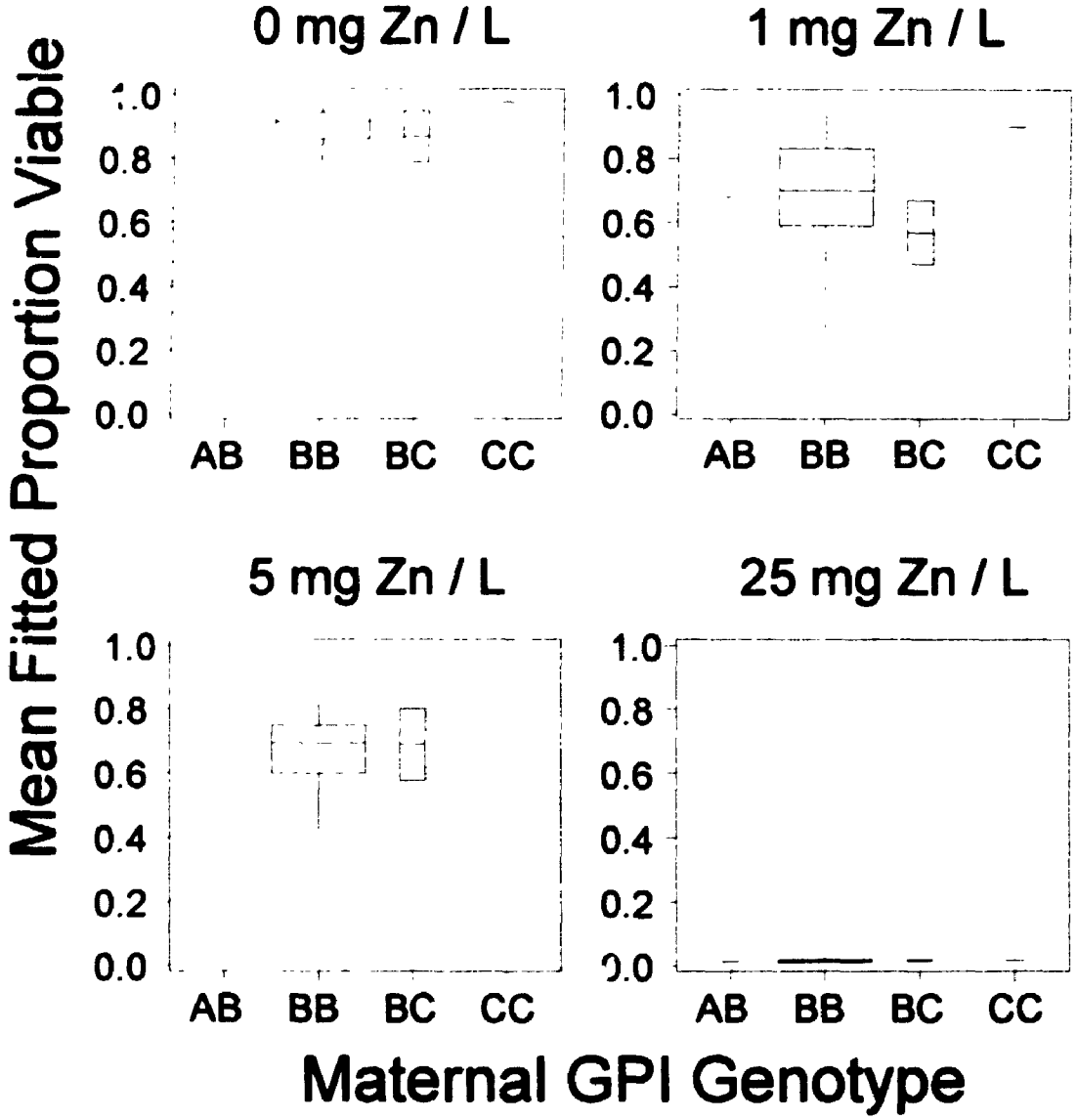


Fig 6.5. Viability of glochidia and maternal allozyme genotype: GPI. Boxplots show quartiles, whiskers extend to adjacent values and points indicate outliers. Box widths are proportional to counts. Values are fitted for effects in ANOVA.

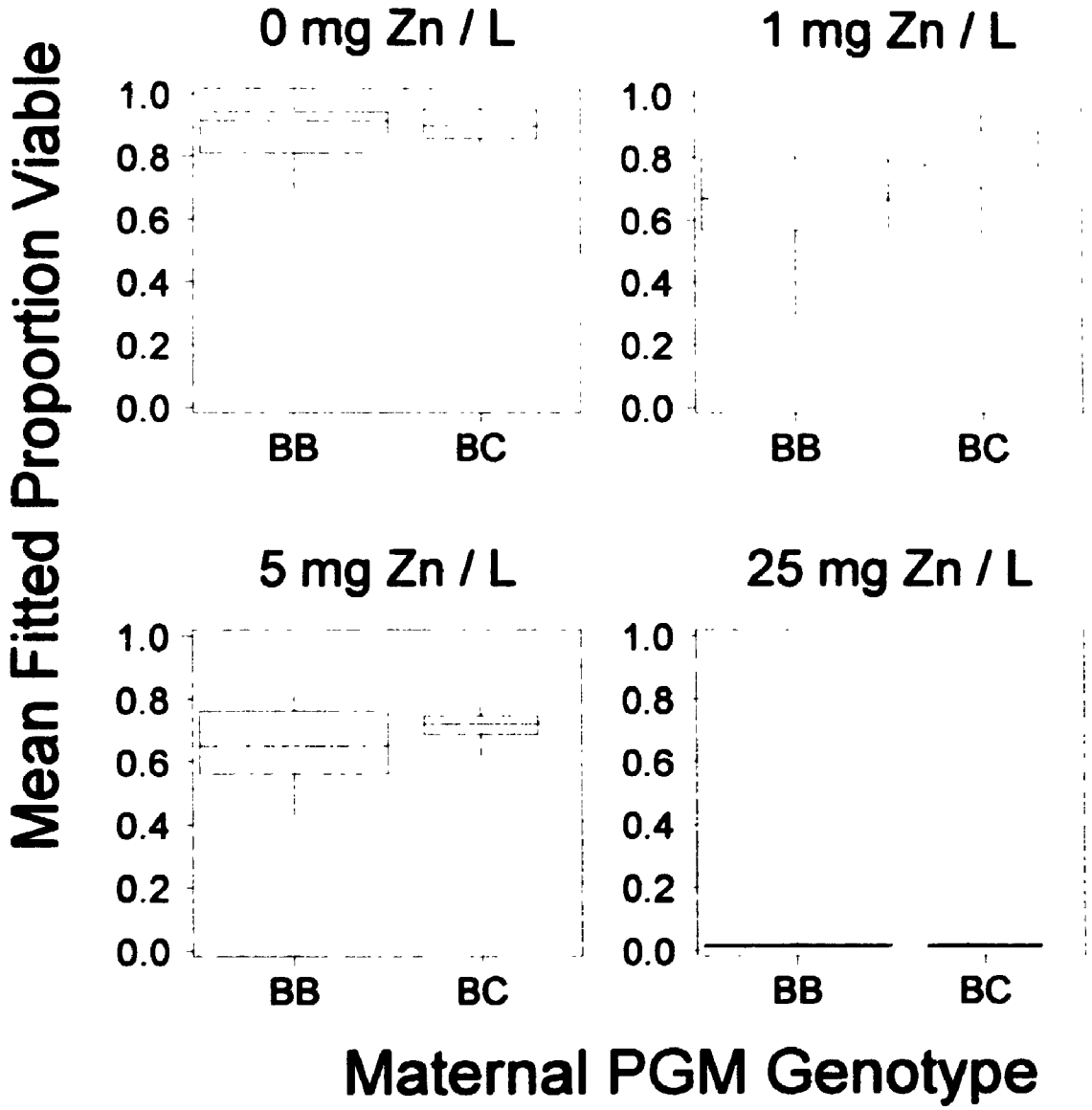


Fig. 6.6. Viability of glochidia and maternal genotype: PGM Boxplots are as described for Fig. 6.5.

accounted for by differences among the parent clams. Glochidia from the same parents showed similar resistance to zinc toxicity. Over time such differential resistance in contaminated sites could result in selection for enhanced tolerance of zinc. However, there was no overall difference between glochidia of clams from site 13 compared to site 15, and thus no evidence of differences in zinc tolerance between clams from upstream and downstream of the contaminant discharge.

The similarity between sites in the response of glochidia to zinc suggests that the degree of selection due to zinc contaminants is the same in both sites. Possibly, glochidia that successfully attach to their host are taken up immediately after expulsion from parent, thereby avoiding exposure to high zinc levels. In *Lampsilis*, modified edges of the mantle in gravid females, as well as the conglutinates (packets) of glochidia that are ejected from the gills, are modified to resemble host prey items such as small fish, leeches and flatworms, adaptations which serve to attract hosts and increase the probability of parasitization (Kat 1984).

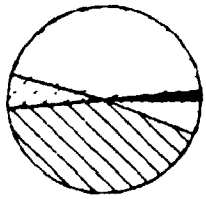
Alternatively, migration of individuals between sites may be sufficient to disperse enough metal - tolerant individuals selected in contaminanted sites to counter the evolution of clam beds that different in mean tolerance. The high degree of similarity in allozyme frequencies observed across eight sites separated by up to several hundred kilometres (Sec. 4), indicates that gene flow among clam beds is likely not restricted.

6.4.3. Lack of GPI and PGM gentotype effects Whereas 50% of a parent's alleles are shared by its offspring, the relationships for specific genotypes are more complex. Genotypic frequencies for GPI and PGM of glochidia depend on the proportions of alleles contributed from each parent. Alleles contributed from the mothers were determined by allozyme analyses (Table 6.3). If the frequencies of alleles in the fertilizing sperm from the water column reflect those in the adult males of the population, genotypic frequencies in the glochidia can be calculated. Based on analyses of clams from the same study area (Cornwall, north channel: sites 2 and 3; Fig. 1.2), proportions of GPI alleles were roughly 3% A, 85% B, and 12% C (Sec.4; Table 4.1). With random fertilizations, expected frequencies for the

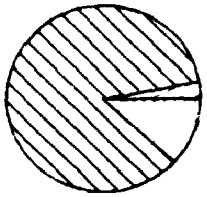
offspring of each female are shown in Fig. 6.7. These genotypic frequencies differ according to the maternal genotype.

If responses of the glochidia to zinc treatments were related to their GPI genotype, mean viability (adjusted for the model and based on roughly 1500 glochidia per clam) should depend on maternal genotype. In fact, they show little diffences among the four genotypes (Fig. 6.5), except for those glochidia from the GPI-CC clam Due to the low numbers of rare genotypes, it is difficult to suppose any allozyme effect on resistance. The difference between glochidia from PGM-BB and PGM-BC mothers is completely nonsignificant (Fig 6 6)

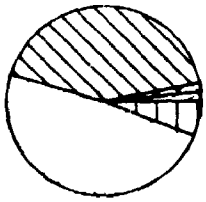
Allozyme - dependent responses to metal toxicity has been observed in several freshwater and marine invertebrates and fishes (Nevo et al. 1984; Chagnon and Guttman 1989; Gillespie and Guttman 1989; Diamond et al. 1989; Benton and Guttman 1990, 1992; Kramer et al 1992; Heagler et al. 1993; Montero et al. 1994). Although not directly comparable, results based on GPI and PGM from the present study suggest a lack of such a response in *I. radiata*.



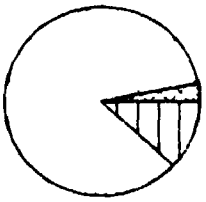
Mother: GPI-AB



Mother: GPI-BB



Mother: GPI-BC



Mother: GPI-CC

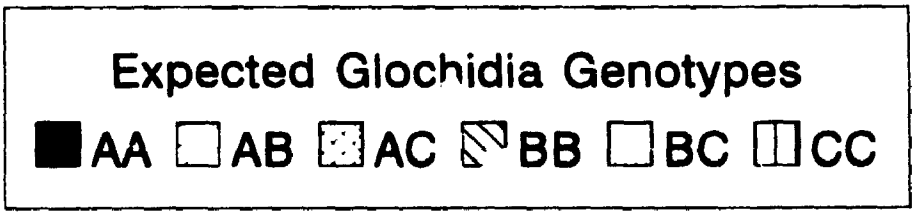


Fig. 6.7. Expected proportions of GPI genotypes in glochidia from mothers of known genotype, assuming that frequencies of alleles among fertilizing sperm are equivalent to those in adult males.

7. SUMMARY AND CONCLUSIONS

7.1. Summary of results

Lampsilis radiata from 13 locations in the St. Lawrence River were examined for evidence of physiological and ecological disturbance due to industrial discharges The main findings are:

A. Growth patterns of shells differed among groups of adult clams sampled from upstream and downstream of four areas receiving discharges. Growth rates of clams from three of the four sites below discharges were depressed relative to clams from corresponding sites above discharges.

B. However, shell growth over a year was not less in adult clams transplanted into sites below discharges compared to that of clams transplanted into sites above discharges. Genetic factors, or possibly irreversible physiological acclimatization, appear to control growth more than enviromental contaminant levels.

C. There was no evidence of selection favoring individuals with specific genotypes of GPI or PGM in contaminated sites, as has been reported in other studies with these allozyme systems. Frequencies of GPI and PGM genotypes were nearly identical in clams from eight sites, suggesting either selective neutrality among allozymes, or sufficient gene flow among sites to counter selective pressure.

D. Accumulation of trace metals in clam tissues was not generally related to sediment metal levels. Only in the most contaminated site were tissue burdens concomitantly higher. Variation in shell growth patterns among sites was not related to metal levels in sediments or clam tissues. Shell growth patterns in individuals show no relationship to specific allozyme genotypes.

E Resistance of glochidia larvae to toxic effects of zinc was highly dependent upon the mother clam, but not on whether the mother was from above or below a discharge, or on the GPI and PGM genotype of the mother. The offspring of clams from below a zinc - enriched discharge were not more tolerant of zinc than glochidia of clams from upstream.

Overall, there was little indication that clam beds located downstream of discharges in the study areas were deleteriously affected. This is in spite of the fact that (i) loadings of trace metals contained in several of the discharges rank them as major sources of contaminants (Environnement Canada 1985; OME 1991, 1992; Metcalfe-Smith 1994), (ii) sediments collected from within clams beds also rank as substantially contaminated relative to other rivers (Leland et al. 1978), (iii) trace metals are toxic to a variety of benthic organsims (Luoma and Carter 1991), and (iv) unionids should be vulnerable to the uptake discharged metals (Sec. 1 6.1).

7.2. Lack of disturbance from industrial discharges Several explanations are possible for the apparent non-response to contaminants shown by *L. radiata*. It is possible that the discharged contaminants are not in fact bioavailable at toxic levels. The limited amount of information on metal accumulation from the present study suggests that burdens in *L. radiata* from the study sites are lower and less indicative of environmental levels than some other unionids (Elder and Collins 1991). If discharges are episodic in their duration, the clams may be able to avoid exposure by closing their valves until after the plume has swept by (Doherty et al. 1987). Metals in the sediments may be in geochemical phases that are not taken up. Regulation by *L. radidata* may be highly efficient. Alternatively, metal concentrations in the sediments may not be toxic even if they were easily taken up. Havlik and Marking (1987) note that despite numerous studies on contaminant dynamics in unionids, little information exists on toxicity

Much of the variance observed in the "responses" examined - shell growth, allozyme frequencies, metal burdens, toxicity of zinc to glochidia - was unrelated to environmental

contaminant levels. Genetic factors (although not GPI and PGM genotypes) and natural environmental conditions, such as water alkalinity and hardness, were more important

7.3. Suitability of *L. radiata* as a biomonitor Although many bivalves species have been useful in assessing effects of environmental disturbances (Bayne 1989; Green et al 1989, Elder and Collins 1991; Johnson et al. 1993; Metcalfe-Smith 1994), the use of *L. radiata* to determine effects of industrial discharges in the St. Lawrence River remains questionable *Lampsilis radiata* has many characteristics of the ideal indicator organism (Johnson et al 1993): easy taxonomic identification, widespread distribution, numerical abundance, large body size, limited mobility, long life history, and well understood ecology. However, the clams examined in this study lacked the required criterion of a relatively narrow range of tolerance for the environmental conditions of interest. Their effectiveness as sentinal indicators (organisms whose bioaccumulation of contaminants is indicative of environmental pollutant levels) may require further study to assess. The species does not appear to be as sensitive of contaminant levels as *Elliptio complanata*, another unionid sympatric with *L. radiata* throughout the study area (Metcalfe-Smith 1994, Metcalfe-Smith et al. 1995)

APPENDIX A: PHYSICOCHEMICAL CHARACTERIZATION OF STUDY SITES, WITH SPECIAL REFERENCE TO CONTAMINANTS

A.1. Water

Between 1980 and 1989, water samples were collected by the Water Quality Branch, Environment Canada, from 25 stations in the St. Lawrence River between Cornwall, Ontario, and Sorel, Quebec, (Fig. A.1) that correspond to sites sampled in the present study (M. Charette, Water Quality Branch, Environment Canada, Ottawa, pers. comm.). Samples from the upper water column were analysed for up to 35 physical and chemical water quality parameters, with sample sizes ranging from 1 to 60. To assess among - area variability of parameters that roughly indicate general physicochemical conditions of the water column, data were plotted by station for specific conductance, alkalinity, calcium concentration, and turbidity (Fig. A.2). Specific conductance, a coarse measure of the concentration of dissolved ions, was about 300 µS/cm, except for stations at the St. Regis River (9207) and Ottawa River (9002) inflows, or near the north shoreline (9008, 9011, 9016, 9018, 9052). Calcium concentration and alkalinity show similar trends. For most stations, Ca concentration was about 35 mg/L, and alkalinity averaged 80-90 mg $CaCO_3$/L. Turbidity showed low variability both among and within sites in the Cornwall area. Further downstream, turbidity was generally higher but also more variable. These data suggest that clam beds in all sites sampled for the present study, except Site 11 at near the infow of the Ottawa River (Fig. 1.2), would have been exposed to similar water column conditions. Concentrations of dissolved Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn were frequently near or below detection limits (generally 1 µg/L, 0.02 µg/L for Hg).

In another study by Anderson and Biberhofer (1991), analyses were conducted for 19 metals on water samples from seven monthly surveys of 15 stations in the Cornwall area during 1988. In general, concentrations were either near or below detection limits (As, Be, Cd, Co, Cr, Hg, Se, V), homogeneous across stations (Ba, Li, Mo, Sr), or more variable among surveys than among stations (Cu, Ni, Pb, Zn). Exceptions were Al, Fe and Mn, which

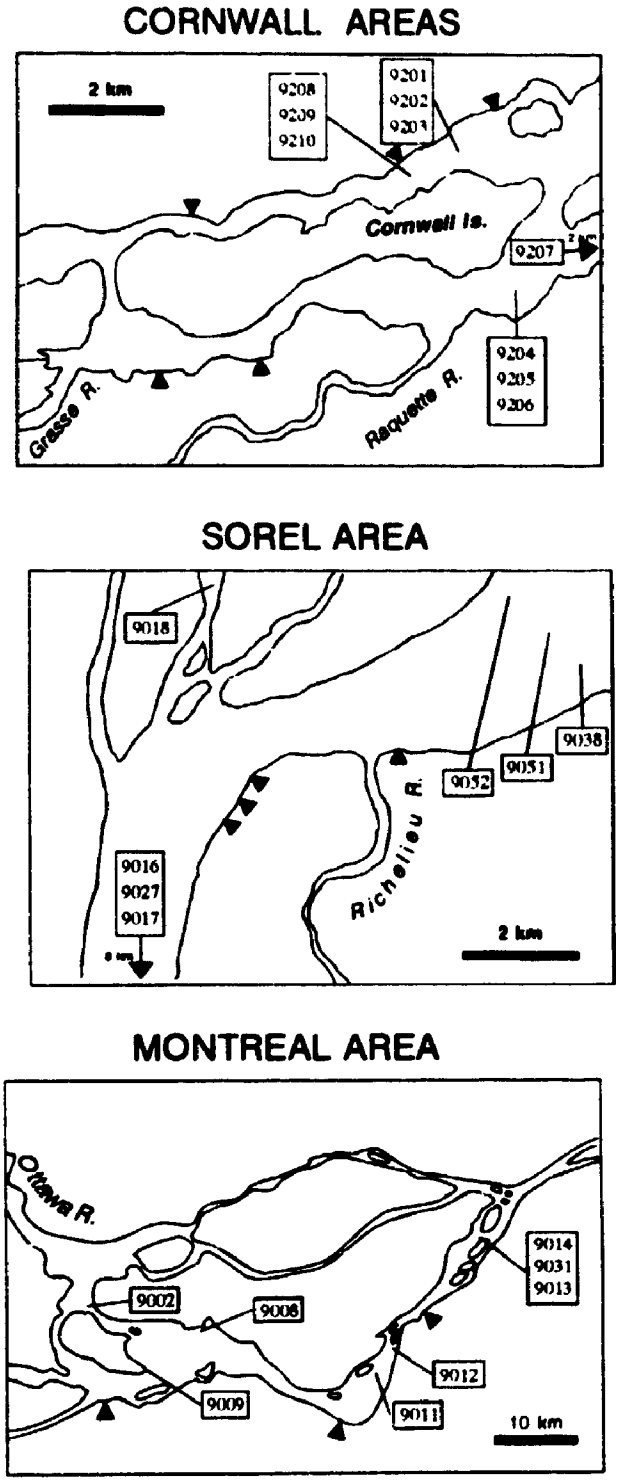


Fig. A.1. Stations in the St. Lawrence River study areas sampled by the Water Quality Branch, Environment Canada, 1980 - 1989. Industrial outfalls are indicated by ▼ . Grouped station numbers represent a transect across the river.

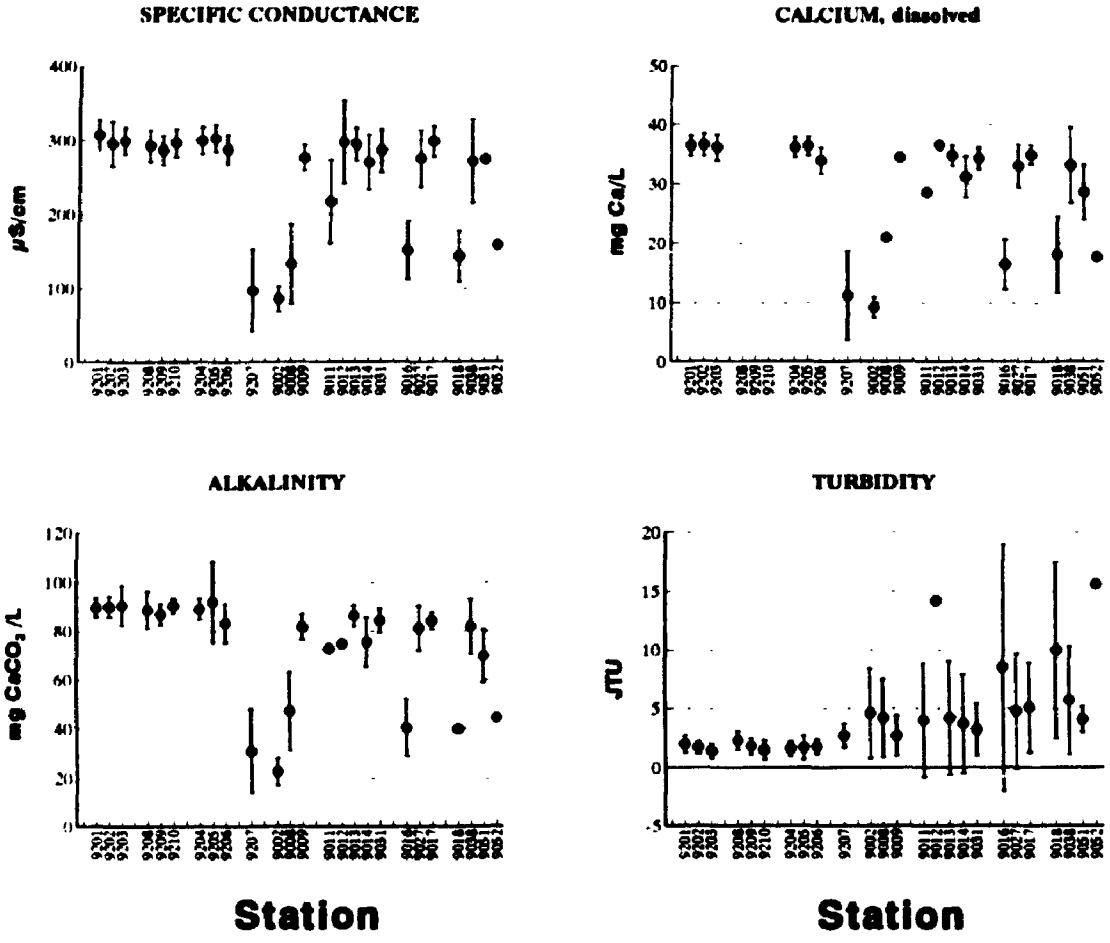


Fig. A.2. Specific conductance, alkalinity, dissolved calcium and turbidity (mean ± S.D.) for 25 stations in the St. Lawrence River sampled by the Water Quality Branch, Environment Canada, from 1980 - 1989. Sample sizes range from 1 to 60. Locations of stations are shown in Fig. A.1. Outlying stations include 9207 at the inflow of the St. Regis River (Cornwall area); 9002 and 9008 near the inflow of the Ottawa River; and 9011, 9016 and 9018 on the north shore side of the river.

(Source: M. Charette, Water Quality Branch, Environment Canada, pers. comm.)

were often elevated in the navigational canal and at the mouths of the Raquette and St Regis Rivers. None of the metals were in concentrations exceeding Provincial Water Quality Objectives for Ontario, except for Fe in the navigational canal.

A.2. Sediment

Surficial sediments were sampled from the study sites (Fig. 1.2) to roughly quantify the degree of pollution, identify the most important contaminants, and to allow comparisions of relative levels of exposure to metals between sites. Sites 1 - 5 and 7 - 11 were sampled in June 1989. Sediment was collected in 500-mL glass jars from the top 5-10 cm thick layer by hand with SCUBA at all sites except 7 and 8 in 1989, at which sediment was taken from an Ekman grab sample. Samples were kept cool with water ice and dry ice in the field (2-8 days), then stored in a freezer (-20°C) until laboratory analyses.

Sediments were assayed for total As, Hg, and Se; total and extractable Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn; particle size distribution; and organic content (loss on ignition). Metals were analysed by the National Laboratory for Environmental Testing (NLET, Environment Canada, Burlington, Ontario) using standard methods, including quality assurance and quality control procedures (NLET 1994). All elements except As, Se and Hg were analyzed by atomic absorption spectroscopy (AAS). Inductively coupled plasma - atomic emission spectroscopy (ICP-AES) was used for As and Se. Mercury was analysed by flameless AAS. The extracting solution for the determination of the extractable or non-residual (ie. not part of the silicate matrix of the rock) fraction of metals was 5% HCl solution. Minimum detection limits (mg/kg) for total concentrations were: Al - 10, As - 0.2, Cd - 1.0, Co - 2.0, Cr - 1.0, Cu - 1.0, Fe - 5.0, Hg - 0.01, Mn - 1.0, Ni - 3.0, Pb - 5.0, Se - 0.2, Zn - 1.0; and for extractable concentrations were: Al - 2.0, Cd - 0.2, Co - 0.4, Cr - 0.2, Cu - 0.2, Fe - 1.0, Mn - 0.2, Ni - 0.6, Pb - 1.0, Zn - 0.2. Sediment particle size distributions and loss on ignition were determined by the Sedimentology Laboratory (NWRI, Burlington) using the methods of Duncan and LaHaie (1979), and provided estimates of gravel, sand, silt and clay percentages, and percentage organic content.

Concentrations of metals in sediments for each site are shown in Fig. A.3 (total) and Fig. A 4 (extractable). Among the sites upstream of outfalls (1,3,5,9,11), concentrations did not tend to increase with distance downstream, which suggests that contaminants are not deposited in reference sites for areas further downstream. The significance of metal contamination in the putative impacted sites (2,4,7,8,10) was assessed by comparing each with the upper 95% confidence limit for the concentrations of the 5 sites upstream of the contaminant sources. Sites with concentrations greater than the upper 95% C.L. would be considered chemically impacted. Thus, based mainly on extractable metal concentrations, which are more toxicologically relevant (Luoma 1989), site 2 (Cornwall, N. Channel downstream) is contaminated with Hg; site 4 (Cornwall, S. Channel downstream) is uncontaminated (by any of the measured metals); site 7 (at Grass R. inflow) is contaminated with Cd, Cu, Mn, Ni, Se and Zn; site 8 (Sorel downstream) is contaminated with Co, Cr, Cu, Fe, Ni, Pb and Zn; and site 10 (Montreal downstream) is contaminated with Hg, Mn and Zn. In all of the cases in which a metal was identified as elevated by the above criterion, total concentrations of the metal exceeded Provincial Sediment Quality Guidelines (Fig. A.3; Persaud et al. 1992).

In 1990, sediments were collected again from sites 3, 4, 7, 8 and 9, and site 13. Analyses were for the same metals and by the same methods as those in 1989. Agreement of extractable metal estimates between years was assessed by regressing the 1990 concentrations on those for 1989 for the five resampled sites. The r^2 for 1990 vs 1989 estimates ranged from 0.80 to 0.9?, except for Zn ($r^2 = 0.63$). Slope was <1 for all metals except Cd, and ranged from 0.522 to 1.03. Therefore, 1990 estimates are, in general, lower than those of 1989, but the relative concentrations in the sediments among sites appears consistent between years for all metals assessed, except Zn, and at site 4.

Particle sizes of sediments differed somewhat among sites. Sites of the Cornwall, N. Channel pair (2 & 3) were both predominantly sand, and both sites at Montreal were characterized as 'sandy mud'. Sites at Cornwall, S. Channel (4,5), and those at Sorel (8,9) were not as similar within pairs ('muddy sand' vs 'sand', and 'sandy mud' vs 'gravelly sand',

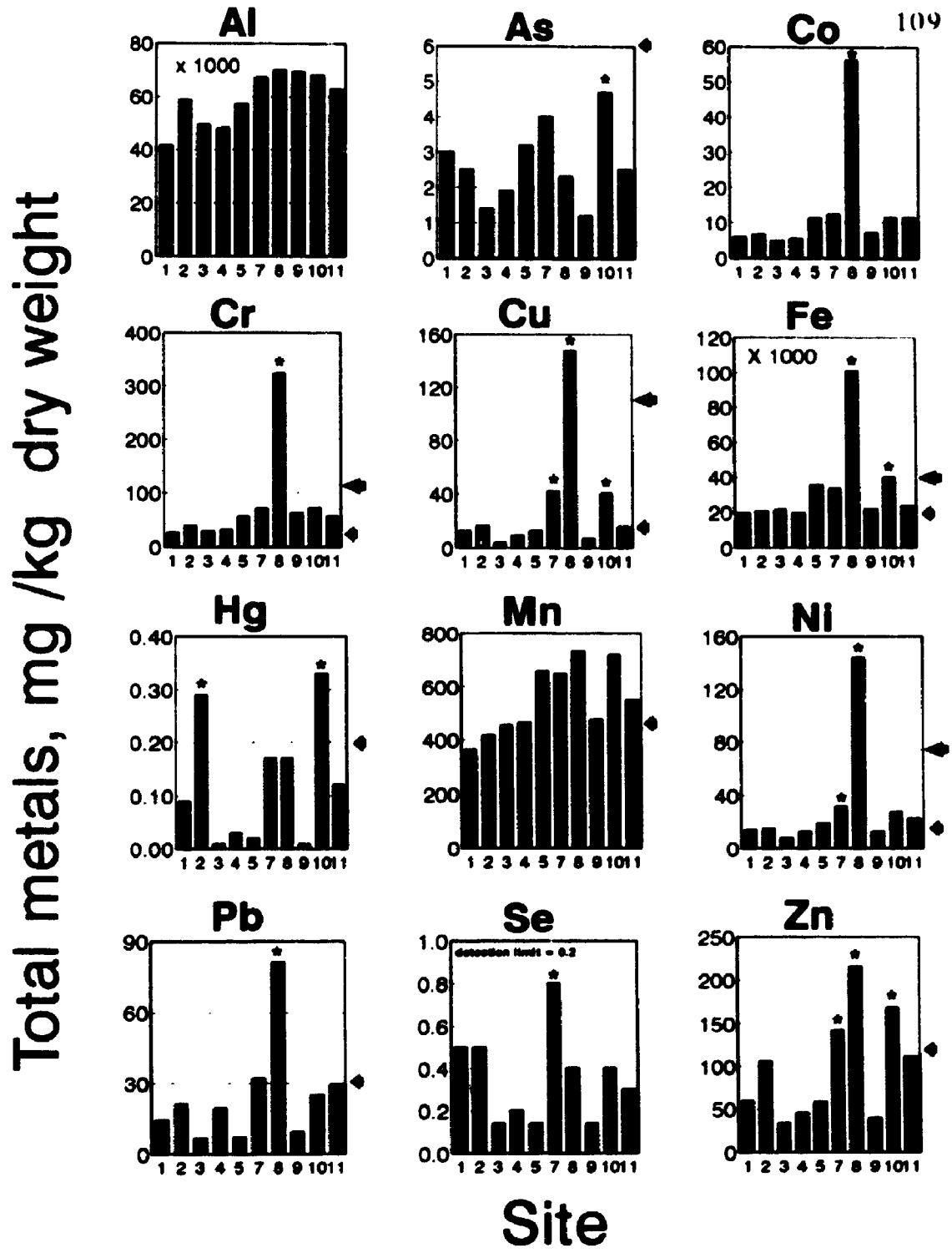


Fig. A.3. Total concentrations of metals in sediments collected from St. Lawrence River study sites in 1989. Sediments of downstream sites in which level is > 95% UCL on mean of concentrations in sites upstream of outfalls (1,3,5,9,11) are indicated by "*". Small and large arrows indicate "lowest" and "severe effect levels", respectively, from Provincial Sediment Quality Guidelines (Persaud et al. 1992).

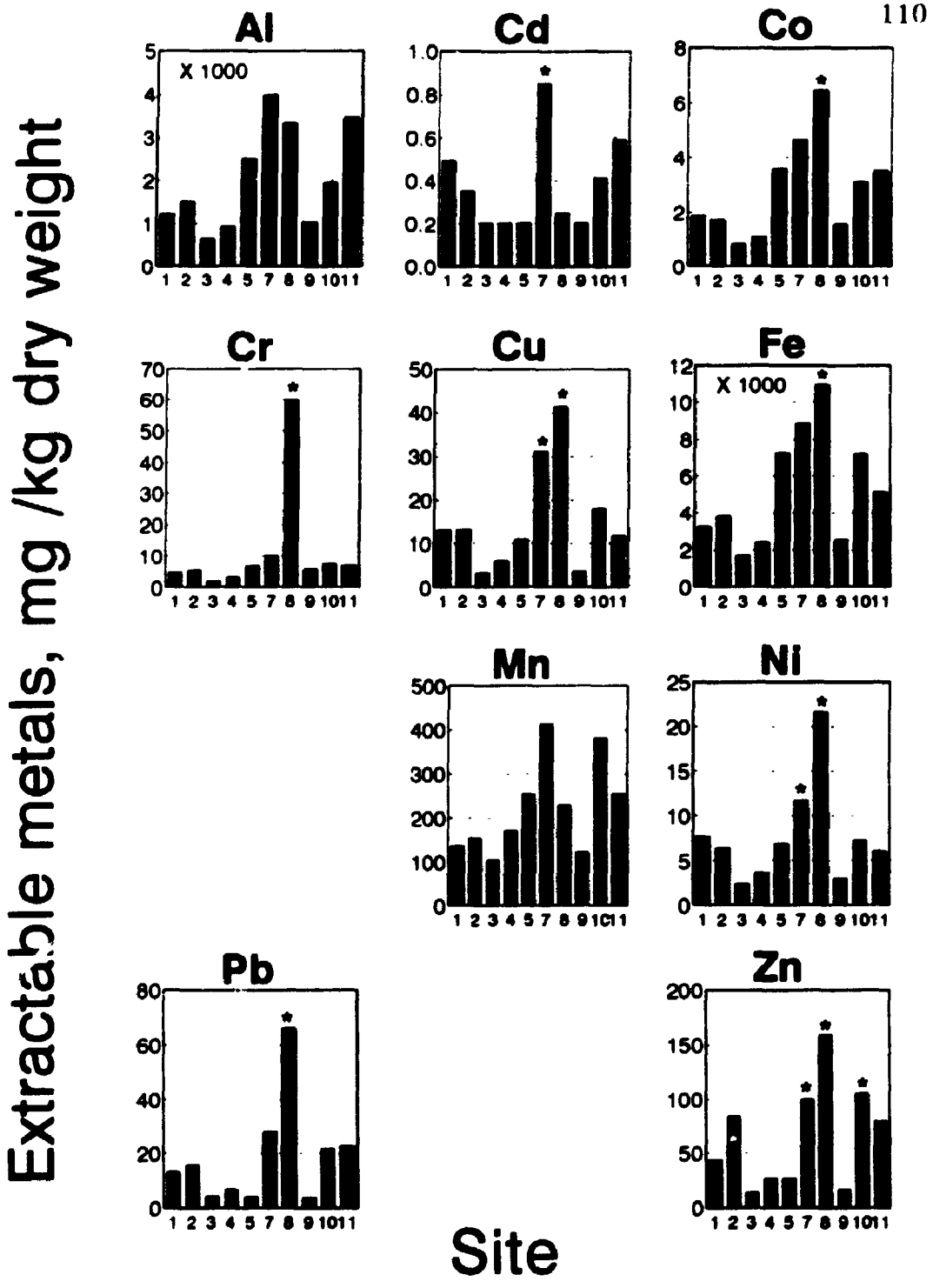


Fig. A.4. Extractable concentrations of metals in sediments collected from St. Lawrence River study sites in 1989. Sediments of downstream sites in which level is > 95% UCL on mean of concentrations in sites upstream of outfalls (1,3,5,9,11) are indicated by "*".

respectively). The 1990 sediment samples gave the same characterizations for sites 7,8 & 9, but site 3 had more gravel, and site 4 more silt and clay than the 1989 samples.

Organic content of sediments, as loss on ignition, was less than 5% for all sites in 1989. The range was from 0.57% (site 9) to 4.3% (site 2). For the 5 sites resampled in 1990, the correlation of estimates between years was very high (0.94), although 1990 values were almost double those 1989.

No strong correlation was found between the concentration in sediments of any metal and sediment particle size frequency or organic content.

Overall degree of trace metal contamination in the primary study sites is shown in Fig. A 5 (total metals) and Fig. A.6 (extractable metals). Concentrations are converted to mmol/kg and grouped by site, with the toxicologically less important Al, Fe and Mn omitted. The Sorel downstream site (8) shows the greatest difference in sediment metal concentrations compared to its upstream reference. It is, by far, the most heavily contaminated of the primary study sites. The downstream sites in the Cornwall, N. Channel and the Montreal areas (2 and 10) appear only slightly more contaminanted that their upstream references Site 4, in the Cornwall, S. Channel, shows no elevated metals in sediments. Mercury concentrations, though higher by far in sites 2 and 10 than in other sites (Fig. A.4), are not well represented here because of their comparatively low molar concentrations.

Multi-metal contaminant levels were also examined using a principal components analysis (PCA) on ln - transformed metal concentrations for the eight primary sites and sites 1 and 7 (extractable concentrations for all except As, Hg and Se). PC1 accounted for 78.6 % of the total variation and was negatively related to a general increase in all metals, especially Hg, Pb, Zn and Cu. PC2 accounted for 11.5 % of the total variation and was related to a decrease in Hg and increases in Cr and Co. Although only one sample per site was analyzed (and therefore samples and sites are confounded), a plot of PC2 vs PC1 scores (Fig. A.7) suggests that the downstream site-samples are generally more contaminated than the reference upstream ones, except for the Cornwall, S. Channel. This is in agreement with the previous assessment based on Fig. A.5 and A.6.

Total Metals

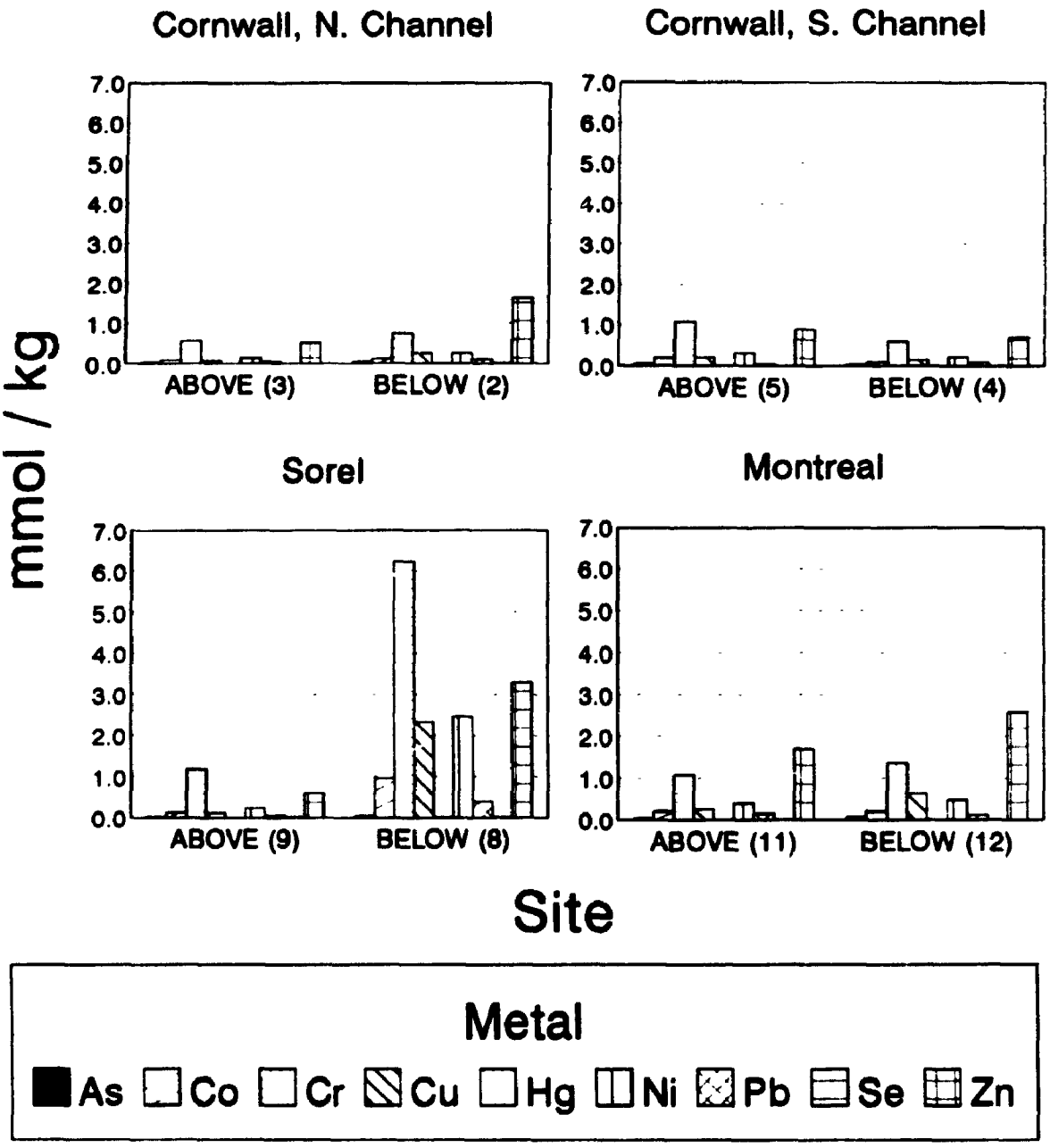


Fig. A.5. Total molar concentrations of toxicologically important metals in sediments from the eight primary study sites in the St. Lawrence River. Sites are paired to be upstream and downstream of four areas that received industrial effluents.

Extractable Metals

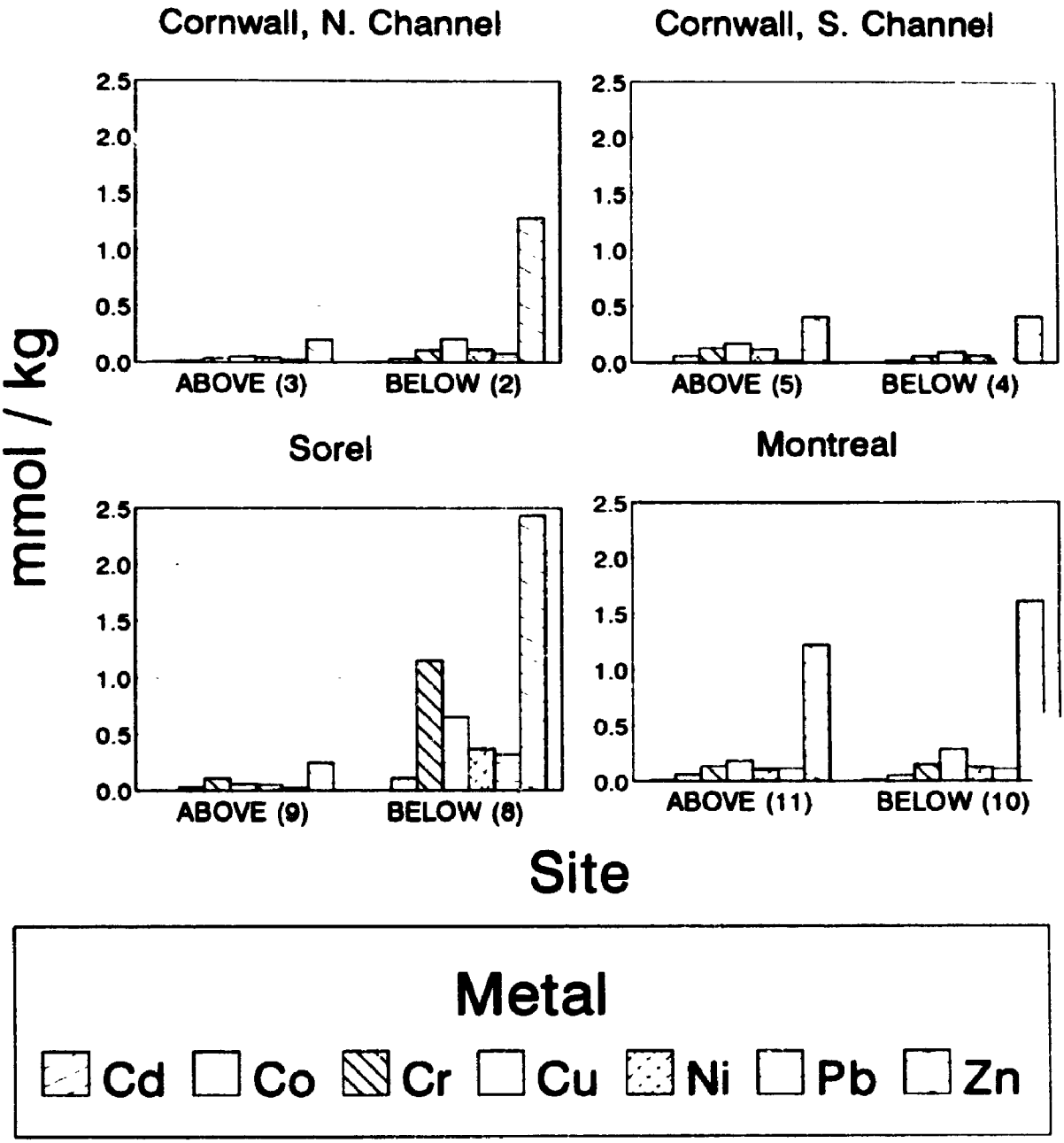


Fig. A.6. Extractable molar concentrations of toxicologically important metals in sediments from the eight primary study sites in the St. Lawrence River. Sites are paired to be upstream and downstream of four areas receiving industrial effluents.

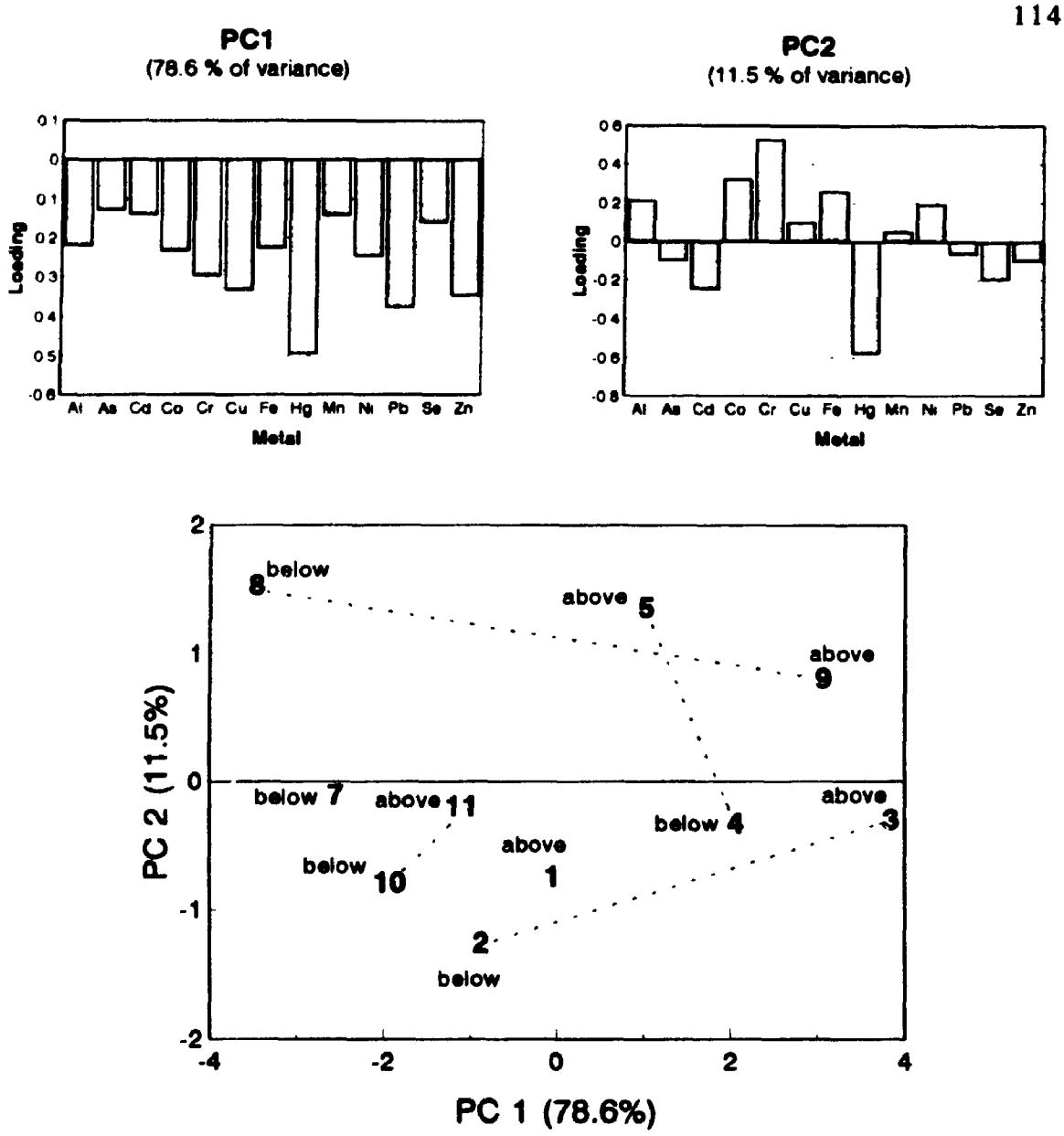


Fig. A.7. Component loadings and plot of scores for PC1 and PC2 from ordination of site-samples from the St. Lawrence River based on sediment metal concentrations (extractable fractions, except for As, Hg and Se, which were total concentrations). Point symbols on plot of PC2 vs PC1 correspond to site numbers. Lines connect upstream and downstream sites in the same area. Generally, the degree of sediment metal contamination increases from right to left on the plot.

APPENDIX B. ESTIMATION AND ANALYSIS OF SHELL GROWTH PATTERNS IN *LAMPSILIS RADIATA*

B.1. Introduction

Growth rates of unionid bivalves such as *Lampsilis radiata* vary seasonally, usually resulting in distinct "disturbance" marks on the shell that correspond to the annual reduction of growth during winter (Tevesz and Carter 1980). These marks are visible in cross sections of the shell as lines running through the nacre and prismatic layers, apparently the result of interrupted calcium carbonate deposition (Lutz and Rhoads 1980). Often, where the prismatic layer marks meet the periostracum, they are visible as concentric rings on the exterior surface of the shell. If the external rings are sufficiently distinct and found to match with the internal lines, and if the annularity of these marks can be established by some sort of dating analysis or mark-recapture experiment, the rings can be interpreted as yearly records of the shell size. In *L. radiata*, external rings are distinct (McCuaig and Green 1983; Day 1984; Podemski 1992), except for the earliest ring or two (which are usually eroded) and the later rings of old clams (which are often closely spaced and poorly defined).

Growth in length of several unionid species has been fitted by the von Bertalanffy model (Seed 1980; McCuaig and Green 1983)

$$(1) \qquad L_t = L_\infty(1 - e^{-kt})$$

which describes growth in length, L_t, over time, t, to an asymptotic final size L_∞, at the initial instantaneous rate of k. With a series of annual shell ring lengths, rate of growth over the lifetime and expected maximum size of individual clams can be estimated.

To estimate L_∞ and k of a shell, McCuaig and Green (1983) used the Walford Plot method. It involves the measurements of multiple (≥ 3) pairs of consecutive ring lengths, L_t and L_{t+1}, from a shell, and the computation of the linear regression

(2) $$L_{t+1} = a + bL_t$$

which relates length in the year t+1, L_{t+1}, to length in the previous year t, L_t. The intercept, a, represents shell length at the first winter's growth ring, and slope, b, the fraction of growth remaining after the first winter ring. The Walford Plot regression parameters allow calculation of parameters L_∞ and k of the von Bertalanffy model by

(3) $$L_\infty = a/(1 - b) \quad \text{and}$$

(4) $$k = -\ln b$$

which can be used to reconstruct each shell's growth pattern.

In the present study, a modification of the Walford Plot method as applied by McCuaig and Green (1983) was used to estimate the shell growth pattern for each of 421 clams collected from eight study sites. Methods of annual ring length measurements, computations of Walford Plot regressions and growth pattern reconstructions, and potential sources of bias and imprecision of estimates are described below.

B.2. Identitification and Measurement of Annual Growth Rings

Shells were cleaned of soft tissue, and attached debris and examined with reflected and transmitted incandescent light. Ages were estimated by counting from the umbo outward the number of dark, usually raised, macroscopic (Day 1984) rings on the periostracum. In *L. radiata*, the first year's growth ring tends to be 10 - 20 mm in length (McCuaig and Green 1983; Day 1984; this study). Therefore, for shells in which a growth ring less than 20 mm in length was not visible due to erosion of the umbo region, the shortest visible ring (in all cases > 25 mm) was assumed to be the second year's annulus.

In several shells, the identification of annuli by external examination was evaluated by preparing mounted cross-sections to view internal growth lines, which are considered more

reliable records of annual growth interruptions (Neves and Moyer 1988). For each clam, the valve with the lesser amount of erosion was cut from the umbo to the posterior margin with a slow speed diamond blade saw. Cut surfaces were bonded to glass microscope slides using clear epoxy glue. A second cut was made of the mounted shell to leave a 0.2 - 0.4 mm thick section, which was polished with lapidary papers if excessively rough. After rinsing and drying, the section surfaces were coated with clear nail polish to enhance transparency. Internal growth lines were identified and counted by the following procedure. Using a dissecting microscope (magnification ≈ 35X) and reflected light, the locations where macroscopic growth lines intersect the periostracum were marked on the mounted section slide. Under a compound microscope (mag. = 40X), the section was examined again for further identification and counting of prismatic layer lines. Marks on the slide were then compared with the valve from which the section was made to confirm that the locations of internal line - periostracum junctions match with those of the the external rings. Lastly, the number of missing prismatic layer lines was noted. These lines are those which should exist based on the external ring pattern, but are not visible due to shell erosion. The age (in years) of the shell was estimated by the number of prismatic layer lines plus the number of missing lines.

For each shell, the lengths (Fig. B.1) of as many external rings as possible (up to 10) were measured with digital or dial calipers (instrument precision = 0.01 mm). Because at least three consecutive ring pairs are required for the regression, a minimum of four rings, all consecutive, must be measureable (L_{t+1} of one pair can be assigned as "L_t" with L_{t+2} as "L_{t+1}" in the next pair). Out of a total of 443 clams collected from the eight major study sites (Sec 2.), 421 had at least three measureable ring pairs.

B.3. Walford Plot Regression and Growth Pattern Derivation

For each clam, Walford Plot regressions (eq. 2) were fitted to the annual ring lengths. Instead of using the ordinary least squares (OLS) method of estimating the regression line, which assumes negligible error in the observations of the X variable, the geometric mean

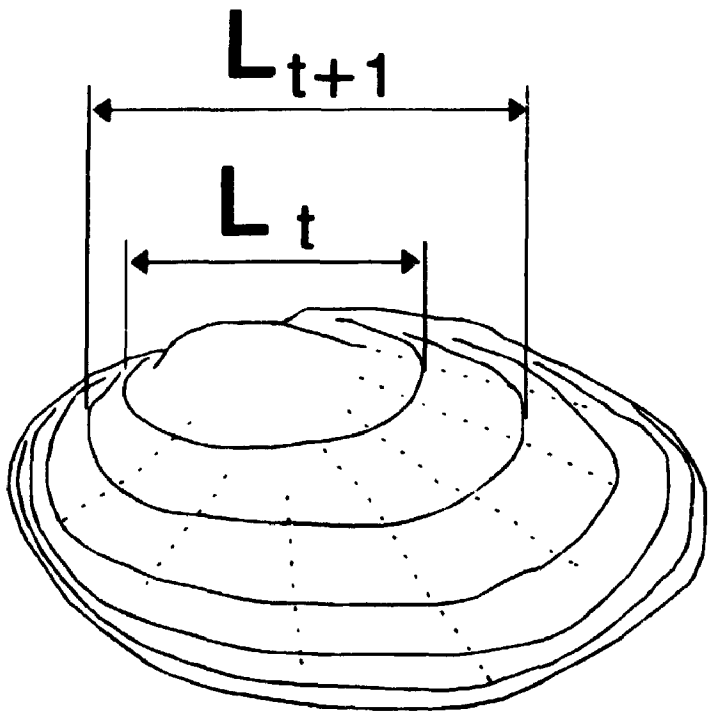


Fig. B.1. Length of a pair ofconsecutive external growth rings on a unionid shell. Measurements are made along the axis of maximum size. L_t and L_{t+1} are lengths at years t and t+1.

(reduced major axis) method was used to compensate for the condition of error in x values being approximately equal to error in y values (McArdle 1988). Rather than minimizing the sum of the squares of the deviations of observed y values from the regression line, as in the OLS method, the geometric mean (GM) method minimizes the sum of the areas of the triangles formed by the data point, the point on the line corresponding to the x value, and the point on the line corresponding to the y value (McArdle 1988). Geometric Mean slope, b_{GM}, was estimated by

(5) $$b_{GM} = s_y / s_x$$ (Jolicoeur 1975)

where s_y = standard deviation of L_{t+1}, and s_x = standard deviation of L_t from a clam. Intercept was calculated by substituting b_{GM}, mean L_{t+1} and mean$_t L$ in eq. 2. Von Bertalanffy parameters, L_∞ and k,were obtained from substitutions of the Walford Plot parameter estimates into eqs. 3 and 4. Thus, an individual von Bertalanffy model was produced for each clam, which was then used to derive a growth curve for ages 0 - 12 years.

B.4. Goodness of Fit of Derived Growth Patterns

To assess how well von Bertalanffy models describe actual growth patterns, total lengths of shells were compared to lengths predicted by the fitted model for the clam at its age of capture. For each shell, total length was measured using digital calipers. Predicted length was calculated with eq. 1, the clam's age, and the estimates of L_∞ and k. Predicted length at time t was then regressed against observed length at time t, and the strength of the relationship evaluated in terms of slope and r^2.

The relationships between predicted length and observed total length of the shells collected from each of the eight study sites are shown in Fig. B.2. Slopes ranged from 0.80 to 1.1 (except for site 10, which had slope equal to 0.69), indicating that total lengths were generally underestimated by the models. Sites 4, 5 and 11 showed the strongest predicted -

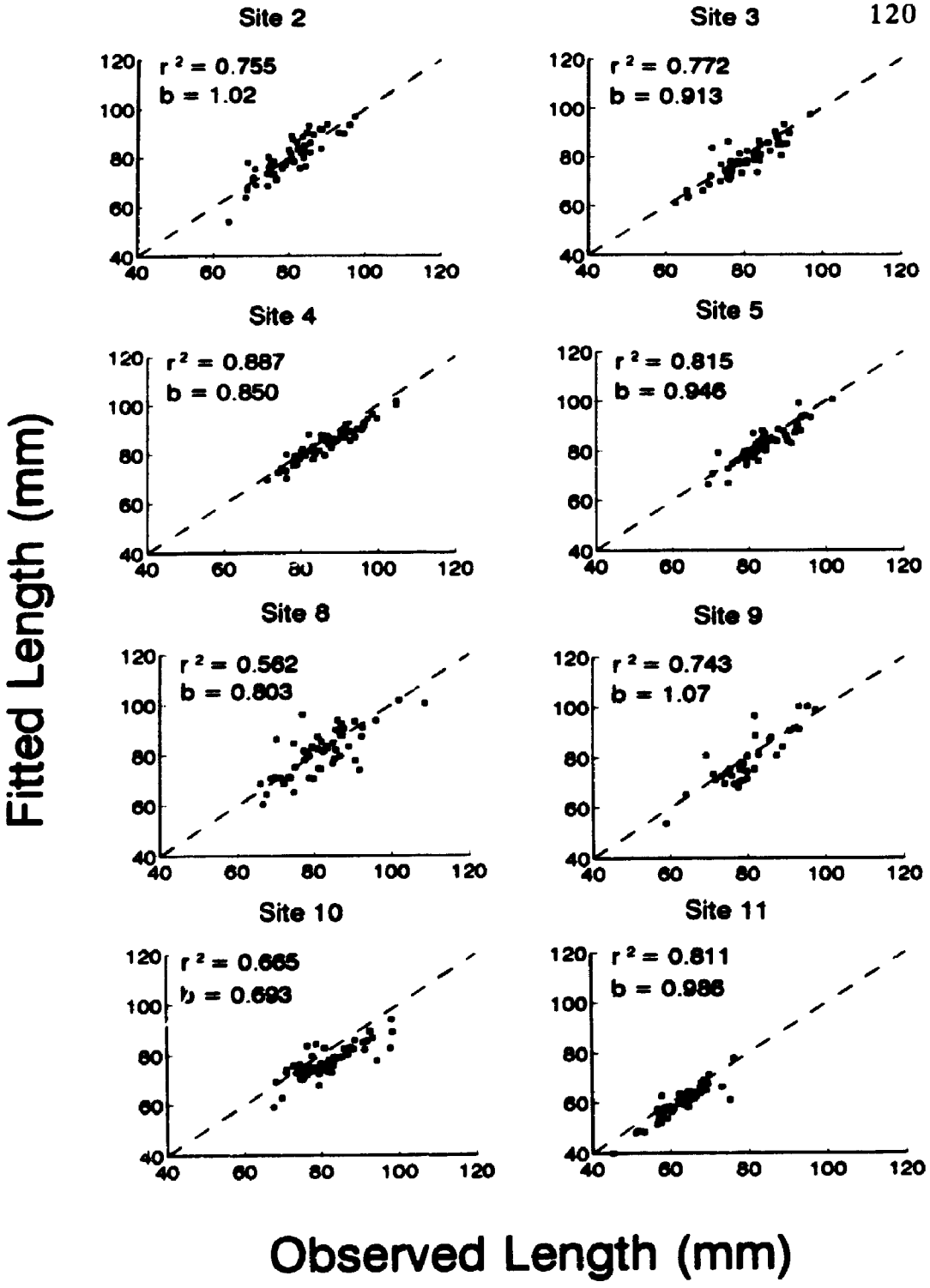


Fig. B.2. Comparison of observed len[illegible] at capture with age-specific lengths fitted from von Bertalanffy m[illegible]els for *Lampsilis radiata* from eight sites in the St. Lawrence River. Estimates of slope (b) and r^2 are from ordinary least square regressions. Dashed lines have slopes = 1, y-intercepts = 0.

to - observed length relationships (r^2= 81 - 88%), and sites 8 and 10 showed the weakest (r^2= 56 and 67%, respectively).

Out of the 421 shells that were old enough for growth analysis, five gave 'invalid' results (estimated $b > 1$ for Walford Plot regression, which implies an ever increasing rate of growth); and eight gave 'unrealistic' estimates ($L_\infty > 200$ mm, which greatly exceeds observed maximum sizes of *L. r. radiata* (105 mm) and *L. r. siliquoidea* (140 mm) according to Clarke 1981)). Estimated and observed lengths were in agreement for 6 out of the 8 sites, based on slope and r^2. Among groups, differences in mean predicted and mean observed length ranged from 0.14 mm (site 2) to 3.98 mm (site 10), which are comparatively small in relation to the range of mean estimated maximum lengths for site groups (74.7 to 133 mm).

B.5. Sources of Bias and Imprecision

Individuals showing poorest agreement between predicted and observed length at capture usually had few ring pairs available for estimation of Walford Plot parameters. Groups from sites 8 and 10, which had the lowest mean proportion of measured-to-total rings, were the worst for predicting shell length at capture (Fig. B.2). Thus, the more ring pairs from a shell measured for growth pattern analysis, the lower the bias for individual estimates and the greater the precision for group mean estimates.

In some applications of the Walford Plot method to fit growth models to a population, only one pair of measurements are made on each individual. A single regression is then calculated for the population, which assumes common L_∞ and k for all individuals. Sainsbury (1980) noted several problems of using this approach with eq. 1 and 2, chief among them being bias in estimates of L_∞ and k. Because the method used in the present study estimates model parameters for each clam (thus avoiding the assumption of common parameters for the populations), such bias should not be important.

Serial correlation of errors resulting from the reuse of rings (L_{t+1} in one pair used as L_t in the next pair) is another potential source of error. Its importance should be proportional to the degree that ring lengths deviate from the growth model.

LITERATURE CITED

Allan, R.J. 1989. Factors affecting sources and fate of persistent toxic organic chemicals: examples from the Laurentian Great Lakes. Pp. 219-248 *in* Boudou, A. and F. Ribeyre [eds.], Aquatic toxicology: Fundamental concepts and methodologies, Vol. 1. CRC Press, Boca Raton, Florida.

Amyot, J.-P. and J.A. Downing. 1991. Endo- and epibenthic distribution of the unionid mollusc *Elliptio complanata*. J. N. Am. Benthol. Soc. 10: 280-285.

Anderson, J. 1990. St. Lawrence River environmental investigations, Vol. 4: Assessment of water and sediment quality in the Cornwall area of the St. Lawrence River, 1985. Report PIBS 1292, Ontario Ministry of the Environment, Water Resources Branch.

Anderson, J. and J. Biberhofer. 1991. Water and suspended sediment quality in the St. Lawrence River at Cornwall/Massena: 1988 Data Report. St. Lawrence River Remedial Action Plan, Technical Report No. 2.

Bailey, R.C. 1987. Variation in the shell morphology and growth rate of *Lampsilis radiata*, a freshwater mussel. Ph.D. Thesis, University of Western Ontario, London, Ontario, Canada.

Bailey, R.C. and R.H. Green. 1988. Within-basin variation in the shell morphology and growth rate of a freshwater mussel. Can. J. Zool. 66: 1704-1708.

Batley, G.E. 1983. Current status of metal speciation studies in natural waters. Pp. 17-30 *in* Leppard, G.G. [ed.], Trace element speciation in surface waters and its ecological implications. Plenum, New York.

Bayne, B.L. 1987. Genetic aspects of physiological adaptation in bivalve molluscs. Pp. 169-189 *in* Calow, P. [ed.], Evolutionary physiological ecology. Cambridge University Press, Cambridge.

Bayne, B.L. 1989. Measuring the biological effects of pollution: the mussel watch approach. Wat. Sci. Tech. Vol. 21: 1089-1100.

Bayne, B.L., D.A. Brown, K. Burns, D.R. Dixon, A. Ivanovici, D.R. Livingston, D.M. Lowe, M.N. Moore, A.R.D. Stebbing and J. Widdows. 1985. The effects of stress and pollution on marine animals. Praeger Publishers, New York.

Bayne, B.L., M.N. Moore, J. Widdows, D.R. Livingstone and P. Salkeld. 1979. Measurement of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs. Philos. Trans. R. Soc. London, Ser.B. 286: 563-581.

Bayne, B.L., P.N. Salkeld and C.M. Worrall. 1983. Reproductive effort and value in different populations of the marine mussel, *Mytilus edulis* L. Oecologia (Berl.) 59: 18-26.

Beanlands, G.E. and P.N. Duinker. 1983. An ecological framework for environmental impact assessment in Canada. Institute for Resource and Environmental Studies, Dalhousie University, Halifax, N.S.

Belanger, S.E., J.L. Farris, D.S. Cherry and J. Cairns, Jr. 1986. Growth of Asiatic clams (*Corbicula* sp.) during and after long-term zinc exposure in field-located and laboratory artificial streams. Arch. Environ. Contam. Toxicol. 15: 427-434.

Belanger, S.E., J.L. Farris, D.S. Cherry and J. Cairns, Jr. 1990. Validation of *Corbicula fluminea* growth reductions induced by copper in artificial streams and river systems. Can. J. Fish. Aquat. Sci. 47: 904-914

Benton, M.J. and S.I. Guttman. 1990. Relationship of allozyme genotype to survivorship of mayflies (*Stenonema femoratum*) exposed to copper. J. N. Am. Benthol. Soc. 9: 271-276.

Benton, M.J. and S.I. Guttman. 1992. Allozyme genotype and differential resistance to mercury pollution in the caddisfly, *Nectopsyche albida*. I. Single-locus genotypes. Can. J. Fish. Aquat. Sci. 49: 142-146.

Berger, E.M. 1983. Population genetics of marine gastropods and bivalves. Pp. 563-596 *in* Russell-Hunter, W.D. [ed.], The mollusca, Vol. 6: Ecology. Academic Press, New York

Brousseau, D.J. 1977. Spawning cycle, fecundity, and recruitment in a population of soft-shell clam, *Mya arenaria*, from Cape Ann, Massachusets. Fishery Bulletin 76: 155-166

Burch, J.B. 1975. Freshwater unionacean clams (Mollusca:Pelecypoda) of North America Malacological Publications, Hamburg, Michigan.

Burky, A.J. 1983. Physiological ecology of freshwater bivalves. Pp 281-327 *in* Russel-Hunter, W.D. [ed.], The mollusca, Vol.6: Ecology. Academic Press, New York.

Cairns, J.,Jr. and J.R. Pratt. 1993. A history of biological monitoring using benthic macroinvertebrates. Pp. 10-27 *in* Rosenberg, D.M. and V.H. Resh [eds.], Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.

Calow, P. 1991. Physiological costs of combating chemical toxicants. ecological implications. Comp Biochem. Physiol. Vol. 100C: 3-6.

Campbell, P G.C. and A. Tessier. 1989. Geochemistry and bioavailability of trace metals in sediments. Pp. 125-150 *in* Boudou, A. and F. Ribeyre [eds.], Aquatic toxicology: Fundamental concepts and methodologies, Vol. 1. CRC Press, Boca Raton, Florida.

Chagnon, N.L. and S.I. Guttman. 1989. Differential survivorship of allozyme genotypes in mosquitofish populations exposed to copper or cadmium. Environ. Toxicol. Chem. 8: 319-326.

Chamberlain, T.K. 1931. Annual growth of fresh-water mussels. Bull. U.S. Bur. Fish. 46: 713-739.

Cherr, G.N., J. Shoffner-McGee and J.N. Shenker. 1990. Methods for assessing fertilization and embryonic/lar al developr ent in toxicity tests using the California mussel (*Mytilus californicus*). Environ. Toxicol. Chem. 9: 1137-1145.

Clarke, A.H. 1981. The freshwater molluscs of Canada. National Museums of Canada, Ottawa, Ontario.

Clarke, A.H. and C.O. Berg. 1959. The freshwater mussels of central New York: with an illustrated key to the species of northeastern North America. Cornell University Agricultural Experiment Station Memoir 367.

Coker, R.E., A.F. Shira, H.W. Clarke and A.D. Howard. 1922. Natural history and propagation of fresh-water mussels. Bull. U.S. Bur. Fish. 37:75-181.

Crow, J.F. 1986. Basic concepts in population. quantitative, and evolutionary genetics. W.H. Freeman and Company, New York.

Cunningham, P.A. 1979. The use of bivalve molluscs in heavy metal pollution research. Pp. 182-221 *in* Vernberg, W.B., A. Calabrese, F.P. Thurnber and F.J. Vernberg [eds.], Marine pollution: functional responses. Academic Press, New York.

Davis, G.M. and S.L.H. Fuller. 1981. Genetic relationships among recent Unionacea (Bivalvia) of North America. Malacologia 20: 217-253.

Day, M.E. 1984. The shell as a recording device: growth record and shell ultrastructure of *Lampsilis radiata radiata* (Pelecypoda: Unionidae). Can. J. Zool. 62: 2495-2504.

Désilets, L. and C. Langlois. 1989. Variabilité spatiale et saisonnière de la qualité de l'eau du fleuve Saint-Laurent. Environnement Canada, Montreal, Quebec.

Diamond, S.A., M.C. Newman, M. Mulvey, P.M. Dixon and D. Martinson. 1989. Allozyme genotype and time to death in mosquitofish, *Gambusia affinis* (Baird and Girard), during acute exposure in inorganic mercury. Environ. Contam. Toxicol. 8: 613-622.

Dickie, L.M., P.R. Boudreau and K.R. Freeman. 1984. Influences of stock and site on growth and mortality in the blue mussel (*Mytilus edulis*). Can. J. Fish. Aquat. Sci. 41: 134-140.

Doherty, F.G., D.S. Cherry and J. Cairns, Jr. 1987. Valve closure responses of the Asiatic clam *Corbicula fluminea* exposed to cadmium and zinc. Hydrobiologia 153: 159-167.

Downing, W.L., J. Shostell and J. Downing. 1992. Non-annual external annuli in the freshwater mussels *Anodonta grandis grandis* and *Lampsilis radiata siloquoidea*. Freshwater Biology 28: 309-318.

Duncan, G.A. and G.G. LaHaie. 1979. Size analysis procedures used in the Sedimentology Laboratory, NWRI, CCIW, Hydraulics Division Manual, September 1979.

Eadie, B.J., C.P. Rice and W.A. Frez. 1983. The role of the benthic boundary in the cycling of PCBs in the Great Lakes. Pp. 213-228 *in* Mackay, D., S. Paterson, S.J. Eisenreich and M.S. Simmons [eds.], Physical behavior of PCBs in the Great Lakes. Ann Arbor Science, Ann Arbor, Michigan.

Eager, R.M.C. 1978. Shape and function of the shell: a comparison of some living and fossil bivalve molluscs. Biol. Rev. 53: 169-210.

Eisenreich, S.J., P.D. Chapel and B.B. Looney. 1983. PCB dynamics in Lake Superior. Pp. 181-211 *in* Mackay, D., S. Paterson, S.J. Eisenreich and M.S. Simmons [eds.], Physical behavior of PCBs in the Great Lakes. Ann Arbor Science, Ann Arbor, Michigan.

Elder, J.F. and J.J. Collins. 1991. Freshwater molluscs as indicators of bioavailability and toxicology of metals in surface-water systems. Rev. Environ. Contam. Toxicol. 122: 37-79.

Environnement Canada. 1985. Inventaire des connaissances sur les sources de pollution dans le fleuve Saint-Laurent, Tronçon Cornwall-Sorel. service de la protection de l'environnement, Montréal, Québec. 68 p.

Foster, R.B. and J.M. Bates. 1978. Use of freshwater mussels to monitor point source industrial discharges. Environ. Sci. Technol. 12: 958-962.

Fuller, S.L.H. 1974. Clams and mussels (Mollusca:Bivalvia). Pp. 215-274 *in* Hart, C.W. and S.L.H. Fuller [eds.], Pollution ecology of freshwater invertebrates. Academic Press, New York.

George, S.G. and A. Viarengo. 1985. A model of heavy metal homeostasis and detoxification in mussels. Pp. 125-143 *in* Vernberg, F.J., F.P. Thurberg, A. Calabrese and W. Vernberg [eds.], Marine pollution and physiology: recent advances. Univ. of South Carolina Press, Columbia, SC.

Gilbertson, M., T. Kubiak, J. Ludwig and G. Fox. 1991. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. J. Toxicol. Environ. Health 33: 455-520.

Gillespie, R.B. and S.I. Guttman. 1989. Effects of contaminants on the frequencies of allozymes in populations of the central stoneroller. Environ. Toxicol. Chem. 8: 309-317.

Green, R.H. 1972. Distribution and morphological variation of *Lampsilis radiata* (Pelecypoda, Unionidae) in some central Canadian lakes: a multivariate statistical approach. J. Fish. Res. Board Can. 29: 1565-1570.

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

Green, R.H., S.M. Singh and R.C. Bailey. 1985. Bivalve molluscs as response systems for modelling spatial and temporal environmental patterns. Sci. Total. Environ. 46: 147-16.

Hallam, A. 1965. Environmental causes of stunting in living and fossil benthonic invertebrates. Palaeontology 8: 132-155.

Harrison, F.L., J.P. Knezovich and D.W. Rice, Jr. 1984. The toxicity of copper to the adult and early life history stages of the freshwater clam, *Corbicula manilensis*. Arch. Environ. Contam. Toxicol. 13: 85-92.

Haux, C. and L. Förlin. 1988. Biochemical methods for detecting effects of contaminants on fish. Ambio 17: 376-380.

Havlik, M.E. and L.L. Marking. 1987. Effects of contaminants on naiad mollusks (Unionidae): a review. U.S. Fisn Wildl. Serv., Resour. Publ. 164. 20 pp.

Heagler, M.G., M.C. Newman, M. Mulvey and P.M. Dixon. 1993. Allozyme genotype in mosquitofish, *Gambusia holbrooki*, during mercury exposure: temporal stabilty, concentration effects and field verification. Environ. Toxicol. Chem. 12: 385-395.

Hebert, P.D.N. 1986. Cellulose acetate methodology. Unpublished monograph. Department of Biology. University of Windsor.

Hellawell, J.M. 1986. Biological indicators of freshwater pollution and environmental management. Elsevier Applied Science Publishers, New York.

Hinch, S.G. and R.C. Bailey. 1988. Within- and among-lake variation in shell morphology of the freshwater clam *Elliptio complanata* (Bivalvia: Unionidae) from south-central Ontario lakes. Hydrobiologia 157: 27-32

Hinch, S.G., R.C. Bailey and R.H. Green. 1986. Growth of *Lampsilis radiata* (Bivalvia. Unionidae) in sand and mud: a reciprocal transplant experiment. Can. J. Fish. Aquat. Sci. 43: 548-552.

Hinch, S.G. and R.H. Green. 1989. The effects of source and destination on growth and metal uptake in freshwater clams reciprocally transplanted among south central Ontario lakes. Can. J. Zool. 67: 855-863.

Hinch, S.G., L.J. Kelly and R.H. Green. 1989. Morphological variation of *Elliptio complanata* (Bivalvia: Unionidae) in differing sediments of soft-water lakes exposed to acidic deposition. Can. J. Zool. 67: 1895-1899.

Huebner, J.D. and K.S. Pynnönen. 1992. Viability of glochidia of two species of *Anodonta* exposed to low pH and selected metals. Can. J. Zool. 70: 2348-2355.

Hummel, H. and T. Patarnello. 1994. Genetics effects of pollutants on marine and estuarine invertebrates. Pp. 425-434 *in* Beaumont, A.R. [ed.], Genetics and evolution of aquatic organisms. Chapman and Hall, London.

Imlay, M.J. 1982. Use of shells of freshwater mussels in monitoring heavy metals and environmental stresses: a review. Malacological Reviews 15: 1-14.

Isley, F.B. 1914. Experimental study of the growth and migration of fresh-water mussels. Appendix III, Report U.S. Commissioner of Fisheries for 1913, Washington, DC.

Jacobson, P.J., J.L. Farris, D.S. Cherry and R.J. Neves. 1993. Juvenile freshwater mussel (Bivalvia: Unionidae) responses to acute toxicity testing with copper. Environ. Co Toxicol. 12: 879-883.

Johnson, R.K., T. Wiederholm and D.M Rosenberg. 1993. Freshwater biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates. Pp. 40-158 *in* Rosenberg, D.M. and V.H. Resh [eds.], Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.

Jolicoeur, P. 1975. Linear regression in fisheries research: some comments. J. Fish. Res. Board Can. 32: 1491-1494.

Jones, D. 1984 Use, misuse, and role of multiple-comparison procedures in ecological and agricultural entomology. Environ. Entomol. 13: 635-649

Karr, J.R. 1993. Defining and assessing ecological integrity: beyond water quality. Environ. Toxicol Chem. 12: 1521-1531.

Kat, P.W. 1982. Effects of population density and substratum type on growth and migration of *Elliptio complanata* (Bivalvia: Unionidae). Malacol. Rev. 15: 119-127.

Kat, P W. 1984. Parasitism and the Unionacea (Bivalvia). Biol. Rev. 59: 189-207.

Kat, P.W. 1986. Hybridization in a unionid faunal suture zone. Malacologia 27: 107-125.

Kauss, P.B., Y.S. Hamdy and B.S. Hamma. 1988. St. Lawrence River Environmental Investigations. Vol. 1. Background: Assessment of water, sediment and biota in the Cornwall, Ontario and Massena, New York section of the St. Lawrence River, 1979 - 1982. Ontario Ministry of the Environment, Toronto, Ontario.

Keller, A. E. and S.G. Zam. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anondonta imbecilis*. Environ. Contam. Toxicol. 10: 539-546.

Koehn, R.K. and B.L. Bayne. 1989. Towards a physiological and genetical understanding of the energetics of the stress response. Biol. J. Linn. Soc. 37: 157-171.

Kramer, V.J., M.C. Newman, M. Mulvey and G.R. Ultsch. 1992. Glycolysis and Krebs cycle metabolites in mosquitofish, *Gambusia holbrooki*, Girard 1859, exposed to mercuric chloride: allozyme genotype effects. Environ. Contam. Toxicol. 11: 357-364.

Lefevre, G. and W. C. Curtis. 1910. Reproduction and parasitism in the Unionidae. J. Exp. Zool. 9: 79-115.

Leland, H.V. and J.S. Kuwabara. 1985. Trace Metals. Pp. 374-415 *in* Rand, G.M. and S.R. Petrocelli [eds.], Fundamentals of aquatic toxicology: methods and applications. Hemisphere Publishing Corp., Washington.

Luoma, S.N. 1983. Bioavailability of trace metals to aquatic organisms - a review. Sci. Total Environ. 28: 1-22.

Luoma, S.N. 1989. Can we determine the biological availability of sediment-bound trace elements? Hydrobiologia 176/177: 379-396.

Luoma, S.N. and J.L. Carter. 1991. Effects of trace metals on aquatic benthos. Pp. 261-300 *in* Newman, M.C. and A.W. McIntosh [eds.], Metal ecotoxicology: concepts and applications. Lewis Publishers, Chelsea, Michigan.

Lutz, R.A. and D.C. Rhoads. 1980. Growth patterns within the molluscan shell. an overview Pp. 203-254 *in* Rhoads, D.C. and R.A. Lutz [eds.], Skeletal growth of aquatic organisms. biological records of environmental change. Plenum Press, New York.

Mackie, G.L. 1984. Bivalves. Pp. 351-418 *in* Tompa, A.S., N.H. Verdonk and J.A.M. van den Biggelaar [eds.], The mollusca, Vol. 7: Reproduction. Academic Press, New York.

Mackie, G.L. and L.A. Flippance. 1983. Relationships between buffering capacity of water and the size and calcium content of freshwater mollusks. Freshw. Invertebr. Biol. 2: 48-55.

Magnin, E. and A. Stanczykowska. 1971. Quelques données sur la croissance la biomasse et la production annuelle de trois mollusques Unionidae de la région de Montréal. Can. J. Zool. 49:491-497.

Mallet, A.L., C.E.A. Carver, S.S. Coffen and K.R. Freeman. 1987. Winter growth of the blue mussel *Mytilus edulis* L.: importance of stock and site. J. Exp. Mar. Biol. Ecol. 108: 217-228.

McArdle, B.H. 1988. The structural relationship: regression in biology. Can. J. Zool. 66: 2329-2339.

McCuaig, J.M. and R.H. Green. 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.

McIntosh, A. 1991. Trace metals in freshwater sediments: a review of the literature and an assessment of research needs. Pp. 243-260 *in* Newman, M.C. and A.W. McIntosh [eds.], Metal ecotoxicology: concepts and applications. Lewis Publishers, Chelsea, Michigan.

McIntyre, A.D. 1984. What happened to biological effects monitoring? Mar. Pollut. Bull. 15. 391-392.

McKim, J.M. 1984. Early life stage toxicity tests. Pp. 58-95 *in* Rand, G.M. and S.R. Petrocelli [eds.], Fundamentals of aquatic toxicology. Hemisphere Publishing Corp., New York.

McMahon, R.F. 1991. Mollusca:Bivalvia. Pp. 315-399 *in* Thorp, J.H. and A.P. Covich [eds.], Ecology and classification of North American freshwater invertebrates. Academic Press, Inc., San Diego, CA.

Metcalfe, J.L. and M.N. Charlton. 1990. Freshwater mussels as biomonitors for organic contaminants and pesticides in the St. Lawrence River. Sci. Total Environ. 97/98: 595-615.

Metcalfe-Smith, J.L. 1994. Influence of species and sex on metal residues in freshwater mussels (family Unionidae) from the St. Lawrence River, with implications for biomonitoring programs. Environ. Toxicol. Chem. 13: 1433-1443.

Metcalfe-Smith, J.L., R.H. Green and L.C. Grapentine. (in press). Influence of biological factors on the bioaccumulation of metals by *Elliptio complanata* and *Lampsilis radiata radiata* (Bivalvia: Unionidae) from the St. Lawrence River. Can. J. Fish. Aquat. Sci.

Millington, P.J. and K.F. Walker. 1983. Australian freshwater mussel *Velesunio ambiguus* (Philippi) as a biological monitor for zinc, iron and manganese. Aust. J. Mar. Freshwater Res. 34: 873-892.

Milstein, C. 1961. Inhibition of phosphoglucomutase by trace metals. Biochem. J. 79: 591-596.

Montero, C., B. Battaglia, M. Stenico, G. Campesan and T. Paternello. 1994. Effects of heavy metals and temperature on the genetic structure and GPI enzyme activity of the barnacle *Balanus amphitrite* Darwin (Cirripedia:Thoracica). Pp. 447-459 *in* Beaumont, A.R. [ed.], Genetics and evolution of aquatic organisms. Chapman and Hall, London.

Moore, J.W. and S. Ramamoorthy. 1984. Heavy metals in natural waters. Springer-Verlag, New York.

Mulvey, M. and S.A. Diamond. 1991. Genetic factors and tolerance acquisition in populations exposed to metals and metalloids Pp. 301-321 *in* Newman, M.C. and A.W. McIntosh [eds.], Metal ecotoxicology: concepts and applications. Lewis Publishers, Chelsea, Michigan.

National Laboratory for Environmental Testing. 1994. Manual of analytical methods. Vol. 2: Trace metals. Environment Canada, Burlington, Ontario.

Negus, C.L. 1966. A quantitative study of growth and production of unionid mussels in the River Thames at Reading. J. Anim. Ecol. 35: 513-532.

Nettleton, P. 1988. Great Lakes Section, Ontario Ministry of the Environment, Toronto, Ontario. Personal communication.

Neves, R.J. and S.N. Moyer. 1988. Evaluation of techniques for age determination of freshwater mussels (Unionidae). Am. Macol. Bull. 6: 179-188.

Nevo, E. 1993. Complex pollution effects of two heavy metals (mercury and cadmium) the genetic structure of populations. Pp. 45-63 *in* MAP Tech. Rep. Series, No. 48: Final Reports on Research Projects (Activity G). UNEP Mediterranean Action Plan, Athens, Greece.

Nevo, E., R. Ben-Shlomo and B. Lavie. 1984. Mercury selection of allozymes in marine organisms: prediction and verification in nature. Proc. Natl. Acad. Sci. USA. 81: 1258-1259.

Nevo, E., B. Lavie and R. Ben-Shlomo. 1983. Selection of allelic isozyme polymorphisms in marine organisms: pattern, theory, and application. Pp. 69-92 *in* Rattazzi, M.C. and G.S Whitt [eds.], Isozymes: current topics in biological and medical research. Vol. 10 Genetics and Evolution. Alan R. Liss, Inc., New York.

Nriagu, J.O. 1989. Preface. Pp. ix-xi *in* Boudou, A. and F. Ribeyre [eds.], Aquatic toxicology: Fundamental concepts and methodologies, Vol. 1. CRC Press, Boca Raton, Florida.

Ontario Ministry of the Environment [OME] 1991. Municiple - Industrial Strategy for Abatement (MISA): Preliminary report on the first six months of process effluent monitoring in the MISA pulp and paper sector (January 1, 1990 to June 30, 1990). Report PIBS 1436. Water Resources Branch, Toronto, Ontario.

Ontario Ministry of the Environment [OME] 1992 Municiple - Industrial Strategy for Abatement (MISA): Six-month monitoring data report: organic chemical manufacturing sector (Period covered October 01, 1989 to March 31, 1990). Report PIBS 1815. Water Resources Branch, Toronto, Ontario.

Patarnello, T., R. Guinez and B. Battaglia. 1991. Effect of pollution on heterozygosity in the barnacle *Balanus amphitrite* (Cirripedia: Thoracica). Mar. Ecol. Progr. Ser. 70: 237-243.

Persaud, D., R. Jaagumagi, and A. Hayton. 1992. The provincial sediment quality guidelines. [draft] Water Resources Branch, Ontario Ministry of the Environment, Toronto, Ontario.

Peterson, C.H. and B.F. Beal. 1989. Bivalve growth and higher order interactions: importance of density, site, and time. Ecology 70: 1390-1404.

Phillips, D.J.H. 1977. The use of biological indicator organisms to monitor trace element pollution in marine and estuarine environments - a review. Environ. Pollut. 13: 281-317.

Phillips, D.J.H. 1980. Quantitative aquatic biological indicators: their uses to monitor trace metal and organochlorine pollution. Applied Science Publishers Ltd., London.

Picard, A. 1989. Whales dying in toxic St. Lawrence. Troubled waters: The St. Lawrence, Part 1. The Globe and Mail. August 14: A1, A9.

Podemski, C.L. 1992. Growth and morphology of a freshwater mussel in relation to benthic infaunal production. M.Sc. Thesis, University of Western Ontario, London, Ontario, Canada.

Rhoads, D.C. and R.A. Lutz. 1980. Skeletal records of environmental change. Pp. 1-19 *in* Rhoads, D.C. and R.A. Lutz [eds.], Skeletal growth of aquatic organisms: biological records of environmental change. Plenum Press, New York.

Richardson, B.J., P.R. Baverstock and M. Adams. 1986. Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, Sydney.

Richman, L.A. 1994. St. Lawrence River sediment and biological assessment, 1991. Report PIBS 3146. Ontario Ministry of Environment and Energy, Environmental Monitoring and Reporting Branch.

Rodgers, J.H. Jr., D.S. Cherry, R.L. Graney, K.L. Dickson and J. Cairns, Jr. 1980. Comparison of heavy metal interactions in acute and artificial stream bioassay techniques for the Asiatic clam (*Corbicula fluminea*). Pp. 266-280 *in* Eaton, J.G., P.R. Parrish and A.C. Hendricks [eds.], Aquatic toxicology. American Society for Testing and Materials, Spec. Tech. Publ. 707. Philadelphia, PA.

Roesijadi, G. and W.E. Robinson. 1994. Metal regulation in aquatic animals: mechanisms of uptake, accumulation, and release. Pp. 387-420 *in* Malins, D.C. and G.K. Ostrander (eds.), Aquatic toxicology: molecular, biochemical, and cellular perspectives. Lewis Publishers, Boca Raton, Florida.

Rosenberg, D.M. and V.H. Resh. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. Pp. 1-9 *in* Rosenberg, D.M. and V.H. Resh [eds.], Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.

Safe, S. 1984. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanism of action. CRC Crit. Rev. Toxicol. 13: 319-395.

Sainsbury, K.J. 1980. Effect of individual variability on the von Bertalanffy growth model. Can. J. Fish. Aquat. Sci. 37: 241-247.

Seed, R. 1980. Shell growth and form in Bivalvia. Pp. 23-67 *in* Rhoads, D.C. and R.A. Lutz [eds.], Skeletal growth of aquatic organisms: biological records of environmental change. Plenum Press, New York.

Simkiss, K. and A.Z. Mason. 1983. Metal ions: metabolic and toxic effects. Pp. 101-164 *in* Hochachka, P.W. [ed.], The mollusca, Vol.2: Environmental biochemistry and physiology. Academic Press, New York.

Silverman, H., W.T. Kays and T.H. Dietz. 1987. Maternal calcium contribution to glochidial shells in freshwater mussels (Eulamellibranchia: Unionidae). J. Exp. Zool. 242: 137-146.

Singh, S.M. and E. Zouros. 1978. Genetic variation associated with growth rate in the american oyster (*Crassostrea virginica*). Evolution 32: 342-353.

Skibinski, D.O.F. and E.E. Roderick. 1989. Heterozygosity and growth in transplanted mussels. Mar. Biol. 102: 73-84.

Soares, A.M.V.M., D.J. Baird and P. Calow. 1992. Interclonal variation in the performance of *Daphnia magna* Strauss in chronic bioassays. Environ. Contam. Toxicol. 11: 1477-1483.

Sprung, M. 1983. Reproduction and fecundity of the mussel *Mytilus edulis* at Helgoland (North Sea). Helgoländer Meeresunters. 36: 243-255.

St. Lawrence River Remedial Action Plan Team [SLRRAPT]. 1992. Remedial action plan for the Cornwall-Lake St. Francis area, stage 1 report: environmental conditions and problem definitions. Environment Canada, Environment Ontario and Ontario Ministry of Natural Resources.

Strayer, D. 1987. Ecology and zoogeography of the freshwater mollusks of the Hudson River basin. Malacological Review 20: 1-68.

Swofford, D.L. and R.B. Selander. 1981. BIOSYS-1: A Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72: 281-283.

Tessier, A. and P.G.C. Campbell. 1987. Partitioning of trace metals in sediments: relationships with bioavailability. Hydrobiologia 149: 43-52.

Tessier, A., P.G.C. Campbell, J.C. Auclair and M. Bisson. 1984. Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area. Can. J. Fish. Aquat. Sci. 41. 1463-1472.

Tessier, A., R. Carignan, B. Dubreuil and F. Rapin. 1989. Partitioning of zinc between the water columns and the oxic sediments in lakes. Geochim. Cosmochim. Acta 53: 1511-1522.

Tevesz, M.J.S. and J.G. Carter. 1980. Environmental relationships of shell form and structure in unionacean bivalves. Pp. 295-322 *in* Rhoads, D.C. and R.A. Lutz [eds.], Skeletal growth of aquatic organisms: biological records of environmental change. Plenum Press, New York.

Thomann, R.V. and J.A. Mueller. 1983. Steady state modeling of toxic chemicals - theory and application to PCBs in the Great Lakes and Saginaw Bay. Pp. 283-309 *in* Mackay, D., S. Paterson, S.J. Eisenreich and M.S. Simmons [eds.], Physical behavior of PCBs in the Great Lakes. Ann Arbor Science, Ann Arbor, Michigan.

Underwood, A.J. and C.H. Peterson. 1988. Towards an ecological framework for investigating pollution. Mar. Ecol. Prog. Ser. 46: 227-234.

Vermeij, G.J. and E.C. Dudley. 1985. Distribution of adaptations: a comparison between the functional shell morphology of freshwater and marine pelecypods. Pp. 461-478 *in* Trueman, E.R. and M.R. Clarke [eds.], The mollusca, Vol. 10: Evolution. Academic Press, New York.

Warren, L.A. and A.P. Zimmerman. 1993. Trace metal-suspended particulate matter associations in a fluvial system: physical and chemical influences. Pp. 127-155 *in* Rao, S.S. [ed.], Particulate matter and aquatic contaminants. Lewis Publishers, Boca Raton, Florida.

Warren, L.W., S.J. Klaine and M.T. Finley. 1995. Development of a field bioassay with juvenile mussels. J. N. Am. Benthol. Soc. 14: 341-346.

Yeats, P.A. and J. M. Bewers. 1982. Discharge of metals from the St. Lawrence River. Can. J. Earth Sci. 19: 982-992.

Yeager, M.M., D.S. Cherry and R.J. Neves. 1994. Feeding and burrowing behaviors of juvenile mussels, *Villosa iris* (Bivalvia:Unionidae) J. N. Am. Benthol. Soc. 13: 217-222.

Zouros, E.E., M. Romeo-Dorey and A.L. Mallet. 1988. Heterozygosity and growth in marine bivalves: further data and possible explanations. Evolution 42: 1332-1341.