AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

Technical Evaluation of Molluscs as a Biomonitoring Tool for the Canadian Mining Industry

AETE Project 2.3.1

TECHNICAL EVALUATION OF MOLLUSCS AS A BIOMONITORING TOOL FOR THE CANADIAN MINING INDUSTRY

Sponsored by:

Canada Center for Mineral and Energy Technology (CANMET)
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on Behalf of:

Aquatic Effects Technology Evaluation (AETE) Program

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AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

Notice to Readers

Technical Evaluation of Molluscs as a Biomonitoring Tool for the Mining Industry in Canada

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments; it is coordinated by the Canadian Centre for Mineral and Energy Technology (CANMET). The program is designed to be of direct benefit to the industry, and to government. Through technical evaluations and field evaluations, it will identify cost-effective technologies to meet environmental monitoring requirements. The program includes three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring.

The technical evaluations are conducted to document certain tools selected by AETE members, and to provide the rationale for doing a field evaluation of the tools or provide specific guidance on field application of a method. In some cases, the technical evaluations include a go/no go recommendation that AETE takes into consideration before a field evaluation of a given method is conducted.

The technical evaluations are published although they do not necessarily reflect the views of the participants in the AETE Program. The technical evaluation should be considered as working documents rather than comprehensive literature reviews.

The purpose of the technical evaluations is to document specific monitoring tools. AETE committee members would like to note that no one single tool can provide all the information required for a full understanding of environmental effects in the aquatic environment.

Any comments concerning its content should be directed to:

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PROGRAMME D'ÉVALUATION DES TECHNIQUES DE MESURE D'IMPACTS EN MILIEU AQUATIQUE

Avis aux lecteurs

Évaluation technique des mollusques comme indicateurs biologiques en vue de son utilisation par l`industrie minière

Le Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ÉTIMA) vise à évaluer les différentes méthodes de surveillance des effets des effluents miniers sur les écosystèmes aquatiques. Il est le fruit d'une collaboration entre l'industrie minière du Canada, plusieurs ministères fédéraux et un certain nombre de ministères provinciaux. Sa coordination relève du Centre canadien de la technologie des minéraux et de l'énergie (CANMET). Le programme est conçu pour bénéficier directement aux entreprises minières ainsi qu'aux gouvernements. Par des évaluations techniques et des études de terrain, il permettra d'évaluer et de déterminer, dans une perspective coût-efficacité, les techniques qui permettent de respecter les exigences en matière de surveillance de l'environnement. Le programme comporte les trois grands volets suivants : évaluation de la toxicité aiguë et sublétale, surveillance des effets biologiques des effluents miniers en eaux réceptrices, et surveillance de la qualité de l'eau et des sédiments.

Les évaluations techniques sont menées dans le but de documenter certains outils de surveillance sélectionner par les membres de l'ÉTIMA et de fournir une justification pour l'évaluation sur le terrain de ces outils ou de fournir des lignes directrices quant à leur application sur le terrain. Dans certains cas, les évaluations techniques pourraient inclure des recommandations relatives à la pertinence d'effectuer une évaluation de terrain que les membres de l'ÉTIMA prennent en considération.

Les évaluations techniques sont publiées bien qu'elles ne reflètent pas nécessairement toujours l'opinion des membres du l'ÉTIMA. Les évaluations techniques devraient être considérées comme des documents de travail plutôt que des revues de litérature complètes.

Les personnes intéressées à faire des commentaires concernant le contenu de ce rapport sont invitées à communiquer avec M^{me} Diane E. Campbell à l'adresse suivante :

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EXECUTIVE SUMMARY

S.1 Introduction

The Aquatic Effects Technology Evaluation Program, AETE has been established to assist the Canadian mining industry in meeting its environmental effects monitoring and related requirements, in as cost-effective a manner as possible. The program is coordinated by the Canadian Center for Mineral and Energy Technology (CANMET). The present report is a technical evaluation of molluscs as biomonitoring tools for the mining industry.

Molluscs are a diverse taxonomic group that include bivalves and gastropods and are found widely distributed throughout Canada. Molluscs have been widely studied in both the laboratory and the field in order to evaluate their ability to serve as biomonitors of metals in the aquatic environment.

S.2 Evaluation

The present evaluation of molluscs as biomonitors is based on published studies conducted in the laboratory and field on mining contaminants of concern listed in the AQUAMIN Report (1996). Published field studies and reports of studies carried out by individual mining companies and the AETE program were consulted. Criteria for a good biomonitor and the work requirements of the contract were used as guidelines for the evaluation process. The following are the conclusions of this evaluation based on individual criteria.

1. **Biomonitors should be relatively non-mobile in order that their exposure be representative of the study area.** Molluscs are relatively sedentary, although some species (i.e. unionids) may migrate short distances (meters) within their habitat. Bivalve molluscs are amenable to transplanting and caging. The sedentary nature and ease of caging of molluscs are advantages for site-specific monitoring not possessed by most fish.
2. **Biomonitors should be abundant, widely distributed, easy to identify and sample at all times of the year.** Molluscs are widely distributed across Canada and can be identified with limited taxonomic expertise. Limited information on the conservation status of molluscs suggests that most unionid bivalve species are currently stable in Canada.
3. **Biomonitors should be large enough in body size to provide sufficient tissue for analysis.** Individual unionid bivalves are large enough to provide sufficient tissue for

metal analyses. Pooling of individuals of the smaller sphaeriids and most gastropods would be required for tissue metal analysis.

4. **Biomonitors should be hardy, tolerating wide ranges of contaminant concentration and physiochemical variables.** Molluscs are relatively hardy and tolerate wide ranges of metal concentrations, but are limited by pH below 4.7-5.0 and [Ca] below 2-2.5 mg L^{-1}.

5. **Biomonitors should be strong accumulators of metals, with a simple correlation between metal concentrations in mollusc tissue and average ambient metal concentrations.** Molluscs strongly accumulate metals (Cd, Cu, Zn, Pb, Ni, Hg, As, Ag and Cr). Field studies suggest that the relationship between mollusc tissue metal concentrations and ambient metal concentrations is influenced by a number of biological, physical and chemical parameters that need to be taken into account. Ultimately, the relationship is metal specific and depends on the availability of the metal from the dissolved and particulate phase.

S.3 Recommendations

1. Molluscs can be used as indicators of exposure to metals such as Cd, Cu, Zn, Pb, Ni, Hg, As, Cr, and Ag. Metal concentrations in bivalve molluscs could be used to:
   - confirm changes in biologically-relevant metal concentrations in the natural environment resulting from mining activities.
   - monitor long-term spatial and temporal trends in biologically-relevant metal concentrations by increases and decreases in tissue concentrations of indigenous or transplanted populations.
   - determine the effectiveness of remedial measures through the use of transplanted and indigenous molluscs.
2. Molluscs are not stand alone tools. Numerous abiotic and biotic measurements must be made in conjunction with metal concentrations in molluscs to interpret effectively the results of field studies.
3. Metal-induced effects in molluscs such as changes in growth, or MT concentrations are not well established. These responses could be measured as part of the monitoring program, but their results should be used with caution as measures of effect until their role has been validated in field studies using mining contaminants.

4. Molluscs could be used in the first steps of a monitoring program in order to assess the extent of contamination in the aquatic environment. In more detailed information stages, molluscs may be used to investigate specific sources of bioavailable metals, improvements to waste-water treatment and effectiveness of remedial actions.
5. For comprehensive biomonitoring, molluscs can be used in conjunction with several other organisms (e.g. invertebrates, fish, plants) to monitor metals and their effects in the aquatic environment. In this way, information on the "biological consequences" of mining operations can be obtained, that is not available from monitoring sediment and water only.

SOMMAIRE

S.1 Introduction

Le Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ETIMA) a pour objet d'aider l'industrie minière canadienne à surveiller les effets de ses activités sur les écosystèmes aquatiques et à s'acquitter de ses obligations connexes, dans une perspective coût-efficacité. Sa coordination relève du Centre canadien de la technologie des minéraux et de l'énergie (CANMET). Le présent rapport rend compte d'une évaluation technique des mollusques comme indicateurs biologiques pouvant être utilisés par l'industrie minière.

Les mollusques, groupe taxinomique diversifié qui inclut les bivalves et les gastéropodes, sont largement répandus au Canada. Tant sur le terrain qu'en laboratoire, les mollusques ont fait l'objet d'un grand nombre d'études visant à évaluer leur efficacité comme indicateurs biologiques d'une exposition environnementale aux métaux.

S.2 Évaluation

La présente évaluation de l'efficacité des mollusques comme indicateurs biologiques est fondée sur une série de comptes rendus publiés d'études sur des contaminants réalisées en laboratoire et sur le terrain et citées dans le rapport du Programme AQUAMIN (1996). Des publications ayant fait l'objet d'une révision par les pairs et des rapports d'études effectuées par des sociétés minières ou dans le cadre du programme ETIMA ont également été consultés. Les critères d'un bon indicateur biologique et les exigences de travail du contrat ont orienté la présente évaluation. Les conclusions de cette évaluation sont présentées dans les paragraphes qui suivent.

1. **Les indicateurs biologiques doivent être relativement peu mobiles, de manière à ce que leur degré d'exposition soit représentatif du degré d'exposition dans la zone à l'étude.** Les mollusques sont des animaux relativement sédentaires, bien que certaines espèces (c.-à-d., les unionidés) peuvent effectuer de courtes migrations (quelques mètres) dans les milieux où elles vivent. Les bivalves tolèrent bien la transplantation et la mise en cage. Pour la surveillance de sites en particulier, le mode de vie sédentaire des mollusques et leur tolérance

à la mise en cage constituent des atouts importants qui font défaut chez la majorité des espèces de poissons.

2. **Les indicateurs biologiques doivent être abondants, largement répandus, faciles à identifier et faciles à échantillonner en tout temps de l'année :** Les mollusques sont largement répandus au Canada, et leur identification s'avère relativement aisée même pour avec une connaissance limitée de la taxonomie du groupe. Les quelques informations disponibles concernant le statut des mollusques donnent à croire que la situation de la majorité des espèces d'unionidés (bivalves) est stable.
3. **Les indicateurs biologiques doivent être d'une taille suffisante pour fournir les quantités de tissus requises aux fins des analyses.** Les unionidés sont suffisamment grands pour fournir les quantités de tissus requises pour le dosage des métaux. En revanche, les sphaeriidés et la majorité des gastéropodes sont de plus petite taille, si bien qu'il faut regrouper les spécimens pour obtenir les quantités de tissus requises aux fins du dosage des concentrations de métaux.
4. **Les indicateurs biologiques doivent être résistants et tolérer un large éventail de concentrations de contaminants et d'autres paramètres physico-chimiques**. Les mollusques satisfont à ce critère, mais ils ne résistent pas à des pH inférieurs à 4,7 à 5,0 et à des concentrations de Ca inférieures à 2,0 à 2,5 $mg.L^{-1}$.
5. **Les indicateurs biologiques doivent accumuler de fortes concentrations de métaux dans leurs tissus, et il doit exister une corrélation simple entre les concentrations de métaux accumulées dans les tissus des mollusques et les concentrations présentes dans le milieu ambiant.** Les mollusques accumulent de grandes quantités de métaux dans leurs tissus (Cd, Cu, Zn, Pb, Ni, Hg, As, Ag et Cr). Des études réalisées sur le terrain portent à croire qu'un certain nombre de paramètres biologiques, physiques et chimiques influent sur la relation entre les concentrations de métaux accumulées dans les tissus et les concentrations présentes dans le milieu ambiant et méritent à ce titre d'être pris en compte. En bout de ligne, la relation varie en fonction du métal considéré et de sa disponibilité à l'état dissous et particulaire.

S.3 Recommandations

1. Les mollusques peuvent être utilisés comme indicateurs biologiques du degré d'exposition aux métaux Cd, Cu, Zn, Pb, Ni, Hg, As, Cr et Ag. Les concentrations de métaux dans les tissus de mollusques bivalves transplantés ou indigènes peuvent être utilisées aux fins suivantes :
   - confirmer des changements de concentrations de métaux ayant des répercussions biologiques dans les écosystèmes naturels exposés aux effluents miniers;
   - suivre les fluctuations spatio-temporelles à long terme des concentrations de métaux ayant une incidence biologique;
   - évaluer l'efficacité de mesures correctrices.
2. Les mollusques doivent être utilisés en conjonction avec d'autres indicateurs. Pour être en mesure de bien interpréter les résultats des études sur le terrain, il faut examiner les concentrations de métaux dans les tissus des mollusques conjointement avec un grand nombre de paramètres biotiques et abiotiques.
3. Les répercussions de l'exposition aux métaux (p. ex., changements dans le taux de croissance) ou dans la concentration en métallothionéine ne sont pas bien établis chez les mollusques. Ces réponses pourraient être mesurées dans le cadre du programme de surveillance, mais tant que le rôle de ces paramètres n'aura pas été déterminé au moyen de contaminants miniers dans le cadre d'études sur le terrain, il faudra faire preuve de prudence dans l'utilisation des résultats comme mesure des effets.
4. Les mollusques peuvent être utilisés avec succès durant les premières étapes d'un programme de surveillance pour déterminer l'ampleur de la contamination d'un écosystème aquatique. Durant les étapes plus détaillées, on peut s'en servir pour étudier des sources spécifiques de métaux biodisponibles, évaluer l'efficacité des améliorations apportées à un procédé de traitement des eaux usées ou de mesures correctrices.
5. Dans une perspective de surveillance biologique globale, on peut utiliser les mollusques conjointement avec plusieurs autres organismes (p. ex., invertébrés, poissons, plantes) pour surveiller les concentrations de métaux et leurs effets dans les écosystèmes aquatiques. Cette façon de faire permet de recueillir des informations sur les effets biologiques des activités minières qu'on ne peut obtenir uniquement par évaluation de la qualité des sédiments et de l'eau.

1. INTRODUCTION

1.1 Goals of the AETE

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program among the Canadian mining industry, several federal government departments and a number of provincial governments. It is coordinated by the Canadian Center for Mineral and Energy Technology (CANMET) of Natural Resources Canada. One mandate of the AETE is to do a field and technical evaluation of molluscs as a biomonitoring tool for the mining industry in Canada. This report represents the technical component of the evaluation.

1.2 Mining in Canada

Mining, smelting and refining of minerals and metals are associated with a number of environmental concerns (The State of Canada's Environment, 1996). Acid-mine drainage and sulfur dioxide, particulate matter, and heavy metal emissions are considered to pose the largest problems. In order to adequately assess the impacts resulting from present mines (active and abandoned) and estimate the potential impacts from future developments, effective environmental monitoring programs are needed.

The Canadian government's Metal Mine Liquid Effluent Regulations (MMLER) require the monitoring of the quality of liquid effluent, but not that of receiving waters. Monitoring of the receiving environment is usually performed as a result of an environmental assessment or through a Provincial and Territorial program (AQUAMIN, 1996). The development of site-specific environmental effects monitoring programs by mine operators was one of the key recommendations in the Assessment of the Aquatic Effects of Mining in Canada (AQUAMIN) report (AQUAMIN, 1996). It was recommended that the monitoring programs be part of a national monitoring program with consistent study objectives, approaches and methods.

The contaminants that might be included in a national monitoring program for mining would consist of those regulated under the current MMLER i.e. arsenic (As), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn), and radium-226 (^{226}Ra) (AQUAMIN, 1996). In addition, cyanide will also be added to the list of MMLER contaminants on the recommendation of the AQUAMIN working

groups. Additional contaminants not listed under the MMLER but recommended for monitoring by the AQUAMIN working groups include aluminium (Al), cadmium (Cd), calcium fluoride, iron (Fe), mercury (Hg), molybdenum (Mo), nitrogen compounds, and thiosalts. The effectiveness of molluscs as potential biomonitoring tools for the Canadian mining industry will be evaluated in reference to these contaminants.

According to the AQUAMIN report, between 103 and 177 active metal mines have been operated in Canada at any one time over the past 25 years. The majority of these mines discharge directly into the freshwater environment. Consequently, this review will focus on molluscs found in freshwater and their effectiveness in monitoring the prescribed contaminants.

2. CONCEPTUAL FRAMEWORK FOR MOLLUSCS AS BIOMONITORS

2.1 The biomonitoring concept

In its broadest sense, the term "biological monitoring" or "biomonitoring" can be defined as "the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program" (Rosenberg and Resh, 1993). There are several types of biomonitoring including surveillance and compliance (Rosenberg and Resh, 1993). Surveillance usually includes surveys before and after a project is completed or before a toxicant is spilled and is used to determine if water management techniques are working or whether conservation measures are successful. Historical biomonitoring or long-term surveillance "can provide the evidence essential to the evaluation of apparent or emerging environmental problems" (Monitoring and Assessment Research Centre, 1985). Compliance monitoring is done either to ensure that regulatory requirements are being met or to maintain the course of long-term water quality goals (Rosenberg and Resh, 1993).

Biomonitoring programs measure different biological responses in order to evaluate changes in the environment including, (1) organism-level responses ranging from biochemical to life-history changes (i.e. growth) and bioaccumulation, and (2) population or community level responses such as presence/absence or numerical predominance of indicator organism populations and species assemblages (Rosenberg and Resh, 1993). Ideally, a biomonitoring program should include organism and population level biological responses using several resident taxa to assess different types of metal stress (Crawford and Luoma, 1993). This report will focus primarily on organism level bioaccumulative biomonitoring (i.e. in molluscs).

Bioaccumulative biomonitors, also called "sentinel organisms" (Goldberg et al. 1978), are used to measure contaminant concentrations in aquatic ecosystems through the accumulation of contaminants in their tissues. Interpreting trace metal concentrations in sediment and water and predicting the threat they pose to aquatic life under variable physico-chemical conditions is difficult. The use of sentinel organisms has many advantages over the analysis of abiotic components in that measurement of a pollutant in the organism signifies that the pollutant is (was) bioavailable and may be a threat not only to the organism itself but also to other parts of the food web and ecosystem (V.-Balogh, 1988). Sentinel organisms also provide a time-integrated measure of pollutant availability, in contrast to the instantaneous nature of pollutant

concentrations measured in water. The primary difficulty faced when using sentinel organisms is separating changes resulting from trends in industrial exposure from changes in exposure resulting from environmental, biological or physico-chemical factors (Phillips and Rainbow, 1993).

In this context, biomonitoring essentially provides information on the variation in time and space of the concentrations of contaminants which are available to a selected biomonitor (Phillips and Rainbow, 1993). Thus, biomonitoring seeks to identify significant differences in contaminant bioavailabilities among samples from different sites, or among samples taken from the site at different times.

2.2 Criteria for a good biomonitor

Organisms are chosen as biomonitors because they possess characteristics that render them useful in monitoring programs (Phillips and Rainbow, 1993; Rosenberg and Resh, 1993):

1. Biomonitors should be relatively non-mobile, either through behavior (e.g. territorial behavior) or because they are sessile or sedentary, in order that their exposure be representative of the study area.
2. Biomonitors should be abundant in study areas, easy to identify and to sample at all times of the year.
3. Biomonitors should be large enough in body size to provide sufficient tissue for analysis of the contaminant of interest.
4. Biomonitors should be hardy, tolerating wide ranges of contaminant concentration and physiochemical variables such as salinity, temperature and acidity, thereby ensuring their ubiquity in the receiving environment and permitting the design of transplant experiments and laboratory studies of contaminant kinetics.
5. Biomonitors should be strong accumulators of the relevant contaminant, with a simple correlation between the metal concentration found in the tissues of the organism and the average ambient bioavailable contaminant concentration. This dose-response relationship should be independent or related to environmental factors and caging in a predictable manner.

Molluscs satisfy many of the above characteristics of a good biomonitor as summarized in Metcalfe-Smith (1994): wide distributions of closely related species maximize data comparability;

sedentary habits maximize site-specific information; large, stable populations permit repeated sampling; specimens are readily sampled, handled, and identified; tolerances to many contaminants are high compared with other aquatic organisms; bioconcentration factors (BCFs) are high; and bivalves provide a measure of contaminant bioavailability near the entry level of the food chain.

Interpreting metal accumulation in the tissues of aquatic biota, including molluscs, can be extremely complex. Some other important factors that will be considered when evaluating the use of molluscs as biomonitoring tools for the mining industry include:

1. Do molluscs occur naturally in mining impacted areas, and if not can they be artificially introduced in cages?
2. Are there differences in metal accumulation patterns between naturally occurring indigenous populations and transplanted molluscs?
3. Are there physiological constraints to using molluscs to monitor areas receiving mining discharge, e.g. pH, Ca concentrations, etc.?
4. Are molluscs useful for detecting short-term pollution events (episodic events) or long-term trends in the receiving environment?
5. Can tissue metal concentrations be related to different metal sources in the aquatic environment?

These questions will be addressed in subsequent chapters and the answers will provide a framework within which to evaluate the usefulness of molluscs as biomonitoring tools for the mining industry in Canada.

2.3 Conceptual framework for molluscs as biomonitors for the mining industry

2.3.1 **Molluscs as sentinels**

Monitoring bioavailable metals over the long-term: Historical Surveillance

This approach mimics that used in the Mussel Watch (O'Connor, 1992). The goal is to utilize the larger slow-growing populations of unionids to monitor changes in bioavailable metal concentrations over time. This approach would be particularly useful in monitoring metal release in areas where mining activities have ceased and to determine if further clean-up is required. This type of monitoring would require the use of indigenous populations of molluscs that are likely to be present in sufficient numbers for the duration of the monitoring program. Transplanted

molluscs could also be used in this case provided they are collected from the same population for the duration of the monitoring program.

Monitoring bioavailable metals over the short-term:

- to determine the spatial extent of metal contamination

  Transplanted molluscs could be deployed at specific sites and their tissue levels compared to characterize the metal contamination throughout the impacted area and identify contamination "hot-spots".

- to determine the effectiveness of remedial measures

  The effectiveness of remedial measures such as "liming" or waste water treatment could be determined with transplanted or indigenous mollusc populations.

2.3.2 **Molluscs as effects-monitors**

Monitoring metal-induced effects:

- Growth measurements (shell length, soft-tissue weights and growth rates) could be used to obtain a estimate of potential metal-induced effects at the organismal level.
- Condition measurements could be used to determine the overall health of the metal-exposed population relative to a control population.

The above measurements would have to be accompanied by measurements of potential confounding environmental variables including temperature and food quality and quantity.

2.4 Suggested readings

For further discussion on the use of benthic macroinvertebrates in biomonitoring programs the reader is referred to Phillips and Rainbow (1993) and Rosenberg and Resh (1993). These books offer additional information on the biological responses at the population and community level used in biomonitoring programs but not covered in this report.

3. OVERVIEW OF MOLLUSC CHARACTERISTICS

The following is a brief description of the important aspects of the diversity and distribution, ecology, physiology, reproduction and growth of molluscs that should be considered when applying these organisms as biomonitoring tools. The review will focus on freshwater bivalves and briefly discuss marine species and freshwater gastropods.

3.1 Taxonomy and Diversity

The Phylum Mollusca is divided into seven living classes: 4 minor ones Monoplacophora, Polyplacophora, Aplacophora, Scaphopoda and 3 major classes Cephalapoda, Gastropoda and Pelecypoda.

Phylum Mollusca		
Classes	Description	Aquatic Environment
Gastropoda	Snails and Slugs	Freshwater and Marine
Pelecypoda = Bivalvia	Mussels and Clams	Freshwater and Marine
Cephalapoda	Octopods, Cuttlefish and Squid	Marine
Monoplacophora	Limpet-like shells, *Neopilina*	Marine, some fossil families
Polyplacophora	Chitons	Marine
Scaphopoda	Tusk Shells	Marine
Aplacophora	Solenogasters , Worm-like bodies with Spicules	Marine

Roughly 110,000 species of freshwater and marine molluscs have been described, of which over 50,000 species of molluscs belong to the marine and freshwater subclass Prosobranchia and another 20,000 belong to the primarily terrestrial subclass Pulmonata (Thorp and Covich, 1991). In North America there are about 49 genera (350 species) of freshwater prosobranch snails and 29 genera (150 species) of pulmonate snails (Thorp and Covich, 1991). Some pulmonate snails have re-invaded the freshwater environment from the terrestrial environment, using a modified portion of the mantle cavity as a lung.

Although, the majority of bivalves are marine (Russell-Hunter, 1983), the North American fauna of freshwater bivalves is the richest in the world, with over 260 species of mussels and

Table 1. Freshwater mollusc species by zoogeographic regions. Bivalve species are shown in bold. Adapted from Clarke (1981).

Zoogeographic Region	Characteristic Mollusc Species
Atlantic Coastal -Atlantic coastal plain to the south	***Margaritifera margaritifera, Anodonta cataracta cataracta, Anodonta implicata, Lampsilis ochracea*** and *Lyogyrus granum*
Lake Erie-Lake St. Clair -south-central Ontario	***Quadrula pustulosa, Cyclonaias tuberculata, Pleurobema coccineum, Ptychobranchus fasiolaris,*** and ***Obliquaria reflexa***
Great Lakes-St. Lawrence	***Valvata perdepressa, Alasmidonta marginata,*** *Pleurocera acuta, Goniobasis livescens,* and *Acella haldemani,*
Red River-Assiniboine River	***Amblema plicata, Fusconaia flava, Quadrula quadrula, Proptera alata,*** and *Cincinnatia cincinnatiensis*
Western Prairie -Manitoba, Saskatchewan and Alberta	*Stagnicola caperata, Promenetus exacuous megas, Planorbula campestris,* and *Helisoma trivolvis subcrenatum*
Pacific Coastal -most of British Columbia	***Margaritifera falcata, Gonidea angulata, Anodonta nuttalliana,*** *Fossaria truncatula,* and *Physa columbiana*
Beringian Refugium -Yukon River system in the Yukon Territory, northern British Columbia, and some small river systems in the northwestern Northwest Territories	***Anodonta beringiana,*** *Lymnaea atkaensis,* and *Stagnicola kennicotti.*
Subarctic -south of tree line from Labrador to the mouth of the Mackenzie River	***Anodonta grandis simpsoniana, Sphaerium nitidum, Pisidium conventus,*** and *Stagnicola catascopium preblei*
Arctic -north of tree line to the southern part of the Arctic Archipelago	*Valvata sincera helicoidea, Stagnicola arctica,* and *Physa jennessi jennessi*

clams (Thorp and Covich, 1991). There are an additional 6 introduced species of bivalves. Most bivalve species belong to the superfamily Unionacea (227 native species, 2 introduced) and the rest belong to the family Sphaeriidae (33 native, 4 introduced species). The unionids are known for their unique morphological adaptations. Many are considered to be endangered or threatened. Sphaeriids are more widely distributed where some species have pandemic distributions. Of the introduced bivalve species, the zebra mussel, *Dreissena polymorpha* inhabiting some of the Great

Lakes, has achieved the most notoriety. The distributions of freshwater molluscs in Canada are related to glacial and postglacial history and to specific characteristics of the species themselves (Clarke, 1981). Based on the distribution of freshwater molluscs throughout Canada, there are several zoogeographic regions (Table 1) (Clarke, 1981). Many freshwater mussel species and all large prosobranch snails require continuous waterways for successful migration and as a result highly endemic species have developed (McMahon, 1991; Clarke, 1981). After periods of glacial cover, re-invasion by molluscs was restricted to the post-glacial stream confluence (Clarke, 1981). Alternatively, smaller molluscs (sphaeriid clams and small snails) are thought to have been transported to different geographical areas by ingestion and regurgitation by migratory birds or attached to their feathers or muddy feet (Clarke, 1981). Sphaeriid clams may also be transported distances attached to the legs of large insects (McMahon, 1991; Clarke, 1981). Transport between drainage systems mediated by human vectors (e.g. boats) has been partially responsible for the successful invasion of the zebra mussel into the Great Lakes and the spread of *Corbicula fluminea* throughout North America. Within drainage systems, migration of these exotic species occurs when water currents transport the actively swimming veliger larvae of *Dreissena polymorpha* and juvenile *Corbicula fluminea* (McMahon, 1991).

The distribution of bivalves species in the Canada's provinces is shown in Table 2. The most abundant of the unionid species are *Elliptio complanata* in eastern Canada, *Lampsilis radiata radiata* on the prairies, and *Anodonta kennerlyi* and *Margaritifera falcata* in British Columbia (Clarke, 1981). Among the gastropods, the pulmonate *Stagnicola elodes*, family Lymnaeidae, is most common species in Canada (Clarke, 1981).

Endangered and Threatened Species

The conservation status of molluscs will play a significant role in their usefulness as biomonitoring tools. Species listed as threatened may have harvesting limits associated with them and/or moratoriums that prohibit their collection for any reason (scientific or otherwise). This factor alone can restrict the usefulness of molluscs as biomonitoring tools. Evaluation of the status of mollusc diversity has only recently been considered in Canada with the formation of the Mollusca and Lepidoptera Subcommittee under the umbrella of the Committee on the Status of

Table 2. Distribution and conservation status of freshwater mussel species in Canada and the provinces in which they occur. Adapted from Williams et al. (1993).

Mussel Species	Conservation Status	Provinces
Margaritifera *falcata*	undetermined	British Columbia
M. margaritifera	special concern	New Brunswick, Nova Scotia, New Foundland, PEI, Quebec
Actinonaias *ligamentina*	currently stable	Ontario
Alasmidonta *heterodon*	endangered	New Brunswick
A. marginata	special concern	Ontario
A. undulata	special concern	New Brunswick, Nova Scotia, Ontario, Quebec
A. varicosa	threatened	New Brunswick, Nova Scotia
A. viridis	special concern	Ontario
Amblema *plicata plicata*	currently stable	Manitoba, Saskatchewan, Ontario
Anodonta *beringiana*	undetermined	Yukon Territory, British Columbia
A. implicata	currently stable	New Brunswick, Nova Scotia, Quebec
A. kennerlyi	undetermined	Alberta, British Columbia
A. nuttaliana	undetermined	British Columbia
Anodontoides *ferussacianus*	currently stable	Manitoba, Ontario, Quebec, Saskatchewan
Cyclonaias *tuberculata*	special concern	Ontario
Elliptio *complanata*	currently stable	New Brunswick, Nova Scotia, Ontario, Quebec
E. dilatata	currently stable	Ontario
Elliptiodeus *torulosa rangiana*	endangered	Ontario
E. triquetra	threatened	Ontario
Fusconaia *flava*	currently stable	Manitoba, Ontario
Gonidea *angulata*	undetermined	British Columbia
Lampsilis *cariosa*	threatened	New Brunswick, Nova Scotia
L. fasciola	currently stable	Ontario
L. ovata	special concern	Manitoba, Ontario, Quebec, Saskatchewan
L. radiata radiata	currently stable	New Brunswick, Nova Scotia, Ontario, Quebec
L. siliquoidea	currently stable	Alberta, Manitoba, Northwest Territories, Ontario
L. complanata complanata	currently stable	Alberta, Manitoba, Ontario, Saskatchewan
L. compressa	currently stable	Manitoba, Ontario, Quebec, Saskatchewan
L. costata	currently stable	Manitoba, Ontario, Quebec

Table 2. Continued.

***Leptodea** fragilis*	currently stable	Ontario, Quebec
L. ochracea	special concern	New Brunswick, Nova Scotia
***Ligumia** nasuta*	special concern	Ontario
L. recta	special concern	Manitoba, Ontario, Quebec, Saskatchewan
***Obliquaria** reflexa*	currently stable	Ontario
***Obovaria** olivaria*	currently stable	Ontario, Quebec
O. subrotunda	special concern	Ontario
***Pleurobema** coccineum*	currently stable	Ontario
***Potamilus** alatus*	currently stable	Manitoba, Ontario, Quebec
***Ptychobranchus** fasciolaris*	currently stable	Ontario
***Pyganodon** cataracta*	currently stable	New Brunswick, Nova Scotia, Ontario, PEI, Quebec
P. fragilis	currently stable	New Brunswick, Nova Scotia, New Foundland, Quebec
P. grandis	currently stable	Alberta, Manitoba, Northwest Territories, Ontario, Quebec, Saskatchewan, Yukon Territory
***Quadrula** pustulosa pustulosa*	currently stable	Ontario
Q. quadrula	currently stable	Manitoba, Ontario
***Simpsonaias** ambigua*	special concern	Ontario
***Strophitus** undulatus*	currently stable	Manitoba, New Brunswick, Nova Scotia, Ontario, Quebec, Saskatchewan
***Toxolasma** parvus*	currently stable	Ontario
***Truncilla** donaciformis*	currently stable	Ontario
T. truncata	currently stable	Ontario
***Utterbackia** imbecillis*	currently stable	Ontario
***Villosa** fabalis*	special concern	Ontario
V. iris	currently stable	Ontario

Endangered - species or subspecies are in danger of extinction throughout a significant portion of its range.

Threatened - species or subspecies likely to become endangered throughout a significant portion of its range.

Special concern - species or subspecies may become endangered or threatened by relatively minor disturbances to its habitat and deserves careful monitoring.

Undetermined - species or subspecies whose historic and current distribution and abundance has not been evaluated in recent years.

Currently stable - species or subspecies whose distribution and abundance may be stable, or it may have declined in portions of its range but is not in need of immediate conservation.

Endangered Wildlife in Canada (COSEWIC). Unfortunately, no comprehensive assessments of the status of molluscs in Canada have been completed and only scattered information exists.

One of the most recent and comprehensive accounting of the conservation status of mollusc species in Canada is provided by the American Fisheries Society (AFS). It provides a list of 297 native freshwater mussels that occur throughout North America, of which 213 taxa (71.7%) are considered endangered, threatened, or of special concern (see end of Table 2 for definitions) (Williams et al. 1993). Of the 52 native species found in Canada, 17 taxa (33%) are endangered, threatened or of special concern and another 5 species have undetermined status (Table 2). The remaining 30 mussel species are considered currently stable by the AFS. The difficulty with this assessment by the AFS is that the conservation status of each mussel species is based on its status across all of the provinces and states in which they occur. Therefore, the results are not specific to the success of mussels in Canada.

In the United States, over 25 unionid species have been put on the Federal Register's Endangered species list, with additional species on state lists and new species being added yearly (McMahon, 1991). Factors affecting their decline include habitat destruction from dams, channel modifications, siltation, and the intoduction of non-indigenous species as well as the freshwater pearling industry (Williams et al. 1993; McMahon, 1991). The consequences of the declining status of mussel species in Canada for the use of molluscs as biomonitoring tools for the Canadian mining industry are significant, especially for long-term biomonitoring programs. The conservation status of individual mollusc species in Canada should be determined prior to their inclusion in a national monitoring program.

3.2 Ecology

The utility of molluscs as biomonitoring tools depends in part on their ability to successfully inhabit areas influenced by mining activities. Although it is impossible to characterize the aquatic environment typical of all mining areas there are some similar traits including high metal concentrations, lower pH due to smelting processes and acid mine drainage (AMD) and high turbidity and silt loads from the under-water disposal of waste rock and till. The range of habitats occupied by molluscs are described in the following section.

3.2.1 General Ecology

Freshwater molluscs have a relatively ubiquitous distribution inhabiting a wide range of aquatic environments, from small ponds to large lakes and rivers. They are usually absent from extremely cold alpine lakes and streams, acidic waters or grossly polluted areas (Elder and Collins, 1991; Clarke, 1981). Mollusc distribution can be limited by very low concentrations of dissolved salts including calcium, needed for shell production (Elder and Collins, 1991). They are restricted to more alkaline waters and are rarely found in waters more acidic than pH 6. Molluscs generally have high oxygen demands that tends to exclude them from anoxic or near-anoxic conditions (Elder and Collins, 1991). Nevertheless, some species of freshwater bivalves have adapted to hypoxic or even anoxia conditions (McMahon, 1991). This allows the bivalves to occur below the thermocline of stratified lakes or above reducing substrata with heavy organic loads or in areas with large numbers of molluscs (McMahon, 1991). Fish are the primary predators of molluscs, although only a few species, such as suckers, perch and whitefish, rely on them for a significant portion of their diet (Elder and Collins, 1991). Snails are consumed by ducks, shore birds, and occasionally amphibians. Both mussels and clams are consumed by terrestrial species, such as muskrats and turtles (Elder and Collins, 1991).

Marine gastropods can be found living between tide marks on the seashore, on the ocean bottom at all depths, and drifting in the plankton near the sea surface (Russell-Hunter, 1983). The gastropod *Littorina* live high on the seashore in zones wetted by sea spray (Russell-Hunter, 1983). Most of the primitive marine bivalves are infaunal, burrowing into soft substrates, but several superfamilies are epifaunal, mostly attached to rocks (Russell-Hunter, 1983).

3.2.2 Gastropods

Gastropods are grazers, feeding on detritus, periphyton covering rocks and macrophytes and algae trapped at the water surface (Brown, 1991). Pulmonates and detrivorous prosobranchs occupy slow-moving, silty habitats, whereas limpets or prosobranch grazers prefer fast-current areas (Brown, 1991). They are found at a wide range of water depths and participate in seasonal migrations to deeper water in the fall and back to shallow littoral regions in the spring. The freshwater pulmonate *Physa gyrina*, found in the St. Lawrence River system, is restricted to

shallow water surfaces on rock and macrophyte where they graze on epiphytic material (Flessas et al., submitted). Alternatively, the freshwater prosbranch *Bithynia tentaculata*, also found in the St. Lawrence River system, is usually found on aquatic macrophytes, but can be associated with rock surfaces, grazing on the periphytic algae/detritus/bacteria complex (Flessas et al., submitted).

The lower pH limit that gastropods can tolerate is around pH 5.5 (Gerhardt, 1993). Nearly half of all freshwater gastropods (~45%) require calcium concentrations greater than 25 mg L^{-1}, and virtually none of them can survive in waters less than 3 mg L^{-1} (Brown, 1991). Prosobranch snails are "oxygen conformers" having less resistance to periods of hypoxia than pulmonates (Brown, 1991).

3.2.3 **Bivalves**

The distribution of freshwater bivalves is determined to some extent by sediment type. Unionids are most often found in stable, coarse sand or sand-gravel mixtures and usually absent from silted areas (McMahon, 1991). Mussel fauna in areas receiving silt loads and acid discharges from mines have been adversely affected or totally extirpated (McMahon, 1991). Areas where water velocities are low enough to allow for sediment stability and high enough to prevent excessive siltation tend to have successful unionid populations (McMahon, 1991). Sphaeriids, on the other hand prefer substrates with fine particles, although there are some species differences on the optimal particle sizes (McMahon, 1991). A few unionids, *Pyganodon grandis*, *Lampsilis anodontes*, and *L. radiata*, are found in sand-mud substrates (McMahon, 1991).

Water depth is a factor in the distribution of freshwater bivalve species. Most unionaceans, as well as *Sphaerium* and *Musculim* sphaeriid species prefer water depths less than 10 m, but can be found deeper if oxygen concentrations are sufficient (McMahon, 1991). Two unionid species, *Elliptio complanata* and *Pyganodon grandis*, that inhabit lakes in northern latitudes are tolerant of low oxygen levels resulting from long periods of ice-cover (McMahon, 1991). Due to their ability to regulate oxygen uptake many species of the sphaeriid *Pisidium* can be found below the epilimnion of lakes in poorly oxygenated areas. Profundal sphaeriids such as *Sphaerium simile* and *Pisidium casertanum* are somewhat oxygen independent; however, long

periods of hypoxia and anoxia have deleterious effects on their growth and reproduction (McMahon, 1991).

Unionaceans grow and reproduce over a pH range of 5.6 - 8.3, with a pH of 4.7-5.0 being the absolute lower limit (McMahon, 1991). Sphaeriid species are somewhat more tolerant of low alkalinity habitats. A pH of 5.0 has been identified to produce maximal growth and reproduction in the sphaeriid *Musculium partumeium* (McMahon, 1991). The pH ranges that limit bivalve success are linked to the availability of calcium. The minimal Ca concentrations tolerated by freshwater bivalves are 2-2.5 mg L^{-1}, with sphaeriid species occurring at the lower concentration ranges (McMahon, 1991).

Bivalve species distributions are limited by temperature. If water temperatures are too high, molluscs may perish or have their reproductive cycles disrupted, but if water temperatures are only slightly elevated over normal, reproduction may actually increase (Clarke, 1981). *Corbicula fluminea* is not found in drainage systems that experience water temperatures less than 2°C (McMahon, 1991). *Dreissena polymorpha* has maximal temperature limits of 25-27°C above which larval development is prevented (McMahon, 1991). No studies describing temperature limits for other unionid species could be found.

3.3 Basic morphology, physiology, reproduction and growth

Life-history characteristics are used to evaluate the different mollusc groups (i.e. unionids, sphaeriids and *Dreissena polymorpha*) on their practical application as biomonitors of spatial and temporal change. Factors such as maturation age, life span and biomass turnover rates are considered. In addition, aspects of molluscan morphology, physiology, reproduction and growth are reviewed in light of the important role they play in determining metal uptake, accumulation and metabolism.

3.3.1 **Gastropods**

Snails have a soft body which is organized into a muscular foot, distinct head region and visceral mass; covering the body is a fleshy mantle that secretes a spiral, univalved shell (Thorp

and Covich, 1991). They possess a file-like radula used in feeding on periphyton covering a wide range of surfaces (Brown, 1991). Respiration in freshwater prosobranchs is through a single gill that is enervated with blood vessels which facilitate oxygen exchange by the usual counter-current mechanism (Brown, 1991).

Freshwater snails have a wide diversity of life history traits (Brown, 1991). They generally have short life cycles and are relatively easy to rear in the laboratory (Brown, 1991). Prosobranch snails are generally dioecious, often iteroparous, with perennial life cycles (Brown, 1991). All pulmonates are monoecious, and are usually annual and semelparous (Brown, 1991). Annual species generally live for a single year, and perennial species live and reproduce for 4-5 years. Reproduction is initiated by increases in temperature and egg development can be internal or external depending on the species (Brown, 1991). Gastropods grow faster and reproduce at an earlier age if the average temperature increases, consequently resulting in more generations per year (Brown, 1991). The freshwater prosobranch *Bithynia tentaculata*, found in the St. Lawrence River, has a life cycle of 6 months to a year, including a single reproduction event in the fall or in some cases June or July (Flessas et al., submitted).

3.3.2 **Bivalves**

Unlike snails, bivalves have limited mobility and their body is encased in two hinged, calcareous shell valves. They possess enlarged gills with elongated, ciliated filaments for filter feeding (McMahon, 1991). Bivalves are laterally compressed and dorso-ventrally expanded, which combined with their spade-like foot make them highly efficient at burrowing into the substrate. North American freshwater bivalves generally have short siphons and as a result are normally found buried to depths where the posterior shell margin is either slightly below or above the sediment surface (McMahon, 1991). The zebra mussel *Dreissena polymorpha* uses byssus threads made of proteinaceous materials to anchor itself to many different types of surfaces.

3.3.2.1 *Life-history traits and reproduction*

A summary of important life-history traits of freshwater bivalves is shown in Table 3. Almost all North American freshwater bivalves are ovoviviparous, brooding embryos through

Table 3. Summary of life-history charcteristics of North American freshwater bivalves, Unionacea, Sphaeriidae, and *Dreissena polymorpha*. Adapted from McMahon (1991).

Life History Trait	Unionacea	Sphaeriidae	*Dreissena polymorpha*
Life span	<6 - >100 yr (species dependent)	<1 - >5 yr (species dependent)	4 - 7 yr
Age at maturity	6 - 12 yr	> 0.17 - <1 yr (1 yr in some species)	1 - 2 yr
Reproductive mode	gonochoristic (a few hermaphroditic species)	hermaphroditic	gonochoristic
Growth rate	rapid prior to maturity, slower thereafter	slow relative to unionids	rapid throughout life
Fecundity (young/average adult/breeding season)	200,000 - 17,000,000	3 - 24 (*Sphaerium*) 2 - 136 (*Musculim*) 3 - 7 (*Pisidium*)	30,000 - 40,000/female
Juvenile size at release	very small 50 - 400 μm	large 600 - 4150 μm	extremely small 40 - 70 μm
Relative juvenile survivorship	extremely low	high	extremely low
Relative adult survivorship	high	intermediate	intermediate
No. of reproductive efforts/yr	1	1 -3 (continuous in some species)	26 - 88 %/yr 1 (2 - 8 months long)
Assimilated energy respired	-	21 - 91 % (avg. = 45 %)	-
Nonrespired energy in growth	85.2 - 97.5 %	65 - 96 % (avg. = 81 %)	96.1 %
Nonrespired energy in reproduction	2.8 - 14.8 %	4 - 35 % (avg. = 19 %)	4.9 %
Turnover time in days (mean standing crop biomass: biomass production/day ratio)	1790 - 2849	27 - 1972 (generally < 80)	53 - 869 (dependent on habitat)

early development stages in the gill. *Dreissena polymorpha* is the only exception, releasing both sperm and eggs externally, leading to a free-swimming veliger larval stage (McMahon, 1991). The majority of unionids are gonochoristic and all sphaeriids are hermaphroditic (McMahon, 1991). Self-fertilization does occur, but most fertilization results when sperm released into the water is taken up in the inhalent currents of surrounding individuals and transported to the unfertilized eggs retained in the gills. Temperature is the main stimulus for initiating reproduction. Unionaceans release a large number of parasitic glochidium larvae once per year that require a fish host prior to metamorphosing into a juvenile (McMahon, 1991). Glochidium fish hosts are species specific (Kat, 1984). If the glochidium is not compatible with the fish species, an immune

reaction in the fish causes the glochidium to be sloughed off (McMahon, 1991). Because of this specificity the reproductive success of certain unionid species depends on the success of their fish host.

Age-specific survivorship among juvenile unionids tends to be low, improving with age (Table 3). Survivorship in adult *Anodonta anatina* (5-7 year olds) was 81-86% (McMahon, 1991). In unionids, the shell growth rate declines exponentially with age, but the rate of tissue biomass accumulation remains constant or actually increases with age (McMahon, 1991). Natural unionid populations tend to be dominated by large adults, due to their high adult survivorship, long life spans and low juvenile survivorship. Biomass turnover times in these types of populations are extremely long ranging from 1790-2850 days (McMahon, 1991). In contrast, turnover times for sphaeriids (27-1970 days) and *Dreissena polymorpha* (53-870 days) are much shorter. These life-history traits make unionids highly susceptible to human perturbations (McMahon, 1991).

Sphaeriid species show a greater degree a variability in life history traits among species compared to the unionids. Sphaeriids are characterized by early maturation, rapid growth and reduced reproductive effort, which equips them to deal with the environmental stresses of their habitat (McMahon, 1991). Fertilized embryos develop in brood chambers in the gills and are supplied with maternal nutrients allowing for considerable growth and development prior to release (McMahon, 1991). In contrast to the unionids, sphaeriids generally produce a small number (3-24 young/adult *Sphaerium sp.*) of large offspring (0.6-4.15 mm) (McMahon, 1991). The production of fewer young normally suggests that a species has adapted to relatively stable habitats. However, sphaeriids are most often found in shallow ponds and profundal portions of lakes which are exposed to extended periods of hypoxia and drying.

3.3.2.2 *Nutrition and filtration rates*

Of the three distinct subclasses that make up the class Bivalvia the most common are the filter-feeding lamellibranchs and deposit-feeding protobranchs. Most freshwater bivalves are suspension feeders, filtering unicellular algae, bacteria and suspended detrital particles from the pallial water flow across the gill. The material filtered on the gill is passed to the labial palps for

Table 4. Bivalve filtration rates and size of particles most efficiently retained. Range of particles retained are shown in parentheses.

Species	Particle	Filtration Rate	Reference
Mytilus edulis		119 L g^{-1} d^{-1}	Widdows et al. 1995
Pyganodon cataracta	8	4 L g^{-1} d^{-1}	Tankersley and Dimock, 1993
Dreissena polymorpha	5	5.5 L d^{-1}	Sprung and Rose, 1988
	(5 - 35)		
Dreissena polymorpha	>1		Jorgensen et al. 1984
Unio pictorum	5-8		Jorgensen et al. 1984
Anodonta cygnea	6-8		Jorgensen et al. 1984
Elliptio complanata	5		Paterson, 1984
Elliptio complanata	(8 - 80)		Tessier et al. 1984

cilia-mediated sorting of food from nonfood before being carried on ciliary tracts to the mouth. The stomach and style have pH levels ranging from 6.0-6.9, style acidity varying with phase of digestion.

Filtration studies on both marine and freshwater bivalves report that most species can retain particles greater than 4 μm in diameter with nearly 100% efficiency (Table 4) (Tankersley and Dimock, 1993). The zebra mussel could retain particles as small as 0.7 μm in diameter, although the preferred size range was considerably larger (5 - 35 μm) (Sprung and Rose, 1988). Particle ingestion appears to be affected by food type with some bivalves selecting for certain algal species (McMahon, 1991). Filtration rates are affected by particle size and particle concentration (Tankersley and Dimock, 1993). For example, filtration rates in the freshwater mussel, *Pyganodon cataracta*, increased over a particle diameter of 2 μm to 9+ μm (Tankersley and Dimock, 1993). Females had significantly higher filtration rates during pre-brooding periods compared to females during brooding.

3.3.2.3 *Shell Production*

The bivalve shell is composed of non-living calcium carbonate ($CaCO_3$) crystals secreted from the mantle tissue layer. A careful pH balance must be maintained inside the bivalve shell in order for shell layers to form. Formation of the $CaCO_3$ crystals requires the release of protons (H^+) ($Ca^{2+} + HCO_3^- \leftrightarrow CaCO_3 + H^+$). However, the protons must also be removed from the extrapallial fluid to maintain the pH range (pH 7.4 - 8.3) necessary for $CaCO_3$ deposition

(McMahon, 1991). Freshwater bivalves have adapted to limiting Ca^{2+} concentrations by maintaining a higher extrapallial pH which favors $CaCO_3$ deposition at a lower Ca^{2+} concentration. Unionid bivalves possess dense calcium phosphate concretions in their tissues that are thought to provide a source of Ca^{2+} for shell deposition, glochidial shell development and to buffer respiratory acidosis during hypoxia (McMahon, 1991). Balancing the amount of energy spent on shell production (up to one-third of total energy related to growth) and that allocated to tissue growth is an adaptive strategy developed by bivalves. Fast-growing, thin-shelled species (most sphaeriids) devote less energy to growth and reproduction compared to slower-growing, thick-shelled species (most unionids) (McMahon, 1991).

3.3.2.4 *Osmotic regulation*

Due to the extremely dilute waters in which some freshwater bivalves live they are constantly gaining water and losing ions. Bivalves maintain their ionic balance in their tissues via active transport across the gills and other epithelial tissues (McMahon, 1991). Most freshwater bivalves cannot osmoregulate above 3 parts per thousand sodium.

3.3.2.5 *Circulation and metabolism*

Bivalves possess an open circulatory system in which the tissues are directly bathed in the blood hemolymph (containing free O_2) before returning to the heart. The gills serve as gas exchangers, brooding chambers for their young and feeding structures. Metabolic rates in bivalves correspond to seasonal changes in temperature, where the highest rates occur in the summer and the lowest in the winter months (McMahon, 1991). Sphaeriids experience variable metabolic rates corresponding to growth and reproductive cycles while unionids do not. Unionids do not provide embryos with maternal nourishment, although the physical presence of the developing glochidia in their gills have been found to reduce filtration rates and particle-retention efficiencies (Tankersley and Dimock, 1993). There is some evidence that bivalves have diurnal activity patterns where the most active periods occur at night, possibly related to feeding and vertical migration cycles of zooplankton that could conceivably increase epilimnetic particle concentrations at night (McMahon, 1991). Distinct annual biochemical cycles have been found in

freshwater bivalves and are thought to be related to reproduction (McMahon, 1991). Maximal levels of whole body protein, glycogen and lipid are reached during gametogenesis and gonad development and then drop to a minimum after glochidial release.

4. RELATIONSHIP BETWEEN CONCENTRATIONS OF METALS IN SEDIMENT AND WATER AND CONCENTRATIONS IN MOLLUSCS

It is important that a biomonitor strongly accumulate the relevant contaminant and that the tissue contaminant concentrations be directly proportional to average ambient bioavailable contaminant concentrations. For many organic contaminants, relatively simple correlations have been determined (e.g. fugacity/hydrophobicity constants) that hold over a range of environmental conditions (Clark et al. 1990). However, the development of "simple" correlations for metals has been more difficult. Metal bioavailability and metal accumulation by molluscs change in response to changing environmental conditions and from one species to the next. Interpreting tissue metal concentrations in the aquatic environment requires an understanding of the factors that influence metal availability and determine metal accumulation in molluscs (Fig. 1).

The availability of a metal is determined by its speciation which may be very sensitive to changing environmental conditions (e.g. pH, presence of natural inorganic and organic ligands). Reactions affecting metal speciation may occur within organisms, on their surface, in solution, and on the surface of particles, making estimates of metal behavior difficult. The availability of metals to molluscs will be a function of both the source of metal exposure (dissolved or particulate phase) and the geochemical processes that control metal availability within each phase.

Metal accumulation in molluscs is also affected by individual biological characteristics and aspects of mollusc physiology. Individual biological characteristics that determine organism response to metal exposure typically include aspects of growth and reproduction, as well as behavior. Biological characteristics are of particular importance when comparing metal accumulation in indigenous populations to accumulation in molluscs that have been transplanted and/or caged. Other factors affecting accumulation that are a function of mollusc physiology include the route of metal uptake, rates of uptake, assimilation/retention efficiencies and detoxification and elimination processes.

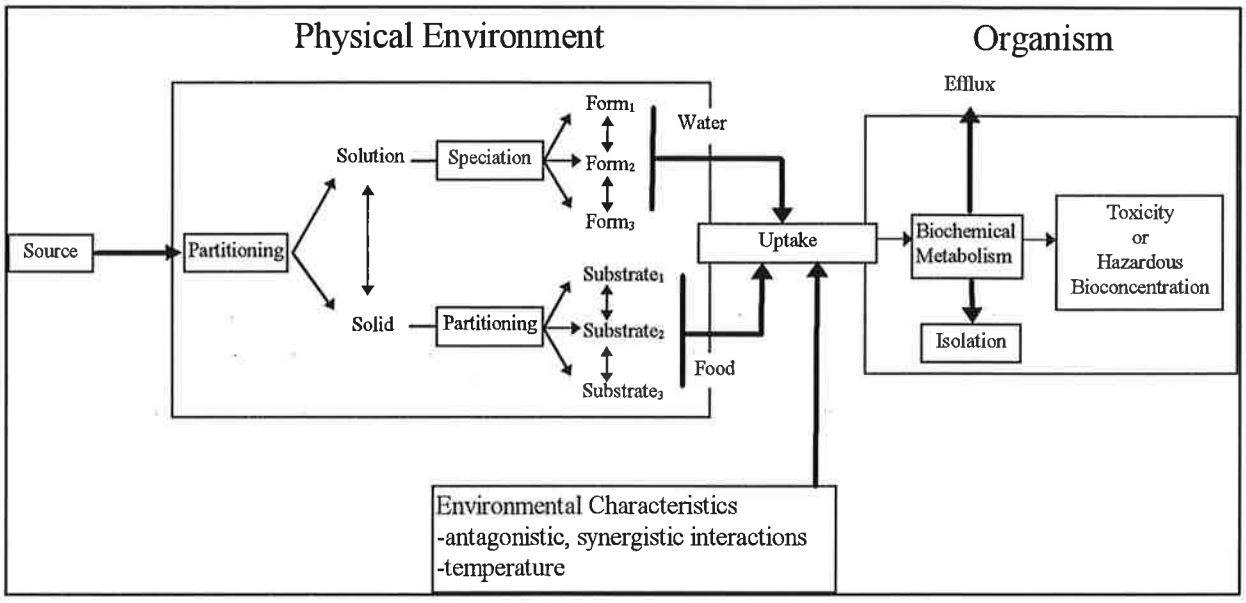

Figure 1. Processes affecting metal availability and accumulation in aquatic organisms. Redrawn from Luoma (1983).

4.1 Bioaccumulation

The accumulation of metals by molluscs can be divided into three phases: 1) metal uptake, 2) metal transport, distribution, and sequestration within the body, and 3) metal excretion (may or may not occur) (Rainbow and Dallinger, 1993). The role that a metal plays in mollusc physiology (essential vs. non-essential) and the ability of the different species to regulate metals influence the accumulation processes (Rainbow and Dallinger, 1993). Molluscs able to regulate metals show no significant change in body metal content over time on exposure to elevated concentrations of bioavailable metal. This may be achieved by excluding all metal (no absolute uptake), a strategy possible for the short-term but not long-term, or by altering the rate of metal excretion to match the rate of metal uptake (Rainbow and Dallinger, 1993). Unlike many other invertebrates, molluscs have limited ability to reduce uptake by reducing permeability of the outer surfaces. Filter-feeding bivalves have huge gill surfaces which are bathed in the external medium to facilitate food uptake and respiration. The molluscs' inability to strongly regulate metals is important characteristic that many argue makes them good biomonitors.

The following is an overview of metal accumulation in molluscs including uptake (kinetics), elimination (assimilation/retention efficiencies) and storage (distribution among tissues, organs and cytosol).

4.1.1 **Uptake**

The rate of metal uptake has consequences for using molluscs as biomonitoring tools. For example, molluscs with rapid uptake rates might be suited to monitoring short-term episodic events (e.g. spills) compared to molluscs with considerably slower uptake rates that would be more suited to monitoring long-term fluctuations in bioavailable metal concentrations. Metal uptake rates are influenced by characteristics of the mollusc species (life strategies, age, growth rate, reproductive status) as well as a series of factors related to the metal species (i.e. route of uptake, partition coefficient (K_d)). Steady state is obtained when uptake rates equal excretion rates.

Metal uptake in bivalves may occur through the transport of dissolved metals across the gill or by ingestion of metal-laden particles filtered out by the gills. Consequently, uptake rates in bivalves are predominantly a function of filtering activity and absorption efficiency from the dissolved phase. Uptake rates vary from one mollusc species to the next and are metal specific. Laboratory studies with *Mytilus edulis* found uptake rates for the different metals in the order Ag>Zn>Am≈Cd>Co (Wang et al. 1996).

The life strategy of the freshwater zebra mussel *Dreissena polymorpha* (small, high biomass turn-over rates, high growth rates) makes it more suitable to monitoring short-term fluctuations in metal concentrations than other older long-lived unionids. Indigenous and caged zebra mussels, used to monitor metal concentrations in a Cu-contaminated reservoir in France, showed marked fluctuations in metal concentrations in their tissues (including decreases) reflecting changes in water contamination on a monthly basis (Mersch et al. 1996).

Larger, slower growing unionids tend to respond more slowly to changes in metal concentrations. Uptake rates calculated for *Pygandon grandis* transplanted from uncontaminated Lake Brompton to contaminated Lake Joannes for 130 days, were 0.11 μg Cd $g^{-1}d^{-1}$ and 0.18 μg Cu $g^{-1}d^{-1}$, assuming a linear increase (Tessier et al. 1987). Based on these rates of uptake it would

take 1 (Cu) and 2 (Cd) years for metal concentrations in the transplanted Lake Brompton mussels to reach the levels found in indigenous Lake Joannes mussels. Tessier et al. (1993) reported that freshwater bivalves (*Pyganodon grandis*) transplanted along a contamination gradient (Cd, Cu and Zn) had not reached steady state with their new environment after 1 year. Similar results were found by Couillard et al. (1995a), except that tissue Zn concentrations in transplanted *Pyganodon grandis* rapidly reached those concentrations in the indigenous population. This may have been due to the essential role of this metal in mollusc physiology. Cadmium concentrations in indigenous *Pyganodon grandis* in Lake 382 in the Experimental Lakes Area (ELA), receiving epiliminetic additions of Cd (average ambient water [Cd] 1987 ~85 ng L^{-1}), continued to increase 120 days after metal additions had begun (Malley et al. 1989). In another experiment, Cd concentrations in *Pyganodon grandis* transplanted from pristine Lake 377 in the ELA and caged on the sediment in Lake 382, were still increasing after 26 days (average ambient water [Cd] 1988 ~100 ng L^{-1}) (A.R. Stewart, unpublished data).

Uptake rates in marine species in the field appear to be much faster than those for freshwater species. Regoli and Orlando (1994) found that the marine mussel *Mytilus galloprovincialis*, transplanted to a metal contaminated site, accumulated Pb, Fe, and Mn (linear increase) and reached steady state metal concentrations in their tissues after 14 days exposure.

Several laboratory studies on the kinetics of metal uptake in freshwater molluscs indicate that uptake rates and times to reach steady-state are much more rapid in the laboratory than in the field. However, these studies utilized unrealistically high water metal concentrations that may have resulted in uptake by different routes with different uptake kinetics than those expected for lower, more realistic metal concentrations in the natural environment (Hemelraad et al. 1986, 5 to 25 µg Cd L^{-1}; Tessier et al. 1994, 10 and 50 µg Cd L^{-1}; Jenner et al. 1991, ~50 µg Cd L^{-1}; Graney et al. 1984, 50 µg Cd L^{-1}).

4.1.2 **Assimilation/Retention efficiencies**

The net accumulation of a metal by molluscs is a function of its assimilation and retention efficiencies from food and water, respectively. Assimilation efficiency (AE) refers to the amount of metal retained from particles relative to total amount ingested. Retention efficiency (RE) is the

amount of metal adsorbed from the dissolved phase relative the amount filtered. Although rarely calculated for freshwater species, Wang et al. (1996) suggest that estimates of AEs and REs for different mollusc species are critical for interpreting bioaccumulation studies. These measurements provide a means to identify important sources of metals to bivalves under natural conditions and help explain tissue metal burdens. Recent work by Wang et al. (1996) and Fisher et al. (1996) using the marine mussels *Mytilus edulis* and *M. galloprovincialis* provide a comprehensive overview of factors affecting assimilation and retention efficiencies and provide comparisons between the laboratory and field. Some of the relevant findings are provided below as well as the concentration ranges used in the experiments.

Fisher et al. 1996	Concentration ranges of dissolved trace elements[1]
Co	0.8 - 1 ng L^{-1}
Zn	3 - 6 ng L^{-1}
Cd	0.6 - 0.7 ng L^{-1}
Ag	860 ng L^{-1}
Pb	3 - 4 ng L^{-1}
Wang et al. 1996	Concentration ranges of dissolved trace elements
Co	0.1 - 10 µg L^{-1}
Zn	0.5 - 300 µg L^{-1}
Cd	0.1 - 10 µg L^{-1}
Ag	0.2 - 100 µg L^{-1}
[1] Values = Concentrations of added metals + background metal concentrations in 0.2 µm filtered Mediterranean seawater (Personal communication, N. Fisher, Stony Brook, New York).	

Retention efficiencies determined for laboratory-exposed *M. galloprovincialis* were generally higher than AEs for Ag, Am, Cd, inorganic Co, organic Co, Pb and Zn (Fisher et al. 1996). Also, variability in REs was generally higher than for AEs for all the metals tested. These results suggest that the accumulation of the tested metals from the dissolved phase is greater than from the ingested solids, but that uptake from solution is more sensitive to changes in water chemistry.

Mytilus edulis fed radioisotope tagged seston showed differences in Ag and Cd assimilation that were found to vary with the nutritional quality of ingested food particles (Wang

et al. 1996). Silver was more efficiently assimilated and Cd was less efficiently assimilated from seston with higher "chlorophyll a" concentrations. Cadmium has been found to be assimilated with lower efficiency from green algae, possibly due to the algae's rigid cell wall and resistance to digestion (Atkinson et al. 1972). Zinc, Am and Co were not appreciably affected by the quality of seston in the experiment. The AEs of ingested metals from natural seston were Ag (5 to 18%), Am (0.6 to 1%), Cd (8 to 20%), Co (12 to 16%) and Zn (32 to 41%) (Wang et al. 1996).

Assimilation and retention efficiencies from the laboratory and field were not appreciably different, except for Ag and Co (Fisher et al. 1996). For Ag, the AEs of mussels held in the laboratory were 5 times greater that those in the field. Silver binds strongly to sulfur in ligands in protein and so different environmental conditions (e.g. temperature) could affect protein metabolism, significantly affecting its absorption in mussels. Only Ag absorption in Mytilus edulis was found to be inversely related to temperature (5 vs. 15°C) (Fisher et al. 1996). This finding suggests that most laboratory determined assimilation efficiencies (AEs) could be used to estimate field situations. Further, efflux rate constants for metals in marine mussels derived from laboratory experiments were consistent with values obtained from field studies (Fisher et al. 1996).

4.1.3 **Elimination**

Metals taken up into organisms are not necessarily incorporated into tissues or utilized in mollusc metabolism. Some metals are released immediately as feces and others are detoxified in the digestive gland and excreted. In the absence of elevated metal concentrations in the environment, metals may be progressively lost from the soft tissues and shell. The elimination of metals in molluscs has consequences for measuring impacts of improvements to waste-water treatment as well as trends in recovery of metal contaminated systems (i.e. will tissue-burdens decrease to reflect lower ambient metal concentrations?).

Elimination rates were very slow in the freshwater bivalve *Pyganodon grandis* transplanted from contaminated Lake Joannes to relatively uncontaminated Lake Brompton. The half-life ($t^{½}$) or time to reduce the metal concentration at time zero by half, for Cu and Cd in *P. grandis* calculated over the 130 day transplant period were 315 and 143 days, respectively

(Tessier et al. 1987). Cadmium elimination rates for several freshwater and marine clams and mussels are shown in Table 5. It appears that elimination rates vary between metals, mussel species and tissues. A very general conclusion from these data is that if losses occur at all they do so slowly over a 100 to 300 day period (for an approximately 50% reduction in metal concentration). Half-lives for some marine species are considerably shorter than 100 days.

In a study that compared the elimination in the marine mussel *Mytilus galloprovincialis* caged in the field and in the laboratory, metal release (Ag, Am, Cd, inorganic Co, organic Co, Pb and Zn) generally followed a two-compartment exponential model (Fisher et al. 1996). Uptake from food (cultured marine phytoplankton) resulted in a rapid release of isotope, primarily in the form of fecal pellets. Alternatively, metals accumulated from water were lost more slowly as metal desorbed from the shell. A discrepancy was evident in the efflux rates for whole mussels and for mussel shells between laboratory and field mussels which was thought to be linked to epiphytic growth on the mussel shells in the field, thus limiting desorption (Fisher et al. 1996). For this reason, the authors suggested that efflux rate constants for soft tissues may be more relevant. The authors also noted seasonal changes in efflux rates in mussels which they suggested might be linked to seasonal variations in food availability (Fisher et al. 1996).

Neither the route of uptake nor exposure time (12 hrs. vs. 6 days) had a significant effect on the efflux rates in *Mytilus edulis* fed radioisotope (Ag, Am, Cd, Co, Se, and Zn) tagged natural seston, suggesting that assimilated radiotracers were able to partition rapidly into even the least labile compartment (Wang et al. 1996). Conversely, Fisher et al. (1996) found that efflux rate constants for radiolabeled Co and Am in *Mytilus galloprovincialis* soft parts were 2-3 times greater following uptake from food than from water, although there were no differences found for the other metals (Zn, Cd, Ag, Pb).

4.1.4 **Storage**

The processes that control the distribution of metals within tissues and cells affect both bioaccumulation itself and any adverse impact as a result of bioaccumulation. Thus, the understanding of metals storage is critical to interpreting possible risk posed by accumulated

Table 5. Depuration of metals in the soft tissues of bivalves.

Species	Metal		Metal loss pattern	Reference
Pyganodon grandis	Cu	field	$t^{1/2}$= 143 d	Tessier et al. 1987
Pyganodon grandis	Cd	field	$t^{1/2}$= 315 d	Tessier et al. 1987
Dreissena polymorpha	Cd	laboratory	0% loss, 50 d depuration	Bias and Karbe, 1985
Unio pictorium	Cd	laboratory		Jenner et al. 1991
kidney			0 % loss, 203 d	
gill			$t^{1/2}$= 203 d	
hepatopancreas			30 % loss(exponential) , 203 d	
Mytilus californianus	^{65}Zn	field	$t^{1/2}$= 76 d	Young and Folsom, 1967
Mytilus californianus	^{65}Zn	field	$t^{1/2}$= 380 d	Seymor and Nelson, 1973
Mytilus edulis	^{65}Zn	field	$t^{1/2}$= 277 d	Seymor and Nelson, 1973
Mytilus edulis	Cd	laboratory	$t^{1/2}$= 14-29 d	Scholz, 1980
Mytilus edulis	Cd	laboratory	$t^{1/2}$= 96-190 d	Borchardt, 1985
Crassostrea virginica	^{65}Zn	field	$t^{1/2}$= 347 d	Wolfe, 1970
Mercenaria sp.	Cd	laboratory	0% loss, 64 d depuration	Robinson and Ryan, 1986

metals. The distribution of metals among tissues and within cells is continuously changing following metal exposure. The route of uptake (solution vs. particulate), the mechanism of transport across the cell membrane (facilitated or diffusive transport; passive or active-transport), the affinity of the metals for intracellular ligands (e.g. metallothionein) and most importantly the role of the metals (essential vs. non-essential) determine where the metals are sequestered and how they metabolized.

The distribution of Cd concentrations in tissues of naturally occurring unionids has been reported to follow the sequence: kidney>midgut gland>gills>>whole animal>mantle=mantle edge=labial palps>guts/gonads complex>foot and adductor muscle (Hemelraad et al. 1986; Salánki et al. 1982; Manly and George, 1977). The kidney and midgut gland comprise approximately half of the total body burden for Cd. This distribution can dramatically change after exposure. Concentrations of Cu, Zn and Pb in *Elliptio complanata* collected from lakes in the Rouyn-Noranda mining region of Quebec, were highest in the gills and mantle, intermediate in the hepatopancreas and lowest in the foot and adductor muscle (Tessier et al. 1984). The gills (Cu, Zn, Mn) and mantle (Fe) had the highest contribution to the metal body burden (Tessier et al. 1984). The highest proportion of the total Cd body burden was also found in the gill (40%) of another bivalve species, *Pyganodon grandis* collected from lakes in the Rouyn-Noranda mining region (Tessier et al. 1993). A different distribution of Cd was found in indigenous *Pyganodon grandis* exposed *in situ* to ^{109}Cd (~83 ng L^{-1}), where the order of concentrations within the tissues followed the sequence: kidney>remains of soft tissues[1]>gill>foot>mantle (Malley et al. 1989). However, the order of Cd in tissues expressed as a proportion of the total body burden followed the sequence: remains of soft tissues>gill>mantle, kidney>foot, which is similar to that found in other indigenous bivalves exposed to elevated metals.

Fisher et al. (1996) found that the distribution of metals within *Mytilus galloprovincialis* was affected by the source of metal exposure (dissolved vs. particulate) in the laboratory. A greater fraction of metal obtained from the dissolved phase was associated with the shell, while a greater fraction obtained from ingested food was enriched in soft parts such as the digestive gland. Within soft-tissues, metal obtained from the dissolved phase was enriched in the gills at the end of the metal exposure and beginning of the depuration period, whereas metals obtained from

[1] Remains of soft tissues include gonads, gut, adductor muscles and digestive gland.

food were most enriched in the digestive gland. There were no significant differences in the distribution of radioisotope from either source in the mantle, gills, or adductor muscle.

The subcellular distribution of metals in molluscs depends on the metal and the duration and degree of exposure. Couillard et al. (1995b) report the subcellular distibution of Cd and Cu in the gills of *Pyganodon grandis* transplanted along a contamination gradient in the Rouyn-Noranda Region of Quebec. After 14 days exposure, Cd was associated primarily with the high molecular weight (HMW, >15 kDa) ligands in the gills. All of the Cd then shifted to the moderate molecular size fraction (15 - 3 kDa, Metallothionein fraction (MT)) after 90 days. After 400 days, a considerable portion (74%) of the Cd was found in the low molecular weight (LMW) fraction. Alternatively, cytosolic Cu, measured only after 90 days exposure, was found primarily bound to the HMW pool (85%). Based on experiments with *Crassostrea virginica*, Roesijadi and Klerks (1989) suggested that Cd binding in the gill is controlled by competition among available ligands displaying varying affinities for the metal, MT having the highest. After a lag period during which the MT is synthesized, the majority of Cd is found associated with the MT fraction. The observed shift from the MT fraction toward the LMW after 400 days exposure may have been an example of "spillover" of excess metals from the MT pool. The reader is referred to Couillard et al. (1995b) and Couillard (1996) for a more detailed discussion of the toxicological significance of the "spillover" effect.

Cytosolic shifts in the distribution of Ag, Cu and Zn were also found in the bivalve *Macoma balthica* collected on a monthly basis from January 1981 to June 1982 in South San Francisco Bay near Palo Alto (cytosols were extracted from whole animals) (Johannson et al. 1986). Copper and Ag remained relatively stable in the HMW pool (>30 kD) over the two year period, but showed temporal variations in the MT (3-25 kD) and LMW (<3 kD) pools. Concentrations of Cu, Ag in the HMW, MT and LMW pool and Zn in the MT metal ligand pool were linearly correlated with corresponding cytosolic levels, although at high cytosolic metal levels the MT pool tended to plateau asymptotically. Johannson et al. (1986) also found that above certain "threshold" cytosolic metal concentrations the amount of metal bound to the LMW pool increased dramatically, coinciding with the plateau of metals in the MT pool. Threshold cytosolic Cu, Ag and Zn concentrations were, ~5 μ g^{-1} tissue wet wt, 200 ng g^{-1} tissue wet wt, and 15 μg g^{-1} tissue wet wt, respectively.

The subcellular distribution of metals within the digestive gland of *Mytilus galloprovincialis* transplanted to a metal contaminated site varied among metals (Regoli and Orlando, 1994). Half of the Cu and Zn was present in the microsomal-cytosolic (M+C) fractions, and the other half distributed equally among the heavy (H; inorganic granular concretions, nuclei, cellular debris and undisrupted tissues) and lysosomal-mitochondrial (L+M) fractions. Lead was found primarily contained within the H fraction (up to 70%) and the L+M fraction (20%, sometimes up to 60%). Both Fe and Mn were mainly associated with the H fraction (50-80%) (Regoli and Orlando, 1994). Lesser amounts were found in the L+M (15 to 30% of Fe) or in the M+C fraction (25 to 40%). The authors found no differences in subcellular distributions with respect to season (within indigenous populations), metal exposure (within transplanted mussels) or recovery period (depurating mussels), contrary to the previous studies. The gravimetric method used in this study to determine subcellular metal distributions may not have been sufficiently sensitive to detect changes.

The lysosomal system is well developed in mussels and also plays a major role in the cellular homeostasis of heavy metals (Regoli, 1992). Metal can frequently be sequestered within these organelles (George, 1990).

4.1.5 **Bioconcentration Factors (BCFs)**

Bioconcentration factors are a unit-less measurement calculated by dividing "steady-state" wet tissue concentration by "steady-state" water concentration of a particular substance. BCFs have been used extensively in the organic pollution literature but have been used less to describe metal pollution. The Canadian Toxic Substances Management Policy uses BCFs to determine the hazard posed by substances that are bioaccumulative and the associated action that should be taken by the government given any particular BCF (trigger levels). Recently, the use of BCFs in assessing metal contamination has been reviewed and given an unfavorable recommendation. Chapman et al. (1996) suggest a number of reasons why BCFs are not appropriate for regulating metal contamination: 1) some metals (e.g. Co, Cu, Fe, Mn, Mo, Zn) are essential for health and organisms regulate these metals over a range of environmental metal concentrations; therefore, BCFs will vary according to the external metal concentration; 2) essential metals have a double "toxicity" threshold (due to shortages and excesses); organisms will concentrate essential metals

when external concentrations are low resulting in high BCFs, and; 3) BCFs assume that tissue metals concentrations are at steady-state, a condition that is difficult to determine in the field.

Corresponding to BCFs are Bioaccumulation factors (BAFs) which are calculated for steady-state environmental concentrations (metal source is not specified - could be sediment, water or food). Table 6 lists the BAFs for Cd, Cu and Zn in a variety of freshwater mussel species and varying sediment concentrations. BAFs for Cd generally ranged from 0.1 to 33 (one BAF of 91) over a sediment concentration range of 0.05 to 90 $\mu g \cdot g^{-1}$ dry weight. In these studies, mussels from relatively uncontaminated habitats had higher BAFs than those from contaminated habitats. Either the bioavailability of Cd varied widely at the contaminated sites or there are some other mechanisms influencing uptake. In the short term, the mussels may have reduced filtration rates and closed their shells as a toxic response to high metal concentrations. Alternatively, other metals present at the contaminated sites may have been in competition with Cd for uptake sites. Stewart (unpublished data) found that *Pyganodon grandis* exposed to Cd and a mixture of metals (Cu, Zn, Pb, and Ni) showed reduced uptake of Cd at the highest concentrations of the metal mixture. This occurred despite the fact that there was more Cd available in the water column for uptake. In any case, these studies indicate that BAFs can be misleading. A more detailed approach is needed to accurately describe the relationship between tissue metal concentrations and metal concentrations in the environment.

4.1.6 **Summary**

1. Metal uptake rates are faster in zebra mussels and sphaeriid species than in unionids. Time for tissue metal concentrations to reach steady-state range from 2 weeks to over 1 year.
2. Laboratory and field studies indicate that *Mytilus* species accumulate metals more efficiently from the dissolved phase and that accumulation of Ag and Cd from food is dependent on the nutritional quality of the food.
3. Elimination rates vary according to the metal, species, target organ and season. A very general estimate for a 50% reduction in tissue concentration is 100 to 300 days.
4. Metal storage varies for different metals, route of uptake (dissolved vs. food) and over the exposure period. Metals accumulated from the water or food are generally accumulated in

the gill and digestive gland, respectively, and then ultimately stored in the kidney. Subcellular metal distributions are metal specific and tend to change in response to cytosolic metal concentrations.

5. Metal BCFs or BAFs can be potentially misleading and should not be used to describe the relationship between tissue-metal concentrations and environmental exposures.

Table 6. Cadmium, Zn and Cu concentrations in mussels, sediments and bioaccumulation factors. Adapted from Metcalfe-Smith et al. (1992)

	Concentrations (total, $\mu g\ g^{-1}$ dry wt.)				
Metal	Sediment	Mussels	BAF	Species	Reference
Cd	0.051	0.8	15.7	*E. complanata*	Campbell and Evans, 1991
	0.072	0.9	12.5	*E. complanata*	Campbell and Evans, 1991
	0.079	1	12.7	*E. complanata*	Campbell and Evans, 1991
	0.09	8.2	91.1	*L. siliquoidea*	Pugsley et al. 1988
	0.093	0.6	6.5	*E. complanata*	Campbell and Evans, 1991
	0.107	1.6	15.0	*E. complanata*	Campbell and Evans, 1991
	0.19	6.3	33.2	*L. siliquoidea*	Pugsley et al. 1988
	0.191	1.4	7.3	*E. complanata*	Campbell and Evans, 1991
	0.197	2.1	10.7	*E. complanata*	Campbell and Evans, 1991
	0.197	2.2	11.2	*E. complanata*	Campbell and Evans, 1991
	0.202	0.6	3.0	*E. complanata*	Campbell and Evans, 1991
	0.205	1.2	5.9	*E. complanata*	Campbell and Evans, 1991
	0.25	1.5	6.0	*E. complanata*	Campbell and Evans, 1991
	0.25	4.8	19.2	*L. siliquoidea*	Pugsley et al. 1988
	0.3	10	33.3	*P. grandis*	Heit et al. 1980
	0.3	9	30.0	*E. complanata*	Heit et al. 1980
	0.3	9	30.0	*L. radiata*	Heit et al. 1980
	0.321	0.6	1.9	*E. complanata*	Campbell and Evans, 1991
	0.361	6.9	19.1	*E. complanata*	Campbell and Evans, 1991
	0.378	5.9	15.6	*E. complanata*	Campbell and Evans, 1991
	0.59	0.57	1.0	*L. ventricosa*	[b]Czarnezki, 1987
	0.6	1.31	2.2	*A. plicata*	[b]Adams et al. 1981
	0.7	0.85	1.2	*L. ventricosa*	[b]Czarnezki, 1987
	2	5.6	2.8	*Q.quadrula*	[a]Mathis and Cummins, 1973
	2	6.9	3.5	*F. flava*	[a]Mathis and Cummins, 1973
	2	3.8	1.9	*A. plicata*	[a]Mathis and Cummins, 1973
	<3	58	19.3	*E. dilatata*	[a]Wren et al. 1983
	5.85	.81	0.1	*L. complanata*	[c]Anderson 1977
	5.85	2.71	0.5	*L. ventricosa*	[c]Anderson 1977
	5.85	5.89	1	*L. siliquoidea*	[c]Anderson 1977
	5.85	3	0.5	*A. marginata*	[c]Anderson 1977
	9.53	1.98	0.2	*A. plicata*	[b]Adams et al. 1981
	16.5	1.44	0.1	*A. plicata*	[b]Adams et al. 1981
	16.8	1.43	0.1	*A. plicata*	[b]Adams et al. 1981
	17.5	2	0.1	*L. ventricosa*	[b]Czarnezki, 1987
	19.6	1.61	0.1	*A. plicata*	Adams et al. 1980
	26.8	10.2	0.4	*L. ventricosa*	[b]Czarnezki, 1987
	29.9	3.96	0.1	*A. plicata*	Adams et al. 1980
	35.4	7.78	0.2	*A. plicata*	[b]Adams et al. 1981
	57.3	11.3	0.2	*L. ventricosa*	[b]Czarnezki, 1987
	89.7	12.5	0.1	*A. plicata*	Adams et al. 1980

Table 6. Con't

Metal	Concentrations (total, $\mu g\ g^{-1}$ dry wt.) Sediment	Mussels	BCF	Species	Reference
Zn	14	267	19.1	*L. radiata*	[d]Friant, 1979
	14	170.5	12.2	*E. complanata*	[d]Friant, 1979
	14	303	21.7	*A. cataracta*	[d]Friant, 1979
	32.2	127	4.0	*A. plicata*	[b]Adams et al. 1981
	33	240	7.3	*E. complanata*	Heit et al. 1980
	33	375	11.4	*L. radiata*	Heit et al. 1980
	33	240	7.3	*P. grandis*	Heit et al. 1980
	78.4	132	1.7	*E. complanata*	Tessier et al. 1984
	81	660	8.2	*F. flava*	[a]Mathis and Cummins, 1973
	81	950	11.7	*A. plicata*	[a]Mathis and Cummins, 1973
	81	480	5.9	*Q. quadrula*	[a]Mathis and Cummins, 1973
	93.3	146.7	1.6	*E. complanata*	Dermott and Lum 1986
	102	173.45	1.7	*L. ventriculosa*	[c]Anderson 1977
	102	319.85	3.1	*L. siliquoidea*	[c]Anderson 1977
	102	182.6	1.8	*A. marginata*	[c]Anderson 1977
	102	251.8	2.5	*L. complanata*	[c]Anderson 1977
	119	180	1.5	*E.complanata*	Tessier et al. 1984
	135	136	1.0	*E.complanata*	Tessier et al. 1984
	185	241	1.3	*A. plicata*	[b]Adams et al. 1981
	217	251	1.2	*A. plicata*	[b]Adams et al. 1981
	236	228	1.0	*A. plicata*	Adams et al. 1980
	242	295	1.2	*E.complanata*	Tessier et al. 1984
	249.5	111.62	0.5	*E.complanata*	Dermott and Lum 1986
	253	259	1.0	*A. plicata*	[b]Adams et al. 1981
	262	785	3.0	*E. dilatata*	[a]Wren et al. 1983
	270	85	0.3	*E.complanata*	Dermott and Lum 1986
	272	388	1.4	*A. plicata*	[b]Adams et al. 1981
	277	883	3.2	*A. plicata*	Adams et al. 1980
	318	320	1.0	*E.complanata*	Tessier et al. 1984
	342	202	0.6	*E.complanata*	Tessier et al. 1984
	423	272	0.6	*E.complanata*	Tessier et al. 1984
	433	535	1.2	*E.complanata*	Tessier et al. 1984
	791	717	0.9	*A. plicata*	Adams et al. 1980

Table 6. Con't

Metal	Concentrations (total, $\mu g\ g^{-1}$ dry wt.) Sediment	Mussels	BCF	Species	Reference
Cu	4	14	3.5	*P. grandis*	Heit et al. 1980
	4	9	2.3	*E. complanata*	Heit et al. 1980
	4	18	4.5	*L. radiata*	Heit et al. 1980
	7.1	15.05	2.1	*A. cataracta*	[d]Friant 1979
	7.1	7.45	1.1	*L. radiata*	[d]Friant 1979
	7.1	19.8	2.8	*E. complanata*	[d]Friant 1979
	19	12	0.6	*A. plicata*	[a]Mathis and Cummins, 1973
	19	17	0.9	*Q. quadrula*	[a]Mathis and Cummins, 1973
	19	17	0.9	*F. flava*	[a]Mathis and Cummins, 1973
	23.7	13.8	0.6	*E. complanata*	Tessier et al. 1984
	26	20	0.8	*E. dilatata*	[a]Wren et al. 1983
	26.4	13.09	0.5	*E. complanata*	Dermott and Lum 1986
	32.7	2.82	0.1	*L. complanata*	[c]Anderson 1977
	32.7	7.41	0.2	*L. siliquoidea*	[c]Anderson 1977
	32.7	9.41	0.3	*A. marginata*	[c]Anderson 1977
	32.7	22.35	0.7	*L. ventricosa*	[c]Anderson 1977
	35	16	0.5	*E.complanata*	Tessier et al. 1984
	40.7	13.6	0.3	*E.complanata*	Tessier et al. 1984
	59.4	5.96	0.1	*E.complanata*	Dermott and Lum 1986
	106	59.1	0.6	*E.complanata*	Tessier et al. 1984
	107	23.3	0.2	*E.complanata*	Tessier et al. 1984
	142	46.8	0.3	*E.complanata*	Tessier et al. 1984
	145	28.2	0.2	*E.complanata*	Tessier et al. 1984
	180	68.2	0.4	*E.complanata*	Tessier et al. 1984
	278	14	0.1	*E.complanata*	Dermott and Lum 1986

[a] concentrations in mussels converted from wet weight basis (x10)

[b] caged mussels

[c] mean concentration for sediment calculated from a range

[d] mean concentrations for sediment and mussels calculated from a range

4.2 Bioavailability

Unlike the case of organic pollutants, relationships between aquatic organisms and environmental concentrations of metals are not easily defined by a few characteristics of the external medium and organism (i.e. organic content of sediments, lipid content of organisms). Bioaccumulation of metals by aquatic organisms cannot be easily related to total pollutant, nor is it possible to measure one chemical fraction that is universally and exclusively the bioavailable fraction for any metal (Luoma, 1996). There is no universally accepted approach to explain how metals in sediments become bioavailable to aquatic organisms (Luoma, 1996). Many different theories and models have been developed and help to explain the bioavailability of different metal groups; free-ion activity correlates with Cu, Cd, Pb or Zn bioavailability; chloro-complexes of dissolved Ag may be able to bypass specific transport pathways and passively diffuse across lipid membranes; methylated forms of Hg and Sn demonstrate enhanced bioavailability via specific organo-complex transport and sequestration (Luoma, 1996). Depending on the metal in question, different theories must be considered. The paper by Campbell and Tessier (1996) provides an excellent overview of the factors controlling metal bioavailability in freshwater systems that will be discussed in the following section.

Metals in contaminated sediments are thought to affect aquatic life in two ways: indirectly (i.e. by partitioning of the metals into the ambient water, followed by their assimilation from the aqueous phase), and directly (e.g. by digestion of the sediments and assimilation of the metals from the gut) (Campbell and Tessier, 1996). In the past there has been considerable debate over which of the above pathways is of greater significance in terms of metal exposure in molluscs. However, recently there has also been a growing acceptance that both pathways must be resolved to avoid underestimating full exposure (Luoma, 1996). There are additional means for contaminant uptake including pinocytosis of metal-rich particles and mixing of the external medium directly with body fluids. However, due to their minimal contribution to body burdens, these mechanisms will not be discussed (Phillips and Rainbow, 1993).

The route of metal exposure in molluscs appears to vary with metal species as well as biological factors pertaining to the food source (particle concentration, nutritional quality, presence of organic coatings) and assimilation efficiencies of the metal by individual mollusc species. Essentially, the relative uptake from ingestion compared to uptake from solution is

geochemistry dependent. It is therefore more advantageous to examine both routes of uptake on a per metal basis and the factors that control the bioavailability of metals to mollusc through both exposure routes. Before discussing the bioavailability of metals from both exposure routes, some laboratory and field data are presented showing the relative dependence of different metals on each pathway and the reasoning behind the data.

4.2.1 **Sources of metal exposure**

The route of uptake for an individual metal species will depend on its particular binding affinities for specific ligands. Metal ions that have Class B or Borderline characteristics (Nieboer and Richardson, 1980) have high affinities for sulfur and nitrogen ligands and as a result bind to proteins and other cellular macromolecules. This high affinity for proteins causes the metals to bind to transport-proteins (i.e. intrinsic proteins) which then traverse the plasma membrane and transfer the metals to ligands of higher affinity within the cell (Phillips and Rainbow, 1993). Ag, Zn, and Cd bind strongly with sulfur groups in protein and show the greatest propensity for carrier protein transport across membranes. In some instances, Class B or borderline metal ions are taken up by "accident" by transport systems designed for other major ions due to similar ionic characteristics. There is evidence for the transport of the Cd ion using a Ca channel (Phillips and Rainbow, 1993). The high demands of molluscs for Ca have been related to atypical high Ca pump activities which may enhance Cd uptake to the point where it becomes the primary route (Phillips and Rainbow, 1993). Comparison of labeled Cd and Ca in the Australian freshwater mussel *Velesunio angasi* showed a strong correlation between the two ions (Phillips and Rainbow, 1993). The metals Mo and vanadium (V), which occur in seawater predominantly as oxygenated anions, use active transport pumps specific for the metal ions or those designed for the uptake of other anions (sulfate, phosphate) (Phillips and Rainbow, 1993). Neutral metal complexes (e.g. $HgCl_2^0$, $CdCl_2^0$) and organometallic compounds (e.g. CH_3HgX) are lipophilic and may be directly transported across the hydrophobic plasma membrane by passive diffusion (Phillips and Rainbow, 1993).

Tessier et al. (1984) suggest that the high levels of Cu, Zn and Pb associated with the gill and mantle and the high contribution of those tissues to the body burden indicates that dissolved

trace metals are an important route of uptake for *Elliptio complanata*. Nevertheless, the authors also suggest that direct uptake of metal particles by the gills and mantle by endocytosis is possible.

Wang et al. (1996) found that trace elements vary widely in their accumulation patterns in marine mussels (*Mytilus edulis*) and that both dissolved and particulate metals are appreciably accumulated. The relative importance of each source is principally related to metal partitioning on food particles, metal assimilation efficiency and to a lesser degree, the seston load. Based on a model, Wang et al. (1996) predicted the primary route of uptake for the different metals under varying environmental conditions (seston load, K_d).

Metal	Source	Percent of total uptake
Cd	water	>50-80%
Ag	water	30-70%
Zn	food	50-72%

4.2.2 **Dissolved phase**

Considerable evidence suggests that the total aqueous concentration of a metal is not a good predictor of its bioavailability (Campbell and Tessier, 1996). Metal speciation greatly affects metal availability to aquatic organisms; only a portion of the total dissolved metal is bioavailable. Of the dissolved metal species, the free metal ion concentration $M^{z+}(H_2O)_n$ has been shown to be linked to biological responses (Campbell and Tessier, 1996). The free metal ion concentration is a function of both the total aqueous metal present and the quantity and nature of ligands present in the water. Consequently, it is not surprising that the free metal ion concentration can vary widely among systems as does the biological response it causes. The free-ion activity model (FIAM) developed by Morel (1983) and reviewed recently by Campbell (1995) has been relatively successful in predicting the bioavailability of dissolved metals that exist in natural waters.

The major concepts of the FIAM will be presented here, but for more detailed description of the underlying equations of the FIAM the reader is directed to Campbell (1995). The FIAM is essentially based on the premise that in a system at equilibrium, the free-metal ion activity reflects the chemical reactivity of the metal (Fig. 2). The reactivity of the metal determines the extent of the reaction of the metal with surface cellular sites, and hence its bioavailability. In order for a

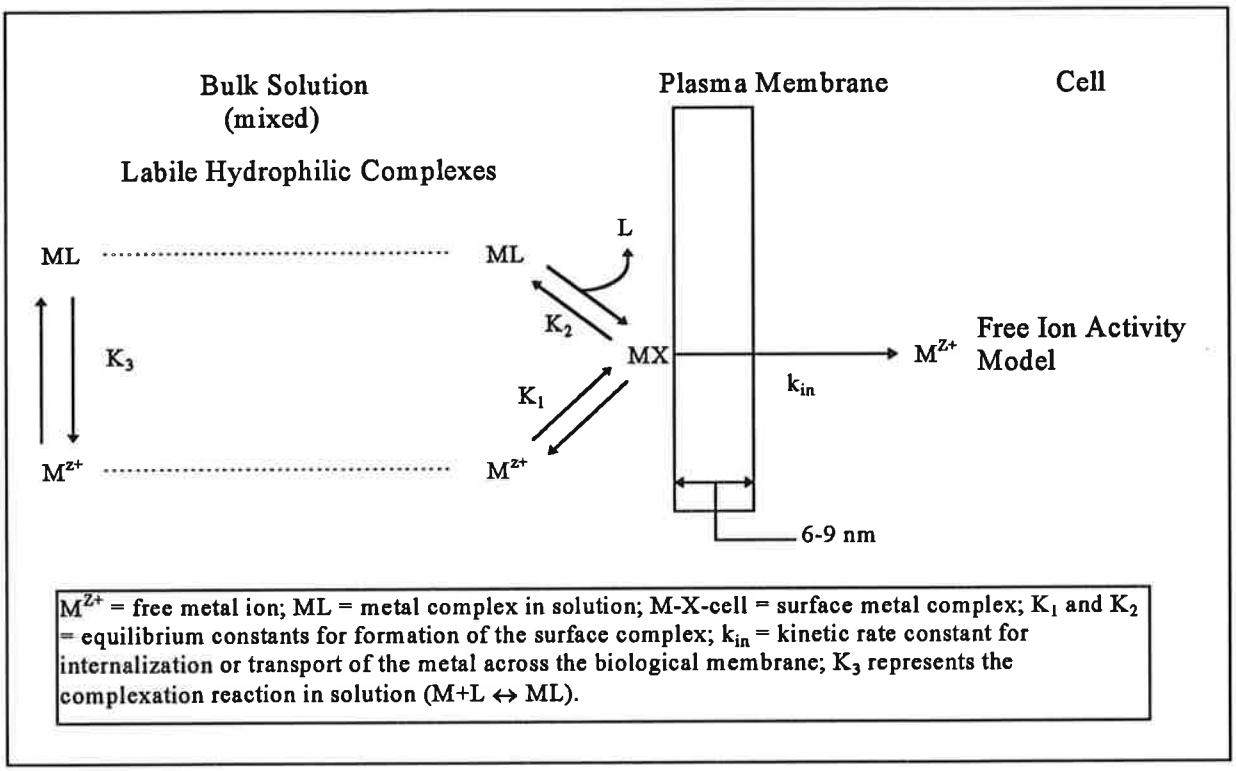

Figure 2. Concept of the Free Ion Activity Model (FIAM). Redrawn from Couillard (1996).

metal to be accumulated by an organism, a metal must first interact with and/or cross a cell membrane (Campbell and Tessier, 1996). This interaction takes place with the free metal ion (M^{z+}) or a metal complex (ML^{z+}) as the reactive species, and results in the formation of M-X-cell surface complex, where X-cell is a cellular ligand present at the cell surface. Assuming the concentration of free -X-cell sites remains approximately constant, the biological response (accumulation, toxicity, etc.) will vary as a function of $[M^{z+}]$. In the case where a metal complex (ML^{z+}) reacts at the cell surface, the reaction must be accompanied by the loss of ligand "L" (i.e. ML + X-cell ⇌ M-X-cell + L, where charges on individual species are not shown). Thus, the idea that the free hydrated metal ion is the only bioavailable species is a misconception, since no single species in a solution can be considered more (or less) available than another (Campbell, 1995). There are a number of key assumptions surrounding the FIAM involving the biological surface and the kinetics of metal-organism interactions (Table 7).

Table 7. Assumptions of the free-ion activity model (FIAM). Adapted from Campbell and Tessier (1996).

Biological surface:
• The key interaction of a metal with a living organism involves the plasma membrane , which is impermeable to the free metal ion, M^{z+}, and to its (hydrophilic) complexes, $ML^{\pm}$.
• The interaction of the metal with the plasma membrane can be described as a surface complexation reaction, forming M^{z+}-X-cell (M^{z+} + $^{-}$X-cell ⇌ M^{z+} - X-cell). The biological response, whether it be metal uptake, nutrition, or toxicity, is proportional to the concentration of this surface complex; variations in {M^{z+}-X-cell} follow those of [M^{z+}] in solution ({M^{z+} - X-cell} = K_{ad}{$^{-}$X-cell}[M^{z+}]).
• The biological surface does not change during the metal exposure experiment (i.e., the FIAM will be more applicable to short-term experiments than to long-term chronic exposure).
Kinetics:
• Metal transport in solution, towards the membrane, and the subsequent surface complexation reaction occur rapidly, such that a (pseudo-) equilibrium is established between metal species in solution and those at the biological surface ("rapid" = faster than metal uptake, faster than the expression of the biological response).
• Thus, the identity of the metal form(s) reacting with the plasma membrane is of no biological significance. No one species in solution can be considered more (or less) available than another.

Table 8. Relationship between metal bioavailability, as determined on indigenous molluscs, and geochemical estimates of the free-metal ion concentration present in the oxic sediment-interstitial water. Adapted from Campbell and Tessier (1996).

Metal	Mollusc	Geochemical predictor	Site	Reference
Cd	filter-feeder *Anodonta grandis*	$[Cd^{2+}]$, estimated from oxic sediment-water equilibria	Rouyn-Noranda; Chibougamau; Eastern Townships, Quebec; Sudbury; Muskoka (N=19); r^2=0.82; p<.01	Tessier et al., 1993
Cu	filter-feeding *Elliptio complanata*	{Fe-OCu}/{Fe-ox}, both extracted with $NH_2OH \bullet HCl$	Rouyn-Noranda(N=8; r^2=0.95; p<.01)	Tessier et al., 1984
Pb	estuarine deposit feeder *Scrobicularia plana*	{Fe-OPb}/{Fe-ox}, both extracted with HCl	U.K estuaries (N=37; r^2=0.88; p<.01)	Luoma and Bryan, 1978
Hg	estuarine deposit feeder *Scrobicularia plana*	{Hg}/{OM}, Hg extracted with HNO_3; organic matter by loss on ignition	U.K estuaries (N=78; r^2=0.63, p<.01)	Langston, 1982
As	estuarine deposit feeder *Scorbicularia plana*	{Fe-OAs}/{Fe-ox}, both extracted with HCl	U.K estuaries (N=75; r^2=0.93; p<.01)	Langston, 1980

If the dissolved phase is the primary exposure vector, the metal concentrations in the molluscs should be correlated with the free-metal ion concentration in the ambient water $[M^{z+}]$ or its surrogate. The review by Campbell and Tessier (1996) of bioassays and field surveys of indigenous benthic organisms supports the idea that benthic organisms respond to the free-metal ion concentration in the ambient water in or near the surficial sediments (Table 8). For field studies, the geochemical gradient (metal bioavailability) has been defined in terms of the free-metal ion (as estimated from sediment-water equilibria) or the ratio of sorbed metal to sorbent (related to the free-metal ion concentration),

$$[M^{z+}] = \frac{\{Fe - OM\}}{K_{M-Fe}[Fe - ox]}.$$

Field-derived equilibrium constants ($K_{M\text{-}Fe}$) for the sorption of trace metals on amorphous Fe (III) oxyhydroxides are pH-dependent - see Table 9.

Table 9. Field-derived equilibrium constants for the sorption of trace metals on amorphous Fe (III) Oxyhydroxides.

Metal	Relation	
Cd	$\text{Log } K_{Fe\text{-}Cd} = 1.03\ pH - 2.44$	($r^2 = 0.80$; n=26)
Cu	$\text{Log } K_{Fe\text{-}Cu} = 0.64\ pH + 0.10$	($r^2 = 0.75$; n=39)
Ni	$\text{Log } K_{Fe\text{-}Ni} = 1.04\ pH - 2.29$	($r^2 = 0.87$; n=29)
Pb	$\text{Log } K_{Fe\text{-}Pb} = 0.81\ pH + 0.67$	($r^2 = 0.81$; n=7)
Zn	$\text{Log } K_{Fe\text{-}Zn} = 1.21\ pH - 2.83$	($r^2 = 0.89$; n=41)

From Tessier, A., 1992. In: Environmental Particles - Environmental, Analytical and Physical Chemistry Series, edited by J. Buffle and H. P. Van Leeuwen, Lewis Publishers, Boca Raton, FL, pp. 425-453.

Two approaches can be used to estimate the concentration of the free metal ion $[M]_I$ depending on the redox potential of the medium (Campbell and Tessier, 1996). Under oxic conditions $[M]_I$ is controlled by sorption reactions on such sorbents as Fe- or Mn-oxyhydroxides or sedimentary organic matter. Alternatively, under anoxic conditions $[M]_I$ is controlled by precipitation-dissolution reactions with reactive amorphous sulfides (Acid Volatile Sulfides, or AVS). For a more complete description of these types of approaches see Campbell and Tessier (1996) and the December 1996 issue of Environmental Toxicology and Chemistry . Determining which of the two approaches is more appropriate depends on the site in question. Molluscs are almost exclusively in contact with the oxic layer since the water they filter is above or near the sediment-water interface, therefore, sorption reactions in oxic sediments are presumably more relevant in determining $[M]_I$ available to molluscs. It is likely that reactions with AVS may control a portion of the metal available to molluscs; however, field studies on a variety of sites are needed to test the validity of this statement.

4.2.3 **Particulate phase**

The bioavailability of metals primarily obtained in particulate form is determined by factors controlling AEs such as particle type and size ingested and digestion chemistry in the gut (pH, pE, residence time). Freshwater bivalves appear to utilize only particles less than 80 μm and have a maximum retention efficiency of particles in the 5 to 35 μm range (see section 3.3.2.2). The amount of metal associated with these particles generally increases as their size decreases (i.e. increased surface area for binding). Digestive processes may also affect the fate of ingested

sediment-bound metals. Metals may be assimilated from particles after their release from particles in the gut (Luoma, 1983). Gut pH levels tend to vary from a pH of 5 recorded for suspension feeders such as oysters to a pH of 6 to 7 for deposit feeding organisms (Luoma, 1983). Uptake in the gut would be expected to increase due to the desorption expected at a low pH, except the concomitant increase in $[H^+]$ competes for available uptake sites on the intestinal membrane (Campbell and Stokes, 1985). Further, the membrane carriers across the gut are reportedly less efficient in complexing the metal for transport than membrane carriers in other tissues such as the gill (Luoma, 1983).

Long residence times of food particles in the digestive tract increase the potential for metals to be assimilated, particularly for those metals with long desorption/dissolution rates (Wang and Fisher, 1996a). Long residence times also improve the extraction efficiency of the metals from the particles. For many bivalves (after food is sorted in the stomach), digestion can be a two step process. Initially, food particles undergo "rapid" intestinal digestion whereby enzymatically degraded solute or colloidal organic material and associated metals may be absorbed across the stomach and intestine. Low metal absorption efficiencies are usually associated with intestinal digestion (Decho and Luoma, 1991). Finer particles are then sorted from the solutes and transferred the digestive gland for further intracellular glandular processing called glandular digestion (Decho and Luoma, 1991). During this process, digestive cells phagocytize the particles and digest them intracellularly. The relative time spent in either intestinal (extracellular) or glandular (intracellular) digestion has significant implications for the relative assimilation efficiency of metals associated with ingested food particles. A recent study by Decho and Luoma (1996) found that *Potamocorbula amurensis* reduced its assimilation efficiency (81 to 51 %) of Cr(III)-labeled bacteria at high Cr(III) concentrations in part by reducing the proportion of bacteria processed by glandular digestion. The relative absorption efficiencies during both intestinal and glandular digestion vary among metals and food particles (e.g. bacteria, diatoms), and can vary between bivalve species (Decho and Luoma, 1996).

Endocytosis is another form of solid phase metal uptake in benthic organisms that involves metal-bearing particles being engulfed by specific amoebocytes and/or digestive vesicles outside the cell membrane in the gut lumen or even outside the gills, forming vesicles that move into the tissues (Luoma, 1983). Digestion within the metal-bearing vesicles that leads to the release of

metals into the cytosol is poorly understood, although processes similar to those present in the intestinal tract may exist. The importance of this form of endocytosis as a route of uptake for molluscs is unknown. Further research into the mobilization of the metals from the vesicles is needed since a metal is not truly assimilated until it crosses the vesicular membrane into the cytosol.

The type of particle ingested has a significant influence on the bioavailability of the particulate-bound metals. Laboratory techniques using radio-labeled substrates have been used to determine the relative bioavailability of metals bound to different sediment and food particles (Table 10). The major conclusion from the sediment experiments was that metal bioavailability varies for each substrate according to the specific binding affinity of the metal for the particle i.e. the stronger the metal binding affinity the less bioavailable the metal (Campbell and Tessier, 1996). Further, the degree of crystallinity played an important role in determining the bioavailability of the different metals; metals associated with older more highly crystalline solid phases tended to be less bioavailable.

Particle coatings also influence the bioavailability of the sediment-bound metals, and again the effect is dependent on the specific metal. Adherent bacteria and bacterial exopolymer (used in bacterial adhesion) had variable effects on the different metals. The exopolymer adsorbed onto the Fe-ox surface resulted in an increase in the availability of particulate-bound metals in the order Ag>Cd>Zn. Adherent bacteria had no effect on metal uptake by *Macoma balthica* (Harvey and Luoma, 1985a). The role of the exopolymer on uptake and availability was thought to be to stimulate enzymes in the digestive tract that enhanced the removal of the metals from the sediment particles (Harvey and Luoma, 1985b). Other organic coatings consisting of humic and fulvic acids also influenced uptake and bioavailability. Humic coatings only slightly enhanced Cr bioavailability over non-living particles to the marine suspension feeder *Potamocorbula amurensis* and deposit feeder *Macoma balthica* (Decho and Luoma, 1994). Assimilation of Cd was generally higher than for Cr in both bivalves, but the presence of organic coatings on particles reduced Cd bioavailability compared to uncoated particles (Decho and Luoma, 1994).

The bioavailability of metals from food particles was found to differ among phytoplankton taxon and the cytoplasmic partitioning of metals within the algae. The effect of food composition on metal assimilation in *Mytilus edulis* was recently examined by Wang and Fisher (1996a).

Table 10. Assimilation of sediment- and algal-bound metals by the clam *Macoma balthica* and mussel *Mytilus edulis*. Adapted from Campbell and Tessier (1996).

Metal	Species	Relative Bioavailability Sequence	Reference
Cd	*Macoma balthica*	uncoated Fe-ox » coated Fe-ox, organic detritus	Luoma and Jenne, 1976
Ag	*Macoma balthica*	calcite > Mn-ox » biogenic $CaCO_3$ > Fe-ox > detritus	Luoma and Jenne, 1977
Zn	*Macoma balthica*	biogenic $CaCO_3$ > detritus > calcite > Fe-ox, Mn-ox	
Co	*Macoma balthica*	biogenic $CaCO_3$ > calcite ~ detritus > Fe-ox > Mn-ox	
Cd	*Macoma balthica*	(exopolymer + Fe-ox) > (bacteria + Fe-ox) ~ uncoated Fe-ox natural sediment » alkaline extracted sediment extracted sediment + exopolymer ~ original sediment	Harvey and Luoma, 1985b
Zn	*Macoma balthica*	(exopolymer + Fe-ox) slightly > uncoated Fe-ox natural sediment » alkaline extracted sediment extracted sediment + exopolymer ~ orignial sediment	
Cr (VI)	*Mytilus edulis*	phytoplankton > oxidized marine sediment (2% loss on ignition)	Wang et al. 1997
Cd	*Mytilus edulis*	diatoms ~ dinoflagellates > prasinophytes ~ inorganic particle > chlorophytes	Wang and Fisher 1996a
Zn	*Mytilus edulis*	diatoms > dinoflagellates > prasinophytes ~ inorganic particle > chlorophytes (*Nannochloris atomus*)	
Ag	*Mytilus edulis*	dinoflagellates > chlorophtyes > diatoms » prasinophytes	

Mytilus edulis were exposed in the laboratory to radio-labeled algae (2 diatoms, 2 dinoflagellates, 2 chlorophytes, 1 prasinophyte) and glass beads (representing extreme end member of inorganic particles). Chromium was not efficiently assimilated from phytoplankton and over 98% was lost from the mussels within 24 h. Assimilation efficiencies were generally lower for Cd and Zn from chlorophytes *Chlorella autotrophica* and *Nannochloris atomus* compared to other algae, but this trend was not observed for Ag, Am, Co or Cr. The cell walls of chlorophytes are more rigid and resistant to enzymatic digestion and physical breakdown (Atkinson et al. 1972). Therefore, the assimilation of essential metals (e.g. Zn) which normally penetrate the cytoplasm of algal cells (Reinfelder and Fisher, 1991) would be expected to decrease within the rigid cell walls. Non-essential elements such as Cd mostly adsorb onto cell surfaces (Reinfelder and Fisher, 1991), suggesting a different mechanism was operating to cause the reduced assimilation of Cd from the chlorophytes. Gut passage times for metals (Am, Cr, Cd) determined in this experiment suggest that extracellular digestion may be responsible for differences in assimilation among food types. The effect of food type was most significant for Ag which showed a 10-fold difference in assimilation efficiencies among algal species. Assimilation efficiencies were significantly correlated with metal cytoplasmic distribution in algal cells (i.e. % penetration into the algal cytoplasm) for Am and Co but not for Ag, Cd and Zn (Wang and Fisher, 1996a). Assimilation efficiencies for metals adsorbed to glass beads were found to be similar to those for algal diets, except for Co which more efficiently assimilated from glass beads.

In another experiment by Wang and Fisher (1996b), diatom protein content was not found to have a major influence on metal assimilation in *Mytilus edulis* (Wang and Fisher, 1996b).

Given the differences in metal bioavailability and assimilation efficiency among sediment and food particles, the composition of ingested particles should be considered when interpreting metal accumulation data and estimating the potential for metal bioaccumulation at mining sites.

4.2.4 **Measuring bioavailability - Practical considerations**

Countless field studies have endeavored to relate tissue metal concentrations to total metal concentrations in the sediment or water with limited success. In many cases, cost-cutting measures and time limitations have lead to the use of less complicated measurements of total metals in water and sediment. However, as the above sections illustrate, the amount of metal

available for uptake varies as a function of a number of environmental variables and thus, total metal concentrations are often misleading.

Some investigators have successfully related tissue metal concentrations to total metal concentrations in sediment. For example, concentrations of Zn in *Elliptio complanata* and Cd, Cr and As in *Lampsilis radiata* and *Elliptio complanata*, were significantly correlated with concentrations of these metals in the sediment (readily extractable or total) (Metcalfe-Smith et al. 1992). Correlations were positive for Cd, Cr, and As and negative for Zn. Cadmium levels in caged *Lampsilis ventricosa* used to monitor pollution from mine tailings in the Big River, Missouri, were highly correlated with total concentrations in sediment (r=+0.89) (Czarnezki, 1987).

Total metal concentrations in the environment might be expected to provide a reasonable estimate of bioavailable metals for the following cases:

- non-essential metals
- over wide ranges of total metal concentrations (i.e. including grossly contaminated and pristine environments).
- over narrow ranges of underlying geochemistry/geology (e.g. for lakes on the Canadian Shield).

In a more general context, it is strongly recommended that an effort be made to collect environmental data (sediment and water) such that detailed estimates of bioavailable metals can be made. The bioavailable fraction in water for most metals can be estimated from water chemistry and surficial sediment concentrations (see Tessier et al., 1993).

4.2.5 **Summary**

1. Bioavailability estimates should consider metal contributions from both routes of uptake (dissolved and particulate) and should be determined for individual metals.
2. The uptake from the dissolved phase can be estimated using the Free Ion Activity Model. The free-metal ion concentration can be estimated from sediment-water equilibria.

Variations in the relative values of the free-metal concentration can be approximated by the ratio of sorbed metal to sorbent, provided that the pH is reasonably constant.

3. The bioavailability of metals from the particulate phase is determined by factors specific to sediment and food particles. Metal assimilation from sediment particles is influenced by chemical composition (Fe-ox, calcite etc.) and presence of organic or bacterial coatings. Differences in metal assimilation from algae result from the relative amount of time spent in extracellular and intracellular digestion, algal cytoplasmic metal distribution and cell wall structure.

4.3 Biological characteristics

There is considerable evidence that individual biological characteristics affect metal accumulation in molluscs. This is not surprising since many metals are essential for growth and reproduction and would be accumulated in varying amounts depending on the biological state of the individual mollusc. Aspects of age, size and growth rate, sex and reproductive status, behavior and inter-specific differences and how they affect metal accumulation are briefly addressed below. In addition, metal accumulation in transplanted or caged molluscs will be compared to that in indigenous populations. Decisions about the usefulness of transplanted or caged molluscs in monitoring programs depend in part on their ability to mimic metal accumulation of indigenous populations from impacted areas.

4.3.1 **Age, size and growth rate**

Age and size were found to be the most significant predictor of tissue metal concentrations (Cd, Cu, Fe, Hg, Mn, Ni, and Zn) in the freshwater bivalves *Elliptio complanata* and *Lampsilis radiata radiata* collected from the Sorel delta in the St. Lawrence River (Metcalfe et al. 1996). The effect of age and size was followed by growth rate and then sex in order of importance in determining metal accumulation. Levels of As, Cd, Mn, Zn, Hg, and Fe were higher in older-larger individuals of both species, whereas Cu, Al, Ni, Cr, and Se were higher in younger, smaller individuals of *Lampsilis radiata radiata*. In *Elliptio complanata* only Cu was higher in younger,

Table 11. Relationship between metal concentrations and size and age (inferred from size) in marine molluscs: metal concentrations increase with size (+), decrease with size (-), or are not related to size (0). Adapted from Hinch and Stephenson (1987).

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

smaller individuals. Lead levels were not affected by age or size in either species (Metcalfe-Smith et al. 1996). In the freshwater prosobranch *Bithynia tentaculata* collected from Lake St. Louis and St. Pierre in Quebec concentrations of Cu and Zn were significantly higher in adults compared to juveniles, whereas no life-stage differences were found for Cd, Pb or Ni (Flessas et al., submitted). Age and size successfully predicted tissue metal concentrations in *Elliptio complanata* collected from an acid-sensitive and circumneutral lake in Ontario, in metal- and tissue-specific manner (Hinch and Stephenson, 1987). For example, mussel age was a better predictor of gill Zn and Mn concentrations, but gill Cd was better predicted by mussel size.

However, the age and size of individuals are not always correlated (Hinch and Stephenson, 1987). Relationships between metal concentrations and size can vary among metals, among species, and among studies (Table 11). The overall effect of age and size on metal concentrations in molluscs also depends on growth rates, feeding habits and reproductive status. For example, Malley et al. (1989) found that growth rates were distinctly different between two populations of *Pyganodon grandis* exposed *in situ* to ^{109}Cd in a Precambrian Shield lake over the ice-free season. The smaller, faster growing bivalves had higher concentrations of Cd in their

tissues compared to the larger, slower, growing bivalves of the same age. The incorporation of Cd into the tissues of bivalves is linked to their filtration rates and metabolism. Thus, even though the faster growing individuals were smaller, it was possible for them to accumulate metals faster. Because it is difficult to predict the effect of age and size, it is recommended that age and size either be standardized in all monitoring programs or tested for age- and size-specific differeneces prior grouping specimens together (Hinch and Stephenson, 1987).

4.3.2 **Sex and reproductive status**

The sex and reproductive status of molluscs may influence tissue metal concentrations in bivalves. Differences in tissue metal concentrations of male and female bivalves collected from a range of contaminated sites along the St. Lawrence River were significant in both *Lampsilis radiata radiata* (Cd, Fe and Zn were higher in males), and *Elliptio complanata* (Cu was higher in females) (Metcalfe-Smith, 1994). Males tended to display less variability in metal concentrations than females. The effect of sex on tissue metal concentrations was less significant than inter-species differences. Lobel et al. (1989) found that in "post-spawn" *Mytilus edulis* collected from Bellevue, Newfoundland As, Cu, Mn and Zn were higher in females, Pb was higher in males and Al and Cd did not differ between sexes. Alternatively, Jones and Walker (1979) found no sex-specific differences in metal tissue levels in freshwater *Velesunio ambiguus* from the River Murray in South Australia.

In general, tissue concentrations of metals in filter-feeding bivalves are highest in males and females just prior to spawning and differences between sexes are minimal at this time compared to other times throughout the year. For biomonitoring programs, Lobel et al. (1991a) suggest that the sexes be analyzed separately and that the sampling be performed prior to spawning to reduce individual differences.

4.3.3 **Behavior**

Recently developed laboratory techniques reveal that unionids may close their valves in response to high metal concentrations (Kramer et al., 1989; Doherty et al., 1987). Kramer et al. (1989) found that *Mytilus edulis* elicited a valve closure response when exposed to 38 $\mu g\ L^{-1}$ Cu

(as copper sulfate). At lower Cu concentrations (<20 μg L^{-1}) the closure response was delayed and less obvious. Similar detection limits, determined when 7 or more out of 10 mussels reacted by closing their valves or changing filtering activity, for Cd (<100 μg L^{-1}), Cu (<10 μg L^{-1}), Zn (<500 μg L^{-1}) and Pb (<500 μg L^{-1}) were found for *Mytilus edulis* and *Dreissena polymorpha* (Kramer et al., 1989). The metal concentrations used in the above study and those used by Doherty et al. (1987) (100 to 400 μg Cd L^{-1}) were well above typical environmental metal concentrations and thus it is difficult to determine their relevance for mollusc biomonitoring studies. If unionids can limit the uptake of metals during periods of shell closure, metal levels in unionid tissues may be underestimated. Further research on the influence of valve closure on metal accumulation at environmentally realistic concentrations is needed.

4.3.4 **Inter-specific differences**

The advantages of using more than one species in a biomonitoring program include broadening the geographic range and increasing the habitat coverage (Metcalfe-Smith, 1994). However, differences in metal accumulation among freshwater and marine bivalve species have been reported and may limit inter-species comparisions. Metcalfe-Smith (1994) found that in freshwater mussels collected from a range of contaminated sites, *Elliptio complanata* accumulated significantly more Al, Cr, Fe, Hg and Ni and less As, Cu, Mn and Zn than the mussel *Lampsilis radiata radiata*. There were no inter-species differences for Cd and Pb, except for Cd when the sexes were separated. *Elliptio complanata* tended to show a broader range of responses across contaminated sites than *Lampsilis radiata radiata* suggesting that the latter species may be more capable of regulating tissue metal concentrations. This would make *Lampsilis radiata radiata* less suitable as a biomonitor since it does not directly reflect the bioavailability of metals in the environment (Metcalfe-Smith, 1994).

Differences between species were generally in the range of 1.2 to 2.5 X which corresponded to other similar studies (Metcalfe-Smith, 1994; Metcalfe-Smith et al. 1992). Although these differences don't appear to be large, if differences among study sites only range between 2 to 10X, the ability to use different species to distinguish between sites diminishes (Metcalfe-Smith, 1994). Tessier et al. (1987) report higher metal concentrations (Cd, 1.7 - 3.0 X; Cu, 1.1 - 2.3 X; Pb, 0.9 - 8.3 X; Zn, 1.2 - 1.9 X) in indigenous *Pyganodon grandis* compared to

indigenous *Elliptio complanata* in both Lake Memphrémagog, Quebec and Lake St. Nora, Ontario. A similar range of differences between species were found for two marine mussels collected from a lagoon in Newfoundland, whereby, concentrations of all the 25 elements analyzed (except Mn) were higher (1.5X) in *Mytilus trossulus* than *Mytilus edulis* (Lobel et al. 1990).

Despite inter-species differences in absolute metal concentrations, some metals in Metcalfe-Smith (1994) were highly significantly correlated (Al, Cd, Cu, Fe and Pb) between the species while others were significantly correlated (Cr, Hg and Ni). Metcalfe-Smith (1994) suggested that *Elliptio complanata* and *Lampsilis radiata radiata* could be used interchangably to monitor site-to-site trends in the bioavailability of Cd and Pb, and after conversion by means of regression equations to monitor Al, Cr, Cu, Fe, Hg and Ni. Where correlations were lacking (As, Mn and Zn), the two species could not be used interchangeably and further research would be required prior to choosing the better species for monitoring those metals. Nevertheless, relative differences in metal concentrations within one species are often more important in biomonitoring programs than absolute differences. Biomonitoring programs should be carefully designed with the knowledge of inter-species differences in mind, ensuring that there will be sufficient availability of the species for the duration of the monitoring program.

4.3.5 **Transplanted/caged vs. indigenous populations**

Transplanted and/or caged molluscs are often used in metal accumulation studies as representatives of indigenous populations. However, metal accumulation patterns in these experimental animals may not reflect those of the free-living indigenous populations. There are two primary questions relevant to the use of transplanted and/or caged molluscs in monitoring programs: (i) do molluscs that are caged in their "source" lake grow and accumulate metals at the same rate as do the free-living molluscs in the same lake, and; (ii) do molluscs that are moved from an uncontaminated environment (whether it be a control lake or an aquaculture facility) to a contaminated environment grow and accumulate metals at the same rate as do the free-living molluscs in the contaminated environment?

Studies by Couillard et al. (1995a,b) suggest that caging molluscs on the sediment in their source lake does not significantly influence metal accumulation patterns. No significant differences were found between *Pyganodon grandis* caged for 400 days in its source lake and free-living individuals outside the cages with regards to tissue Cd, Cu and Zn concentrations (except Zn in digestive gland), condition index and MT (except mantle MT). However, shell growth was significantly reduced in 400 day caged source lake mussels in both the uncontaminated and contaminated lake, which may have had consequences for metal accumulation if the exposure had continued. No significant differences between individuals caged on the sediment for 7 days and those collected outside the cages were found for Zn and Cd concentrations (except Zn in foot and Cd in digestive gland) in the freshwater clam *Amblema plicata*, near an electroplating plant on an Indiana stream (Adams et al. 1981). Similarly, Malley et al. (1996) found that total mercury and methyl mercury concentrations and body burdens in tissues of *Pyganodon grandis* caged on the sediment for 88 days in their source lake were not significantly different from free-living mussels outside the cages.

Transplanted molluscs may grow and accumulate metals at the same rate as do the free-living molluscs in the destination lake depending on the metal. Cadmium concentrations in tissues and whole body of *Pyganodon grandis* transplanted from uncontaminated Lake Opasatica to contaminated Lake Vaudray and caged for 400 days were only one third of those found in the free-living indigenous mussel population (Couillard et al. 1995a). In contrast, Zn tissue and whole body concentrations in the transplanted mussels rapidly reached those of the free-living indigenous population (<100 d). Couillard et al. (1995a) suggest that the rapid uptake of Zn and the rapid attainment of steady-state concentrations was consistent with the essential role of this metal. Based on these data, Zn uptake and accumulation in transplanted mussels could be used as representative of that which occurs in indigenous mussel populations. Hinch and Green (1989) found that both the destination and source or collection site influenced growth rates and tissue metal concentrations (Cd, Cu and Zn) in transplanted *Elliptio complanata* after 1 year. Growth rates have been previously reported to be affected by the source of the mussels rather than the transplant destination, suggesting that growth rates may be under direct genetic control (Hinch et al. 1986). To control for the "source effect"in transplant experiments all specimen collections should be from the same site within a lake or from the same aquaculture facility and the exposure

periods should exceed 1 year to allow the destination lake to become the dominant influence (Hinch and Green, 1989).

4.4 Modeling trace element bioaccumulation in mussels

Recent developments in our knowledge of assimilation efficiencies and uptake rates in freshwater mussels have made it possible to develop models that can be used to estimate bioaccumulation factors (BAFs) to calculate possible concentrations in mussels based on environmental concentrations of trace elements. These models take into consideration the critical environmental variables that influence metal accumulation to make the BAFs more accurate and useful than BCFs. Models offer considerable advantages for biomonitoring in that bioaccumulation data collected in mussels can be interpreted and the source of metals released into the environment can be better ascertained.

Mytilus edulis fed radioisotope-tagged natural seston or put in a radioisotope-labeled water, was then monitored in a clean environment to measure efflux rates (Wang et al. 1996). Trace metal concentrations predicted using a kinetic model were comparable to concentrations measured in various monitoring programs, suggesting that metal accumulation in mussels can be accurately predicted using the physiological and geochemical parameters identified (C_w, water concentration, C_f, food concentration, Total suspended solids and K_d). Two important physiological parameters, metal absorption efficiency from the dissolved phase and assimilation efficiency from food, must however be known if this approach is to be used to assess metal bioavailability to aquatic animals.

5. RELATIONSHIP BETWEEN TISSUE (OR WHOLE BODY) METAL CONCENTRATION AND EFFECTS IN MOLLUSCS[2]

In order to adequately address the relationship between tissues (or whole body) metal concentrations and effects in molluscs a thorough discussion of the factors that influence effects in molluscs must be undertaken. However, allocated time for this report will not allow for a in-depth review of all relevant literature. Instead, a brief discussion of relevant information needed to determine the utility of effects measurements in molluscs as part of a monitoring program will be provided. This should enable the AETE program committee to determine the next course of action regarding effects monitoring in molluscs.

Rather than relate metal effects in molluscs to metal concentrations in the sediment and water they will be related to mollusc tissue-residues. This approach tends to improve the relationships drawn between metal exposure and effects because the concentrations of metals in tissues are implicitly bioavailable; as described in the previous section, determinations of "bioavailable metal" can be complicated.

5.1 Interpreting tissue-residue effects in molluscs

The relationship between tissue (or whole body) metal concentrations ("Dose") and effects in molluscs is dependent on many factors including the metal (i.e. chemical form, essential vs. non-essential), type of effect (i.e. biochemical, physiological, population), exposure history (e.g. development of tolerant populations), environmental conditions (i.e. temperature, food availability, presence of other stressors) and numerous biological characteristics (i.e. species, age, size and reproductive state). These factors must be well described and understood in order to successfully relate dose to effects in molluscs as well as in any other organisms. Luoma and Carter (1991) suggest that attributing a biological change in a natural system to the specific influences of metals requires: 1) demonstrating which processes are sensitive to metals; 2) separating metal-induced changes in a process from background fluctuations, and; 3) unambiguously relating the detected change to metal exposure rather than abiotic (e.g.

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[2] This section includes revisions made in response to a critical review performed by Michael Salazar of Applied Biomonitoring and provided in PART II of this report.

temperature, salinity, oxygen, or physical processes) or biotic (e.g. species interactions, nutritional status) confounding variables.

Effects may occur at different levels of biological organization including biochemical, physiological, population and community (Luoma and Carter, 1991). The "effect" or functional impairment within a level occurs after the compensatory capabilities are overcome (Luoma and Carter, 1991). Effects at lower levels of biological organization (biochemical and physiological) do not necessarily result in observable effects at higher levels of organization (population and community). Typically, as the exposure increases the compensatory capabilities are overcome at the higher levels of organization and changes in the population and community structure results. Effects at the biochemical or physiological level have been recommended for use as early warning signals for effects at higher organizational levels. However, few field studies have attempted to relate effects at the biochemical or physiological level to effects at higher levels of biological organization. Therefore, the ecological relevance of many biochemical or physiological responses is unknown, which severely limits their usefulness in monitoring programs.

The development of effects monitoring in molluscs has benefited from recent collaborative field and laboratory studies. Although effects in molluscs have primarily consisted of laboratory some effects have been successfully identified and quantified in the field. Field studies provide data which are more relevant to biomonitoring programs. Recently there has been a rise in the number of studies that link effects at all levels of biological organization. The effects that have received the most recent attention include changes in growth, condition index, and metallothionein (MT). Changes in MT concentration, although described here as an effect, is actually an indicator of exposure to metals. Molluscs have been included in measurements of community structure (e.g. taxonomic indices), but, the relatively small numbers of species found in Canada render mollusc population measurements not very useful. The following is a brief summary of how growth and MT concentrations can be related to tissue metal concentration in bivalves.

5.2 Growth

Growth is a physiological endpoint measured at the individual organism level that reflects the environmental conditions in which the organism lives. Natural environmental conditions such as food quality and quantity and temperature as well as characteristics of the organism (e.g. size, age, sex) are major determining factors of growth in individuals, although metal exposure can play a role. Growth is one of many physiological responses that are sensitive to metals, although it tends not to be metal-specific (Luoma and Carter, 1991). Nonetheless, effects of metal exposure on growth have been well studied (in the marine environment) and may prove to be a suitable effects measurement as part of a monitoring program.

5.2.1. **General description**

Growth in molluscs can be determined in many different ways including changes in shell length and soft-tissue weight. Changes in absolute growth as well as growth rates of bivalve molluscs can be determined non-destructively using the external rings visible on the shells. It is generally accepted that populations of bivalves in northern climates put down one new external growth ring a year, based on mark and recapture studies (Metcalfe-Smith and Green, 1992 and references therein). A recent study that utilized a similar type of mark and recapture, found that external annual rings were formed less frequently than annually for several *Pyganodon grandis* casting doubt on the process of aging molluscs (Downing et al. 1992). However, the generality of this observation cannot be established at this time without further field studies. There have been numerous unionid species that have been successfully aged using the above technique including *Anodonta piscinalis*, *Anodonta anatina*, *Anodonta grandis simpsionata*, *Elliptio complanata*, *Lamsilis radiata siliquoidea* and *Leptodea fragilis* (Metcalfe-Smith and Green, 1992).

To determine an individual's growth rate external annual growth rings are measured and the total number of growth rings counted. These measurements are then entered into "Walford Plots" to determine the shell growth rate. For example, the length delimited by an annulus for year n+1 is regressed against the length for year n (Fig. 3). Comparison of the regression coefficients for bivalve populations can be then compared.

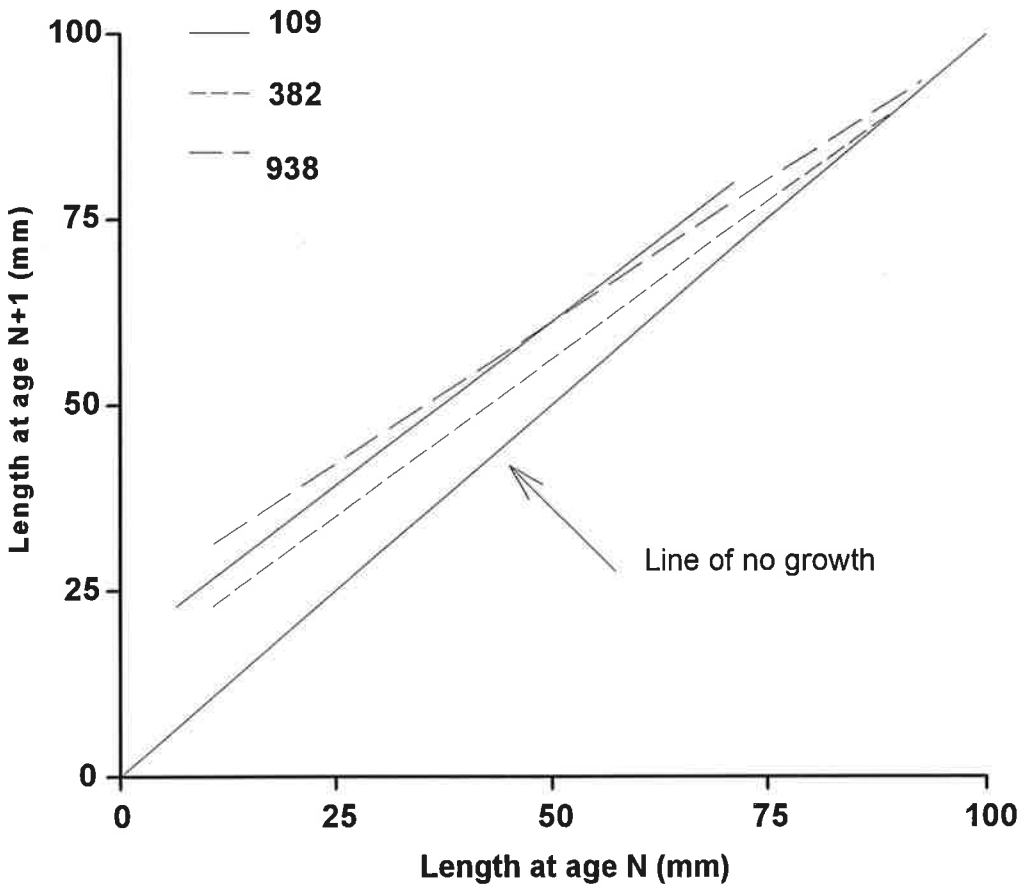

Figure 3 Walford plot for *Pyganodon grandis* collected from Lakes 109, 382 and 938 in the Experimental Lakes Area, Northwestern,Ontario.

Von Bertalanffy growth curves, which relate individual annular length (y-axis) to the age of the bivalve, provide a more qualitative means for comparing relative growth of different bivalve populations (Fig. 4). Using both the Walford Plot and the Von Bertalanffy growth curves similarities between bivalve populations can be determined as well as growth potential for a particular year, such as in Couillard et al. (1995a). Growth of individual bivalves can be measured at the beginning and end of exposure periods, providing the marking technique does not cause excessive stress on the animals. A considerable number of studies have been done by Green et al. (1989) and others (Hinch and Green, 1989; Hinch et al. 1986; Hanson et al. 1988) to quantify the

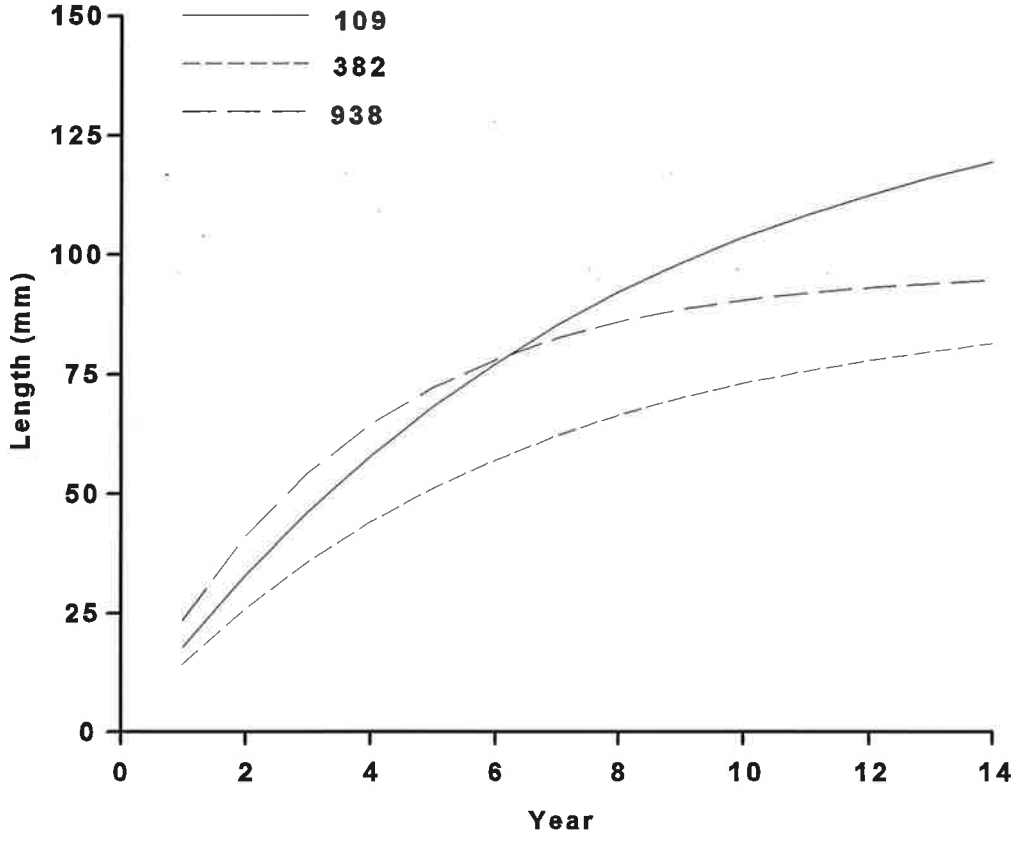

Figure 4. Von Bertalanffy growth curves for *Pyganodon grandis* collected from Lakes 109, 382 and 938 in the Experimental Lakes Area, Northwestern, Ontario.

influence of natural environmental factors on shell growth. Shell growth must be interpreted with caution since hydrodynamic conditions (i.e. wave action) can influence the allometric growth of the shell and thus indirectly influence growth rate measurements (Green et al. 1989).

Changes in soft-tissue weights are another useful measure of mollusc growth. Measurements of individual tissues or the whole organism can be made on sacrificed individuals and compared among composite samples of pre- and post-exposure groups. This destructive techniques prohibits measurements on individuals throughout the experiment, although sample variability caused by intra-specific differences can be reduced by collecting animals of a similar size. Salazar

et al. (1996) recommend a non-destructive measurement of whole-animal-wet-weight, that can be determined for the same individual throughout the exposure period. Care must be taken with this measurement to avoid the introduction of sample error by ensuring that the shell cavity is full of water, to maintain a constant water content, and that the shell is free of debris.

5.2.2. **Dose-Response**

There are significant difficulties in attributing growth impairment in bivalve molluscs to metal exposures. Natural cycles in food availability and differences in temperature among sampling sites can confound metal-induced growth effects. All bivalves, caged and native, marine and freshwater, have shown natural fluctuations in dry soft-tissue weights. For example, Cain and Luoma (1990) found a constant decrease in the soft tissue weight in indigenous *Macoma balthica* in San Francisco Bay for two consecutive years. Couillard et al. (1995a) showed decreases in organism dry weights of indigenous and caged populations of *Pyganodon grandis* over a 400 day exposure period, although the decreases were more pronounced in the bivalves exposed to higher metal concentrations. Salazar et al. (1996) found a significant negative correlation between tissue mercury concentrations and changes in whole-organism wet-weights of *Elliptio complanata* caged for 84 days along a mercury contamination gradient. A significant negative correlation was also found between soft-tissue wet-weights in *Elliptio complanata*, measured at the end of the study, and tissue mercury concentrations. The overall reduction in growth of *Elliptio complanata* could not be attributed entirely to elevated mercury concentration because of other confounding physico-chemical factors (i.e. temperature).

Relationships between shell growth and metal exposure are also sensitive to environmental conditions. Holding the mussel, *Pyganodon grandis* in open enclosures in both high and low metal contaminated lakes for 400 days had a significant negative effect on growth rate. In the first 90 days mussels reached 75% of their annual growth increments. Couillard et al. (1995a) suggested that the caging effect on growth rate could have been an artifact of the temperature range in which the bivalves were caged. Hanson et al. (1988) found that *P. grandis* caged for over a year at 1 and 3 m grew faster than those caged at 7 or 5 m and that the differences were strongly correlated to temperature at either depth. Couillard et al. (1995a) hypothesized that bivalves left in their natural environment grew faster because they were able to migrate vertically

to micro-environments more favorable to their growth. Nevertheless, bivalves caged in the more metal contaminated lake had a significantly lower mean growth rate than bivalves caged in the control lake. Both the Couillard et al. (1995a) and Salazar et al. (1996) studies compared bivalves from the same population, ensuring that the genetic aspects of growth in the bivalves were held constant and thus, could not confound the results.

Metcalfe-Smith and Green (1992) utilized regression relationships between the bivalve age and shell length and shell weight to compare mussel populations from a low and high As and Hg contaminated lake (bottom sediment: As, <detection, 890-3050 $\mu g\ g^{-1}$ dry wt; Hg, <detection, 5.7 $\mu g\ g^{-1}$ dry wt.). *Elliptio complanata* ranging in age from 5 to 17 years were found to be longer (shell length) at a given age in the less contaminated lake than the more contaminated lake, although the growth rates of the bivalves did not differ for bivalves 5 years and older. However, regression intercepts were significantly different indicating that the bivalves from the less contaminated lake had faster growth rates during the first 5 years of their life than those from the more contaminated lake. An identical relationship was found when age was used to predict shell weight. Similar measurements on the unionids *Anodonta implicata* and *Alasmidonta undulata* did not reveal any significant differences between lakes. The authors suggest that the lower initial growth rates and sizes of *Elliptio complanata* may have been due to the arsenic contamination. Tissue-metal concentrations would have been required in order to further evaluate the role of metal contamination in the observed differences in growth.

5.2.3. **Recommendations**

The above examples suggest that bivalve growth (shell length, growth rate, soft-tissue weights) could be used to monitor metal-induced effects in the freshwater environment. However, field studies should be designed to demonstrate that the observed changes in bivalve growth are caused by exposure to metals and not by subtle changes in physico-chemical variables (e.g. temperature and food). Until this is done, the results of growth studies may continue to produce confounding results that will not serve the needs of the Regulators and Canadian mining industry.

5.3 Metallothionein (MT)

For an excellent review of MT the reader is referred to "Technical evaluation of metallothionein as a biomarker for the mining industry" (Couillard, 1996). Here I will highlight the author's recommendations and provide a background on MT based on Couillard (1996).

5.3.1. **General description**

Metallothioneins are low molecular weight metal-binding proteins found within the cytosol. These metal-binding proteins normally bind Group IB and IIB metal ions including Cd, Cu, Zn, and occasionally Ag. Metallothioneins serve as a compensatory mechanism in response to metals, play a role in the regulation of essential metals and the detoxification of metals, and they provide a cellular basis for the bioaccumulation of metals.

Couillard (1996) describes two potential major roles for MT in the intracellular metal distribution: 1) MT induction results in the interception and binding of metal ions taken up by the cell, and; 2) MT removes metals from non-thionein ligands that include cellular targets of toxicity; this redistribution onto MT is suggested to represent a rescue function. Cellular toxicity is expected when these roles are not carried out effectively. Alternatively, cellular toxicity is also expected when there is excessive accumulation of metals beyond the binding capacity of available MT, resulting in their binding to other intracellular ligands, a phenomenon termed "spillover" (Couillard, 1996). According to this model, the degree of metal detoxification, as determined by intracellular metal partitioning, is a better indicator of metal-induced stress than the absolute measure of MT (Couillard, 1996).

5.3.2. **Dose-Response**

Case studies reviewed by Couillard (1996) indicated that MT concentrations increased in a dose-dependent manner along metal contamination gradients (Table 12). Shifts in intracellular metal partitioning towards the very low-molecular-weight metal complexes, typical of metal-induced stress, were detected in contaminated areas. These biochemical effects were accompanied by deleterious effects at higher levels of biological organization (organ, organism,

population). Deleterious effects occurred prior to complete saturation of metal-binding sites by the toxic metal, due to competition for binding sites from essential metals. Thus, the spillover hypothesis based on a complete saturation prior to detection of toxic effects was not strictly observed. Little information was available describing external factors influencing MT concentrations. Although factors related to the basic biology and physiology of molluscs were found to influence MT concentrations, changes in metal bioavailability were deemed more important as sources of variation (Couillard, 1996).

Couillard (1996) noted that most field studies have not been able to "convincingly demonstrate a mechanistic linkage between biochemical responses and adverse effects at higher levels of biological organization" and "further research is needed." Linkages between the hypothesized metabolic costs associated with the activation of tolerance mechanisms such as MT to metal exposure could not be conclusively made at this time, although further research is again recommended.

5.3.3. **Recommendations**

Couillard (1996) recommends the use of MT as a biomarker of exposure to certain metals (notably Cd, Zn, Cu and Ag), but states that MT requires further development as an effects biomarker. In particular, Couillard (1996) recommends further field studies that demonstrate that there are metabolic costs to MT synthesis and/or that the overwhelming of detoxification mechanisms, including MT, is associated with deleterious effects on the host organism.

Table 12. Summary of case studies documenting metallothionein in molluscs. Adapted from Couillard (1996).

Field Site	Species	Tissue	Metal Gradient	Result	Reference in Couillard (1996)
Rouyn-Noranda lakes, Quebec (N=11)	Freshwater mollusc (*Pyganodon grandis*)	gills; whole organism	Cd defined in terms of $[Cd^{2+}]$ at sediment-water interface	MT increased 2.5- to 4-fold in the indigenous populations along the contamination gradient; [MT] correlated with increase in tissue Cd.	Section 4.3.1
Rouyn-Noranda lakes, Quebec (Lake Vaudray exposure period = 400 days)	Freshwater mollusc (*Pyganodon grandis*)	gills; whole organism	Cd defined in terms of $[Cd^{2+}]$ at sediment-water interface	MT increased 2.5- to 4-fold over the first 400 days in molluscs transferred from control lake to highly contaminated Lake Vaudray; increase in tissue [MT] correlated with increase in tissue Cd.	Section 4.3.1
Experimental Lakes Area (ELA), Ont. Lake experimetnally contaminated by Cd over 6 years	Freshwater mollusc (*Pyganodon grandis*)	gills; mantle; foot; kidney; visceral mass	Cd defined in terms of $[M]_d$	All body parts produced MT in response to Cd exposure	Section 4.3.3
Rouyn-Noranda lakes, Quebec	Freshwater mollusc (Pyganodon grandis)	gills	Cd defined in terms of $[Cd^{2+}]$	Overwhelming of the detoxification mechanism including MT (MT levels were very high) was observed along the contamination gradient and appeared to be reproducible under severe metal stress (transplantation experiment). This was associated with toxic effects at cellular-, organ-, individual-, and population levels of biological organization.	Section 4.3.1
San Francisco Bay, California, U.S.A	Marine bivalve (Macoma balthica)	whole organism	Ag, Cu defined in terms of $[M]_d$	Overwhelming of the detoxification mechanism including MT was observed in an indigenous population of M. balthica. Links appeared to exist between these biochemical measurement and adverse effects at the organism- and population-levels of organization.	Section 4.3.2

6. CASE STUDIES ON THE USE OF MOLLUSCS AS BIOMONITORS OF MINING ACTIVIES

The following case studies were chosen because they illustrated the usefulness of bivalve molluscs as monitoring tools of metals in the aquatic environment. An effort was made to use mining related studies. However, other studies (i.e. Salazar et al. 1996) were included because they illustrated how transplanted molluscs could be used to monitor metals at contaminated sites.

6.1 Marine

6.1.1 **Mussel Watch: International and U.S.**

6.1.1.1 *Site description*

Sampling sites were chosen keeping in mind that the Mussel Watch Project was designed to describe chemical distributions over national and regional scales. To avoid "hot spots", or small-scale patches of contamination, no sites were chosen near known waste discharge points. Sites were also chosen that supported a large enough population of indigenous mussels and oysters to provide annual samples (O'Connor, 1992).

Sampling sites were located along the North Atlantic, Middle Atlantic, South Atlantic, Gulf of Mexico and Pacific coasts predominantly near urban areas, within 20 km of population centers in excess of 100,000 people (Figs. 5a-e.) (O'Connor, 1992). In 1986 and 1987, 145 sites were sampled. In 1988 additional sites were added on the East Coast, Hawaii, and Gulf of Mexico. By 1990, 234 sites had been sampled, with further additions made to test how representative the earlier studies were.

6.1.1.2 *Study objectives*

In response to public and scientific concern about the quality of the marine environment and the absence of any long-term national monitoring program in the United States in the early 1980's, the National Oceanic Atmospheric Administration (NOAA) created the National Status and Trends (NS&T) Program in 1984 (O'Connor, 1992). The purpose is to monitor trends of

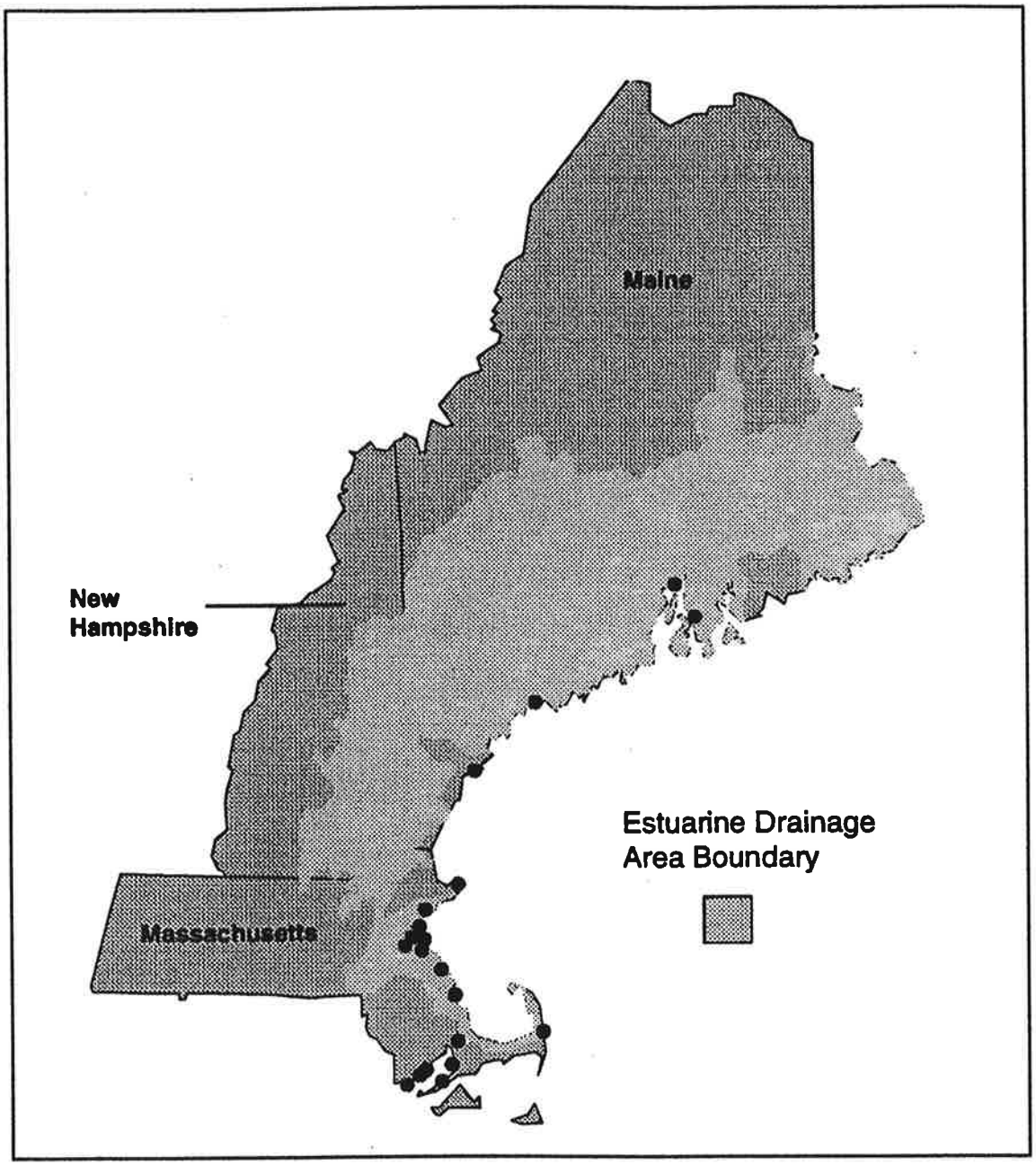

Figure 5a. North Atlantic Mussel Watch Sites. Adapted from O'Connor (1992).

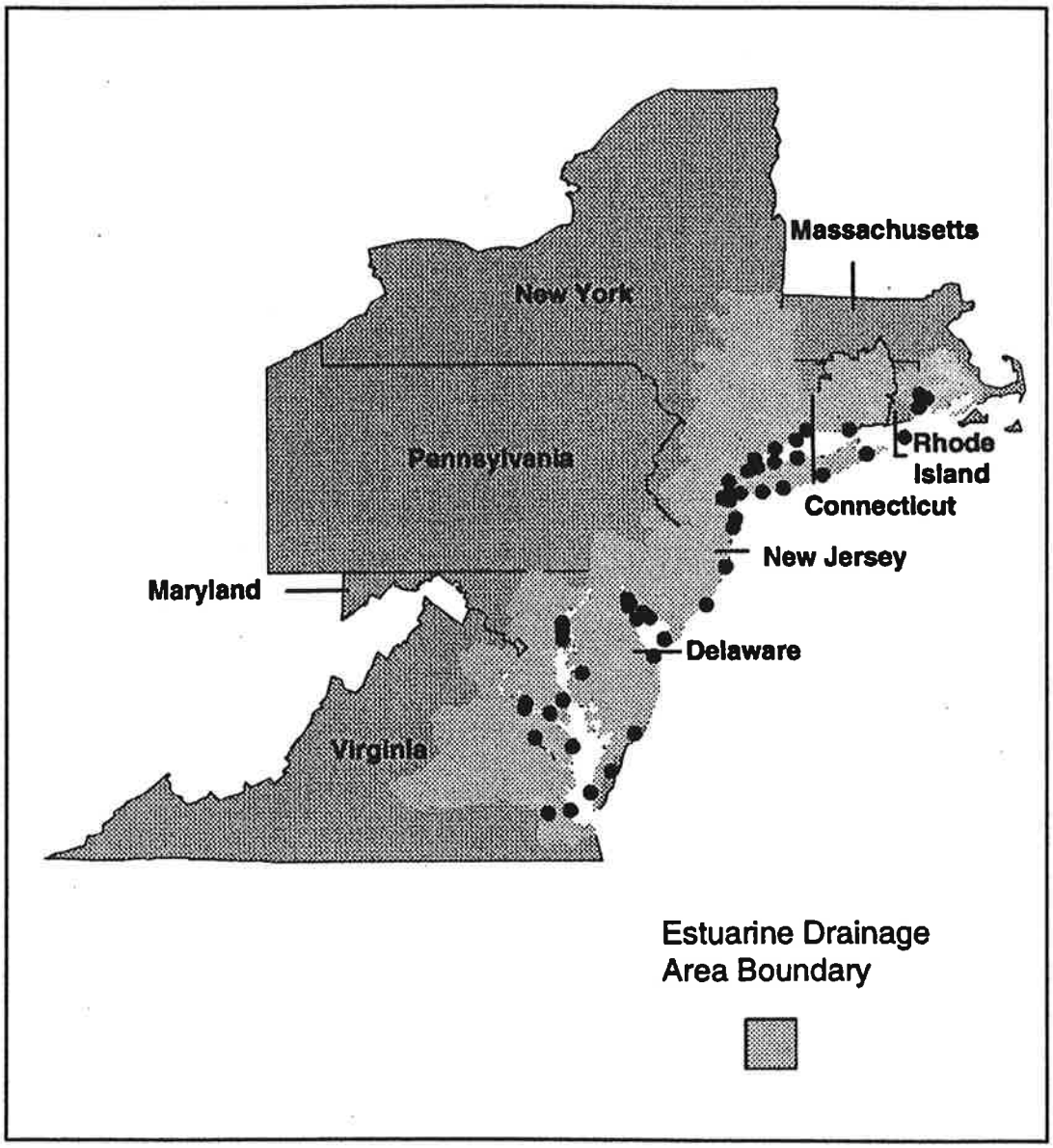

Figure 5b. Middle Atlantic Mussel Watch sites. Adapted from O'Connor (1992).

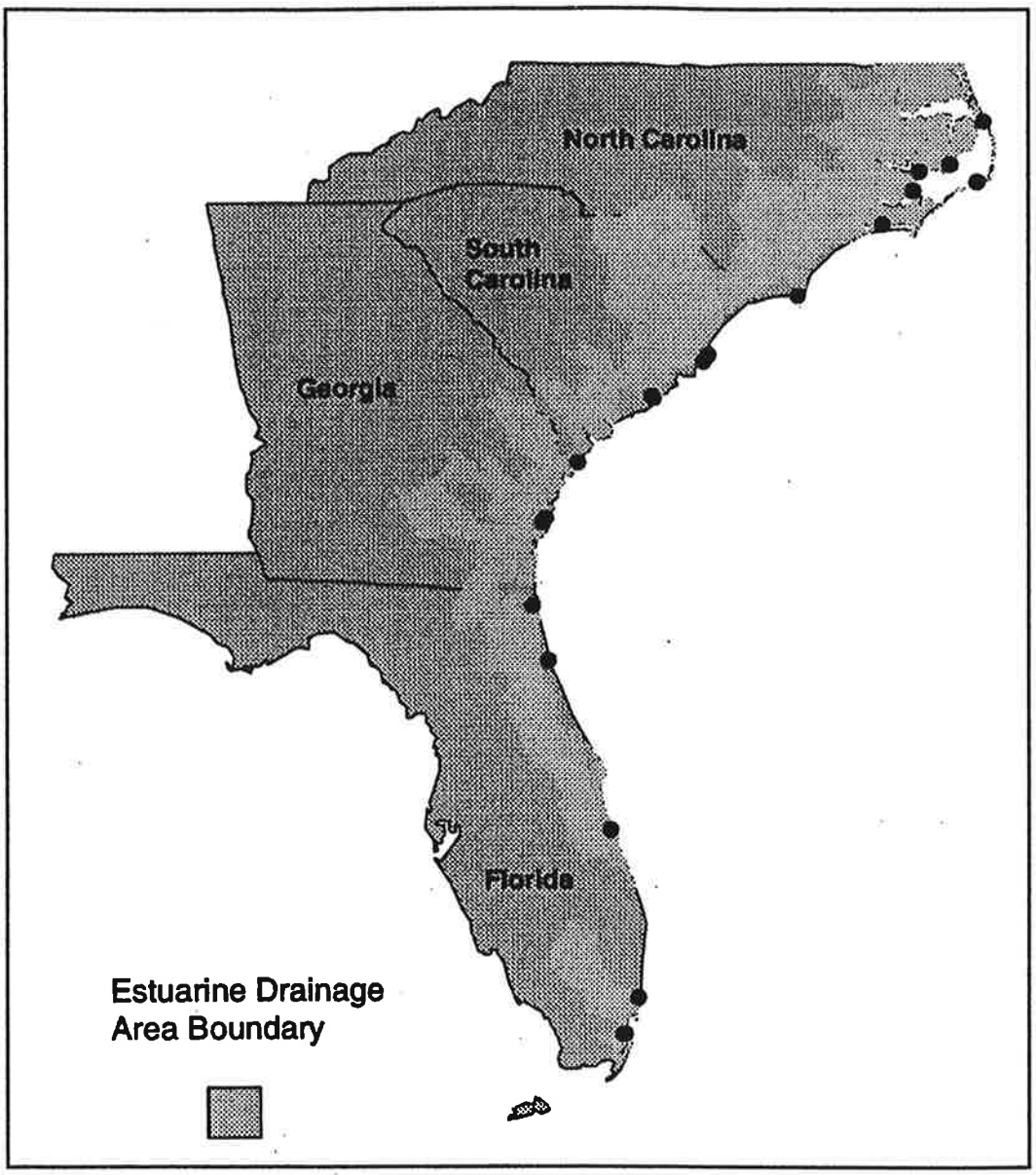

Figure 5c. South Atlantic Mussel Watch sites. Adapted from O'Connor (1992).

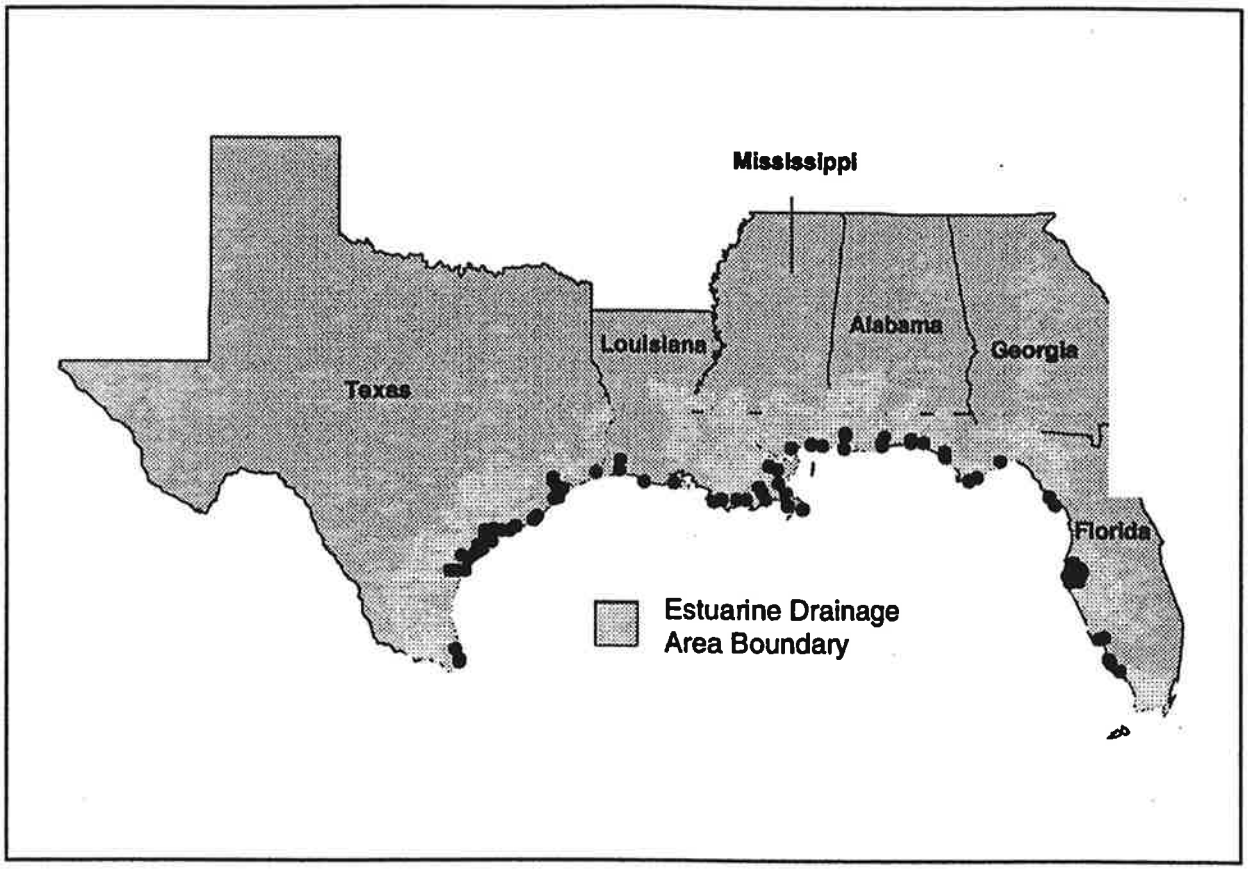

Figure 5d. Gulf of Mexico Mussel Watch sites. Adapted from O'Connor (1992).

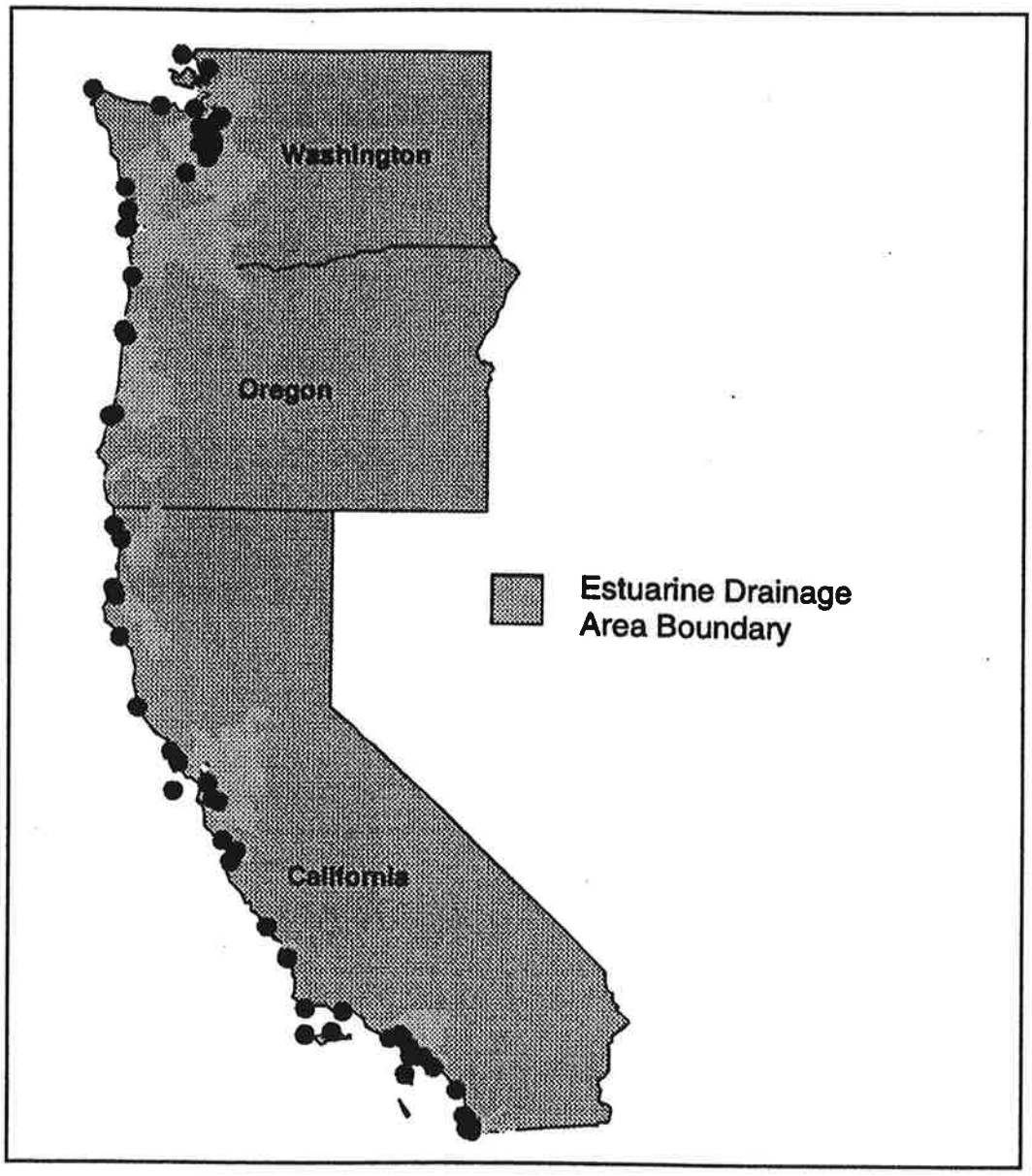

Figure 5e. Pacific Mussel Watch sites. Adapted from O'Connor (1992).

chemical contamination and assess the effects of human activities on coastal and estuarine areas around the nation. The NOAA Mussel Watch has been a major component of the NS&T Program since 1986 and has made chemical measurements on surface sediments and whole soft-parts of mussels and oysters collected from about 200 coastal and estuarine sites. The purpose of the Mussel Watch Project was to describe the spatial distribution of coastal contamination and identify temporal trends in contaminant concentrations. The Mussel Watch concept evolved in 1976, utilizing species of mussels (*Mytilus*) and osyters (*Ostrea* or *Crassostrea*) as surveillance organisms (Goldberg et al. 1978). The U.S. Mussel Watch concept has since developed to include an International Mussel Watch Project (Jernelov, 1996). The International program has just completed its "Initial Implementation Phase", the information from which provides direct experience for introducing this program to other global regions. The following is an overview of the results of the NOAA Mussel Watch Project from 1986 to 1990.

6.1.1.3 *Species and chemical measured*

Since no single species was common to all coasts, four different species were chosen; the mussel *Mytilus edulis* on the East Coast from Maine to Cape May, N.J.; the oyster *Crassostrea virginica* from Delaware Bay southward and throughout the Gulf of Mexico; the mussels *Mytilus edulis* and *M. californianus* on the West Coast; and the oyster *Ostrea sandvicensis* in Hawaii (O'Connor, 1992).

Concentrations of both trace metals and organic compounds were measured as part of the NS&T Program (Table 13) (O'Connor, 1992). "Contamination" is considered to have occurred when chemical concentrations exceed natural levels. All levels of organic chemicals in the environment are considered above natural levels since organic chemicals are man-made. However, in the case of naturally occurring trace metals, distinguishing contaminated levels from natural levels can be difficult. O'Connor (1992) suggests that the levels depend on the species of mollusc as well as many local and regional conditions.

Table 13. Chemicals measured in the National Status &Trends Mussel Watch Project. Adapted

Trace metals	Organic Compounds
Arsenic (As)	Total DDT (tDDT)
Cadmium (Cd)	Total chlordane (tCdane)
Chromium (Cr)	Total polychorinated biphenyls (tPCB)
Copper (Cu)	Total polycyclic aromatic hydrocarbons (tPAH)
Lead (Pb)	Total butyl tin (tBT)
Nickel (Ni)	
Mercury (Hg)	
Selenium (Se)	
Silver (Ag)	
Zinc (Zn)	

6.1.1.4 *Interspecific differences in chemical concentrations*

One of the goals of the Mussel Watch data was to be able to compare contaminant concentrations among sites. Since not all species occur at all sites, interspecies comparisons must be made. However, the data suggest that there are significant differences in contaminant accumulation between species. Comparisons between mussels (*M.edulis*) and oysters (*C.virginica*) at Long Island Sound revealed concentrations of Cu, Zn, and Ag more than 10-fold higher in oysters than in mussels, whereas Cr and Pb were 3-fold higher in mussels (O'Connor, 1992). Small differences between mussels and oysters were found for Hg, Ni, Cd and As, but deemed insignificant and the contaminant concentrations were compared regardless of species. At the mouth of the Columbia River, contaminant concentrations were compared in *M. edulis* and *M. californianus* and it was concluded that the differences were small enough that the two species could be considered interchangeable (O'Connor, 1992).

6.1.1.5 *Distributions of chemical concentrations*

Using the distributions (logarithmic) of contaminants from all of the sites in 1990, a statistically objective definition of a "high" concentration was determined for each of the contaminants (O'Connor, 1992). A "high" concentration is one where the logarithmic value is more than the mean plus one standard deviation of the logarithms of all concentrations. "High" concentrations for the trace elements evaluated in O'Connor (1992) are in Table 14.

A list of States within each region having sites with "High" trace element concentrations is shown in Table 15. High concentrations of Cd were found along the coast of Northern

Table 14. Geometric mean and "High" concentrations from analyses of molluscs collected in 1990 at 214 sites (oysters collected at 107 sites and mussels at another 107). Adapted from O'Connor (1992).

Trace Element	Geometric Mean ($\mu g\ g^{-1}$ dry wt.)	"High" Concentration ($\mu g\ g^{-1}$ dry wt.)
Oysters and Mussels		
As	10	17
Cd	2.7	5.7
Hg	0.094	0.24
Ni	1.7	3.3
Se	2.5	3.5
Oysters Only		
Ag	1.9	3.7
Cu	150	360
Zn	2400	5200
Pb	0.52	0.94
Cr	0.48	0.93
Mussels Only		
Ag	0.17	0.58
Cu	8.9	11
Zn	130	190
Pb	1.8	4.3
Cr	1.7	3.0

All concentrations are for whole soft parts of molluscs.
"High" concentration = mean + one Standard Deviation of the logarithms of individual site means.

California, but have been attributed to naturally high levels in deep-ocean water and upwelling of this water in that area (O'Connor, 1992). Other "High" levels not attributable to natural occurrences include high Cd in oysters at sites in the Gulf of Mexico. Arsenic in mussels and oysters along the Southeast Coast are high, but that may also reflect the natural association of As with phosphate deposits in the area that present in sufficient quantities to support mining activities.

These results emphasize that trace metals in the environment must be interpreted in light of natural sources as well as anthropogenic inputs, unlike organic contaminants that are entirely attributable to anthropogenic activities. Further, the results provide an example of how contaminant distributions can be characterized in a productive manner by identifying the sites that are the most contaminated and should have highest priority for further study and/or clean-up.

Table 15. List of the States having "High" trace element concentrations and the number of sites within each State based on samples taken between 1986 and 1990. Adapted from O'Connor, 1992.

Main Location	State	Species	No. of sites	Trace Element As	Cd	Hg	Ni	Se
North Atlantic	Maine	*M. edulis*	4			1		
	Massachusetts	*M. edulis*	9	1		6		
Mid-Atlantic								
	Massachusetts	*M. edulis*	7	5				
	Rhode Island	*M. edulis*	4				2	
	New York	*M. edulis*	12		1	5	1	1
	New Jersey	*M. edulis*	7			3	2	1
		C. virginica	4		2		4	
	Delaware	*C. virginica*	2		1		2	
	Maryland	*M. edulis*	1					1
		C. virginica	6		2		6	2
	Virginia	*C. virginica*	8		2		3	1
South Atlantic	Virginia	*C. virginica*	1				1	
	North	*C. virginica*	7	2				
	South	*C. virginica*	4	4				3
	Georgia	*C. virginica*	3	3				1
	Florida	*C. virginica*	6	2				1
Gulf of	Florida	*C. virginica*	26	5	2	6		1
	Alabama	*C. virginica*	3		2			
	Mississippi	*C. virginica*	3	1	1			
	Louisiana	*C. virginica*	17		7	1	1	4
	Texas	*C. virginica*	29	2	11	3		14
Pacific	California	*M. californianus*	26	9	6	4	5	3
		M. edulis	11		4	2	3	3
	Oregon	*M. californianus*	2		1			
	Washington	*M. edulis*	15				4	1
	Alaska	*M. edulis*	2				1	1
	Hawaii	*O. sandvicensis*	3	2		1	2	1

The Mussel Watch data does not link "High" concentrations to biological effects. Concentrations are compared with U.S. Food and Drug Administration (FDA) guidelines that warn against human consumption of shellfish with concentrations (by wet weight) above specified levels of mercury and chlorinated hydrocarbons (O'Connor, 1992). No molluscs collected as part of the NS&T Program had trace element concentrations above the specified levels. However, many trace metal concentrations that produce biological effects in aquatic organisms are many factors below those considered harmful to human consumption (HWC, 1987; CCME, 1987).

6.1.1.6 *Temporal trends in trace element concentrations*

The NS&T define temporal trends as; 1) a year-to-year trend that occurs at the vast majority of sites, and; 2) a statistically meaningful relationship between concentration and time ('sign-test"). Since differences over time are measured at the same site, there is no need to consider oysters and mussels separately.

Temporal trends at 141 sites for which contaminants have be measured in at least four of the last five years are shown in Table 16 (O'Connor, 1992). Mean concentrations for most of elements showed decreasing trends for the period of 1986 to 1990 as well as for the bi-annual comparisons (O'Connor, 1992). Geometric means for elemental concentrations among all 141 sites sampled for four or five years indicate that the changes (increases or decreases) were small (O'Connor, 1992).

Given that there is only a maximum of five years of data in which to observe a trend and that contaminant data are generally noisy, statistically significant correlations between concentration and time are rare. Spearman rank correlation coefficients as high as 0.9 would be required. Using the four or five years of data, the 141 sites and 14 chemicals (including organic ones), O'Connor (1992) found 152 sites with a statistically significant decreasing trend and 87 with increasing trends. However, the author points out 99 of the correlations may not be real, but instead a statistical false-positive (i.e. 99 = 0.05% error due to chance x 1,974 combinations (14 chemicals x 141 sites)). However, more "real" trends can be identified by those trends that occur among groups of sites. For example, among nine Long Island Sound sites (data not shown), Cu decreased at six, Cd at five, Ag at four and Zn at three. Nevertheless, these data argue for temporal trends to be measured for longer than five years.

6.1.1.7 *Separating natural and anthropogenic influences*

Strict sampling protocols were established as part of the Mussel Watch Project such as sampling in the same season every year, always collecting the same species at a site, and using molluscs of a certain size. However, there are additional factors such as salinity that cannot be controlled. O'Connor (1992) suggests that interannual variation in contaminant concentration

Table 16. Results of sign test applied to mean chemical concentrations at the 141 sites sampled in at least four of the years between 1986 and 1990. Adapted from O'Connor (1992).

Trace Element	1986-1987	1987-1988	1988-1989	1989-1990	1986-1990
Ag	-	-	-	↑	-
As	↓	-	↓	↑	-
Cd	↓	-	-	-	↓
Cr	↓	-	-	-	-
Cu	-	-	-	-	-
Hg	-	-	-	-	-
Ni	-	↓	↓	-	↓
Pb	-	-	↓	↑	-
Se	↑	↑	↓	-	-
Zn	-	-	-	↑	-

Comparisons made on a year-to-year basis and between 1986-1990. A statistically significant (0.05 level) proportion of changes in the increasing (↑) or decreasing (↓) direction are indicated, as are cases with no significant direction of change (-). Since it was not routinely measured in earlier years.

may be linked to natural factors, but it is difficult to attribute temporal correlations to natural factors.

6.1.1.8 *Summary*

The NOAA NS&T Mussel Watch Project has been used to describe the spatial distribution of coastal contamination and identify temporal trends.

- More decreases than increases were found for trace elements (As, Ag, Cr, Ni, Cd, Cu, Zn, Pb and Hg) at the Mussel Watch sites.
- A few significant correlations (increases < decreases) were noted between concentration and year.
- More years of data (>5) will be required to clearly establish long-term trends for the individual sites.

6.1.2 **Belledune Harbour, New Brunswick**

6.1.2.1 *Site description*

Belledune is located on the Baie des Chaleurs shore of New Brunswick. The two main industries on the man-made harbor are a lead smelter and fertilizer plant. The smelter was established in 1966 as both a lead and zinc smelter, with the process being converted in 1972 to only lead smelting. During the smelting process, the sulfide from the ore is converted to sulfuric acid and piped to the adjacent fertilizer plant to be utilized there.

6.1.2.2 *Study objectives*

Ecological surveys have been conducted by the Noranda Research Center for the Brunswick Mining and Smelting Corporation Limited (Smelting Division) plant operations at Belledune, New Brunswick annually since 1972 (Charron, 1981). The purpose of these surveys is/was to assess the effects of Pb smelter operations on the marine ecosystem. In 1979, concerns were raised by investigators with the Noranda Research Centre regarding the consequences of increased metals levels (Cd in particular) in blue mussels (*Mytilus edulis*) noted in the 1978 sample collection. This study describes the results of the annual surveys by the Noranda Research Centre during the summers of 1979 to1981 and their conclusions. The primary objectives of the studies were to determine:

1. the extent of metal contamination in indigenous mussel colonies located along the coast at varying distances from the smelter.
2. the rate of metal detoxification/depuration by transplanting mussels from the vicinity of the smelter to an unaffected area.
3. the rate of metal accumulation by transplanting mussels from uncontaminated areas to the vicinity of the smelter's operations and approximate contaminant dispersal patterns.
4. the effects of waste treatment and relocation of the main out-fall on bioaccumulation in mussels.

Prairie and Charron (1983) describe the differences in long- and short-term mussel studies:

Types of Studies	
Mussel Cultures	Mussel Colonies
• relocation	• on-site (indigenous)
• short-term	• long-term
• labor intensive	• simple collection
• in and out of intertidal zone (three dimensional)	• intertidal zone only (uni-dimensional)

Objective 1 was determined using mussel colonies, whereas objectives 2-4 were determined using mussel cultures.

6.1.2.2 *Accumulation of metals in indigenous mussels*

The location of sampling stations of indigenous blue mussels is shown in Figure 6. Concentrations in mussels sampled at 20 stations in July are shown in Table 17. Cadmium concentrations in the soft tissues of *M. edulis* (2 to 67 $\mu g\ g^{-1}$ wet wt.) were well above the Canadian Food and Drug Directorate (CFDD) suggested guidelines (1 $\mu g\ g^{-1}$ wet wt.) and above levels found at reference sites (0.5 to 1.6 $\mu g\ g^{-1}$ wet wt.) at stations located from 1 to 20 km (South East, SE) from the smelter (Charron, 1981). Stations closest to the smelter had Cd concentrations similar to those in 1977/78, whereas, Cd concentrations between 5 km and 16 km were generally higher than those collected in 1977/78 (Charron, 1981). Lead levels (10 to 179 $\mu g\ g^{-1}$ wet wt.) in mussels also exceeded the CFDD guidelines on-site and at stations up to 13 km SE of the smelter. Zinc levels were below the CFDD guidelines at all sites, but soft-tissue concentrations (30 to 87 $\mu g\ g^{-1}$ wet wt.) were elevated with respect to the reference stations from on site to 6 km SE of the smelter. Copper, Hg and As were all well below the CFDD limits and showed only marginal elevation near the smelter.

Copper, Pb, Zn and As levels in the <500 μm fraction of the sediments were all above the levels in the mussels, at corresponding stations, with a peak at the smelter (Charron, 1981). Cadmium and Hg levels in sediment peaked 3 to 5 km SE of the smelter, with Cd concentrations

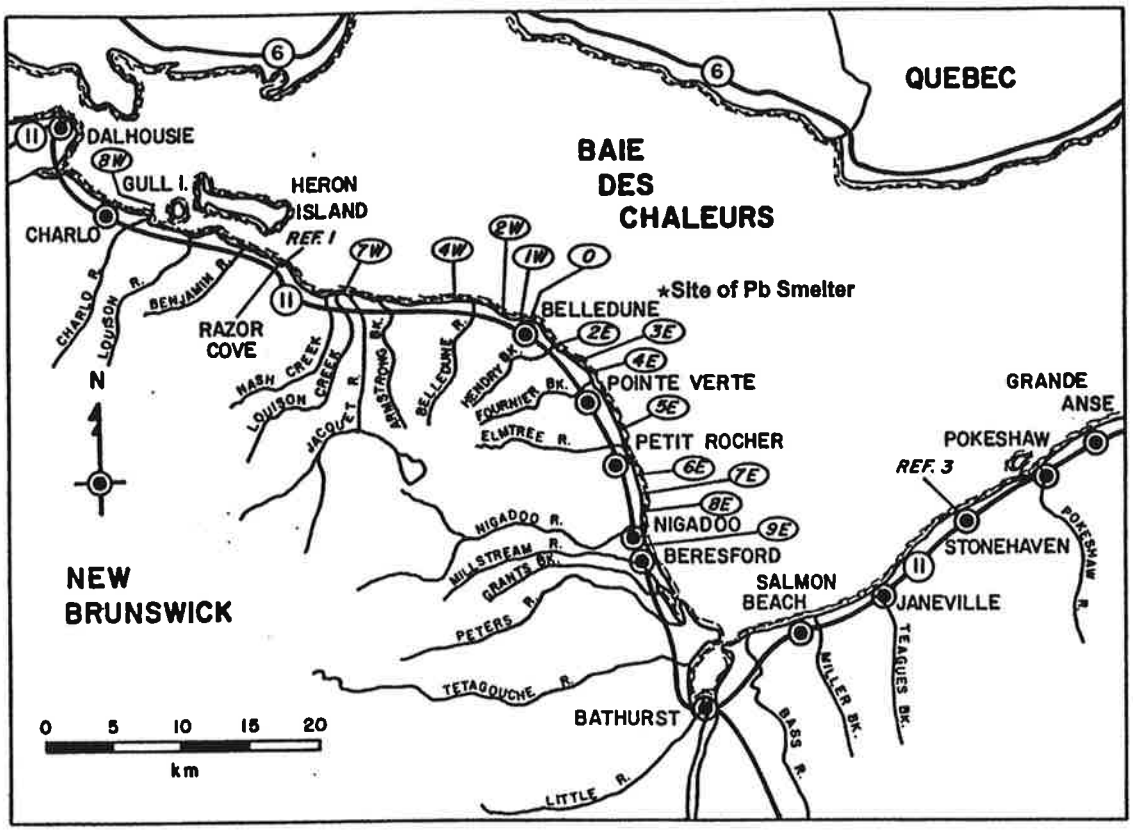

Figure 6. Location of Belledune Harbour and indigenous *Mytilus edulis* sampling stations along the Northeastern Coast of New Brunswick, July 1979. Adapted from Charron (1981).

Table 17. Metal levels in native blue mussels, *Mytilus edulis* sampled along the northeastern coast of New Brunswick, July, 1979. Adapted from Charron (1981).

Distance from Plant (km) E - East W - West	No. of mussels	Length Range (mm)	Weight Range (mg)	Metal Concentrations ($\mu g\ g^{-1}$ wet wt.)					
				Cu	Pb	Zn	Cd	As	Hg
				CFDD : 100	CFDD : 10	CFDD : 100	CFDD: 1	CFDD : 5	CFDD : 0.5
27E	197	30-84	2-73	1.9	2.2	16	0.6	1.9	0.04
22E	459	42-80	8-39	1.9	2.6	12	0.5	1.6	0.04
17E	170	25-83	1-88	1.5	1.2	14	0.8	1.9	0.04
6E	211	30-85	2-70	2.7	2.7	35	1.0	1.9	0.03
1E	193	37-70	5-51	1.7	6.1	17	2.2	1.3	0.08
0.5E	**80**	**31-75**	**2-44**	**1.8**	**14.0**	**38**	**26.8**	**1.0**	**<0.01**
0	252	28-73	3-42	3.2	179	87	67.0	3.1	0.04
3W	263	24-82	1-72	2.5	55.0	72	21.3	2.0	0.02
	375	**23-74**	**1-47**	**1.0**	**53.0**	**60**	**21.5**	**2.1**	**<0.01**
5W	199	41-79	7-67	1.9	34.0	30	11.3	1.4	0.02
	305	**25-74**	**1-56**	**2.0**	**34.0**	**40**	**16.2**	**2.5**	**0.03**
6W	185	28-84	2-54	1.9	40.0	41	11.6	1.8	0.03
	100	**35-84**	**4-125**	**4.0**	**59.0**	**54**	**17.0**	**1.6**	**0.02**
8W	294	24-71	1-40	1.8	17.0	21	4.3	1.3	0.03
13W	114	23-80	1-76	1.8	10.0	19	3.3	1.4	0.02
16W	219	39-80	5-75	1.5	5.7	18	2.4	1.7	0.02
20W	**264**	**23-73**	**1-62**	**2.5**	**7.0**	**23**	**2.0**	**1.8**	**0.03**
23W	30	31-81	5-74	2.6	4.0	20	1.6	1.5	0.02
	207	**25-74**	**1-57**	**2.3**	**4.0**	**19**	**1.5**	**1.3**	**0.02**
Ref. Stn.	233	28-75	2-73	2.7	3.5	24	1.6	1.9	0.03

CFDD : Canadian Food and Drug Directorate suggested guidelines for human consumption.
Non-Bold: Stations accessible any time of year for mussel collection.
Bold : Stations accessible in summer by diving or in winter by walking on ice cover.

lower and Hg concentrations higher in sediments than in mussels. No attempts were made to statistically compare concentrations in sediment to those in the mussels.

6.1.2.3 *Loss of metals in depurating cultures*

Contaminant levels were determined in mussels from the vicinity of the smelter in August of 1978. Specimens were transferred to an uncontaminated station, and contaminant levels were measured again in May, 1979. After 277 days in an uncontaminated environment metal concentrations in mussels decreased 23 % for Pb and 36 % for Zn but Cd levels did not change. Comparison of mussels from the vicinity of the smelter and those allowed to depurate for 277 days revealed As levels 32 % lower and Hg levels 125 % higher (Charron, 1981). It was concluded that recovery to levels similar to background or at least below the CFDD limit would be very slow.

6.1.2.4 *Bioaccumulation rates in mussel cultures*

The bioaccumulation rates in cultures or transplanted mussels were determined in 1980 (for comparison) and in 1981 to assess the effects of changes in waste treatment and location of effluent release into the harbor (Prairie and Charron, 1983). Mussels were held in mesh bags at 18 stations in Belledune harbor surrounding the effluent outfall area and sampled approximately every 25 days for 3 months (3 bags x 9 mussels/bag per collection) (Prairie and Charron, 1983). Concentrations of Pb, Zn and Cd were obtained for mussels at 18 different locations and three different dates (Fig. 7).

Bioaccumulation curves were obtained by examining variations in time at individual stations (Fig. 8) (Prairie and Charron, 1983). Using a student's t-test statistical differences in bioaccumulation between groups of stations were determined (Prairie and Charron, 1983). This statistical technique was not helpful in detecting overall trends in relation to station location. Bioaccumulation patterns were obtained by examining variations in location for each sample date and determining statistical differences among stations using ANOVAs. For the three sampling dates, an integration of Pb, Zn and Cd concentrations can be plotted showing spatial and temporal variations (Fig. 9). Finally, the bioaccumulation rate and pattern data were combined to

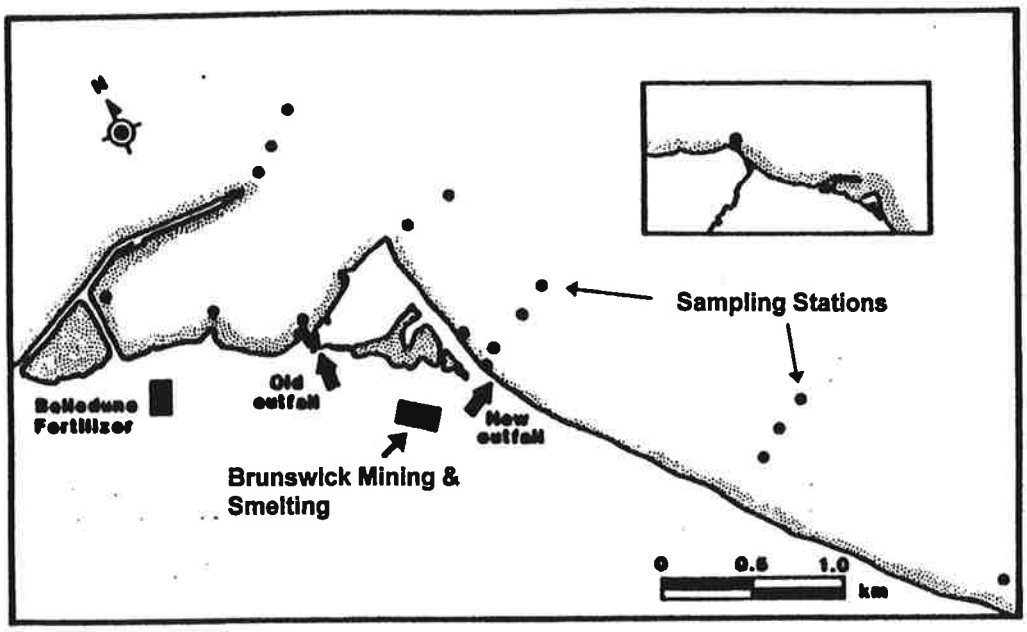

Figure 7. Locations of *Mytilus edulis* cultures near the Brunswick Mining and Smelting Corp. lead smelter in Belledune Harbour. Adapted from Prairie and Charron (1983).

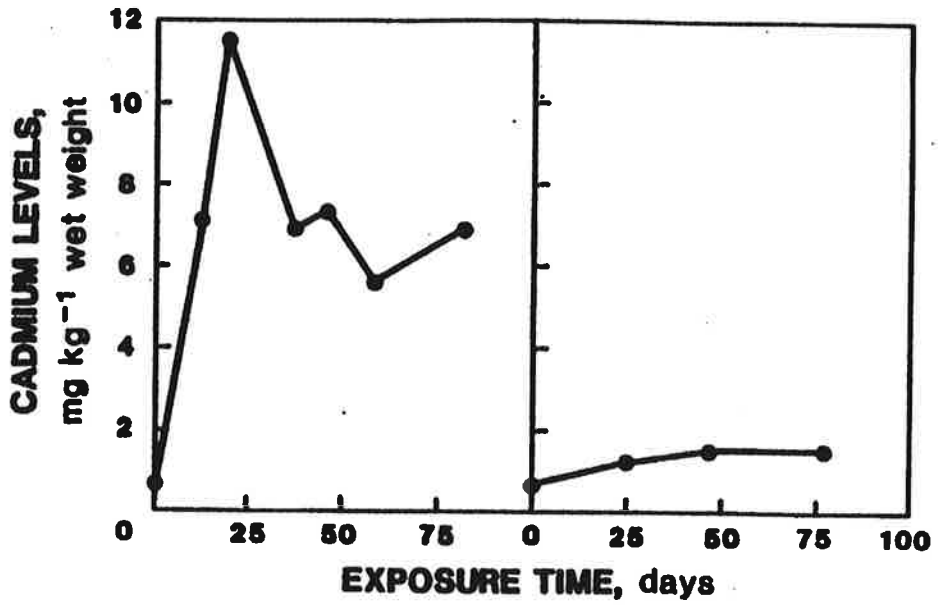

Figure 8. Comparison of cadmium accumulation in *Mytilus edulis* cultures from stations in Belledune Harbour, New Brunswick with mussels possessing high and low accumulation rates. Adapted from Prairie and Charron (1983).

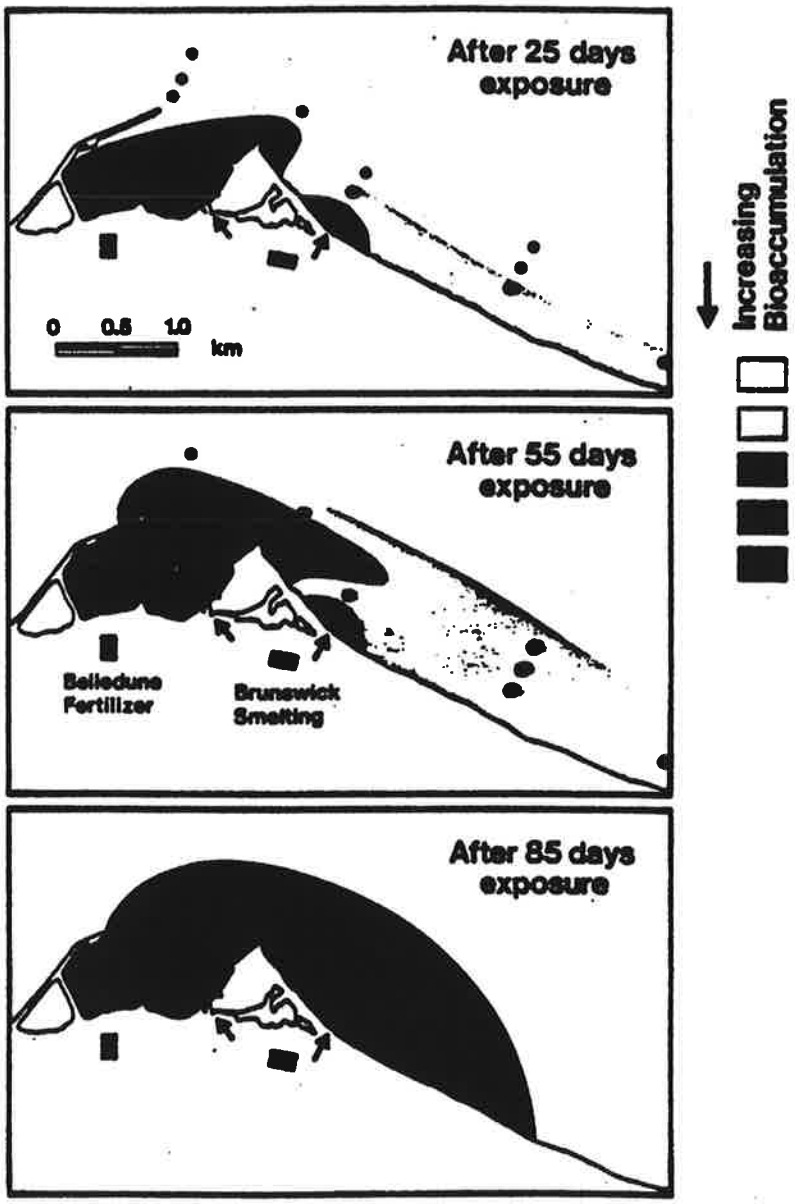

Figure 9. Spatial variations in cadmium accumulation in *Mytilus edulis* cultures over time stationed in Belledune Harbour, New Brunswick. Adapted from Prairie and Charron (1983).

determine (or confirm) the sources of contamination, classify zones of bioaccumulation (highly, moderate, and slightly contaminated) and approximate contaminant dispersal patterns (Fig. 10). Inter-year comparisons (1980 to 1981) revealed decreases in bioaccumulation by cultured mussels in the vicinity of the smelting operation, subsequent to improved waste-water treatment (Fig. 11).

6.1.2.5 *Summary*

This study on the New Brunswick mining and smelting company in Belledune harbor illustrated how the marine mussel *Mytilus edulis* (indigenous and cultured) could be used to:

- identify the extent of metal contamination along the coast surrounding the smelting complex.
- identify the metals of greatest concern due to their levels being higher than the CFDD limits. Since *Mytilus edulis* is a food product for human consumption these guidelines exist.
- quantify differences in bioaccumulation rates and patterns at stations surrounding the smelting complex, enabling investigators to estimate dispersal patterns.
- assess the effectiveness of improved waste-water treatment through inter-year comparisons of metal concentrations in cultured mussels.

The primary drawback in this study is that analytical detection limits, at that time, were not sufficiently low enough to allow comparison of metal concentrations in seawater with metal concentrations in soft-tissues. Further, no attempts were made to compare total concentrations of metals in sediments to soft-tissue concentrations in the mussels.

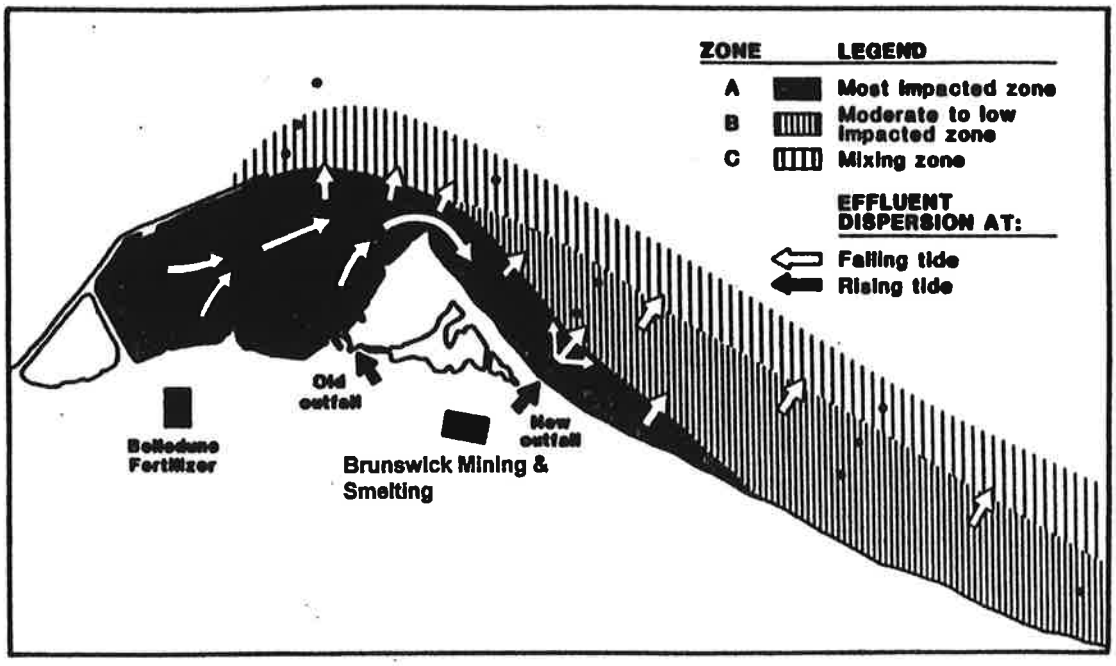

Figure 10. Spatial variations in cadmium accumulation in *Mytilus edulis* cultures and contaminant dispersal patterns in Belledune Harbour, New Brunswick. Adapted from Prairie and Charron (1983).

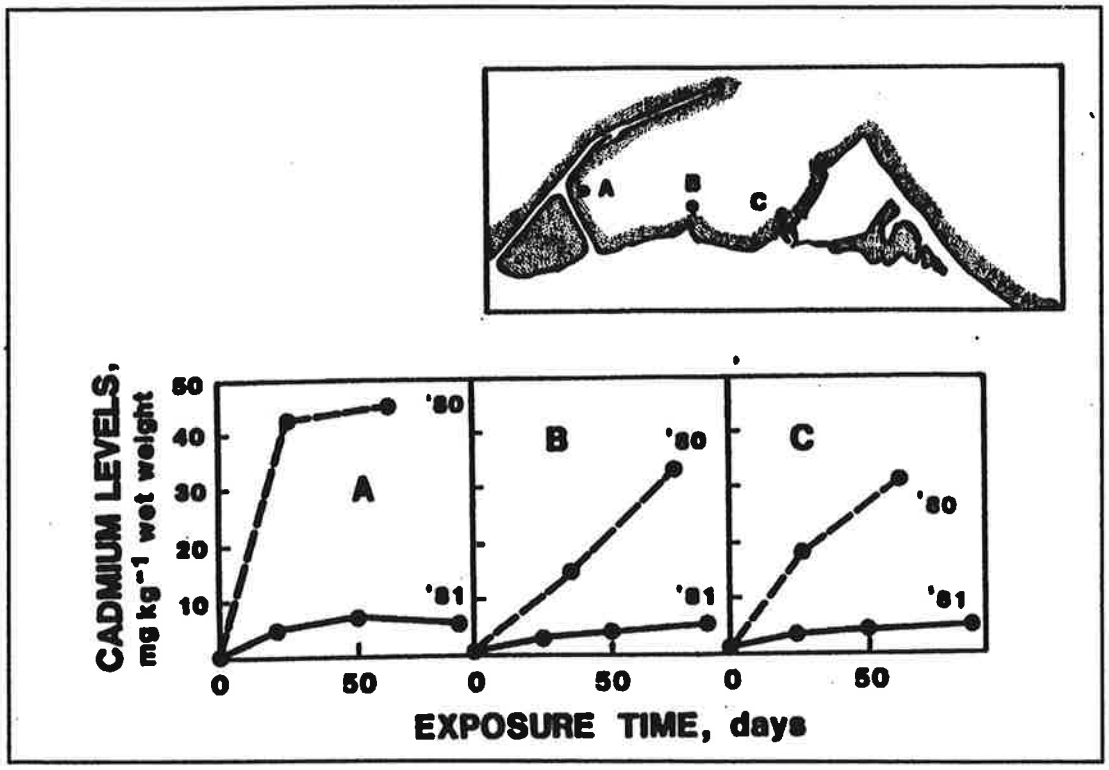

Figure 11. Comparisons of Cd accumulation in *Mytilus edulis* before (1980) and after (1981) changes to waste-water treatment at the Brunswick Mining and Smelting Corp. lead smelter in Belledune Harbour. Adapted from Prairie and Charron (1983).

6.1.3 **Nanisivik, Northwest Territories**

6.1.3.1 *Site description*

The Nanisivik lead and zinc mine is located on the northern tip of Baffin Island, N.W.T, Canada (Fig. 12). Trace metals associated with the operations of the Nanisivik mine (73°02' N, 84°32' W) may enter Strathcona Sound through a number of routes, the most likely of which are tailing pond discharges into Twin Lakes Creek from West Twin lake and accidental spillage of Pb and Zn concentrates during the loading of concentrate-carrying ships (Fig. 13) (Fallis, 1982). Concentrates on land may subsequently be transported to Strathcona Sound via wind action, precipitation or melt water runoff.

6.1.3.2 *Study Objectives*

In 1974 prior to the commencement of milling at Nanisivik, Fisheries and Oceans Canada undertook field studies to obtain an appreciation of the abundance and diversity of biota in the marine environment in proximity to the development site. That work convinced them that the marine ecosystem warranted protection and subsequent negotiations resulted in a major adjustment to the developmental plan with tailings being pumped to a freshwater lake for disposal rather than deposited at depth in Strathcona Sound.

A monitoring program was initiated to evaluate spatial and temporal changes in trace metal concentrations in sediments, sea urchins, seaweed (*Fucus vesiculosis*) and the filter-feeding bivalve mollusc *Mya truncata* over the life of the mine (Fallis, 1982). The investigators were interested in determining the zone of influence due to mining activities and the discharge of effluent from West Twin Lake (the tailings pond for the mine) with respect to biota in Strathcona Sound. Owing to the magnitude of tides, and the wind and wave action, water sampling was not considered to be a useful indicator of spatial and temporal changes in metal distribution, since resources were not available to undertake sampling at the frequency needed to obtain meaningful data.

6.1.3.3 *Accumulation of metals in bivalve tissues*

Sampling undertaken in 1979, 1980 and 1981 revealed marked increases in metal

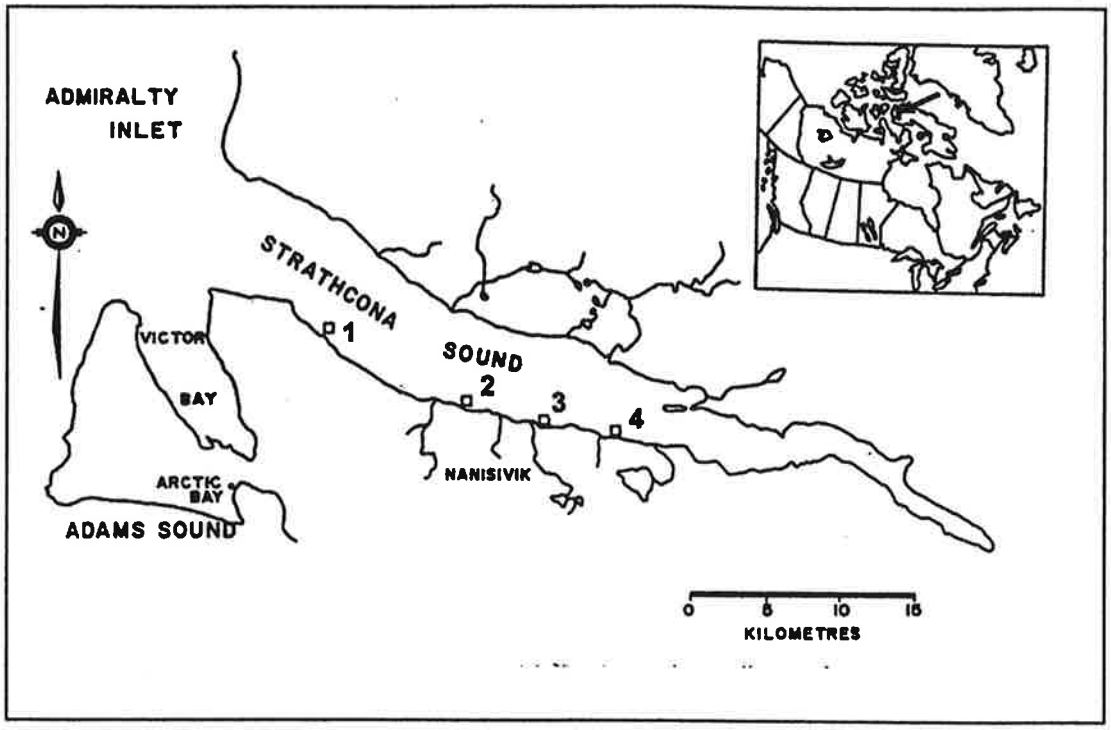

Figure 12. Location map and sampling stations associated with the 1979 Nanisivik, N.W.T. monitoring programme. Adapted from Fallis (1982).

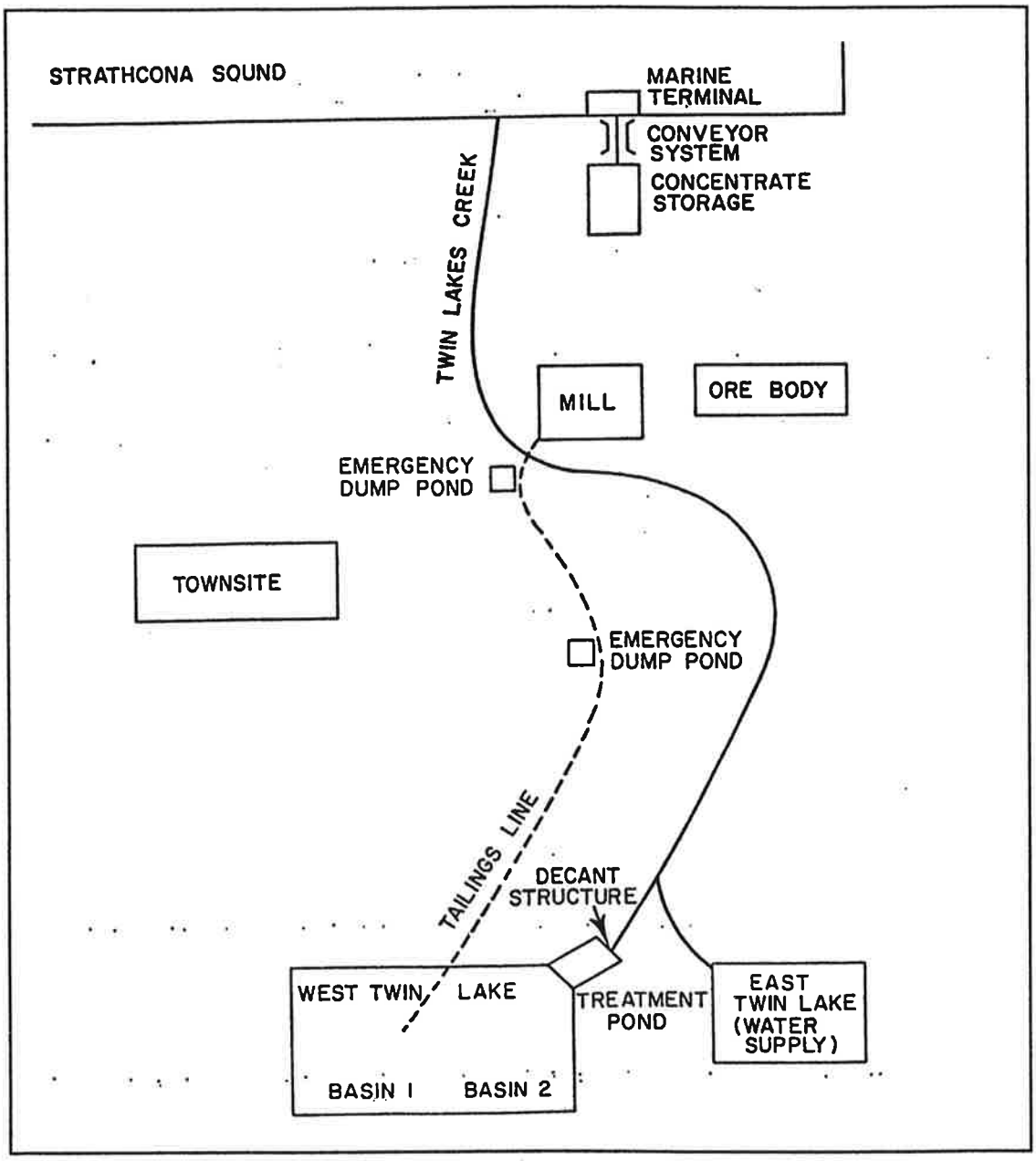

Figure 13. Schematic drawing of Nanisivik, N.W.T. mine tailings disposal system. Adapted from Fallis (1982).

concentrations in the whole-organism soft tissues relative to pre-operational concentrations (Table 18). Concentrations of both Zn and Pb in bivalve tissues tended to be higher closer to the Twin Lakes outfall (Asmund et al. 1991). A similar relationship was found in mussels (*Mytilus edulis*) samples in the vicinity of mines at Maarmorilik and Ivittuut (Greenland), although the concentrations of Zn and Pb were generally lower at Nanisivik (Asmund et al. 1991). Mine tailings from milling operations were deposited on shore at Ivittuut and on shore and at depth in the ocean at Maarmorilik. Based on the metal accumulation in bivalves, seaweed and sediments, Asmund et al. (1991) concluded that the environmental effects were least at Nanisivik where tailings were deposited into a freshwater lake under controlled conditions and where a minimum of waste rock is produced.

Table 18. Lead and zinc concentrations in the soft tissues of *Mya truncata* collected from Strathcona Sound, N.W.T in 1979 and 1980-81. Adapted from Asmund et al. (1991).

Station #	Distance from Mouth of Twin Lakes Creek (km)	Zn[1] $\mu g\ g^{-1}$ dry wt.	Pb[1] $\mu g\ g^{-1}$ dry wt.
Pre-operational (1975) (station 3)	1	105	1.19
1980 and 1981			
3	1	437	65.4
2	4.5	137	6.3
4	5.8	193	22.1
1	15	114	2.7
1979			
3	1	388	2.28
2	4.5	118	1.00
4	5.8	288	6.96
1	15	124	1.42
Sediment concentrations[2] 1979-1981			
3	1	509	73.2
2	4.5	42	9.8
4	5.8	111	25.9
1	15	19	5.4

[1] Values are from one sample consisting of three clams

[2] Values are for the <1mm fraction of surface sediments (0-2 cm).

6.1.3.4 *Rationale for discontinuing the monitoring program*

By 1984 it appeared that many of the metal concentrations had started to plateau, and the program was discontinued since the investigators felt that there was little merit in expending funds

to come up with numbers which could not be meaningfully interpreted with respect to the likely "effects" of such contaminant concentration information to the "health" of the organism (B. Fallis, Freshwater Institute, Fisheries and Oceans, Winnipeg, Manitoba, personal communication).

The investigators concluded that the *M. truncata*, in marine arctic environments, serve as a useful biomonitor of historical exposure to trace metals, but under continued exposure it appears that tissue concentrations plateau. Consequently, at that point, soft tissue metal concentrations may not reflect incremental increases expected under conditions of continuing exposure to metals (Pb, Zn, Cd).

6.1.3.5 *Summary*

This study has several important highlights:

- Lead and zinc concentrations in the marine bivalve *Mya truncata* increased in response to mining activities (run-off from tailings and concentrate spills) and showed a general decrease in concentrations with distance from the mine.
- Comparisons with other bivalves from other sites were used to determine the relative impacts of different methods of disposal of mine tailings and waste rock.
- After five years, metal concentrations began to plateau and not increase relative to the continued release of metals into the environment.
- Investigators discontinued the monitoring program using *Mya* due to the lack of scientific information allowing them to link metal concentrations to "health effects" in the bivalve.

6.2 Freshwater

6.2.1 **Rouyn-Noranda Mining and Smelting Area, Quebec**

6.2.1.1 *Site Description*

The mining area of Rouyn-Noranda, located in northwestern Quebec, 500 km northwest of Montreal, has been the site of several studies using freshwater molluscs to monitor metals in the environment. Mining activities have been carried out in the area since 1927 and potential sources of metal contamination include abandoned mines, current mining operations, accumulated residues from the smelting processes and atmospheric fallout from the smelting processes. Atmospheric emissions in 1977 from the smelting complex included 485,000 t of SO_2, 75 t of Cd, 34 t of Cu, 1,540 t of Pb, 610 t of Zn, and 16 t of Hg (Couillard et al. 1993). Lesser emissions of Bi, Co, Fe, Ni, Sb, Se, and Te were also reported. Metal concentrations found in the sediments are thought to have been introduced both by anthropogenic activities and by natural processes of weathering and erosion (Table 19).

6.2.1.2 *Study Objectives*

The following studies by Tessier et al. (1984, 1993) and Couillard et al. (1993, 1995a) examine the relationship between metal concentrations in the sediment and water and concentrations in molluscs. Tessier et al. (1984) identify factors influencing trace metal bioavailability to freshwater benthic organisms and establish relationships between metal concentrations in bivalve tissue and metals distributed among geochemical phases within sediment. The later work by Tessier et al. (1993) further develops the relationship between Cd in oxic sediments and in overlying water by means of surface-complexation concepts. The authors also relate Cd concentrations in bivalves to dissolved and sedimentary Cd using the FIAM.

Couillard et al. (1993 and 1995a) related tissue metal concentrations (Cd, Cu and Zn) to changes in environmental metal concentrations. In their first study, Couillard et al. (1993) established a relationship between the metal contamination gradient and metallothionein (MT) development. For the second study, bivalves were transplanted and caged in metal contaminated areas and their concentrations of metals and MT were compared with bivalves situated at an

Table 19. Mean metal concentrations ($\mu g\ g^{-1}$dry wt) in the surface sediments of various lakes in the Rouyn-Noranda mining area in the late 1970's (from Couillard, 1996).

Lake	Distance from smelter	Cd	Cu	Zn	Pb	Hg ($ng\ g^{-1}$ dry wt)
Osisko	<1	55	8900	10900	770	2000
Noranda	2.5	115	3400	3600	---	240
Rouyn	5	58	6200	14500	460	260
Dufault	6	17	1200	2200	380	200
Pelletier	6	16	1000	660	250	200
Beauchastel	13	5	100	270	68	110
Montbeillard	20	1	36	100	26	27
Caron	26	8	38	600	88	200
Regional back ground level		<0.2	15-40	60-120	1-10	20-90

uncontaminated site (indigenous and caged). This second study was particularly useful in that its design allowed for comparison of metal uptake among indigenous mussels and transplanted mussels.

6.2.1.3 *Relationship between environmental metal and tissue levels*

Tessier et al. (1984) found that Cu, Pb and Zn concentrations in the tissues (or whole body) of indigenous *Elliptio complanata* were best correlated to one or more of the relatively easily extracted geochemical fractions rather than total sediment metal concentrations. The geochemical extraction technique developed by Tessier et al. (1979) and used in the Rouyn-Noranda studies partitions sediment into five operationally defined fractions: 1) exchangeable metals; 2) metals bound to carbonates or specifically adsorbed; 3) metals bound to Fe-Mn oxides; 4) metals bound to organic matter and sulfides, and; 5) residual metals. This technique cannot isolate specific geochemical fractions, but provides an operational approximation of several sinks for metals in sediments.

Tissue Cu levels in soft tissues (foot, muscle, visceral mass, hepatopancreas, gills and mantle) were significantly correlated with Cu in the carbonate and Fe-Mn oxide fractions of

sediment (Tessier et al.1984). Correlations increased when the sediment Cu concentrations were normalized for organic content, or more importantly, Fe-oxide content. The best predictor of Cu tissue levels was the ratio of the sum of Cu in the first three fractions (exchangable, carbonate bound or specifically adsorbed and bound to Fe-Mn oxides) and the Fe associated with the Fe-Mn oxide fraction ([Fe (Fe-Mn oxides)]). Zinc content in bivalve tissues was best described by the ratio of Zn in the organic matter and sulfide fraction and Fe-Mn oxide fraction (Tessier et al.1984). Normalizing the Zn concentrations with respect to organic matter in sediments tended to reduce the *r* value of the correlation with tissue concentrations. The best relationship between Pb in bivalve tissues and sediment Pb was based on gill tissue concentrations (Tessier et al.1984). The best predictor of Pb in gill tissue was the sum of Pb in the first three fractions (exchangeable, carbonates and Fe-Mn oxide) normalized for [Fe (Fe-Mn oxides)].

The fact that tissue metal concentrations were best related to extractable metals in the sediments supports the contention that the availability of a particular metal is inversely related to its binding strength to the various substrates in sediments. For example, the normalizing capacity of the Fe oxyhydroxides was likely due to their adsorption of Cu, Zn and Pb in the external medium, thus controlling the dissolved metal concentrations to which the organism is exposed. This assumes the primary route of metal uptake for the bivalves used in the study (*Elliptio complanata*) is the dissolved phase.

In the later study by Tessier et al. (1993) the relationship between tissue metal concentrations and bioavailable fractions in the sediment (estimated using extraction techniques) was found to be less straightforward when applied to 38 lakes with varying pH (4.07 - 8.10). Tessier et al. (1993) described the partitioning of Cd among geochemical phases of the sediment and water overlying the sediment using surface complexation concepts (see discussion of bioavailability). Those concepts were then used in conjunction with the FIAM (see discussion of bioavailability) to relate sedimentary Cd concentrations to those found in the tissues of indigenous freshwater bivalve *Pyganodon grandis*. Linear regression analysis found that Cd concentrations in the soft tissues of bivalves, [Cd(Org)] ($\mu g\ g^{-1}$ dry wt.) were related to dissolved Cd concentrations measured above the sediment-water interface: $[Cd(Org)] = 44\ [Cd^{2+}] + 10$ ($r^2 = 0.81$) for $[Cd^{2+}]$ expressed in nmol L^{-1}. Contrary to the previous study with Cu, Zn and Pb,

sedimentary extractable Cd normalized with respect to Fe oxyhydroxide or organic C concentrations in the sediments did not predict Cd concentrations in the bivalve.

The authors suggest that the simple normalizations may be appropriate in systems where pH does not significantly vary (e.g. marine systems, chemically similar lakes) since sorption reactions of metals onto the Fe-oxyhydroxides and organic matter tend to dominate. But in systems where pH gradients exist, metal bioaccumulation as a function of the concentration ratio of sorbed metal to sorbent (Fe-oxyhydroxides or OM) varies with the pH of the system. However, the authors suggest that sedimentary measurements (concentration of organic matter and Fe-oxyhydroxides) could be used to predict [Cd(Org)] using an equation that takes into consideration the pH dependence of Cd sorption reactions:

$$[Cd(Org)] = F \frac{\{Cd\}_T[H^+]^{1.79}}{(10^{-1.30}\{Fe-ox\}[H^+]^{0.97} + 10^{-2.45}\{OM\}[H^+]^{0.82}) + [Cd(Org)]^0}$$

{Fe-ox} = Fe oxyhydroxides (μmol g^{-1}) in sediment

{OM} = organic matter in sediment

F = proportionality factor between [Cd(Org)] and $[Cd^{2+}]$

$$[Cd(Org)] = \frac{\sum[Cd(tissue)]_i W_i}{\sum W_i}$$

W_i - weight of ith tissue

$[Cd^{2+}]$ = calculated using HYDRAQL (Papelis et al. 1988) and $[Cd]_{Total}$ and inorganic ligands (OH^-, CO_3^{2-}, SO_4^{2-} and Cl^-) and stability constants of the inorganic complexes

$[Cd(Org)]^0$ = intercept on the y-axis of regression [Cd(Org)] vs. $[Cd^{2+}]$

The proportionality factor F and $[Cd(Org)]^0$ are determined from linear regressions (with above

equation) using values of $\{Cd\}_T$, pH, {Fe-ox}, and {OM}obtained from the same sediment sample in a lake. Both F and $[Cd(Org)]^0$ are organism-specific and should be determined separately, but the equation is thought to be valid for other aquatic organisms. However, it should be noted that no field studies have been undertaken to prove this hypothesis. Further, this equation requires testing in progressively more complex chemical environments (variable DOC concentrations) that may deviate from the underlying concepts of the FIAM.

The use of sedimentary measurements offers considerable advantages over the direct measurement of dissolved Cd. At low environmental [Cd] there are problems with contamination, and analytical detection of Cd. Further, measurement of sedimentary variables provides a temporally integrated sample of dissolved Cd (Tessier et al. 1993).

6.2.1.4 *Accumulation of metals in transplanted bivalves*

Cadmium concentrations in *Pyganodon grandis* transplanted from an uncontaminated lake (Lake Opasatica) to a lake downwind (Lake Vaudray) from the Rouyn-Noranda metal smelter increased 3.3 fold over 0 to 400 days (Couillard et al. 1995a). After just 90 days, metal concentrations in transplanted mussels were significantly different from caged controls in the Lake Opasatica for Cd in the gills, mantle, digestive gland, remainder and whole organism and for Zn in the digestive gland, remainder and whole organism. After 400 days, mantle Zn concentrations in the Lake Vaudray transplanted mussels were significantly higher than the control mussels in Lake Opasatica. Alternatively, after 400 days Cu concentrations were not different between control mussels in the Lake Opasatica and caged mussels in Lake Vaudray, which was attributed to the similar water Cu concentrations of the two lakes. However, Cu concentrations in indigenous mussels from Lake Vaudray were about twice the concentration of mussels from Lake Opasatica. The overall higher concentrations of Lake Vaudray bivalves were attributed to anomalously high gill Cu levels. The authors suggest that although Cu concentrations were similar at the two sites, Lake Vaudray water chemistry (lower alkalinity (93 meq L^{-1}, Ca concentrations ($0.9x10^{-4}$ M), and pH (6.56)) may have enhanced Cu accumulation.

Cadmium concentrations in transplanted mussels were only one third of the indigenous

mussels from Lake Vaudray after 400 days. However, Zn concentrations in transplanted Lake Vaudray mussels quickly approached the Zn concentrations in all tissues of indigenous Lake Vaudray mussels, suggesting faster uptake kinetics for Zn.

These results suggest that even reasonably long exposure periods of 400 days (2 growing seasons) are not sufficient for the Cd levels in the transplanted bivalves to come the steady state with the contaminated environment. Concentrations of Zn (an essential metal) did however, reach steady-state. Transplanted bivalves provide a time-integrated measure of bioavailable metal for the duration of the exposure period and do not reflect previous years of metal exposure.

6.2.1.5 *Effects of marking and caging*

Bivalves caged in the uncontaminated lake (Lake Opasatica) did not show any effects of caging or marking (engraved number in shell with sharp nail) on condition index (CI = total flesh dry wt (g)/total shell wt (g)) or metal concentrations (except for [Zn(digestive gland)] after 400 days (Couillard et al. 1995a). Marking however, resulted in significant mortality in bivalves from Lake Vaudray and caged in Lake Vaudray (control group). Of the 16 individuals collected after 90 and 400 days only, 7 and 1 were found alive, respectively. The authors report that many of the Lake Vaudray molluscs had their shell punctured during the marking process. In comparison, 14 of the 16 bivalves transplanted from Lake Opasatica and caged in Lake Vaudray and 9 of the 16 caged control bivalves in Lake Opasatica were recovered.

Transplanted Lake Vaudray bivalves showed consistently lower condition indices than caged control or indigenous bivalves in Lake Vaudray or caged control and indigenous bivalves from Lake Opasatica. Organism dry weights did, nevertheless, show a general decrease over time in both indigenous and caged bivalves resulting in lower condition indices in all experimental groups. Couillard et al. (1995a) point out that this effect has been reported in a number of other studies with bivalves (Cain and Luoma, 1990). They call for further field studies to evaluate annual variations in condition index and determine their relationship with the abundance and nutritional quality of suspended particulate matter.

Caged bivalves in both the uncontaminated lake and Lake Vaudray showed depressed shell growth relative to free-living molluscs. Lake Opasatica bivalves grew on average 1.74 ± 0.26 mm (mean ± SE) compared to the expected increment of 5.30 ± 0.20 mm calculated from Von Bertalanffy growth curves for natural bivalve populations in both Lake Opasatica and Lake Vaudray.

6.2.1.6 *Relationship between environmental metal concentrations, tissue metal concentrations, and MT development*

Metallothionein concentrations in bivalves collected along a contamination gradient (Couillard et al. 1993) and compared in transplant studies (Couillard et al. 1995a) revealed highly significant relationships with both Cd concentrations in bivalve tissue and dissolved Cd^{2+} concentrations estimated from sediment-water sorptive equilibrium. Relationships between MT concentrations in indigenous bivalves (along the contamination gradient) and total metal concentrations in the sediment were not statistically significant (Couillard et al. 1993). Metallothionein concentrations measured in transplanted bivalves also showed a temporal increase that corresponded with a temporal increase in tissue Cd concentrations (Couillard et al. 1995a). No significant relationships were found between [MT] and tissue levels of Cu or Zn. Couillard et al. (1995a) indicated that although the field studies showed otherwise, MT may bind other metals than Cd.

Couillard (1996) describes a mechanism of cytotoxicity in bivalves resulting from metal-induced stress. Essentially, non-metallothionein bound metal is expected to enhance the lipid peroxidation process, resulting in increased levels of malondialdehyde (MDA), a by-product of peroxidation. After sustained exposure to non-metallothionein bound metal, depletion of glutathione (GSH), a cellular antioxidant, would be expected. Reactive oxygen species and mobile metals could then alter the Ca-extruding systems of the plasma membrane, e.g. by interacting with -SH groups in the Ca-Mg ATPase. Calcium accumulation in the cytoplasm would occur, thus Ca-mediated functions of the cell would be impaired and this would lead to cell death.

The temporal changes in the distribution of Cd in the gills of transplanted bivalves (Lake Vaudray) suggest that as the pool of MT proteins were exhausted an apparent "spillover" occurred, whereby Cd was associated with low molecular weight (LMW) ligands. The shift of Cd towards the LMW ligands coincided with an increase in oxidative degradation of membranes and disturbed Ca homeostasis (i.e. cytotoxicity). As indicated before, transplanted bivalves also experienced lower condition indices and growth rates.

Natural populations of bivalves collected along a contamination gradient also showed a portion of Cd associated with the LMW ligands that increased markedly above a threshold of 0.9 nM [Cd^{2+}] (Couillard, 1996). Whether or not the increase in Cd associated with the LMW fraction results in toxic effects has yet to be confirmed. However, bivalves with significant proportions of Cd associated with the LMW ligands in the gill appeared necrotic and tended to have lower incidences of gravid females per sample and a lower mean larval dry weight per female relative to the least contaminated population.

These results show promise for linking metal contamination in bivalves (transplanted and indigenous) to deleterious effects at the organism and population levels. Initiatives to further develop MT as a biomarker are currently being taken and are expected to enhance the utility of molluscs as biomonitoring tools to monitor contaminant levels and effects of metals in the natural environment.

6.2.1.4 *Summary*

There are several important conclusions from the studies described above:

- Tissue metal concentrations in transplanted and indigenous bivalves (*Elliptio complanata* and *Pyganodon grandis*) were strongly correlated to bioavailable metals and not total metal concentrations either in the water or sediment. The relationships could be explained using surface-complexation concepts and the FIAM.
- Tissue metal concentrations in *Elliptio complanata* were strongly correlated to the extractable metal fractions, Fe-oxyhydroxides and OM, in the sediment. These results

support the contention that the availability of a particular metal is inversely related to its binding strength to the various substrates in sediments.

- Tissue metal concentrations in indigenous *Pyganodon grandis* from 38 lakes of varying pH were significantly correlated to dissolved [Cd^{2+}] and not to bioavailable fractions in the sediment. Sedimentary measurements (total Cd, pH, Fe-ox, organic matter) could be used to predict tissue concentrations in bivalves using an equation that adapted the ratio of sorbed Cd to sorbent (Fe-oxyhydroxide, OM) to changing pH levels.
- Tissue metal concentrations in transplanted bivalves did not mimic those measured in indigenous bivalves (except for Zn) after 400 days exposure.
- Marking and caging resulted in higher mortality and reduced growth rates compared to indigenous bivalves. No effect on condition indices or metal concentrations (except [Zn(digestive gland)]) were observed as a result of caging.
- Metallothionein concentrations in bivalves were strongly correlated with tissue Cd concentrations, although correlations with tissue Cu or Zn were non-existent. Spatial and temporal variation in MT concentrations in *Pyganodon grandis* closely reflected changes in ambient dissolved Cd^{2+} concentrations as estimated from sediment/water sorptive equilibria.
- Shifts in cytosolic metal distributions were observed along the contamination gradient and they appeared to be reproducible under severe metal stress (transplantation experiment). These biochemical abnormalities have yet to be linked to deleterious effects at higher levels of biological organization.

6.2.2 **Sudbury River, Massachusetts, U.S.**

6.2.2.1 *Site Description*

This site description was adapted from Salazar et al. (1996). The Nyanza Chemical Waste Dump Site (Nyanza) is the former location of several textile dye production companies near the Sudbury River in Ashland, Massachusetts (MA). From 1917 to 1978, mercury and chromium were used as catalysts in the production of textile dyes. Approximately 2.3 metric tons of mercury was used per year from 1940 to 1970, with approximately 45 to 57 metric tons of mercury released to the Sudbury River during this period. On-site treatment of the production wastes from 1970 to 1978 reduced the amount of mercury released to the Sudbury River to 23 and 30 kg per year. Since dye production stopped in 1978, the property has been leased to other light production companies. In 1982, the Nyanza site was added to the National Priorities List and declared a Superfund site.

Land along the Sudbury River ranges from semi-rural to urban-suburban. There are several impoundments, behind intact or partially collapsed dams built for milling operations during the early 1900s. Below the Saxonville Dam, the river is primarily depositional and meanders through an extensive floodplain. The main geographical features of the Nyanza site are shown in Figure 14.

Mercury contamination in sediments ranged from 55 mg/kg at Reservoir #2 to 0.5 mg/kg near the Concord River. Background mercury levels at Reservoir #3 were below the detection limit of 0.1 mg/kg and were 1.6 and 0.5 mg/kg in the downstream section of Reach 1. Tissue mercury levels in fish caught along the Sudbury River were as high as 12 mg/kg in 1971 and 8 mg/kg in 1990 suggesting that contaminant levels were not significantly declining and could still pose a threat to consumers of aquatic biota.

6.2.2.2 *Study Objectives*

As part of an EPA ecological risk assessment of the Sudbury River, the National Oceanic and Atmospheric Agency (NOAA) conducted a study to measure total- and methyl mercury

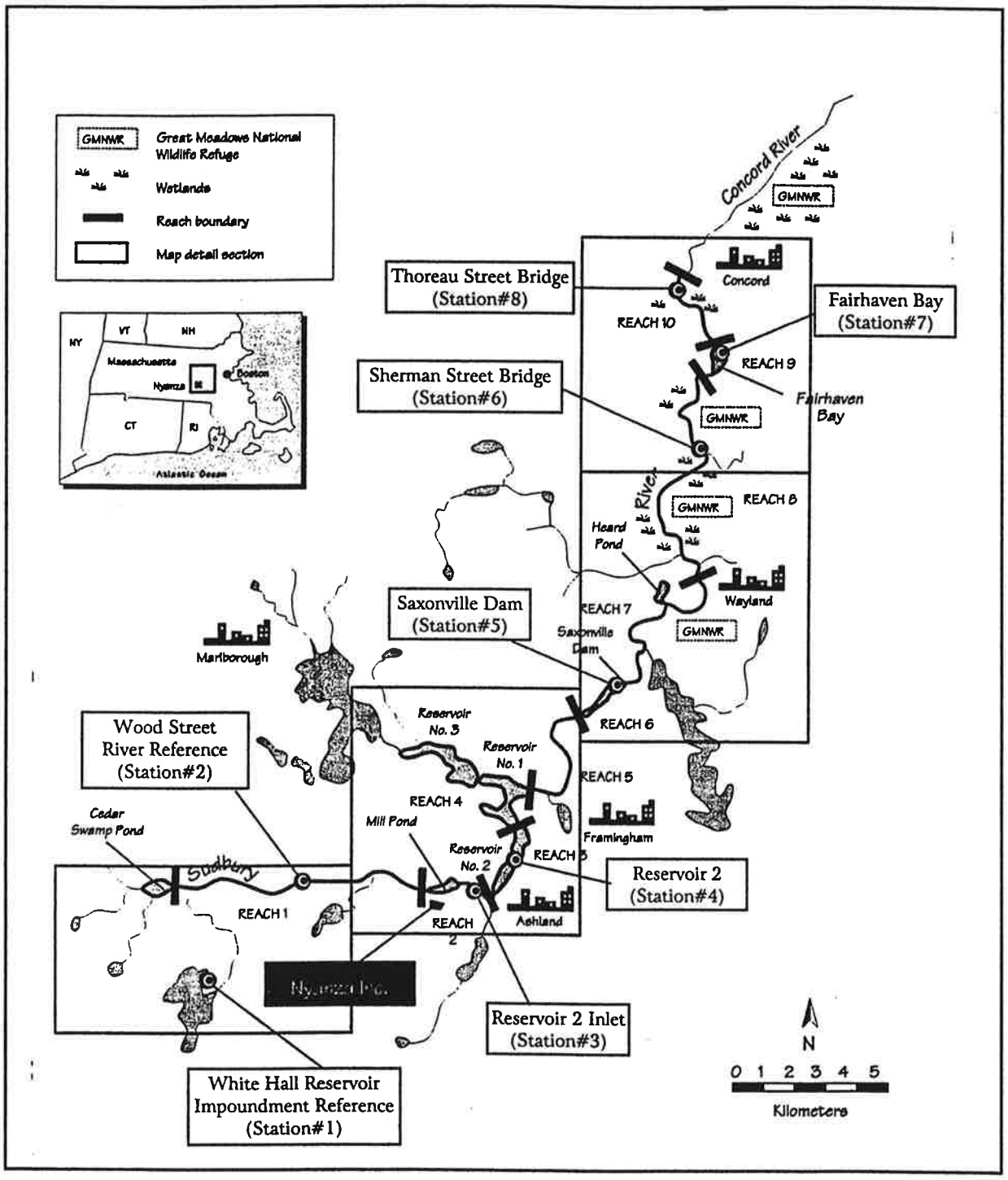

Figure 14. Location of sampling stations at the Nyanza site and the Sudbury River drainage basin in Middlesex County, Massachusetts. Adapted from Salazar et al. (1996).

bioaccumulation and to estimate chronic effects on a resident bioindicator species. The freshwater mussel *Elliptio complanata* was transplanted at selected sites along the Sudbury River and at a reference site in a distant reservoir in order to test effects from exposure to mercury-contaminated water, sediments, and food. Information obtained in the study would also help NOAA assess potential impacts to trustee natural resources.

The primary objectives of the mussel transplant study were to:

- Demonstrate the extent of bioavailable mercury within the downstream reaches of the Sudbury River resulting from operations at the Nyanza site;
- Identify areas that could act as sources of mercury for transport downstream; and
- Determine the effect of mercury exposure on a resident species.

The authors suggested that the data could be used to identify areas that show significant mercury bioaccumulation and biological impacts as candidates for EPA remedial action.

6.2.2.3 *Effects of caging*

Mussels were collected from Lake Massesecum, Bradford, New Hampshire and deployed in cages after one day. Cages were constructed of PVC piping and tube shaped, plastic mesh bags. The cages held the mussels in individual mesh bags which allowed for growth measurements to be taken at the beginning and end of the test periods. The cages were anchored 1 m above the river bottom.

Survival of the mussels ranged from 83 to 95 % at all of the sites, except Station 6, where it was 36 %; growth rates for these mussels were significantly lower than other downstream sites (Table 20). Mussels caged at the reference sites (station #1 and #2) showed high survival rates (83 to 91 %), but also showed losses in whole-animal wet weights and unexpectedly high mercury concentrations. As a result, statistical comparisons between "contaminated sites" and "reference sites" were not made. Instead the reference sites were included as part of the contamination gradient in the experimental design.

Table 20. Mussel measurements at the start of the test (initial) and after 84 days exposure (end of test) in the Sudbury River. Adapted from Salazar et al. (1996).

	Initial (n=75)		Exposed		Increase		%		%
Station	Mean	SD	Mean	SD	Mean	SD	Moisture	N	Survival
	Tissue Weights (g wet)								
1	4.03	0.54	4.29	0.7	0.26	0.49	88.4	62	83
2	4.00	0.50	3.99	0.6	-0.01	0.49	87.1	68	91
3	3.98	0.39	4.26	0.5	0.28	0.50	86.5	70	93
4	3.98	0.46	5.79	0.8	1.81	0.61	85.4	71	95
5	3.94	0.47	5.09	0.9	1.15	0.92	84.9	65	87
6	4.00	0.48	5.33	0.7	1.33	0.68	84.3	27	36
7	3.94	0.43	6.26	1.1	2.32	1.15	82.8	66	88
8	3.97	0.49	6.87	1.2	2.90	1.15	81.3	65	87
	Whole-animal Wet-Weight (g)						Growth (mg/wk)[1]		
1	15.90	2.56	15.62	2.43	-0.28	0.57	-21	62	83
2	15.74	2.34	15.43	2.29	-0.31	0.49	-38	68	91
3	15.67	1.82	15.80	1.62	0.13	0.44	23	70	93
4	15.64	2.16	17.84	2.16	2.20	1.28	185	71	95
5	15.46	2.21	17.62	2.60	2.16	1.99	198	65	87
6	15.74	2.26	16.25	2.37	0.51	1.75	46	27	36
7	15.46	2.02	18.82	2.17	3.36	1.86	270	66	88
8	15.59	2.29	19.26	2.40	3.67	1.51	303	65	87

[1] Growth rate (mg/wk) = weight $_{(t)}$ - weight$_{(t)}$/number of weeks

6.2.2.4 *Accumulation of mercury in mussel tissues*

Concentrations of trace elements at the 8 stations are shown in Table 21. Laboratory analysis of mussel tissues indicated that replicate analyses (duplicates of the same digest and duplicate digestion of mussel composites) had a variance of approximately 16 to 20 % (Salazar et al. 1996). Tissue residue levels in mussels from Lake Massesecum were 640 (± 103) and 140(± 9.29) ng g^{-1} dry wt. for total and methylmercury, respectively. Salazar et al. (1996) indicated that the mercury levels were higher than expected for mussels collected from a relatively pristine lake. Malley et al. (1996) report lower total mercury (368 ± 18 ng g^{-1} dry wt.) and similar methylmercury (161 ± 9) levels for mussels collected from a pristine lake in northwestern, Ontario. After 42 days exposure, mussels had mean tissue total mercury concentrations ranging from 330 to 930 ng g^{-1} dry wt.; mercury concentrations decreased with distance from the Nyanza site (Salazar et al. 1996). Mean total mercury concentrations in mussel tissues at the reference stations #1 and #2 increased approximately 110 to 140 ng g^{-1} dry wt. and closest to the Nyanza

Table 21. Tissue mercury concentrations (± SD) in mussels collected from Lake Massesecum at the start of the test and growth and tissue mercury concentrations in mussels at each station after 42 (Mid Test) and 84 (End Test) days exposure in the Sudbury River. Adapted from Salazar et al. (1996).

Station	Growth Rate (mg/wk)	Total Hg ($ng\ g^{-1}$ wet)	Methyl Hg ($ng\ g^{-1}$ wet)	Total Hg ($ng\ g^{-1}$ dry)	Methyl Hg ($ng\ g^{-1}$ dry)	Inorganic Hg ($ng\ g^{-1}$ dry)	Total Hg Content (ng)	Methyl Hg Content (ng)	Inorganic Hg Content (ng)	% MeHg
Initial	-	120 (20)	25 (1.66)	640 (103)	140 (9.29)	500	510 (125)	110 (18.2)	400	22
Mid Test										
1	-24	99 (22.4)	-	750 (165)	-	-	370 (116)	-	-	-
2	-64	96 (17.7)	-	780 (179)	-	-	330 (66.3)	-	-	-
3	0	120 (9.60)	-	930 (79.4)	-	-	470 (60.0)	-	-	-
4	252	84 (8.02)	-	550 (47.1)	-	-	400 (54.3)	-	-	-
5	255	85 (7.81)	-	550 (70.0)	-	-	440 (56.4)	-	-	-
6	15	67 (13.6)	-	520 (104)	-	-	310 (66.5)	-	-	-
7	281	63 (8.66)	-	370 (60.6)	-	-	330 (45.2)	-	-	-
8	318	58 (6.46)	-	330 (31.9)	-	-	320 (50.7)	-	-	-
End of Test										
1	-21	100 (5.43)	-	**890 (85.5)**	**360 (46.7)**	530	440 (70.4)	**180 (28.5)**	260	40
2	-38	110 (17.3)	-	**850 (71.9)**	**260 (44.3)**	600	440 (90.5)	130 (28.0)	310	30
3	23	130 (5.53)	-	**950 (33.3)**	**320 (29.6)**	640	550 (73.1)	**180 (25.2)**	370	33
4	185	100 (26.3)	-	690 (228)	**260 (24.8)**	430	570 (140)	**220 (33.4)**	350	38
5	198	78 (5.40)	-	520 (56)	**200 (49.8)**	320	390 (64.5)	**150 (31.0)**	240	38
6	46	94 (26.6)	-	590 (127)	170 (42.8)	420	450 (108)	150 (25.5)	350	33
7	270	69 (8.95)	-	**400 (51.1)**	140 (11.0)	**260**	430 (92.5)	**150 (31.2)**	280	34
8	303	62 (5.96)	-	**340 (35.7)**	130 (3.5)	**210**	430 (59.5)	**170 (20.1)**	260	39

Content (ng) = Concentration ($ng\ g^{-1}$) * Animal weight (g)

- = Not measured or Not applicable

Bold - indicates number is significantly different from initial measurement. Station 6 was not included in the analyses due to high mortality, low growth rates and station relocation at Mid Test.

site increased by about 290 ng g^{-1} dry wt. Decreases of approximately 90 to 310 ng g^{-1} dry wt. were observed in mussels at all other stations. A similar decrease in total, inorganic, and methyl mercury concentrations in mussel tissues with distance from the Nyanza site was observed at the end of the exposure period (Salazar et al. (1996). Mean tissue total mercury concentrations at Station 8 through Station 3 showed no significant changes from those determined at mid test, but Stations #1 and #2 increased above mid test levels to 890 and 850 ng g^{-1} dry wt., respectively (Salazar et al. 1996). After 84 days exposure, tissue total mercury concentrations in mussels at Stations 1, 2 and 3 were signficantly higher than mussels at Stations 7 and 8. The lowest methylmercury concentrations in mussels were found at Station 8 and the highest at Station 1. Methymercury concentrations generally mimicked those measure for tissue total mercury.

Much to the investigators surprise mean tissue total- and methymercury concentrations in mussels at the reference sites were not significantly different from the concentrations measured in mussels caged at the Nyanza station. These results suggest that there were sources of bioavailable mercury to the mussels, not obvious from sediment mercury concentrations. Mussels are particularly sensitive to waterborne mercury concentrations. Mussels (*Pyganodon grandis*) transplanted from a pristine lake to cages in two natural wetlands with very low methymercury concentrations (0.09 ng L^{-1} and 0.24 ng L^{-1}) showed significant differences in tissue mercury concentrations after 90 days exposure (Malley et al. 1996).

Mercury levels measured as contents or body burdens showed slightly different results. Total mercury burdens in mussels were not significantly different from initial levels at any of the stations and methylmercury contents were significantly higher in mussels at Stations 1, 3-5, and 7 and 8, but not Station 2. The proportion of methylmercury to total mercury contents increased from 22 to 30 - 40 % after 84 days.

6.2.2.5 *Relationship between mercury levels in tissue and sediments*

There were no significant correlations between total mercury concentrations in tissue and sediments (r = 0.446), nor TOC-normalized sediment mercury concentrations. Correlations with

Table 22. Trace element analyses and conventional parameters for sediments collected from Whitehall Reservoir (Reference Station #1) and the Sudbury River. Adapted from Salazar et al. (1996).

	Station							
	1	2	3	4	5	6	7	8
Trace Elements (mg kg^{-1} dry wt.)[1]								
Mercury	0.17	0.11	17.9	0.17	5.4	0.5	0.07	0.36
Chromium	24.3	10.3	152.3	14	78	22.3	7.9	28
Lead	132.7	107	225	17.7	410	58.7	5.4	40
Antimony	U (0.4)	U (0.3)	1.4	U (0.2)	1.1	U (0.5)	U (0.2)	U (0.2)
Arsenic	5.9	3.7	12.2	3	11.9	8.1	9.2	10.7
Cadmium	U (0.8)	0.6	3.3	0.4	10	3.6	0.3	1
Physical Parameters (%)[2]								
TOC	5.93	3.45	11.7	3.37	7.7	10	1.62	4.58
Total solids	22.87	43.97	18.57	49.43	19.28	18.98	65.98	38.79
Grain size								
Sand	17	82	30	80	38	34	90	85
Silt	70	14	58	16	46	42	6	10
Clay	10	3	10	2	14	22	4	5

U Undetected; concentration in parentheses equals the detection limit

[1] Concentrations were determined as the mean of three replicate samples.

[2] Measurements were made on one sample only at each station, except for grain size at Station 6, determined as a mean of triplicate samples.

tissue methylmercury and total sediment mercury concentrations were equally as poor ($r = 0.381$). However, tissue and sediment mercury concentrations were significantly correlated when the reference stations were excluded from the analysis. Mussels at Station 4 showed the highest methylmercury content on a per-animal basis, despite a low concentration of total mercury in sediment at this station (Table 22). However, additional concurrent studies indicated that Station 4 had high waterborne methylmercury levels which may have caused the high tissue levels (Salazar et al. (1996).

6.2.2.6 *Relationship between mercury levels in tissue and mussel growth*

At mid test, tissue weight, shell length and whole-animal weights showed similar responses, whereby values for mussels increased at Stations 4, 5, 7, and 8 and did not change at Stations 1, 2, 3, and 6 (Salazar et al. 1996). At the end of the test, mussel growth (whole-animal wet weights, lengths, tissue weights, and shell weights) showed a general increase from Station 1

to Station 8. Mussels from Stations 1, 2, and 3 formed a statistical grouping based on very low or negative growth and Stations 7 and 8 formed a second statistical grouping characterized by highest growth, based on changes in tissue weight, whole-animal wet weight, and shell length.

Table 23. Correlations between mussel growth metrics and tissue mercury concentrations (ng g^{-1} dry wt.). Adapted from Salazar et al. (1996).

Comparison		
Growth Metric	Tissue Mercury	*r* value[1]
Whole-animal wet weight	Total	-0.95
Whole-animal wet weight	Methyl	-0.88
Whole-animal wet weight	Inorganic[2]	-0.95
Tissue Weight[3]	Total	-0.93
Tissue Weight	Methyl	-0.88
Tissue Weight	Inorganic	-0.91
Shell Length	Total	-0.94
Shell Length	Methyl	-0.85
Shell Length	Inorganic	0.95
Shell Weight	Total	-0.93
Shell Weight	Methyl	-0.87
Shell Weight	Inorganic	-0.92

[1] critical *r*-value $r_{0.05,(2),5} = 0.755$; all correlations are significant at the 95 % confidence level.

[2] Inorganic mercury = total mercury concentration (ng g^{-1}) - methylmercury concentration (ng g^{-1}).

[3] Only end of test values for tissue weight, shell length and shell weight were used.

Significant correlations were found between a variety of growth metrics (whole-animal wet weight, tissue weight, shell length, shell weight) and tissue mercury concentrations (Table 23). However, there was no relationship between mussel growth and tissue mercury contents. Whether or not the changes in mussel growth are related to elevated mercury in the mussels or just an statistical artifact of "growth dilution" is debatable. Small or negative changes in tissue weight would have resulted in an increase in tissue mercury concentrations, suggesting that the changes in mussel growth were caused by the higher tissue mercury concentrations. However, Salazar et al. (1996) did find that calculations of the amount of growth needed to account for the changes in tissue mercury concentrations could not completely account for the increases in mercury. It is likely that mercury contributed to the reduction in growth along with other variables (other pollutants, temperature, food quality and quantity etc.), although the relative

contribution of each variable is unknown. Temperature was measured throughout this study at the individual stations and showed a general trend of increasing mean temperature away from the Nyanza site. The order of increasing mean temperatures were:

Station2 < Station3 < Station6 < Station5 < Station7 < Station8 < Station4

A positive correlation between tissue temperature and growth was found for the mussels in the study, suggesting that temperature differences may have contributed to the changes in growth.

6.2.2.7 *Summary*

Transplanted mussels were used to measure mercury contamination and possible effects at the organism level. Some of the major conclusions that can be drawn from this study include:

- High levels of mercury were found in *Elliptio complanata* collected from an "uncontaminated" source lake and both "reference" stations had high levels of bioavailable mercury. Choosing reference sites and source populations for mercury studies is critical for interpretation of results at "contaminated" sites.
- Mussel growth has a significant effect on tissue mercury concentrations. Body burden data, which normalizes data for growth is a preferable measure of contaminant bioaccumulation.
- Total mercury body budens of mussels remained constant due to a decrease in inorganic mercury concomitant with an increase in methylmercury at all stations.
- Total mercury concentrations in sediment did not correlate with total mercury in mussel tissue for all stations. However, tissue and sediment mercury concentrations were significantly correlated when the reference stations were excluded from the analysis.
- Waterborne mercury concentrations may have been correlated with tissue levels, but water mercury concentrations were not determined in this study and thus correlations could not be tested. Due to the lack of information on sources of mercury to molluscs, both sediment and water should be analyzed.

- The source of methylmercury to the mussels throughout the study area could not be attributed unequivocally to Nyanza.
- Mussel growth rates decreased from the farthest stations (7 and 8) towards the Nyanza site.
- Changes in growth rate were significantly correlated with tissue total-, inorganic- and methylmercury concentrations, but not tissue mercury contents. Growth rate was also correlated with temperature. It was impossible to attribute the decreases in mussel growth to elevated tissue mercury due to the potential artifact of growth diluation and influence of temperature on growth.

Although it could be concluded that methylmercury was bioavailable throughout the study area, mussel growth effects could not be unequivocally associated with mercury exposure. Environmental variables known to affect growth (e.g. food quality and quantity, temperature) may have contributed to the observed differences at each station.

7. FIELD TESTING AND ANALYTICAL METHODS OF DETECTION AND QUANTIFICATION

This section covers the important factors that should be considered when organizing field studies and analyzing molluscs for metals. Analytical methods of detection and quantification are generally straightforward for molluscs and a number of sources exist from which to establish QA/QC protocols for biomonitoring programs. A number of protocols exist for field studies using molluscs, but aspects of experimental design depend to a large degree on the goals and objectives of the monitoring program and mine site characteristics.

7.1 Experimental Design

Determining the experimental design of any field study is critically important and deserves high priority. A good experimental design can mean the difference between useful and worthless data. Molluscs can be useful tools in biomonitoring programs, but they have certain inherent variables that need to be controlled in order to detect changes resulting from mining activities.

7.1.1 **Statistical considerations**

As discussed earlier in the case study of mercury contamination at the Nyanza textile plant, finding a suitable "reference" site may be difficult (Salazar et al. 1996). Malley et al. (1996) also describe problems in statistically comparing mercury concentrations in mussels held in a flooded wetland to a reference wetland given that the mercury concentrations were significantly different between the two sites prior to flooding (or impact). In recent years there has been significant development in experimental design and statistical approaches for detecting environmental impacts. Most notably is the BACI (Before/After Control/Impact) developed by Stewart-Oaten et al. (1986) and further developed by Underwood (1992). Basically, the BACI approach compares "changes over time" at the reference site to "changes over time" at impacted sites. The question that is asked is "are the changes occurring at the impacted site due to natural variation or due to the impact?" The BACI approach offers an alternative to direct comparisons between a reference site and an "impact" site. For example, many environmental impact studies

compare populations downstream from an industrial plant to reference populations located upstream from the plant. In many cases, there are natural factors that result in differences between the upstream and downstream populations that may mask the true impact resulting from plant operation. The major improvement of the BACI design by Underwood (1992) was to include an asymmetrical sampling design using a randomly-selected set of reference sites. By including more than one reference site the chances of changes occurring at a reference site in the same direction and same intensity as the impacted site (i.e. canceling out the impact) are significantly reduced. For further information on the BACI design the reader is directed to Stewart-Oaten et al. (1983) and Underwood (1992).

The number of samples that should be collected at different sites and on different sampling dates to provide the maximum amount of statistical power is most often limited by analytical costs. The sampling scheme recommended by Crawford and Luoma (1993) for the United States National Water-Quality Assessment (NAWQA) Program is appropriate for the needs of the mining industry. They suggest that 3 composites of 10 individuals be collected from each sample site and sample period. Although 2 or 3 individuals could provide enough material (5 g wet weight) for tissue analysis, 10 individuals account for individual variability.

7.1.1 **Seasonal/spatial variability**

Metal concentrations in molluscs may be affected by the season due to changes in temperature and food availability as well as changes in reproductive status and growth. Controlling for these variables depends on the mollusc species, although certain consistent effects have been documented.

Growth in unionids is generally restricted to the warmer, ice-free months of the year. Therefore, collections are usually restricted to these times if the goal is to measure active uptake in molluscs, such as in transplant studies. Several authors recommend that molluscs be sampled just prior to fertilization, since at this time metal concentrations tend to be highest and no glochidia are present in the gills of unionid species (Metcalfe-Smith et al. 1996; Lobel et al. 1991a; Tessier et al. 1993). Changes in food availability are difficult to control since it may

change on a daily, weekly and monthly scale. The best approach is to sample impacted and reference populations at the same time (within a week) and sample at the same time each year for annual trends.

Spatial variability is an important factor in mollusc studies due to differences in the hydrodynamic conditions, substrate, and micro-habitat (temperature, food availability and quality etc.) that can affect metal uptake. Mollusc populations have been found to differ in genetic composition over short distances, possibly due to their adaptation to a specific habitat (Green et al. 1985). Consequently, mollusc populations collected from different areas of a lake may have inherently different size-age relationships and/or growth rates which can influence metal accumulation. There are several considerations when controlling for spatial variability:

1. Choose sampling sites for comparisons among indigenous populations that share similar hydrodynamic, trophic status and substrates.
2. In mollusc transplant studies all of the individuals needed for the study should be collected from the same area and preferably from the same population.
3. Collect molluscs for transplant studies from an area with similar hydrodynamic, substrate and trophic status as the site of the study. This reduces the effect of "source" on metal uptake and increases the potential for the transplanted molluscs to mimic metal uptake of resident populations (Hinch and Green, 1989). Alternatively, the effect of source can be reduced by extending the transplant studies for periods longer than 1 year (Hinch and Green, 1989).

7.1.2 **Growth and age effects**

Controls for seasonal and spatial effects on metal accumulation will likely also control for growth, although further precautions may be taken to reduce natural variation in metal uptake. By collecting molluscs within a narrow size-age range (e.g. individuals 80-100 mm in length and 7-10 yr.) the investigator can be relatively confident that the individuals possess similar growth rates. Size in bivalves is usually determined by length (Hinch and Stephenson, 1987) and age is

determined by counting external annuli in unionids (Metcalfe-Smith and Green, 1992). Determining the age of marine mussels, gastropods and Sphaeriid sp. is difficult if not impossible; length is therefore used to obtain individuals with similar growth rates. Metcalfe-Smith and Green (1992) recommend that only bivalve species that can be easily aged be used in biomonitoring programs. Size alone in not often a good indicator of growth since bivalves that are 80-100 mm in length may be 4 or 15 years old because they possess fast or slow growth rates, respectively.

7.1.2 **Choosing Species and Sex**

Bivalves and gastropods have many characteristics that make them suitable for biomonitoring studies. Snails are common in freshwater, but they lack supporting scientific information on metal accumulation which makes them less desirable as sentinel organisms. Further, snails are small and extracting them from their shells for tissue analysis may be problematic in routine field assessments. For these reasons gastropods are not recommended for use as sentinel organisms at this time.

Of the bivalve species, the major groups include fingernail clams (Sphaeriidae), long-lived Unionids and introduced *Dreissena polymorpha*. Sphaeriid clams have not been widely used in biomonitoring programs because they are small and their soft tissues are difficult to remove. *Dreissena polymorpha* is limited in distribution to the Great Lakes and there is strong motivation to prevent any further invasion of other parts of the country, ruling out transplant studies. Since there are no other similar species, Dreissena is not recommended for a nation-wide biomonitoring program, but may be suitable for programs specific to the Great Lakes. Dreissena is currently being used in biomonitoring throughout Europe (Kraak et al. 1991; Mersch et al. 1996).

Unionid species offer the greatest number of advantages due to their ubiquitous distributions and large size. Some Unionid species are more widespread than others (e.g. *Pyganodon grandis*, *Elliptio complanata*), making them suitable for a nation-wide program. However, in many cases the choice of species will be site-specific depending on availability and abundance. Unionids are not recommended for collection in the United States National Water-Quality Assessment (NAWQA) Program because many species are considered endangered

(Crawford and Luoma, 1993). Although there are a number of species that have not declined in abundance and would be suitable as biomonitors, organizers of NAWQA Program are concerned that endangered species may be collected accidentally along with the healthy species. The NAWQA Program instead utilizes the introduced asiatic clam *Corbicula fluminea* which is spreading throughout waterways in the U.S. Before a unionid species is chosen for any biomonitoring program its conservation status must be carefully considered, for the present time and the duration of the monitoring program.

The marine mussel *Mytilus edulis* has been chosen for use in the Mussel Watch (O'Connor, 1992) and is recommended for monitoring programs in Canada.

Several authors have found that metal accumulation varies among unionid species in a metal-specific manner (Metcalfe-Smith, 1994; Lobel et al. 1990). Inter-specific differences may exist in absolute metal concentrations if correlations are in the same direction, species could be used interchangeably after conversion by means of regression equations. For example, *Elliptio complanata* and *Lampsilis radiata radiata* could be used interchangeably to monitor site-to-site trends in the bioavailability of Cd and Pb (no inter-specific difference exist), and after conversion by means of regression equations to monitor Al, Cr, Cu, Fe, Hg and Ni (Metcalfe-Smith, 1994). There are no easy answers in this case. Samples will have to be taken to determine if and for what metals inter-specific differences exist at a particular site before concentrations can be compared between species.

There are differences in metal accumulation between the sexes of unionids, but they are less pronounced that inter-specific differences. Determining the sex of unionids from morphological attributes is only possible for one species (*Lampsilis radiata*), making sex-specific collections impossible. Therefore, it is recommended that collections be taken prior to fertilization when sex-specific metal differences are minimal and/or enough samples be collected so brooding females can be rejected from metal analyses.

7.2 Transplanting and caging molluscs

Transplanted molluscs can be useful tools in biomonitoring programs provided the method

of caging does not cause undue stress to the animals and affect normal metal uptake. Many studies have documented the use of transplanted and caged bivalves (Couillard et al. 1993 and 1995a,b; Salazar et al. 1996). The following are some of the approaches and problems associated with transplanting and caging molluscs.

7.2.1 **Methods of caging**

The method of caging should take into consideration a number of factors:

1. Cages in metal accumulation studies should not be made of metals or products that are degradable and release metals or enhance metal adsorption. Plastic is often the material of choice. Colored plastic should be used with caution since many dyes contain Cd and Pb.
2. Cages should allow for good circulation of water and particulate material. Mesh sizes 4 mm and larger appear to a acceptable. Light penetration should also be considered in cage development. Some bivalves show diurnal patterns of valve-openness corresponding with light that may affect metal uptake (McCorkle et al. 1979).
3. Whether or not the cage allows the bivalves contact with the sediment depends on the species and the study objectives. Bivalve behavior will be disrupted less if the cages are in contact with the sediment, particularly for those species (i.e. unionids) normally found in contact with the sediment.
4. Cages or compartments should prove enough space so as to not cause over-crowding. Natural densities of bivalves are species specific and efforts should be made to ensure these densities are not exceeded.
5. Cages that completely enclose the bivalves tend to reduce predation, but they can also reduce water flow past the bivalves as well as light intensity.

The reader is referred to studies by Couillard et al. (1993 and 1995a,b), Salazar et al. (1996) and Malley et al. (1996) for three different methods of caging freshwater bivalves for metal studies.

7.2.2 **Effect of transplanting, marking and caging on molluscs**

Little is known about the effects of transporting and caging molluscs. It is not unlikely that moving to a new environment results in stress, but does it effect survival and metal accumulation; are there consequences for metal accumulation studies? Preliminary studies using the freshwater mussel *Pyganodon grandis* caged in the field and held in the laboratory revealed that blood ion composition (physiological indicator of stress, Malley et al. 1988) of caged mussels was not significantly different from non-caged mussels from the same lake (D.F. Malley, unpublished data). On the other hand, mussels transported and held in the laboratory showed significant differences in blood ion composition from the same non-caged mussels. The changes in blood ion composition may be a reflection of the physical transport process to the laboratory rather than the actual holding the laboratory. If transportation causes stress then caging studies that require mussels to be physically move from one system to another will likely result in stressed animals. Further studies are needed to confirm the causes of stress in mussels and to determine the consequences for metal uptake in bivalves.

Information on the effects of transplanting and caging bivalves is sparse. Results are inconsistent as well as species-, metal- and site- specific. A common result of caging is the loss of soft-tissue weight throughout caging periods ranging from 80-400 days (Couillard et al. 1995a; Malley et al. 1996; Salazar et al. 1996). Metal concentrations will have to be adjusted for weight losses (i.e. expressed as body burdens = concentration ($\mu g\ g^{-1}$) x organism (or tissue) weight (g)). Mortality is also a common effect of many caging studies. Survival rates of up to 85-90 % are possible, but in certain situations (low oxygen, high temperature, toxicity) high mortality results. Sample sizes should take into consideration potential losses and cages should be checked regularly for mortalities. Losses of more than 2 mussels often indicate a larger problem and if they are detected early the cages could be moved to an alternate site.

Marking transplanted or caged bivalves is often done so that the growth of individual specimens can be tracked throughout the exposure period. The results of field studies by Couillard et al. (1995) indicate that marking techniques can effect bivalve mortality. Couillard et al. (1995a) found that scratching a number into the shells of the bivalves with a nail resulted in the death of

several animals due to accidental puncture of the shell. However, subsequent experiments where bivalves were marked with a glued-on tag mortality rates improved (Couillard et al. 1995a). Careful consideration should be given to the mollusc marking technique used in field studies to ensure specimens are not lost.

7.2.2 **Collecting and rearing**

The collecting process should not injure or contaminate the bivalves. Since freshwater bivalves are usually found in depths less than 6 m, collections can be done relatively easily using SCUBA or in shallow instances, snorkeling. Marine bivalves can also be collected using SCUBA. Species can be identified in the field and require only a modest understanding of bivalve taxonomy.

Aquaculture has improved techniques for rearing marine mussels, but rearing techniques for freshwater bivalves are limited and labor intensive. Most unionids require a specific fish host for larval development, a stage that is often difficult to achieve in the laboratory. Rearing of unionid populations would be advantages in that bivalves used in transplant studies would possess the same genetic make-up and their "source" environmental conditions could be controlled. Further research into rearing of freshwater bivalves in the field is recommended, particularly in light of recent information on the declining status of unionids in North America.

7.3 Analysis of mollusc tissue for metals

7.3.1 **Preparation of molluscs for analysis**

It is critical when preparing molluscs for metal analysis that precautions be taken to avoid metal contamination. Animals should be rinsed in lake or stream water to remove attached algae or debris and placed in clean plastic bags. Whether or not the animals should be held in lake or stream water for gut clearing is debatable. There is evidence both for (Lobel et al. 1991b; Uthe and Chou, 1988) and against (Metcalfe-Smith, 1994; Malley et al. 1989) gut clearing on the basis of it's effect on metal concentrations. Crawford and Luoma (1993) recommend as part of the

NAWQA Program that bivalves be held in water from the collection site at approximately 10°C for a 24-hour depuration period. In light of possible comparisons with their data, it might be advantageous to adopt their approach and incorporate a depuration period. It is important that the containers and water used for depuration do not result in contamination. Alternatively, the digestive tracts of dissected animals could be flushed with deionized water to remove the animals' gut contents (Couillard et al. 1995a).

Immediately after the depuration period bivalves should be weighed (valves closed and full of water) and measured (greatest anterior-posterior dimension) to the nearest millimeter (after Crawford and Luoma, 1993). Other morphometrics may be taken depending on the study objectives, but these are the minimum measurements required for metal accumulation studies.

The bivalves may be processed whole or dissected into tissues (i.e. gills, mantle, digestive gland) depending on the study objectives. Specific methods for processing the specimens may also be needed depending on the type of analyses. For example, samples for MT analysis should be frozen immediately in two plastic bags (inner bag vacuum sealed and outer bag filled with nitrogen) and stored at -20°C or lower temperature until analysis. For metal analysis, shells and whole bivalves or individual dissected tissues should be placed in clean plastic bags and immediately frozen and kept frozen (-20°C) until further analysis. All samples should be analyzed within 6 months of the time of collection (Crawford and Luoma, 1993).

7.3.2 **Tissue digestion and metal analysis techniques**

Analysis of mollusc soft-tissue is fairly straightforward. Samples are dried, digested in acid and their metal content determined by a number of techniques depending on the metal and the instruments available. Several methods for drying, digesting and analyzing bivalve tissues exist and it is important that all samples are analyzed with the same methods. The following is a basic description of one method of tissue metal analysis:

Table 24. Instrumental analysis methods used by Battelle laboratories for Mussel Watch Project samples. Adapted from Lauenstein et al. (1993).

Metal	Method
Al	GFAA/ICP-MS
Si	XRF
Cr	GFAA/ICP-MS
Mn	XRF
Fe	XRF
Ni	GFAA/ICP-MS
Cu	XRF
Zn	XRF
As	XRF
Se	XRF
Ag	GFAA/ICP-MS
Cd	GFAA/ICP-MS
Sn	HAA/ICP-MS
Sb	GFAA/ICP-MS
Hg	CVAA
Ti	GFAA
Pb	GFAA/ICP-MS

GFAA - Graphite Furnace Atomic Absorption; ICP-MS - Inductively Coupled Plasma Mass Spectrophotometry; XRF - X-ray Fluorescence; CVAA - Cold Vapor Atomic Absorption; HAA - Hydride Generation Atomic Absorption;

1. **Dry weights** - weigh ~500 mg of wet tissue, oven dry at ~70°C for 24 hr, allow to cool and reweigh. Wet tissues may also be freeze-dried as an alternative to oven drying.

2. **Digestion** - dissolve dried, homogenized tissue using nitric and perchloric acid in a Teflon digestion bomb using a conventional oven. In a microwave oven, dissolve ~300 mg freeze-dried and homogenized tissue in nitric acid in a Teflon bomb.

3. **Instrumental analysis** - Table 24 shows the different instrumental analyses used in the Mussel Watch Program. The choice of instrumental analysis also depends on the availability of the instruments.

7.3.3 **Soft tissue vs. shell**

This report has focused exclusively on soft-tissue metal concentrations and their relationship to environmental metal concentrations. An alternative to soft-tissue metal concentrations are shell metal concentrations. Metals may accumulate in shells by adsorption onto the external shell surface (Clarke et al. 1976) or deposition from the pallial fluid from which each shell layer is precipitated (Fuge et al. 1993). Since bivalves in northern latitudes put down annual shell layers, it is theoretically possible to measure metal concentrations accumulated by the bivalves in previous years. Recent studies have found that the water is the primary source for metals accumulated in shells (Fisher et al. 1996) and that the amount of metals in individual growth layers is correlated to ambient water metal concentrations (Fuge et al. 1993).

Shell metal concentrations offer some advantages over soft-tissue metal concentrations including the ability to determine background tissue metal concentrations prior to the start of a mining operation and in determining "natural", pre-industrial metal levels using museum archived shells. The latter point is particularly important in what constitutes acceptable levels of metals in the environment. However, there are some concerns regarding the use of shell metal concentrations in biomonitoring programs. The relationship between metal levels in shells and metal levels in the environment has not been widely studied and further research is needed to examine the same questions posed for metals in soft-tissues in section 4. Outridge et al. (1995) have detected elemental mobility between shell growth layers which suggests the year-by-year metal accumulation data may be misleading. Techniques that measure metal concentrations in mollusc shells are available (Laser ablation ICP-MS - Outridge et al. 1995; nuclear microscope SLIM-UP - Nystrom et al. 1996), however, suitable solid standard reference materials are lacking (Outride et al. 1995).

Only soft-tissue measurements are recommended at this time, however, mollusc shells should be archived for analysis in the future and research on the use of shells in biomonitoring programs for the mining industry should be continued.

7.3.3.1 *Suggested readings*

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7.3.4 **QA/QC associated with the analytical protocols**

Data quality is critical to the success of any biomonitoring programs. Quality assurance and quality control (QA/QC) procedures can be established at all stages of the data collection process: sample collection, field preparation, sample shipping, laboratory sample processing, chemical analysis, and data storage (Crawford and Luoma, 1993). Many of the field related

QA/QC procedures have been discussed above (e.g. replicate sampling, depuration of bivalves). The use of QA/QC protocols for analytical procedures is particularly important and may include the following:

1. All analyses should include reference materials such as the NRC Certified Reference Materials (CRMs) and NIST Standard Reference Materials (SRMs) similar to the type of tissue being analyzed (e.g. oyster tissue). Reference materials should be run at the beginning and end of each analytical run and should report within the specified limits.
2. All sample should be quantified within the standard calibration range. Quantification based on extrapolation is not acceptable.
3. Method detection limits (MDLs) should be calculated regularly (annually) using a defined formula such as that suggested by EPA.
4. Precision is determined as a function of concentration and tends to be independent of the nature of the analyte or the analytical technique (Lauenstein et al. 1993). The coefficient of variation among analytical sample replicates at the 10 ng g^{-1}(ppb) analyte level should be $\leq$30 % and at 10 mg g^{-1}(ppm) should be $\leq$15 %.

8. RESEARCH NEEDS

At this point, molluscs can be considered useful biomonitoring tools of metal exposure. However, there are several research areas that need to be developed to further improve and extend the use of molluscs as biomonitoring tools.

1. Interpretation of the relationship between metal concentration in the environment and tissue metal concentrations in molluscs would be greatly enhanced by a mechanistic/model approach that outlines each step of the data collection (experimental design, sampling, statistical analysis and interpretation) . A model approach would enable more investigators to successfully relate tissue metal concentrations to metals in the environment by determining the type of data required (i.e. metal sources, physico-chemcial factors affecting uptake, detoxification mechanisms and elimination rates).
2. The relationships between mollusc tissue metal concentrations and effects at the individual, population, and ecosystem level are not well established[3]. Although there have been considerable advances linking metal exposure to MT development and cellular damage (Couillard et al. 1995a,b), disruption of growth (Salazar et al. 1996) and a decline in condition (Luoma, 1996), these tools require further field testing. The recommendation by Couillard (1996) for further research on metal-induced effects (MT) conducted under the auspices of the AETE program is strongly supported. Field data on the effects of metals on growth, condition, reproduction could be obtained if these are included in the initial biomonitoring protocols for the mining industry.
3. A survey of the conservation status of molluscs in Canada is critical to the further development of molluscs as biomonitoring tools. Confidence that molluscs will be available for sampling and collection is a pre-requisite to incorporating them into a long-term national biomonitoring program. Further, permits for transplanting molluscs within or between Provinces are needed.

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[3] The author of the critical review in the companion chapter suggests that knowledge of effects is sufficient for their use in a biomonitoring program for the mining industry.

4. Knowledge of the physiological effects of handling and transplanting molluscs is needed to determine how stress affects metal uptake in transplanted or caged molluscs. These effects could be examined as side-projects associated with primary biomonitoring studies conducted by the mining industry.
5. The use of transplanted molluscs would be enhanced by further research into the rearing of unionids in the laboratory or field.

9. RECOMMENDATIONS ON THE USE OF MOLLUSCS AS A COST-EFFECTIVE BIOMONITORING TOOL FOR THE MINING INDUSTRY

Compared to fish or plants, molluscs offer several advantages as biomonitoring organisms, meeting most of the criteria outlined in section 2.2. In the U.S., the bivalve *Corbicula fluminea* was recommended as the top priority for sample collections in the NAWQA Program, over fish and plants (Crawford and Luoma, 1993). Therefore, molluscs, bivalves in particular, are likely candidates for monitoring programs for the mining industry. Before describing how they might be used it would be useful to review the additional factors outlined in section 2.3 that are specifically relevant to biomonitoring tools for the mining industry:

1. *Do molluscs occur naturally in mining impacted areas, and if not can they be artificially introduced in cages?* Yes, bivalve species occur in mining impacted areas (e.g. Rouyn-Noranda) and they have been successfully transplanted and caged in mining areas.
2. *Are there differences in metal accumulation patterns between naturally occurring indigenous populations and transplanted molluscs?* Yes, in some cases the patterns of uptake are different and thus, metal accumulation in indigenous populations and transplanted populations cannot be considered the same until proven otherwise.
3. *Are there physiological constraints to using molluscs to monitor areas receiving mining discharge, e.g. pH, [Ca]?* Yes, bivalves have absolute lower pH limits of 4.7-5.0 and [Ca] limits of 2-2.5 mg L^{-1} and are restricted to waters above these lower limits. Bivalves would not be suitable to monitoring areas directly receiving acid-mine discharge.
4. *Are molluscs useful for detecting short-term pollution events (episodic events) or long-term trends in the receiving environment?* Bivalves could be used to monitor both short- and long-term trends. Fast-growing, short lived Sphaeriid species or *Dreissena polymorpha* and transplanted unionids could be used to detect short-term trends (e.g. 1 to 6 months) and long-term trends could be monitored by indigenous long-lived unionid species (e.g. years).
5. *Can tissue metal concentrations be related to different sources of metal in the aquatic environment?* There is potential for bivalves to detect different sources of metals (e.g.

water/particulate, point-sources), but this requires extensive sampling of the external conditions and rigorous experimental design.

Based on the answers, the following are recommendations of the use of molluscs as biomonitoring tools for the mining industry. The recommendations will be provided based on a series of questions posed by the AETE Program committee.

1. *Is the tool an indicator of exposure or response?* Molluscs are indicators of exposure to metals such as Cd, Cu, Zn, Pb, Ni, Hg, As, Cr, and Ag. Metal-induced effects in molluscs such as changes in growth, or MT concentration are not well established. These responses could be measured as part of the monitoring program, but the results should be used with caution until their role has been validated in field studies using mining contaminants.
2. *What information does the tool provide?* Bivalve molluscs could be used to:
   - confirm changes in biologically-relevant metal concentrations in the natural environment resulting from mining activities.
   - monitor long-term spatial and temporal trends in biologically-relevant metal concentrations by increases and decreases in tissue concentrations of indigenous or transplanted populations.
   - determine the effectiveness of remedial measures through the use of transplanted and indigenous molluscs.
3. *Does the tool require supporting information for proper interpretation?* Proper interpretation of bivalve tissue metal concentration requires rigorous collection of external physical and chemical variables including metal concentrations in sediment (metals are partitioned into bioavailable fractions, e.g. Fe-ox bound metals, free-ion concentrations), water chemistry (i.e. $[Ca^{2+}]$, pH, DOC etc.), temperature and food availability and quality. These additional variables are critical in order to derive useful relationships between metal concentrations in the environment and tissue metal concentrations.
4. *Level of action for tool?* Molluscs may be used in the first steps of a monitoring program in order to assess to extent of metal contamination in the aquatic environment. In more

detailed information stages, molluscs may be used to investigate specific sources of bioavailable metals, improvements to waste-water treatment and effectiveness of remedial actions.

5. *Best use of the tool in the overall monitoring strategy of the mining industry?* For comprehensive biomonitoring, molluscs can be used in conjunction with several other organisms (e.g. invertebrates, fish, plants) to monitor metals and their effects in the aquatic environment. In this way, information on the "biological consequences" of mining operations can be obtained, that is not available from monitoring sediment and water only.

10. GLOSSARY

Bioaccumulation - a net retention of a contaminant by an organism over time, that is the influx of a contaminant exceeds its efflux. Bioaccumulation may not be associated with an increase in an organism's contaminant concentration, which may be caused by a loss in body weight.

Bioavailability - the portion of a contaminant in the environment whose presence in or mobilization from water, sediment, or diet gives rise to measurable accumulation by an organism.

Bioconcentration - the amount of a metal accumulated by an organism in relation to its concentration in the abiotic environment.

Biomagnification - the accumulation of a substance from food organisms leading to a higher concentration in organisms of a higher trophic level.

Biomarker - a biological response to the exposure to an environmental chemical. This response at the below-individual level, is not necessarily detected at the whole organism level.

Body Burden (=Content)- the total amount of contaminant found in an organism. Usually determined by multiplying the body/tissue metal concentration by the weight of the body/tissues.

Content (=Body burden)- the total amount of contaminant found in an organism. Usually determined by multiplying the body/tissue metal concentration by the weight of the body/tissues.

Endemic - species is restricted to a single geographical area.

Exposure - the amount of contaminant an organism is subjected to in the environment. Exposure is a function of contaminant concentration and bioavailability.

Dioecious - an individual possessing either a male or female reproductive system; the reproductive systems are contained in separate individuals.

Dose - is the concentration of a contaminant in an organism's tissues and is the starting point for adverse effects. Contaminant toxicity is derived from Dose-Response relationships.

Gonochoristic - two sexes; males and females in the same population reproducing by cross-fertilization.

Hermaphroditic - an individual that has both male and female reproductive organs.

Iteroparous - reproducing repeatedly at multiple times during the life of an organism before it dies.

K_d - partition coefficient of a trace element on particles.

Metallothionein (MT) - cysteine-rich, heat-stable, low-molecular weight protein which chelates metals such as Cd, Cu and Zn. Functions attributed to MT include detoxification, and regulation of these metals.

Metal Species - the molecular representation of a physiochemical form of an element.

Monoecious - having both the male and female reproductive systems in one individual.

Ovoviviparous - females that lay eggs which hatch within the body of the female and are released as free-living offspring.

Pandemic species - species occurs throughout the whole country or world.

Pseudofeces - masses of mucous-bound particles rejected from the labial palps of bivalve molluscs before ingestion and released into the mantle cavity to be carried out the siphon on water currents induced by valve clapping (i.e., rapid valve adduction).

Semelparous - breeding only once during life history; one mass reproductive effort.

Steady-state - when used in the context of toxicokinetic studies, refers to the condition in which organism uptake and excretion rates are balanced and further net bioaccumulation does not occur.

Toxicity - capacity of a chemical to cause injury to a living organism. This is usually defined with reference to:

a. the species and its life stage,

b. the dose of the chemical,

c. distribution of dose in time (acute dose, chronic dose),

d. type and severity of injury (lethal response, sub-lethal response e.g., immunotoxicity, genotoxicity, cytoxicity, avoidance),

e. time needed to produce injury (acute, chronic).

Zoogeographic Region - Geographical region identified by a group of species that do not occur anywhere else.

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PART II. CRITICAL EVALUATION OF BIVALVE MOLLUSCS AS A BIOMONITORING TOOL FOR THE MINING INDUSTRY IN CANADA - Michael H. Salazar, Applied Biomonitoring

EXECUTIVE SUMMARY

A critical evaluation was made of bivalve molluscs as a biomonitoring tool for the mining industry in Canada. This critique was based on the technical evaluation conducted by Stewart and Malley (previous chapters), experience as a developer and a practitioner of the methodology, discussions with other scientists and practitioners, and a critical review of the scientific literature. Only literature available in the author's database was used in the review, no actual literature search was conducted. As instructed by Natural Resources Canada, this critical evaluation was conducted independently and without influence from the authors of previous chapters. It was concluded, contrary to the technical evaluation provided by Stewart and Malley, that bivalves can and should be used as indicators of effects associated with metals from mining effluents by measuring sensitive sublethal endpoints like growth and reproduction in addition to their use as indicators of exposure by measuring accumulation of metals in their tissues. Furthermore, it is this synoptic characterization of exposure, dose, and response the makes the use of bivalves such a potentially powerful monitoring tool. It was also determined that transplanting caged bivalves facilitates measuring endpoints like bioaccumulation and growth and, therefore, makes this tool more flexible when used for manipulative field testing and hypothesis testing. Examples are provided to demonstrate how an exposure, dose, response triad, using bivalves can reduce the uncertainty associated with traditional ecological risk assessments. Examples are also provided to give a perspective on the number of locations where this approach has been used successfully and the number of different effects endpoints that have been successfully measured. Finally, it is concluded that an effects-based bivalve mollusc biomonitoring (BMB) program is cost-effective, scientifically defensible, and technically feasible for monitoring mining effluents. It is recommended that a pilot study be conducted at a select number of mill sites in conjunction with other monitoring approaches to validate the method and compare results.

This report represents a critical evaluation of the evaluation from the perspective of a practitioner of the method. It is organized into the following sections:

Section 1: ***Introduction***

Section 2: ***Conceptual Framework for Effects-Based Bivalve Mollusc Biomonitoring***

(BMB)

Section 3: ***Examples of Using Caged Bivalves as a Monitoring Tool to Support an Integrated Risk Assessment Strategy***

Section 4: ***Technical Aspects***

Section 5: ***Practical Aspects***

Section 6: ***Research Needs***

Section 7: ***Recommendations***

1. INTRODUCTION

The technical evaluation of bivalve molluscs as a biomonitoring tool for the mining industry in Canada (Stewart and Malley, previous chapters) provides an excellent overview of the subject. However, there is one major philosophical difference between their suggested approach and current thinking among many practitioners that has a significant bearing on the conceptual framework, the experimental design, and implementation of a monitoring program using bivalve molluscs. Stewart and Malley conclude that bivalves can only be used as indicators of exposure by monitoring chemical concentrations in bivalve tissues because effects measurements are not adequately developed. They have advocated an exposure-based monitoring program that would be inadequate to address the real issue of potentially adverse biological effects. The current chapter (Chapter 8) advocates an effects-based monitoring program that includes elements of exposure, dose, and response. The working premise here is that bivalves can and should be used as indicators of effects. Moreover, their real utility for the mining industry is the combined measurement of exposure and effects endpoints in the same organism at the same time. This approach is consistent with the standard risk assessment format and is directly applicable to monitoring mining effluents in Canada. A preponderance of evidence approach will be used to

demonstrate that using bivalves as a dual monitors (exposure and effects) is cost effective, scientifically defensible, and technically feasible.

The Metal Mine Liquid Effluent Regulations (MMLER) of Environment Canada only require monitoring liquid effluent quality and not receiving waters. Monitoring receiving environments is usually required by Provincial and Territorial programs (AQUAMIN 1996). One of the key recommendations of AQUAMIN report was to develop site-specific environmental effects monitoring (EEM) programs. It was further recommended that the monitoring programs be consistent with a national monitoring program in terms of study objectives, approaches and methods. The scope of AQUAMIN clearly includes biological effects on individuals, populations, and communities. Nevertheless, the AQUAMIN report includes both biological and non-biological changes associated with mining effluents in their definition of effect. One mandate of the Aquatic Effects Technology Evaluation (AETE) is to do a field and technical evaluation of molluscs as a biomonitoring tool for the mining industry in Canada. Metals included in a national monitoring program for mining effluents would probably include those regulated under the current MMLER such as arsenic (As), copper (Cu), lead (Pb), nickel (Ni), zinc, and radium^{226} (Ra^{226}). These chemicals have been measured routinely in marine environments for over 20 years and chemical biomonitoring is becoming more common in freshwater systems. It is only within the last 10 years that effects monitoring has been most fully developed for routine use in marine environmental monitoring programs. More recently, these approaches have been adapted for freshwater systems. In itself, this does not mean that effects monitoring is not well established.

Recently, a meeting was held in Dartmouth, Nova Scotia to evaluate biomonitoring methodologies as an alternative to the adult fish surveys for the pulp and paper mill industry. The following criteria were used to rank the reliability of the various approaches: (1) linkages to toxicity and community responses; (2) scientifically defensible; (3) cost-effective; (4) provide different endpoints; and (5) generate interpretable results. Although the expert working group is still in the process of evaluating the proposed methodologies against these various criteria, monitoring natural populations and/or caged bivalves appear to satisfy most of these criteria. Based on the presentations and discussions at the Dartmouth meeting, mesocosm and bivalve methodologies for exposure and effects monitoring were considered to be the most well-developed for possible implementation in Cycle II EEM for the pulp and paper industry as

alternatives to the adult fish survey when fish collection is problematic. As an example, during Cycle I of EEM for pulp and paper mills in British Columbia condition index was measured as an indicator of effects in either oysters (*Crassostrea gigas*) or mussels (*Mytilus trossulus*) at five different mills (Mike Hagen, personal communication, March 1997). Previously, dioxins and furans were measured in oyster tissues on a yearly basis between 1989 and 1993 to monitor exposure and document the decline in chemical output at one British Columbia mill (Environment Canada EEM web site). The point to remember is that bivalves have already been used to monitor exposure and effects associated with another industry in Canada.

The purpose of this chapter is to critically evaluate the usefulness of bivalve molluscs as a biomonitoring tool based on the Stewart and Malley technical evaluation, experience as a developer and a practitioner of the methodology, discussions with other scientists and practitioners, and a critical review of the scientific literature. Their conceptual framework for bivalve mollusc biomonitoring will be modified where necessary to remain consistent with the goals of the proposed goals for EEM of mining effluents. Recommendations will also be made on the need for (and, if relevant, the scope of) field evaluation of molluscs at mine sites in Canada.

The proposed approach will be evaluated for cost effectiveness as a monitoring tool compared with other environmental effects monitoring techniques against a set of criteria including:

- Relative sensitivity (dose response)
- Ecological relevance (predictive capability of effects, warning systems)
- Availability of individuals and species including natural populations and laboratory cultures
- Validation (peer-reviewed, field and laboratory)
- Site specificity
- Applicability
- Repeatability
- Chemical specificity
- Practical limitations for carrying out field work
- Commercial availability

- Response time (exposure, rapidity)
- Interpretability (statistical evaluation, confidence, clarity)
- Variability (temporal, spatial representativeness, sampling effort and variance

2. CONCEPTUAL FRAMEWORK FOR EFFECTS-BASED BIVALVE MOLLUSC BIOMONITORING (BMB)

It is important to develop a conceptual framework before any bivalve mollusc biomonitoring (BMB) program begins. The conceptual framework for evaluating mining effluents should include both a bioaccumulation and bioeffects component. It is the concern about the effects of metals from mining effluents and not their mere presence in various environmental compartments that is the basis of ecological risk assessments. From an ecotoxicological perspective, the relationships between external exposure from environmental media, accumulation in organisms, and the associated adverse biological effects is the key to assessing ecological risk (Couillard, 1996; Salazar and Salazar, in review). Field bioassays bridge the gap between laboratory bioassays and traditional field monitoring of benthic community assemblages by combining some elements of experimental control commonly associated with laboratory bioassays and other elements of environmental realism commonly associated with field monitoring (Salazar and Salazar, 1997a,b).

During the 1980's when marine mussel monitoring was being developed as a strategic part of many environmental monitoring programs, bivalves were primarily used as a chemical monitoring tool. Many monitoring programs were developed and implemented without a clear definition of purpose or ultimate use of the data. White (1984) coined the term "mussel madness" to reflect improperly formulated objectives and conceptual frameworks with respect to some bivalve monitoring programs. Carpenter and Huggett (1984) used the term "mindless monitoring" in reference to chemical monitoring programs that had no obvious hypotheses testing or decision matrix included as a framework for analyzing and utilizing the monitoring results. Because of the historical use of biomonitoring many still think of pollution in terms of chemicals but the ultimate concern is actually adverse biological effects (Addison, 1996). Waldock et al. (1996) have emphasized the importance of distinguishing the use of bioindicators to quantify exposure and bioindicators to quantify adverse biological effects. It would be easy to continue using traditional monitoring methods that are commonly accepted but laboratory bioassays are not always good predictors of adverse effects in nature (White and Champ, 1983) and the environmental significance of benthic community changes are difficult to interpret (Diamond et al., 1994;

Barbour et al., 1996).

2.1. Risk assessment paradigm

Bivalves can and should be used both as indicators of effects and indicators of exposure. They provide a unique opportunity to measure bioaccumulation and bioeffects in the same organism at the same time as part of routine EEM. Regulatory agencies in Canada, the United States (U.S.), and other countries use a risk assessment paradigm that emphasizes two major elements, characterization of exposure and characterization of effects. The concept is that in order to properly assess ecological risk, one must understand the various pathways of chemical exposure and associate them with adverse biological effects. Traditional approaches using laboratory bioassays and community assemblages have a relatively high degree of uncertainty because the dose has not been adequately characterized. BMB can help to reduce some of that uncertainty through direct, integrated measurements to characterize exposure and effects.

2.2. Exposure-dose-response Triad

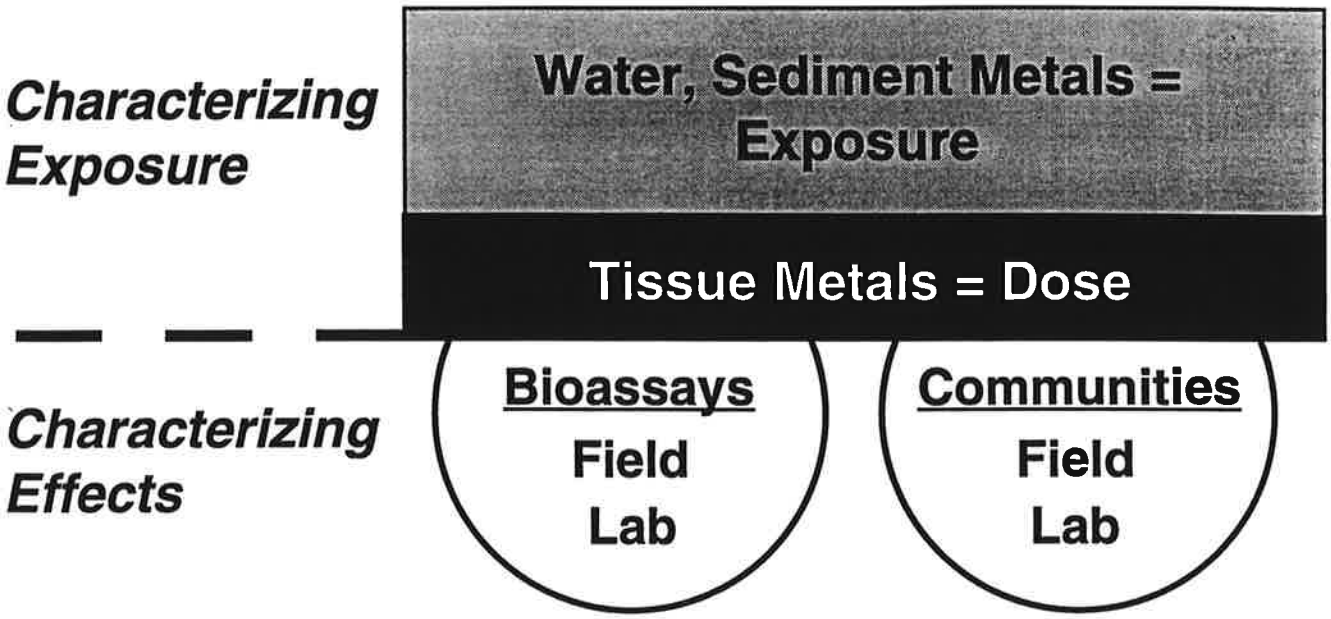
A number of investigators have emphasized the importance of an integrated assessment strategy (Chapman, 1996; Hall, 1996; Diamond et al., 1994). An integrated sediment quality triad (SQT) has been developed for assessing sediment quality (Long and Chapman, 1985). It is surprising that they did not include bioaccumulation as a major element in their triad since it was included in their original discussion regarding the importance of laboratory bioassays in assessing sediment quality (Chapman and Long, 1983). Subsequently, Chapman et al. (1992) have discussed integrated sediment assessments in terms of any group of two or more of the following components: 1) toxicity tests; 2) sediment chemistry; 3) tissue chemistry; 4) pathology; and 5) community structure. There are three problems with this approach. First, including any two elements for the purposes of integration does not guarantee characterizing exposure and effects to be consistent with the risk assessment paradigm. For example, the integration of pathology and community structure would not provide a very good characterization of exposure. Second, even laboratory bioassays do not provide a direct measurement of exposure. Since only effects

endpoints are measured in most laboratory bioassays, exposure is only inferred by the observed effects. This increases the uncertainty of the assessment because the effects endpoints measured in the laboratory bioassay are being used to characterize both exposure and effects, and exposure is not measured directly. It should also be mentioned that metal speciation must be considered when characterizing exposure since speciation has been shown to affect bioaccumulation and bioeffects. Third, the *in-situ* studies they describe as part of the SQT preferentially include resident community alterations but may also include measures of resident organism pathology and bioaccumulation/metabolism. The main drawback of these field approaches is that they are all descriptive. The *in-situ* field bioassays advocated as part of the EDR triad is a manipulative field test that has more of the characteristics of a laboratory bioassay in terms of an experiment than the descriptive information associated with other *in-situ* studies advocated by Chapman et al. (1992). These approaches do not guarantee adequate characterizations of exposure or effects. Chapman (1995) has also emphasized the importance of measuring exposure and dose as a key issue in ecotoxicological approaches. Increasing emphasis is being placed on the use of tissue burdens associated with adverse biological effects and a new meaning to the term dose-response. Increased emphasis is also being placed on the use of manipulative field bioassays (Parrish et al., 1988; Green et al., 1985).

One important caveat to note here is that many laboratory experiments are still routinely conducted at unrealistically high water concentrations that would never occur in nature. As examples, the test concentrations used by Hemelraad et al. (1986) and by Tessier et al. (1994) greatly exceed the dissolved metal concentrations that have been measured, even in the most contaminated receiving waters. Furthermore, since the bioaccumulation of metals is dependent on chemical speciation, uptake routes and mechanisms, intracellular compartmentalization, and other aspects of metal equilibrium within organisms, it is important to understand these processes to accurately predict ecological risk associated with measurement endpoints. Unfortunately, these processes are not well-understood and the necessary measurements are seldom made in either laboratory or field exposures.

Using the risk assessment framework paradigm as a framework, and the SQT from Chapman and Long (1983) as a template, Salazar and Salazar (1995, 1996, in review) developed an *exposure-dose-response* (EDR) triad (Figure 15). The EDR triad emphasizes the importance

Exposure-Dose-Response Triad



Advantages

- Preponderance of evidence
- Laboratory & field
- Individuals & communities
- Bioassays & field monitoring
- Manipulative experiments

Applications

- Lab bioassay validation
- Bioaccumulation calibration
- Inputs to models
- Status & Trends monitoring
- Ecological risk assessment

Figure 15. Exposure-dose-response triad showing the link between toxic metals in the environment, in tissues, and associated adverse biological effects. The triad also shows how toxic metals in tissues establish a link between environment and organism.

of monitoring chemicals in external media, chemicals in tissues, and biological effects to support an integrated risk assessment strategy. The advantages and potential applications of this approach are shown in Figure 15. This effects-based approach, emphasizing measurements of exposure and dose, is the type of approach that should be used for EEM of mining effluents in Canada.

2.3. Bivalve mollusc biomonitoring (BMB) model

A generic conceptual monitoring model, originally developed for marine bivalve biomonitoring (Salazar and Salazar, 1996), is potentially applicable to most monitoring programs, including mining effluents (Figure 16). This conceptual model illustrates the interaction between natural factors and chemical exposure in affecting bioaccumulation and growth, as well as the impacts of man-made factors such as habitat alteration and destruction. This model demonstrates the importance of measuring factors that affect bioaccumulation and growth. These factors act in concert to modify bivalve growth, bioaccumulation, and survival. The key to calibrating the bivalve bioindicator is separating the effects of natural and biological factors from the effects of metals. The measurements recommended here facilitate interpreting the environmental significance of trace metal concentrations in various environmental compartments and their potentially adverse biological effects.

2.4. Transplants vs indigenous

The rapid development of transplant methodologies and corresponding clinical measurements in laboratory bioassays has led to the development of field bioassays with caged bivalves as a potentially powerful tool for environmental monitoring. This approach is a significant refinement and advancement of methodology over traditional monitoring because it includes elements of manipulative experimentation in the field that lends itself to hypothesis testing. This is another important distinction between traditional field monitoring that only includes observational data from enumeration studies of benthic community studies. Caging facilitates the quantification of exposure and effects under natural conditions. The exposure and effects measurements are made synoptically in the same organism, providing a direct way to

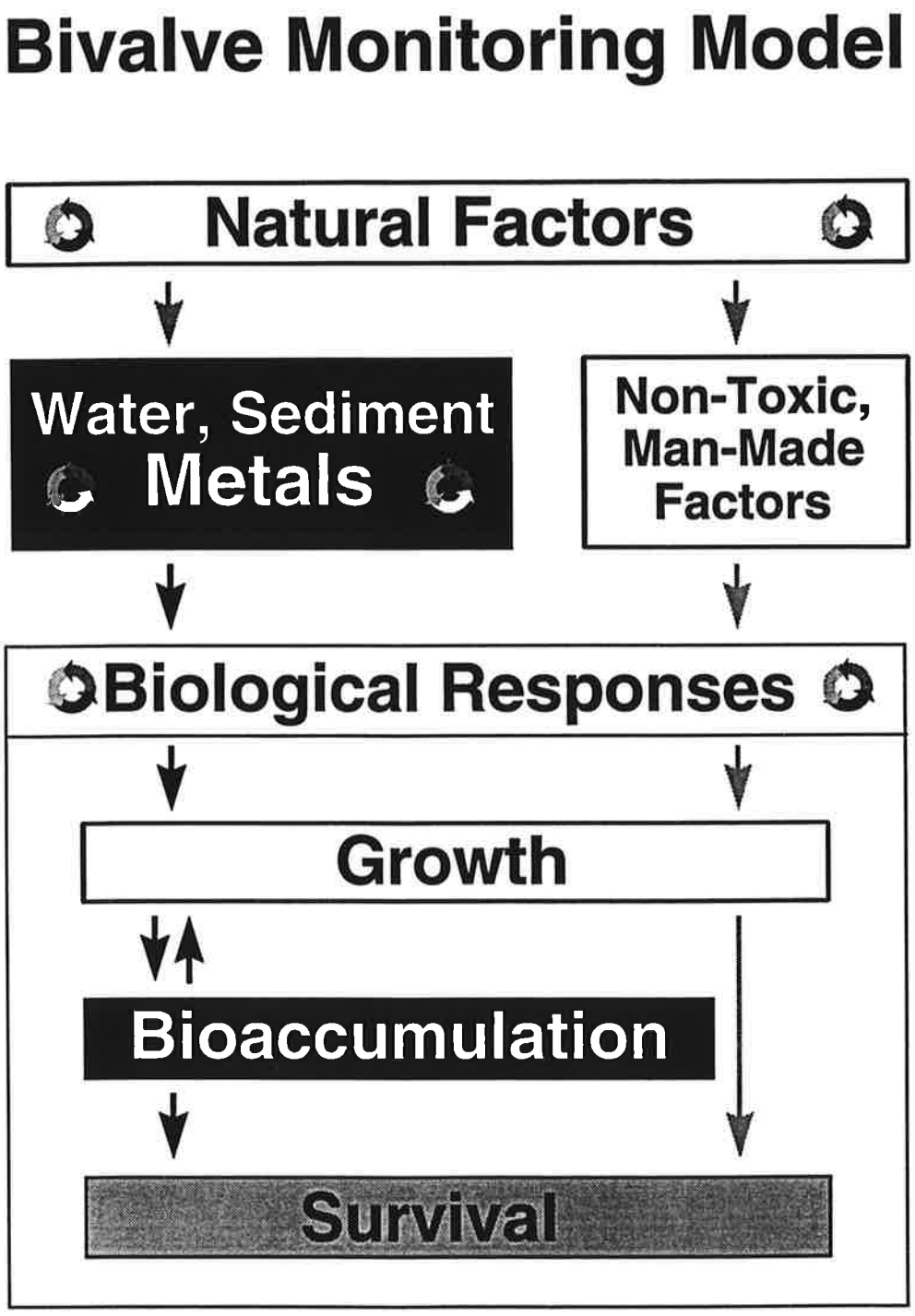


Figure 16. Mussel monitoring model showing the influence of natural factors, toxic metals, and non-toxic man-made factors on biological responses. Natural factors, toxic metal concentrations, and biological responses can also be cyclical. Double arrows are shown between bioaccumulation and growth to indicate interactions.

evaluate exposure conditions. Caging studies are preferred over monitoring resident populations because the measurements are direct, the measurements are integrated, and they represent site-specific exposures and site-specific effects. The primary advantage to conducting transplant studies with caged bivalves over monitoring resident populations is the increased experimental control while maintaining a high level of environmental realism.

A distinction should be made between transplanting and caging bivalves. Transplanting is a methodology where bivalves are taken from an uncontaminated site and transplanted to a contaminated site. Caging is a form of transplanting where the animals are actually held in some form of discrete containment system such as a box enveloped in mesh. Other forms of confinement such as leashes and corrals can be used in transplant studies, depending on the application. These approaches also reduce bivalve mobility but are often more time consuming and difficult to accomplish. Transplantation of organisms constitutes an avenue to evaluate trace metal contamination and induced-stress at a given location while sparing possibly metal-stressed populations at this location. Relocated organisms may originate from an abundant population nearby, or from an aquaculture facility (Couillard, 1996).

Versteeg et al., 1988 offer the following with regard to field utilization of clinical measures for the assessment of xenobiotic stress in aquatic organisms and provide two case studies for pollution episodes involving acid rain and heavy metals; *"Histological, biochemical, and physiological measures of xenobiotic effects on aquatic organisms have been utilized extensively in laboratory exposures to document toxic effects. In spite of the ability of these measures of stress to integrate the effects of multiple stressors, and their utility to instantaneously assess the "health" of a population, to date few studies have used these methods in situ to document adverse effects of environmental stressors. This is not due to the lack of information on appropriate clinical methods. Sufficient laboratory research has developed clinical measures to the extent that they will be useful in field situations. A portion of the lack of field use of these methods is the lack of understanding of the utility and knowledge in the flexibility of these diagnostic tools."*

There are several important points from this citation that are relevant to the present chapter and the critical evaluation of BMB as an assessment tool for mining industry effluents: (1) This citation appeared in 1988 and suggests that these clinical measurements were widely

available almost 10 years ago; (2) Although the authors suggest that the lack of field use is attributable to a lack of understanding of their utility and flexibility, it seems more likely that most of the biochemical measurements to which they refer are actually indicators of exposure and provide little more information than measuring tissue chemistry; (3) the physiological measurements to which they refer and indicators of effects but many have not been linked to effects at the population level.

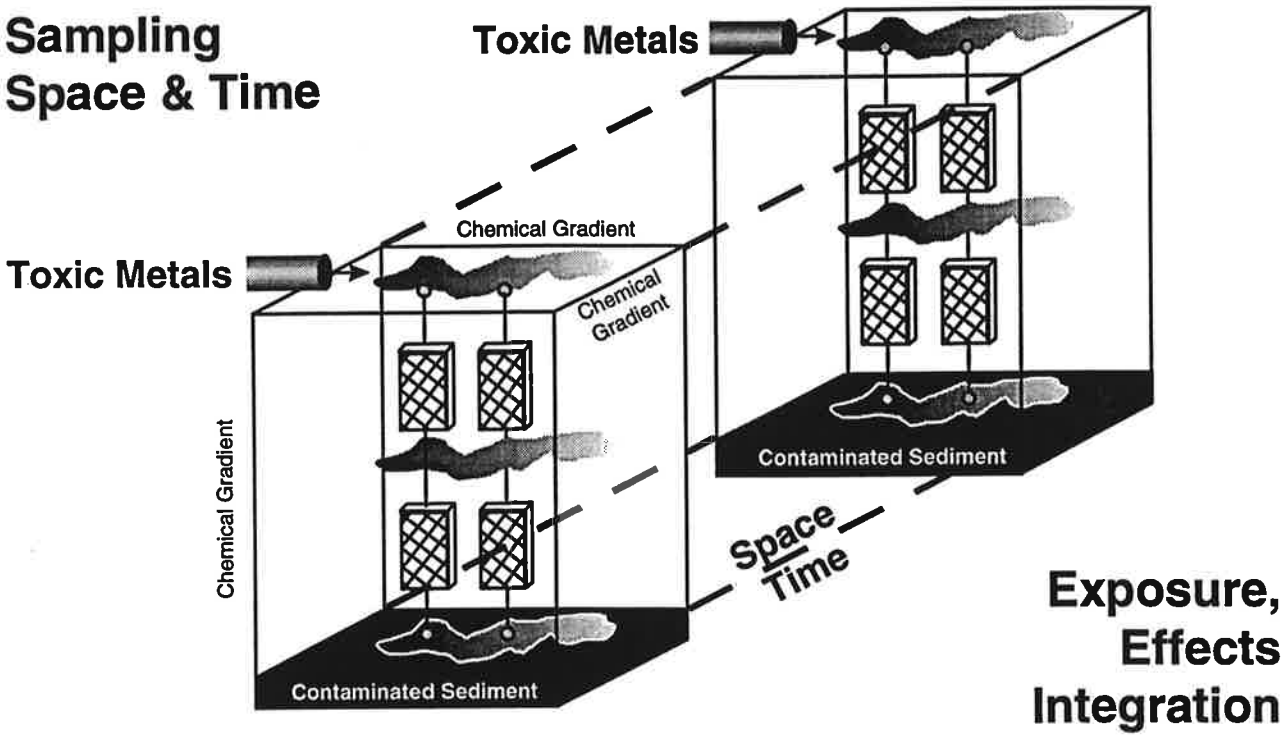
The advantages and disadvantages of using bivalves as *in-situ* test organisms are shown in Table 25. A major advantage to using bivalves over other animal groups is that they will accumulate chemicals in their tissues at concentrations which are orders of magnitude above those found in the environment. In some cases, the chemical may not be detectable by chemical analysis of environmental media but is still accumulated by the bivalves and detectable in their tissues. Tissue chemistry measurements represent the concentrations of chemicals that are biologically available and integrated over time. Therefore, a single tissue measurement can provide more useful information than thousands of water or sediment samples. Further, bivalves can be easily caged and transplanted to various assessment areas for site-specific evaluations. Caged bivalve monitoring is particularly useful in areas where resident populations are not normally found. Similarly, it is difficult to make repetitive measurements on resident individuals from natural populations over time. Another advantage of using caged bivalves is that similar sizes in addition to similar environmental and genetic history reduces variability in bioaccumulation and bioeffects measurements and thereby increases the discriminating power of the test. Utilizing animals with a known history also facilitates data interpretation and risk estimation.

Caged bivalves permit monitoring individual organisms as well as sampling an almost infinite matrix of space and time because the animals can be strategically situated along physical and chemical (metal) gradients associated with both the water column and sediments (Figure 17). Caged bivalves can be placed along known and/or suspected gradients of chemical contamination in three dimensional space and time. The advantages of this approach is combining the advantages of experimental control from laboratory bioassays (placement in areas of concern where they might not normally be found, defined exposure period, facilitation of measurements) and the environmental realism of traditional field monitoring (experiments are conducted *in-situ*). Even if bioaccumulation in natural populations of bivalves were to be measured to characterize

Table 25. Advantages and disadvantages of the *in-situ* field bioassay by category: transplants, bivalves, bioaccumulation, and growth.

	Transplants	Bivalves	Bioaccumulation	Growth — Whole Animal/Tissue
Advantages	Experimental control Environmental realism Defined exposure period Infinite sampling matrix Repetitive, non-destructive sampling Monitoring individuals Field validation Exposure system Captive biochemical sampling Hypothesis testing Low maintenance Complex mixtures	Integrate bioavailable contaminants Bioconcentrate contaminants Easy to collect, cage, measure Large database from field monitoring and lab bioassays Survive sub-optimal conditions Any biochemical measurements Sedentary No feeding required	Concentrations above ambient Integration of contaminants, natural factors, man-made non-toxics Equivalent to 1000's of water samples Link between exposure and response Link between lab and field Link between bioassays and community structure Biologically available chemicals Uptake from food and water	Integration of internal biological processes Environmentally significant response Link to population effects Quantifiable dose-response Related to environmental exposures Repetitive, non-destructive measurements Easy for the public to understand No special equipment No specialized training More sensitive than survival Simple, cost-effective measurements
Disadvantages	Effects of transplanting Loss of cages from acts of nature, inadvertent capture by moving vessels, vandalism Cost of collection, sorting, deployment	Not found in all areas May not be representative of assessment area May not be the most sensitive species May not directly assess community effects Conservation status	Affected by chemical and natural factors Not all contaminants are accumulated equally Some contaminants may be purged May not always accurately represent effective dose	Affected by chemical and natural factors May not be the most sensitive bioeffect Tissue and shell growth occur at different rates and are affected by different factors May not directly assess community effects

Caged Bivalve Monitoring



Advantages

- Control & realism
- Outside natural populations
- Defined exposure period
- Physical or chemical gradients
- Manipulative experiments

Applications

- Site-specific differences
- Temporal/spatial variability
- Short & long-term trends
- Source identification
- Dose-response estimates

Figure 17. Sampling space and time with bivalve transplants along gradients of toxic metal contamination. Two suspected sources, two sites, two depths, and two sampling intervals are shown.

exposure, it would not be clear if the tissue chemistry would represent the last day, week, month, or year. Since the exposure period is clearly defined in a caged bivalve monitoring program, any differences in tissue chemistry between the beginning and end of the test can be attributed to bioavailable metals that have been accumulated during the course of that exposure period. Therefore, there are two comparisons that can be made with respect to metal tissue burdens: (1) beginning and end-of-test; and (2) different sites. It should be added however, that metal concentrations can also change due to changes in tissue weight associated with somatic growth or the addition of reproductive tissue. This is another reason for measuring growth and reproduction endpoints; to calibrate bioaccumulation with changes in tissue weight.

A considerable amount of freshwater BMB has been conducted in both Canada and the U.S. Table 26 provides examples by country, species, chemical and endpoints measured with corresponding references. It is apparent that the vast majority of studies in Canada used *Elliptio complanata* and the vast majority of studies in the U.S. used *Corbicula fluminea*. More importantly however, it should be noticed that most monitoring has only included a characterization of exposure by measuring chemicals in bivalve tissues. This should not be interpreted as evidence that effects-based monitoring is not possible or practical, but that the technology has not been completely transferred to freshwater systems. Space and time preclude including a similar list for marine species, but it would be about an order of magnitude larger for both studies measuring bioaccumulation and those measuring bioaccumulation and effects. As an example, Table 27 includes only those studies measuring bioaccumulation and bioeffects in caged bivalves where the author was a principal investigator or participant..

2.5. Characterizing exposure

Bioaccumulation has been identified as a strategic link between the external environment and the organism (Laughlin, 1986). The concept of exposure (external exposure) versus internal exposure (dose) is an important one in the risk assessment process because all toxic metals in the surrounding media are not always biologically available to organisms. It has been established that toxicity is caused by the dose of toxic chemicals at the receptor site. Therefore, each measurement of accumulated chemicals could be an integral part of the exposure assessment.

Table 26. Examples of freshwater bivalve mollusc biomonitoring, mostly exposure assessments, but some effects measurements.

SITE	SPECIES	CHEMICAL	ENDPOINTS	REFERENCE
Argentina	Corbicula fluminea	PCBs, organics	bioaccumulation	Colombo et al, 1995
Australia	Westralunio carteri	organochlorines	bioaccumulation	Storey & Edward, 1989
Australia	Anadara trapezium	Cd, Cu, Pb, Zn	bioaccumulation	Scanes, 1993
Brazil	Anodontites trapesialis	organochlorines	bioaccumulation	Lopes et al, 1992
Canada	Elliptio complanata	chlorophenol	bioaccumulation	Metcalfe & Hayton, 1989
Canada	Elliptio complanata	PCBs, DDT	bioaccumulation	Creese et al., 1986
Canada	Elliptio complanata	PCBs, DDT	bioaccumulation	Curry, 1977
Canada	Anodonta grandis		growth	Hanson et al, 1988
Canada	Elliptio complanata	dioxins, furans	bioaccumulation	Ontario MEE, 1996
Canada	Elliptio complanata	PAHs	bioaccumulation	Kauss & Hamdy, 1985
Canada	Elliptio complanata	Cu, Zn, Mn, Cd	bioaccumulation + growth	Hinch & Green, 1989
Canada	Lampsilis radiata		growth	Hinch et al, 1986
Canada	Elliptio complanata	organochlorines	bioaccumulation	Ontario MEE, 1996
Canada	Elliptio complanata	organochlorines	bioaccumulation	Richman, 1992
Canada	Elliptio complanata	organochlorines	bioaccumulation	Hayton & Hollinger, 1989
Canada	Elliptio complanata		amino acids + condition index	Day et al, 1990
Canada	Lampsilis radiata, Elliptio complanata	organics	bioaccumulation + length	Muncaster et al., 1990
Canada	Elliptio complanata	organochlorines	bioaccumulation	Kauss et al, 1981
Canada	Pyganodon grandis	Cd, Cu, Zn	bioaccumulation + metallothionein	Couillard et al, 1995

SITE	SPECIES	CHEMICAL	ENDPOINTS	REFERENCE
Canada	Pyganodon grandis	MeHg	bioaccumulation	Malley et al, 1996
Canada	Elliptio complanata	Cu, Zn, Mn, Cd	bioaccumulation + growth	Hinch & Green, 1988
Canada	Lampsilis radiata		growth	Hinch et al, 1986
Canada	Pyganodon grandis	Cd	bioaccumulation	Malley et al, 1989
Canada	Dreissena polymorpha		settlement + growth	Martel, 1993
Canada	Elliptio complanata	organochlorines	bioaccumulation	Kauss & Hamdy, 1985
Finland	Anodonta piscinalis	chlorophenol	bioaccumulation	Herve, 1991
Finland	Anodonta piscinalis	chlorophenol	bioaccumulation	Herve et al., 1988
Finland	Anodonta piscinalis	chlorophenol	bioaccumulation	Herve et al, 1996
France	Dreissena polymorpha	Cd, Cr, Cu, Sn, Pb	bioaccumulation + condition index	Mersch & Pihan, 1993
France	Anodonta cygnea	AOX, EOX	bioaccumulation	Hayer & Pihan, 1996
France	Dreissena polymorpha	Cr, Ni, Pb, Zn	bioaccumulation	Mersch & Johansson, 1993
Hungary	Anodonta cygnea	Cd, Hg	bioaccumulation	V.-Balogh, 1988
Netherlands	Dreissena polymorpha	Cd, Cu, Pb, Zn	bioaccumulation	Kraak et al, 1991
Netherlands	Dreissena polymorpha	Cd	bioaccumulation + histopathology	Bowmer et al, 1991
New Zealand	Hyridella menziesi	resin acids	bioaccumulation	Burgraaf et al, 1996
U.K.	Anodonta anatina, Unio pictorum, Unio tumidus		growth	Negus, 1966
U.S.	Corbicula fluminea		growth, life span, life cycle	McMahon & Williams, 1986
U.S.	Corbicula fluminea	PAH, DDT, DDE	bioaccumulation	Pereira et al, 1996
U.S.	Corbicula fluminea	10 metals	bioaccumulation	Joy et al, 1983
U.S.	Elliptio complanata		growth	Kat, 1982

SITE	SPECIES	CHEMICAL	ENDPOINTS	REFERENCE
U.S.	Corbicula fluminea	organochlorines	bioaccumulation	Prest et al, 1992
U.S.	Sphaerium striatinum	PCBs	bioaccumulation	Rice & White, 1987
U.S.	Elliptio complanata	MeHg, Hg	bioaccumulation + growth + condition	Salazar et al, 1996
U.S.	Corbicula manilensis	organochlorines	bioaccumulation	Hartley & Johnston, 1983
U.S.	Corbicula fluminea	organochlorines	bioaccumulation	Petreas et al, 1992
U.S.	Actinonaias ligamentina, A. pectorosa, Amblema plicata, Fusconaia subrotunda, villosa nebulosa, V. vanuxemensis, Medionidus conradicus		survival & growth	Sheehan et al, 1989
U.S.	Corbicula fluminea	metals	bioaccumulation	Lee et al, 1993
U.S.	Corbicula fluminea	dioxins, furans	bioaccumulation	Hayward et al, 1996
U.S.	Lampsilis radiata		survival + biochemistry	Haag et al, 1993
U.S.	Corbicula fluminea		shell growth, tissue growth, scope for growth, condition index, mortality	Foe & Knight, 1987
U.S.	Corbicula fluminea	PAH	bioaccumulation	ENTRIX, 1993
U.S.	Corbicula fluminea, Anodonta kennerlyi	metals	bioaccumulation + growth	Fishman Environmental Services, 1993
U.S.	Margaritifera falcata	metals	bioaccumulation + growth	Fishman & Johnson, 1989

Table 27. Effects-based caged bivalve mollusc biomonitoring.

Site	Dates	# Sites	Exposure (days)	Species	Initial (mm)	#/Rep	Total #	Deployment Strategy	% Survival
San Diego Bay, CA	1974-75	5	11	*Mytilus galloprovincialis*	35-45	50	1500	1 m below surface	90
Hood Canal, WA	1975-77	4	11	*Mytilus trossulus*	35-45	50	600	1 m below surface	90
San Diego Bay, CA	1987- 90	18	84	*Mytilus galloprovincialis*	10-12 50-70	18 50	2000 200	1 m below surface & 1 m above bottom	93 80
San Diego Bay, CA	1993	6	85	*Mytilus galloprovincialis*	26-29 60-65	88 100	704 800	1 m below surface	90 80
Harbor Island, WA	1991 - 92	14	82	*Mytilus trossulus*	24-30	18, 54; 200, 300	432 3300	1 m above bottom	90 80
Tampa Bay, FL	1993	4	90	*Crassostrea virginica*	17-18	54 60	324 360	1 m below surface	91 98
Delaware Bay, DE	1994	11	30	*Mytilus edulis,* *C. virginica*	40-51 50-100	150 120	1650 1420	Intertidal, 0.3 m above substrate	0 97
Sinclair Inlet, WA	1994	8	90	*Mytilus galloprovincialis*	32-38	300	1500	1 m above bottom	99
Sudbury River, MA	1994	8	90	*Elliptio complanata*	57-63	105	840	Directly on bottom	90
San Diego Bay, CA	1995	6	30	*Mytilus galloprovincialis* *Macoma nasuta*	35-39 30-43	50 50	900 900	1 m above bottom in sediment	95 10
Hylebos Waterway, WA	1995	2	97	*Mytilus galloprovincialis*	44-49	50	300	1 m above bottom	91
Ward Cove, AK	1996a 1996b	7 7	60 60	*Mytilus trossulus* *Mytilus trossulus*	29-37 30-35	100 100	2100 2100	5 m below surface 5 m below surface	95 86
Port Valdez, AK	1997	7	56	*Mytilus trossulus*	31-36	100	2100	70 m depth	Underway

Measurements of environmental media (i.e., water or sediment) only represent external chemical exposure. Bioaccumulation, or uptake of chemicals by the organism, is an internal process, and in itself is not an effect. Bioaccumulation can be used to estimate potential effects at the receptor site because bioaccumulation is related to the external exposure and the metal burden that directly affects the ultimate receptor within the organism. Although measurements of bioaccumulation may not be a perfect estimate of the dose because the concentration at the receptor site may be different than the concentration within all tissues, estimating the dose by measuring accumulated chemicals is a reasonable approximation and another useful tool that can be used to characterize exposure (McCarty and Mackay, 1993).

A major advantage to using bivalves over other animal groups for the exposure characterization is that they will accumulate chemicals of concern in their tissues at concentrations which are orders of magnitude above those found in the environment. In some cases, the chemical may not be detectable by chemical analysis of environmental media but is still accumulated by the bivalves and detectable in their tissues. Tissue chemistry measurements represent the concentrations of chemicals that are biologically available and integrated over time. Therefore, a single tissue measurement can provide more useful information than thousands of water or sediment samples. The relationship between chemicals in the external environment and chemicals in the tissues can be used to determine when biological effects are expected. However, complicating factors associated with biological availability and metal speciation add another level of complexity to the assessment. There is a perception that "regulation" of chemical uptake is interpreted as a weakness of the biomonitor which makes them less useful. On the contrary, it is that very regulation and change in the relationship between exposure and dose that helps define levels of effects. In fact, it is even possible that effects, or at least potential effects can be predicted based on where this relationship changes. Although there is limited data to do this now, continued use of the integrated assessment process by Environment Canada and monitoring conducted by the mining industry in Canada will lead to an extensive database. Through careful planning and execution, predictive models can be developed. As more and more data are entered into the database, the models will become more accurate, and the monitoring programs could become more focused and cost-effective. Four examples of how water chemistry and tissue chemistry can be used to better understand the relationship between exposure, dose, and response

and identify potentially adverse biological effects are provided in the following.

2.5.1. *Mytilus galloprovincialis* and seawater TBT in San Diego Bay

Synoptic chemical measurements of seawater and tissue concentrations of TBT have helped to understand the relationship between exposure, dose, and response in the marine mussel *Mytilus galloprovincialis* in San Diego Bay, California (Salazar and Salazar, 1995, 1996). Figure 18 shows how the relationship between TBT in seawater and bivalve tissues changes dramatically near seawater concentrations of 100 ng TBT/L. It also provided the first estimate of where adverse biological effects might be expected. Statistical analyses of the relationship between tissue TBT and mussel growth rates confirmed that the probable effects threshold was 100 ng TBT/L. The 89 data points in Figure 18 represent means from water samples taken either weekly or every other week during a 12-week exposure period and tissue samples taken at the end of each test during the 3-year study.

Similarities in tissue burdens of TBT associated with adverse biological effects are shown in Figure 19. When similar endpoints are compared across species on a tissue residue basis, it is interesting to note how close the endpoints are, for a variety of species, including bivalves. It is not surprising to see that the mortality endpoint is about an order of magnitude higher, on a tissue residue basis than for the growth endpoint. Recently, tissue burden effect studies on TBT in marine (Meador, 1996; Meador et al.,1996) and freshwater amphipods (Borgmann et al., 1996) have reported almost identical concentrations of approximately 40 μg/g dry wt. as an LC-50 under similar exposure conditions.

2.5.2. *Dreissena polymorpha* and freshwater Cu in laboratory studies

Similar between exposure, dose, and response have been shown for freshwater bivalves as well. *Dreissena polymorpha* exposed to Cu solutions for 48 hours maintained a constant tissue burden up to water concentrations of approximately 35 μg Cu/L (Figure 20) (Kraak et al. 1992, 1993). The tissue concentrations of copper in *D. polymorpha* did not increase until the concentration of Cu in water exceeded this 35 μg/L threshold. The authors suggest that tissue Cu

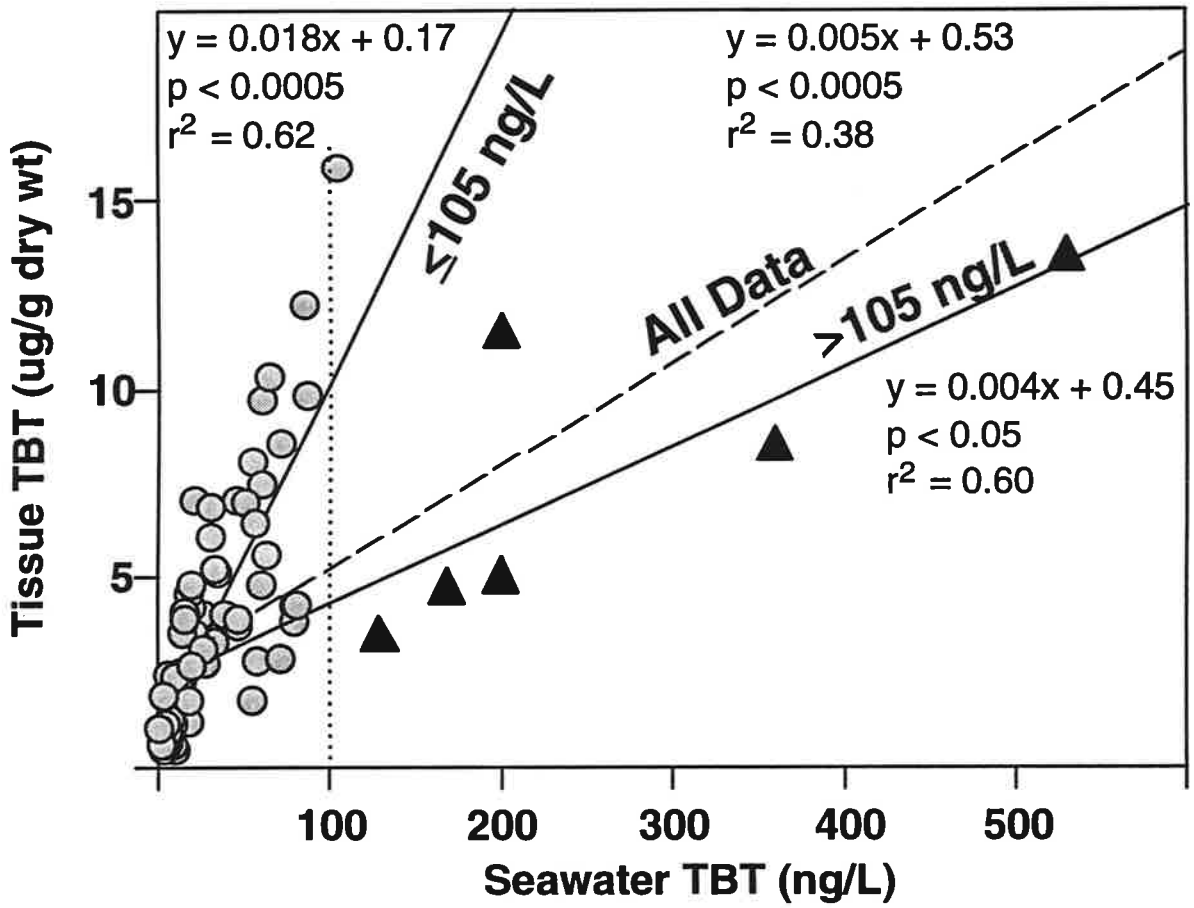


Figure 18. The relationship between seawater TBT and Tissue TBT in *Mytilus galloprovincialis* changes @ 100 ng/L. Redrawn from Salazar and Salazar (1996).

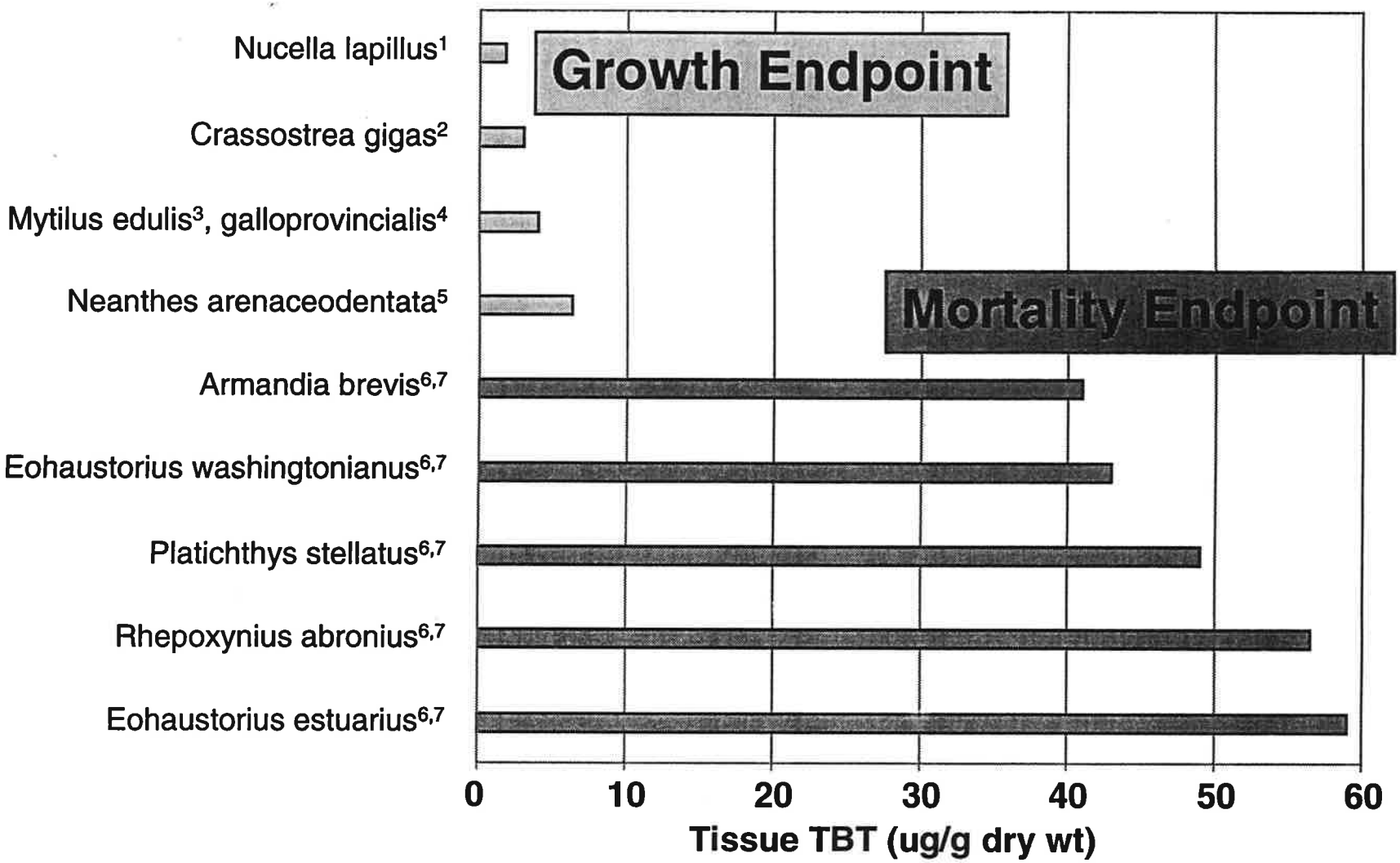


Figure 19. Relationship between tissue concentrations of TBT and adverse effects on growth and survival. 1 = Gibbs and Bryan (1996); 2 = Waldock et al. (1996); 3 = Widdows and Page (1993); 4 = Salazar and Salazar (In review); 5 = Moore et al. (1991); 6 = Meador et al. (1996); 7 = Meador (1997).

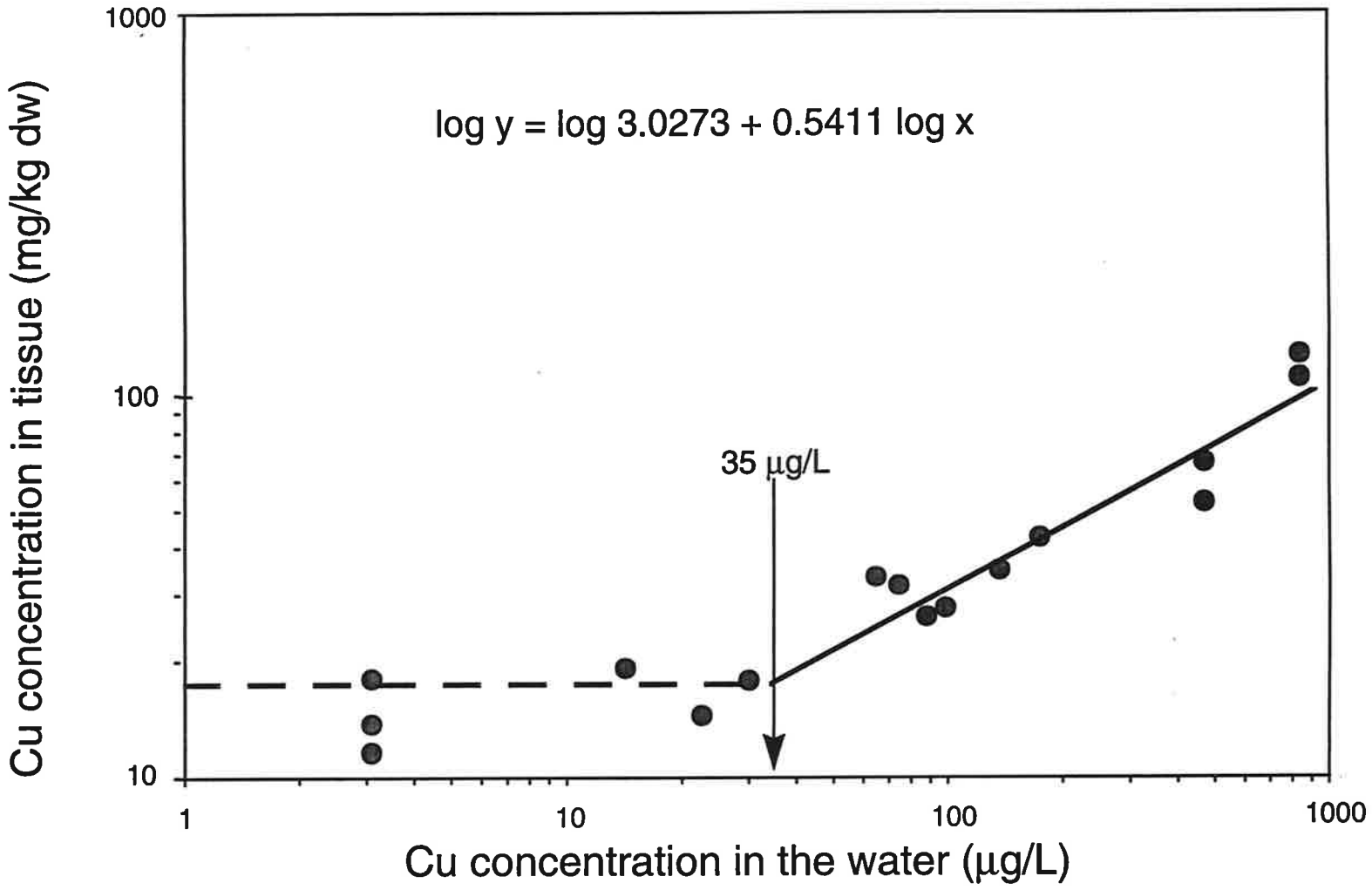


Figure 20. The relationship between freshwater Cu and tissue Cu in *Dreissina polymorpha* changes @ 35 mg/L. Redrawn from Kraak et al. (1993).

concentrations were regulated at the lower aqueous Cu concentrations. The average tissue concentration maintained during exposure to water concentrations <35 μg Cu/L was approximately 20 μg Cu/g dry wt. Since Kraak et al. (1992, 1993) also measured filtration rates and found significant reductions near 35 μg/L, their effects-measurements could also be related to a dose of approximately 20 μg/g dry wt. In other words, the EC-50 or effects concentration for a 50% reduction in filtration rate could have also been expressed as an ED-50 or effects dose concentration in tissues for a 50% reduction in filtration rate. These tissue burdens are within the range already demonstrated as ECs and NOECs for both marine and freshwater bivalves as shown in Tables 28 and 29. This relationship provides an additional comparative tool and another way to express potential effects thresholds in a way that could be more meaningful and more applicable for comparing potential effects of mining effluents. It is much easier to measure tissue burdens, as they would be measured as part of the monitoring program than multiple water samples with an extremely high degree of variability and a high associated cost.

Coincidentally, the largest database with paired dose and effects data for bivalves in the author's database was for Cu. Table 28 shows reported effects concentrations (EC) and no-observable effects concentrations (NOEC) on a tissue residue basis for Cu in marine bivalves. The mean NOEC for these 15 studies was 21 μg/g dw. This is very similar to the copper tissue burden for predicted effects in *D. polymorpha*. The mean probable effects concentration for effects in these 19 studies with the necessary data was 95 μg C/g dry wt. For freshwater bivalves the author's database was very limited but the mean NOEC of 45 μg/g dry wt. and the mean EC of 90 μg/g dry wt. are similar to those predicted for marine bivalves (Table 29). Given the differences in exposure methods (lab, field, microcosm, transplants), species differences (filter feeding water column species vs sediment dwelling deposit-feeders), time of the year, and endpoint (survival, gametogenesis, growth, histopathology) this similarity is surprising. It is even more surprising when considering that it has been suggested that predictions of this type are virtually impossible with essential metals that are regulated, or partially regulated by many species. It is possible then that these relationships may have some utility to make first order approximations of potentially adverse effects across species regardless of whether they are from marine or freshwater habitats.

Table 28. Tissue (μg/g dw) copper effects on marine bivalves.

EC	NOEC	SPECIES	EXPOSURE	REFERENCE	ENDPOINT
16.5 17.0	14.3 16.6	*Mytilus edulis*	Field	Widdows & Johnson, 1988	SFG
21	7	*Mytilus edulis*	Lab	Comber et al., 1988	Inhibited glycine uptake
25	10	*Mytilus edulis*	Lab/Field	Myint & Tyler, 1982	Gametogenesis
26.8 59.0	7.3 16.3	*Mytilus edulis*	Microcosm	Widdows & Johson, 1988	SFG, predicted mortality
27		*Mytilus edulis*	Mesocosm	Viarengo et al, 1988	Metallothionein, protein
40	14	*Mytilus edulis*	Lab	Harrison & Berger, 1982	Enzyme effects
50		*Mytilus edulis*	Lab	Roesijadi, 1980	Enzyme, protein induction
59		*Mytilus edulis*	Lab	Martin, 1979	Lethal
73.2	35.6	*Mytilus edulis*	Lab	Moore et al., 1984	SFG, lysosomal latency
75	25	*Mytilus galloprovincialis*	Field Transplant	Salazar & Salazar, 1995	Growth rate
95	30	*Mytilus edulis*	Lab	Calabrese et al., 1984	Histopathology
100	10	*Mytilus edulis*	Lab	Kaitala, 1988	Survival
100		*Mytilus edulis*	Lab	Viarengo et al., 1980	Biochemical changes
100	10	*Mytilus trossulus*	Field Transplant	Roesijadi et al, 1984	Condition index, proteins
124	6.75	*Mytilus edulis*	Lab	Krishnakumar et al., 1990	Filtration rate, SFG
125	20	*Macoma balthica*	Lab	Kaitala, 1988	Survival
175	20	*Crassostrea virginica*	Field	Frazier et al., 1976	Shell thinning
250	4	*Cerastoderma edule*	Field	Bryan et al., 1987	Survival
300	95	*Mytilus edulis*	Lab	Calabrese et al., 1984	Growth
95	**21**	**MEANS FOR ALL BIVALVES TESTED**			

Table 29. Tissue copper (μg/g dw) effects on freshwater bivalves.

EC	NOEC	SPECIES	EXPOSURE	REFERENCE	ENDPOINT
100	50	*Corbicula fluminea*	Artificial stream	Belanger et al., 1990	Growth: weight, shell length
120	65	*Corbicula fluminea*	Artificial stream	Belanger et al., 1990	Growth: weight, shell length
40	20	*Dreissena polymorpha*	Lab	Kraak et al., 1992	Filtration rate
100	-	*Quadrula quadrula*	Transplant	Foster & Bates, 1978	Mortality
90	**45**	**MEANS FOR ALL BIVALVES TESTED**			

2.5.3 *Mytilus edulis* and tissue Cu/TBT concentrations associated with effects

Widdows and Donkin (1992) pooled data from controlled laboratory and mesocosm studies to develop tissue concentration-physiological response relationships using growth as the effects endpoint. This also facilitated predictions of adverse biological effects associated with environmental concentrations found in water and bivalve tissues. This ecotoxicological approach provides information necessary to establish a database for interpreting tissue residue monitoring programs such as existing Mussel Watch Programs or a monitoring program that might be developed for monitoring mining effluents in Canada. By pooling data from two different studies (Redpath, 1985; Widdows and Johnson, 1988), Widdows and Donkin (1992) predicted that adverse effects would begin to occur near 20 μg Cu /g dry wt. (Figure 21). The other interesting feature to note in Figure 21 is the dramatic decline in growth as tissue concentrations increase above 20 μg Cu/g dry wt. Not only is this dramatic shift similar to the one shown for accumulation of Cu for *Dreissena polymorpha* in Figure 20, but the concentrations where these effects are shown are also similar (20 μg Cu/g dry wt. versus 35 μg Cu/g dry wt.). A similar dramatic decline in scope for growth in *Mytilus edulis* has been demonstrated as tissue burdens increase above about 4 μg TBT/g dry wt. as shown in Figure 22 (Widdows and Page, 1993). This predicted effects level is almost identical to that recently predicted using the pooled data for San Diego Bay field data and end-of-test tissue weights as an estimate of growth (Salazar and Salazar, in review). Collectively, these studies also show that bivalves appear to be changing the way they accumulate chemicals above certain threshold levels and that when these changes occur, they also have an adverse effect on their growth and perhaps other effects endpoints.

2.5.4. *Elliptio complanata* and freshwater sediment Cd in Canadian Lakes

In the absence of synoptic data on sediment concentrations, tissue concentrations, and associated effects (exposure-dose-response triad), a data set with only sediment Cd, tissue Cd, and BCF values (BSAF), were evaluated for changes in the relationship as in the previous two examples. The data shown in Table 30 is from a literature summary for sediment Cd and tissue Cd in freshwater bivalves from Canadian freshwater environments (Metcalfe-Smith et al.,1992).

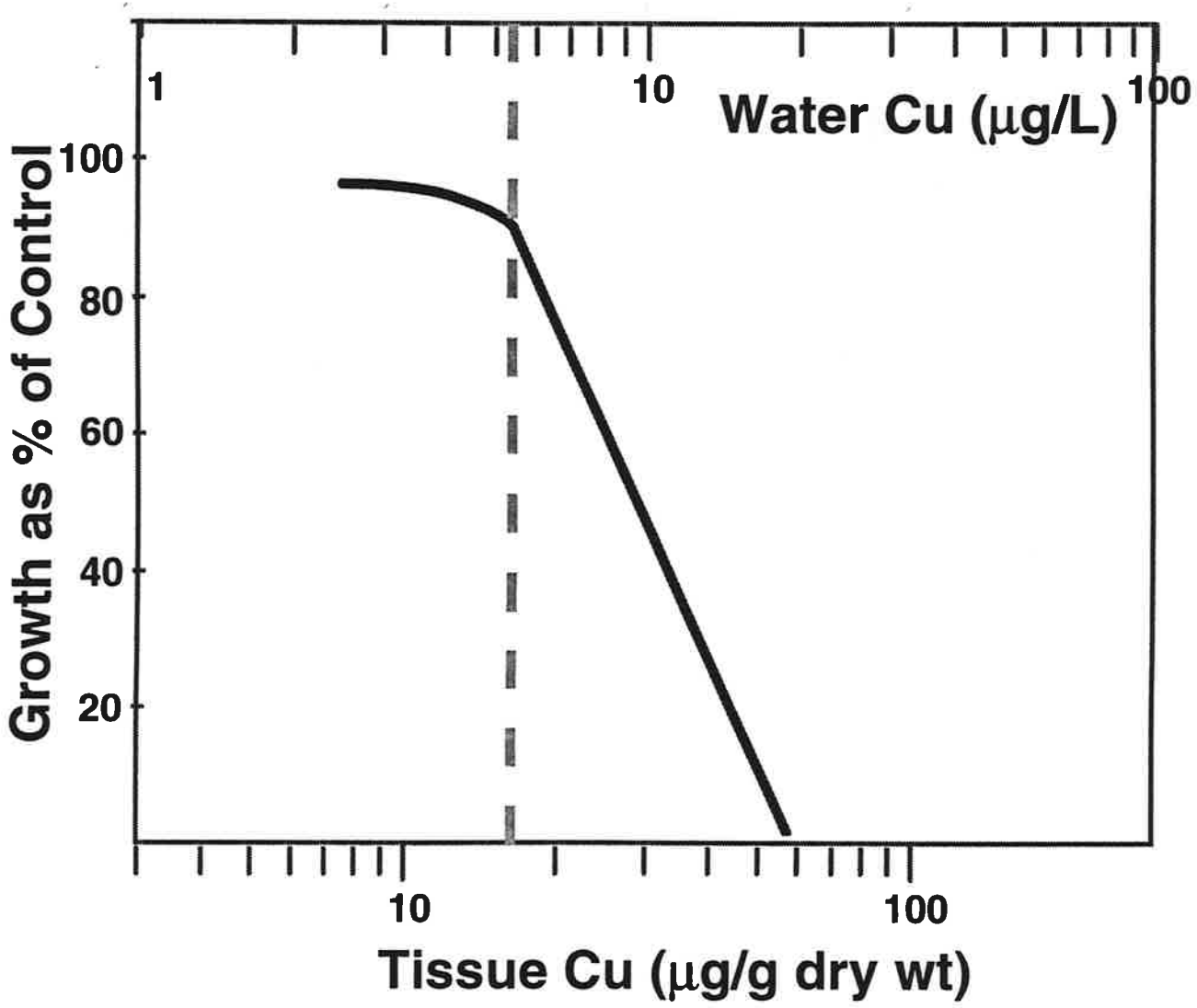


Figure 21. Effects of water Cu and tissue Cu on *Mytilus edulis* growth. Redrawn from Widdows and Donkin (1992).

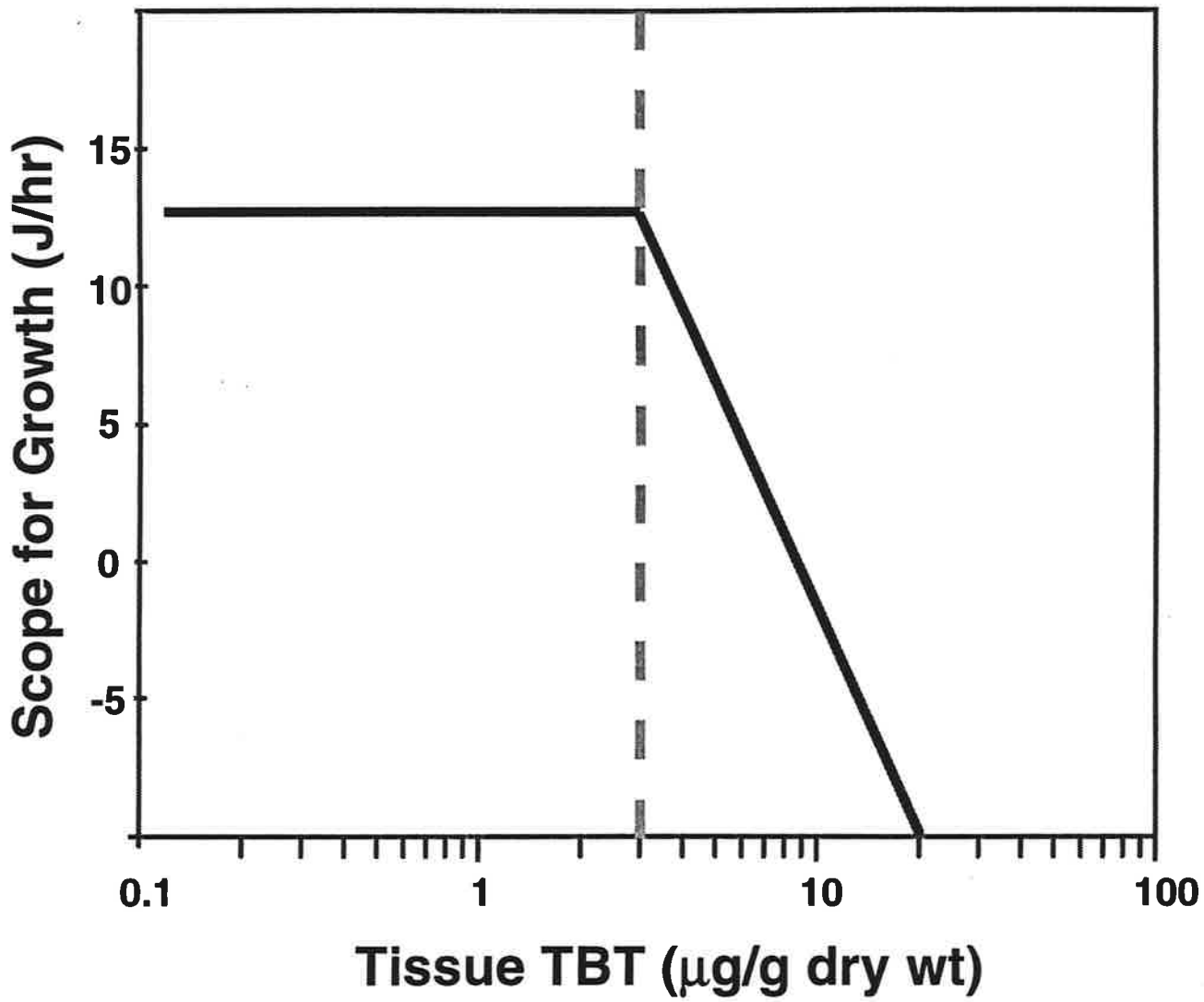


Figure 22. Effects of tissue TBT on *Mytilus edulis* growth. Redrawn from Widdows and Donkin (1992).

Table 30. Literature summary of relationships between concentrations of cadmium in sediment and freshwater mussels. Data for each metal are arranged in order of increasing concentration in sediment. All concentrations are in μg/g dry weight. (Table from Metcalfe-Smith et al., 1992)

Sediment	Muss	BCF	Species	Notes	Reference
0.051	0.8	15.7	*E. complanata*		Campbell & Evans, 1991
0.072	0.9	12.5	*E. complanata*		Campbell & Evans, 1991
0.079	1	12.7	*E. complanata*		Campbell & Evans, 1991
0.09	8.2	91.1	*L. siliquoidea*		Pugsley et al., 1988
0.093	0.6	6.5	*E. complanata*		Campbell & Evans, 1991
0.107	1.6	15.0	*E. complanata*		Campbell & Evans, 1991
0.19	6.3	33.2	*L. siliquoidea*		Pugsley et al., 1988
0.191	1.4	7.3	*E. complanata*		Campbell & Evans, 1991
0.197	2.1	10.7	*E. complanata*		Campbell & Evans, 1991
0.197	2.2	11.2	*E. complanata*		Campbell & Evans, 1991
0.202	0.6	3.0	*E. complanata*		Campbell & Evans, 1991
0.205	1.2	5.9	*E. complanata*		Campbell & Evans, 1991
0.25	1.5	6.0	*E. complanata*		Campbell & Evans, 1991
0.25	4.8	19.2	*L. siliquoidea*		Pugsley et al., 1988
0.3	10	33.3	*A. grandis*		Heit et al., 1980
0.3	9	30.0	*E. complanata*		Heit et al., 1980
0.3	9	30.0	*L. radiata*		Heit et al., 1980
0.321	0.6	1.9	*E. complanata*		Campbell & Evans, 1991
0.361	6.9	19.1	*E. complanata*		Campbell & Evans, 1991
0.378	5.9	15.6	*E. complanata*		Campbell & Evans, 1991
0.59	0.57	1.0	*L. ventricosa*	Caged mussels	Czarnezki, 1987
0.6	1.31	2.2	*A. plicata*	Caged mussels	Adams et al., 1981
0.7	0.85	1.2	*L. ventricosa*	Caged mussels	Czarnezki, 1987
2	5.6	2.8	*Q. quadrula*	[dw] = [ww]*10	Mathis & Cummins, 1973
2	6.9	3.5	*F. flava*	[dw] = [ww]*10	Mathis & Cummins, 1973 a
2	3.8	1.9	*A. plicata*	[dw] = [ww]*10	Mathis & Cummins, 1973
<3	58	19.3	*E. dilatata*	[dw] = [ww]*10	Wren et al., 1983
5.85	0.81	0.1	*L. complanata*	$\bar{x}$ [sed] from range	Anderson, 1977
5.85	2.71	0.5	*L. ventricosa*	$\bar{x}$ [sed] from range	Anderson, 1977
5.85	5.89	1.0	*L. siliquoidea*	$\bar{x}$ [sed] from range	Anderson, 1977
5.85	3	0.5	*A. marginata*	$\bar{x}$ [sed] from range	Anderson, 1977
9.53	1.98	0.2	*A. plicata*	Caged mussels	Adams et al., 1981
16.5	1.44	0.1	*A. plicata*	Caged mussels	Adams et al., 1981
16.8	1.43	0.1	*A. plicata*	Caged mussels	Adams et al., 1981
17.5	2	0.1	*L. ventricosa*	Caged mussels	Czarnezki, 1987
19.6	1.61	0.1	*A. plicata*		Adams et al., 1980
26.8	10.2	0.4	*L. ventricosa*	Caged mussels	Czarnezki, 1987
29.9	3.96	0.1	*A. plicata*		Adams et al., 1980
35.4	7.78	0.2	*A. plicata*	Caged mussels	Adams et al., 1981
57.3	11.3	0.2	*L. ventricosa*	Caged mussels	Czarnezki, 1987
89.7	12.5	0.1	*A. plicata*		Adams et al., 1980

Based on these data the authors identified a dramatic decline in BCF to below 1 as sediment concentrations increased above 5 μg/g dry wt. (There are no data points between <3 and 5.85 μg/g dry wt.) Nevertheless, it appeared that at tissue concentrations somewhere between 3 and 5.85 μg/g dry wt that the animals were so stressed that they were not accumulating much Cd. If the tissue weights or growth rates had been measured they would probably have been extremely low. More interestingly however, there appears to be another threshold in the data between 0.3-0.59 μg/g dry wt. This is where the BCF began to plateau and start its downward trend. This area of possible effects occurs between 0.3-3.0 and is shaded in the Table 30 to indicate possible effects covering a range of about one order of magnitude.

The relationships between freshwater Cd concentrations, sediment concentrations, and tissue concentrations in *Elliptio complanata* are shown in Figure 23A, B, C, D and were derived from the original data of Campbell and Evans (1991) that were a subset of Table 30. Figure 23A shows how the relationship between water Cd and tissue Cd changes dramatically near 0.12 μg Cd/L. Figure 23B shows how the relationship between sediment Cd and tissue Cd changes dramatically above 0.321 μg/g dry wt. These changes in the relationship between exposure and dose are very similar to those given for *Mytilus edulis* and seawater TBT as well as those for *Dreissena polymorpha* and freshwater copper.

Campbell and Evans (1991) derive a regression equation describing the relationship between the four variables they measured (pH, TOC, TIC, and Ca) and Cd concentrations. They point out the effects of these variables and problems associated with attempting to predict tissue concentrations based on either water or sediment concentrations. Nevertheless, if the extreme values of TOC, water cadmium concentrations, and pH are eliminated, the relationships improve significantly. For example, by eliminating data that meet two of three exclusion criteria (i.e., TOC > 5.7, water cadmium $\geq$ 0.1 μg/L, pH > 8.2), the ability to predict tissue Cd concentrations in *Elliptio complanata* from either water or sediment concentrations improves significantly (r^2 = 0.87, 0.88; respectively) as shown in Figures 23C and 23D.

These results are surprising due to expected variability in bioavailability among the sites. It shows promise however, as in the previous three examples, of providing another tool for assessing data sets with paired water or sediment chemistry with tissue chemistry to examine where the relationship changes. Even more surprising is the fact that these threshold sediment

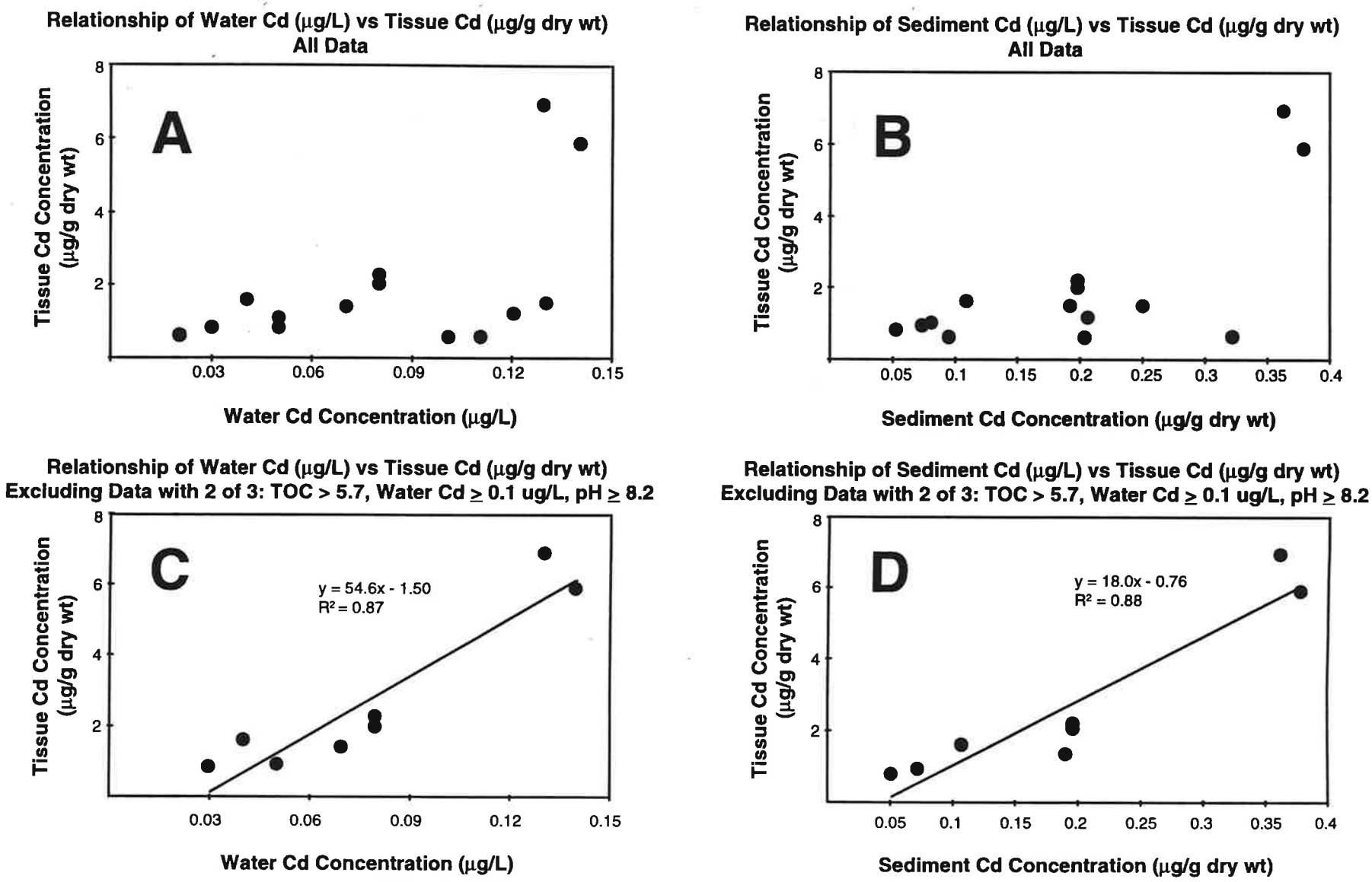


Figure 23. Relationships between Cd concentrations in water, sediment and tissue of *Elliptio complanata* from Canadian lakes. Redrawn using data from Campbell & Evans (1991) and restricting the data set to meet two of the following three criteria: TOC > 5.7, Water Cd ≥ 0.1 ug/L, pH ≥ 8.2.

concentrations are very similar to the interim Canadian freshwater sediment guidelines for Cd (Environment Canada, 1995). The predicted threshold effect level (TEL) is 0.598 μg/g dry wt. and the probable effects level is 3.53 μg/g dry wt.

2.5.5. **Using the relationship between exposure and dose to predict effects**

If the concept of critical body residues hold for all metals, it might be possible to predict first-order approximations of potential effects from where the relationship changes between concentrations in environmental media (water and sediment) and concentrations in bivalve tissues. Again, assessing the effects of metals presents unique problems compared to organic chemicals and the effects of metal speciation on bioaccumulation and bioeffects must be considered in the interpretation of results. A shift in emphasis from exposure concentrations in water and sediment to dose concentrations in tissues has several advantages according to McCarty and Mackay (1993):

- bioavailability is explicitly considered
- accumulation kinetics are considered, which reduces the confounding effect of organism exposure duration when interpreting results
- uptake from food (as distinct from water) is explicitly considered
- toxic potencies are expressed in a less ambiguous manner, facilitating identification of different modes of toxic action
- effects of metabolism on accumulation are considered
- mixture toxicity may be more readily assessed
- experimental verification can be readily sought in the lab and the field

This approach is also well-suited to an EEM program using bivalves such as the one being considered for the mining industry in Canada. Bivalves have long been used for chemical monitoring and the synoptic measurement of effects will help to reduce the uncertainties associated with traditional approaches. Further, once these associations have been made on a site-specific basis and the relationships are understood, it may be possible to apply the large

database that exists in bivalve bioaccumulation from the field and the lab and to eventually terminate the effects monitoring at some sites below some threshold and utilize a tiered monitoring approach as in the EEM for pulp and paper mills. Bioaccumulation establishes a link between environment and organism as well as a link between results from laboratory bioassays, field bioassays, and traditional chemical monitoring of natural populations. As such, this could form the basis of a national EEM program for the mining industry. Most importantly, there is the potential to predict adverse biological effects from tissue burdens. Until these relationships have been proven conclusively however, it will be necessary to measure exposure, dose, and response as suggested previously. This must be done as part of EEM to reduce the uncertainty associated with characterizing ecological risk.

2.5.6. **Characterizing effects**

Traditionally, impacts of chemicals like metals on marine and freshwater systems have been assessed with laboratory bioassays and alterations in benthic community assemblages as discussed previously. The advantages and disadvantages of these approaches have been outlined. The bivalve monitoring model (Figure 16) includes growth because of its utility and cost-effectiveness. Growth is a commonly used indicator of biological effects, it is a sensitive and environmentally realistic biological response, and it is a sublethal effect that shows a dose-response relationship. It is a biological response that represents the integration of all internal biological processes and has been identified as a significant effect to be measured in environmental assessments (Bayne et al., 1985; Widdows and Donkin, 1992). Reductions in growth are easily quantified and correlated with adverse environmental effects. However, since both natural and pollution-related stresses have been shown to reduce bivalve growth rates in marine and freshwater environments, it should be pointed out that establishing correlations by themselves, does not necessarily prove cause-and-effect (Green et al., 1985; Widdows and Donkin, 1992). This is one of the reasons for using integrated studies and a preponderance-of-evidence approach in environmental monitoring and risk assessments. Furthermore, Annex 1 for EEM emphasizes the importance of *in-situ* testing and includes tests on invertebrate survival and growth (Environment Canada EEM web site).

Caging facilitates measuring bivalve growth and utilizing the power of manipulative experimentation in the field for hypothesis testing that help develop cause-and-effect relationships. Several methods have been developed for compartmentalized cages that allow repetitive measurements using the same individuals over the course of the exposure period (Salazar and Salazar, 1995). There is an increasing trend toward measuring sublethal effects like growth in laboratory studies instead of, or in addition to measuring mortality because it is a more sensitive indicator of stress. Reduced growth in marine and freshwater environments has been associated with a variety of chemicals, including metals, in both laboratory and field bioassays (Stromgren, 1982,1986; Stromgren and Bongard, 1987; Widdows et al., 1990; Valkirs et al., 1991; Widdows and Donkin, 1992). Juvenile mussel growth was the most sensitive sublethal indicator of TBT measured in San Diego Bay microcosm experiments (Salazar and Salazar, 1987; Salazar et al., 1987).

Naimo (1995) has recently provided a review of the effects of heavy metals on freshwater mussels. The review demonstrates that exposure to metals can adversely affect growth, filtration, enzyme activity, and behavior. She concluded that understanding how metals affect biological processes (like growth) in freshwater mussels would provide greater insight regarding the ecotoxicological significance of metals in various environmental compartments with respect to natural populations of freshwater mussels and facilitate the development of water quality criteria to protect those mussels. Doherty (1990) reviewed the use of *Corbicula* as a biological indicator of exposure and an indicator of effects in freshwater environments. Bioaccumulation was the most frequently measured exposure endpoint. Several effects endpoints were also used, including: histopathology, adenylate charge, reproduction, condition index, enzyme inhibition, scope for growth, and growth. Of these, growth was the most commonly measured effects endpoint. Transplant studies with caged clams (*Corbicula*) was the most common method used to measure growth. Doherty (1990) emphasizes that the primary advantage of all these effects endpoints is that they require no sophisticated instrumentation and that they provide valuable information on site-specific effects. The review concludes that field studies have provided a greater consistency in results than the laboratory studies in attempting to predict the effects of effects of bivalve exposures under natural conditions.

There are four recurring themes occurring in the following examples relevant to the use of

BMB for mining effluents in Canada: (1) Effects endpoints like growth have been measured successfully in freshwater bivalves for a number of years; (2) These endpoints have been related to metal exposures in freshwater bivalves; and (3) Growth is one of the most commonly measured endpoints in freshwater bivalves; and (4) Transplants are the most commonly used monitoring method to successfully measure bioaccumulation of metals and growth in manipulative field experimentation. Foe and Knight (1986) measured shell growth, condition index, and tissue concentrations of both indigenous and transplanted freshwater clams (*Corbicula fluminea*) exposed to effluents containing Zn and Cu. Subsequently, they (Foe and Knight, 1987) used the caging method to assess the impact of a thermal discharge during a 6-month deployment using the same effects endpoints. Collectively, their work demonstrated the feasibility and utility of measuring effects endpoints in freshwater bivalves under a variety of conditions. More recently, Belanger et. al. (1990) provided another level of sophistication by measuring tissue and shell growth in Corbicula exposed to Cu in artificial streams and river systems. This work is particularly significant from a regulatory perspective. The State of Virginia lowered the site-specific water quality criterion for Cu on the Clinch River as a result of the compelling evidence provided by exposure and effects measurements in transplanted *Corbicula fluminea* in addition to other effects endpoints in three other indigenous species and corresponding laboratory bioassays (Scott Belanger, personal communication, March 1997). Furthermore, of the seven most sensitive species tested, to confirm establishing new site-specific criteria, 6 were bivalves. In addition to *Corbicula*, the seven most sensitive species included 1 snail and five unionids (John Van Hassel, personal communication, March 1997). Similarly, effects endpoints in *Musculium transversum* were used to validate ambient water quality criteria for ammonia (Charles Stephan, personal communication, March 1997). It is interesting to note that freshwater bivalves have consistently been among the most sensitive species tested for a variety of chemicals, including metals (Jerry Farris, personal communication, March 1997).

Given the preponderance of evidence provided by these and other studies, it is misleading to conclude from the technical evaluation provided by Stewart and Malley (previous chapters) that effects endpoints are not well-developed enough to be used in a routine monitoring. Similarly, the primary citation they use to support this argument (O'Connor, 1992) is also misleading. O'Connor was referring only to the use of bivalve biomarkers in terms of a research

program to evaluate the relationships between chemical exposure and associated biological effects. The present author was responsible for directing the bivalve biomarker effort within NOAA between 1993 and 1995. During that time a number of studies were conducted to evaluate the utility of biomarkers and marine bivalve growth was used as the standard to compare the test results. In each case, the biomarkers did not provide any more useful information than that already provided by measuring various growth endpoints.

The State of California has been routinely monitoring bioaccumulation of chemicals in natural populations and transplanted marine mussels since 1977 (Martin and Severeid, 1984; Martin and Richardson, 1991) and occasionally have measured scope for growth or growth as an endpoint (Stephenson et al., 1986; Stephenson, 1991). As part of the U.S. EPA Environmental Monitoring and Assessment Program to evaluate the effects of chemicals (EMAP) (Paul et al., 1992) a combination of transplanted oysters (*Crassostrea virginica*) and clams (*Mercenaria mercenaria*) were used in the Carolinian Province (Ringwood et al., 1995). Additionally, growth rates of clams were measured in laboratory exposures as well as in the deployed clams for comparative purposes. Caged mussels have also been used to routinely monitor dredge disposal operations and even make near real-time decisions regarding project scope and duration by measuring chemical exposure endpoints and effects endpoints like growth and scope for growth (Nelson et al., 1987; Nelson, 1991; Nelson and Hansen, 1991; Nelson et al., 1991). Finally, the environmental relevance of the effects of metals on bivalve growth rates can be determined by associating the effects on individuals measured during the monitoring program to effects on the population (Sibly, 1996). This approach has been used routinely since the 1980's.

3. EXAMPLES OF USING CAGED BIVALVES AS A MONITORING TOOL TO SUPPORT AN INTEGRATED RISK ASSESSMENT STRATEGY

As stated previously, the primary area of disagreement between this evaluation of caged bivalves as a monitoring tool and that provided by Stewart and Malley is the question of effects monitoring. The following two examples will demonstrate how exposure and effects measurements can be easily accomplished and the utility of these synoptic measurements in the final analysis of risk.

3.1. Temporal and spatial trends

Sequential, serial transplant studies with caged bivalves can be used to establish temporal and spatial trends in exposure, dose, and response. Figure 24 shows these relationships developed for two sites, separated by 3 meters vertical distance, in the Shelter Island Yacht Basin, San Diego Bay, over a 3-year period. These studies were conducted to evaluate the effects of tributyltin (TBT) on marine ecosystems. Caged mussels were used because natural populations of mussels were absent in the assessment areas.

The exposure characterization (Graph A) clearly demonstrates a decline in TBT concentration at the surface site associated with the ban on TBT antifouling coatings in 1988. By monitoring dose, i.e., the amount of TBT accumulated in the soft tissues of the mussels, as part of the characterization of exposure, the assessment gains a perspective on chemical changes within the organism that help to understand the process involved. Although there is a steady decline in dose (Graph B) during the first four tests, there is a surprising increase in tissue TBT concentrations between tests 3 and 4 that is equal to the tissue burdens when the seawater concentrations were five times higher (500 ng/L vs 100 ng/L). The measured increase in tissue TBT concentration with decreased seawater TBT concentration has been associated with the change in the relationship between seawater TBT and tissue TBT as discussed in the preceding section on predicting effects from media metal chemistry and bivalve tissue chemistry. One interpretation is that mussels are able to regulate TBT and cannot be used as an indicator of exposure for this chemical. Another is that the amount to TBT in mussel tissues is a function of

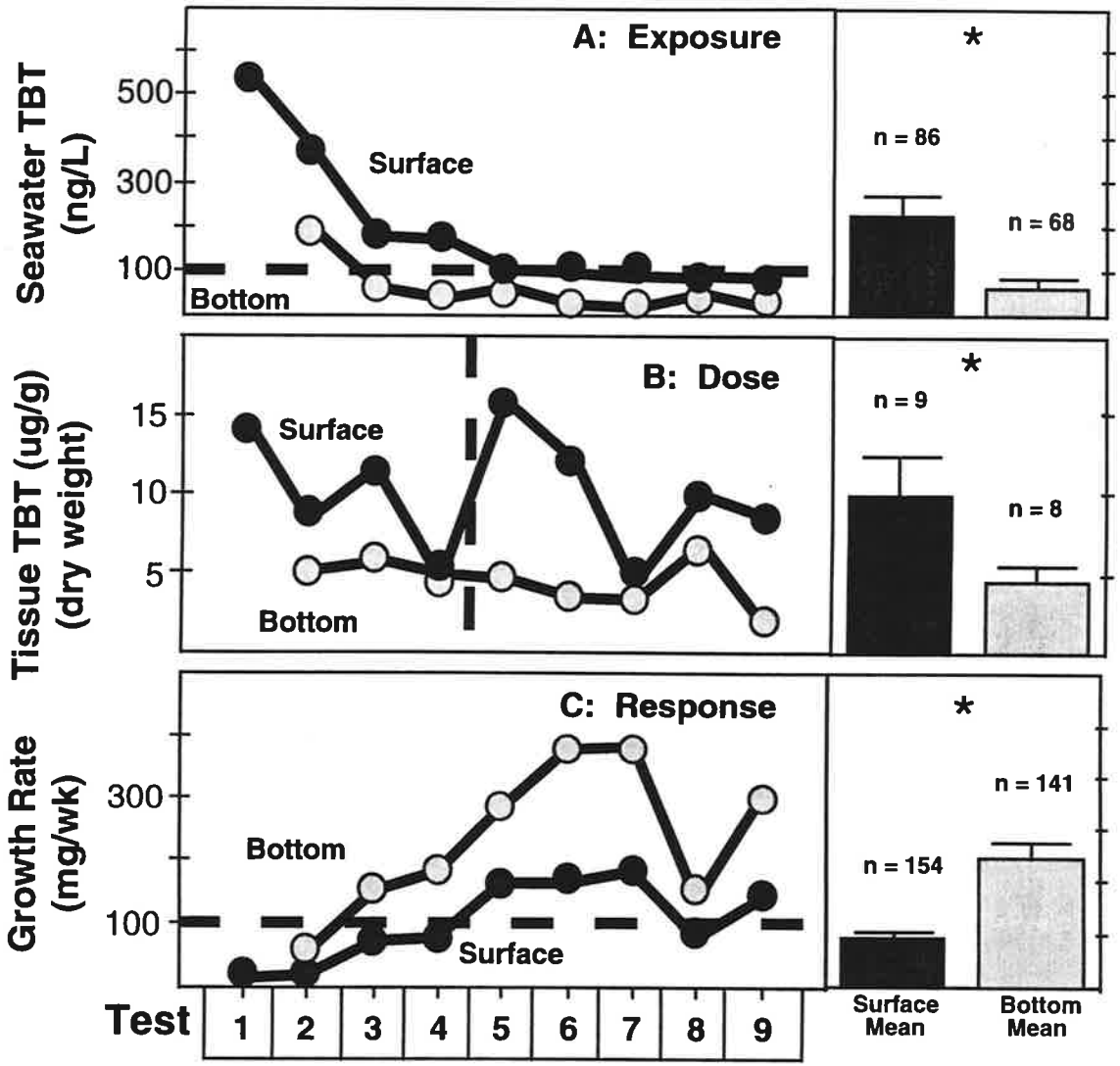


Figure 24. Characterizing exposure and effects with TBT and *Mytilus galloprovincialis* in San Diego Bay at 2 sites separated by 3 meters vertical distance. Shown are seawater TBT concentrations, tissue TBT concentrations, and mussel growth rates by test and pooled across tests to show statistically significant differences between the two sites.

environmental exposure conditions, and that relationship is not constant. The latter interpretation is supported by two events that occurred between Test 4 and Test 5: (1) the seawater concentration of TBT dropped below the critical concentration where the relationship between exposure and dose changes significantly (ca. 100 ng/L); and (2) growth rate measurements were reduced from once/wk to once/every other week when it was discovered that the weekly measurements were also reducing mussel growth. It appears that the critical concentration was the most important factor but the added effect of reducing measurement rate may have been a factor as well. Clearly, growth rates increased steadily during the first 6 tests as seawater TBT concentrations decreased. After Test 6 growth appears to have been more controlled by variability in natural factors than low concentrations of TBT. This is most evident in Test 8, conducted in the winter when all temperatures were below 15°C. Analysis of the pooled data resulted in a statistically significant difference in exposure, dose, and response across tests. Although the surface and bottom stations were separated by only 3 meters, the presence of a thermocline just below the surface of the seawater resulted in very different physical conditions. The identification of differences in TBT availability and its effect on the environment was facilitated by using caged mussel study. It would have been impossible to obtain these results with resident species (since mussels are not found at that depth at that site), or by only conducting bioaccumulation studies. The growth component was necessary to calibrate and explain the bioaccumulation results.

Although this study was primarily designed to assess the affects of TBT, selected metals were measured during four or five of these tests. Tissue concentrations of Cu and Zn were both significantly higher in mussels at the surface site than at the bottom site (Figure 25). Since similar differences were found in tissue concentrations of TBT in mussels from the surface compared to the bottom site (Figure 24), these data suggest that all three metals co-occurred and were associated with the floating ship hulls. Similar differences were found in other metals as well but these differences were not statistically significant. If there were real differences between the other metals, it seems likely that replication was inadequate to detect them.

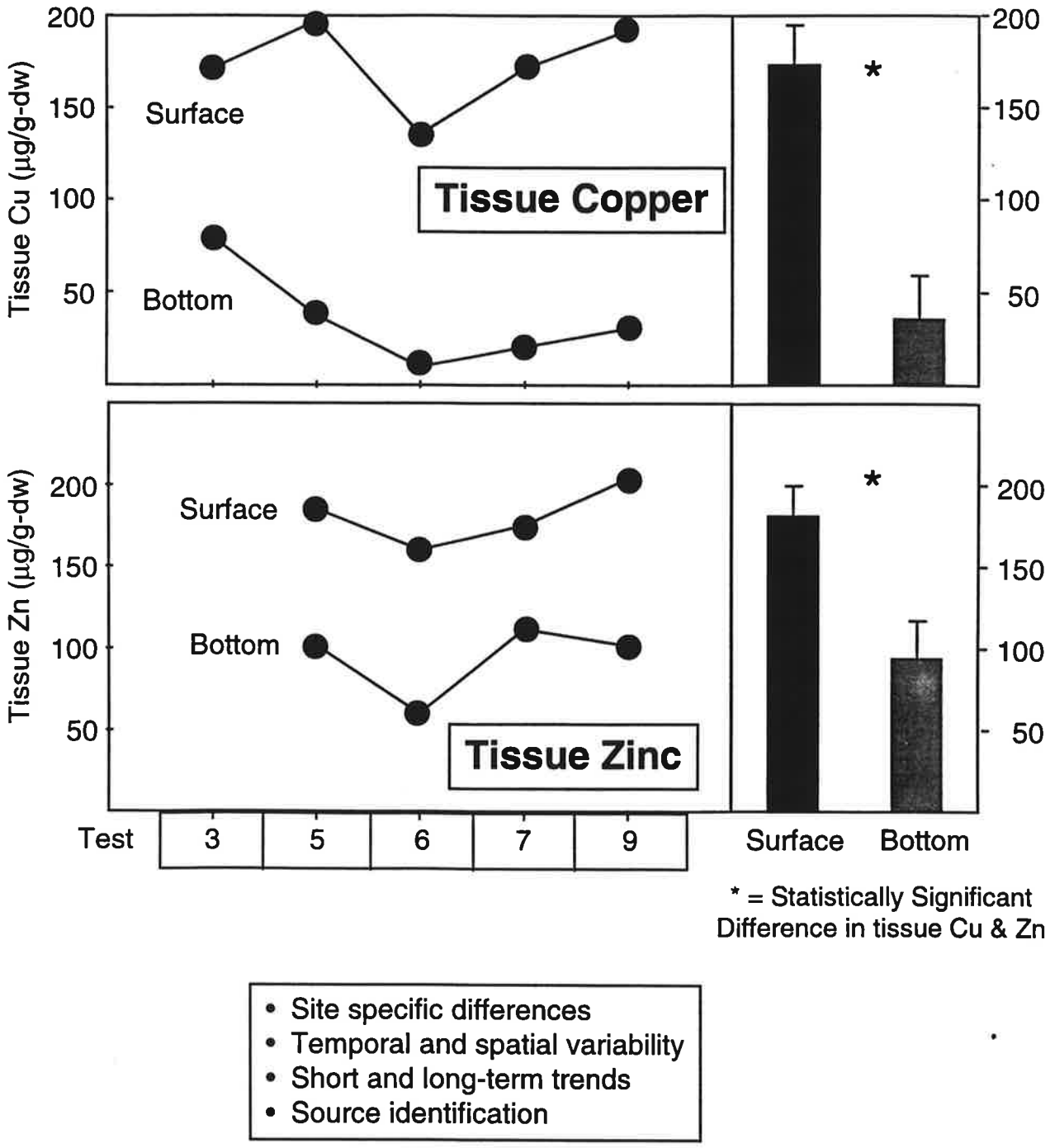


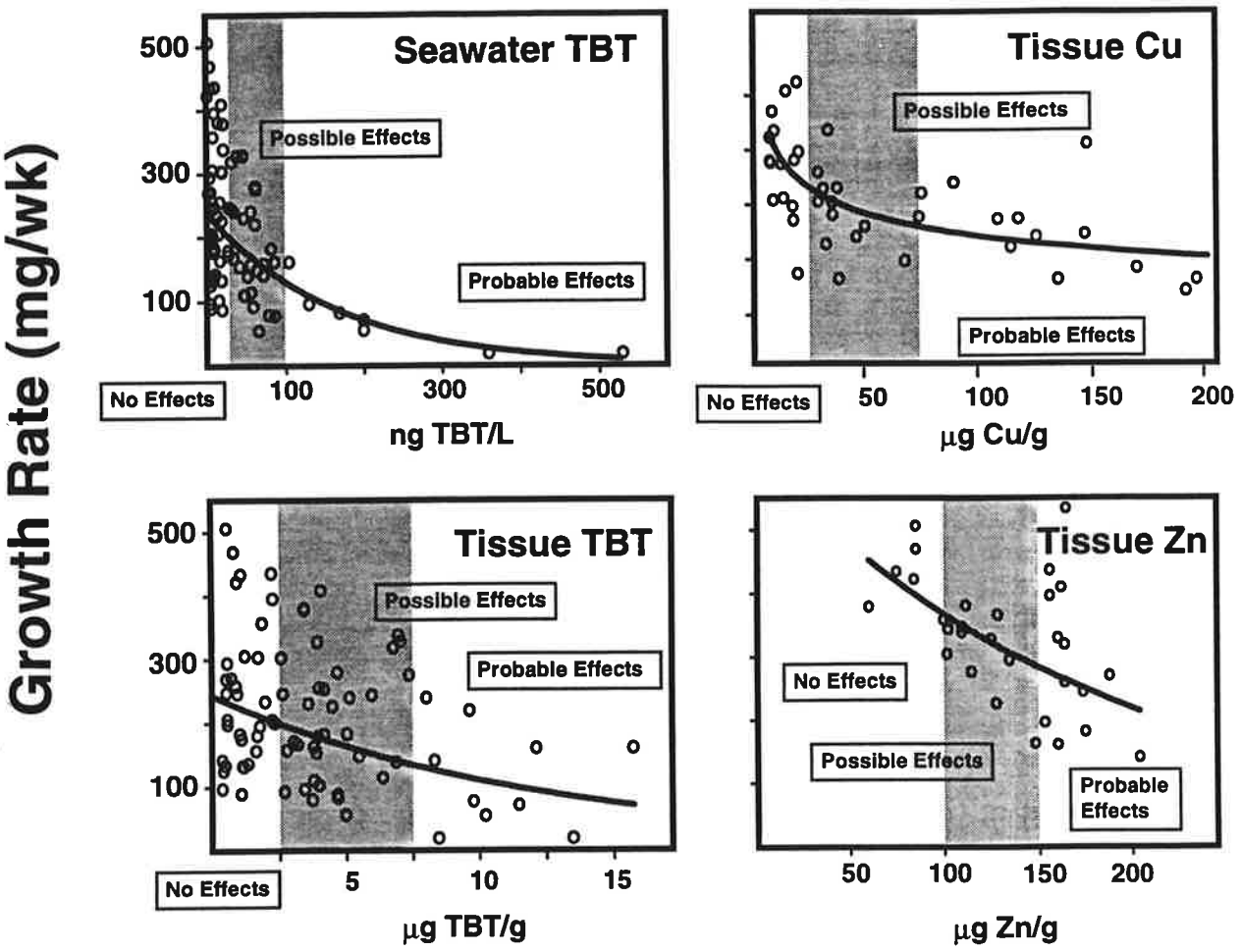
Figure 25. Characterizing metal exposure with caged bivalve monitoring at 2 sites, 3 meters apart. Shown are Cu and Zn concentrations by test, and pooled across tests to show statistically significant differences between the two sites.

3.2. Dose-Response

Dose-response relationships can be developed from synoptic measurements of exposure (water or sediment concentrations), dose (tissue concentrations), and response (growth). These relationships are extremely useful in the risk assessment process because they allow a direct determination of whether the dose can be accommodated by natural processes or if it is beyond this limit and likely to adversely affect the target populations. Dose-response relationships and first order approximations have been developed for water and tissue concentrations of seawater TBT, tissue TBT, tissue copper, and tissue zinc for the caged mussel study conducted in San Diego Bay (Figure 26). The data presented in this figure represent 18 sampling stations, including the Shelter Island Yacht Basin stations previously discussed. Effect concentrations (ECs) and no observable effect concentrations (NOECs) were estimated for these three chemicals based on the concentrations where changes in the relationships appeared (as in the seawater TBT - tissue TBT regressions). Tissue metals were only measured in four of the nine tests resulting in a data set that is smaller than the TBT data set. Nevertheless, these values (Figure 26) are very similar to those from other studies.

Site-specific dose-response relationships were developed for the Shelter Island Yacht Basin (Figure 27). Although the relationships between seawater and tissue TBT were amost identical to those developed for all of San Diego Bay, the significance of the regressions improved for growth versus seawater TBT and growth versus tissue TBT. These stronger relationships allowed us to calculate a site-specific EC value for tissue TBT of 4 μg/g-dry wt (Salazar and Salazar, in review). This value is nearly half of the EC value of 7.5 μg TBT/g-dry wt predicted for all of San Diego Bay. The site-specific EC value of 4 μg TBT/g-dry wt is quite similar to the predicted threshold concentration associated with reductions in mussel scope for growth (Page and Widdows, 1991; Widdows and Page, 1993) as well as oyster growth (Waldock, et al., 1996).

Exposure-Dose-Response: TBT, Cu, Zn



	Seawater TBT	Tissue TBT	Tissue Cu	Tissue Zn
Effect	>100	>7.5	>75	>150
No Effect	<25	<2.5	<25	<100

(Seawater TBT units = ng/L; all tissue units = µg/g dry weight)

Figure 26. Using exposure-dose-response relationships to predict the effects of TBT, Cu, Zn. All San Diego Bay data for *Mytilus galloprovincialis*.

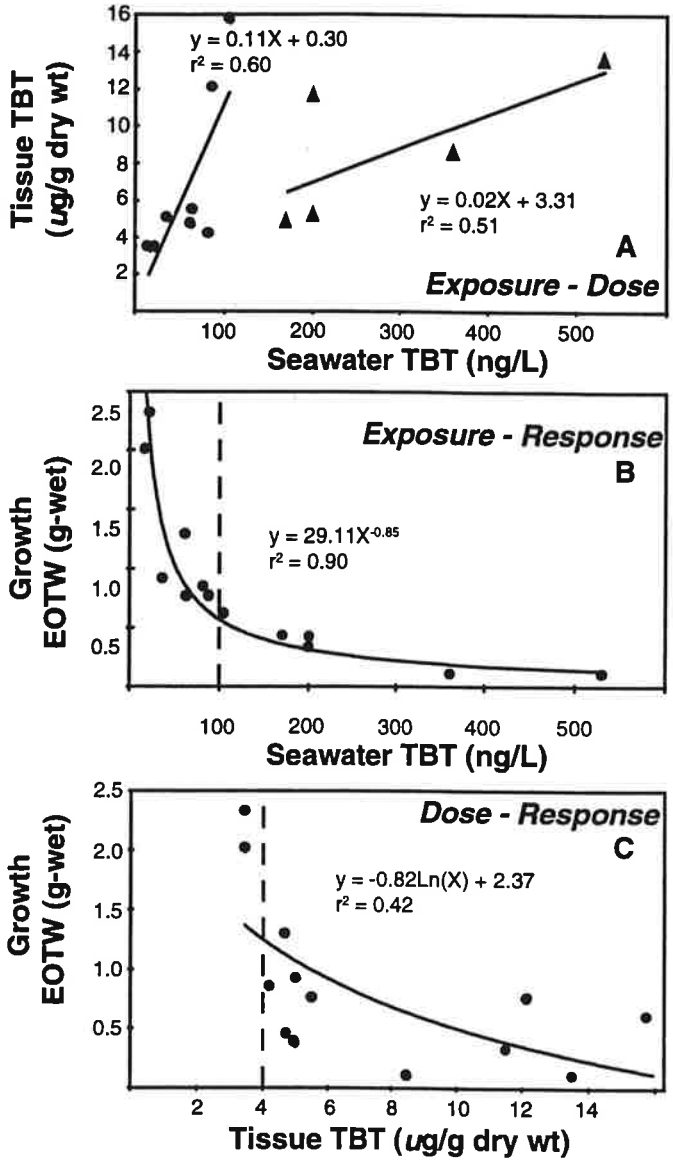


Figure 27. Exposure-dose-response triad showing: exposure-dose (A), exposure-response (B), and dose-response (C). Shown here are the relationships between seawater TBT and tissue TBT; between end-of-test tissue weights(EOTW) and 1) seawater TBT and 2) tissue TBT for a surface (SI) and a bottom (SID) site in San Diego Bay. All regressions are statistically significant. Data from Tests 8 and 9 were excluded because extremely low and high temperatures reduced end-of-test tissue weights. Note: the relationships improved significantly by only using data from the Shelter Island Yacht Basin instead of data for all San Diego Bay sites and removing data from temperature extremes.

4. TECHNICAL ASPECTS

A natural division of the criteria provided by AETE was into technical aspects which included the more scientific or technical aspects of the review and into practical aspects which included the more pragmatic and state-of-the-art equipment, facilities, and resources available for conducting a BMB program.

4.1. Sensitivity (dose response)

There is a perception among many that since bivalves are used in many chemical biomonitoring programs because they can survive high concentrations of chemicals in water, sediment, and in their tissues that they are insensitive. As with standard laboratory bioassays, it is generally acknowledged that acute endpoints like survival are generally not very sensitive in any species. The same is true for bivalves. Four recent studies have shown that bivalves are as sensitive or more sensitive than standard bioassay species with acute endpoints like survival as well as chronic endpoints like growth. Furthermore, these studies have shown that their findings are applicable, in some cases, for water, sediment, and tissue concentrations.

In a laboratory bioassay conducted by the EPA Narragansett Laboratory, the 7-day growth endpoint in the marine bivalve *Mulinia lateralis* was shown to be more sensitive to contaminated sediment than the 10-day mortality endpoint in the amphipod *Ampelisca abdita* (Burgess and Morrison, 1994). In two separate experiments the freshwater bivalve *Anodonta imbecilis* was shown to be: (1) more sensitive using the 10-day mortality endpoint than the 7-day mortality endpoint in *Daphnia* when exposed to pulp and paper mill effluents (McKinney and Wade 1996); and (2) as sensitive to metals as *Daphnia*, *Pimephales*, and *Chironomus* (Keller and Zam, 1991). Cellulolytic enzyme activity in the freshwater clam *Corbicula* was more sensitive to Cu and Zn exposures than laboratory bioassays or measurements of macroinvertebrate diversity (Farris et al., 1988). On a tissue residue basis, the marine mussel *Mytilus edulis* was shown to be an order of magnitude more sensitive than amphipods, polychaete worms and fish mortality endpoints (Salazar and Salazar 1996; in review).

4.2. Ecological relevance - bivalves as a whole and specific measurement endpoints

In general, marine bivalves are ecologically relevant because they make up such a large percentage of the biomass in many intertidal and subtidal communities. Freshwater bivalves are important because of their conservation status as discussed in the previous chapters. As discussed in the previous section, growth and reproduction are two of the most commonly measured sublethal endpoints because of their ecological relevance. Reductions in growth and reproduction have both been related to adverse effects on the population but growth is generally much easier to measure than reproduction. Furthermore, growth has been used as a standard to compare other biomarkers as endpoints and they have generally not provided any more useful information than measuring growth. Scope for growth is one of the more commonly used physiological endpoints in the marine environment (Widdows and Donkin, 1992) and has been used in some Canadian studies (St-Hilaire and Pellerin-Massicotte, 1996; Richard Addison, personal communication, March 1997). Similarly *in-situ* chambers have been used by Canadians to track energy flow through marine bivalves (Cranford and Gordon, 1992; Cranford and Hargrave, 1994; Cranford et al., 1996). Currently this system is being tested in Atlantic Canada to monitor effluents from offshore oil and gas platforms..

Scope for growth (SFG) is another potentially powerful tool in BMB because of its physiological relevance and applications to the population. Where results have been compared with direct measurements of growth, they have been extremely similar. Although SFG is potentially more powerful, it requires specialized equipment and trained personnel. This is a distinct disadvantage in monitoring programs compared to direct growth measurements that can be made with a calipers and balance by most laboratories or contractors. The other disadvantage is that the SFG measurements (clearance rate, filtration, absorption) offer only an instantaneous measurement of animal health or effects. In some applications of intermittent discharges into freshwater systems from mining effluents, it may be more difficult to relate these SFG endpoints to ecological relevance. Direct measurements of growth however, are more integrative over a longer time scale and have a potentially greater relevance for long-term effects.

While it is clear that caged bivalves offer more versatility as a BMB monitoring tool for the mining industry, it may be possible to use growth rings in indigenous populations to assess

growth rates and environmental health (Green et al., 1985, 1989). There are several potential problems with this approach as well in terms of applications for mining effluents: (1) In many areas the natural populations may have been diminished due to the effluents or other causes and are not available; (2) The natural populations may be threatened or endangered so that monitoring them may have a greater adverse effect than the effluents themselves; (3) The time scale of response is generally measured in years and this may not be appropriate for particular mining applications; (4) Measurements of growth rings has more inherent variability than growth measured over two distinct points in time with a known exposure period; and (5) It will be more difficult to relate population growth rates to metals concentrations in water, sediment, and tissues unless they are measured on the same time scale.

Finally, there is another reason for measuring growth and that is to calibrate bioaccumulation. In the example cited by Stewart and Malley (previous chapters), changes in tissue weights in *Elliptio complanata* in the Sudbury River were used to explain the growth dilution of methylmercury by addition of somatic tissue. Without these measurements the ecological relevance would have been misunderstood because the stations furthest from the source had the lowest tissue concentrations (μg/g dry wt) (Salazar et al., 1996). On a content basis however (μg/animal) methylmercury burdens increased at all stations compared to the beginning of the test and suggested that methylmercury was biologically available at all river sites. It should be added that these measurements would not have been possible without transplanting caged bivalves because there were insufficient numbers of *Elliptio complanata* in areas of concern within the various reaches of the river. Similar differences between concentration and content data have been reported for other metals in both marine and freshwater environments (Mersch et al., 1996).

4.3. Validation (peer review)

There have been numerous studies to validate the utility of bivalves as both an indicator of exposure and an indicator of effects for both natural and transplanted populations in both freshwater and marine environments. A search of the author's database revealed over 300 citations on the bioaccumulation of metals in bivalve tissues and over 300 references on mussel

growth. This indicates that these exposure and effects endpoints have been studied and developed to similar levels. Growth has been studied since the early 1900s, initially to support the mariculture industry and later for environmental monitoring purposes. Considerable expertise on bivalve growth in marine waters is available on both Canadian coasts. Although not nearly as well developed due to the lack of a freshwater mariculture industry to support it, considerable expertise also exists in Canada regarding freshwater bivalve growth.

4.4. Site specificity - what kind of sites have been studied and what is their relevance to other studies with regard to data comparisons?

All types of sites have been studied including freshwater, marine, and estuarine environments with a variety of bivalve species. While not all of these studies were specifically conducted to assess metals, it gives some idea of the number of studies that have been done. It should also be recognized that any bivalve could potentially be studied in or transplanted to any area if the site-specific conditions support survival and growth. As described previously, Table 26 provides a list freshwater studies where bivalves have been used for chemical monitoring. The only restrictions are those already described by Stewart and Malley in previous chapters regarding pH, alkalinity, grain size etc. Table 27 provides a listing of caged bivalve studies conducted using a refined methodology with compartmentalized cages. It should also be emphasized that the use of compartmentalized cages facilitates effects measurements like growth. Compartmentalized cages have not been used in many studies, and this could be one of the reasons why effects measurements have not been included in previous studies. It is also important to remember that the significant relationships found between exposure, dose and response, and the statistically significant differences in the two marina sites separated by only 3 meters vertical distance would not have been possible using resident populations because mussels are not naturally found at those depths. Similarly, a recent pilot study in Port Valdez designed to measure bioaccumulation and growth near the bottom diffuser at a depth of 70 meters would not have been possible without the use of caged bivalves. The caged bivalve approach has been used by a number of investigators at varying depths in the marine environment. While freshwater environments provide a unique set of problems such as intermittent flow described by Stewart and Malley (previous chapters), the

approach remains technically feasible for providing site-specific information on exposure, dose, and response at mining sites.

4.5. Repeatability - what is the ability to return to a given site at a given time of the year and reproduce the same results again?

The primary limitation of returning to a given site at a given time of the year is the availability of sufficient numbers of test animals in the area of concern. This is another distinct advantage of the caged bivalve approach; i.e., the monitoring program need not rely on the availability of natural populations in the area of concern. If test animals are routinely transplanted to the test area, there is no limitation on the number of animals available. Caging the bivalves instead of using natural populations also has more flexibility for becoming part of a national monitoring program where similar species and stocks could be used for many sites. It has also been suggested that using non-indigenous species would be beneficial from the standpoint of limiting pressures on the conservation status of many natural populations. All of these factors favor the use of caged bivalves in terms of maximizing the possibility of repeatability

4.6. Response time - how long must the bivalves be exposed to a physical chemical stressor to elicit a particular response - give supporting data and studies?

From the studies provided in previous chapters, it appears that the time necessary to reach chemical equilibrium (steady state) varies from about two weeks to over one year. Bivalves transplanted by Hinch and Green (1989) did not reach the concentrations of natural populations even after one year. It has been suggested in these cases that caged bivalves may not be appropriate as monitors because they did not reach the levels of the natural population during the exposure period. While there is some evidence that this may occur only for some specific metals like silver, where animals continue to accumulate an increasing metal burden over their entire life span, it demonstrates that the use of caged bivalves may not be a perfect surrogate for natural populations after relatively short-term exposures (compared to the lifetime of the natural populations before sampling). It should be emphasized that the single reference cited by Stewart

and Malley (previous chapters) to an equilibration period of over one year was in the comparison to the natural population at one point in time and does not demonstrate that the transplants were not in chemical equilibrium (steady state) in their relative lifetime (Hinch and Green, 1989). It should also be pointed out that the majority of transplant studies demonstrate that chemical equilibrium (steady state) in a variety of freshwater and marine bivalve species is reached between external media (water and sediment) in a period of weeks to months.

In some respects this may almost be a moot point. Considering the numerous advantages of using caged bivalves over natural populations, shown in Table 25, the advantages far outweigh the disadvantages. To put this in the proper perspective, we should also consider the utility and representativeness of laboratory bioassays. Chemical equilibrium (steady state) is not achieved in many laboratory exposures and yet results from these tests have been generally useful. There are some problems however. For example, it has been demonstrated that it takes about 45 days for amphipods to reach chemical equilibrium with TBT in contaminated sediment (Meador et al.,1996) and yet the standard protocol for laboratory exposures is only 10 days. This is one reason for the lack of concordance of sediment chemistry, laboratory toxicity tests and benthic community assemblages during some recent studies in Puget Sound. Why would one expect similar results if the exposures were not similar. Similarly, while there were no significant elevations in laboratory exposures to sediment contaminated with dioxins and furans, there was a significant elevation in mussels exposed in the water column. While it is possible that the dioxins and furans in the sediment were not biologically available and chemicals in the water column were available, it seems just as likely that the laboratory exposures with bivalves were environmentally unrealistic and the animals may have been so stressed that they did not accumulate even biologically available chemicals. This is one of the problems with not measuring effects endpoints in either laboratory or field bioassays; i.e., the health of test animals must be quantified in order to properly interpret test results. Furthermore, it takes bivalves about 60 days to reach chemical equilibrium with highly hydrophobic chemicals like dioxins and furans in sediment. Nevertheless, in practice, only the 28-day exposure is generally used as specified in the standard protocols. In general it appears that metals take longer to reach equilibrium than either organics or organometals. In terms of a routine monitoring program, it appears that 60-90 days would be appropriate in most cases. A longer exposure period would also provide more time for effects

like growth to manifest themselves.

Another problem with almost all studies, whether in the lab or the field, is that animal health is generally not quantified in any way. It is generally assumed that if the animals are fed to excess that they must have been in good health (Tessier et al., 1994). Bivalves appear to be particularly sensitive to feeding since growth measured in the laboratory seldom achieves the rates measured under field conditions (Kiorboe et al., 1981). With field studies it is also assumed that if animals were held under natural conditions that they must be in good health. There is no guarantee that animals held under either exposure regime are necessarily in good health. Even the use of traditional methods like condition index and shell growth are questionable due to the variability associated with measuring the volume of the mantle cavity. This is why some investigators have shifted from condition index to an index based on tissue weights and shell weights. Differences in growth assessments based on whole-animal weights and tissue weights have also been demonstrated. Over 20 studies have been conducted using the application of multiple mussel metrics, and length has never been a more discriminating measure than whole-animal weights or tissue weights (Table 27). This is particularly true for bivalves with variable growth patterns such as oysters.

4.7. Interpretability - how easily are results interpreted and what statistical tests must be employed?

The easiest results to interpret are those designed to detect statistically significant differences among sites during a particular test. Usually this can be accomplished through the use of an analysis of variance (ANOVA) to determine if there are differences and a multiple range test to determine where the differences lie. It is more difficult to detect differences between initial and end-of-test since other conditions have changed as well and the tissue weights have changed. This is another reason for measuring tissue weights at the beginning and end of the test. If the tissue weights are measured, chemical burdens can be compared on a concentration (μg/g) basis as well as a whole-animal basis (μg/animal). If the tissue burdens are compared on a content basis, it is easier to determine statistically significant differences between the beginning and end of the test. Similar comparisons can also be made for effects measurements.

4.8. Variability - In a particular data set from a single study, what type of variability can be expected and how does this affect variability in repetitive studies?

There is usually considerable variability in both exposure and response measurements. This is why it is imperative to reduce the size range between approximately 5-10 mm at the beginning of the test. Reducing the size range reduces the variability in both exposure and response information and increases the discriminating power of the test. Other sources of variability include differences among sites and seasonal differences. Site differences and associated variability in the measurement endpoints cannot be controlled but seasonal differences can be minimized by always monitoring at the same time of the year.

4.9. Predictive Power - How can the dose-response data be used to predict potential problems and characterize environmental risk?

It has been emphasized from the beginning of this chapter that characterizing environmental risk requires accurate characterizations of exposure and effects. Clearly, only characterizing exposure cannot provide a realistic assessment of risk. Effects measurements not only help characterize effects but they help to calibrate the estimates of exposure by explaining changes in tissue weights. For marine mussels and oysters 15-20 individuals per sample from a restricted size range has been recommended to maximize the predictive power with a minimum number of samples (Gordon et al., 1980; Wright et al., 1985; Daskalakis, 1996). Numerous power analyses have been conducted for many data sets and, for effects endpoints like growth, it appears that about 100 individuals are necessary to achieve the power to detect statistically significant differences in growth when the growth rates differ by 25% (Salazar and Salazar, 1995). In the Sudbury River study with *Elliptio complanata*, three replicates of 35 animals each were used (about 100 animals), and statistically significant differences were detected among sites (Salazar et al., 1996).

The real predictive power of this approach is the development of critical body residues associated with adverse biological effects. As mentioned previously, there is mounting evidence

demonstrating that there is less variability in using tissue metal concentrations to predict effects than with metal concentrations in water or sediment. Although this approach has been most useful with hydrophobic organic chemicals, the tissue burdens of copper associated with adverse effects in bivalves compiled as part of this review were surprisingly similar for both freshwater and marine species. Given that copper is one of those essential elements which may be at least partially regulated, these findings are even more surprising. It seems reasonable to assume that if tissue burdens of copper have been useful as a predictive tool, that other metals could possibly be even more useful. Unfortunately, since most previous studies have not included effects endpoints, it is difficult to make these comparisons for other metals. As it was, many of the studies where effects information was extracted were not specifically designed for that purpose and may have only tested a few concentrations and had more concentrations been included, more precise information could have provided an even tighter data set. The added benefit of using this approach as part of environmental effects monitoring for the mining industry is that a large data set will be generated under realistic test conditions that could be used to increase the predictive power and accuracy of this approach. Since this is not the primary reason for advocating such a monitoring program it would only be an additional bonus provided with the data available. Eventually, it may be demonstrated that the most definitive assessments are site-specific and that it is necessary to continue such a monitoring program at each of the mines as described. Alternatively, if the dose-response relationships are confirmed by the combined use of laboratory and field studies, it may be possible to only measure the tissue burdens and discontinue the effects measurements. Until this has been accomplished however, it is imperative to use dose-response monitoring.

5. PRACTICAL ASPECTS - Natural populations versus caged bivalves. As a practitioner of these methods, various practical aspects of conducting these types of monitoring studies will be addressed.

5.1. Availability of animals

For tests requiring marine species, availability of animals is not an issue. Mussels, oysters, and clams can often be acquired from commercial culturing facilities or farms. It is also possible to collect large numbers of marine species from indigenous populations. The advantage of using animals from farms is that they are of more equal size and genetic composition. They also generally have cleaner shells because of their faster growth. Most importantly of all however, since the animals must be uncontaminated for human consumption, sites are rigorously tested before farms were established and then subsequently sampled at regular intervals to assure that animals are always uncontaminated.

This is more of a problem with freshwater species, In general, freshwater bivalve species are more diverse but there appears to be fewer numbers available. In the case of the most commonly used species such as *Elliptio* and *Anodonta,* animals are generally available but not always in the area of concern. In these cases, transplantation again appears to be the most viable option. In the Sudbury River experiment, for example, *Elliptio complanata* was collected in New Hampshire, because animals in the immediate vicinity of the study site co-occurred with other species of freshwater bivalves that are considered threatened or endangered. This is not an issue with marine species.

Another problem that is more unique to freshwater than marine environments is the potential introduction of exotic species. Regulators are becoming more aware of this in the marine environment, not just in terms of exotic bivalves themselves, but subspecies or species carrying diseases, parasites, or other unwanted pests. Regulations regarding the transport of marine and freshwater species have been established in many Canadian provinces. Many states in the U.S. also have regulations for the transport of species to minimize the potential for introduction of exotic species. Many of these states have or will have quarantine protocols to follow before native mussels can be considered zebra mussel-free. In some cases collections by

recognized experts may be sufficient to qualify for a transport permit in the U.S. As a practical matter, it should be mentioned that acquiring a permit for transporting bivalves could take months or longer, particularly if sensitive species are involved. Another point that should be mentioned here regarding availability of test animals is the time of year for conducting the test. It should be made very clear that animal health and reproductive status is of ultimate importance for conducting a successful test. Practitioners of this transplant methodology need to convince potential clients that it is not possible to conduct tests with all species during all times of the year at all sites. The spawning season for example, should be avoided. Often, clients call and want the test conducted within a week or a month without due consideration to animal health and reproductive status or the time required to secure the necessary permits.

One marine species that has already invaded San Francisco Bay is *Potamocorbula*. In freshwater examples of exotic bivalve species include *Corbicula* and the zebra mussel *Dreissena polymorpha*. Unwanted diseases include *Vibrio marinus* in oysters and the unwanted freshwater weed pest *Hydrilla hydrilla*. Any transplant program should be aware of these problems. In the St. Lawrence area, monitoring *Elliptio* has been terminated due to virtual elimination of the species in the area. It is believed that this elimination is attributable to the invasion of *Dreissena*. In fact, the elimination was predicted by modeling and the outcome was almost exactly as predicted. As unfortunate as this has been, if these exotic species are already present in sufficient numbers, they too can be used as a biomonitoring tool. Further, if the indigenous species has been eliminated, the exotic species can be used without interference to the native populations. Some of the most useful information from biomonitoring studies has come from those using either natural populations or transplanted exotic species like *Corbicula* and *Dreissena*.

5.2. Animal collection, transport, storage

Biological suppliers, commercial mussel farmers and universities have been shipping bivalves across country and around the world for decades. The key feature is to keep them cool and moist. This is commonly done with moist towels or newspapers, bags of ice or cold packs and styrofoam ice chests for water column bivalves. They are usually shipped without water to ensure that the animals will not deplete the oxygen supply and putrefy the water. Depending on

the size, sediment dwellers can be shipped in sediment with a small amount of overlying water and oxygen above in a sealed plastic bag. The point is that these methods are commonly used and can be used to ship bivalves overnight to any location in Canada.

5.3. Commercial availability

Dr. Richard Neves has recently compiled a list of laboratories with facilities to hold and/or culture freshwater bivalves in the U.S. as a result of an inquiry on the Internet. This list is provided in Table 31 (Richard Neves, personal communication, March 1997). It demonstrates the large number of facilities available in the U.S. In addition, there are at least six large companies in the U.S. that use freshwater mussels collected by divers for shell export to Japan. About 12 different species are commercially harvested in the U.S. If the species are appropriate for a particular location it would probably be more expedient to purchase live mussels from these commercial suppliers and have them shipped to the study site. It should be emphasized that Table 31 is primarily a list of those currently evaluating and developing handling and holding methods. Currently, the laboratory of Richard Neves is the only one that is routinely culturing freshwater mussels. This culture facility is primarily intended for the propagation of endangered species and not for commercial purposes. While similar culture information was not available for Canadian facilities, it seems logical to assume that they exist. Even if there are not, the fact that these laboratories are functioning in the U.S. demonstrates that such an operation is feasible. Furthermore, as has happened with other consultants in the U.S. and Canada, if the regulations include a requirement for the supply of animals for testing, many firms will adapt and make themselves available to fill the demand. Culture methods have also been developed for some species (Keller and Zam, 1990) and are being developed for others (Anne Keller, personal communication, March, 1997). Similarly, the experience and expertise required to collect animals from indigenous populations and/or conduct transplant studies are simple and straightforward. This does not imply that they are necessarily easy to accomplish and some training may be required to develop the basic skills to perform the tests as they would with any laboratory bioassay.

Table 31. U.S. facilities for holding and/or culturing freshwater bivalve molluscs.

Name	Affiliation	Location
Jerry Farris	Arkansas State University	National Fish Hatchery Mammouth Springs, AR
Teresa Naimo	Biological Resources Division US Geological Survey, La Crosse, WI	Genoa National Fish Hatchery, WI
Jim Layzer	Tennessee Tech	Center Hill Lake, TN Minor Clark Hatchery, KY Elkhorn Station, TN Laurel Hill, TN Birdsong (KY Lake), TN
Dave Hendrix	Neosho National Fish Hatchery	Neosho, MO
Catherine Gatenby	Virginia Tech	Leetown Science Center, WV
Matthew Patterson	Virginia Tech	Leetown Science Center, WV
Rita Villela	Biological Resources Division, US Geological Survey	Leetown Science Center, WV
Tom Watters	Ohio State University	Ohio State, OH
Dick Neves	Virginia	Critz, VA

5.4. Costs

Costs are associated with animal collection, beginning and end-of-test measurements (i.e., length, weight, tissue preparation), deployment and retrieval activities, and chemical analyses. Costs associated with collecting animals from natural populations include the costs of technicians in the field for an appropriate amount of time to collect the desired number. Depending on the water depth at the collection site, SCUBA diving may be necessary to collect the test animals. Analytical costs, which are often the greatest single cost, are well established and can be obtained from any reputable analytical lab.

Although the methods for caging studies are simple, they are not necessarily easy and inexperienced practitioners could make some minor errors which could reduce the reliability of the results. Costs associated with hiring an experienced practitioner to guide the work (excluding travel) and depending on the remoteness of collection and retrieval sites would be approximately $8,000-$10,000 U.S. Only one experienced worker may not be enough however since full concentration is required to maintain the position of each animal for the multiple measurements. It is highly recommended, at a minimum, that two experienced practitioners be used to participate in and oversee the collection, caging, and deployment phases of the work. The cost for two experienced workers would be $16,000 - $20,000 U.S. They would require support from experienced technicians from the agencies, companies or contractors requesting the work. Alternatively, experienced firms could provide a team of experienced workers to conduct the entire study for $30,000-$40,000 U.S. These costs reflect processing about 2400 bivalves (measuring each length twice), with 300 animals per station (three replicates of 100 animals each), as conducted during the last three tests in Alaska shown in Table 27. These three tests were conducted with marine species with no limits on number of animals available. The costs reflect the maximum number that could be processed in one day. Since the number of animals available for testing in freshwater environments may be significantly less, the number of animals and associated costs could also be less. For example, the Sudbury River study with *Elliptio complanata* (Salazar et al., 1996) as shown in Table 27, a total of only 840 animals was used.

5.5. Equipment

The only specialized equipment that is required is the collecting gear, particularly if SCUBA is needed, protective clothing for the collectors if chemical contamination is present, and specially prepared sample jars for chemical analysis that are usually supplied by the firm conducting the chemical analyses. Specialized equipment is required if the growth rings are counted in order to estimate growth rates in the natural population. If a transplant study is conducted, racks can be constructed of inexpensive materials such as PVC and oyster culch netting. Weights and lengths are usually measured in the field with a portable analytical balance and a calipers. An automated system developed by the author uses hardware and software to speed the measurement process considerably. It consists of hardware and software to connect a portable analytical balance and a digital calipers via the RS-232 port on a portable PC. Excluding the cost of the P.C., the cost of this setup is approximately $2,000 U.S.

5.6. Time

On average, a caged bivalve study requires three days to set up and two days to break down, excluding travel time to the site destination. One day is required to collect and sort the animals, one day to weigh, measure, and prepare them for deployment, and one day to deploy caged mussels at the stations. Collection time will vary with the number of animals to collect and the remoteness of the collection site. Water depth can also add significantly to the time required, particularly if SCUBA is necessary for the collections and/or deployments. Similarly, end-of-test activities require one day for retrieval and one day for processing the bivalves (i.e., weights/lengths, and tissue extractions and weights).

5.7. Frequency of sampling

The most important factor to consider in the evaluation of tissue chemistry and growth rates is the necessity for avoiding the spawning period. This is a time when tissue concentrations and growth rates vary dramatically. Most sampling programs only sample once per year and that

approach should be adequate for the mining effluents in Canada.

5.8. Duration of sampling program

One sampling team of experienced field personnel should be able to sample the area in about 2 days using natural populations and 3-4 days if transplants are used - depending on the spatial separation of the sample sites.

5.9. Developing a sampling program - interaction of science, government, industry

Just as the most effective monitoring programs integrate different disciplines, endpoints, and levels of organization, it is also important to integrate science, government, academia, and industry. These types of relationships could be forged during a pilot study to demonstrate the method.

6. RESEARCH NEEDS

Although exposure-effects biomonitoring is relatively new as a science a number of previous studies have demonstrated its utility. However, since this interaction between chemicals in the environment and associated biological is not straightforward there are a number of areas that need better understanding in order to maximize the benefits of this approach. Furthermore, an integrated monitoring strategy should be developed that is consistent with the risk assessment format and the *exposure-dose-response* triad. In that regard, one research element should include a field validation of the monitoring strategy using the traditional sediment quality triad (sediment chemistry, laboratory bioassays, benthic community assemblages) in conjunction with measurements on both indigenous populations and caged bivalves. A second research element should include research on at least three biomarkers that have the potential to indicate effects: (1) metallothionein induction (Couillard, 1996); (2) cellulolytic enzyme induction (Farris et al., 1988); and DNA strand breaks (Black et al., 1996). A third research element should include a comparison of natural populations versus transplanted animals as indicators.

7. RECOMMENDATIONS

Although exposure-effects biomonitoring is a potentially powerful tool and the methods are straightforward, it is important to develop and use standardized protocols in the approach. It is absolutely essential that the monitoring model be developed before proceeding, with agreement between government and industry on data collection and data interpretation. A decision tree would be extremely helpful and an independent review process essential. To foster a working relationship between government, academia, and industry, a pilot study would be extremely helpful. It would also help if the pilot study were conducted in concert with more traditional monitoring methods as described above in order to validate the approach.

8. GLOSSARY

Accuracy - The closeness of a measured or computed value to its true value.

Acute - Having a sudden onset, lasting a short time. Of a stimulus, severe enough to induce a response rapidly. Can be used to define either the exposure or the response to an exposure (effect). For clarity, the length of the exposure (short, medium, or long) and the nature of the effect endpoint (lethal or sublethal) should be specified. The duration of an acute aquatic toxicity test is generally 4 d or less and mortality is the response measured.

Bioaccumulation - General term describing a process b which chemicals are taken up by aquatic organisms directly from water as well as through exposure through other routes, such as consumption of food and sediment containing the chemicals.

Bioaccumulation Factor - A value that is the ratio of tissue chemical residue to chemical concentration in an external environmental phase (i.e., sediment or food). BAF is measured at steady state in situations where organisms are exposed to multiple sources (i.e., water, sediment, food), unless noted otherwise.

Bioassay - "A biological assay (or bioassay) is an experiment for estimating the nature, constitution, or potency of a material (or of a process), by means of the reaction that follows its application to living matter (Finney, 1978). This general definition includes both the aquatic toxicology and the pharmaceutical usage. The pharmaceutical definition of bioassay is a test used to evaluate the relative potency of a chemical or mixture of chemicals by comparing its effect on a living organism with the effect of a standard preparation on the same type of organism. Bioassays are frequently used in the pharmaceutical industry to evaluate the potency of vitamins and drugs.

Bioconcentration - A process by which there is a net accumulation of a chemical directly from water into aquatic organisms resulting from simultaneous uptake (e.g., by gill or epithelial tissue) and elimination.

Bioconcentration factor (BCF) - A term describing the degree to which a chemical can be concentrated I the tissues of an organism in the aquatic environment as a result of exposure to water-borne chemical. At steady state during the uptake phase of a bioconcentration test, the BCF is a value which is equal to the concentration of a chemical in one or more tissues of the exposed aquatic organisms divided by the average exposure water concentration of the chemical in the test.

Biological availability (bioavailability) - the form of various chemicals like metals and metal compounds that affects how and if chemicals are taken up and/or accumulated by organisms.

Biological effect - usually considered an adverse effect measured by changes in physiological condition of test animals in either laboratory or field bioassays. Effect is often used as a synonym for response but many consider effect to be only those adverse biological responses that have been related to effects at higher levels of biological organization like the population or community level.

Biological response - according to Environment Canada regulations, response includes changes in physical, chemical, and biological parameters as a result of the chemical exposure. In common usage however, response generally refers to a biological response endpoint used as an indicator of adverse effects and is often used as a synonym for biological effect.

Biomarker - The use of physiological, biochemical, and histological changes as indicators of exposure and/or effects of xenobiotics at the suborganismal and organismal level. This is an example of a biological response that may not have proven relevance at higher levels of biological organization and therefore could be considered, in that context, as a biological response but not a biological effect.

Biomonitoring (or biological monitoring) - Use of living organisms as "sensors" in water/sediment quality surveillance and compliance to detect changes in an effluent or water body and to indicate whether aquatic life may be endangered.

Biota-sediment accumulation factor (BSAF) - A specific type and form of bioaccumulation factor that is the ratio of lipid-normalized tissue chemical residue to organic carbon-normalized sediment chemical concentration. Note that various other terms for this are used in the literature, including accumulation factor (AF).

Chemical burden - a general term usually referring to the amount of chemical in an organism and usually refers to the chemical concentration in the tissues. However, it could also be used as a synonym for the chemical content or the total amount of chemical within the organism.

Chemical concentration - the amount of chemical per unit of water, sediment or tissue usually expressed in the form μg/L water, μg/g sediment, or μg/g tissue. Generally believed to be the operative toxic unit within various environmental compartments.

Chemical content - the amount of chemical per organism usually expressed in the form μg/organism. Useful for eliminating the effects of growth dilution or degrowth magnification when the tissue weight changes dramatically and effects the chemical concentration.

Chemical speciation - The ionic state or chemical form of metals and metal complexes. It has a direct bearing on biological availability, bioaccumulation, and adverse biological effects.

Chronic - Involving a stimulus that is lingering or continues for a long time; often signifies periods from several weeks to years, depending on the reproductive life cycle of the aquatic species. Can be used to define either the exposure or the response to an exposure (effect). For clarity the length of the response and the nature of the effect endpoint should be specified.

Chronic exposure typically induces a biological response of relatively slow progress and long continuance. The chronic aquatic toxicity test is used to study the effects of continuous, long-term exposure to a chemical or other potentially toxic material on aquatic organisms.

Concentration-response curve - A curve describing the relationship between different exposure concentrations of an agent or material and percentage response of the exposed test population.

Critical body residue - The whole-body concentration of a chemical that is associated with a given adverse biological response. This assumes organisms are a single compartment, rather than the multiple compartments they actually are, but it has considerable utility as a first approximation of dose.

Dose - The quantifiable amount of material introduced into the animal by injection or ingestion is considered to be the exposure dose. The amount of a material at receptor or target sites in the animal that elicits a response is the target dose.

Dose-response curve - Similar to concentration-response curve except that the exposure dose (i.e., the quantity of the chemical administered (e.g., by injection) to the organism is known. The curve is plotted as dose versus response.

Effluent - A complex waste material (e.g., liquid industrial discharge or sewage) that may be discharged into the environment.

Endpoint - In toxicity testing and evaluation it is the adverse biological response in question that is measured. Endpoints vary with the level of biological organization being examined but include changes in biochemical markers or enzyme activities, mortality or survival, growth, reproduction, primary production, and changes in structure (and abundance) and function in a community. Endpoints are used in toxicity tests as criteria for effects.

Environmental availability - The portion of the total chemical material present in all or part of the environment that is actually involved in a particular process or processes and is subject to all physical, chemical, and biological modifying influences. It defines the total amount of material potentially available to organisms.

Environmental bioavailability - The ratio of the uptake clearance divided by the rate at which an organism encounters a given contaminant in a medium (e.g., water, food) being processed by the organism. This is a measure of an organism's extraction efficiency, via respiratory, dietary, and surface absorption processes, from the environmentally available portion of a material.

Equilibrium - In a thermodynamic sense this indicates that both a steady state of flux and an equivalence in chemical activity have been reached in compartments or phases separated by a membrane or boundary across which the chemical fluxes occur.

Equilibrium partitioning (EqP) - An approach for estimating the fate of chemicals (primarily organics) in the aquatic environment that is based on the assumption that a steady-state can be

achieved, and usually is achieved, between the activity of chemicals (usually approximated as a concentration) in the various component phases - water, sediment, organisms. The EqP approach is often exploited, for interpretation and extrapolation purposes, by normalizing chemical concentrations on the lipid content of the aquatic organisms and the organic carbon content of the sediments. These normalized BSAF values are considered to be independent of particular sediments and species.

Exposure - The contact reaction between a chemical or physical agent and a biological system.

Monitoring test - A test designed to be applied on a routine basis, with some degree of control, to ensure that the quality of water or effluent has not exceeded some prescribed criterion range. In a biomonitoring test, aquatic organisms are used as "sensors" to detect changes in the quality of water or effluent. A monitoring test implies generation of information, on a continuous or other regular basis.

Mussel Watch - A generic term used for routine chemical monitoring of bivalve tissues from natural populations and transplanted mussels. Programs exist in Australia, Canada, New Zealand, and the U.S. The state of California has the longest running program and includes both natural populations and transplants. There is even an International Mussel Watch.

Precision - The degree of mutual agreement characteristic of independent measurements resulting from repeated application of a method under specified conditions. The closeness of results.

Quality assurance - A program organized to provide accurate and precise results. Included are selection of proper technical methods, tests, or laboratory procedures; sample collection and preservation; selection of limits; evaluation of data; quality control; and qualifications and training of personnel.

Quality control - Specific actions required to provide information for the quality assurance program. Included are standardization, calibration, replicates, and control and check samples suitable for statistical estimates of confidence of the data.

Reference site - A site that is identical to the treatment sites in all physical, chemical and biotic properties except for the presence of chemicals of concern.

Risk - A statistical concept defined as the expected frequency or probability of undesirable effects resulting from a specified exposure to known or potential environmental concentrations of a material. A material is considered safe if the risks associated with its exposure are judged to be acceptable. Estimates of risk may be expressed in absolute or relative terms. Absolute risk is the excess risk due to actual exposure. Relative risk is the ratio of the risk in the exposed population to the risk in an unexposed population.

Statistically significant effects - Effects (responses) in the exposed population that are different from those in the controls at a given statistical probability level, typically $p \leq 0.05$. Biological endpoints that are important for the survival, growth, behavior, and perpetuation of a species are

selected as criteria for effects. The endpoints differ depending on the type of toxicity test conducted and the species used. The statistical approach also changes with the type of toxicity test conducted.

Steady state - The state in which fluxes of material moving bidirectionally across a membrane or boundary between compartments or phases have reached a balance. An equilibrium between the phases is not necessarily achieved. This is particularly applicable for field monitoring where chemical equilibrium changes with exposure conditions.

Structure-activity relationships (SARs) - Studies relating the structure (and related properties) of chemicals to their activities in biological systems. SARs are used to assist in explaining (and predicting) the occurrence and mechanism of biological responses to chemicals and to aid in prediction of incidence and magnitude. Although lethal and sublethal toxicity test results are often employed, other biological activities, such as bioaccumulation, biodegradation, and biotransformation, may also be used. SARs can also be used to explain or predict physicochemical characteristics of chemicals. Also called quantitative structure-activity relationships (QSARs).

Sublethal - Below the concentration that directly causes death. Exposure to sublethal concentrations of a material may produce less obvious effects on behavior, biochemical, and/or physiological function, and histology of organisms.

Toxicity - The inherent potential or capacity of an agent or material to cause adverse effects in a living organism when the organism is exposed to it. See Direct toxicity and indirect toxicity.

Toxicity test - The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of a chemical).

Toxicodynamics - The phase of toxic action that comprises the uptake (absorption), distribution, metabolism, (and/or biotransformation), and elimination (excretion) of bioavailable chemical by the exposed organism.

Toxicological bioavailability - The classical pharmacological/toxicological definition of bioavailability. It is the fraction of the total exposure dose extracted from the medium that is actually adsorbed/absorbed by the organism, is distributed by the system circulation, and is ultimately presented to the receptor sites of toxic action.

Transplant - Collecting animals from natural populations or cultures in clean environments and placing them in areas of concern with elevated concentrations of chemicals of concern to characterize exposure and/or effects.

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