Mytilus edulis SHELLS AS ENVIRONMENTAL RECORDERS FOR LEAD CONTAMINATION

by

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ABSTRACT

Marine contamination has been the object of a number of scientific studies, including the use of bivalves as biological monitors: This study investigates the feasibility of using *Mytilus edulis* shells as environmental recorders for lead contamination.

Samples of suspended particulate matter, surficial sediments and live mussels were collected from three locations: New Brunswick's northshore, in an industrialized region involved with the refining (Belledune Harbour) and stockpiling (Dalhousie Harbour) of lead/zinc ores; tidal flats near an urbanized area (Rimouski, Québec), and a remote estuary (Negro Harbour, Nova Scotia). Organic and inorganic carbon fractions of the sediments were determined by combustion. Major and trace elements were determined by X-ray fluorescence spectrometry. Sequential extraction techniques were employed to study the partitioning of iron, manganese and lead among various sedimentary phases. Atomic absorption spectrometry was applied for the analyses of these three metals, as well as the lead obtained from the acid digests of the suspended particulate matter, tissue and shell components. A method was developed to separate the outer calcitic and inner aragonitic shell layers. Scanning

electron microscopy was used to define the separation boundary between these two shell layers. The number and thickness of the "annual layers" in the nacreous shell component were determined using acetate peels. The variation of lead contamination occurring during the mussels' lifetime was estimated by subsampling the oldest and most recently deposited nacre in the shells.

The total lead concentrations in the sediments collected from Belledune Harbour, Dalhousie Harbour, Rimouski and Negro Harbour were 941, 108, 21 and 29 µg/g, respectively. Similar amounts of lead were extracted from the sediments except at Negro Harbour where only 13 µg/g lead was extracted. The highest and lowest tissue (shell) lead levels were detected in mussels collected from near Belledune Harbour and Negro Harbour, 456 (49) and 2 (<0.5) µg/g, respectively, whereas mussels sampled from Dalhousie Harbour and Rimouski contained comparable amounts of lead, 9 (0.8) µg/g. The tissue and shell lead concentrations were not influenced by mussel size. The lead levels in the nacreous shell layers were correlated (r:0.966) with those in the tissues, and were best related to the lead obtained from the second fraction of the sequential extraction, lead bound to carbonates. The higher phosphorus concentration at one station in Belledune Harbour, B3 (1.33%), may have suppressed the uptake of lead in the mussels at this site, 150 µg/g tissue Pb and 13 µg/g shell Pb. Significant changes

of lead concentrations within the nacreous shells of mussels collected near Belledune Harbour (19 and 45 µg/g), reflected variations of lead levels in sediments.

This study demonstrates that *Mytilus edulis* shells, reflect the biological uptake of lead during the lifespan of the organisms, and serve as environmental lead recorders.

To the new kid on the block,

Maxime

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Chapter 1: INTRODUCTION

1.0 SURVEYS OF TRACE METAL ABUNDANCE USING WATER, SEDIMENTS, OR BIOLOGICAL INDICATORS.

The pollution of coastal areas by trace metals can be studied by the analysis of trace metals in water, sediments, or some members of the indigenous biota common to all regions. Because of the present knowledge of trace metal cycling in the ecosphere at the present time, any one of these approaches may be criticised. The choice of study method must also take into account the applicability to the final method to other areas, expense, instrumentation and personnel needed, etc.

1.1 Water Analysis

Analysis of seawater is perhaps the most obvious way of assessing contamination-and many authors have reported data specifically concerning the concentrations of trace metals in water from open ocean areas, nearshore or coastal areas or estuaries. Trace metals in water exist partly in

solution (dissolved metal fraction) and partly in suspension bound to organic or inorganic particulate matter (particulate metal fraction). In addition, a certain amount of metal exists in colloids or chelate which may be difficult to assign to either soluble or particulate fraction. This boundary has been arbitrarily based on whether the metal passes through, or is retained by a 0,45 µm pore size filter.

The main disadvantages of identifying polluted areas by the analysis of the metal concentration in water are:

1° Analysis of the low metal concentrations found in most water samples requires the pre-concentration of large volumes of water, either by chelation-extraction (Brooks *et al.*, 1967), by resin (Riley and Taylor, 1968). This is expensive, laborious and the number of steps involved increases the possibility of either positive or negative contamination. Jones (1982) concluded that owing to the use of different methods by different workers, intercomparability of results for even abundant metals such as zinc is unsatisfactory.

2° A knowledge of the total (or dissolved) concentrations of metals in water does not always allow the prediction of possible toxicological effects on the biota. The presence of humic acids or other complexing or chelating agents may render some of the metal unavail-

chelating agents may render some of the metal unavailable to the biota, thus causing over-estimation of metal toxicities based on consideration of the metal concentrations in water (Jenne and Luoma, 1977; Zamuda and Sunda, 1982).

3º Perhaps the main disadvantage of water analyses as a means of comparing locations for their degree of metal pollution is the large variation in concentrations of metals encountered in water with differences in season, the extent of freshwater run-off, depth of sampling, the intermittent flow of industrial effluent and hydrological factors such as tides and currents. The interacting effects of these variables may lead to as much as a 10-fold variation in the concentration of any one metal encountered at any one location (Phillips, 1977). This variation is particularly evident in estuarine areas.

1.2 Sediment Analysis

The use of sediments to define areas of trace metal pollution has been reported in several studies. Earlier studies employed methods for the determination of total metal concentrations and therefore the results included natural variation in the trace metal content of the sediments, as well as concentrations of anthropogenic metals.

It also implied that all forms of a given metal had an equal impact on the environment, which is not always the case (Tessier *et al.*, 1979). This prompted some authors either to report a natural concentration derived from studies of reference areas or pre-colonial sediments (Chester and Stoner, 1975). Sediment grain size was also shown to affect the concentration and distribution of metals (Tessier *et al.*, 1982; Mudroch, 1984).

In the sediment bed, an oxidized layer usually separates anoxic subsurface sediments from the water column. This oxidized interface, operationally definable as a brown layer of sediment with a positive redox potential, has been shown to be the most active zone of metal interaction in the aquatic environment (Johnson, 1974). Several components varying in quantity and metal binding capacity make up this surficial layer (Jenne, 1977). The predominant components of fine-grained, oxidized estuarine and coastal sediments which bind trace metals include hydrous oxides of iron and manganese, organic matter and carbonates (Luoma and Jenne, 1976). Clays and other alumino-silicate mineral surfaces also have some metal binding capacity, but the strongest of metal association with clay surfaces is weak relative to metal associations with other competing components such as organic matter and iron oxides (Jenne, 1977). Furthermore, these clay particles are usually coated with other components such as hydrous oxides or organics and thus unlikely

to have a large number of unoccupied binding sites (Oakley *et al.*, 1981).

Efforts to quantitatively describe metal partitioning in natural sediments have involved a search for extractants which selectively remove forms of metals from sediments. Studies with well-defined components of sediments have demonstrated that few, if any, extractants specifically remove metals from only one component (Luoma and Jenne, 1976; Guy *et al.*, 1978, Tessier *et al.*, 1979,1980). However, some studies have demonstrated the possibility of estimating the metal bioavailability from concentrations of metals in the sediments (Luoma and Bryan, 1978; Langston 1980, 1982; Breteler *et al.*, 1981; Tessier *et al.*, 1984). Others have suggested a modeling approach, similar to those in describing metal adsorption to well-defined component surfaces (Oakley *et al.*, 1980), to help unravel the complexities of the partitioning process, in lieu of direct extraction techniques (Luoma and Davis, 1983; Mackay and Paterson 1981,1982).

As most studies of metal pollution are orientated towards the prediction of effects on the ecosystem or human health, this inability to predict consistently the metal availability would appear to be the greatest disadvantage to the analysis of water or sediments for trace metals.

1.3 Biological Indicators

The use of a biological indicator organism provides, ideally, an estimate of metal availabilities to the biomass of different regions. In studies of the abundance of toxic pollutants, a biological indicator can be generally defined as an organism which may be used to quantify relative levels of pollution by the measurement of the toxicant concentration in its tissues. Either the entire organism, or a part of it, or a single tissue[1] (which may sequester metals from the rest of the organism) may be used.

1.3.1 Trace Metal Uptake Routes

The choice of organism is important, as this defines the particular trace metal load measured in a survey. Metals can be derived by three possible routes: from solution, from the ingestion of food, and from the ingestion of particulate material containing metals. Not all indicator types reflect all three trace metal loads. Filter-feeding bivalves, such as *Mytilus edulis*, obtain trace metals from all three routes (Moore 1971). The exact proportion of the total body trace element content derived from each of the three routes in bivalves is uncertain. Studies have suggested that trace metal uptake from food is the most important route in

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[1] from this point on the term "tissue" will include only the soft parts, and the hard part will be referred to as "shell".

Mytilus edulis (Pentreath, 1973; Phillips 1976). Schulz-Baldes (1974) observed, however, that lead uptake by the same species from solution alone or food alone occurred at a similar rate when exposure concentrations were the same. Several authors have also shown that the uptake of metal by the ingestion of inorganic particulate matter by bivalves is also significant (Raymont, 1972; Preston *et al.*, 1972; Boyden and Romeril, 1974). The ability of bivalves to respond to each of the three possible absorption routes of metals may be an advantage over other types of indicator organisms in terms of their suitability as indicators of total metal pollution. This is especially important in estuarine areas, where variable amounts of inorganic and organic particulate material are present in the water column. Metal uptake by endocytosis, the engulfment of particulate metal by the epithelial cell, has been observed in molluscs (Coombs, 1980). George *et al.*,(1977, 1978) observed the uptake of various iron complexes in *Mytilus edulis* by this mechanism, and suggested it was unlikely such uptake would be confined to a single metal form. The quantitative contribution of endocytosis to metal uptake has not been established (Luoma, 1983).

1.3.2 Criteria for Selecting Biological Indicators

The choice of an organism as biological indicators is quite complex. The properties of an ideal indicator were

reviewed by Butler *et al.* (1971), updated by Haug *et al.* (1974) and Phillips (1977) as follows:

1º The organism should accumulate the pollutant without being killed by the levels encountered.

2º The organism should be sedentary in order to be representative of the area of collection.

3º The organism should be abundant in the study region.

4º The organism should be sufficiently long-lived to allow the sampling of more than one year-class, if desired.

5º The organism should be of a reasonable size, giving adequate material for analysis.

6º The organism should be easy to sample and hardy enough to survive in the laboratory, allowing defecation before analysis (if desired).

7º The organism should tolerate brackish water.

8º The organism should exhibit a high concentration factor for metals, allowing direct analysis without pre-concentration.

9º A simple correlation should exist between the metal content of the organism and the average metal concentration in the surrounding water.

10º All organisms in a survey should exhibit the same correlation between their metal contents and those in the surrounding water, at all locations studied, under all conditions.

Many types of biological indicator organisms studied, were reviewed by Bryan *et al.*, (1985). The best-studied indicators are undoubtedly bivalve molluscs and macroalgae. In the former group, *Mytilus edulis* is probably the most-studied as it satisfies the first eight requirements listed above. Its extensively studied physiology and the amount of accumulated knowledge concerning its uptake of metals may enable us to elucidate the effects of biotic and abiotic factors on the uptake of trace metals, alluded to in the last two requirements. Its increasing commercial importance also makes it economically interesting to study.

2.0 VARIABILITY of TRACE METAL CONCENTRATIONS in TISSUES

Although analyses of mussel soft parts offer a comparatively fast method in assessing environmental quality, the data sets are usually associated with a high degree of variability. Coefficients of variations of metal concentrations in tissues ranging from 40 to 60% were reported by several authors (Shimizu and Tsuji, 1980; Lobel *et al.*, 1982). Gordon *et al.*, (1980) reported that natural populations of mussels often exhibited considerable natural variability which complicated attempts to detect changes resulting from anthropogenetic activity. The factors most frequently influencing the statistical variability in trace metal analysis of bivalve soft tissues are: depuration of the gut contents (i.e., allowing the bivalves to rid

themselves of their gut contents), methods used to homogenize the tissues, seasonal fluctuations of environmental trace metal levels, body weight and physiological condition of the organisms.

2.1 Effects of depuration

There is some disagreement on the effect of depuration on metal analyses. Gordon *et al.* (1980) noted that although the ingestion of suspended sediment by mussels was a major source of variability, much of it could be significantly reduced when the specimens were routinely depurated. La Touche and Mix (1982a) studied the effects of depuration on the concentration of 5 metals (Cd, Cu, Mn, Ni and Zn) in the gonadal and somatic tissues of *Mytilus edulis* and concluded that depuration effects may be more complex than expected. After depuration, manganese levels decreased significantly in both tissues while copper and nickel concentrations increased in the somatic tissues. Although the differential migration of the metal shouldn't affect the variability of trace metal analyses performed on whole specimens, it may enhance the variability when organs or tissues are analysed separately.

2.2 Metal Fractionation Among Various Tissues/Organs

Data in the literature report differences in the accumulation and distribution of trace metals between vari-

ous organs of bivalves. Martincic *et al.*, (1984) observed that trace metal concentrations varied by as much as two orders of magnitude depending on the tissue studied. Dunstand *et al.*, (1980) noted that the kidney was the primary site for metal accumulation in *Mytilus edulis*. Similarly, Schulz-Baldes (1974) found 50 to 70% of total lead in the kidney, whilst the organ formed only 7% of the dry weight of the mussels. These large variations in metal concentrations can pose serious problems of cross-contamination during the dissection of a specific tissue or organ (Patterson and Settle, 1976) thereby increasing the variability. Workers who focus on the analysis of "whole" specimens are not exempt from metal variability. Since the favored mussel size ranges between 4 and 6 cm (shell length) and yields too much material to be totally digested, these studies usually analyse a subsample from the specimen. A limited survey of the literature revealed that the pestle and mortar was the most popular method (>80%) employed to grind the mussel soft parts. It is difficult using this method to completely homogenize the tissues, and one is frequently left with feather-like strands (gills and muscle) and granular material (visceral organs).

2.3 Environmental Fluctuations of Trace Metals

Environmental trace metal levels (dissolved or particulate) can vary considerably during the year, espe-

cially in temperate regions. Buckley and Winters (1983) have calculated that about 85% of the annual supply of suspended particulate matter, and 30-55% of the annual supply of labile metals enter a temperate estuarine system in less than 40 days during the spring run-off. Fowler and Oregioni (1976) suggested that the seasonal maximum of trace metal concentrations observed in mussel samples was partly due to high winter run-off increasing the amount of available metals. Trace metal enrichment of other indicator species linked to climatic changes have also been reported in other studies (Frazier, 1975; Purchase and Fergusson, 1986a). Furthermore, Fowler and Oregioni (1976) found differences between each metal in terms of the amount of their seasonal fluctuation in mussels. They observed that the ratio between the seasonal maximum and seasonal minimum concentrations was greatest for chromium (factor of 8.8) and least for zinc (factor of 2.0) and attributed this to differences in the biological half-lives of metals in mussels. All these effects could introduce considerable variability in monitoring studies which neglect to adequately synchronize yearly sampling programs.

2.4 Trace Metal Concentration/Size Relationships

Boyden (1974) focussed on the relationship between trace metal content and the body weight of indicator organisms and observed that smaller mussels were richer in

trace metal than larger ones. He subsequently showed that the regression coefficient of the relationship between metal content and body weight was generally constant for a given metal and species (Boyden, 1977). Cossa *et al.* (1980) similarly observed that smaller mussels sequestered more trace metals but the regression coefficients they obtained were not uniform and varied with the different sampling sites and different seasons. Phillips (1976) noted that smaller mussels did not consistently contain more trace metals and later reported (Phillips 1980) that cadmium and lead concentrations were higher in larger mussels. De Wolfe (1975) measured higher mercury concentrations in larger mussels at one station but noted that no consistent trend was observed in two other localities. In laboratory controlled experiments, Ritz *et al.*, (1982) noted that the initial cadmium and lead concentrations were higher in larger mussels, but accumulation rates were higher in the smaller specimens. Higher accumulation rates in smaller mussels were also noted by Schultz-Baldes (1974) and agree with observations by Walne (1972) in which filtration rates were negatively correlated with tissue weight of mussels.

2.5 Reproductive and Physiological State of the Organisms

Seasonal changes in the tissue trace metal concentrations associated with the physiological condition of the indicator organisms are the most important source of

variability reported (Pentreath, 1973; Majori *et al.*, 1978; La Touche and Mix, 1982b; Luten *et al.*, 1986). Cossa *et al.* (1980) reported that body weight accounted for most of the variance (>60%) in tissue trace metal concentrations, whereas age accounted for considerably less (≈3%). Dare and Edwards (1975) observed that the dry tissue weight of *Mytilus edulis* varied seasonally up to 50% and was related to spawning. They demonstrated that the individual dry flesh weight was highest in summer and autumn, and decreased through the winter to a post-spawning minimum in spring. This conforms with Phillips (1976) who attributed seasonal changes in metal concentrations to the fluctuations in tissue weights due to gametogenesis and spawning in *Mytilus edulis*. Simpson (1979) indicated that the uptake and loss of both zinc and lead by *Mytilus edulis* were greatly affected by changes in body weight, resulting from the phases of the reproductive cycle and body condition. He not only emphasised the importance of the reproductive state in defining metal concentrations in a mussel, but also pointed out the necessity of using mussels of similar reproductive condition during a monitoring program.

Ritz *et al.* (1982) recognized the effects of extraneous factors and recommended the following precautions when selecting monitoring individual and monitoring periods:

(i) use of immature individuals only,

(ii) use of individual of uniform size,

(iii) use of individual from one population only,

(iv) selection of a relatively uncontaminated area as a source of test animals,

(v) use of a relatively short time period for subsampling,

(vi) allowance for seasonal changes in water such as salinity and water temperature at the monitoring sites.

It seems therefore that tissue trace metal analyses, initially chosen for their convenience over water and sediment analyses, are increasingly difficult to implement adequately. Boyden (1977) remarked that, while it may be possible to minimize variations by careful sampling with reference to biological variables, physiological differences and, more importantly, differences in reproductive state are often unavoidable. Although published information on metal concentrations in organisms collected from the environment is growing rapidly, the lack of attention to sampling techniques may invalidate many of the conclusions concerning the relative abundance of metals in different areas. Much attention is correctly paid to interlaboratory comparisons of analytical techniques in order to maximize the analytical accuracy of the results, but unfortunately no such attention is given to biological variables affecting the organism at the site of collection (Folsom *et al.*, 1972).

3.0 ALTERNATIVE APPROACHES

Zaroogian (1980) casually mentioned relating accumulated trace metal content with a less variable parameter, the protein fraction of the soft body, as an alternative approach for improving the use of bivalves as indicators of environmental trace metals. Fischer (1983) however, specifically focussed on this approach and used the shell weight as an alternative parameter to relate cadmium contamination in *Mytilus edulis*. Rather than attempt to minimize variability in the sampling procedures, utmost heterogeneity of individuals was chosen as a major test criterion in his study. He observed that while soft tissues were related to the physical condition of mussels, the relation of cadmium body burden to shell weight was constant in relation to environmental cadmium concentration. The basic concept to this approach has been to regard metal accumulation as the consequence of organismic immobilization. Therefore to what extent it may apply to other metals will depend on their biological half lives. Some findings suggested that it may be a promising approach for mercury (DeWolf, 1975), lead (Griffin *et al.*, 1980) and zinc (Lobel and Wright, 1982) contamination in *Mytilus edulis*.

3.1 Trace Metal Uptake in Bivalve Shells

Although the biological half-life of a metal varies depending on the particular metal and species studied (Fagerstrom, 1977), Phillips (1977) observed that generally metals have shorter half-lives in the soft tissues of bivalves as compared to other indicator organisms and therefore the time-integration capacity of bivalves is less. Therefore despite improvements concerning tissue trace metal analyses, the information obtained by this method essentially represents environmental metal levels at the time of sampling. Hence, as the tissues are constantly undergoing changes, it is impossible to permanently record any significant changes in environmental trace metal levels. Animal hair, bivalve shells and byssal threads are considered as external "dead" tissues in that they do not participate in any metabolic activity once they are formed[2]. All three concentrate a wide range of elements (Brooks and Rumsby, 1965; Bowen, 1966; Hamilton, 1980). It was not clear at first whether the metals were incorporated directly into or adsorbed onto the surface of the material. Strain and Pories (1966) subsequently demonstrated that the trace element spectrum of hair reflects the nutritional state of the

[2]Cresnshaw and Neff (1969) suggested that the shell serves as an alkali reserve which is dissolved to neutralize acids produced during anaerobic conditions. However, this is a short term process and affects a minor portion of the shell.

animal. George *et al.* (1976) and Unlü and Fowler (1979) similarly concluded that the byssal threads constituted a significant pathway for the elimination of metals. Others have also suggested that the bivalve shell could serve as a receptacle for unwanted chemical species (Comfort, 1949; Bertine and Goldberg, 1972, Ferrel *et al.*, 1973; Talbot *et al.*, 1976). Metal incorporation into bivalve shells has also been demonstrated in the laboratory (Sturesson, 1976, 1978) as well as in field experiments (Frazier, 1976; Carriker *et al.*, 1980).

Coombs and Keller (1981) studied the biological life-time of metals in byssal threads of *Mytilus edulis* and observed that relatively steady metal concentrations were maintained throughout the study. Sturesson (1978) similarly concluded that once metals are incorporated into the bivalve shell, they would be depleted by a process too slow to be of any significance during the lifetime of the animal. Goldberg *et al.* (1978) observed that metal concentrations in mussel shells taken from sites adjacent to highly industrialized areas were markedly higher than those from less polluted environments and that this relationship was not as evident in the tissues. They concluded that, as a consequence of the metals' longer biological half-lives in the shell and perhaps a relatively uniform pumping of metal from the soft tissues to the new shell, the shells may be better environmental recorders of trace metal levels.

3.2 Advantages of Shell Trace Metal Analyses

Koide *et al.* (1982) later noted that bivalve shells offered the following advantages over tissues for the monitoring of trace metals in the environment:

1º shells are easier to handle and store,

2º the problem of whether to depurate the animals before analyses is avoided,

3º shells appear to be more sensitive to environmental trace metal levels (eg. lower variability) over the long term than do the tissues.

3.3 Bivalve Shells as Environmental Recorders

Oddly enough, the analysis of shell material never gained much popularity in trace metal monitoring programs. This may largely be because much of the subsequent information gathered on the trace metal concentration in shells had been obtained incidentally by studies primarily involved in tissue analyses(Keckes, 1968; Romeril, 1971). These studies ignored the complicated nature of the bivalve shells and usually failed to distinguish between the entirely organic periostracum and the underlying carbonate shell layers. The general consensus was that the trace metals were adsorbed onto the shell surface and therefore did not bear any significance to its availability toward the indicator organisms (Bryan and Uysal, 1978; Martincic *et al.*, 1984). It was also

usually concluded that the trace metals were leached out of the shell at variable rates making it difficult to estimate past metal levels (Fang and Shen, 1984).

It is the purpose of this study to quantitatively compare the trace metal concentrations in *Mytilus edulis* shells to the corresponding levels in the tissues, and to establish if past metal levels can be traced from the shells. Lead was the trace metal targeted for this study as it displays a high affinity for shell material (Koide *et al.*, 1982; Dermott and Lum, 1986). Before these goals may be realized however, a satisfactory method for subsampling the shell of *Mytilus edulis* is required. An overview on the principal structures and mechanisms involved with shell secretion would be useful at this point.

4.0 SHELL STRUCTURE AND FORMATION

The shell of *Mytilus edulis* is covered by a thin, pliable, organic layer: the periostracum. The carbonate shell[3] is composed of several layers of calcite and aragonite. Both of these contain varying amounts of organic material, the organic matrix. A brief description of the various structures follows.

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[3] from this point on the term "shell" will exclude the periostracum, but refer specifically to the calcium carbonate components.

4.1 Periostracum

The periostracum consists of quinone-tanned proteins (Degens *et al.*, 1967) and in *Mytilus edulis*, it is composed of three layers which differ in their structure and/or staining properties (Duncachie, 1963). The periostracum may play an important role in the mineralization of the shell, acting as the support and substrate for the initial nucleation and crystal growth of the outer shell layer (Taylor and Kennedy, 1969; Clark, 1976; Petit *et al.*, 1979). The periostracum also has a protective function in preventing the corrosion of the calcified shell.

4.2 Organic Shell Matrix

The molluscan shell contains about 0.3-4.0% (by weight) organic matrix, depending on the species. The content also varies among different forms of structural layers, the *simple prismatic* and *nacreous* structures being the richest (Taylor and Layman, 1972). The organic matrix may be divided into two main components based upon solubility in aqueous solutions (Weiner *et al.*, 1977). The *soluble* matrix has been regarded as intracrystalline (Watabe, 1965; Crenshaw, 1972) while the *insoluble* matrix has been thought to be intercrystalline (Grégoire *et al.*, 1972). The insoluble matrix, conchiolin, is primarily protein with significant amounts of polysaccharides. The principal feature of this matrix is the

preponderance of amino acids having hydrophobic chains (Grégoire, 1972). The soluble matrix is 40 to 80% protein (Krampitz *et al.*, 1983). A water-soluble glycoprotein has been shown to be its primary constituent in both aragonite (Crenshaw, 1972) and calcite (Wheeler *et al.*, 1981). Negatively charged groups predominate in this substance, due to the ester sulfate associated with hexosamine residues (Wilbur, 1976). The soluble matrix has calcium-binding properties (ie, Ca^{2+}-binding polypeptides; Samata and Krampitz, 1982).

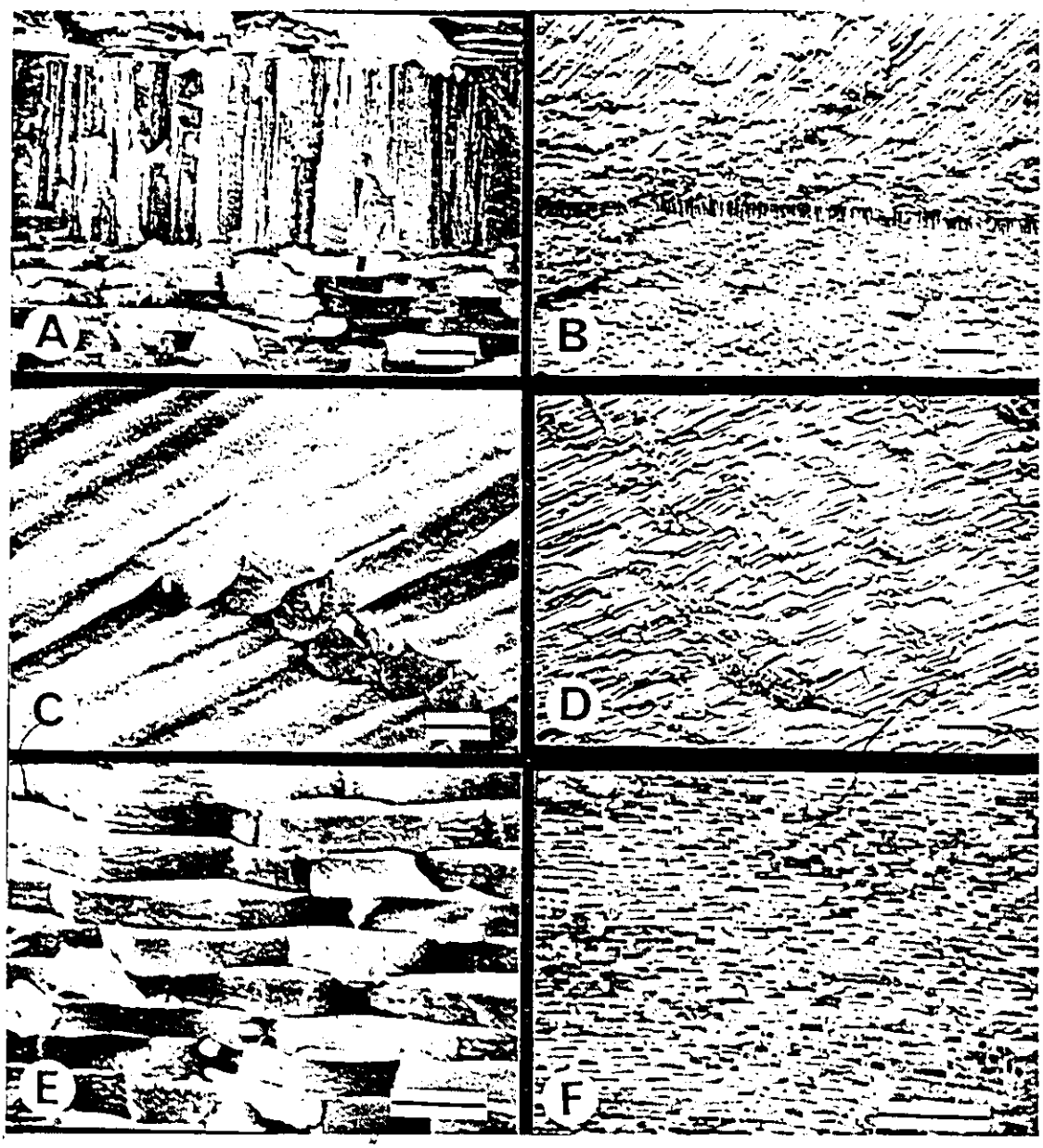
4.3 Shell Mineralogy

Boggild (1930) presented the first comprehensive summary of shell structure types, their mineralogy, and distributions in bivalves and other mollusks. The next major advances came from the use of electron microscopy. Until the work of Taylor *et al.* (1969), which encompassed 22 orders of modern bivalves from 5 subclasses, only a limited number of species and structures had been studied. Aragonite and calcite dominate bivalve shell mineralogy and always occur in separate layers (Boggild, 1930). They occur together consistently in Mytilaceae as well as five other superfamilies of the subclass Pteriomorpha. While certain environmental factors such as salinity and temperature may modify the calcite:aragonite ratio within a shell of a particular

superfamily, the prime control on shell mineralogy is genetic (Kennedy *et al.*, 1969).

Mytilus edulis has a two-layered shell composed of calcite and aragonite. The pallial myostracum consists of irregular aragonite prisms (Fig. 1.1A) and separates the calcite and aragonite layers (Fig. 1.1B). This structure is relatively thin (4 µm) and is therefore considered as a band rather than a distinct shell layer. The outer layer of the shell is categorised as prismatic calcite and consists of columnar prisms (1 -2 µm thick), polygonal in section and up to 50 µm long (Fig. 1.1C), which are arranged in sheet-like rows (Fig 1.1D). A thick wall of conchiolin separates the individual units of calcite. The inner nacreous shell layer is made up of tablet-like aragonite crystallites 5 µm in length and 0.5 µm thick (Fig. 1.1E) which are deposited in regular layers parallel to the shell interior (Fig. 1.1F). The layers of tablets are separated by an interlamellar sheet of organic matrix. Individual nacre tablets are separated and surrounded by an envelope of *intercrystalline* organic matrix. Each nacre tablet or crystal is made up of smaller blocks, each surrounded by *intracrystalline* matrix resembling the intercrystalline matrix in thickness and general structure, while further intracrystalline matrix is present within these smaller blocks (Watabe, 1965).

Figure 1.1. Scanning electron micrograph of a fractured anteroposterior longitudinal section of the calcite and aragonite shell layers of *Mytilus edulis* L. The outer and inner shell surfaces are toward the top and bottom respectively, of the micrographs. Scale bars are 1µm for A,C,E and 10µm for B,D,F. [A] irregular aragonite prisms of the pallial myostracum. [B] myostracal band separating the outer (top) and inner (bottom) shell layers. [C] polygonal calcite prisms of the outer shell layer. [D] sheet-like arrangement of the calcite prisms of the outer shell layer. [E] tabular aragonite crystals of the inner nacreous layer. [F] nacreous tablets arranged in steplike patterns characteristic of bivalve nacre.

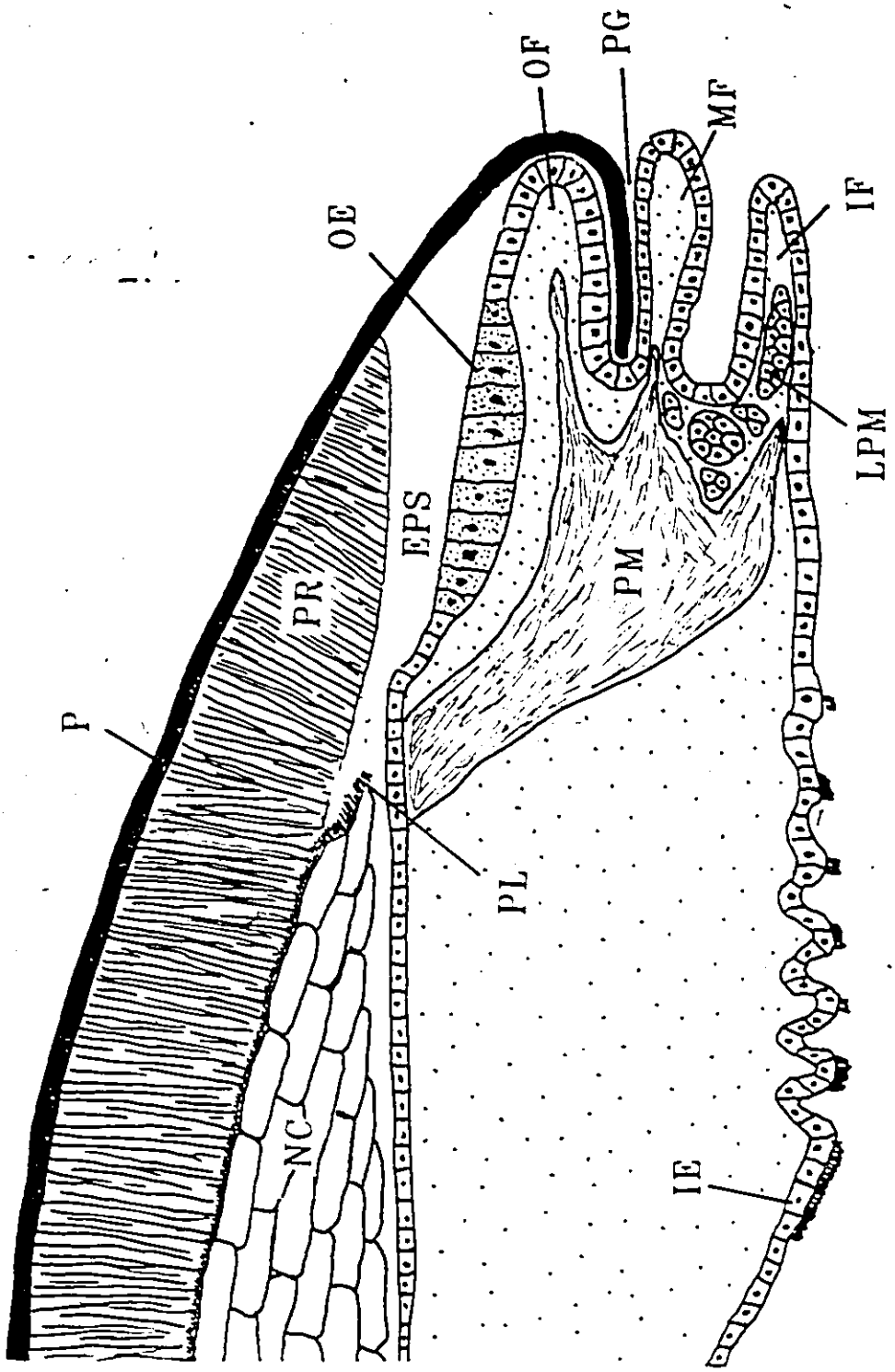

A
B
C
D
E
F

4.4 Shell Formation

The mantle, a thin organ lining the inner shell surface, is directly responsible for the deposition of the crystals and the secretion of the organic matrix of the shell. The mantle edge is divided into three folds---an inner muscular, a middle sensory and an outer secretory fold (Fig. 1.2). The total system of shell formation in bivalves comprises four compartments: (1) the external medium, (2) the hemolymph and body tissues, (3) the extrapallial fluid compartment between the mantle and the inner shell surface, and (4) the shell. Ions from the outer medium are transferred to the hemolymph and body tissues via the body epithelium (inner epithelium). The mantle epithelium (outer epithelium), transfers calcium, other ions and various organic molecules from the hemolyph to the extrapallial fluid which is effectively sealed from the outer medium (Simkiss and Wilbur, 1977).

Shell secretion begins with the formation of the periostracum by the epithelial cells on the inner side of the outer mantle fold, in the groove lying between the middle and outer folds (i.e., periostracal groove). The first calcareous material is laid down from the epithelial cells of the outer part of the outer fold. The rest of the shell is laid down by the cells of the general outer surface of the mantle. Evidencence suggests that there is a generative zone at the

Figure 1.2. Radial section of the mantle edge of a *Mytilus edulis* shell to show the relationship between the shell and mantle (not to scale). EPS, Extrapallial space; IE, inner epithelium; IF, inner fold; LPM, longitudinal pallial muscle; MF, middle fold; NC, nacreous shell layer; OE, outer epithelium; OF, outer fold; P, periostracum; PG, periostracal groove; PL, pallial line; PM, pallial muscle; PR, prismatic shell layer.


OF
PG
MF
IF
OE
LPM
EPS
PM
PR
P
PL
NC
IE

inner part of the periostracal groove, and cells produced there migrate around the mantle edge, changing their chemistry and function as they do so (Beedham,1958; Dunachie,1963). In *Mytilus edulis* each cell in turn secretes periostracum, outer shell layer (i.e., prismatic calcite), becomes an attachment area at the pallial line (i.e., myostracal band), and then secretes the inner nacreous shell layer.

Although the intricate biochemical reactions governing shell calcification have yet to be fully understood, the following mechanism suggested by Krampitz *et al.* (1983) is one of the most popular theories on shell secretion. The insoluble matrix is secreted first and is anchored to the surface of the secreting cells or in the periostracal groove. The soluble, calcium-binding matrix is fitted into the pores of the insoluble matrix. The total assembled matrix presents a hydrophobic field interrupted by specifically placed hydrophillic, calcium-binding sites. The sites bind calcium and concentrate additional calcium from the parent fluid. A secondary layer, specifically enriched in carbonate, is formed around the calcium-enriched site. A local concentration change occurs, such as an increase in pH or a decrease in carbon dioxide, and a crystal seed is formed at the site. Some of the seeds grow, while others dissolve. The survival of a seed may be determined by local transport processes or by the original, local concentration of the calcium-binding matrix. The initial rapid growth of the crystal is along the

vertical c-axis. Growth along this axis is stopped by the next episodic deposition of a complete matrix assembly. The horizontal growth of the crystal continues until the space between adjacent growing crystals is filled.

5.0 ACTIVE *versus* PASSIVE METAL ENRICHMENT

It is important to distinguish between active and passive metal enrichment in the shell material. Incorporation of trace metals into the shell by the process described in the preceding section cannot be performed without energy, this is called active enrichment. Active enrichment means, in this case, the lead uptake in the carbonate fraction and in the organic matrix, those surfaces which are isolated from the seawater by the mantle. A passive enrichment which takes place on surfaces exposed to seawater , particularly the periostracum and unprotected carbonate, probably comprises several adsorption processes and is not governed by metabolic activity (Sturesson, 1976).

Ideally, the periostracum should prevent adsorption of trace metals directly onto the carbonate shell. This proteinaceous film is, however, rarely intact, leaving large portions of the calcite layer exposed to particulate and dissolved trace metals. Several studies have demonstrated how shell surfaces can effectively adsorb lead from solution (Clarke *et al.*, 1976; Sturesson, 1976). Hence whole shell analysis would render little information on trace metal

bioavailability as it would be impossible to distinguish between the actively and passively incorporated fractions. The inner aragonite layer would be more suitable for such analyses and the author has developed a method by which the often corroded calcite layer is separated from the inner nacreous layer (Bourgoin, 1987a).

6.0 BIVALVE AGE DETERMINATION

Before trace metal levels can be traced back in time to a particular event, an accurate method for age determination of bivalves is needed. Such methods may be grouped into the following 5 categories: (1) the use of mean or modal size-frequency distributions; (2) the measurements of the shell dimensions; (3) the interpretation of growth interruption lines on shell surfaces; (4) experimental methods involving the release and recovery of marked individuals and (5) various isotopic methods. Seed (1973, 1976) has reviewed the weakness related to the first four categories. The chemical determination involved with the last one are time consuming and expensive.

Lutz (1974, 1975, 1976) has demonstrated the effectiveness of another age determination method for *Mytilus edulis*. Using acetate peels of polished and etched longitudinal shell sections, he has shown that annual cycles are reflected in growth increment sequences in the nacreous shell layer of this species. This method was preferred in this

study as it relies on the same shell structure used in the trace metal analyses.

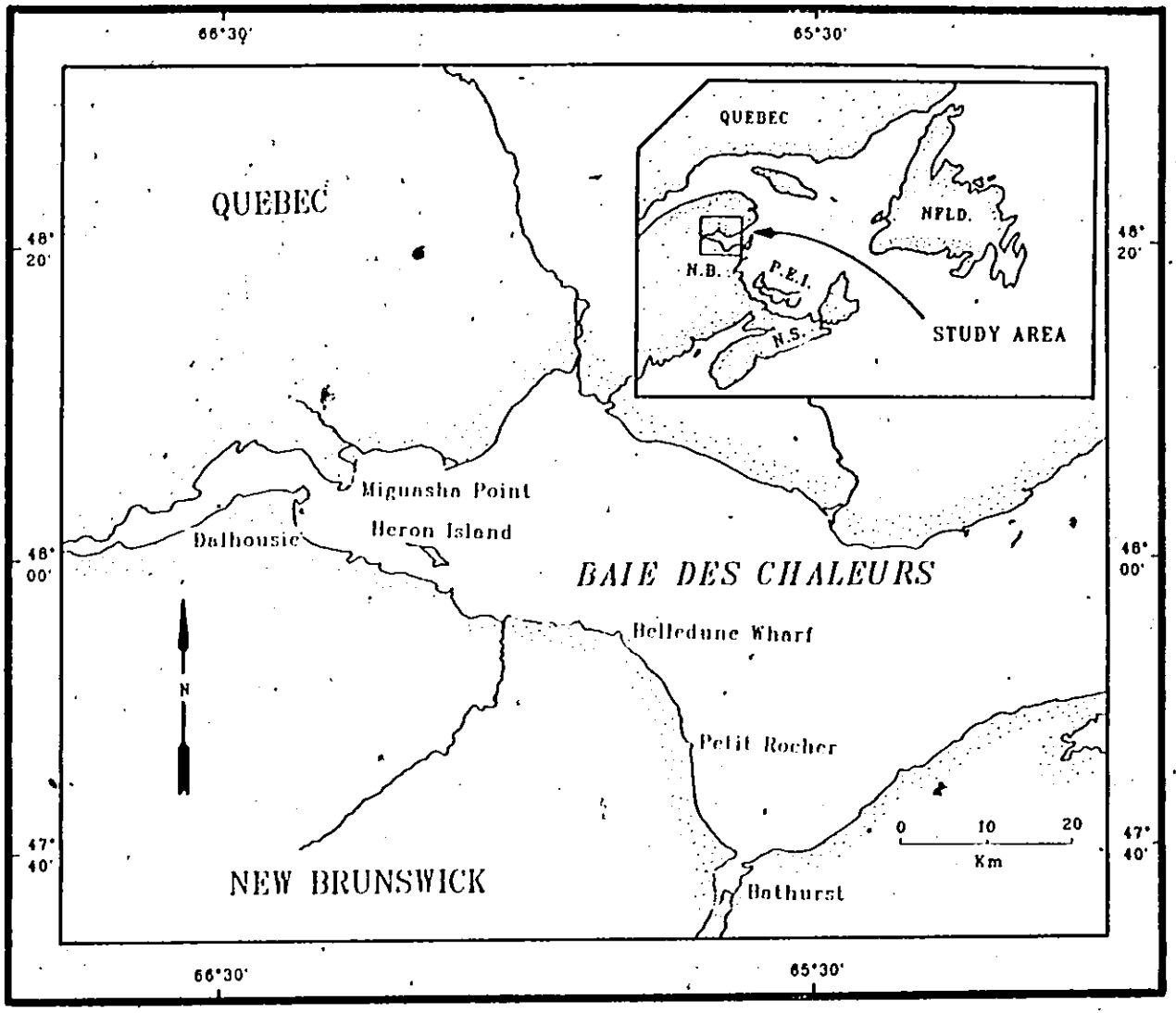
Chapter 2: MATERIALS AND METHODS

1.0 STUDY AREA

Necessary conditions for the present study included the existence of a spatial gradient of lead concentrations in the environment and the presence of an appreciable mussel population along this gradient. Such conditions were met at the Baie des Chaleurs in the Belledune-Dalhousie area (Fig. 2.1). The Baie des Chaleurs opens to the Gulf of St Lawrence and separates the north shore of New Brunswick from the south shore of Québec's Gaspé Peninsula. The bay is approximately 150 km long and 45 km wide at its mouth, where it forms part of the Gulf of the St Lawrence.

The Restigouche River, of which 80% of its watershed is in New Brunswick, is a major freshwater input into the Baie des Chaleurs, draining an area close to 8,000 km^2. The estuary of the Restigouche River extends from Matapedia to Dalhousie and is tidal for about 40 km of its length. Sources of lead contamination are in the tidal portion of the New Brunswick side of the river and include lead/zinc ore loading facilities in Dalhousie and a

Figure 2.1. Location map of the Belledune-Dalhousie study area in the Baie des Chaleurs.


66°30'
65°30'
QUEBEC
48° 20'
46° 00'
47° 40'
QUEBEC
NFLD.
N.B.
P.E.I.
N.S.
STUDY AREA
Miguasha Point
Dalhousie
Heron Island
BAIE DES CHALEURS
Belledune Wharf
N
Petit Rocher
0 10 20
Km
NEW BRUNSWICK
Bathurst

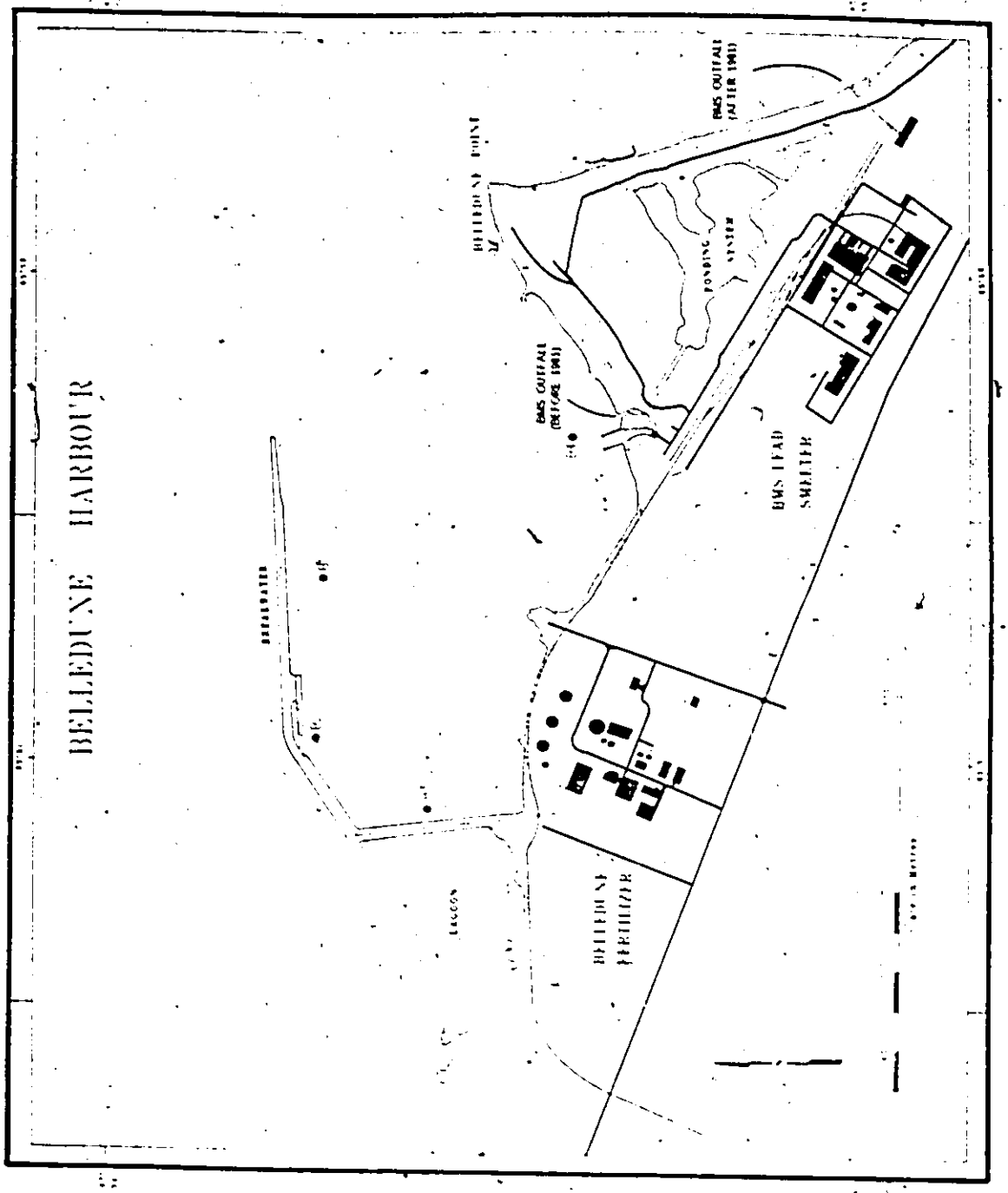
lead/zinc smelting plant and a fertilizer plant in Belledune.

1.1 Belledune Harbour

Belledune Harbour is man-made, bounded by a breakwater to the north and west and by Belledune Point to the east, and is approximately 1 km^2 (Fig. 2.2). There was no industrial activity in the area until 1966, when Brunswick Mining and Smelting Corporation Limited (BMS) began production, followed by the start-up in 1968 of a fertilizer plant (Belledune Fertilizer).

Originally designed to produce both refined lead and zinc, the plant was modified in 1972 to refine only lead. The facility presently consists of sinter and acid plants, a blast furnace and a lead refinery with a nominal annual capacity of 65,000 tonnes of refined lead, 3,500 tonnes of copper matte, 90 tonnes of doré (impure silver) and 200,000 tonnes of sulphuric acid, the latter being piped to the nearby fertilizer plant. Prior to 1981, surface water runoff, process water and slag pond discharge water all contributed significant quantities of cadmium, lead, zinc, arsenic and suspended solids to Belledune Harbour. Significant efforts were made in 1980 toward the control and treatment of liquid discharges with the introduction of effluent treatment and slag water recycle systems. At this

Figure 2.2. Sampling stations (B1 - B4) at Belledune Wharf. Liquid discharges were dumped directly into the harbour near B4 until 1981 when it was moved onto the coast. The gypsum-laden effluent for the fertilizer plant is dumped on the northern side of the breakwater, directly across from B2.


BELLEDUNE HARBOUR
BMS OUTFALL (AFTER 1981)
BELLEDUNE POINT
POOLING SYSTEM
BMS OUTFALL (BEFORE 1981)
BMS LEAD SMELTER
BELLEDUNE FERTILIZER

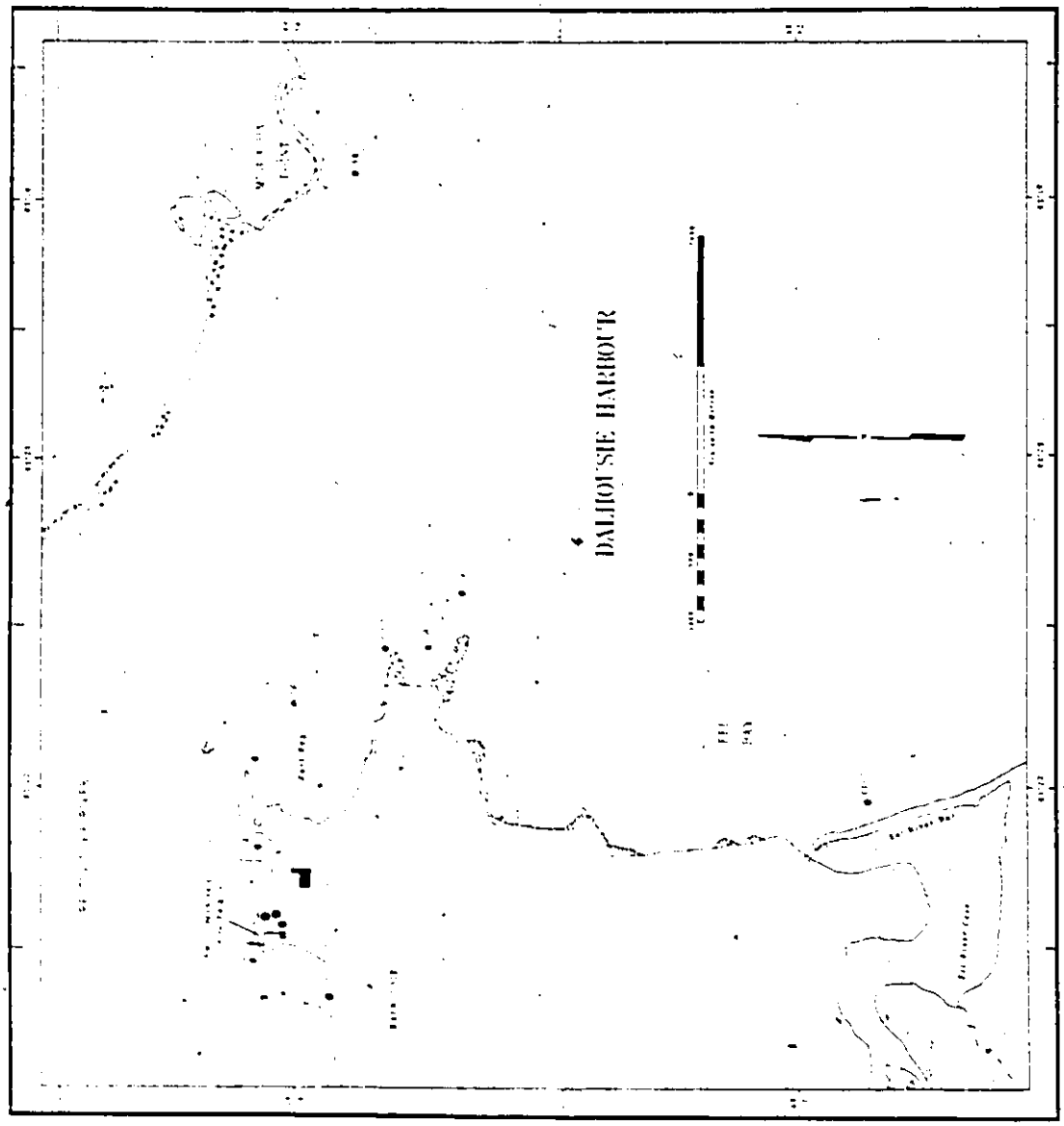
time the smelter's discharge point was also moved to a location outside the harbour (Fig. 2.2).

Belledune Fertilizer began operation in 1968. The sulfuric acid by-product from the smelter is used in combination with ammonia to convert Florida calcium rock (mostly fluorapatite) into diammonium phosphate fertilizer. Approximately 300 tonnes of fertilizer are produced per day. For each tonne of fertilizer produced, 4 tonnes of calcium sulfate (gypsum) is discharged as waste directly in Baie des Chaleurs. The effluent which contains about 4% gypsum also includes significant amounts of trace metals derived from the phosphate rock.

1.2 Dalhousie Harbour

Dalhousie, with a population of approximately 5,000, is a small commercial port situated at the mouth of the Restigouche River. The main source of lead contamination occurs at the Public Wharf which has been used to load zinc and bulk concentrate by Brunswick Mining since 1964 (Fig. 2.3). The lead is mainly introduced into the marine environment from dockside piles due to wind and runoff, as well as during loading (spillage).

Figure 2.3. Sampling stations at Dalhousie harbour (D1 - D9) and at Miguasha Point (MIG) and Eel Bay (EEL). Concentrates are stockpiled at the western part of the harbour and dredging spoils are dumped into "West Bay".


DALHOUSIE HARBOUR

2.0 SAMPLING

2.1 Sampling Stations

In July 1985, a total of 20 stations were sampled as follows:

-- 3 stations at Belledune Harbour (B1-B4, Fig 2.2)

-- 9 stations at Dalhousie Harbour (D1-D9, Fig 2.3)

-- 1 station at Miguasha Point (MIG, Fig 2.3)

-- 1 station at Eel Bay (EEL, Fig 2.3)

-- 4 coastal stations distanced 5 Km apart, southeast of Belledune Harbour (C1-C4, Fig 2.4)

-- 1 station in Negro Harbour (NH, Fig 2.4)

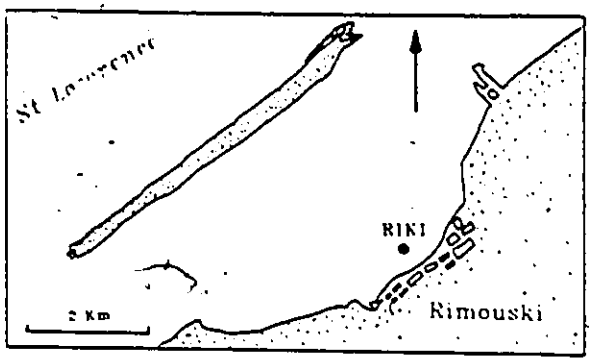
-- 1 station near sewage and storm discharges at Rimouski, Québec (RIKI, Fig 2.4).

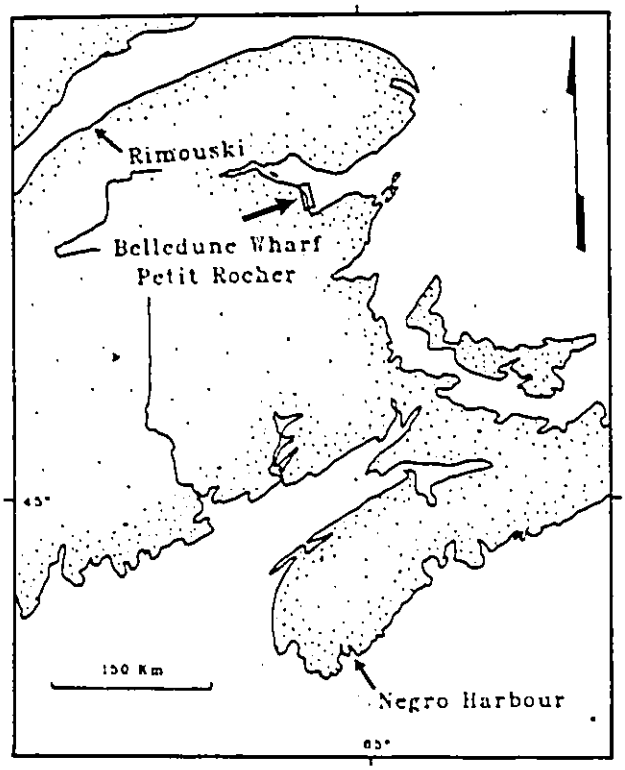
The various types of samples collected along with corresponding depths and bottom salinities are shown in table 2.1.

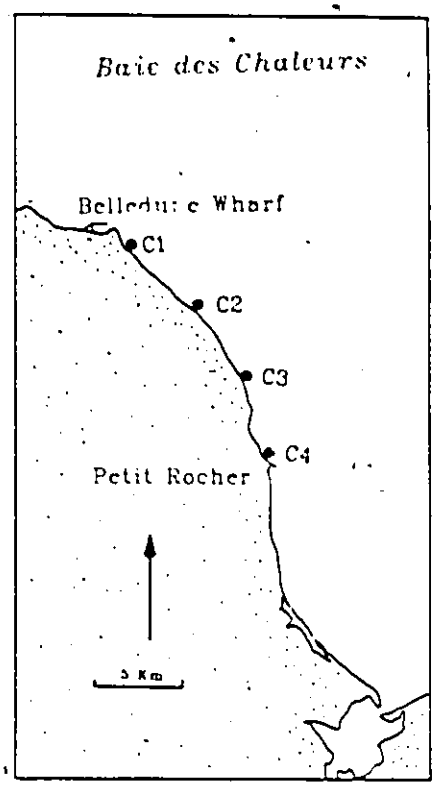
2.2 Suspended Particulate Matter Samples

Water samples were always collected first, at approximately 30 cm from the bottom, during the divers' initial descent to avoid re-suspending the sediments in the water column. An acid-cleaned (Patterson and Settle, 1976) polyethylene bottle (500 ml) was uncapped and closed at the required depth so as to exclude the surface layer. The

Figure 2.4. Location map of sampling stations at Rimouski, Québec (RIKI), the Belledune Wharf-Petit Rocher area (C1-C4), and Negro Harbour, Nova Scotia (NH).


St Lawrence
RIKI
2 Km
Rimouski


Rimouski
Belledune Wharf
Petit Rocher
150 Km
Negro Harbour


Baie des Chaleurs
Belledune Wharf
C1
C2
C3
C4
Petit Rocher
5 Km

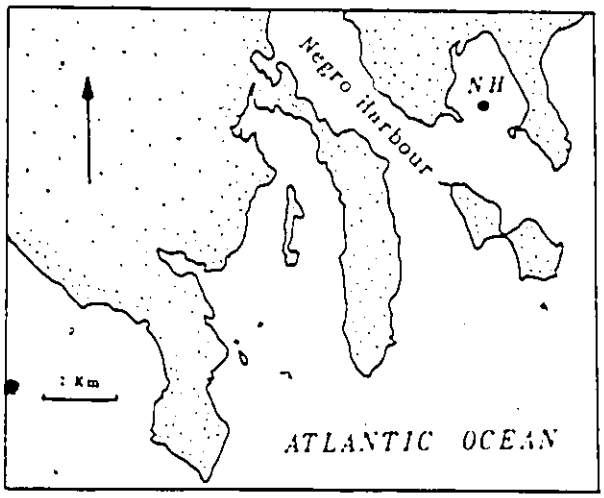

Negro Harbour
NH
1 Km
ATLANTIC OCEAN

Table 2.1 Depth, salinity and types of samples collected at Dalhousie Harbour (D), Belledune Harbour (B), a coastal transect (C), Miguasha Point (MIG), Eel Bay (EEL), Rimouski (RIKI), Negro Harbour (NH).

STATION	DEPTH (m)	SALINITY (%)	TYPES OF SAMPLES sediment	water	mussel
D1	4.1	20	+	+	+
D2	*		+		
D3	3.0	21	+	+	+
D4	3.5	25	+	+	+
D5	3.3	25	+	+	
D6	3.1	25	+	+	+
D7	3.7	23	+	+	+
D8	3.4	27	+	+	+
D9	5.5	26	+	+	+
B1	12.2	24	+	+	+
B2	11.3	24	+	+	+
B3	6.2	24	+	+	+
B4	11.5	24	+	+	
C1	0.5	23		+	+
C2	0.5	24		+	+
C3	0.5	24		+	
C4	4.5	24		+	+
MIG	2.5	20		+	+
EEL	*		+		
RIKI	**		+	+	+
NH	2.5	***	+	+	+

* sampled directly on the intertidal flats
** sampled from tidal pool
*** not measured

volume of water sampled was noted and filtered through pre-weighed Nucleopore filters (47 mm diameter, 0.4 µm pore size) within 48 h of collection. The residue laden filters,

eventually used to determine the suspended particulate matter (SPM), were maintained frozen.

2.3 Sediment Samples

Sediment samples were retrieved by a diver using an acrylic boxcore to avoid trace metal contamination. After the water had been slowly drained from the device, the surficial sediment layer (top 5 mm) was sampled with a clean nylon spatula and transferred into acid cleaned polyethylene bottles. The samples were maintained frozen until further processing in the laboratory (≈60 days).

2.4 Mussel Samples

Mytilus edulis samples were hand collected by divers who avoided any specimens anchored to metallic structures. Except for the mussels attached to the wharf wooden piling at D3, D4 and C4[4] all the other bivalves were attached to rocks. The specimens were allowed to depurate in ambient seawater for 48 h and then frozen pending analyses.

3.0 EXPERIMENTAL

3.1 Fractured Shells for Scanning Electron Microscopy

Dried shell valves were fractured along the desired axis by placing the shell on the edge of a table with the desired transect superimposed directly over the edge and

[4] sample C4 was collected within Petit Rocher Wharf.

applying firm pressure to the unsupported portion. Shell fragments were then mounted on a standard SEM stub and adequate electrical conductivity was assured by tracing a line from the fragment to the stub with silver paint. Loose particles were removed from the fracture surface with clean compressed air and coated with gold-palladium. The detailed experimental procedure is given in Kennish *et al.* (1980).

3.2 Acetate Peels of Shell Sections for Age Determinations

Mussels collected from stations D3, B2 and C1 were used for this study. Clean valves were dried sequentially in 50%, 75% and twice in 100% ethanol, prior to embedding in Spurr's resin (Spurr, 1969). After the resin had hardened, the embedded shells were sectioned along the desired axis, using a diamond rock saw. The cross sections were lightly sanded with a fine grit alumina sandpaper, ground on a glass plate using 600 carborundum powder and then polished with 3000 alumina powder on a high speed lapidary wheel covered with a polishing cloth. The polished sections were etched by immersion for in 1% (v/v) HCl for approximately 45 sec, thoroughly rinsed and air-dried. The etched surface was flooded with acetone to which was then applied a piece of sheet acetate. After the acetone had evaporated (20-30 min), the peel was removed and examined under a microscope.

4.0 ANALYSES

A complete set of glassware was reserved for metal analyses, as recommended by Patterson and Settle (1976), stored in 20% (v/v) nitric acid and washed in deionized water immediately before use. Decomposition vessels were dried in a vacuum oven. Polyethylene gloves and Teflon coated forceps and spatulas were used in handling samples kept in acid cleaned desiccators.

4.1 Pb in Suspended Particulate Matter (SPM)

Water samples were filtered through Nucleopore filters to separate suspended particulates that were dried at 60°C and weighed. The concentration of the SPM was taken to be the weight of the residue divided by the volume of filtered seawater. The Pb content in the SPM was determined by a method developed by Rantala and Loring (1977). The filters were placed in Teflon bombs to which were added 1 ml aqua regia (Ultrex) and 1 ml HF (Ultrex) and tightly covered. The decomposition vessels were heated at 90°C for 1 h. After cooling to room temperature the solution was transfered, through a polyethylene funnel, to a 25 ml polyethylene volumetric flask containing 0.93 g boric acid crystals and 5 ml of deionized water. The filter was washed several times with deionized water, and washings were transferred to the flask. The flask was shaken thoroughly and diluted to

volume. Blanks were prepared the same way but omitting the sample. The solutions were stored in polyethylene bottles and analysed by inductively coupled plasma mass spectrometry (ICP-MS). Quantification of the Pb was achieved by the technique of standard additions.

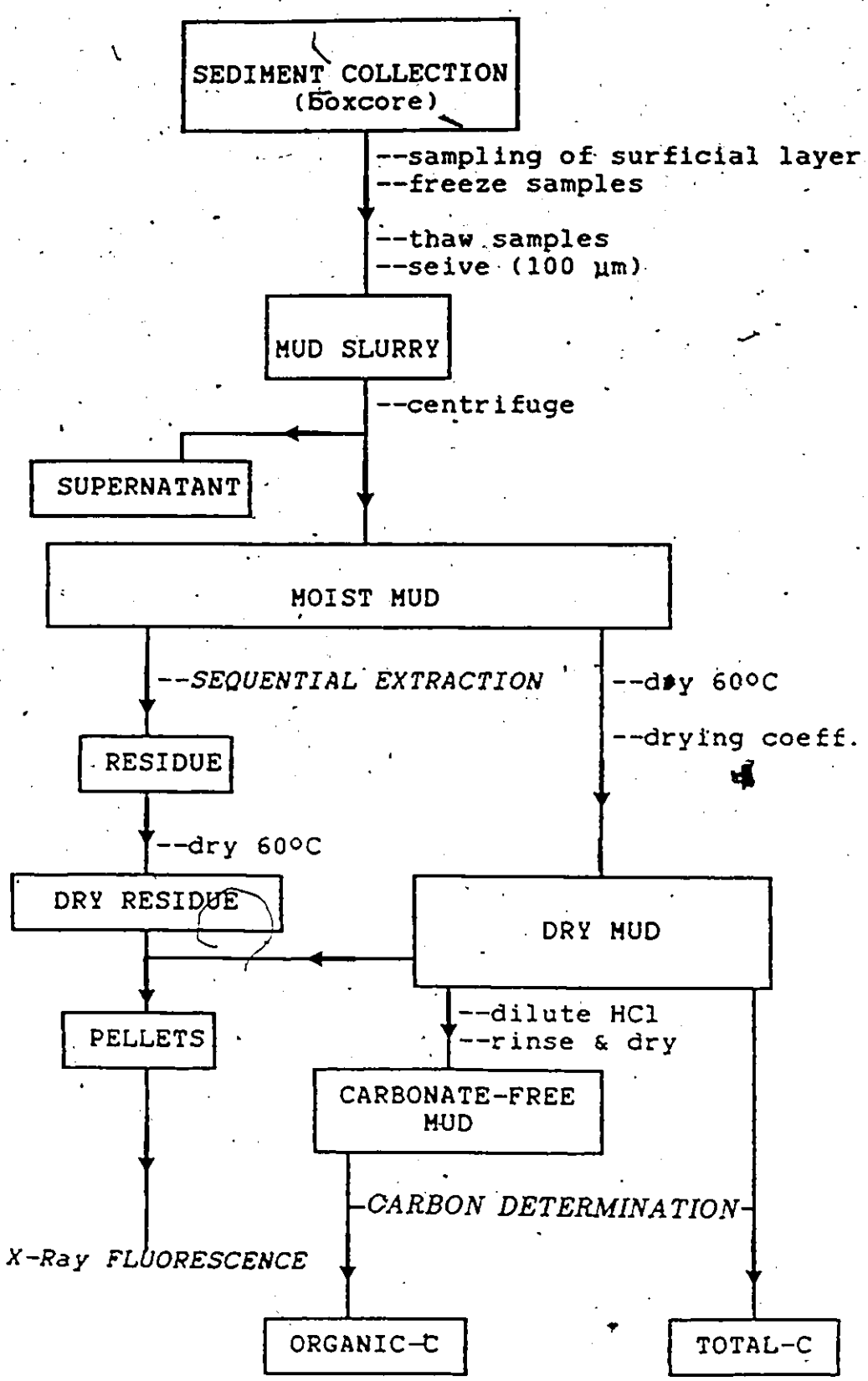
4.2 Metal Determination in the Sediments

Thawed sediments were sieved through 100 µm polyethylene mesh with a minimum amount of deionized water, to remove particle size biases (Mudroch, 1984). The slurry was centrifuged at 12000 g for 20 min. The supernatant was discarded and the sediments were either immediately sequentially extracted for Fe, Mn and Pb or dried at 60°C. Essential sediment analyses are presented in the flow chart (Fig 2.5).

4.2.1 X-Ray Fluorescence Spectrometry (XRF)

Dried sediment subsamples were pressed into pellets to determine the concentration of the major (Si, Al, Fe, S, Ca, Na, Mg, K, Mn, and P) and trace (Cu, Co, Cr, Ni, Pb, V, and Zn) elements by XRF. The analytical precision was tested by analysing five pellets made from a homogenized sediment sample, sample B2. Coefficients of variation of 10% or lower were typically observed (Appendix A.1). The accuracy of the

Figure 2.5. Flowchart for the sediment analyses.


SEDIMENT COLLECTION
(boxcore)
--sampling of surficial layer
--freeze samples
--thaw samples
--seive (100 µm)
MUD SLURRY
--centrifuge
SUPERNATANT
MOIST MUD
--SEQUENTIAL EXTRACTION
--dry 60°C
--drying coeff.
RESIDUE
--dry 60°C
DRY RESIDUE
DRY MUD
PELLETS
--dilute HCl
--rinse & dry
CARBONATE-FREE MUD
CARBON DETERMINATION
X-Ray FLUORESCENCE
ORGANIC-C
TOTAL-C

analyses was checked by running two Canadian Reference standards, BCSS-1 and MESS-1, and a South African standard dard, NIM-G, and comparing the analytical results with the stated references values for major and trace elements.

4.2.2 Sequential Extraction

The sequential extraction procedure was designed to partition elements and compounds, in this study into the following four fractions: (a) M(F1): exchangeable metal. A sediment sample equivalent to 1.5 g dry weight was extracted for 30 min with 8 ml of 0.5 M $MgCl_2$ at pH 7.0; (b) M(F2): metals bound to carbonates. The residue from (a) was leached from 5 h with 8 ml of 1.0 M NaOAc adjusted to pH 5.0 with HOAc; (c) M(F3): metals bound to organic matter. The residue from (b) was extracted at 85°C for 5 h with 5 ml of 30% H_2O_2 adjusted to pH 2.0 with HNO_3 and then at room temperature with 3.0 ml of 3.2 M NH_4OAc in 20% (v/v) acetic acic (HOAc); (d) M(F4): metals bound to Fe-Mn oxides. The residue from (c) was extracted with 20 ml of 0.04M $NH_2OH.HCl$ in 25% (v/v) (HOAc) at 96°C; (e) M(F5): strongly bound metals. The residue from (d) was extracted with 6 ml of aqua regia (3:1 mixture of HCl and HNO_3). The detailed experimental procedures for the first four fractions and that of fraction 5 are given in Tessier *et al.*, (1979, 1980) and Nriagu *et al.*, (1979), respectively.

Extractions were conducted sequentially in Teflon centrifuge tubes (50 ml) to minimize losses of solid material. The refractory nature of the organic matter in some Dalhousie samples, particularly wood particles, caused excessive foaming in certain samples in the third fraction. This problem was overcomed by dipping the tubes in an ice-bath and returning them to the heated water bath once the reaction had subsided. Between each successive extraction, separation was carried out by centrifuging (Sorvall, Model RC2-B) at 12 000 g for 20 min. The supernatant was removed with a pipet and analyzed for the metals, and the residue was washed with 8 ml of deionized water; after centrifugation for 20 min, the washing water was discarded. The residue from extraction "e" was washed with deionized water, dried at 60°C, and analyzed by X-ray fluorescence spectrometry (XRF) to determine the remaining content of the major and trace elements.

For each station, three subsamples of moist sediments were dried at 60°C to determine their water content, thereby establishing an average drying coefficient (Appendix A.2). These coefficients were then used to estimate the dry weight of the sediment samples used in the sequential extraction (Appendix A.3). Dilution factors used to convert metal concentrations in the leachates to metal concentration per gram of dry sediment were calculated by the following formula (Appendix A.4):

$(H_2O_{sediment}$ ml + Extractant ml) ÷ Dry Sediment g

A total of 150 dilution factors (i.e., 30 samples x 5 fractions) were computed (Appendix A.4.1 - A.4.5).

Trace metal concentrations in the leachates were determined by atomic absorption spectrometry (Perking-Elmer Model 603) in flame mode for Fe and Mn and with a HGA-2100 graphite furnace and a deuterium background corrector for the Pb determinations. Quantification was achieved with the following techniques: (i) appropriate calibration curves prepared with the components of the extraction solutions, for Fe and Mn in fractions 1-4; (ii) a standard addition technique for Pb in all fractions and for Fe and Mn in fraction 5.

The coefficients of variation obtained from replicate samples were generally smaller than 10% in all fractions for Fe and Mn, and fraction 5 for Pb (Appendix A.5). The coefficients of variation for Pb varied between 11 and 15% in fractions 2-4.

4.3 Lead Determination in Biological Material

4.3.1 Tissue Lead Analyses

Thawed mussels were shucked as described by Bernhard (1976). A total of 10 mussels per station were analysed separately. Soft parts of every mussel were dried at 70°C,

ground in an agate mortar, weighed and digested with concentrated HNO_3 (BDH, Aristar) in Teflon bombs at 70°C for 2 h. The volume of acid used was proportional to the weight of the mussel analysed; 2 ml of nitric acid per 0.1 g of dry tissue (Cossa *et al.*, 1980). The solution was then transferred to a 25 ml volumetric flask, diluted to volume with deionized water and filtered through a pre-cleaned Whatman glass fiber filter. When larger mussels yielded too much tissue to be entirely digested in the 30 ml bombs, a subsample from a homogenized sample was analysed.

Lead was analysed by atomic absorption spectrometry either in flame mode or by graphite furnace depending on lead concentration in the solutions. The salient analytical conditions are listed in Appendix B.1. Optimum quantification of the metal concentrations was achieved by the technique of standard additions (Borg *et al.*, 1981).The results were expressed in micrograms of metal per gram of dry tissue.

4.3.2 Periostracum Lead Analyses

Mussel valves were cleaned with a nylon brush under running distilled water and dried at 40°c. Periostracal material was sampled directly from three different regions of the shell: 1) near the umbo, 2) the middle portion of the shell, and 3) near the ventral shell margin. The

periostracum shavings were weighed and analysed similarly to the tissues.

4.3.3 Shell Lead Analyses

4.3.3.1 Aragonite

Empty shells were measured (length), cleaned with a nylon brush under running water, dried at 40°C for 24 h and weighed. The calcite layer was separated from the nacreous layer by a method developed by Bourgoin (1987a). Specific areas of the aragonitic shell component were subsampled after having established the spatial distribution of the annual layers through acetate peel examinations.

4.3.3.2 Calcite

Separated calcite layer samples were analysed to compare the relative Pb concentration between the two shell components. This material required an additional cleaning step whereby the top 3 mm of the outer shell surface was removed with an abrasive bit. This procedure assured complete removal of any calcite which might have been directly exposed to dissolved and/or particulate Pb in the water column. A 1 g subsample of previously crushed shell layers, either calcite or nacre, was transferred into a 25 ml volumetric flask to which 2 ml of concentrated HNO_3 (BDH, Aristar) was slowly added, shaken to complete the dissolution, and made up to volume with deionized water. Blanks and

standards were prepared similarly by dissolving reagent grade calcium carbonate powder. The calcium content and the acidic strength of these solutions were the same as those of the shell digests, 4% $CaCO_3$ (w/v) and 1N HNO_3, respectively.

The solutions were analysed by atomic absorption spectrometry (Perkin-Elmer model 603) equipped with a graphite furnace (HGA 2100) and a deuterium background corrector. The furnace assembly had to be cleaned after about 40 determinations because of the high salt content in the solutions. Quantification was achieved with appropriate calibration curves prepared with standard solutions.

4.3.4 Quality Control Assurance

To evaluate possible contamination, reagent blanks and reference materials were processed with each set of tissue or shell samples. A standard reference material, oyster tissue (NBS 1566), accompanied all groups of tissue samples whereas an internal reference consisting of lead-spiked calcium carbonate powder was used with the shell samples. Our results were reasonably close to the NBS values (Appendix B.2). Inductively coupled plasma mass spectrometry was used to cross-check metal levels in the internal reference and agreed reasonably well with those obtained by atomic absorption spectrometry (i.e., ICP-MS: 14.2; GF-AAS: 14.8 µg/g).

Control charts were followed during all the tissue and shell trace metal analyses. The standard deviation (s) estimates were based on 16 different analyses. To develop control limits based on long-term behaviour, no more than two points were obtained on the same day (Taylor, 1985). The control limits observed during the analyses are listed in Appendix B3. Approximately 20 percent of effort was devoted to quality assurance and the measurement schedule followed throughout this study is listed in Appendix B.3.

4.4 Sediment Carbon Determination

The sediment carbon content was determined with a Leco Carbon Analyzer (model WR-12). Inorganic carbon was calculated as the difference between total and organic carbon concentrations, the latter being determined after sediment treatment with 10% (v/v) HCl to dissolve the carbonates. The analysis of five subsamples from a homogenized sediment yielded a coefficient of variation of 2%.

4.5 Shell Organic Matrix Determination

The percentage of organic matrix within the outer calcite and inner nacreous shell components was measured as the weight of dried material after ashing (Paine, 1971). Ten subsamples of each shell layer were collected using a drill equipped with a stainless steel burr. The top 3 mm of the outer shell surface was discarded to exclude any periostra-

cal material. The white nacre could easily be distinguished from the blue calcite powder. Samples were then transferred in a desiccator for 3 days, weighed and heated to 400°C for 18 h. After cooling to room temperature, the samples were returned to the desiccator for another 3 days and re-weighed.

Chapter 3: RESULTS

1.0 Total Organic Carbon (TOC) in sediment samples

Organic carbon data are given in Table 3.1. There were no significant differences in the content of total carbon between samples from Belledune and Dalhousie. The percentage of organic carbon relative to the total carbon was higher in the Dalhousie sediments by approximately 15%, which is likely related to the waste loadings from the pulp and paper mill, New Brunswick International Paper Limited (NBIP), in Dalhousie. Heavy buildups of wood chips were reported for distances of up to 1.5 km from the discharge points (Cook and Hoos, 1971). Macknight and Schafer (1980) reported high concentrations of lignin in the Dalhousie sediments. The lowest percentage of organic carbon was observed in the Eel Bay sample which was mainly composed of sands.

Table 3.1 Concentration of total carbon and its importance as organic and inorganic-carbon in the sediment samples (percent dry weight).

SITES	TOTAL CARBON (%)	ORGANIC CARBON (%)	INORGANIC CARBON (%)
D1	3.53	2.90	0.63
D2	2.37	2.06	0.31
D3	3.90	3.20	0.70
D4	4.17	3.91	0.26
D5	3.25	2.70	0.55
D6	3.53	2.90	0.63
D7	3.59	2.91	0.68
D8	2.32	1.95	0.37
D9	3.01	2.24	0.77
B1	2.61	1.84	0.77
B2	3.52	2.47	1.05
B3	3.65	2.50	1.15
B4	3.28	2.45	0.83
EEL	2.50	1.30	1.20
RIKI	1.50	1.09	0.41
NH	1.71	1.33	0.38

2.0 Major and Trace Element Analyses of Sediments

2.1 X-ray Fluorescence Spectrocoscopy

2.1.1 Major Elements

Except for sulphur and calcium, the total major element content in the sediments collected from the Belledune-Dalhousie area showed little variation in their distribution pattern (Table 3.2). Two sulphur anomalies were displayed: one at stations nearest to the ore loading facilities (D2 & D3) and a second in the Belledune samples. The sharp increase in calcium content was restricted within Belledune Harbour sediments. Sediments at station B3

Table 3.2 Total major and trace element content (dry weight) in sediment samples as determined by X-ray fluorescence spectrometry.

SITES	SiO_2	Al_2O_3	MgO	CaO	Na_2O	K_2O	S	P_2O_5
				(%)				
D1	72.6	12.3	2.4	0.67	2.00	2.40	0.82	0.22
D2	72.2	12.7	2.4	0.74	1.92	2.29	2.51	0.22
D3	71.1	12.4	2.8	0.58	2.00	2.53	2.47	0.37
D4	72.6	12.5	2.8	0.65	2.05	2.47	0.78	0.28
D5	72.9	12.0	2.5	0.61	2.15	2.51	0.73	0.23
D6	71.6	12.4	2.7	0.62	2.05	2.57	0.70	0.22
D7	74.1	12.6	2.5	0.68	2.04	2.45	0.69	0.22
D8	73.3	11.8	2.4	0.77	2.22	2.38	0.68	0.24
D9	71.8	11.6	2.4	0.75	2.18	2.33	0.72	0.24
B1	70.5	11.0	2.9	3.55	1.74	2.38	1.16	0.58
B2	68.7	11.6	3.1	4.02	1.95	2.56	1.15	0.62
B3	68.2	10.5	3.0	5.66	1.74	2.21	1.17	1.33
B4	69.5	11.7	3.1	4.56	1.83	2.41	1.15	0.67
EEL	66.2	13.1	4.3	4.69	1.29	2.72	0.56	0.40
RIKI	64.4	15.3	3.4	2.42	1.88	4.04	0.39	0.22
NH	82.0	9.4	0.2	1.70	2.61	1.4	0.58	0.32

SITES	Ni	Co	Cr	V	Zn	Cu
			(µg/g)			
D1	54	12	120	88	610	17
D2	54	12	122	90	2410	25
D3	57	18	136	92	1930	27
D4	55	15	131	91	820	26
D5	55	12	124	83	570	17
D6	54	12	120	95	1190	38
D7	54	13	125	93	710	21
D8	52	12	117	82	650	16
D9	53	14	121	85	690	24
B1	49	12	120	95	1190	38
B2	52	13	115	89	1120	41
B3	48	12	106	92	1950	139
B4	51	13	111	94	1170	54
EEL	53	12	144	90	145	14
RIKI	39	12	82	91	105	24
NH	19	2	27	37	50	8

contained approximately twice as much phosphorus than the other Belledune samples, all of which are significantly higher than the Dalhousie sediment. Together, the oxides of silicon and aluminum represent approximately 85% of the sediments. Magnesium, sodium and potassium oxides values are between 2 and 3%. Phosphorus, calcium and sulphur compounds are below 1% in most cases.

2.1.2 Trace Elements

The total trace element content in the sediments from the Belledune-Dalhousie region also displayed little variation (Table 3.2) except zinc which displayed the same distribution pattern as detailed for sulphur. Belledune samples were significantly enriched in this element in relation to Dalhousie sediments except for stations D2 and D3 in which exceptionally high contents were observed. Similarly, to phosphorus, copper was higher at station B3.

2.2 Sequential Extraction

Although the last and strongest extraction procedure, fraction 5, of the sequential extraction effectively dissolved the sulphides (Agemian and Chow, 1976); minerals which may hold trace metals within their original lattice were not readily attacked (Tessier *et al.*, 1979). This was illustrated when the total metal concentrations determined by XRF were compared before and after the final

step of the sequential extraction (Appendix A.6-A.7). However, the distribution of a given metal among the various fractions obtained by the sequential extraction procedure (Tables 3.3 - 3.5) did not necessarily reflect the scavenging action of discrete sediment phases, but rather should be considered as operationally defined by the methods of extraction (Tessier *et al.* 1979).

2.2.1 Manganese

The results from the sequential extraction of manganese (Mn) did not show any major differences among the sediment samples (Table 3.3). Except for fractions 3 and 4 which yielded comparable amounts of Mn, progressively more Mn was leached from the sediment as the extractants became more vigorous. Approximately twice as much Mn was extracted in fraction 5 alone, than in the first four fractions, where F5 >> F3 ≈ F4 > F2 > F1. Generally, half of the total Mn was extracted from the sediments.

2.2.2 Iron

The partitioning pattern of iron (Fe) among the 5 fractions closely resembled that of Mn, similarly, no major differences were observed among the various sediment samples (Table 3.4). Comparable amounts of Fe were also obtained in fractions 3 and 4 while the bulk of Fe was extracted in fraction 5, where F5 >> F3 ≈ F4 > F2 > F1. Approximately a third

Table 3.3 Manganese concentration obtained in the various extracted fractions of the sediment samples. Average of duplicate samples and standard deviations (±). All results are in µg/g (dry weight) unless otherwise stated. S(F5) represents the sum of the five fractions; Total: total Mn as determined by XRF; Extracted Mn = S(F5) ÷ Tot Mn.

SITES	F1	F2	F3 [Mn]	F4	F5	S(F5)	TOTAL (%)	EXTRACTED Mn
D1	5.2 ±0.7	16.3 ±1.0	25.7 ±1.9	22.9 ±0.9	108.1 ±6.4	178.2	0.04	45%
D2	4.1 ±0.4	4.4 ±0.2	29.7 ±1.6	20.0 ±2.8	81.1 ±3.3	139.3	0.04	35%
D3	8.9 ±0.8	20.2 ±1.3	47.4 ±5.0	56.9 ±3.5	118.6 ±18.6	251.9	0.06	42%
D4	6.8 ±0.4	10.5 ±1.1	39.0 ±3.6	20.4 ±1.5	102.8 ±10.9	179.4	0.04	45%
D5	4.2 ±0.3	9.6 ±0.6	53.5 ±5.6	20.2 ±1.5	100.8 ±9.9	188.3	0.05	38%
D6	4.7 ±0.8	9.4 ±0.4	70.0 ±5.6	21.8 ±0.8	99.6 ±7.3	205.4	0.04	51%
D7	6.4 ±0.5	20.2 ±1.0	39.1 ±4.8	19.8 ±1.8	107.1 ±16.8	192.5	0.04	48%
D8	8.9 ±0.7	19.4 ±2.4	41.0 ±2.7	20.2 ±1.5	111.4 ±12.3	200.9	0.05	40%
D9	5.5 ±0.5	16.3 ±1.3	57.6 ±3.1	19.7 ±1.2	132.8 ±7.2	231.9	0.04	58%
B1	1.7 ±0.2	30.8 ±1.3	58.9 ±4.4	19.5 ±0.7	122.5 ±17.1	233.4	0.05	47%
B2	3.3 ±0.3	46.7 ±3.8	75.7 ±4.1	23.7 ±3.7	235.3 ±10.3	384.7	0.06	64%
B3	2.7 ±0.4	43.4 ±6.8	42.5 ±6.7	25.2 ±2.3	154.1 ±9.4	267.9	0.05	54%
B4	3.9 ±0.5	48.2 ±4.3	65.6 ±7.2	22.5 ±1.8	198.1 ±11.9	338.3	0.06	56%
EEL	6.6 ±0.8	191.3 ±9.8	58.6 ±6.1	33.9 ±1.8	176.3 ±5.1	466.8	0.12	39%
RIKI	17.6 ±1.3	66.3 ±3.2	117.3 ±8.7	64.4 ±1.9	270.4 ±28.8	536.1	0.08	67%
NH	<1.5	4.0	<10	11.4 ±1.1	82.9 ±2.3	100.3	0.07	14%

Table 3.4 Iron concentrations obtained in the various extracted fractions of the sediment samples. Average of duplicate samples and standard deviations (±). All results are in µg/g (dry weight) unless otherwise stated. S(F5) represents the sum of the five fractions; Tot Fe: total Fe as determined by XRF; Extr Fe = S(F5) ÷ Tot Fe.

SITES	F1	F2	F3	F4	F5	S(F5)	Tot (%)	Extr Fe
			[Fe]					
D1	9.9	185.1	3158	4015	13277	20645.6	6.43	32%
	±1.6	±6.7	±285	±146	±782			
D2	11.1	70.2	3958	3995	9032	17066.0	6.52	26%
	±0.4	±1.9	±110	±297	±372			
D3	28.0	291.4	6131	6410	17960	30820.5	7.32	42%
	±0.8	±19.4	±858	±264	±1094			
D4	13.2	183.4	4375	4018	12939	21527.8	6.90	31%
	±0.4	±11.2	±292	±299	±1168			
D5	75.8	776.5	7775	3618	12231	24476.0	6.22	39%
	±5.6	±76.3	±719	±475	±902			
D6	11.2	304.6	8528	3934	11041	23818.8	6.93	34%
	±0.8	---	±373	±340	±997			
D7	11.9	320.7	7947	4009	13228	25516.4	6.85	37%
	±1.2	±28.9	±327	±218	±382			
D8	11.4	280.1	4457	3558	12631	20938.2	6.77	31%
	±1.5	±13.4	±623	±147	±520			
D9	10.8	328.0	8014	3501	12579	24432.9	6.69	37%
	±1.1	±11.3	±641	±344	±602			
B1	10.1	218.4	5238	5050	14325	24841.6	6.30	39%
	±1.4	±13.3	±319	±372	±519			
B2	22.6	245.2	4014	5722	17305	27309.0	6.44	42%
	±0.6	±18.1	±165	±516	±1153			
B3	8.1	287.1	3413	4839	20745	29292.1	6.45	45%
	±0.7	±25.9	±448	±140	±2047			
B4	13.6	254.1	4516	5210	19865	29858.7	6.43	46%
	±1.2	±17.8	±362	±261	±1589			
EEL	4.1	107.2	2878	3335	10169	16493.8	6.24	26%
	±0.6	±3.9	±138	±115	±1082			
RIKI	9.9	236.4	1545	8435	28538	38764.6	7.36	53%
	±1.5	±19.5	±45	±182	±408			
NH	5.5	10.9	834	1577	4758	7185.5	1.77	41%
	±0.4	±0.6	±92	±168	±392			

of the total Fe was extracted from the samples.

2.2.3 Lead

The superficial sediments collected at Belledune were significantly enriched in lead as compared to the Dalhousie samples, except for stations nearest to the ore loading facilities, D2 and D3 (Table 3.5). The lead content at B3 was considerably higher than the other Belledune samples. Although lead was effectively leached from most of the Belledune-Dalhousie samples, the pattern in which it was extracted differed between these two regions. In Dalhousie sediments, the bulk of the lead was extracted in fractions 3 and 4 followed by fractions 2 and 5, and quantitatively insignificant in fraction 1, F3 ≈ F4 > F2 > F5 >> F1. In Belledune sediments, however, all of the last four fractions yielded comparable quantities of lead except for B3 where the bulk of the lead was obtained in fraction 5.

3.0 SUSPENDED PARTICULATE MATTER (SPM)

The SPM concentrations were higher in Dalhousie water samples than in those collected at Belledune (Table 3.6). The water sample collected at B4 seemed to have a higher SPM content, but this was probably due to the resuspension of sediments during sampling. Samples collected from D2, EEL, and RIKI all showed a significant increase in SPM concentration. All of these stations were intertidal, however, and the

Table 3.5 Lead concentrations obtained in the various extracted fractions of the sediment samples. Average of duplicate samples and standard deviation (±). All results are in µg/g (dry weight) unless otherwise stated. S(F5) represents the sum of the five fraction; Tot Pb: total Pb as determined by XRF; Extr Pb = S(F5) ÷ Tot Pb.

SITES	F1	F2	F3	F4	F5	S(F5)	Tot Pb	Extr Pb
			[Pb]					
D1	<1.5	11.8 ±1.2	28.1 ±4.4	21.2 ±3.3	7.1 ±0.4	68.2	80	85%
D2	<1.5	49.3 ±7.3	98.0 ±12.0	52.9 ±4.9	32.3 ±0.7	232.5	218	107%
D3	<1.5	49.0 ±3.6	135.2 ±21.2	89.8 ±10.3	55.9 ±0.8	329.8	299	110%
D4	<1.5	11.1 ±1.1	23.0 ±4.9	20.6 ±1.7	8.3 ±0.8	63.1	75	84%
D5	<1.5	11.5 ±1.3	45.5 ±7.2	20.8 ±1.3	4.3 ±0.3	82.3	87	95%
D6	<1.5	5.0 ±0.8	26.2 ±3.4	10.0 ±1.6	3.0 ±0.3	44.2	50	88%
D7	<1.5	5.2 ±0.5	20.8 ±4.0	15.3 ±1.6	2.1 ±0.1	43.3	52	83%
D8	<1.5	6.7 ±0.4	27.0 ±4.1	13.1 ±1.9	3.7 ±0.2	50.5	53	95%
D9	<1.5	4.6 ±0.5	20.0 ±3.3	14.8 ±1.8	2.6 ±0.1	42.1	58	73%
B1	<1.5	155.3 ±14.4	149.4 ±23.5	190.3 ±14.0	218.2 ±12.9	713.1	720	99%
B2	<1.5	121.7 ±14.0	176.7 ±21.7	186.5 ±16.8	326.5 ±14.3	811.5	734	111%
B3	<1.5	123.8 ±18.4	118.0 ±15.9	213.2 ±13.0	1025.0 ±49.0	1480.0	1520	97%
B4	<1.5	136.1 ±15.0	124.5 ±17.4	175.7 ±22.8	358.6 ±21.5	794.9	790	101%
EEL	<1.5	4.7 ±0.4	<1.0	8.9 ±2.2	3.6 ±0.2	17.9	24	74%
RIKI	<1.5	2.2 ±0.3	5.1 ±0.8	8.2 ±1.1	5.2 ±0.8	20.7	21	98%
NH	<1.5	3.4 ±0.4	1.4 ±0.2	6.2 ±1.0	1.5 ±0.2	12.5	29	43%

increase is most likely also associated to the resuspension of sediments.

Table 3.6 Lead concentration in suspended particulate matter (SPM) collected approximately 30 cm above the sediment bed.

STATIONS	[SPM] (mg/L)	[Pb] (µg/g)
D1	6.12	161.5
* D2	42.88	118.6
D3	4.34	240.8
D4	5.04	101.7
D5	5.00	195.4
D6	3.56	105.4
D7	6.44	158.5
D8	5.92	135.1
D9	5.14	162.3
B1	1.30	582.1
B2	1.82	696.3
B3	1.86	844.0
B4	5.88	624.6
C1	6.34	974.3
C2	4.87	230.8
C3	5.28	63.9
C4	4.28	55.2
* EEL	25.03	82.5
MIG	3.78	77.7
* RIKI	11.07	26.8
NH	8.91	57.7

* Samples D2, EEL, RIKI were sampled from the intertidal zone.

A strong lead gradient was observed at the Belledune coastal stations with the highest value recorded at C1. Although lead levels were considerably lower at C2, levels at this station remained 4 to 5 times higher than those observed at C3 and C4. Lead levels in the Belledune samples

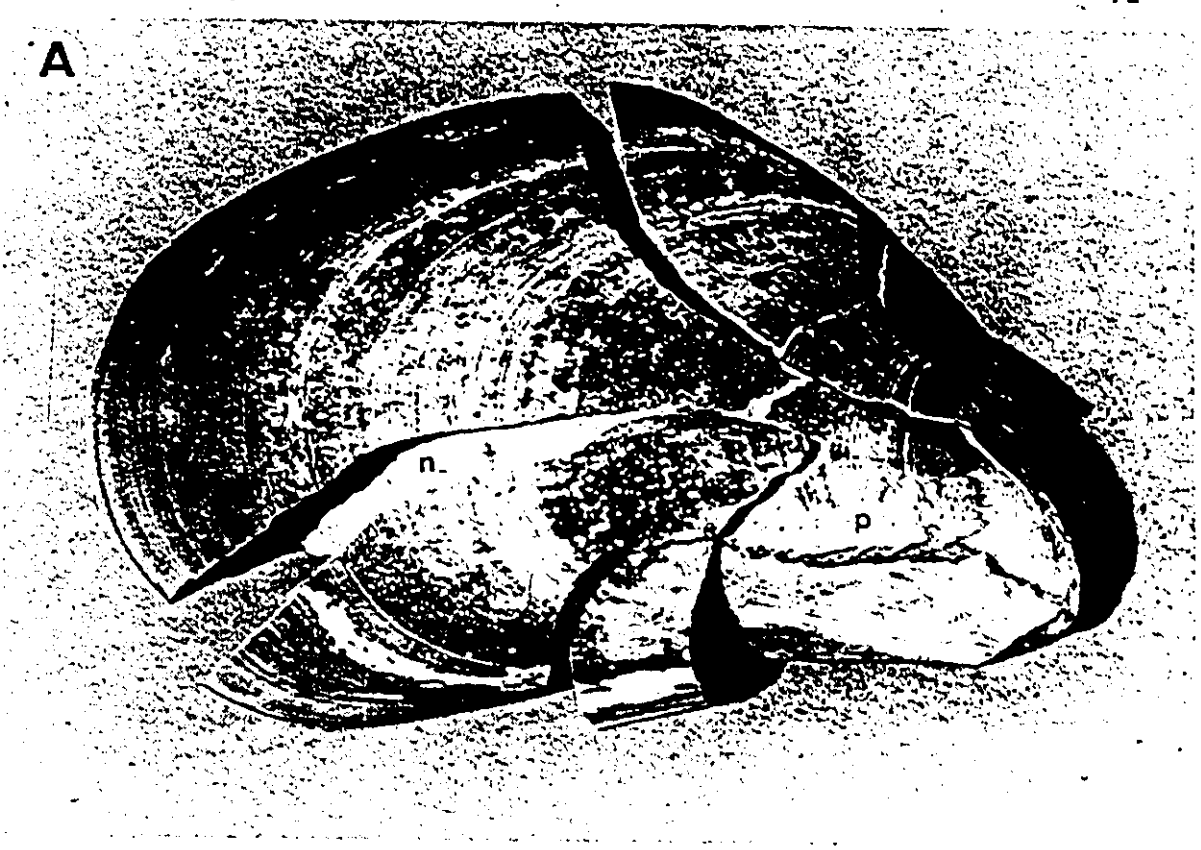
were intermediate to those of C1 and C2, and significantly higher than Dalhousie levels. Although the highest lead level in the Dalhousie samples was recorded at the site nearest to the ore loading facilities, no definite trend of lead contamination was observed.

4.0 BIOLOGICAL MATERIAL

4.1 Separation of the Aragonitic and Calcitic Shell Layers

Calcitic and aragonitic shell layers could consistently be separated, for all shell lengths, when baked at 400°C for 18h. Most of the calcite would crack and readily fall off as the shell cooled (Fig. 3.1A). The remaining calcite fragments could then easily be scraped off the intact nacreous layer (Fig 3.1B). Observation on the SEM showed that parting of the shell layers occurred at the pallial myostracum band which remained associated with the calcitic layer. The calcite and aragonite layers were easily differentiated, the former being dull and brittle and the latter displaying a characteristic pearly lustre. The heating also served to soften the nacre and made possible to subsample distinct regions of the shell material by lightly scraping the surface.

Figure 3.1 A: Fractures in the outer calcitic prismatic component in *Mytilus edulis* L. The underlying aragonitic nacreous shell component (n) is largely intact, despite the extensive fracture propagation within the prismatic calcite (p). B: Intact valve of *Mytilus edulis* (right) and separated shell components after heating at 400°C; reconstructed prismatic component (p) and intact nacreous shell component (n).


A
n
p


B
p
n

4.2 Organic Matrix and Lead Content in Aragonite and Calcite Layers

Preliminary results indicated that there was no significant difference (Mann-Whitney U-test, 95% level of confidence) between the lead content in the right and left valves. The calcite generally contained about 30% more lead than the aragonitic layers (Table 3.7). Aragonite layers however, contained 30% more organic matrix than the calcite.

Table 3.7 Organic matrix and lead concentration in the aragonitic and calcitic shell layers of *Mytilus edulis*.

	[Pb] µg/g		[Org Mtx] %	
	Aragonite	Calcite	Aragonite	Calcite
sample 1	21.2	28.7	3.17	1.94
sample 2	23.7	31.4	3.22	1.85
sample 3	28.5	32.1	3.15	1.86
sample 4	25.7	33.4	3.19	1.79
sample 5	23.3	43.8	3.30	1.84
sample 6	31.3	41.6	3.14	1.78
sample 7	27.4	46.3	3.08	1.87
sample 8	18.5	29.6	2.93	1.69
sample 9	24.3	26.2		
sample 10	29.6	34.4		
AVG	25.4	34.8	3.15	1.83
STD	3.9	6.8	0.10	0.07
CV	15%	20%	3%	4%

4.3 Lead levels in the Periostracum

Analyses of lead from periostracum of selected mussel samples are summarized in Table 3.8. Lead levels

Table 3.8—Lead concentration (µg/g) in the periostracum sampled from three different regions of *Mytilus edulis* shells.

SITES	SAMPLE LOCATION		
	Anterior Shell Margin	Middle of Shell	Umbo
D1	128 ± 77	135 ± 61	150 ± 45
D3	187 ± 88	235 ±114	227 ± 52
D4	198 ± 61	213 ± 57	287 ± 76
D9	93 ± 46	116 ± 63	132 ± 55
B2	327 ±120	491 ± 83	525 ± 77
B3	684 ±117	611 ± 73	795 ± 97
C1	611 ±121	741 ± 95	987 ±132
MIG	62 ± 26	71 ± 23	95 ± 37
RIKI	81 ± 53	85 ± 47	117 ± 38

generally increased from the ventral margin toward the umbo. Or, lead levels were lowest in most recently deposited material. The lead concentrations from the three shell regions were shown to be significantly different (Friedman 2-way analysis by ranks). Samples collected from Belledune and the coastal station nearest to the smelter (C1) had comparable levels of lead. Lead levels measured in Dalhousie mussels were intermediate to those from Belledune and the control sites.

5.0 SHELL AND TISSUE LEAD DETERMINATIONS

The results of the lead determinations in the tissues and shells of *Mytilus edulis* are summarized in table 3.9.

Table 3.9 Average shell size (length and weight) and lead concentration in *Mytilus edulis* tissues and shells (i.e., nacreous layer). Averages of 10 analyses. Confidence intervals (c.i.) calculated using the expression: $\pm ts/\sqrt{n}$; s: standard deviation; t: value at the 95% confidence level and n-1 degrees of freedom; n: number of analyses.

SITES		SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (µg/g)	SHELL [Pb] (µg/g)
D1	AVG	66.7	19.37	17.3	1.3
	c.i.			7.2	0.4
	CV			58.4%	22.8%
D3	AVG	65.4	15.24	10.9	0.9
	c.i.			2.4	0.2
	CV			31.1%	15.2%
D4	AVG	64.8	15.96	8.4	0.7
	c.i.			1.8	0.2
	CV			30.2%	20.8%
D6	AVG	64.2	14.75	6.1	<.5-.82
	c.i.			2.1	< 0.5
	CV			48.0%	---
D7	AVG	66.4	18.02	5.2	<.5-.84
	c.i.			1.9	< 0.5
	CV			50.2%	---
D8	AVG	67.0	17.76	5.9	<.5-.86
	c.i.			1.6	< 0.5
	CV			37.0%	---
D9	AVG	65.0	15.83	6.1	<.5-.78
	c.i.			1.4	< 0.5
	CV			32.3%	---
B1	AVG	60.2	11.91	425.1	31.5
	c.i.			85.2	3.5
	CV			26.0%	13.0%
B2	AVG	59.6	11.22	306.4	25.6
	c.i.			105.3	5.2
	CV			48.0%	28.7%
B3	AVG	59.0	10.40	149.5	12.8
	c.i.			44.8	1.2
	CV			41.9%	13.3%
C1	AVG	59.1	13.66	455.5	48.8
	c.i.			70.0	6.6
	CV			21.5%	18.9%
C2	AVG	56.2	14.15	170.7	5.7
	c.i.			36.8	0.6
	CV			30.2%	14.7%
C4	AVG	65.8	15.16	16.3	2.0
	c.i.			6.2	0.1
	CV			52.4%	8.3%
MIG	AVG	66.3	18.40	3.6	<.5-.63
	c.i.			1.8	< 0.5
	CV			69.5%	---
RIKI	AVG	43.8	4.45	8.5	0.8
	c.i.			1.9	0.1
	CV			31.1%	18.0%
NH	AVG	64.5	16.41	1.8	< 0.5
	c.i.			0.5	< 0.5
	CV			37.6%	---

Each value represents a mean of 10 analyses except for B2 in which 30 mussels were analysed. The data relating to the analyses of all the individual specimens are compiled in Appendix C.1 - C.5.

The relationship between lead levels in the shells and tissues was significant (r: 0.966; Fig 3.2), and was represented by the following linear relationship:

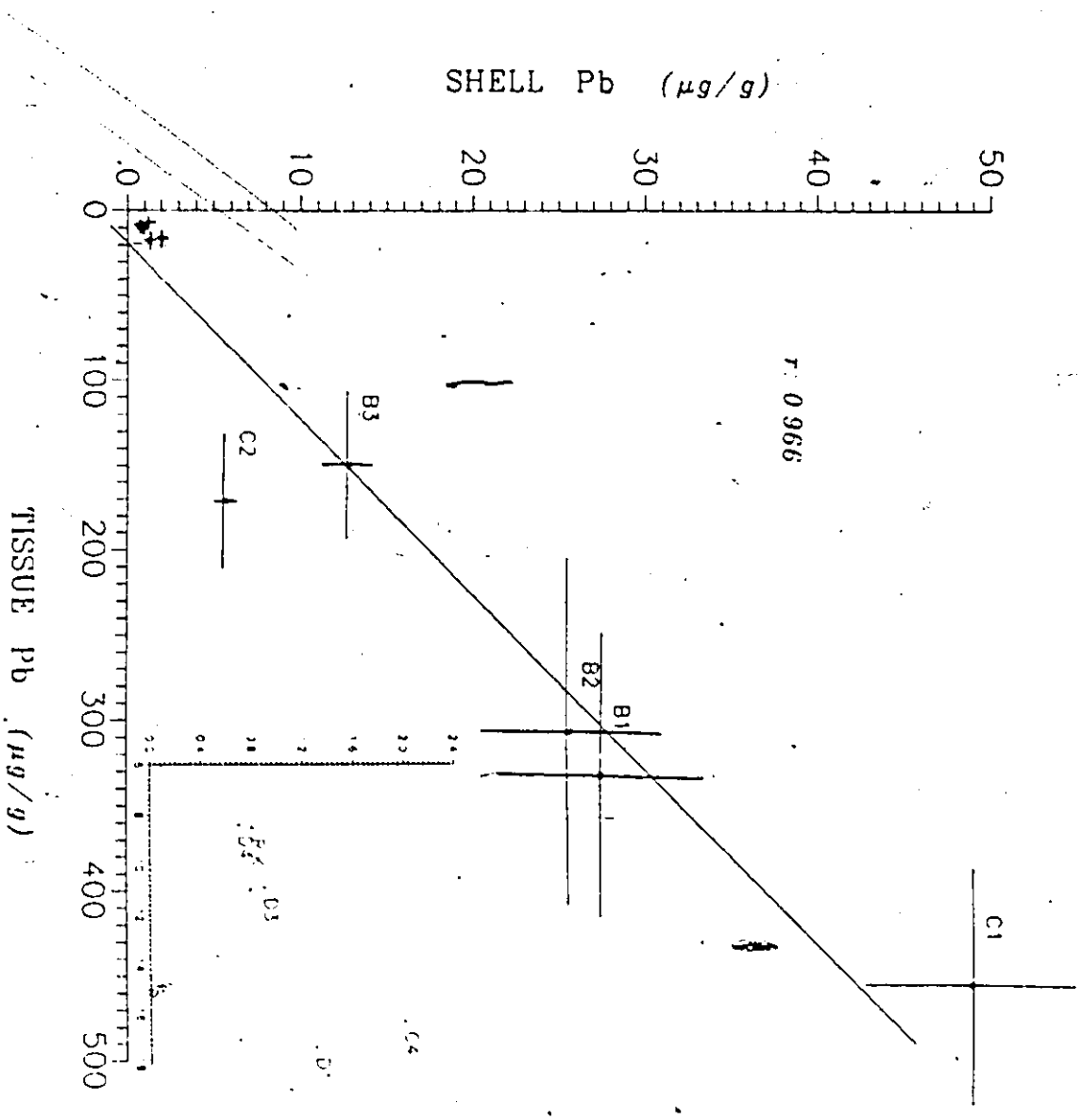
$$Y = 0.1\ X + (-1.8)$$

where Y and X represent the lead concentrations (µg/g, dry weight) in the shells and the tissues, respectively.

The coefficient of variation of tissue lead concentrations ranged between 22 and 70%, whereas values from the shell analyses displayed considerably less variability ranging between 8 and 29%. Because of the strong relationship, the location-dependent metal variations discussed below generally apply to both shells and tissues.

The tissue lead levels observed in the mussels fall within the data range reported in other studies on the Belledune-Dalhousie area (Hildebran, 1984; Packman *et al.*, 1984; Matheson and Bradshaw, 1985). The highest lead concentrations in *Mytilus edulis* were observed in specimens collected nearest to the functional outfall (C1). Mussels collected 5 km south of this site (C2) contained significantly lower lead levels. Although all specimens collected from Belledune Harbour displayed a high degree of

Figure 3.2. Relationship between the lead levels in the aragoinitc shell component and the tissues of *Mytilus edulis*. Averages of 10 determinations, except B2 which compirses 30 determinations. Vertical and horizontal lines represent 2σ confidence intervals.


SHELL Pb (μg/g)
0
10
20
30
40
50
TISSUE Pb (μg/g)
0
100
200
300
400
500
B3
C2
B2
B1
C1
r: 0.966

contamination, B3 mussels consistently contained approximately half as much lead as the other specimens collected within the harbour.

Dalhousie mussel samples could be divided into two distinct groups based on their degree of lead contamination. Lead levels in mussels sampled nearest to the ore loading facilities (D1,D3,D4) were significantly higher (Friedman 2-way analysis by ranks; 95% confidence level) than specimens collected in "East Bay". Shell lead levels in the latter group were also often below the detection limit (Appendix C.2). Coastal mussels sampled furthest from the Belledune area contained lead concentrations intermediate between Belledune and Dalhousie values.

The lead concentrations in the mussels collected from Rimouski were comparable to those in mussels sampled in Dalhousie Harbour. The lead levels from these two mussel populations were approximately 2 and 4 times higher than those collected at Miguasha and Negro Harbour, respectively.

6.0 Bivalve - Sediment Relationships

Tissue lead levels were used to examine the relationship with metal levels in the sediments for the following reasons:

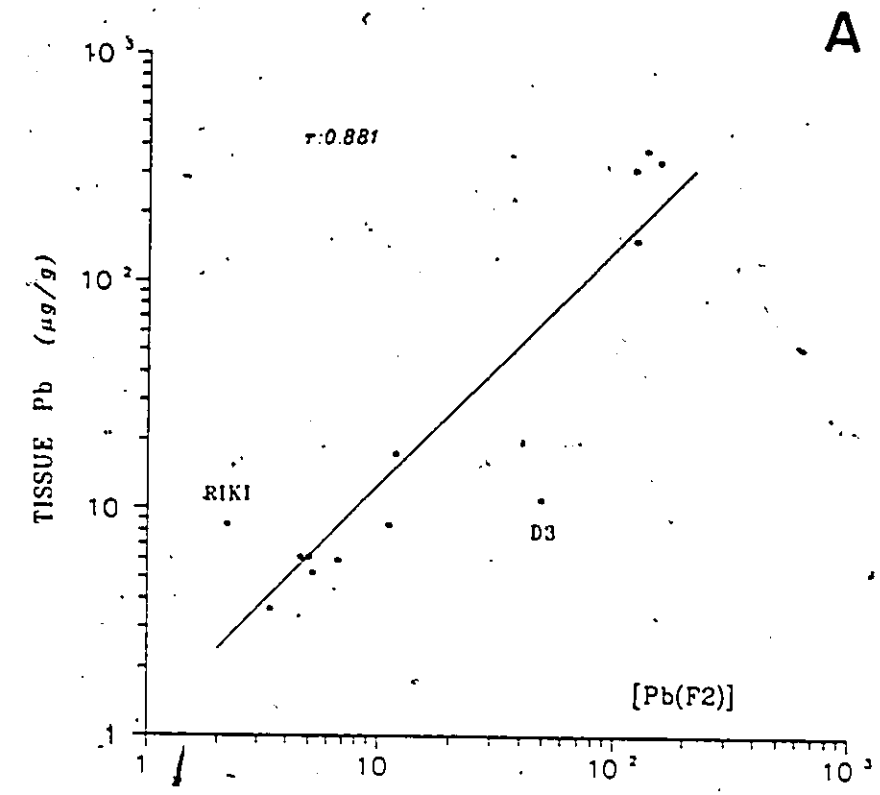
1) tissue lead levels adequately reflect those observed in the shell

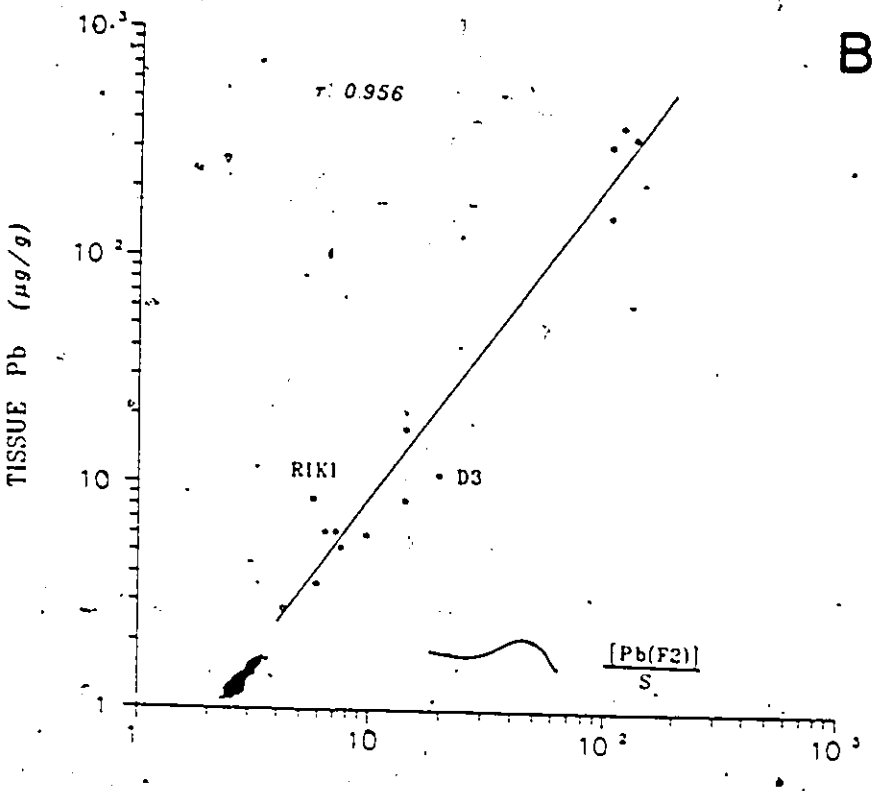
2) lead levels in the tissues were always above the detection limit

Tissue lead levels were first compared with the lead concentrations in each of the five extraction fractions ([Pb(F1)] to [Pb(F5)]). Because high multicolinearity often occurs between the sediment fractions (Tessier *et al.* 1984), multiple regression analyses are usually difficult to interpret. Greater coefficients of determination (r^2) were generally observed when bivariate analyses were performed on lead concentrations in each sediment fraction (or combination) normalized with respect to various other sediment parameters (eg. organic carbon, total sulphur). Since the objective was to obtain the best overall single predictor among the fractions and normalized parameters, all subsequent statistical analyses were bivariate. For all calculations, mean values for the mussels at each site were used. Rather than present all the statistical parameters, the following discussion was limited to the steps undertaken to obtain the best predictor (Fig. 3.3).

When single sediment fractions or combinations were compared to tissue lead levels in *Mytilus edulis*, [Pb(F2)] displayed the best correlation (r: 0.881; Fig 3.3A). Normalizing this parameter with the total sulphur content in the sediments proved to be the best predictor (r^2: 0.914, Fig 3.3B).

Figure 3.3. Relationship between the lead levels in *Mytilus edulis* tissues and various sedimentary components. A: Tissue lead vs. lead obtained from the second fraction of the sequential extraction, "lead bound to the carbonates". B: Tissue lead vs lead obtained from the second fraction normalized by the amount of total sulphur in the sediments as determined by XRF. RIKI: sample collected from Rimouski, D3: sample collected near the ore-loading facility at Dalhousie Harbour.


A
r:0.881
TISSUE Pb (μg/g)
RIKI
D3
[Pb(F2)]
10 3
10 2
10
1
1
10
10 2
10 3


B
r: 0.956
TISSUE Pb (μg/g)
RIKI
D3
[Pb(F2)]
S
10 3
10 2
10
1
1
10
10 2
10 3

7.0 LEAD CONCENTRATION *vs* TOTAL SHELL WEIGHT

Metal levels determined in both the hard and soft parts of mussels should be related to shell measurements as these prove to be less variable than body weight (soft tissues). Before undertaking such relationships, it is essential to define which shell measurements best reflect the age of the mussels.

Shell length and weight are the parameters most commonly used to index the ages of bivalves. Both offer a means to rapidly and inexpensively collect large amounts of data. Seed (1973) observed, however, that linear growth gradually ceases in older mussels, while nacre continues to be deposited on the inner shell surface. Hence, shell weight would better reflect the ages of mussels.

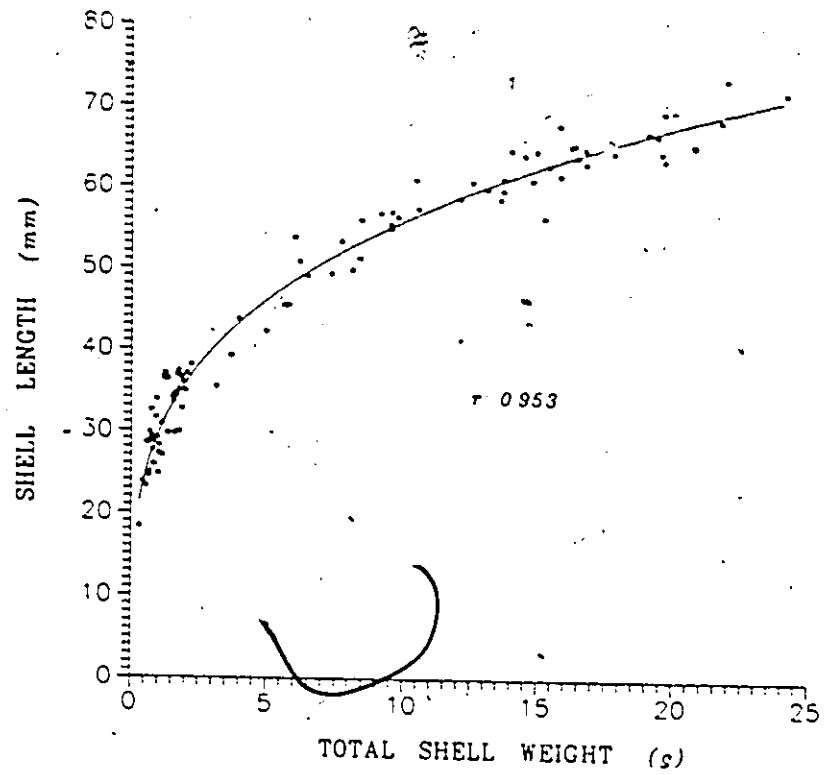
I investigated this relationship by relating the total shell weight to either the shell length or percentage of aragonite (weight basis) in over 70 mussel shells. Both of these functions are illustrated in Fig 3.4 and the data sets are compiled in Appendix D. The relationship between shell length and shell weight is best defined by the power equation:

$$Y = X^{.28} * 29.6$$

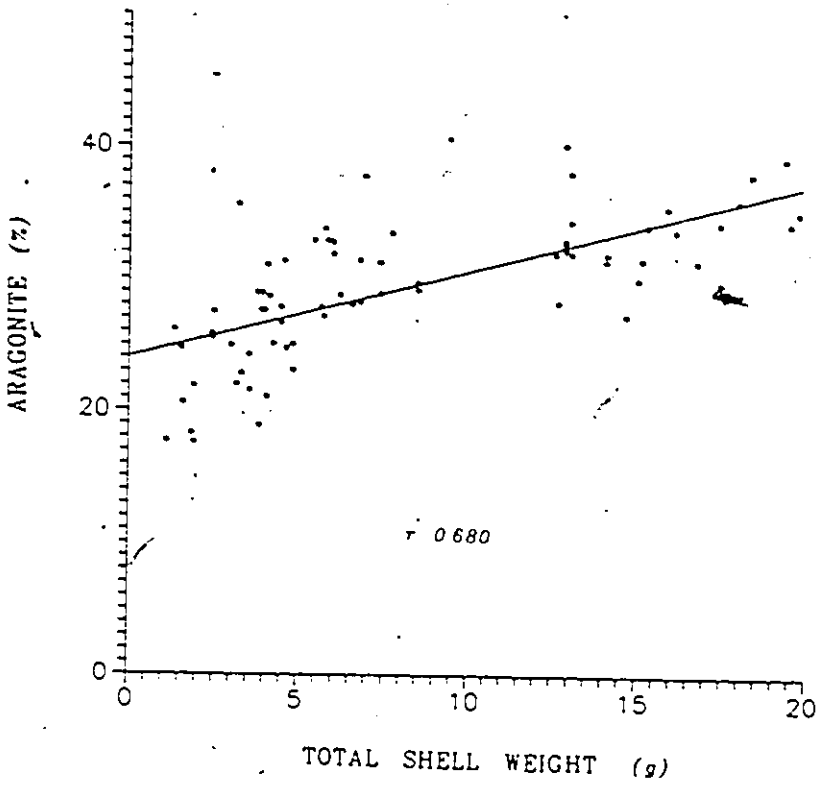
where Y represents shell length (mm) and X the total shell weight (g) (Fig 3.4A). The function illustrates how the initial linear shell growth in the smaller mussels (<40 mm)

Figure 3.4. A: Relationship between shell length (mm) and the corresponding total shell weight (g)in *Mytilus edulis* valves. B: Relationship between shell length (mm) and the percentage of aragonite in *Mytilus edulis* shells.

A


SHELL LENGTH (mm)
80
70
60
50
40
30
20
10
0
r 0 953
0
5
10
15
20
25
TOTAL SHELL WEIGHT (g)

B


ARAGONITE (%)
40
20
0
r 0 680
0
5
10
15
20
TOTAL SHELL WEIGHT (g)

is rapid and then decreases in the larger specimens. When the total shell weight is plotted against percentage of aragonite, a linear function is obtained (Fig 3.4B) which is best defined by

$$Y = 0.65 X + 24$$

where Y represents the percentage of aragonite and X the total shell weight (g). In this case we see that nacreous material is continuously deposited during the mussels' growth and therefore, total shell weight would be a better index of the ages of mussels. Finally, total shell weight was plotted against the tissue dry weight (Fig 3.5) to determine how closely these two parameters were related. The following linear function was

$$Y = 55 X + 40$$

where Y represents dry tissue weight (mg) and X the total shell weight (g). The high correlation coefficient (r: 0.929; $p>0.01$), illustrates how closely these parameter are inter-related.

8.0 SIZE DEPENDENT RELATIONSHIPS

Metal concentrations may alter with size in a given population, so that larger mussels have more or less metal (expressed as a concentration µg/g) than smaller ones, even though all members of the population are exposed to the same environment. In these cases, it is difficult to assess whether observed differences in element concentrations

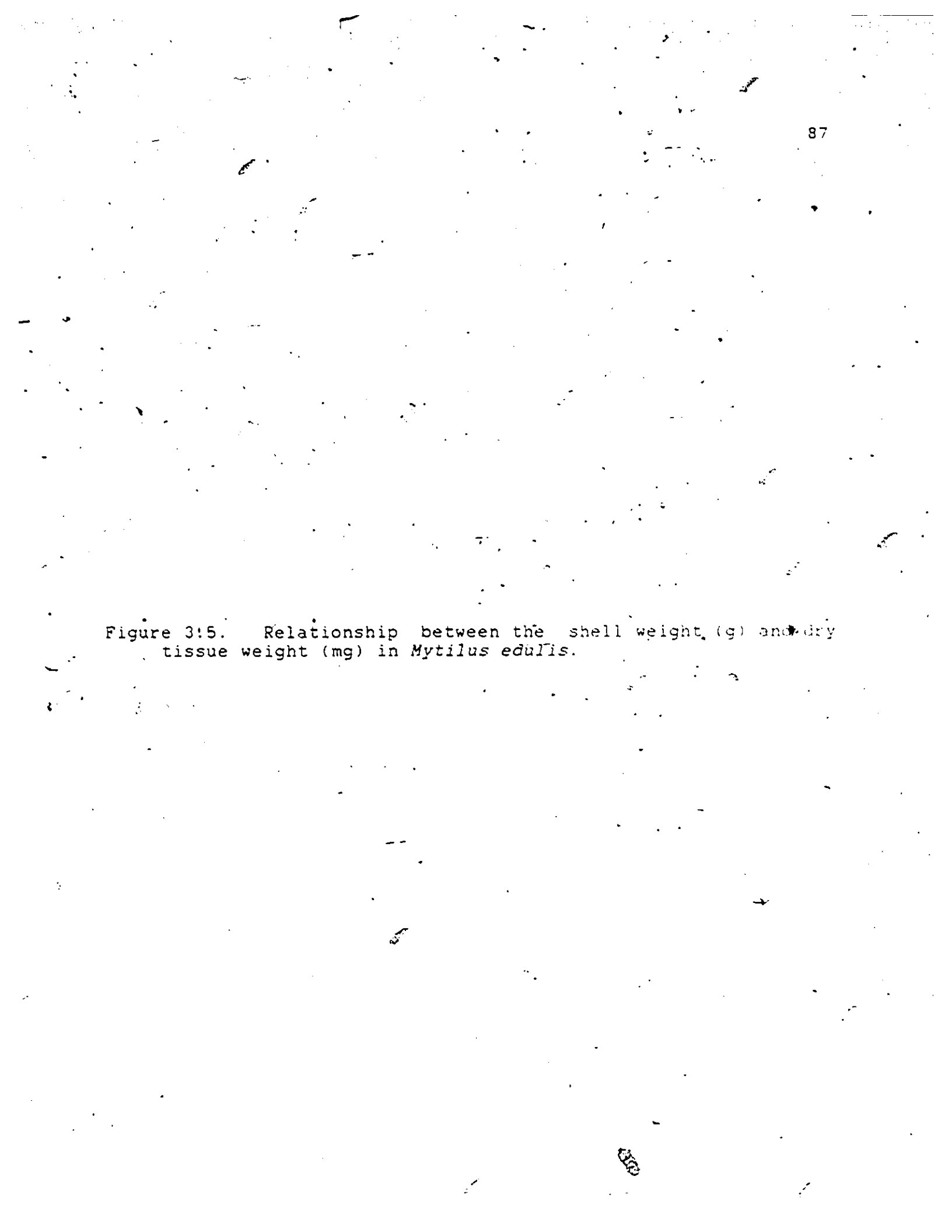
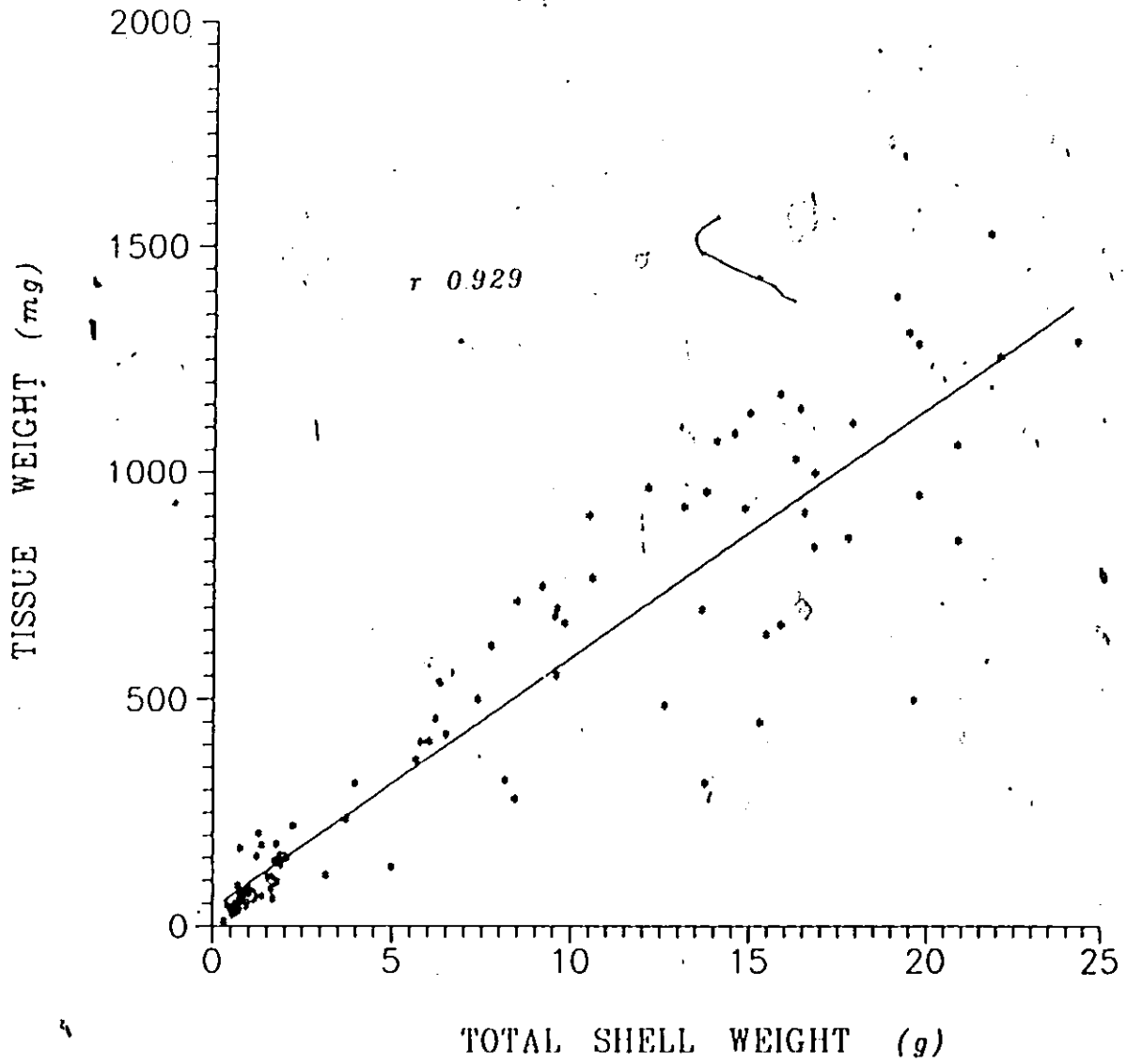


Figure 3:5. Relationship between the shell weight (g) and dry tissue weight (mg) in *Mytilus edulis*.


2000
1500
1000
500
0
TISSUE WEIGHT (mg)
r 0.929
0
5
10
15
20
25
TOTAL SHELL WEIGHT (g)

reflect real differences in environmental trace element concentration, or are merely due to variations in body size. This problem was circumvented in section 5.0, by comparing lead levels in mussels of a similar size, 60 mm ± 5 shell length. This method cannot be applied, however, when the main objective is to compare lead levels in mussels of vastly different sizes/ages (i.e., juvenile vs adults). In this case, it is essential to determine metal concentration over a range of body sizes to evaluate whether the metal levels in the shells and/or tissues are size dependent.

Mussels collected from the B2 site were targeted for this aspect of the study. More than 60 tissue and shell lead determinations were performed on over 30 mussels with a total shell weight ranging between 1 and 35 g. Shell lead levels were plotted against the total shell weight and the relationships then examined by bivariate analysis (Fig 3.6). In both cases, lead levels proved to be independent of mussel size.

9.0 Estimation of "Past" Lead Levels in *Mytilus edulis*

Acetate peel investigations revealed that mussels collected at station C1 were approximately 3 years older than bivalves of similar size ($\bar{x}$: 65 ± 3 µm) obtained from the deeper stations at B2 and D3 (Fig 3.7). The "annual layers" of mussels collected from C1 also proved to be significantly thinner than those from the two other sites (Friedman 2-way analysis by ranks; $p>0.01$).

Figure 3.6. Relationship between shell lead concentrations (µg/g) and total shell weight in *Mytilus edulis*. AM, arithmetric mean; GM, geometric mean; crossed-hatched bar, standard deviation; vertical lines, data range.

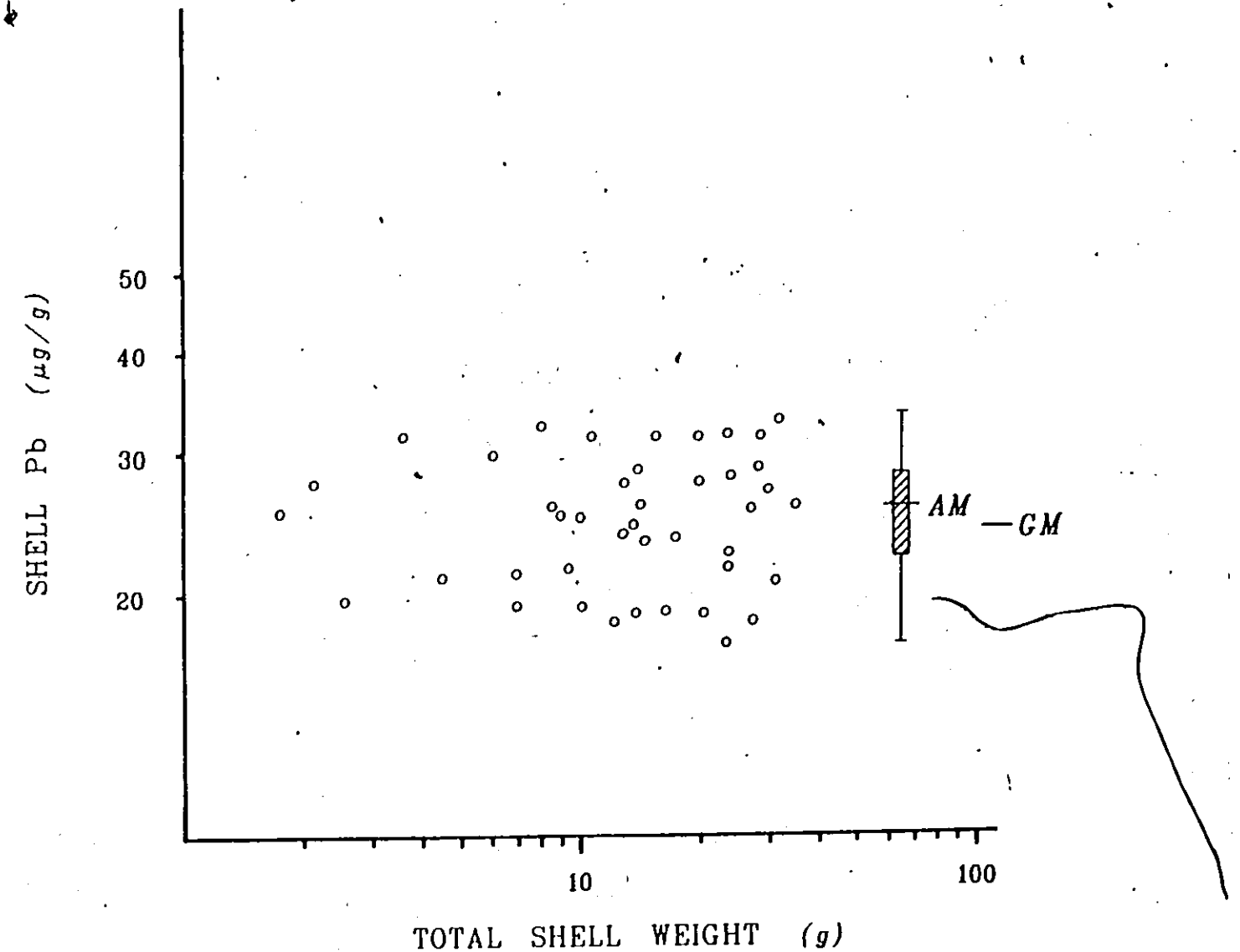
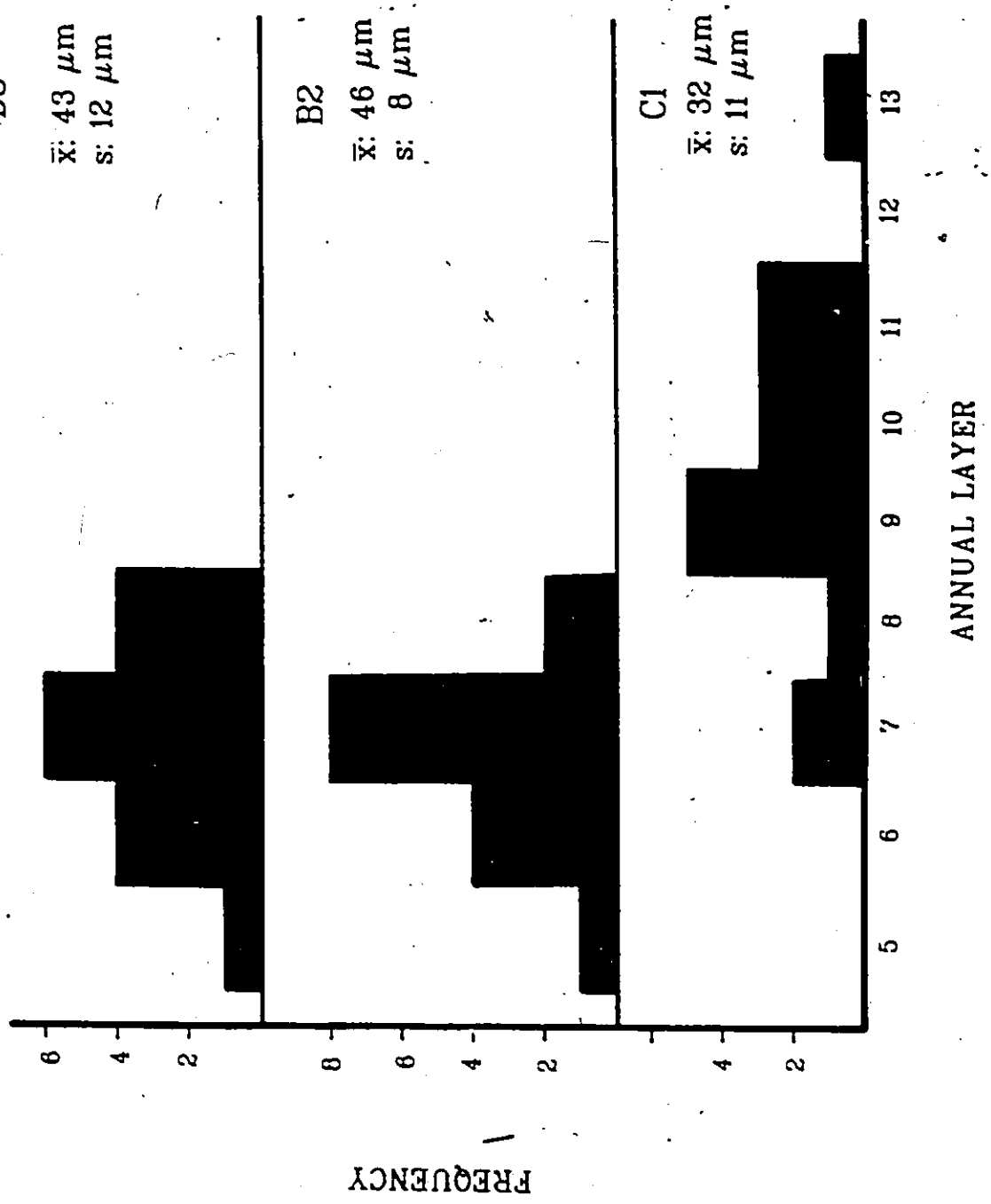

SHELL Pb (μg/g)
50
40
30
20
10
100
TOTAL SHELL WEIGHT (g)
AM
GM

Figure 3.7. Frequency distribution of the "annual layers" in *Mytilus edulis* shells, as determined by acetate peel examinations of longitudinal valve sections (x: 65 ± 3 mm shell length) in samples collected from D3, B2 and C1. x, average thickness of an annual layer; s, standard deviation.


D3
x̄: 43 μm
s: 12 μm
B2
x̄: 46 μm
s: 8 μm
C1
x̄: 32 μm
s: 11 μm
FREQUENCY
ANNUAL LAYER
5
6
7
8
9
10
11
12
13
2
4
6
8

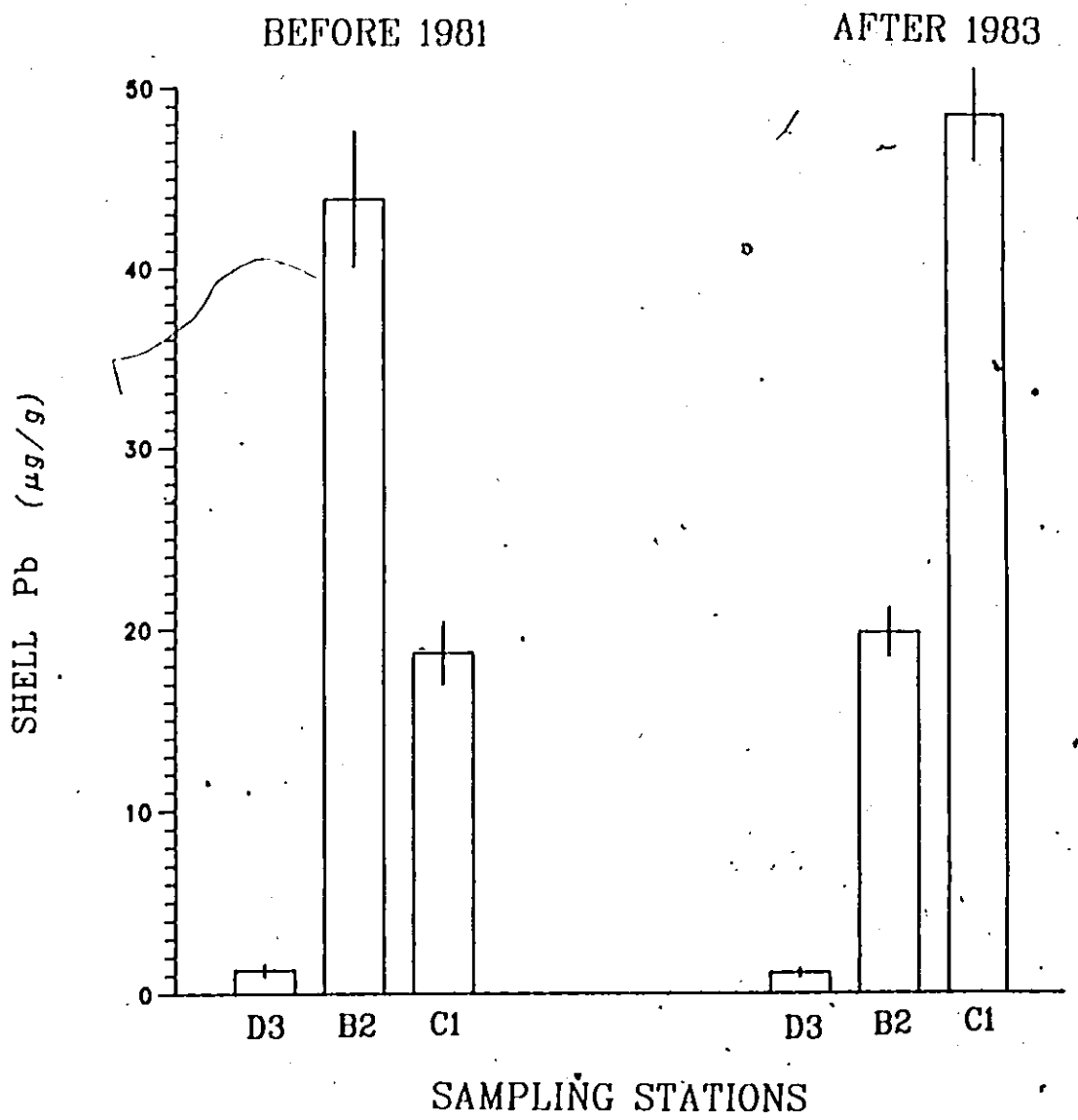
Having established the average thickness of the annual layers, the following areas of the nacreous shell were subsampled:

a-- the top ≈100 µm of the inner shell surface

b-- the top ≈100 µm of the outer shell surface, within 1.5 cm of the umbo

I estimated that nacre subsampled from the outer and inner surfaces of the aragonitic shell component was deposited prior to 1981 ("<1981") and during 1984-85 (">1983"), respectively. Lead determinations from these subsamples suggest that while no significant changes occurred in mussels collected near the ore loading facilities in Dalhousie (D3), specimens sampled within Belledune Wharf (B3) and near the relocated outfall (C1) displayed significant temporal changes which coincide with the relocation of the smelter outfall (Fig 3.8; data sets Appendix C.6). The "<1981" nacre in Belledune mussels contained approximately twice as much lead than the "<1981" nacre in specimens collected at station "C1" (43.8 and 18.7 µg/g, respectively). Conversely, lead measurements in recently deposited nacre (">1983") of these two mussel populations displayed an opposite trend ("B2": 19.8 and "C1": 48.4 µg/g). From the relationship obtained in section 5.0, the tissue lead concentration in mussels from "B2" prior to 1981 was estimated at approximately 510 µg/g.

Figure 3.8. Shell lead concentration (µg/g) determined in discrete regions of the nacreous shell component of *Mytilus edulis*. Each value represents the mean of either 6 (D3) or 31 (B2 and C1) analyses. The vertical lines across the bars represent 2-σ confidence intervals.


BEFORE 1981
AFTER 1983
SHELL Pb (µg/g)
50
40
30
20
10
0
D3
B2
C1
D3
B2
C1
SAMPLING STATIONS

Chapter 4: DISCUSSION AND CONCLUSION

1.0 LEAD UPTAKE *vs* ENVIRONMENTAL LEAD LEVELS

1.1 Sedimentary Components

The results from this work conform with other studies on metal uptake by *Mytilus edulis* in the Belledune-Dalhousie region, in that Belledune mussels sequestered significantly higher amounts of lead than those collected at Dalhousie Harbour. Macknight (1980) reported a reduction in metal bioavailability because of the anoxic conditions encountered in Dalhousie sediments, caused by the decomposition of the waste loadings from the pulp and paper mill. He suggested that the anoxic regime chemically "forces" much of the metals into relatively immobile forms, for example sulphides.

Diving observations in this study revealed that anoxic surface sediments did occur in Dalhousie Harbour, localised near the log boom area. The transparent acrylic boxcore used to sample the sediments enabled us to measure the thickness of the surface oxic layer *in situ*. While this layer was thinner than in the sediments collected in

Belledune, approximately 15 and 50 mm thick, respectively; it was present in all of the sediment samples. Fergusson (1983) suggested that lead compounds from smelter effluent may display a higher bioavailability because they were labile or weakly complexed. The results from the bivariate analyses (chapter 2, section 6) suggested that other sediment components could affect/control the bioavailability of lead.

Any sequential extraction procedure will unavoidably suffer from a certain lack of selectivity, as been shown theoretically (Sigg *et al.*, 1984) and experimentally (Tessier *et al.*, 1979; Rapin and Forstner, 1983). Futhermore, if chemical extractions of oxidized sediments are to be meaningful, preservation of extractable characteristics between the time of sample collection and sample analysis will be an important consideration. In the past, problems of sample handling have been recognized (Luoma and Bryan, 1981), but most often, the time and method of sediment storage before extraction were not mentioned despite the fact that many types of sample storage may result in changes which would greatly alter the chemistry of the sediments (Luoma and Davies, 1983). In an extensive study on the effects of different methods of storing oxidised sediment samples, Thomson *et al.*(1980) concluded that freezing was the best method of preservation when a variety of extractants are to be used.

The lead levels in *Mytilus edulis* were best related to the lead obtained in fraction 2, "lead bound to carbonates". This conforms with Macknight and Schafer (1980) who studied the bioavailability of sediment-bound metals through the use of weak acid and organic leaches. They considered metals released by the former to be the most bio-available and observed that 13% of the sediment-bound lead was released with the weak acid leach as compared to only 2% with the organic leach. In a study of an estuary undergoing lead contamination, Purchase and Ferguson (1986b) noted the following lead species in a sediment profile: $PbCO_3$, $PbSO_4$, PbS and Pb metal. They observed that the carbonate dominated near the top (i.e., oxic sediment layer) and the sulphide form near the bottom of the profile (i.e., anoxic sediments).

Normalizing the lead obtained in fraction 2 with the total sulphur in the sediments essentially reduced the contribution of sulphide-bound lead, thereby further improving the relationship (Fig. 3.3B). This process essentially served to "pull-in" the two data points, RIKI and D3, closer to the general relationship (Fig. 3.3). Mussels from these two sites contained comparable amounts of lead whereas the lead levels in the sediments differed by an order of magnitude. This is explained by the fact that RIKI mussels were mainly exposed to organic-lead species, for example alkyl- and methyl-lead, considered to be much more

biologically available (Schmidt and Hubert, 1976; Wong *et al.*, 1978; Chau and Wong, 1978). Conversely, D3 is nearest to the ore loading facility and its sediment should contain a larger amounts of the less available sulphide-bound lead (Ray *et al.*, 1981).

These findings differ from other studies which suggested a "protective" or "competitive" role for iron in the process leading to the accumulation of lead (Luoma and Bryan, 1978; Tessier *et al.*, 1984) and arsenic (Langston, 1980). This may be related to the lower sulphur contents in the sediments in these studies. Except for one station, the sediment sulphur levels reported by Tessier *et al.*(1984) ranged in between 0.03 and 0.07%---the sulphur levels were not reported in the two other studies. Futhermore, Tessier *et al.*(1982) suggested that metals obtained in fraction 2 were predominantly those specifically adsorbed on iron oxides. The gradients of iron concentration within the sediments were greater in these studies. By way of example, Luoma and Bryan (1978) observed that the amount of iron extracted from the sediments with weak acid (1N HCl) varied by an order of magnitude.

1.2 Total Phosphorus

Although the steps previously discussed improved the correlation between lead levels in mussels and the environment (i.e., sedimentary), it did not correct for an

anomalously low lead level measured in mussels collected at B3. Despite the high sediment lead levels at this site, twice as high than the other Belledune sediment samples, B3 mussels contained half as much lead than bivalves collected from the other Belledune sites (Table 3.9). The exceptionally high phosphorus level also associated with the B3 sediments (Table 3.2) may provide further insight into this problem.

The effects of the coexistence of several metal pollutants on the uptake of any single metal by an organism are difficult to study because of the large number of metal combinations and biological responses possible. Most of the studies dealing with this problem in bivalves focus on antagonistic and/or synergistic effects among trace metals (Roosenburg, 1969; Romeril, 1971; Jackim *et al.*, 1977; Ray and McLeese, 1983; Hemelraad *et al.*, 1987). Although it has been reported more than 45 years ago (Shields and Mitchell, 1941) that the ingestion of phosphorus reduces the absorption of lead in animals, this relationship seems to have been ignored in marine environmental studies.

Jawrowski *et al.* (1985) observed that the lead levels in bones from cows were approximately 5 times lower than the bone lead levels in wild deer in the neighboring area. They suggested that the addition of mineral mixes containing calcium and phosphorous to the fodder of the cows probably decreased the absorption of lead from their alimen-

tary tract, so that their bone lead level was "artificially" lowered. Similarly, Quaterman *et al.* (1978) reported that the uptake of lead from the diet in laboratory rats was reduced by about half when either dietary calcium or phosphate or both was doubled. It is conceivable that the increased level of phosphorus may have reduced the uptake of lead in the mussels at site B3.

1.3 Suspended Particulate Matter

The mechanisms previously discussed occur at the sediment-water interface and may conceivably affect or control the partioning of metals in the water column (Hem, 1977). The SPM may also represent an important source of lead contamination.

In field experiments using a large benthic mesocosm (i.e., Bremerhaven Caisson; 13 m^2 sediment area and 13 m^3 trapped water column), Schultz-Baldes *et al.* (1983) demonstrated that lead was preferably adsorbed onto seston particles (50-70%). Only a moderate enrichment, about 3% of the total lead added, was measured in the top 3 cm of the sediment. The fauna attained much higher accumulation factors than the sediment. Lead uptake rates could be placed in order according to the feeding types, in which the filter-feeders (i.e., *Mytilus edulis*) were placed first.

In a similar study, Loring and Prosi (1986) also demonstrated that 85% of the cadmium injected into the

caisson remained in the soluble phase whereas 90% of the lead was transferred to the particulate phase. Lead was enriched relative to cadmium by a factor of 2 in *Mytilus edulis* despite its low level in the dissolved phase. This conforms with the findings of Schultz-Baldes (1974) who demonstrated that the lead uptake by *Mytilus edulis* from solution alone or food (algae) alone occurred at a similar rate when exposure concentrations were the same. The uptake of lead from food was suggested to be the more important route in the environment, as concentrations of lead in seawater are generally very low.

Prosi (1983) criticised this work because of the "unrealistic" lead levels in the food source (i.e., 600 µg/g, dry weight). The lead uptake rate in *Mytilus edulis* measured by Schultz-Baldes *et al.* (1983) in the field however, agreed well with those obtained in the laboratory by Schultz-Baldes (1974). Futhermore, the lead levels in the food source of this latter study were within the range of the SPM lead levels measured at Belledune (Table 3.6).

A significant correlation (r: 0.870, 95% level of confidence) existed between lead levels in mussels and the SPM. Considering that the method employed to analyse the lead totally dissolved the particulate phase (Rantala and Loring, 1977), this relationship is exceptionally strong. Had a weak acid leach (e.g., acetic acid) been employed, an

even stronger correlation might have been obtained (Rantala and Loring, 1985).

Lead levels decreased significantly with increasing distance from the source of contamination, from station C1 to C2. A similar observation was reported (Ward *et al.*, 1986) in which lead and cadmium levels in biota and sediments were shown to decrease exponentially with increasing distance from a lead smelter.

2.0 LEAD LEVELS in SHELL COMPONENTS

2.1 Periostracum

Lead levels measured in the periostracum were always much higher than those detected in the shells. Westbroek (1983) reported that the periostracum can readily adsorb large amounts of trace metals. Sturesson (1976, 1978) observed that 75% of the lead and 78% of the cadmium in the shell of *Mytilus edulis* were adsorbed onto the periostracum.

The periostracum of molluscs has been the subject of numerous reviews. Wilbur and Simkiss (1968) discussed the amino acid composition. Protein is the major constituent of the periostracum. DOPA (3,4-dihydroxyphenylalanine) is an o-diphenol which serves as a precursor in the formation of the periostracum. DOPA was detected in the periostracum as an integral part of the protein (Waite, 1983). o-Diphenols are capable of chelating various metals with their vicinal aromatic hydroxyls. Waite (1983) suggested that this

property of O-diphenols may contribute to sclerotization of molluscan proteins by fostering a passive mineralization resulting from a selective sequestration of metals from seawater.

Lead analyses of the periostracal material sampled from three different regions of the shell revealed that lead levels increased significantly in the older portions of the periostracum, anterior shell margin towards the umbo (Table 3.8). Sturesson (1976) also observed this and suggested the two following explanations:

1º The newly formed periostracum is exposed to the lead for a relatively shorter time than the older parts, and is therefore less enriched.

2º There are differences in the physical and chemical composition within the periostracum which may give rise to new binding sites for the lead ion.

While both mechanisms may apply, the latter may best explain the temporal increase of lead levels. The tanning of the periostracum is one of the processes leading to changes in its chemical composition (Dunachie, 1963). The chemical changes pertinent to this study were an increase of cystine and sulfhydryl compounds during sclerotization of the periostracum (Roston, 1960). Lead (II) is a B-type metal cation and coordinates preferentially with bases containing iodine, sulfur or phosphorus as donor atoms (Ahrlands *et al.*, 1958).

Shell trace metal analyses are unpopular in

monitoring studies because it is commonly believed that metal uptake by shells is controlled by passive adsorption mechanisms. Works by Romeril (1971) and Keckes *et al.* (1968) are the studies most frequently referred to concerning this subject. I have included the following example to illustrate the misconception of metal uptake in shell. In a review dealing on heavy metal uptake by aquatic organisms, Prosi (1983) writes:

> Romeril (1971) found that the uptake of ^{65}Zn in the shell of *Ostrea edulis* increased with the addition of Fe and Co; a linear uptake of ^{65}Zn in soft organs was however suppressed. In investigations on the enrichment and desorption of ^{65}Zn, Keckes *et al.* (1968) found that the uptake of ^{65}Zn by the shell of *Mytilus edulis* is not greatly affected by biologic mechanisms. Therefore, mussel shells---and thereby probably all mollusc shells--are not suitable as bio-indicators.

Prosi (1983) postulated that a passive uptake occurs by mechanisms of physico-chemical adsorption. Although the studies by Keckes *et al.* (1968) and Romeril (1971) provided added insight on metal accumulation in marine bivalves, both were primarily geared for tissue analyses as the organisms were exposed to ^{65}Zn solutions for short periods of time, generally a few hours. Futhermore, they failed to distinguish between zinc incorporated within the shell and periostracum-bound zinc. Hence, what was referred to as metal uptake <u>in</u> the shell is chiefly metal uptake <u>onto</u> the shell surface.

Mechanical cleaning procedures of the such as brushing the surface under running water (Bryan and Uysal,

1978), scraping the shell (Martincic *et al.*, 1984) and ultrasonic radiation (Fang and Shen, 1984) may remove the extraneous material from the shell, but do not remove the bulk of the periostracum. Taylor *et al.* (1969) observed that the periostracum and the prismatic conchiolin walls of the outer calcite layer in *Mytilus edulis* were completely continuous. The periostracum of this species has been shown to resist digestion by pepsin and trypsin (Stary and Andratschke, 1925; Brown, 1952) as well as chemical dissolution in a variety of strong chemical solutions including concentrated HCl at 55°C (Beedham, 1958)! The periostracum is effectively dissolved, however, in hot concentrated KOH (Beedham, 1958), sodium hypochlorite (Hunt, 1971) and hot tetramethylammonium pentahydrate 20% (Y.K.Chow, National Water Research Institute, pers. comm. 1986).

If trace metal analyses of the periostracum are not foreseen, heating the shell at 400°C may serve as an alternate method to remove/destroy the periostracum from the shell (Purchase and Fergusson, 1986a). This method also has the advantage of ashing the organic matrix within the shell (Dermott and Lum, 1986).

2.2 Aragonite and Calcite Shell Layers

Although the exact mechanism by which trace metals are incorporated within the shell has not been clearly detailed, the mechanism of shell secretion suggested by

Krampitz *et al.* (1983) presented briefly in the first chapter of this work may provide a clue.

Analyses of extrapallial fluid are relatively rare, partly because the volumes that can be collected are small (Crenshaw, 1972) and partly because the interpretation of the results in terms of ionic and protein bound fractions is difficult (Simkiss, 1983). Wada and Fujinuki (1976) observed that heavy metals occurred at much higher concentrations in the extrapallial fluid of marine and freshwater molluscs than in the surrounding water. By the effects of filtration and dialysis, they suggested that the metals appeared bound to phosphorous and sulphate ions. Kitano *et al.* (1982) subsequently reported that the extrapallial fluids contained high concentrations of various organic materials which form complexes with heavy metal ions thereby reducing the activities of heavy metals and decreasing the values of their apparent distribution coefficients in the extrapallial fluid. Wheeler *et al.* (1981) first reported the presence of a calcium-binding soluble protein (Ca-BP) in the extrapallial fluid of bivalves. Samata and Krampitz (1982) extracted a Ca-BP from the soluble fraction of the organic matrix in oyster shells. Krampitz *et al.* (1983) reported that this soluble protein consisted primarily of sulfated, high molecular weight glycoproteins some of which selectively bind calcium even in the presence of excess amounts of other cations.

Krampitz *et al.* (1983) suggested that the Ca-BP act as "vehicles" to transport Ca^{2+} ions to the active sites of the organic matrix. It is conceivable that other trace metals which display a particular affinity for sulphur such as lead, are entrained along and incorporated within the shell. Quaterman (1978) suggested that it seems likely that Ca-BP transport more metals than had been supposed.

Imlay (1982) has noted that trace metals are concentrated in the organic rich part of bivalve shells. Hewitt *et al.* (1983) arrived at a similar conclusion when studying trace metal concentrations in cephalopod shells. They observed high iron and zinc levels in the dorsal shield and suggested that these values correlate with the relatively high content of organic matter in this part of the shell. Similarly, Carriker *et al.* (1980) reported that strontium may have been associated with the mineral component of the foliated calcite shell layer in oysters (*Crassostrea virginica*), while cadmium, copper, manganese and zinc were associated with the organic matrix of the prismatic layer which was characterized by "conspicuously" more organic matter.

Isomorphic substitution of certain metals into the crystal lattice likely does occur as suggested by other studies (Travis, 1968; Carriker, 1978). If this were the main mechanism, however, lead with an ionic radius of 131 A° (in an 8-fold coordination; Nriagu, 1978) would be expected

to substitute for calcium more readily in the aragonite lattice than in the calcite, because lead carbonate (cerussite) and aragonite are isostructural (Deer *et al.*, 1980). Results from the lead analyses on the aragonite and calcite shell components (Table 3.7) showed that lead levels were consistently higher in the calcite. These results conform with other studies which demonstrated that lead levels were approximately 30% higher in the calcitic shell component of bivalve shells (Harris, 1965; Sturesson, 1976).

The aragonite component however, also contains a higher percentage of organic matrix suggesting that the organic matrix in the calcite may bind proportionally higher amounts of lead. Weiner *et al.* (1977) reported that the individual shell components of a particular species contain unique assemblages of proteins. Hare (1963) also reported differences in the amino-acid composition of the shell components in *Mytilus californianus*.

3.0. Relationship Between Lead Levels in the Tissues and Shells

Either process, the accumulation of lead in the tissues as well as the formation of calcareous shells, includes a net transfer of specific organic ligands from the living pool of the soft body to metabolically inert metallo-organic compounds (e.g., metal binding proteins; Coombs and George, 1978; Schultz-Baldes, 1978; Talbot and Magee, 1978)

or to the stable structure of shells. The fact that lead levels in the shells are generally one order of magnitude lower than in the tissues is misleading because shell lead values are additionally normalized to the weight of the aragonite.

Although the organic matrix accounts for about 3% of the dry weight of the aragonite shell, its contribution should not be underestimated. Basing balance calculations on a mean shell weight/tissue weight ratio of 17.8 (Appendix D.3), the organic matrix equals 56% of the weight of the soft tissues.

Palmer (1983) estimated that the production of skeletal organic matrix is more demanding metabolically than the crystallization of calcium carbonate. Borchardt (1985) observed that shell growth continued in starved mussels while the soft body lost weight. Tanaka *et al.* (1986) have subsequently demonstrated that a large percentage (≈ 50%) of the carbon in calcareous tests was metabolic carbon. There must be a considerable potential in the soft bodies' metabolism for providing proteinaceous substances to be transferred to the shells.

The variability of the shell lead values were significantly lower than those in the tissue. This is likely related to the fact that shell lead values are associated with less variable parameters such as metal-complexing proteins and shell weight. Zaroogian (1980) suggested that

relating metal levels to the protein fraction of organisms may reduce metal variability. The lead levels in the shell also display a strong correlation with those in the tissues. It is therefore possible to estimate lead levels in the tissues from shell analyses. These two characteristics provide a powerful tool in environmental studies because of the difficulty in adequately preserving the original integrity of metal levels in the tissues (Schmitt and Finger, 1987).

4.0 ESTIMATION of PAST Pb LEVELS in *Mytilus edulis*

More importantly, the correlation between the lead levels in the tissues and the shells provides a means to estimate past lead levels in the tissues. From the lead levels measured in the nacre labeled "prior to 1981" in B2 mussels (i.e., 43.3 µg/g), it was estimated that the lead levels in the tissues were approximately 450 µg/g. Levaque-Charron (1981) reported that the tissue lead levels in mussels collected from the same vicinity in 1980 were 50 µg/g, wet weight. When converted to a dry weight value of 430 µg/g (Harris *et al.*, 1979), the agreement was remarkably good with the tissue lead levels estimated from the shell. The lower lead levels detected in the nacre labeled "past 1983" from the same specimens (i.e., 19.8 µg/g) suggest that the lead contamination within the harbour has decreased since the relocation of the smelter outfall. Hildebran

(1984) reported that cadmium, zinc and lead levels in mussels collected within Belledune Harbour decreased after the relocation of the outfall.

Unfortunately, data relating to lead levels in C1 mussels prior to the relocation of the outfall were not available. From the shell lead analyses, the tissue lead levels of these mussels were estimated at 205 µg/g. Considering this station's proximity to Belledune Harbour and the circulation pattern in this region, southerly flowing currents, this estimation seems quite plausible.

Fang and Shen (1984) suggested that metal levels in the shell decreased with age making it difficult to reconstruct the history of contamination. Their study consisted of doing point-count analyses by SEM coupled with an X-ray energy dispersive analyzer, along the outer surface of a clam shell (i.e., *Meretrix lusoria*). They observed that relatively high metal levels (i.e., S, Fe; Cu and Zn) decreased exponentially as they moved from the younger portion of the shell (ventral shell margin) toward the older part of the shell (umbo). This observation was misleading, however, as they only focussed on periostracum-bound metals and neglected to consider the extent of the periostracal covering. Other studies suggested that elements incorporated in the shells would only be removed by dissolution or diffusion, the latter occurring too slowly to change the con-

centrations significantly after incorporation into the shell (Dodd, 1966; Sturesson, 1978; Donner and Nord, 1986).

Recent studies have demonstrated that trace metal analyses of molluscan shells are useful in reconstructing the environmental history over the past hundreds (Carell *et al*, 1987) and even thousands of years (Bourgoin, 1987b; Bourgoin and Risk, 1987a). Through instrumental neutron activation analysis (INAA) on the shells of freshwater mussels (i.e., *Margaritifera margaritifera*), Carell *et al.* (1987) measured the elemental concentrations of various metals dating from 1860 to 1985. They observed that silver, gold, iron and cobalt decreased while manganese and sulphur increased since 1940 and attributed this to the acidification of the water, probably by acid rain. Bourgoin and Risk (1987a) demonstrated that the temporal increase of lead contamination observed in the Greenland Ice Sheet (Herron *et al.*, 1977) is also reflected in Arctic marine bivalve shells.

Shell trace metal analyses, however, may not equally be applied to all metals or bivalve species. Bourgoin and Risk (1987b) have observed that although the Arctic propeller clam, *Cyrtodaria kurriana*, sequesters relatively high amounts of vanadium in its tissues (i.e., 25 µg/g), this metal was not detected within the shells. Whether this was related to the particular behaviour of vanadium or to the bivalve species studied remains to established.

5.0 CONCLUSION

Lead levels in the periostracum were about two orders of magnitude higher than in the shell material. Temporal variations of lead levels in the periostracum were probably related to biochemical changes within this layer (the tanning process) rather than to variations of environmental lead levels. Passive physico-chemical adsorption of lead seemed to have been the main mechanism of uptake.

The highest total lead concentrations were measured in the sediment samples near the Belledune area, followed by those collected at Dalhousie Harbour. Lead levels determined in sediment samples from Rimouski and Negro Harbour were significantly lower.

The lead levels in the nacreous shell layers were correlated (r:0.966) with those in the tissues and in both cases showed no relationship with mussel size. The variability of the lead concentrations in the shells was significantly lower than in the tissues (17 and 40%, respectively). The highest and lowest tissue and shell lead concentrations were measured in mussels collected near Belledune Harbour and Negro Harbour. Mussels sampled from Dalhousie Harbour and Rimouski sequestered comparable lead levels.

The lead concentration in the mussels were closely related (r:0.881) to the lead obtained from the second fraction of the sequential extraction, operationally defined as "lead bound to carbonates". This relationship was further improved (r:0.956) by normalizing the lead levels obtained in fraction 2 with the sulphur concentrations in the sediments. Regression analysis suggested that the forms of lead encountered at Rimouski were more bio-available than those at Dalhousie Harbour.

The sedimentary lead concentrations at station B3 in Belledune Harbour were twice as high as those from the other Belledune stations. Tissue and shell lead levels in mussels sampled at B3 however, were significantly lower than those measured in mussels from the other stations. The high phosphorus concentration in the sediments at B3 (1.33%) may have suppressed the lead uptake in mussels from this site.

Significant changes of lead concentrations within the nacreous shells of mussels collected near Belledune Harbour coincided with changes in environmental lead concentrations due to the relocation of the smelter outfall.

Although the results from this study are promising, much work remains to be done with respect to shell trace metal analyses and environmental contamination. While some problems have been clarified, many others remain to be answered. Among these are:

1° To what extent can the trends observed in this study

be applied to other non-essential metals, for example cadmium, mercury?

2º Would essential metals, particularly copper and zinc, behave in a similar fashion?

3º Are the mechanisms involved with the metal fractionation between the shells and tissues species specific (i.e., *Mytilus edulis*) or would it equally apply to other bivalve species?

4º To what extent does the phosphorus in the environment control the uptake of lead and other metals in organisms.

In spite of this, this work has demonstrated the usefulness of *Mytilus edulis* shells relating to environmental contamination of lead. Many problems related to the collection and analyses of the biota (some of which are unsurmountable) are by-passed through shell analyses. The writer does not advocate the sole use of shells in impact studies, but suggests rather that effectively combined with tissue analyses may render a more complete picture. The shell trace metal analyses' greatest power illustrated in this study lies in their ability to estimate past levels of trace metals.

REFERENCES

Agemian,H. and Chau,A.S.Y. 1976. Evaluation of extraction techniques for the determination of metals in aquatic sediments, Analyst, 101: 761-767.

Ahrlands,S., Chatt,T. and Davies,N.R. 1958. The relative affinities of ligand atoms for acceptor molecules and ions. Quaterly Review, Chemical Society, 12: 265-276.

Amiard,J.C., Amiard-Triquet,C., Berthet,B. and Métayer,C. 1986. Contribution to the ecotoxicological study of cadmium, lead, copper and zinc in the mussel *Mytilus edulis*, Mar. Biol., 90: 425-431.

Beedham,G.E. 1958. Observations on the non-calcareous component of the shell of the Lamellibranchia. Quart. J. micr. Sci., 99: 341-357.

Bernhard,M. 1976. Sampling and analyses of biological material (part 3) IN: Manual of Methods in Aquatic Environment Research. FAO Fisheries Technical Paper No. 158, 117pp.

Bertine,K.K. and Goldberg,E.D. 1972. Trace elements in clams, mussels and shrimp, Limnol. & Oceanogr., 17: 877-884.

Boggild,O.B. 1930. The shell structure of the molusks. K. Danske Vidensk. Selsk. Skr., 2: 232-325.

Borchardt,T. 1985. Relationships between carbon and cadmium uptake in *Mytilus edulis*, Mar. Biol., 85: 233-244.

Borg,H., Edin,A. Holm,K. and Sköld,E. 1981. Determination of metals in fish livers by flameless atomic absorption spectroscopy, Water Res., 15: 1291-1295.

Bourgoin,B.P. 1987a. A technique to separate the calcite and aragonite shell layers in *Mytilus edulis* L., submitted for publication to Marine Environmental Research, May 1987.

_____. 1987b. Trace metal concentration in fossil and recent shells of the Arctic infaunal bivalve, *Mya truncata* L., IN: R.Crick (ed) The Fifth International Symposium on Biomineralization: the origin of ocean chemistry and its

significance to biomineralization, Texas, (in press; accepted March 1987).

Bourgoin,B.P. and Risk,M.J. 1987a. Historical changes in lead in the Eastern Canadian Arctic, determined from fossil and modern *Mya truncata* shells, Sci. Total Environm., (in press; accepted for publication July 1987).

_____. 1987b. Vanadium contamination monitored by an Arctic bivalve, *Cyrtodaria kurriana*, Bull. Environm. Contam. Toxicol. (in press; Vol 39 (6) December 1987).

Bowen,H.J.M. 1966. Trace elements in biochemistry. Academic Press, New York, 241 pp.

Boyden,C.R. 1974. Trace element content and body size in molluscs, Nature, 251: 311-314.

_____. 1977. Effect of size upon metal content in shell fish, J. mar. biol. Ass. U.K., 57: 675-714.

Boyden,C.R. and Romeril,M.G. 1974. A trace metal problem in pond oyster culture, Mar. Pollut. Bull., 5: 74-78.

Breteler,R.J., Valiela,I. and Teal,J.M. 1981. Bioavailability of mercury in several north-eastern U.S. *Spartina* ecosystems, Estuar. Coastal & Shelf Sci., 12: 155-166.

Brooks,R.R. and Rumsby,M.G. 1965. The biogeochemistry of trace element uptake by some New Zealand bivalves, Limnol. & Oceanogr., 10: 521-527.

Brown,C.H. 1952. Some structural problems of *Mytilus edulis*, Q. J. Mircrosc. Sci., 93: 487-502.

Bryan,G.W. and Uysal,H. 1978. Heavy metals in the burrowing bivalve *Scrobicularia plana* from the Tamar Estuary in relation to environmental levels, J. mar. biol. Ass. U.K., 58: 89-108.

Bryan,G.W., Langston,W.J., Hummerstone,L.G. and Burt,G.R. 1985. A guide to the assessment of heavy-metal contamination in estuaries using biological indicators, J. mar. biol. Ass. U.K. Occasional Publication 4, 92pp.

Buckley,D.E. and Winters,G.V. 1983. Geochemical transport through the Miramichi Estuary. Can. J. Fish. Aquat. Sci., 40(Suppl.2): 162-182.

Butler,P.A., Andren,L., Bond,G.J., Jernelöv,A. and Reisch,D.J. 1971. Monitoring organisms IN: M.Ruivo (ed.) Food and

Agricultural Organization Technical Conference on Marine Pollution and its Effects on Living Resources and Fishing, Rome. Suppl. 1: Methods of detection, measurement and monitoring of pollutants in the marine environment, Fishing News (Books) Ltd., 101-112.

Carell,B. Forberg,S., Grundelius,E., Henrikson,L., Johnels,A., Lindh,U., Mutvei,H., Olsson,M., Svardstrom,K. and Westermark,T. 1987. Can mussel shells reveal environmental history, Ambio, 16: 2-10.

Carriker,M.R. 1978. Ultra-structural analysis of dissolution of shell of the bivalve *Mytilus edulis* by the accessory boring organ of the gastropod *Urosalpinx cinerea*, Mar. Biol., 48: 105-134.

Carriker,M.R., Palmer,R.E., Lowell,V.S. and Johnson,C.C. 1980. Interaction of mineral elements in sea water and shell of oysters (*Crassostrea virginica* (Gmelin)) cultured in controlled and natural systems, J. exp. Mar. Biol. Ecol., 46: 279-296.

Carter,J.G. 1976. The structural evolution of the bivalve shell, with notes on the phylogenetic significance of crossed lamellar structures, Ph.D. dissertation, Yale University, Connecticut, 255pp.

Chau,Y.K. and Wong,P.T.S. 1978. Occurrence of biological methylation of elements in the environment IN: Organometal and Organo metaloids---Occurrence and Fate in the Environment. ACS Symp. Ser., 82: 39-53.

Chester,R. and Stoner,J.H. 1975. Trace elements in sediments from the lower Severn Estuary and Bristol Channel. Mar. Pollut. Bull., 6: 89-92.

Chétail,M. and Krampitz,G. 1982. Calcium and skeletal structures in molluscs: concluding remarks. Malacologia, 22(1-2): 337-339.

Clark,G.R. 1976. Shell growth in the marine environment: approaches to the problem of marginal calcification. Am. Zool., 16: 617-626.

Clarke,J.H., Clarke,A.N. and Wilson,D.J. 1976. Lead levels in freshwater mollusk shell. J. Environ. Sci. Health, A11: 65-78.

Comfort,A. 1949. Acid-soluble pigments of shells, Biochem. J., 44: 111-117.

Cook,R.H. and Hoos,R.A.W. 1971. New Brunswick industrial survey report, 1971. Department fo Fisheries and Forestry. Resource Development Branch. Atlantic Region: Report 71-3, 15p.

Coombs,T.L. 1980. Heavy metal pollutants in the aquatic environment IN: R.Gilles (ed) Animals and Environmental Fitness, Permagon Press, pp. 283-302.

Coombs,T.L. and George,S.G. 1978. Mechanisms of immobilization and detoxification of metals in marine organisms, IN: D.S. McLusky and A.J.Berry (eds), Proceedings of the 12th European Symposium on Marine Biology, Physiology and Behaviour of Marine Organisms, Permagon Press, New York, 211-218.

Coombs,T.L. and Keller,P.J. 1981. *Mytilus* byssal threads as an environmental marker for metals, Aquat. Toxicology, 1: 291-300.

Cossa,D., Bourget,E., Pouliot,D., Piuze,J. and Chanut,J.P. 1980. Geographical and seasonal variation in the relationship between trace metal content and body weight in *Mytilus edulis, Mar. Biol., 58: 7-14.*

Cranston,R.E., Fitzgerald,R.A. and Winters,G.V. 1974. Geochemical Data: Baie des Chaleurs. Bed. Inst. Ocean. Data Series Rep. BI-D-74-6. 22pp.

Crenshaw,M.A. 1972. The soluble matrix from *Mercenaria mercenaria* shell. Biomineralization 6: 6-11.

Crenshaw,M.A. and Neff,J.M. 1969. Decalcification at the mantle-shell interface in molluscs. Am. Zool. 9: 881-885.

Dare,P.J. and Edwards,D.B. 1975. Seasonal changes in flesh weight and biochemical composition of mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales, J. exp. Mar. Biol. Ecol., 18: 89-97.

Deer,W.A., Howie,R.A. and Zussman,J. 1980. An introduction to the rock-forming minerals, Longman Group Ltd., London, 528pp.

Degens,E.T.,Spencer,D.W. and Parker,R.H. 1967. Palaeobiochemistry of molluscan shell proteins. Comp. biochem. physiol., 20: 553-579.

Dermott,R.M. and Lum,K.R. 1986. Metal concentrations in the annual shell layers of the bivalve *Elliptio complanata*, Environ. Pollut., 12: 131-143.

DeWolf,P. 1975. Mercury content of mussels from West European coasts. Mar. Pollut. Bull., 6: 61-63.

Dodd,J.R. 1966. Diagenetic stability of temperature-sensitive skeletal properties in *Mytilus* from the Pleistocene of California. Geol. Soc. Am. Bull., 77: 1213-1224.

Donner,J. and Nord,A.G. 1986. Carbon and oxygen stable isotope values in shells of *Mytilus edulis* and *Modiolus modiolus* from Holocene raised beaches at the outer coast of the Varanger Peninsula, North Norway. Palaeogeogr., Palaeoclimatol., Palaeoecol., 56: 35-50.

Dunachie,J.F. 1963. The periostracum of *Mytilus edulis*. Trans. Roy. Soc., 65 (15): 383-411.

Dunstan,I.C., de Forest,A. and Pettis,R.W. 1980. *Mytilus edulis* as an indicator of trace metal pollution in naval dockyard waters with preliminary results from Williamstown Naval Dockyard, Victoria, Department of defense, Report MRL-R-781.

Fagerström,T. 1977. Body weight, metabolic rate and trace substance turnover in animals. Oecologia (Berl.). 29: 99-104.

Fang,L.-S. and Shen,P. 1984. Foreign elements in a clam shell: a clue to the history of marine pollution events. Mar. Ecol. Prog. Ser., 18: 187-189.

Ferguson,J. 1983. Concentrations and speciation of lead, zinc and cadmium in seawater-like smelter effluent and adjacent marine environments, Port Pirie, South Australia, Aust. J. Mar. Freshw. Res., 34: 375-385.

Ferrell,R.E., Carville,T.E. and Martinez,J.D. 1973. Trace metals in oyster shell. Environm. Letters, 4: 311-316

Fischer,H. 1983. Shell weight as an independent variable in relation to cadmium content of molluscs, Mar. Ecol. Prog. Ser., 12: 59-75.

Folsom,T.R., Hodge,V.F., Wong,K.M., Kishore,R. and Guinn,V.P. 1972. Some trace element concentration variations observed in marine organisms that suggest caution in sampling. IN: Report for the IDOE Workshop on Baseline Measurements, Upton, New York.

Fowler,S. and Oregioni,B. 1976. Trace metals in mussels from the North-West Mediterranean, Mar. Pollut. Bull., 7: 26-29.

Frazier,J.M. 1975. The dynamics of metals in the American oyster, *Crassostrea virginica*. I. Seasonal effects. Chesapeake Sci., 16: 162-171.

_____. 1976. The dynamics of metals in the American oyster, *Crassostrea virginica*. 2. Environmental effects, Chesapeake Sci. 17: 188-197.

George,S.G., Pirie,B.J.S. and Coombs,T.L. 1976. The kinetics of accumulation and excretion of ferric hydroxide in *Mytilus edulis* (L.) and its distribution in the tissues. J. exp. Mar. Biol. Ecol.23: 71-84.

_____. 1977. IN: T.C.Hutchinson (Ed.) International Conference on Heavy Metals in Environment, Proc. Vol. 2., Toronto, Canada, pp. 887-900.

_____. 1978. Metabolic characteristics of endocytosis of ferritin by gills of a marine bivalve mollusc, Biochem. Soc. Transactions, 5: 136-137.

Goldberg,E.D., Bowen,V.T., Farrington,J.W., Harvey,G., Martin,J.H., Parker,P.L., Risebrough,R.W., Robertson,W., Schneider,E. and Gamble,E. 1978. The mussel watch, Envir. Conserv., 5: 101-125.

Gordon,J. and Carriker,M.R. 1980. Sclerotized protein in the shell matrix of a bivalve mollusc. Mar. Biol., 57: 251-260.

Gordon,M., Knauer,G.A. and Martin,J.H. 1980. *Mytilus californianus* as a bioindicator of trace metal pollution: variability and statistical considerations, Mar. Pollut. Bull., 11: 195-198.

Grégoire,C. 1972. Structure of the molluscan shell. IN: M.Florkin and B.T.Sheer (eds) Chemical zoology. VII Mollusca, Academic Press,NY, 45-102.

Griffin,J.J., Koide,M., Hodge,V. and Goldberg,E.D. 1980. Estimating the ages of mussels by chemical and physical methods. IN: E.D.Goldberg, Y.Horibe and K.Saruhashi (eds.) Isotope Marine Chemistry, , Rokakugo, Tokyo, 193-209.

Guy,R.D., Chakrabarti,C.L. and McBain,D.C. 1978. An evaluation of extraction techniques for the fractionation of copper and lead in model sediment systems. Water Res., 12: 21-24.

Hamilton,E.I. 1980. Concentration and distribution of uranium in *Mytilus edulis* and associated materials. Mar. Ecol. Prog. Ser., 2: 61-73.

Hare,P.E. 1963. Amino acids in the proteins from aragonite and calcite in the shells of *Mytilus californianus*, Science, 139: 216-217.

Harris,J.E., Fabris,G.J., Statham,P.J. and Tawfik,F. 1979. Biogeochemistry of selected heavy metals in Western Port, Victoria, and use of invertebrates as indicators with emphasis on *Mytilus edulis planulatus*, Aust. J. Mar. Freshwater Res., 30: 159-178.

Harriss,R.C. 1965. Trace element distribution in molluscan skeletal material I. Magnesium, iron, manganese and strontium, Bull. Mar. Sci., 15: 265-273.

Haskin,H.H. 1954. Age determination in mollusks. Trans. N.Y. Acad. Sci. 16: 300-304.

Haug,A., Melsom,S. and Omang,S. 1974. Estimation of heavy metal pollution in two Norwegian fjord areas by analysis of the brown alga *Ascophyllum nodosum*. Environ. Pollut., 7: 179-192.

Hem,J.D. 1977. Reactions of metal ions at surfaces of hydrous iron oxides, Geochim. Cosmochim. Acta, 41: 527-538.

Hemelraad,J., Kleinveld,H.A., de Roos,A.M., Howlwerda,D.A. and Zandee,D.I. 1987. Cadmium kinetics in freshwater clams. III Effects of zinc uptake and distribution of cadmium in *Anonta cygnea*. Arch. Environ. Contam. Toxicol. 16: 95-101.

Herron,M.M., Langway Jr.,C.C., Weiss,H.V. and Cragin,J.H. 1977. Atmospheric trace metals and sulphate in the Greenland Ice Sheet, Geochim. Cosmochim. Acta, 41: 915-920.

Hewitt,R.A., Lazell,B.H. and Moorhouse,S.J. 1983. An introduction to inorganic components of cephalopod shells, N. Jb. Geol. Palaont. Abh., 164: 331-361.

Hildebran,L.P. 1984. An assessment of environmental quality in the Baie des Chaleurs. Environmental Protection Service Report Series (Atlantic Region), # EPS-5-AR-848, 191pp.

Holden,A.V., Topping,G. and Uthe,J.F. 1983. Use and revelance of analytical intercomparison exercises in monitoring the marine environment. Can. J. Fish. Aquat. Sci., 40(Suppl. 2): 100-110.

Hunt,S. 1971. Comparison of three extracellular structural proteins in the gastropod mollusc, *Buccinum undatum* L.: The periostracum, egg capsule, and operculum, Comp. Biochem. Physiol. B 40B: 37-46.

Imlay,M.J. 1982. Use of shells of freshwater mussels in monitoring heavy metals and environmental stresses: A review, Malacological Review, 15: 1-140.

Jackim,E., Morrison,G. and Steele,R. 1977. Effects of environmental factors on radiocadmium uptake by four species of marine bivalves, Mar. Biol., 40: 303-308.

Jawrowski,Z., Barbalat,F. and Blain,C. 1985. Heavy metals in human and animal bones from ancient and comtemporary France, Sci. Total Environ., 43: 103-126.

Jenne,E.A. 1977. Trace element sorption by sediments and soils---sites and processes IN: W.Chappel and K.Petersen (eds), Symposium on Molybdenum in the Environment, pp.. 425-553.

Jenne,E.A. and Luoma,S.N. 1977. Biological implications of metals in the environment, R.E.Wilding and H.Drucker (eds), CONF-750929, NITS, Springfield, VA., pp 110-143.

Johnson,R.G. 1974. Particulate matter at the sediment-water interface in coastal environments, J. Mar. Res., 32: 313-329.

Jones,P.G.W. 1982. A review of nutrient salt and trace metal data in U.K. tidal waters. Aquatic monitoring report, MAFF Directorate of Fisheries Research, No. 7, 22pp.

Keckes,S., Ozretic,B. and Krasnovic,M. 1968. Loss of ^{65}Zn in the mussel *Mytilus galloprovincialis*, Malacologia, 7: 1-6.

Kennedy,W.J., Taylor,J.D. and Hall,A. 1969. Environmental and biological controls on bivalve shell mineralogy. Biol. Rev., 44: 499-530.

Kinrade,J.D. and Van Loon,J.C. 1974. Solvent extraction for use with flame atomic absorption spectrometry. Anal. Chem., 46: 1894-1898.

Kitano,Y., Kasai,K. and Wada,K. 1982. Heavy metals in extrapallial fluid and shell of *Saxidomus purpuratatas and Crassostrea gigas*, Proceedings of a Pacific Regional Workshop on Assimilative Capacity of the Oceans for Man's Wastes, Taipei, 232-237.

Koide,M., Lee,S.D. and Goldberg,E.D. 1982. Metal and transuranic records in mussel shells, byssal threads and tissues. Estuar. Coastal & Shelf Sci., 15: 679-695.

Krampitz,G., Drolshagen,H., Hasle,J. and Hof-Irmscher,K. 1983. Organic matrices of mollusc shell. IN: Biomineralization and Biological Metal Accumulation. (eds, P.Westbroek and E.W.de Jong), 231-247.

Langston,W.J. 1980. Arsenic in U.K. estuarine sediments and its

availability to benthic organisms, J. mar. biol. Ass. U.K., 60: 869-881.

_____. 1982. The distribution of mercury in British estuarine sediments and its availability to deposit-feeding bivalves. J. mar. biol. Ass. U.K., 62: 667-684.

La Touche,D.Y. and Mix,M.C. 1982a. The effects of depuration, size and sex on trace metal levels in Bay Mussels. Mar. Pollut. Bull., 13(1): 27-29.

_____. 1982b. Seasonal variations of arsenic and other trace elements in Bay mussels (*Mytilus edulis*), Bull. environm. Contam. & Toxicol., 29: 665-670.

Levaque-Charron,R.L. 1981. Marine environmental impact survey of the Belledune Harbour area, New Brunswick, for the period May, 1979, to April, 1980, Noranda Research Centre, Internal Report No. 389.

Lobel,P.B. and Wright,D.A. 1982. Total body zinc concentration and allometric growth ratios in *Mytilus edulis* collected from different shore levels. Mar. Biol., 66: 231-236.

Lobel,P.B., Mogie,P., Wright,D.A. and Wu,B.L. 1982. Metal accumulation in four molluscs, Mar. Pollut. Bull., 13: 170-174.

Loring,D.H., Bewers,J.M., Seibert,G. and Kranck,K. 1980. A preliminary survey of circulation and heavy metal contamination in Belledune Harbour and adjacent areas, p. 35-47 In: Uthe,J.F. and V.Zitko (Eds.) Cadmium Pollution of Belledune Harbour, New Brunswick, Canada. Can. Tech. Rep. Fish. Aquat. Sci. 963: 35-47.

Loring,D.H. and Prosi,F. 1986. Cadmium and lead cycling between water, sediment, and biota in an artificially contaminated mud flat on Borkum (F.R.G.). Wat. Sci. Tech., 18: 131- 139.

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

Luoma,S.N. and Bryan,G.W. 1978. Factors controlling the availability of sediment-bound lead to the estuarine bivalve *Scrobicularia plana*, J. mar. biol. Ass. U.K., 58: 793-802.

_____. 1981. A statistical assessment of the form of trace metals in oxidized estuarine sediments employing chemical extractants, Sci. Total Environ., 17: 165-196.

Luoma,S.N. and Jenne,E.A. 1976. Estimating bioavailability of sediment-bound trace metals with chemical extractants, IN:

D.D.Memphill (ed) Trace Substances in Environmental Health-X, University of Missouri Press, Columbia, 343-353.

Luoma,S.N. and Davis,J.A. 1983. Requirements for modeling trace metal partitioning in oxidized estuarine sediments. Mar. Chem., 12: 159-181.

Luten,J.B., Bouquet,W., Burggraaf,M.M., Rauchbaar,A.B. and Rus,J. 1986. Trace metals in mussels (*Mytilus edulis*) from the Waddenzee, Coastal North Sea and the estuaries of Ems, Western and Eastern Scheldt, Bull. environm. Contam. & Toxicol., 36: 770-777.

Lutz,R.A. 1974. Annual periodicity and its relation to the internal shell morphology of *Mytilus edulis*. Proc. Natl. Shellfish. Assoc., 64: 5.

_____. 1975. *Mytilus edulis* L.: age determination, peal incidence, and commercial raft cultivation implications. Ph.D. Thesis, University of Maine, Orono, Maine, 133p.

_____. 1976. Annual growth patterns in the inner shell layer of *Mytilus edulis* L., J. mar. biol. Ass. U.K., 56: 723-731.

Lutz,R.A. and Rhoads,D.C. 1980. Growth patterns within the molluscan shell: an overview. IN: D.C.Rhoads and R.A.Lutz (eds) Skeletal Growth of Aquatic Organisms, Plenum press, New York, 203-254.

Mackay,D. and Paterson,S. 1981. Calculating fugacity. Environ. Sci. Technol., 15: 1006-1014.

_____. 1982. Fugacity revisited. Environ. Sci. Technol., 16: 654A-660A.

MacKnight,S.D. 1980. A study of cadmium geochemistry in New Brunswick estuaries and its effect upon dredging, Proceeding of WODCON IX (9th World Dredging Conference) Vancouver, British Columbia, 745-759.

MacKnight,S.D., and Schafer,C.T. 1980. Geochemistry and benthic ecology at the Dalhousie, New Brunswick Ocean Dump Site. Proceedings of WODCON IX (9th World Dredging Conference) Vancouver, British Columbia, 760-781.

Majori,L., Negoclan,G. Modonietti,G.B. and Darris,F. 1978. Study of the seasonal variations of some trace elements in the tissues of *Mytilus galloprovincialis* taken in the Gulf of Trieste, Rev. Int. Oceanogr. Med. XLIX: 37-40.

Martincic,D., Nurnberg,H.W., Stoppler,M. and Brancia,M. 1984.

Bioaccumulation of heavy metals from Lim Fjord (North Adriatic Sea), Mar. Biol., 81: 177-188.

Matheson,R.A.F. and Bradshaw,V.I. 1985. The status of selected environmental contaminants in the Baie des Chaleurs ecosystem. Environmental Protection Service, Atlantic Region, EPS-5-AR-6, 65p.

McGonigle,R.M. 1940. Report of an investigation of alleged pollution from certain mills in northern New Brunswick. Fish. Res. Bd. Canada, Manuscript Report, Biol. Sta., No. 250, 13p.

Moore,H.J. 1971. The structure of the latero-frontal cirri on the gills of certain lamellibranch molluscs and their role in suspension feeding, Mar. Biol., 11: 23-27.

Mudroch, A. 1984. Particle size effects on concentration of metals in Lake Erie bottom sediments. Water Poll. Res. J. Canada 19: 27-35.

Nriagu,J.O. 1978. Properties and the biogeochemical cycle of lead, IN: J.O.Nriagu (ed) The Biogeochemistry of Lead in the Environment (part A), Elsevier/North-Holland Biomedical Press, New York, 1-14.

Nriagu,J.O., Kemp,A.L., Wong,H.K.T. and Harper,N. 1979. Sedimentary record of heavy metal pollution in Lake Erie,Geochim. Cosmochim. Acta, 43: 247-258.

Oakley,S.M., Delphey,C.E., Williamson,K.J. and Nelson,P.O. 1980. Kinetics of trace metal partitioning in model anoxic marine sediments, Water Res., 14: 1067-1072.

Oakley,S.M., Nelson,P.O. and Williamson,K.J. 1981. Model of trace-metal partitioning in marine sediments. Environ. Sci. Technol., 15: 474-480.

Packman,G.A., Tay,K.L. and Berman,C. 1984. Environmental monitoring at the Heron Island dump site in the Bay of Chaleur near Dalhousie, New Brunswick. Environmental Protection Service, Atlantic Region, EPS-5-AR-84-6, 91p.

Paine,R.T. 1971. The measurements and application of the calorie to ecological problems, Ann. Rev. Ecol. Syst., 2: 145-164.

Palmer,A.R. 1983. Relative cost of producing skeletal organic matrix versus calcification: evidence from marine gastropods, Mar. Biol., 75: 287-292.

Patterson,C.C. and Settle,D.M. 1976. The reduction of orders of magnitude errors in lead analysis of biological materials

and natural water by evaluating and controlling the extent and sources of industrial lead contamination introduced during sample collecting, handling and analysis IN: Accuracy in Trace Analysis: sampling, sample handling, and analysis. Proceedings of the 7th IMR Symposium, National Bureau of Standards Special Publication 422, 321-351.

Pentreath,R.J. 1973. The accumulation from water of Zn^{65}, Mn^{54}, Co^{58} and Fe^{59} by the mussel *Mytilus edulis*, J. mar. biol. Ass. U.K., 53: 127-143.

Petit,H.,Davis,W.L., and Jones,R. 1979. Morphological studies on the periostracum of the freshwater mussel *Amblena* (Unionidae). Tissue Cell 11, 633-642.

Phillips,D.J.H. 1976. The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals, Mar. Biol., 38: 59-69.

_____. 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments--a review, Environ. Pollut., 13: 281-317.

_____. 1980. Quantitative aquatic biological indicators, Applied Science Publishers, Barking, 480pp.

Preston,A., Jefferies,D.J., Dutton,J.W.R., Harvey,B.R. and Steele,A.K. 1972. British Isles coastal waters: The concentrations of selected heavy metals in sea water, suspended matter and biological indicators---a pilot survey, Environ. Pollut., 3: 69-82.

Prosi,F. 1983. Heavy metals in aquatic organisms IN: U.Forstner and G.T.W.Whittmann (eds) Metal Pollution in the Aquatic Environment, Springer-Verlag, New York, 271-322.

Purchase,N.G. and Fergusson,J.E. 1986a. The distribution and geochemistry of lead in river sediments, Christchurch, New Zealand, Environ. Pollut., 12: 203-216.

_____. 1986b. *Chione (Austrovenus) stutchburyi*, a New Zealand cockle, as a bio-indicator for lead pollution, Environ. Pollut., 11: 137-151.

Quaterman,J., Morrison,J.N. and Humphries,W.R. 1978. The influence of high dietary calcium and phosphate on lead uptake and release. Environ. Res. 17: 60-67.

Rantala,R.T.T. and Loring,D.H. 1977. A rapid determination of 10 elements in marine suspended particulate matter by atomic absorption spectrophotometry, At. Abs. Newslett., 16:51-52.

_____. 1985. Partion and determination of cadmium, copper, lead and zinc in marine suspended particulate matter, Intern. J. Environ. Chem., 19: 165-173.

Rapin,F. and Forstner,U. 1983. Sequential leaching techniques for particulate metal speciation: the selectivity of various extractants. Proc. Int. Conf. Heavy Metals Environ., Heidelberg, Vol. 2: 1074-1077.

Ray,S. and McLeese,D.W. 1983. Factors affecting uptake of cadmium and other trace metals from marine sediments by some bottom-dwelling marine invertebrates IN: D.R.Kester, B.H.Ketchum, I.W.Duedall and P.Kilho Park (eds) Wastes in the Oceans---dredged-material disposal in the ocean (Vol 2), John Wiley and Sons, New York, 185-197.

Ray,S., McLeese,D.W., Peterson,M.R. 1981. Accumulation of copper, zinc, cadmium and lead from two contaminated sediments by three marine invertebrates---a laboratory study. Bull. environm. Contam. & Toxicol., 26; 315-322.

Raymont,J.E.G. 1972. Some aspects of pollution in Southampton water. Proc. R. Soc. Lond., Series B, 180: 451-468.

Reddy,M.P.M. 1968. Wave conditions and littoral drift near Belledune Point, Chaleur Bay. Bedford Institute of Oceanography. Report BI-R-68-2, 6p.

Riley,J.P. and Taylor,D. 1968. Chelating resins for concentration of trace elements from seawater and their analytical use in conjunction with atomic absorption spectrophotometry, Analytica chim. Acta, 40: 479-485.

Ritz,D.A., Swain,R. and Eliott,N.G. 1982. Use of the mussel *Mytilus edulis planulatus* (Lamarck) in monitoring heavy metal levels in seawater, Aust. J. Mar. Freshwater Res., 33: 491-506.

Romeril,M.G. 1971. The uptake and distribution of ^{65}Zn in oysters, Mar. Biol., 9: 347-354.

Roosenburg,W.H. 1969. Greening and copper accumulation in the American oyster, *Crassostrea virginica*, in the vicinity of a steam electric generating station. Chesapeake Sci. 10: 241-252.

Roston,S. 1960. Reaction of the sulfhydryl group with an oxidation product of β-3,4 dihydroxylalanine, J. Biol. Chem., 235: 1002-1004.

Samata,T. and Krampitz,G. 1982. Ca^{2+}-binding polypeptides in oyster shells. Malacologia, 22 (1-2): 225-233.

Schmidt,U. and Hubert,F. 1976. Methylation of organolead and lead (II) compounds to $(Ch_3)_4Pb$ by microorganisms. Nature, 259: 157-158.

Schmitt,C.J. and Finger,S.E. 1987. The effects of sample preparation on measured concentrations of eight elements in edible tissues of fish from streams contaminated by lead mining, Arch. Environm. Contam. Toxicol., 16: 185-207.

Schulz-Baldes,M. 1974. Lead uptake from sea water and food, and lead loss in the common mussel *Mytilus edulis*, Mar. Biol., 25: 177-193.

_____. 1978. Lead transport in the common mussel *Mytilus edulis*, IN: D.S.McLusky and A.J.Berry (eds), Proceedings of the 12th European Symposium on Marine Biology, Physiology and Behaviour of Marine Organisms, Permagon Press, New York, 211-218.

Schulz-Baldes,M., Rehm,E. and Farke,H. 1983. Field experiments on the fate of lead and chromium in an intertidal benthic mesocosm, the Bremerhaven Caisson. Mar. Biol., 75: 307-318.

Seed,R. 1973. Absolute and allometric growth in the mussel *Mytilus edulis* L. (Mollusca: Bivalvia). Proc. Malac. Soc. Lond., 40: 343-357.

_____. 1976. Ecology. IN: B.L.Bayen (ed) Marine Mussels: Their Ecology and Physiology, Cambridge University Press, 13-65.

Serne,R.J. 1977. Geochemical distribution of selected trace metals in San Francisco Bay sediments IN: Biological Implications of Metals in the Environment. Energ. Res. Div. Admin. CONF-750929, 280-296.

Shields,J.B. and Mitchell,H.H. 1941. The effect of calcium and phosphate on the metabolism of lead. J. Nutr. 21: 541-552.

Shimizu,M. and Tsuji,S. 1980. Heavy metal content of the common mussel, *Mytilus edulis*, and its variation. IN: B.Patel (ed). Management of the Environment, Wiley Eastern, New Delhi.

Sigg,L., Stumm,W. and Zinder,B. 1984. Chemical processes at the particulate-water interface: implications concerning the form of occurrence of solute and adsorbed species. In Complexation of trace metals in natural waters. C.J.M. Kramer and J.C. Duinker (eds.), Nijhoff & Junk Publishers, The Hague, pp. 251-266.

Simkiss,K. 1983. Trace elements as probes of biomineralization IN: P.Westbroek and E.W.deJong (eds) Biomineralization and Biological Accumulation, D.Reidel Publishing Company, Boston, 363-371.

Simkiss,K. and Wilbur,K.M. 1977. The molluscan epidermis and its secretions, Symp. zool. Soc. London, 39: 35-76.

Simpson,R.D. 1979. Uptake and loss of zinc and lead by mussels (*Mytilus edulis*) and relationships with body weight and reproductive cycle, Mar. Pollut. Bull., 10: 74-78.

Spurr,A.K. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., 26: 31p.

Stary,Z. and Andratschke,I. 1925. Bertage zur kennthiss einiger skleroproteine, Hoppe-Seyler's Z. Physiol. Chem., 148: 83-98.

Stephenson,M.D., Martin,J.H. and Martin,M. 1978. State Mussel Watch: trace metal concentrations in the california mussel at areas of special biological significance. Ann.Rep. submitted to the California State Water Resources Control Board, 38pp.

Strain,W.H. and Pories,W.J. 1966. Zn levels of hair as tools in Zn metabolism. IN: A.S.Prasad, C.C.Thomas (eds.) Zinc metabolism. Springfield, Ill., 363-377.

Sturesson,U. 1976. Lead enrichment in shell of *Mytilus edulis*, Ambio., 5: 253-256.

_____. 1978. Cadmium enrichment in shell of *Mytilus edulis*, Ambio., 7: 122-125.

Subramanian,K.S. and Méranger,J.C. 1979. Ammonium Pyrrolidinedithiocarbamate-Methyl Isobutyl ketone-Graphite furnace atomic absorption system for some trace metals in drinking water, Intern. J. Environ. Anal. Chem., 7: 25-40.

Talbot,V. and Magee,R.J. 1978. Naturally-occurring heavy metal binding proteins in invertebrates, Arch. Environm. Contam. Toxicol., 7: 73-81.

Talbot,V., Magee,R.J. and Hussain,M. 1976. Lead in Port Phillips Bay mussels. Mar. Pollut. Bull., 7: 234-237.

Tanaka,N., Monaghan,M.C. and Rye,D.M. 1986. Contribution of metabolic carbon to mollusc and barnacle shell carbonate. Nature, 320:520-523.

Taylor,J.D. and Kennedy,W.J. 1969. The influence of the periostracum on the shell structure of bivalve mollusks. Calcif. Tissue. Res., 3: 274-283.

Taylor,J.D., Kennedy,W.J., and Hall,A. 1969. The shell structure and mineralogy of the bivalvia. Introduction. Nuculea-Trigonacea. Bull. Br. Mus. (Nat. Hist.) Zool. Suppl. 3, 1-125.

Taylor,J.D. and Layman,M. 1972. The mechanical properties of bivalve (Mollusca) shell structures. Palaeontology 15 (part 1): 73-87.

Taylor,J.K. 1985. Standard Reference Materials: Handbook for SRM Users, NBS Special Publication 260-100, 85pp.

Tessier,A., Campbell,P.G.C. and Bisson,M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem., 51: 844-851.

_____. 1980. Trace metal speciation in the Yamaska and St-François rivers (Québec). Can. J. Earth Sci., 17: 90-105.

_____. 1982. Particulate trace metal speciation in stream sediments and relationships with grain size: implications for geochemical exploration, J. Geochem. Explor. 16: 77-104.

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

Thomson,E.A., Luoma,S.N., Cain,D.J. and Johansson,C. 1980. The effect of sample storage on the extraction of Cu, Zn, Fe, Mn and organic material from oxidized estuarine sediments. Water, Air, and Soil Pollut. 14: 215-233.

Travis,D.F. 1968. The structure and organization of, and the relationship between, the inorganic crystals and the organic matrix of the prismatic region of *Mytilus edulis*, J. Ultrastruct. Res., 23: 183-215.

Unlü,M.Y. and Fowler,S.W. 1979. Factors affecting the flux of arsenic through the mussel, *Mytilus galloprovincialis*. Mar. Biol., 51: 209-219.

Uthe,J.F. and Zitko,V. 1980. Cadmium pollution of Belledune Harbour, New Brunswick, Canada. Can. Techn Rep. Fish. Aquat. Sci., 963, 107pp.

Wada,K. and Fujinuki,T. 1976. Biomineralization in bivalve molluscs with emphasis on the chemical composition of the extrapallial fluid IN: N.Watabe and K.M. Wilbur (eds) The Mechanisms of Mineralization in the Invertebrates and Plants, University of South Carolina Press, Columbia. 175-190.

Waite,J.H. 1983. Quinone-tanned scleroproteins IN: P.W.Hochachka, K.M.Wilbur (eds) The Mollusca (vol 1), Academic Press, New York, 467-504.

Walne,P.R. 1972. The influence of Current speed, body size and water temperature on filtration rate of five species of bivalves, J. mar. biol. Ass. U.K., 52: 345-374.

Ward,T.J., Correll,R.L. and Anderson,R.B. 1986. Distribution of cadmium, lead and zinc amongst the marine sediments, seagrasses and fauna, and the selection of sentinel accumulators, near a lead smelter in South Australia, Aust. J. Mar. Freshw. Res., 37: 567-585.

Watabe,N. 1965. Studies on shell formation---XI. Crystal-matrix relationships in the inner layers of mollusc shells. J. Ultrastruct. Res., 12: 351-370.

Weiner,S., Lowenstam,H.A. and Hood,L. 1977. Discrete molecular weight components of the organic matrices of mollusc shells. J. Exp. Mar. Biol. Ecol., 30: 45-31.

Westbroek,P. 1983. Biological accumulation and biomineralization in a geological perspective IN: P.Westbroek and E.W.deJong (eds) Biomineralization and Biological Metal Accumulation, 1-11.

Wheeler,A.P., George,J.W. and Evans,C.A. 1981. Control of calcium carbonate nucleation and crystal growth by soluble matrix of oyster shell, Science, 212: 1397-1398.

Wilbur,K.M. 1976. Recent studies of invertebrate mineralization. IN: N.Watabe and K.M.Wilbur (eds) Mechanisms of Mineralization in the Invertebrates and Plants, Columbia University of South Carolina Press, 79-106.

Wilbur,K.M. and Simkiss,K. 1968. Calcified shells IN: M.Florkin and E.H.Stotz (eds) Comprehensive Biochemistry, Elsevier, Amsterdam, 26A: 229-295.

Wong,P.T.S., Chau,Y.K. and Luxon,P.L. 1978. Toxicity of a mixture of metals on fresh water algae. J. Fish. Res. Board. Can. 35: 479-481.

Zamuda,C.D. and Sunda,W.G. 1982. Bioavailability of dissolved copper to the American oyster *Crassostrea virginica*. I. Importance of chemical speciation. Mar. Biol., 66: 77-82.

Zaroogian,G.E. 1980. *Crassostrea virginica* as an indicator of cadmium pollution. Mar. Biol., 58: 275-284.

APPENDIX A

SEDIMENT ANALYSES DATA

APPENDIX A.1
Coefficients of variation from sediment metal determination by X-ray fluorescence

MAJOR ELEMENTS
(%)

	SiO_2	FeO_3	Ca	K_2O	Al_2O_3
	68.73	6.44	4.02	2.56	11.63
	65.98	6.31	4.14	2.51	10.93
	70.15	6.54	3.96	2.64	12.10
	67.36	6.63	4.10	2.63	12.04
	70.79	6.34	3.94	2.48	11.28
X:	68.6	6.5	4.0	2.6	11.6
s:	2.0	0.1	0.1	0.1	0.5
c.v.:	2.9%	2.0%	2.2%	2.8%	4.3%

	MgO	Na_2O	MnO	TiO_2	P_2O_5
	3.12	2.84	0.05	0.86	0.62
	2.80	2.71	0.05	0.84	0.62
	3.44	2.97	0.05	0.83	0.60
	2.79	3.01	0.04	0.86	0.62
	3.46	2.66	0.04	0.84	0.59
X:	3.1	2.8	0.05	0.9	0.6
s:	0.3	0.2	0.01	0.01	0.01
c.v.:	10.2%	5.5%	20.0%	1.6%	2.3%

TRACE ELEMENTS
(µg/g)

	Ni	Co	Cr	V	Zn	Cu	Pb
	52	13	115	89	1120	41	700
	44	12	101	86	1030	45	633
	58	15	125	94	1221	37	765
	60	12	107	81	1266	36	750
	46	11	119	90	1053	40	678
X:	52	13	113	88	1138	40	705
s:	7	2	10	5	103	4	54
c.v.:	14%	12%%	8%	6%	9%	9%	8%

APPENDIX A.2
Average drying coefficient

STATIONS	WET MUD (g)	DRY MUD (g)	DRYING COEFF [dc]	AVG DRYING COEFF
D1	7.1707	4.4364	0.619	0.58
	7.2629	3.5868	0.494	
	4.8732	2.9989	0.615	
D2	12.3102	7.7011	0.626	0.61
	7.5897	4.1714	0.550	
	10.3236	6.7036	0.649	
D3	4.4041	2.7243	0.619	0.53
	5.1257	2.0855	0.407	
	7.0424	3.9199	0.557	
D4	4.5152	2.8351	0.628	0.53
	5.0146	2.0744	0.414	
	7.2539	3.9564	0.545	
D5	4.9143	2.9007	0.590	0.53
	4.7886	1.9332	0.404	
	5.9739	3.6583	0.612	
D6	4.0884	1.8433	0.451	0.53
	5.5847	2.9058	0.520	
	6.9198	4.3395	0.627	
D7	6.2457	3.6850	0.590	0.60
	7.9814	4.7888	0.600	
	7.2947	4.5227	0.620	
D8	6.2964	3.6795	0.584	0.61
	6.7847	4.1868	0.617	
	7.0697	4.4730	0.633	
D9	6.1853	3.5999	0.582	0.61
	6.8958	4.1967	0.609	
	7.2897	4.6830	0.642	
B1	6.4296	4.1042	0.638	0.60
	9.4049	4.7608	0.506	
	6.7073	4.4534	0.664	
B2	4.4600	2.2997	0.516	0.50
	4.4909	1.7586	0.392	
	3.4426	2.0532	0.596	
B3	7.3572	3.4813	0.473	0.55
	5.5706	3.3615	0.603	
	6.1546	3.5540	0.577	
B4	6.8541	3.5367	0.516	0.54
	6.2906	3.1893	0.507	
	7.3411	4.3606	0.594	
EEL	1.6149	0.8865	0.548	0.52
	1.3116	0.6364	0.485	
RIKI	8.3391	5.7550	0.690	0.66
	10.2680	6.1704	0.601	
	8.6495	5.8352	0.675	
NH	8.4521	5.6212	0.665	0.61
	7.4580	3.7444	0.502	
	10.8046	7.0867	0.656	

drying coeff.= dry mud (g) ÷ wet mud (g)

APPENDIX A.3

Dry weight estimation of the sediment samples

STN	SAMPLE ID	MOIST MUD (g)	AVG DRY. COEFF	ESTMTD DRY WEIGHT (g)	SED. H_2O CONTENT (ml)
D1	.1	3.34	0.58	1.94	1.40
	.2	2.65		1.54	1.11
D2	.1	2.33	0.61	1.42	0.91
	.2	2.01		1.23	0.78
D3	.1	4.35	0.53	2.31	2.05
	.2	3.61		1.91	1.70
D4	.1	3.49	0.53	1.85	1.64
	.2	3.97		2.10	1.87
D5	.1	4.16	0.53	2.21	1.96
	.2	3.29		1.74	1.55
D6	.1	3.62	0.53	1.92	1.70
	.2	3.63		1.92	1.71
D7	.1	4.01	0.60	2.41	1.60
	.2	3.88		2.33	1.55
D8	.1	3.68	0.61	2.25	1.44
	.2	4.50		2.75	1.76
D9	.1	3.41	0.61	2.08	1.33
	.2	4.16		2.54	1.62
B1	.1	4.73	0.60	2.84	1.89
	.2	3.55		2.13	1.42
B2	.1	3.24	0.50	1.62	1.62
	.2	3.10		1.55	1.55
B3	.1	4.09	0.55	2.25	1.84
	.2	4.56		2.51	2.05
B4	.1	3.86	0.54	2.08	1.78
	.2	4.27		2.31	1.96
EEL	.1	2.33	0.52	1.21	1.12
	.2	2.01		1.05	0.97
RIKI	.1	3.88	0.66	2.56	1.32
	.2	3.04		2.01	1.03
NH	.1	3.24	0.61	1.98	1.26
	.2	3.99		2.43	1.56

estimated dry weight= moist mud x average drying coefficient

APPENDIX A.4.1

The dilution factor for all 5 Fractions has been calculated by the following formula:

(Vol. of $H_2O_{sediment}$ + Vol. of Extractant) ÷ Sediment Dry Weight

FRACTION 1

D1.1	(1.40ml + 8.01ml) ÷ 1.94g	= 4.85 ml/g
D1.2	(1.10ml + 8.35ml) ÷ 1.54g	= 6.14 ml/g
D2.1	(0.91ml + 8.10ml) ÷ 1.42g	= 6.35 ml/g
D2.2	(0.78ml + 8.00ml) ÷ 1.23g	= 7.14 ml/g
D3.1	(2.05ml + 8.02ml) ÷ 2.31g	= 4.36 ml/g
D3.2	(1.70ml + 8.58ml) ÷ 1.91g	= 5.38 ml/g
D4.1	(1.64ml + 8.31ml) ÷ 1.85g	= 5.38 ml/g
D4.2	(1.87ml + 8.70ml) ÷ 2.10g	= 5.03 ml/g
D5.1	(1.95ml + 8.00ml) ÷ 2.21g	= 4.50 ml/g
D5.2	(1.55ml + 8.00ml) ÷ 1.74g	= 5.49 ml/g
D6.1	(1.70ml + 8.02ml) ÷ 1.92g	= 5.06 ml/g
D6.2	(1.71ml + 8.85ml) ÷ 1.92g	= 5.50 ml/g
D7.1	(1.60ml + 8.02ml) ÷ 2.41g	= 3.99 ml/g
D7.2	(1.55ml + 8.00ml) ÷ 2.33g	= 4.10 ml/g
D8.1	(1.43ml + 8.03ml) ÷ 2.25g	= 4.20 ml/g
D8.2	(1.75ml + 8.00ml) ÷ 2.75g	= 3.55 ml/g
D9.1	(1.33ml + 8.00ml) ÷ 2.08g	= 4.49 ml/g
D9.2	(1.62ml + 8.10ml) ÷ 2.54g	= 3.83 ml/g
B1.1	(1.89ml + 8.03ml) ÷ 2.84g	= 3.49 ml/g
B1.2	(1.42ml + 8.00ml) ÷ 2.13g	= 4.42 ml/g
B2.1	(1.62ml + 8.46ml) ÷ 1.62g	= 6.22 ml/g
B2.2	(1.55ml + 8.81ml) ÷ 1.55g	= 6.68 ml/g
B3.1	(1.84ml +10.04ml) ÷ 2.25g	= 5.28 ml/g
B3.2	(2.05ml + 8.62ml) ÷ 2.51g	= 4.01 ml/g
B4.1	(1.78ml + 8.03ml) ÷ 1.78g	= 5.51 ml/g
B4.2	(1.96ml + 8.54ml) ÷ 1.96g	= 5.36 ml/g
EEL.1	(1.12ml + 8.00ml) ÷ 1.21g	= 7.54 ml/g
EEL.2	(0.97ml + 8.27ml) ÷ 1.05g	= 8.80 ml/g
RIKI.1	(1.32ml + 8.00ml) ÷ 2.56g	= 3.64 ml/g
RIKI.2	(1.03ml + 8.03ml) ÷ 2.01g	= 4.51 ml/g
NH.1	(1.26ml + 8.00ml) ÷ 1.98g	= 4.68 ml/g
NH.2	(1.56ml + 8.42ml) ÷ 2.43g	= 4.11 ml/g

APPENDIX A.4.2

FRACTION 2

D1.1	(1.40ml	+ 9.01ml)	÷ 1.94g	= 5.37 ml/g
D1.2	(1.11ml	+ 9.00ml)	÷ 1.54g	= 6.56 ml/g
D2.1	(0.91ml	+ 9.25ml)	÷ 1.42g	= 7.15 ml/g
D2.2	(0.78ml	+ 9.00ml)	÷ 1.23g	= 7.95 ml/g
D3.1	(2.05ml	+ 9.31ml)	÷ 2.31g	= 4.92 ml/g
D3.2	(1.70ml	+10.82ml)	÷ 1.91g	= 6.55 ml/g
D4.1	(1.64ml	+ 9.09ml)	÷ 1.85g	= 5.80 ml/g
D4.2	(1.87ml	+ 9.17ml)	÷ 2.10g	= 5.26 ml/g
D5.1	(1.95ml	+ 9.02ml)	÷ 2.21g	= 4.96 ml/g
D5.2	(1.55ml	+11.08ml)	÷ 1.74g	= 7.26 ml/g
D6.1	(1.70ml	+ 9.00ml)	÷ 1.92g	= 5.57 ml/g
D6.2	(1.71ml	+ 9.02ml)	÷ 1.92g	= 5.59 ml/g
D7.1	(1.60ml	+ 9.45ml)	÷ 2.41g	= 4.59 ml/g
D7.2	(1.55ml	+ 9.62ml)	÷ 2.33g	= 4.79 ml/g
D8.1	(1.43ml	+ 9.22ml)	÷ 2.25g	= 4.73 ml/g
D8.2	(1.75ml	+ 9.18ml)	÷ 2.75g	= 3.97 ml/g
D9.1	(1.33ml	+ 9.08ml)	÷ 2.08g	= 5.00 ml/g
D9.2	(1.62ml	+ 9.14ml)	÷ 2.54g	= 4.24 ml/g
B1.1	(1.89ml	+ 9.00ml)	÷ 2.84g	= 3.83 ml/g
B1.2	(1.42ml	+ 9.02ml)	÷ 2.13g	= 4.90 ml/g
B2.1	(1.62ml	+ 9.12ml)	÷ 1.62g	= 6.63 ml/g
B2.2	(1.55ml	+ 9.60ml)	÷ 1.55g	= 7.19 ml/g
B3.1	(1.84ml	+ 9.00ml)	÷ 2.25g	= 4.82 ml/g
B3.2	(2.05ml	+ 9.00ml)	÷ 2.51g	= 4.40 ml/g
B4.1	(1.78ml	+ 9.25ml)	÷ 1.78g	= 6.20 ml/g
B4.2	(1.96ml	+ 9.18ml)	÷ 1.96g	= 5.68 ml/g
EEL.1	(1.12ml	+ 9.69ml)	÷ 1.21g	= 8.93 ml/g
EEL.2	(0.97ml	+ 9.41ml)	÷ 1.05g	= 9.89 ml/g
RIKI.1	(1.32ml	+ 9.00ml)	÷ 2.56g	= 4.03 ml/g
RIKI.2	(1.03ml	+ 9.00ml)	÷ 2.01g	= 4.99 ml/g
NH.1	(1.26ml	+ 9.02ml)	÷ 1.90g	= 5.19 ml/g
NH.2	(1.56ml	+ 9.31ml)	÷ 2.43g	= 4.47 ml/g

APPENDIX A.4.3

FRACTION 3

D1.1	(1.40ml	+ 20.02ml) ÷ 1.94g	= 11.04	ml/g
D1.2	(1.11ml	+ 20.43ml) ÷ 1.54g	= 13.98	ml/g
D2.1	(0.91ml	+ 20.00ml) ÷ 1.42g	= 14.73	ml/g
D2.2	(0.78ml	+ 20.00ml) ÷ 1.23g	= 16.89	ml/g
D3.1	(2.05ml	+ 20.33ml) ÷ 2.31g	= 9.69	ml/g
D3.2	(1.70ml	+ 20.56ml) ÷ 1.91g	= 11.65	ml/g
D4.1	(1.64ml	+ 20.82ml) ÷ 1.85g	= 12.14	ml/g
D4.2	(1.87ml	+ 21.06ml) ÷ 2.10g	= 10.92	ml/g
D5.1	(1.95ml	+ 20.77ml) ÷ 2.21g	= 10.28	ml/g
D5.2	(1.55ml	+ 20.00ml) ÷ 1.74g	= 12.39	ml/g
D6.1	(1.70ml	+ 20.00ml) ÷ 1.92g	= 11.30	ml/g
D6.2	(1.71ml	+ 20.26ml) ÷ 1.92g	= 11.44	ml/g
D7.1	(1.60ml	+ 20.09ml) ÷ 2.41g	= 9.00	ml/g
D7.2	(1.55ml	+ 20.04ml) ÷ 2.33g	= 9.27	ml/g
D8.1	(1.43ml	+ 20.00ml) ÷ 2.25g	= 9.52	ml/g
D8.2	(1.75ml	+ 20.24ml) ÷ 2.75g	= 8.00	ml/g
D9.1	(1.33ml	+ 21.00ml) ÷ 2.08g	= 10.74	ml/g
D9.2	(1.62ml	+ 20.00ml) ÷ 2.54g	= 8.51	ml/g
B1.1	(1.89ml	+ 20.00ml) ÷ 2.84g	= 7.71	ml/g
B1.2	(1.42ml	+ 20.00ml) ÷ 2.13g	= 10.06	ml/g
B2.1	(1.62ml	+ 20.74ml) ÷ 1.62g	= 13.80	ml/g
B2.2	(1.55ml	+ 20.44ml) ÷ 1.55g	= 14.19	ml/g
B3.1	(1.84ml	+ 20.02ml) ÷ 2.25g	= 9.72	ml/g
B3.2	(2.05ml	+ 20.01ml) ÷ 2.51g	= 8.79	ml/g
B4.1	(1.78ml	+ 22.60ml) ÷ 1.78g	= 13.70	ml/g
B4.2	(1.96ml	+ 20.25ml) ÷ 1.96g	= 11.33	ml/g
EEL.1	(1.12ml	+ 21.38ml) ÷ 1.21g	= 18.60	ml/g
EEL.2	(0.97ml	+ 20.00ml) ÷ 1.05g	= 19.97	ml/g
RIKI.1	(1.32ml	+ 20.02ml) ÷ 2.56g	= 8.34	ml/g
RIKI.2	(1.03ml	+ 20.00ml) ÷ 2.01g	= 10.46	ml/g
NH.1	(1.26ml	+ 20.00ml) ÷ 1.90g	= 10.74	ml/g
NH.2	(1.56ml	+ 20.81ml) ÷ 2.43g	= 9.21	ml/g

APPENDIX A.4.4

FRACTION 4

D1.1	(1.40ml	+ 20.00ml) ÷ 1.94g	= 11.03	ml/g
D1.2	(1.11ml	+ 20.02ml) ÷ 1.54g	= 13.71	ml/g
D2.1	(0.91ml	+ 20.00ml) ÷ 1.42g	= 14.73	ml/g
D2.2	(0.78ml	+ 20.68ml) ÷ 1.23g	= 17.45	ml/g
D3.1	(2.05ml	+ 20.00ml) ÷ 2.31g	= 9.55	ml/g
D3.2	(1.70ml	+ 21.25ml) ÷ 1.91g	= 12.02	ml/g
D4.1	(1.64ml	+ 20.09ml) ÷ 1.85g	= 11.75	ml/g
D4.2	(1.87ml	+ 20.35ml) ÷ 2.10g	= 10.58	ml/g
D5.1	(1.95ml	+ 20.88ml) ÷ 2.21g	= 10.33	ml/g
D5.2	(1.55ml	+ 20.61ml) ÷ 1.74g	= 12.74	ml/g
D6.1	(1.70ml	+ 20.00ml) ÷ 1.92g	= 11.30	ml/g
D6.2	(1.71ml	+ 20.00ml) ÷ 1.92g	= 11.31	ml/g
D7.1	(1.60ml	+ 21.34ml) ÷ 2.41g	= 9.52	ml/g
D7.2	(1.55ml	+ 20.64ml) ÷ 2.33g	= 9.52	ml/g
D8.1	(1.43ml	+ 20.09ml) ÷ 2.25g	= 9.56	ml/g
D8.2	(1.75ml	+ 20.02ml) ÷ 2.75g	= 7.92	ml/g
D9.1	(1.33ml	+ 21.92ml) ÷ 2.08g	= 11.18	ml/g
D9.2	(1.62ml	+ 20.35ml) ÷ 2.54g	= 8.65	ml/g
B1.1	(1.89ml	+ 21.10ml) ÷ 2.84g	= 8.10	ml/g
B1.2	(1.42ml	+ 20.13ml) ÷ 2.13g	= 10.12	ml/g
B2.1	(1.62ml	+ 21.46ml) ÷ 1.62g	= 14.25	ml/g
B2.2	(1.55ml	+ 20.00ml) ÷ 1.55g	= 13.90	ml/g
B3.1	(1.84ml	+ 20.00ml) ÷ 2.25g	= 9.71	ml/g
B3.2	(2.05ml	+ 20.00ml) ÷ 2.51g	= 8.78	ml/g
B4.1	(1.78ml	+ 20.00ml) ÷ 1.78g	= 12.24	ml/g
B4.2	(1.96ml	+ 25.50ml) ÷ 1.96g	= 14.01	ml/g
EEL.1	(1.12ml	+ 20.12ml) ÷ 1.21g	= 17.55	ml/g
EEL.2	(0.97ml	+ 20.00ml) ÷ 1.05g	= 19.97	ml/g
RIKI.1	(1.32ml	+ 20.21ml) ÷ 2.56g	= 8.41	ml/g
RIKI.2	(1.03ml	+ 20.00ml) ÷ 2.01g	= 10.46	ml/g
NH.1	(1.26ml	+ 21.43ml) ÷ 1.98g	= 11.46	ml/g
NH.2	(1.56ml	+ 20.00ml) ÷ 2.43g	= 8.87	ml/g

APPENDIX A.4.5

FRACTION 5

D1.1	(1.40ml	+ 6.32ml)	÷ 1.94g	= 3.98	ml/g
D1.2	(1.11ml	+ 6.22ml)	÷ 1.62g	= 4.75	ml/g
D2.1	(1.91ml	+ 6.00ml)	÷ 1.42g	= 4.87	ml/g
D2.2	(0.78ml	+ 6.01ml)	÷ 1.23g	= 5.52	ml/g
D3.1	(2.05ml	+ 6.68ml)	÷ 2.31g	= 3.78	ml/g
D3.2	(1.70ml	+ 7.93ml)	÷ 1.91g	= 5.04	ml/g
D4.1	(1.64ml	+ 6.54ml)	÷ 1.85g	= 4.42	ml/g
D4.2	(1.87ml	+ 6.09ml)	÷ 2.10g	= 3.79	ml/g
D5.1	(1.95ml	+ 6.00ml)	÷ 2.21g	= 3.60	ml/g
D5.2	(1.55ml	+ 6.00ml)	÷ 1.74g	= 4.34	ml/g
D6.1	(1.70ml	+ 6.00ml)	÷ 1.92g	= 4.01	ml/g
D6.2	(1.71ml	+ 6.00ml)	÷ 1.92g	= 4.02	ml/g
D7.1	(1.60ml	+ 6.07ml)	÷ 2.41g	= 3.18	ml/g
D7.2	(1.55ml	+ 6.27ml)	÷ 2.33g	= 3.36	ml/g
D8.1	(1.43ml	+ 6.86ml)	÷ 2.25g	= 3.68	ml/g
D8.2	(1.75ml	+ 6.00ml)	÷ 2.75g	= 2.82	ml/g
D9.1	(1.33ml	+ 7.05ml)	÷ 2.08g	= 4.03	ml/g
D9.2	(1.62ml	+ 6.13ml)	÷ 2.54g	= 3.05	ml/g
B1.1	(1.89ml	+ 6.26ml)	÷ 2.84g	= 2.87	ml/g
B1.2	(1.42ml	+ 6.19ml)	÷ 2.13g	= 3.57	ml/g
B2.1	(1.62ml	+ 6.00ml)	÷ 1.62g	= 4.70	ml/g
B2.2	(1.55ml	+ 6.16ml)	÷ 1.55g	= 4.97	ml/g
B3.1	(1.84ml	+ 6.00ml)	÷ 2.25g	= 3.48	ml/g
B3.2	(2.05ml	+ 6.00ml)	÷ 2.51g	= 3.21	ml/g
B4.1	(1.78ml	+ 7.00ml)	÷ 1.78g	= 4.93	ml/g
B4.2	(1.96ml	+ 6.92ml)	÷ 1.96g	= 4.53	ml/g
EEL.1	(1.12ml	+ 6.00ml)	÷ 1.21g	= 5.88	ml/g
EEL.2	(0.97ml	+ 6.01ml)	÷ 1.65g	= 6.65	ml/g
RIKI.1	(1.32ml	+ 6.00ml)	÷ 2.56g	= 2.86	ml/g
RIKI.2	(1.03ml	+ 6.64ml)	÷ 2.01g	= 3.82	ml/g
NH.1	(1.26ml	+ 6.59ml)	÷ 1.98g	= 4.23	ml/g
NH.2	(1.56ml	+ 6.71ml)	÷ 2.43g	= 3.63	ml/g

APPENDIX A.5

COEFFICIENT OF VARIATION OF SEQUENTIALLY EXTRACTED METALS

SITES	M(F1) %			M(F2) %			M(F3) %		
	Fe	Mn	Pb	Fe	Mn	Pb	Fe	Mn	Pb
D1	16%	14%		4%	6%	10%	9%	7%	16%
D2	4%	10%		3%	5%	15%	3%	5%	12%
D3	3%	9%		7%	7%	7%	14%	11%	16%
D4	3%	5%		6%	11%	10%	7%	9%	21%
D5	7%	8%		10%	7%	11%	9%	10%	16%
D6	7%	16%			4%	16%	4%	8%	13%
D7	10%	7%		9%	5%	10%	4%	12%	19%
D8	13%	7%		5%	12%	6%	14%	7%	15%
D9	10%	10%		3%	8%	11%	8%	5%	17%
B1	14%	13%		6%	4%	9%	6%	7%	16%
B2	3%	8%		7%	8%	11%	4%	5%	12%
B3	9%	15%		9%	16%	15%	13%	16%	13%
B4	9%	13%		7%	9%	11%	8%	11%	14%
EEL	16%	12%		4%	5%	9%	5%	10%	6%
RIKI	16%	7%		8%	5%	14%	3%	7%	16%
NH	7%			5%		13%	11%		13%
AVG	9%	10%		6%	8%	11%	8%	9%	15%

SITES	M(F4) %			M(F5) %		
	Fe	Mn	Pb	Fe	Mn	Pb
D1	4%	4%	16%	6%	6%	5%
D2	7%	14%	9%	4%	4%	2%
D3	4%	6%	11%	6%	16%	1%
D4	7%	7%	8%	9%	11%	10%
D5	13%	7%	6%	7%	10%	6%
D6	9%	3%	16%	9%	7%	9%
D7	5%	9%	10%	3%	16%	5%
D8	4%	7%	15%	4%	11%	5%
D9	10%	6%	12%	5%	5%	5%
B1	7%	4%	7%	4%	14%	6%
B2	9%	16%	9%	7%	44%	4%
B3	3%	9%	6%	10%	6%	5%
B4	5%	8%	13%	8%	15%	6%
EEL	3%	5%	25%	11%	3%	6%
RIKI	2%	3%	13%	1%	11%	16%
NH	11%	10%	16%	8%	3%	11%
AVG	7%	7%	12%	6%	11%	6%

APPENDIX A.6

BEFORE SEQUENTIAL EXTRACTION

	SiO2	AL2O3	MGO	CaO	Na2O	K2O	S	P2O5
D1	72.61	12.31	2.42	0.67	2.00	2.40	0.82	0.22
D2	72.21	12.67	2.42	0.74	1.92	2.29	2.51	0.22
D3	71.10	12.36	2.79	0.58	2.00	2.53	2.47	0.37
D4	72.59	12.45	2.84	0.65	2.05	2.47	0.78	0.28
D5	72.87	11.99	2.54	0.61	2.15	2.51	0.73	0.23
D6	71.63	12.39	2.67	0.62	2.05	2.57	0.70	0.22
D7	74.06	12.56	2.47	0.68	2.04	2.45	0.69	0.22
D8	73.25	11.84	2.42	0.77	2.22	2.38	0.68	0.24
D9	71.79	11.60	2.37	0.75	2.18	2.33	0.72	0.24
B1	70.54	10.98	2.34	3.55	1.74	2.38	1.16	0.58
B2	68.73	11.63	3.12	4.02	1.95	2.56	1.15	0.62
B3	68.19	10.5	3.03	5.66	1.74	2.21	1.17	1.33
B4	69.51	11.7	3.12	4.56	1.83	2.41	1.15	0.67
EEL	66.16	13.08	4.25	4.69	1.29	2.72	0.56	0.4
RIKI	64.35	15.29	3.41	2.42	1.88	4.04	0.39	0.22
NH	82.02	9.44	0.18	1.7	2.61	1.4	0.58	0.32

AFTER SEQUENTIAL EXTRACTION

	SiO2	AL2O3	MGO	CaO	Na2O	K2O	S	P2O5
D1	80.83	12.23	0.13	0.16	1.32	3.02	< 0.01	0.03
D2	79.10	13.68	0.18	0.19	1.10	3.22	< 0.01	0.05
D3	80.26	12.62	0.13	0.14	1.22	3.17	< 0.01	0.05
D4	81.87	12.87	0.13	0.14	1.24	3.23	< 0.01	0.05
D5	80.76	12.30	0.13	0.16	1.22	3.02	< 0.01	0.04
D6	79.41	13.37	0.17	0.13	1.03	3.46	< 0.01	0.04
D7	81.00	13.64	0.17	0.13	1.05	3.53	< 0.01	0.04
D8	81.26	11.82	0.12	0.19	1.39	2.88	< 0.01	0.04
D9	79.63	11.58	0.12	0.19	1.36	2.82	< 0.01	0.04
B1	81.33	11.67	0.13	0.23	0.94	2.99	< 0.01	0.06
B2	80.40	12.39	0.15	0.17	0.84	3.20	< 0.01	0.09
B3	81.87	11.17	0.12	0.23	0.92	2.94	< 0.01	0.09
B4	80.93	11.35	0.13	0.21	0.89	2.99	< 0.01	0.08
EEL	79.63	13.33	0.25	0.19	0.44	3.01	< 0.01	0.03
RIKI	70.81	17.57	0.92	0.42	1.25	5.29	< 0.01	0.06
NH	84.44	9.24	0.03	1.33	2.30	1.47	< 0.01	0.04

DIFFERENCE BETWEEN BEFORE AND AFTER

	SiO2	AL2O3	MGO	CaO	Na2O	K2O	S	P2O5
D1	11%	-1%	-95%	-76%	-34%	26%	>99%	-86%
D2	10%	8%	-93%	-74%	-43%	41%	>99%	-77%
D3	13%	2%	-95%	-76%	-39%	25%	>99%	-86%
D4	13%	3%	-95%	-78%	-39%	31%	>99%	-82%
D5	11%	3%	-95%	-74%	-43%	20%	>99%	-83%
D6	11%	8%	-94%	-79%	-50%	35%	>99%	-82%
D7	9%	9%	-93%	-81%	-49%	44%	>99%	-82%
D8	11%	-0%	-95%	-75%	-37%	21%	>99%	-83%
D9	11%	-0%	-95%	-75%	-37%	21%	>99%	-83%
B1	15%	6%	-96%	-94%	-46%	26%	>99%	-90%
B2	17%	7%	-95%	-96%	-57%	25%	>99%	-85%
B3	20%	6%	-96%	-96%	-47%	33%	>99%	-93%
B4	16%	-3%	-96%	-95%	-51%	24%	>99%	-88%
EEL	20%	2%	-94%	-96%	-66%	11%	>99%	-93%
IKI	10%	15%	-73%	-83%	-34%	31%	>99%	-73%
NH	3%	-2%	-83%	-22%	-12%	5%	>99%	-88%

PERCENTAGE OF METAL EXTRACTED:

$$\frac{\text{conc. after extractions} - \text{conc. before extractions}}{\text{conc. before extractions}} \times 100$$

N.B.: The value obtained is QUALITATIVE and does not represent the actual percentage of metal extracted

i.e., POSITIVE values represent elements which were not readily leached during the sequential extraction; NEGATIVE values represent elements which were partly lost during the extraction process.

APPENDIX A.7

BEFORE SEQENTIAL EXTRACTION

	Ni	Co	Cr	V	Zn	Cu
D1	54	12	120	88	610	17
D2	54	12	122	90	2410	25
D3	57	18	136	92	1930	27
D4	55	15	131	91	820	26
D5	55	12	124	83	570	17
D6	54	12	120	95	1190	38
D7	54	13	125	93	710	21
D8	52	12	117	82	650	16
D9	53	14	121	85	690	24
B1	49	12	120	95	1190	38
B2	52	13	115	89	1120	41
B3	48	12	106	92	1950	139
B4	51	13	111	94	1170	54
EEL	53	12	144	90	146	14
RIKI	39	12	82	91	105	24
NH	19	2	27	37	50	8

AFTER SEQUENTIAL EXTRACTION

	Ni	Co	Cr	V	Zn	Cu
D1	7	1	90	93	12	4
D2	8	1	90	104	17	6
D3	11	< 1	66	87	30	< 1
D4	9	1	92	88	15	5
D5	8	1	98	95	13	5
D6	7	1	96	107	17	5
D7	7	2	93	95	13	4
D8	8	2	87	88	15	6
D9	7	1	90	89	14	5
B1	9	2	90	102	22	7
B2	8	1	102	108	22	6
B3	6	1	85	103	23	6
B4	6	1	89	106	22	6
EEL	6	2	98	98	26	9
RIKI	7	2	78	112	19	5
NH	4	< 1	14	23	< 1	4

DIFFERENCE BETWEEN BEFORE AND AFTER

	Ni	Co	Cr	V	Zn	Cu
D1	-87%	-92%	-25%	6%	-98%	-76%
D2	-85%	-92%	-26%	16%	-99%	-76%
D3	-81%	< 1	-51%	-5%	-98%	< 1
D4	-84%	-93%	-30%	-3%	-98%	-81%
D5	-85%	-92%	-21%	14%	-98%	-71%
D6	-87%	-92%	-20%	13%	-99%	-87%
D7	-87%	-85%	-26%	2%	-98%	-81%
D8	-85%	-83%	-26%	7%	-98%	-63%
D9	-87%	-93%	-26%	5%	-98%	-79%
B1	-84%	-83%	-25%	7%	-98%	-82%
B2	-91%	-92%	-11%	21%	-99%	-85%
B3	-88%	-92%	-20%	12%	-99%	-96%
B4	-88%	-92%	-20%	13%	-98%	-89%
EEL	-89%	-83%	-32%	9%	-82%	-36%
RIKI	-82%	-83%	-5%	23%	-82%	-79%
NH	-79%	< 1	-48%	-38%	< 1	-50%

PERCENTAGE OF METAL EXTRACTED:

$$\frac{\text{conc. after extractions} - \text{conc. before extractions}}{\text{conc. before extractions}} \times 100$$

N.B.: The value obtained is QUALITATIVE and does not represent the actual percentage of metal extracted.

i.e., POSITIVE values represent elements which were not readily leached during the sequential extraction: NEGATIVE values represent elements which were partly lost during the extraction process.

APPENDIX B

INSTRUMENTAL SETTINGS AND QUALITY CONTROL SPECIFICATIONS

APPENDIX B.1

Instrumental settings for lead determination by graphite furnace – atomic absorption spectrometry.

Instrumental Parameters	Pb
wavelength	283.3 nm
spectral band width	0.7 mm
light source	EDL
background corrector	ON
HGA Parameters	
sample aliquot	10 µl
sample introduced	manual
purge gas	Ar
gas flow interrupt	ON
DRY	180°C (20s)
CHAR	500°C (20s)
ATOM	1800°C (13s)
CLEAN	2700°C (3s)

APPENDIX B.2

Reference Materials

MATERIAL	Pb (µg/g)
Oyster Tissue (NBS 1566)	0.48 ± 0.04
This work (> 50 determinations)	0.55 ± 0.10
Spiked $CaCO_3$ (internal standard)	
- ICP-MS	14.2 ± 0.30
- GF-AAS	14.8 ± 0.40

Values obtained in this study were compared with certified values. The limits for the NBS certified values were equal to the entire range of observed values or two standard deviations (95% confidence level). The limits for work reported in this study refered to confidence intervals for a mean calculated using the expression: $\pm ts\sqrt{n}$; s: standard deviation, t: value at the 95% confidence level and n-1 degrees of freedom; n: mumber of determination.

Control Chart Limits

Central Line	X (mean 16 sets of measurements)
UCL	$X + 3s \div \sqrt{n}$
UWL	$X + 2s \div \sqrt{n}$
LWL	$X - 2s \div \sqrt{n}$
LCL	$X - 2s \div \sqrt{n}$

In this study n represented the number of repetitive measurements of the reference sample. The mean was plotted on the X-chart. Rejection of data occurred when more than 5 percent of the points lied outside of the warning limits (UWL/LWL) or when values fell outside of the control limits (UCL/LCL).

APPENDIX B.3

QUALITY ASSESSMENT USING REFERENCE MATERIALS

Daily-Event Schedule

Calibration - full expected range
* SRM/IRM
Test samples - Group 1
* Calibration - midrange point
* SRM/IRM
Test samples - Group 2
* Calibration - midrange point
* SRM/IRM

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Test samples - Group N
* SRM/IRM
* Calibration - midrange point

NOTES

*: Decision points
1. Maintain control charts
2. System must be in control at decision point
3. At least 2 groups:
   -- maximum of 10 samples per group
   -- minimum of 1 reagent blank per group
4. At least 2 SRM/IRM measurement should be made each sequence/day.

APPENDIX C

LEAD DETERMINATIÓN DATA IN BIOLOGICAL MATERIAL

sample code: D1-6.1

D1: sampling station
6 : bivalve size class (cm)
.1: bivalve identification No.

APPENDIX C.1
Dalhousie mussel samples

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
D1-6.1	63.5	10.1758	9.6	1.5
D1-6.2	69.1	22.0983	15.7	1.2
D1-6.3	62.3	14.1981	19.5	1.3
D1-6.4	68.0	22.3724	19.6	0.8
D1-6.5	66.2	17.7214	9.5	1.5
D1-6.6	68.1	22.1875	7.1	1.4
D1-6.7	69.9	20.3536	42.9	1.5
D1-6.8	67.4	21.6226	19.2	1.1
D1-6.9	65.9	21.2002	12.8	2.0
D1-6.10	66.1	21.7645	17.0	1.2
AVG	66.7	19.3694	17.3	1.3
STD	2.4	4.1301	10.1	0.3
CV	3.6%	21.3%	58.4%	22.8%
D3-6.1	66.1	18.5627	14.1	1.0
D3-6.2	64.7	11.6742	13.9	0.9
D3-6.3	64.8	10.6599	13.0	1.1
D3-6.4	68.1	20.7654	5.1	0.8
D3-6.5	62.5	10.0091	10.4	1.1
D3-6.6	62.4	12.9163	12.0	1.0
D3-6.7	64.4	12.1861	6.0	0.8
D3-6.8	66.7	15.9123	14.9	0.7
D3-6.9	65.1	17.0562	8.6	0.8
D3-6.10	68.8	22.6933	10.9	0.8
AVG	65.4	15.2436	10.9	0.9
STD	2.1	4.4304	3.4	0.1
CV	3.2%	29.1%	31.1%	15.2%
D4-6.1	68.9	21.9854	12.3	0.6
D4-6.2	65.7	18.6297	6.1	0.6
D4-6.3	66.2	19.6243	5.9	0.6
D4-6.4	68.1	21.8123	8.3	1.0
D4-6.5	61.9	11.7878	8.9	0.7
D4-6.6	62.6	10.6277	10.5	0.7
D4-6.7	63.7	13.4563	5.0	0.6
D4-6.8	64.4	14.7684	7.0	0.6
D4-6.9	64.8	16.0987	8.0	0.8
D4-6.10	61.2	10.8122	12.0	0.5
AVG	64.8	15.9603	8.4	0.7
STD	2.5	4.3609	2.5	0.1
CV	3.9%	27.3%	30.2%	20.8%

APPENDIX C.2

Dalhousie mussel samples

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
D6-6.1	62.8	11.8345	1.0	0.7
D6-6.2	62.7	10.1239	8.5	< 0.5
D6-6.3	67.9	19.8854	5.9	0.6
D6-6.4	61.5	12.2134	2.9	< 0.5
D6-6.5	66.8	18.6122	9.8	0.8
D6-6.6	65.1	15.9234	6.9	< 0.5
D6-6.7	62.9	11.5543	8.9	< 0.5
D6-6.8	62.3	13.9966	5.9	0.5
D6-6.9	61.9	12.0547	8.0	< 0.5
D6-6.10	68.2	21.2987	8.1	< 0.5
AVG	64.2	14.7497	6.1	<.5 - .82
STD	2.5	3.9431	2.9	< 0.5
CV	4.0%	26.7%	48.0%	---

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
D7-6.1	64.4	14.1380	3.3	< 0.5
D7-6.2	68.9	21.2855	5.0	0.5
D7-6.3	67.4	22.4402	4.8	0.8
D7-6.4	64.2	15.4944	2.7	< 0.5
D7-6.5	69.2	20.8939	7.2	< 0.5
D7-6.6	67.7	16.7494	2.8	0.6
D7-6.7	67.5	19.8349	6.0	< 0.5
D7-6.8	64.5	18.9656	3.7	< 0.5
D7-6.9	68.1	18.9367	11.4	0.8
D7-6.10	61.7	11.4371	4.6	< 0.5
AVG	66.4	18.0176	5.2	<.5 - .84
STD	2.5	3.4942	2.6	< 0.5
CV	3.7%	19.4%	50.2%	---

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
D8-6.1	69.1	19.9546	6.9	0.9
D8-6.2	66.6	17.1889	7.0	< 0.5
D8-6.3	65.0	14.2765	2.0	< 0.5
D8-6.4	68.2	20.9985	5.9	< 0.5
D8-6.5	69.3	21.9249	6.1	0.7
D8-6.6	69.9	17.3930	5.9	< 0.5
D8-6.7	66.2	16.9074	5.9	< 0.5
D8-6.8	63.9	13.9856	8.6	0.5
D8-6.9	68.2	17.5182	8.7	0.6
D8-6.10	64.0	17.4859	2.6	0.7
AVG	67.0	17.7634	5.9	<.5 - .86
STD	2.2	2.5896	2.2	< 0.5
CV	3.3%	14.6%	37.0%	---

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
D9-6.1	66.9	18.3654	6.8	< 0.5
D9-6.2	61.4	11.7645	5.3	< 0.5
D9-6.3	64.9	16.4398	5.6	< 0.5
D9-6.4	61.4	12.5676	8.1	0.6
D9-6.5	68.8	20.6649	3.9	< 0.5
D9-6.6	63.5	13.9321	6.1	0.8
D9-6.7	61.9	10.7322	7.0	0.6
D9-6.8	67.5	19.6287	8.2	0.8
D9-6.9	65.9	14.3336	8.0	< 0.5
D9-6.10	68.1	19.8865	2.1	< 0.5
AVG	65.0	15.8315	6.1	<.5 - .78
STD	2.8	3.6524	2.0	< 0.5
CV	4.4%	23.1%	32.3%	---

APPENDIX C.3

Belledune mussel samples

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
B2-4.1	44.3	2.1466	218.0	n.m.
B2-4.2	48.1	3.5589	802.5	31.4
B2-4.3	43.8	2.7453	347.4	n.m.
B2-4.4	49.7	7.7829	366.8	20.9
B2-4.5	42.7	1.7413	81.3	n.m.
B2-4.6	46.1	4.4410	277.5	19.8
B2-4.7	42.7	2.5332	321.4	27.4
B2-4.8	40.3	2.1226	155.0	n.m.
B2-4.9	40.4	1.6526	94.6	25.2
B2-4.10	49.6	6.1317	377.1	n.m.
B2-5.1	58.6	7.1148	277.1	25.9
B2-5.2	54.7	10.0410	109.1	22.0
B2-5.3	50.7	6.2721	282.1	39.8
B2-5.4	59.4	9.9906	118.8	19.2
B2-5.5	50.3	6.8467	83.6	10.9
B2-5.6	57.3	9.3262	217.4	25.0
B2-5.7	51.4	6.4580	544.5	19.3
B2-5.8	52.4	3.6084	110.0	21.6
B2-5.9	54.5	9.8654	120.6	25.8
B2-5.10	54.4	6.8566	236.0	21.8
B2-6.1	64.8	14.5011	219.6	21.2
B2-6.2	62.1	12.6785	385.3	23.3
B2-6.3	64.5	13.8734	460.8	23.7
B2-6.4	69.6	15.5183	283.0	28.5
B2-6.5	62.8	14.1294	357.1	31.3
B2-6.6	68.7	8.8590	766.1	25.7
B2-6.7	66.9	12.8363	348.1	40.7
B2-6.8	61.9	11.9527	247.0	27.4
B2-6.9	63.7	13.5436	316.8	18.5
B2-6.10	66.5	15.8008	304.0	24.3
B2-7.1	71.2	17.3487	282.2	39.0
B2-7.2	71.8	19.6573	466.5	23.6
B2-7.3	72.0	19.8780	253.5	31.2
B2-7.4	76.0	28.3752	418.5	31.3
B2-7.5	77.9	21.1877	388.1	31.4
B2-7.6	70.7	19.6806	437.8	14.6
B2-7.7	70.7	18.9856	490.8	36.2
B2-7.8	71.3	15.8438	449.1	15.9
B2-7.9	70.2	20.3137	150.5	18.9
B2-7.10	73.5	22.7453	501.4	29.6
AVG	59.6	11.2236	306.4	25.6
STD	10.9	6.8551	147.2	7.3
CV	18.4%	61.1%	48.0%	28.7%

n.m.: not measured

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
B1-6.1	69.1	19.3487	425.1	31.5
B1-6.2	65.2	15.2147	299.7	27.5
B1-6.3	66.4	17.3468	365.6	33.1
B1-6.4	67.1	17.7784	345.6	28.8
B1-6.5	63.4	12.4555	285.4	21.3
B1-6.6	62.2	10.2441	320.7	24.7
B1-6.7	61.4	11.6538	396.5	24.6
B1-6.8	64.9	14.9697	395.3	26.1
B1-6.9	64.7	16.4578	463.1	27.4
B1-6.10	65.5	15.4327	222.5	29.1
AVG	65.0	15.0902	332.0	27.4
STD	2.2	2.7332	80.8	3.3
CV	3.3%	18.1%	24.4%	12.0%

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
B3-5.1	56.3	6.7240	109.1	13.6
B3-5.2	54.9	4.1244	96.7	11.2
B3-5.3	57.1	8.8433	138.9	12.2
B3-5.4	58.4	8.6621	88.7	12.3
B3-5.5	57.1	7.3327	86.8	10.1
B3-5.6	52.0	6.8625	190.5	13.2
B3-5.7	58.5	9.3780	137.3	14.5
B3-6.1	65.6	17.7536	173.1	16.2
B3-6.2	67.7	22.6117	288.8	12.8
B3-6.3	62.3	11.0933	185.4	11.9
AVG	59.0	10.3986	149.5	12.8
STD	4.8	5.6186	62.6	1.7
CV	8.2%	54.0%	41.9%	13.3%

APPENDIX C.4

Coastal mussel samples

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
C1-6.1	60.2	12.1707	360.9	42.5
C1-6.2	60.1	10.4980	440.3	66.1
C1-6.3	63.5	15.6484	541.9	31.6
C1-6.4	60.3	18.8449	428.9	49.0
C1-6.5	60.3	14.6236	492.2	44.4
C1-6.6	64.8	19.3606	392.7	51.2
C1-6.7	64.7	19.8752	538.0	43.0
C1-5.2	50.5	6.2370	572.1	55.6
C1-5.3	53.6	9.1654	260.5	51.9
C1-5.4	52.9	10.1760	528.0	52.9
AVG	59.1	13.6600	455.5	48.8
STD	5.1	4.7440	97.9	9.2
CV	8.6%	34.7%	21.5%	18.9%
C2-5.1	59.9	16.5887	235.1	5.2
C2-5.2	54.2	14.4079	145.0	5.4
C2-5.3	58.2	17.7075	116.9	6.0
C2-5.4	57.9	11.5823	159.7	5.3
C2-5.5	53.2	14.7145	154.1	5.3
C2-5.6	57.1	10.1547	104.2	5.4
C2-5.7	51.5	12.4981	202.3	5.8
C2-5.8	53.3	14.8539	258.2	4.4
C2-5.9	58.5	16.0667	202.0	7.1
C2-5.10	58.0	12.9242	129.3	7.1
AVG	56.2	14.1499	170.7	5.7
STD	2.9	2.3557	51.5	0.8
CV	5.1%	16.6%	30.2%	14.7%
C4-6.1	65.6	17.7426	26.4	1.9
C4-6.2	64.7	14.9538	28.1	2.1
C4-6.3	64.8	14.6493	24.3	1.7
C4-6.4	69.7	18.4389	9.0	2.1
C4-6.5	67.4	14.4931	8.3	2.2
C4-6.6	66.2	13.3309	8.8	2.2
C4-6.7	66.9	15.0669	10.9	1.9
C4-6.8	61	15.1206	11.3	2.0
C4-6.9	67.5	15.7895	25.7	1.8
C4-6.10	64.5	12.0169	10.4	2.1
AVG	65.8	15.1594	16.3	2.0
STD	2.3	1.8800	8.6	0.2
CV	3.5%	12.4%	52.4%	8.3%

APPENDIX C.5

Miguasha, Rimouski and Negro Harbour mussel samples

SITE & BIVALVE ID	SHELL LENGTH (mm)	TOTAL SHELL WEIGHT (g)	TISSUE [Pb] (ug/g)	SHELL [Pb] (ug/g)
MIG-6.1	65.9	17.7830	3.2	< 0.5
MIG-6.2	68.1	18.4659	8.5	< 0.5
MIG-6.3	66.3	22.5568	1.6	< 0.5
MIG-6.4	65.6	17.6990	2.2	< 0.5
MIG-6.5	66.7	20.3109	2.5	< 0.5
MIG-6.6	66.8	15.6079	1.6	0.6
MIG-6.7	67.3	14.3474	4.6	< 0.5
MIG-6.8	64.1	20.7364	7.7	< 0.5
MIG-6.9	66.2	19.2009	1.8	0.6
MIG-6.10	66.2	17.2413	2.6	< 0.5
AVG	66.3	18.3950	3.6	<.5 - .63
STD	1.1	2.4374	2.5	< 0.5
CV	1.6%	13.3%	69.5%	---
RIKI-4.1	45.2	3.8767	8.4	0.8
RIKI-4.2	41.3	3.9395	5.1	0.9
RIKI-4.3	39.1	2.9879	9.2	0.6
RIKI-4.4	42.1	5.0368	9.4	0.9
RIKI-4.5	42.6	5.3682	10.7	0.7
RIKI-4.6	45.6	4.6676	5.9	0.8
RIKI-4.7	42.7	2.8375	11.9	0.7
RIKI-4.8	48.3	5.2719	4.1	1.1
RIKI-4.9	42.7	4.7969	9.5	1.0
RIKI-4.10	48.6	5.6670	11.2	0.8
AVG	43.8	4.4450	8.5	0.8
STD	3.0	0.9910	2.7	0.2
CV	7.0%	22.3%	31.1%	18%
NH-6.1	61.5	12.5162	0.8	< 0.5
NH-6.2	66.9	21.2894	3.0	< 0.5
NH-6.3	64.3	15.8419	2.6	< 0.5
NH-6.4	69.3	25.3264	1.7	< 0.5
NH-6.5	62.5	15.4539	1.8	< 0.5
NH-6.6	65.8	13.4139	1.8	< 0.5
NH-6.7	61.0	10.6718	2.0	< 0.5
NH-6.8	61.8	13.5552	0.9	< 0.5
NH-6.9	64.5	16.2785	1.7	< 0.5
NH-6.10	67.2	19.7686	1.5	< 0.5
AVG	64.5	16.4116	1.8	< 0.5
STD	2.8	4.4853	0.7	< 0.5
CV	4.3%	27.3%	37.6%	---

APPENDIX C.6

Lead concentrations (ug/g) in nacreous material sampled from the top and inner shell surfaces

D3		B2		C1	
< 1981	> 1983	< 1981	> 1983	< 1981	> 1983
Pb (ug/g)					
1.4	1.1	25.4	17.3	14.7	42.5
1.3	1.0	51.1	16.4	26.3	66.1
1.1	0.9	60.2	25.3	13.8	31.6
1	1.3	65.2	17.4	13.9	49.0
1.6	1.1	51.3	20.4	27.4	44.4
1.6	1.3	46.2	14.2	13.9	51.2
		24.8	17.1	16.5	43.0
		27.6	20.4	10.5	48.9
		33.4	20.8	16.5	55.0
		57.1	21.3	9.9	49.4
		37.1	24.9	24.0	46.7
		56.9	20.2	15.3	52.6
		39.2	18.2	21.5	45.6
		51.3	19.2	19.0	47.3
		40.0	21.1	26.6	55.6
		53.3	17.5	16.5	60.0
		46.2	16.0	17.6	52.9
		43.6	19.8	23.1	40.5
		61.0	29.9	21.0	47.3
		35.6	18.5	13.2	41.6
		46.3	21.6	20.5	55.0
		36.2	12.3	16.6	35.3
		44.2	21.6	18.9	47.3
		39.3	16.5	15.5	45.8
		47.9	18.2	21.4	56.0
		40.1	23.5	26.4	49.5
		43.7	13.2	24.4	46.8
		43.6	25.9	17.6	55.5
		30.5	23.5	19.5	45.8
		27.9	19.8	17.9	47.6
		35.7	20.1	19.5	45.7
AVG	1.3	1.1	43.3	19.8	18.7	48.4
STD	0.3	0.2	10.9	3.7	4.6	6.8
CV	23%	17%	24%	19%	5%	7%

APPENDIX D

BODY SIZE PARAMETERS

APPENDIX D.1

Relationship between shell length and shell weight

SHELL LENGTH (mm)	SHELL WEIGHT (g)		SHELL LENGTH (mm)	SHELL WEIGHT (g)
18.4	0.307	*	45.5	5.798
23.8	0.426	*	53.7	6.023
23.3	0.543	*	50.8	6.201
28.6	0.590	*	49.0	6.488
24.6	0.623	*	49.3	7.369
28.5	0.648	*	53.3	7.742
24.6	0.670	*	49.8	8.147
25.0	0.672	*	51.2	8.407
29.8	0.691	*	55.9	8.467
32.5	0.762	*	56.7	9.163
29.2	0.768	*	55.4	9.532
27.7	0.815	*	54.9	9.581
25.9	0.841	*	56.9	9.590
28.6	0.858	*	56.3	9.807
31.6	0.920	*	60.9	10.490
33.8	0.946	*	57.3	10.578
29.1	0.956	*	58.6	12.143
29.1	0.961	*	60.6	12.601
24.8	1.003	*	59.8	13.156
27.3	1.024	*	58.6	13.646
28.2	1.036	*	59.6	13.751
30.8	1.116	*	61.1	13.772
27.0	1.147	*	64.6	14.058
36.4	1.224	*	64.0	14.560
37.0	1.266	*	60.9	14.851
36.3	1.350	*	64.5	15.001
29.7	1.360	*	56.3	15.296
34.1	1.550	*	62.7	15.463
33.6	1.576	*	67.7	15.848
29.7	1.600	*	61.5	15.876
34.5	1.608	*	65.1	16.271
34.2	1.659	*	65.3	16.404
36.8	1.719	*	63.8	16.523
37.3	1.757	*	64.8	16.787
35.0	1.762	*	63.0	16.820
29.9	1.779	*	65.9	17.748
32.7	1.866	*	64.4	17.878
36.5	1.869	*	66.9	19.122
35.9	1.912	*	66.5	19.466
34.9	2.002	*	64.4	19.630
37.0	2.054	*	69.3	19.741
38.1	2.231	*	63.5	19.748
35.4	3.146	*	65.4	20.818
39.2	3.681	*	65.2	20.845
43.7	3.958	*	68.4	21.802
42.2	4.973	*	73.5	22.051
45.5	5.651	*	71.9	24.240

APPENDIX D.2

Relationship between total shell weight and percentage of aragonite (by weight)

TOTAL SHELL WEIGHT (g)	PERCENTAGE OF ARAGONITE		TOTAL SHELL WEIGHT (g)	PERCENTAGE OF ARAGONITE
1.13	18%	*	6.02	32%
1.34	26%	*	6.22	29%
1.54	25%	*	6.59	28%
1.60	21%	*	6.81	31%
1.86	18%	*	6.83	28%
1.92	22%	*	6.94	38%
1.93	18%	*	7.39	31%
2.44	26%	*	7.41	29%
2.47	26%	*	7.74	33%
2.51	27%	*	8.51	30%
3.01	25%	*	8.53	29%
3.18	22%	*	9.43	41%
3.23	36%	*	12.62	32%
3.33	23%	*	12.71	28%
3.55	24%	*	12.90	40%
3.57	22%	*	12.91	33%
3.80	29%	*	12.91	33%
3.86	19%	*	12.92	32%
3.91	28%	*	13.07	34%
3.94	29%	*	13.07	38%
4.02	28%	*	13.09	32%
4.08	21%	*	14.12	31%
4.09	31%	*	14.15	32%
4.15	29%	*	14.72	27%
4.27	25%	*	15.05	30%
4.49	28%	*	15.16	31%
4.50	27%	*	15.33	34%
4.59	31%	*	15.91	35%
4.65	25%	*	16.17	34%
4.83	25%	*	16.80	31%
4.85	23%	*	17.46	34%
5.47	33%	*	17.48	30%
5.67	28%	*	18.00	36%
5.74	27%	*	18.38	38%
5.77	34%	*	19.39	39%
5.86	33%	*	19.55	34%
6.01	33%	*	19.79	35%

APPENDIX D.3

Relationship between shell and tissue weight

SHELL WEIGHT (g)	DRY TISSUE WEIGHT (mg)		SHELL WEIGHT (g)	DRY TISSUE WEIGHT (mg)
0.307	12	*	13.751	316
0.543	27	*	8.147	322
0.672	33	*	5.651	366
0.623	33	*	5.798	406
0.670	40	*	6.023	407
0.590	42	*	6.488	423
0.815	44	*	15.296	450
0.426	45	*	6.201	457
0.920	45	*	12.601	487
0.841	52	*	7.369	500
0.648	53	*	19.630	500
0.961	55	*	9.581	552
1.147	59	*	7.742	616
0.956	59	*	15.463	642
1.659	61	*	15.876	663
1.360	66	*	9.807	666
0.768	67	*	9.532	681
1.003	67	*	13.646	696
1.116	74	*	9.590	698
1.036	75	*	8.467	714
0.946	75	*	9.163	746
0.858	77	*	10.578	764
1.024	79	*	16.787	834
1.608	83	*	20.845	850
0.691	88	*	17.748	855
1.757	97	*	10.490	902
1.779	98	*	16.523	910
1.576	104	*	14.851	918
1.600	108	*	13.156	921
1.550	111	*	19.748	950
3.146	112	*	13.772	954
4.973	130	*	12.143	963
1.866	135	*	16.820	998
1.719	144	*	16.271	1029
2.054	150	*	20.818	1063
1.912	152	*	14.058	1068
2.002	153	*	14.560	1085
1.224	153	*	17.878	1109
1.869	156	*	15.001	1130
0.762	172	*	16.404	1141
1.350	179	*	15.848	1174
1.762	181	*	22.051	1258
1.266	205	*	19.741	1286
2.231	222	*	24.240	1293
3.681	236	*	19.466	1311
8.407	281	*	19.122	1389
3.958	315	*	21.802	1530

RATIO [shell (g)/tissue (g)]: 17.8