TRACE METAL ANALYSIS OF MARINE ZOOPLANKTON FROM CONCEPTION BAY, NEWFOUNDLAND

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ABSTRACT

Stripping Voltammetry has been used in the determination of the concentration, speciation, and for toxicity studies of trace metals in the marine environment. Anodic Stripping Voltammetry (ASV) and Cathodic Stripping Voltammetry (CSV) are two highly sensitive techniques that have frequently been used in the study of trace elements at environmental (or trace) concentrations in both water, sediment, and biota. Due to the information that may be obtained through the use of stripping voltammetry, the cycling and fate of trace elements may be determined. The determination of the speciation of metals provides knowledge of what forms are present in the water column. Upon use of stripping voltammetric analysis it is determined what ASV- and CSV-labile concentrations of metals, such as Zn, Cd, Pb, and Cu are present in sea water. This information is extremely valuable since it correlates with most of the dissolved fraction, that is the free ionic and weakly bound (both organic and inorganic), in sea water which is believed to be the bioavailable fraction. An ASV method was developed for the analysis of zooplankton for Zn, Cd, Pb, and Cu which could be used to study the cycling of trace metals in the Labrador Current.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS AND SYMBOLS USED

ASV - anodic stripping voltammetry

Cd - cadmium

CSV - cathodic stripping voltammetry

Cu - copper

DPASV - differential pulse anodic stripping voltammetry

e^- - electron

Hg - mercury

HMDE - hanging mercury drop electrode

M - metal ion

Pb - lead

μ - 10^{-6}

V - volts

Zn - zinc

CHAPTER 1

Introduction

1.1 Trace Metals in the Marine Environment

The composition of marine organisms with respect to trace metals began receiving increased attention from the scientific community when it was discovered that some marine organisms, such as tuna, were capable of accumulating potentially hazardous levels of mercury. While most of the attention was focused on determining whether trace metals could accumulate in organisms to levels that posed a threat to human health, increased attention began to focus on determining the mechanisms of the cycling of trace metals in the marine environment and the possible damage they might cause marine biota.

In order to determine the mechanisms of cycling of trace metals in the marine environment, the reservoirs of trace metals had to be identified and the quantities of metals present in reservoirs had to be determined. Wolfe and Rice (1972) have stated that nearly all the metal content of the aquatic environment resides in sediments and water and that only a fraction resides in biota. Despite this, Wolfe and Rice (1972) have also stated that zooplankton and phytoplankton constitute the most important living elemental reservoirs, in terms of turnover, total physical transport, and re-distribution processes. Most of the metals present in the periodic table have been detected in many plankton species.

Marine zooplankton play a major role in determining the fate of metals, especially particle reactive metals, in the water column. The ability of plankton to concentrate large quantities of metals from sea water has been reported by many authors (Nicholls *et al.*, 1959; Martin and Knauer, 1973). Metals concentrated by plankton can be passed on to higher levels of the food chain or re-distributed in the marine environment through different pathways, such as the sinking of fecal pellets, organic detritus, crustacean exoskeletons, and by vertical migration (Martin, 1970; Fowler, 1977). By accumulating some elements and dispensing them through rapidly sinking fecal pellets, crustacean zooplankton, such as copepods, can effectively remove metals from surface waters. Metals which are ingested but not accumulated are mostly defecated, but metals which are accumulated enter the organic cycle in the ocean.

Despite the little attention given zooplankton, their study may provide evidence on the cycling of trace metals in the marine environment. The reasons for the lack of information on zooplankton are: (i) the difficulty in collecting sufficient material for analysis; (ii) difficulties in separating zooplankton from phytoplankton, making it impossible to determine which group contains which elements; and (iii) contamination problems faced during sampling, preparation, and analysis. These problems can be overcome in part using an electroanalytical procedure called Anodic Stripping Voltammetry (ASV) for the analysis of sea water and zooplankton. ASV requires only small amounts (typically 10 mL) of organic material or sea water for analysis and, when carried out in a trace metal-free environment, is capable of fulfilling the contamination-free requirements of trace metal analysis. In addition to determining the total levels of

certain trace metals present in zooplankton and sea water, ASV is capable of ascertaining information on the levels of bioavailable trace metals, thus providing information that could be used for toxicity studies.

The goals of this research were to develop a method of collecting, preparing, and analyzing zooplankton from Conception Bay. Newfoundland using ASV for the determination of zinc, cadmium, lead, and copper which may be used to obtain information about the levels of trace metals in the Labrador Current. The main factor to be considered was mainly the minimization of contamination of the zooplankton samples; this included sampling, preparation, and analysis. For this project to succeed, sampling devices and storage bottles had to be cleaned, a digestion method that was free of contamination had to be developed, and the voltammetric analysis procedure had to be worked out and tested.

1.2 Trace Metals in Sea Water

Since the mid-1970s, the sea water concentrations of many trace elements have been found to be factors of 10 to 1000 lower than what was previously detected, due to improvements in instrumental analysis and the control of contamination during sampling, storage, and analysis (Bruland, 1983). In more recent years, there has been a continuation of this trend, resulting in the improvement of analytical techniques, and sample collection, including an increase in the information obtained from such analysis (i.e. speciation). It is from these advances that the types of distributions and reliable estimates of the concentrations of trace elements have been determined.

Table 1.1: Range of metal concentrations in sea water (Burton and
   Statham, 1982)

Metal	Range (μg L^{-1})
Zn	0.0007 - 0.588
Cd	0.0002 - 0.025
Pb	0.006 - 0.015
Cu	0.025 - 0.64

Table 1.2: Concentrations of metals in Atlantic Slope Water from Halifax
   (Bewers *et al.*, 1979)

Metal	Concentration(μgL^{-1})
Zn	0.72
Cd	0.034
Pb	no data
Cu	0.24

The most important information, following the relative distributions, that can be
obtained on trace metals, is that of speciation, or the physico-chemical forms of individual
elements that make up the total concentration. The determination of metal species
provides information on the bioavailability of metals and their interaction with sediments
and suspended particulates. Such information plays an integral role in the study of the
toxicity of metals to aquatic organisms and to the understanding of the transport of trace
metals.

Table 1.3: The possible physico-chemical forms of metals in sea water
(adapted from Florence, 1982)

Physico-chemical form	Possible example	Diameter (nm)
Particulate	Retained by 0.45 μm filter	>450
Simple hydrated ions	$Cd(H_2O)_6^{2-}$	0.8
Simple inorganic complexes	$Pb(H_2O)_4Cl_2$	1
Simple organic complexes	Cu-glycinate	1-2
Stable inorganic compounds	$ZnCO_3$	1-2
Stable organic complexes	Cu-fulvate	2-4
Adsorbed on inorganic colloids	$Cu^{2+}-Fe_2O_3$	10-500
Adsorbed on organic colloids	Cu^{2+}-humic acid	10-500
Adsorbed on mixed colloids	Cu^{2+}-humic acid-Fe_2O_3	10-500

In order to distinguish between the different metal species present in sea water, one must divide the total dissolved metal into seven classes: free metal ion, labile metal complexes (organic and inorganic), inert metal complexes (organic and inorganic), and metals associated with colloidal particles (organic and inorganic) (Florence and Batley, 1980). By convention, the dissolved fraction of metals present in sea water is that which passes through a 0.45 μm filter, however, this would include the colloidal fraction.

Salinity and pH have a significant effect on the speciation of trace metals. The relatively high pH of sea water can cause the adsorption of metal ions onto organic and inorganic colloids. Despite this, complexation with chloride is the principal inorganic form of most trace metals in sea water.

1.3 Trace Metals in Sediments

The distribution of trace metals in the various fractions of sediment is of interest to sediment and water chemists for several reasons. The distribution of trace metals in sediments may be helpful in evaluating the relative availability of trace metals to biological communities.

In unpolluted aquatic systems, such as coastal Newfoundland, the primary source of trace metals is due to geologic weathering. In areas characterized by metal-bearing formations, trace metals often occur at elevated levels in water and bottom sediments. Sea water in Conception Bay would not be expected to contain elevated levels of trace metals due to the circulation of the Labrador Current, but it would, however, contain background levels derived from the geological formations of the particular area. The concentrations of Zn, Cd, Pb, and Cu in regions with similar geology are given in Table 1.4.

Table 1.4 : Concentrations of total Zn, Cd, Pb. and Cu (in $\mu g \ g^{-1}$) in fine sediments from various eastern Canadian locations (Loring, 1988)

Location	Zn	Cd	Pb	Cu
Bay of Fundy	77	0.24	30	19
Placentia Bay	80	0	32	34
Arctic Region	61	0.16	15	29
Gulf of St. Lawrence	84	0.26	21	25

Since it is expected that Conception Bay sediments show elevated concentrations of trace metals, it may be an important factor in determining the source of accumulated

metals in marine organisms in that region. In order to do so, some explanation of the sources of metals to the sediment-water interface is necessary.

1.3.1 Sedimentation

The adsorption of trace metals by suspended particles plays an important role in the fate of metals in the marine environment since the latter are dependent upon the distribution and transport of the particles which in turn is dependent upon the transport rate and the transport of the particle relative to the movement of water. Dissolved metals are transported as the water moves; however, particulates have a greater specific gravity than water and, therefore, a lower net transport rate. This causes the larger and heavier particles to have different transport patterns from the water they are transported in. The result of the transport of particle-bound trace metals is sedimentation. Despite this, sea water is generally considered to be undersaturated in trace metals. Trace metals are scavenged from the water by the adsorptive particulate matter which settles out in the estuarine and near-shore regions (Pagenkopf, 1978).

1.3.2 Release of Trace Metals from Sediments

Naturally occurring trace metal levels in sediment are well known in many regions of the world. The geological setting of east-coast Newfoundland is particularly important in relation to the concentration of trace metals in coastal sediments. An important question about these concentrations must be asked: how long do these trace metals remain in the sediments? This question is extremely important when considering the toxic effects of metals to marine organisms.

The rate of trace metal desorption from suspended particles is not a rapid process and complete desorption rarely occurs (Pagenkopf, 1978). Therefore, the release of trace elements from sediments through dissolution is not an important process. Physical disruption of the sediments may expose more surface, thereby facilitating the release of trace metals. Reactions that change the chemical nature of sediments may also have some effect. When the oxygen concentration in the sediments is depleted and the system becomes reducing, some adsorbed metals may be released. The most important process according to Pagenkopf (1978) in the release of metals from sediments is biological activity. The biologically-mediated alkylation of mercury is an extremely important process in the cycling of mercury.

1.3.3 Chemical Weathering

The dissolution of trace metals may also occur from the chemical oxidation of their sulfide or oxide ores, or from the acidic dissolution of their carbonates (Faust and Aly, 1981). Weathering of the sulfide ore is believed to be oxidation with gaseous oxygen as the electron acceptor. The following reaction may occur:

$$ZnS \text{ (s)} + 1.5O_2 \text{ (g)} + H_2O \text{ (l)} = SO_4^{2-} \text{ (aq)} + Zn^{2-} \text{ (aq)} + 2H^- \text{ (aq)}$$
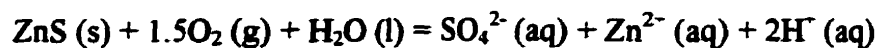
Sulfide ore contains high concentrations of Cu, Zn, Mo, Ag, Hg, and Pb and often contains Se, As, and Cd. Since these ores weather rapidly, they can often give rise to local high concentrations of dissolved trace elements (Drever, 1988). Similar reactions are possible for many other trace metals. Such reactions may be important processes in the release of trace metals to not only sea water, but to marine organisms as well. The release of the ionic forms of these metals from geological formations may explain much of the

bioaccumulation of Zn, Cd, Pb, and Cu that is found in marine animals in unpolluted regions.

1.4 Trace Metals in Plankton

The routes for uptake of trace metals by marine plankton include uptake from solution and uptake from food. Metals dissolved in sea water occur as different chemical species (ions, complexes, etc.) in equilibrium with organic and inorganic complexing agents including chlorides and carbonates. Free metals are considered to be the bioavailable form of the metal, although in sea water, the free species represents only a small percentage of the total dissolved fraction. The uptake of free metals may occur when ions bind to a membrane-associated transport protein and subsequently with a series of metal-binding ligands according to the stability constants of the ligands for the metals and their relative concentrations. The metal ions would then continue to enter the cell by passive diffusion in the free ion form along a diffusion gradient. The uptake of metals into the body via permeable surfaces might occur all over the body, especially in small and soft bodied invertebrates. The uptake of metals from solution can occur in the alimentary tract when swallowed during drinking or food ingestion. Metals associated with particles usually become available to plankton after ingestion and digestion in the alimentary tract.

Trace metals are taken up by plankton even when total metal concentrations are low, therefore plankton have the capacity to accumulate metals to high body concentrations, depending on the relationship between uptake and excretion and the rate of diluting body growth. Some species are net accumulators in which metal excretion does not balance metal uptake. Decapod crustacens, for example, accumulate the non-essential

metal cadmium at all dissolved cadmium exposures, with little significant excretion. In most coastal waters, cadmium concentrations are such that, growth rates cause the dilution of increasing accumulated cadmium content and body concentrations fall into a typical range (Rainbow, 1990).

Table 1.5: Whole Body Metal Concentrations from different

marine regions of the world ($\mu g \ g^{-1}$ dry weight)

Taxa	Region	Zn	Cd	Pb	Cu
Euphausiacea	Mediterranean	108	0.6	-	33
Euphausiacea	N.E. Atlantic	97	0.4	-	72
Amphipoda	Mediterranean	150	6.6	-	24
Amphipoda	North Sea	81 - 562	-	-	35 - 200
Copepoda	Antarctic	135	0.75	1.0	76
Copepoda	North Pacific[1]	62 - 170	1.6 - 7.5	1 - 9	10 - 207
Copepoda	North Pacific[2]	62 - 158	1.6 - 7.5	1.4 - 9.0	9.0 - 22.6

(References: N.E. Atlantic - Rideout *et al.*, 1989; North Sea - Rainbow and
Moore, 1986; Mediterranean - Fowler, 1986; Antarctic - Zauke and Petri,
1993; North Pacific[1] - Martin, 1970; North Pacific[2] - Martin and Knauer, 1973)

The relationship between sea water concentrations of trace metals and the ability of trace metals to cause toxic effects in an organism is not yet fully understood. This problem is generally considered in terms of metal speciation and the condition of the organism.

1.4.1 The Speciation of the Metal

Whether a metal can interact with biota or not is dependent upon the exact form of the metal in the marine environment as well as the concentration of that metal. Metals

are not considered bioavailable simply because they are present. It is generally considered that the bioavailable fraction consists of free metal ions and metals that are labile, that is weakly bound, in inorganic and organic complexes.

1.4.2 The Condition of the Organism

There are many factors which may influence the effect a metal might have on an organism. These include the stage of development (egg, larva, etc.), the age, size, and sex, the previous history of the organism, its location, and its diet (Preston, 1989). More general factors which also affect the toxicity of metals to biota are temperature, salinity, pH, Eh, light intensity, and dissolved oxygen concentration.

1.5 The Electroanalysis of Trace Metals

The great understanding of electrochemical techniques has led to the development of procedures which can detect many elements, both organic and inorganic, in sea water at very low concentrations. Voltammetry makes use of the measurement of the current while the potential is varied under potentiostatic control. The sensitivity of Anodic Stripping Voltammetry (ASV) for Zn, Cd, Pb, and Cu ranges from 10^{-5} to 10^{-11} M while Cathodic Stripping Voltammetry (CSV) ranges from 10^{-5} to 10^{-12} M for Cu, Pb, Cd, V, Fe, U, Zn, Mo, Ni, Co, Sb, Pt, Sn (Van Den Berg, 1989). These concentration ranges represent the free, uncomplexed ions.

Stripping voltammetry often makes use of a stationary, hanging mercury drop electrode (HMDE). The advantage of this type of electrode is that a pre-concentration

step can be included in the measurement, which lowers the limits of detection from 10^{-7} - 10^{-8} to 10^{-10} - 10^{-11} M (Van Den Berg, 1989). The analyte is then stripped from the electrode surface during the potential scan. In ASV, the current is applied in an increasingly negative potential direction and involves oxidation (therefore anodic), while in CSV the opposite occurs. In ASV, the pre-concentration step consists of the formation of an amalgam on the HMDE. This is necessary as the dilution of metal ions in mercury improves the reversibility of the oxidation of the concentrated metal during the stripping process.

For ASV: **Deposition:** Applied potential more negative than $E_{1/2}$ of M^{n-}

$$M^{n-} + ne^- \rightarrow M(Hg)$$
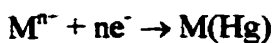
Stripping: Scan in the positive direction, peak current is proportional to the concentration of M

$$M(Hg) \rightarrow M^{n-} + ne^-$$
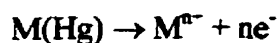
For CSV: **Deposition:** At a positive potential where Hg^- ions can be produced

$$Hg \rightarrow Hg^- + e^-$$
then
$$2Hg^- + 2X^- \rightarrow Hg_2X_2 \text{ (insoluble film)}$$

Stripping: Scan in the negative direction, peak current is proportional to the concentration of X^-

$$Hg_2X_2 + 2e^- \rightarrow 2Hg + 2X^-$$
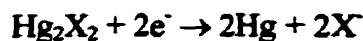
During the measuring step, the potential is scanned, to more positive (for ASV) or negative (for CSV) potentials; while the elemental (oxidation state 0) form of the metal is re-oxidized or re-reduced, and the oxidation or reduction currents are determined. The

potential is applied from a potentiostat to the working electrode relative to a reference electrode and via a counter electrode. The current produced during the scan has two components: a capacitive and a faradaic. The capacitive current is not of interest, but the faradaic current, caused by the oxidation or reduction of the analyte, is. Discrimination between the capacitive and faradaic currents is possible if a modulated potential is superimposed on a linear scan, in which the potential is varied at a constant rate (50 mv/s). This creates a pulsed waveform in which the current is measured immediately before and after each pulse, and the difference is measured by the recorder. The time delay between the measurements (40 - 50 ms) is sufficient to allow the capacitive current to fall almost to zero. This type of voltammetry is called differential pulse ASV or CSV (DPASV or DPCSV).

The same amount of deposited metal is effectively deposited and re-oxidized as the pulse waveform is scanned past its reduction potential, thus increasing its contribution to the faradaic current. The large pulse height (25 mV) coupled with a scan rate of 10 mV s^{-1} and a pulse rate of 10 s^{-1} causes a repeated reduction-oxidation step, re-oxidizing the metal 25 times during a single scan. It is for this reason that DPASV has a much greater sensitivity than linear scan ASV. The same effects occur in DPCSV.

The procedure must be calibrated using a series of standard additions (or "spikes") to ensure that a fixed portion of the electroactive component is being accurately recovered. The concentration of the metal in the sample (C_x) can be calculated from the concentration of the standard (C_s) using the following equation (Whitfield, 1975):

$$C_x = \frac{i_1 v C_s}{(i_2 v + (i_2 - i_1)V)}$$

where i_1 is the original peak height, i_2 is the "spiked" peak height, v is the volume of the standard added and V is the original sample volume.

The application of ASV is limited to metals which can be reduced to the metallic state at potentials between 0 and -1.5 V and which are at the same time soluble in mercury. The reduction potentials of metal ions to their metallic state are given in Figure 1.1.

Figure 1.1: The reduction potentials of uncomplexed metal ions to the metallic state between -1.2 and + 0.4 V (Van den Berg, 1989)

		Bi			Tl		Ga	
Ag	Hg	Cu	Sb	Pb	GeSn InCd		Zn Ni	Co

0.2	0.0	-0.2	-0.4	-0.6	-0.8	-1.0	-1.0	V
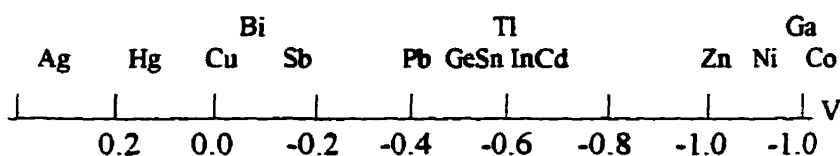
Generally, only Zn, Cd, Pb, and Cu are determined in unpolluted sea water by ASV. Other elements are either present at concentrations below the detection limits or are subject to interferences. Cu, Pb, and Cd can be determined simultaneously in acidified samples. At low pH, Zn suffers interference with hydrogen; therefore Zn is best determined at pH 4 in a buffered solution. Zn, Cd, Pb, and Cu may be simultaneously determined at this pH.

Figure 1.2: Stripping voltammetry detection limits ($\mu g\ L^{-1}$) (Copeland and Skogerboe, 1974)

Element	Differential Pulse	Linear Sweep
Zn	0.04	0.04
Cd	0.005	0.01
Pb	0.01	0.02
Cu	0.005	0.01

Comparison of the scans of Zn, Cd, Pb, and Cu in fresh water and sea water shows that some of the peaks are shifted to more negative potentials in sea water due to complexation with chloride ions (Whitfield, 1975). This effect is especially problematic for the determination of Cu, in which the peak is smaller and broader due to the formation of Cu(I)Cl complexes. The Cu(II) reduction occurs in two steps: the Cu(II)/Cu(I) reduction potential is shifted in a positive direction past that of Hg(I)/Hg(0), which in turn is shifted in a negative direction by chloride complexation. The Cu(I)/Cu(0) reduction potential is shifted in a negative direction due to chloride complexation of the Cu(I), giving a different peak for sea water. It is for these reasons that Cu determinations are acquired with some difficulty using a hanging mercury drop electrode; the oxidation of the mercury itself interferes with the detection of the Cu peak, particularly at low concentrations of Cu.

Organic compounds present in sea water may interfere with the determination of metals by ASV by adsorption onto the surface of the mercury drop. Most notable is the diminished response of Cu in the presence of low concentrations of organic compounds such as glycine, which traps the intermediate, Cu(I), of the reduction to Cu(0), as $CuCl_2^-$ on the surface of the electrode (Nelson, 1985). Organic compounds in sea water behave as chelating agents, interfering by maintaining the metals in non-labile forms. The complexes

are strong enough that the reduction potential is irreversible, thus making it impossible to re-oxidize the metal back into solution, making the stripping step impossible. In contrast, a labile complex is one in which the metal is loosely and reversibly complexed, therefore it can be deposited at the applied potential along with the ionic (uncomplexed) metal. Organic complexing compounds may be destroyed by acidification and UV-irradiation of the sample, thus making it possible to determine the total metal present in solution by ASV.

In DPCSV the potential scan is carried out in a negative direction and the reduction current of the deposited metal is measured. The deposition step differs from that in ASV as the metals are chelated with a chelating agent and then are adsorbed rather than directly deposited on the HMDE. For this process to occur the element must be able to form a complex with the chelating agent which then adsorbs onto the electrode and the chelate must be reducible. The process used in this project for the determination of Cu in sea water makes use of the fact the Cu ions in the adsorbed complex are reducible. The reduction potential is specific for the metal analyzed, though shifted in a negative direction from that of the uncomplexed metal. This procedure was developed by van den Berg (1986) and makes use of an oxine compound (8-hydroxyquinoline) and a HEPES buffer (4-(2-hydroxyethy)-1-piperazineethanesulfonic acid) in the pH range of 6 to 9.

CHAPTER 2

Zinc, Cadmium, Lead, and Copper

in the Marine Environment

2.1 Introduction

The scope of this research only includes Zn, Cd, Pb, and Cu. Nevertheless, this group of metals is of particular importance when investigating trace metal cycling in the environment, especially since they present a somewhat higher degree of ease in sampling due to detectable (but still very low!) concentrations in sea water, sediment, and biota, unlike most of the other trace metals. An important thing to note about these four metals is that two are known to be essential elements (Zn and Cu) and two are considered to be toxic (Cd and Pb) to marine organisms. Zn and Cu also display toxic effects when present at elevated concentrations in sea water, and often at only slightly elevated levels.

2.2 The Distribution and Speciation of Zn

Zn is a member of Group IIb in the periodic classification of the elements, resembling the transition elements in its ability to form complexes, particularly with ammonia, amines, halide ions, and cyanide. Zn is between hard and soft acceptors in its chemical interaction with ligands, thus it is capable of forming hard and soft bases, as is reflected by its natural occurrence as sulfide and carbonate ores. Zn is an essential element

for marine organisms and is present in many metalloenzymes for carbohydrate, lipid, and

protein metabolism, and plays a vital role in the synthesis of nucleic acids, RNA, and DNA

polymerases (Moore and Ramamoorthy, 1984).

In aqueous solution, Zn^{2+} binds halide ions as a hard acid metal. It hydrolyzes at

pH 7 to 7.5 to form relatively stable $Zn(OH)_2$ at pH>8.0. At pH 6.7, Zn is present as

divalent Zn and is available for sorption onto suspended colloids and complexation with

organic matter. Zn-chloride complexes do not form until the chloride concentration is ≥

0.4 M (89 000 mg L^{-1}) (Moore and Ramamoorthy, 1984). Florence and Batley (1980)

suggest that dissolved inorganic Zn in sea water is divided between Zn^{2-} (27%), chloro

complexes (47%) and $ZnCO_3$ (17%).

Figure 2.1: Distribution of Zn and silicate in the ocean (Bruland, 1983)
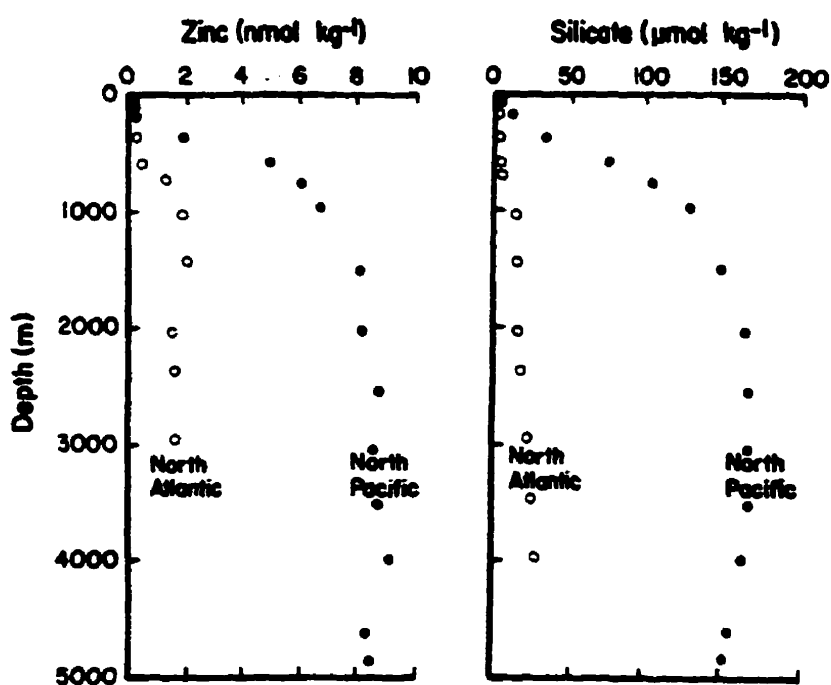
Zn has a nutrient type distribution in sea water with a concentration ranging from 0.05 to 9 nmol kg^{-1} (Bruland, 1983). Zn and silicate follow a similar trend, suggesting that Zn is removed from surface waters and transported down the water column as a trace constituent of biogenic particles. The low concentration of Zn in the surface photic zone (~0.1 nmol kg^{-1}) indicates that it is a biolimiting nutrient for photosynthetic marine organisms, such as phytoplankton.

2.3 The Distribution and Speciation of Cd

Cd is another member of the Group IIb elements. The stable state of Cd in the environment is Cd^{2-} and has a medium class b character, giving it a moderate covalency in bonds and a high affinity for sulfhydryl groups, leading to increased lipid solubility, bioaccumulation, and toxicity. Metalloenzymes, such as metallothionein, bind and transport Zn and Cd at the same time due to the similarities in the metabolism of these two elements (Moore and Ramamoorthy, 1984).

Cd undergoes hydrolysis at natural pH values. At pH's up to 8 it is present as the divalent species, except in the presence of phosphate and sulfide. In the absence of such precipitating anions, Cd^{2+} is available for sorption onto suspended particles and complexation with organic matter. Chlorides are the most prevalent complexing agents that interact with Cd. It is believed to exist as $CdCl^+$ and $CdCl_2$ complexes (92%) in sea water (Florence, 1982).

Figure 2.2: Distribution of Cd and phosphate in the ocean (Bruland, 1983)
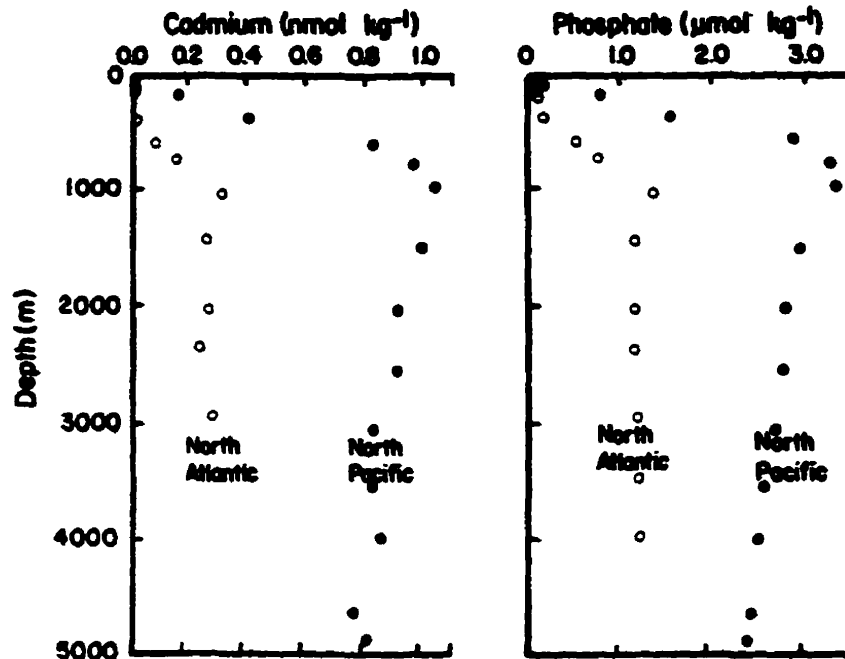
Cd also exhibits a nutrient-type distribution with oceanic concentrations ranging from 1 pmol kg^{-1} to 1.1 nmol kg^{-1} (Bruland, 1983). This distribution is regulated by its involvement in a shallow regeneration cycle similar to those of phosphate and nitrate. The surface depletion, however, is not due to biochemical demand, but due to the efficiency of Cd scanvenging onto organic-rich biogenic particles (Bruland, 1992).

2.4 The Distribution and Speciation of Pb

Pb is a member of the Group IV elements and is characterized as an intermediate acceptor between hard and soft acids in complexation with ligands. Pb resembles the divalent alkaline earth group metals in chemical behaviour more than its own Group IVA metals, except for the poor solubility Pb salts, such as halides, hydroxides, sulfates, and

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phosphates (Moore and Ramamoorthy, 1984). Its behaviour in natural waters is a combination of precipitation equilibria and complexing with inorganic and organic ligands.

The two main inorganic species of Pb in sea water are $PbCO_3$ (83%) and $PbCl_2$ (11%) (Florence, 1982). The distribution of Pb in sea water is greatly influenced by man's activities. Open ocean concentrations ranges from 5 to 175 pmol kg^{-1} (Bruland, 1983) with concentrations in the upper end of this range representing surface water concentrations and the lower end represent concentrations at depth.

Figure 2.3: The distribution of Pb in the ocean (Bruland, 1983)
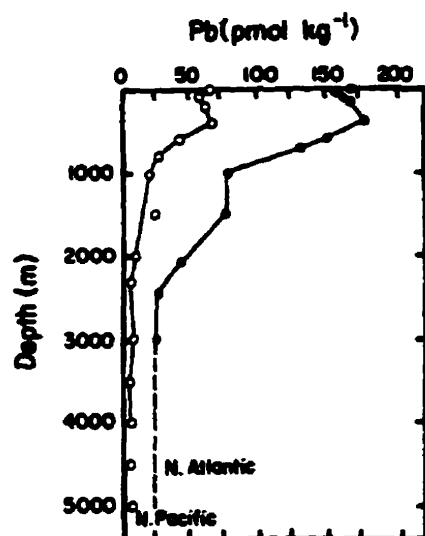
Florence (1986) notes that Pb has a stronger affinity for some inorganic adsorbents, especially iron oxide, than for organic ligands. Therefore it is likely that in most waters with a pH above 7, most of the Pb that is present is associated with hydrated Fe_2O_3, the inorganic colloid fraction.

2.5 The Distribution and Speciation of Cu

Cu belongs to the first transition metal series. Its properties (complexation and oxidation states) result from a partially filled d subshell. The known oxidation states of Cu are +1, +2, and +3, where Cu^{2+} is the most common. Cu is an essential element and plays important roles in many enzyme systems.

Cu exists in the marine environment in three categories: particulate, colloidal, and soluble. The dissolved phase contains both the free ionic form as well as organically- and inorganically-complexed forms. The speciation of Cu in sea water is dependent upon the physico-chemical, hydrodynamic characteristics, and the biological state of sea water.

Cu forms complexes with hard bases, such as carbonate, nitrate, sulphate, chloride, ammonia, and hydroxide (Moore and Ramamoorthy, 1984). Since Cu is an intermediate acceptor between hard and soft acids, Cu complexes with nitrogen and sulfur containing ligands, such as those in humic materials.

Florence (1986) suggests that the dominant inorganic forms of Cu in sea water are $CuCO_3$ (82%), $CuOH^-$ and $Cu(OH)_2$ (6.5%), $Cu(OH)(CO_3)^-$ (6.3%), $CuHCO_3^-$ (1.0%), and Cu^{2+} (2.9%). However, he also suggests that of the total sea water copper concentration, up to 98% may be associated with organic matter and a high percentage with inorganic colloidal particles.

The distribution of Cu in sea water is intermediate between that of nutrient-type and that of elements such as Mn and Pb. Oceanic Cu concentrations range from 0.5 to 6 nmol kg^{-1} (Bruland, 1983).

2.6 The Toxicity of Zn, Cd, Pb, and Cu

The presence of metals in the aquatic environment does not mean that there are adverse effects on biota. For adverse effects to occur, there must be accumulation by biota plus toxicity, an adverse reaction between a chemical and a biological tissue. Many metals may be present but are not taken up, since they are not biologically available. Similarly, a given level of metal in biota may not always have the same effect. The toxic effects of metals are controlled by the conditions in the marine environment which are mostly related to exposure.

Exposure to a specific metal that is sufficiently high to cause accumulation and toxicity may be prevented by circulation and mixing. The natural turnover of coastal waters may provide sufficient dilution, so that most metals are below levels that cause toxic effects.

The complexation and precipitation of metals by sediments exert a large degree of control over the presence of metals in the water column. Organic particulates, both biotic and abiotic, will bind waterborne metals and reduce their uptake by biota. Starodub *et al.* (1987) have demonstrated that organic complexing agents extracted from sediments can significantly reduce metal toxicity to algae.

However, precipitation on sediments may also have the opposite affect on toxicity. The complexation and precipitation of metals by particulates causes sediment enrichment. This results in unavoidable exposure to benthic organisms. Feeding in the benthos by fish can transfer metals directly to fish, by ingestion of sediments or disturbance of pore water enriched with metals.

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Luoma (1986) showed that biominification occurs with metals, as opposed to biomagnification with organic pollutants. Therefore, as one goes up the food chain, there is a decrease in metal concentrations, unlike the special cases of organomercurial compounds.

Toxic effects occur when excretory, metabolic, storage, and detoxification mechanisms are no longer capable of matching uptake rates. This capacity varies between phyla, species, populations, and individuals and even depends on the life stage history of the organism. These factors account for the range of sensitivities observed in toxicity studies. Toxic effects are linked to bioaccumulation and metal burdens in organisms are usually a function of environmental concentrations; thus, chemical speciation of metals in sea water can greatly modify their bioavailability and toxicity. Bioavailability is reduced by the presence of natural organic chelating agents for metals like Cu. Therefore it is the free ion that appears to be the most biologically active species.

2.6.1 Zinc

Zinc is a micronutrient for most organisms. Rainbow (1990) estimates the essential requirement of Zn for marine invertebrates to be 34.5 μg g^{-1}. Spear (1981) has shown that Zn is readily accumulated by aquatic biota, but mechanisms are present for its elimination. Zn concentrations are highest in the lowest trophic levels, but can exceed 100 times their normal levels in polluted areas.

Lethal sea water concentrations range from 72 to 60 000 μg L^{-1} for invertebrates, depending on the water quality and the species studied (Spear, 1981). Zinc lethality to fish varies considerably, from about 70 to 40 000 μg L^{-1}, again depending on the water quality,

the species tested, and the life stage tested. The most sensitive life stages are post-hatch (alevin or larval stage) when fish have high metabollic rates per unit of body mass, large developmental changes are occurring, and there is rapid uptake of waterborne ions.

2.6.2 Cadmium

Unlike Cu and Zn, Cd is not an essential nutrient. Eisler (1985) stated that Cd is readily accumulated by aquatic organisms to levels that increase with proximity to urban or industrial development. Cd is concentrated in the viscera of fish, particularly the liver and kidneys, and particularly with increasing age.

Levels of Cd that are acutely toxic to most aquatic biota are as low as 0.8 to 10 µg L^{-1} (Eisler, 1985). These levels may impair growth, development, and reproduction of invertebrates and fish during chronic exposures.

2.6.3 Lead

Lead can enter the aquatic environment in two forms: as inorganic lead salts and, although rarely, organolead compounds. Inorganic Pb is rapidly precipitated on sediments by organic and inorganic complexing agents. The results of this enrichment of sediment Pb, by organisms such as algae who have bioconcentration factors up to 20 000 (Hodson, 1986), is shown by core profiles. Pb accumulation exhibits biominification with increasing trophic level (Luoma, 1986).

Waterborne and sediment Pb are both biologically available, however, the processes that cause Pb precipitation and enrichment in sediments also reduce uptake from water by biota, so that high organic and inorganic complexing capacity will reduce the uptake and toxicity of Pb.

2.6.4 Copper

Copper is an essential micronutrient for most organisms. It plays a vital role in some metalloenzymes and in the respiratory pigments of invertebrates. Cu is readily accumulated from water, retained when supplies are limited, and actively excreted when intake rates approach toxic levels. The high organic and inorganic complexing capacity of sea water can reduce the availability of Cu to very low levels.

The uptake of Cu has been suggested by Florence (1986); Cu (II) ions bind initially to phytoplankton with a stability constant ($\log \beta_1$) ranging from 10 to 12, complexing with amino acid and carboxylic groups. Cu ions are then transported across the membrane by a carrier protein by facilitated diffusion, where they react with thiol in the cytosol or the interior surface of the membrane, and are reduced to Cu (I). Although most of the evidence points towards the ionic metal form being the most toxic species of Cu, there is some evidence that other inorganic Cu complexes are also toxic, such as Cu hydroxy complexes (Magnusson *et al.*, 1979) and, more importantly, lipid soluble Cu complexes.

The chronic effects of Cu on invertebrates are evident at concentrations greater than 5 to 10 μg L^{-1} (Hodson *et al.*, 1979) and include adverse effects on rates of mortality, growth, and reproduction. Newly hatched organisms are more sensitive than eggs or adults and resistance increases with body covering (ie. impermeable exoskeleton). Other chronic effects may include reduced feeding and pumping rates by bivalves, impaired reaction to food, and changes in diurnal vertical migrations of invertebrates (Hodson *et al.*, 1979).

CHAPTER 3

Oceanography and Biology

3.1 The Labrador Current

Several surface water bodies exist on the North American continental shelf, such as the northeastward flowing Gulf Stream which makes up the Florida Current flowing north of the Bahamas, the Atlantic Slope Water which separates the coastal waters from the Gulf Stream and flows slowly southeastward along the western boundary of the Gulf Stream, the low salinity flow of the Gulf of St. Lawrence which flows southwestward against the continental margin, the Sargasso Sea Water confined to the interior of the large subtropical gyre formed by the Antillean and Gulf Stream Currents at the subtropical convergence, and, finally, the Labrador Current which flows southward in and around the Grand Banks which contains elements of the West Greenland Current, the Canadian Current, and runoff components derived from Hudson Bay and the coasts of Baffin Island and Labrador (Bewers, 1979).

The Coriolis force causes the great volume of arctic water pouring out of the Arctic Ocean through Smith, Jones, and Lancaster Sounds and the Hudson Straight directly against the east coasts of Baffin Island, Labrador, and Newfoundland. Due to its low density, this homogeneous mass of cold water starts off as a surface current, but the forces which push it against the coast cause it to become a bottom current when it reaches

inshore areas as it flows south. The Labrador Current occupies much of the fishing grounds of Newfoundland and Labrador and has temperatures that are below 0°C, reaching an extreme of -1.4°C (Robe, 1971).

Off Labrador, the Current is at its deepest near the coast. In the northerly bays of Newfoundland, it tends to build up as a total mass of cold water except where it is overlain by an insolated layer in the bays. As it moves southward around capes such as Cape Bonavista, it has a structure similar to that off Labrador (Robe, 1971). The seaward portion of this current may overlie but does not descend to the bottom on the continental slope, except near the coast, and is, in turn, overlain by a warm layer due to summer insolation. Two thermoclines often occur: the upper one is usually more shallow and stable, and the lower one shows evidence of mixing or temperature transfer, so that low temperatures persist from Baffin Island to the Grand Banks (Robe, 1971).

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Figure 3.1: Newfoundland and Atlantic Canada (Sinclair *et al.*, 1987)
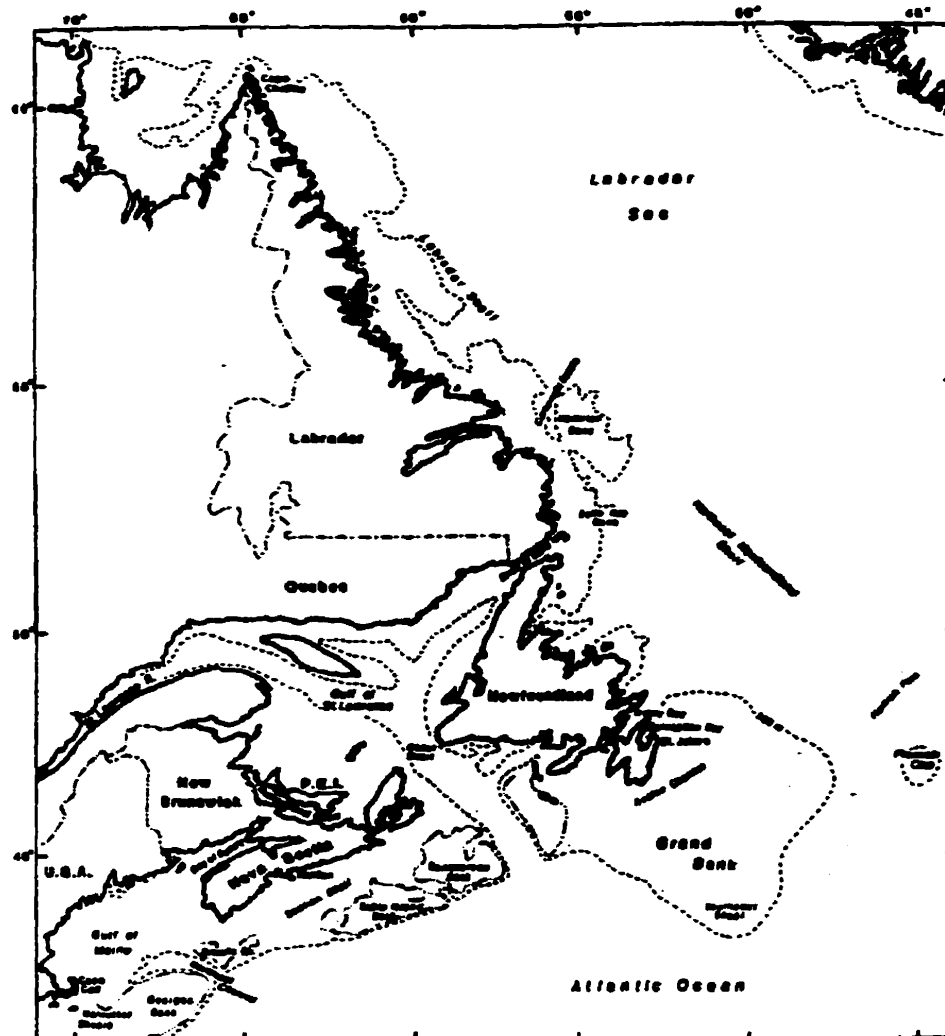
3.2 Conception Bay

Conception Bay is a long and narrow bay (70 to 100 km long, 20 to 30 km wide at the mouth) on the east coast of the island of Newfoundland (de Young *et al.*, 1993). Its maximum depth is 300 m in the central basin, but only 150 m at the mouth, closed off by isobaths restricting the inflow of deep water (DeYoung *et al.*, 1993). There are three

islands, Bell, Little Bell, and Kelley's, of various sizes on the southeast side of the Bay. The Bay is usually covered by pack ice from mid-March to late April. It derives primarily from ice over the shelf which is driven in by onshore winds the source of which is primarily from the shelf offshore and the presence of onshore winds (DeYoung *et al.*, 1993).

Figure 3.2: Sampling site in Conception Bay
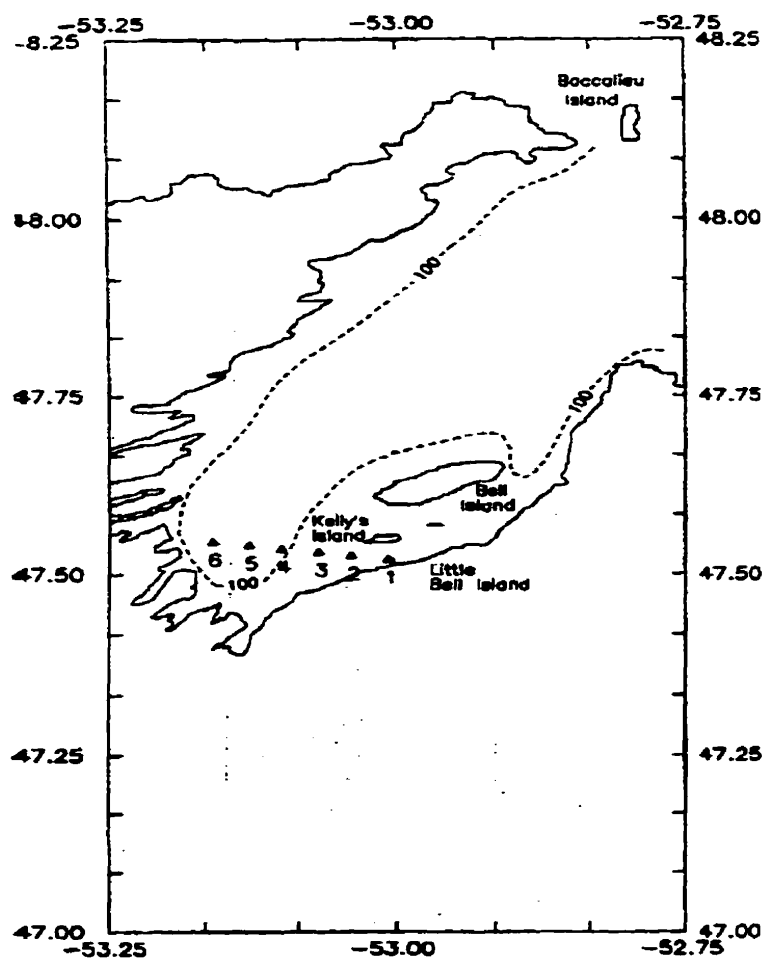
3.3 Zooplankton: Copepods

An important group of marine organisms, the plankton, are separated into two groups, phytoplankton (plants) and zooplankton (animals). Plankton are organisms that drift or swim weakly and are powerless to exercise any significant control over their location in a horizontal direction (Pinet, 1992). Despite the fact that they tend to drift slowly with the currents, they undergo regular vertical migrations; ascending and descending through the water column. Plankton can be further subdivided on the basis of size:

Table 3.1: Classification and size of marine plankton (Pinet, 1992)

Classification	Size	Example
picoplankton	<2 μm	bacteria
ultraplankton	2-5 μm	flagellates
nanoplankton	5-20 μm	dinoflagellates
microplankton	20 μm-2 mm	diatoms, copepods
macroplankton	>2 mm	salps, krill

Living organisms are directly affected by changes in their environment, particularly with respect to chemical composition. In the marine environment, the oceans are subject to large-scale geographical differences (low latitudes versus high latitudes) and seasonal variations that occur in high latitudes. Although most environmental variables change more slowly in the oceans than on land, the marine environment is not a static system, especially the pelagic zone, where the production of microalgae and microheterotrophs determines the trophic conditions for herbivorous zooplankton (Bamsted, 1986).

Table 3.2: Relationships among marine organisms (Pinet, 1992)

Group	Trophic Level	Body Size (cm)	Relative Abundance
Fish	3 and 4	11-200	0.1
Zooplankton	2 and 3	10^{-1}-10	11
Phytoplankton	1	10^{-4}-10^4	100

Zooplankton represent an important part of the food web and of oceanic biomass, therefore contributing to a significant portion of the composition of sea water. Zooplankton, however, are not just biomass, they are considered to be animals who have definite patterns of growth, reproduction, and mortality.

Most studies indicate that copepods constitute the greatest portion of the zooplankton community (Grainger, 1965; Steele and Mullin, 1982; Pinet, 1992). Copepods (tiny crustaceans) are herbivorous and graze on phytoplankton, such as diatoms and dinoflagellates. Food is directed into the mouth region of the copepod by the creation of localized currents created by the motions of their anterior feeding appendages (Pinet, 1992). Particles of the size 5 to 50 μm are selectively removed by a filtering net formed by a pair of bristles, called setae. The rate at which copepods filter is governed by increasing body size. Adult copepods can filter as much as 1 L of water per day (Pinet, 1992). The extraction of nutrients from sea water, even at low concentrations, is accomplished by fluid ingestion, attraction to surface secretions on the organism, and absorption through the epidermal cells (Pinet, 1992). Therefore, many zooplankton species not only derive

nutrients directly through their diets, but also by the direct uptake of nutrients from seawater.

Figure 3.3: Copepods (Pinet, 1992)
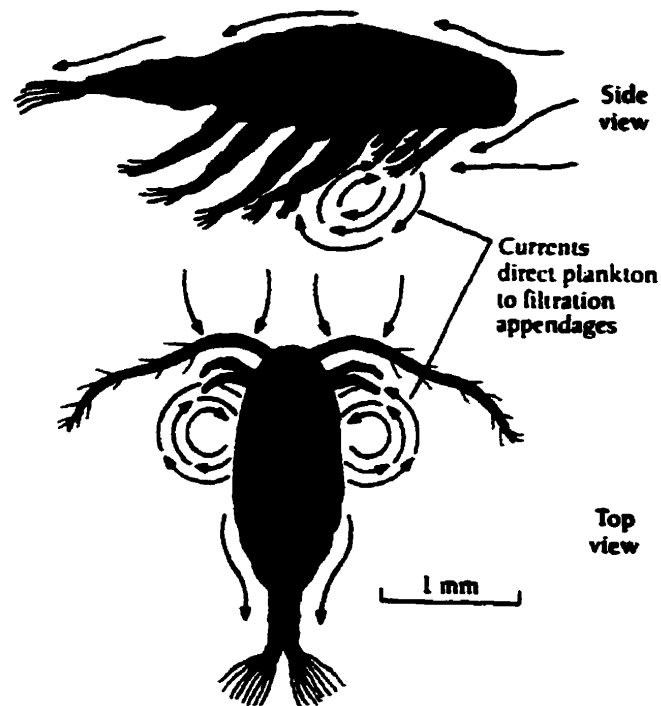
The life cycle of copepods is well known and is shown in Figure 3.4 . Most copepods produce eggs which hatch to become nauplii, which are tiny larvae with a distinct set of appendages. From egg to adult, copepods undergo six naupliar stages and six copepodite stages; each stage separated by a molt during which the exoskeleton is shed for a newer and larger one. Passage from the first naupliar to the last copepodite stage may take fewer than 30 days under optimal environmental conditions (water temperature and food supply) (Pinet, 1992). When they reach the late copepodite stages, copepods

sink to greater depths during the winter months and become dormant; returning to the surface layer to breed in late spring when food supplies are abundant.

Figure 3.4: Copepod life history (Pinet, 1992)
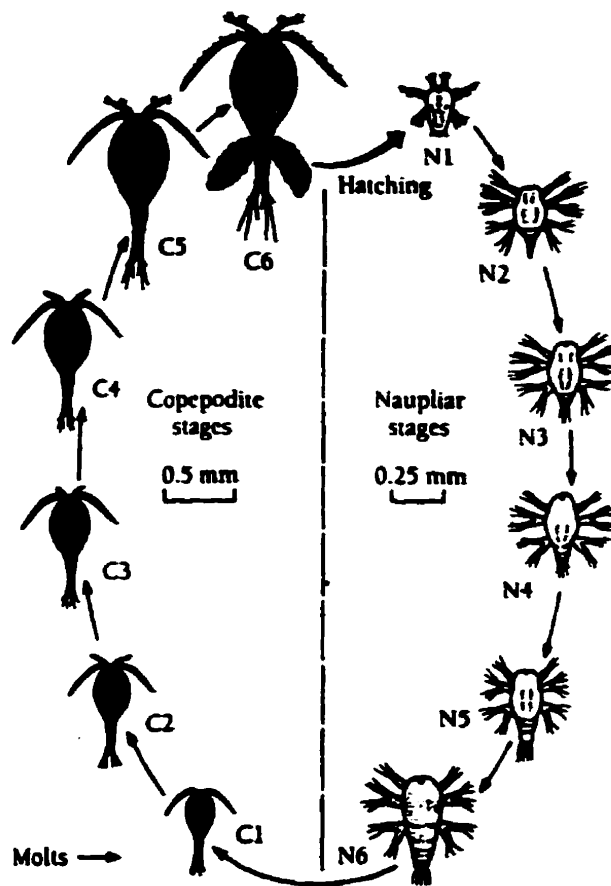
Upon microscopic investigation of the plankton samples collected in this project, mostly copepod naupliar stages were found, including some cyclopoid copepods and dinoflagellates. Since these were early developmental stages, identification of species was impossible. It will be assumed for the sake of this project that the zooplankton sample collected for analysis consists mainly of copepods.

CHAPTER 4

Materials and Methods

4.1 Reagents and Standards

The standards that were used for analysis were made using Atomic Absorption Spectrometry (AAS) standards for Zn, Cd, Pb, and Cu, diluted to specific concentrations for use in the standard addition method. Stock solutions were initially made by diluting 500 µL of the 1000 ppm AAS standards to 10 ppm with 49.50 mL of Nanopure water. Following this, two standards were made from these stock solutions for use in the ASV analyses. For the 0.1 ppm Zn, Cd, Pb, and Cu standard, 100 µL of each stock solution were diluted with 9.60 mL of Nanopure water. For the 0.4 ppm Zn, 0.1 ppm Cd, Pb, and Cu standard, 400 µL of Zn stock and 100 µL of Cd, Pb, and Cu stocks were diluted to 10 ml with Nanopure water. A 0.1 ppm standard was prepared for use in the CSV sea water analyses using a similar dilution. All standard solutions were prepared daily.

Table 4.1: Standards used for ASV and CSV Analyses

Standard Solution	Use
10 ppm Metal	Stock Solution
0.1 ppm Zn, Cd, Pb, and Cu	ASV
0.4 ppm Zn and 0.1 ppm Cd, Pb, and Cu	ASV
0.1 ppm Cu	CSV

All acids and bases used in analyses, digestions, and neutralizations were Seastar™ double-distilled in quartz acids and bases. The buffer used in ASV sea water analyses was made by adding 13.61 g of sodium acetate (NaAc) to 5.72 mL of glacial acetic acid (HAc) and diluting to 100 mL with Nanopure water (pH 4.5 to 4.6), followed by purification by ion exchange. The buffer used in CSV sea water analyses was made by dissolving 9.54 g of 4-(2-hydroxyethy)-1-piperazineethanesulfonic acid (HEPES) in 40 mL of 0.5 M NaOH (pH 7.45 to 7.55), followed by purification by MnO_2 adsorption. The details of the purification procedures can be found in Li (1996).

Table 4.2: Reagents used for ASV and CSV Analyses

Reagent	Grade	Use
Concentrated HNO_3	Seastar	Digestion
0.1 M HCl	Seastar	Digestion
Concentrated NH_3	Seastar	Pre-Analysis
1 M KCl	Suprapur	ASV
1 M HAc/1 M NaAc	Suprapur	ASV
2 M KCL	Suprapur	CSV
1 M HEPES (purified)	Analytical	CSV
0.1 M Oxine	Analytical	CSV

Nanopure water was made using a NANOpure™ II filtration system. Nanopure water was used for dilution, for rinsing at all stages of acid-cleaning, and for analyses.

4.2 Acid-Cleaning:

Polyethylene apparatus, which includes Nalgene™ bottles, pipette tips, and microcentrifuge tubes, were cleaned in the following manner. They were placed in a hot 5% micro-detergent (Cole-Palmer Instrument Co.) bath for 24 hours, rinsed three times with Nanopure water, placed in a hot 3 M HCl bath for 48 hours, rinsed three times, placed in a hot 7.5 M HNO_3 bath for 48 hours, rinsed three times, then filled with 6 M HCl and immersed in 3 M HCl for a minimum of two weeks. The bottles were not, however, placed in the 3 M HCl and 7.5 M HNO_3 baths, but were placed directly in the 6 M/3 M HCl soak. After this period, the bottles were rinsed three times with Nanopure water and filled with 0.1 M HNO_3, placed in Ziploc freezer bags, and left for one week. Before sampling, the bottles were rinsed five times with Nanopure water and placed back into Ziploc bags until ready to use. Pipette tips and centrifuge tubes were rinsed well and placed in 0.1 M HNO_3 for one week. Before use, the pipette tips were placed in Nanopure water and the centrifuge tubes were rinsed well and placed on the clean bench to air dry, then capped until used.

The quartz digestion beakers were placed in a hot detergent bath for 24 hours, rinsed 3 times, placed in a hot 3 M HCl bath for 48 hours, rinsed 3 times, placed in a hot 7.5 M HNO_3 bath for 48 hours, then rinsed 5 times and placed on the clean bench where they were filled with 1 M HCl until ready to use.

The sieve cup was constructed from a 1 L acid-cleaned Nalgene polyethylene bottle. After construction, the cup was cleaned again using the same procedure as for the bottles. The nylon mesh used in the cup was rinsed well with Nanopure water and stored in 1 M HCl on the clean bench. Both the cup and the mesh were rinsed and dried on the clean bench prior to use and then rinsed and stored in 0.1 M HCl after use.

The GO-Flo[®]'s that were used for sea water sampling were washed out thoroughly with tap water and rinsed well with Nanopure water. They were then filled with 1 M HCl and placed in bags overnight. Following this, they were rinsed three times, filled with 0.1 M HNO$_3$, bagged, and left for one week. Before sampling, the GO-Flo[®]'s were rinsed and left bagged until they were attached to the hydrowire for sea water collection.

The EG&G 303A electrode was kept on the clean bench and a sample cell filled with 0.1 M HCl was kept in place until an analysis was prepared. At that time, the electrode was rinsed with nanopure water. All Teflon sample cells, after having been cleaned in the same manner as for the polyethylene bottles, were kept on the clean bench filled with 0.1 M HCl and rinsed five times prior to use.

4.3 Sea Water Collection and Analysis

Sea water samples were collected at the BRLP5 site (47° 32.5' N, 53° 07.8' W) along the Brigus-Long Pond transect in Conception Bay, Newfoundland at depths of 30 m, 60 m, and 100 m using 5 L GO Flo[®] bottles. This station was chosen because it is in the deepest part of the bay and yet is located such that travel times to the laboratory are relatively short. Once collected, the sea water was stored frozen in clean polyethylene bottles.

Simultaneous analyses for Zn, Cd, Pb, and Cu were carried out using 10 mL portions of thawed sea water using a PAR 164A voltammetric analyzer connected to an EG&G 303 hanging mercury drop electrode. CSV analysis was also carried out on BRLP5 sea water (60 m) for the determination of free and total Cu. The procedure and parameters for the CSV analyses can be found in Li, 1996. All laboratory preparation and analyses were carried out on a class 100 clean bench.

Parameters For ASV Analysis:

> Scan Rate: 5 mV sec^{-1} in positive direction, 1.5 V range
> Initial Potential: -1.2 mV
> Modulation Amplitude: 25 mV
> Deposition Time: 10 min
> Equlibration time: 30 sec

Sea water was also collected at the Marine Station Research Lab (MSRL) in Logy Bay, Newfoundland for comparison.

4.4 Plankton:

Plankton samples were collected on June 13, 1996 in Conception Bay at the BRLP5 site using a 202 µm Nitex® mesh ring net with a 50 cm diameter at the mouth of the net. A sampling depth of 60 m was chosen based on the maximum density of plankton. The following CTD information was collected:

> Salinity: 32.5
>
> % Transmission: 85
>
> Light (PAR): 211.14
>
> Temperature: -0.6709°C
>
> Density: 26.4978
>
> Fluorescence: 5.42 x 10^{-1}

This site suited the purpose of collecting the maximum number of animals.

After collection, the samples were kept in sea water and stored frozen in 1 L polyethylene bottles. When ready to use the samples, they were thawed in their bottles then removed from the sea water onto a 12 - 16 μm nylon mesh using a sieve cup. The mesh was rinsed briefly with Nanopure water to remove adhering salt and groups of animals were removed, placed in microcentrifuge tubes, and left to air dry for 24 hours on the clean bench.

The dried plankton and centrifuge tubes were weighed using a Perkin-Elmer Autobalance AD-2Z. The dried plankton were placed into 15 mL quartz beakers on a hot plate in a laminar flow hepafilter box and were digested for 1 hour using 3 mL of concentrated HNO_3. The resulting solution was evaporated to dryness and re-dissolved in 10 mL of 0.1 M HCl.

Zn, Cd, Pb, and Cu determinations were carried out with a PAR 164A voltammetric analyzer connected to an EG&G 303 hanging mercury drop electrode. The analyses were carried out using a 1 in 10 dilution using 1 mL of neutralized digestion solution diluted with 9 mL of Nanopure water; the purpose of this was to bring the concentration of trace metals present in the sample down to levels suitable for the analytical procedure. Analyses were also performed using 1 mL of digestion solution and in 9 mL of filtered MSRL sea water. All analyses were carried out using simultaneous calibrations for Zn, Cd, Pb, and Cu concentrations using the method of standard additions.

Blanks were also performed for the digestions. 3 mL of concentrated HNO_3 was

digested in the same way and the resulting 10 mL solution was neutralized and analyzed.

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CHAPTER 5

Results and Discussion

5.1 Introduction

Given the short period of time to carry out this project, an attempt has been made to obtain some information about trace metals in sea water and plankton from Conception Bay. The procedures for the analysis of sea water and plankton and the procedure for the digestion of the plankton were applied. Although much of the information obtained may be suspect due to contamination from a variety of sources, such as during sampling, some information has been obtained. A discussion of the sampling and sampling devices is given in this chapter and are included in an evaluation of the analytical method used.

Three aliquots of sea water collected from Conception Bay at a depth of 60 m were analyzed using the ASV method outlined in Section 4.3 for all four metals. Two more aliquots were then acidified, irradiated, and analyzed in the same manner. The results from the regression of the analyses are shown in Tables 5.1 and 5.2. The CSV procedure was then applied to 6 more aliquots of sea water and are displayed in Table 5.3. The CSV method improved the detection of Cu in BRLP5 sea water. The overall results of both the ASV and CSV analyses are shown in Table 5.4 and are comparable to those shown in Table 5.5 for MSRL sea water from Logy Bay.

The procedure for performing blanks for the digestion procedure that was used in preparing the plankton samples, referred to in Section 4.4, was also performed in order to ascertain the level of contamination for this procedure. The results for the analysis of the final 0.1 M HCl solution are given in Table 5.6 and are followed by the results of the plankton analysis procedure in Table 5.7. These results represent the ASV analysis of the final solution of digested plankton minus the average concentration of Zn, Cd, Pb, and Cu present in the blanks.

5.2 Sea Water

Performing a regression of the results (Table 5.1) for an aliquot of sea water provides the concentration of the original sea water sample (X intercept). The result for aliquot 3 appears somewhat suspect and will not be considered. The average of the x intercepts gives the average concentration present in BRLP5 sea water (0.29 μg L^{-1}) and a standard deviation (0.00701). These results represent the ASV-labile (free) portion of metals present in BRLP5 sea water.

Table 5.1: Regression of ASV of BRLP5 sea water (60 m)

Aliquot	Metal (Free)	Standard Concentration	Peak Height	X Intercept	Standard Deviation
1	Zn	0.00	1.45	-0.29	0.036519
		0.50	5.10		
		0.99	8.10		
		1.48	10.40		
2	Zn	0.00	0.75	-0.28	0.003614
		0.50	2.30		
		0.99	3.50		
		1.48	5.00		
3	Zn	0.00	1.50	-0.11	0.031150
		0.50	5.20		
		0.99	10.60		
		1.48	15.40		

Irradiation and acidification of the same sample gives the total metal concentrations present in the sample. The results of this procedure are given in Table 5.2. These results give an average total-Zn value of 0.19 μg L^{-1} (SD = 0.04246). This value is unexpectedly lower than the average value for free-Zn in the same sample since irradiation and acidification of the sea water sample would be expected to release more of the complexed Zn than is present. However, both values are virtually the same within a small margin of error, which is due to the sensitivity of the instruments.

Table 5.2: Regression of BRLP5 sea water (60 m) after UV irradiation

Aliquot	Metal (Total)	Standard Concentration	Peak Height	X Intercept	Standard Deviation
1	Zn	0.00	1.40	-0.16	0.012631
		0.50	4.60		
		0.99	8.30		
		1.48	12.30		
	Cd	0.00	0.30	-0.06	0.009485
		0.50	4.70		
		0.99	8.80		
		1.48	12.30		
	Pb	-	-	-	-
	Cu	-	-	-	-
2	Zn	0.00	1.60	-0.22	0.061685
		0.50	3.80		
		0.99	6.80		
		1.48	11.00		
	Cd	0.00	0.30	-0.02	0.037760
		0.50	3.80		
		0.99	6.60		
		1.48	11.20		
	Pb	0.00	0.30	-0.12	0.002783
		0.50	1.20		
		0.99	2.20		
		1.48	3.30		
	Cu	-	-	-	-

Very small concentrations of Cd and Pb were detected after irradiation and acidification. These concentrations, however, are very close to the detection limits for the ASV procedure and are therefore not considered to be detected, except the value for Pb in the second aliquot, which may have been the result of contamination during the irradiation procedure. Tables 5.1 and 5.2 indicate concentrations for Zn, Cd, and Pb in Labrador Current sea water which are comparable to those reported in Tables 1.1 and 1.2 from Section 1.2.

Table 5.3: Results of CSV analysis of BRLP5 sea water (60m)

Aliquot	Metal	Standard Concentration	Peak Height	X Intercept	Standard Deviation
1	Free-Cu	0.00	1.10	-0.35	0.308938
		0.98	8.40		
		1.95	14.20		
		2.91	17.60		
2	Free-Cu	0.00	1.10	-0.35	0.380938
		0.98	9.50		
		1.95	15.60		
		2.91	19.40		
3	Free-Cu	0.00	2.10	-0.53	0.183163
		0.98	9.50		
		1.95	13.60		
		2.91	17.40		
1	Total-Cu	0.00	0.90	-0.44	0.045850
		0.98	4.10		
		1.95	6.50		
		2.10	8.70		
2	Total-Cu	0.00	1.00	-0.49	0.075262
		0.98	4.40		
		1.95	6.90		
		2.10	9.00		
3	Total-Cu	0.00	1.45	-0.58	0.130067
		0.98	5.60		
		1.95	8.70		
		2.91	11.00		

The concentration of total and free Cu were not detected by ASV for the reasons that were described at the end of Section 1.5; however, CSV provides adequate results for both free and total Cu as is shown in Table 5.3. Oxine has been used effectively to determine Cu concentrations in sea water that contains high concentrations of organic matter due to the highly productive waters of the region.

Table 5.4 shows the results for the sea water analysis conducted in July, 1996. These results are representative of the expected levels of Zn, Cd, Pb, and Cu in unpolluted coastal sea water. However, it is important to point out that these are only the values for water collected at 60 m, the other two depths that were sampled resulted in a high degree of contamination, possibly due to improper functioning of the GO-Flo®'s. Either the teflonized inner coating has degraded over the years for the GO-Flo®'s used at 30 and 100 m, or the fact that they opened as they entered the water, therefore being contaminated by the surface layer, might be possible explanations of why these samples became contaminated. Another explanation for this contamination, which stems from the poor state of the GO-Flo®'s, is the fact that insufficient time was available for proper cleaning of these devices. Not only was the Teflonized inner surface (probably) degraded, but several components were rusting and only a week-long acid soak was possible; whereas, based on their condition, the GO-Flo®'s should have been taken apart and properly cleaned following the same cleaning procedure as in Section 4.2. One last point to be made about sampling is the fact that the weather may have had some effect due to the high winds present that day; dust particles and paint chips may have entered the collection devices and bottles. All of these factors, although presenting only tiny degrees of contamination individually, may have amounted to a significant degree of contamination to the GO-Flo®'s used at 30 and 100 m.

Why, then, did the GO-Flo® used at 60 m not have the same degree of contamination? Perhaps the answer is as simple as the fact that it was the only one that

functioned properly. Proper functioning may be indicative of the slightly better condition of this device.

Table 5.4: Results of BRLP5 sea water analysis (60 m)

Metal	Average Free-Metal Concentration ($\mu g\ L^{-1}$)	Standard Deviation	Average Total-Metal Concentration ($\mu g\ L^{-1}$)	Standard Deviation
Zn	0.29	0.00701	0.19	0.04246
Cd	<0.005	-	0.04	0.028284
Pb	<0.01	-	0.06	0.084853
Cu (by CSV)	0.41	0.103923	0.50	0.046224

The results for ASV sea water analysis (Table 5.5), with respect to the free metal concentrations, from Logy Bay, another unpolluted system, are very close to those for BRLP5. The samples were taken from the pump system that supplies water to the fish holding tanks. This system is constantly supplying water to the tanks and, based on previous sampling and analysis, provides adequate samples, probably due to the well-rinsed state of the pump components.

Table 5.5: Results of MSRL ASV sea water analysis

Sample	Metal	Concentration ($\mu g\ L^{-1}$)	Standard Deviation
1	Zn	0.12	0.009197
2	Zn	0.15	0.014504
3	Zn	0.22	0.019462
4	Zn	0.09	0.123165
5	Zn	0.16	0.007255
6	Zn	0.11	0.002500

(Avg. = 0.14 $\mu g\ L^{-1}$, SD = 0.046224)

Analysis of the MSRL sea water samples showed no detection of Cd, Pb, or Cu, therefore none of these metals are present in Table 5.5; although CSV analysis would probably have detected Cu. The MSRL samples served the purpose of determining the concentrations of trace metals in sea water from an unpolluted bay in coastal Newfoundland that could be used to confirm the concentrations detected at the BRLP5 site. The results of the analysis of BRLP5 water would be expected to be somewhat higher than those for MSRL water due to the greater degree of human activity in the Conception Bay region; these values support this statement.

5.3 Blanks

From a series of eight blanks performed for the digestion procedure, all resulted in small quantities of Zn, four showed Pb, and two showed detectable levels of Cu. These

results, as shown in Table 5.6, are good enough for the purpose of this project and it can be reasonably stated, that any contamination of the plankton samples could not have resulted during the digestion procedure.

Table 5.6: Results of analysis of the final solution from the blanks

Blank	Zn (μg L^{-1})	Cd (μg L^{-1})	Pb (μg L^{-1})	Cu (μg L^{-1})
1	0.25	<0.005	0.14	0.14
2	0.32	<0.005	<0.01	0.29
3	0.44	<0.005	0.26	<0.005
4	0.14	<0.005	0.21	<0.005
5	0.10	<0.005	<0.01	<0.005
6	0.23	<0.005	<0.01	<0.005
7	0.25	<0.005	<0.01	<0.005
8	0.38	<0.005	0.05	0.43
Average	0.26	0	0.08	0.11

5.4 Zooplankton

Nine aliquots of one sample of plankton were analyzed using the previously described ASV procedure (Table 5.7). The results for whole body Zn concentrations exhibit a fairly broad range (Table 5.8). The lower end of this range is comparable with the values reported in Table 1.5 in Section 1.4, although somewhat higher. This fact might be

expected of plankton from Conception Bay which feed on phytoplankton that are subject to high concentrations of metals in the sediment-water interface due to the geology of the region. The upper end of this range is highly indicative of sample contamination; which may be caused by several factors: surface layer contamination, environmental conditions (wind, dust, etc.), and the fact that the net was never cleaned prior to sampling.

Despite these assumptions, other possible factors could be considered. Very little information was available on the plankton, therefore it is not known where the plankton spend most of their time. It is possible that the plankton that were sampled inhabited areas of Conception Bay, such as harbours and wharf areas, which would have been subject to the effects of a much higher degree of contamination from human activities.

Table 5.7: BRLP5 Plankton samples

Aliquot	Dry Weight	Metal Concentration (μg g^{-1})			
		Zn	Cd	Pb	Cu
1	0.87	920	<0.005	48	<0.005
2	1.91	340	4.5	<0.01	<0.005
3	0.57	610	40	36	180
4	0.87	790	<0.005	67	<0.005
5	0.70	560	<0.005	62	<0.005
6	0.63	580	<0.005	<0.01	410
7	1.18	540	<0.005	<0.01	130
8	0.38	240	7.9	32	<0.005
9	0.32	240	3.1	<0.01	<0.005

The results for Cd, Pb, and Cu from the nine aliquots can be grouped into ranges that are to similar to that for Zn. Comparison with Table 1.5 proves that the lower end of the range for these values (Table 5.8) is probably correct. It would be expected that these metals would be present, not because of any great degree of accumulation, but because of the underlying geology of the region. Despite this, one or more of these metals were detected in several of the aliquots. This lack of homogeneity of the sample may be indicative of contamination during the separation or analytical procedures.

Table 5.8: Results of ASV plankton analysis

Metal	Minimum Dry Weight Concentration ($\mu g \, g^{-1}$)	Maximum Dry Weight Concentration ($\mu g \, g^{-1}$)	Average Dry Weight Concentration ($\mu g \, g^{-1}$)
Zn	240	920	640
Cd	3.1	40	6.2
Pb	32	67	27
Cu	62	410	80

It has been assumed that the sample contained primarily copepods (Section 3.3). This may in fact have been an incorrect assumption, since the lack of homogeneity for these results may be indicative that a greater portion of the sample might have contained other planktonic organisms which would have quite a significant effect of on the concentrations of metals in the sample. Species-specific uptake patterns in different

epibenthic invertebrate species would account for this and would be an important factor in future studies.

5.5 Conclusion

Sea water collected at site 5 on the BRLP station in Conception Bay, Newfoundland showed comparable levels of Zn, Cd, Pb, and Cu to those reported in the literature. The values for the free (or ASV-labile) concentrations are: 0.29 and 0.41 μg L^{-1} for Zn and Cu respectively, while no Cd or Pb were not detected at a depth of 60 m. The values for the total concentrations of Zn, Cd, Pb, and Cu are: 0.19, 0.04, 0.06, and 0.50 μg L^{-1} respectively. The ASV and CSV procedures used for the analysis of sea water were performed successfully and indicate that Labrador Current water is unpolluted with respect to Zn, Cd, Pb, and Cu. Unfortunately, improper functioning of the sampling devices and the possibility of other sources of contamination made it impossible to include the results of the water collected at 30 and 100 m. However, since the plankton were collected from 60 m up, the 60 m water sample is sufficient for this study.

The procedure for the blanks was developed and was then applied to the digestion of the plankton sample. It may be possible that some contamination had occurred during the separation of the plankton prior to digestion, which, when included with the possibility of contamination during sampling, resulted in a broad range of concentrations for Zn, Cd, Pb, and Cu. The lower end of this range indicates concentrations for Zn, Cd, Pb, and Cu in whole body copepods of 240, 3.1, 32, and 62 μg g^{-1} for Zn, Cd, Pb, and Cu respectively; indicating that plankton in Conception Bay have accumulated some trace metals which may have been caused by the geology of the region. The presence of certain rock

formations make high concentrations of metals available to benthic organisms which may be passed up the food chain.

This report may provide valuable information for future studies of the Conception Bay area and the marine organisms that inhabit the region. It has been proven that ASV can effectively be used to determine trace metal concentrations in biota and yield reasonable results, despite the possibility of contamination from various sources. This study may provide some answers towards what is necessary for future studies.

References

Bamstedt, U. Chemical composition and energy. In: *The Biological Chemistry of Marine Copepods*. E.D.S. Corner and S.C.M. O'Hara (eds.), Clarendon Press, Oxford, 1986, pp.1-58.

Batley, G.E. Interferences in the determination of copper in natural waters by anodic stripping voltammetry. *Anal Chim Acta* **189**: 371-377 (1986).

Bewers, J.M. *Trace Metals in Waters Within the Jurisdictional Area of the International Commission for the Northwesrt Atlantic Fisheries*. Bedford Institute of Oceanography Report Series BI-R-79-2 (1979).

Bruland, K. Trace elements in sea water. In: *Chemical Oceanography*. Vol. 8. J.P. Riley and R. Chester (eds.), Academic Press, London, 1983, pp.157-220.

Bruland, K. Complexation of cadmium by natural organic ligands in the Central North Pacific. *Limnol. Oceanogr.* **37**: 1008-1017 (1992).

Burton, J.D. and Statham, P.J. Occurrence, distribution and chemical speciation of some minor dissolved constituents in ocean waters. In: *Environmental Chemistry*. Vol. 2, H.J.M. Bowen (ed.), Royal Society of Chemistry, London, 1982, p. 234.

Copeland, T.R. and Skogerboe, R.K. *Anal. Chem.* **46**: 1257A (1974).

DeYoung, B. and Sanderson, B. The circulation and hydrography of Conception Bay, Newfoundland. *Atmospheric-Ocean* 33(1): 135-162 (1995).

Drever, J.I. *The Geochemistry of Natural Waters*. Second Edition, Prentice Hall, New Jersey, 1988, pp. 326-346.

Eisler, R.. Chromium hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Dept. of Interior, Fish, and Wildlife Service, Biological Report 85 (1.2), 1985, p.46.

Faust, S.D. and Aly, O.M. Chemical weathering reactions. In: *Chemistry of Natural Waters*, Ann Arbour Science, Michigan, 1981, pp. 209-243.

Florence, T.M. The speciation of trace elements in waters. *Talanta* **29**: 345-364 (1982).

Florence, T.M. Electrochemical approaches to trace element speciation in water. *Analyst* **111**: 489-505 (1986).

Florence, T.M. and Batley, G.E. Chemical speciation in waters. *CRC Critic. Rev. Anal. Chem.* **9**: 219-296 (1980).

Fowler, S.W. Trace elements in zooplankton particulate products. *Nature* **269**: 51-53 (1977).

Fowler, S.W. Trace metal monitoring of pelagic organisms from the open Mediterranean Sea. *Environ. Monitor. Assess.* **7**: 59-78 (1986).

Grainger, E.H. Zooplankton from the Arctic Ocean and adjacent Canadian waters. *J. Fish. Res. Board. Can.* **22**: 543-564 (1965).

Hodson, P.V., Borgmann, U., and Shear, H. Toxicity of copper to aquatic biota. In: *Copper in the Environment. Part 2. Health Effects.* J.O. Nriagu (ed.), Wiley Interscience, New York, 1979, p. 489.

Hodson, P.V. The effects on aquatic biota of exposure to lead. In: *Pathways, Cycling and Transformation of Lead in the Environment.* P.M. Stokes (ed.), Commission of Lead in the Environment, Royal Society of Canada, 1986, p. 415.

Li, J. *Personal Communication.* M.Sc. Thesis, Chemistry Department, Memorial University of Newfoundland (In Progress).

Loring, D.H. Trace metal geochemistry of Gulf of St. Lawrence sediments. In: Chemical Oceanography of the Gulf of St. Lawrence. *Canadian Bulletin of Fisheries and Aquatic Sciences* **220**. P.M. Strain (ed.), Department of Fisheries and Oceans, Ottawa, 1988, pp.99-122.

Luoma, S.N. Cycling of lead into food webs in aquatic environments. In: *Pathways, Cycling and Transformation of Lead in the Environment.* P.M. Stokes (Ed.), Commission of Lead in the Environment, Royal Society of Canada, 1986, p. 415.

Magnusson, V.R., Harris, D.K., Sun, M.S., Taylor, D.K., and Glass, G.E. In: *Chemical Modeling in Aqueous Systems.* E. A. Jenne (ed.), ACS Symposium Series 93, American Chemical Society, Washington, D.C., 1979, p. 635.

Martin, J.H. The possible transport of trace metals via moulted copepod exoskeletons. *Limnol. Oceanogr.* **15**: 756 (1970).

Martin, J.H. and Knauer, G.A. The elemental composition of plankton. *Geochim. Cosmochim. Acta* **37**: 1639-1653 (1973).

Moore, J.W. and Ramamoorthy, S. *Heavy Metals in Natural Waters: Applied Monitoring and Impact Assessment.* Springer-Verlag, New York, 1984, 253 pp

Nelson, A. Voltammetry of copper species in estuarine waters. *Anal. Chim. Acta* **169**: 273-298 (1985).

Nicholls, G.D., Curl, H., and Bowen, V.T. Spectrographic analyses of marine plankton. *Limnol. Oceanogr.* **4**: 472 (1959).

Pinet, P.R. *Oceanography: An Introduction to the Planet Oceanus.* West Publishing Co., St. Paul, 1992, pp.290-384.

Pagenkopf, G.K. Transport of trace elements in natural water systems. In: *Introduction to Natural Water Chemistry.* Marcel Dekker, Inc., New York, 1978, pp. 220-238.

Preston, M.R. Marine Pollution. In: *Chemical Oceanography.* Volume 9, J.P. Riley (ed.), Academic Press, San Diego, 1989, pp. 53-196.

Rainbow, P.S. and Moore, R.G. Comparative metal analyses in amphipod crustaceans. *Hydrobiol.* **14**: 273-289 (1986).

Rainbow, P.S. Heavy metal levels in marine invertebrates. In: *Heavy Metals in the Marine Environment.* R.W. Furness and P.S. Rainbow (eds.), Boca Raton: CRC Press, 1990, pp. 67-79.

Rideout, P.S., Rainbow, P.S., Roe, H.S.J., and Jones, H.R. Concentrations of V, Cr, Mn, Fe, Ni, Co, Cu, Zn, As, and Cd in mesopelagic crustaceans from the North East Atlantic Ocean. *Mar. Biol.* **100**: 465-471 (1989).

Robe, R.W. Oceanographic observations; North Atlantic Standard monitoring sections A1, A2, A3, and A4; 1967-1968. *U.S. Coast Guard Oceanographic Unit Oceanographic Report*, 1971.

Sinclair, M., Loder, J.W., Gascom, D., Horne, E.P., Perry, I., and Sandeman, E.J. Fisheries needs for physical oceanographic information within the Atlantic Zone. *Canadian Technical Report of Fisheries and Aquatic Sciences. No. 1568.* Department of Fisheries and Oceans, 1987, p. 2.

Spear, P.A. *Zinc in the Aquatic Environment: Chemistry, Distributions, and Toxicology.* Associate Committee on Scientific Criteria for Environmental Quality. National Research Council of Canada. Publication No. 17589, 1981, p. 145.

Starodub, M.E., Wong, P.T.S., Mayfield, C.I., and Chau, Y.K. Influence of complexation and pH on individual and combined heavy metal toxicity to freshwater alga. *Can. J. Fish. Aquat. Sci.* **44**: 1173-1180 (1987).

Steel, J.H. and Mullin, M.M. Zooplankton dynamics. In: *The Sea: Ideas and Observations in the Study of the Seas.* Volume 6. E.D. Goldberg, I.N. McCave, J.J. O'Brien, and J.H. Steele (eds.). John Wiley and Sons, New York, 1982, pp. 857-890.

Van den Berg, C.M.G. Determination of copper, cadmium, and lead in sea water by cathodic stripping voltammetry of complexes with 8-hydroxyquinoline. *J. Electroanal. Chem.* **215**: 111 (1986).

Van den Berg, C.M.G. Electroanalytical chemistry of sea water. In: *Chemical Oceanography.* Volume 9, J.P. Riley (ed.), Academic Press, San Diego, 1989, pp. 199-245.

Waldichuk, M. Global Marine Pollution: An Overview. *Intergovernmental Oceanographic Commission Technical Series* No. 18, UNESCO, 1978, pp.14-32.

Whitfield, M. The electroanalytical chemistry of sea water. In: *Chemical Oceanography.* Vol. 4. J.P. Riley and G. Skirrow (eds.), Academic Press, New York, 1975, pp.1-154.

Wolfe, D.A. and Rice, T.R. Cycling of elements in estuaries *Fish Bull.* **70**: 959-972 (1972).

Zauke, G.-P. and Petri, G. Metal Concentrations in Antarctic Crustacea: The Problem of Background Levels. In: *Ecotoxicology of Metals in Invertebrates.* R. Dallinger and P.S. Rainbow (eds.), Lewis Publishers, London, 1993, pp. 73-101.