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STUDIES ON THE BIOGEOCHEMISTY OF ZINC IN THE SUBARCTIC NORTH PACIFIC

By

Maeve Carroll Lohan

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ABSTRACT

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Studies on the biogeochemistry of zinc in the subarctic North East Pacific Ocean

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Trace metals are of fundamental importance to all living organisms and to marine phytoplankton because of their role even at very low concentrations in essential enzymes. Zinc has an essential role as the catalytic centre in carbonic anhydrase and thus its availability greatly affects inorganic C acquisition by phytoplankton. This research involves a study on the distributions and speciation of Zn in the NE Pacific Ocean and its interactions with phytoplankton species present. Total dissolved Zn and Zn speciation was measured using AdCSV. A clear gradient is evident in the total dissolved Zn concentrations within the mixed layer, decreasing offshore along an E –W transect from a shelf station (0.9 nM) out into the High Nutrient Low Chlorophyll (HNLC) area at Ocean Station Papa (OSP; 0.04 nM) in both summer and winter. Dissolved Zn/Si ratios in the upper 200m decreases with distance from shore, which infers a decoupling between dissolved Zn and silicic acid in the upper ocean. It is hypothesised that the profile of dissolved Zn in deeper waters is a result of recycling from relatively biologically resistant organic particulate phases that leads to profiles very similar to those of silicic acid.

Zinc speciation was dominated by complexation to a natural organic ligand reducing the free Zn^{2+} concentration to 0.72 pM at OSP. Below 200m the total dissolved Zn concentration exceeds the concentration of the Zn-binding ligand and therefore the speciation of Zn is not dominated by organic complexation in the deep waters of the NE Pacific. No relationship between the Zn-binding ligands and chlorophyll fluorescence was observed.

The addition of Fe to surface waters containing a natural phytoplankton assemblage dramatically increased the growth rate and abundance of diatoms and other phytoplankton species at OSP. Addition of Fe increased the uptake of total dissolved Zn and in combination with the production of organic ligands reduces the $[Zn^{2+}]$ to 0.2 pM, well below the limit suggested in laboratory cultures that can limit phytoplankton growth. At these low bioavailable Zn concentrations ligand production may be a mechanism by which phytoplankton can access Zn bound to these ligands. Zinc concentrations observed at OSP in this study were not limiting phytoplankton growth as the addition of Zn showed no significant increase in phytoplankton biomass. Production of Zn-binding ligands was observed on the addition of Zn, indicating a rapid response from phytoplankton and specificity for Zn. Production of these ligands by phytoplankton at high Zn concentrations is hypothesised as mechanism for luxury uptake by phytoplankton. Both production and destruction of these ligands occurred on a time scale of less than a day suggesting that these ligands may be regulated by phytoplankton and aid in controlling the uptake of Zn.

Mesoscale eddies are an important source of trace metals to the open ocean in the NE Pacific transporting on the order of 2.2 m mol m^{-2} yr^{-1} of Zn from coastal waters into to the intermediate HNLC waters (100-400m). Upwelling and vertical diffusion of this trace metal enriched water enhances biological production. Total dissolved Zn concentrations decreased as the eddy aged due to mixing with the surrounding waters and after 19 months the Zn concentrations resemble those observed in surrounding HNLC waters. Lower Zn-binding ligand concentrations were observed where both the total dissolved Zn and chlorophyll *a* concentrations were low providing further evidence for uptake of these ligands by phytoplankton.

Graduate School of the Southampton Oceanography Centre

This Ph.D. dissertation by

Maeve Carroll Lohan

has been produced under the supervision of the following persons

Supervisors: Dr. Peter Statham
Dr. Duncan Purdie

Chair of Advisory Panel: Professor Bill Jenkins

Member of Advisory Panel: Dr Toby Tyrell

DECLARATION

This thesis is the result of work done by the author whilst in registered postgraduate candidature at the University of Southampton. The work is entirely original except where due reference is made and no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification at this or any other university or institute of learning.

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Glossary of Thesis Abbreviations

Abbreviation	Definition
CCM	Carbon Concentrating Mechanisms
HNLC	High Nutrient Low Chlorophyll
CA	Carbonic Anhydrase
AdCSV	Adsorptive Cathodic Stripping Voltammetry
IOS	Institute of Ocean Sciences
OSP	Ocean Station Papa
SBDW	Sub-Boiled Distilled Water
PVC	Poly Vinyl Chloride
UPLA	Ultra Low Penetration Aie
LDPE	Low Density Polyethylene
UHMW	Ultra High Molecular Weight
HEPA	High Efficiency Particle Air
APDC	Ammonium PyrrolidineDithioCarbamate
DDC	Diethylammonium DiethylDithioCarbamate
GFAAS	Graphite Furance Atomic Adsorption Spectroscopy
NASS	National Research Council Canada
HMDE	Hanging Drop Mercury Electrode
SMDE	Static Mercury Drop Electrode
PTFE	PolyTetraFluroEthylene
EDTA	EthyleneDiamine Tetra-Acetic acid
NTA	NitrilotriAcetic acid
IRMS	Istope Ratio Mass Spectrometer
CCAR	Colorado Centre for Astrodyamics Research
	Zinc speciation
C_{Zn}	Total dissolved Zn concentration
C_L	Zn-binding ligand concetration
Zn^{2+}	Free Zn ion concentration
Zn'	Labile inorganic Zn species

Chapter 1

Introduction

1.1 Introduction

Oceanic primary production is important as the biogeochemical properties of the biota and the evolution of global climate are inextricably linked (Lovelock, 1991). The oceans exert a major influence on climate and the properties of the surface ocean waters and the marine atmosphere are modified by the biochemical and optical properties of the marine organisms, in particular phytoplankton (Holligan, 1991). For example, marine phytoplankton strongly influence the air-sea exchange of carbon dioxide (CO_2) as CO_2 is incorporated into organic matter (OM) and calcium carbonate by phytoplankton (Holligan, 1991). Much of the organic matter is rapidly re-oxidised within the euphotic zone, but a small proportion (~10% of net primary production) is transferred to deep water and sediments thereby maintaining an atmosphere-to-deep water gradient in CO_2 concentrations, which represents the ocean carbon pump (Holligan, 1991). The flux of OM to deep water and sediments depletes surface CO_2 levels, leading to uptake from the atmosphere at least on the time scale of ocean ventilation (Sarmiento *et al.*, 1988).

1.2 The role of zinc in phytoplankton

Zinc has attracted considerable attention in marine biogeochemical studies due to its requirement as a micronutrient. During the last 30 years, it has been established that Zn is an integral component of numerous functional proteins (Vallee & Falchuk, 1993). Zinc is essential for phytoplankton growth as it is a cofactor of nearly 300 enzyme systems, such as alcohol dehydrogenase, carbonic anhydrase and carboxypeptidase, which are involved in nearly all aspects of metabolism (Anderson & Morel, 1978). These enzymes encompass the synthesis and /or degradation of all major metabolites (Vallee & Auld, 1990). Among the Zn enzymes there are oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Vallee & Falchuk, 1993). Zinc is also important in structural cross-linking and in the organisation of chromosomes (Barnes *et al.*, 1998). It is the most common catalytic metal ion in the cytoplasm, it is involved in digestive enzymes outside the cell or in vesicles but is rarely in contact with the cell membrane (Whitfield, 2001). In contrast, there are fewer Fe metalloproteins or enzymes (Vallee

& Falchuk, 1993) and Fe is established as a limiting parameter for phytoplankton growth. Zinc also has a role in exported hydrolytic enzymes that break down external organic debris (Whitfield, 2001).

In biological systems very little, if any, Zn is free in solution (Vallee & Falchuk, 1993). Zinc carries out its biochemical functions as a divalent cation primarily when bound to enzymes and other proteins. Under physiological conditions, Zn does not undergo reduction or oxidation (Butler, 1998). This lack of redox changes renders it stable in a biological medium. However, Zn^{2+} has a variable co-ordination sphere and the stereochemical adaptability to assume multiple co-ordination geometries and these unusual features contribute to its biochemical versatility. The co-ordination number of Zn^{2+} can vary from 2 to 8, although 4, 5 and 6 are the most frequently encountered in enzymatic and biological functions of Zn (Vallee & Falchuk, 1993). Zinc therefore represents a versatile metal centre for different donor groups of varying ligand types, resulting in a broad range of stability constants, mobilities, reactivities and function (Vallee & Falchuk, 1993). These chemical properties form the basis for the extensive participation of Zn in protein, nucleic acid, carbohydrate and lipid metabolism as well as in the control of gene transcription and other fundamental biological processes (Vallee & Falchuk, 1993).

Essential life processes such as the equilibrium of CO_2 and bicarbonate ion (HCO_3^-) are hydrolytic transformations that are catalysed by metalloenzymes containing active-site transition metal ions which do not undergo oxidation state changes, such as Zn, but which function as Lewis acid type catalysts (Butler, 1998). In phytoplankton, Zn has a particularly important role in carbonic anhydrase. Laboratory studies on carbon acquisition by phytoplankton have revealed Carbon Concentrating Mechanisms (CCMs) (Raven, 1990). CCMs involve the active uptake of HCO_3^- and/or CO_2 from the environment and generally require the enzyme carbonic anhydrase, which is a Zn metalloenzyme, to catalyse the conversion of these inorganic carbon species (Tortell *et al.*, 2000). Carbonic anhydrase is one of the most catalytically active enzymes known and catalyses the exchange between the gaseous CO_2 and carbonic acid, which is the rate limiting step in the transport of molecular CO_2 to the photosynthetic CO_2 fixing enzyme Ribulose-1,5 bisphosphate carboxylase/oxygenase (RubisCo) (Whitfield, 2001). This ubiquitous enzyme accelerates the reaction scheme:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \qquad \text{(Fast)}$$

$$H_2CO_3 \leftrightarrow CO_2 + H_2O \qquad \text{(Slow)}$$

A CCM appears to be essential for sustaining maximum phytoplankton growth rates over a wide range of CO_2 concentrations (Tortell *et al.*, 2000). Laboratory studies have revealed that at the extremely low Zn concentrations of open-ocean surface waters microalgae cannot produce sufficient functional carbonic anhydrase; this, in turn, may limit carbon acquisition and consequently, the rate of algal growth. This limitation may be particularly important for diatoms, which are responsible for fixing the bulk of carbon that is exported from surface waters to the deep ocean (Morel, *et al.*, 2000).

To gain an understanding of the interactions between trace metals and marine algal growth and productivity, several levels of complexity must be considered (Fig. 1.1). The first and foremost is the chemistry of trace metals in seawater, which controls their biological availability. Both total metal concentrations and chemical speciation are important and both exhibit wide spatial and temporal variations leading to large differences in availability.

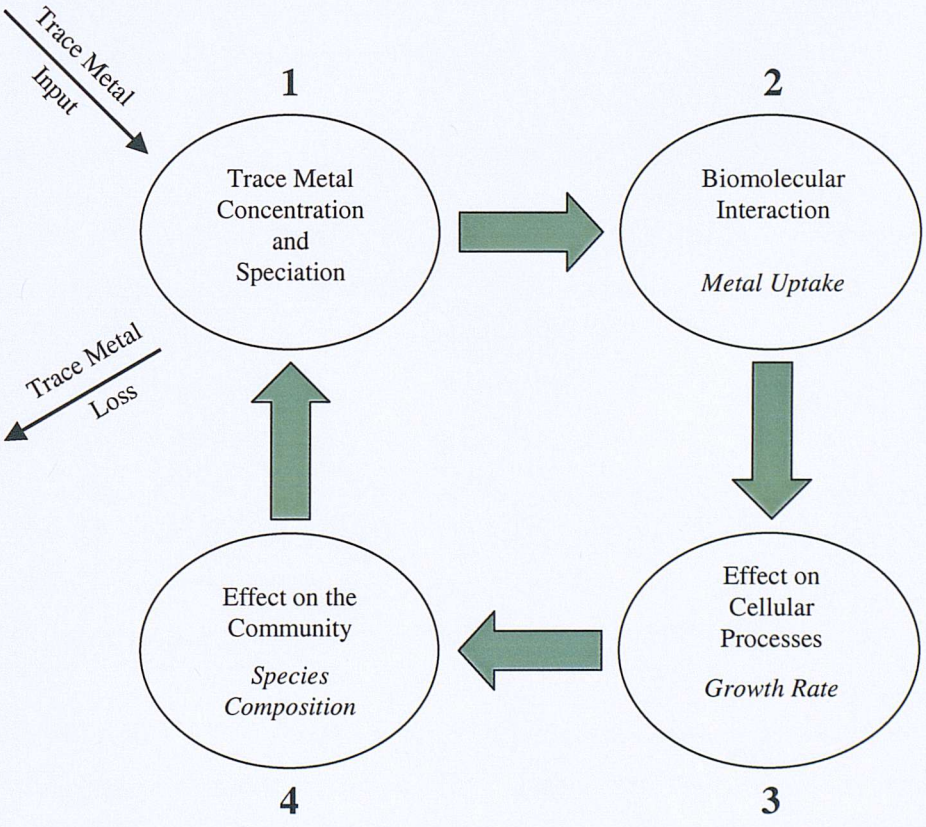


Figure 1.1 Conceptual diagram for the interaction of trace metals with marine algae. Modified from Sunda (1991).

1.3 The concentration and distribution of zinc in the oceans

Zinc is a trace element in seawater, with concentrations in the surface euphotic zone of oceanic waters ranging from 2.7 nM down to values as low as 70 pM in surface waters of High-Nitrate, Low Chlorophyll (HNLC) regions (Table 1.1). The nutrient-type distribution of dissolved Zn in seawater indicates that it is removed from surface waters and transported to depth as a trace constituent of biogenic particles (Bruland, 1989). Hudson & Morel (1993) state that the existence of stable oceanic trace nutrient profiles reflects the end result of competition among plankton for major and trace nutrients, as one or the other became limiting. Zinc distributions in the nutricline appear to be controlled by algal uptake and regeneration as supported by algal uptake models (Sunda & Huntsman, 1995a). Dissolved Zn concentrations are approximately five times greater in the old, nutrient-rich deep waters of the North Pacific than in the young, nutrient-poor North Atlantic deep waters (Bruland *et al.*, 1994; Table 1.1). Its distribution in ocean basins is governed by an internal cycle with rapid removal from surface waters coupled with extensive remineralisation at depth. The efficiency with which Zn is recycled in the ocean leads to its relatively long oceanic residence time (3000-6000yrs) (Bruland *et al.*, 1994).

1.3.1 Sources and sinks for metals in the oceanic euphotic zone

The surface water distributions of reactive trace metals such as Zn can be affected by a number of physical and chemical processes. These include vertical mixing with deep waters by advection (i.e. upwelling) and eddy diffusion (or convection), horizontal advection and diffusion, atmospheric fallout and particulate removal (Bruland, 1980). Vertical mixing and atmospheric fallout provide the dominant transport to oceanic surface waters while vertical particle flux is the major removal mechanism (Bruland, 1980). Due to the isolation of the open ocean from allochthonus sources, atmospheric deposition is the dominant source of dissolved Zn to the North Pacific. Within the subarctic Pacific, the supply of atmospheric Zn exhibits a strong longitudinal pattern with high fluxes in the west and low fluxes in the east (Duce & Tindale, 1991).

New production in the surface waters must be balanced by a particulate flux of organic material from the surface waters. An indirect estimate of the input of nutrients to surface waters via vertical mixing can therefore be obtained by using the particulate flux of phosphate leaving the surface zone and the rate of new production (Bruland, 1980). The change in metal:phosphate ratio observed in the upper part of the thermocline multiplied by the phosphate flux then provides an

estimate of the rate at which the various trace metals are brought to the surface by vertical mixing (Bruland, 1980). The supply of phosphate by vertical mixing can range from 0.1 mmol/cm^2/yr in oligotrophic waters to more than 100 times higher in coastal upwelling or neritic regions with corresponding variations in trace metal inputs (Bruland, 1980). Due to the relative importance of its atmospheric input, Zn may be enriched in the central gyre of North Pacific waters compared to the predicted concentration based on vertical mixing input alone (~0.07nmol/kg observed vs. ~0.02nmol/kg predicted) (Bruland, 1980).

The North Atlantic Ocean is characterised by both a large river input relative to the North Pacific Ocean and a higher atmospheric delivery rate of many trace metals (Duce & Tindale, 1991). This is particularly evident in shelf waters, where the average concentration of Zn is 2.4nM compared to a concentration of 0.34nM Zn in the upwelling zone off the coast of California (Bruland & Franks, 1983). Young deep waters of the Atlantic are markedly nutrient poor relative to older North Pacific deep waters (1.5 nM Zn in the Atlantic compared to 8-9 nM Zn in the Pacific). In the Indian Ocean deep water Zn concentrations (5-6 nM) are intermediate between those of the North Atlantic and North Pacific which reflects the age of deep water and the thermohaline circulation through the ocean basins (Morley *et al.*, 1993).

In contrast, nearshore and coastal environments have much higher concentrations of dissolved Zn due to the impact of adjacent landmasses. Surface water concentrations are an order of magnitude higher than those reported for the open North Atlantic Ocean. The North Sea has many adjacent estuaries and is therefore a mixing zone of high concentration continental sources of Zn with the lower oceanic endmember Zn concentrations (Nolting *et al.*, 1999). Tappin *et al.* (1995) reported high dissolved Zn concentrations in the southern North Sea and observed a clear seasonality in Zn concentrations, with remarkably higher concentrations in spring than autumn. Although Zn develops a nutrient-like profile in the stratified oceanic water column, the coupling of this cycle to the utilisation and regeneration of nutrients in the North Sea is not detectable (Burton *et al.*, 1993).

Table 1.1 shows the concentration of total dissolved Zn determined by various workers in different oceanic environments. Although there have been many studies on total dissolved Zn there is a distinct lack of studies on the speciation of Zn in oceanic environments due to the difficulty in carrying out these measurements.

Dissolved Zn Concentrations					
Ocean	Surface (0-50m) Total (nM)	[Zn^{2+}] (pM)	Inorganic (pM)	Deep (>1500m) Total (nM)	Reference
Pacific					
N.E.Pacific	0.06-0.08				Martin et al., 1989
N. Pacific gyre	0.10-0.30	1.8-3	3-8	9	Bruland et al., 1989
C. N. Pacific	0.15	2-14			Donat & Bruland 1990
C. N. Pacific	0.07			8	Bruland, 1980
California Upwelling	0.34			8	
Atlantic					
Sargasso Sea	0.06			1.7	Bruland & Franks 1983
W. N. Atlantic (Shelf)	2.4				
N. Atlantic	0.13-0.32	6.8-20			Elwood & Van den Berg 2000
Southern Ocean	0.2				Martin et al., 1990
	1.4-12.4				Nolting et al., 1994
	1.1-8.0				Loscher, 1990
Indian Ocean	0.4-0.6			6	Morley et al., 1993
North Sea	1.9-6.1				Nolting et al., 1999 Tappin et al., 1995
Mediterranean	2.7			4.7	Ruiz-Pino et al., 1991

Table 1.1 Concentrations of total dissolved zinc and zinc speciation in the oceans.

1.4 Zinc speciation

Evidence suggests that the distribution and speciation of trace metals in the upper water column plays an important role in the species composition and physiology of phytoplankton assemblages (Sunda, 1991). Trace metals exist in a variety of chemical species in seawater, strongly influencing their availability to marine algae (Sunda, 1991). Trace metals dissolved in seawater can exist in different forms: free hydrated ions, inorganic complexes and organic complexes. Knowledge of the various chemical species is extremely important as the different forms enter into very different biological and geochemical interactions (Bruland *et al.*, 1991). Trace metal uptake is usually controlled by the concentration of free aquated ions or kinetically labile inorganic species (Hudson & Morel, 1990).

Inorganic Zn is complexed to chlorides, carbonates, hydroxides and sulphates in surface waters. Temperature and pH may significantly alter the inorganic speciation of Zn in seawater (Byrne *et al.*, 1988). According to the inorganic speciation models of Byrne *et al.* (1988), the free Zn ion contributes the largest fraction (62 %) of the total inorganic Zn (at pH 8.2, 25 °C). However, as

pH and temperature decrease (e.g. as depth increases), the relative contribution of the free Zn ion to the inorganic forms increases to 72% (pH 7.6, 5°C).

In the upper water column, speciation of many biologically active trace metals is controlled by complexation with strong organic ligands (Bruland *et al.*, 1991). Organic complexation can markedly influence the availability of a metal to biota, reduce or eliminate its toxicity and effect its transport and cycling by decreasing or potentially increasing its adsorption onto suspended particles (Neubecker & Allen, 1983).

The speciation of dissolved Zn in the upper water column is dominated by the organically complexed fraction (Bruland, 1989). 98.7% of the total dissolved Zn in surface waters shallower than 200m is reported as complexed with a relatively Zn-specific organic ligand present at a concentration of ~1.2nM (Bruland, 1989). The concentration of this ligand appears relatively uniform and long-lived (Ellwood & van den Berg, 2000). This ligand exceeds the concentration of total dissolved Zn from the surface down to ~350m (Bruland *et al.*, 1991). The excess ligand concentration relative to that of dissolved Zn causes the high degree of organic complexation observed in the upper 300m (Bruland *et al.*, 1991). While little is known about the chemical structure of these ligands, the high degree of organic complexation reduces the amount of free metal ion and hence biological availability (Sunda & Huntsman, 1992). At depths greater than 500m the dissolved Zn concentration exceeds that of the ligand and organic complexation is relatively unimportant (Donat & Bruland, 1990). It has been hypothesised that these ligands are of biological origin and are either produced directly by phytoplankton or released indirectly by grazing and cell lysis (Bruland, 1989; Ellwood & Van den Berg, 2000; Muller *et al.*, 2003). However, the biological function of these Zn-binding organic ligands, if any, is unknown (Croot *et al.*, 2000; Whitfield, 2001).

The concentration of total dissolved Zn in surface waters of the Pacific Ocean is ~0.1nM and the high degree of organic complexation reduces the concentration of inorganic Zn species to ~2pM and free $[Zn^{2+}]$ to values as low as 1pM (Bruland *et al.*, 1991). The domination of dissolved Zn speciation by organic complexes may suggest a biological influence. Bruland (1989) speculated that organic complexation may help keep Zn in solution as opposed to being adsorbed onto particle surfaces. Another hypothesis is that some phytoplankton may be able to assimilate organically complexed Zn to the exclusion of other phytoplankton in a manner analogous to the siderophore-mediated uptake of Fe (Bruland *et al.*, 1991). Brand *et al.* (1983) examined the

limitation of marine phytoplankton reproductive rates by Zn, Mn and Fe and suggested that differences among species in their abilities to grow in the presence of low free ion concentrations could result in species shifts in phytoplankton communities subjected to changes in trace metal or organic complexation regimes.

1.5 Vertical distribution of zinc and its relationship with silica

The close correlation between the vertical distributions of specific trace metals and those of major nutrients provides evidence for the biological control of trace metals concentrations in the sea (Sunda, 1991). For a trace element such as Zn to have a distribution that is strongly correlated with one of the major nutrient elements requires that: (Bruland, 1980)

1. It is involved in similar internal cycles
2. It has an oceanic residence time that is long with respect to the time scale of ocean mixing i.e. at least 5000 years
3. That any deep water scavenging be insignificant compared to the time scale of ocean mixing.

The vertical distribution of Zn correlates most closely with that of silicic acid in oceanic water (Donat & Bruland, 1994). In the Pacific and Antarctic Oceans, Zn concentrations correlate with those of the nutrient Si ($r^2 = 0.99$) suggesting that Zn is taken up and regenerated similarly to Si (Bruland, 1980). In the Indian Ocean, Morley *et al.* (1993) observed a significant correlation between dissolved Zn and Si ($r^2=0.88$). Morley *et al.* (1993) concluded from their data that the cycling of Zn involves dissolution deeper in the water column than other trace metals such as Cd, and suggested that it is transported down the water column primarily with skeletal material rather than soft tissue. However, no such relationship is observed in Atlantic waters as they contain an excess of Zn relative to Si (Bruland & Franks, 1983; Martin *et al.*, 1993).

Investigations into the chemical composition of phytoplankton have revealed that Zn is incorporated into biogenic opal. However, the amount is less than would be predicted from deep Pacific Zn profiles (Ellwood & Hunter, 1999). The amount of Zn incorporated into the opal represented only 1-3% of the total Zn taken up by the diatom. Thus, most Zn within diatoms appears to be associated with their cellular organic tissue rather than with their exoskeleton (Collier & Edmond, 1984). Ellwood & Hunter (2000) demonstrated that Zn:Si increased in the frustule of a diatom, *Thalassiosira pseudonana,* with increasing free Zn^{2+} concentration and when

the free Zn^{2+} concentrations were low (~0.1 pM) the rate of uptake of Si decreased. Ellwood & Hunter (2000) also carried out a study on the dissolution of diatom frustules separated from marine sediments and demonstrated that Zn is released at the same rate as Si during dissolution, suggesting that Zn is evenly distributed throughout the opal structure. This also suggests that the Zn:Si ratio may be a useful indicator of oceanic free Zn^{2+} concentrations (Ellwood & Hunter, 2000).

1.6 Uptake of zinc by phytoplankton

The uptake of trace metals by phytoplankton has been modelled by rigorous thermodynamic and kinetic theory in which speciation chemistry can be varied systematically and quantified by chemical equilibrium or kinetic calculations (Hudson & Morel, 1990; Sunda, 1991; Hudson & Morel, 1993). Both short-term and long-term uptake of Zn by marine phytoplankton has been investigated using ^{65}Zn in culture experiments (Sunda & Huntsman, 1992; Hudson & Morel, 1993; Hutchins & Bruland, 1995; Sunda & Huntsman, 1995a). These studies rely on the addition of artificial chelators such as EDTA to maintain the $[Zn^{2+}]$ and concentration of inorganic Zn complexes within the cultures at the low levels characteristic of natural seawater. However, such experiments do not provide a true representation of the oceanic environment as EDTA affects the equilibria and kinetics of various natural chemical forms of trace metals (Gerringa *et al.*, 2000).

Uptake of all necessary trace metals by phytoplankton occurs via binding to a surface ligand and subsequent transfer across the cell membrane (Morel *et al.*, 1991). Metal transport into the cell is determined by the interplay between the metal speciation in the medium and ligand exchange reactions at specialised transport sites on the cell membrane (Sunda, 1991). Bi-layer membranes are virtually impermeable to charged or polar species and metal ions are usually taken up into cells by binding to specialised transport ligands associated with the plasmalema. The uptake of micronutrients, such as Zn, requires many transport ligands working far from saturation (Hudson & Morel, 1993). Zinc is required by phytoplankton intracellularly and as such, Zn must first form co-ordination complexes with specialised transport sites on the plasmalema. Following binding of a metal ion (M) to the transport ligand (L), the metal may either dissociate back into the medium or be transported across the membrane and transferred to ligands in the cytoplasm (Sunda, 1991). Once inside the cell, Zn affects cellular metabolism by forming co-ordination complexes with various biomolecules, including numerous enzymes that require Zn as essential cofactors (Sunda, 1991).

The transport of metal ions into the cell should follow the classical saturation kinetics equation for facilitated or active transport, which has been observed experimentally in phytoplankton for the uptake of Zn (Sunda & Huntsman, 1992). Kinetically, the simplest scheme representing this uptake is:

$$M + L \longrightarrow ML \qquad (1)$$

$$ML \xrightarrow[k_{in}]{} M_{cell} \qquad (2)$$

Where M is the metal ion in the medium to be taken up by cells via the cellular ligand L and k_{in} is the constant for the transport of the metal across the membrane and subsequent transfer to the cytoplasm (Sunda, 1991). As in Michaelis-Menton enzyme kinetics, the steady state assumption for ML concentration is given by:

$$\frac{d[ML]}{dt} = k_f [M]\ [L] - (k_d + k_{in})\ [ML] = 0 \qquad (3)$$

$$[ML]_{ss} = \frac{k_f}{k_d + k_{in}}\ [M]\ [L] = K_s^{-1}\ [M]\ [L] \qquad (4)$$

$$K_s = \frac{k_d + k_{in}}{k_f} \quad \text{(half-saturation constant)} \qquad (5)$$

Where k_f, is the kinetic rate constant for formation of the metal ion transport ligand complex and k_d is the rate constant for the dissociation of the metal from the complex back into the medium. K_s is defined by equation 5, and is the half-saturated constant, equal to the metal ion concentration at which half of the transport molecules are bound.

Since the total ligand concentration (L_T) is given by:

$$L_T = [ML] + [L] \qquad (6)$$

Equation 4 can be rewritten as:

$$[ML]_{ss} = \frac{L_T[M]}{K_s + [M]} \quad (7)$$

From equation 2, the cellular metal uptake rate (V) is given by:

$$V = k_{in}\,[ML]_{ss} \quad (8)$$

From equation 7, when $[M] >> K_s$, $[ML]_{ss} = L_T$ and $V = V_{max}$. That is:

$$V_{max} = k_{in}L_T \quad (9)$$

Where V_{max} is the maximum rate achieved when the transport ligands are fully saturated. Substituting equation 7 and 9 into equation 8 gives the uptake rate of metal ion into the cell:

$$V = \frac{V_{max}[M]}{K_s + [M]} \quad (10)$$

Under usual circumstances, trace metal uptake ligands are far from saturated in natural waters; most of the ligands are free and available to react with essential metals in seawater ($[M] << K_s$). Equation 10 then simplifies to:

$$V = \frac{V_{max}[M]}{K_s} \quad (11)$$

and the uptake rate is then a function of the metal concentration.

As surface seawater is essentially a constant ionic medium at a stable pH, the concentration of Zn^{2+} and the concentration of kinetically labile inorganic species are related to each other by constant ratios (Sunda, 1991). For nutrient ions such as Zn^{2+}, free metal ions represent more than half of the kinetically labile inorganic species (Byrne *et al.*, 1988).

As discussed earlier, the concentration of Zn in oceanic surface waters is low, which implies that a large number of transport ligands are important. At steady state, the complex formation rate must at least equal the uptake rate (Hudson & Morel, 1993). Since the complexation rate is proportional to the inorganic metal concentration [M′] and the total free transport ligands [L′], a low [M′] necessitates a high [L′] to achieve an uptake sufficient for cellular requirement (Hudson & Morel, 1993). For Zn the combination of slow reaction kinetics and low metal concentrations requires the cellular concentration of free transport ligands be comparable to the cellular concentration of the metal (Hudson & Morel, 1993).

Uptake rates can be increased as long as the cell can synthesise more surface ligands. There is however, a physical limit to this due to the development of a diffusive boundary around the cell (Hudson & Morel, 1993). Hudson & Morel (1990) have shown that an organism increases its transport system to the point where diffusion limitation begins. Zn requires enough ligands (2-5 x 10^{-18}mol cell^{-1}) so that these ligand may cause crowding even under kinetic control (Hudson & Morel, 1990). Zn concentrations in the surface ocean occur at values where diffusion limitation is of concern. Oceanic species have a lower requirement for Zn than neritic species where the concentration of Zn is higher (Sunda, 1991). Zn uptake appears to be most strongly influenced by diffusion limitation since uptake rates reach a significant fraction of the diffusion limited rates and the growth of the diatoms becomes limited at concentrations near the calculated diffusion limit (Hudson & Morel, 1993).

Organism size is an important factor in controlling diffusion limited uptake rates. Oceanic Zn concentrations may be low enough that diffusion limits growth rates of cells less than 4 μm in radius. This may explain the dominance of smaller phytoplankton (pico- and nanoplankton) within oceanic ecosystems (Hudson & Morel, 1993) such as that observed in the subarctic North Pacific (Boyd et al., 1996).

1.6.1 Cellular zinc uptake from culture experiments

Culture experiments have shown that relationship between cellular Zn uptake rate and [Zn^{2+}] has a sigmoidal shape which indicates two separate uptake systems for Zn with widely different stability constants (Sunda & Huntsman, 1998).

Short-term Zn uptake rate experiments with an oceanic species, *Emiliania huxleyi* have indicted that cellular Zn is actively regulated by an inducible high-affinity uptake system, the capacity for Zn uptake increases as $[Zn^{2+}]$ in the medium is decreased (Sunda & Huntsman, 1995a). At $[Zn^{2+}]$ below the half-saturation constant (0.25 nM), nearly constant uptake rates are maintained by negative feedback regulation of the transport capacity. However, at $[Zn^{2+}]$ above these values, constant uptake rates are primarily related to saturation of the uptake system (Sunda & Huntsman, 1995a). Once $[Zn^{2+}]$ falls below 10 pM, regulation of cellular Zn transport is no longer possible as Zn uptake approaches the physical limits imposed by diffusion of kinetically labile inorganic species (Zn^{2+}, ZnCl and $ZnCO_3$) to the cell surface (Sunda & Huntsman, 1992; Hudson & Morel, 1993). In cultures of *E. huxleyi* the concentration at which Zn^{2+} becomes diffusion limited (39 pM) is higher than observed for diatoms (5 pM) (Sunda & Huntsman, 2000). At low $[Zn^{2+}]$ (3.9 pM), the smallest species *E. huxleyi* (3.3 μm cell diameter) had a 16 fold higher uptake rate per unit of cell volume than the larger species *Thalassiosira weissflogii* (12 μm cell diameter) (Sunda & Huntsman, 2000).

Culture experiments on a coastal diatom, *T. pseudonana* also showed that at low free Zn^{2+} concentrations, the rate of Zn uptake is limited by diffusion (Ellwood & Hunter, 2000). At higher Zn^{2+} concentrations *T. pseudonana* is able to regulate the uptake of Zn and thus the rate becomes relatively constant at 2-5 x 10^{-17}mol Zn $cell^{-1}$ day^{-1} (Ellwood & Hunter, 2000). When the concentrations of free Zn^{2+} were increased above 10^{-9}M, the uptake of Zn increased as a secondary Zn uptake system became dominant (Sunda & Huntsman, 1992).

Hutchins & Bruland (1995) designed experiments to measure both the extracellular and intracellular transfer of ^{59}Fe, ^{65}Zn and ^{54}Mn in different size fractionated phytoplankton. Net accumulation of ^{65}Zn was observed in large cells. Extracellular transport revealed that ^{65}Zn and ^{59}Fe were transferred with equal efficiency with 74% of ^{65}Zn found in the large size fraction. Extracellular ^{65}Zn was far more efficiently transferred to the large size class than intracellular Zn (Hutchins & Bruland, 1995).

1.7 Trace metal interactions

Trace metal interactions will affect different members of the phytoplankton community in different ways leading to potentially large changes in productivity and species dominance (Bruland *et al.*, 1991). Hudson & Morel (1985) hypothesised that a combination of

macronutrients and trace metals, some of which are essential while others are toxic, may be simultaneously controlling biological production in the oceans. At the same time, biological processes may largely control cycling of these elements. This feedback system between the biological and chemical systems of the ocean may be very important in controlling and maintaining productivity and influencing species composition in high-nutrient areas of the open ocean (Bruland *et al.*, 1991).

1.7.1 Substitutions for zinc in phytoplankton

Substitution of Zn by other metals or use of alternate biochemical pathways must occur as oceanic species are capable of growing maximally at concentrations of Zn well below those which limit coastal species (Brand *et al.*, 1983) and at diffusion limited rates (Hudson & Morel, 1993). Previous experiments have shown that oceanic phytoplankton have adapted to low levels of Zn by reducing their cellular requirement for Zn (Brand *et al.*, 1983; Sunda & Huntsman, 1992; Sunda & Huntsman, 1995a). The mechanism involved remains unknown but one possibility is the replacement of Zn in enzymatic sites by other chemically similar metals (Sunda & Huntsman, 1995a). Culture experiments carried out by Price & Morel (1990) have demonstrated that both Co and Cd can substitute for Zn in *T. weissflogii*. The growth-promoting properties of Co and Cd are only evident when Zn is absent from the culture medium and the distributions of Co, Cd and Zn among the cellular constituents is remarkably similar (Price & Morel, 1990). There is however, a difference in the growth efficiency of the two metals, Cd stimulates the growth at 90% while Co can only maintain 60% of maximum growth rate (Price & Morel, 1990).

Sunda & Huntsman (2000) have demonstrated using a culture of *Thalassiosira oceanica* that both Cd and Co uptake rates were similar when the $[Zn^{2+}]$ was decreased from 31 to 3.1 pM, where cellular Zn was depleted toward growth-limiting values. This was also observed in *T. pseudonana* (Sunda & Huntsman, 1998) and suggests that both metals are taken up by a common inducible transport system that is under negative feedback regulation by cellular Zn (Sunda & Huntsman, 2000). In cultures of *E. huxleyi*, taking a linear regression of log cell Cd uptake rate vs. log $[Zn^{2+}]$ within the $[Zn^{2+}]$ range of 39 pM – 5 nM yielded a slope of –1.11 ($r^2 = 0.999$) close to the theoretically predicted slope of negative unity. A similar observation was made for Co over a similar $[Zn^{2+}]$ range of 19 pM- 6.3 nM (Sunda & Huntsman, 2000).

Yee & Morel (1996) studied Carbonic Anhydrase (CA) using gel electrophoresis and have shown that Co and Cd appear in different bands indicating that they substitute in different CA isoforms. This has been used to explain the difference in growth responses of the two metals, as Cd is able to fully restore growth in Zn-limited cells. The difference in function of Co-CA and Cd-CA may arise from differences in the locations of the enzymes (internal and external to the cell) (Yee & Morel, 1996).

Cobalt

Surface water concentrations of Co are low and highly variable in the North Pacific (Martin *et al.*, 1989) and regions where Co depletions occur in surface waters coincide with areas where Zn concentrations are very low (Sunda & Huntsman, 1995a). Depletion of Co from surface seawater seems to be chiefly determined by both low Zn and CO_2 concentrations (Yee & Morel, 1996). The only known absolute requirement for Co in eukaryotic phytoplankton is as a cofactor in vitamin B_{12} (da Silva & Williams, 1991). Recent studies have suggested that in cyanobacteria Co is essential for carbonic anhydrase activity (Saito *et al.*, 2002). The addition of Co in Zn-limited cultures of oceanic diatoms results in partial restoration of growth (Yee & Morel, 1996). However, not all diatom species behave in the same way and recent studies have shown that there is no apparent substitution of Co for Zn in *Chaetoceros calcitrans* (Timmermans *et al.*, 2001). The affinity for Zn in *T. weissflogii* CA is much higher than for Co, which indicates why adding Co improves, but does not eliminate, the growth limitation caused by low Zn concentration (Yee & Morel, 1996). Cobalt replacement of Zn could provide an adaptive strategy for growth in the open ocean (Sunda & Huntsman, 1995a). However, the low concentrations of Co relative to Zn in the subarctic North Pacific (Martin *et al.*, 1989) should limit the usefulness of this strategy.

Culture experiments with elevated concentrations of Zn have revealed that Zn toxicity is related to an induced Co deficiency brought on by suppression of Co uptake by elevated $[Zn^{2+}]$, due to competition between the two metals for internal metabolic sites (Sunda & Huntsman, 1995a).

Cadmium

The distribution of Cd in the oceans is similar to the algal nutrient phosphate below the surface layers (Boyle, 1976). The biological reason for this was unknown as unlike other surface depleted trace metals Cd is not required by organisms and was considered toxic at high levels (Price &

Morel, 1990). Cullen *et al.* (1999) proposed that biological removal of Cd is related to its utilisation in CA and is regulated by dissolved CO_2 and Zn concentrations. Total Zn concentrations in surface waters of the ocean are generally an order of magnitude higher than Cd, however, Zn is more extensively complexed by strong organic chelators than Cd (Bruland, 1989).

Previous studies on the marine diatom *T. weissflogii* have demonstrated that Cd can act as an algal nutrient under conditions of Zn limitation by replacing Zn in certain macromolecules (Price & Morel, 1990; Lee & Morel, 1995). Inorganic Cd restores the activity of CA in Zn-limited cells (Lee *et al.*, 1995) but other algal species studied revealed that the beneficial role of Cd is only observed over a narrow range of inorganic Cd and Zn concentrations and this can depend on the species studied (Lee & Morel, 1995).

In *E. huxleyi*, the $[Zn^{2+}]$-dependent relationships for Cd and Zn uptake are consistent with both metals being taken up by a single inducible transport system that is under negative feedback regulation by cellular Zn (Sunda & Huntsman, 2000). At $[Zn^{2+}]$ below $10^{-10.4}$M, Zn-uptake rate is proportional to $[Zn^{2+}]$. However, at higher $[Zn^{2+}]$, Zn uptake rates are constant and independent of changes in ionic Zn (Sunda & Huntsman, 2000). Within the region of constant Zn uptake rate ($>10^{-10.4}$M), the uptake rates of other metals transported by the same system should be inversely proportional to $[Zn^{2+}]$ which occurs for the uptake of Cd in *E. huxleyi* (Sunda & Huntsman, 2000).

Cd could be toxic to *T. weissflogii* at the concentrations measured in the open ocean if the concentration of Zn is low enough (Lee *et al.*, 1995). Although Cd is toxic to phytoplankton, the inactivation of Zn enzymes by Cd/Zn substitution under conditions of severe Zn limitation appears to reduce Cd toxicity and allows uptake of Cd to occur (Lee & Morel, 1995). Production of metal-binding polypeptides by phytoplankton plays an important role in the ability of Cd to replace Zn (Lee & Morel, 1995). Ahner *et al.* (1998) demonstrated that decreasing $[Zn^{2+}]$ enhanced cellular metal-binding polypeptide concentrations and this enhancement has been linked to Cd present in the medium.

1.8 Study area

The subarctic North Pacific was chosen as the research area for this study. The subarctic North Pacific, equatorial Pacific and the Southern Ocean are three large oceanographic regions, where high concentrations of the major nutrients and low chlorophyll concentrations persist in the

surface layer throughout the year. High Nutrient Low Chlorophyll (HNLC) regions are characterised by low stable phytoplankton stocks which rarely deplete nitrate, phosphate or silicic acid in the upper water column to growth-limiting levels (Strom *et al.*, 2000). In these areas primary production and chlorophyll levels are lower than might be expected given the high ambient nutrient concentrations in surface waters.

In the subarctic Pacific average chlorophyll *a* concentrations are $0.5\mu gL^{-1}$ and the highest concentration recorded over 6 spring cruises at Ocean Station Papa (OSP) was $\sim 1\mu gL^{-1}$ in May 1984 which does not correspond to a typical oceanic phytoplankton bloom (Welschmeyer *et al.*, 1991). Seasonal variation in the rates of primary production with little variation in chlorophyll concentrations suggests that there are concurrent variations in C flux or grazing pressure that maintain the constant low phytoplankton biomass (Coale, 1991). In the surface waters of the subarctic Pacific the concentrations of NO_3^- are $\geq 6\mu M$, PO_4^{3-} $\geq 0.2\mu M$ and Si $\geq 14\mu M$ year round (Miller *et al.*, 1991). Despite the high ambient nutrient concentrations, measured nutrient assimilation rates suggest that phytoplankton from this area are nutrient stressed and therefore not growing at their maximum rate (Falkowski, 1980).

Iron availability is known to be limiting phytoplankton growth in HNLC areas (Martin & Gordon, 1988; Martin *et al.*, 1990; Johnson *et al.*, 1997). Other micronutrients such as Zn have also been hypothesised to limit phytoplankton growth in these regions (Morel *et al.*, 1991; Morel *et al.*, 1994). The subarctic North East Pacific is an ideal region to study total dissolved Zn concentrations and Zn speciation and thereby examine the impact of Zn speciation on phytoplankton communities. Only one previous study has reported total dissolved Zn concentrations for this region (Martin *et al.*, 1989) and no previous studies have investigated Zn speciation in the subarctic North East Pacific.

1.9 Research objectives

The main aim of this research was the investigation of both total dissolved Zn concentrations and Zn speciation in the subarctic North Pacific. Trace metal speciation has become a subject of increasing interest in recent years as different trace metal species exhibit different biogeochemical behaviour and are involved in biological cycles. The more specific objectives of this project were firstly to establish a technique for measuring low concentrations of total dissolved Zn (40 pM) and for measuring the speciation of Zn in order to determine the concentration of bioavailable Zn

in seawater. A second objective was to investigate the concentrations of total dissolved Zn in both winter and summer in the subarctic North Pacific and examine changes with respect to silicic acid concentrations.

The complexation of Zn in the subarctic North Pacific was investigated to gain an understanding of the chemical species of Zn in this region. Once the bioavailable concentration of Zn was known, incubation experiments using water from the study area, subarctic North East Pacific were designed to investigate if the natural phytoplankton assemblage at OSP are limited by Zn availability.

Total dissolved Zn concentrations and Zn speciation within mesoscale eddies were also investigated to examine the impact and fate of Zn on the time scale of one to two years for which these eddies persist. Mesoscale eddies have the potential to transport trace metals from coastal water out into the open ocean and this process was also investigated.

Chapter 2

Experimental Procedures

2.1 Introduction

As can be seen from Table 1.1, there are very few measurements of total dissolved Zn in surface oceanic waters due to the difficulty of obtaining uncontaminated samples. As Zn is a ubiquitous contaminant, accurate data can only be obtained using strict precautions during sampling and analytical procedures. The surface waters of the subarctic North East Pacific Ocean are known for their low concentrations of Zn although this data is restricted to only a few key stations within this region (Bruland *et al.*, 1978; Martin *et al.*, 1989).

Analytical methods for measuring trace metals in seawater have improved significantly over the past two decades, contributing to important advances in our understanding of oceanic biogeochemistry. The development of electrochemical speciation methods has led to the idea that biogenic ligands may in fact be controlling the residence time of trace metals in surface waters (Johnson *et al.*, 1997).

This section will cover the critical issue of the need to use clean procedures during sampling and the subsequent handling of the samples together with the analysis of Zn in samples. Two techniques were used for total Zn measurements, graphite furnace and adsorptive Cathodic Stripping Voltammetry (AdCSV). As organic speciation of trace metals in seawater appears critical to understanding its interaction with phytoplankton (Rue & Bruland, 1997) a competitive ligand AdCSV was also used to determine Zn speciation in seawater and is described here. Electrochemical methods are very suitable for the study of speciation because of their high sensitivity and the fact that the electrochemical response is species specific.

2.2 Aspects of contamination control

Trace metal analysis of environmental samples has shown improvements in both sample handling procedures and the performance of electronic and optical analytical systems since the 1970s (Howard & Statham, 1997). With these improvements, detection limits have dropped thus

allowing low and accurate trace metal concentrations in open ocean environments to be obtained. Therefore, in this work rigorous contamination control procedures were used throughout the sampling and analytical procedures with minimum exposure to potential contamination sources.

The two most common forms of contamination that can occur during the collection of environmental samples are adventitious and systematic. Adventitious contamination can be caused by, e.g., boats used in the collection of samples, the collection vessel itself and improper handling of samples in the laboratory. This type of contamination is often indicated by data that has random errors. Systematic contamination is often caused by the use of impure reagents in the analysis of samples and is indicated by errors that are non-random in nature (Howard & Statham, 1997).

In this study all laboratory apparatus contacting the samples was thoroughly cleaned using the following protocol:

(i) A detergent wash for one week (2% v/v Micro (International products Corporation, Burlington NJ))
(ii) A Milli-Q (Q) water rinse followed by a soak for one week in 50% (v/v) HCl
(iii) A Q-water rinse followed by a soak for one week in 50% (v/v) HNO_3
(iv) A further Q-water rinse and a rinse in Sub-Boiled Distilled Water (SBDW) and then air-dried in the clean room suite/class 100 laminar flow bench.

Some sample equipment such as pipette tips were stored in 10% (v/v) Sub-Boiled Distilled nitric acid and rinsed in SBDW before use. The quartz UV digestion tubes were washed in detergent (2% Micro) for 1 week, followed by a two-step wash in HCl (1 M) and rinsed with SBDW. Both the 0.4 μm polycarbonate membrane filters (Nuclepore) and the Opticap filters (Millipore) used for the filtration of seawater were soaked for one week in 2N HCl and then stored in 2% (v/v) Ultrapure HNO_3 solution. Before use, they were rinsed in Q-water and seawater. The Go-Flo (General Oceanics) bottles were pre-cleaned in the laboratory by sequential soaking with 5% Extran (VWR Canlab) for 1 day and 0.1% HCl for 5 days. Once at sea the bottles were filled with open ocean water for a 1-day soak prior to use. All critical handling steps onboard ship and onshore were carried out in either a Class-100 laminar flow hood or in a clean room.

2.3 Sample collection and treatment

The Institute of Ocean Sciences (IOS) on Vancouver Island, Canada managed a time series at Ocean Station Papa (OSP), which is one of the longest records of oceanographic data and involves three research cruises a year along line P (Fig 2.1). Occasionally another line Z is also sampled as part of the line P program (Fig 2.1).

Samples for total dissolved Zn analysis were collected from the subarctic North East Pacific during two cruises on the C.S.S. John P. Tully in February (cruise no. 1999-01) and in August/September (referred to as September) 1999 (cruise no. 1999-21). In February, stations P4 to Ocean Station Papa (OSP) (P26) (Fig. 2.1) were sampled at depths of 10m, 25m, 40m, 100m, 200m, and 400m (stations sampled 9^{th} - 23^{rd} of February 1999). Both the line P and line Z transects were sampled in September (Line P sampled 25^{th} August - 1^{st} September and Line Z 3^{rd} - 8^{th} September 1999). Due to time constraints and the number of stations sampled, the sampling frequency in September was reduced and samples were collected at depths of 10m, 40m, 100m and 200m.

In August of 2001 samples for total dissolved Zn analysis were collected along Line P aboard the C.C.S John Tully (cruise no. 2001-29). Only stations P4 to P16 (stations sampled 22^{nd} – 30^{th} of August) (Fig. 2.1) were sampled at the depths of 0m, 10m, 25m, 40m, 75m, 100m, 200m and 400m. Samples were also collected for Zn speciation from 10m, 40m, 75m, 100m and 400m and analysis was carried out on-board the C.C.S John Tully within an hour of sampling.

For depths ≤ 40m water was pumped onboard, via a trace metal clean pneumatically driven Teflon pump and Teflon tubing, and filtered directly through in-line 0.4 μm acid cleaned polycarbonate membrane filters. These samples were collected in an on deck Poly Vinyl Chloride (PVC) Ultra Low Penetration Air (ULPA) clean hood. The filtered samples were placed in a 0.5 or 1 L Low-Density acid cleaned PolyEthylene (LDPE) bottle, doubled bagged and stored in a cold room. Open ocean samples from ≥ 40m were collected with acid-cleaned 30L Teflon-coated Go-Flo bottles attached to a Kevlar® line and closed with Teflon messengers (Bruland & Franks, 1979). A plastic (Ultra High Molecular Weight (UHMW) polyethylene) spooler wheel and rollers were used to feed out the Kevlar® to an Electro-polished stainless steel wheel (freshly coated with fibreglass resin). Lead weights encased in epoxy resin were attached to the end of the Kevlar® and separated from the samplers by 30 to 35m.

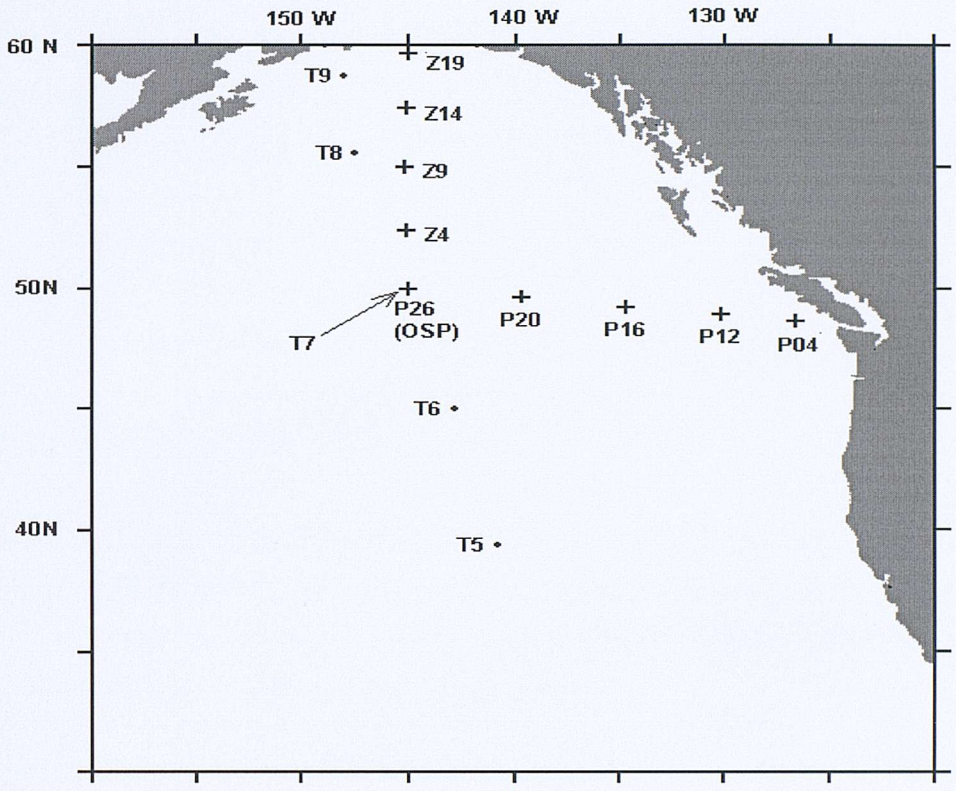


Figure 2.1. Sampling stations along Line P and Line Z from this study and VERTEX stations T5-T9 from Martin *et al.* (1989).

The Go-Flo bottles were sub-sampled on deck using boxes with extended sides and roof to minimise air disturbances and contamination. A Teflon tube was attached to the Teflon valve on the Go-Flo and attached to the end of the Teflon tube was a bell jar, which covered the sample bottle during sub-sampling. To avoid potential contamination of the clean 30L Go-Flo bottles with higher Zn concentration waters, the shelf station samples (Station P4) were collected with separate acid-cleaned 10L Teflon-coated Go-Flo bottles. Due to adverse weather conditions, no depths greater than 40m could be sampled at P16 in February 1999. Upon recovery, seawater in the Go-Flo samplers were filtered under gravity through 0.4μm acid cleaned polycarbonate membrane filters. The filtered samples were collected into acid cleaned 0.5 or 1 L LDPE bottles.

Samples were stored as described above. All samples for total dissolved Zn analysis were acidified to a pH of 2 with SBD-HCl prior to rebagging and storage in sealed plastic. In August 2001 all samples were filtered through an acid cleaned 0.2 μm Opticap cartridge (Millipore).

At sea, 'a clean area' was constructed in the main laboratory area using plastic sheeting and one High Efficiency Particle Air (HEPA) (Class –100) to provide a positive pressure filtered air supply. This area was utilised for the Zn speciation analysis.

2.4 Analytical procedures

2.4.1 Preparation of reagents

Sub-boiled distilled water (SBDW)

Milli-Q water was poured into a quartz flask and heated with two infra-red lamps. The water evaporates, condenses on a quartz cold finger and then drips through a collection tube and into a clean Teflon bottle. To reduce any possible contamination problems this was carried out in a clean room environment under a laminar flow hood.

Sub-boiled distilled acids

Aristar grade hydrochloric (HCl) and nitric (HNO_3) acids were purified in a similar to SBDW.

Isothermally distilled ammonia solution (ID-NH_4OH)

A Fluorinated Ethylene Polypropylene (FEP) beaker containing SBDW was placed in an airtight container with a beaker of analytical grade ammonia and left for two days in a laminar flow hood. During this time the ammonia partitions between the SBDW and the ammonia solution and forms ID-NH_4OH. This was then decanted into a FEP bottle, capped and stored until use.

Chloroform

Ultra-pure trace metal clean chloroform (Fisher) was used for solvent extractions. No Zn in the blank was detected and therefore no cleaning stages were required.

Mixed complexant

The mixed complexant used in the extraction of metals from seawater samples was prepared using a 2% (w/v) analytical grade Ammonium PyrrolidineDithioCarbamate (APDC) (Sigma) and 2% (w/v) Diethylammonium DiethylDithioCarbamate (DDDC) (Sigma) in SBDW. The solution was filtered through a Whatman #1 filter paper, the filtrate was collected and poured into a FEP separatory funnel. 5ml of chloroform was added and the funnel rotated for 6 minutes, the chloroform was then discarded. This process was repeated three times to ensure complete removal of metals. The complexant was stored in a Teflon bottle, refrigerated and used within 4 days.

Complexant for AdCSV

A 0.01M stock solution of APDC (Sigma) was prepared every four days in a 1% (v/v) ID-NH_4OH and was cleaned by extraction with two 2-ml aliquots of trace metal clean chloroform (Donat & Bruland, 1990).

Borate buffer

A 1.5M stock borate solution was prepared by dissolving boric acid in a 0.4M NaOH solution and the buffer was cleaned by passing it through a chelex-100 column followed by UV digestion (Ellwood & Van den Berg, 2000). Addition of 60µl of buffer to 10ml of seawater gave a pH of 8.2.

Standards

Zinc standards were prepared in 0.05% SBD-HCl by serial dilution of a 1000µg/ml standard (BDH).

A mixed stock standard of Co, Cd, Zn and Fe was prepared in 0.05% SBD-HCl by serial dilution of a 1000µg/ml standard (BDH). On a daily basis, working stock standards were prepared to a concentration of 10 µg/l.

2.4.2 Solvent extraction

In open ocean waters, many trace metals occur at low concentrations (~10^{-9} M) and these concentrations are below the detection limit of numerous techniques and therefore a preconcentration step is required before analysis. This preconcentration step isolates the metal from the matrix and thereby enhances the selectivity of the analysis and also increases the concentration by 1-2 orders of magnitude. The solvent-extraction procedure is based on the formation of metal-dithiocarbamate complexes. This technique involves chelation with APDC and DDDC, a triple extraction into chloroform and a back-extraction into nitric acid (Fig. 2.2). This technique is similar to that of Bruland & Franks (1979) but modified with a higher complexant concentration to allow the simultaneous additional extraction of Mn (Statham, 1985).

Solvent extractions were carried out in a Class 100 clean room at the Southampton Oceanography Centre (SOC). A mechanical rotating table was used for the extraction step as described in Statham (1985) with some modifications and upgrades.

Prior to any extractions four 500ml FEP separating funnels were cleaned internally using a rinse step, which involves 4ml of complexant and 3ml of chloroform being rotated for 5 minutes. The wash from this step was discarded and this rinse was repeated between each sample to prevent sample carryover.

A 200ml sample of seawater was placed in a FEP separatory funnel. As the seawater samples were acidified it was necessary to adjust the pH to the optimum range for the trace metal complexation (8-8.5). A 20ml sub-sample of seawater was removed, and the volume of ID-NH_4OH to be added to achieve optimum pH of 7.8-8.2 determined. The amount of ID-NH_4OH required to neutralise the samples was then calculated for 200ml samples. Operational blanks of SBDW do not require a pH change and therefore no ID-NH_4OH was added. The funnels were shaken to ensure total pH equilibrium. Complexant (3ml) and chloroform (3ml) were added to the sample and the samples rotated for 6 minutes. The funnels were then left for 5 minutes for the aqueous layers to separate. The chloroform layer was drawn off into a screw top 15ml taper bottomed FEP pots (Savillex) and care was taken to ensure there was no transfer of the seawater. A further 2ml of chloroform was added to the sample and the metal complexes removed, and this procedure was repeated twice more. In all 3 extractions were carried out.

The chloroform extracts were subjected to a back extraction process to remove the metal ions from the solvent and leave them in an acidic solution in which they are more stable and provided a better matrix for the Atomic Adsorption Spectroscopy (AAS) step. Back extraction was performed by adding 100µl of concentrated SBD-nitric acid to the vials containing the solvent phase. The pots were then placed on a hot plate (50-55 °C) in a clean wet station and allowed to evaporate down to a dry pellet. Another addition of 100µl of concentrated nitric acid was added to the pellet and allowed to evaporate once more. A final addition of 100µl of concentrated nitric acid was added followed by 400µl of SBDW. This solution was then transferred into a 2 ml vial and a further 500µl of SBDW was added to the FEP pot to rinse the inner surfaces and then transferred into the vial making a final volume of 1ml, and producing a concentration factor of 200 relative to seawater. The vials were labelled and closed, placed in a tray, bagged and stored in a refrigerator to reduce the potential for evaporative loss and contamination. All sample extracts were analysed for their metal content within a week of completing the solvent extraction procedure. This procedure is show diagrammatically in Figure 2.2.

Four blanks were analysed at the beginning and end of each day. Blanks were determined using SBDW, mixed complexant and chloroform. The limit of detection for the procedure is calculated as three times the standard deviation value for the blank. To ensure data quality, analysis of certified reference material NASS 5 (National Research Council, Canada) was carried out in a similar manner to the seawater samples. A summary of data for the quality control programme for this study is shown in Table 2.1.

Element	NASS-5 Certified Value (µg/L)	NASS-5 Measured Concentration (µg/L)	Blank Data (nM)	Detection Limit (nM)
Iron (Fe)	0.207 ± 0.035	0.206 ± 0.02	0.008 ± 0.004	0.024
Cadmium (Cd)	0.023 ± 0.003	0.025 ± 0.01	0.009 ± 0.003	0.027
Zinc (Zn)	0.102 ± 0.039	0.106 ± 0.03	0.008 ± 0.002	0.024

Table 2.1. NASS-5 certified values, measured concentrations, blank data and detection limits (3 times the standard deviation of the blank) for the determination of trace metals in seawater (n=4, ± standard deviation).

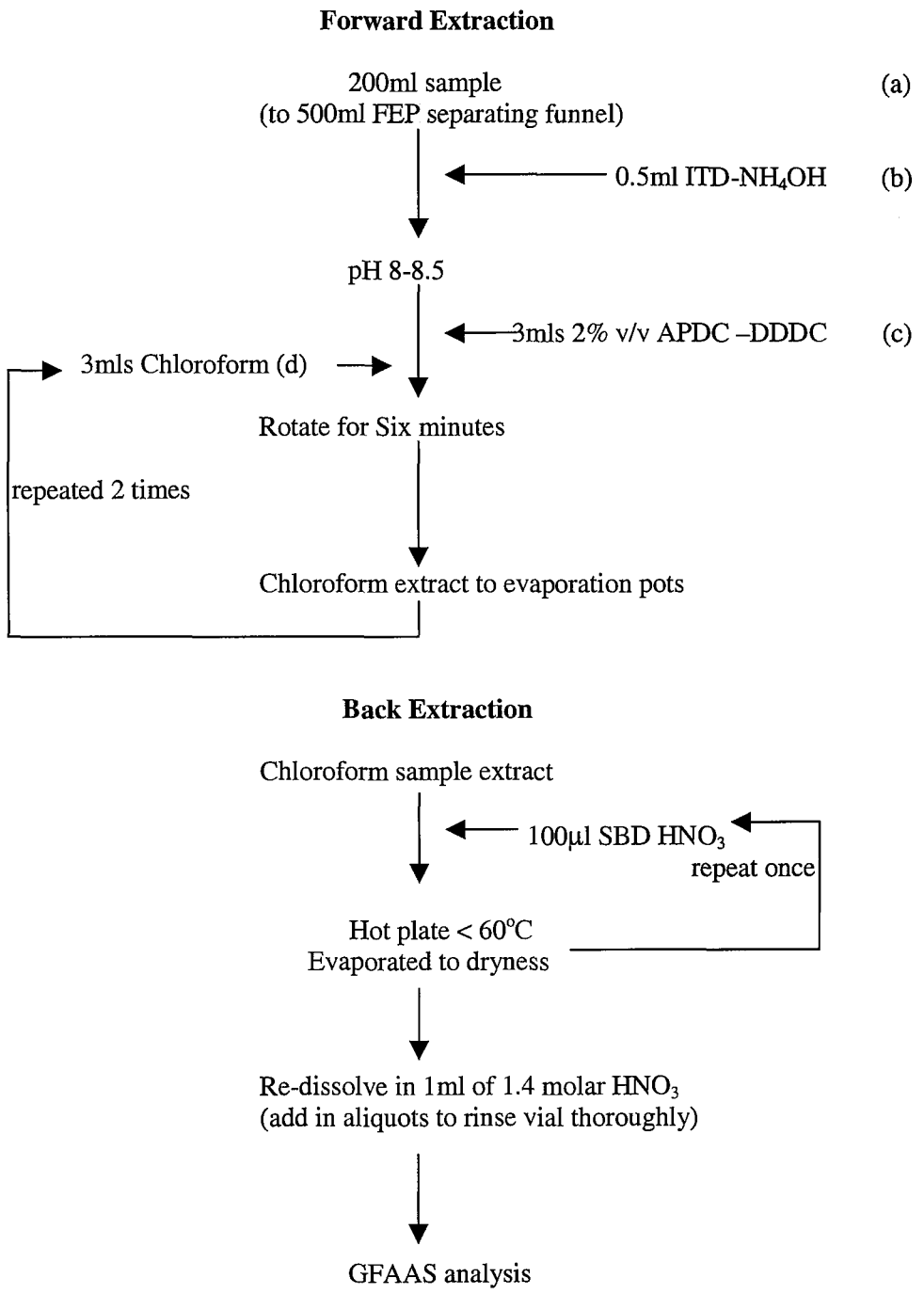


Figure 2.2 Flow diagram indicating the steps involved in the chelation and solvent extraction procedure.

2.4.3 Determination of seawater trace metals extracts by Graphite Furnace Atomic Adsorption Spectroscopy (GFAAS)

All of the samples collected during the incubation experiment (Chapter 4) were analysed for Fe, Cd and Zn using GFAAS. A Perkin-Elmer 1100B AAS equipped with an HGA-700 graphite furnace and an AS-60 autosampler was used. All analyses were performed using a pyrolytically coated L'Vov platforms and tubes. The programmes used in the determination are shown in Table 2.2.

On a daily basis, working stock standards were prepared to a concentration of 10 μg/l. The working stock standards were used for calibration of the instrument. All analyses were performed with deuterium arc lamp background correction. Each concentrate was analysed in triplicate and any sample that had a relative standard deviation greater than 10% was re-analysed. The concentrations in extracts from the GFAAS were given in μg/l and converted to nM in the seawater sample using a computer spreadsheet program.

Element	Iron (Fe)	Cadmium (Cd)	Zinc (Zn)
Wavelength (nm)	248.3	228.8	213.9
Lamp energy (ma)	20	4	10
Drying °C	140	140	140
Ramp (sec)	30	30	30
Hold (sec)	10	10	10
Ashing °C	1400	700	700
Ramp (sec)	1	1	1
Hold (sec)	15	15	15
Standing °C	20	20	20
Ramp (sec)	3	3	3
Hold (sec)	5	5	5
Atomisation °C	2400	1650	1800
Ramp (sec)	0	0	0
Hold (sec)	3	3	3
Clean °C	2700	2300	2400
Ramp (sec)	3	3	3
Hold (sec)	3	3	3

Table 2.2. Programmes used for GFAAS analysis

2.4.4 Adsorptive Cathodic Striping Voltammetry (AdCSV)

Stripping voltammetric techniques do not have the multi-element analysis capabilities of GFAAS after separation by chelation and solvent extraction, but does have the advantage of allowing determinations directly in seawater as the preconcentration step is performed in the voltammetric cell itself.

Voltammetry is based on the measurement of a current response as a function of the potential applied to an electrochemical cell (Achterberg & Braungardt, 1999). In stripping voltammetry a preconcentration step is combined with a stripping step, thereby enhancing sensitivity and selectivity. During the preconcentration step, the trace metal of interest is collected onto or in a working electrode and during the stripping step the collected metal is oxidised or reduced back into solution (Wang, 1996).

Adsorptive cathodic stripping voltammetry was chosen due to its ability to measure both the total dissolved Zn and the speciation of Zn and also due to the reduced sample handling, which minimises the risk of sample contamination. AdCSV makes use of a specific added ligand (AL), which is added to the water sample and forms an adsorptive complex with the trace metal (M) (Eq. 1).

$$yM^{n+} + zAL^{m-} \leftrightarrow M_y(AL)_z^{(yn-zm)} \quad (1)$$

$$M_y(AL)_z^{(yn-zm)} \leftrightarrow M_y(AL)_z^{(yn-zm)} \text{ adsorbed} \quad (2)$$

$$M_y(AL)_z^{(yn-zm)} \text{ adsorbed} + e^- \leftrightarrow {}_yM^{(n-1)+} + {}_zAL^{m-} \quad (3)$$

A minute fraction of the metal-ligand complex is adsorbed on the surface of a Hanging Mercury Drop Electrode (HMDE) (Eq.2) and a potential scan towards more a negative potential is carried out (Achterberg & Braungardt, 1999). The adsorption potential is chosen to be more positive (ca 0.1V or more) than the reduction potential of the metal ligand complex. The current produced is a result of the reduction of a reducible group on the ligand or the metal itself in the adsorbed complex (Eq. 3) (Achterberg & Braungardt, 1999).

Detection limits of 0.07 nM have been reported using this technique (van den Berg, 1985). However, Zn concentrations as low as 0.06 nM have been determined in the subarctic North East Pacific using a chelation and solvent extraction method (Martin *et al.*, 1989) and therefore the detection limits of the AdCSV technique would have to be lowered.

2.4.5 Interferences in AdCSV

Oxygen causes interference because the potential of the second O_2 wave is situated under the Zn peak and is the main cause of irreproducibility of its determination (van den Berg, 1999). The sample must be completely purged with N_2 both prior to analysis and in between analytical steps. Other metal ions can interfere with the Zn if their complexes with APDC are adsorbed on the Static Mercury Drop Electrode (SMDE) and their reduction peaks are close to that of Zn. At concentrations of 10^{-7} M Cu, Fe, V, U, and Pb do not interfere with this Zn analysis (van den Berg, 1999). APDC produces a wave between –0.3 and –0.8V, which masks any peaks produced by these elements. Ni (II) and Co (II) do produce peaks close to that of Zn and are potential interferents when present at high concentrations (van den Berg, 1999). As the planed research was to carried out in the open ocean both Ni and Co are present in low (nM- pM) concentrations and therefore no interferences were expected.

Dissolved organic material (DOM) may interfere with the physical and electrochemical processes occurring at the electrode surface as a result of its surface activity or by binding and thus inactivating some, or all of the metal ions (Achterberg *et al.*, 2001). The nature of DOM in seawater is complex, but is thought to include humic acids, fulvic acids, glycollic acid, peptides, proteins, amino-acids, lipids and polysaccharides (Buffle, 1988). Seawater samples were acidified to a pH value of 2.5 to prevent trace metals adsorbing onto container walls. The interference caused by organic complexation of metals is to a large extent eliminated by carrying out the measurements at low pH (2-3), but experimental evidence has demonstrated that not all the organic complexed metal is released by acidification (van den Berg & Nimmo, 1987). However, both organic complexing material and surfactants are destroyed by treatment of the sample with ultra-violet (UV) radiation prior to trace metal determination (van den Berg, 1988). UV digestion is effective and can be readily incorporated in flow injection manifolds, allowing stand alone trace metal analysis (Achterberg *et al.*, 2001).

2.4.6 Design of a new UV-sample irradiation system

A new UV-system was designed and built in-house, based on the model of Achterberg & Braungardt, (1999). The new system has a stainless steel housing, rather than aluminium, to reduce contamination from metal corrosion (Fig. 2.3). The system uses a 400W medium pressure Hg vapour lamp (Photochemical Reactors). The power supply for the lamps (Photochemical Reactors) was switched from a high output during the initiation of the Hg arc lamp to a reduced output for continuous operation. Quartz vials with Teflon screw caps were used to hold discrete seawater samples (50ml), where the vial holder was made of polytetrafluoroethylene (PTFE), again to reduce contamination (Fig. 2.4). This system was also designed to be capable of in-line UV-digestion of samples using a silica coil in place of the vials. Another modification to the UV-system is that the orientation of the lamp is the reverse of Achterberg & Braungardt (1999). This allows easier access and ensures that the sample tubes would not be in contact with metal. The centre-to-centre distance between the light source and the vials was ca. 5 cm. The UV unit was air-cooled using a fan, resulting in a sample temperature during digestion of ca.70°C. Safety protocols were also in-built into the instrument. A safety switch ensures the lamp will not turn on until the lid is firmly closed and the housing was light tight to prevent exposure to harmful UV radiation. If the fan fails a thermal fuse will automatically turn the instrument off once the temperature reaches 75°C. Any ozone produced is vented away through an open window.

Batch experiments were carried out over different time periods to investigate the optimum time needed to UV digest seawater samples. The digestion efficiency as reflected by the total metal values was unchanged with irradiation times longer than 6 hours. This digestion treatment is therefore expected to free Zn existing in the form of organic complexes or incorporated in organic colloids as well as metal species adsorbed on particles (Muller *et al.*, 2001). UV-digestion was therefore performed on discrete samples for a period of 6 hours. Samples were UV-irradiated at a pH of 2 – 2.5 to ensure no metal is lost by adsorption onto the fused silica vials. Rigorous tests were performed on the system to ensure minimal contamination was occurring. Low metal seawater, Sub-Boiled Distilled water and NASS-4 were analysed both before and after digestion. As expected the concentration of low metal seawater increased after UV-digestion and the concentration of Sub-Boiled Distilled water remained the same. The concentration of NASS-4 increased slightly but was in the specified range. Before UV-digestion the concentration was 1.91 nM ± 0.02 and after UV-digestion was 1.95nM ± 0.01 where 0.02 and 0.01 is the standard deviation on three separate measurements.

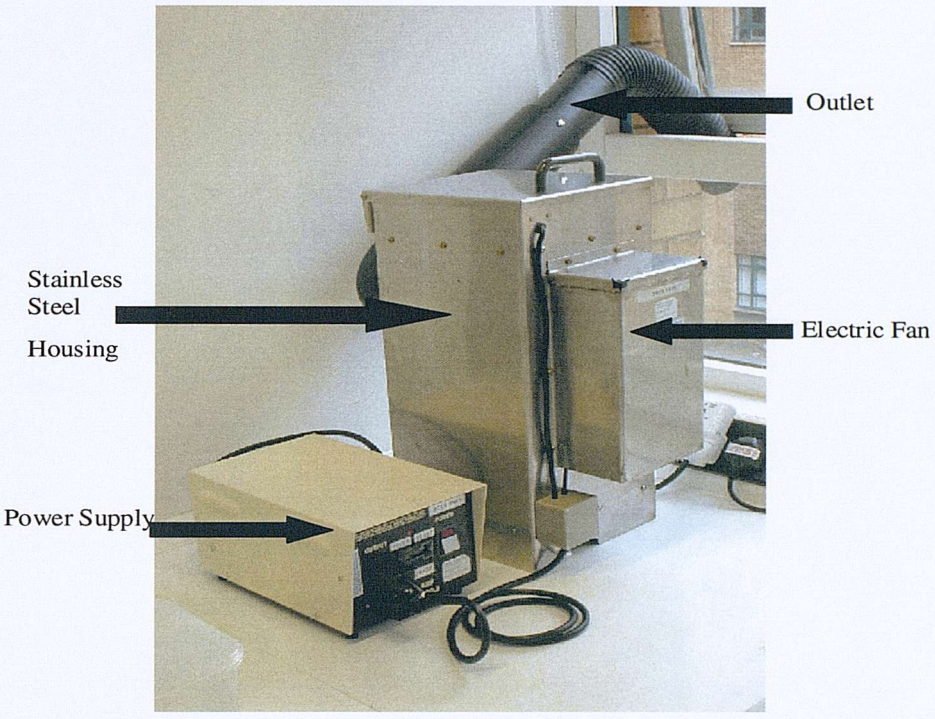


Figure 2.3. UV-digestion system

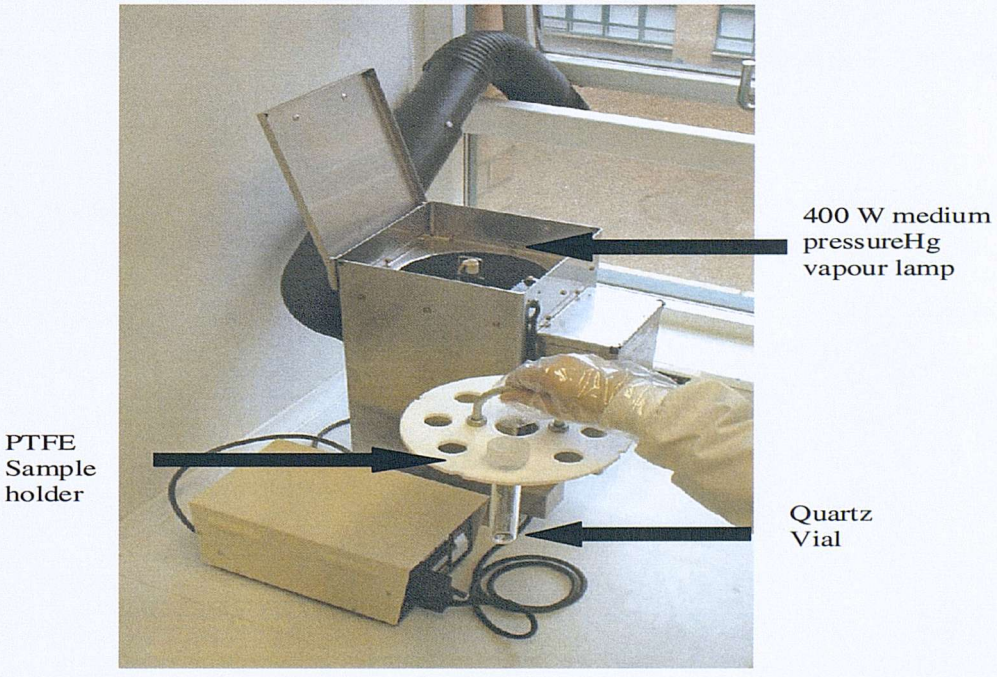


Figure 2.4. UV-Digestion system and sample holder

2.4.7 Analysis of total dissolved zinc

Total dissolved Zn was determined by cathodic stripping voltammetry (van den Berg, 1985; Donat & Bruland, 1990). The voltammetric system consisted of a Metrohm (663 VA Stand) static mercury drop interfaced with a μAutolab voltammeter (Eco Chemie) which was controlled by a Viglen 486 computer. The reference electrode was a double-junction, Ag/AgCl, KCl (3M), saturated AgCl electrode and the counter electrode was a glassy carbon rod.

Acidified seawater samples (pH 2) in silica tubes were UV digested using a high-pressure mercury lamp (400W) for 6 hours. Total Zn measured by AdCSV should include all organic and inorganic Zn, because samples were exposed to pH <2 and were UV oxidised before analysis. Following UV digestion, 10ml of the sample were transferred to the voltammetric cell and neutralised with ~ 9M ID-NH_4OH prior to addition of 20μl APDC and 60μl borate buffer. Voltammetric conditions were: deoxygenation time 4min; adsorption potential –1.3V; adsorption time 60 to 240s; reoxidation potential –0.8V for 10s and a potential scan using square-wave modulation (50Hz) with a step potential of 2.5mV. After 3 replicate scans the procedure was repeated for each of two standard additions of Zn, where the amount of Zn added was adjusted to be approximately equal to the concentration of the sample.

To measure the analytical blank, Zn-free seawater was created by UV-irradiating North Pacific open ocean water followed by equilibration with clean Chelex-100 resin beads as described in (Price *et al.*, 1989). This seawater was then UV-irradiated again to degrade any iminodiacetate groups, which may have been released from the Chelex. This final irradiation step was important at these picomolar levels despite the rigorous protocol used to prepare the chelex. The analytical blank of the reagents and equipment determined using twice UV-irradiated seawater was close to the detection limit (0.025 nM). Procedural blanks were performed on a daily basis. The detection limit calculated from three times the standard deviation of the blank was 0.02 nM.

To assess the accuracy and precision of the entire analytical procedure, the open ocean reference material NASS-4 (National Research Council of Canada, NRCC) was analysed in triplicate. NASS-4 was UV-irradiated for 4 hours in covered quartz vessels, then transferred to sterlin vials and the pH raised to 8.1 using ID-NH_4OH. Standard addition analysis was then carried out on

NASS-4 and yielded a concentration of 1.95nM ± 0.01, where 0.01 is the standard deviation on three separate measurements of the same sample. These results fell within the range reported by NRCC for Zn of 1.76 ± 0.28nM. The precision of the technique is ± 4% RSD.

2.4.8 Comparison of solvent extraction and AdCSV

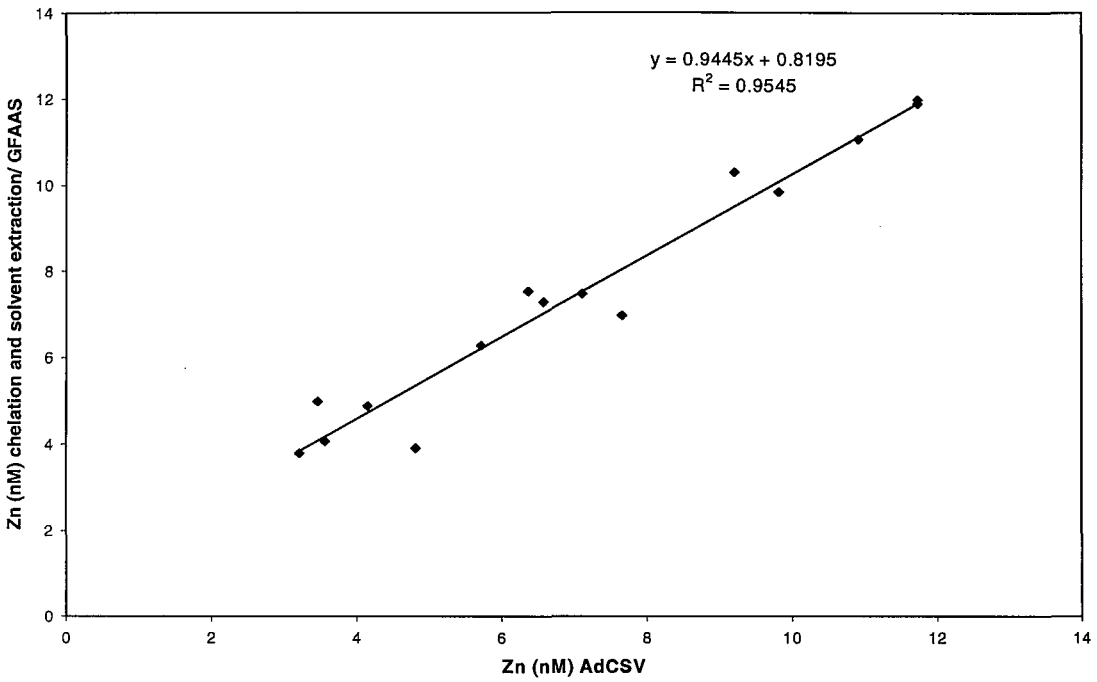
Total dissolved Zn concentrations from the incubation experiment (Chapter 4) were measured over a concentration range of 34pM to 11.7 nM using both AdCSV and chelation and solvent extraction. Results from both techniques were in clear agreement. As the concentration range is large, the lower concentrations are considered separately from the higher concentrations in order not to bias the data. By plotting these values against each other and applying a 'least-squares' linear fit a r^2 value of 0.909 and 0.954 and a slope of 1.47 and 0.945 for the lower and higher concentration range is achieved (Figure 2.5 a, b). As both the X and Y values are independent variables neither are "controlled" but both are measured, a Model II regression was carried out. In Model II regressions, the offsets are measured along a line perpendicular (or normal) to the regression line. Thus, the line is fit by minimising the sum of the squares of the normal deviates. This changes the slopes to 1.54 and 0.966 for the lower and higher concentration range. A slope of close to 1 is near to unity. This regression implies that total dissolved Zn concentrations by both techniques produce similar results.

2.5 Zinc Speciation

2.5.1 Introduction

It has now been widely recognised that total dissolved metal concentrations do not yield sufficient information about the toxicity, bioavailability and geo-chemical behaviour of trace metals in natural waters and therefore the importance of metal speciation has come to the forefront of trace metal studies. Speciation analysis involves the determination of different physico-chemical forms of trace metals (Achterberg & Braungardt, 1999) that are anticipated to behave differently in biological and physio-chemical interactions in the water column. The high sensitivity and selectivity of stripping voltammetry make this technique suitable for trace metal speciation studies.

(a)



(b)

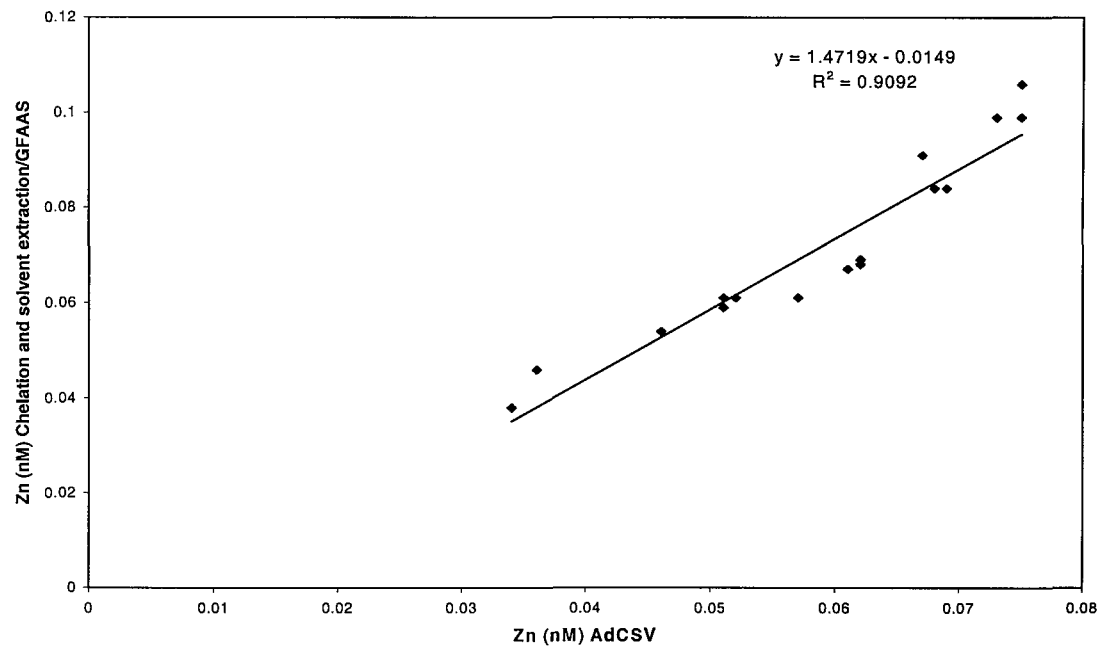


Figure 2.5. Comparison between Zn analysed by adsorptive cathodic stripping voltammetry and chelation and solvent extraction. Two different concentration ranges used (a) 0.1 – 0.034 nM (b) 11.9 - 3.2 nM.

2.5.2 Theoretical considerations

The speciation of dissolved Zn is determined using competitive ligand equilibrium-cathodic stripping voltammetry. This CSV-CLE method involves the establishment of a competitive equilibrium between Zn and Zn-complexing ligands naturally present in the sample and a competing organic ligand, APDC added to the sample (van den Berg, 1985; Donat & Bruland, 1990). Once APDC has been added to the sample containing natural zinc-complexing organic ligands (L), the mass balance for total dissolved Zn (C_{Zn}) in the sample is:

$$C_{Zn} = [Zn'] + [ZnPDC] + [ZnL] \quad (2.1)$$

Where [Zn′] is the concentration of the inorganic Zn (free and inorganically complexed), [ZnPDC] is the concentration of Zn complexed by PDC and [ZnL] is the concentration of Zn complexed by natural ligands. C_{Zn} is calculated after UV irradiation of acidified samples (see above).

The equilibrium expression for the formation of ZnL is:

$$K'_{ZnL} = [ZnL] / ([Zn^{2+}] [L']) \quad (2.2)$$

where K'_{ZnL} is the conditional stability constant of the Zn complex in seawater. The measured conditional stability constants reflect the strength of the ZnL complex between the added Zn and excess ligand present in seawater. [L′] is the concentration of the L not complexed by Zn, which includes free L as well as L complexed by H^+, Ca^{2+}, Mg^{2+} and Na^+ and $[Zn^{2+}]$ is the free Zn ion concentration. The total ligand concentration (C_L) is defined as

$$C_L = [ZnL] + [L'] \quad (2.3)$$

assuming a 1:1 stoichiometric complex with PDC. Both of these equations (2.1 and 2.2) form the basis of a linear relationship between the Zn ion concentration, $[Zn^{2+}]$ and [ZnL]. The following relationship is obtained by substitution for [L′] in equation (2.3) into equation (2.2) and rearranging to give (Ruzic, 1982; van den Berg & Dharmvanij, 1984; Ellwood & van den Berg, 2000)

$$[Zn^{2+}]/[ZnL] = [Zn^{2}]/C_L + 1/(K'_{ZnL}C_l) \quad (2.4)$$

Plotting $[Zn^{2+}]/[ZnL]$ as a function of $[Zn^{2+}]$ gives a linear result, providing Zn complexation is predominantly controlled by a single organic ligand (van den Berg & Dharmvanij, 1984). The linear result is due to the limited range of metal concentrations covered by the titration and detection of only a certain group of natural ligands due to the type and concentration of the ligand added (Zhang *et al.*, 1990).

Data for the calculation of the complexing ligand concentrations were obtained from a titration of each sample with added Zn. The labile Zn concentration $[Zn_{labile}]$ was measured using AdCSV and the concentration of the [ZnL] was calculated from

$$[ZnL] = C_{Zn} - [Zn_{labile}] \quad (2.5)$$

The labile Zn concentration is that which equilibrates with the added ligand (APDC) and this is measured by AdCSV. Substitution of equation 2.1 into equation 2.5 gives an expression for the labile Zn concentration

$$[Zn_{labile}] = [Zn'] + [ZnPDC] \quad (2.6)$$

The AdCSV peak height is related to the $[Zn_{labile}]$ via the sensitivity, S (peak current/Zn concentration (na/nM or pM).

$$ip = S[Zn_{labile}] \quad (2.7)$$

S is estimated from the linear portion of the Zn titration curve after effectively all of the Zn-complexing organic ligands have been saturated with Zn. Ligand saturation is verified by comparison with the slope obtained from further Zn standard additions to a sample aliquot in which the Zn-complexing ligands were previously saturated by a Zn increment greater than the ligand concentration. The concentration of labile Zn concentration (2.6) includes [Zn′] as a small constant fraction of the added Zn remains uncomplexed by ADPC. $[Zn^{2+}]$ is directly related to the $[Zn_{labile}]$ concentration by α'

$$[Zn^{2+}] = [Zn_{labile}]/\alpha' \quad (2.8)$$

α' is the overall α-coefficient (Ringbom & Still, 1972), excluding complexation by L. Substitution for Zn^{2+} in equation 2.4 using equation 2.6 gives

$$[Zn_{labile}]/[ZnL] = [Zn_{labile}]/CL + \alpha'/(C_L K'_{ZnL}) \quad (2.9)$$

This equation is conveniently used for the van den Berg/Ruzic plot as it is the $[Zn_{labile}]$ which is measured by AdCSV rather than $[Zn^{2+}]$. α' is obtained from

$$\alpha' = \alpha_{Zn} + \alpha_{ZnPDC} \quad (2.10)$$

where α_{Zn} is the inorganic side-reaction coefficient for Zn^{2+} (Ringbom & Still, 1972), which was calculated to be 2.1 in seawater at pH 8, salinity 35 using an ion-pairing model with constants from (Turner *et al.*, 1981).

$$\alpha_{Zn} = 1 + \Sigma(K^*_i [Lj]^i) + \Sigma(K^*_{a,i}/[H^+]^i) \quad (2.11)$$

where K^*_i is the stepwise stability constant for complexes of Zn^{2+} with L_j (Cl^-, CO_3^{2-}, SO_4^{2-}, $B(OH)_4^-$) and Ka,i is the stepwise acidity constant of Zn^{2+} (Taken from van den Berg, 1985).

α_{ZnPDC} is the side-reaction coefficient for Zn complexed with the added competing ligand PDC, which is fixed by the concentration of PDC added to the sample.

$$\alpha_{ZnPDC} = K'_{ZnPDC} [PDC'] \quad (2.12)$$

where K'_{ZnPDC} is the conditional stability constant and [PDC'] is the concentration of PDC not complexed to Zn. As the concentration of PDC added to solution is much greater than that of Zn, the total concentration of PDC can be used instead of [PDC']. A value of 4.4 was used for K'_{ZnPDC} for a salinity of 36 and a pH of 8.2, was taken from (van den Berg, 1985; Donat & Bruland, 1990; Ellwood & van den Berg, 2000) where this value had been determined experimentally by equilibrium competition between PDC and the ligand NTA (whose side reaction coefficient under

seawater conditions can be reliably calculated) using the assumption that Zn formed a one to one stoichiometric complex with PDC.

The values for $[Zn_{labile}]$ in equation 2.9 are obtained directly from the AdCSV peak current as $[Zn_{labile}] = ip/S$ (equation 2.7) and the concentration of ZnL from equation 2.5.

Values for C_L and K'_{ZnL} are calculated by linear least-squares regression of equation 2.9 where the slope is equal to 1/CL and the intercept yielding $1/(K'_{ZnL}C_L)$.

Finally once C_L and K'_{ZnL} have been determined the concentration of Zn^{2+} in solution is calculated by solving the following quadratic equation:

$$[Zn^{2+}]^2 \alpha_{Zn} K'_{ZnL} + [Zn^{2+}] (K'_{ZnL}C_L - K'_{ZnL}C_{Zn} + \alpha_{Zn}) - C_{Zn} = 0 \quad (2.13)$$

The electrochemically 'labile' species of the metal are those which influence the voltammetric signal to the same extent as the free metal ion M^{2+}. Provided the deposition step is carried out at a suitable potential, most metal-organic complexes are not labile because their dissociation rate constants do not enable significant dissociation to M^{2+} to take place at the electrode (Muller *et al.*, 2001).

In voltammetric analysis, it must be assumed that organic ligands with a weak affinity for metal are included in the labile fraction (Coale & Bruland, 1988). Ligands of a very strong affinity that are fully titrated initially do not equilibrate with added metal and have no contribution to the estimation of the metal affinity of the organic complexes in solution. This concept is known as the analytical window and refers to the metal affinity range of the measurement (Van den Berg & Donat, 1992).

2.5.3 Zn titrations

The determination of Zn speciation was performed on-board ship, in order to minimise possible changes to chemical equilibria in samples caused by storage and transport of the samples and to prevent loss of analyte due to changes in redox speciation or adsorption on the container walls.

The above method therefore involves a titration procedure to determine the concentration of natural metal complexing ligands (L) and their conditional stability constants (K'_{ML}). Before use, the Teflon vials were acid cleaned and then conditioned (3x with seawater and Zn additions) to minimise the effects of Zn-PDC adsorption onto the vial walls. Filtered seawater was divided into 10 x 10ml sub-samples in Teflon vials, to which increasing amounts of metal were added. 6 µl of APDC and 100 µl of borate buffer were added to each teflon pots which were then spiked with the appropriate concentration of Zn. Samples were allowed to equilibrate for 12 hours at room temperature (~20°C). This establishes a competitive equilibrium for Zn between the PDC ligand added and the organic ligands naturally present in the sample. The Zn-PDC complexes formed in each aliquot were then determined using AdCSV.

The voltammetric cell was de-aerated for 4 minutes using ultra clean N_2, subsequently 4 new mercury drops were made and discarded and after the extrusion of the fifth mercury drop the adsorption period was initiated. Adsorption of the ZnPDC complex on the HMDE at a potential of –0.8 V was carried out for 3-6 minutes. The stirrer was then stopped and a quiescent period of 8 seconds was allowed followed by a potential scan from –0.8V to –1.2V using square wave modulation (50 HZ), with a potential step of 2.5 mV. Square wave modulation was utilised to minimise noise on-board ship to minimise noise as a result of engine vibration and ship motion.

The deposition potential of –0.8 V was used rather than the more negative one used for total metal determination (-0.95 or –1.3V) to prevent possible dissociation of ZnL complexes. In this way a possible dissociation of natural organic complexed zinc during the deposition step is prevented (van den Berg, 1999). The sensitivity in seawater containing organic mater is generally somewhat less than in seawater subjected to UV digestion and is possibly not constant. The sensitivity is therefore calibrated regularly by using a standard addition of Zn to a sample aliquot in which the Zn-complexing ligands were previously saturated by a Zn increment greater than the ligand concentration.

2.6 Collection of ancillary data

Conductivity-Temperature-Depth (CTD) profiles were generated using a Seabird CTD probe attached to a rosette frame. Fluorescence and transmissivity profiles were generated from *in situ* fluorometer and transmissometer units attached to the CTD.

Salinity samples from the Go-Flo bottles were collected into 250ml borosilicate glass bottles. The screw thread was wiped with a low lint tissue after sampling to reduce the chance of salt crystal formation around the cap, which could result in evaporation of the sample due to an improper seal. The bottles were then tightly capped and stored at room temperature until analysis, generally within 2 days. A Guideline model 84000 Autosal salinometer was used for the determination of salinity. The salinity is determined by conductivity using 4 conductivity cells. Calibration was performed using IAPSO standard seawater. This analysis was carried out onboard by Hugh Mac Lean.

Nutrients

Nutrient samples were collected in 15 ml plastic tubes from Niskin bottles mounted on a CTD-rosette system. Nitrate, phosphate and silicate was analysed upon collection on a Technicon AutoAnalyzer by Frank Whitney using the methods described in Strickland & Parsons (1972) and Barwell-Clarke & Whitney (1996).

Chlorophyll a

Samples were collected in 500 ml High Density Polyethylene bottles rinsed three times with ambient seawater and gently vacuum filtered (pressure < 1000 nm Hg or 5 Psi) onto 45mm Glass Fibre Filters (GF/F). The filters were folded and placed into glass vials and frozen for several days until analysis. Chlorophyll *a* was extracted from the filters by adding 10ml 90% acetone to the vials, capping and leaving for 24 hours in the freezer. Analysis was carried on-board ship using a Turner Designs model 10 fluorometer against an acetone blank and calibrated against a pure chlorophyll *a* standard (Sigma) standardised on a spectrophotometer (Strickland & Parsons, 1972). Concentration of chlorophyll *a* was then calculated using standard formulae.

Chapter 3

Total dissolved zinc and zinc speciation in the upper water column of the subarctic North East Pacific

3.1 Introduction

Total dissolved Zn concentrations collected from the subarctic North Pacific from the upper water column during two cruises in February (1999-01) and in September (1999-21) (Chapter 2) has been reported by Lohan *et al.* (2002). This data has been extended to include both total dissolved Zn concentrations and Zn speciation from a cruise in August 2001 (2001-29) and is reported in this chapter.

3.1.1 Dissolved Zinc in the North East Pacific

Dissolved Zn concentrations in the North East Pacific have been reported to be as low as 0.06 nM (Martin *et al.*, 1989). Organic complexes of dissolved Zn in surface waters accounts for about 98% of the total dissolved Zn and these organic forms therefore, dominate the dissolved speciation of Zn in seawater shallower than 200m (Bruland, 1989). This reduces the bioavailable fraction of Zn, the free metal ion [Zn^{2+}], to values as low as 2 pM (Bruland *et al.*, 1991). Our knowledge of the routes by which metals, such as Zn cycle through the natural phytoplankton community is very limited.

It is generally inferred from previous experiments, particularly those carried out in cultures that biological uptake of metals is a function of the Free Metal Ion (FIAM) concentration (Anderson & Morel, 1978; Sunda & Huntsman, 1992; Campbell, 1995). In a system at equilibrium, the free-metal ion activity reflects the chemical reactivity of the metal. It is this reactivity that determines the extent of the metal's reactions with surface cellular sites, and hence its bioavailability (Campbell, 1995). If however the surface equilibrium is untenable e.g. if the reaction of Zn^{2+} or ZnL at the cell surface is slower than the rate of internalisation of the metal or if the metal

transport to the cell surface is the rate limiting step then the FIAM will no longer apply. In this case the uptake will not be determined by thermodynamic equilibrium but by the kinetics of the complexation reaction or the rate of metal transport the reaction layer outside the cell (Hudson & Morel, 1993). Therefore it is imperative to take into account the limitations of FIAM (Morel, 1983; Campbell, 1995) when studying metal-biota interactions.

The largest fraction of dissolved Zn in the upper ocean is considered to be unavailable for direct uptake by phytoplankton. This is due to organic complexation, occlusion of the metal in particulate material, or formation of an insoluble oxide phase (Bruland, 1989; Muller *et al.*, 2001). Therefore, the physiochemical speciation of Zn is an important consideration in an attempt to quantify and describe its biogeochemical cycle.

As Zn is a ubiquitous contaminant, accurate data can only be obtained using strict precautions during sampling and analytical procedures (Ellwood & Van den Berg, 2000) (see Chapter 2). The surface waters of the North East Pacific Ocean are known for their low concentrations of dissolved Zn although this data is restricted to only a few key stations within this region (Bruland *et al.*, 1978; Martin *et al.*, 1989). To date, there has been limited information on dissolved Zn in relation to biological activity and there has been only one paper published on the seasonal variability in dissolved Zn concentrations, which forms the main focus of this chapter (Lohan *et al.*, 2002). Little is know about the organic complexation of Zn or the speciation of dissolved Zn in open-ocean waters. Two previous studies have investigated Zn complexation in open-ocean waters of the Pacific (Bruland, 1989; Donat & Bruland, 1990) and one in the Atlantic (Ellwood & Van Den Berg, 2000).

The present study area of the subarctic North East Pacific Ocean is classified as a High Nutrient Low Chlorophyll (HNLC) region. HNLC regions are characterised by low stable phytoplankton standing stocks which rarely deplete nitrate, phosphate or silicic acid in the upper water column to growth-limiting levels (Strom *et al.*, 2000). Iron is known to limit phytoplankton growth in this area (Martin *et al.*, 1989; Cullen, 1991; Boyd *et al.*, 1996) but data on Zn limitation is limited. Enrichment experiments on water from Ocean Station Papa (OSP) have indicated that Zn had only a minor role in stimulating phytoplankton growth (Coale, 1991). However, only 0.75 nM Zn was added to the enrichment experiment which was lower than the concentration of Zn specific ligands (1.25 nM) observed in surface waters in neighbouring regions of the North East Pacific Ocean (Bruland, 1989; Coale, 1991). Prior to this investigation, no speciation studies have

been carried out either in the water column or in conjunction with enrichment experiments carried out at OSP. Information on the impact of Zn additions to the subarctic North East Pacific waters are presented in Chapter 4.

In this chapter seasonal dissolved Zn data on samples collected along W-E and S-N transects extending from OSP (50°N 145°W) to the respective coasts, and changes with respect to silicic acid (Si) is investigated. Zinc speciation measurements were also carried out along the W-E transect to investigate the complexation of Zn and to gain an understanding of the chemical species of Zn in this region which is crucial to improving our understanding of how phytoplankton acquire this micronutrient.

3.2 Methods

3.2.1 Sampling

Samples for total dissolved Zn were collected on three cruises on the C.S.S. John Tully in February 1999, September 1999 and August 2001 as outlined in Chapter 2. Total dissolved Zn concentrations were measured in the laboratory in Southampton using UV digestion to break down complexing and interfering organics in the sample. Zinc speciation samples were collected in August 2001 from P4, P12, P16 (Fig. 2.1) and analysed on-board within an hour of sampling, using the techniques described in Chapter 2.

Nutrient samples were collected using Niskin water samplers on a CTD-rosette system. Nitrate, phosphate and silicic acid were analysed upon collection on a Technicon AutoAnalyzer by Frank Whitney using the methods described in Chapter 2.

3.3 Results

3.3.1 Total Zinc Concentrations

The total dissolved Zn concentrations are in good agreement with previous data (Martin *et al.*, 1989) analysed by chelation and solvent extraction, for three stations (T7, T8, T9) in this region (Fig. 2.1). Surface water (10m) concentrations range from 0.04-0.62nM (this study) compared to 0.06-0.52nM (VERTEX) (Martin *et al.*, 1989). Below 100m the dissolved Zn concentrations range from 1.8 to 2.1nM (this study) compared to 0.99-3.71nM (VERTEX) (Martin *et al.*, 1989).

Zinc results for the subarctic North East Pacific transects are shown in Fig. 3.1 a, b, c, d and in Appendix 1. These dissolved Zn profiles are oceanographically consistent showing strong similarities to the Si distribution, with Zn depletion in surface waters and increases in concentration with depth (Martin *et al.*, 1989). No obvious trends were observed in dissolved Zn concentrations along the line Z transect in September, although a strong depletion was observed in the surface waters at the coastal stations Z14 and Z19. Along the line P transect, highest concentrations of dissolved Zn were observed at the shelf station P4 for both seasons. There is a clear gradient of dissolved Zn concentrations decreasing with increasing distance from the coast on the line P transect in both February and September. Conversely, on the line Z transect, higher concentrations of dissolved Zn were not observed at the station closest to the shore but at Z9 which is an open ocean site. During February, the mixed layer depth was around 100m and this shoaled to 40m in September. However, no significant seasonal variation was observed between the February and September samples, other than the depth of the nutricline and associated Zn profile increased with the depth of the mixed layer in winter. In August 2001, Zn concentrations followed the same trends as in 1999 but were lower than those observed in September 1999 below the mixed layer. Surface water concentrations along line P in August 2001 are similar to those observed in September 1999.

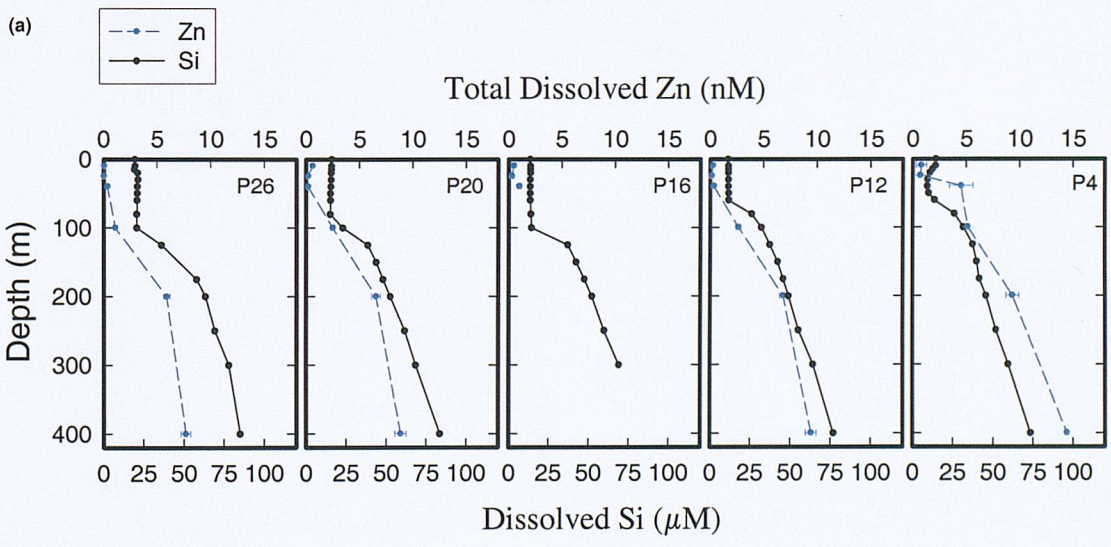


Figure 3.1 Profiles of total dissolved Zn concentrations (nM) and Si (μM) along line P in (**a**) February 1999.and Error bars indicate the standard deviation of three replicate scans on the same sample.

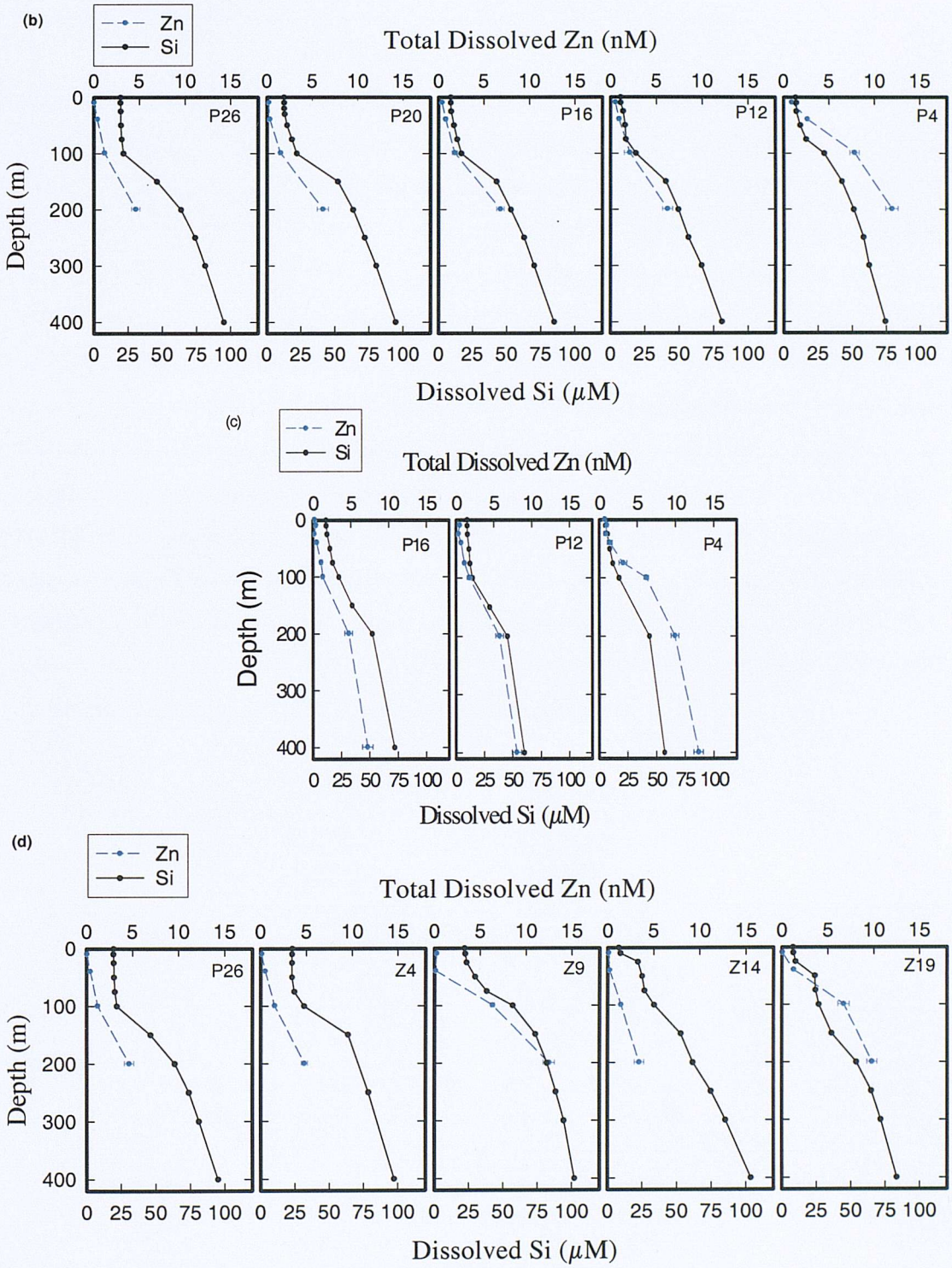


Figure 3.1 Profiles for total dissolved Zn concentrations (nM) and Si (μM) along (**b**) line P transect in September 1999(**c**) line P transect in August 2001 and (**d**) line Z transect in September 1999. Error bars indicate the standard deviation of three replicate scans on the same sample.

3.3.2 Zinc and Silicic acid

As widely reported for the ocean environment (Bruland *et al.*, 1978; Bruland & Franks, 1983; Donat & Bruland, 1990) the full water column dissolved Zn profile is similar that of Si in this region (Fig. 3.1 a, b, c, d). However, the surface water Si concentrations are homogenous down to a depth of 100m at ca. 20μM, whereas the Zn concentrations approach zero, which might suggest that Zn will become limiting before Si, assuming that the $[Zn^{2+}]$ will also decrease. As observed with Zn there is little variation in Si below the mixed layer on a seasonal basis along the line P transect. A surface depletion of Si (several μM) was observed at all stations between February and September with the exception of OSP. Silicate concentrations increased westward from P4 (5.6 μM) to OSP (19.2 μM) in both winter and summer. Surface water concentrations of Si in the shelf station, P4 deviate from the other Si profiles in this region as the concentration decreases from 20-50m. Temperature and salinity data indicate that this station, P4 has warmer surface waters combined with lower salinities (Fig. 3.2). Along the line P transects in both February and September a clear trend is observed in the dissolved Zn/Si ratio, with Zn/Si decreasing with distance from the shore, with stations P12 and P16 (mid-transect) having similar Zn/Si (Fig. 3.3). No pattern is observed in Zn/Si ratios along the line Z transect.

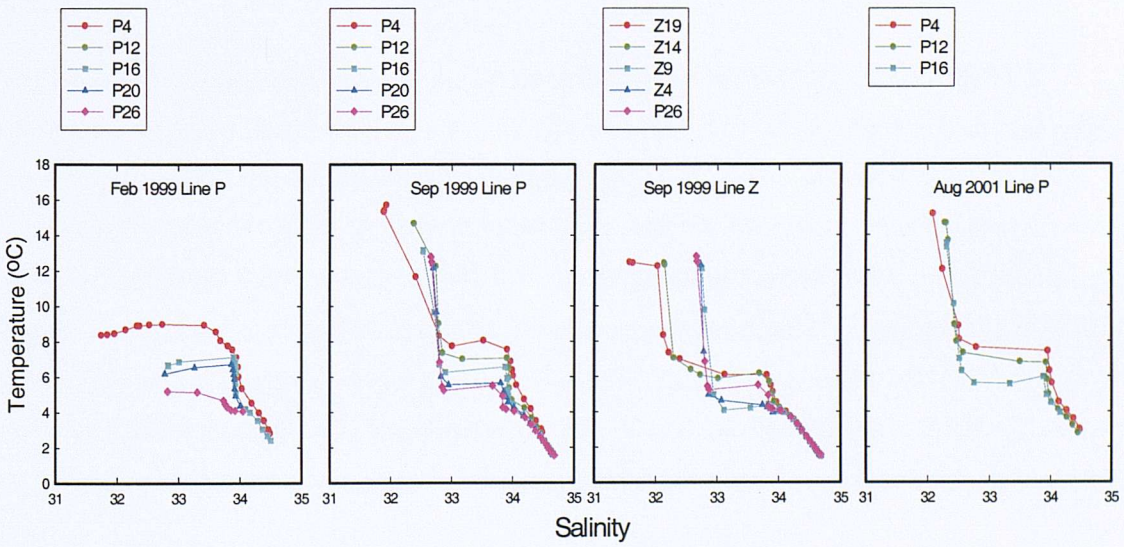


Figure 3.2 Temperature and Salinity along line P transect in February 1999 and along line P and Z transects in September 1999 and along Line P in August 2001.

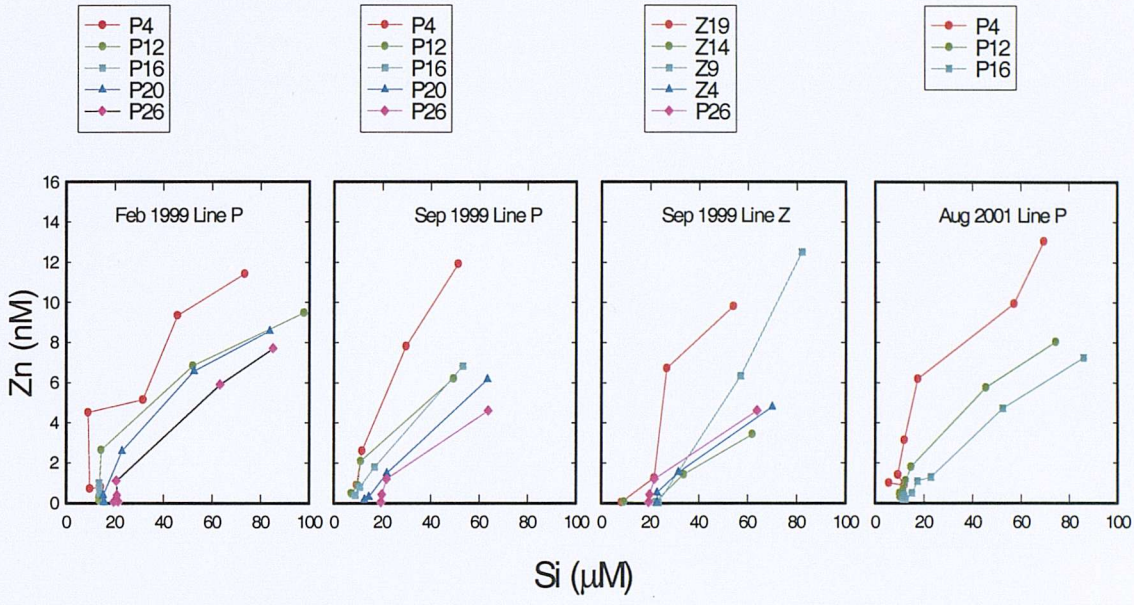


Figure 3.3 Zn/Si ratios alone the line P transect in February 1999 and along line P and Z in September 1999 and along Line P in August 2001.

3.3.3 Zinc Speciation

The Zn titration data and the corresponding linearised data obtained for station P4 at 0m is presented as a typical example (Fig. 3.4 a, b). The clear curvature in the titration data at low Zn concentrations indicates the presence of a Zn binding ligand. As the Zn concentration was increased the electrode response became linear indicating that effectively all of the Zn complexing ligands had been titrated with Zn. Figure 3.4 a was transformed using Equation 2.4 to yield a ligand concentration of 2.36 ± 0.03 nM with a conditional stability constant (log K'_{ZnL}) of 10.5. Plots of $[Zn_{labile}]/[Zn_L]$ vs $[Zn_{labile}]$ were linear indicating that a simple one-metal model could be applied (Ruzic, 1982; Van den Berg, 1985; Ellwood & Van den Berg, 2000). The one-ligand one-metal model was applied to all the sample titrations carried out in this study.

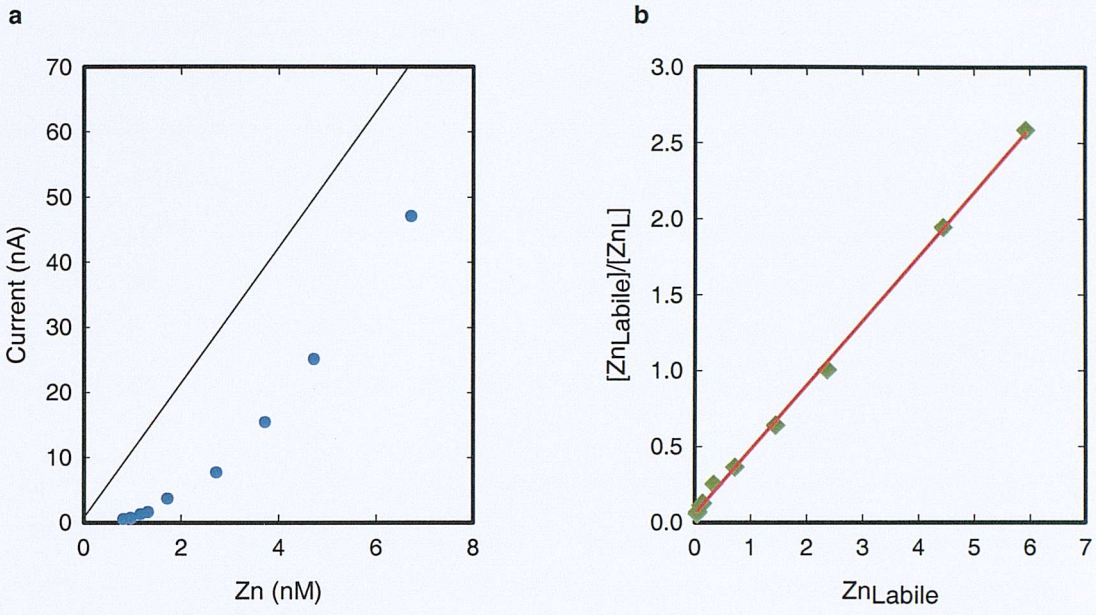


Figure 3.4 Zinc titration data for transect sample P4 0m. (a) Peak current vs. added zinc concentration. (b) Linearisation of data from which a ligand concentration of 2.36 nM and a log K'_{ZnL} = 10.5 was calculated.

A number of replicate titrations were carried out to determine method reproducibility. Replicate titrations were in good agreement with an average variation in ligand concentration of ~ 0.09 nM and in the conditional stability constant of 0.1 (Table 3.1).

Station	Depth	Zn_T (nM)	S.D.	Ligand (nM)	S.D.	log K'_{ZnL}	S.D.
P4	0	0.72		2.36		10.5	
	0	0.73		2.31		10.5	
	0	0.68	0.026	2.4	0.045	10.4	0.058
P12	40	0.70		2.88		10.4	
	40	0.64		2.78		10.4	
	40	0.72	0.042	2.84	0.050	10.4	0.000
P16	100	1.30		2.62		10.6	
	100	1.35		2.60		10.5	
	100	1.31	0.026	2.57	0.025	10.5	0.058

Table 3.1 Triplicate analysis of zinc speciation data from line P transect. S.D. is the standard deviation on three measurements and Zn_T is the total dissolved Zn concentration.

Natural ligand concentrations, conditional stability constants for the Zn-ligand complex and Zn^{2+} concentrations for P4-P16 are shown in Figure 3.5 a, b, c and in Appendix 2. In speciation studies it is essential to avoid sample contamination as this may lead to an underestimation of the natural ligand concentration (Achterberg, 1993). These profiles were measured using the same precautions against sample contamination during collection, storage and sample processing as applied for total dissolved Zn samples. Sample analysis occurred between 12-14 hours after collection to ensure changes in chemical equilibria were kept to a minimum. Stability constants in this study are in good agreement with those of previous Zn studies (Donat & Bruland, 1990; Ellwood & Van den Berg, 2000) and as the stability constants are calculated using the concentration of the Zn binding ligand, sample contamination is not thought to be a factor.

For samples collected from depths above 400m, the stability constants for the Zn-ligand complex changed little with sampling depth or location, with log K'_{ZnL} values ranging between 10.1 and 10.6 (Fig. 3.5 a, b, c; Appendix 3). However samples collected at 400m had either a higher stability constant of 10.8 or a lower stability constant of 9.8. At 400m the Zn binding ligand concentration is lower than total dissolved Zn concentration and therefore the Zn′ concentrations increase dramatically exceeding the Zn-complexing capacity of organic ligands. At the shelf station, P4 no Zn-complexing ligand was observed due to the large excess of dissolved Zn over the complexing organic ligand.

In surface waters i.e. samples collected at depths less than 100m, ligand concentrations varied between 0.72 and 3.63 nM but were always in excess of the total dissolved Zn concentration. Highest ligand concentrations in surface waters were observed at the shelf station, P4 (2.83 nM) and no real difference was observed between P12 and P16 (Appendix 2). However at OSP the ligand concentration in surface waters decreased to 0.72 nM. The Zn-complexing ligand concentrations follow those of total dissolved Zn, decreasing offshore. At 100m the ligand concentrations at all stations were the highest observed in this study. Zn^{2+} concentrations shown in Figure 3.5 a, b, c, d were calculated by solving equation 2.9. Surface Zn^{2+} concentrations decrease from 13.1 to 0.97 pM with increasing distance from the shelf. Vertical profiles of Zn^{2+} at P4, P12 and OSP show a similar pattern to total dissolved Zn, increasing with depth while at P16 the Zn^{2+} concentration decreases at 40m before increasing with depth. For open-ocean waters between 94 and 99% of Zn is complexed by strong organic ligands.

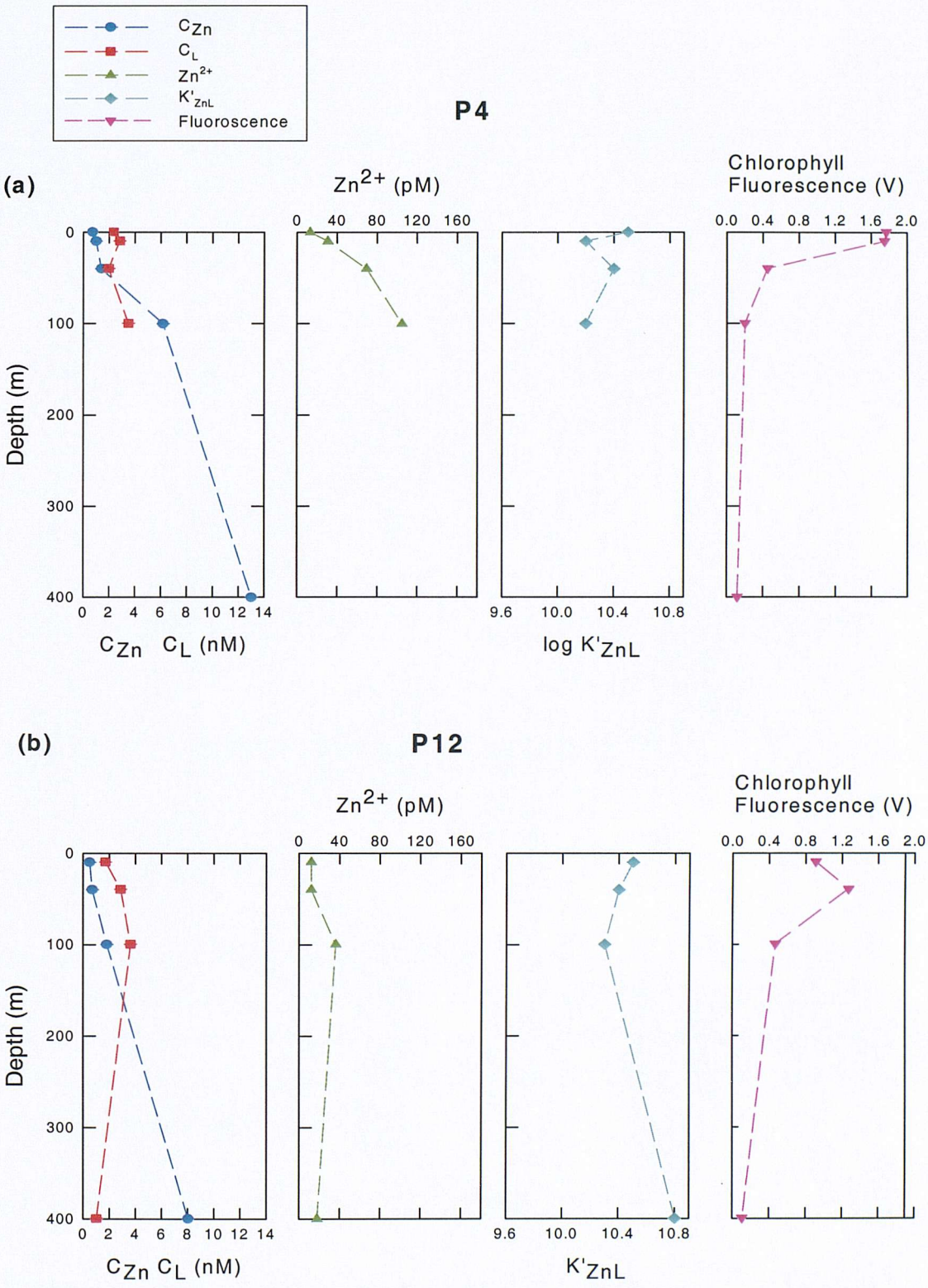


Figure 3.5 Zinc profiles data for **(a)** P4 and **(b)** P12 in August 2001.

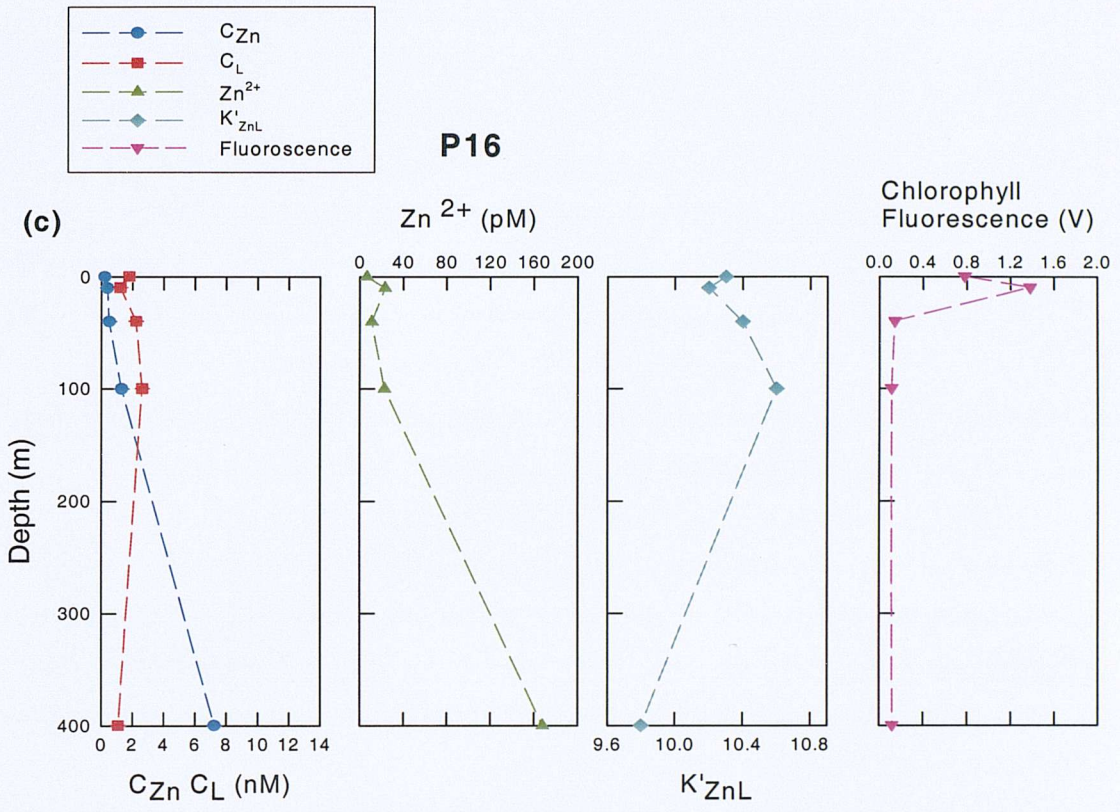


Figure 3.5 (c) Zinc speciation profiles for P16 in August 2001.

3.4 Discussion

Results from the total dissolved Zn profiles agree well with previous studies on Zn in the subarctic North East Pacific (Bruland, 1980; Bruland, 1989; Martin *et al.*, 1989; Donat & Bruland, 1990). However, the much more extensive data set for total dissolved Zn in the upper water column from both February and September 1999 in the current study provides additional information on both spatial and temporal scales.

3.4.1 Horizontal Zn distributions in the mixed layer

Highest concentrations of Zn along the line P transect in September were observed at the shelf station P4, and concentrations rapidly decrease offshore. Upwelling has been observed close to the coast in summer (Whitney & Freeland, 1999), and is evident here from the temperature and

salinity data (Fig. 3.2) and in the relatively high concentrations of Zn typical of deeper waters. The increased vertical mixing at the eastern boundaries from wind-induced coastal upwelling leading to increased surface water Zn has been reported off the Californian coast where the Zn concentration was 0.34 nM (Bruland, 1980). The elevated concentrations of Zn observed at P4 are 0.8 and 1 nM in winter and summer respectively compared to 0.07 and 0.04 nM respectively observed at OSP, 913 km offshore. Advective inputs from coastal and shelf processes may also increase the surface Zn concentrations at the eastern end of the line P transect, and contribute to the trend of dissolved Zn concentrations increasing from west to east.

Temperature and salinity profiles indicate that the mixed layer depth in winter is approximately 100m in winter and shoals to 40m in summer as a result of seasonal warming. Whilst a lateral increase in Zn concentrations is evident in both seasons as the coast is approached along the line P transect, vertically within the upper mixed layer only limited variability in concentrations of Zn with depth was observed at each station. There is no clear horizontal correspondence between total dissolved Zn and chlorophyll *a* or primary production (Lispen and Crawford, unpublished data) at these stations, although at Z14 and Z19 there may be an indirect relationship.

Conversely, on the line Z transect highest concentrations of Zn are not seen at the station closest to the shore but at Z9 which is an open ocean site. The lowest surface water Zn concentration was observed at shelf station Z19 (0.04 nM) and the Zn concentration observed at Z14 (0.07 nM) is comparable to OSP (0.04 nM). Both Z14 and Z19 have different water mass characteristics when compared to the open ocean stations along line Z (Fig. 3.2). At these stations the highest levels of primary production were observed (807 mg C m^{-2} d^{-1}at Z 14 and 789 mg C m^{-2} d^{-1}at Z19; Lispen and Crawford, unpublished data), the concentrations of PO_4^{3-} and Si were depleted and the concentration of NO_3^- was not detectable. The higher primary production observed would be expected to increase the flux of organic matter down the water column, and if Zn is associated with oceanic particulate matter, as has been reported (Collier & Edmond, 1984), depletion in total dissolved Zn is expected.

3.4.2 Upper water column distributions of Zn below the mixed layer

The vertical distribution of Zn in oceanic waters is governed by an internal cycle with rapid removal from surface waters coupled with almost complete recycling to dissolved forms at depth

(Bruland *et al.*, 1994). Although the mixed layer depth is 40m in summer, the presence of a permanent halocline at 120m has been reported (Tabata, 1975). Within the halocline in both seasons recycling of Zn is occurring resulting in increasing Zn concentrations with depth below the mixed layer. This is evident along line P on time scales similar to that of ocean scale mixing.

The results from this study show very little variation in the Zn profiles in the upper ocean on a seasonal basis, other than a slight increase in concentrations of Zn at 100 and 200m in February. The mixed layer depth indicates a seasonal change in water column structure and the increase in vertical mixing in winter increases the nutrient concentrations (Appendix 1). The macronutrient cycle follows an annual pattern in which maximum concentrations are observed February/March when the mixed layer is deepest and primary production is slowest and minima occur in late summer following the spring/summer period of reduced winds and elevated phytoplankton activity (Wong *et al.*, 1995). Martin *et al.*, (1989) have hypothesised that Zn to a greater extent than Fe would be introduced to surface waters by vertical mixing implying a seasonal variation. However, this was not observed, suggesting that the demand by phytoplankton for Zn may be high even in winter. Intensive scavenging of ^{234}Th in the surface mixed layer along the P transect in August 1996 compared to the near secular equilibrium observed in February 1996, indicates a higher export flux of POC in summer (Charette *et al.*, 1999) but no significant change in Zn was observed on the time scales in this study. However, unlike the bioactive metals such as Zn, ^{234}Th strongly interacts with a wide range of non-specific adsorption sites and therefore has little potential use for estimating the removal rates of dissolved Zn in oceanic surface waters (Wells *et al.*, 2000).

Zinc is an end-member example of a "Si-type" trace metal in the oceans where dissolved Zn concentrations are approximately five times greater in the old, nutrient-rich deep waters of the North Pacific than they are in the young nutrient-poor Atlantic deep waters (Bruland *et al.*, 1994). However, this view of Zn biogeochemistry in the ocean is dominated by the full water column distributions, with apparent tight coupling between Zn and Si in the deep water. There is little reliable data to investigate this Zn-Si relationship in the upper water column where total dissolved Zn concentrations are typically very low.

3.4.3 Zinc/Silicic acid relationship in the upper water column

The general vertical distribution of Zn described here is similar to Si, as has been widely reported for the North East Pacific (Bruland *et al.*, 1978; Bruland & Franks, 1983; Donat & Bruland, 1990). Linear correlations between Zn and Si have been reported by Bruland, (1980) for waters off the coast of California, and using data from VERTEX (Martin *et al.*, 1989) correlations have been determined for stations Z9, a station close to Z19, and OSP. These data are reported in Table 3.2, together with the slope and r^2 values for Zn/Si from the stations in this study. However, variations are observed in slopes from each station for these data, which range from 0.069 to 0.269 (nM/μM). At OSP the Zn/Si relationship observed in the present study is comparable to that of Martin *et al.* (1989) for the upper 200m. Stations Z9 and Z19 indicate a difference in the slopes observed in this study compared to VERTEX where the slope of 0.21 (nM/μM) in this study is higher than 0.1 (nM/μM) observed by Martin *et al.* (1989). This is due to higher Zn concentrations observed at 200m in the present study. At the shelf station P4, Zn/Si ratio is higher in summer (0.269 (nM/μM)) than in winter (0.138 (nM/μM)).

Zn/Si Relationships

February 1999			Spetmeber 1999			August 2001			Previous Work			
Station	Slope	r^2	Station	Slope	r^2	Station	Slope	r^2	Station	Slope	r^2	Author
P4	0.158	0.84	P4	0.269	0.98	P4	0.242	0.91	OSP	0.116	0.98	1
P12	0.132	0.85	P12	0.135	0.99	P12	0.147	0.98	Z9	0.110	0.98	1
P16			P16	0.143	0.99	P16	0.101	0.99	Z19	0.099	0.99	1
P20	0.127	0.94	P20	0.118	0.99				California	0.070	0.86	2
OSP	0.117	0.99	OSP	0.094	0.96							
Z4				0.094	0.98							
Z9				0.207	0.99							
Z14				0.069	0.94							
Z19				0.220	0.85							

Table 3.2 Comparison between Zn/Si relationships in the mixed layer from this work and from published data. 1. Martin et al. (1989) and 2. Bruland et al. (1994).

The increased spatial density of data in the present study allows trends in Zn/Si ratios to be observed. Along the line P transect in both seasons the Zn/Si ratio for the mixed layer demonstrates a clear trend of Zn/Si decreasing with distance from shore (Fig. 3.6). In February the Zn/Si ratios also decrease offshore along Line P but to a lesser extent than that observed in September. The coastal stations have elevated concentrations of dissolved Zn and lower concentrations of Si compared to the open ocean. At the shelf station, P4 upwelling and coastal inputs would increase the Zn concentration, while biological (diatom) production would lower Si concentrations. De La Rocha et al. (2000) has observed that Zn is required by diatoms for

silicification and therefore the increased dissolved Zn concentrations close to shore could potentially increase the rate of Si uptake if limited by Zn. Conversely the offshore low dissolved Zn concentrations could inhibit diatoms from taking up Si leading to higher Si concentrations within this region.

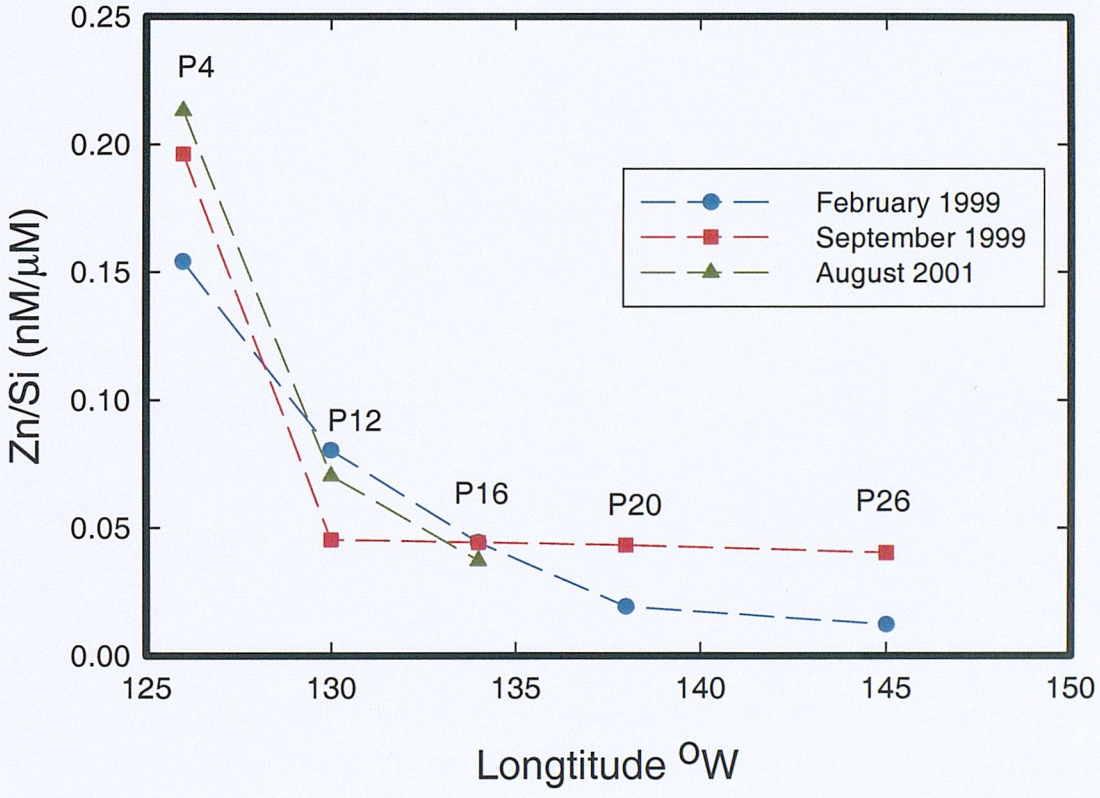


Figure 3.6 Zn/Si ratios along line P transect with stations labelled P4 to P26 in both February and September 1999 and in August 2001.

The difference in Zn/Si ratios on moving offshore infers a decoupling between Zn and Si concentrations in the upper ocean. The increased surface Si concentrations offshore could reflect either an increase in Si input to surface waters, or a decrease in the biological uptake and removal of Si. Decreasing Zn concentrations in surface waters reflect reduced atmospheric inputs with increasing distance from shore, and /or more effective removal of Zn from surface waters than Si. Any input of Zn and Si to the mixed layer by upwelling will reflect the pre-formed deep-water Zn/Si ratio, and not explain the different ratios seen in surface waters.

The similarity of the oceanic dissolved Zn and Si profiles has led to the hypothesis that a significant fraction of Zn is taken up by, and regenerated from, phytoplankton, in some biogenic carrier phase, in a manner similar to Si (Bruland, 1980; Bruland & Franks, 1983; Yeats & Campbell, 1983; Morley *et al.*, 1993). However, the amount of Zn incorporated into opal represents only 1-3% of the total amount of Zn taken up by diatoms (Ellwood & Hunter, 2000). Additionally, a significant amount of Zn in phytoplankton is associated with organic tissue that is not rapidly recycled in seawater, unlike, P, Cd, Cu, Ni and Mn which are rapidly remineralised (Collier & Edmond, 1984). Thus it appears that Zn is associated with a fraction of organic matter that degrades slowly in relation to that fraction which contains Cd and P. This comparatively Zn-rich material would therefore be expected to spend longer in the POM pool, as it is less readily recycled, and show a deeper regeneration pattern that appears similar to the dissolution-driven Si profile. Thus the implication is that the Zn/Si relationship in the deep ocean is not solely a result of Zn released from biogenic opal.

The atmospheric supply of Zn to the surface Pacific is small. Bruland *et al.* (1994) calculated that the dissolved flux of Zn to the North Pacific gyre was 1.1 μmol m^{-2} yr^{-1} and estimated that the increase in concentration in the upper 25m was only 0.01 nmol kg^{-1}. Bruland *et al.* (1994) also reported low summertime scavenging of ^{234}Th, for the central Pacific gyre, thus allowing time for the atmospheric input of dissolved metals to accumulate. This situation does not hold true for the subarctic North Pacific where relatively high rates of ^{234}Th scavenging have been reported along the line P transect in August (Charette *et al.*, 1999). If the elevated rates of scavenging reported for this area correspond to removal of dissolved Zn to deeper waters, the aeolian input of Zn will be depleted from surface waters and would be less than the 0.01 nmol kg^{-1} increase of Zn reported by Bruland et al. (1994).

From the data of Duce *et al.* (1991), and assuming 10% of the silicon present will dissolve in seawater, it can be estimated that the aeolian input of dissolved Si to the North Pacific is 3.16 mmol m^{-2} yr^{-1}. Approximately 60% of Si produced by diatoms in the photic zone dissolves in the upper 100m (Nelson *et al.*, 1995). Therefore, there is a significant aeolian input of Si to these waters compared to Zn. As stated earlier Zn is associated with organic matter, which is not readily remineralised (Collier & Edmond, 1984). Therefore, in contrast to Zn, Si is more efficiently recycled in the upper water column and thus maintains the dissolved Si in surface waters that has been released from aeolian inputs, and supplied by upwelling.

Thus an hypothesis that limited Zn inputs and removal in particulate organic fallout, in combination with more efficient recycling of dissolved Si and more significant aeolian supply, lead to the pattern of decreasing Zn/Si ratios offshore observed in the current study is made.

3.4.4 Zinc Speciation

3.4.4.1 Zinc binding ligand

At present little is known about Zn speciation in open-ocean regimes due to the difficulty in collecting uncontaminated samples and determining Zn-complexing ligand concentrations. The results in this study for the Zn-binding ligand are similar to those reported in the Atlantic (Ellwood & Van den Berg, 2000) and the North Pacific (Donat & Bruland, 1990) for P4 – P16 suggesting that the samples were uncontaminated with Zn. However, lower concentrations were observed at OSP in June 2001 (0.72 nM) and are the lowest reported for open ocean work. The Zn-complexing ligands have been shown to be selective for Zn, as addition of Cu roughly equivalent to the concentration of the Zn-complexing ligands could be added to the sample without changing the results (Bruland, 1989). However, if sufficient Cu was added (> 2nM) to completely complex the strong Cu-binding organic ligands the Zn′ sensitivity is severely depressed due to the formation of a Cu-Zn intermetallic compound (Bruland, 1989).Van den Berg (1995) has shown that the stability constants of natural organic complexes increases strongly in the order Zn < Pb < Cu << Co < Ni << Fe and that the natural organic ligands show an increased specificity for metals in seawater in the above order.

The low Zn-binding ligand concentration observed at 10m at OSP could be due to photochemical breakdown of the natural ligand in the surface euphotic zone. This has been previously reported for Cu (Moffett *et al.*, 1990; Achterberg, 1993;) and for Fe (Van den Berg, 1995). However, at P4 and P16 the concentration of the Zn-binding ligand is higher in the surface waters, indicating at these stations photochemical breakdown is unlikely. This low concentration could also reflect the low total dissolved Zn concentration observed (0.05 nM) or due to low primary productivity in near-surface waters.

Highest concentrations of Zn-binding ligand have been observed in surface waters (40-100m). Enhanced ligand production in open ocean surface waters for Cu and Zn has previously been observed (Coale & Bruland, 1988; Moffett *et al.*, 1990; Ellwood &Van den Berg, 2000). The

source of these Zn-binding ligands is thought to be by direct production by phytoplankton or a metabolic by-product released indirectly by grazing and cell lysis (Bruland, 1989; Ellwood & Van den Berg, 2000; Muller *et al.,* 2003). The role of bacteria in the production of these Zn-binding ligands has yet to be investigated but the producing of ligands would be expected as this has been observed for Fe (Butler, 1988; Maldonado & Price, 1999). It has been hypothesised that these Zn-complexing ligands are produced by certain phytoplankton to reduce the Zn^{2+} concentrations at the expense of other phytoplankton (Bruland, 1989). The fluorescence maximum (35-50m) corresponds with the area of highest primary production causing nitrate depletion. In the mixed layer (~40m) there appears to be no relationship of the Zn-binding ligand with fluorescence, a proxy for phytoplankton concentration. However, only four stations were analysed in this study and only three points in the upper water column were sampled for Zn-binding ligands. A previous study in the North Atlantic demonstrated no relationship between Zn-binding ligands and fluorescence (Ellwood & Van den Berg, 2000) but only one vertical profile was sampled in that study.

The relationship between phytoplankton and natural Cu-binding ligands was investigated by Robinson & Brown (1991), who observed an increasing Cu complexing ligand concentration with increasing concentration of *Gymnodinium sanguineum.* Copper and Co-binding ligands are related to both the chlorophyll *a* and particle maxima (Hanson *et al.,* 1988; Saito & Moffett, 2002). One problem with ligand production by phytoplankton is the energy required to produce these ligands would lower the return each individual cell would obtain from its production (Bruland, 1989). However, algae are known to produce large quantities of these ligands in response to toxic Cu^{2+} concentrations and for Fe when cells are Fe limited (Moffett & Brand, 1996; Rue & Bruland, 1997; Leal & Van den Berg, 1999). Complexing ligand titrations have indicated that natural ligands in seawater tend to pass through C-18 columns unless complexed by Cu (Donat *et al.*, 1986) or Fe (Gledhill & Van den Berg, 1994) when they are retained to various extents, suggesting that the free ligands tend to be rather hydrophilic whist complexes are more hydrophobic. This could suggest a pathway by which hydrophilic free ligands can be exuded from hydrophobic phytoplankton cells and the hydrophobic complexes re-absorbed (Van den Berg, 1995).

Another hypothesis is that the Zn-binding ligand is a degradation product or an enzyme from dead and decaying organisms sinking out of the water column (Ellwood & Van den Berg, 2000). Evidence for this theory comes from the relative constant spatial temporal distribution of the Zn

ligand in deep waters of the Atlantic. However, in the Pacific the high concentration of total dissolved Zn is in excess of the Zn-binding ligand and this has not been observed in the Pacific in this study or in one carried out off the coast of California (Bruland, 1989; Donat & Bruland, 1990). Muller *et al.* (2003) have demonstrated that increased Zn ligand concentrations was related to the death of a *Emiliania huxleyi* bloom and attributed this to the break-up or bacterial decomposition of algal cells. Zinc ions are known to stabilise proteins structurally by forming a "zinc finger" which allows polypeptide loops to fold around the Zn ion thus holding the polypeptide molecules in a rigid domain (Barnes *et al.*, 1998; Ellwood & Van den Berg, 2000). Therefore the Zn-binding ligands found in oceanic waters could be polypeptide molecules produced during the decay of phytoplankton. Further studies are necessary to understand the exact nature of the Zn-binding ligands.

The binding of Zn by dissolved organic ligands which form strong complexes is thought to render Zn less particle reactive, thereby extending its residence time in oceanic surface waters (Albergoni & Piccinni, 1983). This would help alleviate any limitation of phytoplankton growth by low Zn concentrations, if phytoplankton can take up Zn from the organic ligand complexes. This has been demonstrated for Fe, where specific Fe mediated uptake by desferrioxamine B, a siderophore produced by microorganisms complexes Fe and transports this Fe across the cell membrane (Maldonado & Price, 1999; Martinez *et al.*, 2001). In this case an organism, which can produce these types of organic ligands and utilise the complexes of Fe directly would have an advantage of obtaining Fe for growth without having to alter the speciation of Fe.

3.4.4.2 Zinc ion concentrations

It has been argued that trace metal uptake in organisms is controlled by the concentration of free aquated ions or kinetically labile inorganic species (Hudson & Morel, 1990). If this theory is correct, the profiles for $[Zn^{2+}]$ are therefore of greater biological significance than those for total Zn. Minimum Zn^{2+} concentrations were observed in the euphotic zone due to Zn complexation with organic ligands, and uptake by phytoplankton. In addition maxima were observed in the surface layer below the euphotic zone (100m). This could be due to remineralisation of organic material at these depths, with subsequent release of zinc ions. Zn^{2+} concentrations showed a much larger variation with depth than total dissolved Zn; i.e. total dissolved Zn varied by a factor of 18, whereas Zn^{2+} varied by two orders of magnitude.

The Zn^{2+} concentrations observed at P4, P12 and P16 ranged from 5.25 pM in surface waters to 0.1 nM at 400m are similar to those observed in the Pacific and Atlantic (Bruland, 1989; Ellwood & Van den Berg, 2000). However, at OSP the observed Zn^{2+} concentrations (0.97 – 4.14 pM) in the top 100m are the lowest reported for open ocean studies, and these values could have implications for phytoplankton growth.

The present study of Zn speciation in the North East Pacific has demonstrated that between 94 and 99.5% of the dissolved Zn was bound to organic ligands throughout the upper water column (Appendix 3.2). These percentages of organically complexed Zn agree well with other literature values for open ocean work. Bruland (1989) observed values in the central North Pacific ranging between 96 –99% in the upper 200m, whilst Ellwood & Van den Berg, (2000) found values ranging between 96-99% in the surface waters (15m) of the North Atlantic.

3.4.5 Implications of Zn speciation on phytoplankton growth

In the subarctic North East Pacific there are relatively constant standing stocks of phytoplankton year round (Boyd *et al.*, 1995a), as is reflected in the relatively constant chlorophyll *a* concentrations in February and September. Phytoplankton require Zn for growth, and Zn^{2+} is reported as the major Zn species taken up by phytoplankton (Anderson & Morel, 1978; Sunda & Huntsman, 1992). Using a C : Chlorophyll *a* ratio of 50 μg : μg and the Zn : C ratio of 0.4 μmol : mol for *Emiliania huxleyi* (Sunda & Huntsman, 1995a), an estimate of the Zn required by phytoplankton was obtained for OSP. On this basis an estimate that 0.4 pM is required by *E. huxleyi* for a growth rate of 0.8 d^{-1} is calculated. This is 100 times lower than the concentration of total Zn (0.04 nM) in the surface water column. However, Zn is complexed by Zn specific ligands which acts to reduce the concentration of Zn^{2+} to 0.72 pM. Culture experiments have shown that at concentrations of Zn^{2+} of 1 pM, growth of some species of phytoplankton, particularly diatoms, are limited by Zn concentrations (De La Rocha *et al.*, 2000; Sunda & Huntsman, 1995a). Therefore it is possible that Zn concentrations at OSP are limiting phytoplankton growth. This would not be the case for P4, P12 and P16 where Zn^{2+} concentrations ranged from 5.26 – 69.6 pM and as such are not considered limiting to phytoplankton growth. However, culture experiments are carried out using artificial chelators such as EDTA to maintain the free Zn^{2+} ion concentration at low levels and artificial seawater. These culture experiments rely on the free metal ion theory and assume that phytoplankton cultures are similar to those in the open ocean

environment. While much has been learnt from these culture experiments more work is needed in open ocean water with natural phytoplankton assemblages in order to investigate if Zn is limiting phytoplankton growth.

Both Co and Cd are reported as being able to substitute for Zn in the enzyme carbonic anhydrase in some marine diatoms and maintain growth rates at low available Zn levels (Price & Morel, 1990; Sunda & Huntsman, 1995a). As the Zn^{2+} concentration approaches 10^{-12}M, the uptake of Co^{2+} and Cd^{2+} increases rapidly until its supply is limited by diffusion across the cell boundary layer (Sunda & Huntsman, 1995a; 1998). Cobalt replacement for Zn has been hypothesised as an adaptive strategy for growth in the open ocean where Zn levels are low (Sunda & Huntsman, 1995b). However, surface water concentrations of Co are even lower than Zn and highly variable in the North Pacific (Martin *et al.*, 1989) and much of that inorganic Co may not be bioavailable (Zhang *et al.*, 1990). Sunda & Huntsman (1995a) concluded that the concentrations of both Co and Zn within this region are low, thereby limiting the growth of some fraction of the phytoplankton community. This weakens the case for a long-term adaptive strategy of Co substituting for Zn (Whitfield, 2001). At OSP the concentration of total dissolved Cd is higher than Co (Martin et al., 1989) but is still lower than the concentration of Zn reported in this study. It has been reported that despite the lower Cd levels, Cd uptake may be higher in the open ocean than coastal waters due to the low ambient levels of Zn and Mn, which would induce the additional pumps necessary for Cd uptake (Whitfield, 2001).

Although Cd and Co may be able to substitute for Zn, the low concentration of all three elements in the North East Pacific combined with the high Si concentration support the hypothesis that phytoplankton growth could be limited by these micronutrients, in addition to Fe. Incubation studies at OSP are needed to fully investigate if Zn is limiting phytoplankton growth in this region. Unlike previous incubation studies at OSP (Coale, 1991) knowledge of total dissolved Zn concentrations are not enough to investigate the role of Zn in oceanic phytoplankton and therefore Zn speciation studies will be required.

3.5. Summary

Total dissolved Zn profiles for the upper ocean are consistent with earlier work, and extend our knowledge of Zn in the subarctic North East Pacific Ocean. Zinc is depleted in surface waters and increases with depth in close correlation with dissolved Si concentrations. Concentrations of

Zn in the upper mixed layer decrease offshore along the line P transect (0.8- 0.04 nM). No major differences were observed in Zn concentrations during this study between winter and summer surveys, contrary to what was predicted by Martin *et al.* (1989), on the basis of seasonal changes in mixed layer depths in this region. However, sampling only took place in February and September and more extensive sampling at different months would be necessary to confirm the lack of seasonality in Zn concentrations, as episodic events such as blooms could influence the concentrations of Zn over shorter time scales. Surface Zn concentrations at OSP in winter are very low (0.07 nM).

Zn/Si ratios in the mixed layer show a decreasing trend with distance from shore, which infers a decoupling between Zn and Si concentrations in the upper ocean. It is therefore hypothesised that limited Zn inputs and removal in particulate organic fallout in combination with more efficient recycling of dissolved Si and more significant aeolian supply, lead to the observed trend.

The concentration of the Zn-complexing ligand is higher than total dissolved Zn in the upper water column. In the surface water column there is no relationship between ligand concentration and fluorescence. Zinc-complexing organic ligands strongly buffer free Zn ion activity in surface seawater with respect to perturbations in total dissolved Zn. At OSP the concentration of Zn^{2+} is 0.72 pM which may limit phytoplankton growth according to the current literature. Organic ligands existing at low nanomolar concentrations and forming strong Zn complexes effectively control the speciation of Zn in surface waters, with 93-99% of total dissolved Zn complexed. At depth Zn concentrations are much higher than the ligand concentration and therefore, the dissolved Zn is mainly present as inorganic species of Zn. This study has shown that Zn speciation is controlled by a number of dynamic processes resulting in considerable spatial variability.

Chapter 4

Influence of zinc and iron enrichments on phytoplankton growth in the subarctic North Pacific

4.1 Introduction

Over the past two decades there has been increasing evidence to suggest that the low total dissolved Zn concentrations observed in some oceanic environments may limit phytoplankton growth (Anderson & Morel, 1978; Morel *et al.*, 1991; Sunda & Huntsman, 1992; 1995a). At these low Zn concentrations microalgae are unable to make enough functional carbonic anhydrase which may limit carbon acquisition and consequently the rate of algal growth (Morel *et al.*, 1994; Tortell & Price, 1996; Tortell *et al.*, 2000). This limitation is particularly important for diatoms, which are responsible for fixing the bulk of carbon, which is exported from the surface to the deep ocean (Tortel *et al.*, 1997; 2000). This however, remains a controversial theory as there is conflicting evidence as to whether or not Zn is limiting phytoplankton growth in the open ocean. Experiments on Zn speciation in the North Atlantic indicate that Zn is not limiting phytoplankton growth in this region (Ellwood & Van den Berg, 2000). Conversely, in High Nutrient Low Chlorophyll (HNLC) regions such as the subarctic North Pacific phytoplankton growth is known to be limited by Fe (Martin & Gordon, 1988; Coale, 1991; Boyd *et al.*, 1996; Boyd *et al.*, 2000). As the total dissolved Zn concentrations are extremely low (0.04 nM, Chapter 3) phytoplankton growth may also be limited by Zn in the subarctic North Pacific.

Different phytoplankton groups can strongly affect the export of trace metals, nutrients and particulate carbon from the surface to deeper waters (Wassmann, 1998). Phytoplankton also have the ability to exude organic substances and metal ligands into the surface ocean (Bruland, 1989). Phytoplankton affect trace metal chemistry in natural waters through surface reactions, by taking up the metal directly and by production of extracellular organic matter with metal complexing properties (Vasconelos *et al.*, 2002). Depending on their physiochemical properties, these ligands can influence metal availability to the algal species that produce them and also to their competitors (Whitfield, 2001) by lowering the concentration of the free metal ion. At any point

during the cycle of ligand production and degradation, different micro-organisms will seek to optimise their use of essential metals according to their cellular quotas (Morel *et al.*, 1991), uptake rates (Hudson & Morel, 1993) and concentration mechanisms (Hudson & Morel, 1993; Sunda & Huntsman, 1998) leading to changes in community structure with time (Muller et al., 2003).

Extensive field studies of trace metal biota interactions are lacking in HNLC regions. Previous enrichment studies at OSP have examined the impact of additions of trace metals on phytoplankton growth (Martin *et al.*, 1989; Coale, 1991; Boyd *et al.*, 1996; Crawford *et al.*, 2003). While these studies have revealed that Fe strongly limits phytoplankton growth they have not assessed the production of complexing ligands by phytoplankton and the impact that this has on the speciation and availability of Fe and Zn to phytoplankton. Most of the laboratory culture studies for Zn using in vitro cultures (e.g. Brand *et al.*, 1983; Sunda & Huntsman, 1992) have been carried out using natural seawater supplemented with trace metal buffer systems (e.g. EDTA and NTA), where the natural organic ligands (including those released during growth) have been ignored. Coale (1991) studied the effect of added Cu on the production of Cu complexing ligands and the free Cu^{2+} ion concentration although only 3 out of the 7 days were examined.

To date, the few field studies where incubations of surface HNLC waters have been supplemented with Zn have suggested minimal effects in phytoplankton growth (Coale, 1991; Shareck, *et al.,* 1997; Franck et al., 2000; Gall *et al.*, 2001). A previous study at OSP investigated the effect of addition of Zn to HNLC waters, and demonstrated that Zn has a minor effect in stimulating growth and production of phytoplankton and increased the short term carbon fixation rates (Crawford *et al.,* 2003). The addition of Fe and Zn in combination compared to Fe alone resulted in a significant shift in physiology and size distribution of chlorophyll within the population (Crawford *et al.,* 2003).

The Institute of Ocean Sciences (IOS) on Vancouver Island, Canada manage a time series at Ocean Station Papa (OSP), which is one of the longest records of oceanographic properties and involves three research cruises a year along Line P (Chapter 2, Fig. 2.1; Whitney & Freeland, 1999). This provided the opportunity to carry out a shipboard study on the influence of Zn and Fe enrichments on incubations of the natural oceanic phytoplankton population. A previous incubation experiment was carried out in September 1999, where the total dissolved Zn concentrations were measured on two of the eight days (Crawford *et al.*, 2003). This study

focuses on the influence of total dissolved Fe and Zn and Zn speciation on chlorophyll *a*, nutrient concentrations and species composition over an 8 day shipboard incubation carried out during a research cruise in the NE Pacific in June 2001. In one treatment both Fe and Zn were added as Fe is known to increase phytoplankton growth and it could potentially therefore stimulate release of more Zn specific organic ligands.

4.2 Methods

4.2.1 Introduction

An experimental strategy was designed to incubate a series of 4L aliquots of natural surface High Nutrient Low Chlorophyll (HNLC) NE Pacific water supplemented with Fe, Zn plus Fe and Zn and a control (no additions) and incubate this water on deck onboard ship. One 4L aliquot from each set was removed and subsampled on a daily basis for changes in trace metals and biological parameters over an 8 day period. The water samples were not pre-filtered to exclude grazing organisms prior to incubation.

4.2.2 Acid Cleaning

The 4L low density polyethylene (LDPE) flexible cubic containers ('cubitainers') (VWR Canlab) were acid cleaned by soaking for 24 hours in 5% 'Extran' followed by a rinse in MQ. The cubitainers were then soaked for 24 hours in 10% HCl and rinsed with MQ. This was followed by soak in a 1% ultra-pure HCl solution (Seastar) for a further 24 hours and rinsed with MQ. Finally the cubitainers were soaked in 0.1% acetic acid (Seastar) for seven days, emptied but taken to sea wet with acid. They were finally rinsed in open ocean water and left to soak in oceanic water for 2 days before arriving at OSP, in order to permit equilibration and thus minimise adsorption of trace metals onto the container walls. Once arriving on station the cubitainers were rinsed three times with OSP surface (15m) seawater 3 times prior to filling. For cleaning and rinsing, the flexible LPDE cubitainers were maintained in the flattened shape in which they were supplied. This minimised internal volume and thus reduced the amount of soaking solution required.

4.2.3 Sampling

Samples for the incubation experiment were collected on cruise 2001-08 of the research vessel *C.S.S. John P. Tully*. Seawater was collected from OSP during the hours of darkness between the 17th and 18th of June 2001. Samples were collected from 15m using a Teflon pump (PFD-1 Asti) and Teflon tubing. The tubing was attached with plastic tape to a Kevlar line weighted with resin coated lead weights and deployed over the side of the ship. Samples were pumped directly into 32 acid-cleaned LDPE 4-litre cubitainers in an on-deck PVC Ultra Low Penetration Air (ULPA) clean hood. Rinsing and filling the cubitainers took most of the night and therefore the species composition could have changed during that time. In order to minimise any bias, the filled cubitainers were randomly assigned to one of the 4 treatments.

4.2.4 Experimental Procedure

Once filled, additions of Zn as $ZnCl_2$ equivalent to 10 nM Zn were made to 8 cubitainers, another 8 filled cubitainers were spiked to 10nM Fe ($FeCl_3$), and 10 nM Fe plus 10nM Zn added to another 8 cubitainers. The remaining 8 cubitainers were utilised as a control (Fig 4.1). In many previous incubation experiments Fe is added in a 1:1.5 molar ratio with EDTA solution to ensure initial solubility of Fe and it had been reported that this does not affect the overall speciation (Coale, 1991; Boyd *et al.*, 1996). Upon the addition of EDTA it is argued that the supply of Fe to the cell is then automatically related to the concentration of free Fe, as the EDTA bound Fe is not directly available (Boye & Van den Berg, 2000). However, as the natural speciation of Zn was to be measured in all treatments the addition of EDTA was not possible, as this would affect the speciation measurements. All cubitainers were double bagged in clear heavy-duty polyethylene bags to minimise contamination from the incubator coolant water and were placed in incubators before dawn on deck in a running surface seawater bath to maintain mixed layer temperatures (~ 7°C). Neutral density screening provided spectrally unmodified shading to 30% of the ambient light level, estimated to correspond to the light level at the depth of collection. Cubitainers were randomly placed in the incubators to minimise any shading bias due to position on-deck.

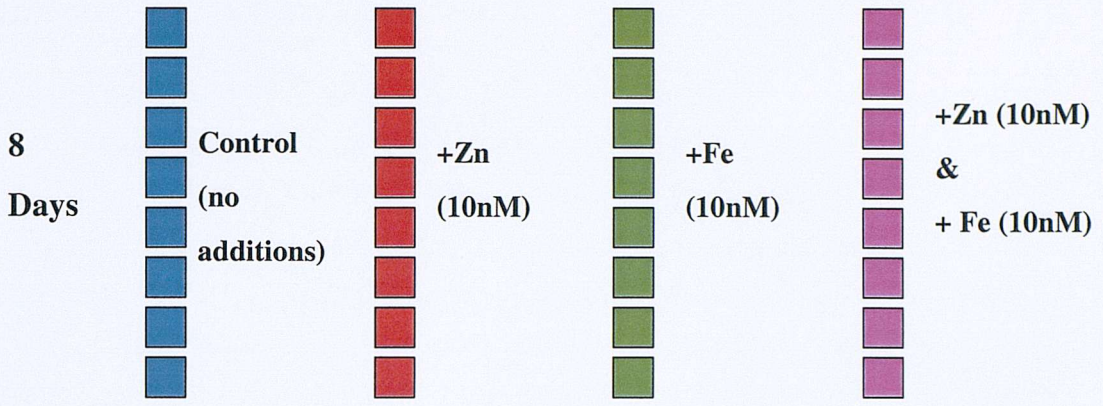


Figure 4.1 Experimental design for the incubation experiment. Each coloured block represents a cubitainer which was removed and sampled, one for each treatment and each day of the experiment.

Each cubitainer was sub-sampled once only. These cubitainers were not repetitively sampled thereby avoiding contamination during the subsampling procedure. Filtered samples were taken from pumped supply at the time of collection (t_0) for total Zn, Fe and Cd analysis using an acid cleaned 0.22 μm acid cleaned polycarbonate membrane filter (Nuclepore). Samples were also taken from the pumped supply at t_0, for chlorophyll *a*, nutrients, total CO_2 and total alkalinity (data for the latter are not shown here).

During the course of the experiment, one cubitainer from each of the four treatments was randomly removed each evening at 6 pm after the light period and was sampled for Chlorophyll *a*, nutrients (N, P, Si), phytoplankton counts, total alkalinity, total CO_2, POC, total dissolved Zn and Zn speciation. Due to the limited water volume available (4L), no replication of any samples was possible. In a previous incubation experiment carried out in 1999 each sample was triplicated and the results for the measured parameters showed very little difference between each triplicate (Crawford *et al.*, 2003). It was decided that having daily measurements (without replication) over 8 days would allow a more detailed study of trace metal interaction with biota rather than having only three time samples with replication.

4.2.5 Methods

Chlorophyll *a* and nutrient samples were collected and analysed by David Crawford (SOC) and Wendy Richardson (IOS) as outlined in Chapter 2.

Phytoplankton samples (250ml) for cell counts were preserved in formalin (0.4%; buffered with hexamethylenetetramine) according to Booth *et al.* (1993). In the onshore laboratory, 100ml subsamples were concentrated to 2ml in settling chambers (Utermohl, 1958) for the first few days of the experiment. However, in the Fe enriched treatments after day 3, only 10-50ml was needed. Phytoplankton cells were counted by Tawyna Peterson (UBC) using an inverted light microscope (Zeiss) counting a minimum of 400 cells per sample and a minimum of 5 fields (UNESCO, 1978).

Samples for POC (750 ml) were collected on precombusted GF/F filters (4h, 450° C) and then stored at –20° C. After drying, the filters were combusted in-vacuum and the resultant CO_2 gas was isolated by cryogenic trapping into glass vials. The glass vial was then attached to an Isotope Ratio Mass Spectrometer (IRMS). Once the glass vessel was opened to IRMS, the mass 44 (^{12}C $^{16}O^{16}0$) beam was measured and converted using a calibration curve to moles of POC. By repeating this procedure with a number of different concentration POC samples a calibration curve was drawn of the size of the mass 44 beam signal against moles of POC. This analysis was carried out by Hillary Kennedy at the University College of North Wales, Bangor.

Seawater samples for Zn analysis were immediately vacuum-filtered through acid-cleaned 0.22 μm polycarbonate membrane filter (Nuclepore) into 2 replicate 500ml LDPE bottles. Sample collection, filtration and analysis were carried out using clean techniques. One 500ml aliquot of each sample was acidified to pH 2 with 0.5ml ultra pure HCl (SeaStar) for total Zn analysis. The other 500ml sample was used for speciation studies, which were analysed within 3 hours after collection, while samples for total dissolved Zn analysis were analysed back at the SOC using AdCSV as described in Chapter 2. Measurements of total dissolved Zn, Fe and Cd were also carried out at the SOC using a chelation solvent extraction method, which is also described in Chapter 2.

4.2.6 Quantifying Adsorption of Zn and Fe during shipboard experiments

Previous experiments had reported a problem of adsorption onto the container walls during an incubation experiment (Martin *et al.*, 1989; Coale, 1991). Therefore, to ensure that any removal of trace metals in the enrichment experiment was an effect of adsorption onto particles or onto

phytoplankton or uptake by the phytoplankton, and was not an effect of adsorption onto the container walls, an experiment to measure adsorption was carried out. Seawater was collected at the same time as the samples collected for the shipboard incubation experiments. This seawater was not filtered and was stored in the cool and under darkness before analysis, several months later at SOC. Therefore, no live phytoplankton or zooplankton would be expected to be present in the samples. Additions of 10nM Fe ($FeCl_3$) and 10nM Zn ($ZnCl_2$) were made to the cubitainer and the experiment was run for eight days. No additions were made to the control sample. Each day, 250ml of sample was removed and analysed for total dissolved Fe, Cd and Zn by chelation and solvent extraction. At the end of 8 days the remaining 2L of sample in the cubitainer was removed, and a wash of the inside of the cubitainer done with sub-boiled distilled water and then 10% HCl. These solutions were then analysed to assess if any material had adsorbed to the container walls on this timescale.

4.3 Results

4.3.1 Chlorophyll a and Nutrients

Chlorophyll *a* was similar in all treatments (0.47-0.44 μg/L) initially but results diverged dramatically between days 3 and 4 (Fig 4.2; Appendix 3). The Fe enriched treatments showed a large stimulation of phytoplankton growth with a sixfold higher final concentration of chlorophyll *a* (up to 9.9 μg/l) compared to the control, which increased only by a factor of 3. There is no difference in chlorophyll *a* concentrations between the control and the Zn enriched treatment with the concentration increasing by a factor of three (1.45 μg/l) during the course of the experiment. In the Fe enriched treatments there is a small increase in chlorophyll *a* concentrations between days 1 and 3 after which a large increase in chlorophyll *a* is observed.

Changes in the nutrient concentrations reflected the above changes in chlorophyll *a* (Fig. 4.3 a, b, c; Appendix 3). Iron addition removed all measurable nitrate after 8 days (13.3μM to concentration below detection limits) and phosphate concentrations decreased from 1.32 - 0.29 μM. Phytoplankton were able to remove only 18% (13.3 - 10.9 μM) of the available nitrate and 15% (1.32 – 1.12 μM) of the phosphate in the control and the added Zn treatment. Silicic acid showed a strong removal between day 6 and the end of the experiment in the Fe enriched treatments resulting in 38% (19.2 – 11.9μM) uptake of available silicic acid by phytoplankton

(Fig 4.2 c). The control and added Zn treatments showed only a slight removal of silicic acid and only between day 6 and 8.

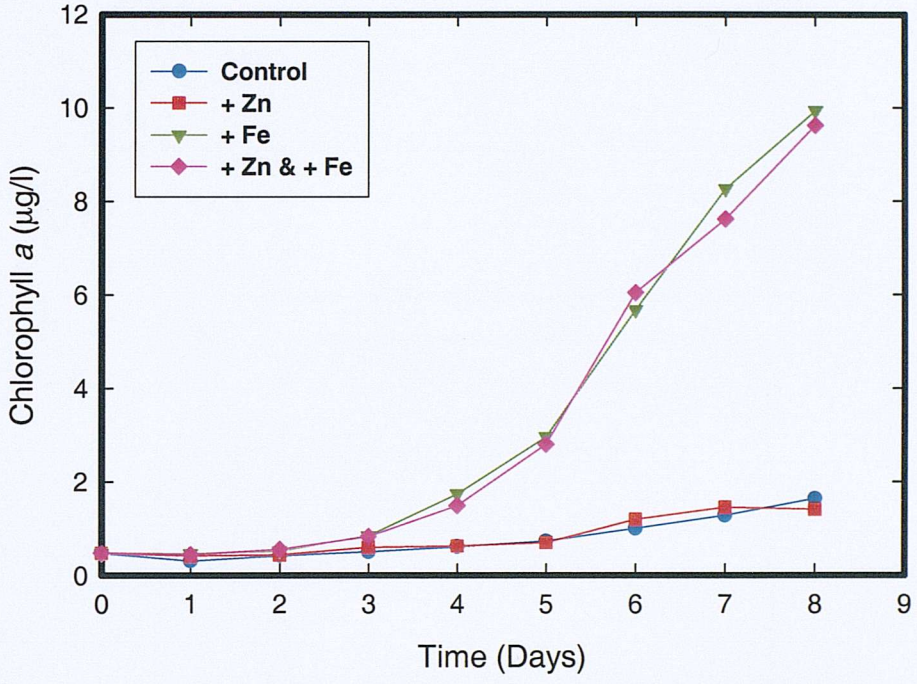


Figure 4.2 Chlorophyll *a* concentrations throughout the eight days. Note the chlorophyll *a* concentrations are based on one replicate only.

4.3.2 Particulate Organic Carbon

POC data shows a similar trend to the chlorophyll *a* data (Fig 4.4; Appendix 3). A small increase was observed in both the control, with a slightly larger increase observed in the Zn treatment (23.4-29.0 µM in the control and 23.4 – 34.6 µM for added Zn). The POC increase in the control and Zn enriched treatment is proportionately much less than the increase in chlorophyll *a*. The addition of Fe and Fe plus Zn produced considerably more POC after 5 days of incubation than in the control. Between days 7 and 8 the POC concentration approximately doubled in both treatments with added Fe (Appendix 4.1).

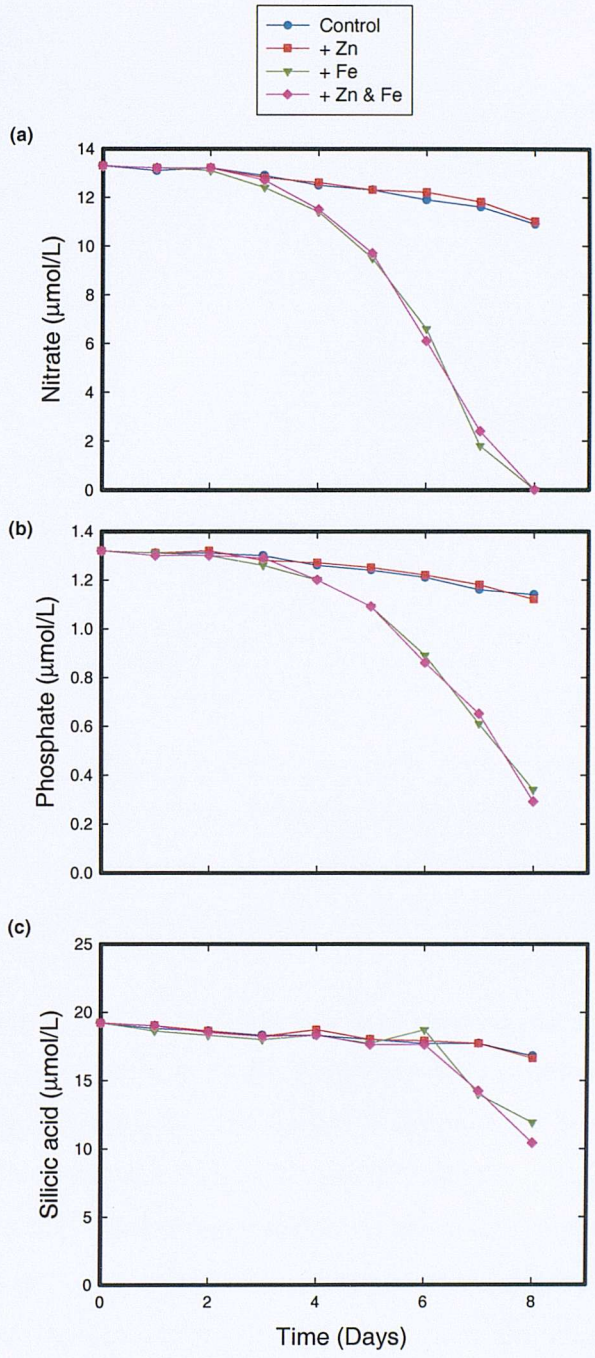


Figure 4.3 Nutrient concentrations throughout the eight day incubation (a) Nitrate (b) Phosphate (c) Silicic acid.

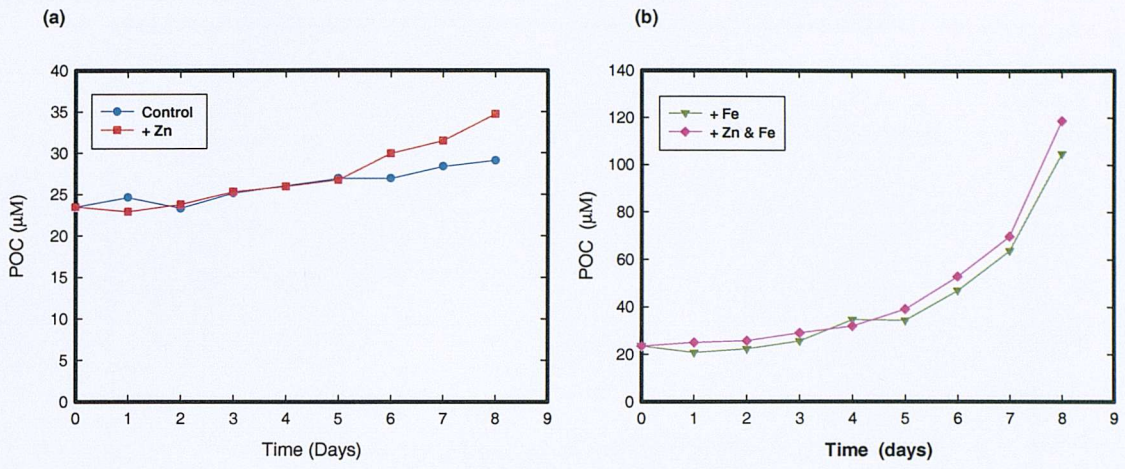


Figure 4.4 Particulate Organic Carbon (POC) concentrations in (a) the Control and Zn enriched treatment and (b) in the Fe enriched treatments.

4.3.3 Trace metal concentrations

Despite precautions taken to avoid contamination, a small increase in total trace metal concentrations was observed between day 0 and day 1 in the control (Appendix 4). The increase is very small (0.02 nM Fe, 0.03nM Cd and the change in Zn is below the detection limit for this technique) and as such does not represent a large degree of contamination.

Although problems with adsorption and desorption have been suggested in previous incubation experiments, the adsorption experiment in this study showed no evidence for significant adsorption and/or desorption (Fig 4.5). The amount of added 10 nM Fe plus 10 nM Zn adsorbed onto the wall of the cubitainers after 8 days was only 0.34 nM Zn and 0.26 nM Fe. This is less than the 9.3% adsorption of Fe that was reported by Martin *et al.* (1989). Coale (1991) reported that 39% of Zn added was adsorbed onto the container walls by assuming that all the Zn removed from the dissolved phase throughout the 7-day experiment was adsorbed onto the container walls and no uptake by organisms occurred. In the present experiment, in the control there was no detectable removal of either Zn or Fe with concentrations remaining relatively constant at 0.08 nM Zn and 0.1 nM Fe. This is similar to results reported by Takeda & Obata (1995) in the equatorial Pacific. These seawater samples had not been acidified and no bactericide was added so some bacteria will be present and could have a small effect.

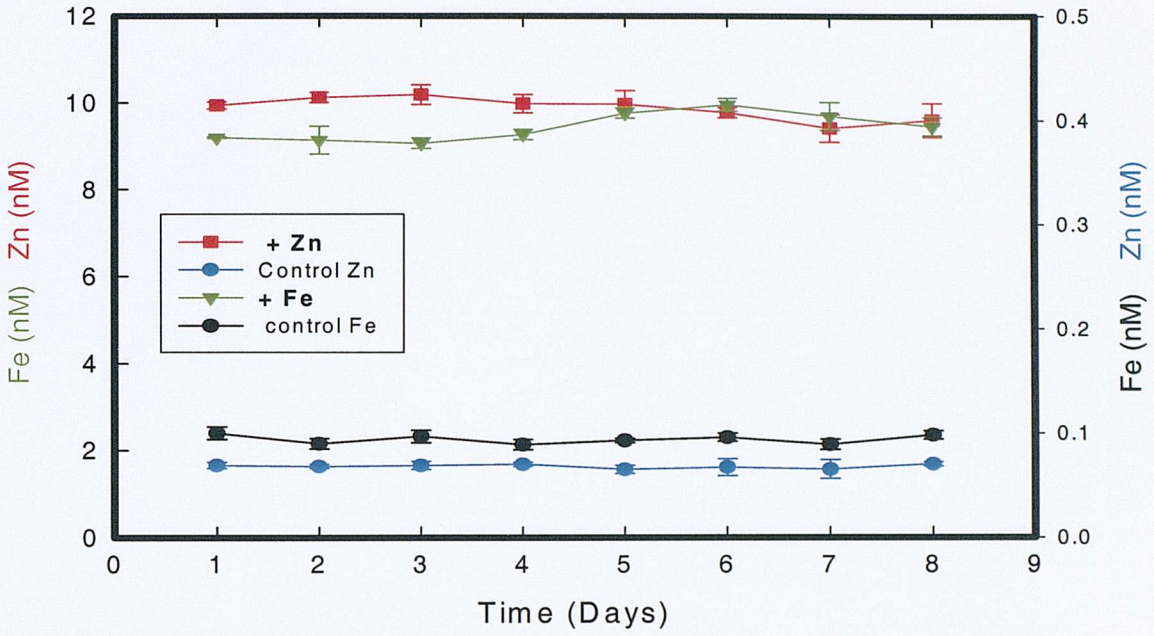


Figure 4.5 Adsorption/desorption of total dissolved Zn and Fe from walls of 4L cubitainers.

Results from this experiment suggest that no phytoplankton were present in the sample as only small changes of Fe and Zn were detected when compared to the incubation experiments. Only 250ml was removed for analysis each day for the eight days and therefore the surface area available for adsorption decreased to half (2L) in the eight days. Vasconelos *et al.* (2002) measured metal concentrations in algae (extracellular adsorption plus intracellular uptake) and found that the metal concentration in the algae balanced the metal lost from seawater, thus indicating adsorption onto container walls was negligible. Therefore in this study, given the minimal removal of trace metals to the walls of containers, the loss of metal from the dissolved phase can be assumed to be predominately through extracellular adsorption onto the and uptake into the cells.

4.3.4 Total Dissolved Trace Metal data

Cadmium concentrations, which were not manipulated in this experiment, were variable in all 4 treatments throughout the 8 days (Fig. 4.6) (Appendix 4). Some general trends can be observed. In the added Zn treatment Cd concentrations doubled between day 2 and 4 and decreased after day 6 to similar concentrations observed at day 1. At the end of the incubation, the lowest Cd concentrations (0.04 ± 0.01nM) were observed in the treatment with added Fe.

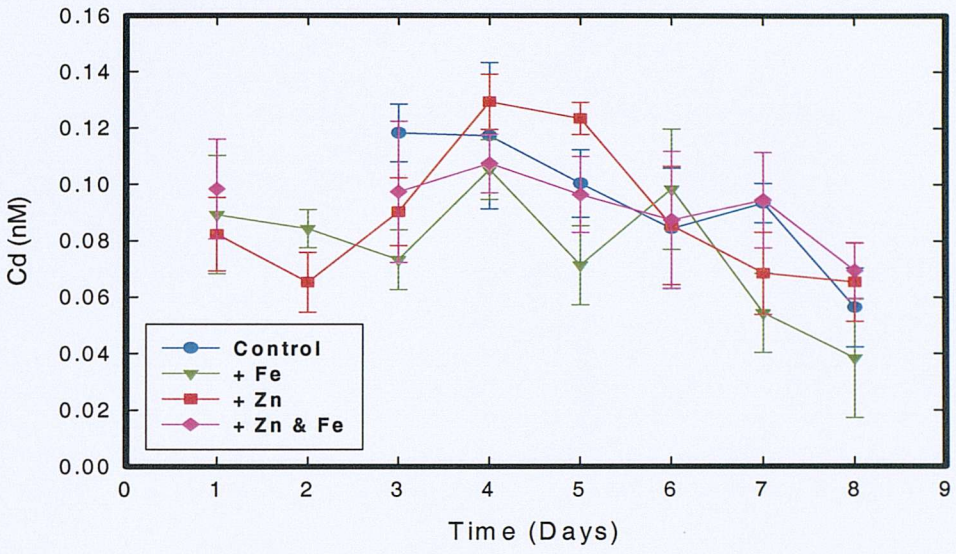


Figure 4.6 Cadmium concentrations throughout the eight day incubation. Error bars represent the standard deviation on three measurements of the same sample.

In contrast both total dissolved Fe and Zn concentrations showed a general trend of decreasing concentrations as the experiment progressed in all 4 treatments (Fig. 4.7 a, b & Fig. 4.8 a, b) (Appendix 4). In the Fe enriched treatments a rapid removal of Fe was observed until day 5, reducing the concentration of Fe from 11.3 nM to 3 nM after which less Fe removal was observed despite the increase in phytoplankton biomass and continued nutrient uptake (Fig 4.8 b). The rapid uptake of Fe observed between days 3 and 5, coincided with the start of the diatom bloom (see later). The Zn enriched treatments indicated an increased uptake or removal of Fe than in the control. The control showed relatively constant Fe concentrations until day 4 after which a slight decrease in Fe concentration was observed until day 6 and than a final decrease between day 7 and 8.

A similar trend was observed in the total dissolved Zn concentrations, where additions of Fe increased the uptake of Zn compared to the control. However, unlike the rate of Fe removal the Zn uptake during the start of the growth phase (days 1 to 3) was relatively small (11.9 – 10.3 nM). Between days 3 and 6 the samples show large decreases in Zn concentrations in all treatments except for the control, which remained constant until day 6 (Fig 4.7 a, b). There was a 16 pM removal of Zn in the Fe treatment compared to the control and an extra 2.5 nM uptake of

Zn in the Zn plus Fe treatment compared to Zn added alone. This difference is larger than the 0.51 nM difference observed in Fe uptake in the Zn plus Fe enriched treatment (Fig 4.8 b).

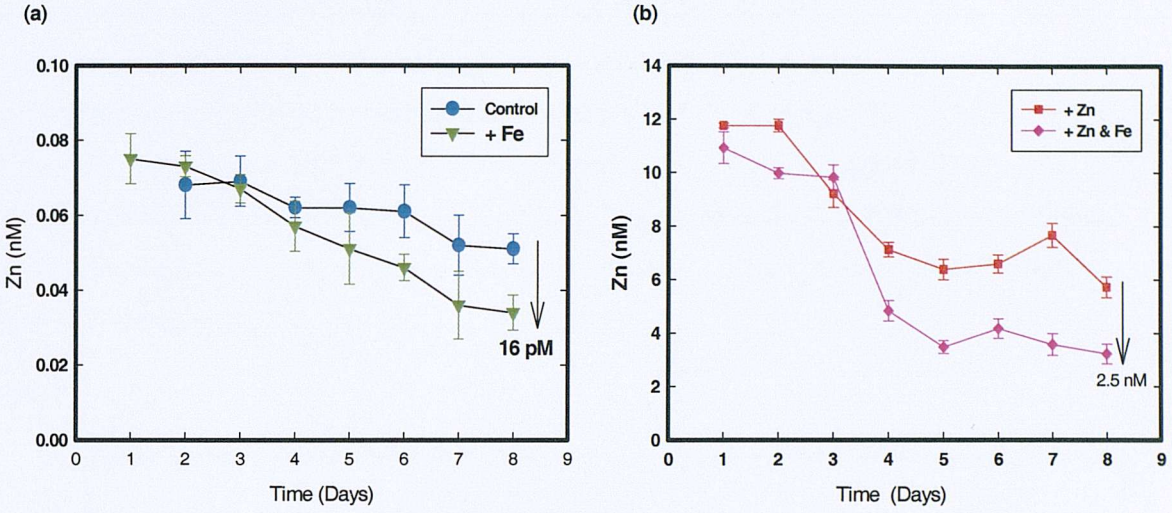


Figure 4.7 Total dissolved Zn concentrations in (a) the control and the Fe enriched treatment and (b) the Zn enriched treatments. Error bars represent the standard deviation on three separate measurements on the same sample. The arrow indicate the difference at the end of the experiment.

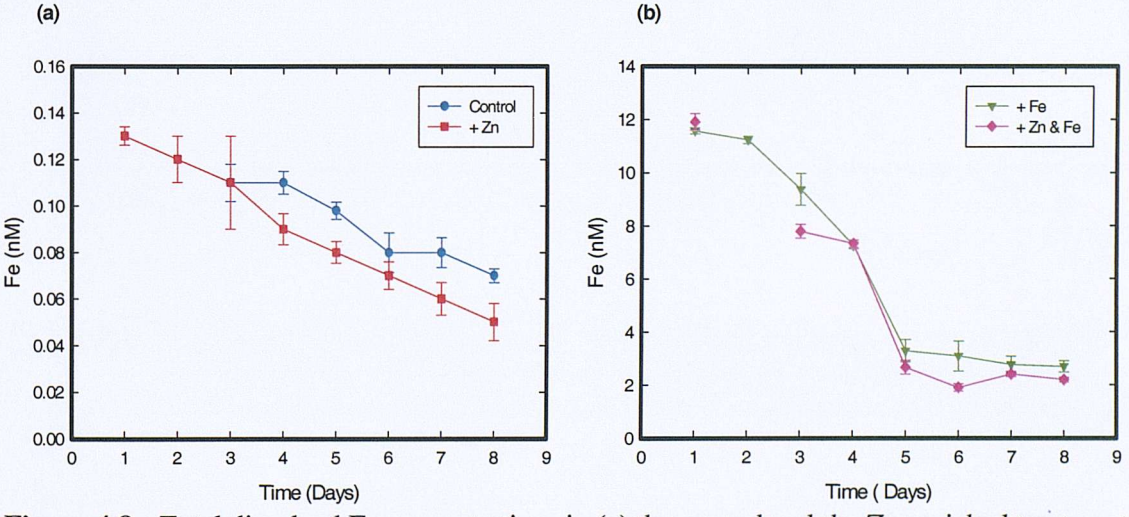


Figure 4.8 Total dissolved Fe concentrations in (a) the control and the Zn enriched treatment and (b) the Fe enriched treatments. Error bars represent the standard deviation on three separate measurements on the same sample.

4.3.5 Zinc Speciation Data

A typical ligand titration curve is presented in Figure 4.9 a. The clear curvature in the titration data at low Zn concentrations indicates the presence of a zinc binding ligand. As the total Zn concentration was increased, the electrode response became linear indicating that effectively all of the Zn complexing ligands had been titrated with Zn. Plots of $[Zn_{labile}]/[ZnL]$ vs $[Zn_{labile}]$ were linear (Fig 4.10 b) indicating that a simple one-metal model could be applied (Ruzic, 1982; Van den Berg, 1985; Ellwood &Van den Berg, 2000). For example, the data from Figure 4.9 was transformed using Eq. (2.4) to yield a ligand concentration of 0.26 ± 0.002 nM with a conditional stability constant (log K'_{ZnL}) of 10.8.

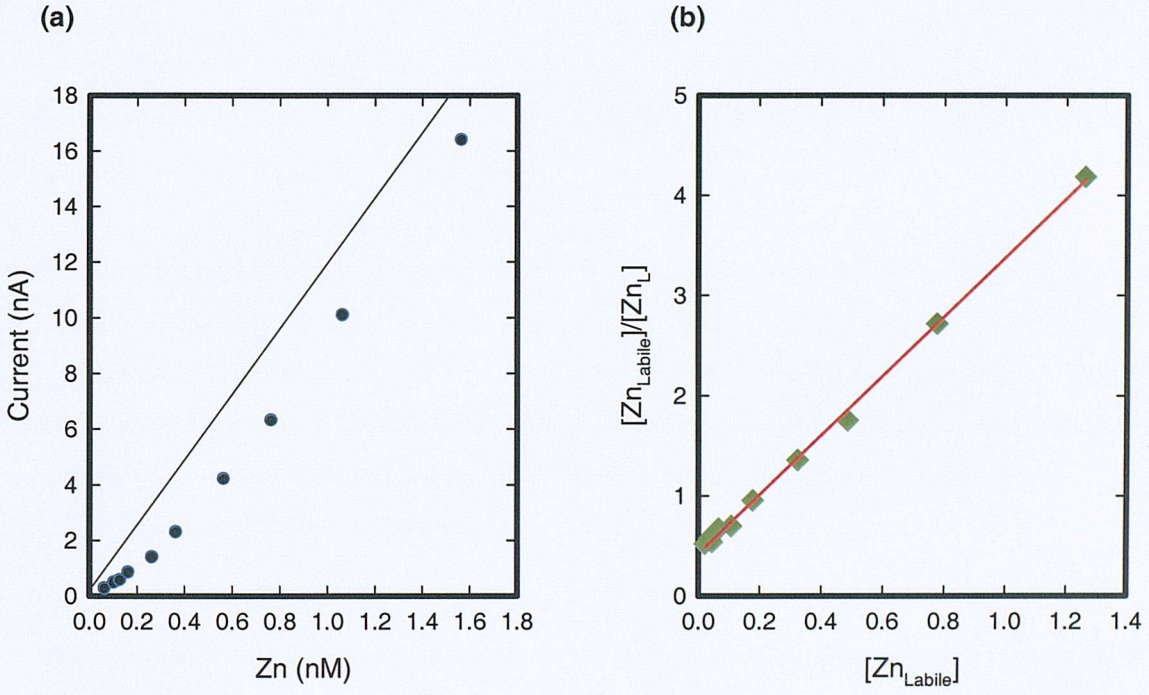


Figure 4.9 Example of Zn titration data for Day 5, Control. (a) Peak current vs. added Zn concentration. The line in indicates the response if no ligand were present. (b) Linearisation of data from which a ligand concentration of 0.26 nM and a log K'_{ZnL} of 10.8 was calculated.

Figure 4.10 a and b show changes in the concentration of the natural Zn-binding ligand concentration in the 4 treatments (Appendix 5). The concentration of the Zn-binding ligand was much lower in the control and the added Fe treatment than previously reported in Pacific (Bruland, 1989; Donat & Bruland, 1990) or Atlantic (Ellwood & Van den Berg, 2000) waters. For speciation studies it is essential to avoid sample contamination as this may lead to an

underestimation of the natural ligand concentration (Achterberg, 1993). However, the stability constants in this study are in good agreement with those of previous Zn studies (Bruland, 1989; Donat & Bruland, 1990; Ellwood & Van den Berg, 2000) and as the stability constants are calculated using the concentration of the Zn binding ligand, sample contamination is not thought to be a factor.

Addition of 10nM of Zn to the Zn enriched treatments rapidly increased the ligand concentration by an order of magnitude to 3nM on day 1 (Fig 4.10 a, b). The ligand concentration in the Zn amended treatments was much lower than the concentration of total dissolved Zn in the sample, but there is no evidence of a second weaker ligand. Extracellular production of Zn-binding ligands was observed to increase on days 4 and 5 in treatments with added Zn, which coincided with a rapid removal of total dissolved Zn, and a concomitant decrease in the bioavailable Zn^{2+} ion. These ligands were then partially removed or broken down and by day 8 were lower than observed on day 1 (Appendix 5).

In the Fe enrichment treatment and the control, Zn-binding ligands decreased over the first 3-4 days followed by a rapid increase between days 4 and 6 and continued to increase until the end of the experiment. A higher biomass was observed in the Fe amended treatment and the Zn-binding ligand increased to a larger extent in this treatment than in the control. This coincided with the decreasing total dissolved Zn concentrations (75pM to 34pM), which reduced the free Zn concentration to 0.2pM (Figure 4.10 a).

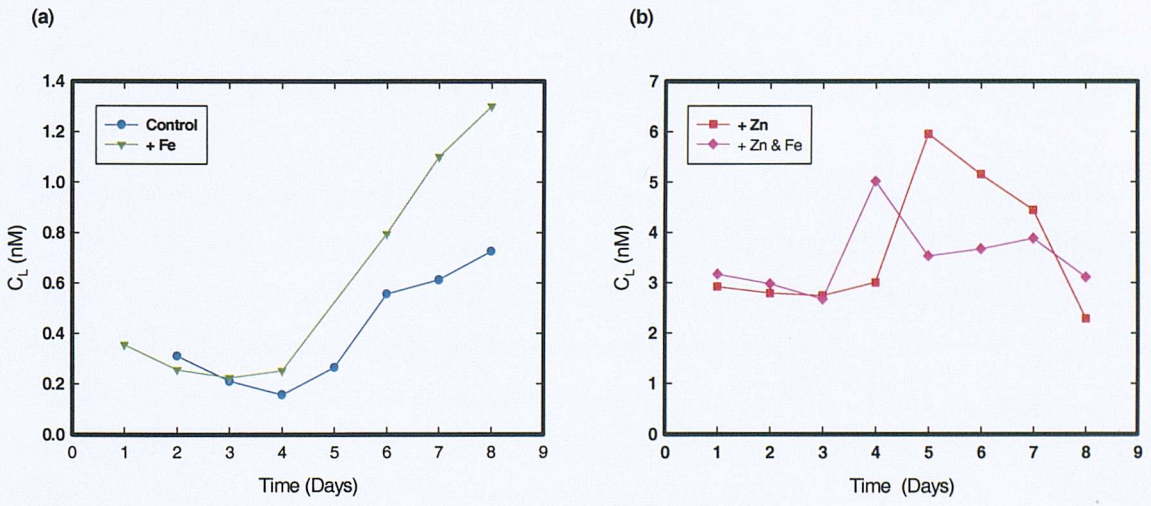


Figure 4.10 Zinc-binding ligand concentrations in (a) the control and Fe enriched treatment and in (b) the Zn enriched treatments.

The free Zn^{2+} ion concentration shown in Figure 4.11 a, b (Appendix 5) was calculated using Eqn. 2.9 and reflected the changes in the concentration of the total dissolved Zn and the Zn-binding ligand. Between days 3 and 5 there was a large reduction in free Zn^{2+} available to phytoplankton in all 4 treatments. After day 5 the Zn^{2+} concentration in the added Zn treatment increased reflecting the decrease in ligand concentration. In both the control and the added Fe treatment there was an initial increase in the concentration of Zn^{2+} concomitant with the decrease in ligand production mentioned earlier. By day 5 the Zn^{2+} concentrations in both the control (1.8 pM) and added Fe treatment (0.94 pM) are the lowest concentrations observed in field studies.

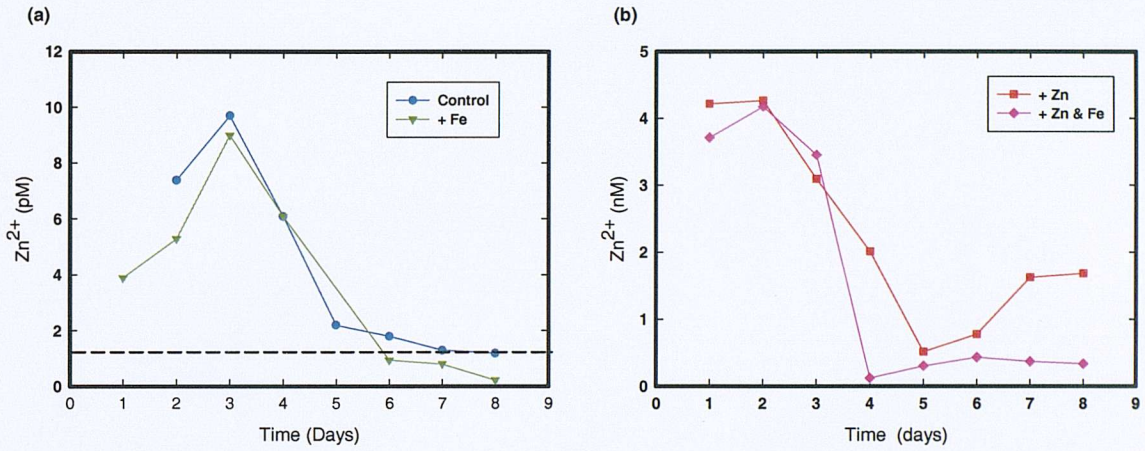


Figure 4.11 Free Zn^{2+} concentrations in (a) the Control and the Fe enriched treatment and (b) the Zn enriched treatments. The dashed line indicates the concentration at which Zn^{2+} becomes limiting to some phytoplankton growth (Sunda & Huntsman, 1992).

4.3.6 Effects of Zn and Fe enrichment on phytoplankton species composition

Phytoplankton species composition data up until day 5 is presented in Figure 4.12 and Appendix 4.4. Phytoplankton counts were analysed by Tawyna Peterson at University of British Columbia (UBC) Canada, data from days 6-8 is not yet available. An increase in phytoplankton cell numbers was observed in all four treatments over the 5 days (Fig 4.13). The number of cells in broad taxonomic groupings of phytoplankton are shown in Appendix 6. Coccolithophores were most abundant in the control after 5 days. The abundance of flagellates only doubled in response to the addition of both Fe and Zn while in the presence of added Fe they increased five-fold. With

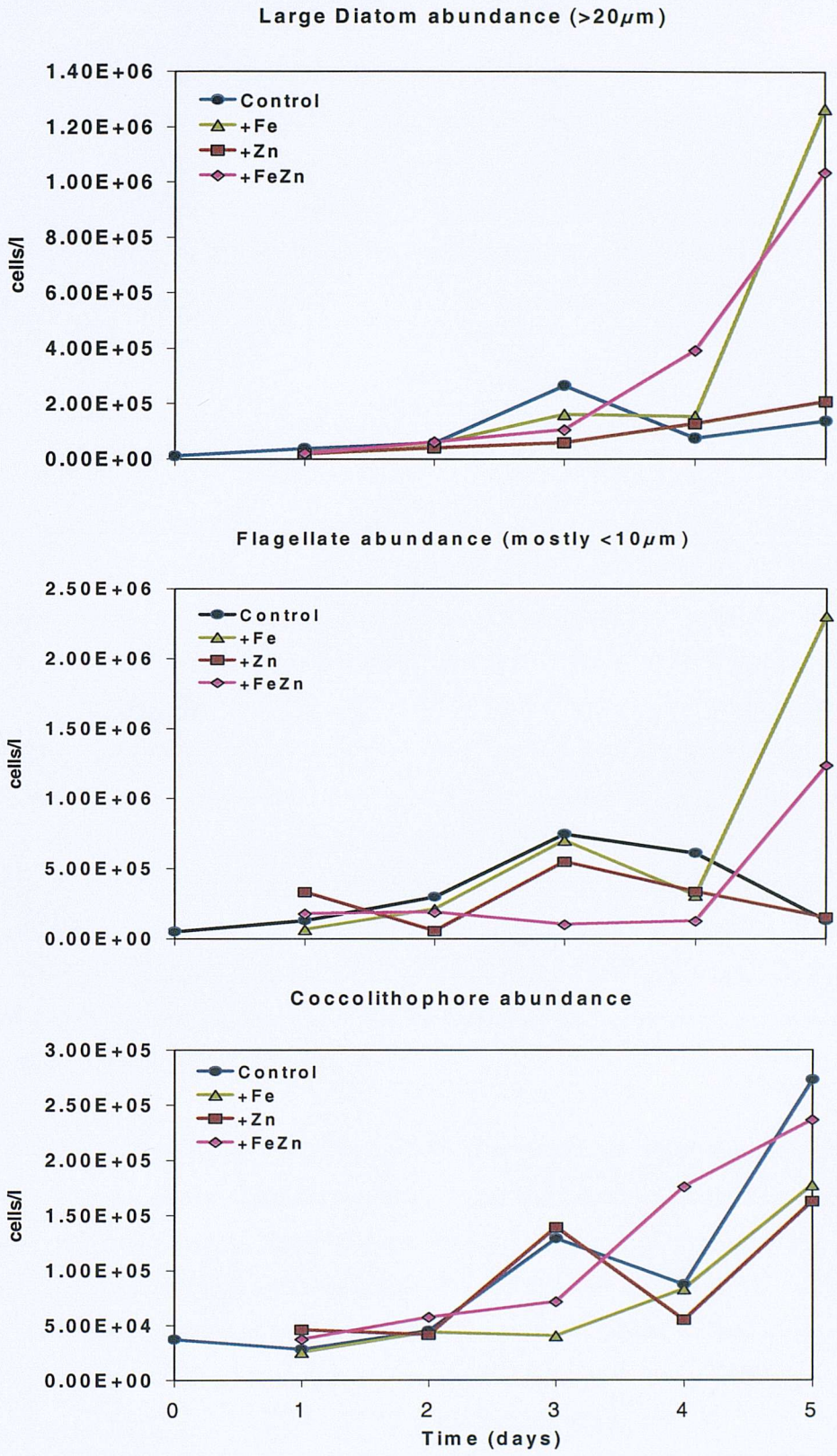


Figure 4.12 Phytoplankton count data up to day five in all 4 treatments

the exception of coccolithophores, the Fe enriched treatments showed a ten-fold increase in cell numbers compared to the control. A small increase in large diatoms (> 20 μm) occurred in the control on day 3 (Fig 4.12) which subsequently decreased by day 4. A large increase in diatom cell number started on day 4 in the Zn plus Fe enriched treatment but did not appear in the Fe enriched treatment until day 5. These results are similar to earlier studies at OSP indicating that the addition of Fe favours the growth of diatoms (Martin *et al.*, 1989; Coale 1991; Boyd *et al.*, 1996; Crawford et al., 2003).

4.4 Discussion

Both the biological and chemical parameters measured in this study show smooth clear changes throughout the eight days. This lack of noise in the data gives confidence in the experimental design employed in this study and the quality of the information. Factors other than the metal additions have the potential to influence the experiment. These experiments were performed on deck in a closed system whilst in transit and therefore it was not possible to compare changes with those truly occurring *in-situ*. The absence of some grazers, particularly migrating zooplankton, from the samples during the incubation experiments has been hypothesised as a cause of increased biomass in the control experiments (Martin *et al.*, 1989; Coale, 1991). Small variations between in-situ and on deck irradiance may also have been an influence (Crawford *et al.*, 2003). However, such factors are not expected to significantly impact the interactions between phytoplankton, bacteria and metals present in the cubitainers used. All samples were collected at a similar time and under similar conditions and therefore relative changes observed are due to trace metal enrichment.

In the previous experiment carried out in 1999 (Crawford *et al.*, 2003) very little growth was observed in the control, which was attributed to the low concentrations of Fe and Zn. In this experiment total dissolved Fe and Zn were measured daily and the concentrations of both trace metals decreased throughout the eight days (Fig. 4.8 and 4.9) whereas in the previous experiment the Fe and Zn concentrations in the control at the end were slightly higher than at the start (Crawford et al., 2003). The growth in the control in the present study is similar to that observed by Martin *et al.* (1989) and Coale (1991) in similar incubations, and also to reported growth rates of the natural phytoplankton assemblage at OSP in spring (Boyd & Harrison, 1999). This indicates that growth in the control is consistent with expected *in-situ* and incubation growth rates and supports the view that the incubations were a reasonable reflection of *in situ* processes.

4.4.1 Macronutrients and phytoplankton changes

Nutrients

As observed in all previous incubation experiments nitrate was depleted to below detection limits ($\leq$ 0.2 μM) in the Fe enriched treatments (13.3 μM over 8 days) which corresponds with phytoplankton uptake rates observed in productive upwelling regions (Dugdale & Wilkerson, 1991). Similar enhancement of nitrate uptake by Fe has been observed at OSP in incubation experiments (Boyd *et al.*, 1996). In the control, the removal of nitrate (2.4 μM over 8 days) was significantly higher than 84 nMd^{-1} reported for HNLC areas (Dugdale & Wilkerson, 1991). However, both values are low and different organisms will be present and phytoplankton numbers can vary greatly.

Laboratory evidence indicates that Zn is required for Si uptake (Rueter & Morel, 1981; De La Rocha *et al.*, 2000;). Franck *et al.* (2000) reported that the absence of a stimulatory effect of Zn addition on Si uptake rates was due to saturating *in-situ* total dissolved Zn concentrations (2nM). This was not the case in this study where the Zn concentration in the surface water at OSP is very low (0.075nM, Lohan *et al.*, 2002), while Si concentrations are very high (19μM). However, the addition of Zn did not increase the removal of Si in comparison to the control. The removal of Si in the control and Zn enriched treatment coincided with the low diatom growth observed in these treatments. In the Fe enriched treatments a large increase in diatom growth was observed with a concomitant decrease in Si. There was, however, a difference between the Fe enriched treatment and the Zn plus Fe enriched treatment in Si removed from solution. In the latter case the removal of Si higher in the treatment with added Zn and Fe. The difference between these 2 treatments suggests that Zn does aid the uptake of Si but the uptake is only observed in the presence of added Fe, which is required to stimulate the growth of Fe-limited large diatoms.

Phytoplankton Communities

Addition of Fe causes a dramatic increase in phytoplankton biomass (Fig 4.12, Appendix 6) by day 5. The large increase in diatom growth is observed between day 4 and 5 for the Fe enriched treatments. At this stage in the incubations the uptake of Fe has decreased substantially indicating that the phytoplankton require time to assimilate Fe and to stimulate growth. In the control, the phytoplankton communities on day 5 were different from those initially present, which is

reflected in an increase in chlorophyll *a* concentration and the small increase in POC. The increase in cell numbers of some large phytoplankton species in the control could result from a lack of sinking loss or a decrease in grazing pressure in the incubation cubitainers. This feature has been observed in all previous incubations experiments (Coale, 1991; Takeda *et al.*, 1995; Boyd *et al.*, 1996). The lack of Fe present in the control and Zn enriched treatment is reflected in the smaller numbers of diatoms present. Coccolithophores have been observed to have high relative growth rates under low Fe concentrations (Brand, 1991), which is indicated in this study by the high cell counts of this organism observed in the control and Zn enriched treatments. Although species counts from day 5 until day 8 are not yet available the concentration of chlorophyll *a* increases during this time indicating that a higher biomass would be expected.

The addition of Zn alone increased phytoplankton biomass of large diatoms compared to the control on day 7 and day 8. This is evident from the POC data even though the chlorophyll *a* concentrations are similar in both these treatments. In a previous study the addition of Zn also resulted in an increase in small diatoms (Crawford *et al.*, 2003). Although the addition of Zn did not stimulate a large bloom in phytoplankton in this study, a small shift in phytoplankton species composition was observed. The small increase in phytoplankton biomass in the Zn enriched treatment compared to the large increase in growth which was observed upon the addition of Fe indicates that Zn is not limiting the biomass of phytoplankton in this region. However, the addition of Zn could influence the growth of some phytoplankton species as has been reported in the previous experiment in 1999 (Crawford, *et al.*, 2003).

4.4.2 Changes in total dissolved trace metals

As stated in the results adsorption onto the container walls is minimal, and therefore the loss of trace metals from solution is predominately through extracellular adsorption onto the cell and uptake into the cell. Cadmium was measured to investigate if it can substitute for Zn when Zn concentrations are low. The Cadmium concentrations in all four treatments is low and therefore any benefit for phytoplankton substituting Cd for Zn is unlikely.

In all 4 treatments trace metal removal from solution was observed throughout the eight days. The highest relative uptake of Zn was observed in the Zn plus Fe enriched medium (70%) compared to the control (32%). The Zn uptake in the Fe enriched treatment is similar to that of the Zn enriched treatment (55% compared to 51% respectively). A large uptake of Zn was reported in an

earlier incubation experiment (Crawford *et al.*, 2003). The results of both studies are in line with an experiment carried out by Coale (1991) who reported 39% removal of Zn in a treatment with only 0.75 nM of added Zn. This is a similar uptake to the control in this study, although the Zn concentration is higher than in our control, the removal is comparable. While there have been a few incubation experiments that have studied the effect of added Zn and Zn plus Fe (Cochlan *et al.*, 2002; Franck *et al.*, 2000) there were no measurements of Zn concentrations at the end of these experiments and therefore no comparison on Zn uptake can be made. Coale *et al.* (2000) observed a 2nM removal of Zn through the course of a season in the Ross Sea, Southern Ocean as part of the AESOP's study suggesting that the Zn requirement for algal growth controls Zn concentrations in that region.

The highest relative uptake of Fe was also highest in the Zn plus Fe enriched treatment (82%) and lowest in the control (32%). A significant difference ($P < 0.001$) is observed between the Fe uptake in the control and in the Zn enriched treatment (32% and 60% respectively). The addition of Zn therefore corresponds to an increased removal of Fe from solution. This is also observed in Zn removal upon the addition of Fe. However, a larger biomass is observed in the Fe enriched treatment compared to the control and the relative uptake of Zn by phytoplankton would be expected to increase.

The reduction in total dissolved Zn concentrations in the Zn enriched treatment does not appear to be related to phytoplankton biomass, as there is no relationship to chlorophyll *a* concentration. However, both Fe and Zn-deficient phytoplankton have been reported to accumulate surplus metals in response to episodic metal inputs such as dust events or upwelling of higher trace metal concentrations (Sunda & Huntsman, 1992; Takeda & Obata, 1995). This hypothesis of 'luxury uptake' is supported by this data, where the removal of added total dissolved Zn and Fe concentrations is observed upon their addition to a natural phytoplankton assemblage.

In the treatments with added Zn, added Fe and added Zn plus Fe, a large removal in the total dissolved Zn and Fe concentration was observed in the first five days (Fig 4.8 b & 4.9 b). The rapid uptake of Fe and Zn in the first few days is consistent with observations from culture studies, which indicate that initial fast uptake rates of Fe or Zn-deficient cells is followed by a decrease in trace metal uptake similar to those observed for cells growing under replete Fe and Zn concentrations (Harrison & Morel, 1991; Sunda & Huntsman, 1992). This is supported by the fact that final microplankton communities in the Fe enriched incubation had higher Fe:C ratios

(102-109 μmol:mol for the Zn plus Fe treatment and Fe treatment respectively) compared to control (7.48 μmol:mol). In the case of Zn, Zn:C ratios are also higher (81-537 μmol:mol for the Zn plus Fe treatment and Zn treatment respectively) compared to 3.5 μmol:mol observed in the control. In the control both Zn:C and the Fe:C ratios are within the range reported from culture studies for phytoplankton cells grown under low Fe (Fe:C 6-9 μmol:mol) and Zn (Zn:C 0.9-4.2 μmol:mol) concentrations (Sunda, 1991; Sunda & Huntsman, 1992). The higher Zn:C and Fe:C ratios suggest that phytoplankton cells in the metal enriched incubations have elevated Zn and Fe quotas during the incubation when the respective metal is present in higher concentrations.

The Zn:C ratios in the control is 3.5 μmol:mol while in the Fe enriched treatment the ratio is lower at 0.5 μmol:mol. This indicates that Fe limited cells take up more Zn:C than Fe replete cells. As shown in Chapter 3, the concentration of Zn at OSP is low in the summer and winter and as standing stock of phytoplankton are Fe stressed throughout the year, this may explain the low concentrations of Zn observed in winter.

4.4.3 Zn^{2+} ion concentration

The high concentration of organic ligands in the 4 treatments reduces the concentration of the free Zn^{2+} ion, $[Zn^{2+}]$. $[Zn^{2+}]$ rather than organically complexed Zn is argued to be the major Zn species taken up by phytoplankton (Anderson & Morel, 1978; Sunda & Huntsman, 1992). In this discussion of the data, the Zn enriched treatments are considered separately as the addition of Zn increases the $[Zn^{2+}]$ to nM levels. The increase in phytoplankton growth in the Zn plus Fe enriched treatment begins at day 4 with a concomitant reduction in $[Zn^{2+}]$. Culture studies have demonstrated that $[Zn^{2+}]$ of 10^{-9} M are not toxic and the growth rate of *E. huxleyi* or *T. oceanica* is increased at these concentrations, although $[Zn^{2+}]$ was controlled though the addition of EDTA and NTA (Sunda & Huntsman, 1992). An increase in both coccolithophore and diatom growth in the Zn and Fe enriched treatment compared to the control was observed indicating that the $[Zn^{2+}]$ is probably not toxic to phytoplankton species observed in this region.

The only previously reported values for $[Zn^{2+}]$ in the Pacific Ocean range from 2 to 14 pM (Donat & Bruland, 1990) and $[Zn^{2+}]$ concentrations along line P range from 2 – 13 pM (Chapter 3). The concentrations in the control for the first 4 days of the experiment fall within this range (Appendix 4). The concentration of $[Zn^{2+}]$ decreases to 1.2 pM as phytoplankton growth occurs.

In the Fe enriched treatment the final [Zn^{2+}] concentration was 0.2 pM. This value is the lowest reported from field studies. Despite the very low [Zn^{2+}] present in this natural phytoplankton population, it is clear that in the Fe enriched treatment high levels of growth are occurring. Therefore, there must be an adequate supply of Zn to phytoplankton. Oceanic species of phytoplankton have adapted to the low levels of available Zn rather than evolving more effective transport systems due to the limits permitted by diffusion (Sunda & Huntsman, 1992). The mechanisms by which phytoplankton reduce their cellular growth requirements is not known. Phytoplankton are hypothesised to have developed more efficient use of internal metal micronutrient pools or the replacement of enzymes containing scarce metals and /or having no metallic cofactors have been suggested as mechanisms by which they can survive at these low concentrations (Sunda, 1991). Both Cd and Co can substitute for Zn (Price & Morel, 1990; Sunda & Huntsman, 1995a; Yee & Morel, 1996). The concentration of Cd however in these incubation experiments is extremely low and not much higher than Zn in the control or added Fe by day 8 where the [Zn^{2+}] is lower than 1pM. Although the concentration of Co was not measured in this study, the concentration of Co at OSP is lower than either Zn or Cd (Martin *et al.*, 1989). This would limit any benefit of substitution of either Cd or Co for Zn in phytoplankton cells within this study.

The effect of organic complexation on Zn appears to be detrimental as it reduces the accessible level of [Zn^{2+}] to the point where uptake requirements can only be met by taking up ions at the maximum rate allowed by physical diffusion (Whitfield, 2001). Culture experiments have shown that at a [Zn^{2+}] of 1 pM the growth of some phytoplankton is limited (Sunda & Huntsman, 1995a). The [Zn^{2+}] in the Fe enriched treatment in which both diatom and coccolithophore growth is observed, is much lower at 0.2 pM and therefore the uptake rate by diffusion into the cell would be expected to be depressed further. Increasing evidence has demonstrated that along with the free metal ions and lipid soluble fraction of the metal-complexes (Croot *et al.*, 2000), the labile fraction of metal-complexes in the diffusion layer of the organisms is also bioavailable (Herring *et al.*, 1990). This would act to alleviate the low concentrations of [Zn^{2+}]. In the Fe enriched treatment the lowest [Zn^{2+}] is 0.2 pM while the labile concentration [Zn′] is higher at 0.42 pM but is lower than that established to limit the growth of some phytoplankton species from culture studies.

4.4.4 Physical and Chemical limits on bioavailable Zn uptake

As the [Zn′] in the control and the added Fe treatment are the lowest reported from field studies and this is theoretically low enough to limit the growth of phytoplankton larger than 4 μm (Hudson & Morel, 1993), uptake of Zn′ by diffusion was investigated. Bioavailable Zn′ was used to estimate the diffusion of Zn into the cell instead of $[Zn^{2+}]$ as this $[Zn^{2+}]$ is accounted for in Zn′. Diffusion limitation by Zn and Fe has been hypothesised to explain why HNLC ecosystems are dominated by pico- and nanoplankton (Sunda & Huntsman, 1992). The maximum rate for diffusion (ρ) of labile inorganic Zn species to the cell surface is computed from

$$\rho = 4\pi rD[Zn'] \quad (1)$$

by assuming that the cells are spherical in shape with a radius of r (Hudson & Morel, 1990). D is the diffusion rate constant for Zn′ at 10°C (5.6×10^{-6} cm^2 s^{-1}; Li & Gregory, 1974) and [Zn′] is the inorganic Zn species calculated from the speciation measurements taken each day throughout this experiment. A number of diameters (5, 10, 25, 50 μm) were chosen to represent the different size fractions of phytoplankton observed in the incubation experiments from phytoplankton counts. For this calculation, 4 different scenarios were calculated by assuming that all of the phytoplankton biomass measured was made up of cells 5 μm, 10 μm cells, 25 μm cells or 50 μm cells. The daily chlorophyll *a* concentration was converted to carbon using a C: Chlorophyll ratio of 50 (Booth *et al.*, 1993) and this carbon was then converted to total cell volume by using a C:volume ratio of 0.2 pg C μm^{-3} (Sunda & Huntsman, 1992). The maximum daily diffusion flux of [Zn′] to the cells was subtracted sequentially from the initial total Zn to give modelled changes over the eight days in the control and the Fe enriched treatment as shown in Figure 4.13 a and b.

This modelled uptake indicates that if all the biomass were present as smaller cell sizes (5 and 10μm), they would be able to take up Zn by diffusive process rapidly. However, the larger cells do not appear to be able to take up Zn′ by diffusion to support the growth rates observed in the phytoplankton population. This supports previous modelled work by Sunda & Huntsman (1992) and Hudson & Morel (1993) that theoretically only smaller cell sizes should be capable of dividing once a day and acquire sufficient Zn by diffusion. One of the assumptions used in this model is that all the chlorophyll *a* present is in one cell size and therefore a larger number of smaller cells would be required to achieve the level of chlorophyll *a* observed. This model does

however, show that the removal of total dissolved Zn from solution can be due to uptake by phytoplankton and that a phytoplankton population of 25μm would account for the removal of dissolved Zn.

Although no size fractionated chlorophyll *a* concentrations were measured in this study, values for size fractionated chlorophyll *a* was obtained for day 4 and day 8 from a similar study carried out in 1999 (Crawford *et al.*, 2003). Using the percentage of chlorophyll *a* in different size fractions, 0.2 – 5 μm, 5 – 20 μm and > 20 μm a phytoplankton population of different cell sizes was calculated and the maximum uptake of [Zn′] by diffusion computed using equation 1 (Fig. 4.13 a, b). In the Fe enriched treatment the maximum uptake of [Zn′] by diffusion calculated using equation 1 is 20.1 pMd^{-1} on day 8. However, by day 8 the concentration of Zn′ is 0.42 (Appendix 5). This implies that the concentration of Zn′ is lower than the physical limits permitted by diffusion for Zn uptake. Culture studies combined with kinetic models for diffusion indicate that a Zn′ concentration of 1.2 pM, the specific uptake rate for an oceanic diatom *Thalassiosira oceanica* is only 40% of the computed maximum rate for diffusion while for *Emiliania huxleyi* it is only 32% (Sunda & Huntsman, 1995a). Using 32% of the computed maximum rate for diffusion for the phytoplankton population on day 8, in the Fe enriched treatment an uptake of Zn′ of 6.4 pMd^{-1} would be required for growth, which is higher than the concentration of Zn′ available. Applying a similar calculation to the day 8 in the control, a Zn′ uptake of 3.2 pMd^{-1} is required for growth, which is also higher than the Zn′ concentration of 2.5 pM measured in this study.

The results from this model imply that the concentration of bioavailable Zn is not sufficient to maintain phytoplankton growth. However, high phytoplankton biomass, particularly large diatoms were observed in the Fe enriched treatment. Therefore, phytoplankton are either able to access the Zn-binding organic ligands or the model calculations of kinetic theory based on uptake rates from culture experiments are not sufficient to account for the changes observed in this study. The culture experiments in which the uptake of Zn′ is calculated utilise artificial chelators to control the concentration of Zn′ and do not measure the speciation of Zn directly (Sunda & Huntsman, 1992; 1995a; 1998). These culture experiments add Zn in high concentrations with equivalent amounts of EDTA or NTA and argue that this would not alter the free ion concentrations of Zn or other trace metals and do not measure the initial Zn concentrations in the

culture medium. While much has been learnt from these studies, they differ greatly from the natural environment.

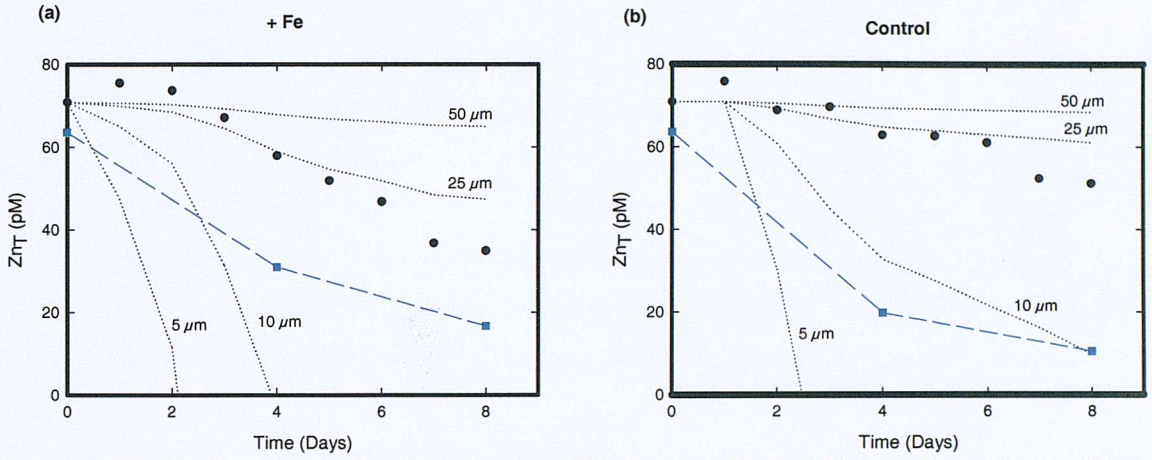


Figure 4.13 Total dissolved Zn (Zn_T) decrease (Black dots). Dashed lines indicate the modelled uptake of Zn′ assuming phytoplankton biomass is divided into 4 different cell sizes. Blue line is modelled total dissolved Zn decrease assuming that phytoplankton biomass is divided up according to size fractionated measurements made during a similar experiment in 1999.

In this model, no estimate of the dissociation of organic complexed Zn or Zn′ is made as little is known about this type of dissociation in natural waters. Total dissolved Zn concentrations decrease each day and therefore the equilibrium between the different forms of Zn present will also change. If the removal of Zn′ by diffusion is a rapid process compared to re-equilibration with total dissolved Zn concentrations then a form of kinetic limitation may be observed. The ligand concentration increases from day 3 to day 8 in the Fe enriched treatment thus decreasing the concentration of bioavailable Zn. Kinetic experiments from culture studies have indicated that phytoplankton cells take several hours to adjust to the changes in ambient Zn^{2+} concentrations (Sunda & Huntsman, 1992). Therefore, the best estimate from this study is that the Zn′ diffusive supply is inadequate to provide the necessary Zn required by larger cells for growth.

4.4.5 Zn-binding ligands

The production of the Zn-binding ligands was a common feature in all four treatments either at low or high total dissolved Zn concentrations. This ligand production is similar to that of Cu-

complexing ligands observed at low and high Cu concentrations (Leal & Van den Berg, 1999). It has been speculated that Zn complexing ligands are of biological origin (Bruland, 1989). Ellwood & Van den Berg (2000) demonstrated no relationship between Zn binding ligands and fluorescence and therefore, hypothesise that these ligands are polypeptide molecules, produced during the decay of phytoplankton. However, in this study upon the addition of Zn a rapid production of the Zn-binding ligands was observed with ligand concentrations increasing by an order of magnitude. The rapid production of these ligands indicates a specific response from the phytoplankton to the supply for additional Zn and that this process can occur on a time scale of hours. Two different hypotheses can be put forward to explain the production of these ligands by phytoplankton: either detoxification or luxury uptake and storage for future growth via this Zn ligand into the cells.

Although there is no laboratory evidence for Zn toxicity to phytoplankton at the concentrations observed in this study, Zn is potentially toxic due to the ability to bind to the sulphur sites and interfere with the acid-base chemistry of the cell (Whitfield, 2001). Complexation of trace metals with organic ligands often ameliorates trace metal toxicity and influences the biogeochemistry of these elements in aquatic environments as observed for Cu (Moffett *et al.*, 1990). Phytoplankton in this region are adapted to the low levels of Zn in solution and the addition of 10 nM Zn gives concentrations more than two orders of magnitude greater than Zn concentrations observed at OSP. Evidence from this study suggests that even with the high concentration of ligands produced, the $[Zn^{2+}]$ was an order of magnitude higher than in the control and at $[Zn^{2+}]$ levels of ~ 4nM, phytoplankton growth is still observed. On day 5 in the Fe plus Zn enriched treatment the concentration of $[Zn^{2+}]$ was 0.5 nM and phytoplankton biomass was increasing dramatically (Fig 4.13). Although phytoplankton biomass was lower in the Zn enriched treatment, phytoplankton growth was also observed. Therefore it appears that ligand production by phytoplankton in this study does not reflect a detoxification mechanism.

It is more tenable to explain ligand production as a mechanism for luxury uptake. Oceanic species may be able to assimilate and utilise organically bound Zn as has been observed for Fe (Rue & Bruland, 1997; Butler, 1998). The current dogma that biological response to metals such as Zn reflects changes in the free metal ion activities is based primarily on studies that have used the synthetic chelators EDTA and NTA to set the free metal ion activities specified by the experiments (Bruland, 1989). In the experiment described here no artificial chelators were utilised and the large increase in ligand production followed by a decrease suggests that these ligands are

very labile. In the Zn plus Fe treatment the large increase in ligand production on day 4 coincides with a large decrease in total dissolved Zn concentrations (9.8 – 4.81 nM). The removal of total Zn would imply that the Zn-ligand complex was readily available and might be like a Zn-siderophore rather than being non-available like a Cu-ligand complex. In culture experiments with high concentrations of Cu, the Cu-binding ligand increased for the duration of the experiment and only when Cu^{2+} levels were no longer toxic was an increase in cell numbers observed (Leal & Van den Berg, 1999). Rue & Bruland (1997) observed a rapid production of Fe (III)-binding ligands upon the addition of small amounts of Fe (0.4-2 nM) in a mesoscale Fe addition experiment in the HNLC area of the equatorial Pacific. In particular, the strong class of ligands, thought to be siderophores was produced increasing the uptake of Fe. Therefore, under Fe-deficient conditions a small Fe input would lead to an immediate increase in siderophore production so that phytoplankton take advantage of such a pulsed input. A similar response to the supply of Zn can be applied in this study.

In the Fe enriched treatment Zn binding ligands were observed to increase from day 3 and by day 8 the concentration had increased from 0.35 to 1.3 nM. It has been hypothesised that there appears to be no immediate advantage to the plankton community in reducing $[Zn^{2+}]$ through complexation by organic exudates (Muller *et al.*, 2003). There are no immediate solubility constraints on the Zn concentration and it does not undergo redox cycling so that the benefits conferred by complexation upon Fe availability do not apply to Zn (Whitfield, 2001). A shortcoming of ligand production by phytoplankton is that the energy required by phytoplankton to produce ligands would lower the return each individual cell would obtain from its production (Bruland, 1989; Ellwood & Van den Berg, 2000). However, if these Zn-complexing ligands were produced by specific phytoplankton to lower $[Zn^{2+}]$ at the expense of other phytoplankton (Bruland, 1989) a competitive advantage would occur. The hypothesis from this study is that phytoplankton can utilise the Zn-binding ligand for Zn uptake could explain how at the low bioavailable Zn concentrations in the Fe enriched treatment phytoplankton growth is occurring.

Figure 4.14 show a schematic diagram of the interactions between Zn speciation, Zn-binding ligands and phytoplankton in the mixed layer. The role of bacteria in the production of these Zn-binding ligands or aiding Zn uptake is as yet unknown. Zn-binding ligands may undergo photochemical degradation and/or polymerisation although this was not investigated in this study. As shown in Chapter 3, Zn-binding organic ligands are observed at 100m and 400m and the concentration at 100m is higher than in the mixed layer. The stability constant does not show a

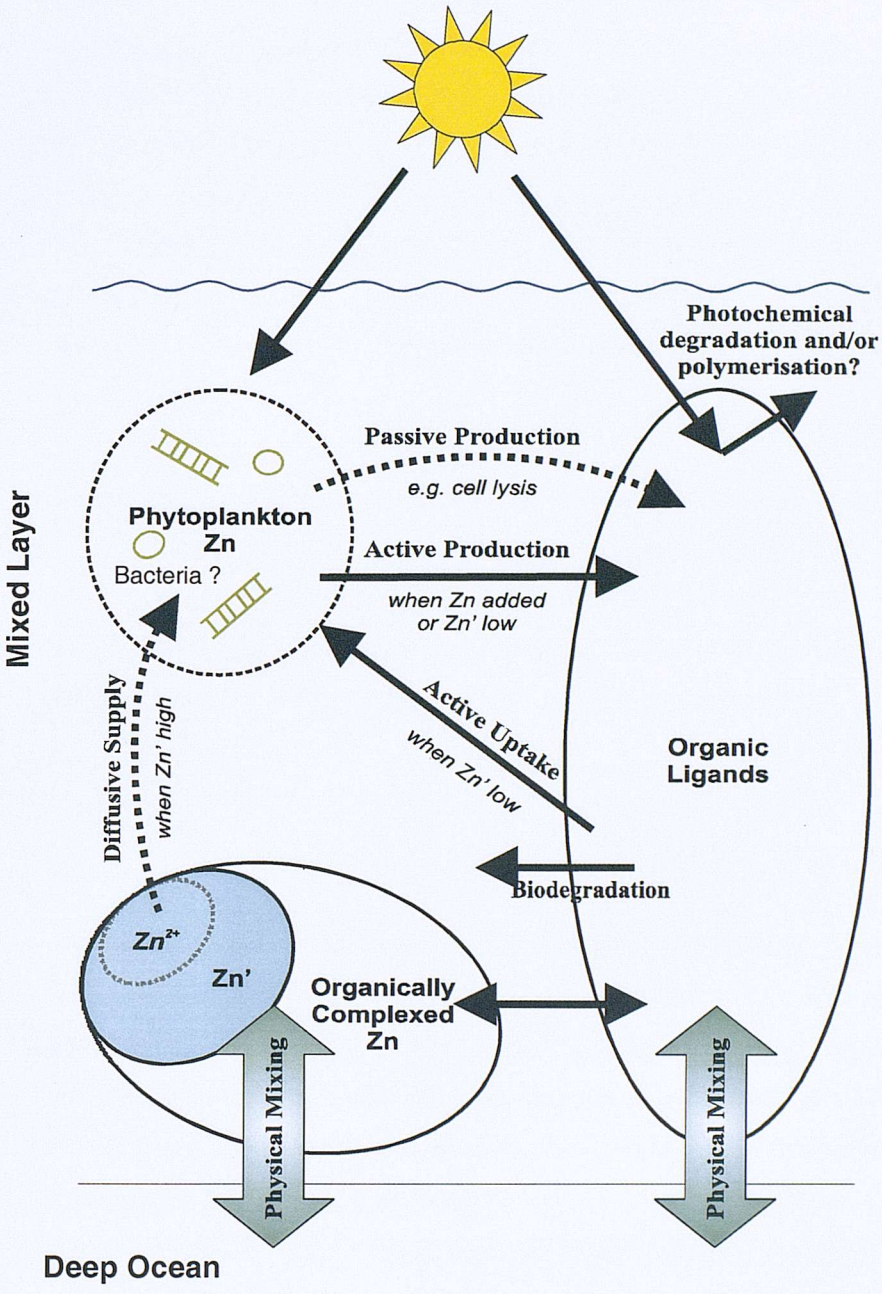


Figure 4. 14 Schematic diagram indicating the interactions between Zn and phytoplankton in the mixed layer. The size of the Zn pool is scaled to reflect the Zn speciation and organic ligands on day 1 in the control. Active process are indicated by filled arrow while passive processes are indicated by dashed arrows.

systematic variation with depth indicating that a similar Zn-complexing ligand is observed throughout the water column. This has been observed down to 1000m in the Atlantic (Ellwood & Van den Berg, 2000). The systematic increases and decreases in Zn-binding ligand concentration throughout the water column suggest that these ligands could be subjected to biological uptake and biodegradation and be removed from the mixed layer to depth. The presence of these ligands at depth indicates that some fraction of these ligands are stable and long-lived. In this study the decrease in total dissolved Zn in combination with the production of these Zn-binding ligands reduces the bioavailable Zn (Zn′).

Rapid production and decline in the concentration of Zn-binding ligands infers that the Zn-binding ligands are labile and can change on a daily basis. Thus, a dynamic balance could exist for this class of ligands between production and consumption, resulting in the relatively uniform and ubiquitous presence of the Zn complexing ligand in the North Pacific as seen in Chapter 3 and observed by Bruland (1989) and in the Atlantic (Ellwood & Van den Berg, 2000).

4.5 Summary

This study supports previous work in demonstrating that the availability of dissolved Fe strongly limits phytoplankton growth at OSP. The addition of Zn to the incubations showed no major phytoplankton biomass change in comparison to the control. Therefore it appears that Zn is not directly limiting phytoplankton growth at OSP. The concentration of Zn^{2+} and Zn' in the Fe enriched treatment is the lowest levels observed in field studies and is below the limit established to limit phytoplankton growth. An estimate of the diffusive flux of Zn′ to phytoplankton cells suggests that the concentration of Zn′ is insufficient to maintain phytoplankton growth rates in the Fe enriched treatments. However, phytoplankton growth was observed both in the control and the Fe enriched treatment, despite the extremely low concentrations of bioavailable Zn.

Production of Zn-binding organic ligands was observed on the addition of Zn, indicating a rapid response from phytoplankton to this addition Zn supply indicating that they are specific for Zn. The production of these ligands is hypothesised to be a mechanism for luxury uptake and storage for future use by phytoplankton. The observed production and destruction of these ligands on a time scale of a day indicates that a dynamic balance could exist for this class of ligands between production and consumption, resulting in the relatively uniform and ubiquitous presence of the Zn

complexing ligand in the North Pacific. In the Fe enriched treatment the increase in ligand production may be a mechanism by which phytoplankton can access Zn bound to these ligands when the Zn′ concentration is low. This study indicates that Zn bound to ligands can have an important role in the uptake of Zn by phytoplankton.

Chapter 5

Processes influencing the form and fate of zinc in mesoscale eddies in the NE Pacific

5.1 Introduction

The time series at Ocean Station Papa (OSP) in the subarctic north-east Pacific is one of the longest records of oceanographic data in existence (Banse, 1991). Sampling over the past 40-50 years reveals a lack of seasonal variability in biological standing stock (Boyd *et al.*, 1995a) despite seasonal oscillations in primary productivity (Boyd *et al.*, 1995b; Wong *et al.*, 1995) and the presence of plentiful macronutrients (Whitney & Freeland, 1999). Iron limitation has been shown to control the growth of phytoplankton within this region (Martin *et al.*, 1989; Coale, 1991; Boyd *et al.*, 1999) and Chapter 4. Superimposed on a relatively constant standing stock of phytoplankton are peaks in chlorophyll *a* concentrations and large vertical fluxes of carbon as detected by deep-ocean sediment traps (Wong *et al.*, 1999). It has been hypothesised that dust deposition events increasing Fe concentrations (Boyd *et al.*, 1998) or lateral advection of nutrients, organisms and trace metals by mesoscale eddies could be the cause of the variation in phytoplankton stocks at OSP.

In the Gulf of Alaska, anticyclonic mesoscale eddies have been found in hydrographic data (Tabata, 1982). Until satellite altimetry became available (1992), these eddies could not be tracked moving away from the coast into the open ocean. Advances in satellite technology has led to the use of data from TOPEX/Poseidon and ERS to map and track eddies containing warmer, fresher, coastal water (Crawford & Whitney, 1999) as they move into the NE Pacific. As the temperature increases, seawater expands and TOPEX/Poseidon can measure the corresponding change in sea surface elevation. Although most of the water in the body of these eddies is warmer than the surrounding ocean, the waters near the surface are either similar or even slightly cooler than the surrounding seas. For this reason satellites that sense ocean surface temperature seldom find these eddies. When the Colorado Centre for Astrodynamics Research (CCAR) began posting

near-real-time altimetry maps in 1998, it became possible to locate and direct research vessels to individual eddies.

Each year from 1993-2002, the period for which winter TOPEX/Poseidon satellite altimetry is available, it has been observed that at least one eddy forms at the mouth of Hecate Strait, every winter, often separating from the coast in February and moving westward into the Gulf of Alaska (Crawford *et al.*, 2002). These eddies are named Haida eddies, the name "Haida" comes from the local native Indian nation, which refers to its homeland as "Haida Gwaii", a region denoted "Queen Charlotte Islands" on most maps (Fig. 1). Haida eddies are formed near Cape St. James in a region where coastal flows on either side of the cape are directed toward the south and initial eddy formation is therefore set up by a combination of rectification of tidal currents and the outflow around the cape (Crawford *et al.*, 2002). The flow through this region persists over several months to create Haida eddies (Whitney & Robert, 2002).

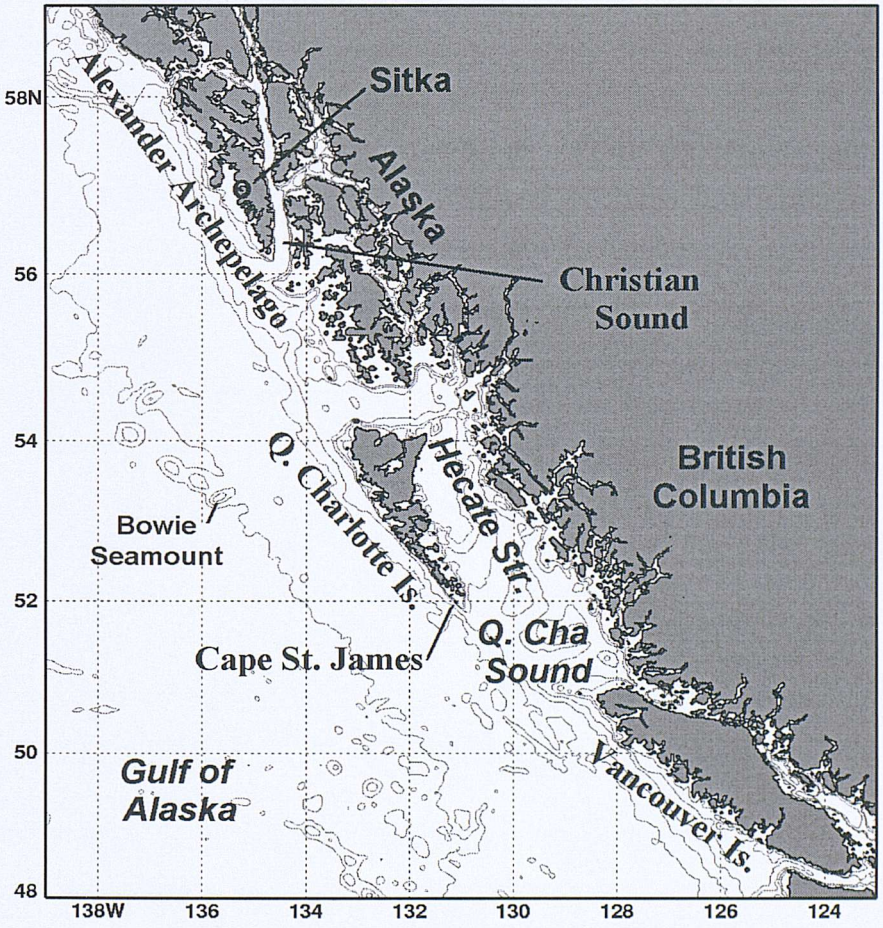


Figure 5.1 Map showing the region in which Haida eddies are formed the contour line indicates the depth of 200m and represents the edge of the shelf.

Haida eddies are typically slow moving and anticyclonic. These rotating masses of water average 200km in diameter and a large eddy can contain 6,000 Km^3 of coastal water. Each eddy contains a core of anomalously warm, low salinity water of coastal origin that depresses isothermal surface in and below the halocline and produces an upward doming of the sea-surface of 10-30cm (Crawford *et al.*, 2000). These eddies persist for one to several years. Haida eddies are typical of anticyclonic eddies found in other oceanic realms (Nelson *et al.*, 1985; Tomosada, 1986; Pingree & Le Cann, 1992; Savidge & Williams, 2001; Zhang *et al.*, 2001) in that they contain a core of buoyant water which distorts the density field in their vicinity. However, unlike other anticyclonic eddies which form along western boundaries of oceans, Haida eddies transport coastal waters up to a 1000km into the open ocean (Fig 5.2). These eddies have revealed water properties and larvae that are typical of winter coastal water normally found in Queen Charlotte Sound and Hecate Strait. As a result these eddies are thought to play a significant role in the regional ecosystem as they sweep larvae and nutrients offshore, thereby reducing the biological productivity on the shelf and increasing the offshore productivity as the eddy decays and releases its contents to the ambient ocean (Mackas *et al.*, 2002). This offshore flow could affect the reproductive success of any organisms that were planktonic between November and February (Whitney & Robert, 2002).

Tracks of the dominant eddy formed each year show that these water masses move westwards away from the coast and travel in a more northerly or southerly track depending on regional climatology, occasionally reaching OSP (Fig 5.2; Whitney & Robert, 2002). The majority of these eddies can be tracked into the High Nutrient Low Chlorophyll (HNLC) region. En route the eddy waters are enriched with nutrients and warmed while crossing the large area of the continental shelf (Whitney & Robert, 2002). Each eddy is named in the year it was formed, e.g., Haida 2001 (H-2001). The H-2000 eddy of approximately 100km in diameter moved in a north-west direction into the open ocean typically at speeds of 3-5km per day while the H-2001 eddy moved in a more west south-west direction. Haida 2000 lingered near the Bowie Seamount from May to October 2000 before moving seaward (Fig 5.2).

The TOPEX/Poseidon altimeter and SeaWifs chlorophyll satellite data substantially complement measurements made in these eddies and allow us to examine its influence on the biological production and spatial variability in the study area. Figure 5.3 shows H-2000 spinning off the coast transporting with it a smaller eddy offshore. This figure also shows a SeaWifs chlorophyll

and thermal image taken at the same time. The centre of the eddy is much warmer and chlorophyll concentrations are higher (3.2 mg m^{-3}) indicating a higher biomass in these eddies than in the surrounding waters. Satellite images reveal that Haida eddies deflect seaward the high chlorophyll *a* surface waters found in shelf waters.

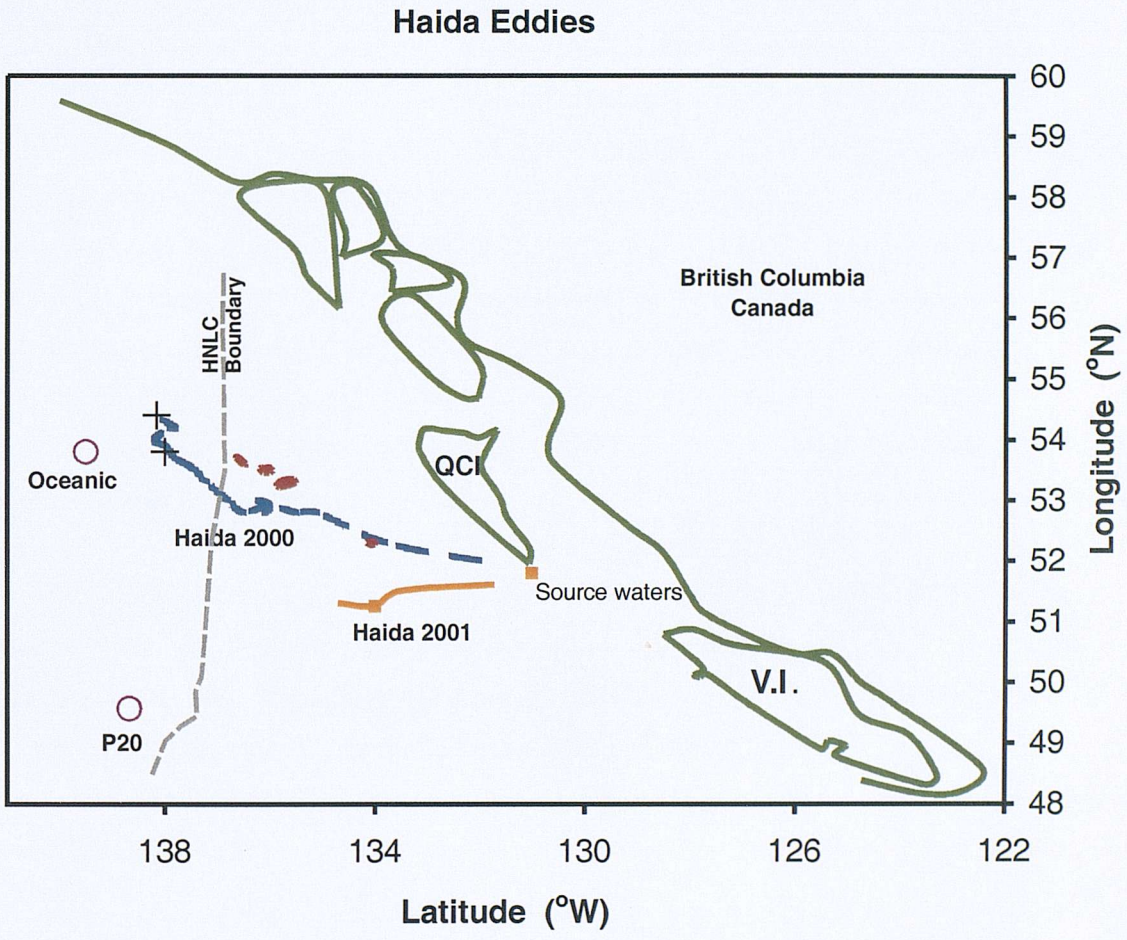


Figure 5.2 Tracks of Haida eddies which formed off the Queen Charlotte Islands (QCI) in 2000 and 2001 as discerned from satellite altimetry. Boundaries of the HNLC waters from Whitney & Welsh (2002). Haida 2001 track is indicated in orange while H-2000 is indicated in blue. The oceanic station is the reference station. Also indicated is P20, which is a station along the Line P program (see Fig 2.1)

The biogeochemical cycles of trace metals in these eddies and other mesoscale eddies have not been considered in detail. To date there have been no studies of total dissolved Zn concentrations in eddy environments. There are only two studies on trace metal distribution in eddy systems and

these studies have been undertaken in warm-core rings in the Sargasso Sea and the Gulf Stream (Sakamoto-Arnold *et al.*, 1987; Hanson *et al.*, 1988). Eddy systems are dynamic and have the potential to transport significant amounts of trace metals and other elements from the coastal environment out into oligotrophic or HNLC areas. Therefore, it is important to understand the impact that these eddies have on supplying additional trace metals to HNLC areas. This study formed part of the Haida Eddy Programme run by Institute of Ocean Sciences (IOS) to study eddy dynamics in the Gulf of Alaska. In this study, data on total dissolved Zn and Zn speciation in H-2000 and H-2001 eddies was obtained and complement additional trace metal studies on Fe and Cd carried out by other workers within this programme. Initially, Haida eddies possess high nutrient concentrations (Whitney & Robert, 2002) and high Fe concentrations (Sutherland *pers. comm.*) which will be transported off shelf. Over time it would be expected that these eddy waters will eventually dissipate and they will come to resemble the HNLC waters into which they travel. However, due to their initial discrete nature and reduced exchange with adjacent water masses eddies therefore, offer the opportunity to study a mini-mesocosm experiment to investigate the time-scale of changes of dissolved Zn within a parcel of water as it moves offshore, and what the dominant process involved are.

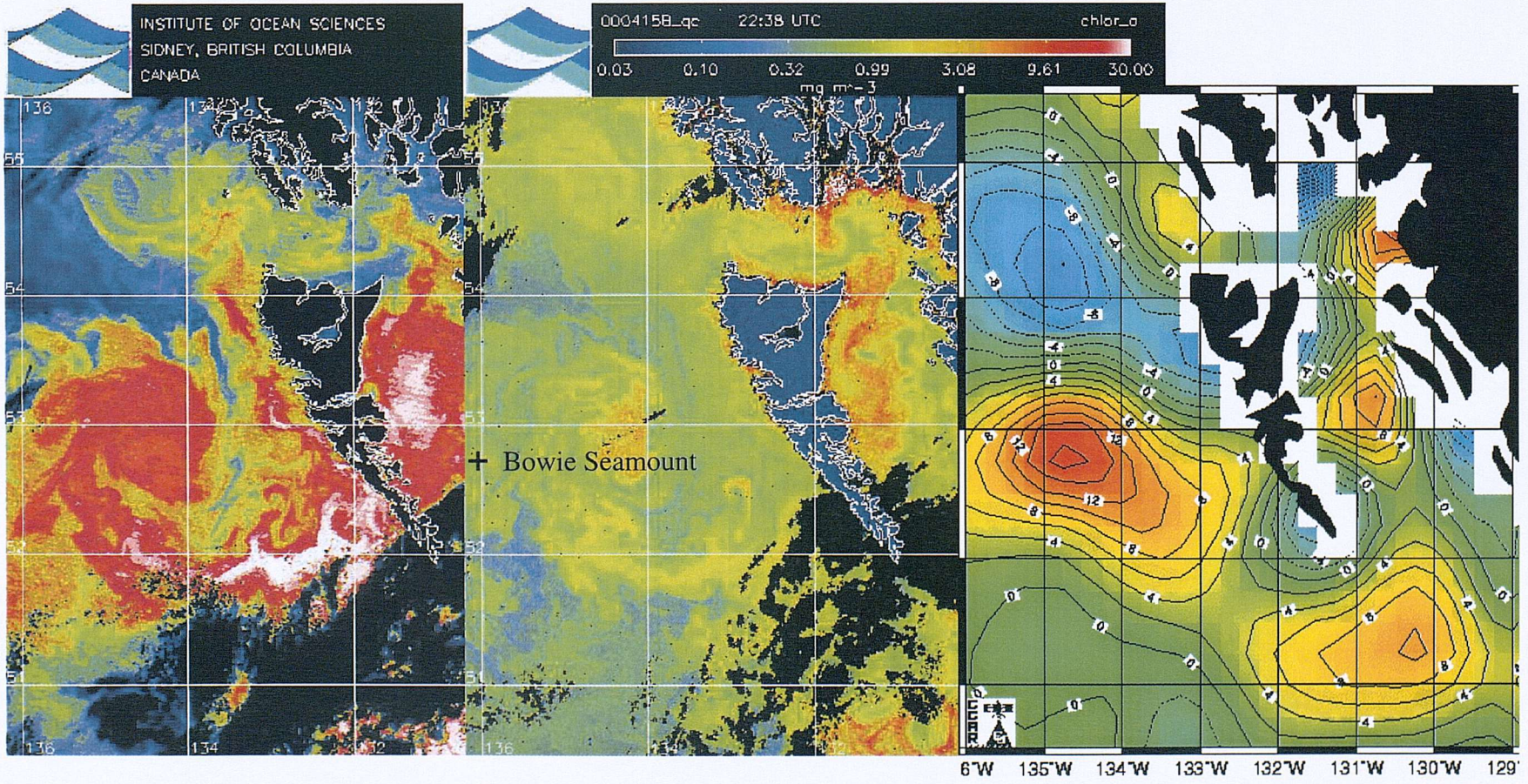
Thus the overall objectives of the Haida studies reported here were to investigate the lateral transport of Zn from coastal waters to the open ocean and examine the change in Zn concentration over time as the eddy ages and to determine the change in speciation of Zn over eddy time-scales and its impact on primary production within these eddies.

5.2 Methods

5.2.1 Sample collection and treatment

Samples for total dissolved Zn and Zn speciation measurements were collected on two cruises, 2001-08 and 2001-31 onboard the C.S.S. John P. Tully in May/June and September/October 2001 respectively as part of the Haida eddy dynamics study. The cruises utilised TOPEX/Poseidon and ERS satellite data to track two eddies, H-2000 and H-2001. Five major stations were sampled on each cruise; the edge and centre of each eddy and a reference station on each cruise. The reference station chosen was never located east of the eddy, where the passage of the eddy may have influenced the water characteristics. Both surveys sampled across the eddy at closely spaced stations to resolve the cross-eddy structure with CTD profiles to depths of a least 1000m, and in

Haida-2000, April 15 2000



NOAA 15 Thermal **SeaWiFS Chlorophyll** **TP/ERS Altimetry**

Figure 5.3 H-2000 leaving the coastline captured by three different satellites taken at the same time.

some cases to 3000m. The centre and edge of the eddy were identified using both satellites and the CTD transects. In May/June 2001 a north-south and an east-west transect of H-2001 was carried out and the centre and edge identified. In September/October a north-south transect of H-2000 was carried out and the centre and edge identified. The centre station was chosen to be the station where the depressed isopycnals were horizontal at the base of the eddy core; the edge station represented the location where the isopycnals were sloping most dramatically. On both surveys it has been estimated that using satellite altimetry locates the centre of an eddy to within 25km (Whitney & Robert, 2002). Figure 5.4 and 5.5 show the cruise track and major stations for both cruises.

	May/June 2001 (2001-08)					September/October (2001-31)				
Depth	H-01Centre	H-01Edge	H-00Centre	H-00Edge	Reference	H-01Centre	H-01Edge	H-00Centre	H-00Edge	Reference
10	xa	xa	xa	xa	xa	xa	x	xa	xa	xa
25	x	x	x	x	x	x	x	x	x	x
40	xa	xa	xa	xa	xa	xa	x	xa	xa	xa
75	xa	xa	xa	xa	xa	xa	x	xa	x	x
100	x	x	x	x	x	x	x	x	x	x
200	xa	xa	xa	xa	xa	xa	x	xa	xa	xa
400	xa	x	xa	x	x	x	x	x	x	x
600	x	x	x	x	x	xa		xa	xa	xa
800	xa	xa	xa	xa	xa			x	x	x
1000	x	x	x	x	x			x	x	x

Table 5.1 Sampling depths for total dissolved Zn (x) and Zn speciation (a) samples analysis collected on both cruises (2001-08 and 2001-31).

Table 5.1 shows the major stations and sample depths at which samples for both total dissolved Zn concentrations and Zn speciation were collected. Along each transect from both cruises samples for total dissolved Zn and Zn speciation were collected at 10m (stations indicated by blue diamonds in Figures 5.4 and 5.5). Samples were collected and analysed as outlined in Chapter 2. Due to adverse weather conditions in September 2001, the sampling program focused on the older eddy H-2000 in order to assess the impact of ageing Haida eddies on processes in the Gulf of Alaska. Therefore, samples were only collected down to a depth of 600m in the centre station and down to 400m at the edge station of H-2001 in September/October and Zn speciation was not determined at the edge station of H-2001 in September/October.

Samples for nutrients and their supporting data were collected as outlined in Chapter 2, and were analysed by Frank Whitney and Janet Barnwell-Clarke at IOS.

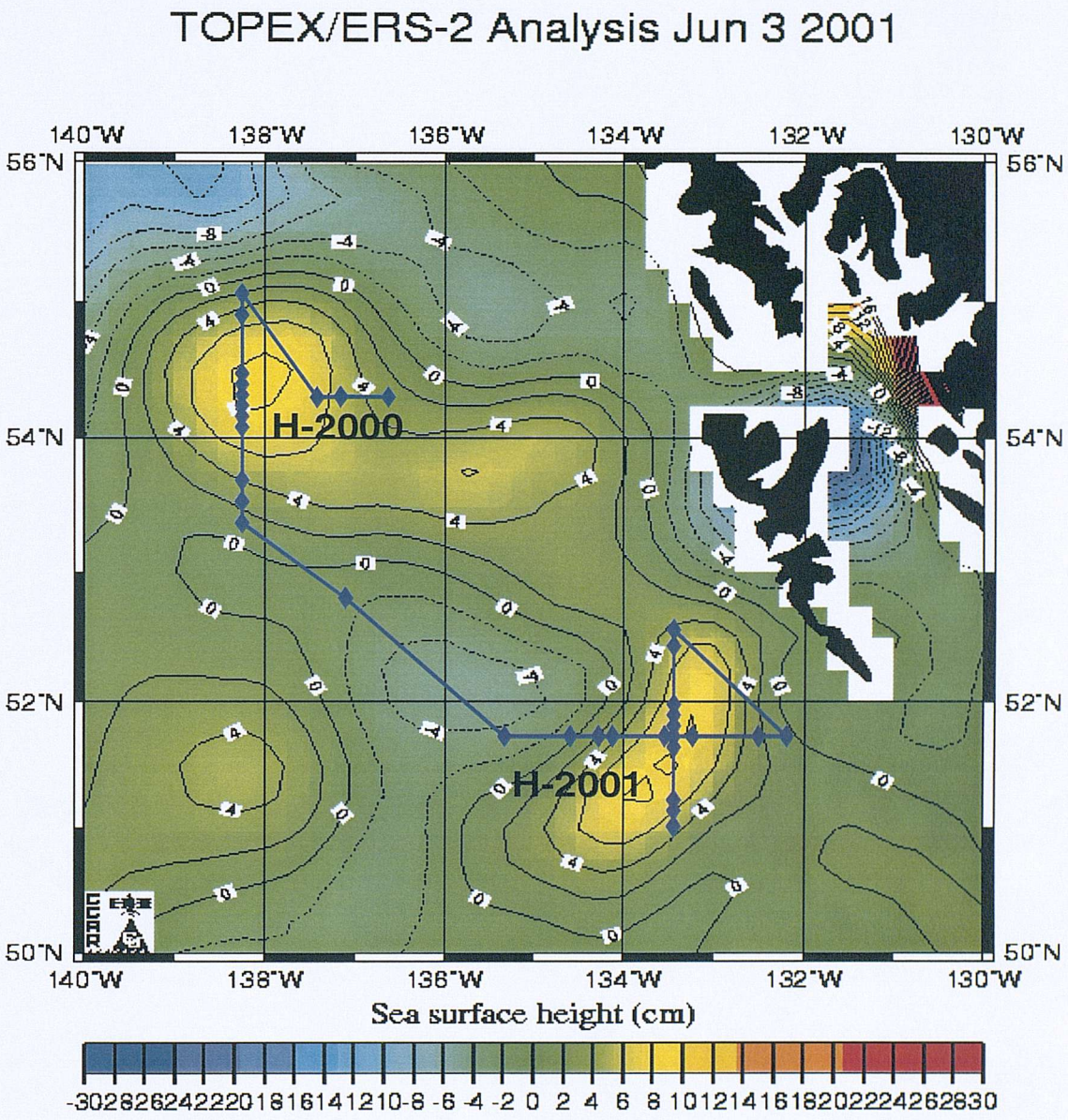


Figure 5.4 Satellite image of the study area for the June 2001 cruise, showing both H-2000 and H-2001 and the cruise track in blue and the stations in blue diamonds.

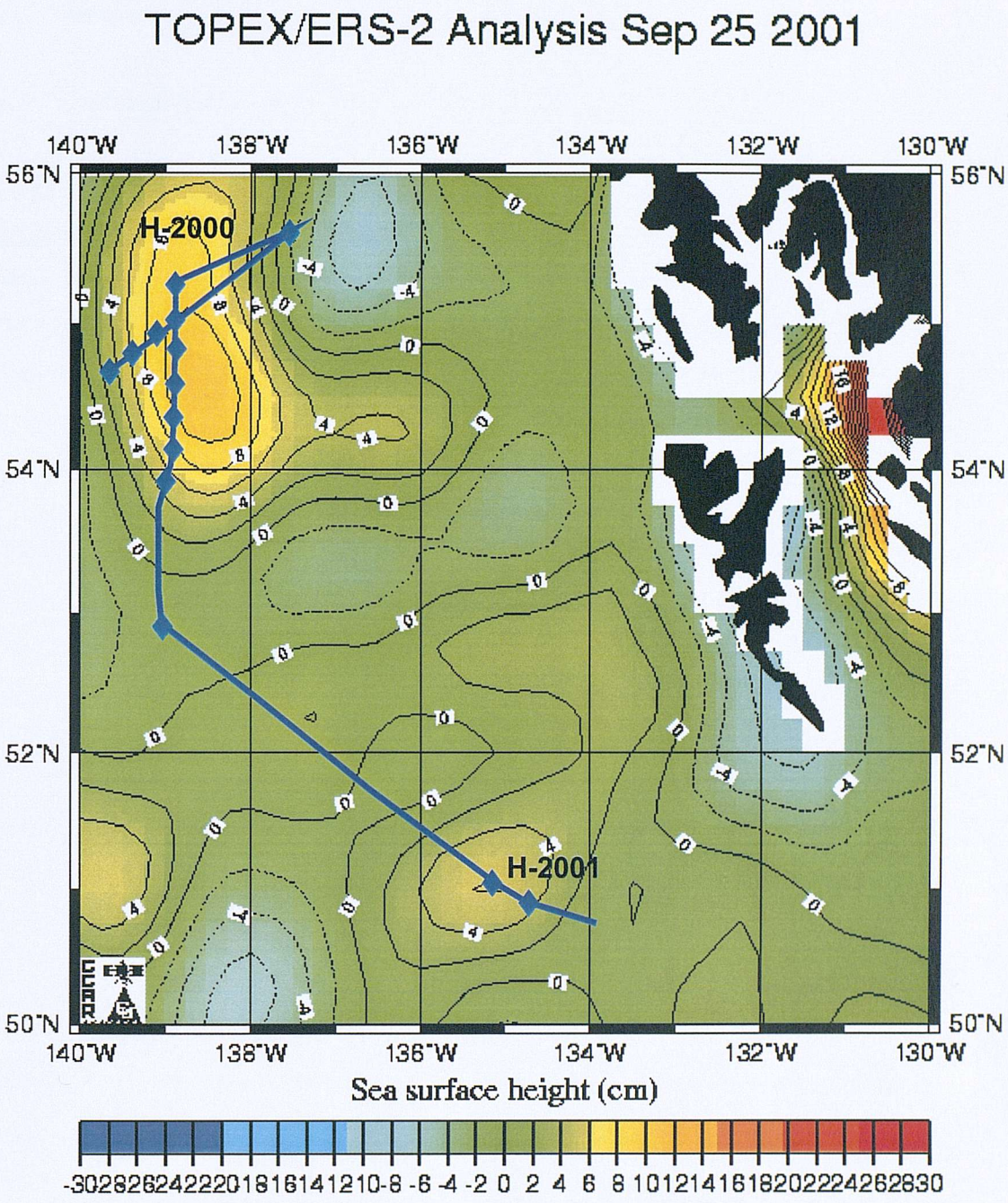


Figure 5.5 Satellite image of the study area for the September 2001 cruise, showing both H-2000 and H-2001 and the cruise track in blue and the stations in blue diamonds.

5.3 Results

5.3.1 Evolution of the eddies

H-2000 was spawned in late January/February 2000 and from satellite images it is clear that it travelled in a west north-west direction out into the open ocean. This eddy was retained on a shallow seamount, Bowie Seamount (53° 20' N, 135° 40' W) for some time during the first summer and broke away by October/ November 2000. H-2001 was spawned in February 2001 and from satellite images it travelled in a south-west direction out into the open ocean.

5.3.2 Physical Characteristics

Both eddies contain a core of warm low salinity water that depresses the isothermal surfaces in and below the halocline. At salinities between 32.6 and 34 (base of the mixed layer to the base of the eddy core waters), eddy waters were 2 °C warmer than adjacent waters in both June 2001 (Fig 5.6 a, b). A similar pattern was observed in H-2000 surveyed in June 2000 where the isotherms were deepened by more than 200m (Whitney & Robert, 2002). When H-2000 was surveyed in June and September 2001 (16 and 19 months after formation) the core eddy waters were still warmer than surrounding waters, although this difference had decreased by 1 °C. The core temperature of Haida eddies reflects conditions that existed along the coast of British Columbia during eddy formation (Crawford, 2002).

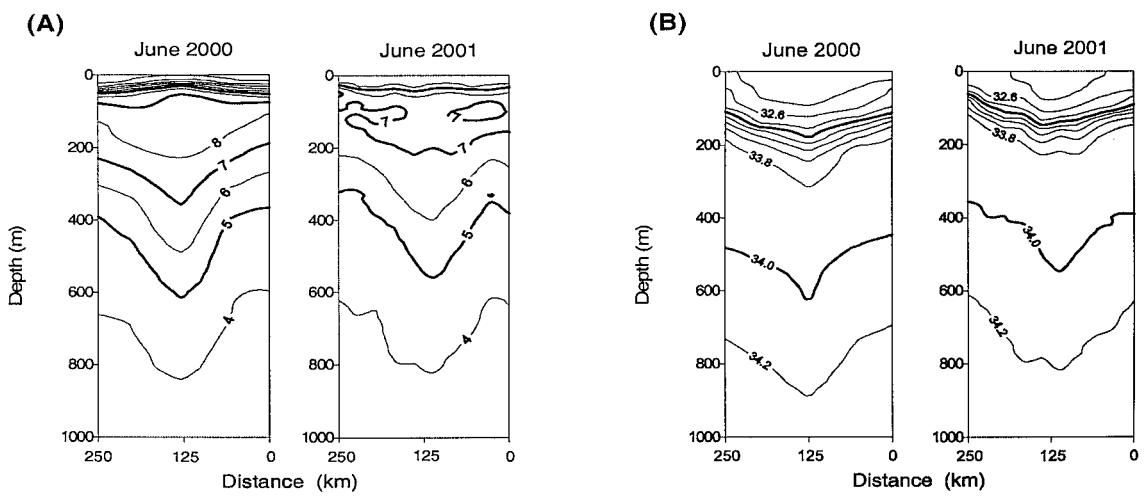


Figure 5.6 (**A**) Temperature and (**B**) Salinity sections through H-2000 and H-2001 Eddies, 4 months after formation. Data from H-2000 taken from Whitney & Robert (2002).

5.3.3 Nutrients

In H-2001 nutrients were homogenous in mixed layer and increased with depth. In June the depth of the nutricline was 75m, which shoaled to 40m by September. Nitrate, phosphate and silicic acid concentrations are higher in the reference station than in eddy indicating a higher biological demand for these nutrients within the eddy. Average nitrate and silicic acid concentrations in the surface water column at the reference station are 10.2 μM and 16.2 μM respectively, which were double those found at the centre of the eddy (5.8 μM and 8.5 μM; Appendix 3.1). Phosphate concentrations are also lower within the eddy with a concentration of 0.83 μM compared to 1.09 μM observed at the reference station. This indicates a higher degree of biological activity within these eddy systems. The concentrations of nitrate, phosphate and silicic acid were lower in the centre of the eddy compared to edge. The northern and southern regions of the eddy appear to be different, with higher concentrations of nitrate and phosphate to the north of centre. A small decrease in nutrients was observed between June and September 2001.

Nutrient concentrations in H-2000 were higher than those observed in H-2001 and although they are lower than observed in the reference station they were approaching background levels. A small decrease was observed in phosphate and silicic acid concentrations between June and September. However, the nitrate concentrations at the edge station in September were half those observed in June (4.1 μM and 8.4 μM respectively), which corresponds to a zone of higher chlorophyll *a* concentrations.

Haida eddies can appear either nutrient enriched or impoverished when compared to ambient waters of similar salinities. If an eddy heads southwards as in H-2001 then its core waters below the nutricline are relatively nutrient rich by as much as 6 μM nitrate and 10 μM silicic acid above background over a salinity range of 32.8 to 33.9 (Fig 5.7 a). However, eddies which travel westward into HNLC waters of the Gulf of Alaska appear nutrient poor, containing 5 μM nitrate and 11 μM silicon (Fig 5.7 b). A small decline in nutrient levels was observed between June and September in both eddies with the exception of nitrate in H-2000 where the concentration in June (8.8 μM) was double that observed in September (4.1 μM). An earlier study carried out on H-1998 revealed a steady decline in nutrient levels over the period of one year (Whitney & Robert, 2002).

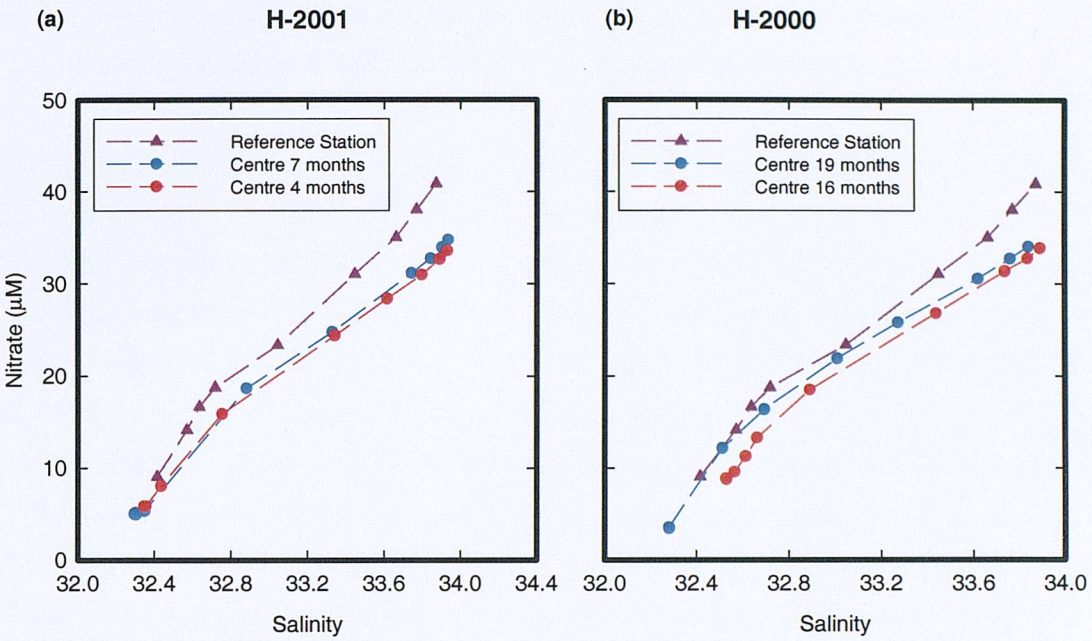


Figure 5.7 Nitrate vs. salinity in core waters of (a) H-2001 and (b) H-2000 over time. The reference station represents HNLC waters.

5.3.4 General features of total dissolved zinc concentrations

Total dissolved Zn concentrations for both reference stations are in good agreement with previous data for this region (Martin *et al.*, 1989; Lohan *et al.*, 2002). These Zn profiles are oceanographically consistent, showing similarities to the silicic acid distribution, with Zn depletion in surface waters and increased concentration with depth. However, Zn concentrations at the edge or in the centre of H-2001 in June 2001 (4 months after formation) showed no similarities to either silicic acid or phosphate for both surface waters and at depth. By September (7 months after formation) Zn concentrations were similar to silicic acid. In H-2000 the Zn profiles are similar to that of silicic acid in both June and September 2001. The water column was stratified in June and September with mixed layer depths of 38m and 46m respectively.

5.3.5 Total dissolved Zn concentration in H-2001

Total dissolved Zn concentrations are shown in Fig 5.8 (a) and Appendix 3.1. There is a clear gradient of dissolved Zn concentrations decreasing within the centre of the eddy and at the edge, at depth over time as the eddy ages (Fig 5.8 a). This is most evident at 400m with Zn concentrations decreasing from 9.74 nM in the centre at 4 months to 5.46 nM after 7 months. Below 600m there is no difference in Zn concentrations between samples taken from the eddy or

at the reference station indicating that the eddy does not influence Zn concentrations in deeper waters. This is also reflected in the nutrient concentrations within this eddy (Appendix 3.1).

In the surface water column, down to a depth of 100m, Zn concentrations are similar to the reference station. The shelf station P4, just south of the region where the Haida eddies were formed has higher Zn concentrations in the surface ocean (Fig 5.8 a). Iron concentrations behave in a similar manner to Zn. Iron data from the Hecate Strait indicate higher Fe concentrations in surface waters in February (2.35 nM) as the eddy is formed and this decreases over the first 4 months to 0.2 nM, which is similar to Fe concentrations observed at the reference station (Sutherland *pers. comm*). In June 2001 the Zn concentration in the top 75m in the centre of the eddy is lower than observed in September. At 40 and 75m Zn concentrations in the centre of the eddy increased by 0.5 and 0.4 nM respectively in September compared to June 2001. A large storm occurred just before sampling H-2001 in September and these results may reflect increased mixing of Zn into the upper water column.

Below 100m, Zn concentrations decreased between June and September 2001. However due to the adverse weather conditions it was not possible to sample below 600m. The Zn concentration at 600m in September is only slightly higher than observed in June (6.54 nM compared to 6.30 nM).

Dissolved Zn concentrations along the S-N transect are lowest at either edge of the transect where the two edge stations were situated just outside the eddy. Highest Zn concentrations were observed at the station adjacent the centre.

5.3.6 Total dissolved Zn concentrations in H-2000

Total dissolved Zn concentrations for H-2000 are shown in Fig 5.8 (b) and Appendix 3.1. Zinc concentrations 16 months after formation were approaching concentrations observed at the reference stations. At 400m Zn concentrations are similar to background Zn concentrations in both June and September. However, compared with H-2001 at depths below 600m, Zn concentrations in the centre of the eddy are higher than those observed in the reference station.

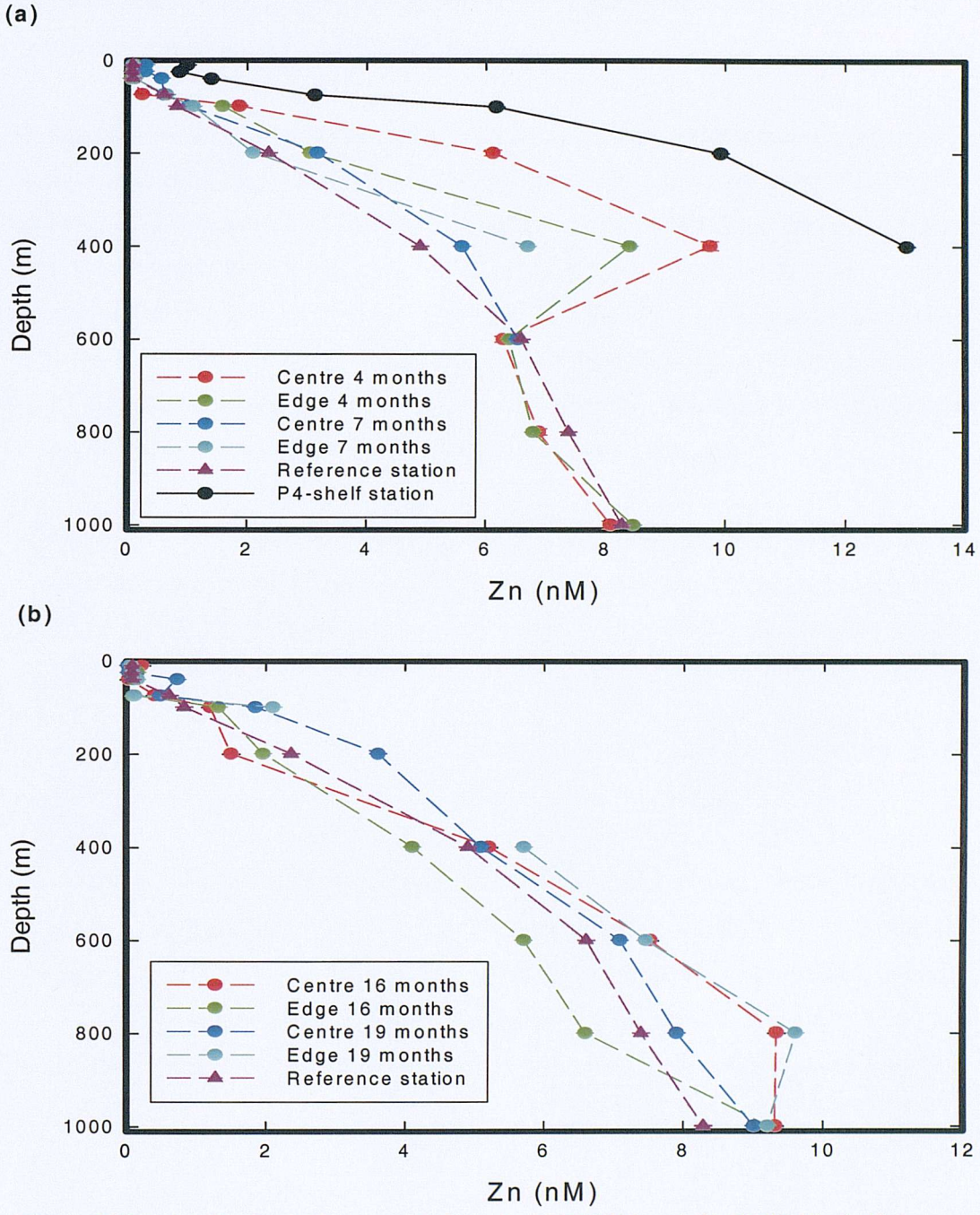


Figure 5.8 Total dissolved Zn concentrations in (a) H-2001 and (b) H-2000 in both June and September 2001. Error bars represent the standard deviation on three separate measurements on the same sample. Months refer to the months after formation i.e. 4 months is June.

The 400m water sample at the of edge H-2000 (16 months after formation) had a salinity value the same as at 300m and therefore explains the low Zn concentrations due to deep water mixing observed at 400m compared to the other stations. However, at 600m and 800m the Zn

concentration at this edge station are lower than the reference station and at the edge station sampled in September (19 months after formation).

In the upper water column the Zn concentrations in the centre are similar to the reference station in June 2001. The edge of the eddy however, has lower Zn concentrations than the centre at 75m (0.1nM compared to 0.41nM). This most probably reflects the biological demand for Zn at the edge stations as the chlorophyll *a* concentrations at 75m at the edge station is 0.53 mg m^{-3} compared to 0.15 mg m^{-3} in the centre of the eddy in June 2001. By September 2001 (19 months after formation) the Zn concentrations in the upper 25m at the eddy centre are lower than those at either the edge station of the reference station and lower than those reported at OSP (Chapter 3). This is the lowest Zn concentration reported for the open ocean.

Along the S-N transect highest Zn concentrations were observed at the southern end decreasing towards the centre, where the lowest Zn concentration was observed, and then increasing slightly as the transect approached the northern end of the eddy. The two stations at either side of the centre of the eddy had lower Zn concentrations than observed at the reference station (Appendix 3.1).

5.3.7 Zinc Speciation

The Zn titration data and the corresponding linearised data for the edge station H-2001 in June 2001 at 25 m is presented as a typical example in figure 5.9 a, b, respectively. The clear curvature in the titration data at low Zn concentrations indicates the presence of a Zn binding ligand. Figure 5.9 (a) was transformed using equation 2.4 (Chapter 2) and yielded a ligand concentration of 1.130 ± 0.023 nM with a conditional stability constant (log K'_{ZnL}) of 10.3. As in Chapters 3 and 4 the one-ligand one-metal model was applied to all the sample titrations carried out in this study.

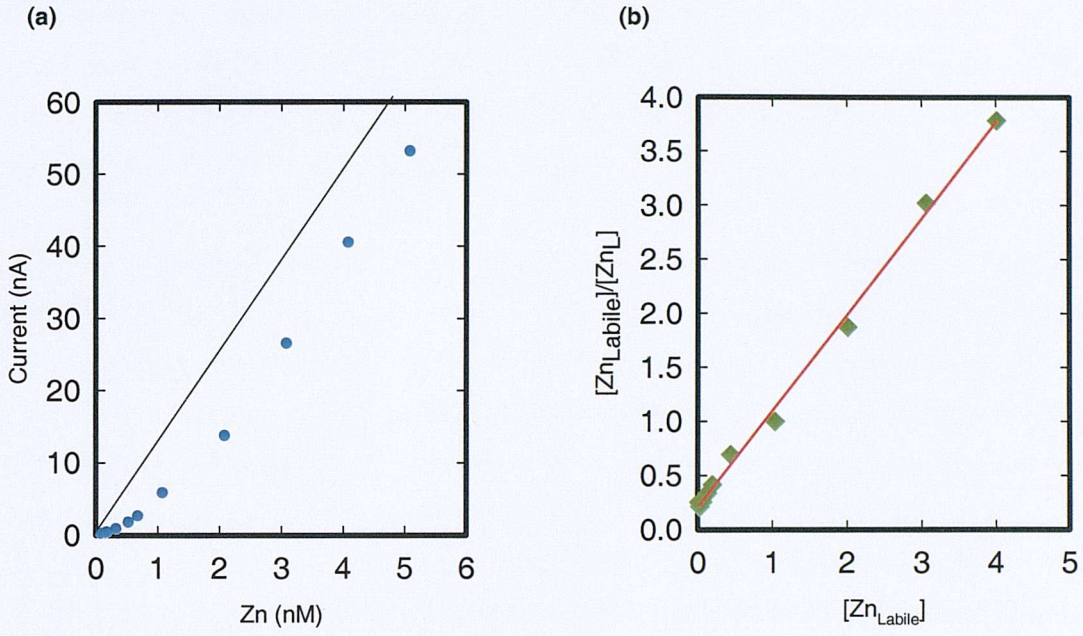


Figure 5.9 Zn titration data for Edge station H-2001 (June 2001) at 25m. (a) Peak current vs. added Zn concentration. (b) Linearisation of data from which a ligand concentration of 1.13nM and a log K'_{ZnL} of 10.3 from the slope was calculated.

A number of replicate titrations were carried out to determine method reproducibility. Replicate titrations were in good agreement with a standard deviation in ligand concentrations of ~ 0.2 nM and in the conditional stability constant of 0.1 (Table 5.2).

Station	Depth	Zn_T (nM)	S.D.	Ligand (nM)	S.D.	log K'_{ZnL}	S.D.
Centre 4 months	40	0.08		1.41		10.3	
	40	0.09		1.45		10.4	
Centre 7 months	75	0.62		2.20		10.4	
	75	0.61		2.20		10.4	
	75	0.60	0.01	2.40	0.12	10.3	0.058
Reference Station	10	0.09		1.10		10.4	
	10	0.09		1.14		10.4	
	10	0.08	0.006	1.20	0.05	10.4	0

Table 5.2 Replicate analysis of Zinc speciation from H-2001 and the reference station. S.D. is the standard deviation on the three measurements and Zn_T is the total dissolved Zn concentration.

Natural ligand concentrations, conditional stability constants for the Zn-ligand complex and Zn^{2+} concentrations for H-2001, H-2000 and the reference station are shown in Figure 5.10 a, b, c, d, e, f, g and are given in Appendix 3.2. Stability constants in this study are in good agreement with

those of previous Zn studies (Bruland, 1989; Donat & Bruland, 1990; Ellwood & Van den Berg, 2000) and data in Chapter 3 and 4. As reported in Chapter 3 the stability constants for the Zn-ligand complex changed little with sampling depth or location, with log K'_{ZnL} values ranging between 10.1 and 10.5 (Fig 5.10 a, b, c, d, e, f, g).

Ligand concentrations observed are also similar to those reported for the Atlantic (Ellwood & Van den Berg, 2000) where surface waters (< 75m), ligand concentrations varied between 0.75 and 2.2 nM and were always in excess of the total dissolved Zn concentration. However, the ligand concentration at 200m in the centre of the H-2001 eddy in June was only half the total Zn concentration but the stability constant was within the range of other measurements made within this study. At all other stations the concentration of the ligand at 200m is greater than the dissolved Zn concentration. Increased Zn concentrations at and below 400m have fully 'titrated' the Zn-binding ligand and therefore the presence of the ligand could not be observed. Below 400m the predominant form of Zn is inorganic Zn. In the upper water column between 96 and 99% of Zn is complexed to the organic ligand.

5.3.8 Zn speciation in H-2001

Along the S-N transect, ligand concentrations ranged from 1.02 to 2.21 nM and the lowest concentrations were observed at either end of the transect (Table 5.3). Similarly, the Zn^{2+} concentrations are also lowest at either end of the transect and range from 2.8 to 10.3 pM. From the depth profiles at the centre of the eddy, a general trend of increasing ligand concentrations is observed as the eddy ages. Due to adverse weather, no speciation measurements were carried out at the edge station in September 2001 and therefore a similar trend in the edge station cannot be established. In June 2001 the ligand concentration was higher at the edge compared to the centre resulting in lower Zn^{2+} concentrations in the upper water column. The fluorescence maximum was observed at 40m in the centre and the edge in both June and September 2001. This maximum coincides with the highest ligand concentration observed in the mixed layer in both June and September

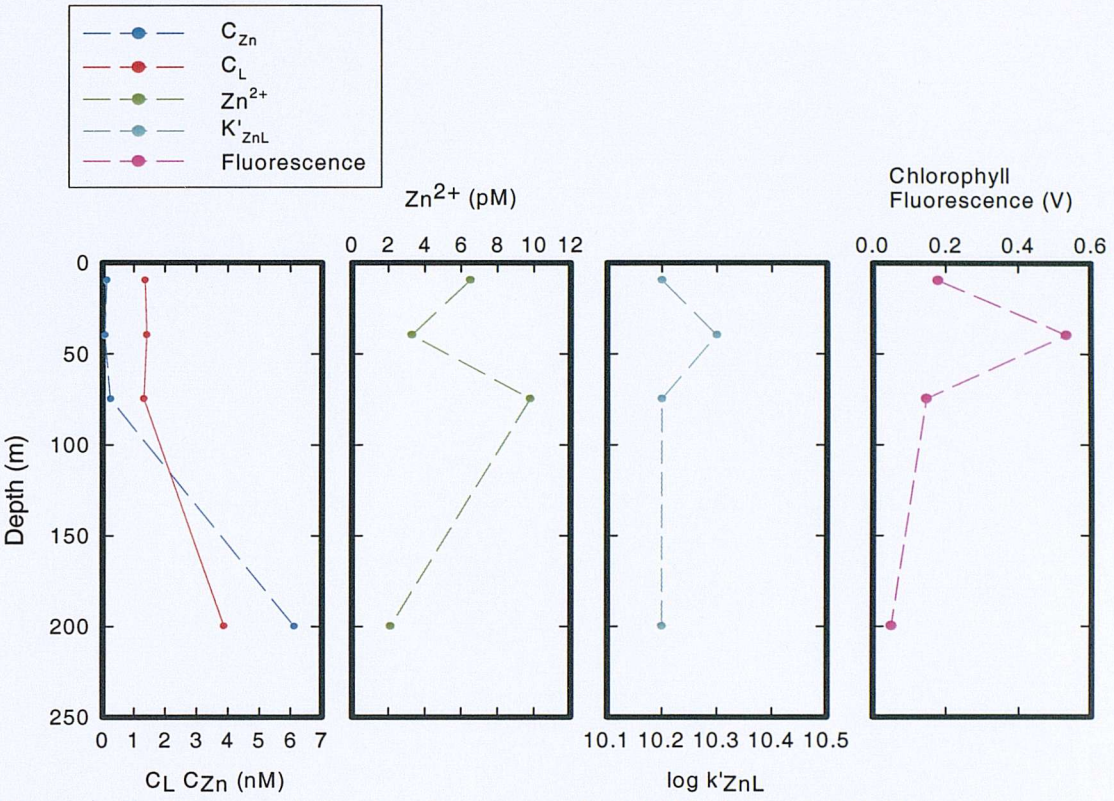


Figure 5.10 (a) Zinc speciation data for centre station H-2001 in June 2001 (4 months).

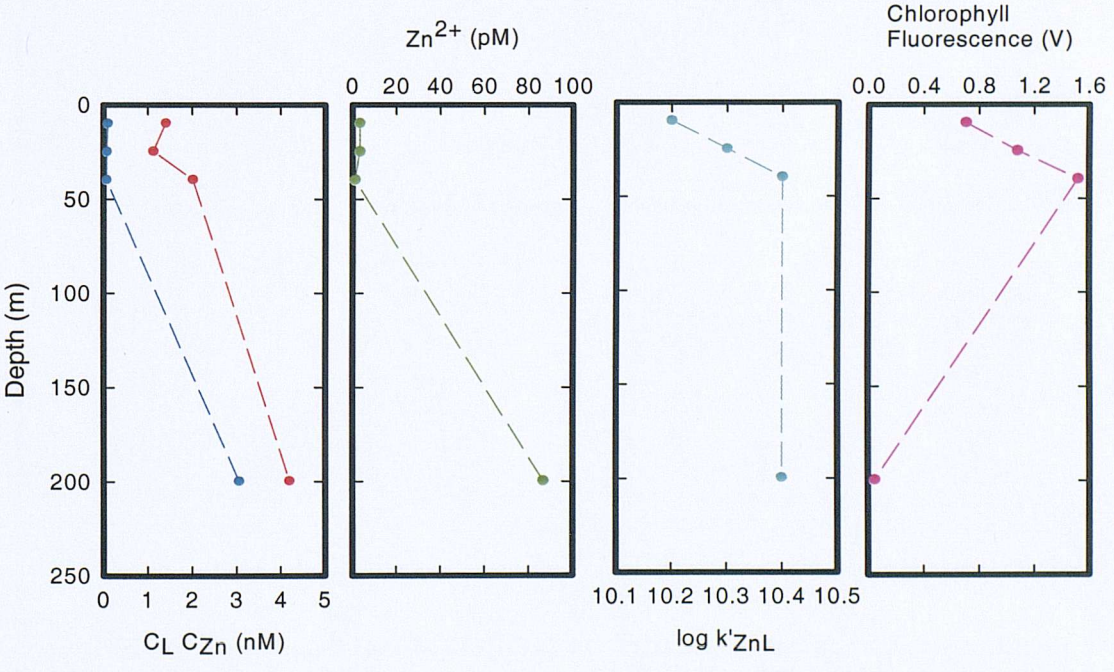


Figure 5.10 (b) Zinc speciation station for edge station H-2001 in June 2001 (4 months).

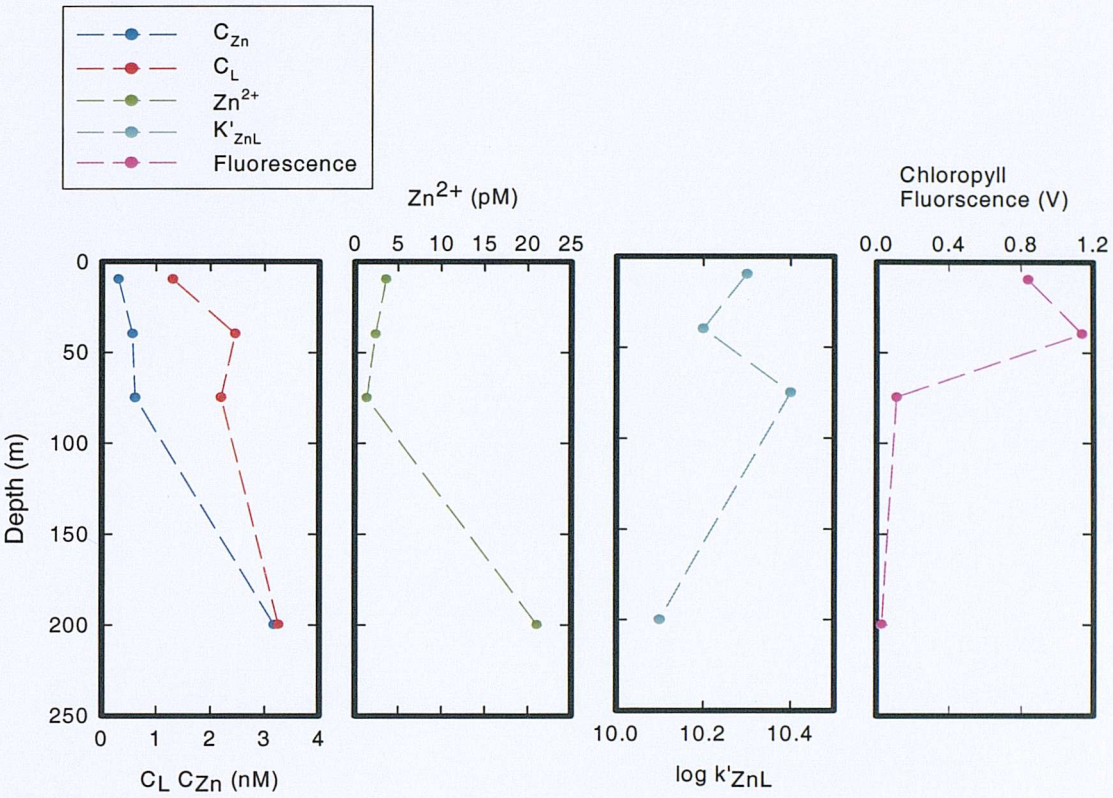


Figure 5.10 (c) Zinc speciation data for centre station H-2001 in September 2001 (7 months).

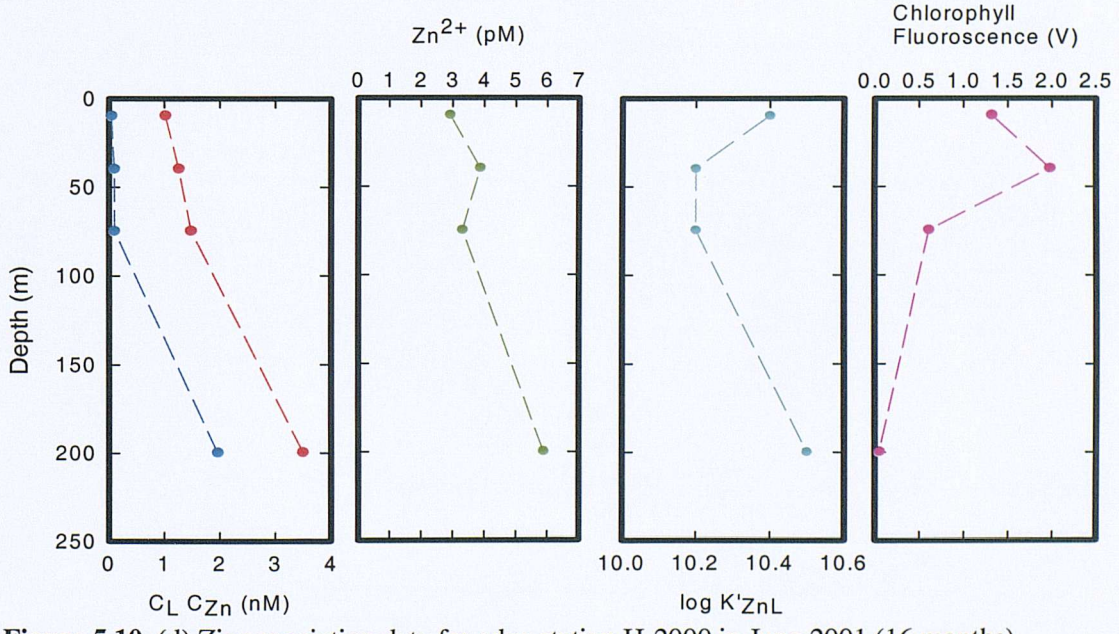


Figure 5.10 (d) Zinc speciation data for edge station H-2000 in June 2001 (16 months).

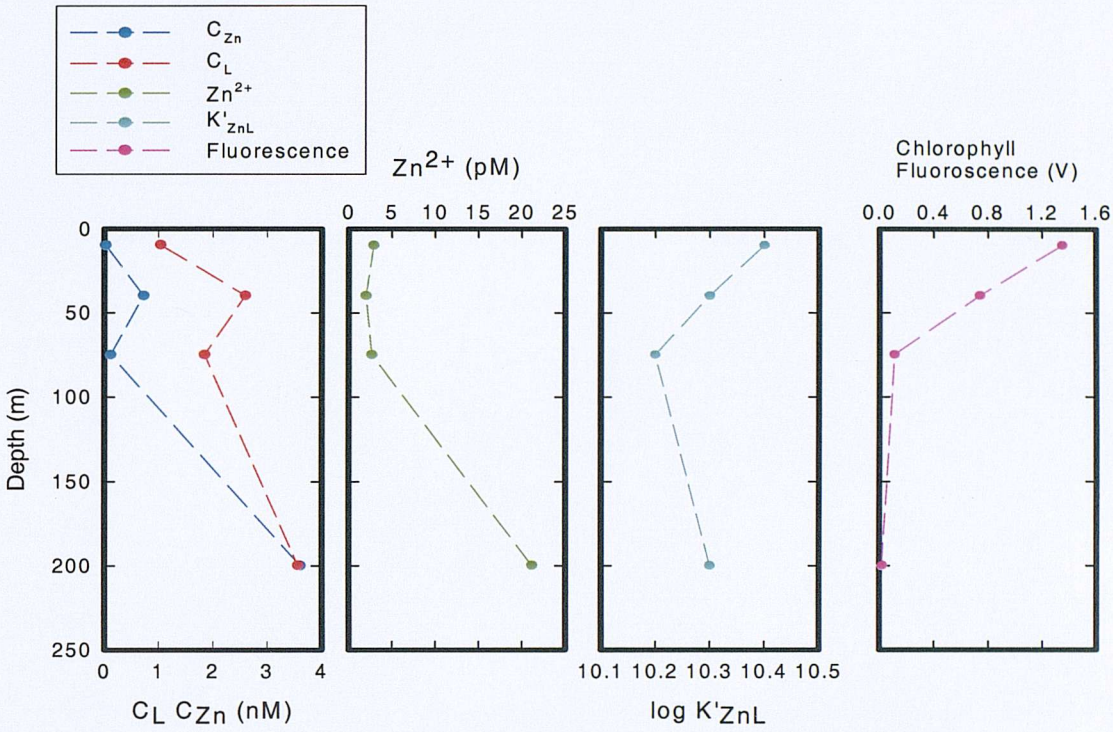


Figure 5.10 (e) Zinc speciation data for Centre station H-2000 in September 2001 (19 months).

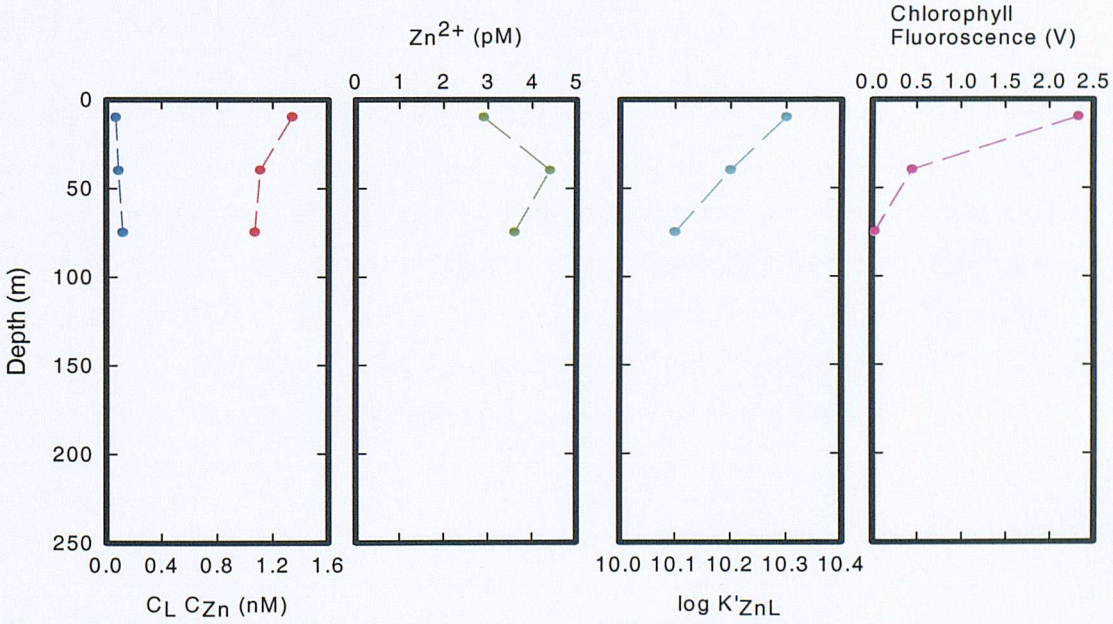


Figure 5.10 (f) Zinc speciation data for edge station H-2000 in September 2001 (19 months).

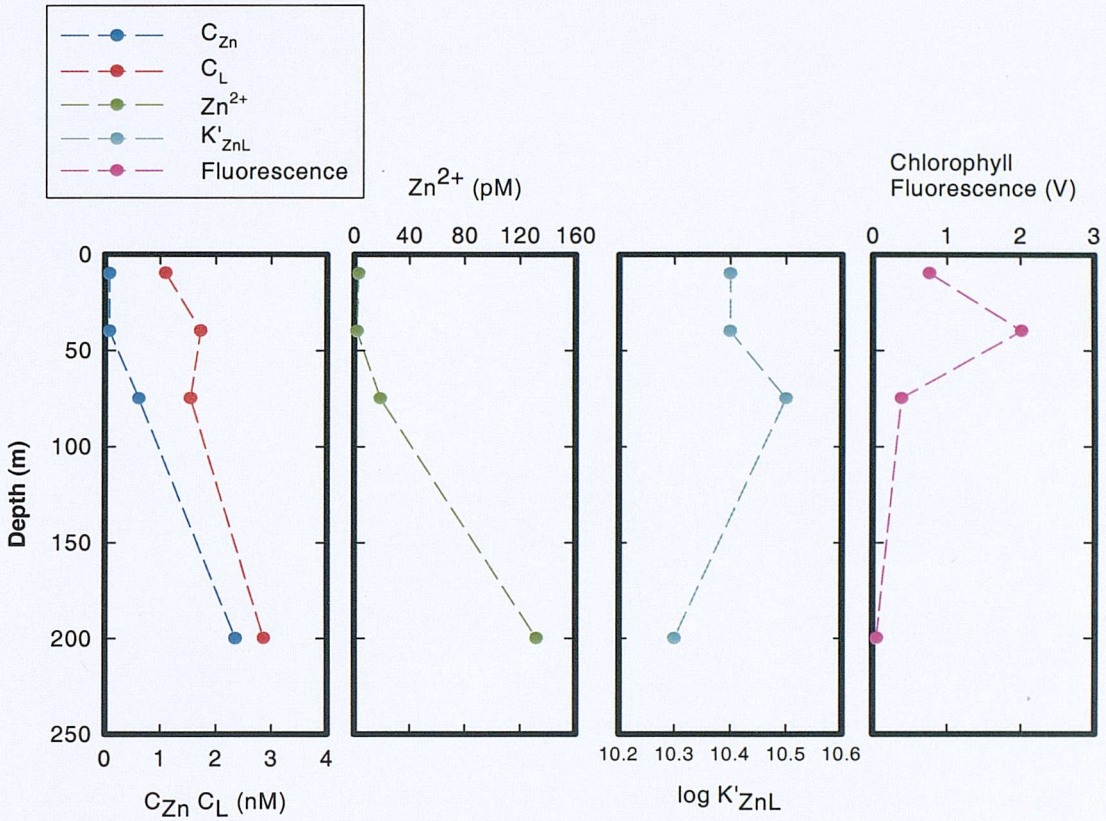


Figure 5.10 (g) Zinc speciation profiles for reference station in September 2001.

5.3.9 Zn speciation in H-2000

Unlike H-2001 the highest ligand concentrations observed on the S-N transect are at the southern end of the eddy and decrease in a northerly direction with the lowest concentration observed at the eddy centre (HOOF) (Table 5.3). Neither the total dissolved Zn nor the Zn^{2+} concentrations observed display this pattern. The Zn^{2+} concentration is lower than H-2001 and is relatively constant throughout the eddy at 10m with values ranging from 1.75 to 3.3 pM. The ligand concentration decreased in the edge station between June and September 2001 with the exception of the sample at 10m where an increase in ligand concentration was observed.

H-2001 transect 10m

Date	Station Number	Position	C_{Zn} (nM)	C_L (nM)	log K'_{ZnL}	Zn^{2+} (pM)
2/6/01	Ed 02	50° 15'N 133° 50'W	0.09	1.09	10.4	3.28
2/6/01	Ed 03	50° 30'N 133° 50'W	0.10	1.32	10.2	4.63
2/6/01	Ed 04	50° 45'N 133° 50'W	0.25	1.34	10.3	10.3
2/6/01	Ed 05	50° 55'N 133° 50'W	0.21	2.21	10.3	4.97
2/6/01	Ed 06	51° 05'N 133° 50'W	0.14	1.36	10.2	6.50
3/6/01	Ed 07	51° 14'N 133° 50'W	0.16	1.44	10.4	4.64
3/6/01	Ed 08	51° 33' N 133° 50'W	0.10	1.41	10.2	3.50
3/6/01	Ed 09	51° 45'N 133° 50'W	0.07	1.13	10.2	3.68
3/6/01	Ed 10	50° 00'N 133° 50'W	0.06	1.02	10.3	2.80

H-2000 transect 10m

Date	Station Number	Position	C_{Zn} (nM)	C_L (nM)	log K'_{ZnL}	Zn^{2+} (pM)
21/9/01	HOOA	53° 30'N 138° 20'W	0.11	2.5	10.2	2.74
21/9/01	HOOB	53° 45'N 138° 20'W	0.15	2.36	10.4	2.61
21/9/01	HOOC	53° 60'N 138° 20'W	0.12	2.14	10.3	2.82
21/9/01	HOOD	54° 10'N 138° 20' W	0.06	1.53	10.2	2.35
21/9/01	HOOE	54° 20'N 138° 20'W	0.05	1.10	10.4	1.75
22/9/01	HOOF	54° 30'N 138° 20'W	0.03	1.04	10.4	2.91
25/9/01	HOOH	54° 50'N 138° 20'W	0.07	1.01	10.3	3.34
25/9/01	HOOJ	55° 15'N 137° 37'W	0.08	1.29	10.2	3.74
26/9/01	HOOL	54° 00'N 138° 50'W	0.09	1.34	10.3	2.95

Table 5.3 Zinc speciation data from the S-N transects of H-2001 and H-2000.

5.4 Discussion

5.4.1 Distribution of Zn below the mixed layer in H-2001

Below 600m Zn concentrations are similar to the reference station and indicate that the eddy is not influencing the distribution of Zn at these depths. A similar pattern is observed for the nutrients, temperature and salinity indicating that 4 months after formation the eddy is not penetrating to depths below 600m. The top 500m of Haida eddies typically transport seaward about 20 to 50% of the fresh water input into the continental margin along the British Columbia Coast (Crawford, 2003). Total dissolved Zn concentrations from 600-1000m in this study are similar to those observed by (Martin *et al.*, 1989). The dissolved Zn concentrations below 400m are determined by the nutrient-type vertical profile and the advective flow of deep-water along

the global conveyor belt, which has evolved over long time scales. After 4 months H-2001 is still relatively young compared to the 2 years that these eddies typically persist for.

Unlike H-2000 which is still strongly evident on the satellite image in September, H-2001 appeared to be a weaker eddy and both its sea surface elevation and size had decreased over time (Fig 5.5). The decrease in Zn concentrations in the centre (100m- 400m) of H-2001 over the three months from June to September 2001 may be due to eddy mixing with surrounding waters. The abundance of phytoplankton and zooplankton in the H-2001 is much lower than those observed in H-2000 (Batten & Crawford, 2003). Nutrient concentrations within H-2000, 4 months after formation were lower than those observed in H-2001. H-2000 shows 3 times higher biological consumption of C and was a significant sink of CO_2 than H-2001 over the same time period (Chierici *et al.*, 2003). However, H-2000 was sampled for dissolved Fe, 4 months after formation and the Fe concentration is similar to that observed in the weaker, H-2001 eddy over the same time period (Wong *et al.*, 2003). A large decrease in Fe was also observed in the centre of H-2001 between June and September. Therefore, although a larger biological activity was observed in H-2000, this appears to make little difference to the trace metal distribution observed in these eddy systems.

5.4.2 Distribution of Zn in H-2000

Higher total dissolved Zn concentrations are observed in H-2000 in September 2001 compared to June 2001 from 40 to 400m. An animation of 3-day satellite altimeter images for 1992 to November 2002 revealed that during spring and summer 2001, H-2000 merged with a newly formed eddy in late June 2001 (circa June 29). This new eddy was formed near the West Coast of Graham Island (the largest of the Queen Charlotte Islands) (Fig 5.1) in April 2001 and not at Cape St James where the eddies in this study originated from. In July 2001, this eddy had caught up with and fully merged into H-2001 (Compare Fig 5.11 and 5.5). H-2000 gained heat between 16 and 19 months when it merged with the new eddy (Crawford, 2003). As the older eddy had entrained water from the new eddy, this may have been a mechanism of entraining higher dissolved Zn concentrations into H-2000. In addition to this, the distribution of dissolved Zn concentrations could reflect the enhanced wind-induced mixing due to a series of storm events, which occurred during sampling. Evidence for the storm events comes from nutrient and chlorophyll *a* data. A lower concentration of both nutrients and chlorophyll *a* was observed at station HOOE, which was sampled just prior to the storm and was located at a distance of ~ 17

km from the centre station HOOF. High biological activity as indicated by the higher chlorophyll *a* data (Appendix 3.1) at this edge station in September would result in increased particle flux from the water column and remineralisation of Zn at depth. Zn concentrations at depth (100-400 m) in the centre and edge station of H-2000 in September are higher than those observed in the reference station or in the centre of H-2000 in June 2001.

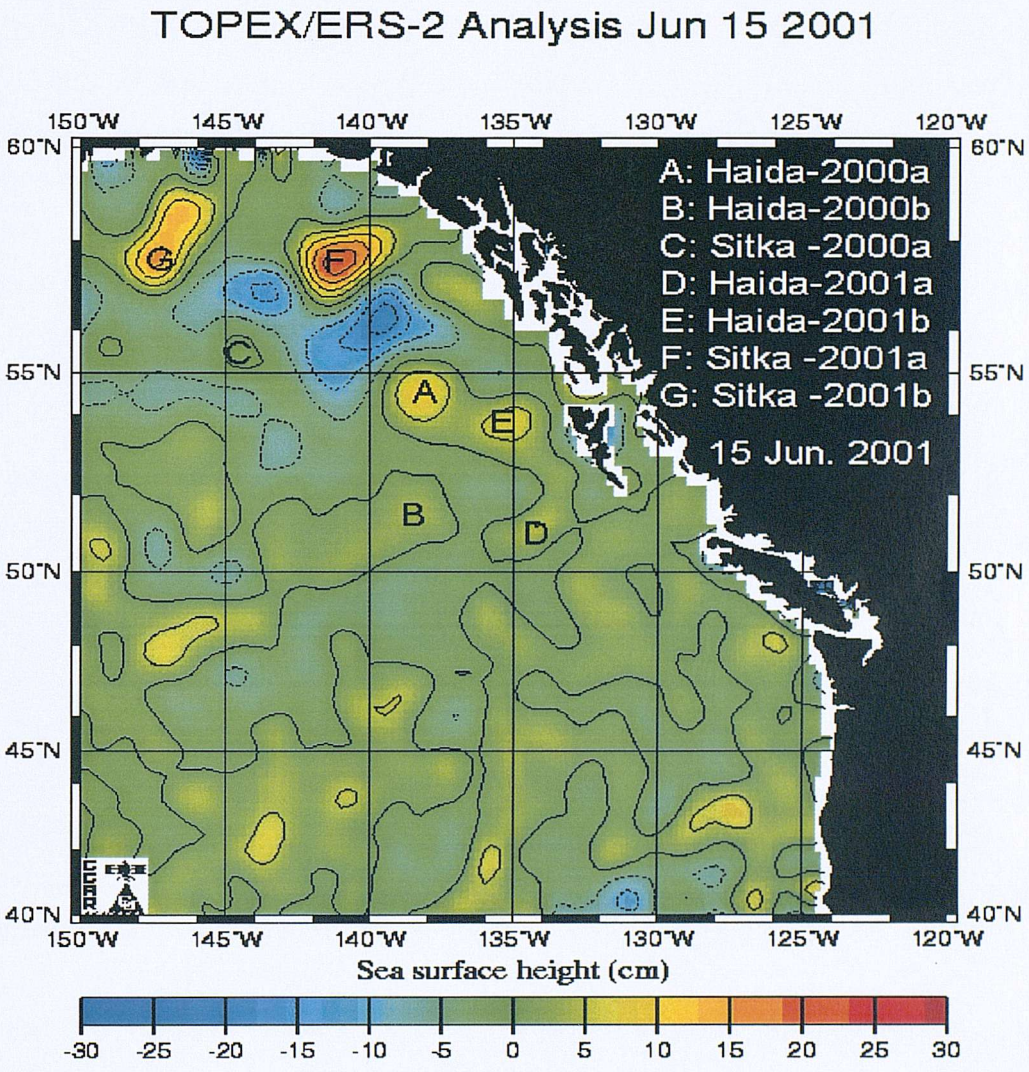


Figure 5.11 Satellite picture showing H-2001b (E) heading towards H-2000 (A) in June 2001.

5.4.3 Mixed layer distribution of dissolved Zn in Haida eddies

Dissolved Zn concentrations in the mixed layer are similar to those observed at the open ocean station P20 (Chapter 3) but higher than those observed at the reference stations. In general Zn concentrations decreased as the eddies aged, although Zn concentrations increased between June and September in the centre of H-2001. This increase may reflect a series of storm events, which occurred after sampling in June and prior to sampling in September. The Zn concentration immediately below the mixed layer is higher than that observed in the open ocean. Thus a storm event could mix these relatively Zn enriched waters up into the surface layer. Along line P (Chapter 3) no difference was observed between winter and summer total dissolved Zn concentrations. However, this data suggests that within eddies, autumn/ winter storm events can increase the concentration of Zn in surface waters. In H-2000 the dissolved Zn concentrations in the centre of the eddy are very low and similar to those observed at OSP. At the eddy edge the Zn concentrations are lower than that observed at the eddy centre with the exception of H-2000 in September 2001. With respect to the centre stations, the edge stations are more dynamic and subjected to increased mixing through entrainment with the surrounding waters.

Three major processes can effect the total dissolved Zn concentrations observed within the mixed layer of Haida eddies; (1) atmospheric input, (2) biological activity and (3) mixing. As discussed in Chapter 3, atmospheric input to this region is small (0.01 nM) (Bruland *et al.*, 1994) and is expected to have a negligible impact on total dissolved Zn concentrations compared to other processes in Haida eddies. Within Haida eddies, primary productivity is significantly higher than surrounding waters (Fig 5.3; Peterson *et al.*, 2003). Higher concentrations of dissolved trace metals such as Zn and Fe and macronutrient concentrations have been hypothesised for this increase in primary productivity (Whitney & Robert, 2002). A large phytoplankton bloom was observed to occur in May and therefore by June the decrease in Zn and nutrient concentrations in the surface water column would suggest a biological demand for dissolved Zn.

The surface layers of Haida eddies undergo frequent and rapid wind-driven horizontal exchange with the surrounding non-eddy ocean water due to Ekman transport during wind events and strong inertial currents following wind events (Mackas *et al.*, 2003). It has been observed that zooplankton taxa retained in the eddy were those that avoided the surface layer for some or all of the day (Mackas & Galbraith, 2002). The physical spinning motion of the eddy itself has been hypothesised to remove nutrient-rich waters from the surface water to depth as indicated by the

depressed isopleths of temperature, salinity and nutrients (Whitney & Robert, 2002) (Fig 5.6 a, b). This process would also remove trace metals such as Zn.

After 19 months, the eddy does not reflect true HNLC conditions, as the concentrations of nitrate and silicic acid are still significantly lower than surrounding waters. This could reflect the fact that higher nutrient utilisation due to the higher trace metal concentrations in these environments created ambient conditions for phytoplankton growth and the signature of this uptake is still evident in the centre of the eddy. Therefore, the distribution of Zn in the surface mixed layer will be the result of the uptake by phytoplankton and loss to the surrounding ocean by mixing processes.

5.4.4 Processes influencing vertical Zn distributions in H-2001

Dissolved Zn concentrations between 100 and 400m are significantly higher than those observed in the reference station. The only obvious processes that can lead to the increased concentrations are removal of Zn from the mixed layer through biological processes and subsequent remineralisation at depth or dilution of the higher Zn concentrations in the source waters of Haida eddies with adjacent oceanic waters. Both processes were investigated to elucidate the mechanism for the higher Zn concentrations observed.

5.4.4.1 Biological Processes

Phytoplankton require Zn for growth and many studies have been carried out on the uptake of Zn by phytoplankton (Chapter 1 & 4). Both phytoplankton and zooplankton are present in larger numbers in Haida eddies compared to the open ocean waters (Mackas & Galbraith, 2002; Peterson *et al.*, 2003). Additionally zooplankton vertically migrate on a daily basis and at different stages of their life histories and can also remove Zn from the upper water column and contribute to remineralisation of Zn at depth. Hutchins *et al.* (1994) has demonstrated that remineralisation of Zn from living plankton cells can be significantly enhanced by the grazing activities of zooplankton and suggested that biologically mediated regeneration and recycling could be an important process in the biogeochemical cycling of Zn. However, as yet little is known about uptake of trace metals by zooplankton in the natural environment.

In order to estimate the impact on dissolved Zn concentrations via uptake by phytoplankton and subsequent removal from the mixed layer, an assumption can be made that all Zn removed from the surface layer over time has been utilised by phytoplankton, and then remineralised at 400m. As there are no available dissolved Zn concentrations from the source waters of the Haida eddies (Hecate Strait), Zn concentrations from a shelf station P4 (Chapter 3) just south of the Hecate Strait was utilised as the source water. Total dissolved Zn concentrations at both P4 and the centre station (4 months) were integrated to a depth of 75m. The maximum addition of dissolved Zn at 400m over a period of 4 months is 106 μ mol m^{-2} which represents 8.1% of the total Zn observed at this depth. If 40m is applied instead of 75m as the mixed layer is 45m, this values decreases to 32.5 μ mol m^{-2} or 2.5% of the total.

Another method for estimating the remineralisation of Zn at 400m uses a simple one-dimensional model of oxygen utilisation. Two assumptions were made for this calculation; (1) that minimal vertical (diapycnal) mixing occurs, which appears too reasonable for eddy systems (Martin & Richards, 2001) (2) that all the oxygen utilised was for carbon oxidation. Therefore, the change in oxygen concentration over time as the eddy ages will equal the carbon oxidised. The particulate Zn concentrations at OSP show a Zn:C ratio of 15 μmol: mol (Martin *et al.*, 1989) while those from Kuss & Kremling (1999) for the Atlantic of 11 μmol: mol. In this calculation the Zn: C ratio from Kuss & Kremling (1999) is utilised, as both particulate Zn and C in the water column was calculated whereas Martin *et al.* (1989) calculated particulate Zn in the water column and *assumed* that 33% of suspended particulate matter was organic carbon. Opal dissolution is not considered as a major process for the release of Zn (Chapter 3). Using this approach, the change in O_2 concentrations between the shelf station and that in the centre of the eddy is 6.3 μM, which corresponds to an oxygen utilisation of 0.05 μM d^{-1}. The maximum Zn released from particles contributes only a very small fraction (5.6 pM) 0.06%, of the total Zn concentration observed at 400m in the centre of the eddy in June 2001.

Therefore it appears that biological process can not account for the higher total dissolved Zn concentrations observed at 400m compared to 'background' oceanic levels.

5.4.4.2 Dilution/mixing processes

It is more tenable to explain the Zn concentrations at 400m in centre of the eddy, 4 months after formation as a consequence of dilution of the higher Zn concentrations present in the source

waters of Haida eddies with the surrounding oceanic waters into which they travel. With the limited data set available, an estimate of Zn removal by isopycnal mixing in the eddy at 400m over time was calculated using a simple one-dimensional box model.

$$\frac{\partial C}{\partial t} = \mu[C_0 - C] \qquad (1)$$

where C is the concentration of Zn at 400m in the eddy centre in the eddy, μ is the dilution/mixing parameter and C_0 is the concentration of Zn in the shelf waters. Equation 1 simplifies to:

$$\ln C - C_0 = -\mu t \qquad (2)$$

As there is no available dissolved Zn concentrations from the source waters of the Haida eddies (Hecate Strait) Zn concentrations from a shelf station P4 just south of the Hecate Strait taken in June 2001 was used for C_0. This approach was also used for silicic acid and phosphate concentrations, as like Zn nutrients are not considered conservative tracers but would be subjected to a similar mixing process.

Zinc, silicic acid and phosphate all indicate a similar order of magnitude in their mixing coefficient, $4x10^{-8}$ nMs^{-1}, $6x10^{-8}$ and $8x10^{-8}$ μMs^{-1} respectively. As all three parameters indicate similar mixing coefficients it gives confidence in approaching the minimal data set in this manner.

The higher dissolved Zn concentration at 400m compared to the reference station and subsequent decrease as the eddy ages is also observed in dissolved Fe profiles. The distribution of dissolved Fe strongly resembles that of dissolved Zn in the centre of H-2001 after 4 months of formation throughout the water column. A strong correlation between Fe and Zn is observed in the centre station, 4 months after formation with r^2 values of 0.9885. As Fe is a scavenged-type trace metal and Zn exhibits nutrient-type profile (Bruland & Franks, 1983), these elements would not be expected to be correlated from a conventional viewpoint. This suggests that similar processes are influencing the distribution of trace metals within the eddy and the mixing with the surrounding waters would affect trace metals in a similar manner.

5.4.5 Decreases in concentration of Zn in the Haida eddies over time

Although H-2000 and H-2001 moved offshore in different directions, Fe concentrations after 4 and 7 months were similar in both eddies (Wong *et al.*, 2003). Assuming that the concentration of Zn would be similar after 4 and 7 months in both H-2000 and H-2001, the removal of Zn from the eddy over 19 months is shown in Figure 5.12. As the eddies only affect trace metal concentrations down to a depth of 400m, the removal of Zn was calculated by integrating dissolved Zn concentrations from 10 to 400m. A rapid decline in Zn concentrations is observed from 4 to 7 months with a smaller decline between 7 and 16 months and almost no change between 16 and 19 months. After 16 months, the Zn concentration down to a depth of 400m is similar to those values found in open ocean which are not influenced by eddies. Sixteen months after formation, both Zn and Fe concentrations were approaching background levels throughout the water column and the coastal zooplankton signal was no longer evident in H-2000 (Mackas *et al.*, 2003). The steady erosion of nutrients in Haida eddies over time has been observed (Whitney & Robert, 2002). As the eddy ages and losses kinetic energy, surface water leaks outwards and depressed isopycnals return to the sea surface.

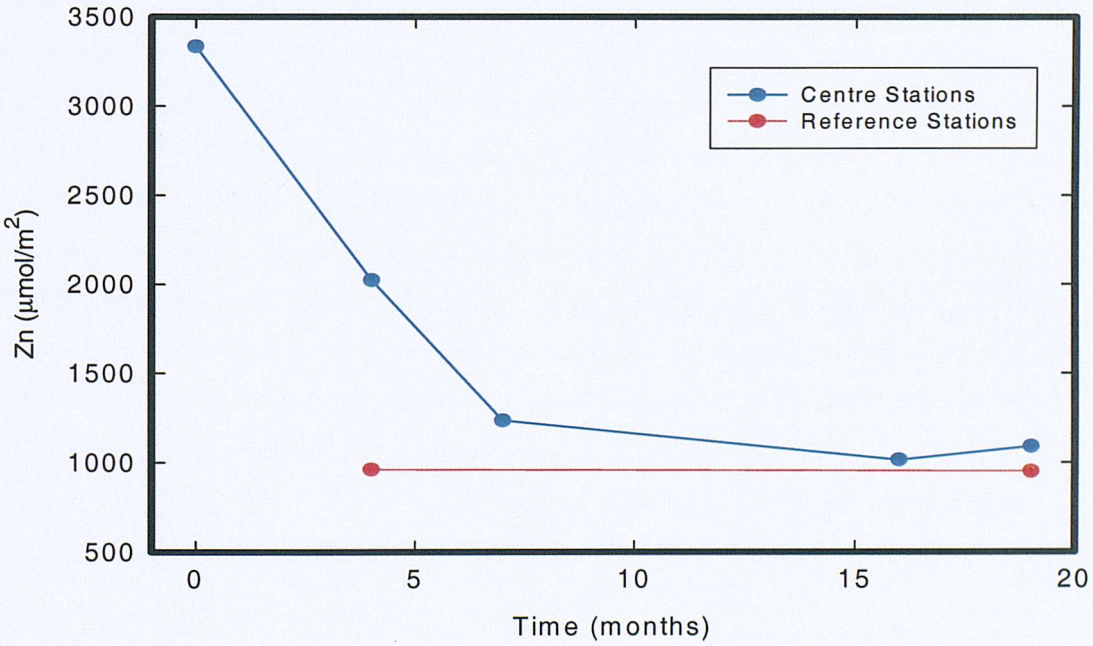


Figure 5.12 Zinc concentrations in the water column from 10 to 400m calculated as µmol/m^2 over time as the eddy ages.

Therefore, the elevated Zn concentrations in the eddy compared to the 'background' oceanic levels is due to the higher Zn concentrations present in the source waters being retained within the eddy centre. The subsequent loss of Zn from the eddy centre over time is primarily due to mixing processes. Using both salinity and silicic acid concentrations Peterson *et al.* (2003) estimated that that a net inflow from surrounding oceanic waters occurred between 150 and 200m and a net outflow of waters from the Haida eddy occurred between 200 and 300m. No Zn concentrations are available for 300m, but at 400m from dissolved Zn and Fe concentrations, it appears that a net outflow from the eddy is also occurring at this depth. Therefore, mixing controls the distribution of Zn in the eddy systems and although biogeochemical processes are occurring, these are overprinted at this depth by isopycnal mixing.

Over time, losses due to biological processes in combination with the outflow of core waters and subsequent replacement with downwelled nutrient depleted waters may create a Fe limited system such that phytoplankton growth, nutrient utilisation and trace metal distribution may closely resemble HNLC conditions (Peterson *et al.*, 2003).

In order to estimate the supply of total dissolved Zn to from Haida eddies to the open ocean, Zn concentrations were integrated over the mixed layer and intermediate layer (75-400m). Haida eddies represent a significant supply of total dissolved Zn, transporting 2203 μ mol m^{-2} yr^{-1} of Zn from coastal waters out into the open ocean. However, Haida eddies are only 200km in diameter and when compared to the size of the North Pacific Ocean into which they travel are considered to be small. Therefore in order to create a simplified box model to compare the eddy transport flux with the aeolian flux (the only other significant supply of Zn to the subarctic North Pacific) the area of the ocean must be taken into account. As Haida eddies have been observed as station P20 (Whiteny & Robert, 2002; Fig 2.1) the maximum distance they travel is 1800km. As the diameter of the Haida eddies is 200km an assumption that the area into which they travel is 720000 km^2 (by assuming the maximum area the eddy can influence is 400 km). Therefore the flux of total dissolved Zn into the intermediate layer is 692 M yr^{-1}, which is comparable to an aeolian flux of 704 M yr^{-1}. Therefore, Haida eddies represent a significant supply of total dissolved Zn to the subarctic North East Pacific/

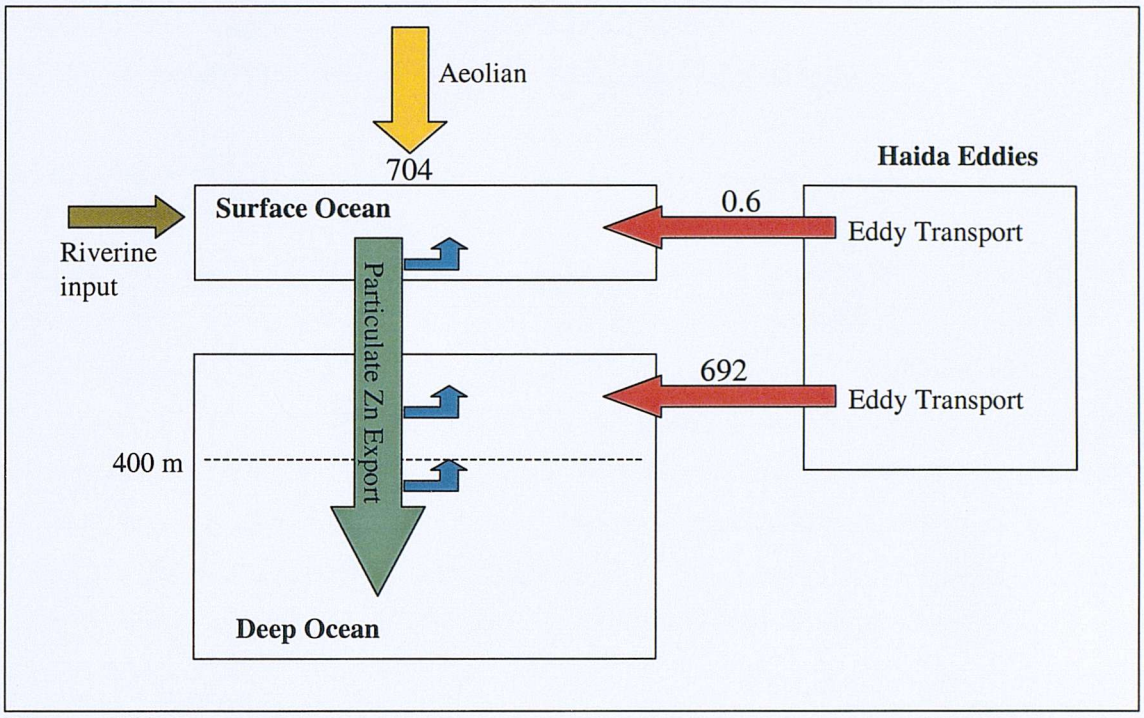


Figure 5.13 Box model indicating the aeolian flux and the flux of total dissolved Zn from Haida eddies into the open ocean. All fluxes are in M yr^{-1} and are calculated as explained above. Blue arrows represent remineralisation.

5.4.6 Zn Speciation

Low total dissolved Zn concentrations at the edge station in H-2001 in June 2001 are similar to those observed at OSP in June 2001. The Zn-binding ligand concentration is however, two times higher in H-2001 than the open ocean. The higher productivity observed at the eddy edge is presumably the source of the higher ligand concentrations observed.

Free Zn concentration in the H-2000 transect is very low and varied little ranging from 2.82 pM to 1.75 pM. However, the lowest Zn^{2+} concentration of 1.3 pM was observed at the edge station in H-2001 in June 2001. This is the lowest free Zn^{2+} concentration observed in this region and is comparable to values reported at OSP where nutrient concentrations are higher but primary production is lower. Peterson *et al.* (2003) observed that when eddy waters are mixed with open ocean waters and incubated for seven days that large diatoms (dominated by *Pseudo-nitzschia* spp.) dominate the phytoplankton population. The Zn uptake by diatoms is higher than other phytoplankton species (Sunda & Huntsman, 1992) and this could account for the low Zn^{2+} concentration observed. However, lower concentrations of Zn^{2+} were reported in incubation

experiments in Chapter 4 where primary production is higher and it is unlikely that this low concentration of Zn would be limiting phytoplankton growth in this region.

5.4.6.1 Depth Profiles

Similar to the results from line P (Chapter 3) there appears to be no relationship between the Zn-binding ligand and fluorescence in the mixed layer. There is no significant difference in the Zn binding ligand concentration between stations sampled within the eddy system and those taken at the open ocean reference station. A relatively similar Zn ligand concentration is observed at 200m throughout this study area, which is compatible with a ligand, that is either a stable molecule or is produced in situ by bacteria or decaying phytoplankton. As the ligand concentration is much higher than the total dissolved Zn in the surface water column, small increases in total Zn due to inputs from aeolian deposition or upwelling will have only a minimal effect of the Zn^{2+} concentration.

5.4.6.2 S-N transects

The surface transect of H-2000 revealed a distinct physico-chemical signature associated with the increase in salinity in and surrounding the eddy centre. A distinct minimum was observed in both the dissolved Zn and the Zn-binding ligand concentration, which coincided with maximum salinity values (Fig 5.14 a). At the southern end of H-2000 the converse was observed with highest dissolved Zn and Zn-binding ligand concentrations coinciding with lowest salinity values. The strong correlation between the concentration of dissolved Zn and the Zn-binding ligand is reflected in the r^2 values of 0.872 and 0.924 respectively. A similar but weaker trend was observed in H-2001 with r^2 values of 0.817 and 0.783 respectively (Fig 5.14 b.). Speciation is probably influenced by factors such as light intensity, water column mixing and the composition of phytoplankton species in the water column (Moffett *et al.*, 1990).

This trend of increased total dissolved Zn concentrations corresponding with increased Zn-binding ligands could reflect mixing in the surface waters or upwelling of higher ligand concentrations from depth. In general, the depth profiles indicate higher Zn ligand concentrations with depth and at the edges of the eddy upwelling is observed in both nutrient concentrations and salinity. Upwelling of higher ligand concentrations and Zn concentrations at the edge station is evident in both eddies (Fig 5.14 a. b). A similar trend was observed along a surface transect of Zn

speciation carried out in the Black Sea (Muller *et al.*, 2001). Muller *et al.* (2001) state that this was due to a low salinity plume that propagated in the direction the transect sampled. However, in this study the transect was sampled in 2 days and so the signal that was observed could reflect mixing that occurred prior to sampling.

Zinc binding ligand concentrations in the water column are thought to have a biological origin and help to maintain trace metals in the dissolved form (Bruland, 1989). Trace metal concentrations can be toxic to phytoplankton and high ligand concentrations coinciding with high trace metal concentrations could alleviate the toxic effect. This has been observed for Cu in coastal waters (Moffett & Brand, 1996; Moffett *et al.*, 1997). There is as yet no evidence for Zn concentrations observed in this study to have a toxic effect on phytoplankton reproduction as demonstrated in Chapter 4, Zn concentrations of 10 nM did not prevent phytoplankton growth. Culture studies have demonstrated that at total dissolved Zn concentrations and the $[Zn^{2+}]$ observed along the transect should not be toxic to phytoplankton growth (Sunda & Huntsman, 1992).

If phytoplankton can take up metals bound to these ligands as observed for the siderophores produce to aid Fe uptake (Rue & Bruland, 1997) and hypothesised in Chapter 4, then perhaps the higher ligand concentrations observed at high Zn concentrations are to aid phytoplankton uptake. In H-2000 a general trend of decreasing total dissolved Zn and ligand concentrations and increasing chlorophyll *a* concentrations is observed moving northwards along the transect. Along either transects the higher Zn ligand and Zn concentrations correspond to lower chlorophyll *a* concentrations and once phytoplankton growth occurs it appears to decrease the concentration of both (Fig 5.14 a, b). The lower ligand concentrations corresponding to higher chlorophyll *a* concentrations may reflect the biological uptake of these ligands. The variation in ligand across the eddy along this 10m transect suggests that microorganisms in general may be responsible for this distribution by excretion of this ligand directly or from lysed cells. The role of bacteria in the production of Zn-binding ligands is unclear and not yet been quantified and could be a source for the Zn-binding ligands.

Another process, that may be having an impact on the distribution and concentration of the Zn-binding ligands, is photochemical reactions in the upper ocean. Organic Zn complexes may also be subject to photochemical degradation in a manner similar to organic complexes of other trace metals such as Cu, Fe, and Mn (Moffett *et al.*, 1990; Barbeau *et al.*, 2001; Kaczynski & Kieber,

1993). However, the Zn-complexing ligands show relatively conservative distribution with depth in the photic zone of Haida eddies and along line P (Chapter 3), suggesting a higher degree of stability. Furthermore, photoreduction of Zn(II) is unlikely, as Zn(I) is not a thermodynamically stable oxidation state . Another photochemical mechanism which may indirectly affect Zn mobility is the potential release of Zn^{2+} by photoreduction of common carrier phases, including Fe(III) and Mn(IV) oxides and POM (Stumm & Morgan, 1996). Photoreduction of Mn oxides has been observed in surface oceanic waters (Sunda & Huntsman, 1988). Recent studies have shown that photolysis of siderophores, a component of the Fe(III) organic ligands present in seawater, leads to the reduction of Fe(III) increasing the availability of siderophore-bound Fe for uptake by planktonic assemblages (Barbeau *et al.*, 2001). Little is known about the impact of photochemical process on organic complexation of Zn.

Salinity is a conservative tracer of water masses and therefore, although this transect was taken at 10m, the salinity of the water indicates the water mass origin. Low salinity water in the centre of H-2001 reflects the lower salinity water where the eddy was formed. The highest Zn concentrations were observed in the core waters of H-2001 (Fig 5.8) indicating that the transport of higher Zn concentrations from the shelf waters was retained in the eddy centre after 4 months due to less mixing with the surrounding open ocean water. Along the edges of the eddy, both the Zn concentrations and salinity were similar to the reference station reflecting the higher degree of mixing at the eddy edge. The salinity is lower along the transect in H-2000 compared to H-2001 reflecting the increased mixing with surrounding waters as the eddy aged. The 10m transect of H-2001 was sampled in June 2001, 4 months after formation compared to H-2000 which was sampled in September 2001 (19 months after formation). Zinc concentrations were also lower in H-2000 than in H-2001. In September, the highest extracted chlorophyll *a* concentrations were found at the eddy centre (Peterson, *pers. comm.*) indicating a higher primary production in the centre of H-2000 and as phytoplankton require Zn for growth this would reduce the already low Zn concentrations observed in the eddy centre in June 2001.

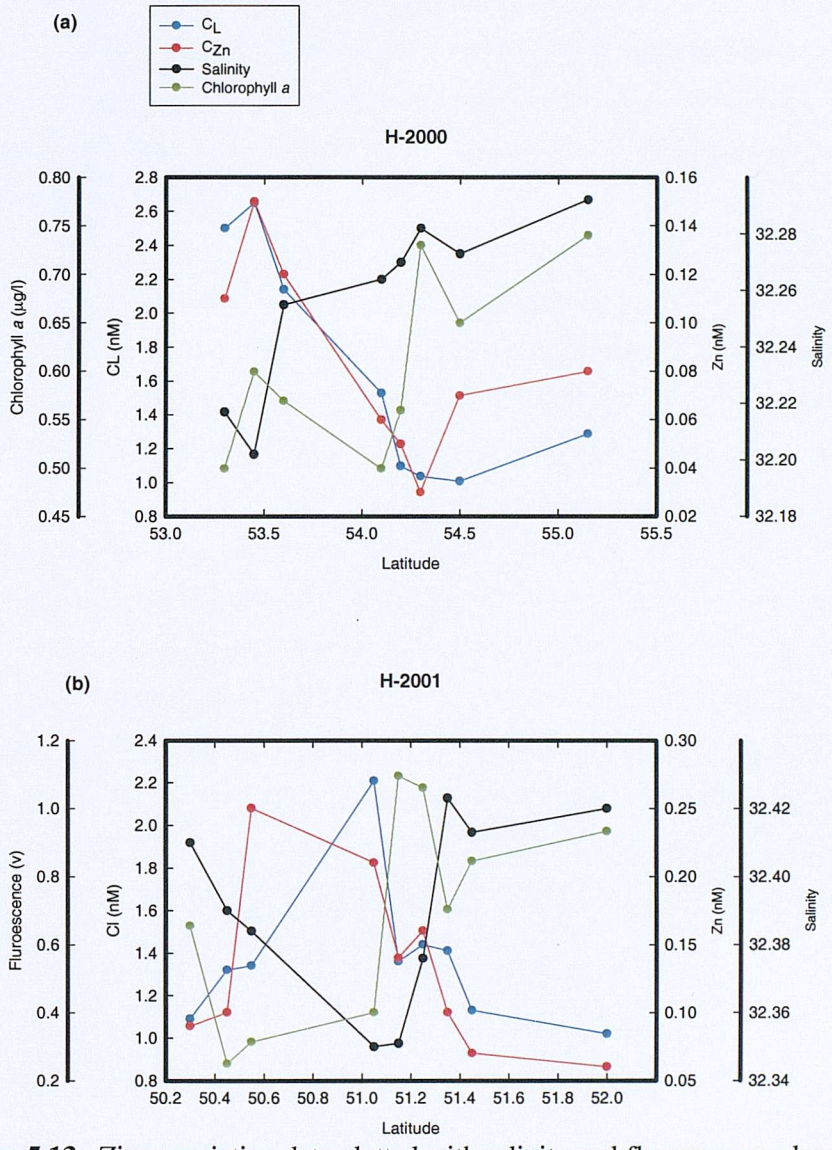


Figure 5.13 Zinc speciation data plotted with salinity and fluorescence along the S-N transects for (a) H-2000 and (b) H-2001.

5.5 Summary

Haida eddies represent a significant supply of total dissolved Zn concentrations to the subarctic North Pacific, transporting 2203 µ mol m^{-2} yr^{-1} of Zn from coastal waters into the intermediate water column (75-400m). Total dissolved Zn concentrations in the euphotic zone are also higher than those observed in the oceanic waters and in combination with increased nutrients and Fe are depleted by biological activity. Although the transport of significant concentrations of dissolved

Zn into the open ocean was observed the time scale for this mixing is much shorter (on the order of 1-2 yrs) than overturning of ocean (10^3 yrs). Thus, mesoscale eddies represents a significant source of trace metals into HNLC areas resulting in an increase in phytoplankton biomass. Therefore, mesoscale eddies should be considered in the biogeochemical cycling of Zn in the subarctic North Pacific.

In general Zn concentrations decrease as Haida eddies age and move offshore. This feature is most prominent at 400m and is most likely due to mixing and dilution with surrounding waters. After 16 months the total dissolved Zn concentrations within the Haida eddies resemble those observed in the HNLC oceanic environment into which they travel.

Organic complexation dominates the speciation of Zn in the upper 200m. Ligand concentrations with Haida eddies are higher than those observed in the reference station concomitant with higher biomass. The lowest ligand concentrations along the 10m transect were observed where chlorophyll *a* concentrations were highest, suggesting that these organic ligands may be available for uptake by phytoplankton. Free Zn^{2+} concentrations are lower in the newer eddy, H-2001 and are similar to those reported at OSP where dissolved Zn concentrations are lower than those observed here. Primary production is higher in this eddy and the low [Zn^{2+}] reflect the uptake by phytoplankton. Although the bioavailable fraction of Zn is low in Haida eddies, phytoplankton growth does not appear to be limited.

Chapter 6

Conclusions and Future Work

6.1 Conclusions

The recent developments in analytical techniques such as adsorptive cathodic stripping voltammetry (AdCSV) have provided the methods necessary to investigate the speciation of trace metals in oceanic environments. However, previous studies on Zn speciation were carried out in oceanic realms where the total dissolved Zn concentrations were higher than those observed in HNLC region of the subarctic North Pacific. Therefore, detection limits were lowered by increasing the deposition time on varying mercury drop sizes, stirring time and varying the concentration of competitive ligand (APDC) and the use of a borate buffer. A new UV digestion system was also designed in this work to reduce contamination. Detection limits were reduced from the reported values of 0.07 nM to 0.02 nM. This method was applied to samples, where the lowest total dissolved Zn concentration was 0.04 nM. Total dissolved Zn samples analysed by chelation and solvent extraction were shown to be similar to those analysed by this method.

This study has greatly increased the knowledge of total dissolved Zn concentrations in the subarctic North Pacific and provided the first measurements of Zn speciation at the low concentrations of Zn observed in this area (40 pM). These speciation measurements are also the first to be carried out in this region, providing a further insight into biogeochemical cycling of Zn.

6.1.1 Impact of Si cycling on Zn concentrations and distributions in surface and deep waters

Total dissolved Zn profiles for the upper ocean are consistent with earlier work Martin *et al.* (1989), where Zn is depleted in surface waters and increases with depth in close correlation with dissolved Si concentrations. It has been hypothesised that as Zn distributions resemble those of Si a similar process is involved in the removal of Zn from surface waters and Zn is transported down the water column primarily with skeletal material (Bruland, 1980; Morley *et al.*, 1993).

However, Zn/Si ratios in the mixed layer along line P show a decreasing trend with distance from shore, which infers a decoupling between Zn and Si concentrations in the upper ocean. It is therefore hypothesised that limited Zn inputs and removal in particulate organic fallout in combination with more efficient recycling of dissolved Si and more significant aeolian supply of Si lead to the observed trend. The apparent association of Zn with non-labile organic phases, and minimal association with biogenic opal, infers that the similarity between Si and Zn profiles is largely coincidental with the Zn profile, mainly reflecting recycling from relatively biologically resistant organic matter.

6.1.2 Mixing vs. biological activity as control on zinc distributions in surface waters of the NE Pacific

Concentrations of Zn in the upper mixed layer decrease offshore along the line P transect (0.8-0.04 nM). No major differences were observed in Zn concentrations during this study between winter and summer surveys, contrary to what was predicted by (Martin *et al.*, 1989), on the basis of seasonal changes in mixed layer depths in this region. However, sampling only took place in February and September and more extensive sampling during different months would be necessary to confirm the lack of seasonality in Zn concentrations, as episodic events such as phytoplankton blooms and upwelling could influence the concentrations of Zn over shorter time scales.

The total dissolved Zn deep water profiles along line P are primarily due to the long time scales of ocean mixing (~10^3 yrs). Within this study it was also possible to investigate the shorter time-scales in the cycling of dissolved Zn by investigating the processes and fate of total dissolved Zn concentrations in mesoscale eddies over time. Although total dissolved Zn concentrations in the mixed layer were significantly higher in the shelf region where the mesoscale eddies formed, after 4 months, total dissolved Zn concentrations were only slightly higher than those observed along line P. This decrease in Zn concentrations is accompanied by a decrease in both nutrients and Fe concentrations reflecting the increased biological demand for these elements. As demonstrated in Chapter 4, phytoplankton within this region are limited by Fe and the presence of increased Fe concentrations and other micronutrients such as Zn from coastal waters within these eddies is hypothesised for the increased phytoplankton biomass observed. Mixing of the surface waters of Haida eddies with the surrounding open ocean water is also occurring. However, the removal of Zn from the mixed layer of Haida eddies is predominately due to the increased

productivity observed. The large increase in primary productivity observed within these eddy systems could account for the periodic increase in chlorophyll *a* concentrations and the corresponding increase in the vertical fluxes of carbon observed at OSP.

Below the mixed layer, total dissolved Zn concentrations within the Haida eddies are significantly higher than that observed in the 'background' subarctic North Pacific. The increase in Zn concentrations is a result of both remineralisation due to an increase in productivity and the presence of the higher Zn concentrations from shelf waters retained within the eddy. In general total dissolved Zn concentrations decrease as Haida eddies age and move offshore. This feature is most prominent at 400m in this study. The decrease in total dissolved Zn concentrations has been shown here to be predominantly due to mixing and dilution with surrounding waters over time. After 16 months the total dissolved Zn concentrations within Haida eddies resemble those observed in the HNLC oceanic environment into which they travel.

As the open ocean is remote from allochthonus sources, atmospheric deposition is the dominant source of Zn to the North Pacific (Bruland, 1980). However, the results from this study show that Haida eddies transport on the order of 2200 μmol m^{-2} yr^{-1} of Zn from coastal waters into the intermediate oceanic water column (75-400m), in comparison to a aeolian flux of 1.1 μ mol m^{-2} yr^{-1} (Bruland *et al.*, 1994). This represents the transport of significant concentrations of dissolved Zn into the open ocean on the time scale of 1-2 years which is much shorter than the overturning time of the ocean (~10^3 yrs). Thus Haida eddies represent a significant source of trace metals into HNLC region of the subarctic North Pacific resulting in an increase in phytoplankton biomass. Therefore, mesoscale eddies should be considered in the biogeochemical cycles of Zn in the subarctic North Pacific.

6.1.3 Interaction of Zn with phytoplankton and the microbial community in the upper NE Pacific Ocean

In order to investigate the interaction of Zn with oceanic phytoplankton, quantification of the different forms of dissolved Zn present in the water column was required. Prior to this research only three studies on Zn speciation in oceanic environments had been reported. No previous studies of Zn speciation had been undertaken in HNLC areas, where the total dissolved Zn concentrations are lower than in other ocean surface waters. This data set includes stations along line P, Haida eddies and trace metal enrichment experiments. The results from this work indicate

that organic complexation dominates the chemical speciation of Zn in surface seawater. At OSP, organic complexation reduces the bioavailable fractions of Zn, the labile inorganic complexes and the $[Zn^{2+}]$ to 0.72 pM and 1.5 pM respectively. The $[Zn^{2+}]$ concentration measured is the lowest concentration reported from field studies and is below the limit established from culture studies to limit phytoplankton growth.

6.1.3.1 Origin, role and fate of Zn ligands

The concentration of the Zn-complexing ligand is higher than total dissolved Zn in the mixed layer of the subarctic North Pacific. This has also been observed in the Atlantic and it has been argued that these Zn-complexing organic ligands strongly buffer free Zn ion activity in surface seawater with respect to perturbations in total dissolved Zn (Ellwood & Van den berg, 2000; Whifield, 2001). Trace metal enrichment experiments from this study have demonstrated that the addition of dissolved Zn causes a rapid production of these Zn-binding ligands from the phytoplankton and microbial community. In contrast to the theory of ligand production as a metabolic by-product released indirectly by grazing and cell lysis (Ellwood & Van den Berg, 2000), this study indicates that the phytoplankton community are capable of producing these ligands directly. The rapid production of these ligands at high Zn concentrations is hypothesised to be a mechanism for luxury uptake and storage for future use by phytoplankton and not as a mechanism to reduce toxicity due to the presence of larger numbers of phytoplankton cells in these treatments.

These Zn-binding ligands were also observed to increase when the bioavailable Zn concentration is low. A higher production of Zn-binding ligands at low Zn′ was observed when phytoplankton biomass is increased. From a model of diffusion uptake of Zn′, it appears that phytoplankton would not be able to acquire sufficient Zn for growth. As continued growth was observed at these low concentrations, phytoplankton may be able to utilise Zn-binding ligands as a source of Zn. A possible mechanism by which the Zn ligand would be available to phytoplankton is via a cell surface binding to the plasma membrane and subsequent uptake.

In the open ocean, along line P, the trend of organic ligand concentrations is similar to that of total dissolved Zn concentrations, decreasing offshore. The ligand concentration profiles observed in both Haida eddies and along line P are similar, with ligand concentrations increasing at 200m

where the concentration of dissolved Zn is higher. Photochemical and biological uptake or degradation of these compounds could reduce the surface concentrations and the more inert material is then transferred to deep waters via downwelling. This resistant material will be recycled to the surface ocean and would provide a background against which new ligands produced by microorganisms can be added. This background of ligands and the variable addition of new ligands would be expected to have a poor correlation with fluorescence which is observed in this study and in a previous study in the Atlantic (Ellwood & Van den Berg, 2000).
The presence of a relatively similar Zn ligand concentration observed at 200m in both the mesoscale eddy and open ocean indicates that some fraction of these ligands are long lived. This is compatible with a ligand, that is a stable molecule, which has been produced insitu by phytoplankton and/or bacteria or from decaying phytoplankton. Evidence from the trace metal enrichment experiment indicates that these ligands are labile as they can be produced and destroyed on a time scale of a day. This indicates that a dynamic balance could exist for this class of ligands between production and consumption, resulting in the relatively uniform and ubiquitous presence of the Zn complexing ligand observed in the North Pacific.

This study has shown that Zn speciation is controlled by a number of dynamic processes resulting in considerable spatial variability. Speciation is most likely influenced by water column mixing and the composition of primary producers and bacteria in the water column.

6.1.3.2 Zinc as a potentially limiting nutrient

Trace metal enrichment experiments on natural phytoplankton assemblages have previously been carried out at Ocean Station Papa (OSP). However, this research represents the first study of Zn speciation in trace metal enrichments experiments and the first speciation measurements to be carried out on a daily basis throughout an incubation experiment. The data obtained provides valuable information and insight into the partition of Zn between inorganic and organic forms during phytoplankton growth. The [Zn^{2+}] ion at OSP has been established in this study to be lower than the reported concentrations established by Sunda & Huntsman (1995) in cultures to limit phytoplankton growth. Modelling the diffusive transport of Zn across the cell wall indicates that the concentration of the bioavailable Zn (Zn′) observed in the incubation experiment are so low that it would not be possible for any but the pico-nanoplankton species to divide once a day and acquire sufficient Zn.

Although the concentrations of bioavailable Zn are low, phytoplankton growth was observed. Upon the addition of Zn to a natural phytoplankton population no significant increase in phytoplankton growth was observed. This indicates that Zn concentrations measured are not limiting phytoplankton growth in this region. The ability of phytoplankton to utilise Zn-binding ligands as a mechanism for Zn uptake may indicate why Zn concentrations are not limiting the growth of phytoplankton. Culture studies carried out with EDTA cannot investigate the role these ligands have in Zn uptake.

During the previous incubation experiment however, an increase in some smaller phytoplankton groups was observed when Zn was added indicating that Zn supply can have subtle effects on the phytoplankton species composition and may be limiting to the smaller cells (Crawford *et al.*, 2003). It has also been observed that addition of Zn increases the short-term carbon fixation rates of phytoplankton species (Crawford *et al.*, 2003). Therefore it is important to consider the concentration of Zn when Fe is added in Fe fertilisation experiments, as it may be that Zn limits the carbon fixation rates and therefore the export of carbon from the euphotic zone.

6.2 Future Work

The presence of a Zn^{2+} uptake system as demonstrated by Sunda & Huntsman (1992; 1995a; 1998) and the possible presence of Zn ligands that are involved with Zn uptake suggest a duality for Zn acquisition. Conclusive evidence for this hypothesis will require isolation of the ligand and an analysis of its chemical structure, as well as a mechanistic understanding of its production and regulation. The fact that phytoplankton may be able to utilise organically complexed Zn is a process that warrants further investigation. Bacteria are known to produce siderophores, which have been demonstrated to be a fraction of the Fe (III) organic ligands. Siderophores aid the uptake of organically complexed Fe (III) by phytoplankton. Therefore the role of bacteria in the production of Zn-binding ligands should also be investigated. Increased spatial resolution of the Zn-binding ligands is needed in the surface euphotic zone in order to investigate the relationship with the biological productivity.

Trace metal enrichment studies on natural phytoplankton populations have proven to be a useful tool to gain an understanding of Zn speciation in relation to phytoplankton growth. Future work on developing these types of experiment in combination with the use of radioisotopes in order to

investigate the uptake of Zn into the cell would provide a more detailed examination of the role of Zn in phytoplankton.

Zinc is known to be required in many enzyme systems within phytoplankton cells. The concentration of Zn within the cell and within the enzymes has yet to be quantified. Analysis of phytoplankton species at a molecular level is needed to gain a better understanding of the processes involved in uptake, and where the Zn in stored in the phytoplankton. This future work is necessary to elucidate the role of Zn in oceanic phytoplankton and the biogeochemical cycles of Zn in the upper water column.

Future research is also needed on development of a stand-alone monitor that can be used as an underwater chemical sensor. The aim of this research could be to build a chemical sensor that will be able to continuously monitor electrochemically labile trace metals in the upper water column (down to 100m) in the open ocean environment, applying flow-analysis techniques. This would elucidate the stability of ligand within the productive photic layer and establish any variations in the production of these ligands.

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Station P4 (48.39.0' °N; 126.40.0' °W)						
Feb-99						
Depth (m)	**Zn (nM)**	**Temperature °C**	**Salinity**	**PO_4 (μM)**	**NO_3 (μM)**	**Si (μM)**
0		8.34	31.75	0.94	8.89	14.4
10	0.8	8.37	31.85	0.92	8.62	14.2
15		8.43	31.96	0.88	7.87	12.3
20		8.64	32.15	0.83	7.17	10.7
25	0.7					
30		8.87	32.33	0.83	7.05	9.44
40	4.51	8.87	32.37	0.82	6.84	9.08
50		8.92	32.53	0.90	8.22	10.0
60		8.95	32.74	1.09	11.2	13.5
80		8.90	33.42	1.74	21.7	26.0
100	5.13	8.52	33.61	1.96	25.2	31.6
125		8.02	33.69	2.09	27.3	37.4
150		7.73	33.80	2.19	29.2	40.0
175		7.50	33.88	2.22	30.2	41.6
200	9.33	7.07	33.94	2.32	31.7	45.8
250		6.53	33.97	2.45	33.8	51.9
300		5.98	33.98	2.60	36.0	59.6
400	14.4	5.32	34.03	2.85	39.2	73.4
Sep-99						
0		15.67	31.93	0.29	0.00	8.91
10	0.9	15.37	31.90	0.30	0.00	9.46
25		11.62	32.41	0.60	1.98	9.47
40	2.59					
50		8.29	32.79	0.98	9.34	12.2
75		7.71	33.01	1.25	13.9	16.6
100	7.8	8.03	33.52	1.90	24.7	29.8
150		7.50	33.91	2.33	31.9	42.7
200	11.9	6.84	33.96	2.50	34.1	51.3
Aug-01						
0	0.72					
10	1	15.14	32.09	0.37	0.0	5.6
25	0.85	12.00	32.24	0.75	3.3	7.2
50	1.4	8.81	32.50	0.90	6.0	9.3
75	3.13	8.05	32.52	1.06	9.9	11.9
100	6.17	7.59	32.79	1.33	14.9	17.4
200	9.92	7.38	33.95	2.28	31.9	43.9
300		6.28	33.98	2.56	35.8	57.1
400	13.02	5.59	34.02	2.82	39.4	69.5

Appendix 1 (a) Total dissolved Zn concentrations, temperature, salinity and nutrient concentrations for Station P4 in February and September 1999 and August 2001.

Station P12 (48.58.2' °N; 130.40.0' °W)						
Feb-99						
Depth (m)	**Zn (nM)**	**Temperature °C**	**Salinity**	**PO_4 (μM)**	**NO_3 (μM)**	**Si (μM)**
0		7.53	32.73	0.95	9.21	11.2
10	0.26	7.53	32.73	0.96	9.31	11.3
15		7.52	32.73	0.96	9.21	11.3
20		7.52	32.73	0.95	9.26	11.4
25	0.13					
30		7.53	32.73	0.95	9.21	11.4
40	0.33	7.53	32.73	0.95	9.31	11.3
50		7.56	32.74	0.95	9.21	11.6
60		7.61	32.77	0.97	9.52	11.6
80		7.98	33.33	1.67	21.1	25.8
100	2.65	7.76	33.60	1.92	25.9	32.0
125		7.49	33.78	2.10	28.9	37.2
150		7.19	33.88	2.20	31.1	42.2
175		6.90	33.92	2.28	32.2	45.6
200	6.84	6.65	33.94	2.37	33.7	49.0
250		6.17	33.96	2.51	35.8	55.3
300		5.61	33.98	2.66	37.8	64.4
400	9.47	5.10	34.04	2.89	41.2	77.0
Sep-99						
0		14.64	32.38	0.42	0.6	7.2
10	0.5	14.64	32.38	0.42	0.6	7.2
25		12.22	32.74	0.62	3.5	9.1
40	0.9					
50		9.00	32.79	0.84	6.1	10.5
75	2.1	7.33	32.85	1.00	8.4	11.1
100		6.97	33.18	1.28	14.6	18.5
150		7.03	33.91	1.98	27.1	40.2
200	6.22	6.48	33.93	2.23	31.0	49.4
Aug-01						
0		14.64	32.28	0.70	3.9	10.0
10	0.5	14.63	32.30	0.71	4.0	10.0
25	0.32	13.66	32.33	0.73	4.4	10.2
40	0.7	8.89	32.44	0.93	7.2	11.5
75	1.12	7.93	32.47	1.05	9.2	12.4
100	1.81	7.28	32.58	1.21	12.5	14.6
150		6.76	33.50	1.71	23.1	29.8
200	5.75	6.73	33.93	2.09	29.6	45.3
300		5.78	33.94	2.42	34.7	60.2
400	8.03	4.96	33.97	2.74	38.9	74.3

Appendix 1 (b) Total dissolved Zn concentrations, temperature, salinity and nutrient concentrations for Station P12 in February and September 1999 and August 2001.

Station P16 (49.58.2' °N; 134.40.0' °W)						
Feb-99						
Depth (m)	**Zn (nM)**	**Temperature °C**	**Salinity**	**PO_4 (μM)**	**NO_3 (μM)**	**Si (μM)**
0		6.64	32.84	0.97	9.21	13.5
10	0.5	6.65	32.84	0.99	9.41	13.7
15		6.65	32.84	0.98	9.36	13.7
20		6.66	32.84	0.99	9.41	13.7
25	0.3					
30		6.64	32.84	1.00	9.41	13.6
40	1.01	6.64	32.84	1.00	9.46	13.6
Sep-99						
0		13.13	32.53	0.58	3.14	8.96
10	0.4	13.14	32.53	0.59	3.14	8.96
25		13.07	32.53	0.58	3.13	9.29
40	0.8					
50		9.63	32.72	0.88	6.90	11.3
75		6.67	32.80	1.11	9.70	13.9
100	1.8	6.24	32.90	1.25	13.0	16.8
150		6.52	33.88	2.07	28.6	42.7
200	6.82	5.88	33.91	2.34	32.1	53.2
Aug-01						
0	0.22	13.49	32.31	0.86	6.3	11.6
10	0.4	13.29	32.31	0.88	6.4	11.8
25	0.21	10.07	32.43	1.00	7.8	12.5
40	0.51	6.97	32.52	1.28	12.3	15.1
75	1.09	6.28	32.55	1.40	14.5	17.3
100	1.3	5.59	32.76	1.58	18.0	22.8
150		5.54	33.34	1.90	24.4	34.6
200	4.72	5.94	33.88	2.29	31.9	52.3
300		4.93	33.93	2.68	38.2	72.0
400	7.24	4.48	34.01	2.91	41.1	85.9

Appendix 1 (c) Total dissolved Zn concentrations, temperature, salinity and nutrient concentrations for Station P16 in February and September 1999 and August 2001.

Station P20 (49.17.0' °N; 138.40.0' °W)						
Feb-99						
Depth (m)	**Zn (nM)**	**Temperature °C**	**Salinity**	**PO_4 (µM)**	**NO_3 (µM)**	**Si (µM)**
0		6.18	32.78	1.11	10.7	16.1
10	0.62	6.18	32.78	1.09	10.7	15.9
15		6.17	32.78	1.09	10.7	15.8
20		6.16	32.78	1.09	10.7	15.6
25	0.18					
30		6.16	32.78	1.09	10.8	15.6
40	0.18	6.16	32.78	1.08	11.0	15.4
50		6.16	32.78	1.07	10.7	15.3
60		6.16	32.78	1.06	10.6	15.3
80		6.16	32.78	1.07	10.7	15.1
100	2.5	6.52	33.26	1.38	16.5	23.0
125		6.69	33.86	1.91	26.8	38.5
150		6.40	33.89	2.03	28.7	43.8
175		6.13	33.90	2.14	30.2	48.0
200	6.56	5.86	33.91	2.28	31.2	52.6
250		5.31	33.92	2.52	34.7	61.6
300		4.91	33.93	2.69	36.7	68.5
400	8.86	4.34	34.01	2.85	40.2	83.7
Sep-99						
0		12.38	32.69	0.76	7.6	12.7
10	0.19	12.38	32.69	0.79	7.8	12.7
20		12.37	32.69	0.80	7.6	12.7
30		12.12	32.70	0.82	7.7	13.1
40	0.31					
50		9.65	32.75	0.91	8.6	14.8
75		6.00	32.82	1.19	11.8	18.4
100	1.5	5.53	32.95	1.39	15.5	21.9
150		5.63	33.81	2.25	30.9	52.1
200		5.13	33.85	2.50	34.2	63.4
250	6.16	4.83	33.89	2.69	37.1	71.9

Appendix 1 (d) Total dissolved Zn concentrations, temperature, salinity and nutrient concentrations for Station P20 in February and September 1999.

Station OSP (50.00.0' °N; 145.00.0' °W)						
Feb-99						
Depth (m)	**Zn (nM)**	**Temperature °C**	**Salinity**	**PO_4 (µM)**	**NO_3 (µM)**	**Si (µM)**
0		5.18	32.82	1.30	14.3	19.6
10	0.07	5.18	32.83	1.30	14.3	19.7
15		5.17	32.83	1.30	14.3	19.3
20		5.17	32.83	1.31	14.3	21.4
25	0.05					
30		5.18	32.83	1.30	14.4	21.5
40	0.39	5.17	32.83	1.30	14.5	21.0
50		5.17	32.83	1.31	14.4	21.1
60		5.17	32.83	1.30	14.5	20.9
80		5.17	32.83	1.30	14.5	20.8
100	1.1	5.16	32.83	1.30	14.4	20.7
125		5.12	33.31	1.75	22.5	36.0
150		4.65	33.74	2.00	24.3	92.5
175		4.44	33.76	2.34	32.5	57.9
200	5.9	4.28	33.79	2.45	34.6	63.3
250		4.10	33.86	2.57	36.2	69.3
300		4.07	33.92	2.75	39.4	77.9
400	7.7	4.04	34.05	2.83	40.4	85.1
Sep-99						
0		12.76	32.66	1.05	11.3	19.7
10	0.04	12.74	32.67	1.07	11.2	19.5
25		12.47	32.67	1.08	11.4	19.7
40	0.42					
50		6.81	32.80	1.25	12.8	19.9
75		5.40	32.85	1.37	14.5	20.5
100	1.2	5.21	32.87	1.41	15.9	21.8
150		5.47	33.67	2.14	28.9	46.2
200	4.61	4.89	33.84	2.56	34.9	63.8

Appendix 1 (e) Total dissolved Zn concentrations, temperature and salinity and nutrient concentrations for Ocean Station Papa (OSP) in February and September 1999.

Station Z4 (52.39.03' °N; 144.59.84' °W)						
Sep-99						
Depth (m)	**Zn (nM)**	**Temperature °C**	**Salinity**	**PO_4 (µM)**	**NO_3 (µM)**	**Si (µM)**
0		12.33	32.72	1.14	12.6	23.1
10	0.13	12.34	32.72	1.16	12.7	22.9
25		12.29	32.72	1.15	12.6	22.9
40	0.53					
50		7.38	32.79	1.29	13.9	22.9
75		4.93	32.86	1.50	17.2	24.5
100	1.54	4.61	33.08	1.71	21.0	31.7
150		4.35	33.74	2.57	34.8	63.7
200	4.79	4.22	33.82			

Appendix 1 (f) Total dissolved Zn concentrations, temperature, salinity and nutrient concentrations for Station Z4 in September 1999.

Station Z9 (54.59.91' °N; 144.59.94' °W)						
Sep-99						
Depth (m)	**Zn (nM)**	**Temperature °C**	**Salinity**	**PO_4 (µM)**	**NO_3 (µM)**	**Si (µM)**
0		12.11	32.74	1.04	11.0	21.9
10	0.24	12.08	32.75	1.07	11.2	22.4
25		9.74	32.79	1.13	11.9	23.3
40	0.12					
50		4.93	32.95	1.55	17.3	29.6
75		4.06	33.12	1.85	22.9	38.1
100	6.34	4.18	33.54	2.40	32.0	57.0
150		4.39	33.83	2.76	38.4	73.6
200	12.5	4.16	33.89	2.91	40.5	82.0

Appendix 1 (g) Total dissolved Zn concentrations, temperature, salinity and nutrient concentrations for Station Z9 in September 1999.

Station Z14 (57.29.64' °N; 145.00.51' °W)						
Sep-99						
Depth (m)	Zn (nM)	Temperature °C	Salinity	PO_4 (µM)	NO_3 (µM)	Si (µM)
0		12.39	32.13	0.34	0.00	7.87
10	0.07	12.27	32.14	0.39	0.63	9.15
25		7.02	32.30	1.41	15.7	21.8
40	0.21					
50		6.36	32.58	1.44	16.3	25.0
75		6.04	32.73	1.47	17.0	26.8
100	1.44	5.83	33.02	1.70	20.9	33.8
150		6.13	33.69	2.30	31.4	53.0
200	3.43	5.67	33.85	2.50	34.7	61.8

Appendix 1 (h) Total dissolved Zn concentrations, temperature, salinity and nutrient concentrations for Station Z14 in September 1999.

Station Z19 (59.26.47' °N; 144.59.91' °W)						
Sep-99						
Depth (m)	Zn (nM)	Temperature °C	Salinity	PO_4 (µM)	NO_3 (µM)	Si (µM)
0		12.42	31.57	0.34	0.0	8.13
10	0.04	12.39	31.62	0.36	0.0	8.32
25		12.20	32.03	0.48	1.5	9.87
40	1.26					
50		8.30	32.13	1.40	15.2	23.9
75		7.31	32.22	1.49	16.4	24.5
100	6.71	6.93	32.40	1.54	17.4	26.8
150		6.05	33.13	1.82	22.6	36.0
200	9.8	6.05	33.81	2.40	32.9	54.1

Appendix 1 (i) Total dissolved Zn concentrations , temperature, salinity and nutrient concentrations for Station Z19 in September 1999.

Depth (m)	Salinity	Temp (°C)	Fluor (v)	C_{Zn} (nM)	C_L (nM)	K'_{ZnL}	Zn^{2+} (pM)	Zn' (pM)	Zn (org) (%)
Station P4									
0	32.11	15.08	1.77	0.72	2.36	10.5	13.1	27.5	96.1
10	32.09	15.14	1.75	1.00	2.83	10.2	30.9	64.9	93.5
40	32.50	8.81	0.45	1.40	1.97	10.4	69.6	146	93.6
100	32.79	7.59	0.20	6.17	3.50	10.2	105	221	96.4
400	34.02	5.59	0.12	13.02					
Station P12									
10	32.30	14.63	0.91	0.50	1.71	10.5	12.0	25.2	94.9
40	32.44	8.89	1.27	0.70	2.88	10.4	12.1	25.4	96.4
100	32.58	7.28	0.47	1.81	3.63	10.3	36.2	76.0	95.8
400	33.97	4.96	0.11	8.03	1.02	10.8	17.9	37.6	99.5
Station P16									
0	32.31	13.49	0.78	0.22	1.79	10.3	6.49	13.6	93.8
10	32.43	10.07	1.38	0.40	2.73	10.5	5.25	11.0	97.3
40	32.76	5.59	0.14	0.51	2.23	10.4	11.1	23.3	95.4
100	33.88	5.94	0.11	1.30	2.62	10.6	22.9	48.1	96.3
400	34.01	4.48	0.12	7.24	1.10	9.8	168	353	95.2
OSP									
10	32.56	7.37	0.334	0.05	0.72	10.5	1.79	3.8	99.5
40	32.59	6.57	1.118	0.21	1.81	10.5	0.97	2.0	99.9
100	32.72	4.88	0.71	1.4	2.37	10.4	4.14	8.7	99.4
400	34.06	3.92	0.06	7.38	1.21	9.9	138	290	96.1

Appendix 2 Zinc speciation data, temperature, salinity and fluorescence for Station P4, P12, P16 in August 2001 and for Ocean Station Papa (OSP) in June 2001. C_{Zn} is the total dissolved Zn concentration, C_L is the Zn-binding ligand concentration, Zn^{2+} is the free Zn ion, Zn′ is the labile inorganic species, K'_{ZnL} is the stability constant Zn_{org} is the percentage of Zn, which is organically complexed.

Time (days) Treatment	0	1	2	3	4	5	6	7	8
					Chlorophyll *a* (µg/l)				
Control	0.47	0.31	0.42	0.50	0.61	0.73	1.00	1.28	1.64
+ Zn		0.41	0.43	0.59	0.62	0.69	1.19	1.45	1.40
+ Fe		0.46	0.52	0.84	1.74	2.96	5.68	8.26	9.93
+ Zn & Fe		0.44	0.55	0.82	1.48	2.79	6.04	7.61	9.61
					Nitrate (µM)				
Control	13.3	13.1	13.2	12.9	12.5	12.3	11.9	11.6	10.9
+ Zn		13.2	13.2	12.8	12.6	12.3	12.2	11.6	10.9
+ Fe		13.2	13.1	12.4	11.4	9.50	6.60	1.80	0
+ Zn & Fe		13.2	13.2	12.7	11.5	9.70	6.10	2.40	0
					Phosphate (µM)				
Control	1.32	1.31	1.31	1.3	1.26	1.24	1.21	1.16	1.14
+ Zn		1.31	1.32	1.28	1.27	1.25	1.22	1.18	1.12
+ Fe		1.31	1.30	1.26	1.20	1.09	0.89	0.61	0.34
+ Zn & Fe		1.3	1.30	1.29	1.20	1.09	0.86	0.65	0.29
					Silicic acid (µM)				
Control	19.2	18.8	18.6	18.3	18.3	18.0	17.7	17.7	16.8
+ Zn		19.0	18.6	18.2	18.7	18.0	17.9	17.7	16.6
+ Fe		18.6	18.3	18.0	18.3	17.7	18.7	14.0	11.9
+ Zn & Fe		19.0	18.5	18.2	18.3	17.6	17.6	14.2	10.4
					Particulate Oragnic Carbon (µM)				
Control	23.40	24.50	23.20	25.00		26.8	26.8	28.2	29
+ Zn		22.80	23.70	25.20	25.8	26.6	29.8	31.3	34.6
+ Fe		20.50	22.10	25.30	34.4	34.1	46.8	63.6	104
+ Zn & Fe		24.80	25.40	28.70	31.6	38.6	52.7	69.6	118

Appendix 3 Chlorophyll *a*, nitrate, phosphate, silicic acid and particulate organic carbon concentrations from all four treatments over the eight day trace metal enrichment experiment.

	Treatments			
Time days	Control nM	Fe nM	Zn nM	FeZn nM
0	**0.069**			
1	0.075	0.075	11.7	10.9
2	0.068	0.073	11.7	9.95
3	0.069	0.067	9.19	9.80
4	0.062	0.057	7.10	4.81
5	0.062	0.051	6.36	3.46
6	0.061	0.046	6.57	4.15
7	0.052	0.036	7.65	3.56
8	0.051	0.034	5.71	3.21

Appendix 4 (a) Total dissolved Zn concentrations (nM) analysed by adsorptive Cathodic Stripping Voltammetry (AdCSV) in all 4 treatments for the eight day trace metal enrichment experiment.

	Treatments			
Time days	Control nM	Fe nM	Zn nM	FeZn nM
0	**0.084**			
1	0.099	0.106	12.0	11.1
2	0.084	0.099	11.9	
3	0.084	0.091	10.3	9.84
4	0.068	0.061	7.48	3.92
5	0.069	0.061	7.54	5.00
6	0.067	0.054	7.29	4.89
7	0.061	0.046	6.97	4.08
8	0.059	0.038	6.28	3.80

Appendix 4 (b) Total dissolved Zn concentrations (nM) analysed by chelation and solvent extraction by Graphite Furnace Atomic Adsorption Spectroscopy (GFAAS) in all 4 treatments for the eight day trace metal enrichment experiment.

	Treatments			
Time days	Control nM	Fe nM	Zn nM	FeZn nM
0	**0.12**			
1	0.115	11.6	0.133	11.9
2	0.116	11.2	0.124	
3	0.115	9.37	0.106	7.78
4	0.106	7.25	0.098	7.30
5	0.098	3.27	0.081	2.64
6	0.087	3.07	0.071	1.87
7	0.080	2.75	0.062	2.38
8	0.078	2.68	0.053	2.17

Appendix 4 (c) Total dissolved Fe concentrations (nM) analysed by chelation and solvent extraction by Graphite Furnace Atomic Adsorption Spectroscopy (GFAAS) in all four treatments for the 8 day trace metal enrichment experiment.

	Treatments			
Time days	Control nM	Fe nM	Zn nM	FeZn nM
0	0.070			
1	0.100	0.089	0.082	0.098
2	0.129	0.084	0.065	
3	0.118	0.073	0.090	0.097
4	0.117	0.105	0.129	0.107
5	0.100	0.071	0.123	0.096
6	0.084	0.098	0.085	0.087
7	0.093	0.054	0.068	0.094
8	0.056	0.038	0.065	0.069

Appendix 4 (d) Total dissolved Cd concentrations (nM) analysed by chelation and solvent extraction by Graphite Furnace Atomic Adsorption Spectroscopy (GFAAS) in all four treatments for the 8 day trace metal enrichment experiment

Time (days)	C_{Zn} (nM)	C_{Fe} (nM)	C_L (nM)	$[Zn^{2+}]$ (nM)	Zn' (nM)	log K'_{ZnL}
				Control		
1	0.075	0.12				
2	0.068	0.11	0.31	0.007	0.015	10.4
3	0.069	0.11	0.21	0.009	0.019	10.5
4	0.062	0.11	0.15	0.006	0.013	10.9
5	0.062	0.10	0.26	0.002	0.004	11.1
6	0.061	0.08	0.56	0.001	0.002	10.8
7	0.052	0.08	0.61	0.001	0.002	10.8
8	0.051	0.07	0.73	0.001	0.002	10.7
				+ Fe		
1	0.075	11.57	0.35	0.004	0.008	10.8
2	0.073	11.23	0.26	0.005	0.011	10.8
3	0.067	9.37	0.23	0.009	0.019	10.5
4	0.057	7.25	0.25	0.006	0.013	10.5
5	0.051	3.27				
6	0.046	3.07	0.79	0.0009	0.002	10.8
7	0.036	2.75	1.1	0.008	0.017	10.6
8	0.034	2.68	1.3	0.002	0.004	10.8
				+ Zn		
1	11.7	0.13	2.91	4.21	8.84	10.2
2	11.7	0.12	2.78	4.26	8.95	10.1
3	9.19	0.11	2.73	3.09	6.49	10.3
4	7.10	0.09	2.99	2.01	4.22	10.1
5	6.36	0.08	5.94	0.51	1.07	10.2
6	6.57	0.07	5.14	0.77	1.62	9.92
7	7.65	0.06	4.43	1.62	3.40	10.1
8	5.71	0.05	2.28	1.68	3.53	10.1
				+ Zn & + Fe		
1	10.9	11.9	3.16	3.71	7.79	10.1
2	9.95		2.97	4.17	8.76	10.2
3	9.80	7.78	2.66	3.45	7.25	10.2
4	4.81	7.30	5.00	0.12	0.25	10.1
5	3.46	2.64	3.52	0.30	0.63	9.85
6	4.15	1.87	3.66	0.428	0.90	10.4
7	3.56	2.38	3.87	0.365	0.77	10.1
8	3.21	2.17	3.1	0.33	0.69	10.2

Appendix 5 Zinc speciation data for all four treatments throughout the 8 day trace metal enrichment experiment. C_{Zn} and C_{Fe} is the total dissolved Zn and Fe concentrations, CL is the concentration of the Zn-binding ligand, Zn^{2+} is the free Zn ion concentration Zn′ is the labile inorganic species and log K'_{ZnL} is the stability constant.

Time days	Control cell/l	+ Fe cell/l	+ Zn cell/l	+ Zn & +Fe cell/l
		Coccolithophores		
0	37094			
1	27934	25319	45858	37163
2	44906	43800	41185	57111
3	128696	40260	138790	71161
4	86715	82733	54506	175632
5	272533	177146	162317	236195
		Flagellates		
0	50342			
1	129453	65371	331494	177559
2	297309	212186	54913	187366
3	745681	699915	547590	99928
4	610103	309033	334826	125668
5	130072	2302907	150294	1235484
		Large Diatoms > 20 μm		
0	10598			
1	37473	24859	184343	20646
2	57293	55480	40168	61119
3	264963	161042	58039	104471
4	74327	153300	126533	390631
5	136266	1267280	206403	1035627

Appendix 6 Phytoplankton cell count data for the major taxonomic groupings for all 4 treatments for 5 of the 8 days in the trace metal enrichment experiment.

H-2001 Centre 4 months								
Depth (m)	Salinity	Temp (o C)	Fluor (V)	oxygen (μM)	NO_3 (μM)	PO_4 (μM)	Si (μM)	C_{Zn} (nM)
10	32.35	9.11	0.179	298	5.8	0.83	8.4	0.14
25	32.36	8.78	0.729	299	5.8	0.83	8.4	0.27
40	32.44	7.97	0.532	293	8	0.99	11.2	0.08
75	32.76	7.36	0.146	249	15.8	1.4	21.7	0.25
100	33.34	7.62	0.059	163	24.3	1.89	35.1	1.87
200	33.93	6.9	0.051	94	33.5	2.42	53.2	6.12
400	34.02	5.48	0.056	53	39.7	2.86	75.4	9.74
600	34.11	4.64	0.057	32	42.9	3.07	92	6.3
800	34.22	4.06	0.069	15	44.5	3.24	110	6.89
1000	34.34	3.53	0.06	12				8.10

Appendix 7 (a) Total dissolved Zn concentrations, salinity, temperature, fluorescence, oxygen and nutrient concentrations for the Centre Station H-2001 4 months after formation in June 2001.

H-2001 Edge 4 months								
Depth (m)	Salinity	Temp (° C)	Fluor (V)	oxygen (μM)	NO_3 (μM)	PO_4 (μM)	Si (μM)	C_{Zn} (nM)
10	32.41	8.51	0.703	299	7.2	0.58	11	0.10
25	32.47	8.37	1.077	300	8.3	0.63	12.8	0.08
40	32.53	7.88	1.52	304	8.8	0.68	13.9	0.07
75	32.57	7.25	0.391	297	10.6	0.78	15	
100	32.90	6.92	0.092					3.59
200	33.92	6.71	0.048	108	32.9	2.07	49.9	3.06
400	34.02	5.02	0.056	54.5	40.8	2.72	78	8.40
600	34.14	4.3	0.056	25.7	43.7	3.02	95.9	6.40
800	34.27	3.77	0.059	13.8	45.1	3.13	119	6.80
1000	34.37	3.39	0.06	12.8	45.1	3.15	133	8.47

Appendix 7 (b) Total dissolved Zn concentrations, salinity, temperature, fluorescence, oxygen and nutrient concentrations for the Edge Station H-2001 4 months after formation in June 2001.

H-2001 Centre 7 months								
Depth (m)	Salinity	Temp (° C)	Fluor (V)	oxygen (μM)	NO_3 (μM)	PO_4 (μM)	Si (μM)	C_{Zn} (nM)
10	32.31	11.55	0.84	274	5	0.83	8.9	0.31
25	32.31	11.56	1.29	273	4.9	0.83	8.7	0.30
40	32.35	10.88	1.14	272	5.3	0.83	9.4	0.57
75	32.81	7.21	0.11	223	18.1	1.5	22.4	0.62
100	33.33	7.45	0.03	165	24.7	1.91	32.8	1.03
200	33.94	6.75		89.4	34.7	2.46	54	3.18
400	34.01	5.25		52.5	40.5	2.84	72.6	5.61
600	34.13	4.41		23.8	43.7	3.1	95.7	6.54

Appendix 7 (c) Total dissolved Zn concentrations, salinity , temperature, fluorescence, oxygen and nutrient concentrations for the Centre Station H-2001 7 months after formation in September 2001.

H-2001 Edge 7 months								
Depth (m)	Salinity	Temp (° C)	Fluor (V)	oxygen (μM)	NO_3 (μM)	PO_4 (μM)	Si (μM)	CZn (nM)
10	32.301	11.49	1.81	283	4.8	0.81	10.1	0.09
40	32.534	8.37	1.31	266	11.7	1.2	17.5	0.12
75	32.814	7.1	0.03	230	17.9	1.53	27.7	0.65
100	33.142	6.76	0.01	203	22.7	1.79	30.8	1.1
200	33.928	6.51		102	34	2.4	52.6	2.1
400	34.012	4.8		52.3	41.4	2.9	80.8	6.7

Appendix 7 (d) Total dissolved Zn concentration, salinity, temperature, fluorescence, oxygen and nutrient concentrations for the Edge Station H-2001 7 months after formation in September 2001.

H-2000 Centre 16 months								
Depth (m)	Salinity	Temp (° C)	Fluor (V)	oxygen (μM)	NO_3 (μM)	PO_4 (μM)	Si (μM)	CZn (nM)
10	32.53	8.68	0.799	309	8.7	1.01	15.1	0.22
25	32.57	7.42	2.159		9.5	1.06	15.7	0.05
40	32.61	6.89	0.974	303	11.2	1.18	17.3	0.04
75	32.66	6.51	0.334		13.2	1.29	19	0.41
100	32.89	6.2	0.134	258	18.4	1.56	25.5	1.2
200	33.89	6.39	0.056	103	33.8	2.38	34.2	1.5
400	34.01	4.72	0.056	49.4	41.5	2.91	84.5	5.2
600	34.15	4.08	0.058	25.4	44	3.09	108	7.51
800	34.26	3.55	0.059		44.7	3.16	127	9.33
1000	34.35	3.16	0.059	15.2	44.9	3.17	142	9.31

Appendix 7 (e) Total dissolved Zn concentrations, salinity, temperature, fluorescence, oxygen and nutrient concentration for the Centre Station H-2000 16 months after formation in June 2001.

H-2000 Edge 16 months								
Depth (m)	Salinity	Temp (° C)	Fluor (V)	oxygen (μM)	NO_3 (μM)	PO_4 (μM)	Si (μM)	CZn (nM)
10	32.51	9.34	1.319	301	8.3	1	13.9	0.05
25	32.53	8.64	2.294	304	8.3	1.01	14.5	0.16
40	32.57	7.33	1.978	305	9.7	1.11	15.4	0.1
75	32.60	6.97	0.611	301	11.3	1.2	16.5	0.1
100	32.70	6.33	0.184	287	14.8	1.37	20.1	1.32
200	33.89	6.2	0.05	116	33.2	2.39	53.3	1.96
400	34.00	4.64	0.056	53.8	41.5	2.96	83.6	4.1
600	34.16	4.03	0.059	25.2	44.3	3.16	107	6.1
800	34.27	3.56	0.058	17.2	45.4	3.21	125	7.2
1000	34.36	3.13	0.06	15.1	45.3	3.25	140	9.21

Appendix 7 (f) Total dissolved Zn concentrations, salinity, temperature, fluorescence, oxygen and nutrient concentrations for the Edge Station H-2000 16 months after formation in June 2001.

H-2000 Centre 19 months								
Depth (m)	Salinity	Temp (° C)	Fluor (V)	oxygen (μM)	NO_3 (μM)	PO_4 (μM)	Si (μM)	CZn (nM)
10	32.28	12	1.35	274	3.5	0.74	11.7	0.03
25	32.27	12	2	274	3.5	0.77	11.7	0.04
40	32.51	7.62	0.74	280	12.1	1.24	17.3	0.38
75	32.69	6.83	0.11	259	16.3	1.46	21.9	0.12
100	33.01	6.85	0.02	208	21.8	1.76	31.4	1.85
200	33.84	6.68		92.2	34	2.46	53.6	3.61
400	33.99	5.3			38.9	2.79	73.4	5.09
600	34.09	4.27		33	43.1	3.08	95.5	7.08
800	34.24	3.68	0.01	17.4	44.4	3.18	117	7.9
1000	34.34	3.24	0.01	16.5	45.8	3.23	131	9.01

Appendix 7 (g) Total dissolved Zn concentration, salinity, temperature, fluorescence, oxygen and nutrient concentration for the Centre station H-2000 19 months after formation in September 2001.

H-2000 Edge 19 months								
Depth (m)	Salinity	Temp (° C)	Fluor (V)	oxygen (μM)	NO_3 (μM)	PO_4 (μM)	Si (μM)	CZn (nM)
10	32.29	11.7	2.33	275	4	0.76	11.7	0.07
25	32.29	11.7	2.49	273	4.1	0.77	12.1	0.09
40	32.58	7.29	0.45	278	13.2	1.78	17.9	0.16
75	32.75	6.93	0.03	291	18.3	1.57	26.3	0.12
100	33.09	6.67	0.01	210	22	1.77	31.3	2.1
200	33.87	6.27		123	32.3	2.31	51.2	
400	34.01	4.69		53	40.7	2.9	83.3	5.7
600	34.15	4.13	0.01	23.5	43.8	3.12	103	7.45
800	34.27	3.64	0.01	16.1	45	3.2	120	9.6
1000	34.36	3.16	0.01	14.3	44.8	3.23	134	9.21

Appendix 7 (h) Total dissolved Zn concentrations, salinity, temperature, fluorescence, oxygen and nutrient concentrations for the Edge Station H-2000 19 months after formation in September 2001.

H-2000 Edge 19 months								
Depth (m)	Salinity	Temp (° C)	Fluor (V)	oxygen (μM)	NO_3 (μM)	PO_4 (μM)	Si (μM)	CZn (nM)
10	32.29	11.7	2.33	275	4	0.76	11.7	0.07
25	32.29	11.7	2.49	273	4.1	0.77	12.1	0.09
40	32.58	7.29	0.45	278	13.2	1.78	17.9	0.16
75	32.75	6.93	0.03	291	18.3	1.57	26.3	0.12
100	33.09	6.67	0.01	210	22	1.77	31.3	2.1
200	33.87	6.27		123	32.3	2.31	51.2	
400	34.01	4.69		53	40.7	2.9	83.3	5.7
600	34.15	4.13	0.01	23.5	43.8	3.12	103	7.45
800	34.27	3.64	0.01	16.1	45	3.2	120	9.6
1000	34.36	3.16	0.01	14.3	44.8	3.23	134	9.21

Appendix 7 (i) Total dissolved Zn concentrations, salinity, temperature, fluorescence, oxygen and nutrient concentrations for the Reference Station in June 2001.

H-2001 Centre 4 months							
Depth (m)	Salinity	Temp (o C)	Fluor (V)	C_{Zn} (nM)	C_L (nM)	Zn^{2+} (pM)	K'ZnL
10	32.35	9.11	0.179	0.14	1.36	6.5	10.2
40	32.44	7.97	0.532	0.08	1.41	3.3	10.3
75	32.76	7.36	0.146	0.25	1.32	9.8	10.2
200	33.34	7.62	0.059	6.12	3.88	2.13	10.2

Appendix 8 (a) Zinc speciation data for H-2001 Centre Station 4 months after formation in June 2001.

H-2001 Edge 4 months							
Depth (m)	Salinity	Temp (o C)	Fluor (V)	C_{Zn} (nM)	C_L (nM)	Zn^{2+} (pM)	K'ZnL
10	32.41	8.51	0.703	0.10	1.41	3.5	10.2
25	32.47	8.37	1.077	0.08	1.13	3.4	10.3
40	32.53	7.88	1.52	0.07	2.02	1.3	10.4
200	33.92	6.71	0.048	3.06	4.2	86.7	10.4

Appendix 8 (b) Zinc speciation data for H-2001 Edge Station 4 months after formation in June 2001.

H-2001 Centre 7 months							
Depth (m)	Salinity	Temp (o C)	Fluor (V)	C_{Zn} (nM)	C_L (nM)	Zn^{2+} (pM)	K'ZnL
10	32.301	11.49	1.81	0.31	1.31	3.6	10.2
40	32.534	8.37	1.31	1.53	2.46	2.4	10.2
75	32.814	7.1	0.03	1.53	2.2	1.5	10.5
200	33.928	6.51		2.9	3.26	21.1	10.1

Appendix 8 (c) Zinc speciation data for H-2001 Centre Station 7 months after formation in September 2001.

H-2000 Centre 19 months							
Depth (m)	Salinity	Temp (o C)	Fluor (V)	C_{Zn} (nM)	C_L (nM)	Zn^{2+} (pM)	K'ZnL
10	32.28	12	1.35	0.03	1.1	2.7	10.3
40	32.51	7.62	0.74	0.38	2.6	2.02	10.3
75	32.69	6.83	0.11	0.12	1.85	2.7	10.2
200	33.84	6.68		3.61	3.57	21.2	10.3

Appendix 8 (d) Zinc speciation for H-2000 Centre Station 19 months after formation in September 2001.

H-2000 Edge 19 months							
Depth (m)	Salinity	Temp (o C)	Fluor (V)	C_{Zn} (nM)	C_L (nM)	Zn^{2+} (pM)	K'ZnL
10	32.29	11.7	2.33	0.07	1.34	2.9	10.3
40	32.58	7.29	0.45	0.16	1.11	4.4	10.2
75	32.75	6.93	0.03	0.12	1.07	3.6	10.1
200	33.87	6.27		2.1	2.69	80.6	10.5

Appendix 8 (e) Zinc speciation data for H-2000 Edge Station 19 months after formation in September 2001.

Reference Station							
Depth (m)	Salinity	Temp (o C)	Fluor (V)	C_{Zn} (nM)	C_L (nM)	Zn^{2+} (pM)	K'ZnL
10	32.58	8.41	0.76	0.09	1.2	4	10.2
40	32.61	6.98	1.81	0.09	1.01	5.8	10.1
75	32.65	6.58	0.38	0.62	2.3	1.8	10.4
200	33.87	5.07	0.057	2.36	3.28	9.4	10.5

Appendix 8 (f) Zinc speciation data for the Reference Station in June 2001.