Arsenic Speciation in Estuarine and Oceanic Waters by Hydride Generation – Atomic Fluorescence Spectroscopy

by

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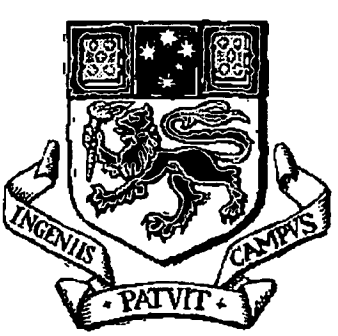

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ABSTRACT

A method has been developed for the routine determination of four arsenic species (arsenite, arsenate, monomethylarsenic and dimethylarsenic) in natural water, and has been applied to a study of arsenic cycling in open ocean (Atlantic and Southern Ocean) and estuarine (Huon Estuary, Tasmania) waters.

The method uses hydride generation and cold trapping of the hydrides, coupled with atomic fluorescence detection (AFS) at 193.7 nm. With the optimised system, detection limits for As(III), As(V), MMA and DMA are 2.3, 0.9, 2.4 and 3.7 ng L^{-1}, respectively, in a 5-mL sample. The precision for nine sample replicates was better than 3.5% for all the arsenic species. Accuracy of the method was determined by analysis of a seawater certified reference material (NASS-4) and by recovery studies on natural samples.

The manual method was semi-automated and modified for shipboard use by automating several of the procedures in the analytical sequence, including addition of $NaBH_4$ to samples, cooling and heating the U-trap used for preconcentration and separation of the arsines, and logging the AFS output. The semi-automated method has a number of advantages over the earlier manual HG-AFS method, namely, shorter sample throughput time, increased precision and, most significantly, ease of use under shipboard conditions.

The absence of pure standards for MMA and DMA was identified and methods were developed to purify and characterise suitable standards.

Samples were collected from tropical and subtropical regions in the western basin of the Atlantic Ocean during an Intergovernmental Oceanographic Commission (IOC) baseline contaminant survey. The biogeochemical cycling of arsenic in this environment is discussed briefly. In addition, an interlaboratory comparison has been carried out to compare the results determined by HG-AFS analysis of stored samples, with those obtained during the IOC voyage by hydride generation-gas chromatography-photo ionisation detection (HG-GC-PID). In the interlaboratory comparison particular emphasis was placed on the results for the methyl arsenic species, for which no certified reference materials are currently available in seawater.

Surface and vertical seawater profiles were obtained in the Subantarctic Zone of the Southern Ocean along a transect between 42° and 55° S along 141° 30' E, south of Australia. The transect line passes through four different oceanic environments: the Subtropical Convergence Zone (STCZ), Subantarctic Zone (SAZ), the Subantarctic

Front (SAF) and the Polar Frontal Zone (PFZ). These represent some of the first measurements of arsenic in the Southern Ocean. Cycling of arsenic is interpreted using the chemical and biology data obtained from the cruise, and the shipboard performance of the semi-automated method is also discussed.

A detailed study of arsenic cycling in the Huon estuary, in south east Tasmania, was undertaken. Arsenic species data were obtained during eight 3-monthly spatial surveys, and weekly sampling from a single site in the estuary over a six-month period. The data has been correlated with other chemical measurements, including nutrients, salinity and dissolved oxygen, and also with biological information about the phytoplankton species present.

Arsenic concentrations in the Huon estuary are very low, even when compared with other pristine systems. The seasonal cycle of arsenic is similar to that found in other temperate estuaries of the Northern Hemisphere. Arsenic cycling is almost entirely biologically influenced and the uptake of arsenic, particularly by the phytoplankton *Pseudo-nitzschia*, is discussed in detail.

Chapter 6 provides a final summary of the work presented in this thesis, and also recommendations for further development of the analytical method and research opportunities revealed in the estuarine study of arsenic.

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LIST OF PUBLICATIONS

Journal Articles

1 Featherstone, A.M., Butler, E.C.V., O'Grady, B.V. & Michel, P. (1998). *Determination of arsenic species in sea-water by hydride generation atomic fluorescence spectroscopy.* Journal of Analytical Atomic Spectrometry. **12**(12) 1355-1360. *(Chapter 3)*

2 Featherstone, A.M., Boult, P.R., O'Grady, B.V. & Butler, E.C.V. (1999). *A shipboard method for arsenic speciation using semi-automated hydride generation atomic fluorescence spectroscopy.* Analytica Chimica Acta, accepted for publication. *(Chapter 3/4)*

3 Featherstone, A.M., Butler, E.C.V. & O'Grady, B.V. (1999). *Distribution of arsenic species in the Subantarctic Zone of the Southern Ocean*, in preparation. *(Chapter 4)*

4 Cutter, G. A., Cutter, L. S. & Featherstone, A. M. (1999) *Antimony and arsenic biogeochemistry in the western Atlantic Ocean* Deep-Sea Research II, submitted for publication. *(Chapter 4)*

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Chapter 1: Arsenic in the Environment

1.1 SOURCES OF ARSENIC TO THE ENVIRONMENT

Arsenic is a Group V element which has four oxidation states (-3, 0, +3, and +5). It is classified as a metalloid as it exhibits both metallic and non-metallic character. It is the 20th most abundant element in the earth's crust [1], and is ubiquitous in the environment, present in the atmosphere, sediments, soils, water and organisms. Therefore, the arsenic cycle is a complex balance of biological, chemical, physical and geological processes.

Arsenic is associated particularly with sulfidic ore bodies [2], and as a result is commonly released into the environment through processes such as mining, smelting and fossil fuel combustion [1]. Natural processes like weathering and volcanic emissions [3] also release arsenic into the atmosphere and waterways. Arsenic concentrations are often elevated in groundwaters, either as a result of natural geothermal activity or because of its release from sulfidic ores. Oxidation of sulfidic ores and subsequent mobilisation of arsenic is a common problem that may occur as the water table is lowered through the use of groundwater for irrigation. This is particularly prevalent in some developing countries such as Bangladesh [2] and India [4] where arsenic has seriously contaminated supplies of drinking water. Additionally, various arsenic compounds have entered the environment through their use in agriculture as insecticides, fungicides and herbicides [5].

Currently, anthropogenic release of arsenic to the environment is estimated to be 30 000 t per year [6], although uncertainties exist in the total arsenic budget and it is unclear whether anthropogenic release exceeds natural processes [1, 7]. It has been estimated, however, that arsenic concentrations in the ocean will increase by between 1 and 15% by the year 2000 as a result of anthropogenic activity [8, 9].

1.2 WHY MEASURE ARSENIC IN THE ENVIRONMENT?

As it is associated with ore bodies, measurements of arsenic in natural waters and soils can be useful as markers for geological prospecting ([1] and references therein), indicating the presence of valuable mineral deposits. More commonly, arsenic is measured in the environment because of its potential toxicity to animal and plant life.

Arsenic is isoelectronic with the Group V element, phosphorus, and is known to enter the biochemical pathways of both phosphorus and another essential nutrient of the same group, nitrogen. There is some limited evidence to support the fact that arsenic is an

essential micronutrient [1]([10] and references therein), but, depending upon environmental conditions, arsenic can be a toxic element even at near natural concentrations. In the environment, arsenic is present as a number of different inorganic and organic species. This will be discussed further in the next section (1.3). Inorganic arsenic species are much more toxic than organic forms. As(V) is known to uncouple oxidative phosphorylation while As(III) has a high affinity for sulfhydryl groups, having the ability to deactivate many enzymes [11], and is also a suspected human carcinogen [10].

In the aquatic environment it has been suggested that the energy requirements for arsenic detoxification in phytoplankton may limit productivity in light limited environments. Also, in culture, Sanders *et al.* [12] have observed that at least in the short-term, elevated As(V) concentrations cause changes in the phytoplankton species composition. They predict these changes may be sufficient to alter species composition further up the trophic chain, and thereby, decrease carbon transfer to the sediments.

Because a variety of arsenic species are found in the environment, and because their relative toxicity varies, it is important that analytical methods are able to measure all species accurately and reliably [13].

1.3 DISSOLVED ARSENIC SPECIATION IN THE AQUATIC ENVIRONMENT

A number of reviews have been published which detail various aspects of arsenic cycling in the aquatic environment [1, 5, 7, 10, 14-18].

Arsenic is typically found in open ocean and coastal seawater at concentrations of 1-2 $\mu g\ L^{-1}$ [1]. Its concentration often falls within a similar range in freshwater environments but is more variable, depending upon the regional geology and industrial inputs. It is predominantly present as four hydride-forming species: two redox states of inorganic arsenic, arsenite [As(III)] and arsenate [As(V)], and two methylated forms, monomethylarsenic (MMA) and dimethylarsenic (DMA).

Arsenate is the major species found in oxic seawater [7, 19]. It is thought to occur as the tetrahedral anion $HAsO_4^{-2}$ ($pKa_1 = 2.2$, $pKa_2 = 6.9$ [1, 20]), hence, its similarity to phosphate ($pKa_1 = 2.2$, $pKa_2 = 7.2$ [1]). Nevertheless, work in rivers in the southeast of the United States [21] suggests that there may be significant interaction of As(V) with low molecular mass organic material (<10 000) in systems with high organic loads.

Arsenite is a thermodynamically unstable species, and the predicted As(III)/As(V) ratio in seawater is $10^{-26.5}$ [19]. Arsenite occurs in seawater predominantly as undissociated *o*-arsenious acid, $As(OH)_3$ (pKa = 9.21 [1]). In practice, the concentration of As(III) found, particularly in surface waters, is greater than that predicted by thermodynamics as a result of biologically mediated reduction of As(V). As(III) produced by abiotic reduction may also be found in surface waters in regions where upwelling of oxygen-depleted bottom water occur [22].

Methylated arsenic species account for 5-20% of the dissolved arsenic found in ocean surface waters [16] and up to 62% [22, 23] in productive estuarine waters, although more usually in the range 5 – 15% [24-26]. These species are not predicted to be present from thermodynamic calculations, and their production is often closely linked to indicators of biological activity such as chlorophyll *a* concentrations [22, 27, 28]. Reduction of arsenate to arsenite, followed by methylation, is thought to be a detoxification pathway [10] which protects phosphate and nitrate biochemical pathways. MMA and DMA are relatively stable species, and in almost all cases the concentration of DMA is greater than that of MMA [27].

The actual form in which the methyl species occur in the water column is somewhat unclear. It is often assumed that MMA and DMA occur as the anions $CH_3AsO(OH)O^-$, and $C_2H_6AsOO^-$. Howard has commented that many of the analytical techniques used are incapable of distinguishing the MMA or DMA anions from other molecules containing these moieties [28] and therefore the more generic terms monomethyl arsenic and dimethyl arsenic should be used to describe these species. In a recent article, however, Howard *et al.* [29] have presented evidence to support the presence of DMA in estuarine waters as the dimethylarsinate anion.

The arsenic species discussed above are all hydride forming, that is, they are able to be reduced to their corresponding arsines by reaction with sodium borohydride, and can subsequently be detected using spectroscopic techniques. Other, more complex, organoarsenic species, which are resistant to hydride generation, have been identified in marine organisms, but are usually present at very low concentrations in the water column. These species include trimethylarsine oxide (TMAO) and the arsenic analogues of betaine and choline, arsenobetaine and arsenocholine, as well as various other arsenosugars and arsenolipids [1, 30, 31]. Recent evidence suggests that hydride-refractory arsenic species may under some circumstances be present in appreciable quantities in estuarine waters [32-36] and sediment pore waters [13]. The determination

of non-hydride forming organoarsenics at low concentrations presents a number of analytical difficulties [37] and so the species that are present are yet to be conclusively identified [37, 38].

Under more reducing conditions, in oxygen depleted or anoxic waters, thermodynamic calculations predict that As(III) will be the dominant arsenic species [39-41]. However, like in surface waters, the As(III)/As(V) ratio in natural systems is quite different to that predicted, with As(V) concentrations significantly exceeding calculated values [39-41]. Mechanisms to explain the presence of As(V) in oxygen depleted and anoxic systems include release from sinking detritus [42], advection of high As(V) surface waters [43], and, where sulfur is present, the formation of thioarsenate complexes [42]. Calculations predict that thioarsenic (thioarsenate and thioarsenite) species probably represent a very small proportion of the arsenic present in anoxic systems [1]. Therefore, it is more likely that interactions between iron, manganese, arsenic and sulfur chemistry, under reducing conditions, lead to dissolution of iron and manganese colloids [40, 44] and subsequent As(V) release.

There is little experimental evidence for the formation of sulfur containing methyl arsenic species, although Bright *et al.* [13] present some indirect evidence to support the presence of mono- di- and trimethylated arsenic(III) thiols in sulfate reducing sediments.

1.4 BIOLOGICALLY MEDIATED ARSENIC CYCLING

Most of the evidence for biological cycling of arsenic species in the aquatic environment comes from work done in the estuarine/coastal environment and sediments, or from laboratory studies of organisms isolated from these environments. From the current literature, it is clear that biological arsenic cycling is highly complex and not easily related to one single physical, chemical or biological parameter. Also, in the natural environment, biological cycling is often superimposed on the physicochemical cycling, making elucidation of biochemical interactions even more difficult. In this chapter only the biological cycling of arsenic will be discussed, but the influence of physicochemical processes will be discussed further in Chapter 4 and Chapter 5.

It has been shown that the biological cycling of arsenic is different in marine algae and animals. In 1977, arsenobetaine was the first arsenic species to be identified in a marine animal, the tail of the western rock lobster [30], and has subsequently been shown to be

the major arsenic compound in all marine animals. Arsenobetaine is a highly stable compound, requiring rigorous digestion to release As(V). More recently the trimethylarsonium ion has been identified [45]. There are also several reports of arsenocholine [46-48], a likely precursor of arsenobetaine, although according to a review by Francesconi and Edmonds [17], this species is yet to be positively identified in marine animals. Trimethylarsine oxide, a possible breakdown product of arsenobetaine, has also been shown to occur in some fish, and increases in frozen fish flesh [49].

Brown algae have been shown to contain significantly higher concentrations of arsenic than either green or red algae [50]. In algae, arsenic is incorporated as As(V) and transformed into a variety of water- and lipid-soluble compounds. These compounds can easily interconvert [51] and it is thought that only a single arsenic detoxification mechanism has evolved [52], as all algae produce a similar group of arsenic compounds. The water-soluble arsenic species were the first to be identified in algae as the lipid soluble species are unstable and readily hydrolyse upon extraction [53]. A summary of the intracellular arsenic compounds that have been identified is given in a recent review by Francesconi and Edmonds [17].

The first water soluble arsenic species to be identified were dimethylarsinylribosides [17]. These species were initially found in brown algae [54], but later shown to be the major constituent of all unicellular algae. In fact, a number of dimethylarsinylribosides have been identified in marine algae as well as a trimethylarsonioriboside [55]. Arsenic containing lipids in marine algae have been identified [55] and are thought to be phospholipids associated with the cell membrane [56].

There is conflicting evidence regarding uptake of As(V) by the cell, although general agreement that it is linked with the phosphate carrier system [52]. Sanders and Windom [57] claim arsenic and phosphate uptake is antagonistic, while Andreae and Klumpp [58] find uptake to be independent of phosphate concentration.

While the exact means by which arsenic enters the cell and the biochemical pathways in which it is involved are unknown, it is clear that within the cell it is transformed into a series of di- or tri- methylated compounds with ribosidyl moieties. Based on this information, Francesconi and Edmonds [17] have proposed a mechanism for arsenic cycling within the algal cell. In the cycle they propose arsenic is progressively reduced and methylated, or reduced and adenosylated by *S*-adenosylmethionine, although the sequence of methylation and adenosylation is unclear, and in some cases adenosylation

may not occur. The adenosylated compounds may subsequently undergo glycosidation, giving di- and tri- methylated arsenoribosides. They predict that the relative abundance of the particular compounds produced is related to taxonomy and the different enzyme groups used to perform glycosidation. In this review, Francesconi and Edmonds suggested that the methylribosides are probably detoxification end-products which have no physiological role in algae, and are excreted to the sea upon conversion to lipid derivatives which can pass though the cell membrane. There is no direct evidence supporting this assumption, and the arsenic compounds may well be redistributed to the water column once the algae die [26]. How these compounds are converted into As(III), MMA and DMA, the species identified in seawater, is yet to be established. Their instability suggests that the phospholipids might be chemically or microbially degraded quite quickly, to give DMA (Benson indicates that DMA is an excretion product commonly identified in phytoplankton culture media [52]). The mechanism for MMA production is less clear although it often follows that of DMA, suggesting either species specific production or production via degradation of DMA (mediated either chemically or microbially) [59-61]. In the estuarine environment it has also been shown that phosphate concentrations [27] and temperature [27, 62] can influence the production and release of methyl arsenic species.

Francesconi and Edmonds [17] also proposed a cycle to explain the presence of arsenobetaine in higher marine animals. They suggested that marine animals obtain arsenic from the food chain rather than the water column and that arsenoribosides are converted to arsenobetaine via the food chain. They suggested that the di- and trimethylated arsenoribosides released by phytoplankton are rapidly degraded in either the sediments or the water column to dimethylarsinyl ethanol and arsenocholine. These species are rapidly taken up by marine animals and converted to arsenobetaine.

Benson has shown [52] that arsenic excretion by algae is rapid and that very low accumulation occurs. It is also apparent that no accumulation of arsenic occurs via the food chain.

Further discussion on the cycling of As(V), As(III), MMA, DMA and the hydride refractory fraction in relation to biological, chemical and physical conditions in the estuarine and open ocean environments are presented in Chapter 4 and Chapter 5.

1.5 HOW IS ARSENIC SPECIATION MEASURED?

1.5.1 Analytical methods

Generally, only analytical methods utilising a hydride generation step have sufficient sensitivity to determine arsenic species at the concentrations they occur in natural waters. Other methods applied to the analysis of natural waters include colorimetry [63-65] and electrochemistry [66-68]. These have almost equivalent sensitivity to hydride generation techniques, and are often simpler methods that enable greater sample throughput. However, they are unable to provide species specific information and may suffer from matrix interferences. Hydride generation methods are particularly advantageous as they allow the arsenic species to be separated from the sample matrix, limiting interferences from this source, and also allow arsenic in the sample to be preconcentrated, giving extremely sensitive methods. Further analytical details on the variety of hydride generation methods are given in the literature review in Chapter 3.

Despite their sensitivity, hydride generation methods do have a number of analytical problems as outlined below.

1.5.2 Problems with the current methods

1.5.2a Analytical problems

As stated in section 1.3, many analytical methods are incapable of distinguishing the exact form of the methyl arsenic species. This point is made by Howard *et al.,* in their discussion of DMA and whether it occurs in waters as the dimethylarsinate anion, or whether other species containing this moiety react to produce the same signal as the anion [28, 29]. Although they developed an experimental technique to confirm the presence of DMA as dimethylarsinate, evidence by Bright *et al.* [13] from the analysis of sediment pore waters has indicated the presence of other arsenic species containing a DMA moiety. They showed that when hydride generation was conducted at pH 1, dimethylarsinate and monomethyl arsonate formed hydrides, whilst at pH 6 no signal occurred. However, at pH 6 species such as dimethylarsinoglutathione and monomethylarsinocysteine gave rise to MMA and DMA peaks. In the analysis of sediment pore waters they found DMA and MMA peaks when hydride generation was conducted at pH 6 and concluded that either the species mentioned above, or some other similar compounds, must have been present.

Another major analytical difficulty with the hydride generation methods is their inability to determine hydride-refractory arsenic species. As mentioned in section 1.3, it

is currently proving very difficult to find a suitable way to identify these species in seawater because of difficulties associated with analysing such low concentrations in a high chloride matrix, often with standards which do not reflect the speciation found in natural systems [37]. Current attempts to identify these non-hydride forming organoarsenic species in seawater have used highly specialised techniques such as electrospray mass spectrometry, and require preconcentration and extraction of large volumes of seawater (200 L) [69].

Often, hydride refractory arsenic species have been identified by the increase in arsenic concentration following UV irradiation of the sample [32]. In sediment pore waters, this has been shown to increase the arsenic concentration of the sample by between 18 and 420% [13], while in estuarine waters the amount varied between and 4 and 26% [32, 36] during summer months. While a useful technique, UV irradiation does not allow identification of the hydride refractory species found and may even still underestimate the total refractory arsenic pool in the dissolved phase. de Bettencourt *et al.* [35] commented that UV oxidation gave incomplete digestion when compared with alkaline digests and total ashing of the sample. Evidence by Bright *et al.* [13] comparing UV oxidation with microwave persulfate digests suggested that the latter liberated more arsenic, however, they indicated that this still may not represent total dissolved arsenic. Hasegawa *et al.* [36] also found that seawater and freshwater contain both 'UV-labile' and 'UV-resistant' (defined as the microwave persulfate digestible fraction) hydride-refractory arsenic species.

A further analytical problem is that it is difficult to obtain very pure samples of MMA and DMA to use as standard materials [70, 71] and currently no seawater or freshwater reference materials are commercially available for the methyl arsenic species. This is discussed further in Chapter 2.

1.5.2b Sample storage problems

In addition to the difficulties associated with developing suitable analytical methods are the issues associated with maintaining the integrity of sample speciation between sample collection and analysis. It is generally agreed that As(V) and the methyl arsenic species are stable for several months in samples which have been filtered, acidified and refrigerated [70, 72-74]. Nevertheless, there is some indication that upon storage hydride refractory organoarsenic species or arsenic colloids may degrade and cause the total arsenic content of the sample to increase slightly [75].

Arsine is the most toxic arsenic species in found in the aquatic environment, but is thermodynamically unstable and therefore only found in highly reducing sediments [76]. In oxic, and even oxygen depleted waters [39, 77, 78], As(III) is the most toxic species. Despite its toxicity and its role in the arsenic biogeochemical cycle, many studies in oxic seawater and estuarine systems [21, 26, 33, 79-91] report results for only total inorganic arsenic [As(V+III)], because of difficulties in preserving speciation. As(III) is a less stable species which can be oxidised rapidly after sampling. A variety of opinions exist regarding the rate of oxidation of As(III) and the importance of immediate sample analysis or preservation after collection [72, 92-98]. In general, samples must be analysed immediately after sampling or quick frozen in liquid nitrogen and stored below the temperature [99] at which any residual brine might exist, otherwise unreliable results may be obtained [88, 100]. Often immediate analysis is not possible as the most sensitive techniques incorporate a hydride generation step and use an element specific detector such as AAS or ICP. Therefore, the instrumentation is large and complex, and does not easily lend itself to making in-field measurements.

1.6 AIMS OF THE THESIS

This thesis had two main aims. The first was to develop an analytical method, suitable for determining the four hydride-forming arsenic species in seawater. The characteristics required of the method were:

- accuracy and reliability
- adequate sensitivity for coastal and open ocean seawater
- potential for shipboard use

The second aim was to study the estuarine cycling of dissolved arsenic through spatial and temporal surveys of a relatively pristine system with no history of major industrial arsenic inputs.

An important aspect of the initial method development was to find and characterise suitable standards for methyl arsenic species, as the literature suggested that it might be difficult to obtain reliable and pure sources of DMA, and particularly MMA, standards. To complement this work, an interlaboratory method comparison exercise was conducted, to assess the comparability of the analytical method and standards, with particular attention to the methyl arsenic species.

Development of the analytical method was carried out in two stages. Initially, a manual hydride generation-atomic fluorescence spectroscopy method, based on the well-established batch HG-AAS methods first published by Andreae [70, 101] in the late 1970's, was developed. It was intended that this method would subsequently be modified/automated and used to make shipboard arsenic measurements during a voyage in the Australasian sector of the Southern Ocean. Although not a new technique, a commercial atomic fluorescence spectroscopy (AFS) instrument has only recently become available [102, 103]. This instrument has been shown to have detection limits intermediate between those of more traditionally used AAS and ICP detectors, but has clear advantages with respect to size, and was therefore considered more appropriate for use as a shipboard method.

Estuarine arsenic cycling will be studied in the Huon River Estuary, Tasmania, forming part of a larger project examining seasonal nutrient cycling and algal dynamics in the river. It is intended that arsenic data will be complemented by detailed ancillary information, such as, phytoplankton species and relative abundance, nutrient concentrations and temperature, providing an opportunity to gain some insights into the biological cycling of arsenic and allowing comparisons to be made with other temperate estuaries in the Northern Hemisphere.

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Chapter 2: Purification and Characterisation of Arsenic Standards

2.1 INTRODUCTION

To enable accurate quantification of As(III), As(V), MMA and DMA, it is important to obtain pure standard materials from a reliable source. Although reliable standards are readily available for the inorganic arsenic species, As(III) and As(V), it has been noted by several authors [1, 2] that methyl arsenic standards are very often not pure or stoichiometric. Therefore, these standards require either further purification, or characterisation of their impurities, before they can be used for quantification of arsenic species in environmental samples.

This chapter discusses in detail the standards that were chosen for use in all subsequent arsenic analyses. It also describes the sources of methyl arsenic standards, particularly MMA, and the procedures used to purify and characterise them.

2.2 STANDARDS FOR As(III), As(V) AND DMA ANALYSIS

2.2.1 Sources of As(III), As(V) and DMA standards

Standards for each of these arsenic species are readily available from a number of commercial sources.

In the literature, standards for As(III) are prepared using either arsenic trioxide (As_2O_3) or sodium arsenite ($NaAsO_2$). Although the sodium salt is easier to dissolve, the As_2O_3 available is of a higher purity, making it most suitable for use as a primary standard. The standard chosen for use was As_2O_3, ACS primary standard (99.995%), supplied by Aldrich Chemical Co. (Milwaukee, WI, USA).

The salt most commonly used for preparation of As(V) standards is disodium arsenate, heptahydrate ($Na_2HAsO_4.7H_2O$). A few references can be found in the literature on the use of arsenic(V) oxide (As_2O_5), however this salt was not considered, as it is deliquescent and its hydration may vary. The $Na_2HAsO_4.7H_2O$ (98.0 – 102.0%) used for As(V) standard preparation was also supplied by Aldrich Chemical Co. Before use, its concentration was verified against the As(III) primary standard.

Cacodylic acid ($C_2H_7AsO_2$) or its sodium salt, dimethylarsinate ($C_2H_6AsNaO_2.3H_2O$), are commonly used as standards for DMA. Both of these salts were obtained from Fluka Chemie (Buchs, Switzerland) for further investigation. The standards are analytical

grade, however the specifications indicate that the acid is of a higher purity (puriss, >99%) than the sodium salt (purum p.a. >98%), based on a total arsenic analysis.

2.2.2 Purification of As(III), As(V) and DMA

The standards for As(III) and As(V) described in section 2.2.1 were used as supplied, without any further purification.

A preliminary investigation into the purity of the DMA standards indicated that both the cacodylic acid and sodium dimethylarsinate contained impurities of inorganic, and particularly monomethyl arsenic. A preliminary comparison of the two standards, by HG-AFS (described in Chapter 3), indicated that the impurities were lowest in the cacodylic acid sample, and so this was used in all subsequent purification and characterisation steps.

An attempt was made to remove the inorganic and methyl arsenic impurities from the cacodylic acid sample based on a column chromatography procedure described by Dietz and Perez [3]. Despite a number of modifications to this method, including the use of different eluents, the separation was insufficient to prevent co-elution of MMA and DMA. The standard manufacturer suggested that further improvements in purity could be achieved by precipitation of the impurities, however, were unable to recommend a suitable method. Therefore, the remaining option was to quantify the inorganic and monomethyl arsenic impurities present in the cacodylic acid, and thereby determine the contribution of DMA to the total arsenic content.

2.2.3 Characterisation of As(III), As(V) and DMA standards

The total arsenic content of the As(V) and DMA standards were determined using flame AAS (nitrous-acetylene), and the results confirmed with ICP-OES, in both cases using As(III) as the primary standard.

The results for the As(V) standard indicated that it was 100% pure, within the limits of experimental error for these two techniques. Further tests of this standard for As(III) using the HG-AFS method described in Chapter 3 indicated that there was none detectable.

Despite the fact that the DMA standard was known to contain inorganic and monomethyl arsenic impurities, it was also found to be 100% pure for total arsenic analysis. This is because the impurities were a small proportion of the total arsenic

concentration and have similar molecular weights to cacodylic acid, and so did not cause obvious differences to the total arsenic content of the standard.

Impurities in the DMA standard were characterised using HG-AFS. Two separate analyses were carried out to quantify inorganic and monomethyl arsenic, with DMA then determined by difference. Using standard additions, the amount of inorganic arsenic in the standard was found to be 1.13% of the total arsenic concentration and MMA 14.19% of total arsenic (using the characterised MMA standard described in section 2.3.3).

2.3 STANDARDS FOR MMA ANALYSIS

2.3.1 Sources of MMA standards

A number of compounds can be used as a standard for MMA. These are: methylarsonic acid (MAA - CH_5AsO_3), monosodium methylarsonate (MSMA – $CH_4AsNaO_3.1½H_2O$) and disodium methylarsonate (DSMA – $CH_3AsNa_2O_3.6H_2O$).

Initially, a literature search was carried out to determine the sources of methyl arsenic used by other researchers, and thereby identify a commercially available analytical grade salt. A summary of the search is shown in Table 2.3 at the end of this chapter. The literature survey indicated that methyl arsenic standards were not readily available. A number of authors cited their standard material as having been donated by another laboratory (1-4), synthesised in their own laboratory (5-6), or obtained from an alternative source such as medical grade strychrotonin (7) or methylarsine dibromide (8-10).

Several publications did, however, refer to a commercial supply of DSMA from either Pfaltz and Bauer (11-14) or Carlo Erba (15-17). Some other methyl arsenic sources cited include, the BCR Measurement and Testing Programme (18-19), Ansul Corporation (20), Quimilado (21), Aldrich (22), Vineland Chemical Company (23), Battelle NW (24), and Chem Service (25).

Initially the Aldrich, Carlo Erba and Pfaltz and Bauer catalogues were consulted, but none were found to carry monomethyl arsenic compounds. DSMA was found in an old Alfa catalogue, however the company advised that they no longer supplied the product. The BCR Measurement and Testing Programme, although cited as a methyl arsenic source by some European groups, does not have this standard commercially available. Both Ansul and Vineland Chemical Company are no longer trading.

A search was instigated through Global Market Services, however this also failed to reveal a commercial supplier. After a delay, Carlo Erba was contacted *via* a local agent and it was found that they did still make DSMA, but were not prepared to supply quantities under 50 kg. They later agreed to supply 5 kg, but at a prohibitively expensive price ($395 kg^{-1}).

At the same time, several authors currently publishing work on arsenic speciation were contacted to determine whether they knew of any commercial methyl arsenic source. As suggested by the literature survey, most authors had not bought a methyl arsenic standard for quite some time or had received a donation from another research group. Dr Alan Howard, University of Southampton, and Professor Bill Cullen, University of British Columbia, though, suggested that Chem Service (West Chester, PA, USA) was a current supplier.

Another possible source of methyl arsenic compounds is agricultural herbicides [4], which are more commonly used for the control of annual grass species [5].

At this point there were three approaches to obtaining a methyl arsenic standard. The first was to buy the commercially available salt, second to synthesise the compound, and the third to prepare methyl arsenic from a herbicide grade material.

2.3.1a Commercial sources

Alltech, the Australian agent for Chem Service, advised that the methyl arsenic standard was DSMA which had been purified from technical grade pesticide material, although would not specify how this was done. The standard specifications recommended that it was suitable for analytical use and had a guaranteed purity of 98%, for total arsenic determination. No specifications were available regarding possible inorganic arsenic impurities in the product.

2.3.1b Synthesis via the Meyer reaction

Sodium salts of methylarsonic acid are easily synthesised *via* the Meyer reaction and several references to this can be found in the literature and synthetic organic chemistry texts [6-8]. The synthesis involves the addition of sodium arsenite to a methyl halide. The reaction is allowed to proceed for a day in a closed vessel and following this monomethyl arsonic acid can be isolated *via* the sodium salt. Preparations of methyl arsenic compounds by this reaction mechanism may contain a number of impurities, including the sodium halide salt, unreacted sodium arsenite and, if the reaction proceeds

too far, dimethyl arsenic compounds. Therefore, any methyl arsenic compound synthesised would require purification before it would be suitable for use as a standard.

2.3.1c Purification of herbicide material

A herbicide methyl arsenic source, was available in the laboratory. Methar Paspalum Killer, henceforth referred to by it's tradename 'Methar', contains DSMA but required purification as it was 45% NaCl, and also contained some insoluble sand-like material. Methar was supplied by Colin Campbell (Chemicals) Pty Ltd. (NSW Australia), but is no longer available as the company has ceased trading. Therefore, before embarking on a purification procedure, a further source of impure material needed to be identified, to avoid running out of starting material while developing the purification method.

Through the National Registration Authority (Canberra, Australia), four companies were identified in Australia as selling products containing MSMA (see Table 2.1).

Table 2.1 Australian sources of monomethyl arsenic herbicides

Product Name	Applicant	Address
Agchem Daconate Post-Emergence Herbicide	ICI Crop Care Aust. P/L	PO Box 167, Hamilton Central, QLD 4007
Rhone-Poulenc MSMA Herbicide	Rhone-Poulenc Rural Aust. P/L	Private Bag 19, Baulkham Hills, NSW 2153
Barmac MSMA Herbicide	Barmac Industries P/L	14 Annie St, Rocklea, QLD 4106
ISK Daconate Post Emergence Herbicide	ISK Oceania P/L	5 Hamilton St, Gisborne, VIC 3437

Both ICI Crop Care and Rhone Poulenc were approached for samples. Rhone Poulenc advised that they no longer sold any MSMA containing products, however ICI Crop Care were able to send a 500 mL sample of 58.2% (w/w) MSMA.

2.3.1d Conclusions

Therefore, the commercially available methyl arsenic standard from Chem Service was ordered. However, because it was suspected this standard might contain impurities, while waiting for it to arrive, Methar was purified for comparison, as described in section 2.3.2.

2.3.2 Purification of MMA

2.3.2a Introduction

The only method found in the literature referring to bulk purification of MMA was that of Dietz and Perez [3]. This method uses column chromatography with a strong acid cation exchange resin and water eluent for the purification of both methylarsonic acid and dimethylarsinic acid and claims to give 100.0% pure reference samples of both these acids.

An ion exclusion mechanism was expected for the separation of a relatively strong acid (As(V) pKa_1 2.2) from a weak acid (MMA, pKa_1 3.6) on a strong cation exchange resin. In fact, it would be expected that arsenic species would elute from the column in the sequence As(V), MMA, DMA (pKa_1 6.2) then As(III) (pKa_1 ~9), but the authors found that this was not the case. As expected, they found that As(V) behaved like a completely ionised solute (e.g. HCl) and was excluded from the resin particles, meanwhile, As(III) behaved like a neutral species (e.g. methanol) which can penetrate the pores of the resin matrix. The retention of MMA and DMA, however, did not fit in between these two as predicted. Instead, MMA and DMA were much more strongly retained than either of the inorganic species, prompting the suggestion that the amphoteric nature of MMA and DMA allowed them to act as bases toward the resin. As MMA is less basic than DMA this also explains why the resin retains DMA more strongly than MMA. The possibility of non-polar attraction of the methyl groups to the hydrocarbon matrix of the resin is excluded as a possible mechanism.

This method was designed for use with relatively clean samples of MMA and was modified to include some preliminary steps for cleaning up the herbicide material Methar.

2.3.2b Experimental

i) Preparation of the column

As described by Dietz and Perez [3], before packing the column, 200 g of the resin (Dowex 50W-X8 (100-200 mesh H^+ form), BioRAD, Australia), was purified by successive washings with: Milli-Q water, 6 M HCl, Milli-Q water, methanol, Milli-Q water, 6 M HCl and Milli-Q water.

Following this the resin was slurry packed into a 32 (i.d.) x 500-mm glass column in preparation for separation of the semi-purified Methar.

ii) Preliminary purification of Methar

The technical grade herbicide, Methar, was a mixture of 55% DSMA and 45% NaCl, but also contained some insoluble material. Therefore, a solution of the herbicide could not be added straight onto the column, and required some additional purification steps. The best approach was to prepare a bulk amount of semi-purified Methar, however, the description below refers to the preparation of ~5 g, which was the maximum amount that could be purified on the column.

About 11 g of Methar was dissolved in 20 mL of Milli-Q water and the solution filtered through a 0.45 μm filter to remove all the particulate material. The resulting solution was yellow in colour, rather than clear, indicating that there were contaminants other than NaCl and sodium arsenite in the solution. Further purification was achieved by precipitation of the DSMA by addition of acetone, redissolving the crystals in water and allowing the solution to stir overnight with activated charcoal. The solution was then filtered to remove the charcoal and DSMA precipitated again (note that the crystals are often very fine and best retained on a 0.5 μm Teflon filter). The activated charcoal procedure was repeated if necessary.

This procedure gave reasonably pure technical grade material which was used in further purification steps, however the appearance of the crystals and their melting point indicated a lack of purity, probably because NaCl was present.

iii) Column chromatography

Five grams of semi-purified Methar was dissolved in 12 mL of Milli-Q water. Bromine water (0.5 N) [9] was added drop-wise until a perceptible yellow colour persisted, indicating the complete oxidation of all As(III) to As(V). Following oxidation, the solution was gently heated to remove any excess bromine.

Once cool, the solution was run onto the column — note that the colour of the resin changed from green to yellow. This was followed by washing with several small volumes of water, taking care not to disturb the resin. The column was filled with water and eluted at 2.8 mL min^{-1}. The following fractions were collected: 0-100 mL, 100-200 mL, 200-250 mL, 250-300 mL, 300-350 mL, 350-400 mL and 400-450 mL.

Water was removed from each fraction by rotary evaporation, however MMA crystals were only found in the 200-250 mL, 250-300 mL and 300-350 mL fractions. The crystals were removed from the round bottom flask with acetone, the fractions combined and the crystals ground with acetone in a mortar and pestle. They were then

filtered through a 0.5 µm Teflon filter and oven dried overnight at 85 °C. Approximately 3 g of MAA was obtained following column purification.

2.3.3 Characterisation of MMA standards

After the purification process, there were four different methyl arsenic samples to be characterised. These were, a sample of semi-purified Methar (Methar), a preparation from the column chromatography procedure (P1) and the Chem Service commercial standard (Chem Service-old). After initial investigations, however it was clear that there was a problem with the purity of the Chem Service sample, and a replacement from another batch (Chem Service-new) was received from the supplier.

Methar and the two commercial standards from Chem Service contained arsenic as DSMA. No molecular weight was supplied by Chem Service, but it was assumed to be 292.03 (DSMA.$6H_2O$). The column purified preparation (P1) was assumed to be MAA which had a molecular weight of 139.970.

2.3.3a Total arsenic determination by flame AAS

Initially the methyl arsenic samples were characterised by determining their total arsenic content with flame AAS, using As(III) as a primary standard. The results for repeated analyses are shown in the following table. Percentage purity was calculated, based on the measured and expected arsenic concentrations.

Table 2.2 Purity of MMA standards determined by total arsenic analysis using flame AAS

	Methar	P1 Preparation	Chem Service-old	Chem Service-new
AAS-1	93.3%	96.8%	121.2%	
AAS-2	92%	101%	129%	101%
AAS-3		98.98%		
AAS-4		100.26%		

The results from AAS-1 represent initial measurements of total arsenic concentrations calculated from a calibration curve. Because the AAS method suffered from considerable instrument drift, these values should be regarded as approximations. The AAS-2 results were obtained using bracketing standards, recalibrated after every third sample, and represent a more accurate determination of concentration than AAS-1. The Chem Service-old sample is an exception though, as its concentration was outside the range spanned by the standards.

Two further total arsenic determinations were made on sample P1 by flame AAS. AAS-3 and AAS-4 were carried out on separately prepared standard solutions three months apart. In each case the instrument was recalibrated after every sample, and each sample was analysed four times.

Total arsenic determinations were also performed by ICP-OES for comparison with flame AAS. The results indicated the same trends as found by AAS, however no internal standard was used to calibrate for instrument drift, so the accuracy of these results is poor. This was confirmed by reanalysing the As(III) standards at the end of each run. Using ICP-OES, the concentrations were found to vary by up to 10%, compared with 0.3% for flame AAS.

Table 2.2 shows that Methar had a total arsenic concentration of ~93%, indicating the presence of impurities, probably NaCl, as was discussed earlier. From this initial analysis Methar was discarded as being a possible analytical standard and was not characterised any further, except to screen for inorganic arsenic contamination.

Results for the Chem Service-old sample suggest that its purity is ~125%. This does not match the specifications for this product, which the manufacturer claims is 98.0 ± 0.5%, for total arsenic determination. Purity of 98% is only achieved if the molecular mass for DSMA.$3H_2O$, rather than DSMA.$6H_2O$ is used when calculating the expected concentration. Thermal analysis (section 2.3.3b), however, showed that the compound was hexahydrate. More information was requested from the manufacturer, but they simply supplied a new sample (Chem Service-new), which was found to have a purity of around 100% for total arsenic determination, therefore confirming a contamination problem with the original standard.

2.3.3b Thermogravimetric analysis

Simultaneous differential thermal (DTA) and (TGA) thermogravimetric analyses were recorded (Fig. 2.1 & 2.2) for the P1 preparation and the Chem Service samples between 25 and 500 °C.

AAS analysis of the P1 preparation indicated that it had a high purity, around 100%. This is confirmed by differential thermal analysis (DTA) (Fig. 2.1a) as there is a sharp peak (characteristic of a pure compound) representing the melting point at ~160 °C. This is followed by a second peak which indicates the onset of a two-stage mass loss. The mass losses are shown in the TGA trace (Fig. 2.2a) and indicate that complete decomposition of the sample has occurred. The first stage of mass loss represents an

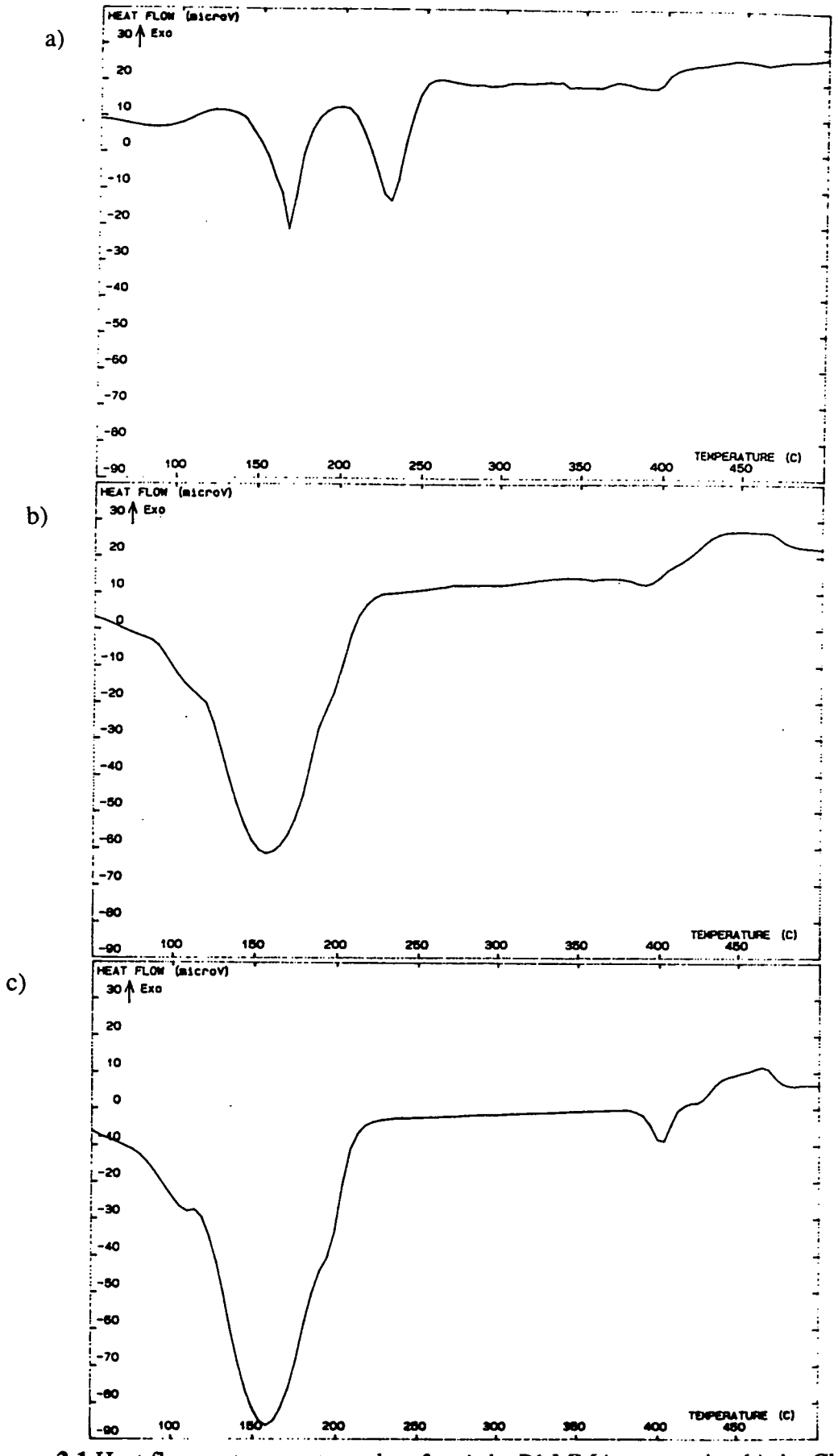

Figure 2.1 Heat flow vs temperature plots for a) the P1 MMA preparation b) the Chem Service-old sample and c) the Chem Service-new sample

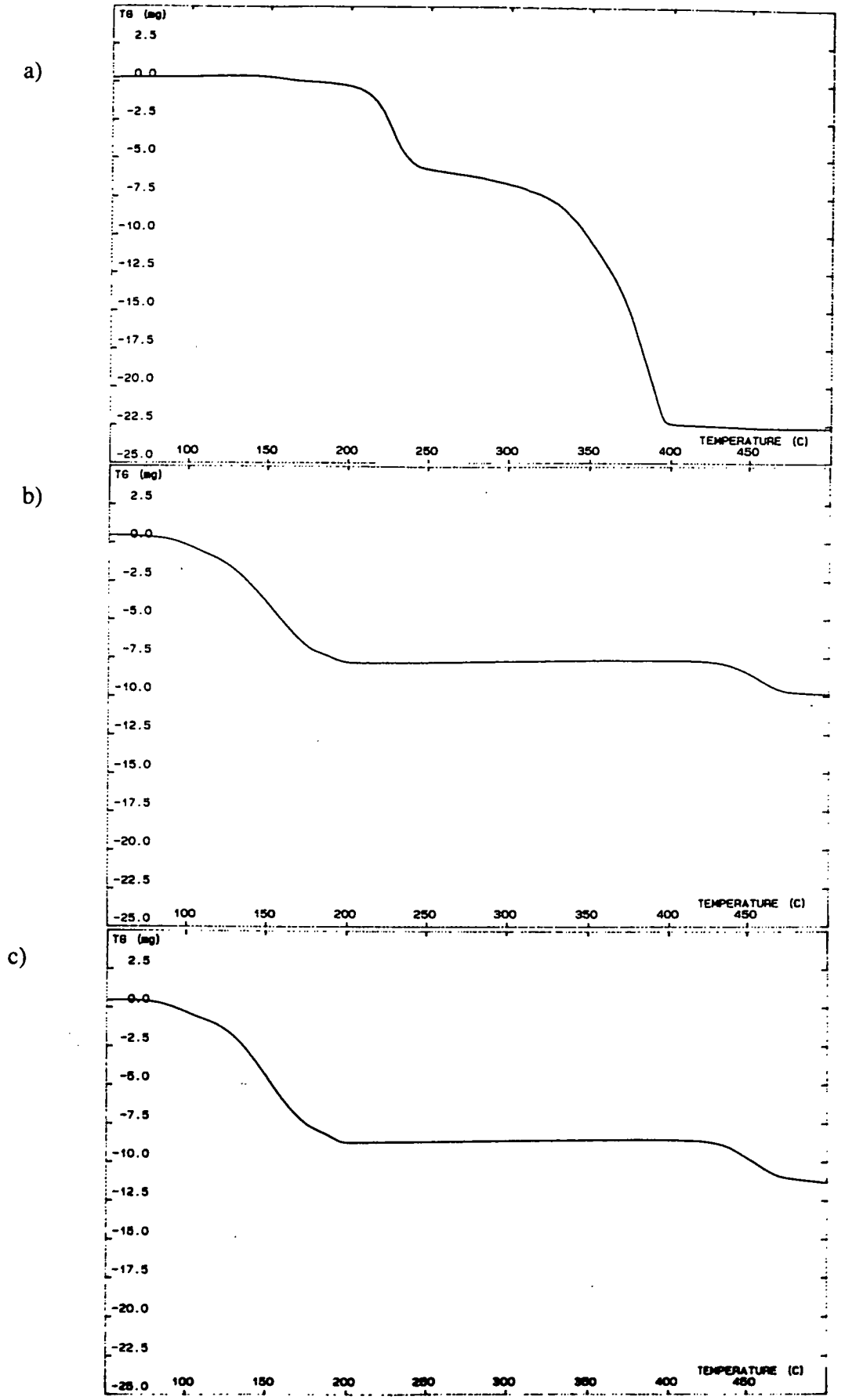

Figure 2.2 Mass loss vs temperature plots for a) the P1 MMA preparation b) the Chem Service-old sample and c) the Chem Service-new sample

equivalent weight of ~36 and the second, ~104. From these two fragments, it is difficult to determine how the decomposition proceeds, however arsenic must be lost in the second stage.

From this data it can be inferred that there is no water of hydration associated with the compound and, because there is no residual mass, that no sodium is present in the sample.

DTA traces for the Chem Service-old and Chem Service-new samples (Fig. 2.1b & 2.1c) both show a very broad peak, with several shoulders, at about the expected melting point (132-139 °C). In the TGA traces (Fig. 2.2b & 2.2c) a large mass loss is observed in the range 100-150 °C. This mass loss corresponds to the equivalent weight of six water molecules, confirming that the original compounds were hexahydrate. The broadness of the peak in the DTA trace is attributed to the step-wise loss of water from the compound. Some small peaks are evident in the DTA traces between 400 and 450 °C, and represent an ~10% mass loss in the TGA trace. This is thought to be caused by partial decomposition of the DSMA, with the mass loss representing an equivalent weight of ~15 or one methyl group.

The Chem Service-old and new TGA and DTA traces are not significantly different from one another, and could not be used to detect any impurity in the Chem service-old sample.

2.3.3c Arsenic species determination by HG-AFS and carbon analysis

All of the MMA standards were screened by HG-AFS to compare the amount of inorganic arsenic present in each preparation. The amount of inorganic arsenic was about the same in all preparations, but was lower in the Chem Service-new and P1 preparation than in the Chem Service-old and Methar samples. Note, however that although the Methar sample had a low purity in terms of total arsenic concentration, the improvement in the inorganic arsenic to MMA ratio following column purification was small for the amount of effort required. It appeared that the major improvement in purity following the column procedure was the removal of excess sodium chloride from the sample.

The MAA produced by the column chromatography procedure had a purity that was comparable to that of the commercially available standard and it was chosen as the standard for analytical work. This was because we had a greater quantity of this

standard and also, we could avoid having to use different batches of the commercially available standard, which had been shown to vary in purity.

Quantification of inorganic arsenic in the P1 preparation was undertaken using HG-AFS. These results indicated that 6.3% of the total arsenic in the sample was present as inorganic arsenic.

This result was confirmed by a carbon analysis on the P1 preparation which found the sample to contain 7.9% C compared with the expected value of 8.6%. Hydrogen analysis was also carried out, but because of the small amount of hydrogen in the sample and the problems associated with contamination by water these results could not be used. From the carbon analysis (using the total inorganic arsenic concentration as 100%), the concentration of methyl arsenic in the sample is calculated to be 92.4%. This is in agreement with the measurements made by HG-AFS.

2.3.4 Discussion

As discussed in the section 2.3.3, in this work the method of Dietz and Perez [3] for purification of methylarsonic acid, was found most effective for removing NaCl from the sample, rather than separation of inorganic and methyl arsenic species.

There are a number of possible reasons why the purification is apparently more effective in the original paper. The most likely explanation is that the detection limit of the analytical methods used to determine impurities is not as low as HG-AFS and is, therefore, unable to pick up low concentrations of inorganic arsenic. Therefore, the column chromatography may not be as efficient at separating the inorganic and methyl arsenic as described in the paper. This is entirely possible as ion exclusion chromatography is characterised by poor peak shapes (peak fronting), particularly when water is used as an eluent. It could then be expected that peak fronting would be exacerbated when using such a large column so as to cause overlap of the inorganic and methyl arsenic peaks. The presence of high NaCl concentrations in the semi-purified sample added to the column, may also have contributed to poor peak resolution.

A second possible problem may be that we added methyl arsenic to the column as DSMA (i.e. a fully dissociated species) while Dietz and Perez add it to the column as MAA, claiming that it acts as a neutral or even a positively charged species. While it is clear that DSMA exchanges sodium for protons on the column, it is uncertain how it would be protonated to acquire a positive charge, allowing it to be retained by the column longer than any neutral species. It seems that this would be difficult unless the

pH is less than *ca.* 1.5 (pKa DMA^+ = 1.57 [6]) at which point, As(V) would be expected to act as a neutral species (pKa_1 = 2.2 [10, 11]), which, according to the authors, it does not.

It is possible, however, that improvements in the separation of inorganic arsenic from methyl arsenic may be achieved by converting the methyl arsonate salt to the acid before addition to the column. Also improved separations may be achieved by using a better eluent, either a buffer or low concentration acid.

In conclusion, the purity of the P1 preparation is comparable to that of the commercially available Chem Service standard. Because of the amount of material prepared, the P1 material was chosen for use as an analytical standard, following characterisation of its inorganic arsenic content.

2.4 SUMMARY

A table summarising the purity of the standards chosen for use in all further arsenic analyses is shown below:

Table 2.3 Concentrations of impurities in the characterised arsenic standards

	As(III) Standard	As(V) Standard	MMA Standard	DMA Standard
Inorganic As content	100% (As(III))	100% (As(V))	6.3%	1.1%
MMA content			93.7%	14.2%
DMA content				84.7%

Reliable standards for the determination of inorganic arsenic species, As(III) and As(V), were easily sourced from chemical suppliers.

This was not the case for MMA or DMA, despite analytical standards for both these arsenic species being commercially available. Although the manufacturers claimed that these materials were of a high purity, this was based on total arsenic analyses, which did not take into account impurities of other arsenic species.

A standard for MMA was prepared by purification of a technical grade herbicide containing DSMA. Following purification, the material still contained some inorganic arsenic that was quantified using HG-AFS and carbon analysis. Inorganic arsenic was found to account for 6.3% of the total arsenic content, and no DMA was observed.

Cacodylic acid, from a commercial supplier, was chosen as the standard for DMA analyses. This material was found to contain impurities of both inorganic and methyl arsenic. An attempt was made at purification using column chromatography, however this was unsuccessful. Therefore, the standard was used as supplied, but the impurities quantified. Using HG-AFS, the DMA standard was found to contain 1.1% inorganic arsenic and 14.2% monomethyl arsenic, with the previously characterised MMA standard used to determine methyl arsenic in the DMA standard.

The proportion of MMA to DMA in the DMA standard is similar to that observed in natural waters. Therefore, in all subsequent analyses, the DMA standard was used for quantification of both MMA and DMA.

Finally, it is recommended that great care be taken when doing the MMA purification in the same laboratory as the analytical work. MMA seems to be difficult to remove from the surfaces of laboratory equipment and causes subsequent contamination problems, evident as a persistent MMA blank in the HG-AFS chromatogram.

Table 2.3 Literature survey of MMA standard sources

	Reference	MMA source	Supplier	Comment
1	[12-14]		Stock solution obtained from Dr J S Thayer, Department of Chemistry, University of Cincinnati	
2	[15]	DSMA.$6H_2O$	Dr K J Irgolic, Texas A & M University, USA	99% pure
3	[16]	DSMA.$6H_2O$	Dr A G Howard, University of Southampton, UK	
4	[17]	Monomethyl-arsonic acid	Gift from Dr K J Irgolic Karl-Franzens University of Graz, Austria	
5	[2]	Not stated	Synthesised in authors laboratory – unpublished data	Trace impurities (<5%) of inorganic arsenic found
6	[18]	DSMA.$6H_2O$	Synthesised by Quick's method [8]	
7	[19, 20]	Methylarsonic acid sodium salt	Chinoin, Budapest	Obtained from medical grade strychrotonin solution. Not stated if the mono- or di-sodium salt was used.
8	[21]	Methylarsine dibromide	Alfa	Soluble in benzene and toluene but aliquots are dissolved in water to prepare standards

9	[22]	Methylarsine dibromide	Not stated	Prepared by hydrolysis of methylarsine dibromide to give MAA solution. Purity confirmed by chromatography and AAS
10	[23]	Methylarsine dibromide	Alfa	Prepared by oxidation of methylarsine dibromide
11	[24-27]	DSMA.$6H_2O$	Not stated	
12	[28]	DSMA.$6H_2O$	Pfaltz & Bauer	
13	[29]	DSMA.$6H_2O$	Pfaltz & Bauer via Phase Separations and ICN Pharmaceuticals	
14	[1]	Not stated	Pfaltz & Bauer	Methyl arsenic salts are generally not very pure or stoichiometric
15	[30, 31]	DSMA.$6H_2O$	Carlo Erba, Italy	
16	[30, 31]	DSMA.$6H_2O$	Carlo Erba, Italy	
17	[32-37]	DSMA.$6H_2O$	Carlo Erba, Italy	
18	[38]	Not stated	BCR Measurement and Testing Programme	
19	[39, 40]	DSMA.$6H_2O$	BCR Measurement and Testing Programme	
20	[41]	Monomethyl-arsonic acid	Ansul Corporation, Texas	99.9% pure
21	[42]	Monomethyl-arsenic acid	Quimilado	99.4%
22	[43]	MMAA	Aldrich, USA	Improbable that it was obtained from this source

23	[44]	Monomethyl arsinic(?) acid	Vineland Chemical Co., USA	
24	[45]	Sodium monomethylarsonate	Battelle NW, Sequim, WA	Mono- or di- sodium salt not stated
25	[46]	DSMA.$6H_2O$	Chem Service, USA	
26	[47]	Sodium salt of monomethyl arsonic acid	No stated	Mono- or di- sodium salt not stated

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Chapter 3: Development of a Hydride Generation Atomic Fluorescence Method

3.1 INTRODUCTION

In general, analysis for dissolved and particulate arsenic is not carried out in the field and samples are usually preserved for later analysis. For shipboard samples, the time elapsed between sampling and analysis may be extensive and although it has been reported that As(V), MMA and DMA are stable for many months when acidified and stored at 4 °C [1], this may not always be the case for surface waters. Cutter [2] has observed decreases in the concentration of MMA and DMA with time in some stored surface water samples from the Atlantic Ocean.

As(III) is a less stable species which can be oxidised rapidly after sampling. Differing opinions exist regarding the rate of oxidation of As(III) and on whether samples should be analysed immediately after collection or can be satisfactorily preserved [3-9]. Several means of preserving As(III) speciation can be found in the literature. However, they are not applicable to shipboard studies as they are not appropriate for the low concentrations involved (e.g. solid phase extraction [10-18]), are unnecessarily time consuming and complex (e.g. solvent extraction [19-24], flotation [25], coprecipitation [26-28]), or, where cryogenic preservation is employed (e.g. quick freezing and in-field hydride generation [29]), require transport and storage of large volumes of liquid nitrogen. Michel *et al.* [30] and Cutter and Cutter [31] both report the use of shipboard methods as a means of avoiding changes in speciation.

To avoid the problem of sample preservation, a method for the shipboard analysis of arsenic species was developed. The method design was based on a number of factors including, the need for a system which was compact, portable, met the safety requirements for shipboard use, was automated for ease of use in difficult conditions, and had the sensitivity required for analysis of open ocean samples.

This chapter reviews the methods for determining arsenic speciation and then describes the two-stage development of a hydride generation atomic fluorescence spectroscopy method for the shipboard determination of As(III), As(V), MMA and DMA. In the first stage a manual HG-AFS method was developed, optimised and validated in the laboratory. In the second stage the system was semi-automated and modified for shipboard use.

3.2 LITERATURE REVIEW

The scope of this review is to detail the variety of methods used for determining arsenic speciation. In this case, speciation refers to the arsenic species, As(III), As(V), MMA and DMA, however, methods which also determine the non-hydride forming organoarsenic species, particularly arsenobetaine and arsenocholine, are included as well. Methods which determine total arsenic, or As(III) and As(V) only are not included. The suitability of each of the techniques reviewed to the shipboard analysis of arsenic species in seawater samples will be assessed.

Although the determination of non-hydride forming organoarsenic species in natural waters was of interest to this project, current attempts at developing methods for these species are highly complex [32-34], and were beyond the scope of this investigation.

Further information regarding methods for determining arsenic speciation can be found in a recent review by Burguera and Burguera [35].

3.2.1 Hydride generation

Almost all the methods reviewed incorporate a hydride generation step in the analytical procedure. As(III), As(V), MMA and DMA all readily form volatile hydrides, which are shown in Table 3.1. As(III) and As(V) both form the same hydride, arsine, however they can still be differentiated from one another, either by separation prior to hydride generation or by manipulation of the pH at which the hydride is generated. Other organoarsenic species, such as arsenobetaine and arsenocholine, are not hydride-forming, but may be determined using hydride generation, by including a UV or microwave oxidation step, to first break them down into hydride forming species.

Table 3.1 Arsenic hydrides and their boiling points

Arsenic Species	Hydride Formed	Hydride Boiling Point (°C)
As(III)	Arsine (AsH_3)	-55 [36]
As(V)	Arsine (AsH_3)	-55 [36]
MMA	Monomethyl arsine (CH_3AsH_2)	2 [36]
DMA	Dimethyl arsine [$(CH_3)_2AsH$]	35.6 [36]

Early hydride generation methods used a zinc-acid reduction for the generation of arsines [37], however this method often had large blanks [38] and was generally cumbersome [39]. More recently, reduction with $NaBH_4$ has been used almost exclusively, as it tends to be a faster reaction and is more easily automated [40]. Initially, $NaBH_4$ was added to the sample solutions as a solid, but later, freshly prepared solutions were found to give higher hydride formation efficiencies, and also allowed the analytical method to be automated more readily [41].

There are several advantages to using a hydride-generation step in the analytical procedure. From Table 3.1 it can be seen that the arsenic hydrides are volatile and therefore easily separated from the original sample matrix. This allows high efficiency introduction of the analyte to the atomizer, and the simple matrix avoids most of the spectral interferences common to liquid sample introduction. Moreover, the hydrides undergo catalytic decomposition at relatively low temperatures [42], and even in relatively cool flames, have a high atomization efficiency. This is advantageous because in atomic spectrometry the useful resonance lines for arsenic are below 200 nm, where there is considerable background absorbance from higher temperature flames [39] — especially when using atomic absorption detection.

Hydride generation is subject to interferences from high concentrations of other hydride forming elements (e.g. Bi, Ge, Pb, Sb, Se, Sn, Te) and transition metal ions [42]. However, these effects are not expected to be significant in unpolluted coastal and open ocean seawater.

There has been much less focus on the potential for interference from organic matter (pollutants or humic material) during hydride generation [43]. It has been shown that organic material in natural water interferes with the release of selenium hydrides from solution [41], while a variety of organics, including humic material, added during organotin analysis was shown to only effect reproducibility [44]. No detailed studies can be found in the literature describing the effect of organic material on the analysis of arsenic species, therefore organics should be considered as potential interferents prior to embarking on studies in natural waters.

To enable speciation, the arsenic species of interest must be separated before hydride generation, or the hydrides separated after their generation. Chromatography or capillary electrophoresis has been used for the former, while cold trapping the arsines followed by differential thermal desorption, has been used for the latter.

3.2.1a Continuous flow hydride-generation systems

Analytical methods employing continuous flow hydride generation are characterised by the continuous addition of both sample and $NaBH_4$ solution [41]. They are the most commonly used because they are simple to interface to detection systems and are easily automated. The ways in which arsenic speciation can be determined using continuous flow systems are discussed in the sections below.

i) Chromatographic separation

A popular approach to pre hydride-generation separation of the arsenic species is to use HPLC, most commonly anion exchange chromatography which exploits the different pKa values of the arsenic species, shown in Table 3.2.

The advantages of using chromatographic separation are the ability to determine non-hydride forming arsenic species (if an oxidation step is included prior to hydride generation), and the possibility of separating the arsenic species from interferents in the sample matrix prior to hydride generation. This is of particular interest when analysing samples containing high levels of transition metals which otherwise interfere with hydride generation.

Table 3.2 Dissociation constants for arsenic species

Arsenic Species	Dissociation Constant	Reference
Arsenite/As(III)	$pKa_1 = 9.21$	[45]
Arsenate/As(V)	$pKa_1 = 2.2$	[45, 46]
	$pKa_2 = 6.9$	
	$pKa_3 = 11.6$	
Methylarsonic acid/MMA*	$pKa_1 = 2.6\text{-}4.58$	[47-49]
	$pKa_2 = 7.8\text{-}8.2$	
Dimethylarsinic acid/DMA	$pKa_1 = 6.19$	[47]
Arsenobetaine	$pKa = 2.18$	[50]
Arsenocholine	Cationic	[50]

* pKa values quoted in the literature for MMA vary considerably

The problems associated with these methods though include, an intolerance of high chloride concentrations, sometimes quite slow separations, and when trying to determine all the species listed in Table 3.2, difficulty in getting sufficient peak resolution to determine all species in a single run. As chromatographic methods can only use small sample volumes, they often have relatively poor detection limits.

In general, these systems are most suited for determining the higher concentrations of arsenic species found in biological matrices, such as urine and seafood, rather than seawater.

ii) Electrophoretic separations

More recently capillary electrophoresis (CE) has been used as a pre hydride-generation separation method for continuous flow systems. Separations with CE are based on electrophoretic mobility and are often quite efficient when compared with IC. CE uses very small sample volumes compared with chromatography though, making it less sensitive and more difficult to interface. In addition, high ionic strength matrices, such as seawater, require long separation times to avoid overheating the capillary.

iii) pH selective hydride-generation

pH selective hydride generation is a direct method of determining arsenic speciation with continuous flow systems. Instead of employing a separation step prior to hydride generation, the sample is mixed with different pH reaction media to achieve selective generation of the different arsines. This method is faster than chromatographic separations and free from the interferences of a seawater matrix, but cannot determine non-hydride forming organoarsenic species. It also requires several analyses of the same sample to determine all the arsenic species of interest, and often some species have to be determined by difference.

3.2.1b Batch hydride generation

Batch hydride generation systems differ from continuous flow systems in that $NaBH_4$ is usually added to a sample aliquot, and then the hydride generated is trapped in one of a variety of ways [40, 41], rather than flowing straight to the detector. This enables the hydrides to be preconcentrated and results in improved detection limits. A U-shaped trap cooled in liquid nitrogen is often used to trap the arsines. Warming the trap allows them to be separated by boiling point, thereby allowing not only preconcentration, but also speciation of the arsines. Although the arsines separate primarily on the basis of their boiling point, the use of an inert packing loaded with a non polar stationary phase is shown to improve peak resolution and reproducibility [51].

Hydride generation is typically carried out at pH 1-2, after addition of acid to the sample. At this pH, both arsenate and arsenite are reduced to arsine, and so total inorganic arsenic is determined. At near neutral pH, however, only arsenite is reduced to

arsine, allowing arsenate to be determined by difference [1]. MMA and DMA both form their respective arsines at pH 1-2.

Batch hydride generation systems have a number of advantages over continuous flow methods. They are capable of using relatively large sample volumes (up to 100 mL [1, 52]), and this, combined with hydride preconcentration, makes them extremely sensitive techniques for analysis of environmental samples, including seawater. The separation used is quite simple and therefore is often faster than methods using chromatography or CE.

One of the disadvantages of batch methods is that they are difficult to automate, and therefore are usually labour intensive. In addition, they are often less tolerant of interferents in the sample matrix [41] than continuous flow methods.

3.2.1c Electrochemical hydride generation

There are also a few reports of electrochemical arsine generation in the literature [53-59]. Electrochemical hydride generation is obviously desirable as it avoids the reagent blank and excess hydrogen production associated with chemical arsine generation. However, initial indications are that only As(III) is hydride forming under these conditions.

Further details on all aspects of hydride generation can be found in a number of papers and reviews [39-43, 51, 60-64].

3.2.2 Methods of detection

The following sections discuss the variety of detectors that have been coupled with the separation schemes described previously. In the methods reviewed the separation is almost always coupled to an element specific detector. This improves the method sensitivity, and is essential for methods determining trace levels of arsenic species.

3.2.2a Detection using AAS

AAS is the detector most commonly coupled to hydride generation, partly because it is simple to interface but also, it is a low-cost detector which is readily available in most laboratories. In addition, AAS has some advantages over more sensitive methods using ICP detectors, including a robust flame that is not subject to spectral interferences from chloride in the sample matrix and is not extinguished by gas fluxes.

i) Continuous flow hydride-generation AAS methods

HPLC-HG-AAS methods

As mentioned in section 3.2.1a, the most commonly used HPLC approach to separating the arsenic species is anion exchange ion chromatography. In typical systems the sample (100 – 500 μL) is injected onto a polymeric or silica strong anion exchange column and eluted with a phosphate buffer. The column effluent is then acidified and mixed with $NaBH_4$, before passing through a gas-liquid separator and being detected by AAS at 193.7 nm in a heated quartz cell. Typical separation times for these systems vary between 6 – 20 minutes, however the actual analysis time may be longer, as some methods require reconditioning of the column after each sample [65], particularly where a difficult matrix is involved. Detection limits vary considerably, depending on the experimental conditions, but are in the order of 200 – 9000 ng L^{-1}. In many cases the detection limits for the four hydride forming species are sacrificed by the need to also separate arsenobetaine and arsenocholine in a single run. Often this is simply not possible as both As(III) and arsenobetaine co-elute in the void volume. However, many published methods manage to avoid this problem by using a microwave [66], UV [67, 68] or thermo-oxidation [69] step to convert arsenobetaine to inorganic arsenic. In this case, two runs are required; where As(III) is determined in one run and arsenobetaine+As(III) in the second. Two authors, however, note that on-column oxidation of As(III) commonly occurs [70, 71], so that often it cannot be determined when using anion exchange chromatography. To improve separations a number of techniques have been employed and include: gradient elution [65, 70, 72, 73], the use of heated columns [65, 70], and binary acid mobile phases, including tartaric acid for the separation of As(III) and arsenobetaine [50].

Polymeric anion exchange columns are the most commonly used stationary phase as resin stability over a wide pH range allows greater flexibility in the selection of the mobile phase and therefore, improved peak resolution. Nevertheless, silica columns give adequate resolution when just the four hydride forming arsenic species are being determined, and have been shown to have a greater tolerance to high concentrations of electrolytes in the sample [74]. These columns also enable decreased retention times through the use of higher ionic strength mobile phases, without peak splitting. They are, however, less robust columns and cause the method performance to deteriorate more quickly.

The only two HPLC-HG-AAS methods applied to determining arsenic species in seawater are by López-Gonzálvez *et al.* [66, 71]. In their earlier paper [66], an anion exchange precolumn is used to separate arsenobetaine and arsenocholine from the hydride-forming arsenic species before they are separated on a polymeric anion exchange column using 17 nM phosphate buffer (pH 6.0). The method was applied to a seawater sample, but the sensitivity was too low to detect any of the arsenic species present, except, interestingly, arsenobetaine. This result was unable to be reproduced by the authors. In a later paper [71], a more sensitive method is presented which uses a high-capacity anion exchange precolumn, capable of preconcentrating up to 1 L of sample, prior to anion exchange separation. Nevertheless, the method still has insufficient sensitivity for natural waters, and the authors also note that the precolumn does not quantitatively retain arsenic when seawater samples are preconcentrated.

Ion interaction (or ion-pair) chromatography is often a more accessible technique than ion chromatography as it uses a lower cost C18 column for separation of the arsenic species. It also enables a different selectivity to anion exchange and allows separation of six arsenic species in a single run [75]. It tends to have poorer sensitivity than anion exchange chromatography, and is still subject to the interferences from samples with high chloride matrix [76], — including column overloading, peak splitting and peak broadening.

Le *et al.* [76] have indicated that anion exchange chromatography is the best method to use when determining only four arsenic species, but, they recommended the use of ion interaction chromatography when trying to separate more than four species in a single run. They used heptanesulfonic acid as the ion-interaction reagent and claimed better resolution and similar selectivity to anion exchange chromatography. In another study by Martín *et al.* [75], however, it was shown that compared with anion exchange, ion interaction chromatography gave poor peak resolution and also had detection limits which were ~50 times higher. A paper by Ko *et al.* [77] reported detection limits of 700 - 3000 ng L^{-1} and a 25 minute separation time for four arsenic species using an ion interaction HPLC-HG-AAS system with tetrabutyl ammonium phosphate.

Detection limits can be improved slightly for AAS detection using some of the suggestions in the paper by Howard and Hunt [67], including the use of an extended UV response photomultiplier, use of electrically heated furnaces for atomisation and the use of high power excitation sources, including boosted discharge hollow cathode lamps (HCL) and electrodeless discharge lamps (EDL). These do not boost the sensitivity of

the detector sufficiently to be able to determine seawater arsenic concentrations following a chromatographic separation though.

A more sensitive AAS technique is electrothermal vaporisation coupled with AAS (ET-AAS) detection, a method which often gives detection limits comparable with ICP-MS. In the case of arsenic analysis it has been shown to give a 10-fold improvement in the detection limit when compared with flame AAS detection [78].

In the literature, only a few examples of HPLC coupled to ET-AAS are given [78-82], because of the difficulties associated with the interface. In all cases it is used as an off-line detector to analyse collected chromatographic fractions, making it a laborious method with often intensive sample work up. The HPLC methods also require modification from those used for flame AAS detection as PO_4 buffers have been shown to suppress the signal, and matrix modifiers such as Zr, Pd and Ni are required to prevent loss of volatile arsenic species from the graphite tube.

Even with ET-AAS detection though, these methods lack sensitivity and the chromatography is still not robust enough to cope with seawater samples, particularly as the optimum separation conditions often require the use of low buffer concentrations.

pH selective HG-AAS methods

Only a few examples of pH-selective hydride generation are found in the literature for the determination of multiple arsenic species. One of the most comprehensive is a two-part study by Anderson *et al.* [83, 84], in which As(III), As(V), MMA and DMA were determined using a continuous flow HG-AAS system which had no reaction coil between the addition point for $NaBH_4$ and gas-liquid separator. The authors' stressed that the signal response obtained with different analytical systems using the same reaction media may vary, as reaction kinetics were often an important factor in the signal obtained. The reaction media tested include a variety of inorganic and organic acids, buffers, oxidising and reducing agents and masking agents. The final selection of reaction media to determine all four arsenic species was:

i) 5M HCl and 0.1% KI for As(III)+As(V),

ii) 0.4 M sodium citrate-citric acid buffer (pH 6.0) for As(III),

iii) 0.16 M acetic acid for As(III)+DMA

iv) 0.1 M mercaptoacetic acid for all four species (MMA determined by difference).

This method gave results that compared well with the determination of these arsenic species by batch hydride generation AAS. The time required for each analysis was approximately one minute, however, four analyses were required to determine each species. No matrix problems were reported for the analysis of seawater samples, but the detection limits (200 – 700 ng L^{-1}) are too high to determine these species in unpolluted seawater.

A similar four-step scheme is described by Rüde and Puchelt [85] for determining arsenic species by flow injection HG-AAS, in a system with a reaction coil. The reaction media used are as follows:

i) 4 M HCl for As(III)

ii) 0.165 M HCl + 1 mg L^{-1} $KMnO_4$ for MMA and DMA (1:1)

iii) 0.025 M HCl + 1 mg L^{-1} $KMnO_4$ for MMA and DMA (0.75:1)

iv) 0.85 M tartaric acid + 1 mg L^{-1} $KMnO_4$ all species

In step ii) of this reaction scheme the sum of MMA and DMA present in the sample is determined. Reaction iii) is also used to determine both MMA and DMA, but in this case the response of MMA is different to DMA, allowing the actual amount of MMA and DMA present to be estimated. As(V) must be calculated by difference
The detection limits for this method are in the range 200 – 500 ng L^{-1}.

Two papers by Torralba *et al.* [86, 87] describe pH-selective hydride generation using HCl, acetic acid and citrate buffers. This method does not achieve complete selectivity, but instead describes the choice and validation of multivariate calibration models to resolve the species. In this case the detection limits are 4000 – 5000 ng L^{-1}.

A recent paper by Bermejo-Barrera *et al.* [88] describes on-line coupling of ET-AAS and pH-selective hydride generation where the hydrides are trapped in situ in a Zr-coated graphite tube, resulting in a more sensitive method (detection limits 56 – 80 ng L^{-1}). The reaction media chosen in this case are:

i) 0.02 M thioglycollic & 1% $NaBH_4$ acid for all four species

ii) 4 M HCl & 2% $NaBH_4$ for As(III)+As(V)

iii) pH 5 citrate buffer & 0.2% $NaBH_4$ for As(III)

iv) 0.14 M acetic acid & 0.2% $NaBH_4$ for As(III)+DMA (As(V) and MMA determined by difference)

However, despite being coupled on-line to a highly sensitive detector, pH-selective hydride generation still did not have sufficient sensitivity for determining the low

concentrations of MMA, DMA and As(III) occurring in seawater. Nevertheless, it is sensitive enough to determine high methyl arsenic concentrations and As(V) in seawater.

ii) Batch hydride generation AAS methods

Most of the batch HG-AAS methods reported in the literature are based on one of the first reports of this method by Braman *et al.* [36], which uses a DC discharge cell and the arsenic atomic emission line at 228.81 nm for element-specific detection.

In this method, a sample (volume up to 70 mL), was placed in a glass reaction vessel. Potassium biphthalate was added to adjust the sample to pH 3.5-4.0. $NaBH_4$ (2 mL, 2% m/v at pH 1-1.5) was manually injected into the sample and the solution stripped with helium, sweeping the arsines onto a U-trap packed with glass beads (60/80 mesh) and cooled in liquid nitrogen. Following sample stripping, the liquid nitrogen was removed and the U-trap heated using a chromel wire wound around the glass surface. The trap was then cooled again, and oxalic acid added to the sample to adjust it to pH 1-1.5. Four aliquots of $NaBH_4$ (4 x 2 mL) were then injected into the sample at 30 second intervals, and the arsines stripped with helium for six minutes. Again the liquid nitrogen trap was removed and heated. The carrier flow initially passed through a NaOH trap to remove CO_2, which otherwise interfered with the detector. After the arsine peak, however, the flow was diverted to bypass the NaOH trap, otherwise losses of the methyl arsenic species occurred. Heating continued until water evaporated from the trap and quenched the dc discharge.

With this method the detection limits achieved were 4-20 ng L^{-1} of As for the four hydride forming arsenic species, and no interferences were observed for analysis in a seawater matrix.

Subsequent to this publication, numerous methods have been developed based on this hydride trapping technique but using flame or electrically heated quartz tubes with atomic absorption detection, allowing a more robust and sensitive system.

The first batch HG-AAS method was published by Andreae [89], using an air-acetylene heated quartz cuvette mounted in the beam of a hollow cathode AAS lamp. Other variations from the procedure by Braman *et al.* [36] included using TRIS buffer (pH 6.0) for As(III) determination and HCl to lower solution pH for As(V), MMA and DMA analysis. Also, the gas stream was passed through a CO_2-isopropanol bath to remove water vapour prior to hydride trapping. The detection limit for this system was

0.5 ng L^{-1} of As and the analysis time *ca.* 20 minutes per sample. The U-trap packing used in this case was silane-treated glass wool.

In a later publication [1], Andreae, replaces the original trap packing with 3% OV-3 Chromosorb W-AW DMCS (60/80 mesh) to improve peak resolution and recovery. The sensitivity of the detector was also improved by the use of flame in tube AAS and an EDL which also increased the linear range [1]. The detection limit for this method was ~3 ng L^{-1} for a 50-mL sample. Several other similar methods have been published by other groups more recently [90, 91].

To avoid the manual addition of reagents used in the methods of Andreae [1, 89] and Braman [36], Howard and co-workers [92-96] have published a number of papers based on the pumped addition of reagents with the aim of improving reproducibility.

In this slightly different approach [92], the sample was mixed on-line with acid and then $NaBH_4$. This was followed by gas-liquid separation and drying of the gas stream with NaOH pellets, after which the arsines were swept by the nitrogen carrier gas onto a U-trap packed with glass beads (40 mesh) cooled to liquid nitrogen temperature. When stripping was complete, the liquid nitrogen was removed and the trap allowed to warm to room temperature in ambient air. The arsines were then swept into an air-acetylene heated quartz tube for detection. $NaBH_4$ and HCl were pumped continuously through the volatilisation and detection of the arsines, to maintain an air-hydrogen flame at the ends of the quartz tube, improving sensitivity. The analysis time for this system was approximately 10 minutes, and because of the low sample volumes used (~1 mL) the detection limit was slightly higher (46 - 124 ng L^{-1}) than for the batch hydride generation methods mentioned previously.

Subsequent improvements to this method have been realised by Comber and Howard [93, 94], where the number of mixing coils in the system has been reduced, the $NaBH_4$ solution is stabilised with NaOH, HF-etched glass beads (40 mesh) are used as a trapping material and an electrically heated quartz tube is used for atomisation, leading to improvements in the sample throughput, precision, peak resolution and sensitivity.

Later papers by Howard and Salou [95, 96], indicated further improvements in the method by replacement of the glass beads used as trapping material, with the gas chromatographic packing 15% OV-3 on Chromosorb W-HP. In these papers, they also investigated the use of L-cysteine and thioglycollic acid as agents for reducing transition metal interferences and obtaining equivalent signals from each of the arsenic species.

As also observed by Le *et al.* [97], Howard and Salou [95] found that pretreating samples with L-cysteine was a way to reduce interferences caused by transition metals species in solution. In addition, the use of L-cysteine gave equivalent signals for all the arsenic species present, and reduced the amount of background noise and the arsenic blank, through the use of lower acid concentrations. Thioglycollic acid was found to have similar effect and be better for use in continuous flow systems, but had the problem of being quite toxic. The detection limits for this system were 57 - 98 ng L^{-1}, a little high for the analysis of methyl arsenic species in seawater.

van Elteren *et al.* [98] have published a method based on continuous flow addition of reagent and sample with cold trapping, similar to that of Howard *et al.*, but for the analysis of MMA and DMA only. The experimental set-up incorporated a Nafion dryer tube to remove water prior to hydride trapping and a stainless steel U-tube that allowed rapid heating of the trapped arsines. According to other authors [36, 52] this is not always advantageous as dimethyl arsine undergoes thermal decomposition if the trap temperature becomes too high. Interestingly in this paper, using radiotracer experiments, the authors found that DMA gave rise to two peaks. One peak represented 85% of the DMA present, and was followed by a later peak which formed either during hydride generation or in the U-tube. The authors suggested that this method was simple to automate, although details on how this was done were not provided.

A number of improvements to the batch-type method of Andreae, where reduction of the arsenic species occurred in a glass reaction vessel, have been presented in some more recent publications. A systematic study by van Cleuvenbergen *et al.* [99] of a method based on that by Andreae, resulted in a number of improvements. Firstly, the authors found that the use of 10% poly-*m*-phenylether on Chromosorb W AW DMCS (60/100 mesh) was superior to the use of other commonly used stationary phases, including OV-101, OV-3 and OV-225, in terms of recovery of the arsenic species. These authors also noted the observation made by Howard and Arbab-Zavar [92], that addition of hydrogen to the carrier stream improves the atomisation efficiency of the hydrides. No drying step was included in this method, as all the chemical drying agents used were found to interfere with the arsenic signal.

Ng *et al.* [52], have recently published a method for determining arsenic species in urine, based on the method of van Cleuvenbergen *et al.* [99]. Their paper supports the conclusions drawn in that publication, but also found that the removal of water from the U-trap after each analysis is essential. They also observed improved results for DMA

when the U-trap was allowed to warm in ambient air, rather then being heated by a Nichrome or chromel heating wire.

The method of Hasegawa *et al.* [22] is also similar to that published by Andreae, but uses a peristaltic pump to add $NaBH_4$ continuously to the sample during stripping. The detection limit for this procedure was 1 ng L^{-1}; a 10-fold improvement was also possible using solvent extraction to initially preconcentrate the sample.

A paper by Michel *et al.* [100], also indicated a number of improvements, including pumped addition of $NaBH_4$ to the sample over two minutes, similar to Hasegawa *et al.* [22]. In this paper the authors used H_2SO_4, rather than HCl, to acidify the sample, and claimed to obtain equal sensitivity for all the arsenic species as a result. Further improvements included the use of hydrogen as a carrier gas to increase the atomisation efficiency, and argon, also as a carrier, replacing helium to reduce the baseline noise. The method was automated to improve precision but the details of this were not given.

Recently, Burguera *et al.* [101] have described a novel, miniaturised automated method, which deviated from the usual cold trapping procedures as it used a microwave oven for volatilisation of the arsines. In this paper, a continuous flow procedure was used where sample was acidified and reacted with $NaBH_4$. The sample stream passes through a gas liquid separator and then "drierite" (to remove water) and NaOH traps (to remove CO_2 and any acid vapours), before cold trapping. In this system, the cryogenic trap consisted of 1 m of knotted PTFE tubing in a polystyrene tube that contained liquid nitrogen, suctioned in by negative pressure from an air pump. This was contained inside the microwave oven. When all the sample had reacted with $NaBH_4$, the microwave was activated. As PTFE, polystyrene and liquid nitrogen are all microwave transparent, only the arsines were volatilised.

Separation of the species did not occur as for normal trap warming where arsines separate on the basis of their boiling points. Instead, the extent of microwave heating was dependent mostly on their dielectric constant and the species separate in the order DMA, MMA, As(V). The system used a 10 mL sample volume and the detection limits were in the order 50 – 150 ng L^{-1}. The entire sequence was computer automated, but again the details of how this was achieved were not given.

In summary, there are a number of reasons why batch hydride generation AAS is an ideal method for the analysis of arsenic in seawater. Firstly, the batch method allows preconcentration from quite large volumes of seawater and therefore is highly sensitive.

Also, because the species of interest are removed from the seawater matrix, there is no chloride interference at the detector. Also, although the method is subject to interferences from transition metal ions, these are not expected to be a significant part of the matrix in the unpolluted coastal and open ocean seawater environment. One of the main disadvantages of these methods is their inability to determine non-hydride forming organoarsenic species, although there is yet to be any routine method developed for the identification and determination of these species in seawater. Many authors have claimed that the batch hydride generation methods are difficult to automate, however a number of papers suggest that this is possible [98, 100, 101].

3.2.2b Detection using ICP and MIP

More recently, hydride generation has been coupled with ICP and MIP detectors, but these instruments are generally more difficult to interface than AAS. This is particularly the case for determining speciation using batch hydride generation, where the fluctuations in gas flow rates are often sufficient to extinguish the plasma.

i) Continuous flow hydride generation with ICPand MIP detection methods

Optical ICP detectors

A number of publications by Rubio and co-workers describe the coupling of anion exchange chromatography and hydride generation with ICP-AES [102-104]/ICP-OES [105] detection. The chromatographic separations described in these papers are similar to those in earlier papers with anion exchange coupled to HG-AAS [68, 72], the only major difference being that ICP detectors are limited to using lower $NaBH_4$ concentrations, as the plasma is extinguished by excessive hydrogen production. Careful consideration of the gas-liquid separator design was also needed to minimise gas fluctuations. The detection limits reported for the arsenic species were in the order 2600 – 21 000 ng L^{-1}, similar to the results for AAS detection. In an earlier publication by Low *et al.* [106] reverse phase and anion exchange chromatography were used to separate arsenic species. In this method the HPLC outlet was directly coupled to an ICP-AES, without hydride generation, and consequently has poorer detection limits.

Optical MIP detectors

As discussed earlier, carrying out separations by ion-interaction chromatography is attractive because of the low cost of C18 columns and the different selectivity. This method though has been shown to give poor peak resolution and sensitivity and so is not ideal.

Two recent papers [107, 108] have used vesicle-mediated and micellar chromatography on a C18 column with low pressure microwave-induced plasma – atomic emission spectroscopy (MIP-AES) detection, with improved results. In both cases, a low power MIP was used because of its stability toward gas fluxes and increased tolerance toward organic solvents in the mobile phase, which allowed an increase in the selectivity of the chromatographic separation.

The vesicle mobile phase [107] was found to give detection limits of 5000 – 10 000 ng L^{-1}, with a 10-minute separation. The authors report that this is a lower cost method than anion exchange chromatography which causes little column deterioration, but its disadvantages include the viscosity of the mobile phase and problems with foaming. The method of Costa-Fernández *et al.* [108] using micellar separation also reported good detection limits (1000 – 6000 ng L^{-1}), but still too high for the analysis of arsenic in seawater.

Mass-spectrometry ICP detectors

ICP-MS is a more sensitive detector than ICP-AES, and so improved detection limits are expected for arsenic analyses. This detector is also useful because it has a wide linear working range.

The literature for arsenic speciation studies with this detector falls into two distinct categories: -those methods that use conventional nebulisation and those using hydride generation for sample introduction. Most of the methods found in the literature employ a chromatographic separation coupled directly to the ICP-MS *via* conventional nebulisation. A variety of nebuliser designs are used to try and increase the sample introduction efficiency and consequently these systems use a cooled plasma spray chamber in an effort to reduce the solvent loading to the plasma. Thermospray nebulisation increased transport of the analyte to 50%, compared with 1-2% for conventional nebulisation, but only increases the sensitivity by a factor of 10 [109]. The major advantage of direct coupling of the separation to the detector is the ability to directly determine non-hydride forming species without an on-line oxidation step. The disadvantage is that all the arsenic species must be well resolved from one another during the chromatographic separation. Nevertheless, as for AAS, the most sensitive methods incorporate hydride generation prior to ICP-MS detection because it improves the efficiency of sample introduction and removes the analyte from interferences in the sample matrix.

A variety of chromatographic methods are coupled with direct ICP-MS detection, but as for AAS and ICP-AES, anion exchange is the most common. Coupled anion exchange suffers from many of the same problems discussed previously, such as peak broadening and splitting [110], but also some that are unique to ICP-MS.

The most common of these is polyatomic interference from argon chloride forming in the plasma. This species has the same mass as arsenic and at high concentrations completely swamps the arsenic signal, even in high resolution instruments. This problem may arise if a chloride eluent is used or if chloride is present in the sample matrix. A number of ways to avoid this interference have been published and include the use of helium [111] or nitrogen plasmas [112]. The use of an organic modifier in the mobile phase, has been shown to inhibit the formation of polyatomic species such as ArCl [110], although organics need to be used carefully as at high concentrations polyatomic carbon species form, causing clogging and a decrease in sensitivity. Therefore, it is important to ensure that chloride is separated from the arsenic species of interest on the chromatographic column [111, 113-116]. The methods published by Sheppard *et al.* [117] and Saverwyns *et al.*, [109] are relatively robust with respect to chloride and can tolerate levels of <1% and 1 mg L^{-1}, respectively.

A further problem is that the sodium phosphate buffers used in HPLC-HG-AAS analysis are not always suitable for the ICP-MS detector. One paper has shown that co-elution of sodium with arsenic peaks causes reduction in the peak height [110]. Other papers have noted that, particularly at high mobile phase concentrations, the cones in the detector can become clogged by salt build-up [109, 118]. This is overcome by the use of ammonium phosphate buffer [118], flushing the analytical system with HNO_3 between samples [109], or using a high solids [112] or cross flow nebuliser [114].

The detection limits for different anion exchange ICP-MS methods vary, but are generally in the range 100 – 2600 ng L^{-1}. A comparison of HPLC-ICP-MS and HPLC-HG-AAS by Moldovan *et al.* [119] showed that the sensitivity of ICP-MS was approximately twenty times better than that for AAS, but only in low chloride matrices.

Ion-interaction chromatographic methods coupled with ICP-MS are also reported in the literature [120, 121], but like those reported for HG-AAS and HG-AFS, suffer from poor peak resolution and detection limits. Micellar chromatography has also been directly coupled with ICP-MS detection, as a way to avoid clogging of the detector by proteins present in a urine matrix [122]. The method is still subject to chloride interferences including poor sensitivity and altered retention times.

Despite the increase in sensitivity of direct ICP-MS detection when compared with hydride generation coupled to optical ICP's and AAS, it still has insufficient sensitivity to determine arsenic species in seawater and also suffers from argon chloride interferences in the plasma in such a high chloride matrix. Post-column hydride generation can be used to improve detection limits. It is a more efficient means of transporting samples to the atomiser, and also an efficient way to separate the sample from the chloride matrix. In addition, higher mobile phase concentrations can be used, improving the separation times, without salt build up on the torch.

Three recent papers in the literature have described methods for HPLC coupled to hydride generation with ICP-MS detection [123-125]. The method by Hwang and Jiang [123] separates As(III), As(V), MMA and DMA on a C18 column by ion-pair chromatography using tetrabuytlammonium phosphate. The use of HCl for acidifying the sample prior to hydride generation was found to cause ArCl interference in the plasma and was replaced by HNO_3. To avoid excessive hydrogen production, low concentrations of acid and $NaBH_4$ were required. The detection limits were in the range 11-15 ng L^{-1} and the separation time was 10 minutes, but no chloride matrix samples were analysed.

Magnuson *et al.* [124] have also recently published an HPLC-HG-ICP-MS method, using anion exchange chromatography to separate the four hydride forming arsenic species. In this method HCl is used to acidify the sample prior to hydride generation. To overcome the problems with chloride entering the plasma, the conventional glass gas-liquid separator is replaced by PTFE tube. This acts to remove chloride and also removes gas flux problems, allowing higher concentrations of $NaBH_4$ to be used. The effect of gas fluctuations is also reduced by continuous use of a high flow rate sweep gas. The detection limits achieved with this system are 12 – 62 ng L^{-1}, with a separation time of seven minutes. The method was applied to the seawater reference sample NASS-4, which has a salinity of ~31. In this matrix, As(V) gave a much broader peak with a later retention time, but the result obtained compared well with the certified value. Unfortunately, with this method chloride was found to co-elute with MMA and DMA, causing peak splitting and a decrease in signal sensitivity. In a lower salinity reference standard (SLEW-2, salinity ~12) chloride had a narrower elution profile and so peak splitting was less of a problem.

Another paper by Magnuson *et al.* [126] describes the coupling of capillary electrophoresis with hydride generation and ICP-MS detection. Because of the small

sample volumes used in CE, an interface was built to match the small CE output with the uptake requirements of the ICP-MS. Electrokinetic injection was used to increase the volume of sample injected and hydrodynamically modified electroosmotic flow was used to reduce peak broadening. The detection limits were 6 – 58 ng L^{-1}. Another CE system by van Holderbeke *et al.* [127] used on-column stacking to improve the sensitivity of a CE-HG-ICP-MS method, and gave detection limits between 1300 and 2100 ng L^{-1}. CE was also interfaced to HG-ICP-AES, in a novel method using a moveable $NaBH_4$ reduction tape [128] for hydride generation, however the detection limits were much greater than for pumped $NaBH_4$ solution (320 000 ng L^{-1}).

The detection limits of these methods suggest that they may be suitable for the analysis of arsenic in seawater, but as stated previously, CE methods are unsuitable for analysis of high ionic strength matrices because of the long analysis times required.

ii) pH selective and batch hydride-generation with ICP detection

No examples are given in the literature of interfacing pH selective hydride generation and ICP detection. Detection limits, particularly when coupled with ICP-MS detection, are expected to be better than for flame AAS detectors, but they will still probably lack the sensitivity required for analysis of seawater samples.

Batch hydride generation interfaced with ICP-MS detection has the potential to be the most sensitive technique available for analysis of arsenic species in seawater. Unfortunately, the large volumes of hydrogen produced and rapid changes in gas flow rates are difficult to interface with the plasma, which is easily extinguished. The interfacing of batch hydride generation with ICP-MS as a method for arsenic speciation is not mentioned in the literature, except for a paper by Santosa *et al.* [129], which mentions this as one of the methods used for determining arsenic species in ocean waters. No published information describing the method can be found in the literature though.

3.2.2c Detection using AFS

i) Introduction to AFS

Atomic fluorescence spectroscopy (AFS) is suitable for the analysis of arsenic hydrides in low temperature hydrogen flames since there is little background emission in the UV spectrum [39] and theoretically, it enables lower detection limits than AAS [130]. However, AFS has not been commonly used until the recent release of a commercial instrument by PS Analytical [131, 132]. This is a non-dispersive AFS instrument, which

uses a hydrogen-argon diffusion flame as the atom cell, and has advantages in terms of linearity, detection limit and size when compared with AAS [131]. Use of the commercial instrument must incorporate a hydride generation step as it does not have a nebuliser for the volatilisation of liquid samples, and the hydrogen-argon flame has poor vaporisation characteristics.

Further details regarding the theoretical aspects of AFS and construction of the PSA instrument can be found in the following references [133, 134].

ii) Continuous flow hydride-generation AFS methods

Several papers have been published in which AFS is coupled with HPLC to allow the determination of arsenic speciation [135-140]. The papers by Gomez-Ariza *et al.* [138] and Šlejkovec *et al.* [140] describe the coupling of anion exchange chromatography with HG-AFS. Both papers used a polymeric strong anion exchange column and phosphate eluent. The method of Gomez-Ariza *et al.*, however, has a shorter separation time (10 minutes) as it used gradient rather than isocratic elution. As with previously published HPLC-HG-AAS methods [70, 72, 141], these methods were unable to determine As(III), As(V), MMA, DMA, arsenobetaine and arsenocholine in a single run. In the paper by Gomez-Ariza *et al.*, arsenobetaine was determined in a second run using photooxidation and in the Šlejkovec paper a second cation exchange separation incorporating on-line UV decomposition was used to determine four hydride refractory arsenic species, including arsenobetaine and arsenocholine.

Two groups have published several papers regarding the development of ion-interaction HG-AFS methods. Two early papers by Le *et al.* [136, 142] described the separation and analysis of seven arsenic compounds on a C18 reverse phase column using mixed ion-interaction reagents to increase selectivity, and a column heated to 70 °C to improve peak resolution. Two runs were required, one with and one without microwave oxidation, to determine all seven species. The separation time for this method was ~14 minutes and detection limits were high, 10 000 ng L^{-1} in urine samples. Also, changes in retention time were observed when analysing for the arsenic species in a urine matrix.

More recently, the same group [139] have published an ion-interaction method which allows rapid separation (2 minutes) of the four hydride forming arsenic species on a 3-cm reverse phase guard column heated to 70 °C. Urine samples required the use of two guard columns in sequence, the first acing as a guard column and the second to perform the separation. Even in urine, using two columns the four species can be

baseline separated on a small particle size column in four minutes. No detection limit data was given.

An initial publication by Woller *et al.* [135], described the separation of the four hydride-forming arsenic species by ion-interaction chromatography using a C18 column and didodecyldimethylammonium bromide(DDAB) as the ion-interaction reagent. Hydride generation was not used, but instead the arsenic species were nebulised using a commercial ultrasonic nebuliser (USN) prior to detection by AFS. In this case the detection limits were quite poor, 80 000 – 200 000 ng L^{-1} and the peaks were incompletely separated. In later publication [137] USN was used as a gas-liquid separator, but was followed by hydride generation prior to detection, improving the detection limits by a factor of 10.

As found for HPLC-HG-AAS, there are many disadvantages with HPLC-HG-AFS methods, including complexity, long analysis times and low sensitivity, which make them best suited to determining the higher concentrations of arsenic species found in biological samples. HPLC-HG-AFS methods have detection limits between 10^4 – 10^6 less sensitive than those described by Andreae [1] for batch HG-AAS. The methods therefore do not have sufficient sensitivity to be applied to the determination of arsenic species at the concentrations found in coastal and open ocean seawater.

A continuous flow HG-AFS method has been shown to have sufficient sensitivity for the determination of arsenic in open ocean seawater samples and had a sample throughput of 40 h^{-1} [143] but without hydride trapping it was unable to determine the methylated species important in arsenic cycling. No examples of pH-selective HG-AFS have been reported in the literature.

iii) Batch hydride-generation AFS methods

The only reference to a batch HG-AFS method in the literature prior to publication of the manual method described in section 3.3 [144], is in the paper by Šlejkovec *et al.* [140]. The method described is similar to that by Andreae for HG-AAS [1]. A glass reaction vessel was used for the sample which can have a volume of up to 100 mL. Three manual injections of 2% $NaBH_4$ were made to the sample, and the arsines were purged for twelve minutes onto a U-trap with a Chromosorb stationary phase. Once all the arsines had been stripped from solution the trap was heated in ambient air by removing it from the liquid nitrogen. Use of a heating wire was found to result in losses of the methyl arsenic species. The method had similar sensitivity to HG-AAS, with the detection limit being 2.5 ng L^{-1}, and is, therefore, suited to arsenic determination in

seawater. However, the authors reported such erratic and significant problems with the arsenic blank that they were unable to use the method to quantify As(V) or As(III).

3.2.2d GC methods

Arsenic compounds require derivatisation before analysis by GC, due to their low volatility. The most commonly used derivatisation is hydride generation, and therefore these methods often differ only slightly from batch cold trap methods using a GC packing. The main differences are that the column is heated at a higher temperature and, often, GC detectors (non-element specific) are used.

A GC method for determination of As(III), As(V), MMA and DMA with has been published by Andreae [89]. This method uses essentially the same experimental design as described for the HG-AAS method [1], but when the U-trap is heated the arsines desorb onto a GC column, with subsequent detection by ECD, FID or AAS. In general he notes that this method is more complex and slower than batch HG-AAS, but when coupled with the ECD detector gives better detection limits.

Batch and continuous flow HG-GC-AAS methods are also described by Ebdon *et al.* [145] and Le *et al.* [97]. In both cases there are no performance characteristics given for the methods but they are applied to the analysis of arsenic species in estuarine sediment pore waters and human urine, respectively.

A batch HG-GC method using a PID detector is described by Cutter *et al.* [6] for the determination of arsenic and antimony in seawater samples. In this case (like Andreae [89]) the detector is not element specific, but specific to all ionisable compounds, which must be taken into account when doing the GC separation. Although this method was not applied to analysis of MMA and DMA, the method is very sensitive (detection limit = 0.8 ng L^{-1} As), and sufficiently rugged for the shipboard analysis of arsenic in seawater.

3.2.3 Conclusions

Previously published methods coupling hydride generation and cryogenic trapping of the hydrides with AAS or anion exchange chromatography with HG-ICP-MS have been shown to have the sensitivity required for arsenic analyses in seawater, but for several reasons they are not suitable for shipboard use. Firstly, AAS and particularly ICP-MS instruments have large space requirements compared with AFS. Although this does not effect the choice of detector for a laboratory-based method, it is of much greater importance when considering shipboard use where space is always limited. In addition

to the physical size of the detector its portability must also be considered, and again the compact nature of the AFS detector has distinct advantages over ICP-MS and AAS, as it can easily by carried by one person.

ICP-MS is a significantly more complex instrument compared with AFS. It requires a stable platform for operation and also high use of consumable gases. Despite being successfully used for the determination of arsenic in a standard seawater sample [124], it is also considered unlikely that an HPLC-HG-ICP-MS method would be able to be used routinely for the analysis of seawater samples without the HPLC column becoming overloaded by chloride.

AFS is also a low cost instrument when compared with ICP-MS and even AAS detectors, therefore more easily able to be used as a dedicated instrument for field work studies. When compared with AAS, AFS has been shown to have improvements in terms of both linearity and sensitivity in the UV range where the arsenic line is found.

For a shipboard method safety is also a key consideration, as most ships will not allow the use of flammable gases. Although an AFS detector requires the use of hydrogen, the small quantity necessary can be produced on-site as required by a hydrogen generator — an acceptable alternative to the use of pressurised cylinders. Many AAS methods require the use of acetylene, which cannot be produced on site, or larger quantities of hydrogen which cannot be produced easily by a hydrogen generator.

3.3 DEVELOPMENT OF A MANUAL HG-AFS METHOD

3.3.1 Introduction

The following section describes the first stage in the development of an automated shipboard method for determining arsenic speciation in seawater; the establishment and validation of a manual method. The method is based on an existing HG-AAS method [100] and couples batch hydride generation, and cryogenic hydride trapping, with atomic fluorescence spectrometry detection. This section describes the design of the analytical system, it's optimisation and application to seawater samples.

3.3.2 Experimental

3.3.2a Preparation of reagents and standards

All reagents used were of analytical reagent grade or better. Ultrapure water from a Millipore (Milford, MA, USA) Milli-Q system was used throughout.

i) Preparation of reagents

A 9 M H_2SO_4 solution (AnalaR Grade, BDH Chemicals, Kilsyth, Victoria, Australia) was prepared and used for acidification of samples prior to hydride generation [100].

A 2.5 M TRIS-HCl buffer was prepared by dissolving 30.29 g of tris(hydroxymethyl)aminomethane (TRIS) (Mallinckrodt, St Louis, MO, USA) in 100 mL of water and adjusting to pH 6.5 with HCl.

A 2% (m/v) $NaBH_4$ solution (BDH Spectrosol, Merck Ltd., Poole, Dorset, UK) was prepared fresh daily in 0.1 M NaOH (Ajax Chemicals, Auburn, NSW, Australia). As(V) is a known contaminant of $NaBH_4$ [1, 42], and the reagent and grade chosen were those with the smallest blank value. The same $NaBH_4$ batch was obtained from suppliers for as long as possible.

ii) Preparation of standards

Stock solutions (1000 mg L^{-1} as As) of all the arsenic species were prepared in 125-mL HDPE bottles (Nalgene, Rochester, NY, USA) which had previously been soaked in 10% (v/v) HNO_3 (Ajax Chemicals), rinsed with water and dried. The stock solutions were stored in darkness at 4 °C, and under these conditions were considered stable for several months [1]. Dilute solutions were prepared daily as required. Care was taken to avoid cross-contamination of the standards with other arsenic species during preparation.

A stock solution of arsenite was made by dissolving 0.132 g of As_2O_3 (Aldrich Chemical Co., Milwaukee, WI, USA) in 10 mL of 0.1 M NaOH. The solution was then neutralised with HCl (Mallinckrodt), and diluted to 100 mL with 0.1 M HCl.

The standard arsenate solution was prepared by dissolving 0.414 g of $Na_2HAsO_4.7H_2O$ (Aldrich) in 0.1 M HCl, and diluting as for the arsenite standard.

A standard MMA solution was prepared as described for arsenate, using 0.190 g CH_5AsO_3 purified from the herbicide Methar Paspalum killer (550 g kg^{-1} disodium methylarsonate) [Colin Campbell (Chemicals) Pty Ltd., Alexandria, NSW, Australia] as described in Chapter 2.

A DMA standard solution was prepared as for arsenate using 0.184 g of $C_2H_7AsO_2$ (Fluka Chemie, Buchs, Switzerland).

iii) Verification of standard purity

The methyl arsenic compounds are often not completely pure, so before use their arsenic content and purity was characterised as described in Chapter 2.

3.3.2b Apparatus

A schematic diagram of the hydride generation atomic fluorescence system is shown in Fig. 3.1. The detector used was a PSA Excalibur atomic fluorescence spectrometer (PS Analytical, Orpington, Kent, UK) fitted with a boosted discharge hollow cathode lamp (Photron, Victoria, Australia). Measurements were performed using the arsenic resonance line at 193.7 nm.

All glassware used was constructed "in-house" of borosilicate glass. The internal surfaces of the glassware were deactivated by silylation with 5% (v/v) dimethylchlorosilane (DMCS) (Princeton Applied Research, Trenton, New Jersey, USA) in CCl_4. First, the glassware was washed by soaking it in 2% (v/v) Extran (BDH Chemicals, Kilsyth, Victoria, Australia) solution for 48 h, then, after a water rinse, 10% (v/v) HNO_3 for 24 h. The glassware was then rinsed thoroughly with water and allowed to dry. The internal surfaces of the glassware were coated with DMCS solution and allowed to air dry for 2 hours before being placed in an oven overnight at 180 °C. A small amount of quartz wool, used for holding the packing in the U-tube, was treated in a similar manner. Silylation of the glassware was repeated whenever performance of the method deteriorated.

The glassware was joined using threaded connectors. The reaction vessel has a single port into which the transfer tube connects *via* a threaded connector with a silicone washer to seal it to the glass surface. The transfer tube has two ports through which high purity hydrogen (BOC Gases, Chatswood, NSW, Australia) and the $NaBH_4$ solution were delivered to the reaction vessel. Hydrogen was introduced into the reaction vessel *via* a GC septum in the top port of the transfer tube and bubbled through the sample at a rate of 70 mL min^{-1}. This hydrogen flow acts as both a carrier for the hydrides and a support for the flame in the detector. Similarly, $NaBH_4$ solution was delivered to the reaction vessel through the side port at 2 mL min^{-1}. During hydride generation the sample solution is stirred constantly using a spherical magnetic stirrer. To remove water from the gas stream prior to trapping, gases from the hydride generation procedure pass through the glass transfer tube and into a model MD 110-12E sheathed Nafion dryer tube (Perma Pure, Toms River, New Jersey, USA), which has a countercurrent air flow of 2.6 L min^{-1}. Following drying, the gases are swept into the U-trap (height 21 cm, i.d. 7 mm), which is packed with 2 g of 3% OV-101 on Chromosorb W AW-DMCS 45/60 mesh (Alltech Associates, Baulkham Hills, NSW, Australia) and held in place by two small plugs of silylated quartz wool. At liquid nitrogen temperature the arsines are

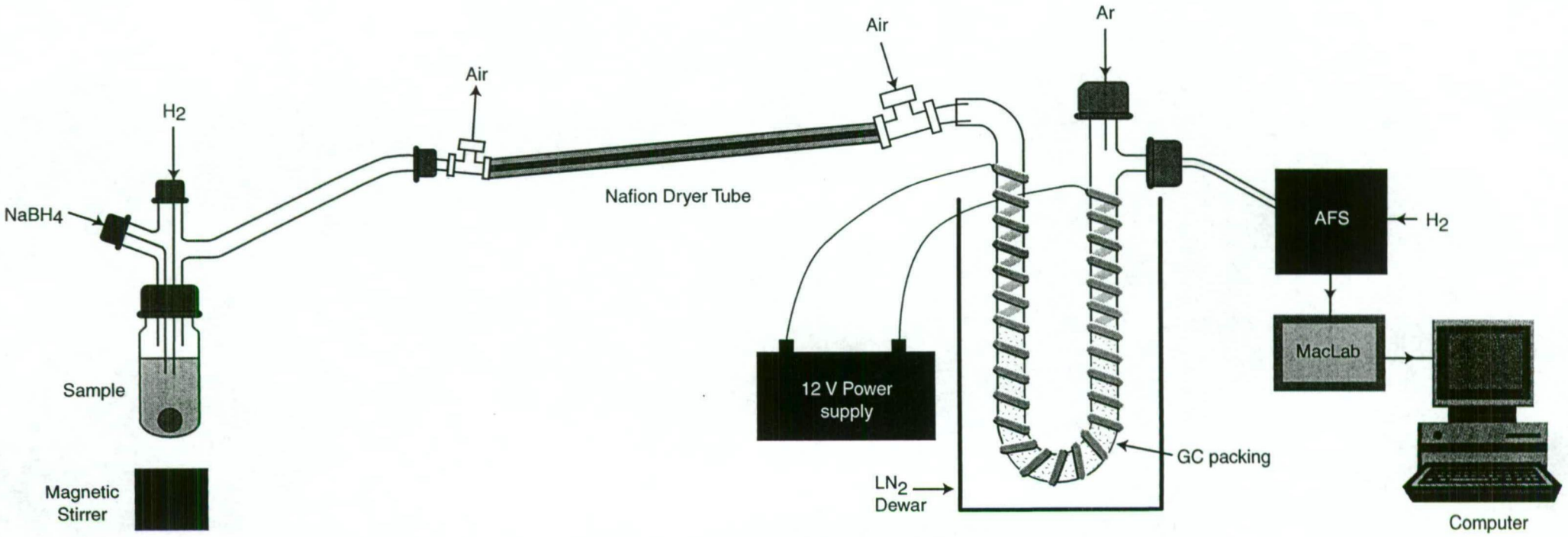

Figure 3.1 Schematic diagram of the manual HG-AFS apparatus

retained by this packing and are preconcentrated. Around the outside of the U-trap is wound 2 m of Nichrome wire (1.76 Ω m^{-1}). This is attached to a variable power supply (Variac) *via* a 12 V transformer, giving an output of approximately 4 V. The cold trap is cooled using a dewar of liquid nitrogen. Ultra-high purity argon (BOC Gases) is supplied to the detector side of the cold trap at 400 mL min^{-1} to act as a carrier gas and assist in supporting the hydrogen-argon diffusion flame in the detector.

A supplementary hydrogen flow of 20 mL min^{-1} is supplied directly to the flame *via* a stainless steel tube in the back of the AFS instrument. This supports the flame while the reaction vessel is disconnected from the system.

Gas flow rates for hydrogen were controlled and measured by Porter gas flow meters (Cole Parmer, Vernon Hills, IL, USA), which have high resolution valves and use stainless steel fittings. Similarly, the argon flow rate was controlled and measured by the gas flow meter supplied with the PSA hydride generation unit.

Data was acquired using a MacLab 2E (ADInstruments Pty. Ltd., Castle Hills, NSW, Australia) in Chart mode. Peaks were integrated using the software package Origin v. 4.1 (Microcal Software Inc., Northampton, MA, USA).

3.3.2c Procedure

To ensure reproducibility of the results, the timing of the hydride-generation reaction and heating cycle was kept as constant as possible.

For the determination of total inorganic arsenic and the organoarsenic species, a 10-mL sample is placed in the reaction vessel. After 0.2 mL of 9 M H_2SO_4 had been added, the vessel was attached to the hydride generation apparatus and the stirrer started.

The U-tube was cooled in liquid nitrogen for 20 s and then the peristaltic pump delivered the $NaBH_4$ solution for 80 s. During this period the hydrides passed from the reaction vessel, through the dryer tube, and into the U-trap, where they were trapped at liquid nitrogen temperature.

Following the addition of $NaBH_4$, there was a 30 s delay to allow hydride generation to cease. The liquid nitrogen was then removed quickly, the Variac heater switched on to warm the U-trap, and data acquisition begun. The hydrides were detected within 2 min of heating the trap.

An identical procedure to that described above was followed for determination of arsenite, using 0.2 mL of 2.5 M pH 6.5 TRIS-HCl buffer in place of H_2SO_4. The concentration of arsenate was determined by difference.

3.3.3 Results and discussion

3.3.3a Optimisation of hydride generation conditions

The procedure was optimised by a univariate approach, changing the parameters involved in hydride generation and detection. All experiments were carried out using an approximately 0.8 $\mu g\ L^{-1}$ aqueous solution of As(V), MMA and DMA.

A summary of the optimum conditions is given in section 3.3.2.

i) Effect of liquid nitrogen level and cooling time

The length of time that the U-trap was allowed to cool in liquid nitrogen, prior to the addition of $NaBH_4$, was investigated. The minimum possible cooling time was the time required for the U-trap to reach liquid nitrogen temperature (20 s).

The minimum cooling time was found to result in a slightly higher analytical signal for each of the arsenic species, and so this was the length of time adopted. In addition, it was also found that a constant liquid nitrogen level was required to ensure optimum reproducibility.

ii) Effect of sample volume

Sample volume was varied over the range from 2.5 to 15 mL, the maximum possible volume with the current glassware design. Peak area was found to increase linearly over the full volume range for both MMA and DMA, and over the range 2.5 to 10 mL for As(V), with a decrease in sensitivity at 15 mL, where it appears the signal might be reaching saturation. Although the current method, using a 10 mL sample, had sufficient sensitivity to be able to determine arsenic in unpolluted systems, further increases in the sample capacity to allow larger sample volumes would give an improvement in the signal-to-noise ratio for very low concentration samples.

iii) Addition time and concentration of $NaBH_4$

For each of the arsenic species, the effect of $NaBH_4$ addition time {using a 2% (m/v)/[0.5 M] solution} on peak area was determined. The results of the experiment are shown in Fig. 3.2, and indicate that there is little improvement in the signal when $NaBH_4$ is added for more than 80 s. The effect of $NaBH_4$ solution concentration on peak area (Fig. 3.3) was investigated over the concentration range 0.5 – 4% (m/v) [0.13 – 1.1 M]. Similar responses were observed for As(V) and MMA, with a maximum

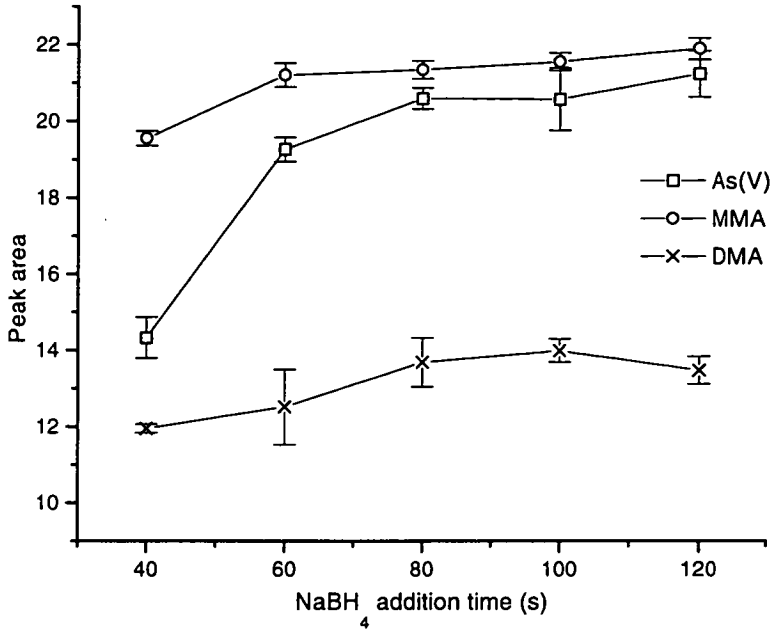

Figure 3.2 Influence of $NaBH_4$ addition time on the analytical signal for arsenic species (n = 3)

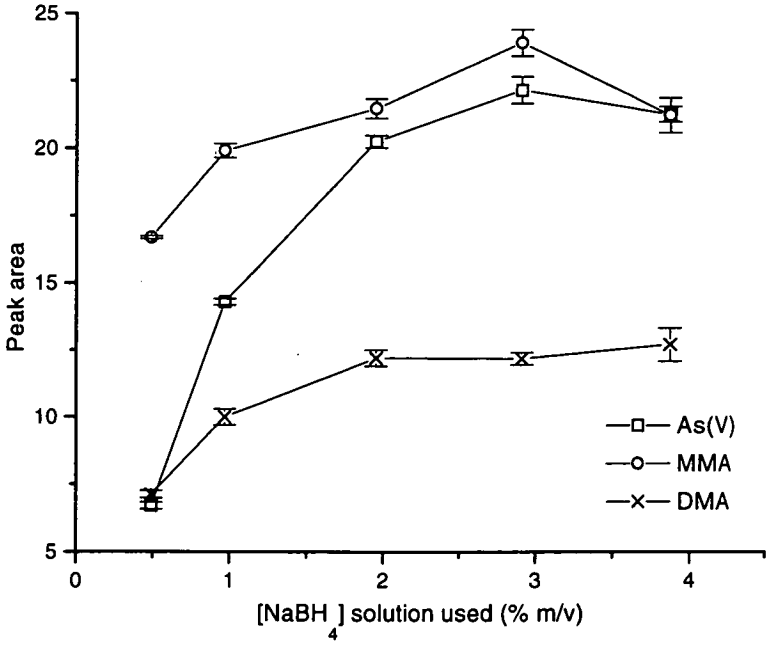

Figure 3.3 Influence of the $NaBH_4$ concentration used on the analytical signal for arsenic species (n = 3)

response at 3%, while DMA gave a maximum signal at 2%. The optimum concentration chosen was 2%, as As(V) and MMA already had sufficient sensitivity, and a further increase in the lower DMA sensitivity could not be achieved with a higher $NaBH_4$ concentration.

iv) Carrier gas flow rates

The effects of the hydrogen (Fig. 3.4) and argon (Fig. 3.5) carrier gas flow rates were determined.

For all the arsenic species the analytical signal increased as the hydrogen carrier flow rate increased. The responses reached a maximum at 70 mL min^{-1} for As(V) and MMA, and at 80 mL min^{-1} for DMA. The optimum flow rate chosen was 70 mL min^{-1}, because the increase in signal for DMA which occurs at flow rates greater than 70 mL min^{-1} also results in greater baseline noise at low concentrations.

An increase in the Ar carrier flow rate over the range 200 to 500 mL min^{-1} resulted in a significant increase in the analytical signal for all the arsenic species. An optimum flow rate of 400 mL min^{-1} represented the best compromise between signal sensitivity and resolution of the MMA and DMA peaks.

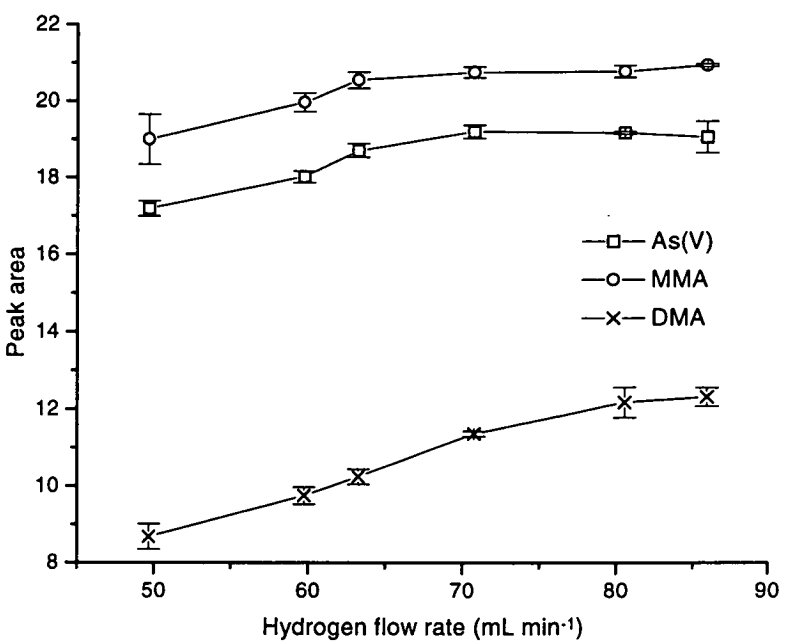

Figure 3.4 Effect of hydrogen carrier flow rate on peak area for arsenic species (n = 3)

v) Position of supplementary hydrogen flow relative to flame

The position of the stainless steel tube delivering the supplementary hydrogen flow to the hydrogen-argon flame was found to be important, the optimum position being when it just touched the flame.

3.3.3b Analytical performance

A typical chromatogram showing the separation of As(V), MMA and DMA in a standard solution is shown in Fig. 3.6. The three peaks are well resolved from one another and have respective retention times of 50, 89 and 110 s, following the removal of liquid nitrogen and the start of heating. The retention times were reproducible to ±0.5 s.

The analytical performance characteristics obtained for 5 mL individual standard solutions of each arsenic species are presented in Table 3.3. This evaluation of the method was performed using the optimum conditions determined previously.

Table 3.3 Analytical characteristics of the HG-AFS method (using 5 mL samples)

	RT[a] (s)	DL[b] (ng L^{-1})	RSD[c] (%)	Equation	RC[d] (R^2)	Linear range (ng L^{-1})
As(III)	53	2.3	3.3	y = 1.59x - 0.010	0.9996	13 – 74
As(V)	53	0.9	2.0	y = 1.40x + 0.005	0.9994	10 – 69
MMA	87	2.4	3.4	y = 1.51x + 0.005	0.9992	8 – 57
DMA	108	3.7	3.5	y = 1.03x - 0.002	0.9985	8 – 53

[a]Retention time. [b]Detection limit (3σ). [c]Relative standard deviation at 50 ng L^{-1} (n=9). [d]Regression coefficient (n = 6).

The 3σ criterion was applied to nine blank measurements, giving a detection limit of 0.9 ng L^{-1} for As(V). Similarly, the detection limits for As(III), MMA and DMA were 2.3, 2.4, and 3.7 ng L^{-1}, respectively, for nine replicates of a 10 ng L^{-1} standard solution. These results compare favourably with those reported for similar hydride generation systems using AAS detection as shown in Table 3.4.

The reproducibility of the method was determined as the percentage relative standard deviation of nine measurements of each arsenic species at 50 ng L^{-1}. The reproducibility of peak areas, shown in Table 3.3, is better than 3.5% for all species. It should be noted that measurement of peak area gives slightly improved precision compared with peak height, but, the improvement is not as significant as that noted by other authors [1, 99].

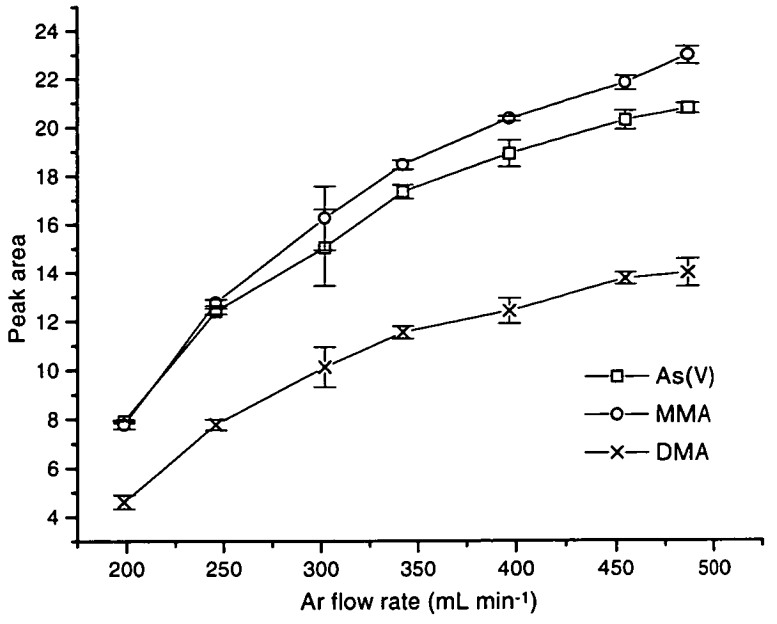

Figure 3.5 Effect of argon carrier flow rate on peak area for arsenic species (n = 3)

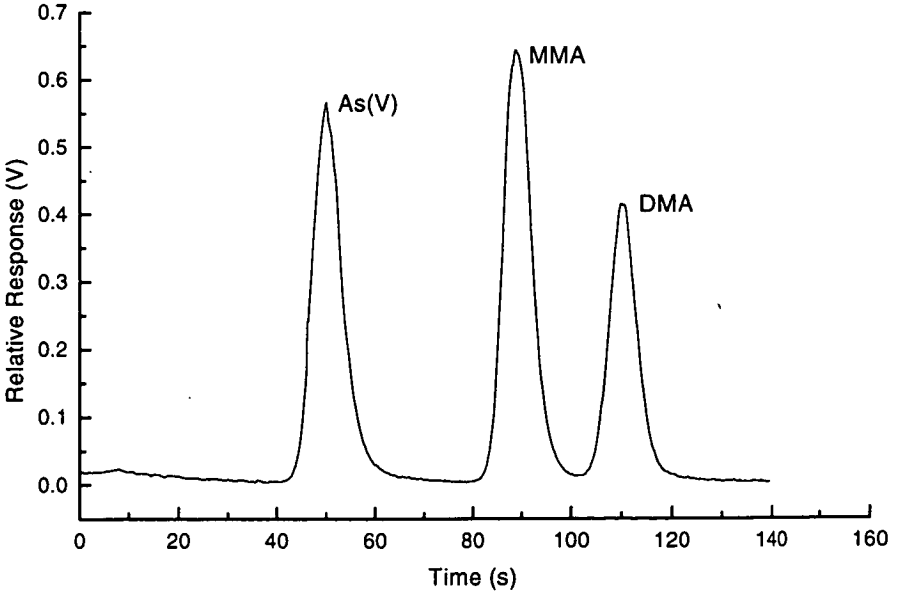

Figure 3.6 Typical chromatogram of a solution containing As(V), MMA and DMA at ~0.8 μg L^{-1} in Milli-Q water

Table 3.4 Comparison of the detection limits of HG-AFS (this work) with HG-AAS methods

Reference	This Work	Ref. [93]	Ref. [98]	Ref. [1]	Ref [34]	Ref. [89]
Method	HG-AFS	HG-AAS	HG-AAS	HG-AAS	HG-AES	HG-AAS
Sample Volume (mL)	5	1	25	100	50	75
No. of standard deviations	3σ	3σ	2σ	Not given	Not given	2σ
As(III) (ng L^{-1})	2.3[a]	30[a]	1.5[b]		4[d]	
As(V) (ng L^{-1})	0.9[b]	57[a]	7.6[b]	0.005[d]	4[d]	26[c]
MMA (ng L^{-1})	2.4[a]	98[a]	0.5[c]		4[d]	
DMA (ng L^{-1})	3.7[a]	42[a]	0.5[c]		20[d]	

[a]Low concentration standard [b]Blank measurement [c]Baseline noise [d]Not specified

The best precision is obtained for the As(V) peak, with poorer precision for subsequent peaks.

Linearity was confirmed for the concentration ranges shown in Table 3.3, using the Fowlis-Scott nonlinear function [146]. Although the calibration graphs for all species are observed to be linear over a wide range of concentrations (~0.01 to 2.5 $\mu g\ L^{-1}$), in practice the working concentration range is limited by the range settings of detector.

The slopes of the calibration graphs for As(III), As(V) and MMA were similar, and under acidic pH conditions both As(III) and As(V) calibrations have the same slope. The DMA calibration has a lower sensitivity, as indicated by its slope. In previous work [36, 52] it has been suggested that lower DMA sensitivity can be caused by thermal decomposition of dimethyl arsine if the trap temperature becomes too high during warming. In this case, DMA still showed lower sensitivity when the trap was allowed to warm in ambient air, without using the heating wire. Therefore the reasons for its lower sensitivity are not clear. Recent evidence suggests that the addition of L-cysteine prior to hydride generation may be able to improve the response of DMA [95, 97]. It is thought that addition of L-cysteine reduces all the arsenic species from the +V to the +III oxidation state, and subsequently reacts with them to form As(III)-cysteine complexes [97]. This gives rise to neutral, three-coordinate species which are less sterically hindered than the parent compounds and therefore react in a faster and more uniform way with $NaBH_4$ [95].

The accuracy of the method was evaluated by analysis of the certified reference material, NASS-4. The inorganic arsenic concentration of the reference seawater was experimentally determined to be $1.18 \pm 0.01\ \mu g\ L^{-1}$ ($n = 4$), which was in good agreement with the certified value of $1.26 \pm 0.09\ \mu g\ L^{-1}$. No methyl arsenic species were observed in the reference material.

Accuracy of the MMA and DMA analyses could not be determined using a certified reference material, as none are currently available for seawater. Instead accuracy for these species was assessed by an interlaboratory comparison of the results for seawater samples from the Atlantic Ocean. This data will be discussed further in Chapter 4.

The HG-AFS system described allows analysis of As(V), MMA, and DMA in six minutes, with the use of the Nafion dryer tube removing the need to have a longer heating period after each analysis to remove water from the U-trap. No memory effects are observed with the system described and the only blank signal is due to As(V)

present as a contaminant in the sodium borohydride. In the grade of borohydride chosen, however, this is a minimal blank source.

3.3.3c Application to environmental water samples

Fig. 3.7 shows a typical chromatogram of an estuarine sample from the Huon River, Tasmania. As with the application of a number of other methods to the analysis of arsenic in natural waters, the difference in concentration between total inorganic arsenic and the methyl species necessitates that these analyses are carried out at different instrument sensitivities.

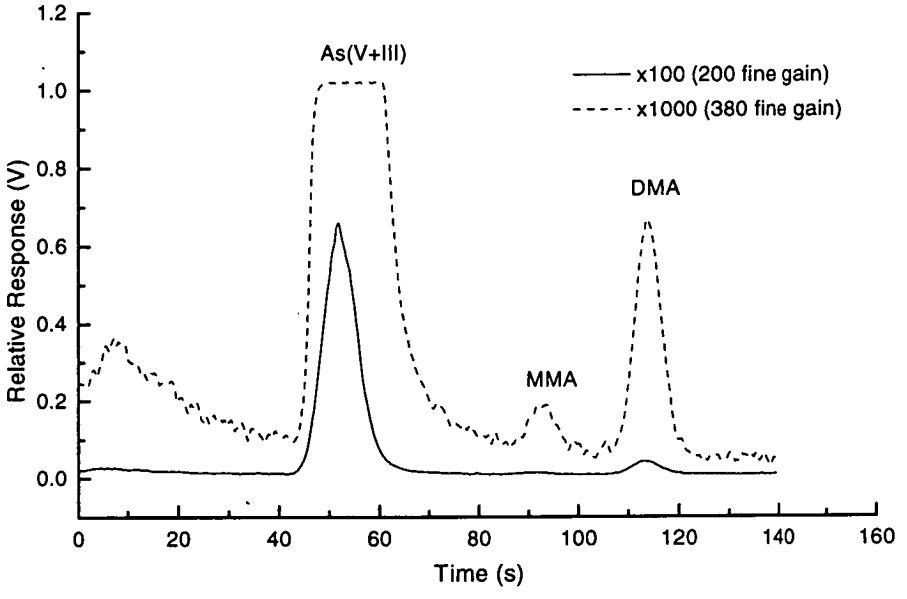

Figure 3.7 Typical example of a coastal seawater sample from the Huon River estuary, Tasmania (salinity 28.9)

To ensure that matrix effects do not interfere in the application of this method to the analysis of environmental samples, each of the arsenic species was spiked in duplicate into three typical matrices and the spike recoveries calculated. Each spike was allowed to equilibrate for at least two hours prior to analysis in triplicate. The three matrices chosen were: fresh river water with a high humic load, collected from the Huon River, Tasmania; coastal seawater, collected from the mouth of the Huon River; and open ocean seawater, collected from the Southern Ocean (42° S, 142° E). The freshwater

sample contained low concentrations of total inorganic arsenic and required no further treatment before performing recovery experiments. The coastal and open ocean seawater samples contained much higher concentrations, and so were stripped of arsenic using $Fe(OH)_3$, according to the method of Portmann and Riley [147]. All samples were spiked with approximately 0.5 $\mu g\ L^{-1}$ of arsenic as As(V), MMA and DMA.

The results of the recovery experiment, summarised in Table 3.5, indicate that there are no problems with matrix effects for most of the arsenic species, with the exception of DMA in the open ocean seawater sample. The reasons for this result are not clear, although it is possible that excess iron remains in the sample following the arsenic stripping process, and this may interfere with formation of the DMA hydride. Better recoveries can be obtained for DMA when using unstripped seawater samples.

As the application of this method is the analysis of unpolluted coastal and open ocean waters, the effects of interferents other than naturally occurring matrix elements, *i.e.* some transition metal ions and other hydride-forming elements, were not investigated.

Table 3.5 Recovery of arsenic species from some typical environmental waters (n = 2)

	Open ocean seawater[a]	Coastal seawater[b]	Fresh river water[c]
As(V)	97.1%	99.8%	95.4%
MMA	97.7%	95.5%	94.4%
DMA	78.0%	108.5%	94.4%
	[a]Southern Ocean	[b]Mouth Huon River	[c]Huon River

3.3.4 Conclusions

The method of hydride trapping with atomic fluorescence detection has been demonstrated to have the sensitivity required for the analysis of arsenic species in open ocean and unpolluted coastal waters. This method exhibits a number of advantages over similar methods using AAS detection. These include slightly improved detection limits, a decrease in overall analysis time, and a system which is compact enough to consider using in shipboard analysis, thereby avoiding speciation changes which may occur when samples are preserved for later analysis.

3.4 DEVELOPMENT OF THE SEMI-AUTOMATED HG-AFS METHOD

3.4.1 Introduction

This section describes the modifications made to the manual HG-AFS method (section 3.3), to give a semi-automated shipboard method. The advantages over the manual method are described in this section, while its shipboard performance on a one-month voyage aboard the RSV *Aurora Australis* in the Southern Ocean, will be described in Chapter 4.

3.4.2 Experimental

3.4.2a Apparatus and instrumentation

A schematic diagram of the semi-automated system is shown in Fig. 3.8. The apparatus and operating conditions are generally the same as described section 3.3.2a and 3.3.2b for the manual method, with the exception of the changes detailed below.

The U-trap is wound with Nichrome wire, as previously, but is now connected to a pulsed power supply to give greater control over the heating cycle. The liquid nitrogen dewar used to cool the U-trap is raised and lowered using a motorised Lab Jack.

A pressurised cylinder supplies high purity argon to the detector side of the U-trap at 400 kPa. For safety reasons, hydrogen cylinders cannot be used with a shipboard method, so hydrogen was produced as required by a Balston model 75-33 hydrogen generator operating at *ca.* 400 kPa.

3.4.2b Modifications for automation

To allow easy operation of the method at sea, a number of previously "operator performed" steps in the analytical sequence were automated. The automated tasks included raising and lowering of the liquid nitrogen dewar around the U-trap, controlling the peristaltic pump used for $NaBH_4$ delivery, turning on and off the Nichrome heating wire to provide a range of heating program options, initiating data logging and overall timing of the analytical sequence.

i) Automating the liquid nitrogen platform

A stepper motor driven Lab Jack operating between two cut out switches was used to automate the process of raising and lowering the liquid nitrogen dewar. Motion up and down was regulated by a signal from WorkBenchMac™.

A standard laboratory jack was used, with some modification. First the whole assembly

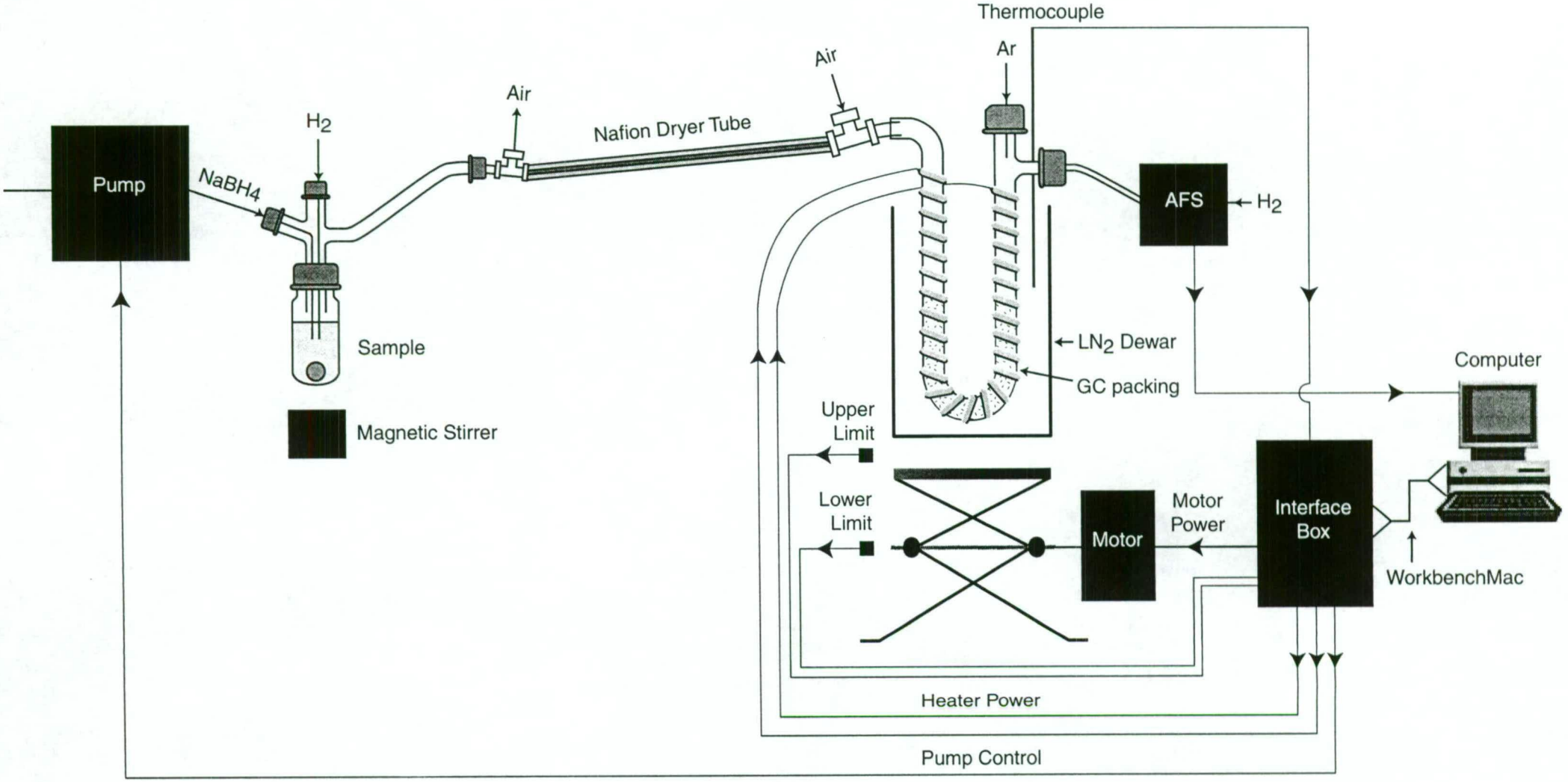

Figure 3.8 A schematic diagram of the semi-automated HG-AFS apparatus

was overhauled to ensure free movement of the mechanism. In place of the screw knob, a motor was attached. A DC motor was selected, as it would enable the computer to drive the jack in both directions. Micro switches were placed at the top and bottom of the jack as safety switches, thus physically defining the upper and lower limits.

With commands from the computer, the jack raised and lowered the dewar, immersing the U-trap in liquid nitrogen. To account for liquid nitrogen loss through evaporation, a thermocouple was placed in the nitrogen dewar. This sensed when the U-trap was immersed in the nitrogen and stopped the jack from being driven any higher. A circuit diagram for the platform drive is shown in Appendix 1a.

ii) Controlling the heating cycle

Prior to automation, the Nichrome heating wire was attached to a 4 V power supply which supplied a constant voltage and caused it to heat rapidly. To enable greater control of the heating cycle, with the objective of improved separation of the arsine peaks, a pulsed power supply was developed.

Power for the heater was supplied from a 15 V, 4 A DC source which also supplied power for the digital circuits. The circuit used is shown in Appendix 1b. To enable precise control over the heating cycle, the computer supplied pulses which could have the mark-to-space ratio varied. To protect the computer from any voltage spikes an opto isolator was used. The output from the opto isolator controlled a hexfet that provided the high power switching for the heater. By adding this feature we have limited the amount of heating during the experiment and thus the amount of liquid nitrogen loss.

3.4.2c Control of the automated system

A Macintosh IIsi computer was fitted with an ACM2 12 8A card having 12-bit analog inputs (A/D) and 12-bit analogue outputs (D/A) at a speed of 10 kHz. Using WorkBenchMac™ data acquisition and control software v. 4.01 (Strawberry Tree, Sunnyvale, CA, USA), a program was written which used six channels on the interface board to control the automated components, time the analytical sequence and log the analytical data.

Pressing a start button begins the computer-controlled sequence. The dewar is driven up on to the U-trap and the peristaltic pump is turned on, adding $NaBH_4$ to the sample for 60 s. The pump then turns off and a 30-s wait period is initiated, after which the dewar is removed from the U-trap. When the dewar is removed, voltage to the heater around the U-trap is turned on for 140 s. The start of the heating cycle is the trigger for

Workbench to begin logging the output voltage from the AFS as a tab-delimited text file. The rate of signal input and the data-logging rate can be varied.

Once the analytical cycle is complete the computer stops logging and waits for then next start command. A logic diagram showing the timing of the overall analytical sequence and a flow chart of the computer program are given in Figs. 3.9 and 3.10. A diagramatic representation of the computer program is given in Appendix 2.

Progress of the analysis can be viewed as an electronic chart on the computer.

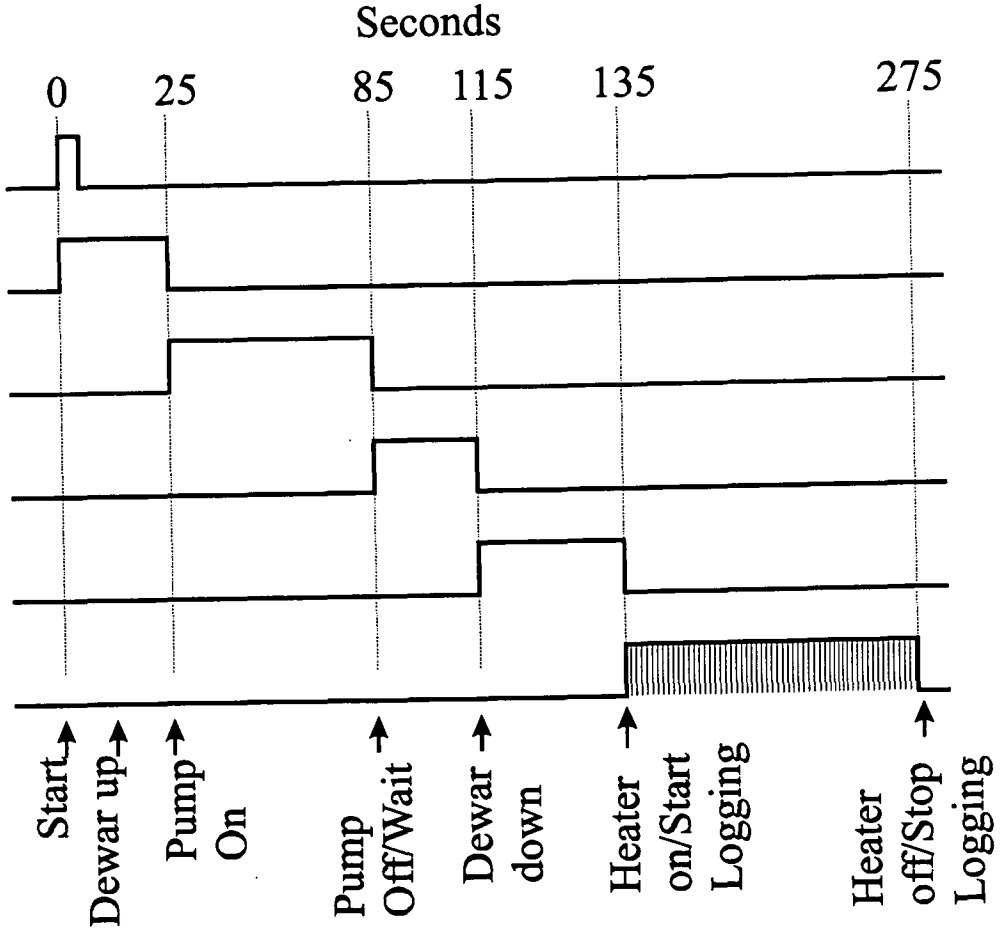

Figure 3.9 Logic diagram indicating the timing of the analytical sequence used for the semi-automated HG-AFS method

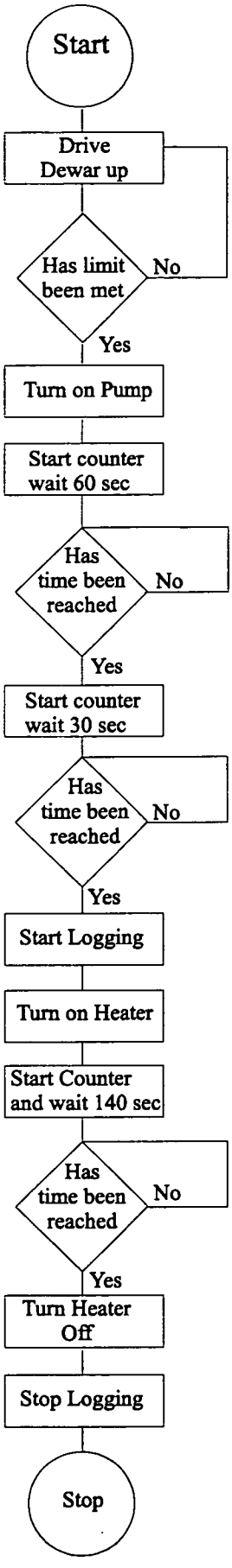

Figure 3.10 Flow chart of the computer program used to control the analytical sequence

3.4.2d Other modification required for shipboard use

Apart from using the hydrogen generator mentioned previously, few other changes to the method were necessary for shipboard use. The most significant alteration was to replace the gas flow meters used for regulating argon and hydrogen flows. The rotameter-type flow gauges, used to measure flow, require a level surface to operate correctly. These were replaced by model VCD-1000 mass flow controllers (Porter Instrument Co., Hatfield, PA, USA), which were preset to give the required flow rates. The only other changes were to make the system as compact as possible by minimising the lengths of tubing used, and to secure all of the components to a board which could then be attached to a laboratory bench.

3.4.2e Analytical procedure

For analysis of As(V+III) and the methyl arsenic species, 10 mL of sample was placed in the reaction vessel with 0.2 mL of H_2SO_4. Higher instrument sensitivity was generally required for determination of the methyl species.

For As(III) analyses, 15 mL of sample and 0.2 mL of TRIS-HCl buffer was used, with As(V) determined by difference.

The WorkBench program sequentially appends the data files for each experimental run to a master data file. To avoid having to save a different data log for each sample run, an MS Excel macro is used to divide the final data file into individual sample runs. Peak area is determined using the software package Origin v. 4.1 (Microcal Software Inc.)

3.4.3 Results and Discussion

3.4.3a Optimisation of the semi-automated method

The heating profile was optimised by incrementally altering the timing of the pulsed heating cycle. The best separations were obtained when the wire power was on for 0.5 s and off for 0.5 s. The maximum temperature reached by the heating wire was noticeably less when using a pulsed heating cycle. This did not effect the width of the arsine peaks, and gave only a very slight visual improvement in separation of the MMA and DMA peaks.

Some slight changes were made to the timing of the analytical sequence, but in general the optimum conditions established for the manual method [144] were found to be satisfactory when applied to the semi-automated procedure.

3.4.3b Advantages of semi-automated HG-AFS

It has been suggested recently that on-site analysis using hydride generation techniques is difficult due to their complexity [101]. However, in the present work, WorkBenchMac™ has been used to control modified components and automate almost all the analytical steps required to routinely carry out a batch hydride generation analysis. Although this method is not sufficiently portable to be carried or used for small boat work, it can be used to perform on-site analyses on larger vessels with laboratory facilities and in basic laboratories located close to field work sites.

The most significant advantage of the semi-automated method is its ability to perform repetitive tasks, such as timing, in a reproducible manner. This represents a major improvement in the method's ease of use, particularly under rough shipboard conditions, where accurate and precise timing of events by an operator would be impossible.

The semi-automated HG-AFS method requires the presence of an operator, but the only tasks that have to be performed manually are sample preparation, starting the analytical sequence, changing the reaction vessel at the end of a sequence and topping up the liquid nitrogen dewar at the start of each sequence.

Apart from the convenience of automation in a difficult work environment, the semi-automated shipboard method showed a number of improvements over the original manual HG-AFS method. Performance characteristics such as accuracy, linearity and detection limit were unaffected, but the automated method gave improved and more consistent precision than the manual method. Using the manual method, the average precision for As(V+III) analysis performed in triplicate on fifty seawater samples was ~2.5% compared with ~1.5% for the same number of samples analysed using the automated procedure. This is because the timing of events in the analytical sequence was more consistent once automated.

Automation of the method also led to a reduction in the total analysis time for each sample from 6 minutes to 4 minutes 35 seconds. This is as a result of the operator being able to perform tasks such as sample preparation during rather than after the analytical sequence.

3.4.3c Field application

The automated method has been successfully used to perform shipboard As(V+III) and As(III) measurements in the Southern Ocean. However, because of time restrictions, MMA and DMA measurements were performed on return to the laboratory.

Fig. 3.11 shows an example trace for the shipboard determination of As(V+III) and laboratory-based determination of MMA and DMA in surface water at 42° S, 142° E. This trace illustrates the difference in concentration between total inorganic arsenic and the methyl species, and the need to determine them at two different instrument sensitivities. The results and further discussion of the method performance on this voyage will be presented in Chapter 4.

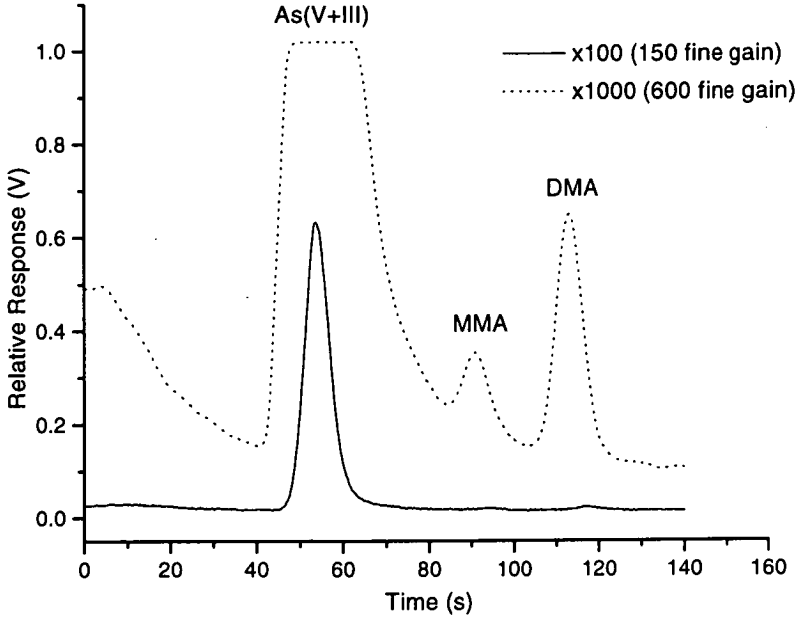

Figure 3.11 Chromatogram of As(V+III), MMA and DMA in surface waters of the Southern Ocean (42° S, 142° E), February 1998 (salinity 34.89; temperature 12.94 °C)

3.4.4. Conclusions

This section has described a semi-automated hydride generation method for the analysis of arsenic species in environmental samples. The method is sufficiently portable, robust and sensitive to be used for the shipboard analysis of arsenic species found in the open ocean environment. This means that there is minimal need to transport samples back to the laboratory for analysis, thereby avoiding speciation changes that might occur if samples are not stored satisfactorily during freighting.

Automation of the method results in a number of improvements that include, increased reproducibility, shorter analysis times, and improved ease of use because of reduced operator intervention. These improvements mean that the method is also equally useful as a laboratory-based instrument for analysis of samples from coastal waters.

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Chapter 4: Application of the HG-AFS Method to Arsenic Speciation in the Open Ocean

4.1 INTRODUCTION

As discussed in Chapter 1, total dissolved arsenic concentrations in open ocean seawater are in the range 1 – 2 $\mu g\ L^{-1}$ or 13 – 27 nM. In this chapter, to be consistent with most of the published data, arsenic concentrations will be expressed in nM (nmol L^{-1}), deviating from the units ($\mu g\ L^{-1}$) used in other parts of the thesis.

There are a number of arsenic sources to the open ocean. In surface waters these include inputs of dust from the atmosphere, arsenic derived from weathering and erosion brought to the sea *via* rivers, and upwelling of arsenic-rich waters from the deep ocean. There are also inputs of arsenic to the deeper waters from a number of sources including: hydrothermal activity [1], pore water infusions from the sediments [2, 3], and from anoxic brines [4]. It should also be noted that the association of arsenic with iron oxide phases is an important process in regulating arsenic concentrations in both the surface and deep ocean [5].

Much of the initial interpretation on arsenic cycling in seawater was published by Andreae in the late 1970's and early 1980's [6-8]. This work related to near-shore and open ocean samples collected from the southern Californian Bight and the northeast Pacific Ocean.

Andreae showed that, within the limits of experimental error, As(V), As(III), MMA and DMA are the only arsenic species found in open ocean seawater [8], despite the presence of both lipid and water-soluble non-hydride forming arsenic species in the cell fractions of marine phytoplankton. He concluded that the higher arsenic species were either short-lived outside the cell or that they were broken down in the cell to MMA and DMA before being excreted into the surrounding environment. In culture, the relative amounts of As(III), MMA and DMA produced were shown to vary considerably between phytoplankton species [6].

In the water column, arsenic was shown to exhibit distinctly different behaviour in the photic zone compared with deeper water. In the photic zone, all four arsenic species were found, while in the deep water essentially only As(V) was present. MMA and DMA were found only in the photic zone and at concentrations of less than 2 nM at all the open ocean sites. Their concentration was relatively constant above 30 – 40 m, but decreased rapidly to below the detection limit at 100 m. Both the methyl species

displayed similar profiles with depth, but the concentration of DMA was approximately an order of magnitude higher than that of MMA. In coastal waters, the MMA and DMA profiles correlated well with the indicators of primary production, chlorophyll *a* and ^{14}C-uptake [6]. In the waters of the Pacific Ocean, though this was not the case, and the methyl arsenic species did not correlate with a deep chlorophyll maximum [7]. The maximum, however, had little associated photosynthetic activity and the methyl arsenic species still correlated well with actual photosynthetic activity, measured as ^{14}C-uptake.

As(III) concentrations at the Pacific Ocean stations varied between 0.2 and 3 nM. The distribution of As(III) was found to be less regular than the methyl species and exhibited two different kinds of depth profile. One type of profile was the same as that observed for the methyl species, but in the other, As(III) increased at the bottom of the photic zone, reaching a maximum at about 200 m, before declining with depth. Although no differences in productivity could be found at the two types of site, the difference was thought to be caused by heterotrophic organisms [7]. The highest As(III) concentrations observed by Andreae were at those sites with lowest filterable reactive phosphate (here on referred to as phosphate) concentrations, where the As/P ratio was greater than 1. These observations were confirmed by culture experiments with marine coccolithophorids, which showed greatest conversion of As(V) occurring as phosphate depletion was approached [6].

As(V) was found to show little variation with depth, although it was depleted in the surface compared with the deep water. The extent of As(V) depletion at the surface was found to be a combination of both biological uptake and release and the rate of upward mixing of deep water with higher arsenic concentrations. The deep water of the Northeast Pacific was found to be quite uniform in As(V), with a concentration of *ca.* 24 nM. Andreae found that the transport of arsenic from the surface to the deep ocean with particulate matter was small compared with nitrate, phosphate and silicate bound in phytoplankton, and that it was regenerated at shallow depths because it was not readily retained by the cellular biomass.

The work of Statham *et al.* [9] in Cape Basin, in the South Atlantic, supports the finding of Andreae regarding As(V) cycling. They found that As(V) concentrations in the deep water were 21.2 nM, indicating only a small increase in the arsenic content of the deep water with water mass age compared with macronutrients such as phosphate. This is further supported by the findings of Middelburg *et al.* [10] which indicate slightly lower

deep water arsenic concentrations (18.3 – 20.3 nM) in the younger North Atlantic Deep Water (NADW) of the Northeast Atlantic compared with the South Atlantic. They calculate that the deep water $\Delta As/\Delta P$ is 2.5×10^{-3} from the Atlantic to the Pacific Ocean, indicating the reduced transport of arsenic from the surface to the deep water compared with phosphate. Further work by Cutter and Cutter [11] also supports these findings.

Much of the surface water cycling of arsenic in the Atlantic Ocean has been described by Cutter and Cutter from work in the eastern basins of the North and South Atlantic [12] and in the high latitudes of the North Atlantic [11]. In the eastern basins, four vertical profiles and a surface transect were collected between 31° N and 24° S. In this region, there is atmospheric deposition from North Africa and North America, and significant freshwater input from the Amazon and the Zaire Rivers.

Similar to Andreae, in this region, Cutter and Cutter found As(V+III) to be depleted in the surface compared with the deep water (*ca.* 20 nM). As found by Statham *et al.*, As(V+III) correlated poorly with phosphate for the whole data set, but good correlation was found when plotting vertical profiles of As and phosphate at each individual site. Surface distributions of arsenic were quite variable, but average concentrations were the same north and south of the equator, as found by Santosa *et al.* [13] for the North and South Pacific. Higher concentrations of arsenic were observed in the surface waters in regions where there was significant coastal upwelling.

No results were obtained for MMA and DMA as the concentrations in the water column were below the detection limit of the analytical method (0.4 nM). As(III) was also not detected, probably because the frozen samples had partially thawed during return to the laboratory [12].

In a second data set, from the North Atlantic [11], eight vertical profile stations and a surface transect were sampled between 54° and 68° N. In the higher latitudes, very little variation in As(V+III) concentration was found between the surface and deep water and the surface concentrations found (15.7 ± 1.3 nM) were higher than in lower latitudes of the North Atlantic. As(III) concentrations in the surface waters were *ca.* 0.45 nM, less than lower latitude waters [7], but similar to the values previously found by Middelburg *et al.* [10] in the high latitudes of the North Atlantic. DMA concentrations were *ca.* 0.14 nM (note the analytical method was more sensitive than that used previously [12]), which were also low compared with higher temperature, low nutrient waters of

the Pacific Ocean studied by Andreae [6]. In the cold waters of the Greenland icefields (-1.5 °C – 0.8 °C), DMA concentrations were below the detection limit.

Santosa *et al.* have conducted a number of cruises sampling mostly surface waters from the Pacific, Indian and Southern Oceans [13-15]. They found that As(V+III) increased with latitude from the Indian to the Southern Ocean along 110° E, particularly when crossing the Subtropical Convergence Zone at *ca.* 42° S. They also found that on crossing this zone, the proportion of total arsenic converted into As(III) and the methyl species decreases significantly, from 48% in the Indian Ocean to 2% in the Southern Ocean, where surface water concentrations of the arsenic species were found to be: As(V) = 13.3 nM, As(III) = 0.04 nM, MMA = 0.09 nM, DMA = 0.31 nM. In general, this dataset indicated that there was poor correlation between chlorophyll *a* and production of the methyl arsenic species between different oceanic environments, but that temperature is an important factor. A steep linear relationship was found between methylation and temperature in waters above 25 °C, but in cooler waters, the proportion of methylation was found to be essentially independent of temperature.

Santosa *et al.* [14] also found that greatest methylation of arsenic occurred in high phosphate environments close to continental land masses, in disagreement with the hypothesis that greatest arsenic uptake occurs in low phosphate environments [6]. They suggested that coastal and open ocean primary producers may employ different strategies toward available nutrients, with those in open ocean environments having a greater affinity for low nutrient conditions, and, therefore, best able to discriminate between arsenate and phosphate. In one sample, from the Tasman Sea at *ca.* 40° S, the authors found that MMA was the only methylated species produced, unlike all other sites where DMA was the dominant methyl species. They suggested that this was because of the different phytoplankton species present and speculated that DMA may be the dominant methyl species produced in low phosphate seawater, while MMA might be the dominant species in low silicate seawater.

In vertical profiles, this group found unusual behaviour in the southwest Pacific [14], where DMA was found at concentrations of 0.12 nM in the water column down to 5000 m and As(V+III) concentrations decreased slightly at depth, which was suggested to be a result of iron oxyhydroxide scavenging.

Aside from those made by Santosa *et al.* [13], few other arsenic measurements have been made in the Southern Ocean. Mentasti *et al.* [16] measured total arsenic concentrations in Ross Bay off the coast of Antarctica, in summer 1987/88 and found

surface water concentrations of 14.7 – 19.5 nM. An early paper by Sugawara *et al.* [17] reported As(V) and As(III) data for the Pacific, Indian and Southern Oceans. They found highest arsenic values in the Southern Ocean, and indicated that surface As(V) concentrations were 13.7 – 18.7 nM north of the Subtropical Convergence and 15.3 – 22.5 nM to the south. They also quoted As(III) values of *ca.* 3 nM in surface waters, which were approximately 100 times higher than those measured more recently by Santosa *et al.* [13].

A summary of arsenic concentrations in different oceanic regions is given in Table 4.1.

A variety of sampling and analytical protocols have been used for the analysis of arsenic in seawater. Most papers report data for samples which have been collected without using clean techniques, except Cutter and Cutter [11, 12] who report using 'trace-metal-clean' techniques. Little comment has been made in the literature about the potential for contamination with arsenic, except by Andreae [6] who indicated that the results obtained for three different types of sampling device were the same within the limits of experimental error.

Another difference between many of the protocols is whether or not samples are filtered. Andreae [6] deals comprehensively with this issue, and finds no difference within experimental error between filtered and unfiltered samples.

Sample storage is where the greatest difference lies between the techniques of different groups. For As(III), Andreae suggests that concentrations are stable for up to 10 days if the sample is kept cool and stored in the dark. For longer storage, however, the sample must be quick-frozen [7] and stored at a low enough temperature to prevent brine formation, otherwise spurious results are obtained, as reported by Cutter and Cutter [12] for As(III) samples which thawed during transportation. Andreae reported that samples for the methyl arsenic species must be acidified otherwise they are converted to As(V) upon storage. Meanwhile, Santosa *et al.* [14] have reported that no speciation changes occurred for samples that were filtered and frozen.

A number of analytical methods do not require speciation to be preserved in the original sample. Statham *et al.* [9] generated the arsines from samples onboard ship and trapped them in KI solution, in which they were reported to be stable for up to six months. Middelburg *et al.* [10] have used a similar procedure, where the arsines were generated onboard ship and trapped onto activated carbon which was then stored for neutron activation analysis (NAA). However, trapping the hydrides for NAA has the disadvantage of being unable to determine the methyl arsenic species.

Table 4.1 Summary of arsenic concentrations reported in the open ocean environment

Reference	Author(s)	Location	Surface As(III) concentration	Surface As(V) concentration	Deep water As(V) concentration	Surface MMA concentration	Surface DMA concentration
[6-8]	Andreae	NE Pacific	0.1 – 11.6 nM	10-30% depletion compared with deep	24 nM	0 – 2 nM	0 – 2 nM
[9]	Statham *et al.*	S Atlantic (Cape Basin)	0.2 – 3 nM	17.6 – 22.8 nM	17.6 – 23.8 nM	0 – 1.5 nM	0 – 1.5 nM
[10]	Middelburg *et al.*	NE Atlantic	0.05 – 4.3 nM[a]		18 – 20.3 nM	nd	nd
[12]	Cutter and Cutter	E Atlantic (N-S Transect)	nd	12.9 ± 1.8 nM	*ca.* 20 nM	<dl (dl = 0.4 nM)	<dl (dl = 0.4 nM)
[11]	Cutter and Cutter	N Atlantic (high latitude)	*ca.* 0.45 nM	15.7 ± 1.3 nM	17.6 ± 2.0 nM		Av. 0.14 nM[b]
[13-15]	Santosa *et al.*	E Indian Ocean	3.1 nM	6.0 nM		0.4 nM	0.7 nM
		N Indian Ocean	2.6 nM	6.3 nM		0.4 nM	2.3 nM
		Southern Ocean	0.04 nM	13.9 nM		0.09 nM	0.31 nM
[16]	Mentasti *et al.*	Antarctic Coast (Ross Bay)	nd	14.7 – 19.5 nM	nd	nd	nd
[17]	Sugawara *et al.*	Southern Ocean			nd	nd	nd
		(North of STC)		13.7 – 18.7 nM			
		(South of STC)		15.3 – 22.5 nM			

[a]As(III) includes MMA and DMA [b]this is methyl As (MMA+DMA) nd - not determined <dl – below detection limit

Finally, Cutter and Cutter [11] reported the use of a shipboard method to make measurements of As(III) and As(V+III), requiring preservation of only the methyl arsenic species. Michel *et al.* [18] also report using a shipboard method to avoid sample speciation changes.

This chapter presents the results of arsenic measurements from two oceanographic cruises. It is not intended to be a rigorous analysis of the behaviour of arsenic in the open ocean, however, some interpretation of the results is given. The main purpose of both cruises was to contribute to the development of a reliable, reproducible and robust shipboard method for determining the low concentrations of arsenic species found in a variety of seawater matrices.

The first set of data presented is from an Intergovernmental Oceanographic Commission (IOC) Baseline Contaminant survey in the western basin of the Atlantic Ocean. These samples were analysed as an interlaboratory comparison exercise for the analytical method. A particular focus of this work was to compare the results obtained for MMA and DMA, as there are currently no standard reference materials for these species in seawater.

The second set of data is from an Australian Joint Global Ocean Flux Study (JGOFS) biogeochemistry cruise in the Subantarctic Zone of the Southern Ocean. The shipboard method described in Chapter 3 (section 3.4) was taken on this voyage and tested to determine its ability to make reliable arsenic measurements in a shipboard environment.

4.2 IOC CRUISE

4.2.1 Introduction

The Intergovernmental Oceanographic Commission (IOC), is an UNESCO organisation which organises the Contaminant Baseline Survey programme to determine the concentration and distribution of trace elements and synthetic organic compounds in the major water masses of the world's oceans. As well as providing current baseline concentrations of potential contaminant species, these surveys also enable intercomparison of sampling techniques and analytical methods [19], and insights into the biogeochemical cycling of these species.

The third IOC baseline contaminant survey was conducted in the tropical and subtropical region of the western Atlantic Ocean, complementing the previous baseline surveys in the east [20] and North [21] Atlantic.

Samples were obtained from the third IOC contaminant baseline survey, in the western Atlantic Ocean. The samples were collected from both vertical profiles and a surface transect and represented a range of oceanographic conditions and water masses.
The primary purpose of collecting these samples was to conduct an interlaboratory comparison to assess the accuracy of the manual HG-AFS method (Chapter 3, section 3.3) using the standards selected in Chapter 2 for calibration. Although the IOC programme has previously been used to conduct interlaboratory comparisons for trace elements [19], arsenic species were not included. The intercomparison was performed with particular attention to the methyl arsenic species, for which the standards have been shown to be cross-contaminated (Chapter 2 and [22, 23]), and currently there are no certified reference materials in seawater. As the samples collected were from a range of different oceanographic environments, it was possible to assess whether variations of nutrient concentrations and salinity could interfere with the arsenic measurements.

The arsenic measurements were made completely independently using two different methods and independent standards. The shipboard method, applied to unpreserved samples, employed hydride generation followed by separation on a GC column with photo-ionisation detection (HG-GC-PID) [24]. In this work, analyses were conducted by HG-AFS on samples from the same location which had been preserved by acidification and stored.

4.2.2 Experimental

4.2.2a Sample collection

Samples were collected from the US Research Vessel *Knorr*, which departed Montevideo, Uruguay on 18 May 1996 and arrived in Bridgetown, Barbados on 20 June 1996. The sampling stations are shown in Fig. 4.1.

Six vertical profile stations were occupied and underway-surface samples were collected twice daily while steaming between stations, starting at the southern-most station, IOC-10. Samples for arsenic analysis were collected only at five of the six IOC sites. These were: IOC-10 (33.0° S, 40.00° W), IOC-8 (17.0° S, 25.00° W), Romanche Fracture Zone (0.59° S, 20.03° W), Amazon 1 (5.72° N, 48.09° W), and Amazon 2 (6.49° S, 48.80° W).

Samples used for standard hydrographic measurements were collected from a plastic-coated General Oceanics rosette with twelve 10-L Niskin bottles. Samples for arsenic analysis were collected using contamination-free procedures. A 5600-m Kevlar

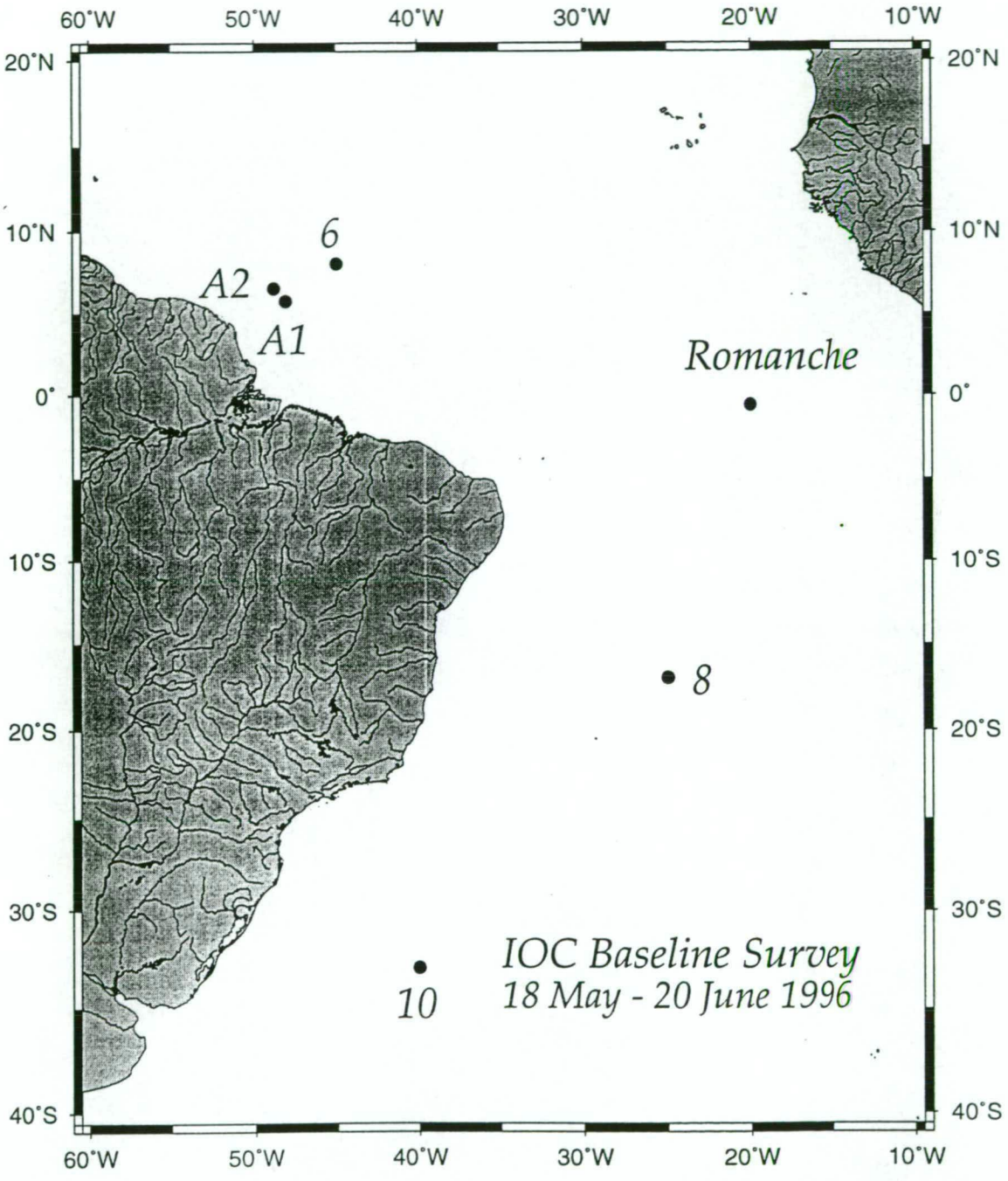

Figure 4.1 Map showing the location of vertical profile station on the IOC Baseline Contaminants Cruise, 16 May – 20 June 1996.

hydrographic cable was used and the Go Flo bottles were tripped with plastic messengers. Both 12-L and 30-L Go Flo bottles were used for sampling, with samples from the 30-L bottle being filtered through 0.4-μm filters before storage or analysis. All sample processing was performed in portable clean laboratories.

Underway surface samples were pumped by peristaltic pump into the clean container and were left unfiltered.

4.2.2b Sample treatment and storage

Samples for analysis by HG-AFS were collected into 250-mL HDPE Nalgene bottles. The bottles had been cleaned by soaking for one week in 2% (v/v) Extran (BDH Chemicals, Kilsyth, Victoria, Australia), followed by rinsing with distilled water, then soaking for one week in 10% (v/v) HCl (Ajax Chemicals, Auburn, NSW, Australia), followed by a thorough rinse with Milli-Q water. The bottles were stored individually in polyethylene bags until use.

Filtered or unfiltered samples were collected into the bottles in the clean container. The samples were acidified to pH 1.6 with HCl and refrigerated until the end of the cruise. The samples were subsequently shipped unrefrigerated to the USA and then sent to Australia (also unrefrigerated) by three-day air express. Upon arrival the samples were stored at 4 °C until analysis (several months).

4.2.2c Analytical methods used for arsenic analysis

i) HG-AFS method

The samples were analysed within six months of arrival using the manual HG-AFS described previously in Chapter 3, section 3.3.

ii) HG-GC-PID method

HG-GC-PID was used for the shipboard analysis of samples during the cruise. Samples for As(III) and As(V) were analysed by the method of Cutter *et al.* [24], which has previously been described in Chapter 3, section 3.2.2d. An identical method was used for analysis of the methyl species, except that an OV-3 GC column replaced the Carbopack B HT column. The detection limit using this method is 0.8 ng L^{-1} and the precision 3% at 2 ng L^{-1} and 0.5% at 70 ng L^{-1}.

4.2.2d Additional analyses

At each vertical and surface transect site temperature, salinity, phosphate, nitrate+nitrite, silicate, dissolved oxygen and chlorophyll *a* were also determined. These results are presented in Appendix 4.

4.2.3 Results and discussion

4.2.3a Laboratory intercomparison

It should be noted that the laboratory intercomparison is not a strict intercalibration exercise because each laboratory used samples that had been stored in different ways and for varying lengths of time and also used independent calibration standards and calibration methods. Only data for the three oceanic stations (IOC 10, IOC 8, and Romanche Fracture Zone) and the underway-surface samples are presented here. In all cases results have only been presented where both laboratories analysed the same sample. Samples analysed using only one of the methods have been excluded from this section.

For several reasons samples from the two Amazon plume stations have been excluded from the laboratory intercomparison. Firstly, it is known that in the Amazon plume significant removal of inorganic arsenic occurs through efficient scavenging into iron oxyhydroxides [3, 25]. This is advantageous as it results in lower arsenic concentrations at these stations, and increases the range of concentrations investigated in the intercomparison. However, the potential for storage artifacts (*i.e.* arsenic remobilisation from dissolved colloids upon acidification and storage) coupled with the small number of samples analysed using both analytical methods, meant these sites were excluded from comparison. Also, no comparative methyl data are presented for these sites, as at Amazon 1 there were no methyl species determined by HG-GC-PID, and at the second site (Amazon 2), there were only three common data points.

As there was a large amount of experimental data, with considerable scatter, to compare, a simple comparison of the plotted profiles was undertaken.

i) Filtered vs unfiltered samples

Although both filtered and unfiltered samples were collected, any differences between the two were unable to be distinguished from experimental error, especially as none were collected at corresponding depths. A typical plot of As(V+III), MMA and DMA in filtered and unfiltered samples from the Romanche Fracture station is shown in Fig. 4.2.

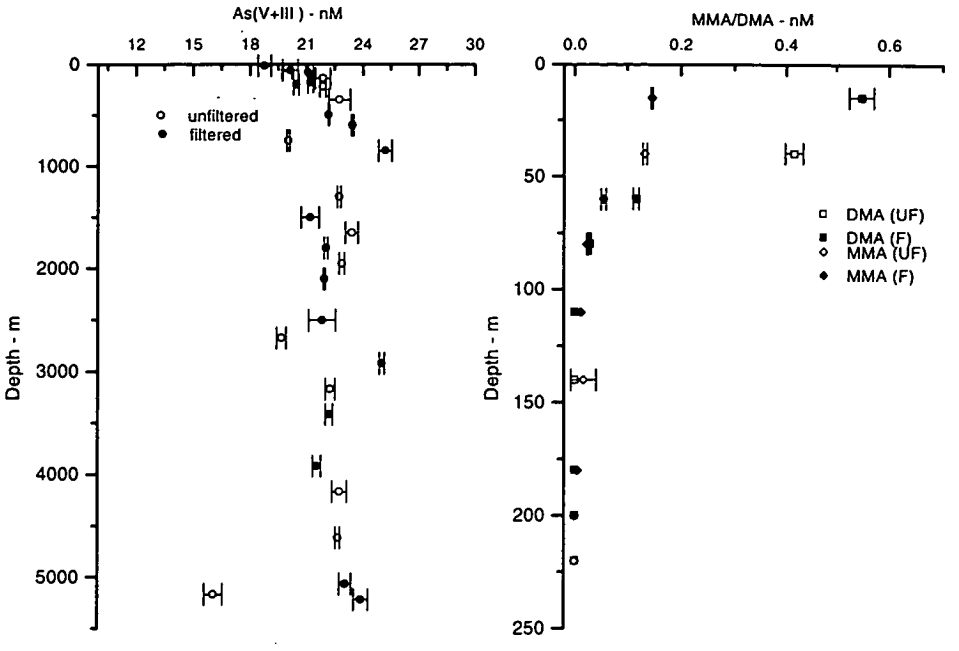

Figure 4.2 Vertical profiles of As(V+III), MMA and DMA at the Romanche Fracture Station in filtered and unfiltered samples. Measurements were performed on stored samples using HG-AFS.

ii) As(V+III) measurements

Plots comparing As(V+III) data obtained using the two analytical methods are shown in Figs. 4.3 and 4.6 (surface underway data). In general, there is a small difference between the HG-GC-PID values and those obtained using HG-AFS, even in deep waters where regenerative effects would be expected to be negligible. Considering the interbasin fractionation of arsenic with respect to phosphate in the deep water masses with age (*i.e.* between the Atlantic and Pacific Oceans), and also previous measurements in the Atlantic Ocean [10, 11], the HG-AFS results are a higher than expected. An offset was calculated as the average difference between the As(V+III) concentrations obtained using each analytical method for samples collected at depths below 1000 m, thereby excluding any potential regenerative effects. A small but significant difference of 2.8 ± 1.6 nM was calculated. In the surface transect the offset is less significant (2.3 ± 1.7 nM), but it is much more difficult to compare the results from the surface where biological and chemical processes are more significant.

These measurements were made early in the development of the manual HG-AFS method prior to analysis of the open ocean certified reference material (CRM), NASS-4.

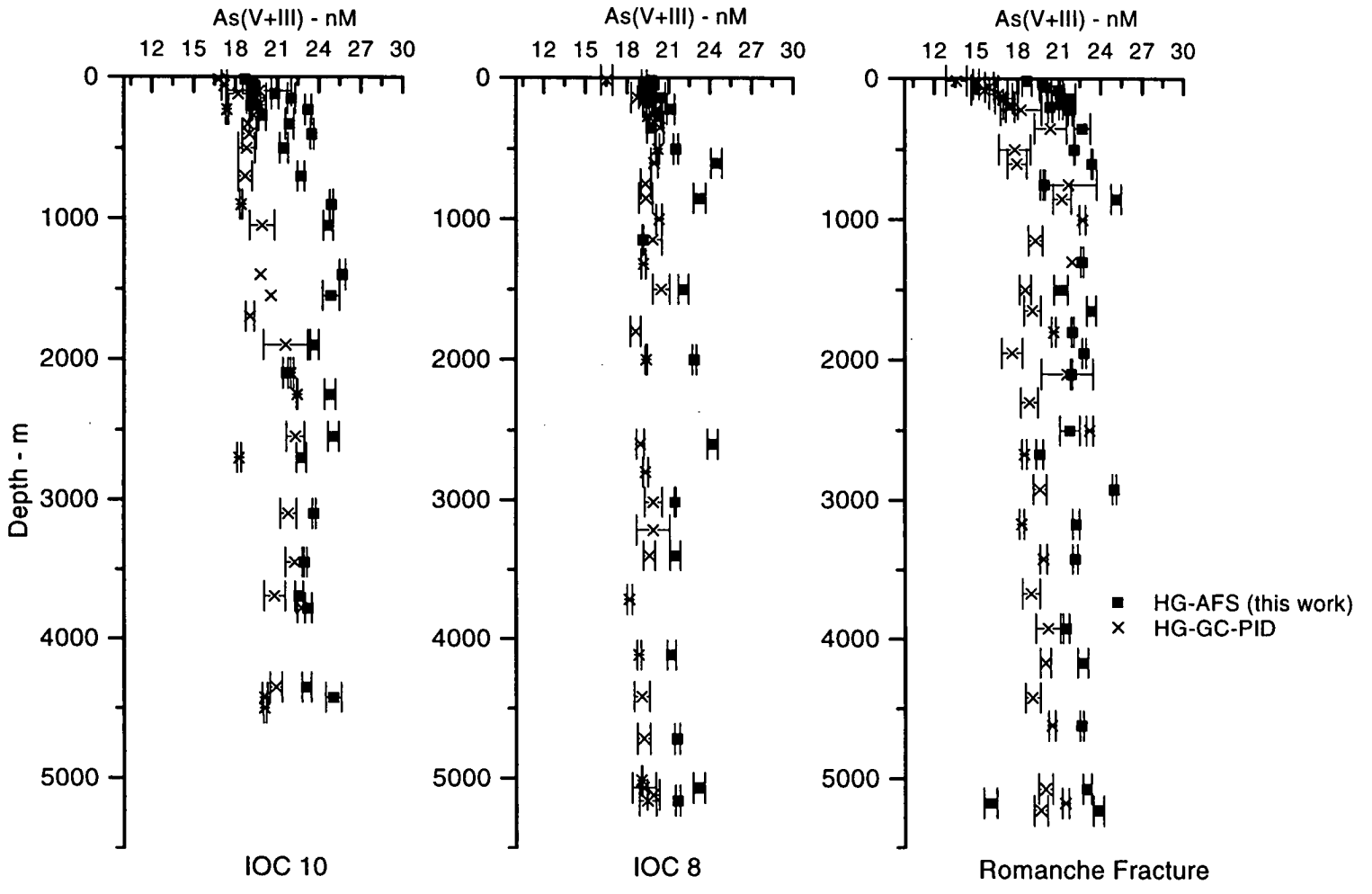

Figure 4.3 Comparison of As(V+III) concentrations, measured using HG-AFS and HG-GC-PID, in vertical profiles from IOC 10, IOC 8 and Romanche Fracture Zone stations in the Atlantic Ocean

Later analyses of this CRM indicated no problem with the accuracy of the HG-AFS method when analysed on several different occasions.

Further investigations revealed that there was an arsenic blank in the acid used for acidification of the stored samples [26]. Therefore, the most probable explanation for the offset is a blank from the acid used to preserve speciation in the stored samples, but not used in the shipboard samples. Because of the time elapsed it was impossible to check this and therefore an interlaboratory comparison of As(V+III) obtained using HG-AFS and HG-GC-PID cannot be considered. Fortunately the accuracy of As(V+III) analyses was able to be confirmed by the analysis of a certified reference material and the intercomparison for methyl arsenic species was unaffected by the inorganic arsenic content of the acid used for sample preservation.

In general, despite the offset, both the surface and vertical As (V+III) profiles show similar trends. An exception is the IOC-10 profile. In this case, the As(V+III) profile using the HG-AFS data is similar to that for phosphate (all other vertical profiles mirror phosphate), but this is not found for the HG-GC-PID data. Reasons for this are unclear, but perhaps reflect a slower rate of regeneration of arsenic from sinking biogenic material at this site.

iii) Methyl arsenic species measurements

As stated in the original aims, the main reason for conducting a laboratory intercomparison was to assess the accuracy of MMA and DMA determinations using the HG-AFS method. The reasons for this were twofold: firstly there are currently no commercially available certified reference materials and secondly, there is a problem sourcing species-pure MMA and DMA standards.

The results comparing DMA concentrations in the vertical profiles and surface transect are shown in Figs. 4.4 and 4.6 respectively. Given that one set of samples had been stored and also that different standards were used, the two analytical methods show remarkably similar concentrations and features in the horizontal and vertical profiles.

Results for MMA analyses are shown in Figs. 4.5 (vertical profiles) and 4.6 (surface underway samples). For this species, neither the concentrations nor the general features of the profiles match well, prompting speculation that a storage artifact is involved.

In general, the concentrations of MMA found are lower using the HG-AFS method than HG-GC-PID. This is particularly the case for the surface sample in the vertical profiles *i.e.* either the surface peak is not being detected by HG-AFS, or has degraded upon storage. Similar observations are made for the surface transect, with HG-AFS

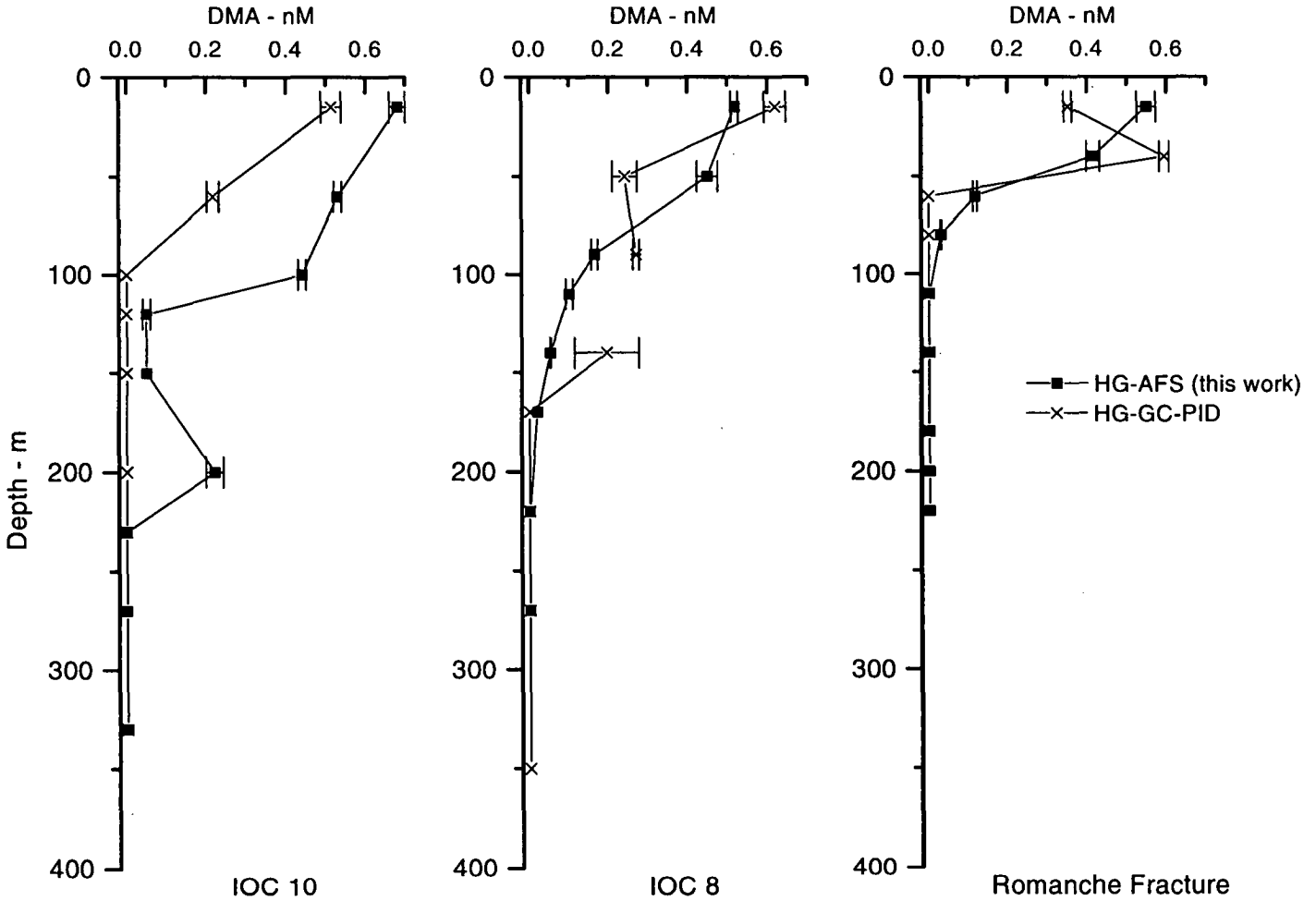

Figure 4.4 Comparison of DMA concentrations, measured using HG-AFS and HG-GC-PID, in vertical profiles from IOC 10, IOC 8 and Romanche Fracture Zone stations in the Atlantic Ocean

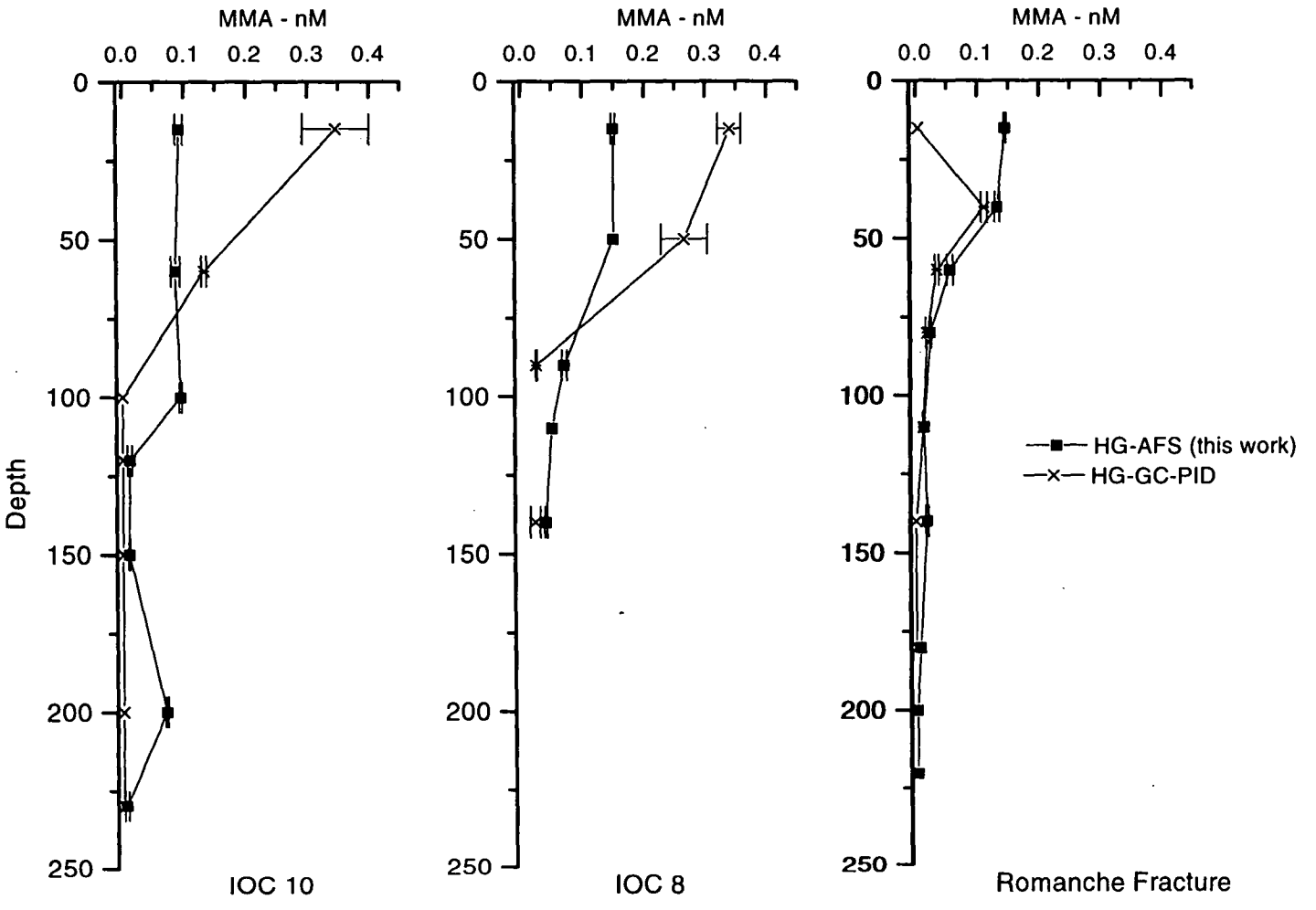

Figure 4.5 Comparison of MMA concentrations, measured using HG-AFS and HG-GC-PID, in vertical profiles from IOC 10, IOC 8 and Romanche Fracture Zone stations in the Atlantic Ocean

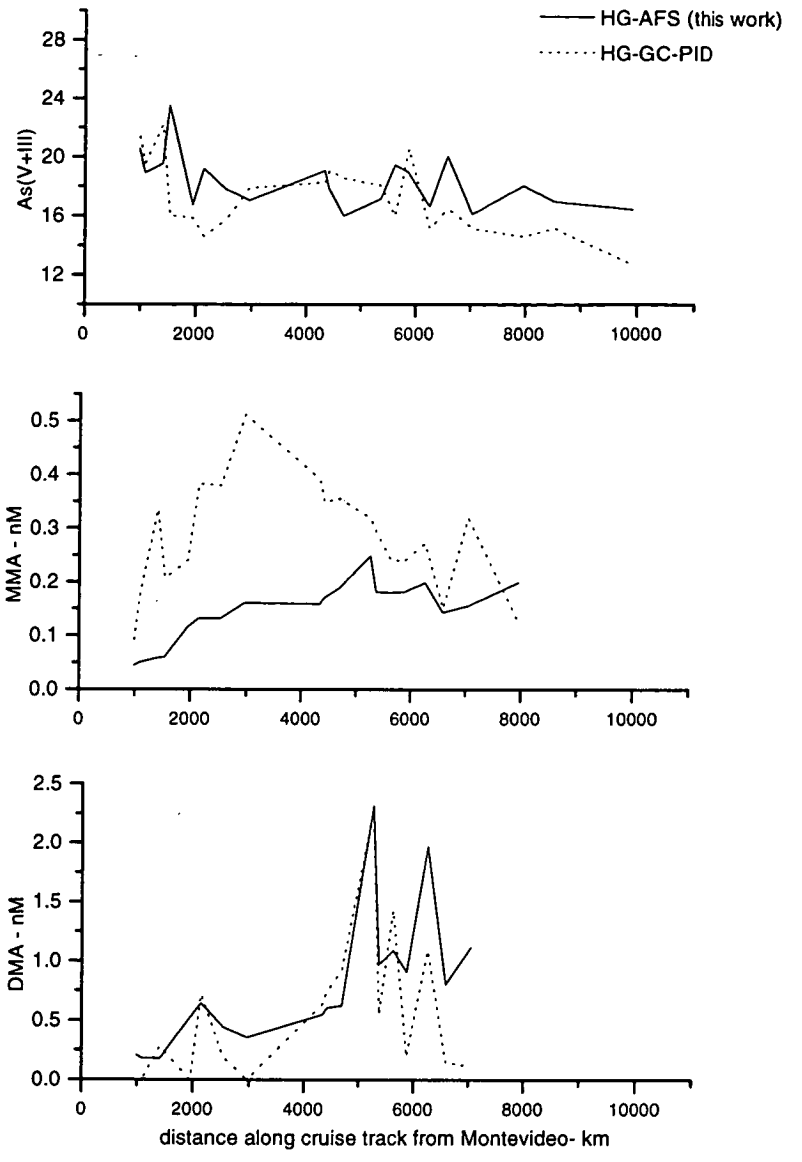

Figure 4.6 Comparison of arsenic species measurements made using HG-AFS and HG-GC-PID on samples from a surface transect in the Atlantic Ocean

consistently giving lower results. This does not appear to be a function of the analytical standard, as the surface data do not even follow the same trends as the shipboard HG-GC-PID measurements. It is suspected that this difference is a result of either biotic or abiotically mediated MMA degradation during storage, although the inconsistent nature of the offset may be suggestive of biological degradation. It is impossible to tell if MMA has degraded to As(V+III) in the samples though, as MMA concentrations are so low that the differences between the shipboard and stored MMA concentrations are smaller than the experimental precision of the As(V+III) measurements.

Unfortunately, planned analyses by HG-GC-PID on stored samples from the cruise have yet to be performed. When these results become available they will be able to confirm whether or not MMA has degraded upon storage. In the meantime, priority should be given to determining concentrations of the methyl arsenic species as soon as possible after sample collection.

4.2.3b Data interpretation

Given the uncertainty associated with the As(V+III) results, any discussion of this species will be limited to the trends observed. Also, in the absence of the HG-GC-PID data for MMA in stored samples, little more can be resolved for this species. Therefore, while no further interpretation of the data will be given here, the uncorrected HG-AFS arsenic profiles, some further hydrography data and a brief interpretation of the trends along the surface transect are provided in the Appendices.

Appendix 3 shows the potential temperature-salinity diagrams, taken from Cutter and Measures [27], for the upper water column and deep waters at each of the four oceanic stations occupied during the IOC cruise. Appendices 4.1 – 4.5 presents the As(V+III) depth profiles at IOC 10, IOC 8, the Romanche Fracture Zone and Amazon 1 and 2 plotted with the corresponding nutrient data. Appendix 4.6 presents the MMA and DMA profiles at Amazon 1 and Amazon 2, while in Appendix 4.7, all of the arsenic species, nutrients, salinity and chlorophyll *a* are plotted as a function of the distance travelled from Montevideo along the voyage track. Appendix 4.8 provides a brief interpretation of the trends in the surface transect shown in Appendix 4.7.

4.3 SUBANTARCTIC ZONE (SAZ) BIOGEOCHEMISTRY CRUISE

4.3.1 Introduction

The Southern Ocean is the oceanic region bounded by the Antarctic continent to the south, and the Subtropical Convergence Zone (STCZ), a permanent frontal zone, to the north. The position of the Subtropical Convergence Zone varies seasonally, but is generally found between 38 and 40° S. The Southern Ocean can be divided into broad regions which are separated by oceanic fronts. The fronts are regions in which there are strong temperature and salinity gradients [28]. These complex hydrological characteristics drive processes such as upwelling and downwelling, thereby often bringing together water masses which independently do not favour phytoplankton growth, but together may result in optimal conditions [29].

As stated above, the Subtropical Convergence Zone marks the northern boundary of the Southern Ocean at around 40° S. This is a region where Subtropical waters mix with Subantarctic water over a broad zonal band. South of the STCZ between 40 and 50° S is a region known as the Subantarctic Zone (SAZ), which is bounded to the south, at about 50 – 51° S, by the Subantarctic Front (SAF). South of the SAF is the region known as the Polar Frontal Zone (PFZ) which has the Polar Front (PF), located at about 54° S, as its southern boundary. Between the PF and the Antarctic continent is a region known as the Polar Zone (PZ) which extends south to the Seasonal Sea Ice Zone (SSIZ) [30].

The entire Southern Ocean is a high nutrient-low chlorophyll (HNLC) area. This means that while there is low growth of algal communities, it is not as a result of nitrate or phosphate limitation. A number of theories exist to explain the low chlorophyll concentrations and these include: the presence of high winds (deep mixing), low light and low iron (an enzymatic cofactor in diatoms for the metabolism of nitrogen [31]). Differences in the nutrient distribution and algal communities do exist in the different oceanic environments of the Southern Ocean, with the fronts often acting as major boundaries [32]. Macronutrients generally increase going south. Nitrate and phosphate concentrations increase strongly across the SAF, however, silicate does not increase until south of the PF. Chlorophyll *a* generally decreases heading south, but increases again after crossing the PF. These changes in nutrient distribution are also reflected in the algal communities, with coccolithophorids only found north of the SAF and diatoms dominating the region south of the PF.

The region being studied in this case was that found between the Subtropical Convergence Zone and the Polar Front, southwest of Tasmania, which has previously been characterised as having low algal growth rates and biomass [33]. This work was a piggyback project carried out as part of the Australian Joint Global Ocean Flux Study (JGOFS) program. The overall focus of this biogeochemical study was to determine the factors regulating phytoplankton production in the Subantarctic Zone and therefore, the control over carbon transfer from the atmosphere to the ocean surface and ultimately the deep ocean.

Within the boundaries of the overall JGOFS project there were three objectives for the current study.

The first objective was to trial the semi-automated HG-AFS method, previously only used in the laboratory, under shipboard conditions, and to make arsenic measurements at sea. It was also intended to compare the results for samples collected under trace-

metal-clean conditions with those obtained from the standard CTD cast, and assess the possibility of contamination. Thirdly, there have been very few measurements of arsenic, particularly MMA and DMA, made previously in the Southern Ocean, with the exception of those by Santosa *et al.* [13, 14] mentioned in the Introduction (4.1). Therefore, the intention was to measure arsenic species in the four distinct oceanic environments of the Subantarctic Zone, and to relate the concentrations found to the physical, chemical and biological conditions. Of particular interest was, to determine whether production of methyl arsenic falls to a background level in waters below 12 °C, as has been suggested for North Atlantic and British coastal marine systems [34, 35] and to investigate whether MMA is the dominant arsenic species in waters where silicate is limiting [14]. Methylation of arsenic is often thought to occur at appreciable levels in seawater when concentrations of its chemical analogue, phosphate, limit phytoplankton growth, and it becomes more difficult for organisms to discriminate between arsenate and phosphate. The Subantarctic Zone presents a unique environment where neither phosphate or nitrate concentrations are limiting, however, phytoplankton growth is thought to be limited by low concentrations of iron and also silica [36].

4.3.2 Experimental

4.3.2a Southern Ocean samples

Seawater samples were obtained on a voyage of the RSV *Aurora Australis* in the Southern Ocean between 28 February and 1 April 1998 (austral autumn). A map of the region and sampling sites is shown in Fig. 4.7. Surface samples were collected every degree of latitude along the transect 42 - 55° S, 141° 30' E, which passed through four different oceanic regions, namely, the Subtropical Convergence Zone (STCZ), Subantarctic Zone (SAZ), Subantarctic Front (SAF) and Polar Frontal Zone (PFZ).

Temperature and salinity contour diagrams used to identify the position of the fronts are shown in Appendix 5.

In addition, one vertical profile was collected in each oceanic environment at: 42° S, 142° E (STCZ), 47° S, 142° E (SAZ), 51° S 143° 30' E (SAF) and 54° S 142° E (PFZ). To check the possibility of contamination, samples were collected from a shallow CTD cast and a trace-metal-clean Kevlar cast at 53° S, 142° E, and the results compared.

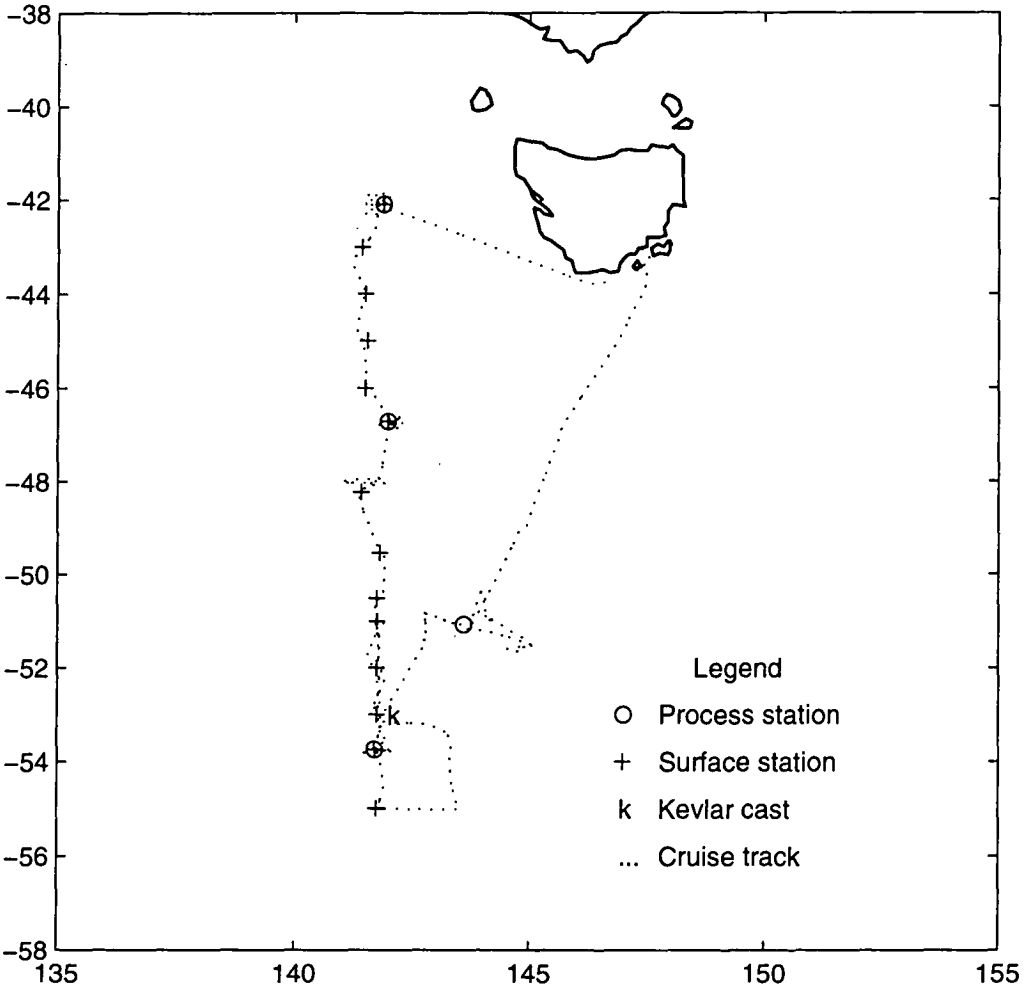

Figure 4.7 Map showing the location of vertical profile and surface transect sites on the Subantarctic Zone Biogeochemistry Cruise, March 1998.

For the standard CTD casts, seawater was sampled using 10-L Niskin bottles. Sub-samples for arsenic analysis were collected into 250-mL HDPE Nalgene bottles (Nalgene, Rochester, NY, USA) which had previously been soaked in 10% (v/v) HNO_3 and rinsed with Milli-Q water. Each bottle was rinsed twice with sample before filling.

A detailed description of the sampling and sample processing protocol for the Kevlar cast can be found in a recent paper by Sedwick *et al.* [37]. The samples for arsenic were collected as described above, but stored individually wrapped in polyethylene bags.

4.3.2b Analytical methods used for arsenic analysis

Shipboard analyses were carried out for As(III) and As(V+III) using the semi-automated HG-AFS method described in Chapter 3, section 3.4. Because of time limitations, methyl arsenic analyses were unable to be completed on board. Samples for MMA and DMA analysis were preserved by acidification to pH 1.4 with HCl (Mallinkrodt, AR Select, St Louis, MO, USA) followed by storage at 4 °C, as described by Andreae [22].

Method blanks were performed during the voyage in the usual sampling area, by pouring Milli-Q water, from storage containers, into clean sample bottles, and acidifying and storing them as for the CTD samples.

4.3.2c Additional analyses

At each surface transect and vertical profile station temperature, salinity, filterable reactive phosphorus (phosphate), nitrate+nitrite (referred to from here on as nitrate), filterable reactive silicon (referred to from here on as silicate) and dissolved oxygen were also determined. Further analyses presented here also include chlorophyll *a*, algal pigments, modelled primary productivity, flow cytometric identification of phytoplankton groups and electron transport system (ETS) activity measurements, an indicator of biomass respiration.

4.3.3 Results and Discussion

4.3.3a Features of the shipboard method

The final dimensions of the semi-automated instrument were 2200-mm (l) x 600-mm (w) x 850-mm (h), compact enough to fit across the width of a laboratory container. Some further space was required, however, for the hydrogen generator [330-mm (l) x 270-mm (w) x 850-mm (h)] and argon and air (if required) cylinders.

The relatively short analysis time of the semi-automated procedure allows *ca.* 12 samples to be analysed per hour in practice. This translates into the ability to analyse samples for a full 24-bottle cast in triplicate in a 12-hour period.

Another advantage of the automated method was that it consumed less liquid nitrogen. This was partly because there was less evaporation during the shorter analysis time, but also because the Nichrome heating wire was being heated less and therefore evaporated less liquid nitrogen when it was cooled during the next analytical sequence. Minimising liquid nitrogen consumption is important, as it is often difficult to store the volumes required for a long voyage.

4.3.3b Shipboard performance of the semi-automated method

The semi-automated method was used during the one-month voyage. The conditions at sea represented a significant test of the method, the Southern Ocean being characterised by low productivity, and therefore, low concentrations of As(III) and the methyl species, combined with rough conditions to challenge instrument operation.

At sea, neither the semi-automated method nor operation of the hydrogen generator was affected by the ship's motion. The method was used successfully to perform shipboard As(V+III) and As(III) measurements in the Southern Ocean. However, because of time restrictions, MMA and DMA measurements were performed on return to the laboratory. As stated in Chapter 3, the difference in concentration between total inorganic arsenic and the methyl species, required that they be determined separately at two different instrument sensitivities.

During the voyage, the As(III) concentrations measured were too low to be reliably quantified. As(III) was only observed in samples collected north of the Subantarctic Zone (SAZ), located at *ca.* 47° S, and the concentration of As(III) was less than 0.04 nM at all sites. Despite being unable to quantify As(III) on this occasion, the sensitivity of the method is still considered sufficient for the study of arsenic in open ocean environments, as the concentration of As(III) found in the Southern Ocean is considerably lower than in other parts of the world's ocean [13] (See Table 4.1).
As discussed in Chapter 3, there is also potential to further increase the method sensitivity through a small modification of the glassware design, allowing use of larger sample volumes.

4.3.3c Comparison with a trace-metal-clean cast

A small set of samples was collected to compare the results obtained for inorganic arsenic analyses from a standard CTD cast and from a trace-metal-clean Kevlar cast. These samples were only analysed for As(V+III), as contamination of the samples by MMA and DMA was considered highly unlikely. Although the small number of samples collected do not provide a rigorous comparison of the two sampling techniques, they can be used to demonstrate that there is no appreciable contamination problem associated with the standard CTD cast. A vertical profile of the As(V+III) data from both casts is shown in Fig. 4.8.

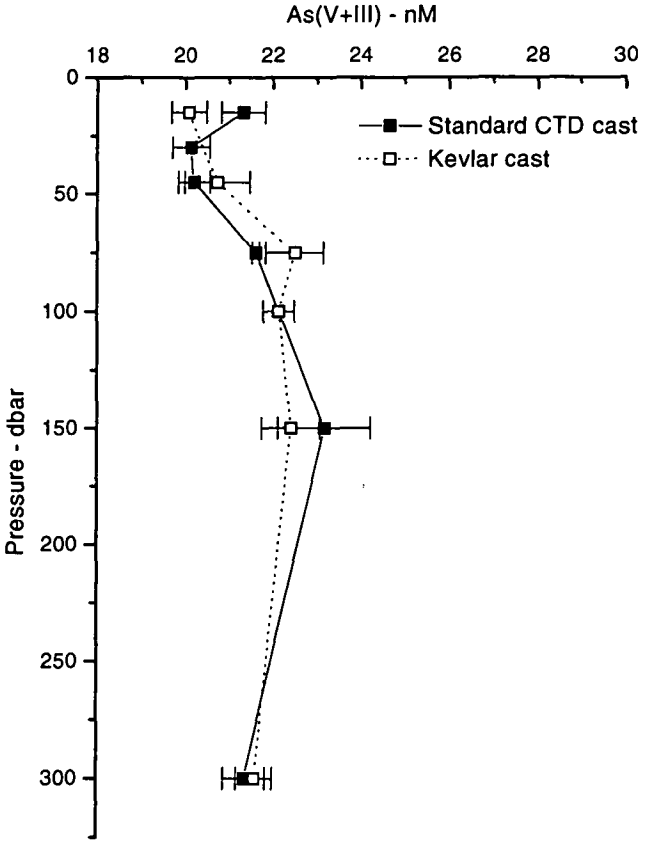

Figure 4.8 Vertical profiles of As(V+III) with depth from a standard CTD cast and a Kevlar cast at *ca.* 53° S, 142° E

The error bars represent one standard deviation, where each analysis has been performed in triplicate on a single sample. In all cases, except the sample at 15 m, the results are in good agreement. Therefore, within the bounds of experimental error, sampling from the standard CTD cast does not appear to represent a source of contamination. The slight difference between the two values obtained at 15 m may be

because the two casts were performed about four hours apart and the sea surface conditions were quite rough at the time of sampling.

Analysis of method blanks collected on the voyage also confirmed that the bottles and acid used for sample storage and preservation did not constitute a blank source.

4.3.3d Southern Ocean transect

i) Hydrochemistry

The general trends in temperature, salinity, dissolved oxygen, phosphate, nitrate and silicate along the surface transect are shown in Fig. 4.9.

Similar trends are observed for salinity and temperature, and both decrease heading south. Because sampling was conducted during late summer conditions, neither the SAF nor the PF are particularly distinct at the surface, but are identified from temperature isotherms below the mixed layer. The mixed layer depth is 90 – 100 m in the SAZ, but increases to 110 m in the SAF, before decreasing to *ca.* 80 m in the PFZ.

Phosphate, nitrate and silicate are plotted on the same scale in Fig. 4.9. All three species tended to increase heading south along the transect line. Presenting the nutrient data in this way it is clear that both phosphate and nitrate occurred at higher concentrations than silicate, and that neither were depleted at the surface. Silicate concentrations were low north of the SAF, and can be considered limiting (<3 μM) north of 47° S [36].
The concentrations increased in the PF, but are still depleted in the mixing zone between the SAF and the PF.

Plots showing the variation of temperature, salinity, phosphate, nitrate, silicate and dissolved oxygen at the vertical profile sites are included in Appendix 6 (6.1 – 6.6).

The vertical profile site at the STCZ had a complex structure in the euphotic zone, because of the interleaving of high salinity subtropical water with lower salinity Subantarctic waters over a broad zonal band. The upper water column is weakly stratified with a well-defined pycnocline at 60 – 90 m.

The vertical profile at the SAZ site is characterised by a shallow surface mixed layer (20 – 50 m), with a second pycnocline at *ca.* 100 m. The salinity below the pycnocline is characteristic of Subantarctic waters (34.5 – 34.6).

Above the pycnocline at the SAF the water column was well mixed, but below this it was dominated by sharp salinity and temperature gradients. Similarly, at the PF, the water column above the pycnocline was well mixed.

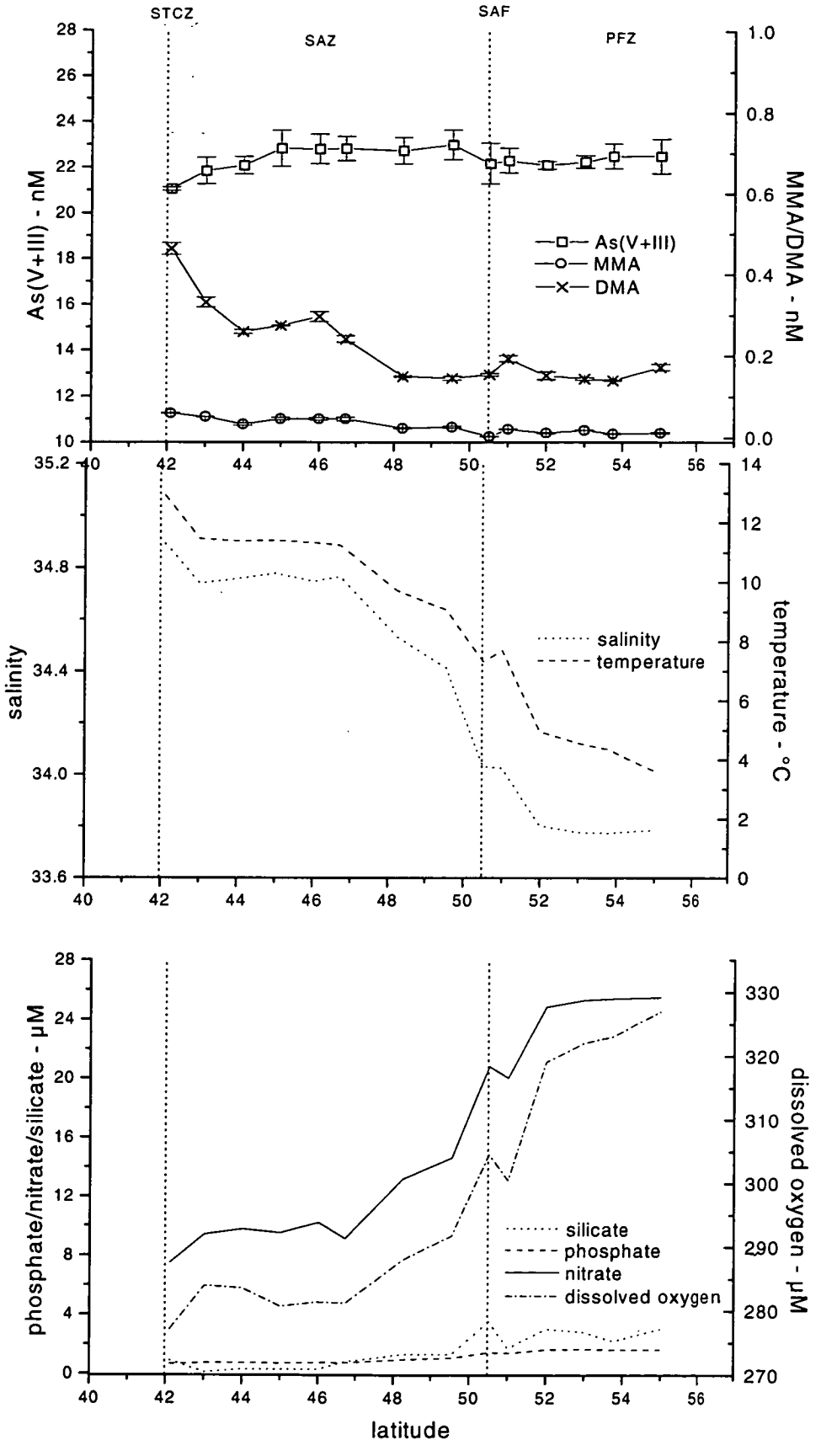

Figure 4.9 As(V+III), MMA, DMA, temperature, salinity, dissolved oxygen, phosphate, nitrate and silicate measurements surface samples along the transect 42 - 55° S, 142° E.

ii) Biology

During the voyage the dominant biological feature was the sub-surface (90 – 100 m) fluorescence maximum, found only south of the SAF. The fluorescence measured was as a result of these waters being populated by heavily silicified diatoms with high chloroplast density. Overall, low algal biomass was observed during the voyage, with sediment trap experiments indicating that the summer bloom had recently finished.

Maximum chlorophyll *a* values were 0.5 – 0.3 $\mu g\ L^{-1}$. North of the SAF chlorophyll was evenly distributed in the mixed layer, however to the south, it was very low at the surface, but had a well-defined subsurface maximum. Cell counts also confirmed that phytoplankton were found deeper in the water column south of the SAF.

Algal pigment analysis indicated that haptophytes dominated the communities north of the SAF. Cryptophytes have a patchy distribution, while cyanobacteria had a very strong signal north of the SAF. The pigment signal for cyanobacteria was particularly strong at the 42° S site and correlated well with cell counts. At 42° S, their numbers reached a maximum at 90 m. At 47° S, the number of cells was lower and the maximum was found deeper. Their numbers were almost non-existent at the 51 and 54° S sites. Cyanobacterial numbers in the Southern Ocean are believed to be temperature-dependent and cell numbers relate exponentially with temperature. South of the front a strong sub-surface maximum was found for the diatom markers.

In general, modelled primary productivity decreases with latitude, with the decrease in growth rate related to temperature and light availability.

ETS measurements are indicators of live cell respiration. As found for primary production, these measurements also indicated the greatest activity at 42° S and the lowest activity at 54° S.

iii) Arsenic speciation results

The results of arsenic analyses for the surface transect are shown in the top panel of Fig. 4.9.

As has been previously noted in section 4.3.3b, As(III) measurements were made on all samples, but at all sites were too low (<0.04 nM) to be reliably quantified. This is in agreement with the observation made by Santosa *et al.* [13], based on analysis of stored samples, that As(III) concentrations in the Southern Ocean were exceptionally low when compared with other parts of the world's ocean. They reported an average As(III) concentration of 0.04 nM for Southern Ocean waters sampled in late spring/early

summer. These values for the Southern Ocean are an order of magnitude lower (*ca.* 0.45 nM) than those reported by Middelburg *et al.* [10] and Cutter and Cutter [11] for similar temperature waters [21] in the high latitudes of the North Atlantic Ocean.

The concentrations of As(V+III) in surface waters along the transect lay within a narrow range (21.1 – 23.0 nM) and showed little depletion compared with the deep waters (*ca.* 24 nM). These values are high compared with other measurements made at high latitudes, where little surface depletion occurs [11], but are similar to those reported by Santosa *et al.* (18.6 nM) for the Tasman Sea at 40° S [14]. The lowest concentrations were recorded at the northern end of the transect between 42 and 44° S, with little variation occurring south of the station at 45° S.

Concentrations of the methyl arsenic species were generally low, approximately 10% of that observed in temperate Australian coastal water (see Chapter 5). The highest DMA concentrations were recorded in the warmer northern waters of the Subtropical Convergence (STCZ) Zone and decreased to a minimum at 48° S. The behaviour of MMA paralleled the trends observed for DMA, although, as reported by Andreae [6], its concentrations were approximately an order of magnitude lower. DMA concentrations were in the range 0.15 – 0.6 nM and MMA 0.02 – 0.07 nM. Despite the region north of 47° S being considered silicate-limiting for phytoplankton growth [36], these results indicate similar DMA/MMA ratios to those reported elsewhere for phosphate-limited conditions. Therefore, these results do not support the observation made by Santosa *et al.* [14] that MMA is the dominant species under silicate-limited conditions, such as in the Tasman Sea. However, the DMA/MMA ratio (Table 4.2) does change slightly on crossing the SAF, which has been indicated to be the border between different phytoplankton communities.

Santosa *et al.* [13] reported that the methyl arsenic species represented only 2% of the total dissolved arsenic present in Southern Ocean water. This is confirmed by experimental data presented here which shows that maximum conversion to methyl arsenic species occurs in the STCZ at 42° S (2.5%) and gradually decreases along the transect to less than 1% south of the SAF.

In the vertical profiles from each of the process sites, MMA (Fig. 4.10) and DMA (Fig. 4.11) concentrations were relatively constant in the surface 75 m, but below this decreased to less than the detection limit. The depth at which MMA and DMA can still be measured decreases heading south, with the methyl species detectable down to 600 m in the STCZ, but only to *ca.* 150 m in the PFZ. The highest methyl arsenic

concentrations are at the STCZ, which also has highest chlorophyll *a* and algal cell counts. The DMA/MMA ratio was similar at the SAZ site (47° S), but the actual concentrations found were lower, corresponding to biological measurements that indicated the presence of a similar algal population but a lower concentration of cells.

Table 4.2 DMA/MMA ratio on a N-S transect along 141° 30'E in the Southern Ocean

Latitude	DMA/MMA
42.097°	7.7
43.011°	6.4
43.998°	7.7
44.994°	5.8
46.000°	6.3
46.714°	5.2
48.232°	6.2
49.541°	5.4
51.002°	9.1
51.996°	12.6
52.998°	7.7
53.740°	13.4
54.991°	14.3

South of the SAF the concentrations of MMA and DMA are approximately 1/3 that measured to the north. This was also found for the ETS measurements, which decrease by about the same proportion crossing the front. The deeper chlorophyll *a* maximum found south of the front is not reflected in the MMA and DMA measurements. As found by Andreae [7], this is supported by carbon uptake data, which suggests there is no primary production associated with the chlorophyll *a* maximum.

The relationship between the methyl arsenic species and the hydrochemistry in the surface transect and in the vertical profiles above 200 m is discussed below.

Both MMA and DMA showed significant correlation with temperature along the surface transect at sites north of the SAF [MMA, $r = 0.905$, $P>0.001$; DMA, $r = 0.955$, $P>0.001$]. South of this, a static level of MMA and DMA production was found. Similar (but negative) correlation is found for MMA and DMA with phosphate, nitrate, silicate and dissolved oxygen, in the region north of the SAF. The reasons for a static level of MMA and DMA production across the front are unclear. It is either related to production being deeper in the water column south of the front, or the presence of a

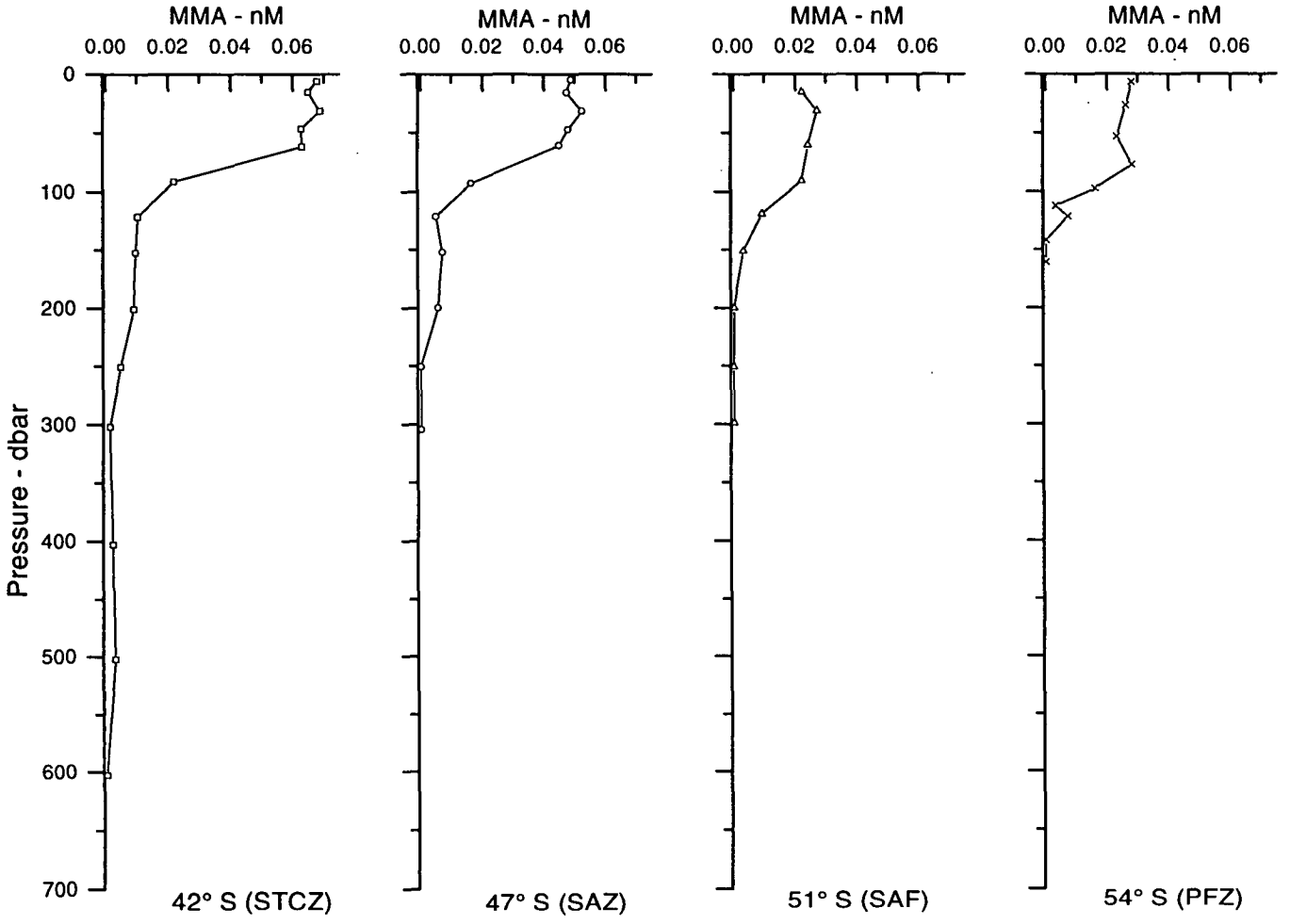

Figure 4.10 Vertical profiles of MMA with depth at the Subtropical Convergence Zone, Subantarctic Zone, Subantarctic Front and Polar Frontal Zone (n = 3).

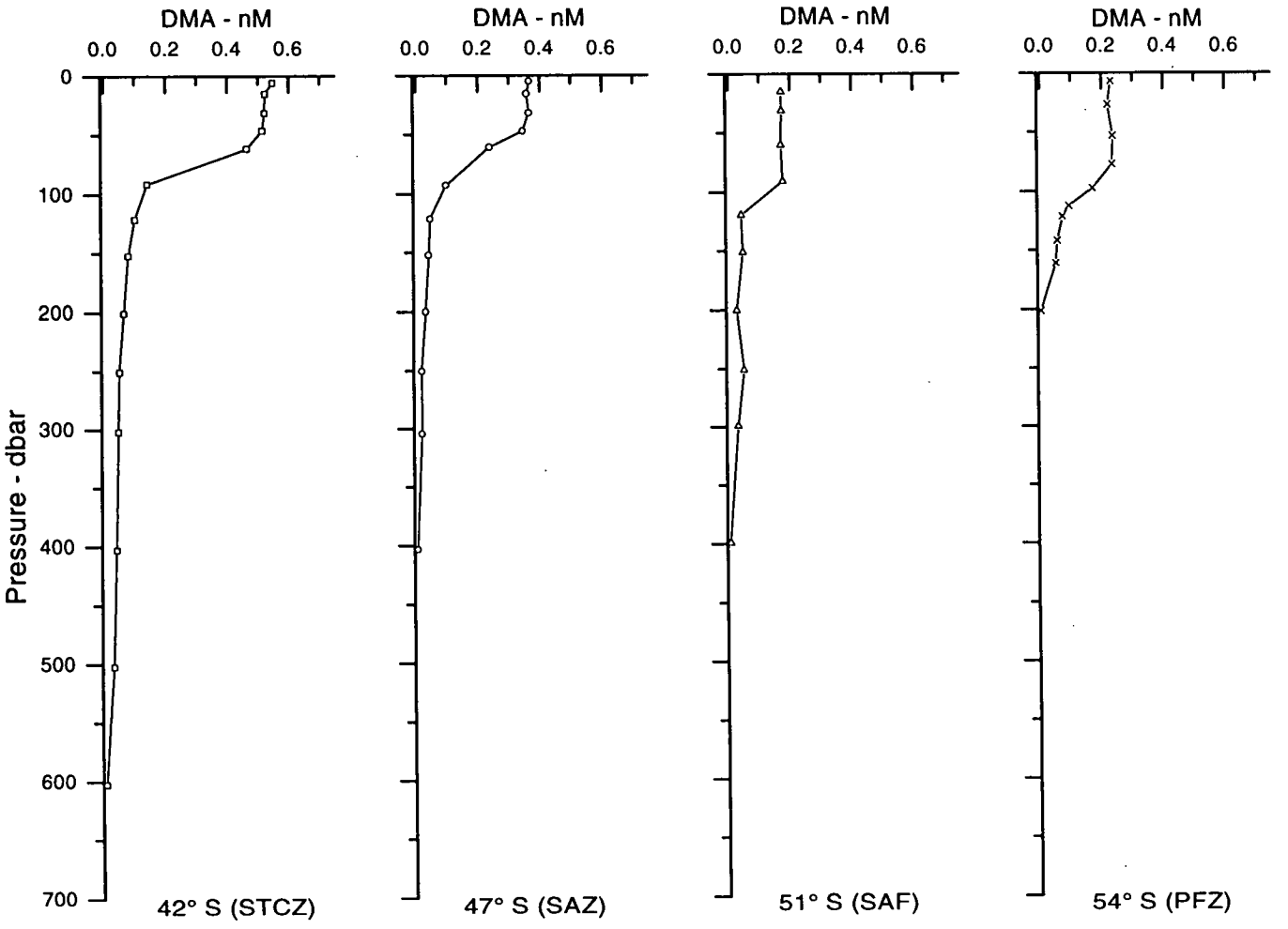

Figure 4.11 Vertical profiles of DMA with depth at the Subtropical Convergence Zone, Subantarctic Zone, Subantarctic Front and Polar Frontal Zone (n = 3).

different phytoplankton community. It is more difficult to establish any relationship between arsenic species and the hydrochemistry across the front.

Combining methyl arsenic data above 200 m from the four vertical profile stations, a clear correlation between MMA and DMA production was found ($r = 0.976$; $P>0.0001$). In general, the relationship between MMA and DMA and the hydrochemistry measurements showed no clear correlation when the data for the four profiles was combined, however, significant correlation exists for each individual data set, with the exception of salinity and dissolved oxygen. The linear regression for the methyl species and temperature has a positive slope, while that for the nutrients is negative.
An example of the relationship between MMA and phosphate is shown in Fig. 4.12. This is typical of the relationship found for DMA or MMA with phosphate or nitrate. The slope of the linear regression is greatest at 51° S, but otherwise 42 > 47 > 54° S. The correlation coefficient between MMA and phosphate is greater than -0.93 for each individual profile, but is poor for the overall data set ($r = -0.612$). Similar results are also obtained for silicate, but the magnitude of the slope decreases with latitude (42 > 47 > 51 > 54° S).

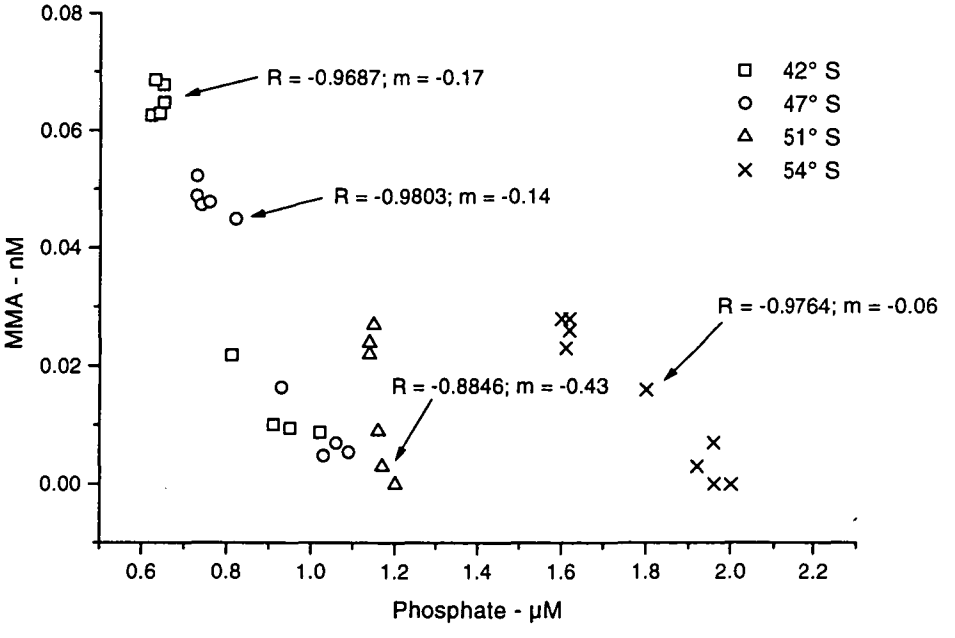

Figure 4.12 The relationship between MMA and phosphate at the vertical profile stations in the SAZ at depths less than 200 m.

No clear correlation can be established between arsenic species and chlorophyll *a* concentrations at either the vertical profile stations or along the surface transect, despite the general trend of decreasing methyl arsenic production and chlorophyll *a* concentrations going south. This perhaps suggests that a reasonable fraction of methyl arsenic production may be as a result of heterotrophic rather than autotropic activity.

In vertical profiles (Fig 4.13), As(V+III) shows only very slight depletion at the surface [As(V+III) = 22.2 ± 1.3 nM, n=21], with the greatest depletion at 42 and 54° S. Below 600 m, As(V) is the only arsenic species which can be identified in the water column and below 1000 m, there is little difference in the concentrations measured at each site [As(V) = 24.1 ± 0.5 nM, n=32]. These concentrations are high compared with those measured in NADW [9-12], but are similar to the values measured by Andreae [7] in deep waters of the Pacific Ocean. Using the $\Delta As/\Delta P$ ratio proposed by Middelburg *et al.* [10], the predicted arsenic concentration is *ca.* 22 nM. This prediction assumes a constant global As/P ratio though, and has been determined principally from data obtained in the North Atlantic Ocean. However, in these waters, unlike in Atlantic Ocean waters [9], there is no clear As-P relationship in the vertical profile and no obvious similarity in the features of the two profiles. There are no other comprehensive deep ocean measurements in this region and since the deep circulation in the Southern Ocean is quite different to deep water transport from the Atlantic to the Indian and Pacific Oceans, different behaviour of arsenic with respect to phosphate might be expected.

4.4 CONCLUSIONS

The first part of this chapter describes the results of a laboratory intercomparison using two entirely independent analytical methods (HG-AFS and HG-GC-PID) for the determination of MMA and DMA in stored (preserved) and unstored (shipboard analysis) samples. This was considered an important exercise because there are currently no certified reference materials for MMA and DMA in a seawater matrix, and also it is difficult to source arsenic species pure standards for these analytes.

The results indicated that the two methods gave good agreement for the determination of DMA on stored and unstored samples from a variety of oceanic environments. For MMA the HG-AFS methods tended to be lower, but this was not a consistent trend and suggests that some biologically mediated degradation of MMA may be occurring upon storage. This can be investigated further once MMA results from the stored samples (analysed by HG-GC-PID) become available.

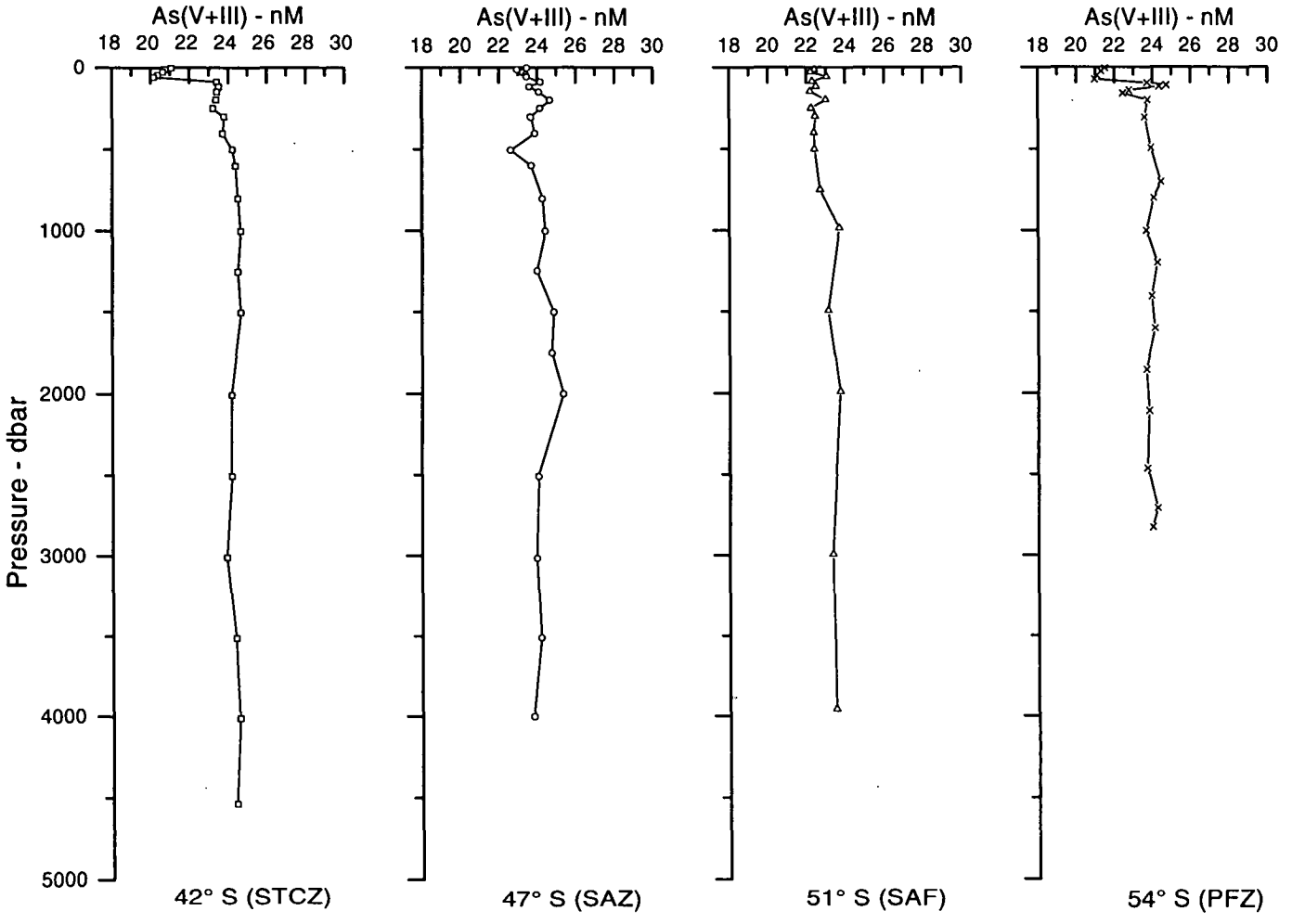

Figure 4.13 Vertical profiles of As(V+III) with depth at the Subtropical Convergence Zone, Subantarctic Zone, Subantarctic Front and Polar Frontal Zone (n = 3).

The sea trial of the semi-automated HG-AFS method (described in Chapter 3) showed that it was sufficiently compact, rugged and reliable to be used for making routine, low level measurements of arsenic even during quite challenging conditions. On the voyage there was only a single operator for the instrument and so MMA and DMA samples had to be stored for later analysis, however, with a second operator these analyses could also be easily carried out at sea.

On the Southern Ocean voyage, a comparison between trace-metal-clean sample collection and handling and sampling from the standard CTD rosette was conducted. This showed that within the limits of experimental error there were no contamination problems associated with sampling directly from the standard CTD bottles and rosette.

The arsenic measurements made in the Southern Ocean are some of the first from this region. While in the Atlantic Ocean, arsenic and phosphate profiles have similar features and correlate well with one another [9], in the Subantarctic Zone of the Southern Ocean this is not the case. It is not clear why this occurs, but perhaps is related to different circulation and biological processes in the two regions. As there are few arsenic measurements from the Southern Ocean, particularly the deep water, this needs further investigation.

In the surface waters of the Southtern Ocean arsenic shows little depletion compared with the deeper waters, partly because very little appears to be converted into "biological forms". In this region, unlike lower latitudes, as little as 1 – 2.5% of arsenic is present as MMA and DMA, and the concentration of As(III) is below the detection limit at all sites. The concentrations of methyl species are approximately an order of magnitude lower than found at sites with similar chlorophyll *a* concentrations in the western Atlantic Ocean during the IOC cruise. It would appear that this results from either the low water temperature or abundance of phosphate in the photic zone.

Despite the observations from the North Atlantic and British coastal systems that MMA and DMA production falls to background levels when water temperature drops to below 12 °C, this is not found in Southern Ocean waters. Here production of the methyl arsenic species is correlated with temperature at least north of the SAF (water temperature *ca.* 4 – 12 °C). South of the front production of the methyl species is unrelated to temperature, possibly because production moves lower in the water column in this region or because the ratio of heterotrophic to autotrophic production becomes significant.

It is also clear from this data that MMA is not the dominant methyl arsenic species produced in silicate-limited waters as proposed by Santosa *et al.* [14]. The results from the surface transect show the ratio of MMA to DMA in the silicate-limited waters north of the SAF to be similar to that found by Andreae [6] in phosphate-limited environments. The hypothesis put forward by Santosa *et al.* [14] was based upon a single sample from the Tasman Sea, and it is suspected that the particular phytoplankton community present at that time produced MMA in preference to DMA, but that this is not a particular feature of silicate-limited waters.

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Chapter 5: Arsenic Cycling in the Huon River Estuary

5.1 INTRODUCTION

5.1.1 Chapter overview

This chapter presents a discussion of the behaviour of arsenic species in a relatively unpolluted, temperate estuary, the Huon River, over a two-year period. This study formed part of a broader interdisciplinary project, The Huon Estuary Study, which examined estuarine circulation, nutrient cycling, algal dynamics and related matters in the waterway. The aim of the study was to provide the scientific framework for development of a management plan for the aquaculture industries operating in the lower estuary. This knowledge of estuarine circulation, nutrient cycling and algal dynamics was used in the interpretation of arsenic species results.

The results presented in this chapter also represent some of the first measurements of arsenic speciation in Australia and the Southern Hemisphere. The distribution of organometallic species, including arsenic, in the Australian coastal environment is documented in a review by Maher and Batley [1]. Previous speciation measurements in Australian waters are limited to those made for As(V) and As(III) by Maher [2] in nearshore South Australian coastal waters, by Butler and Smith [3] in anoxic holes of the Yarra River, Victoria, and Smith *et al.* [4] in Port Hacking, NSW. Some total arsenic measurements have been made in the Derwent [5] and Tamar [6] Rivers as well as in several locations along the north West Coast of Tasmania [7].

Total arsenic measurements have been made in surveys of New Zealand rivers, but in geothermal systems with naturally elevated concentrations [8-12]. Other measurements of arsenic in the Southern Hemisphere include: As(V) and As(III) measurements during mixing of the Porong and Solo Rivers with the Java Sea, Indonesia, in the wet and dry seasons [13], and total arsenic measurements in the Olifants [14] and Crocodile [15] Rivers, South Africa, the Rio Tercero [16], Argentina and the Manati River [17], Puerto Rico. To date, however, no comprehensive measurements of methyl arsenic species have been made.

Therefore, the results obtained for the Huon River are compared with those for other temperate estuaries from the Northern Hemisphere, particularly Europe and the United States. In many ways, the Huon catchment differs from these northern regions because it receives relatively low concentrations of nutrients and other pollutants, and is populated by different phytoplankton species.

Initially, the literature pertaining to arsenic cycling in the estuarine environment will be reviewed.

5.1.2 Estuarine chemistry of arsenic: A review

5.1.2a Arsenic concentrations in seawater and freshwater end-members

It has been well documented that arsenic concentrations in coastal and open ocean seawater fall within the range 1 – 2 $\mu g\ L^{-1}$ [18]. The coastal environment surrounding Australia is no exception, with arsenic concentrations of 1.1 – 1.61 $\mu g\ L^{-1}$ in South Australian coastal waters [2] and 1.4 $\mu g\ L^{-1}$ in the surface 100 m of the southern Tasman sea, east of Tasmania [19]. Arsenic concentrations in freshwater tend to be more variable, but generally also fall within the range 1 – 2 $\mu g\ L^{-1}$. Higher or lower values may be reported depending upon the geology of the catchment region and the impact of anthropogenic activity. Froelich *et al.* [20] estimate that the average total arsenic concentration in pristine rivers is *ca.* 0.8 $\mu g\ L^{-1}$. However, it has been suggested by Maher and Butler [21], that riverine arsenic concentrations probably now represent a historic baseline, because of significant anthropogenic arsenic input to many estuaries. The lowest arsenic concentrations in fresh water are reported for rivers that are far from industrial arsenic sources. In addition, Waslenchuk [22] indicated that in pristine systems, the lowest arsenic concentrations were found for rivers draining an igneous/metamorphic catchment, rather than a sedimentary region, that is subject to chemical weathering. Thus, the lowest reported arsenic values are 0.3 $\mu g\ L^{-1}$ for rivers of the southeast United States [23], 0.2 $\mu g\ L^{-1}$ in the River Beaulieu, England [24], and 0.1 $\mu g\ L^{-1}$ for the Krka River in Yugoslavia [25].

Total dissolved arsenic concentrations have been determined as part of routine State government environmental monitoring programmes in two Tasmanian rivers, the Derwent River Estuary and the Tamar River.

Arsenic data for the Derwent River can be found in a number of State Government Annual Reports [26-32] and are summarised in a recent review by the Office of the Supervising Scientist [5]. Concentrations in the Derwent range between 1 and 6 $\mu g\ L^{-1}$ and were elevated above background levels mid-estuary, in the vicinity of an electrolytic zinc refinery. In the freshwater region, upstream of the refinery, concentrations declined to below the detection limit of the method (0.1 $\mu g\ L^{-1}$) in two surveys [27, 31], indicating low arsenic in the uncontaminated freshwater region. It should be noted that the sites at which samples were collected were geographic

locations, and arsenic concentrations would be expected to vary depending on river flow and tidal conditions.

Total dissolved arsenic concentrations in the Tamar River (Tasmania) have been measured approximately every 2-3 months at up to 16 sites between 1988 and the present, as part of a routine monitoring programme conducted by the local council [6]. There is a history of mining and tailings dumping in the Tamar catchment as well as considerable urban impact [33], therefore elevated arsenic concentrations might be expected in the estuary. In general the concentrations of arsenic found are below the method detection limit (1 $\mu g\ L^{-1}$), however, concentrations of 2 $\mu g\ L^{-1}$ have been reported in the freshwater region (near old mine works) and up to 4 $\mu g\ L^{-1}$ mid-estuary, in the vicinity of a closed municipal refuse site. At the seawater end, typical concentration are 1 $\mu g\ L^{-1}$ [6].

Despite anthropogenic activity in the catchments of these two Tasmanian rivers, they still remain minimally affected by arsenic contamination compared with estuaries in Europe, and particularly southwest England. In rivers like the Thames [34] and the Carnon [35] (UK), maximum arsenic concentrations of 7 and 240 $\mu g\ L^{-1}$, respectively, have been recorded — a legacy of centuries of mining in the catchment.

The Huon region is relatively unaffected by either industry or urbanisation, with a population of *ca.* 13 000 living in the catchment area. There is no indication of mineralisation in the catchment geology and currently no mining or manufacturing industries are operating in the region [36]. Therefore, it might be expected that the freshwaters would have arsenic concentrations as low, if not less, than those reported for other pristine systems.

5.1.2b Behaviour of dissolved arsenic with salinity

i) Property-salinity relationships

Estuaries often carry elevated total dissolved arsenic loads and are therefore potential sources of arsenic to the ocean if no mechanism exists for their removal during estuarine mixing.

A common approach to obtain a simple visual representation of the behaviour of a chemical species during estuarine mixing is to plot its concentration against salinity. A property-salinity plot showing a linear relationship with salinity indicates that the species of interest is behaving conservatively, and neither addition nor removal is occurring. Non-conservative mixing may take one of two forms. Removal, whereby the

species of interest is removed from the dissolved phase by mechanisms including: adsorption onto particulate material, precipitation onto or coprecipitation with particulate phases (particularly iron oxyhydroxides), and uptake by phytoplankton. Addition processes include: point-source discharges, remobilisation from sediments and desorption of arsenic from seaward fluxing particles. As will be shown, this method may have limitations where the freshwater concentration of the chemical species of interest is variable on a time scale less than the flushing time of the estuary [37].

ii) Conservative arsenic-salinity relationships

Low arsenic freshwater end-members

Arsenic exhibits wide variation in its behaviour with salinity in different river systems. Obviously the most straightforward case is where simple physical mixing of the freshwater and seawater end-members occurs giving rise to conservative mixing curves. The mixing curves may have either a positive or negative slope depending on which end-member has the highest arsenic concentration. Conservative mixing is most commonly reported for river systems that are free from industrial inputs, and have low suspended particulate matter and low arsenic concentrations in the freshwater end-member. Examples of this were reported by Tremblay and Gobeil [38] in the St Lawrence Estuary (Canada) and Seyler and Martin for the Krka River (Yugoslavia) [25]. In two other papers, Waslenchuk [22] and Waslenchuk and Windom [23] reported that arsenic behaved conservatively during summer snapshot surveys of several low arsenic southeastern US rivers as they mixed with South Atlantic Bight water. They found that up to 40% of the As(V) present was associated with low molecular mass (<10 000) dissolved organic matter (DOM) and was unable to interact with the suspended particulate matter or iron colloids because of this association. This was supported by evidence from low DOM rivers in the northern US where arsenic behaved non-conservatively with salinity [23]. Arsenic concentrations in the northern US rivers were slightly higher than those in the southern US, but this was a result of different catchment geology, and the rivers were still considered pristine. In the St Lawrence Estuary (also pristine) [38], however, iron was removed during estuarine mixing and despite low DOM concentrations, arsenic still behaved conservatively.

A later paper by Byrd [39] repeated some of the work done on mixing of the Savannah River with South Atlantic Bight water by Waslenchuk [22]. In the summer period, arsenic was shown to behave conservatively with salinity, however, this paper highlights the need to survey over a range of seasonal conditions, as in both spring and

autumn non-conservative behaviour was found. During spring, windy conditions resulted in resuspension of near-shore sediments, causing arsenic removal through adsorption onto the suspended particulate matter. In autumn, arsenic was released again from the sediments during regenerative processes. In this case, adsorption and release processes were approximately equal and so the net result was conservative behaviour. This may not be the case for all estuaries and so seasonal variation must be taken into account before arsenic fluxes are calculated.

High arsenic freshwater end-members

Conservative arsenic-salinity mixing plots have also been reported for estuarine mixing, where the arsenic concentration was highest in the freshwater end-member. This was reported in winter and summer surveys of the Po River (Italy) [40], a system characterised by low suspended particulate matter (SPM). Van der Sloot *et al.* [41] reported conservative mixing in the Rhine River, which has high SPM. They indicated that this unexpected behaviour was because the river has a very short flushing time, thereby limiting interaction with particulate material.

In systems with high arsenic in the freshwater end-member, seasonal variations can result in changes to both the behaviour of arsenic during estuarine mixing, and also its concentration in the freshwater end-member. A study of two Indonesian rivers by van der Sloot *et al.* [13] during the tropical wet and dry seasons showed conservative mixing and high arsenic concentrations during the wet season and non-conservative mixing and low arsenic concentrations in the dry season. High arsenic concentrations in the wet season (*ca.* 15x those measured in the dry season) were associated with arsenic being mobilised from volcanic soils by high rainfall, while adsorption onto higher concentrations of SPM appeared to be the mechanism occurring in the dry season. Different behaviour was found during flood and low-flow periods in the Yellow River, China [42]. Under low-flow conditions, the river has low concentrations of SPM and arsenic behaves conservatively during mixing, but when in flood the flux of SPM is high. During floods, non-conservative behaviour occurred as a result of arsenic being remobilised from the SPM in the mid-estuary region where salinity increases. This resulted in increased dissolved arsenic concentrations, possibly as a result of ion-exchange mechanisms.

As mentioned earlier, a simple picture of the mixing processes occurring may be difficult to obtain in situations where arsenic concentrations in freshwater vary on a time scale less than the flushing time of the estuary. One such example is the Rhône

River (France). In this river, the arsenic concentration is highly dependent on the river flow. This, combined with the short flushing time of the estuary (*ca.* 1 day [43]) and variable flow, makes interpretation of the mixing process difficult [43], although Elbaz-Poulichet *et al.* [44] have classified the behaviour of arsenic here as conservative.

iii) Non-conservative arsenic salinity relationships

Low arsenic freshwater end-members

This is a more unusual situation and only one case is reported in the literature, [24] for the River Beaulieu in England. Although free of industrial contamination, this river contains naturally high levels of iron. It is thought that arsenic is co-removed with iron as it flocculates during estuarine mixing.

High arsenic freshwater end-members

Non-conservative behaviour of arsenic during mixing is commonly reported in estuaries that contain elevated levels through industrial discharges. Arsenic removal is a mechanism which has been reported in several UK rivers including Restronguet Creek, the Avon, Humber, and Tamar Rivers [45]. In each case, these rivers have been polluted by mining activity, and therefore, also have a high iron load. Iron is known to flocculate and form colloidal species at low salinity. The colloidal species readily adsorb other dissolved metals, including arsenic, effectively removing them from the dissolved phase [46]. In each of these rivers, arsenic was being co-removed with iron at low salinity. This removal mechanism is highly effective and was shown to remove up to 80% of the dissolved arsenic load in the Humber River [45]. The case of the Tamar is slightly more complex than the other three rivers [47, 48]. The other three systems have relatively low river flows and/or weak tidal regimes (therefore tend to be stratified), mechanisms which tend to prevent sediment resuspension, and therefore, arsenic remobilisation. By contrast, the Tamar River has a macrotidal regime, which results in arsenic addition when high concentrations of arsenic in interstitial waters from tidal mud flats are released. The amount of arsenic remobilised was greatest during spring tides [49]. Arsenic co-removal with iron has also been observed for the Schelde Estuary (Belgium) leading to pronounced arsenic depletion in the mid-estuary [50].

Non-conservative behaviour in an estuary may also occur as a result of a mid-estuary addition. In the case of the Scheldt[1] (Netherlands) [41] and Tagus (Portugal) [51] Rivers, both exhibit a mid-estuary addition of arsenic as a result of industrial inputs, but

[1] It should be noted that the Schelde River (Belgium) and the Scheldt River (Netherlands) are the same river.

this is followed by arsenic removal onto the SPM during subsequent mixing with seawater. In the Gironde Estuary (France) – a macrotidal system - addition of arsenic occurs as arsenic transfers from the particulate to the dissolved phase at the freshwater-seawater interface, which is also the location of the turbidity maximum [43].

Because arsenic co-removal with iron occurs in the low salinity region in most estuaries it is important that when defining arsenic behaviour during estuarine mixing, samples from a complete salinity range are used. Significant arsenic removal (geochemical) generally occurs in the low salinity (5-15) range. Therefore, samples collected between salinity 20-35 will exhibit conservative behaviour, and may not reflect removal processes occurring earlier.

A summary of the literature discussed is given in Table 5.1.

In conclusion, it is well established that the behaviour of total arsenic with salinity is dependent on many factors including industrial inputs, the nature and quantity of suspended particulate matter in the river and the interaction of these parameters with variations in river conditions. This means that arsenic behaviour during estuarine mixing is unique to a particular estuarine system. A generic model for arsenic cycling has been proposed by Sanders [52], but requires a considerable amount of information about geochemical and biological budgets for arsenic to be able to predict fluxes to the ocean.

5.1.2c Seasonal cycling of arsenic

In addition to geochemical processes, the cycling of arsenic in estuaries is also influenced by biological uptake, giving rise to reduced, methylated and also hydride-refractory or "hidden" arsenic species. The relative importance of physical or biological processes on estuarine arsenic cycling is a function of the individual estuary, and often is seasonally dependent. In many estuaries, particularly those influenced by anthropogenic inputs, the effects of physical processes dominate arsenic cycling.

There are two reports in the literature of seasonal biological arsenic depletion. Seyler and Martin [25] reported that arsenic behaved conservatively with salinity in the Krka River in both winter and summer, but that during summer the As(V+III) concentration in the seawater end-member was lower as a result of biological removal. Andreae and coworkers [20] also reported evidence of biological removal of arsenic coinciding with the chlorophyll maximum in a phosphorus-enriched estuary with high productivity.

Table 5.1 summarises the literature discussed and shows the arsenic end-member concentrations and also the behaviour of arsenic with salinity in estuaries.

Location	Reference	Fresh water [As(V+III)] - $\mu g\ L^{-1}$	Seawater [As(V+III)] - $\mu g\ L^{-1}$
Rivers with low fresh water arsenic concentrations and conservative arsenic-salinity mixing curves			
SE United States rivers	[23]	av. 0.3	1.1
Ogeechee River (+others), US	[22]	0.2	1.5
River Krka, Yugoslavia	[25]	0.13	1.80
St Lawrence River, Canada	[38]	0.47	1.42
Saguenay River, Canada		0.08	1.40
Rivers with high fresh water arsenic concentrations and conservative arsenic-salinity mixing curves			
Po River, Italy	[40]	0.96 - 2.07	0.93 - 1.42
Rhine River, Netherlands	[41]	0.75 - 0.97	2.47 - 3.00
River Solo, Indonesia (wet)[a]	[13]	0.82 - 2.4	*ca.* 1.1
Huang He River, China (low flow)	[42]	1.95	0.75 - 1.80
Rhône River, France	[44]	1.95 (highly variable)	1.65
[a]conservative mixing only in the wet season, during the dry season non-conservative behaviour is found			
Rivers with low fresh water arsenic concentrations and non-conservative arsenic-salinity mixing curves			
River Beaulieu, UK	[24]	*ca.* 0.2	*ca.* 1.0
Savannah River, SE US[b]	[39]	0.19	0.22
[b]but annually net conservative behaviour			
Rivers with high fresh water arsenic concentrations and non-conservative arsenic-salinity mixing curves			
Restronguet Creek	[45]	33 - 209	1 - 2
Avon River		3 - 6	2 - 3
Humber River		1.4 - 1.5	1.4 - 1.5
Tamar River		50 - 10000	not given
Tamar River, UK	[48]	2.7 - 8.8	*ca.* 1.0
Schelde River, Belgium	[50]	0.75 - 3.7	*ca.* 1.9
Tejo Estuary, Portugal	[51]	*ca.* 4.0	*ca.* 1.8
Scheldt River, Netherlands	[41]	7.5 - 41.2	0.75 - 0.97
River Solo, Indonesia[c]	[13]	0.82 - 1.65	0.45 - 0.82
River Porong, Indonesia[c]		1.05 - 1.72	0.45 - 0.90
Huang He River, China (high flow)	[42]	1.95	0.75 - 1.80
Gironde Estuary, France	[43]	1.36 - 3.00	1.46
[c]data for the dry season			

In general, the concentrations of biologically produced arsenic species, As(III), MMA and DMA, have no clearly defined relationship with salinity, but are thought to relate to other physical, biological and chemical parameters like temperature, phosphate concentration, primary production, phytoplankton species composition and sediment porewater concentrations.

i) Temperature dependence of methyl arsenic production

A temperature threshold for the production of methyl arsenic species was first proposed by Howard *et al.* [53], based on seasonal variation of arsenic species in the River Beaulieu. They found that As(III), MMA and DMA were not produced during winter, and were not evident in the water column until after the first spring phytoplankton blooms and when the water temperature was greater than 12 °C. They also observed that As(III) production was limited to periods when the water temperature was within the range 15-18 °C, probably as a result of competing rates of biological production and chemical oxidation. By contrast, in the Po River (Italy), As(III) production was observed only at 26-27 °C [40].

This trend for methyl arsenic production at water temperatures greater than 12 °C has been demonstrated in the River Beaulieu in subsequent seasons [24] and has also been observed in studies of other UK rivers [34, 54-57]. In fact, in each of these studies it is also apparent that methyl arsenic production increased as water temperature increased through spring to summer. Millward and coworkers reported that DMA is the only arsenic species measured in spring, while both MMA and DMA were present in summer [55] with the concentration of DMA doubling between spring and summer conditions. Additionally, these studies reported a rapid decrease in methyl arsenic concentrations at the onset of autumn. Similar annual cycles of methyl arsenic production have been found in other European rivers [40, 50].

Some slightly different observations have been made by Sanders *et al.* in field studies of methyl arsenic production in Chesapeake Bay (USA) [58-60]. In 1983/84 surveys, methyl arsenic production was high during summer, with up to 62% of total dissolved arsenic methylated [58]. Similar cell densities were found in winter and summer, but methyl arsenic species were not detectable in winter. In contrast, in 1988/89 and 1990/91 surveys [60], significant DMA concentrations were found in the water column during winter, although not when the water temperature was <12 °C.

ii) The As/P ratio ("arsenic stress") and methyl arsenic production

As discussed in Chapter 1 (section 1.2), arsenate, the dominant form of dissolved arsenic in seawater, is isoelectronic with phosphate, and has the ability to enter biochemical pathways. Therefore, it has been predicted that phytoplankton might experience increasing arsenic stress during the course of blooms as the As/P ratio increases, resulting in greater production of methyl arsenic species. This assumption, however, is based upon phytoplankton having a poor ability to discriminate arsenate from phosphate [54].

A number of examples exist of quite high arsenic methylation (10-60%) in eutrophic regions where phosphate is not limiting, and therefore, high levels of primary production and low arsenic stress are expected. These include the Po River (Italy) [40], Charlotte Harbor (Florida) [20], the Tamar River (UK) [48], the Itchen estuary (UK) [61], and the southern North Sea (UK) [56]. In contrast are observations by Millward *et al.* [56] (southern North Sea) and Howard and coworkers [62, 63] (closed ecosystem experiments), where no methyl arsenic production occurs under phosphate-depleted conditions (As/P = 3/1 [62]) in early spring. In the natural environment, there is also little correlation found between phosphate depletion and methyl arsenic production [56, 64, 65], or As/P ratios and methyl arsenic concentrations [65]. A number of authors claim that this is as a result of different phytoplankton species having varying abilities to discriminate between arsenate and phosphate [61, 64] as equimolar concentrations are approached. Sanders *et al.* [58] indicate that in general, oceanic phytoplankton have a high nutrient selectivity while estuarine phytoplankton are, by comparison, fast growing and opportunistic. Therefore, estuarine phytoplankton have lower nutrient selectivity and are more likely to take up and methylate arsenic. Work by this group also suggests that differences in arsenic uptake and incorporation also exist within phytoplankton groups [64], making arsenic cycling a complex interplay of species composition and temporal species succession within an estuary.

From their work in Charlotte Harbor, Froelich and Andreae [20] indicated that the methyl arsenic concentrations found were not significantly elevated, despite extremely high productivity. They suggested this was because there was little arsenic stress and that methylation was related more to regeneration processes, and it was more likely to occur under phosphate-depleted, nitrate-enriched conditions. This was supported further by the work of Sohrin *et al.* [66], in mesotrophic and eutrophic basins of the freshwater Lake Biwa, Japan. While higher production was found in the eutrophic basin, this did

not result in greater production of methyl arsenic species. They concluded that although there was no strict correlation between the two parameters, it was obvious that greater methyl arsenic production was occurring in the mesotrophic basin where greater arsenic stress would be expected.

The complexity of factors involved in arsenic methylation is demonstrated by the work of Sanders *et al.* [67], Howard *et al.* [63] and Millward *et al.* [56] with the phytoplankton species *Skeletonema costatum.* In laboratory cultures, this species was shown to methylate arsenic at various phosphate concentrations [67] and apparently did not discriminate between As and P [56]. Under phosphate-depleted conditions, in a closed ecosystem experiment at lower temperature, no methylation occurred during a *S. costatum* bloom, although high DMA concentrations were found in the phytoplankton detritus [63]. Also, in incubations [56], *S. costatum* has been shown to remove inorganic arsenic without subsequent production of methyl arsenic species during the growth phase.

Even further complexity has been shown by Sanders [68] in culture experiments with diatoms of the species *Thalassiosira.* He showed that of five clones of the same genus and similar morphology, grown in phosphate deficient culture, three produced significant amounts of MMA, one DMA, and two produced no methylated compounds. Therefore, factors such as phytoplankton ecotypes, physiological state, temperature and light availability must play a role in methylation and release of methyl arsenic species by the cell, even during periods of arsenic stress.

iii) Correlation between indicators of primary productivity and methyl arsenic production

Despite significant evidence that arsenic methylation is biologically mediated, there are also numerous examples where methyl arsenic production cannot be correlated with commonly used indicators of primary production such as chlorophyll *a* and ^{14}C-uptake.

Work by Andreae in coastal seawater [69] showed that MMA and DMA profiles correlated well with both chlorophyll *a* and ^{14}C-uptake. However, chlorophyll *a* concentrations are often not indicative of actual photosynthetic activity. This was shown by Andreae [70] in the Pacific Ocean and again from our own work in the Southern Ocean (Chapter 4), where deep chlorophyll *a* maxima with little associated photosynthetic activity (based on ^{14}C-uptake) exhibit low methyl arsenic production. This may provide one explanation for the many observations of poor correlation between methyl arsenic concentrations and chlorophyll *a* in the estuarine environment

[48, 56, 57, 61, 65]. However, results by Sohrin *et al.* [66], where good correlation was obtained on a day-day basis but not overall, suggest daily fluctuations in phytoplankton activity and species could be responsible. Poor correlation may also indicate that methyl arsenic species are not produced as a direct result of phytoplankton activity [65] but are released by grazing, breakdown of non-hydride forming organoarsenics, or a combination of both. This is supported by evidence from several studies which show that there is often a lag between the first spring phytoplankton bloom and methyl arsenic production. In a region of recycled production, however, chlorophyll *a* and primary production have been shown to correlate well with DMA production [71], coming back to the earlier arguments about arsenic stress.

Although this seems quite confusing, it reflects the fact that there is not a single major factor influencing methyl arsenic production, but rather it is a mix of several factors which might include phytoplankton class, temperature, and nutrient availability.

iv) Evidence for production of As(III), MMA or DMA by specific phytoplankton species
There is little evidence relating the production of As(III), MMA and DMA to detailed information about phytoplankton species and abundance. This may be partly because there is often a delay between the bloom and appearance of methyl arsenic species.

A lack of biologically produced arsenic species has been observed in spring blooms of *Rhizoselenia* and *Phaeocystis* [56] and a mixed bloom of *Skeletonema, Nitzschia* and *Thalassiosira* [63] species. However, high MMA and DMA concentrations have been observed in a mixed summer bloom of *Chaetoceros debile*, *Thalassiosira nordenskioldii* and unidentified flagellates [56].

In addition, peaks in As(III) concentrations have been associated with *Rhizoselenia* succeeding *Skeletonema* [72] and also with cyanobacteria populations [25]. Sanders [58] reported that the presence of a *Chroomonas* (cryptophyte) species in Chesapeake Bay coincided with MMA being the dominant methyl arsenic species. In the bloom, cell numbers were highly correlated with MMA concentrations, indicating that some phytoplankton species may produce MMA directly.

A recent study by Howard *et al.* [72], has attempted to correlate temporal changes in production of As(III), MMA and DMA with detailed information about phytoplankton and bacterioplankton occurrence and abundance. They were unable to conclusively correlate changes in arsenic species with phytoplankton activity, because of the delay in methyl arsenic appearance in the water column.

v) "Hidden" arsenic species in the estuarine environment

Further complicating the cycle of arsenic is evidence of "hidden" or hydride-refractory arsenic species in estuarine waters [73-79]. These compounds cannot be detected by conventional hydride generation techniques and are yet to be conclusively identified [73, 77, 78]. At present, they are identified as the arsenic fraction produced upon UV irradiation of the sample (UV-labile As). In several cases, UV oxidation has been shown to give incomplete digestion of the samples and a further fraction yielded by more aggressive digestion has also been found (UV-refractory As) [76, 77, 79]. Howard and Comber [74] have found that UV oxidation results in increases in As(V+III) and DMA concentrations (not MMA). They assigned the increases in inorganic arsenic to breakdown of colloidal arsenic species and trimethylated compounds (like arsenobetaine), while the increase in DMA was attributed to breakdown of dimethyl arsenoribosides. MMA concentrations did not change upon UV oxidation supporting the idea that its presence in the water column was as a result of DMA demethylation.

Both Howard and Comber [74], and Hasegawa *et al.* [79] found that the hydride-refractory arsenic fraction had a seasonal cycle coinciding with methyl arsenic production, with a maximum (*ca.* 20 – 25%) occurring in summer. This was in contrast to de Bettencourt and Andreae, who found a constant level of hydride-refractory arsenic (20%), with no seasonal component, probably reflecting particulate rather than biologically produced arsenic species [75].

vi) Some conclusions about arsenic cycling in estuaries

As discussed at the beginning of this section (5.1.3), the biologically mediated cycling of arsenic in estuarine waters is highly complex.

With the exception of work by Sanders [58], circumstantial evidence suggests that MMA is not usually produced directly by phytoplankton, but is more commonly a breakdown product of DMA. Supporting this are a number of studies in which the appearance of MMA and As(III) lagged behind production of DMA in the water column [55-57, 59, 61, 72]. Also, in almost all cases the concentration of MMA was much less than that of DMA in the water column. It is not clear whether demethylation of DMA occurs abiotically or whether in some cases it might be biologically mediated. Evidence by Sanders [80] suggested that bacteria may play an important role in arsenic demethylation in surface waters.

Although DMA in the water column has been identified as being dimethylarsinate [81], there is also evidence to suggest that this is not the form in which it is released by

phytoplankton. One piece of circumstantial evidence to support this is that DMA is observed in the water column following, rather than during the first spring phytoplankton blooms [24, 53]. It has been proposed by Howard *et al.* [74, 81] that dimethylarsinate found in the water column is first released from phytoplankton cells as dimethyl arsinoribosides ("hidden" arsenic species). Therefore, the lag period between the bloom and DMA in the water column is a result of the time taken for release and breakdown of the arsenosugars. He also suggests that DMA sugars are good carbon sources and therefore probably have relatively short lifetimes, although significant on the time-scale of bloom development [81]. Whether the release of dimethylarsinoribosides is as a result of excretion by the cell or occurs upon grazing/cell death is unknown.

Howard *et al.* [72] also suggest that although arsenic uptake may be strongly coupled with phosphate transport, the similarity may be unimportant once arsenic enters the cell, where different chemistries dominate to convert arsenic into nitrogen analogues.

The relationship of methyl arsenic production with other external factors such as light, temperature and phosphate concentrations is unclear, although some evidence does point to increased methylation under conditions where recycled production might be occurring [20, 71]. Whether this is as a result of different physiological processes occurring within phytoplankton cells, succession by different phytoplankton species with different arsenic tolerance, or a mixture of phytoplankton and bacterial influences is not clear.

The role of temperature in methyl arsenic release is also yet to be determined. It may be an important factor in controlling the rate of arsenic uptake, metabolism or excretion by the cell, or may control the rate at which DMA sugars are broken down to give dimethylarsinate in the water column.

As well as phytoplankton, macroalgae have been identified as a potential source of methylated arsenic species in the Tamar estuary (UK) [54, 82]. In this estuary, methyl arsenic species were also identified in the sediment pore waters; however, fluxes to the water column from this source were considered negligible at most times of the year [54].

5.1.3 Setting of the Huon project

A brief summary of the conditions in the Huon River and it catchment area are given in this section. Further details can be obtained from a recent State Government report [36]

and the final report from The Huon Estuary Study conducted by CSIRO Division of Marine Research [83].

5.1.3a The Huon catchment area

The Huon River catchment is located in Southern Tasmania, Australia. The catchment area is located between latitude 42° 45' S and 43° 45' S with a total area of *ca.* 3890 km^2 (Fig. 5.1). A more detailed map of the Huon River is given in Fig. 5.2. The climate in the region is described as being changeable, cool temperate. Annual rainfall varies from more than 2000 mm in the west to just under 1000 mm in the east, with maximum rainfall during winter months. The summer months are the warmest and driest with a mean temperature range of 9 – 21 °C. The winter temperature range is 2 – 13 °C.

A significant proportion of the catchment (1600 km^2) lies within a World Heritage Area. Of the remaining land, 1250 km^2 is used for forestry, 1000 km^2 is freehold land and

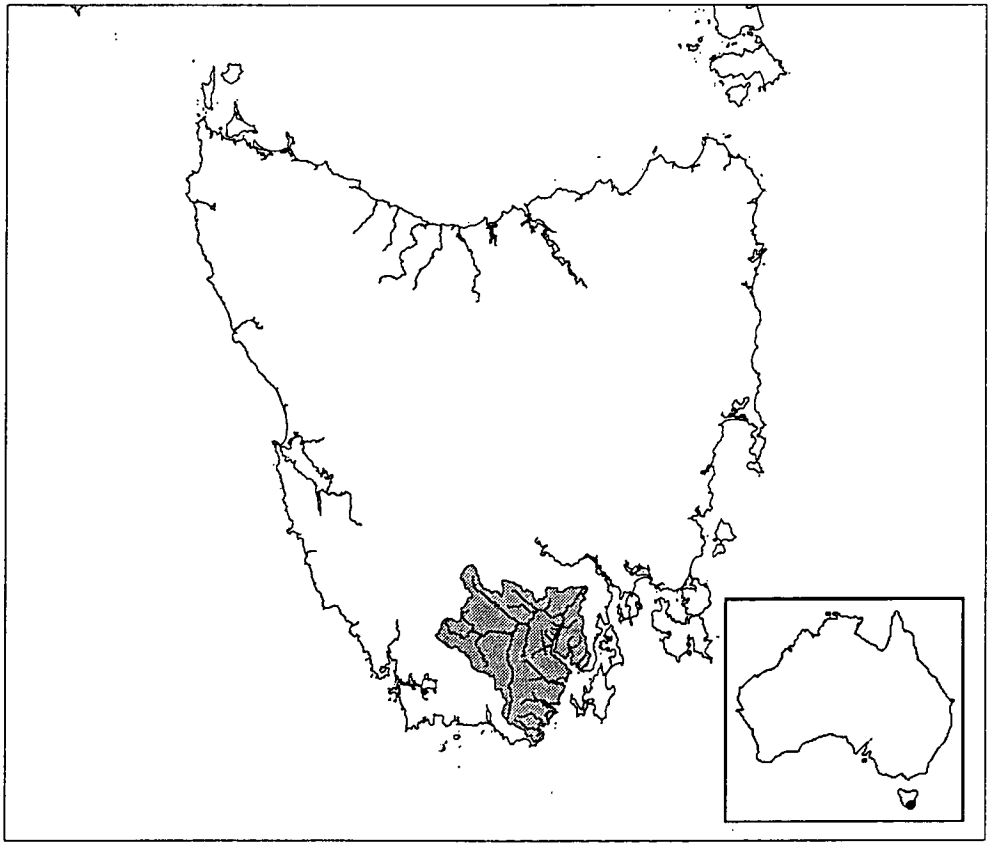

Figure 5.1 Map of Tasmania showing the Huon catchment area (shaded)

50 km^2 is State Reserve. Therefore, the vegetation is dominated by native forests and sub-alpine vegetation in the west and mainly agricultural use in the east.

Activities that may degrade water quality in the region are sewage treatment, forestry, quarrying, fish and fruit processing, agriculture and aquaculture. Currently, there is no secondary industry in the region, although a pulp and paper mill operated in Port Huon between 1962 and 1982, and also re-opened briefly between 1986 – 1989, as a woodchip mill [36]. Agriculture in the region consists mainly of dairying and orchards, which in the past have had a history of intensive pesticide use. The major primary industry in the region is aquaculture in the Huon River. The industry is expanding and is currently the centre for salmonid production in Tasmania. In 1998, 138 ha of the river were leased for salmon production, and a further 21 ha for shellfish production.

5.1.3b The Huon River Estuary

The Huon River is a typical drowned river valley. Water from the estuary flows into the D'Entrecasteaux Channel between mainland Tasmania and Bruny Island. The entrance to the estuary is 20 km from the Southern Ocean, to the south, and 40 km from Storm Bay in the north. Bathymetry of the estuary and channel indicate that the deep marine waters of the Huon are directly influenced by coastal water originating from the Southern Ocean.

The Huon River is characterised as a microtidal salt-wedge estuary where salt water can penetrate as far upstream as Ranelagh (*ca.* 40 km) under most conditions of river flow. Mean flows in the river are 30-40 cumecs in the summer months (January-March) and 125-130 cumecs in winter (July-August), with one in seven year flood events of 1300 cumecs. The river has a relatively short flushing time ($\leq$ 7 days). It has a low suspended particulate load but is characterised by high concentrations of coloured dissolved organic matter. There are no anoxic regions in the water column, but dissolved oxygen depletion is a feature up-river at the N transect (Fig. 5.2).

The estuary can be partitioned into three geographic regions: i) the deep marine zone in the lower part of the estuary, ii) the Port Cygnet arm, and iii) a shallow brackish zone in the upper half of the estuary. The lower half of the estuary is the region between the estuary mouth at Huon Island and Port Huon. It is about 21 km long and ranges in depth from about 40 m at the mouth to 10 m at Port Huon. The Port Cygnet arm is situated mid-way up the lower estuary. It is shallow and marine-dominated, receiving little freshwater input from the Agnes and Nicholls rivulets. Above Port Huon, the main estuary shallows rapidly from 10 m at Port Huon to a mean depth of 4 m on the western

side, and 2 m on the eastern side of Egg Islands. In the upper estuary, the deep salt water layer penetrates upstream for about 19 km to near Ranelagh, while the surface water ranges from brackish to freshwater under the influence of the Huon River. Rapids just above Ranelagh provide an upstream constraint on salt water penetration.

5.1.3c Potential arsenic sources in the Huon region

Despite the absence of mining or manufacturing industries operating in the Huon River catchment, there is still a potential source of historic arsenic contamination to the river from arsenic used as a pesticide in primary industry.

Lead arsenate has been used in the past as a broad insect control, recommended for routine spraying of apples throughout the growing season [84]. The last registration for this chemical in Tasmania was 1983 [85], following Germany setting a zero tolerance for lead in imported apples.

Theoretically, arsenic should bind strongly to soils, preventing leaching, although rainfall simulations have shown arsenic can be removed from soils. Additionally, phosphate, added as fertilizer, may compete with and desorb arsenic from soil binding sites [88]. Therefore, some anthropogenic arsenic might be expected in the Huon River, particularly after periods of high rainfall.

5.2 EXPERIMENTAL

5.2.1 Sampling sites and program

Samples for arsenic analysis were collected from the Huon estuary over a two-year period. The sampling program was designed to reflect both spatial and temporal changes in arsenic speciation. Spatial distribution of arsenic species was determined in samples collected from seven three-monthly surveys of the river. The dates of the spatial surveys are given in Table 5.2. The sites used for making physical, chemical and biological measurements are shown in Fig. 5.2. Transects are labelled alphabetically (A to R) from the sea to the freshwater ends (Port Cygnet transects are V, X and Y), and numbered west to east crossing the page, apart from R1 – R5 which progress upstream in the Huon Estuary; R6 is on the Kermandie River below Geeveston. Note that site X3 was moved further south (closer to the shell fish farms) after HES 5, and from HES 6 onward a new site, closer to the shellfish farm (X3B), was used instead.

At chemical and biological stations, discrete samples were collected for the determination of arsenic species [As(V+III), MMA and DMA], salinity, dissolved oxygen, filterable reactive phosphorus (phosphate), total dissolved phosphorus (TDP),

total phosphorus (TP), nitrate+nitrite, nitrite, total dissolved nitrogen (TDN), total nitrogen (TN), silicate, ammonia/ammonium (ammonia), dissolved organic carbon (DOC), suspended particulate matter (SPM) and phytoplankton species counts (10 m net haul; 20 μm mesh). At biological stations, an integrated water column sample (12 m) was collected for phytoplankton cell counts. At all stations (physical, chemical and biological), CTD profiles of salinity, temperature and fluorescence were obtained, as were Secchi disk depths. Samples were collected at the surface at all sites, at the bottom, when a saline layer was found, and at a mid-depth, corresponding to the fluorescence maximum, when the site was sufficiently deep. A small number of samples (*ca.* 20) were collected from the estuary during HES 10A, a trace metal survey.

Table 5.2 Survey numbers and dates of the Huon Estuary spatial surveys

Survey Number	Dates	Season
HES 3	7 – 11 October, 1996	Spring
HES 4	24 – 28 February, 1997	Late Summer
HES 5	16 – 20 June, 1997	Winter
HES 6	7 – 9 October, 1997	Spring
HES 7	2 – 4 December, 1997	Early Summer
HES 8	17 – 20 February, 1998	Late Summer
HES 9	18 – 21 May, 1998	Winter
HES 10A (trace metal survey)	31 August – 4 September, 1998	Early Spring

The variation of arsenic species [As(V+III), As(III), MMA and DMA] at a single site in the estuary was also determined, as part of the Huon continuous monitoring program. These samples were collected over the summer phytoplankton bloom period at Killala Bay (Site F1, Fig. 5.2), a site with both oceanic and freshwater influences. A surface sample was collected from this site at approximately weekly intervals, although sampling was less frequent during the winter period. The sampling dates are given in Table 5.3.

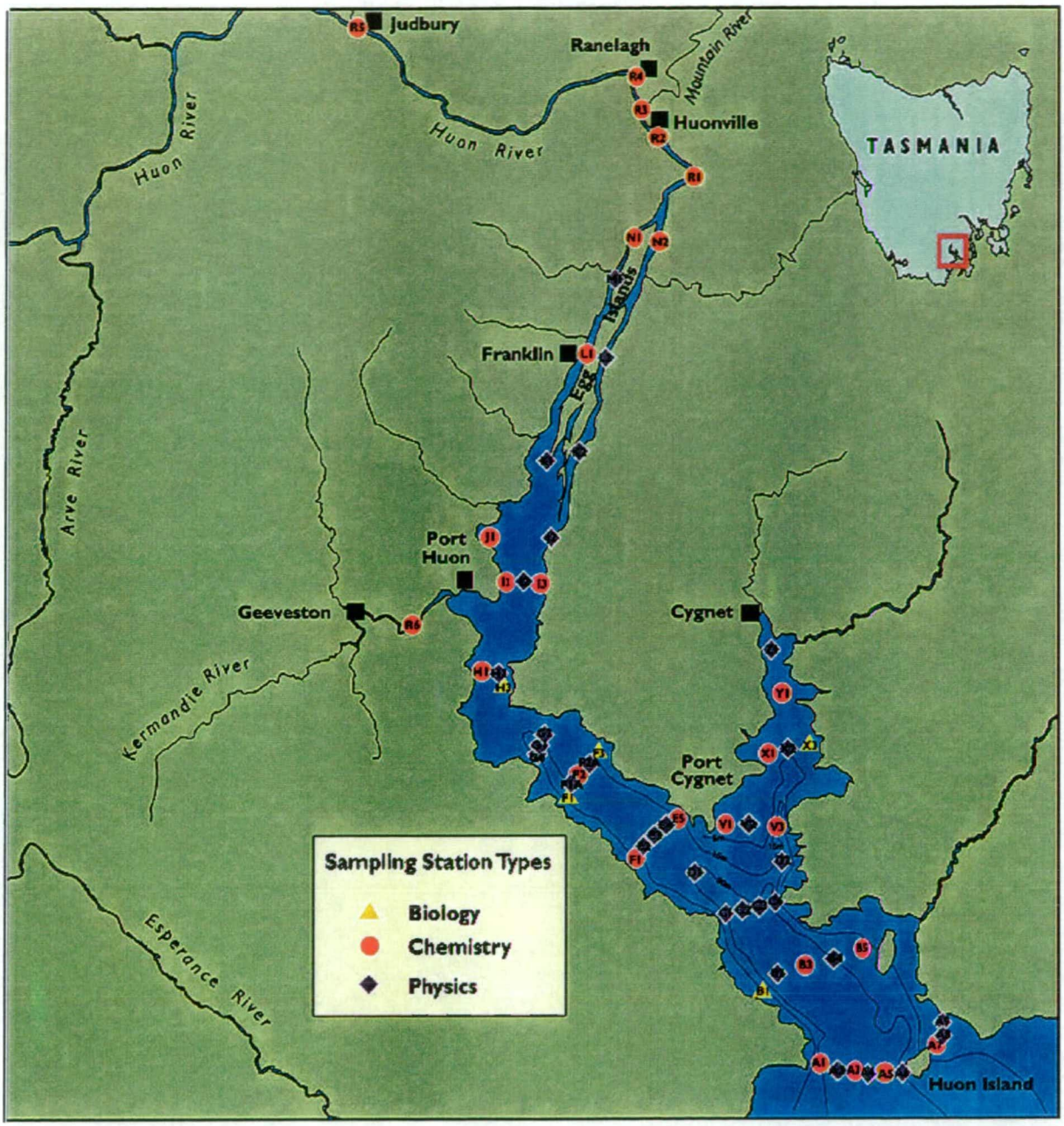

Figure 5.2 Map of the Huon River showing the location of physical, chemical and biological stations used during the spatial and continuous monitoring surveys

5.2.2 Sampling protocols

5.2.2a Preparation of sample bottles

Samples for analysis of As(V+III), MMA and DMA were collected into 250-mL HDPE (Nalgene, Rochester, NY, USA) bottles. These bottles were previously cleaned by soaking for one week in 2% Extran solution, followed by a rinse with distilled water, then soaking for a further week in 10% HCl, and rinsing again with distilled water. Each bottle was rinsed with sample prior to filling.

Table 5.3 Dates of sample collection from Killala Bay, Huon estuary.

Continuous monitoring survey	Date
CM 43	9 December, 1997
CM 44	16 December, 1997
CM 45	30 December, 1997
CM 46	6 January, 1998
CM 47	13 January, 1998
CM 48	23 January, 1998
CM 49	27 January, 1998
CM 50	3 February, 1998
CM 51	9 February, 1998
CM 52	24 February, 1998
CM 53	3 March, 1998
CM 54	10 March, 1998
CM 55	17 March, 1998
CM 56	24 March, 1998
CM 57	7 April, 1998
CM 58	14 April, 1998
CM 59	21 April, 1998
CM 60	28 April, 1998
CM 61	6 May, 1998
CM 62	13 May, 1998
CM 63	26 May, 1998
CM 64	3 June, 1998
CM 65	18 June, 1998
CM 66	14 July, 1998

Samples for the analysis of As(III) were collected into 125-mL HDPE Nalgene bottles that had been cleaned as described above. A small hole was drilled in the lid of each bottle and covered with strong tape, allowing the bottles to be frozen in liquid nitrogen without exploding.

5.2.2b HES 3 – HES 9 spatial surveys

Samples were obtained using 5- or 10- L Niskin bottles. Samples from the surface were collected with the Niskin bottles held horizontally, just below the surface microlayer. Samples from the mid and bottom depths were collected with the Niskin bottle in a vertical position. These bottles were inverted after sampling for dissolved oxygen, and before sampling for any other chemical parameters, to homogenise the sample. From the Niskin bottle, a bulk 1-L sample was collected into acid-cleaned bottles (HDPE-Nalgene or Kartell). This sample was used for nutrient and arsenic analyses, and was stored in ice after sampling until return to the laboratory. Samples were returned to the laboratory after each half-day of fieldwork.

In the laboratory, the samples were filtered through acid-cleaned [10%(v/v) HCl] 47-mm Millipore HA 0.45-µm filters (Millipore, Bedford, MA, USA), using acid-cleaned polycarbonate filter units (Sartorius AG, Goettingen, Germany), under a slight vacuum (*ca.* 5 mm Hg). The filtered sample was transferred to an acid-cleaned 250-mL bottle (HDPE-Nalgene), acidified with 750 µL HCl (Mallinkrodt, AR Select, St Louis, MO, USA) and stored at 4 °C until analysis. For the HES 7 survey, 1000 µL, not 750 µL of HCl, was used to acidify the samples.

The same protocol was followed for each survey, but in HES 3 a smaller number of samples were collected from the surface only. Field blanks (Milli-Q water) were collected on each morning of the surveys. The blanks were stored, filtered and acidified in the same way as normal samples.

5.2.2c HES continuous monitoring

Samples from Killala Bay were obtained using a 10-L Niskin bottle fired horizontally just below the water surface. A bulk sample (5 L) was collected from the Niskin bottle and stored on ice for transport back to the laboratory. In the laboratory 250 mL was sub-sampled for arsenic analysis.

The 250-mL sample was filtered under pressure (14 kPa) through an acid-cleaned [10% (v/v) HCl] 47-mm Millipore HA 0.45-µm filter (Millipore) using an acid-washed Sartorius filter unit (Sartorius).

For As(III) analysis *ca.* 60 mL of sample was transferred to a 125-mL bottle and either analysed immediately or quick frozen in liquid nitrogen and stored at -80 °C.
The remaining filtered sample was acidified to pH 1.4 with 375 µL HCl (Mallinckrodt, AR Select) and stored at 4 °C. Frozen samples were thawed in a microwave oven prior to analysis, ensuring that their temperature remained below 30 °C.

5.2.2d HES trace metal survey

A special survey was conducted to determine the distribution of trace elements in the Huon estuary, using samples from 21 sites. The sampling protocol employed was the same as for the spatial surveys, except trace-metal-clean sampling techniques were used.

Samples for As(V+III) analysis were collected to compare the behaviour of arsenic with other trace metals (Cu, Cd, Co, unfiltered with a double pH 5 solvent extraction, and filtered using single pH 5 solvent extraction, and Zn, Mn, Fe, filtered and unfiltered by direct analysis). At the time of writing all the trace metal analyses except those for unfiltered iron were available for comparison.

5.2.3 Sample analysis

Samples were analysed in triplicate using either the manual or semi-automated HG-AFS method, as described in Chapter 3.

5.2.4 Discussion on quality control issues

One field blank was collected for each morning of fieldwork performed during the Huon spatial surveys. No arsenic was detected in any of the field blanks, with the signal from this sample always being less than three standard deviations of the method blank.

In one survey, four samples were collected from the same Niskin bottle into different sample bottles, with each sample subsequently analysed in triplicate. The results obtained for the four samples were the same within one standard deviation.

5.2.5 UV oxidation experiments

A small UV-oxidation experiment was carried out to estimate the extent of production (if any) of hydride-refractory arsenic species in the estuary. The samples that were UV-oxidised were those taken from the surface at station X3/X3B in Port Cygnet during spatial surveys HES 4 – HES 9. This site was chosen because it was identified as the one where greatest depletion of inorganic arsenic occurred during the late summer

period. Two control samples of 1 μg L^{-1} As(V) and 0.1 μg L^{-1} DMA were also UV-oxidised. An untreated portion of each sample was retained for comparison.

Approximately 60 mL of sample was placed in an acid-cleaned quartz tube and 150 μL of 30%(w/v) H_2O_2 (BDH, Aristar, Kilsyth, Victoria, Australia) added. The quartz tubes were covered with a polyethylene cap, placed around an 1100 W Hg lamp and irradiated for six hours. Following oxidation they were analysed for changes in speciation using the semi-automated HG-AFS method. Samples were weighed before and after UV oxidation to correct for evaporation.

5.3 RESULTS AND DISCUSSION

5.3.1 General overview of conditions during the spatial surveys

5.3.1a Seasonal conditions

The range of seasons corresponding to the HES spatial survey dates is detailed in Table 5.2. Water temperature, as expected, was seasonally influenced.

In any given survey, the highest surface water temperatures tended to be at the seawater end. The seawater was warmest during the two February surveys, HES 4 (1997) and HES 8 (1998), but slightly higher in HES 4 (16.8 °C) than HES 8 (16.2 °C). The period between February and March is when water temperature is expected to reach its maximum. Earlier in the Austral summer (HES 7, December 1997) surface water temperature is somewhat lower (*ca.* 14 °C). During the two spring (October) surveys, HES 3 and HES 6, the surface seawater temperature was *ca.* 12.2 °C, and a similar temperature was found during late autumn (HES 9, May 1998). Unfortunately, no surface temperatures were recorded during the winter survey (HES 5), but temperatures of around 11.7 °C were reported in CTD files from the surface mixed layer (depth *ca.* 0.65 m) near the river mouth.

Similar trends are seen in the freshwater region, although temperatures are slightly lower than in the seawater. During HES 4 and HES 8, surface temperatures were 14.2 and 13.7 °C, respectively. In the December (HES 7) survey, they were *ca.* 10 °C, and in October 97 (HES 6) *ca.* 11 °C, compared with 9 °C in 1996 (HES 3). Temperatures were slightly cooler in May (HES 9), *ca.* 8.2 °C, but warmer than in winter (HES 5), *ca.* 7.2 °C. Temperature between the two end-members is somewhat variable, with localised warming occurring in some areas.

The windiest months tend to be around January/February, while as stated previously, greatest rainfall is experienced during the winter months.

5.3.1b Nutrients, dissolved oxygen and suspended particulate material

In the Huon, dissolved oxygen concentrations are highest in the freshwater (270-350 mM kg^{-1}) and lowest in the seawater (180-290 mM kg^{-1}). Lowest dissolved oxygen concentrations are reported in summer. Particularly low values were measured in the Port Cygnet arm during HES 8 (180-240 mM kg^{-1}), lower than in the previous summer (HES 4) when the minimum was *ca.* 245 mM kg^{-1}. As mentioned earlier, bottom water at the N stations tends to become oxygen depleted. There are a number of possible reasons for oxygen depletion at these sites. On isolation of the bottom water through stratification, for instance the subsequent input of organic matter or reductants from the sediment will lead to the consumption of oxygen, or more simply the supply of DO in marine waters may become depleted as they move upstream over the organic-rich sediments found mid-estuary.

The source of filterable reactive phosphate (hereon referred to as phosphate) in the Huon River Estuary is seawater. Phosphate concentrations at the seawater end fall in the range 0.02 μM (summer) - 0.6 μM (winter). The concentrations in freshwater are much lower, and range between 0 and 0.1 μM. Highest phosphate concentrations are seen in winter months and lowest in summer, corresponding with the period of highest phytoplankton activity. Greatest phosphate depletion occurs in the Port Cygnet arm during summer months, as is evident in February 1997, although in the lower estuary, phosphate concentrations never fall below the method detection limit (0.02 μM).

Nitrate is similar to phosphate, in that its source is the seawater. However, unlike phosphate, in the lower estuary it is depleted to below the method detection limit at almost all sites following the first spring phytoplankton blooms. During HES 8, nitrate concentrations were below the detection limit (< 0.03 μM) at all sites in the lower estuary, while during HES 4 and HES 7 depletion was less extensive. Nitrate is restored to concentrations of 3 – 5 μM in winter.

Nitrite [0 (summer) – 1.6 μM (winter)] exhibits similar trends to nitrate, with highest concentrations found in winter and elevated concentrations occurring mid-estuary between the E and J transects.

Ammonia levels in the Huon Estuary are variable, but generally quite low (20 - 300 nM). Ammonia has a point source in the Kermandie River (up to 1 μM), arising from the Geeveston sewage treatment plant. There are also some signs of ammonia input from the sediment, for example, in HES 8 after fallout from phytoplankton blooms, and on some other sporadic occasions. There is a consistent

input from the N stations, where there are anoxic sediments and oxygen-depleted overlying waters.

Highest silicate concentrations are recorded in the freshwater (50 – 100 μM) and lowest in the seawater (*ca.* 0.2 μM). In general, mixing between these two end-members is conservative. Higher concentrations in the Kermandie River mean this tributary occasionally influences silicate concentrations in the salinity range 5 – 15.

As stated in the introductory section (5.1.3b), the Huon Estuary is characterised by a low suspended particulate load. The highest values reported are from the freshwater region, where the concentration is most variable, ranging between 0.5 and 4.5 mg kg^{-1}. In the seawater very low suspended particulate loads are measured. There are no distinct seasonal changes, but concentrations are perhaps slightly higher in both February surveys (still below 2 mg kg^{-1}) when phytoplankton density is greatest.

5.3.1c Phytoplankton activity

Although not always well correlated with primary production or methyl arsenic concentrations, one measure of phytoplankton activity is chlorophyll *a*. Chlorophyll *a* concentrations are greatest in the lower estuary (0.2 – 3 μg L^{-1} in the surface) and low in the freshwater upper estuary (*ca.* 0.2 μg L^{-1}). As might be expected, lowest concentrations are found in the June survey (HES 5), although in the high salinity region there is significant scatter in the October, December and February surveys, with some quite low concentrations also reported in February. In contrast to the nutrient drawdown, which suggests greatest phytoplankton activity in Port Cygnet, the highest chlorophyll *a* concentrations are at the E and F transects, with more activity in February 1998 than February 1997.

At almost all sites in the lower estuary, there is an obvious sub-surface fluorescence maximum, corresponding to the highest chlorophyll *a* concentrations. The difference between surface and sub-surface chlorophyll *a* concentrations is most distinct in winter and spring surveys.

Phytoplankton biomass in the Huon River is dominated by two diatoms (*Pseudo-nitzschia* spp. and *Chaetoceros* spp.), two dinoflagellates (*Ceratium* spp.and *Gymnodinium catenatum* (toxic)), and small flagellates. The two summer seasons sampled in the spatial surveys have quite different phytoplankton bloom dynamics.

The 1996/97 season is characterised by lower overall biomass. In December, there is a *Chaetoceros* bloom. In January, this is succeeded by small flagellates, and

Pseudo-nitzschia becomes dominant through February and March. The diatom bloom is followed by the dinoflagellate, *Ceratium*, which dominates until small flagellates take over during winter.

In 1997/98, the toxic dinoflagellate *G. catenatum* blooms through November and into late January. In this season, the diatom blooms occur later and their cell numbers are lower compared with the previous season. Although diatoms dominate the cell counts through February, *Ceratium* (dinoflagellate) is the most prominent species in terms of biomass from early February through to late April.

Actual algal counts and biomass show little variation between the biological sites at Port Cygnet (X3/X3B), F1, F3 and B1, although chlorophyll levels are proportionally lower for the counts in Port Cygnet.

5.3.2 Results from the HES spatial surveys

5.3.2a Arsenic concentrations

Arsenic concentrations in the seawater end-member of the Huon River are similar to those reported elsewhere for Australian coastal waters [2], ranging between 1.1 and 1.6 $\mu g\ L^{-1}$. As seen in other relatively clean estuaries, the source of arsenic to the river is predominantly the seawater. Therefore, arsenic concentrations are highly dependent on salinity and consequently increase with depth where the salt wedge exists, as seen elsewhere [53]. The concentrations found in the freshwater end-member are extremely low, 0.023 – 0.057 $\mu g\ L^{-1}$, despite historic use of arsenic in the catchment area. The mean concentration at site R4 (Ranelagh) was $0.035 \pm 0.010\ \mu g\ L^{-1}$ for HES surveys 3-10A. These concentrations are among the lowest reported for arsenic in freshwater. They are an order of magnitude lower than those in the River Beaulieu [24] and unpolluted rivers of the SE United States [22], and about one third that reported by Seyler and Martin [25] (*ca.* 0.1 $\mu g\ L^{-1}$) for a pristine river system draining a national park in Yugoslavia (See Table 5.1). The freshwater concentrations are also lower than those reported for the Tamar River (Tasmania), where the influence of industrial and urban activity is significantly greater. Similar concentrations would probably be expected in the upper reaches of the Derwent River, above industrial sources, where results less than 0.1 $\mu g\ L^{-1}$ (the method detection limit) have been reported [27, 31].

MMA and DMA are also found in the Huon River and their concentration (MMA 0.001 – 0.07 $\mu g\ L^{-1}$; DMA 0.03 – 0.17 $\mu g\ L^{-1}$) and distribution is similar to that found in other temperate estuaries where there is a lag between DMA production and the

appearance of MMA in the water column [57, 61, 71]. MMA and DMA concentrations vary seasonally, with peak production being in the summer months when they may represent as much as 10-15% of total arsenic. DMA is always the dominant methyl arsenic species, although the DMA/MMA ratio does vary both seasonally (decreases through summer, Table 5.5) and at different sites in the estuary (no defined pattern). During winter, methyl arsenic species can still be measured in the water column, but represent a very small proportion of total arsenic (1-3%).

The lowest concentrations of MMA (0.001 – 0.002 $\mu g\ L^{-1}$) and DMA (0.003 – 0.05$\mu g\ L^{-1}$) are found in the freshwater region, and highest at the seawater end of the estuary, particularly in the salinity range 33-35. However, the percentage of total arsenic methylated is similar in the freshwater and seawater end-members.

Little methyl arsenic production occurs where there is low light penetration. The highest percentage arsenic methylation is found at the surface and the lowest at depth, indicating that production is associated predominantly with phytoplankton activity, not remobilisation from sediments. Interestingly, methyl arsenic production is higher at the surface than at the *in situ* chlorophyll fluorescence maximum (mid) depth, although in the Huon, maximum chlorophyll is sometimes found at the surface, with the sub-surface fluorescence maximum being a secondary peak (coloured dissolved organic matter may also effect this peak). This observation may be a consequence of the fact that the chlorophyll maximum does not necessarily reflect a maximum in primary production.

5.3.2b As(V+III) distribution during spatial surveys

As discussed in the literature review (5.1.2b), provided that there is little change in the freshwater end-member concentration with time and river flow, one of the simplest ways to represent arsenic distribution during estuarine mixing is to use arsenic-salinity plots. In the Huon, the freshwater arsenic concentration is very low and exhibits only small changes in concentration over the entire study period (2 years). Therefore, arsenic-salinity plots are a reasonable approach to studying arsenic behaviour during mixing.

From the literature for a variety of different river systems, it is expected that rivers like the Huon, with very low arsenic concentrations in the freshwater, a low suspended solids load, high concentrations of organic matter and a relatively short flushing time, will exhibit conservative arsenic-salinity mixing curves. That is, a linear relationship, representing simple dilution, is expected between the high arsenic concentrations found in seawater and the very low freshwater concentrations, provided there are no point

source inputs from tributaries, sediment remobilisation, or removal *via* biological processes.

The arsenic-salinity plots for all sample depths in HES surveys 3 to 10A are shown in Figs. 5.3 – 5.10. When plotting these results the estuary has been divided into four regions to allow further interpretation. The regions are: lower estuary (A and B transect samples), Port Cygnet (X, Y, Z transects), mid-estuary (E, F, H, I, J transects) and upper estuary (L, N, R transects). The lower estuary and Port Cygnet arm are the most marine influenced sites, while the middle estuary is more brackish, dependent upon river flow. The upper estuary sites are predominantly fresh water, but under low flow conditions salt water can be found at the bottom as far up-river as station R1. Similar plots, but with the samples divided into groups on the basis of the depth at which they were collected (surface, F_{max}, bottom), are given in Appendix 7.

All the arsenic-salinity plots indicate that seawater is the source of arsenic to the estuary. To determine the reactivity of arsenic within the estuary the fresh water and seawater end-members must be designated (Table 5.4). The freshwater end-member is simple to designate, and in this case is the concentration found in the surface sample taken at the furthest up-river site, R5 at Judbury (See Fig. 5.2). Selection of a marine end-member is not so straight forward. For the purposes of this work, the marine end-member has been designated as the As(V+III) concentration in the bottom sample from site A3, one of the most marine sites. In fact, the Huon Estuary opens into the D'Entrecasteaux Channel, which feed into Storm Bay, which subsequently opens into the Tasman Sea. Therefore, there is a variation in the marine characteristics of waters at the mouth of the Huon Estuary, which is dependent on the discharge of several rivers into the channel, the direction and strength of currents within the channel, and the changing influence of the main coastal currents around southeastern Tasmania [89]. Therefore, as expected, the arsenic concentration in the seawater end-member is more variable.

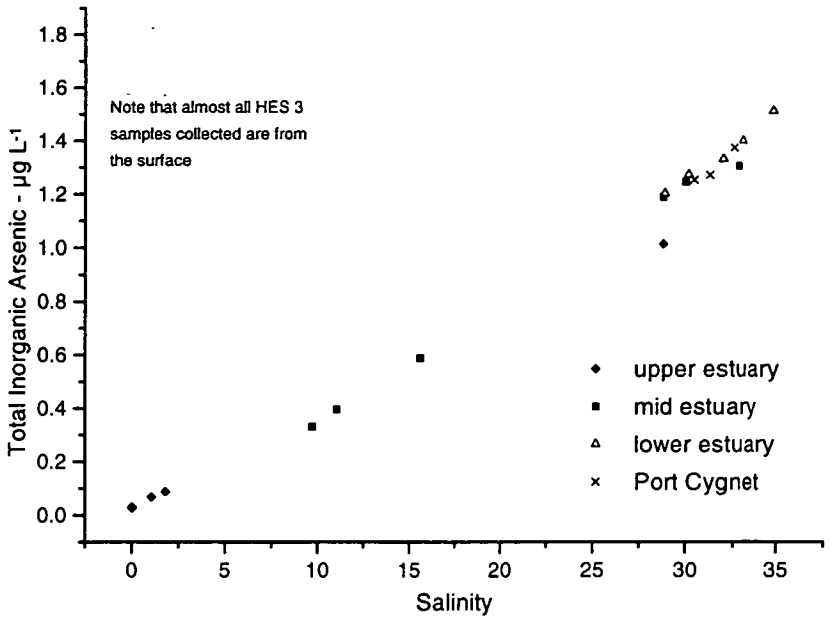

Figure 5.3 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 3 survey (October 1996)

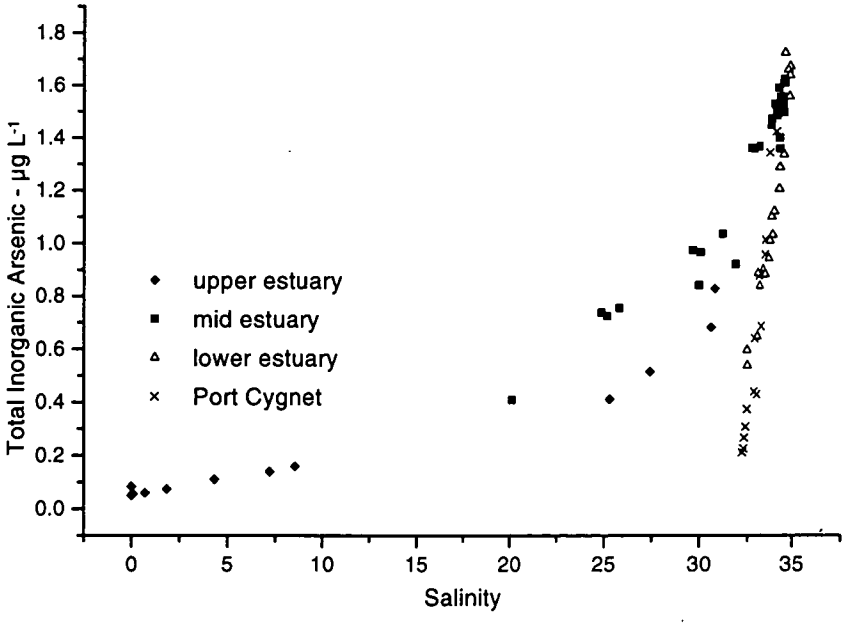

Figure 5.4 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 4 survey (February 1997)

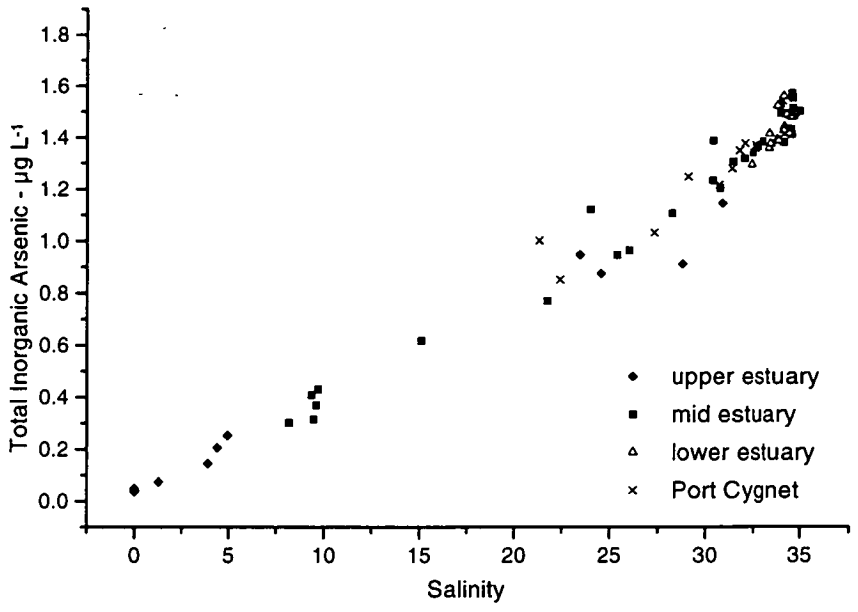

Figure 5.5 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 5 survey (June 1997)

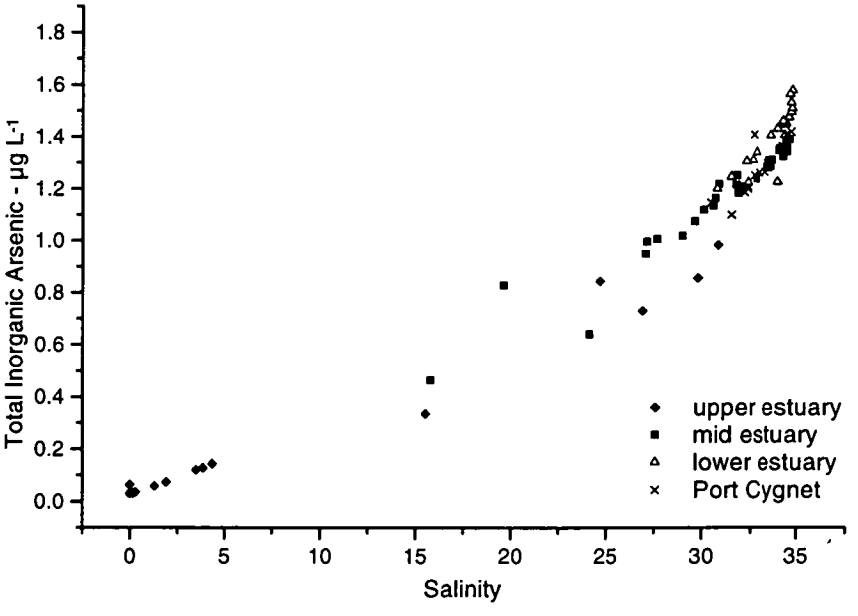

Figure 5.6 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 6 survey (October 1997)

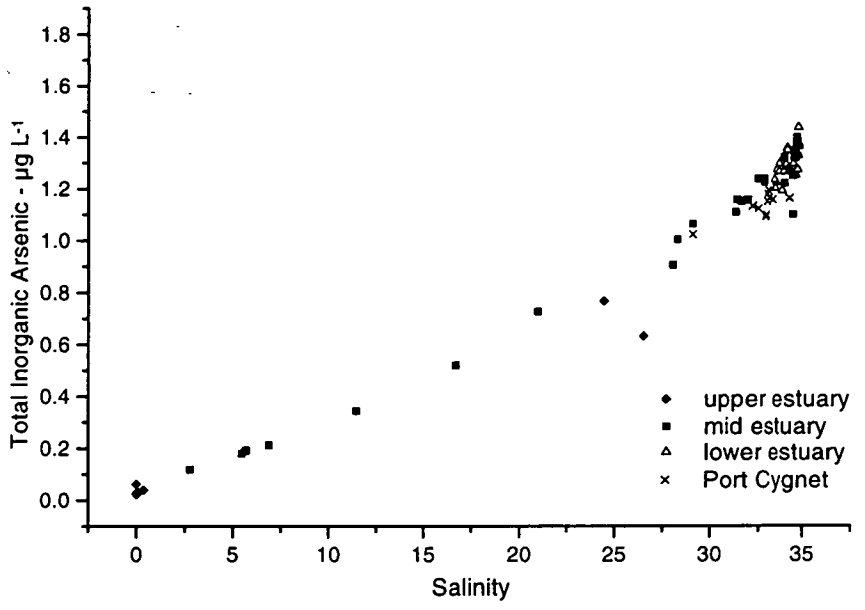

Figure 5.7 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 7 survey (December 1997)

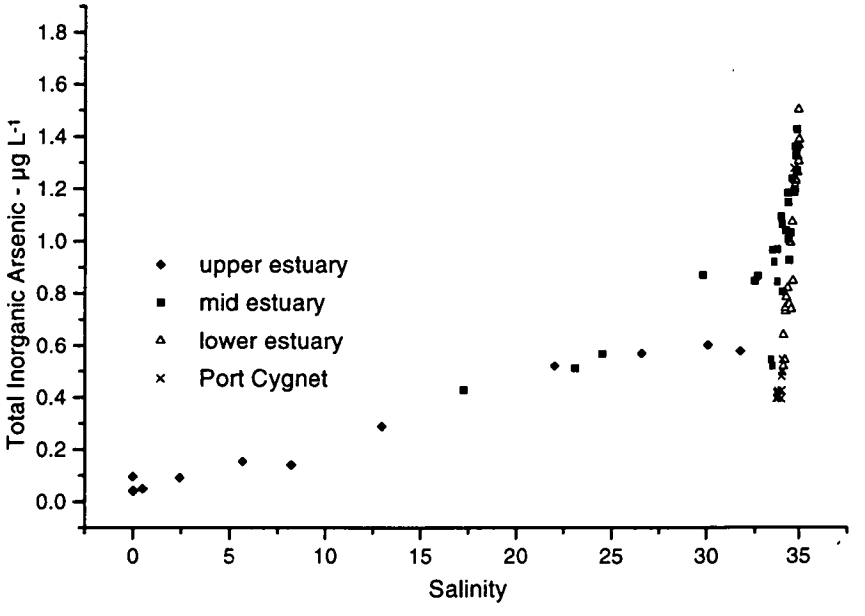

Figure 5.8 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 8 survey (February 1998)

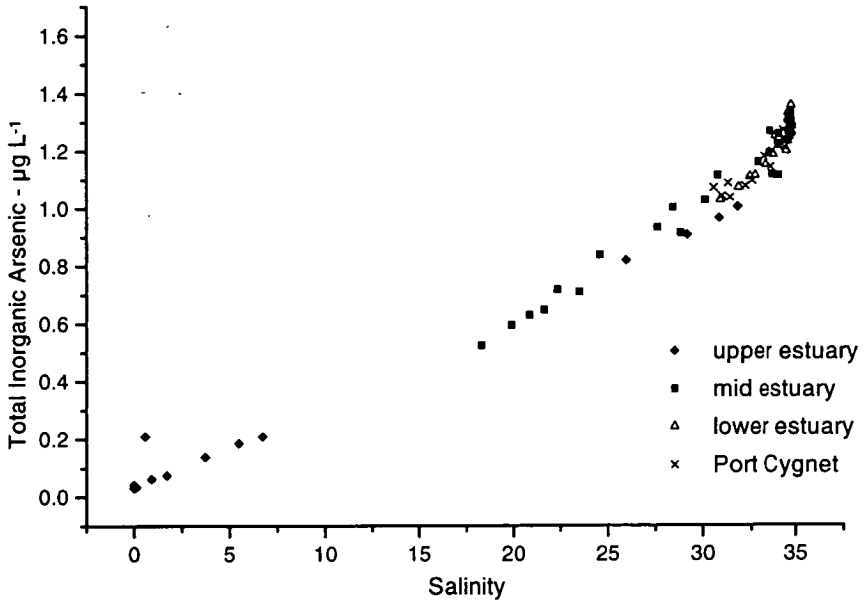

Figure 5.9 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 9 survey (May 1998)

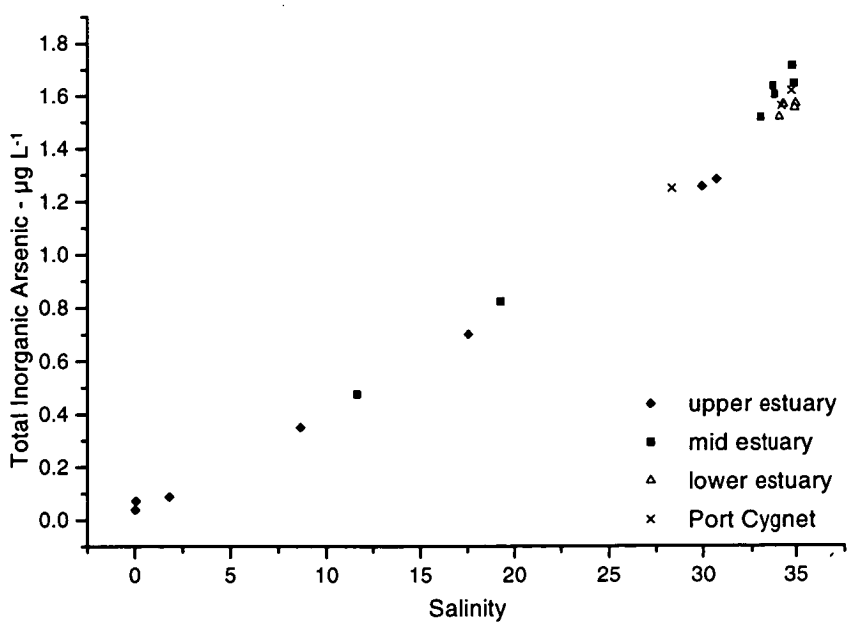

Figure 5.10 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 10A survey (September 1998)

Table 5.4 Fresh water and seawater end-member concentrations during HES 3-10A

Survey Number	[As(V+III)] at R5 (fresh water end-member)	[As(V+III)] at A3 (bottom) (seawater end-member)
HES 3*	0.027 $\mu g\ L^{-1}$	1.515 $\mu g\ L^{-1}$
HES 4	0.050 $\mu g\ L^{-1}$	1.671 $\mu g\ L^{-1}$
HES 5	0.040 $\mu g\ L^{-1}$	1.497 $\mu g\ L^{-1}$
HES 6	0.030 $\mu g\ L^{-1}$	1.492 $\mu g\ L^{-1}$
HES 7	0.025 $\mu g\ L^{-1}$	1.437 $\mu g\ L^{-1}$
HES 8	0.040 $\mu g\ L^{-1}$	1.364 $\mu g\ L^{-1}$
HES 9	0.031 $\mu g\ L^{-1}$	1.299 $\mu g\ L^{-1}$
HES 10A	0.038 $\mu g\ L^{-1}$	1.565 $\mu g\ L^{-1}$

*in HES 3 R4 and B3(bottom) were used as the fresh- and sea water end-members

As predicted, with the exception of both February 1997 and 1998, the behaviour of arsenic with salinity is close to conservative, although in all cases there is some suggestion of removal in the mid-estuary region. Two sites show small but distinct deviation from the typical mixing behaviour observed. The first is R6, in the Kermandie River, where inorganic arsenic concentrations are still very low (0.045 – 0.1 $\mu g\ L^{-1}$), but are higher than at other freshwater sites (R1 – R4) and exhibit greater variability. This may be as a result of anthropogenic input from sewage treatment or a nearby refuse site, but may also simply reflect that the river drains an area with slightly different geology to the upper Huon River. The second site is at the N transect (site N2), where the bottom sample is from an oxygen-depleted, ammonia-enriched environment, and arsenic concentrations are consistently lower than at other sites with similar salinity. This is unlike other oxygen-depleted systems where arsenic concentrations have been reported to increase [90] and the Tamar River (UK) where high ammonia concentrations coincide with the release of sediment interstitial waters with high arsenic concentrations [49]. In this part of the river the bottom sediments are composed of quite fine silt-like mud, while further upstream the bottom is scoured by faster flows and downstream silty muds persist until near the river mouth where sand is found. The smell of hydrogen sulfide in the sediments from the N transect suggests that they are anoxic. When the salt wedge penetrates up to the N transect it has a relatively long residence time in contact with the particulate material at the bottom, that might be sufficient for slight arsenic

removal to occur. It was thought that this might be through adsorption onto Fe-S or other sulfidic phases as seen elsewhere [91]. However, low iron concentrations in these sediments [92] do not favour this mechanism. From the trace metal analyses it is clear that the behaviour of unfiltered iron in the estuary, like arsenic, is close to conservative, except that the highest concentrations are found in the freshwater. There is no apparent anomaly in the iron concentration at N2 either which would explain the low arsenic conentrations. Without further investigation, the mechanism by which arsenic removal is occurring at this site cannot be elucidated.

i) As(V+III) depletion during the spatial surveys

In both February 1997 and 1998 spatial surveys, arsenic markedly departs from conservative estuarine mixing and significant depletion occurs, mostly in Port Cygnet but also at the A and B transects. When sample depth is considered (see Appendix 7 for As(V+III) *vs* salinity by depth plots), most removal is found to be occurring at the surface and fluorescence maximum. As other biogeochemical factors, such as suspended solids and water mass residence time, are similar to other spatial surveys, this depletion is attributed to 'bio-utilisation'. It is, however, unusual that this effect is not also observed in the early summer survey HES 7 (December 1997).

The greatest arsenic depletion is during HES 4, when the arsenic concentration is only 30% of that predicted if conservative mixing was occurring (using a linear regression between the fresh water and seawater end-members identified in Table 5.4 to predict As(V+III) from salinity). HES 4 is also the survey when greatest phosphate depletion occurs, with Port Cygnet being the most depleted site. The extent of arsenic depletion seems to radiate out from Port Cygnet and along the northern side of the river, with the greatest depletion at Y1, X3, and V3, moderate depletion at X1 and B5 and least at V1, B3, A7, and A5 (see Fig. 5.4). In HES 8, the extent of arsenic depletion is less, but the effect is more widely dispersed (Fig. 5.8). Once again, depletion is greatest at the Port Cygnet sites, but also occurs upstream at F and H to a lesser extent, and right across the river at the A and B transects.

5.3.2c MMA and DMA distribution during the spatial surveys

The methyl arsenic species are indicators of biological activity, and are thought to be the degradation products of non-hydride forming arsenic species that form when As(V) inadvertently enters phosphorus biochemical pathways. The Huon Estuary provides a good opportunity to study biological cycling of arsenic because the suspended solids load is very small and residence times in the lower salinity regions of the estuary are

short. Therefore, this is essentially a 'biochemical' estuary where geochemical effects upon arsenic are expected to be almost absent from the water column; biological mediation of arsenic speciation is effectively the sole influence.

DMA concentrations are highest in the seawater and tend to decrease linearly with salinity going upstream, if only surface samples are considered. The relationship is more complicated if samples from all depths are included as concentration decreases with depth as a result of changes in light availability, and therefore, phytoplankton density and activity. To simplify the relationship, only surface samples are considered in this section. However the full set of data for all depths can be found in Appendix 8 (MMA) and 9 (DMA).

The typical seasonal cycle of DMA production in temperate estuaries is well documented [55-57, 59, 61, 72]. Very low concentrations occur over winter. After the first spring phytoplankton blooms, and once the water temperature increases above 12 °C, DMA begins to appear. Its concentration generally builds up during the summer months before falling sharply once the water temperature drops after the onset of autumn.

In the Huon, the seasonal cycle of DMA production is best illustrated with results from the spatial surveys from winter 1997 (HES 5) through to late autumn 1998 (HES 9) (Fig. 5.11). During the winter survey (HES 5) DMA concentrations are very low, but by October (HES 6), when the first phytoplankton blooms have occurred and the water temperature is 12.2 °C, the concentration has increased by about 50%. It then builds up over the summer months, increasing from October to December (HES 7) and further from December to February, when the concentrations are about an order of magnitude higher than in winter. By May, the water temperature has dropped back to about 12 °C and DMA decreases to concentrations similar to those measured in spring, with the exception of some sites in the lower estuary. DMA distribution is significantly more patchy in autumn compared with spring, with some high concentrations, reminiscent of summer, in Port Cygnet and along the northern side of the river at the A and B transects where some residual phytoplankton activity remains. Along the southern side of the river, where more marine waters enter, productivity and DMA production have declined. This is further illustrated in Fig. 5.12 where the results for DMA in HES 9 surface samples are shown by location.

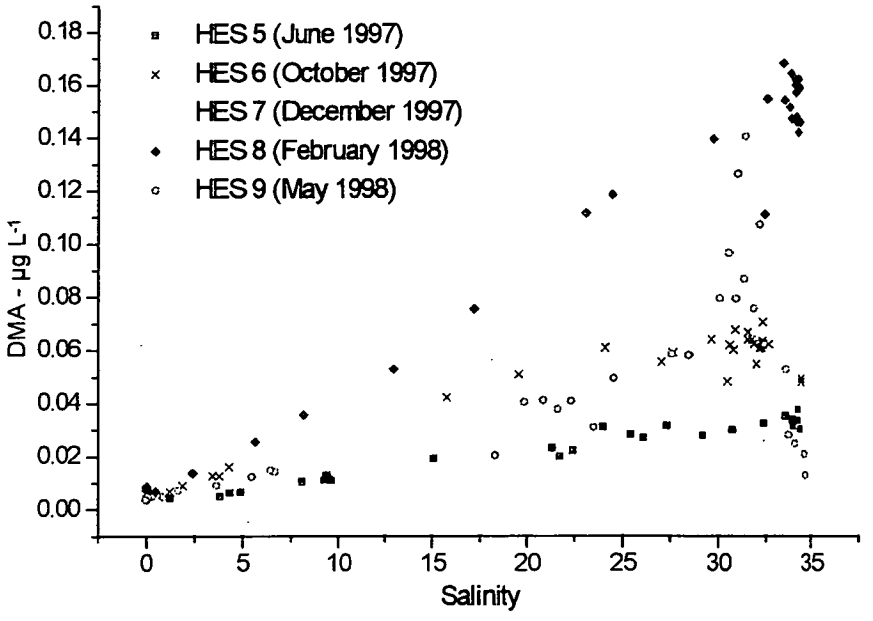

Figure 5.11 Plot showing an annual cycle of DMA production in surface samples from HES 5 (June 1997) through to HES 9 (May 1998) in the Huon River

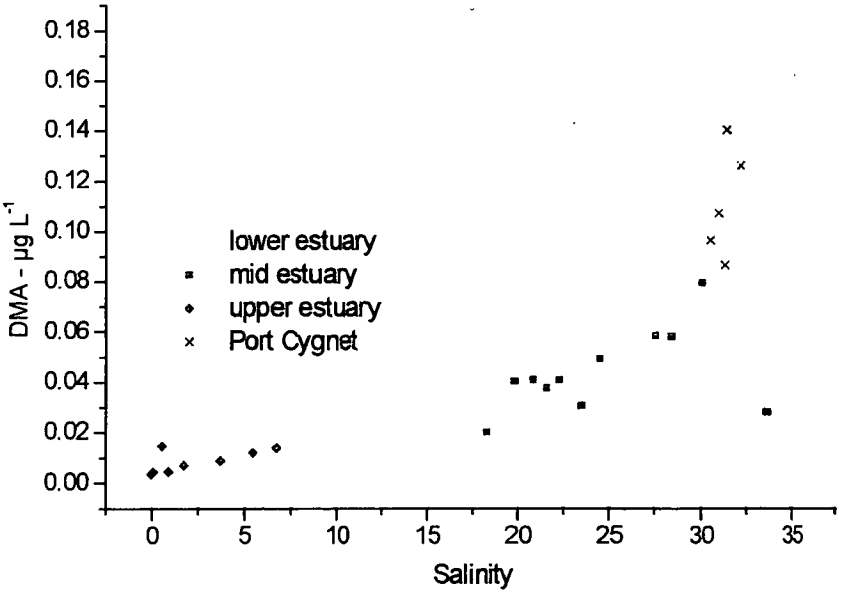

Figure 5.12 Plot showing DMA *vs* salinity data by location for surface waters during the autumn 1998 survey (HES 9)

As seen in Southampton water [72], the Huon also shows interannual variability in DMA production, with October and February of the 1996/97 season distinctly lower than the 1997/98 season, corresponding with observations of greater biomass in the second year. Quite high scatter in DMA concentrations is seen at the high salinity end of the transect, with higher levels in Port Cygnet and at the A and B transect compared with the E ands F transects. In general, the DMA-salinity mixing curves are near-linear, suggesting that dilution is the only process occurring upstream in the estuary.

The seasonal cycle of MMA is different to DMA. As seen by Millward and coworkers [55] in the Humber River, its production lags behind that of DMA. MMA concentrations only increase from baseline levels during the two February surveys (Fig. 5.13), with all other surveys approximately the same ($< 0.01\ \mu g\ L^{-1}$) (Fig. 5.14). Similar to the trend for DMA, less MMA (approximately half) is produced in the 1996/97 season, as would be expected if, as is commonly proposed [55, 72], MMA is formed *via* degradation of DMA.

The highest values for MMA, during both summer seasons, are reported for surface samples at the A and B transects (A > B), with slightly lower values in Port Cygnet. Interestingly, in the February surveys the MMA-salinity mixing curves are different to the essentially linear mixing curves otherwise observed for DMA (the proposed substrate) and MMA. This indicates that there is either an active input or removal process occurring. Input may occur if there is direct production of MMA, a faster rate of DMA breakdown, or a slower rate of MMA removal occurring at the river mouth. Conversely MMA removal may be occurring in the mid-estuary if it is being broken down more quickly. At present there is insufficient evidence to support either mechanism.

Correspondingly, the ratio DMA/MMA decreases through both seasons from *ca.* 20 in winter to as low as 3 in February (Table 5.5). Despite different phytoplankton populations, the DMA/MMA ratio is the same in both February surveys, and varies little between the sites at the mouth of the river, supporting the proposal that MMA is a degradation product rather than a direct product of a specific phytoplankton species.

In the Kermandie River, MMA concentrations are high compared with other freshwater sites, and represent a large proportion of the total arsenic present (up to 44%). This is thought to be associated with high bacterial numbers, a result of effluent from a sewage treatment works, mediating either MMA production or rapid demethylation of DMA.

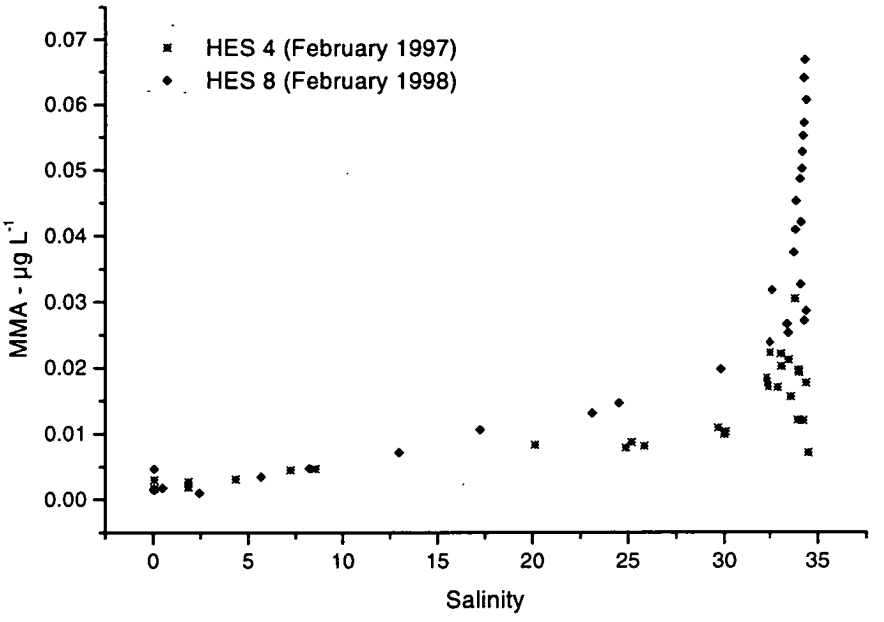

Figure 5.13 Surface MMA concentrations in the Huon River during the two summer surveys, HES 4 and HES 8

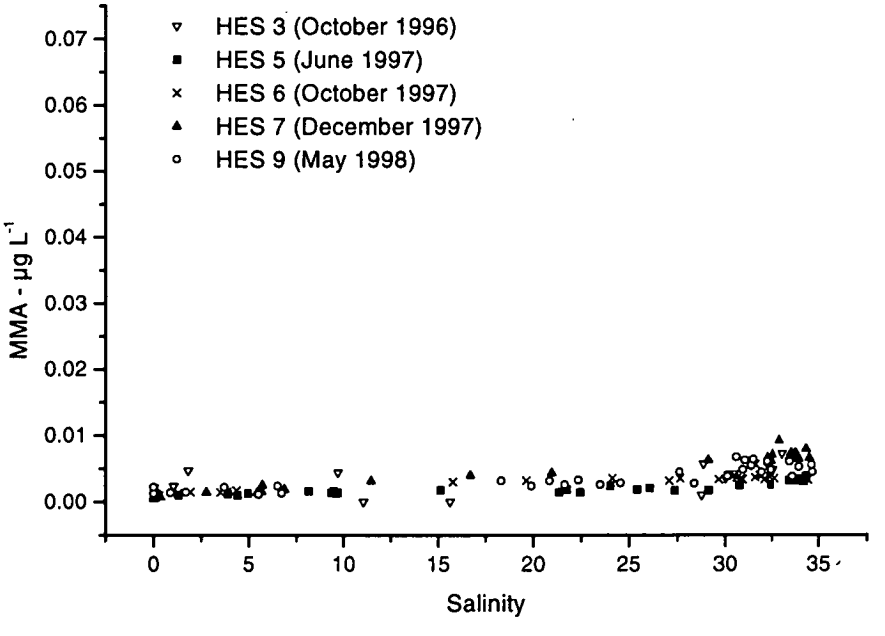

Figure 5.14 Surface MMA concentrations in the Huon River during winter, spring and autumn surveys

Table 5.5 Seasonal variation in the DMA/MMA ratio in the high salinity regions of the Huon Estuary

HES Survey	DMA/MMA
HES 4	3 - 4
HES 5	8 – 20
HES 6	13 – 21
HES 7	8 – 14
HES 8	3 – 5
HES 9	3 – 22

Although high concentrations of methyl arsenic species are found in summer (February), they are not sufficient to account for the arsenic depletion observed at the Port Cygnet and A and B transect sites. That is, the sum of measured As(V+III), MMA and DMA is still significantly less than the concentration predicted if conservative mixing occurred.

There are a number of possible explainations for this depletion. The first is that arsenic species are being converted to hydride-refractory or 'hidden' arsenic species that cannot be measured using the hydride-generation method. Another possibility is that arsenic has been incorporated into biological organisms. This might occur if there is increased arsenic uptake by phytoplankton, or if it is being incorporated by shellfish (predominantly mussels) at nearby lease sites.

5.3.2d UV-labile arsenic species at site X3/X3B (Port Cygnet)

To determine the role of hydride-refractory arsenic species in summer arsenic depletion, surface samples from one of the most depleted sites (X3/X3B) in Port Cygnet were UV-oxidised. Samples were analysed before and after UV-oxidation to determine changes in As(V+III), MMA and DMA concentrations. The results are shown in Fig. 5.15. It should be noted that no MMA or DMA results were obtained for HES 7, HES 9 and the DMA standard as a result of an analytical problem. Subsequent to running the UV-oxidised HES 7 sample the arsenic signal was completely quenched and no further results could be obtained. The reason for this is unclear, although apparently related to changes in the HES 7 sample following UV-oxidation. After the packed U-trap was baked in the oven overnight, normal instrument sensitivity was returned.

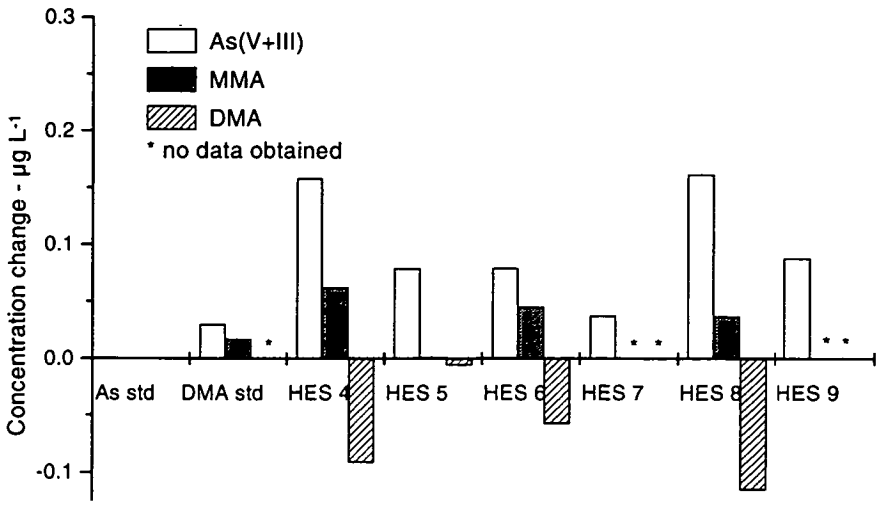

Figure 5.15 Change in arsenic species concentration upon UV oxidation of samples from site X3/X3B in Port Cygnet

The results show that in all cases, As(V+III) and MMA concentrations increase, and DMA concentrations decrease after UV-oxidation of the sample. The increase in As(V+III) and MMA is greater than the decrease in DMA suggesting that some breakdown of hydride-refractory species has occurred. As expected, the largest changes are for the HES 4 and HES 8 samples, with more UV-labile arsenic in the HES 4 sample than HES 8. When the UV-labile arsenic fraction is added to the sum of the hydride-forming species, there is still up to 60% of the expected arsenic missing (Fig. 5.16). In the absence of a total digest on the samples, it is unclear whether the 'missing' arsenic is a fraction that is refractory to UV-photolysis or whether it is no longer in the water column, having been incorporated into the biota.

Indications from the paper by Hasegawa *et al.* [79] are that UV-labile arsenic is the major fraction of refractory arsenic. Therefore, it is likely that only a small fraction of the calculated 'missing' arsenic would be hydride-refractory species. It is more likely that some form of bio-utilisation is occurring.

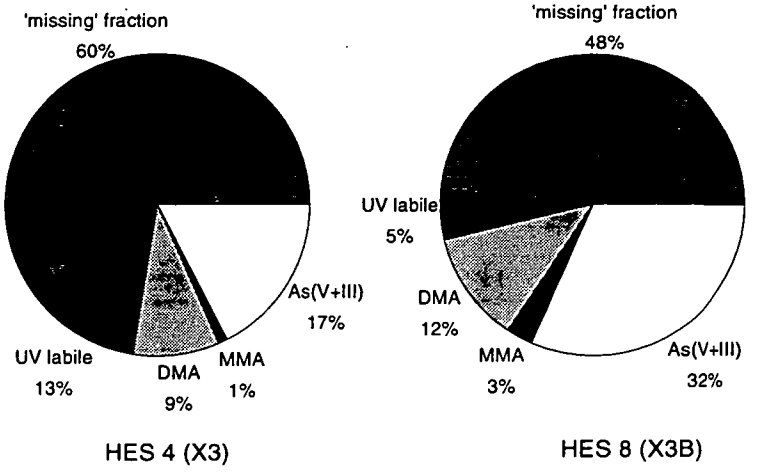

Figure 5.16 Fractionation of arsenic at site X3 and X3B in the two February surveys, HES 4 and HES 8. Total arsenic has been determined on the basis of estimated 'conservative' concentration.

5.3.2e Relationship of arsenic species with nutrients and biological parameters

i) Relationship with nutrients

Similar to As(V+III), in the Huon estuary the main source of phosphate is seawater, although there are also some inputs from sewage. Cycling of phosphate is predominantly influenced by phytoplankton uptake, therefore, correlation between As(V+III) and phosphate can be used to indicate whether arsenic is being taken up during phytoplankton blooms.

As might be expected, for all surveys (using samples from all depths) there is a general positive trend between phosphate and arsenic, however, the degree of correlation is variable between surveys as shown in Table 5.6.

The best correlation is found for the winter survey (HES 5), when there is little biological activity occurring. Poorest correlation is in the late spring/early summer survey (HES 7), while good correlation is established again for the summer surveys. The poor correlation in December is apparently because phosphate is being depleted relative to arsenic, while in summer both phosphate and arsenic are depleted by biological activity, indicating that arsenic-phosphate discrimination might decrease as the season progresses.

Table 5.6 Correlation between As(V+III) and phosphate for each Huon spatial survey

	Correlation (r)	n	Significance
HES 3	0.8893	21	P<0.0001
HES 4	0.8750	76	P<0.0001
HES 5	0.9640	75	P<0.0001
HES 6	0.8063	77	P<0.0001
HES 7	0.5340	74	P<0.0001
HES 8	0.8695	74	P<0.0001
HES 9	0.8068	74	P<0.0001

Looking more closely at surface samples from the arsenic depleted sites (A, B, E, V, X, and Y transects) in HES 4 and HES 8 also shows some interesting trends. During HES 4, there is good correlation between As(V+III) and phosphate ($r = 0.93603$, $n = 14$, $P<0.0001$) and arsenic depletion and phosphate ($r = -0.90573$, $n = 14$, $P<0.0001$) at these sites, but insignificant correlation for HES 8. For the HES 8 samples, there is significant correlation between arsenic depletion and the As/P ratio ($r = -0.8373$, $n = 14$, $P<0.0002$), but not for HES 4.

This evidence suggests that arsenic uptake and methylation is dependent upon the phytoplankton species present and their relative ability to discriminate between arsenic and phosphate. The results from HES 7 indicate that there is little arsenic uptake and perhaps faster arsenic turnover occurring in early summer. This may be because there is a lower As/P ratio and therefore less arsenic stress at this time of year, or because the dominant phytoplankton species at this time, *Chaetoceros*, has a high nutrient selectivity and does not readily take up arsenic. The different arsenic-phosphate relationship in the two summer surveys most likely reflects the different species composition of the phytoplankton blooms in each year. In the first summer, when there is significant As-P correlation, phytoplankton biomass is dominated by the diatom *Pseudo-nitzschia*. In the second summer, when this relationship breaks down, *Pseudo-nitzschia* still dominates cell counts, but the dinoflagellate *Ceratium* dominates the biomass. On the basis of these results, it seems that *Pseudo-nitzschia* has a poor ability to discriminate between arsenic (presumably arsenate) and phosphate. Therefore when it dominates the summer bloom there is a clear correlation between phosphate and arsenic depletion. In the second summer there is a bloom of mixed phytoplankton species that differ in their ability to discriminate between arsenic and phosphate, and thus in this season there is no clear correlation. In fact, in the second summer the

correlation between the As/P ratio and arsenic depletion suggests that arsenic uptake occurs because of reduced ability to discriminate between arsenic and phosphate under increased arsenic stress rather than a complete inability to As-P discriminate.

The poor correlation between the methyl arsenic species and phosphate utilisation is similar to findings in other studies [56, 64, 65]. These results support the theory that phytoplankton do not immediately release DMA (or its precursor) into the water column, but initially release it as a hydride-refractory species that must first be broken down before it can be detected using hydride generation techniques.

No significant correlation was found for As(V+III) or the methyl arsenic species with total dissolved phosphorus, total phosphorus, dissolved oxygen, ammonia, or any of the other nitrogen species measured. The poor relationship of the methyl arsenic species with ammonia supports the observations made earlier, that the sediments have little role in methyl arsenic release. Also, production of the methyl arsenic species is not strongly linked with the remineralisation of organic nitrogen as ammonia.

ii) Relationship with chlorophyll a

No significant relationship was found for As(V+III), MMA or DMA with chlorophyll *a* concentrations. This was the case for all the surveys, even when only surface samples were considered.

This observation is similar to that made for other temperate estuaries [48, 56, 57, 61, 65], and is hardly surprising given that different phytoplankton vary in their As-P tolerance and that DMA and MMA occurrence has been shown to lag behind cell growth. Also, in the Huon it has been shown that chlorophyll *a* concentrations were not always indicative of cell counts. This was seen in Port Cygnet, where chlorophyll *a* concentrations were proportionally lower for the cell counts compared with other parts of the estuary, and also in *G. catenatum* blooms, where chlorophyll *a* was often not indicative of high cell numbers.

iii) Relationship with major phytoplankton species

No clear relationship could be established between cell counts (from integrated water column samples) of the major phytoplankton species, and production of the methyl arsenic species. Again, this is most likely because at each site there is a mixture of different phytoplankton species, which vary in their ability to take up and methylate arsenic, and moreover methyl arsenic production often lags behind phytoplankton growth.

A significant correlation (r = 0.95262, n = 15, P>0.0001) exists between cell counts of the phytoplankton species *Pseudo-nitzschia* and surface As(V+III) depletion, using data from the biological stations in HES surveys 4, 7, and 8 (Fig. 5.17). This finding complements the observations made earlier with regard to arsenic-phosphate relationships during blooms of this species, and provides further evidence of the poor ability of this species to arsenic-phosphate discriminate. There is little evidence in the literature that suggests a unique set of physiological requirements for these species and the nutrient conditions which might trigger blooms, in fact they are quite successful over a wide range of physiological conditions. In the Northern Hemisphere, *P. pseudodelicatissima,* the dominant species found in the Huon, has been shown to prefer warmer seasons [93]. Interestingly, *Pseudo-nitzschia* species are studied in the Northern Hemisphere because they produce domoic acid, which has been responsible for outbreaks of amnesic shellfish poisoning. However to date, species found in Australian waters have not been shown to produce this toxin [94].

Pseudo-nitzschia only became dominant in the Huon River under the nutrient-depleted, higher temperature conditions of late summer, explaining why arsenic depletion only occurred in the two February surveys, rather than during early summer or spring conditions. Also, the different distribution of arsenic depletion in the two summers was directly correlated with *Pseudo-nitzschia* numbers.

No similar correlation could be established using cell counts of the other major phytoplankton species or bacterial counts.

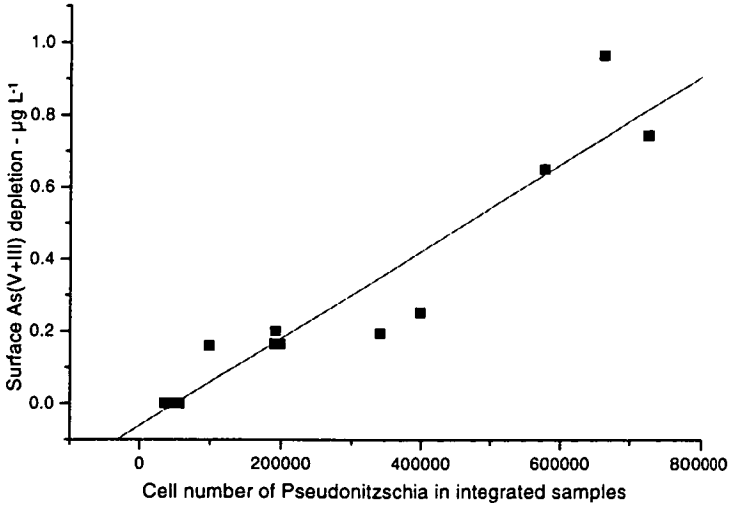

Figure 5.17 Calculated arsenic depletion (surface) *vs* number of *Pseudo-nitzschia* spp. cells in integrated water column samples

5.3.3 Results from continuous monitoring at Killala Bay

The following results are for the weekly time series collected at the continuous monitoring site at Killala Bay during the 1997/8 summer.

5.3.3a Arsenic species results

The variation of As(V+III)), As(III), MMA and DMA in the surface at station F1 during the 1997/98 summer are shown in Fig. 5.18. Ancillary data including the nutrient concentrations and phytoplankton counts are shown in Appendix 10.

The Killala Bay site is subject to both estuarine and oceanic influences depending upon river flow. The resulting variation in salinity is therefore the major influence on As(V+III) concentrations at this site, although the three lowest values in late January, early February and early March corresponded with high *Pseudo-nitzschia* cell counts. As found for the summer spatial surveys, there was again significant correlation between cell counts of this diatom species and As(V+III) depletion ($r = -0.745$, $n = 23$, $P<0.0001$).

As(III) concentrations were quite erratic at this site and as found in other studies [61], do not show the same seasonal trends as the methyl arsenic species. There are two distinct peaks in As(III) concentration. They occur mid-January (0.12 $\mu g\ L^{-1}$) and early May (0.21 $\mu g\ L^{-1}$). Otherwise concentrations are quite low (< 0.05 $\mu g\ L^{-1}$), and below the detection limit after the onset of winter (June). The timing of the January and early May As(III) peaks correspond well with large *G. catenatum* blooms, but the cell counts were not correlated with the measured As(III) concentrations. It does appear, though that As(III) is produced by *G. catenatum* when its bloom corresponds with low phosphate concentrations.

As observed in the spatial surveys, MMA production lagged behind that of DMA. MMA concentrations peaked in February/March after the January DMA peak. Following this, both species declined slowly through autumn, with a sharp decline from autumn conditions in May to winter conditions in late June/early July. Peaks in DMA concentration corresponded with peaks in cell numbers of the dominant phytoplankton species, but because DMA does not break down rapidly in the water column, and because different species vary in their ability to produce DMA, no correlation was found.

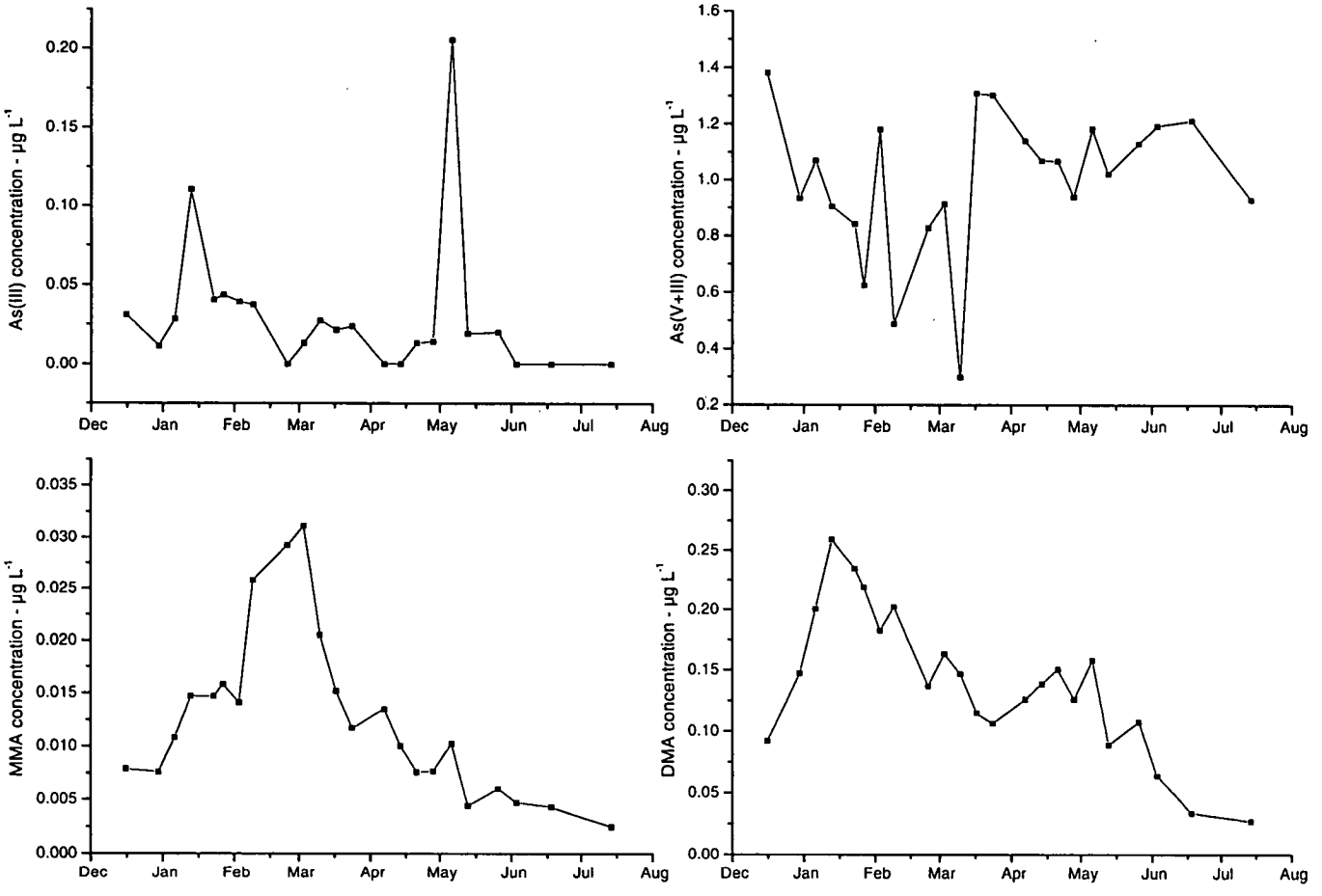

Figure 5.18 Arsenic species variation at the Killala Bay (surface) continuous monitoring site during 1997/98 (see Appendix 10 for the corresponding nutrient, DO, and phytoplankton count data)

5.3.3b Relationship with nutrients

Similar to the spatial survey, no significant correlation was found for any of the arsenic species with any of the nitrogen species. A general trend was observed where As(III), MMA and DMA increased as dissolved oxygen decreased. This was not significantly correlated, but suggests As(V) reduction may be associated with recycled production.

Unlike the spatial surveys, As(V+III) concentration did not correlate strongly with phosphate in the time-series record, although it did generally decrease as phosphate decreased. This was thought to be a result of the different arsenic-phosphate tolerance of successive blooms over the summer period, as indicated in the spatial survey results.

There was no correlation between As(III) or MMA and phosphate, but a negative correlation between DMA and phosphate was found ($r = -0.77493$, $n = 23$, $P<0.0001$), indicating increased production as phosphate becomes depleted. It is unclear why this effect is observed in the time series and not in the spatial surveys, but perhaps reflects the localised effects of phytoplankton grazers and their role in release of arsenic species to the water column.

A high correlation was found between the As/P ratio and As(III) production ($r = 0.8580$, $n = 23$, $P<0.0001$), indicating that reduction of As(V) to As(III) occurs under phosphate-limited conditions. Presumably this applies to nutrient limited conditions generally, with As(V) reduction occurring as recycled production becomes important.

5.3.3c Relationship with chlorophyll a

As observed in the spatial surveys, there was no significant correlation between chlorophyll *a* concentrations and any of the arsenic species, although there is a general positive trend between chlorophyll *a* and concentrations of the methyl arsenic species. The basis for this loose relation has been discussed in subsection 5.3.2e.

5.3.3d Relationship with cell counts of the major phytoplankton species

As discussed above, cell counts of the diatom species *Pseudo-nitzschia* show significant correlation with As(V+III) depletion, but not with any other arsenic species.

None of the other major phytoplankton species cell counts were directly correlated with the arsenic species.

5.4 CONCLUSIONS

Overall, inorganic arsenic concentrations found in the Huon River are low, even when compared with other pristine systems (Table 5.7). Despite the historic use of arsenic

compounds in the catchment region, there is no evidence from this work to suggest that they are present in the river, even though work by Merry *et al.* [87] indicated that arsenic had been depleted from contaminated orchard soils in the Huon region. This suggests that arsenic from orchard soils might have been released as volatile species by microbiological action, but it still remains strongly bound in either soils or sediments, or has been completely flushed from the river system. Even in regions of the river where sediment remobilisation is occurring, there is no apparent mobilisation of arsenic.

Table 5.7 Fresh water arsenic concentrations in the Huon River and some pristine and heavily polluted river systems

Location	Reference	Fresh water [As(V+III)]
Tasmanian Rivers		
Huon River, Tasmania	this work	0.025 - 0.050 $\mu g\ L^{-1}$
Derwent River, Tasmania	[5, 26-32]	<0.1 - 6 $\mu g\ L^{-1}$
Tamar River, Tasmania	[33]	1 - 2 $\mu g\ L^{-1}$
Pristine river systems		
SE United States rivers	[23]	0.3 $\mu g\ L^{-1}$
River Beaulieu, UK	[24]	0.2 $\mu g\ L^{-1}$
River Krka, Yugoslavia	[25]	0.1 $\mu g\ L^{-1}$
Saguenay River, Canada	[38]	0.08 $\mu g\ L^{-1}$
Heavily polluted river systems		
Thames River, UK	[34]	7 $\mu g\ L^{-1}$
Carnon River, UK	[35]	240 $\mu g\ L^{-1}$

Concentrations of the methyl arsenic species and their cycling within the estuary show a similar pattern to that observed in other temperate estuaries. Production of these species is not directly related to chlorophyll *a* concentrations or arsenic-phosphate interactions, but as in other studies, their concentration and distribution is significantly influenced by water temperature and the nutrient strategies of the particular phytoplankton species present.

The Huon River Estuary is an ideal system in which to study the biological cycling of arsenic species, given that it is free from arsenic contamination and, as discussed earlier, because of the low suspended particulate load and short flushing time, is essentially free of geochemical influences on arsenic cycling. Therefore, it represents a quite unique system in which biochemical interactions overwhelmingly dominate arsenic cycling.

Although there are slight indications of arsenic bio-utilisation during the Austral spring and early summer months, the effect becomes extremely marked in both late summer seasons, with high inorganic arsenic and phosphate depletion observed. This effect was most marked in the Port Cygnet region and appeared to radiate out along the A and B transects at the mouth of the Huon Estuary. Initially, it was thought that this might be occurring as a consequence of arsenic uptake through phytoplankton grazing and subsequent arsenic incorporation by mussels on shellfish lease sites. The reason for this was that the effect was most striking at the stations closest to shellfish farms and greatest depletion was occurring closest to the lease with highest stocking. Also, the low methyl and hydride-refractory arsenic concentrations indicated arsenic uptake without subsequent release. However, the continuous monitoring results at Killala Bay (a fin fish lease site) also showed several instances of arsenic depletion and these were highly correlated with *Pseudo-nitzschia* spp. cell counts. When considering the *Pseudo-nitzschia* cell counts at the biological stations in HES 4, 7 and 8 there was also a high degree of correlation with arsenic depletion. Therefore, the observation of marked arsenic depletion only in late summer is directly attributed to the late season dominance of this diatom. It should be noted that the cell counts refer to the phytoplankton genus *Pseudo-nitzschia*. In the Huon Estuary the most common species is *P. pseudodelicatissima*, but other species including *P. pungens* and *P. subpacifica* are also present.

From the observations of co-depletion of arsenic and phosphate, particularly during HES 4 when *Pseudo-nitzschia* is the dominant species, it would seem that this species is unable to discriminate between arsenic and phosphorus. Previous studies on arsenic uptake by phytoplankton have often returned contradictory information about the mechanism of arsenic cycling. Furthermore, although studies of several diatom species have been conducted (e.g. *Skeletonema*, *Nitzschia*[2], *Chaetoceros* and *Thalassiosira* spp.), this is the first example of the role of *Pseudo-nitzschia* species in arsenic cycling, either in culture or field studies. Therefore, the mechanism of arsenic cycling by *Pseudo-nitzschia* and the reason why it does not rapidly recycle arsenic and return it to the water column as methylated species or hydride-refractory species, as observed during blooms of other species, is unclear.

The biological cycling of arsenic in the Huon Estuary is still further complicated by the fact that highest *Pseudo-nitzschia* numbers (and greatest arsenic depletion) occur closest

[2] Note that while *Nitzchia* and *Pseudo-nitzschia* are structurally similar they are distinct genera.

to the mussel leases, as mussels are known grazers of phytoplankton. A map of the aquaculture lease sites in the lower estuary is shown in Fig. 5.19, with shell fish leases (predominantly mussels) circled. The site at Port Cygnet has by far the greatest biomass, with other sites being significantly smaller operations.

It has been shown that mussels are unable to remove arsenic directly from the water column as As(III), As(V), MMA or DMA; however; radiolabeled (^{3}H) inorganic and methylated arsenic species were found to occur in the mussel tissue as arsenobetaine and dimethylarsinoribosides [95]. As arsenic uptake did not occur directly, it was proposed that it most likely happened *via* the food chain (but from aquatic microorganisms not phytoplankton). As yet, there is no evidence for the conversion of arsenosugars to arsenobetaine within a marine food chain [96], although there are growing indications that mussels differ from other marine animals, and even other species within the molluscan group, as they contain arsenic in the form of dimethylarsinoribosides (the major species found in phytoplankton) as well as arsenobetaine [97-99].

Other experiments have shown that mussels can incorporate arsenobetaine from the water column and subsequently release it during depuration [100]. No experiments have been done with the dimethylarsinoribosides, but it is possible that they are also incorporated into mussel tissue directly from the water column.

Although mussel growth rates are about the same throughout the year in Tasmanian waters [101], February is the period of greatest stocking density at the Port Cygnet mussel farms, as this is when large numbers of small mussels are introduced [102]. The numbers of mussels stocked at this site were approximately the same in 1997 and 1998. Therefore, it is expected that greatest grazing of phytoplankton would occur during February when there is highest stocking density. As the dominant species at this time, *Pseudo-nitzschia*, appears to recycle arsenic slowly, it is likely that the mussels will be eating a diet with higher arsenic content at this time of year. If the cells were not eaten, they would be released as mucus bound packages called 'pseudofaeces'. Evidence from nutrient cycling, indicates that material contained within these packages is readily recycled back to the water column [103]. Therefore it is suspected that arsenic from the phytoplankton has the potential to be eaten by the mussels and possibly incorporated into their tissue. It may be the role of mussels in Port Cygnet, grazing phytoplankton before they are able to release DMA to the water column, that explains

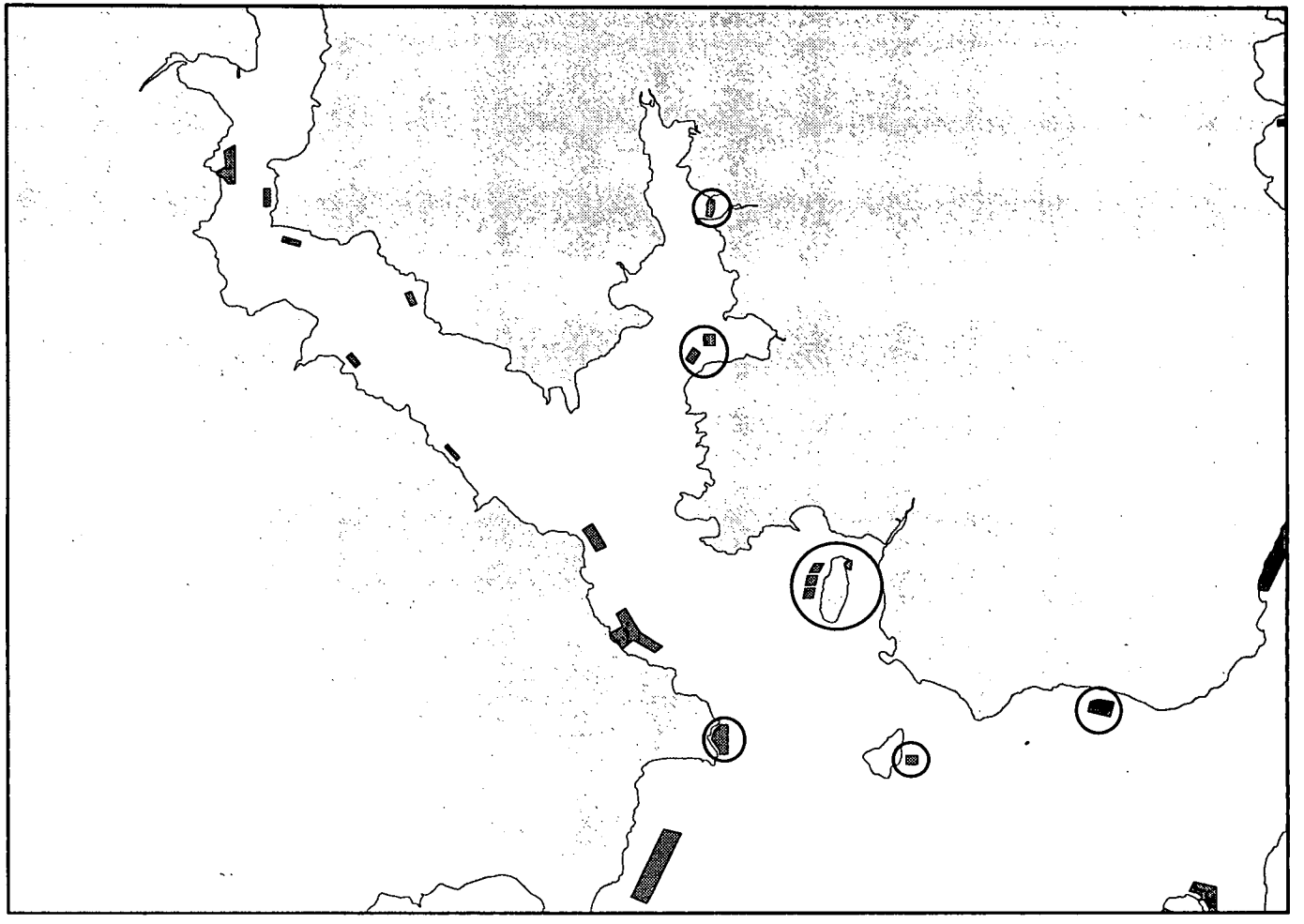

Figure 5.19 Map of the lower Huon Estuary showing the location of fish farm lease sites (shellfish leases are circled)

why there is a clear DMA-P relationship at the fin fish lease site at Killala Bay, but not at the Port Cygnet and A and B transect sites during the spatial surveys.

In conclusion, the historical use of arsenic in the Huon catchment has not influenced water quality in the Huon Estuary. In fact, arsenic concentrations in the freshwater region are among the lowest values ever recorded in natural aquatic systems.

This work in the Huon Estuary represents an important study of seasonal arsenic cycling in a "biological" estuary (with this estuary also well characterised in a broader study of nutrient cycling and algal dynamics). In the Huon Estuary there is clear evidence of different arsenic-phosphate selectivity in a succession of phytoplankton species. This is most striking in late summer, when *Pseudo-nitzschia* is found to be removing arsenic from the water column, because of an apparent inability to discriminate arsenic from phosphate. The cycling of methyl arsenic species in the Huon is similar to that observed in other temperate estuaries. Greatest production of MMA and DMA occurs in the summer months, but like in other systems, production of MMA lags behind that of DMA.

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Chapter 6: Conclusions and Recommendations

6.1 FINAL CONCLUSIONS

As detailed earlier, the aim of the project was to develop a technique for the routine determination of arsenic speciation in natural water samples and to apply it to the study of arsenic cycling in estuarine and open ocean systems. This has been achieved and a compact system using HG-AFS has been developed for both shipboard and more routine laboratory use. The precision and detection limits have been found to be suitable for studies of the ultra-low concentrations of arsenic species found in the Southern Ocean and a relatively pristine river estuary, with no history of major industrial arsenic input.

An important aspect of the initial method development was to find and characterise suitable compounds to use as standards for the various arsenic species. Although there was no difficulty in finding high purity commercial sources of the inorganic arsenic species, the same was not true for the methyl species. Commercial standards for both MMA and DMA were found, but despite manufacturers' claims that the materials were of high purity, this was for total arsenic content only. The commercial MMA standard was shown to contain impurities of inorganic arsenic, while the DMA standard was contaminated with both inorganic arsenic and MMA. In addition, the total arsenic content of the commercial MMA standard was found to be unreliable. As there was only one commercial source of this material available, an alternative source was found (a herbicide) and purified using a column chromatographic procedure. This product was of higher purity than the standard available commercially, and was thus adopted as an appropriate analytical standard for MMA following characterisation of its inorganic arsenic content. A number of DMA standards are produced commercially, and so a number of different brands were investigated before selecting the one least contaminated by other species. The As(V+III) and MMA content of the chosen material were also characterised before its use as an analytical standard.

Development of the analytical method was conducted in two stages. Initially, a method based on the batch hydride generation atomic absorption method of Andreae [1] was developed, but using atomic fluorescence spectroscopy, a detector which has only recently been available commercially. This detector was chosen because of its compact size and its ability to be adapted for use as a dedicated shipboard instrument. Following optimisation, HG-AFS was shown to compare favourably with similar systems using AAS detection [2]. The analysis time was six minutes, the precision for nine replicates

of a 50 ng L^{-1} sample was better than 3.5% for all species, and the detection limits for each of the arsenic species in a 5 mL sample were as follows: As(III) - 2.3, As(V) - 0.9, MMA - 2.4 and DMA - 3.7 ng L^{-1}.

The interlaboratory comparison using Atlantic Ocean samples analysed by HG-AFS and HG-GC-PID, indicated good agreement between MMA and DMA results. A problem with the arsenic blank in the acid used to preserve speciation in the stored samples, precluded the As(V+III) results from the comparison. However, the As(V+III) concentration determined by HG-AFS in the open ocean certified reference material NASS-4 gave excellent agreement with the certified value (HG-AFS = 1.18 ± 0.01 µg L^{-1}, certified value = 1.26 ± 0.09 µg L^{-1}). This work represents one of the first examples of atomic fluorescence spectroscopy being directly coupled with hydride generation, and is the first example of its successful use for the routine analysis of hydride-forming arsenic species in seawater.

The initial manual HG-AFS method was subsequently semi-automated using a commercially available data acquisition and control package, WorkBenchMac™, to control automated components and log the AFS signal. This system had some further advantages over the manual method which included a further decrease in the analysis time (to under 5 minutes) and, because the analytical sequence was automated, improved reproducibility and ease of use. Following some additional minor alterations, mainly related to shipboard safety requirements, the analytical method was tested on a one-month Southern Ocean voyage in the Subantarctic Zone (SAZ). The method was sufficiently robust to be used for making reliable, routine As(V) and As(III) measurements at sea, even during quite challenging (for the operator anyway!) weather conditions. Time restrictions meant that the methyl arsenic analyses were performed on returning to the laboratory, although with two operators more analyses would be possible at sea.

Results for the methyl arsenic species during the Southern Ocean cruise were extremely low, with biologically produced arsenic species representing less than 2.5% of total arsenic at all sites. Results also showed that despite the Subantarctic Zone being a silicate-limited environment, DMA was always the dominant methyl arsenic species. This disproves the hypothesis of Santosa *et al.* [3] that MMA is the dominant methyl arsenic species in silicate-limited waters. As(III) concentrations during the voyage were below the method detection limit at all sites.

This data set represents some of the lowest concentrations of biologically produced arsenic species, with the exception of some other Southern Ocean measurements [4] and some high latitude measurements in the Atlantic Ocean [5]. This was to be expected as not only is the region characterised by extremely low productivity, but the voyage was conducted several weeks after the decline of the summer phytoplankton bloom.

Unlike other oceanic studies of arsenic, little arsenic depletion was found at the surface (*ca.* 1 - 2 nM), with As(V+III) displaying a virtually uniform vertical profile. Deep water arsenic concentrations (below 1000 m) were relatively constant along the entire cruise track (24.1 ± 0.5 nM). The arsenic concentrations found in the deep water are high compared with the high latitude North Atlantic [5], but similar to concentrations reported by Andreae [6] in the northeast Pacific.

The semi-automated HG-AFS method was also found to be useful for routine laboratory analysis of samples from coastal waters. A combination of this and the original manual method was used for the analysis of arsenic species in the Huon River Estuary, Tasmania.

The work in the Huon represents a comprehensive series of measurements where the seasonal cycle of arsenic was determined in both spatial and temporal surveys. The Huon River system is characterised by low suspended solids, a short flushing time and arsenic concentrations lower than those previously reported for other pristine systems. Therefore, this river represents a system in which arsenic cycling is dominated by biological rather than geochemical effects.

In general, biological production of As(III) and methyl arsenic species in the Huon Estuary was shown to be similar to that in other temperature estuaries in the Northern Hemisphere, and particularly those in the south of England, which have also been well characterised. MMA and DMA exhibit quite different seasonal cycles, with DMA appearing first, following the first spring phytoplankton blooms and gradually increasing through summer until peak production in January. After summer its concentration gradually decreases to a background level which is maintained through winter. MMA production is different, and as seen in previous studies, lags behind DMA. Its concentration is static through winter, and increases slightly in January, before a short peak through February, following maximum DMA production. There was no evidence of MMA being produced by a specific phytoplankton species, and despite an interannual variability in methyl arsenic production (related to biomass), the DMA/MMA ratio was similar in both years. Therefore, as in the English studies, it is

plausible that MMA is produced by DMA degradation. This process is most likely biologically mediated, and may correspond with the high bacterial counts also found at this time. Unfortunately, too few sites have been counted to be able to easily relate bacterial numbers with this process. However, the relationship between the DMA/MMA ratio and bacterial counts is an area for further work.

Similar to work in other temperate estuaries there is also no clear relationship between arsenic uptake, phosphate depletion, chlorophyll *a* concentrations and production of the methyl arsenic species, indicating that methyl arsenic production is at least delayed, if not uncoupled, from the process of arsenic assimilation.

The Huon Estuary differs from other systems that have been studied as it shows a marked arsenic depletion during late summer (February) when the diatom *Pseudo-nitzschia* becomes dominant. No previous work on arsenic uptake by this species can be found, although data from the field suggests that this species has little, if any, ability to discriminate between arsenic and phosphate, as evidenced by the high correlation of *Pseudo-nitzschia* cell numbers with arsenic depletion. Although inorganic arsenic is depleted during these blooms, there is no corresponding recycling of arsenic as methyl or hydride-refractory arsenic species. Therefore, it seems that this species has a limited ability to recycle arsenic to the water column. A further complication is that the *Pseudo-nitzschia* blooms occur close to mussel lease sites, which also have their greatest stocking density during the summer. As mussels feed on phytoplankton, there is potential for arsenic to be removed from the water column, at least semi-permanently. However, there is little experimental work available to support this at present.

There is no experimental evidence to support uptake of arsenic (as As(V+III), MMA or DMA) from the water column by mussels, although there is evidence that they can directly take up arsenic as arsenobetaine. There is, however, no clear evidence in the natural environment for the presence of arsenobetaine in the water column or the production of this species by phytoplankton. Conversely, although mussels are known to have arsenic present in their tissue as both ribose sugar analogues (major arsenic products in phytoplankton) and arsenobetaine, there is no evidence that they obtain the sugars from dietary sources (although this might well be suspected), and no evidence in any marine food chain for the conversion of the ribose sugar analogues to arsenobetaine.

The contribution of aquaculture activities to estuarine arsenic budgets has not been considered previously. In the case of the Huon the process of harvesting mussels may represent a significant net removal of arsenic and should be included in any models of

arsenic flux (*e.g.* Millward *et al.* [7]) to determine its importance. Mussels may not be the only aquaculture activity which is important in this regard. Harvesting of seaweed is currently a popular industry in Australia and macroalgae are known to uptake arsenic directly from the water column [8]. Therefore, this industry also has the potential to affect arsenic budgets.

6.2 RECOMMENDATIONS FOR FUTURE WORK

There are several potential directions for future research with regard to both the analytical method development and the study of arsenic cycling in the environment.

Arsenic measurements in the oceanic environment are always limited by the ability to collect, store and transport enough samples to obtain a reasonable size dataset while maintaining the integrity of sample speciation. To avoid this, shipboard measurements must be made. However, it is often difficult to develop a method that is sufficiently sensitive, while also being compact and robust enough for shipboard use. In this thesis, it has been shown that by using HG-AFS for detection, such a system is possible for the hydride-forming arsenic species. One of the factors limiting the possible number of shipboard analyses is the volume of liquid nitrogen that must be transported to perform the hydride trapping step. To overcome this, further miniaturisation needs to be carried out, perhaps based on the recently published method by Burguera *et al.* [9] which was described earlier in Chapter 3 (p. 51-52). Using the existing method there is also some potential for improvement in the detection limits, through a modification of the glassware design to allow the use of larger sample volumes. This would be particularly important if further As(III) measurements are to be made in the Southern Ocean, although during the summer bloom current detection limits would probably be adequate.

The particular characteristics of the Huon Estuary have shown it to be an ideal system in which to study the biological cycling of arsenic. The observations of late summer arsenic depletion and the role of *Pseudo-nitzschia* spp. in this process is an obvious area for future work through both field studies and culture experiments. In addition, the interaction of this diatom with mussels and the potential for arsenic biotransformations in this food chain, also requires further work. The removal of arsenic by mussels may also need to be examined in terms of total arsenic flux, because if it is not released it may represent a net removal of arsenic from the estuary when the mussels are harvested. In conclusion, the results from this work clearly demonstrate the need for further investigations to understand the mechanism of arsenic uptake and release by *Pseudo-nitzschia* and the potential for arsenic transfer *via* the food chain from this

species into mussels, particularly in the Huon Estuary. Additionally, there is potential to investigate further the role of bacteria in arsenic cycling within this estuary.

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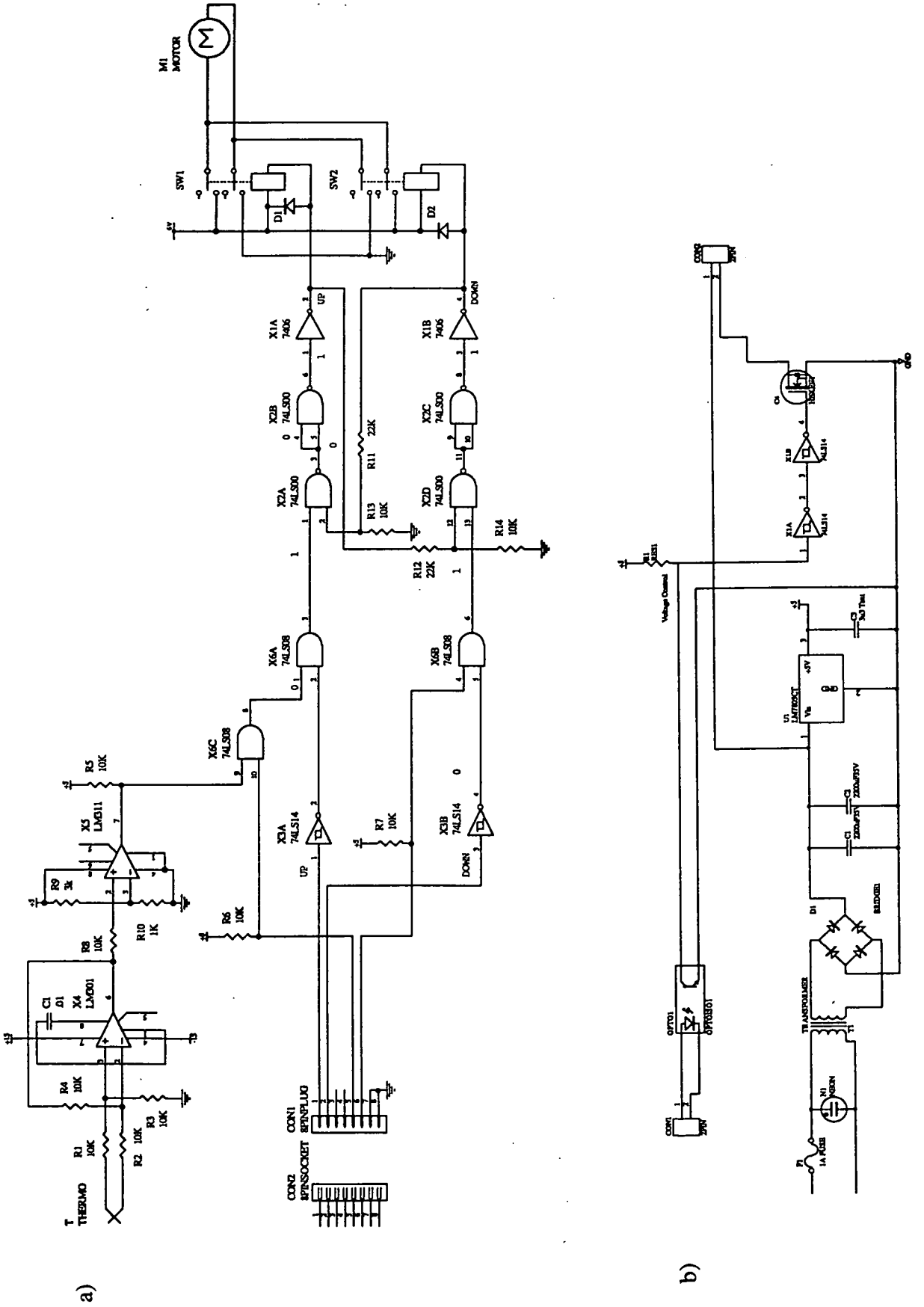
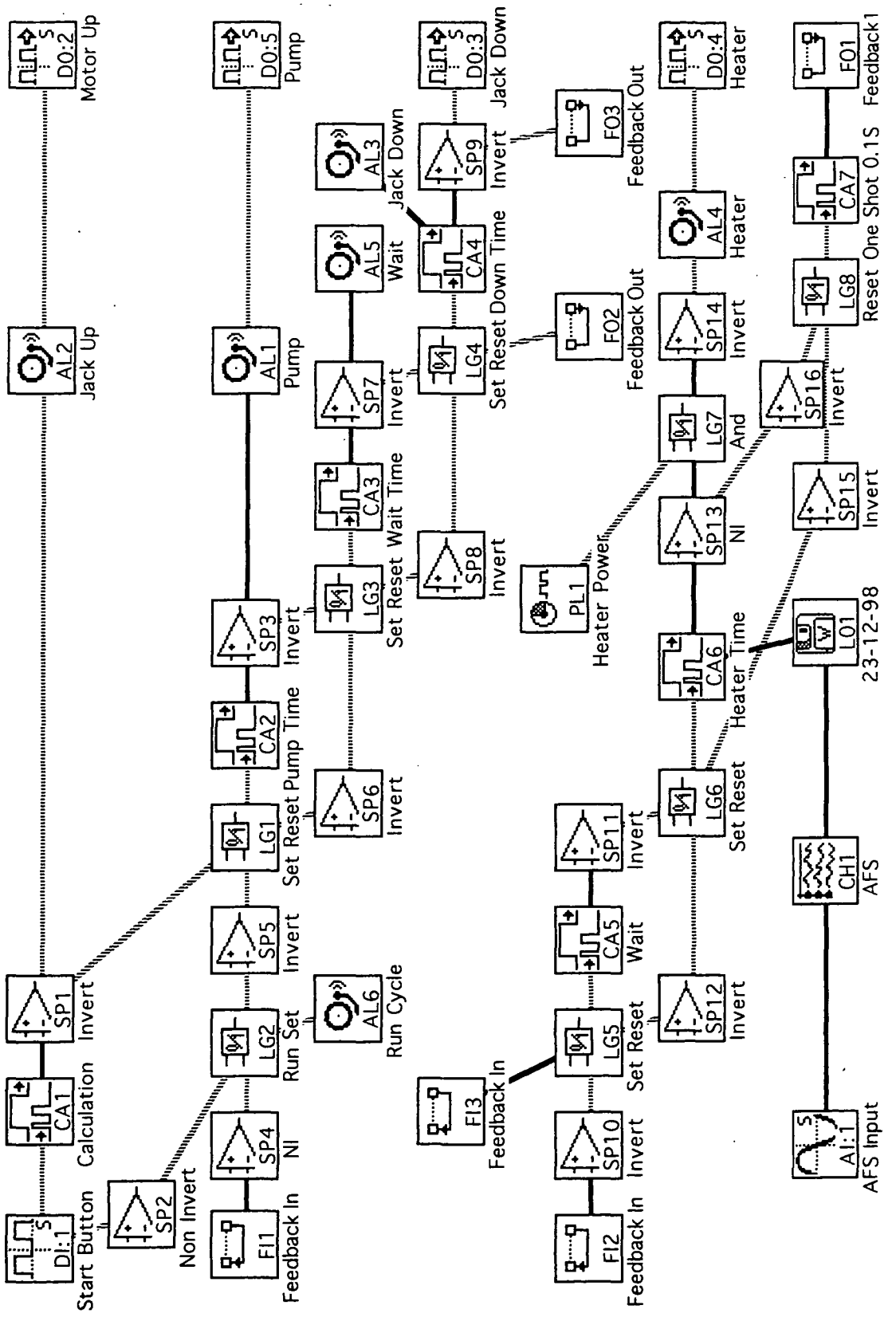
APPENDIX 1: CIRCUIT DIAGRAMS FOR THE SEMI-AUTOMATED METHOD



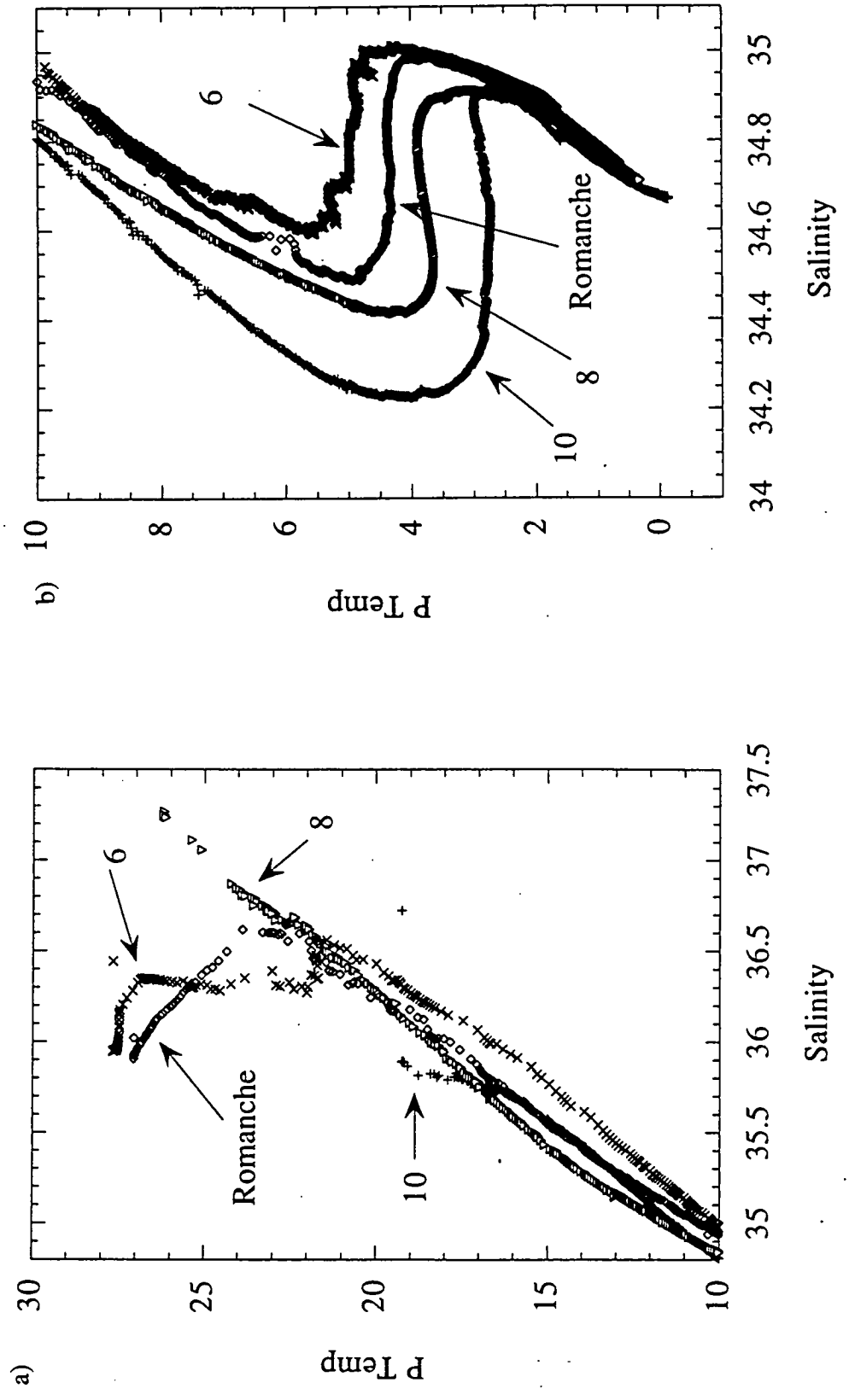
Appendix 1 Circuit diagram for a) the labjack platform drive and b) the heater power supply used to heat the U-trap

APPENDIX 2: COMPUTER PROGRAMME FOR THE SEMI-AUTOMATED HG-AFS METHOD



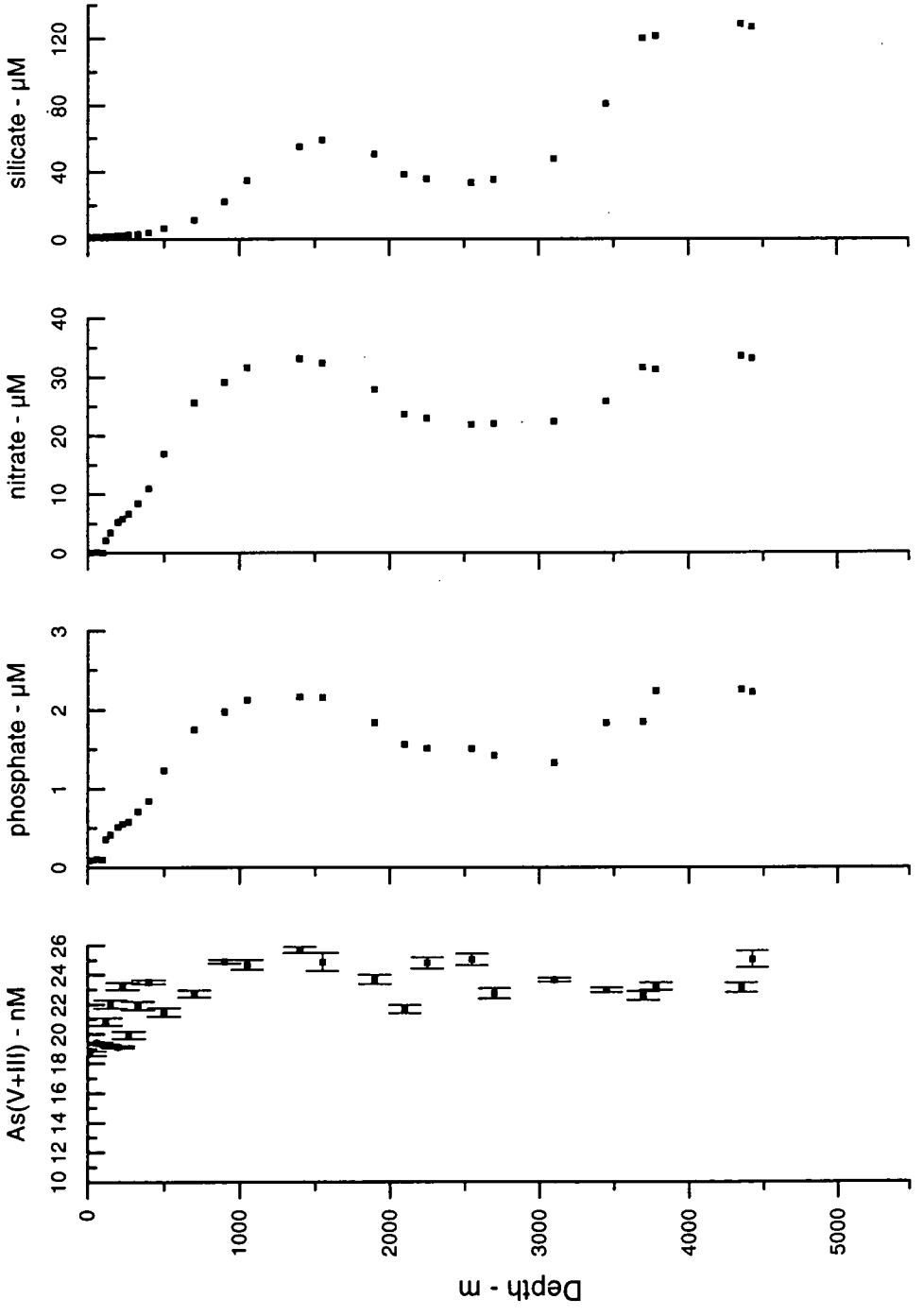
Appendix 2 Diagramatic representation of the computer program used to control the semi-automated HG-AFS method

APPENDIX 3: TEMPERATURE-SALINITY DIAGRAMS FOR THE IOC CRUISE

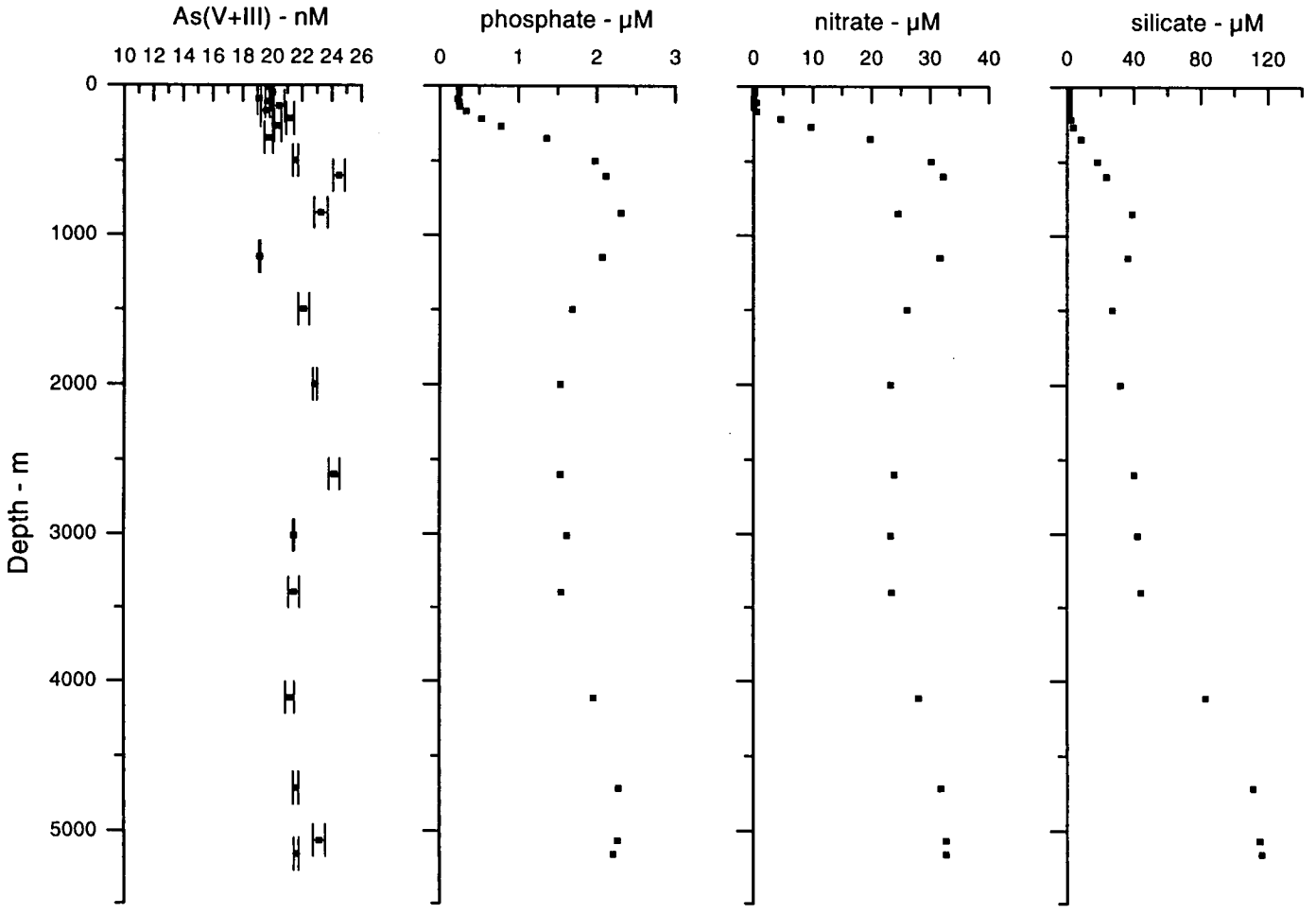


Appendix 3 Temperature-salinity diagrams for the IOC Cruise vertical profiles a) surface and b) deep waters (taken from Cutter and Measures (1999) Deep-Sea Res. II **46**(5), 867-884)

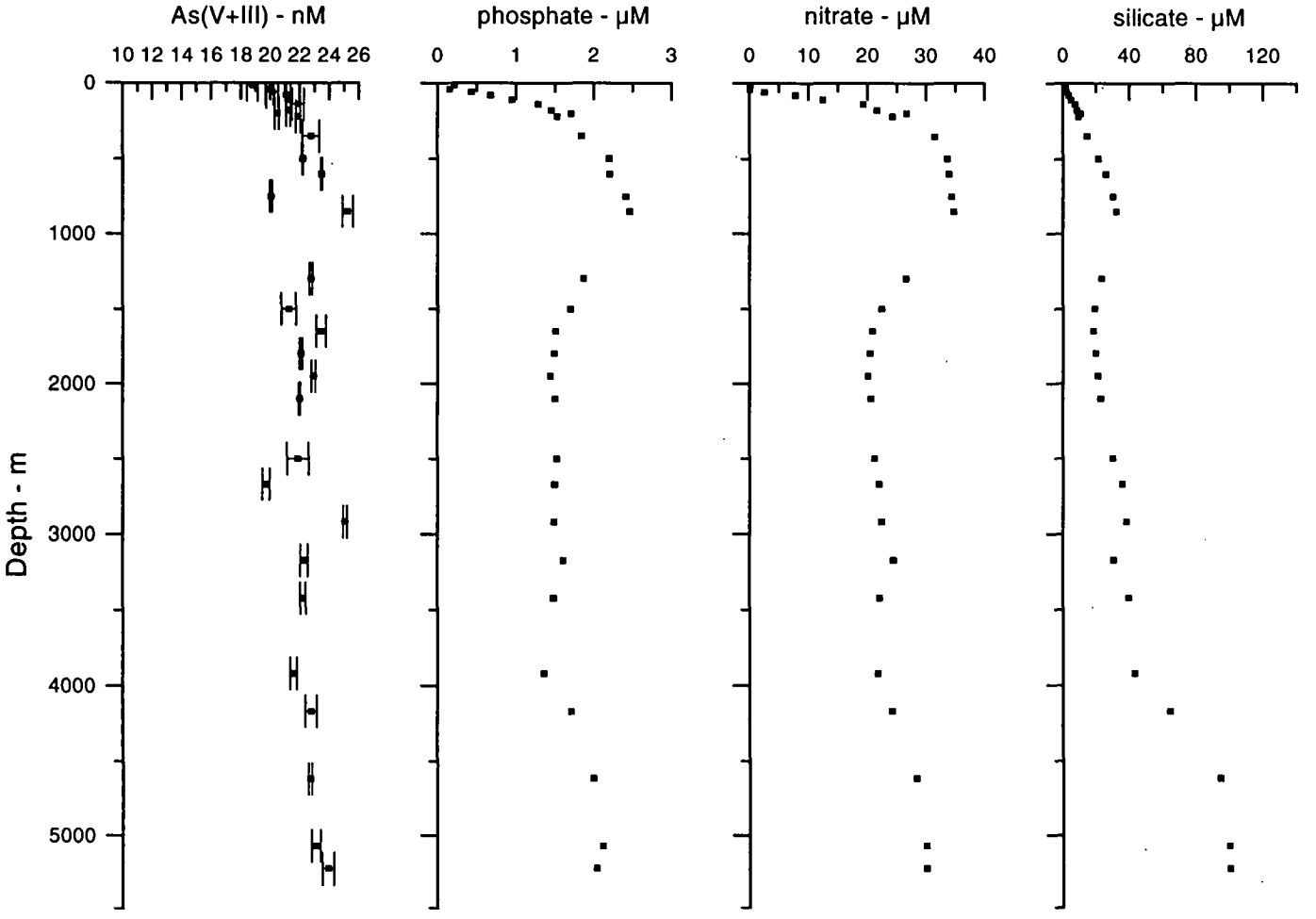
APPENDIX 4: ANCILLARY IOC CRUISE DATA



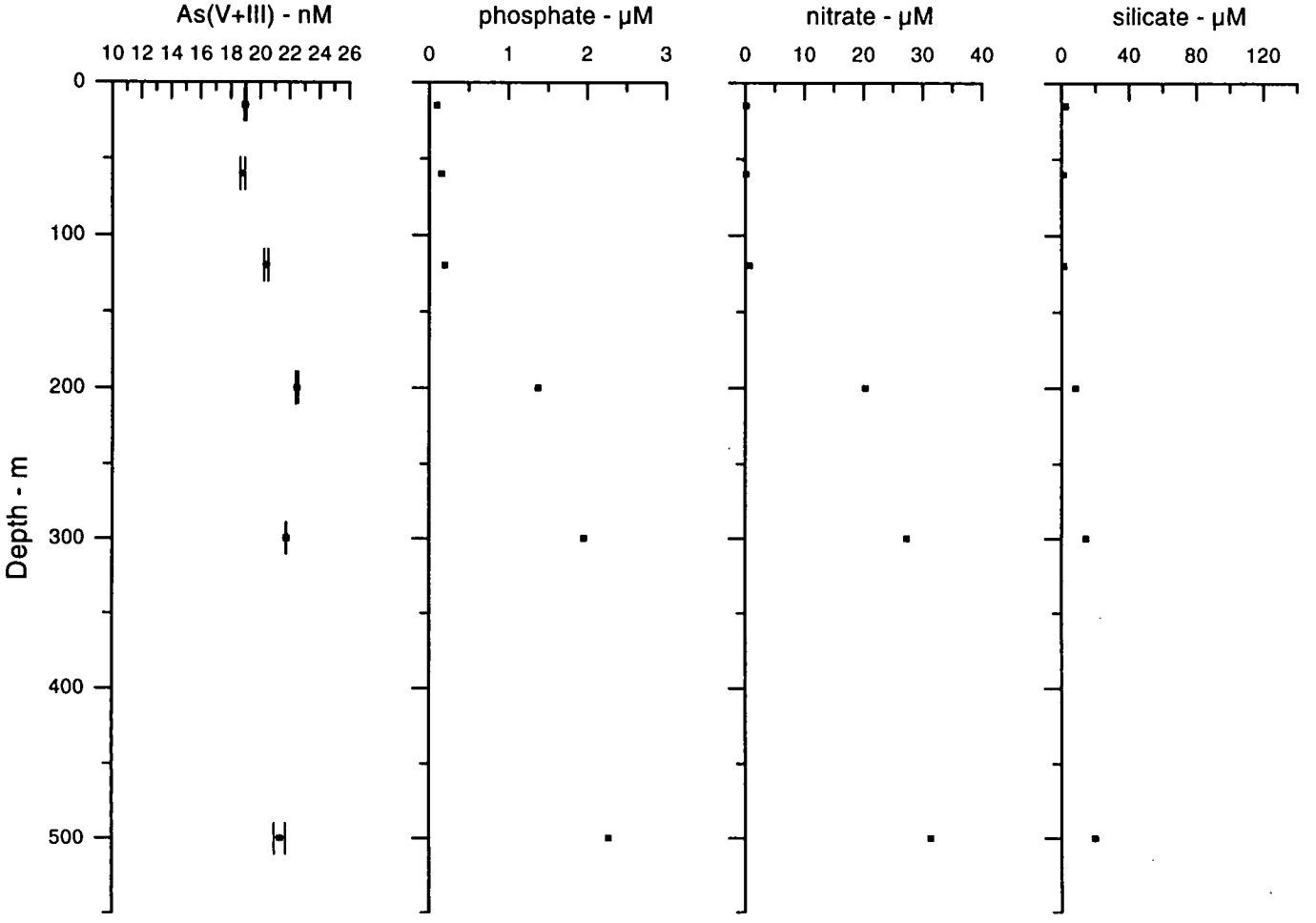
Appendix 4.1 Vertical profiles of As(V+III), phosphate, nitrate and silicate at station IOC 10 in the Atlantic Ocean



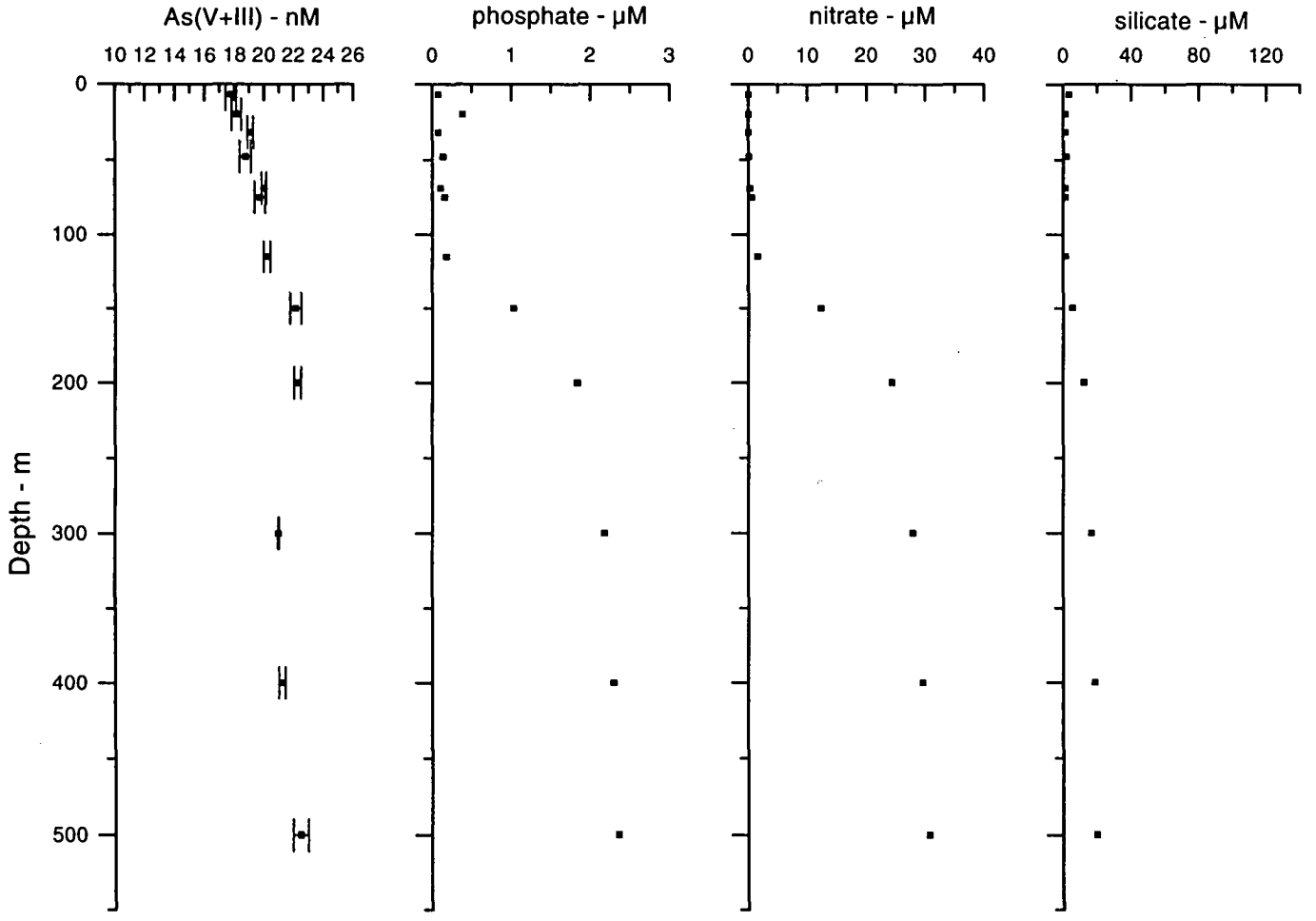
Appendix 4.2 Vertical profiles of As(V+III), phosphate, nitrate and silicate at station IOC 8 in the Atlantic Ocean



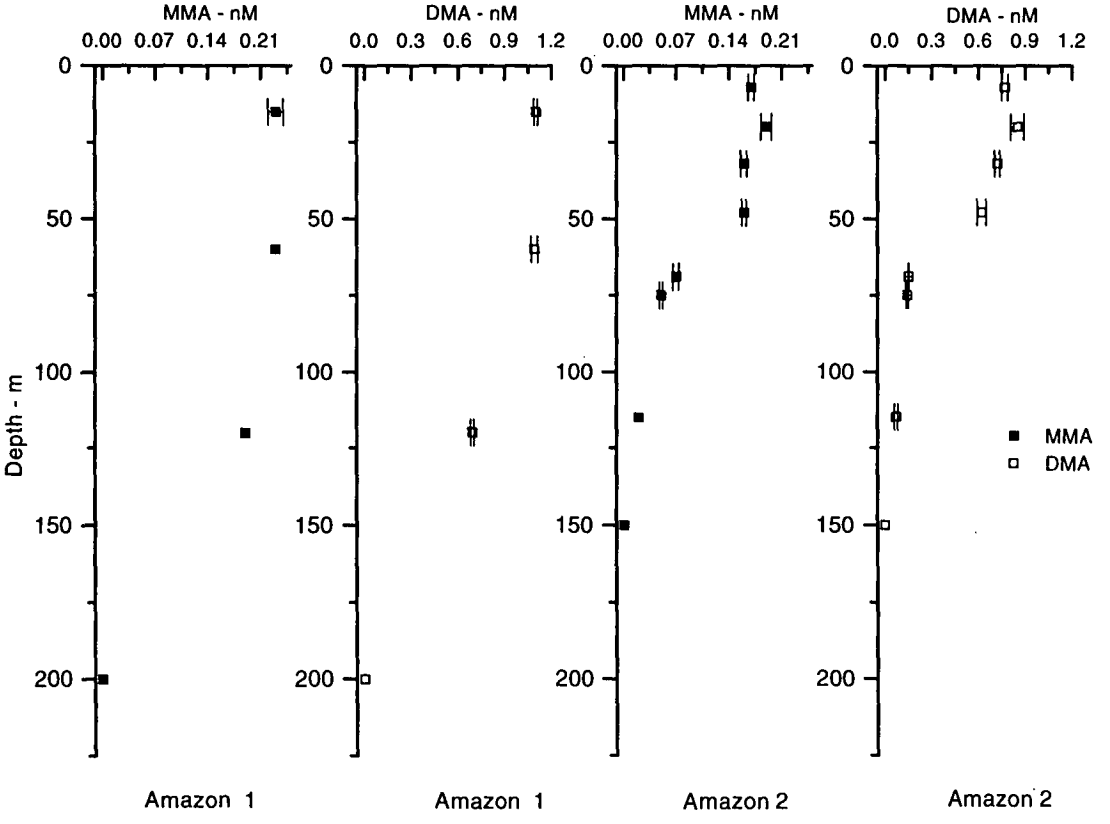
Appendix 4.3 Vertical profiles of As(V+III), phosphate, nitrate and silicate at the Romanche Fracture station in the Atlantic Ocean



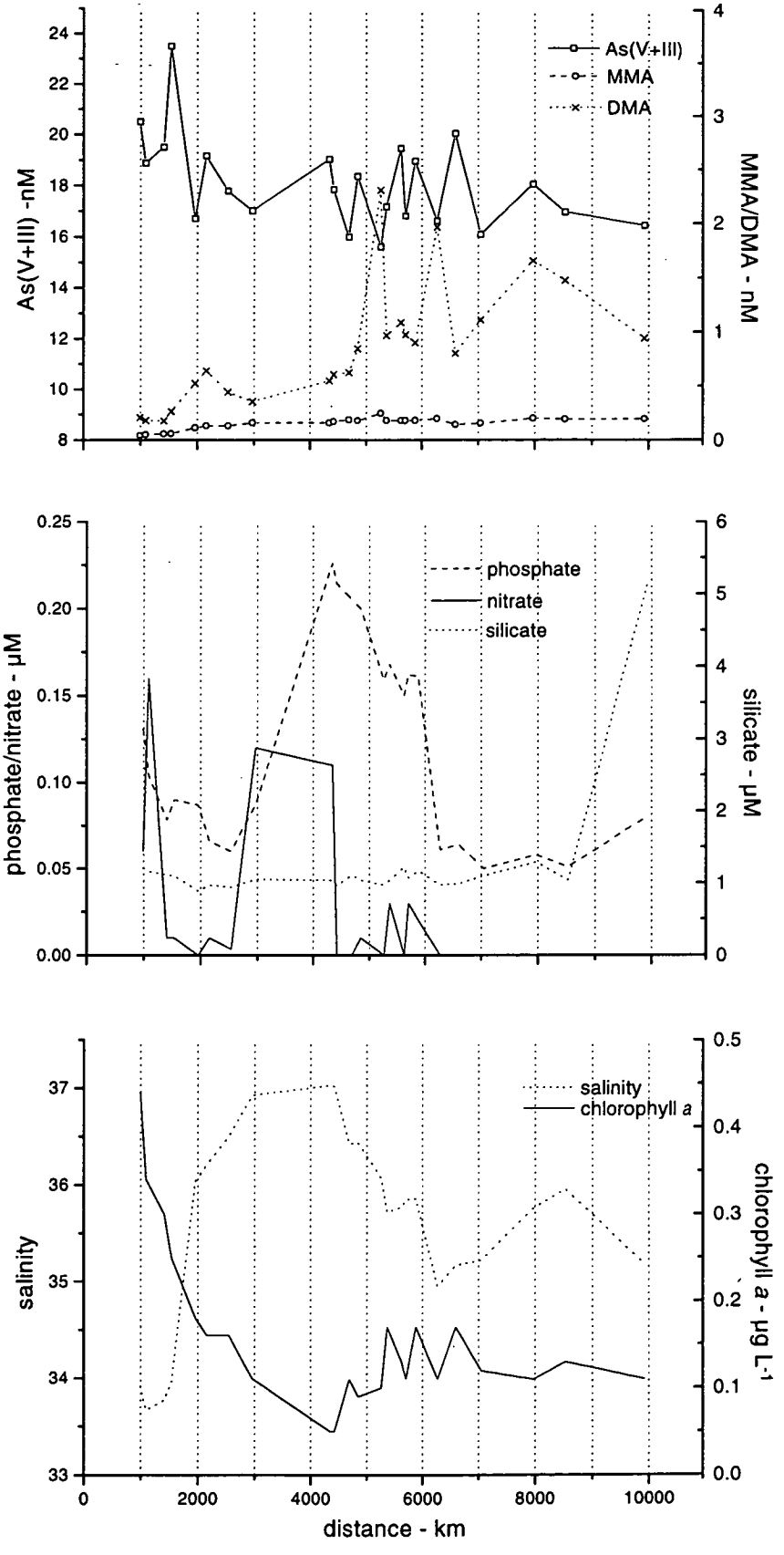
Appendix 4.4 Vertical profiles of As(V+III), phosphate, nitrate and silicate at Amazon 1 station in the Atlantic Ocean



Appendix 4.5 Vertical profiles of As(V+III), phosphate, nitrate and silicate at Amazon 2 station in the Atlantic Ocean



Appendix 4.6 Vertical profiles of MMA and DMA at the Amazon 1 and Amazon 2 stations



Appendix 4.7 As(V+III), MMA, DMA, nutrient, salinity and chlorophyll *a* distribution along the IOC Cruise surface transect in the Atlantic Ocean

Appendix 4.8 Data Interpretation - IOC Cruise surface transect

A plot showing the As(V+III), MMA and DMA data with the nutrients, salinity and chlorophyll *a* is shown in Appendix 4.7.

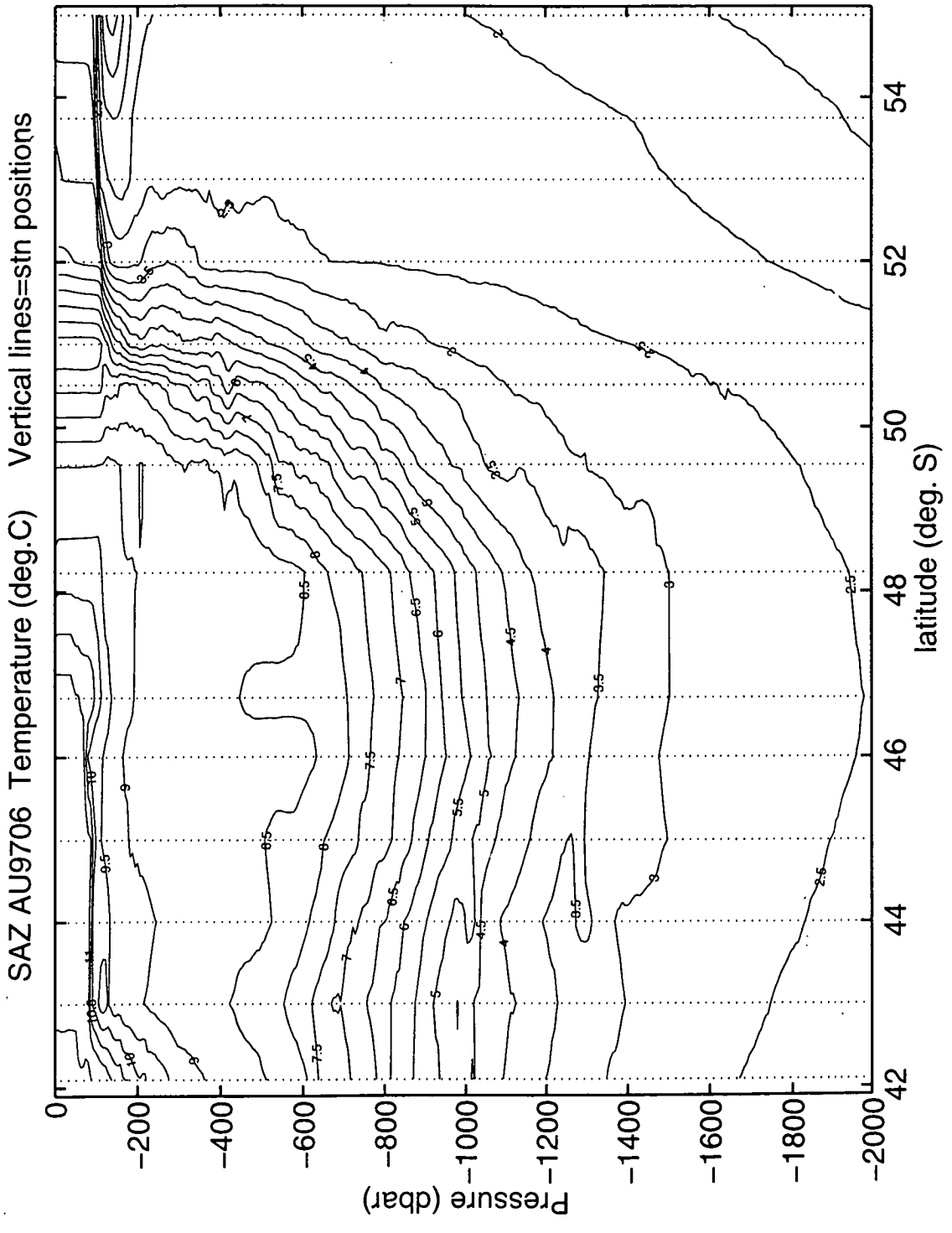
Unlike the vertical profiles, As(V+III) was not well correlated with phosphate (or nitrate) in the surface transect, reflecting the different involvement of both species in biological processes. In general, As(V+III) concentrations decreased between the start of the cruise track at 33° S to the end at 8° N. The decrease was not smooth though, particularly in the equatorial region (5000 – 7000 km) where there was considerable deviation as a result of variations in biomass (arsenic uptake) as well as slight upwelling (arsenic input). Peaks in nitrate and chlorophyll *a* in the equatorial region corresponded with increases in As(V+III), most likely a result of upwelling.

MMA concentrations tended to increase along the cruise track from more southern waters, reaching a maximum in the equatorial region. There was a distinct difference between the MMA results on the stored and unstored samples: stored being higher at the equator and unstored highest further south in the subtropical gyre region. As the reason for this discrepancy has not yet been investigated, these results will not be discussed further at this stage.

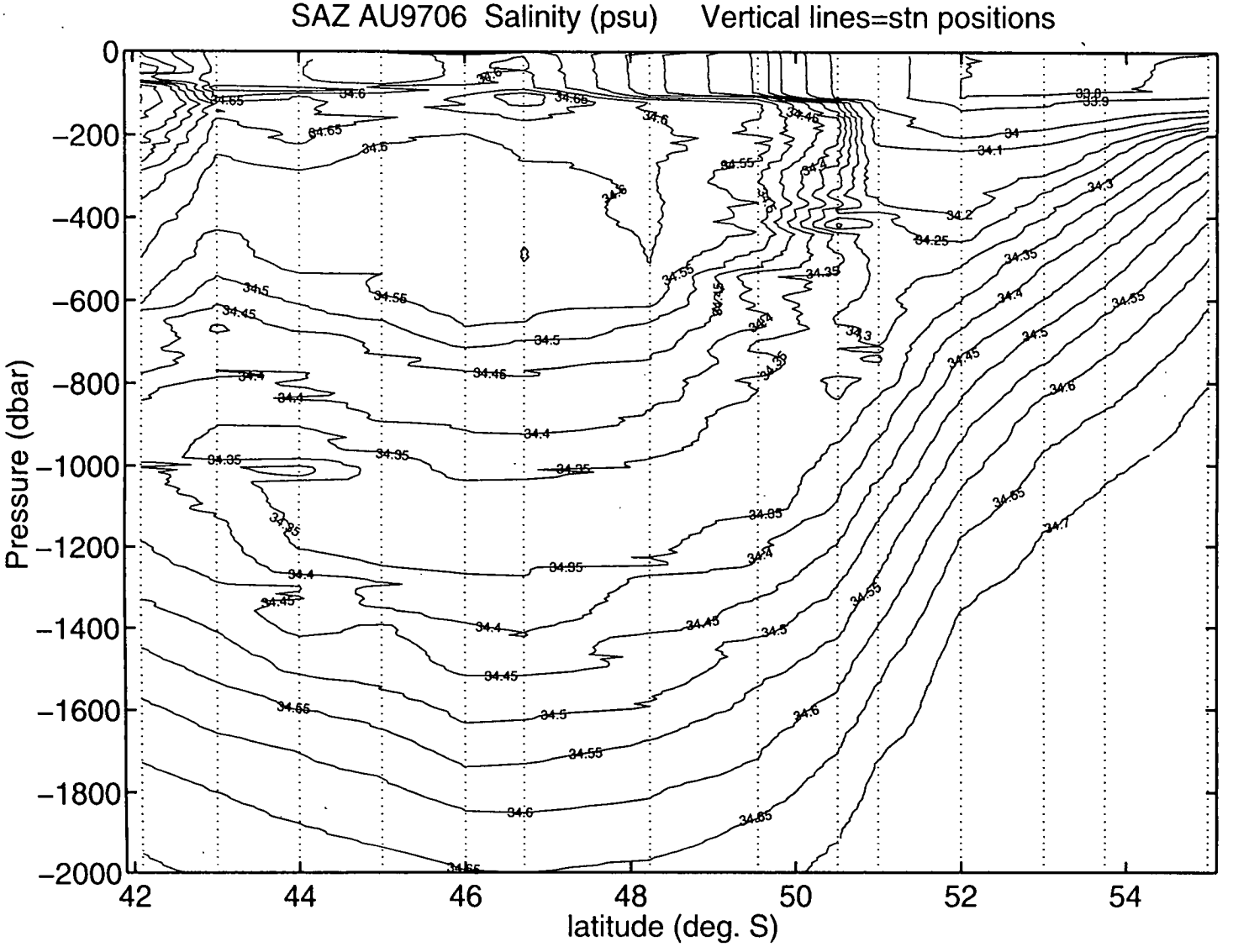
DMA concentrations were lowest in the cooler waters at the start of the transect. No data is given here, but surface water temperature varied from about 15 °C at the southern-most site to about 30 °C in the equatorial region. Apart from a small DMA peak in the subtropical gyre region where nitrate and phosphate concentrations were depleted, DMA concentrations did not increase significantly until the equator. Like As(V+III), the DMA concentration was variable, with several large peaks. In the equatorial region, DMA peaks coincided with decreases in As(V+III), suggesting that there was a short-term conversion under the influence of phytoplankton. Interestingly, the DMA peaks did not correspond with the nitrate and chlorophyll *a* peaks and greatest DMA production was occurring when nitrate was depleted to below the detection limit. The nutrient-rich waters are produced by upwelling, and therefore are slightly lower in temperature. Temperature is known to affect methyl arsenic production, therefore this may be why, despite higher chlorophyll a concentrations, these waters have lower DMA concentrations.

In the region of the Amazon plume and subtropical gyre, unlike the equator, the areas of highest As(V+III) also had greatest DMA concentrations, indicating that there might be regional differences in phytoplankton nutrient strategies. Some species may be more opportunistic, with poor nutrient discrimination, especially in regions of upwelling, while others are better able to discriminate, and therefore the amount of DMA produced is dependent on the concentration of As(V+III) in the water column.

APPENDIX 5: TEMPERATURE-SALINITY DIAGRAMS FOR THE SAZ CRUISE

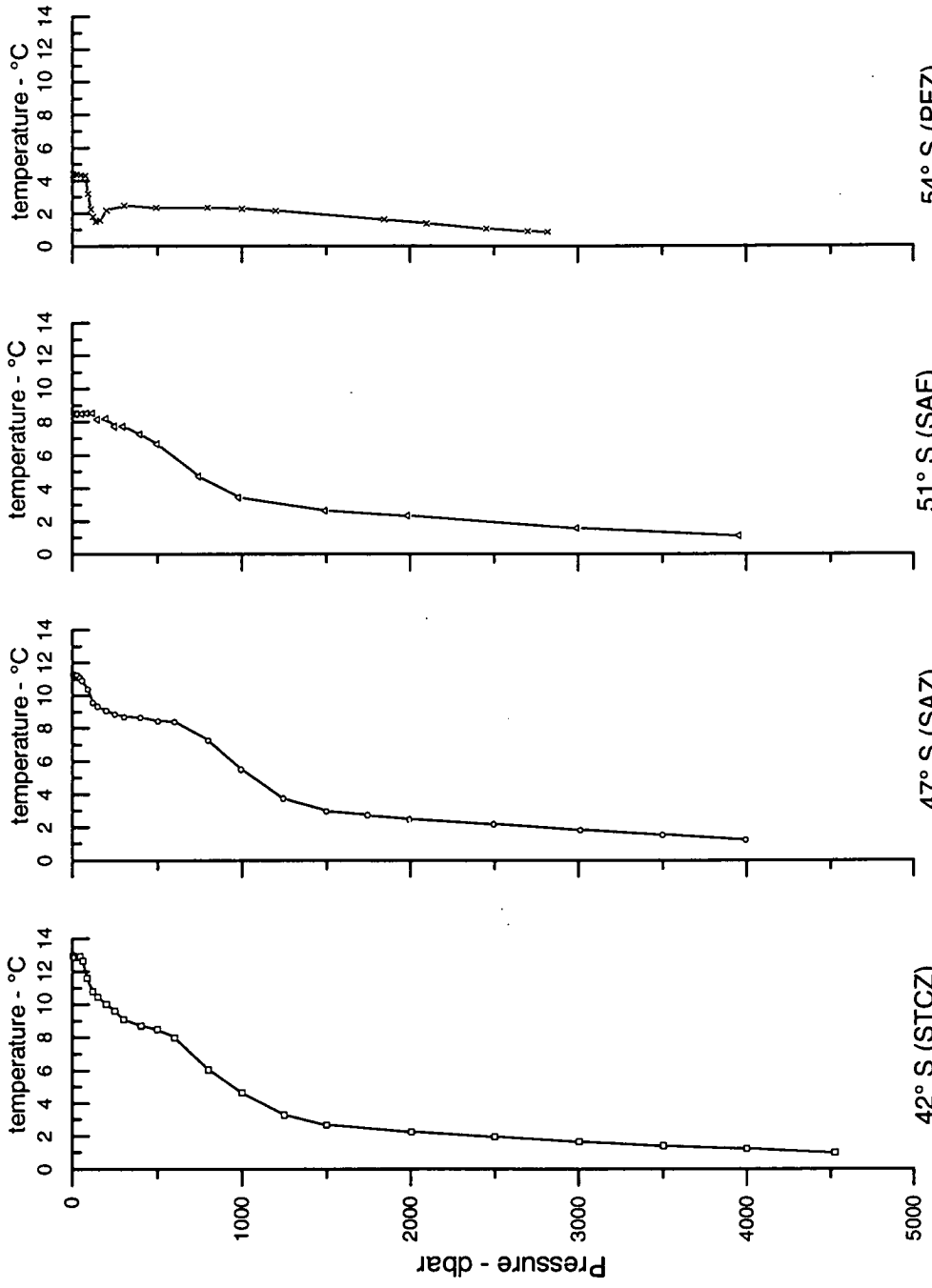


Appendix 5.1 Temperature contour diagram to 2000 m for the SAZ Cruise transect in the Southern Ocean along 141° 30' E

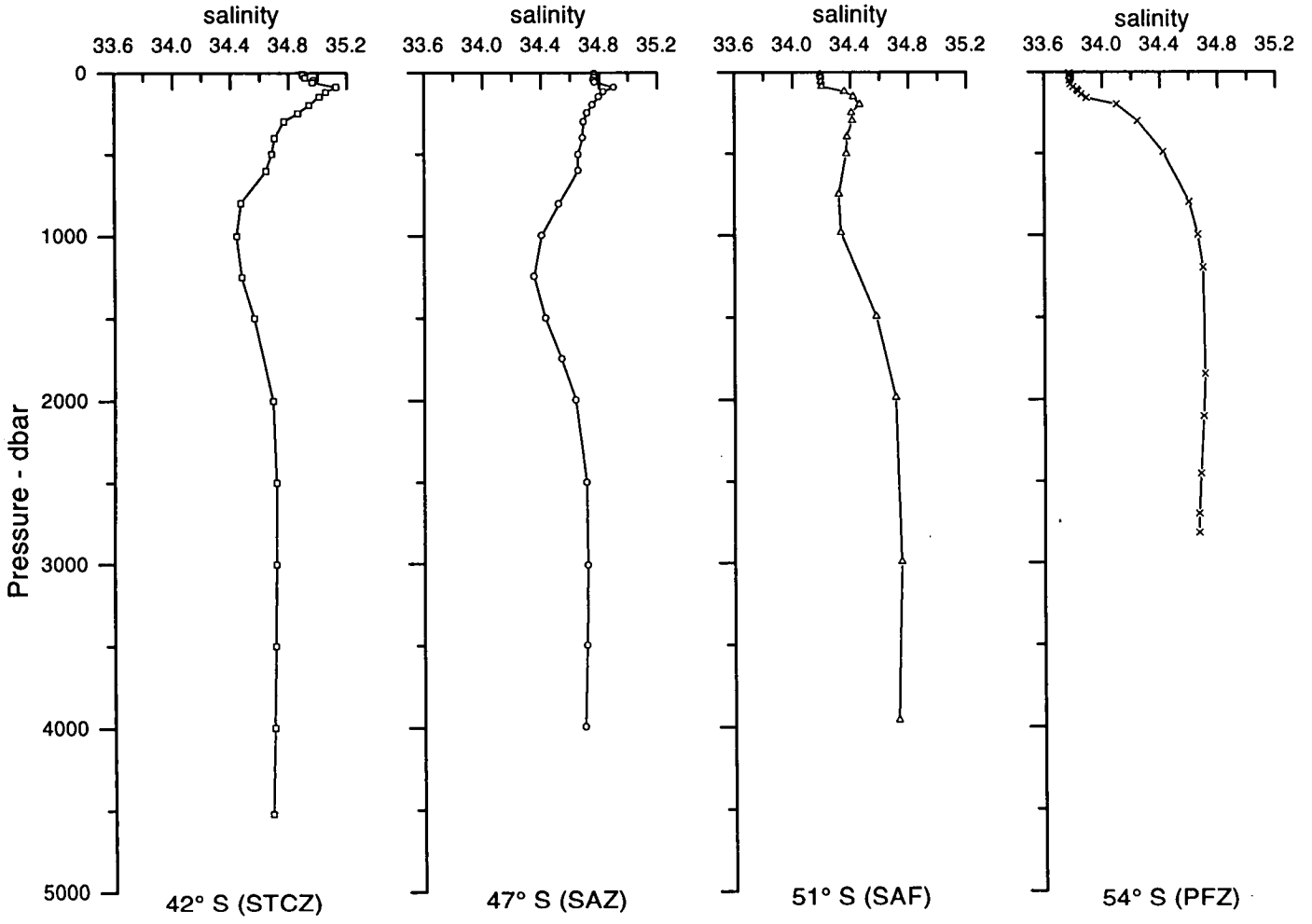


Appendix 5.2 Salinity contour diagram to 2000 m for the SAZ Cruise transect in the Southern Ocean along 141° 30' E

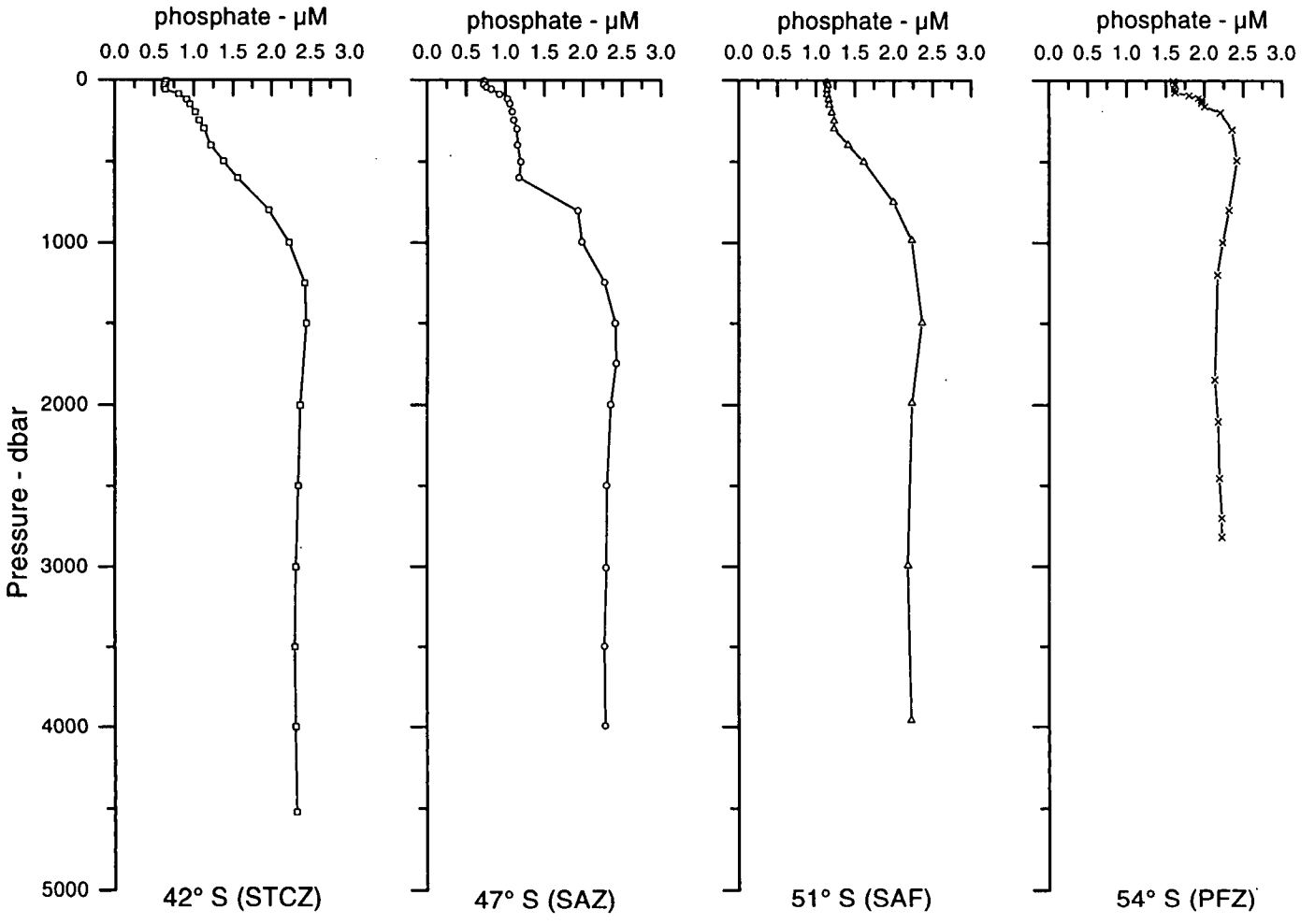
APPENDIX 6: ANCILLARY SAZ CRUISE DATA



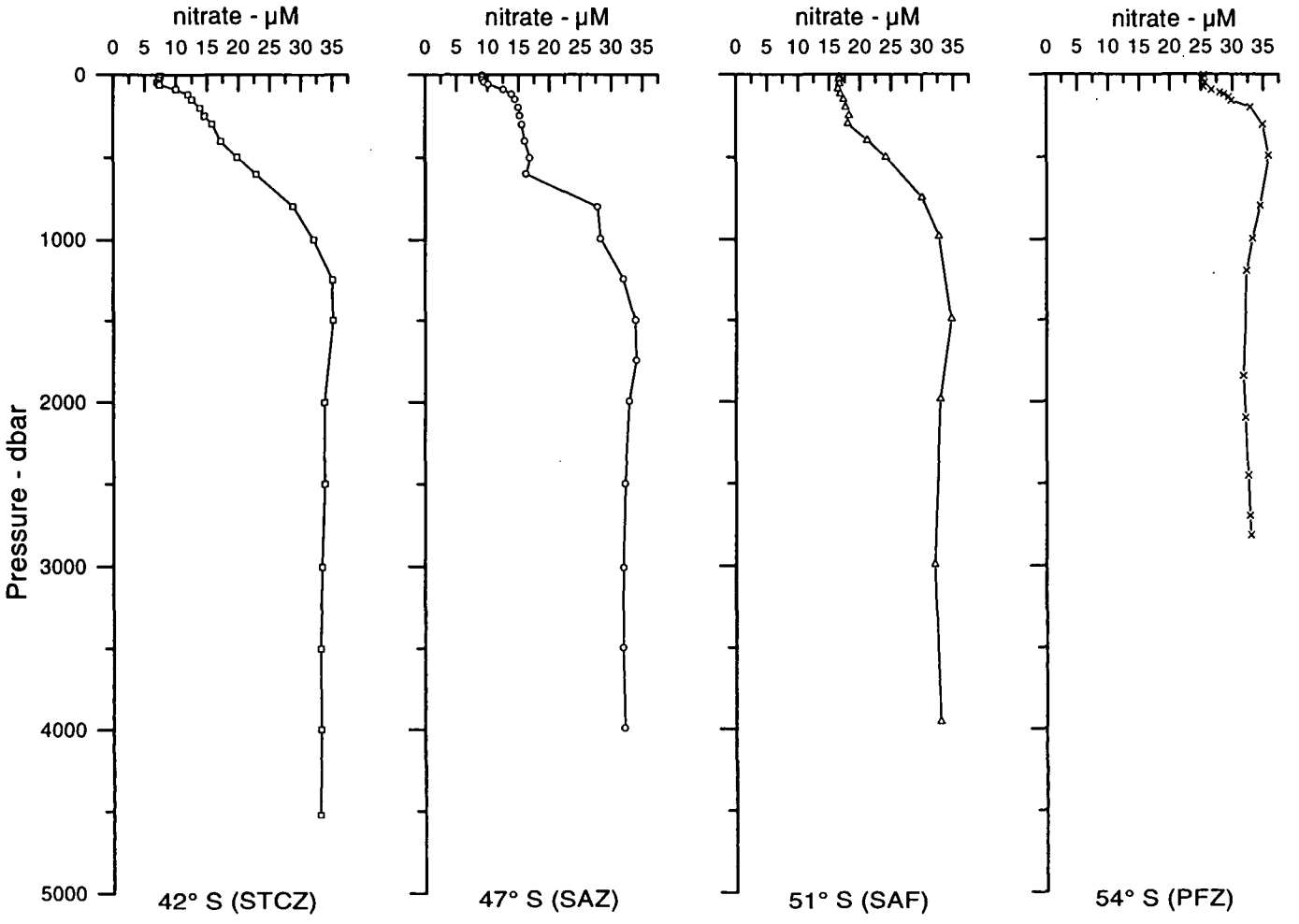
Appendix 6.1 Vertical profiles of temperature with depth in the Subtropical Convergence Zone, Subantarctic Zone, Subantarctic Front and Polar Frontal Zone.



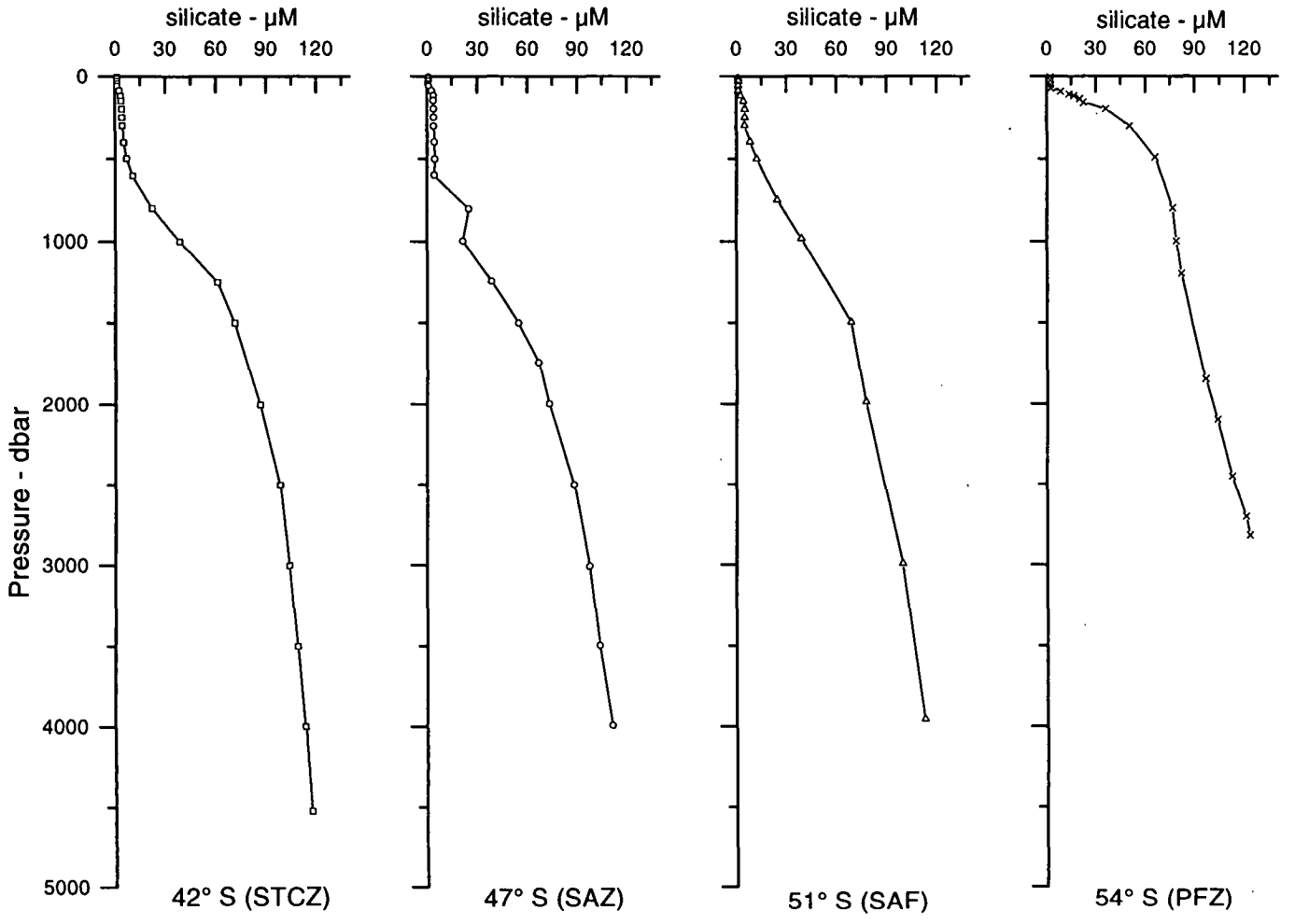
Appendix 6.2 Vertical profiles of salinity with depth in the Subtropical Convergence Zone, Subantarctic Zone, Subantarctic Front and Polar Frontal Zone.



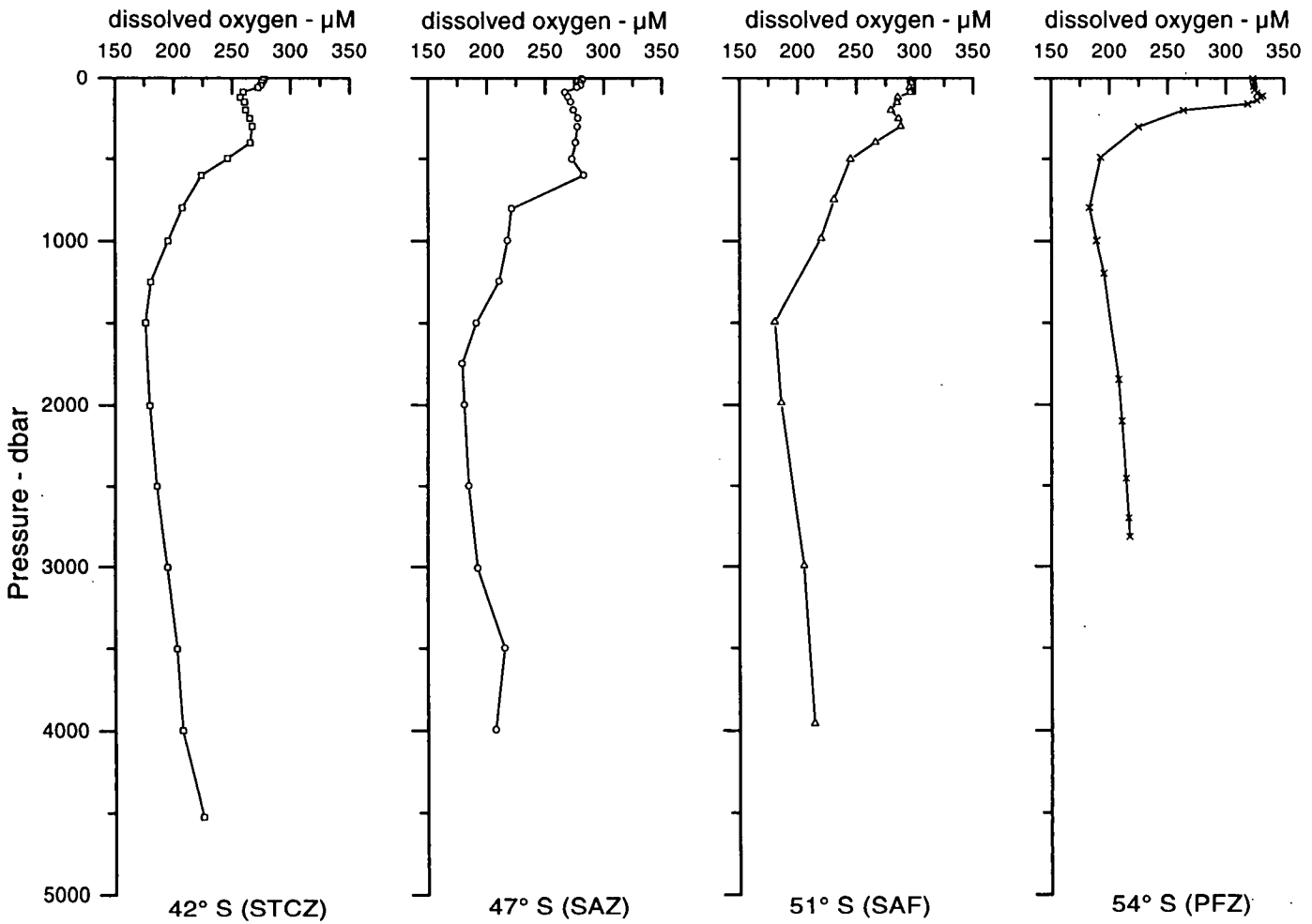
Appendix 6.3 Vertical profiles of phosphate with depth in the Subtropical Convergence Zone, Subantarctic Zone, Subantarctic Front and Polar Frontal Zone.



Appendix 6.4 Vertical profiles of nitrate+nitrite with depth in the Subtropical Convergence Zone, Subantarctic Zone, Subantarctic Front and Polar Frontal Zone.

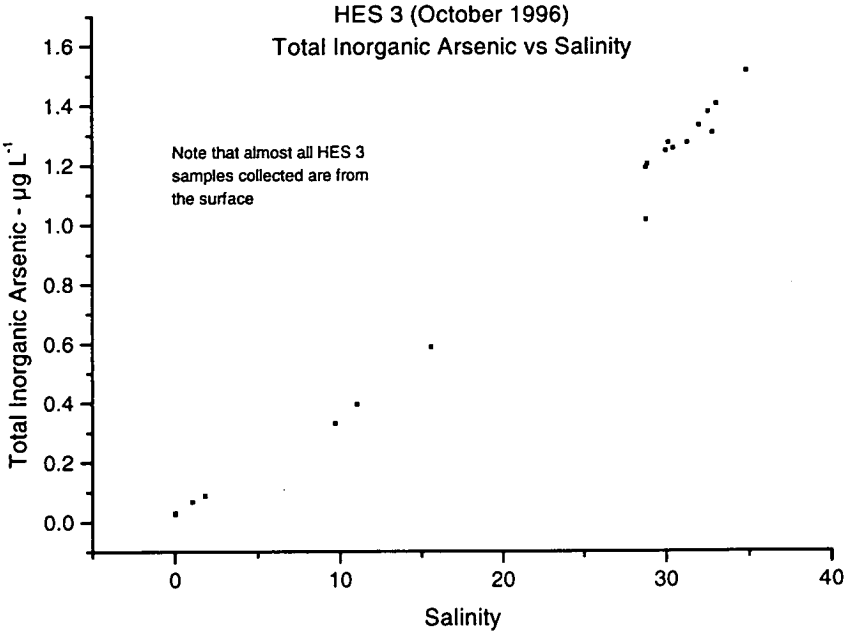


Appendix 6.5 Vertical profiles of silicate with depth in the Subtropical Convergence Zone, Subantarctic Zone, Subantarctic Front and Polar Frontal Zone.

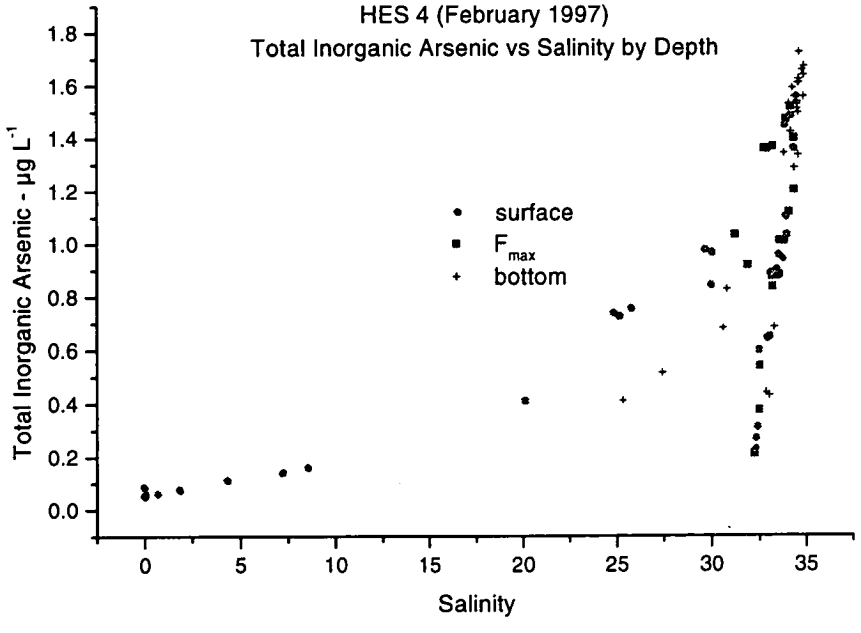


Appendix 6.6 Vertical profiles of dissolved oxygen with depth in the Subtropical Convergence Zone, Subantarctic Zone, Subantarctic Front and Polar Frontal Zone.

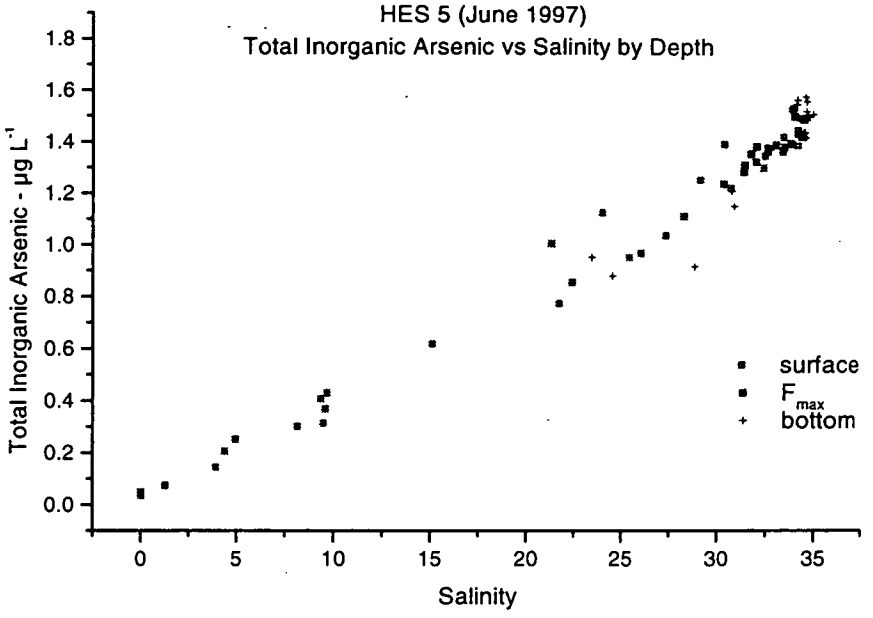
APPENDIX 7: INORGANIC ARSENIC *vs* SALINITY BY DEPTH (HES 3 - HES 10A)



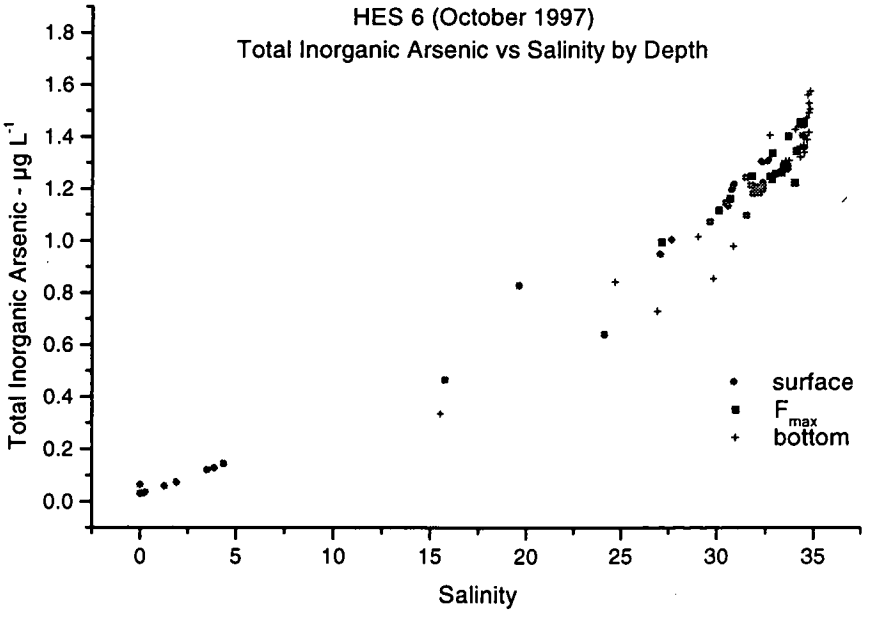
Appendix 7.1 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 3 survey (October 1996)



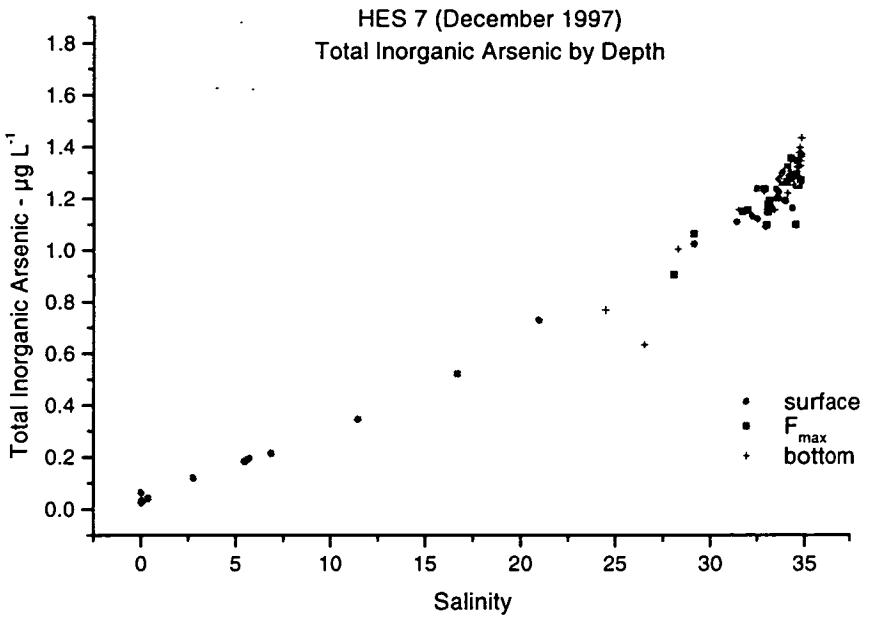
Appendix 7.2 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 4 survey (February 1997)



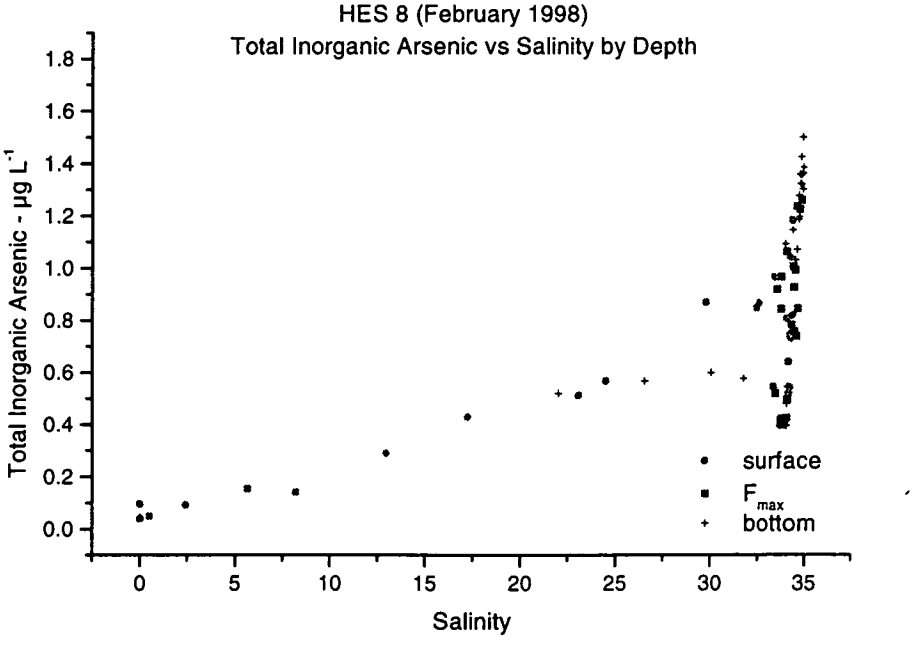
Appendix 7.3 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 5 survey (June 1997)



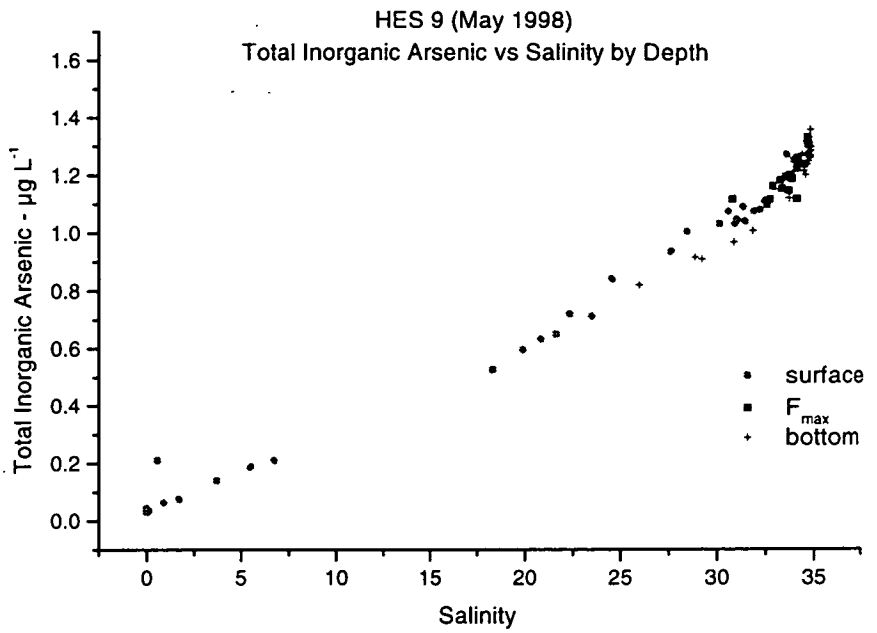
Appendix 7.4 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 6 survey (October 1997)



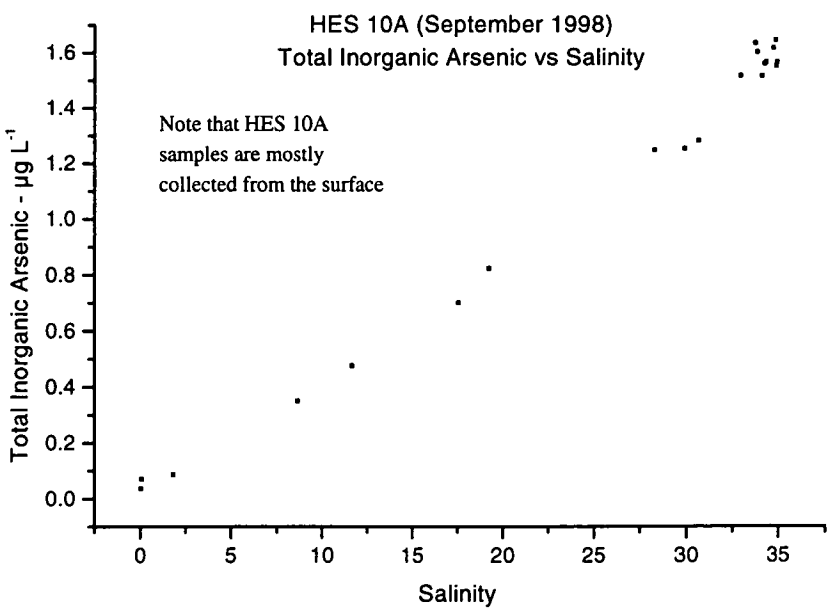
Appendix 7.5 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 7 survey (December 1997)



Appendix 7.6 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 8 survey (February 1998)

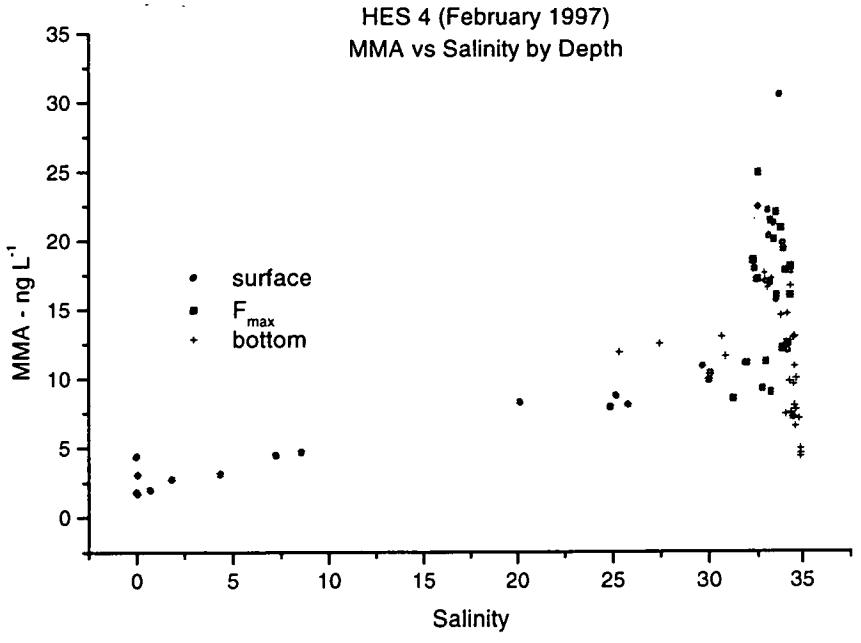


Appendix 7.7 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 9 survey (May 1998)

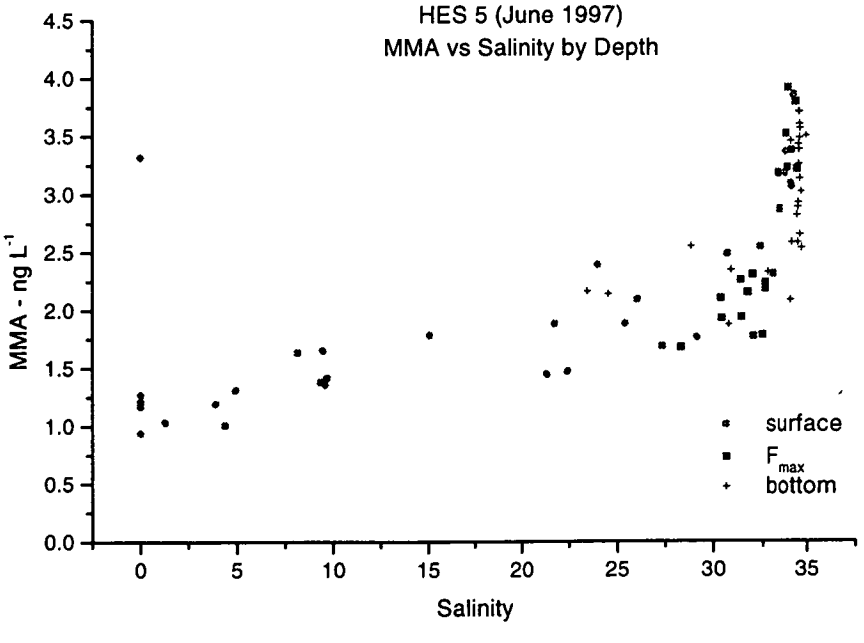


Appendix 7.8 Plot of total dissolved arsenic (As(V+III)) concentration *vs* salinity in the Huon River during the HES 10A survey (September 1998)

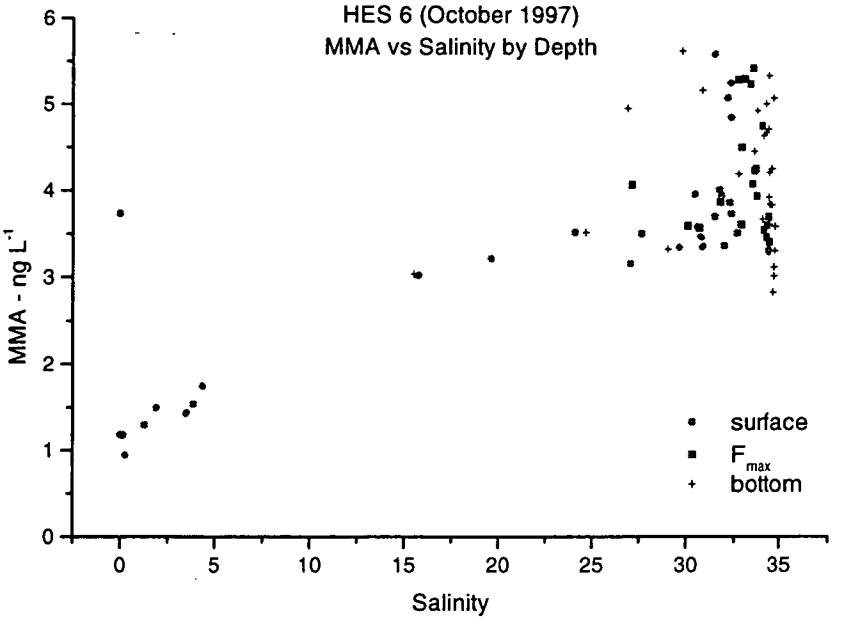
APPENDIX 8: MMA *vs* SALINITY BY DEPTH (HES 4 - HES 9)



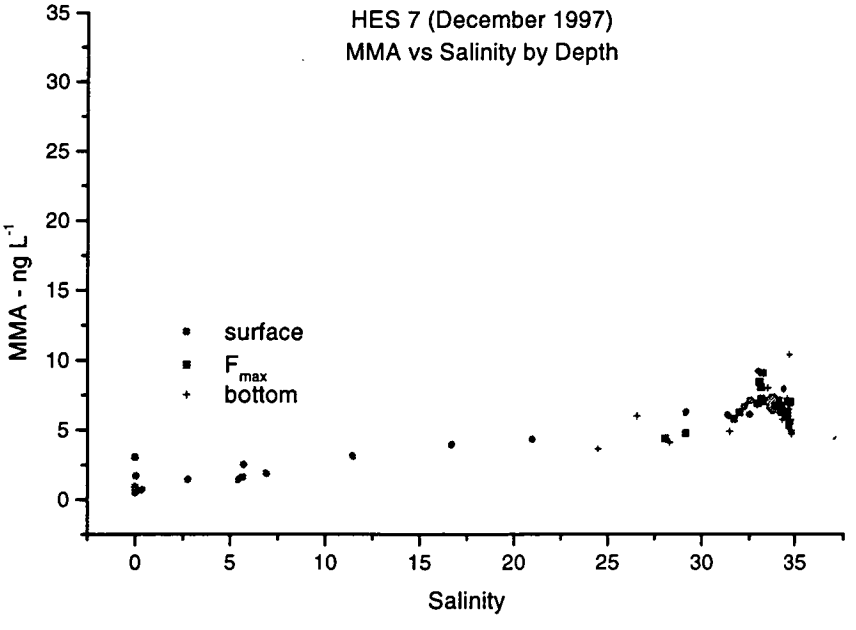
Appendix 8.1 Plot of MMA concentration *vs* salinity in the Huon River during the HES 4 survey (February 1997)



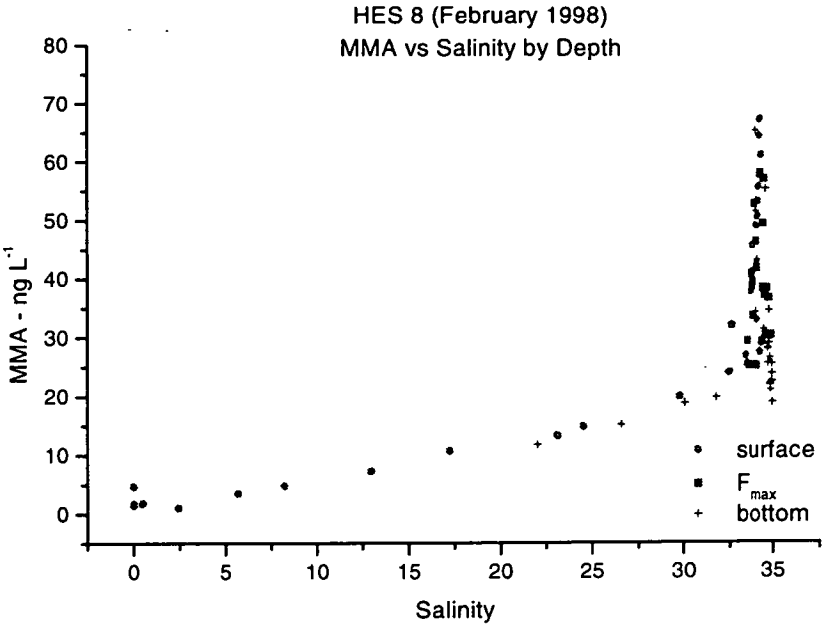
Appendix 8.2 Plot of MMA concentration *vs* salinity in the Huon River during the HES 5 survey (June 1997)



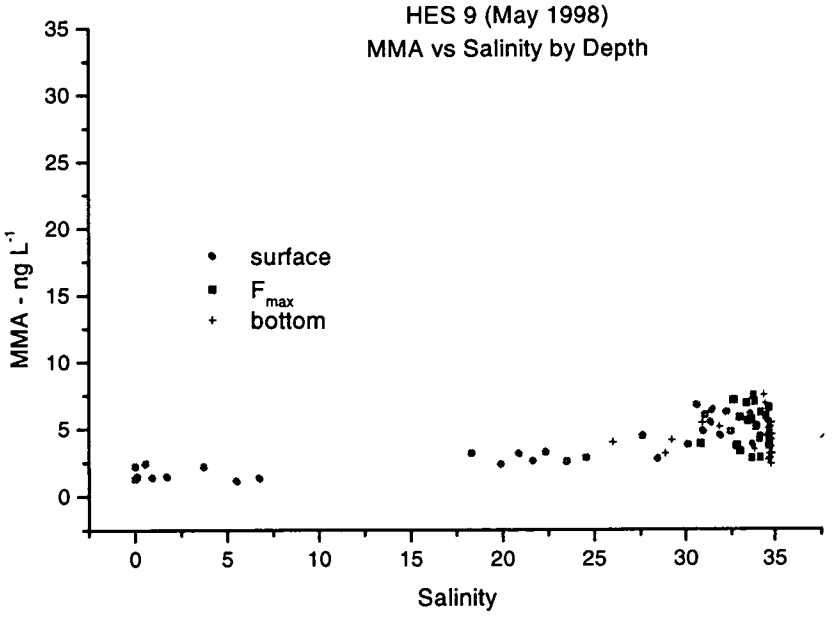
Appendix 8.3 Plot of MMA concentration *vs* salinity in the Huon River during the HES 6 survey (October 1997)



Appendix 8.4 Plot of MMA concentration *vs* salinity in the Huon River during the HES 7 survey (December 1997)

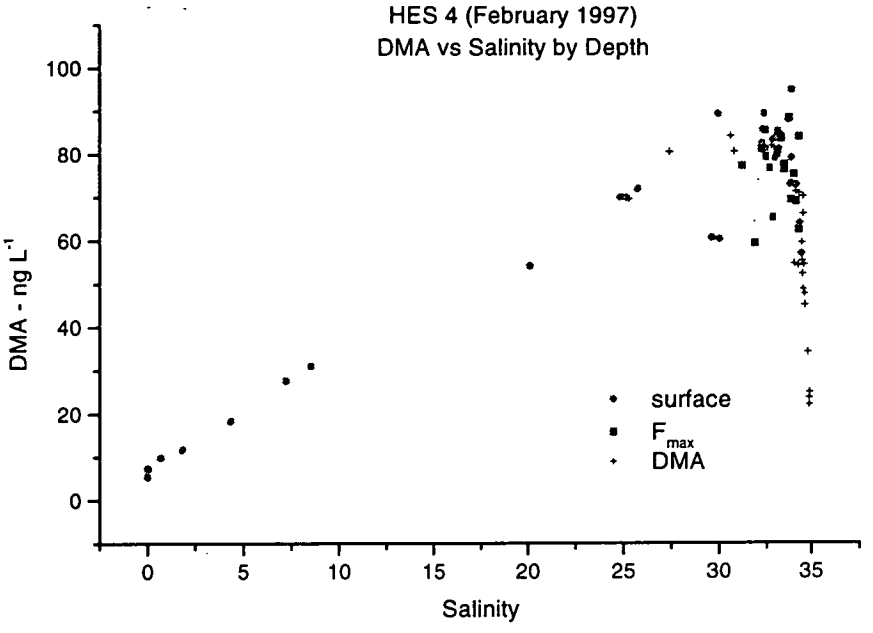


Appendix 8.5 Plot of MMA concentration *vs* salinity in the Huon River during the HES 8 survey (February 1998)

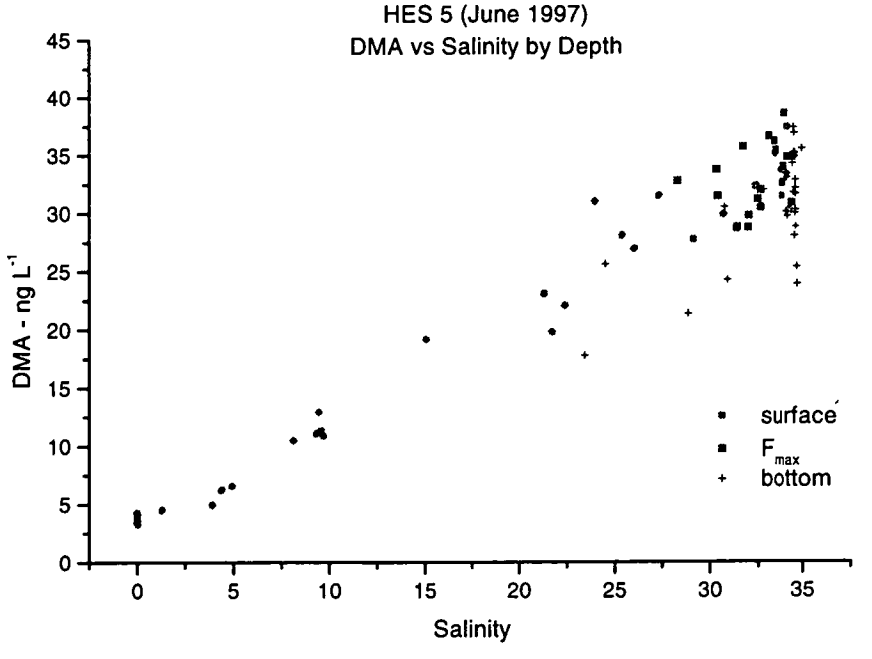


Appendix 8.6 Plot of MMA concentration *vs* salinity in the Huon River during the HES 9 survey (May 1998)

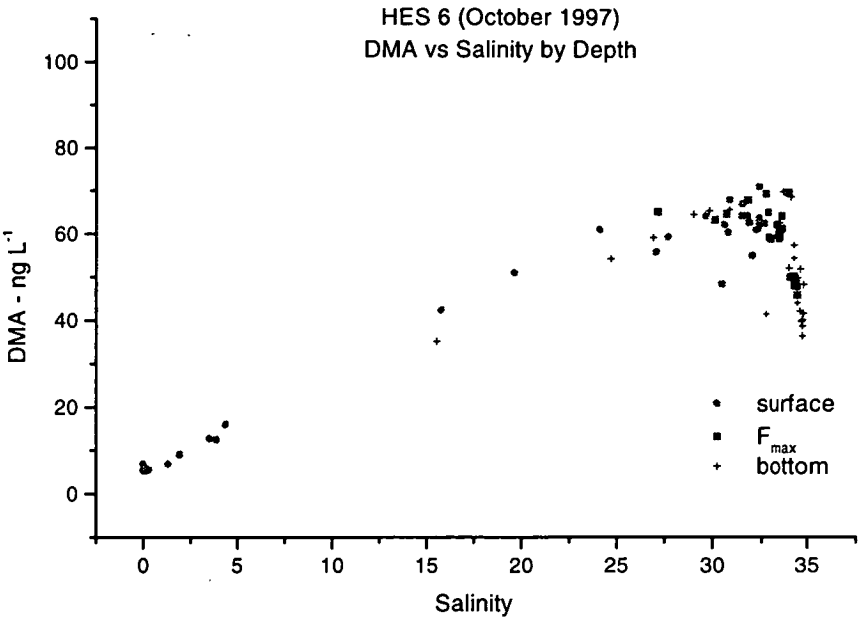
APPENDIX 9: DMA *vs* SALINITY BY DEPTH (HES 4 - HES 9)



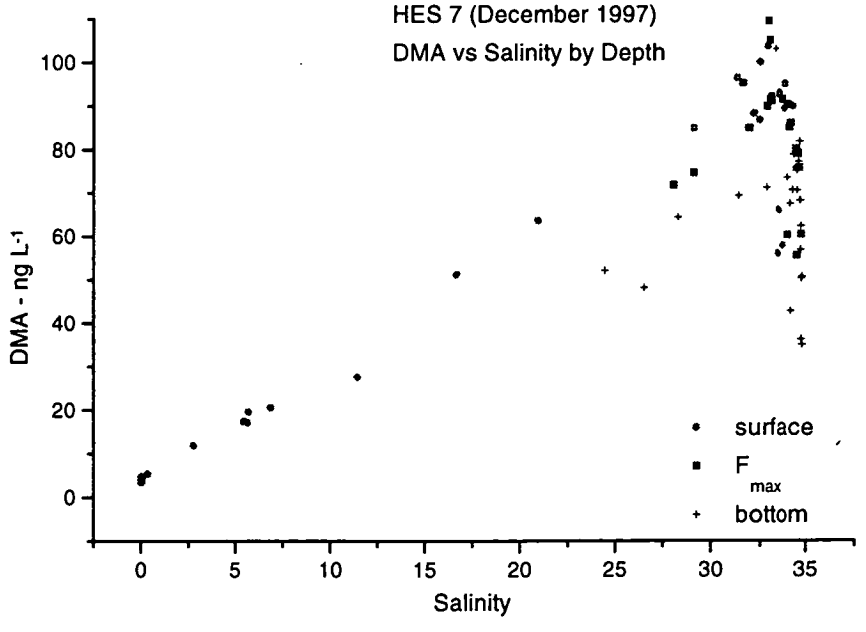
Appendix 9.1 Plot of DMA concentration *vs* salinity in the Huon River during the HES 4 survey (February 1997)



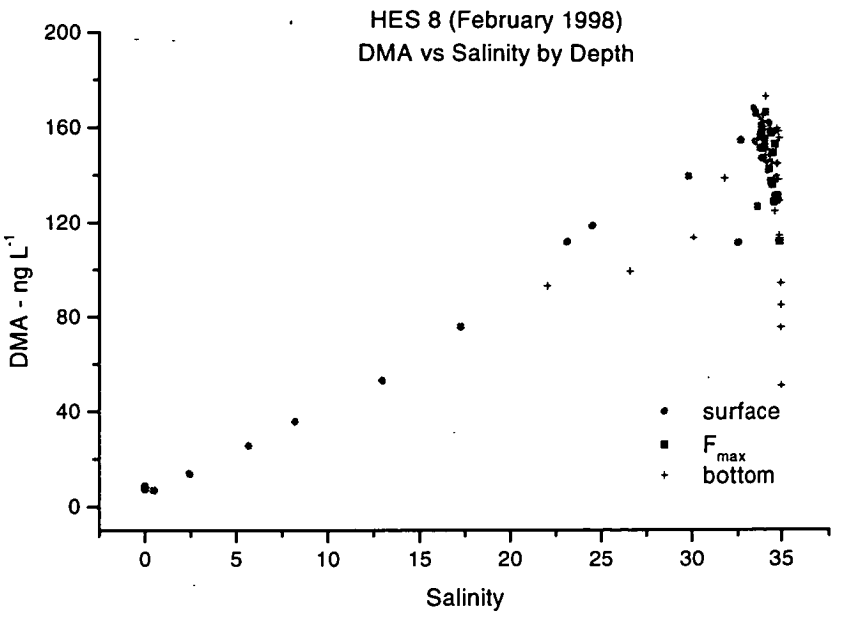
Appendix 9.2 Plot of DMA concentration *vs* salinity in the Huon River during the HES 5 survey (June 1997)



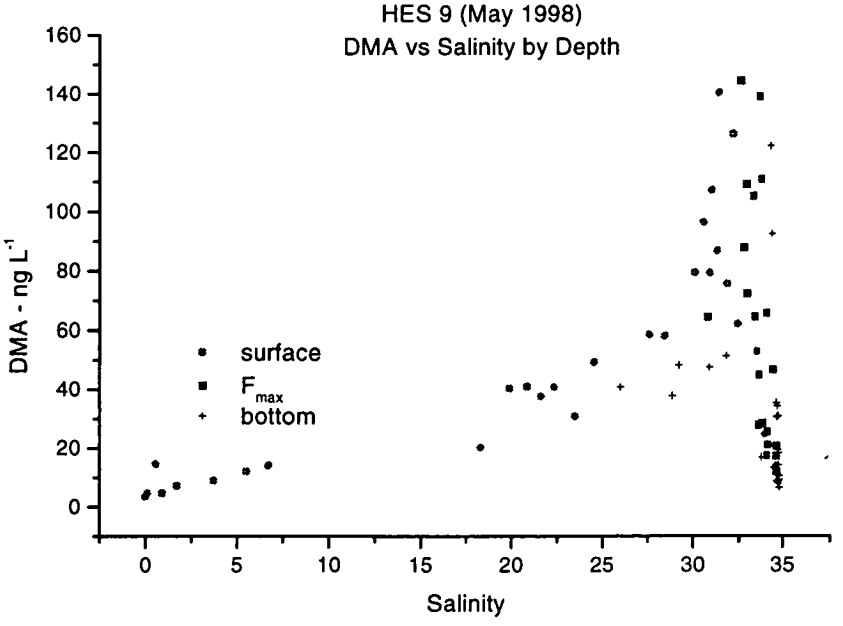
Appendix 9.3 Plot of DMA concentration *vs* salinity in the Huon River during the HES 6 survey (October 1997)



Appendix 9.4 Plot of DMA concentration *vs* salinity in the Huon River during the HES 7 survey (December 1997)

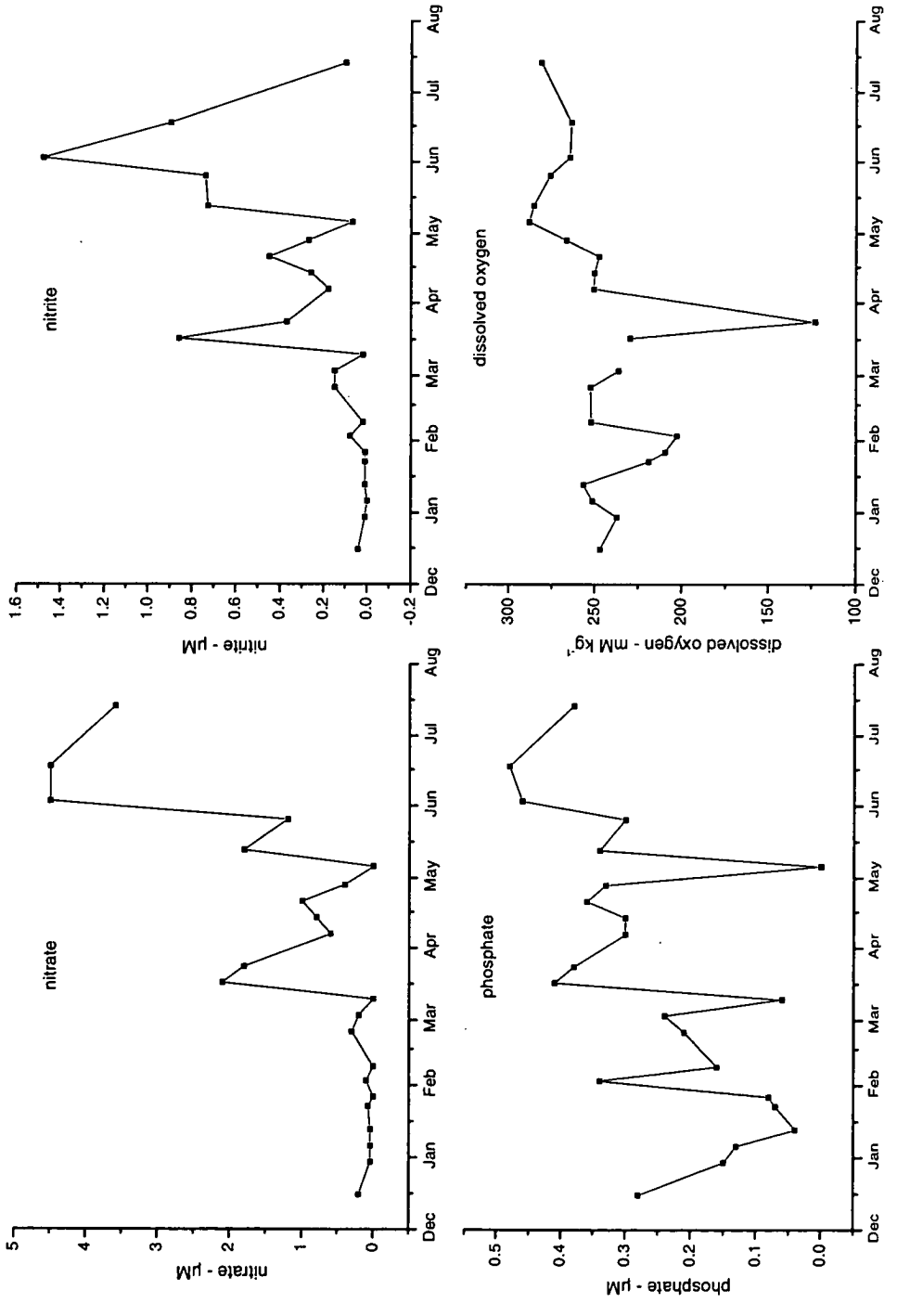


Appendix 9.5 Plot of DMA concentration *vs* salinity in the Huon River during the HES 8 survey (February 1998)

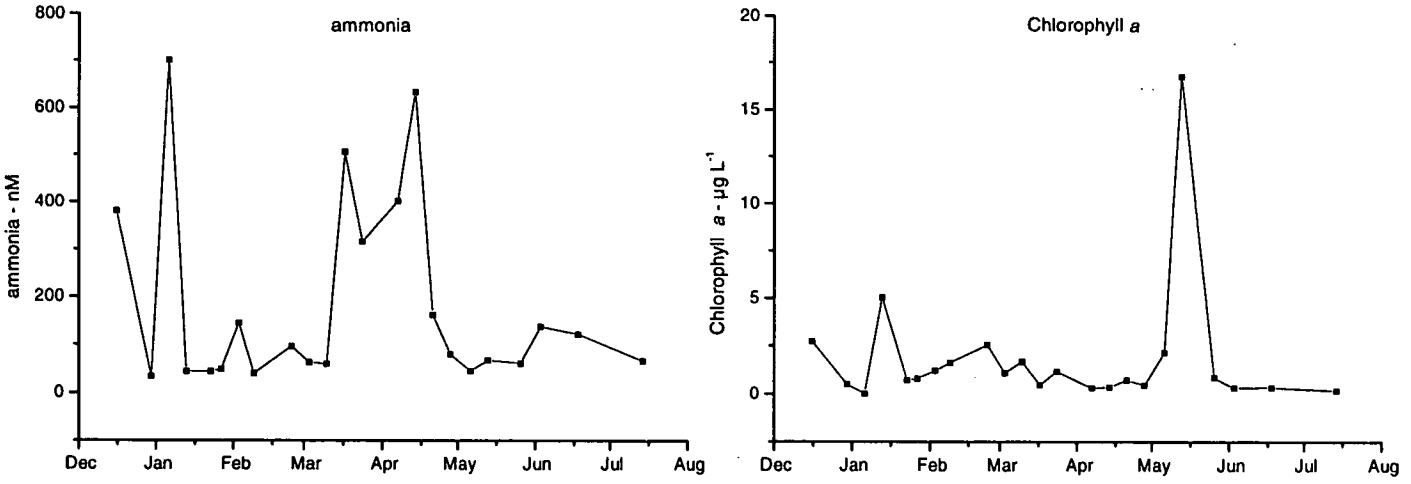


Appendix 9.6 Plot of DMA concentration *vs* salinity in the Huon River during the HES 9 survey (May 1998)

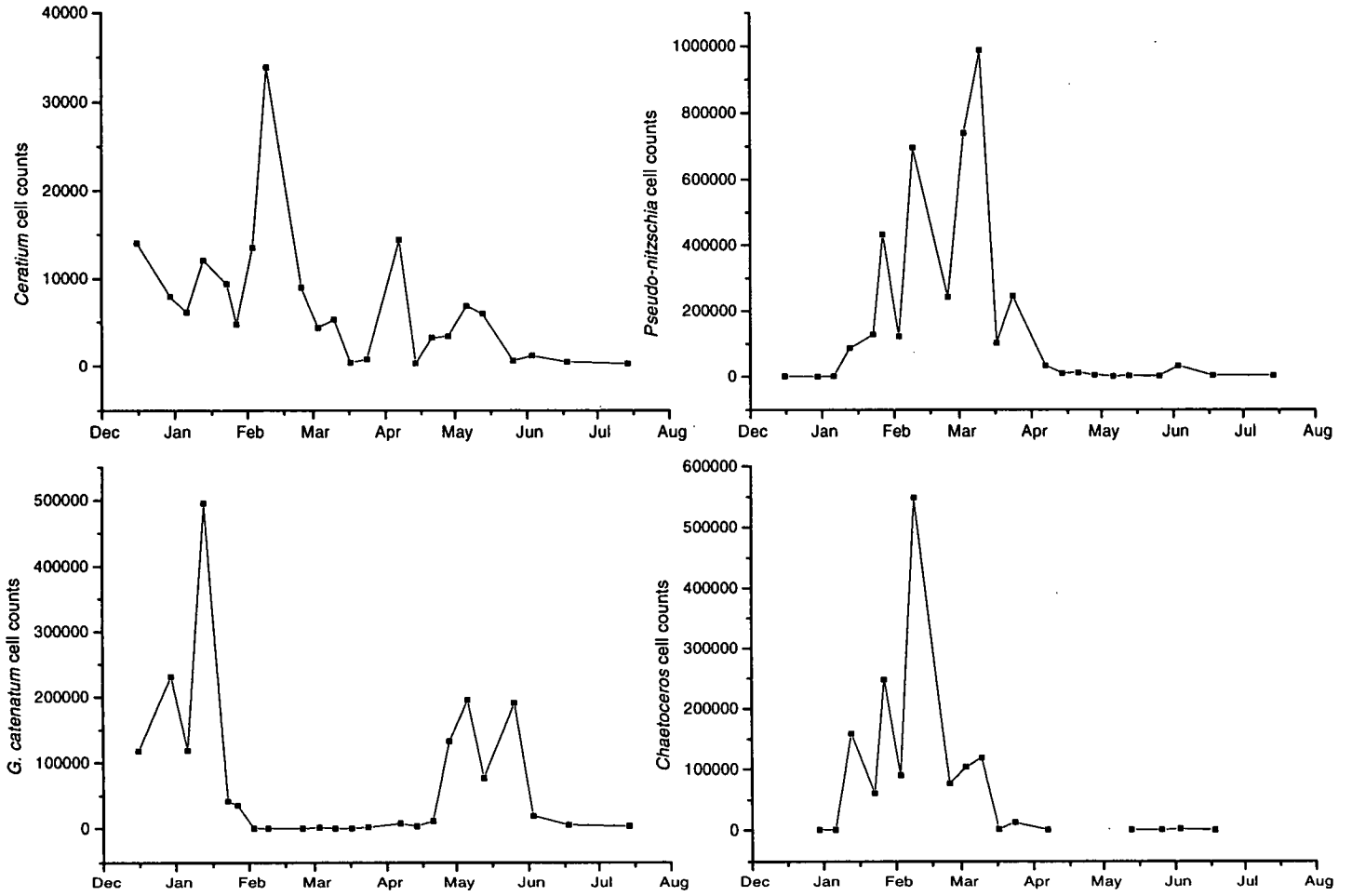
APPENDIX 10: ANCILLARY HUON ESTUARY CONTINUOUS MONITORING DATA



Appendix 10.1 Variation of nitrate+nitrite, nitrite, phosphate and dissolved oxygen at the Killala Bay (surface) continuous monitoring site during 1997/98



Appendix 10.2 Variations in ammonia and chlorophyll *a* concentrations at the Killala Bay (surface) continuous monitoring site during 1997/98



Appendix 10.3 Variation in cell counts of the major diatom (top) and dinoflagellate (bottom) species at the Killala Bay (surface) continuous monitoring site during 1997/98