THE UNIVERSITY OF LIVERPOOL

THE GEOCHEMISTRY OF THE METHYLAMINES IN RECENT MARINE AND LACUSTRINE SEDIMENTS

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy (Ph.D)

by

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Abstract

The methylamines (MAs) are a ubiquitous fraction of organic nitrogen in the environment, and are present at significant concentrations in the recent sediments and porewaters of the geosphere. They play an important role therein, as both organic osmolytes and substrates for sedimentary bacteria. The present study (in which all three MAs were determined concurrently) was carried out in order to gain an insight into the concentrations of the MAs in different sedimentary environments, all of which had a high organic content. The results presented show concentrations of the MAs in both the porewaters and sediments of these cores which are the highest reported to date. The MAs are also persistent with depth, unlike many other organic nitrogen volatiles such amino acids.

Given the reported involvement of the MAs in marine processes such as osmoregulation, it was attempted to investigate this further by sampling an intertidal salt marsh periodically through a tidal cycle. This study was carried out in a salt marsh on the Mersey Estuary, in order to investigate variations in the distributions of the MAs through a tidal cycle, and the speciation between the dissolved and particulate pools. Variation in concentration was observed in areas of different maturity, while speciation between the porewaters and sediments was different for all three MAs. Depletion of the porewater MAs in the UM during tidal inundation suggested a role in osmoregulation of salt marsh macrophytes, while the high concentrations observed in the MM and LM were also thought to be biologically mediated. Depletion of MAs adsorbed to the sediments suggested irreversible adsorption or chemical alteration with burial depth, as little evidence for desorption to the porewaters was observed.

Concentrations of the MAs in the porewaters of Loch Etive (Scotland) correlated nicely with reported population density of the resident infauna, and possibly gives further evidence for the reported positive correlation of trimethylamine with densities of infauna in shallow sediments. Sediments from a lacustrine sediment (Priest Pot, Lake District, England) were also examined for comparison with those of

marine origin. All three MAs were detected at significantly high concentrations, suggesting that a number of different processes may govern the distribution of the MAs in sediments where osmoregulation is not an important physiological process.

A ketoamine (1-aminopropanone, APR) was identified in environmental water samples. As a specific constituent of untreated sewage, its potential as a dissolved marker for sewage pollution was evaluated in water samples from different environments. APR was detected at significant concentrations in all samples and shows definite potential as a sewage marker, despite the analytical problems encountered as a consequence of the volatility of the compound.

Acknowledgements

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Go raibh maith agaibh go leir.

List of abbreviations

AA	amino acid
AMM	ammonia
CHO	choline
DFAA	dissolved free amino acids
DMA	dimethylamine
ECD	electron capture detector
FID	flame ionisation detector
GBT	glycine betaine
GC	gas chromatography
GC-MS	gas chromatography coupled with mass spectrometry
GLY	glycine
HPLC	high performance liquid chromatography
MMA	monomethylamine
NO_3^-	nitrate
NO_2^-	nitrite
NPD	nitrogen phosphorus detector
QA	quaternary amine
SAR	sarcosine
SO_4^{2-}	sulphate

TDFAA	total dissolved free amino acids
TMA	trimethylamine
TMAO.	trimethylamine oxide
[X]	concentration of X
$[X_{pw}]$	concentration of X in the porewaters
$[X_s]$	concentration of X in the sediment

Table of Contents

This thesis is dedicated to my late father, who loved the sea.

Correction

Page 119 Figure 5.5: Distributions of the MAs in **Core Set 2** *should read* Distributions of the MAs in **Core Set 3.**

Page 120 Figure 5.6: Distributions of the MAs in **Core Set 3** *should read* Distributions of the MAs in **Core Set 2.**

CHAPTER 1

INTRODUCTION

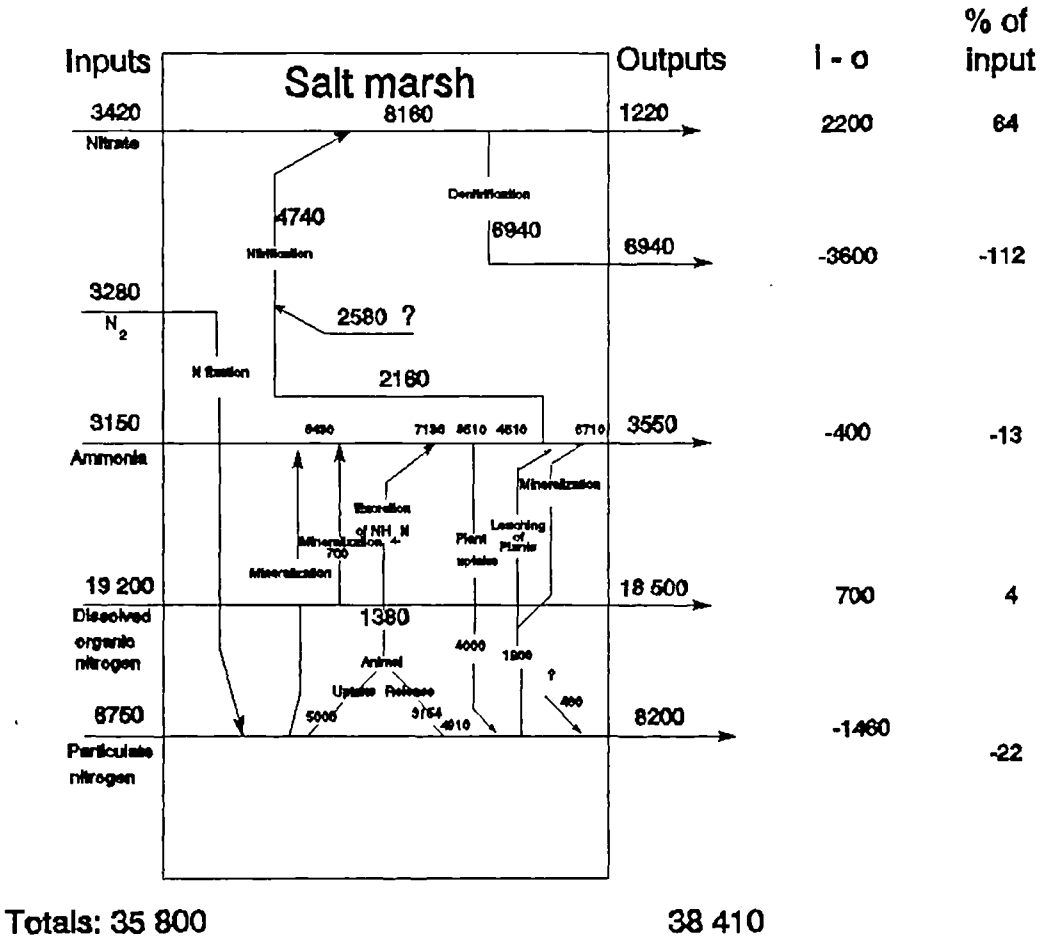
Section 1.1: THE NITROGEN CYCLE

The nitrogen cycle is perhaps the most interesting, the most complex and the least understood of the major nutrient cycles in natural waters and sediments. The geocycle of nitrogen is largely biochemically controlled; in natural waters it is nearly wholly so (Riley and Chester, 1971; Brezonik, 1972). Thus, the nitrogen cycle, like the carbon and phosphorus cycles, is inextricably linked to aquatic organic productivity.

Nitrogen occurs both in the biosphere and the geosphere in a variety of forms, ranging in oxidation state from +5 (NO_3^-) to -3 (NH_4^+). In the past NO_3^-, NO_2^- and NH_4^+ have been determined routinely using colorimetric methods, whereas analytical techniques and instrumentation suitable for the determination of organic nitrogen species (see Chapter 3) have only recently become available. To date, therefore, the nitrogen cycle has been largely described in terms of inorganic species, whilst the quantitatively significant organic nitrogen fraction has received little attention.

Figure 1.1 (Valiela, 1983) describes the net export of nitrogen from a salt marsh. Organic nitrogen represents the highest percentage of nitrogen input and output, but can only be described in bulk terms, even though it consists of many different groups of compounds of different reactivities. Figure 1.2 (Brezonik, 1972) is a simplified version of the overall nitrogen cycle, but shows the interaction between the inorganic and organic nitrogen fractions, and demonstrates the importance of considering the occurrence and behaviour of individual groups of organic nitrogen compounds in both the water column and sediments. Most of the

Fig. 1.1 Inputs, outputs and transformation of nitrogen within Great Sippewissett Marsh, MA, USA (after Valiela, 1983).



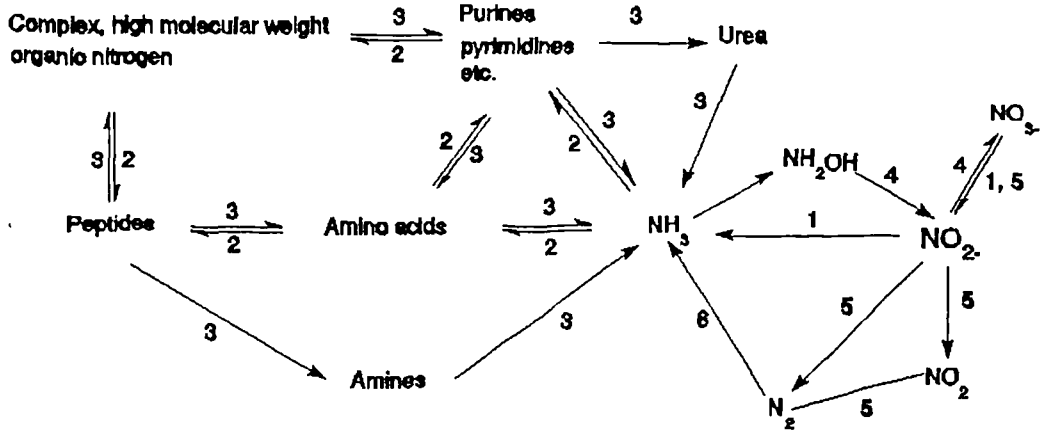
species comprising the environmental organic nitrogen fraction are shown in Fig. 1.2. Chitin, which forms part of the skeletal structure of many aqueous fauna, is included, along with chlorophyll and its derivatives. Chlorophyll is commonly determined by colorimetry or high performance liquid chromatography (HPLC) and, being a product of photosynthesis, is used as a measure of primary production.

It has been reported that marine phytoplankton release as much as 10% of the organic carbon they fix in the form of low molecular weight organic compounds (Mague et al., 1980). Subsequent studies suggested that as much as 45% of this organic carbon is released as dissolved free amino acids (Jorgensen, 1982; Carlucci et al., 1984; Nagata and Kirchman, 1991). Other nitrogen volatiles such as quaternary and aliphatic amines have been reported in the culture media of marine algae and within the intracellular solutions of marine organisms (Yancey et al., 1982; King, 1988a; Fitzsimons, 1989; Abdul-Rashid, 1990; Abdul-Rashid et al., 1991). Furthermore, Blackburn and Henriksen (1983), having studied rates of ammonia production in sediments off the coast of Denmark, reported that the rate of ammonia production in these sediments was equal to the rate of organic nitrogen mineralization. This, in turn, was a function of primary production by marine phytoplankton and benthic algae.

In the water column, organic nitrogen compounds are preferentially removed by bacteria, relative to organic carbon species. This characteristic is reflected in an increase in the C/N ratio of particulate material with depth (Gordon, 1971; Lee and Cronin, 1982; Wakeham et al., 1984). In sediments, however, organic carbon decreases faster than nitrogen, and it was thought that this may be a consequence of the production of low-molecular weight organic nitrogen compounds which are

persistent with burial depth (Lee and Olsen, 1984). The production and reactivity of organic nitrogen species in the marine environment are briefly reviewed in the following section. The reader is referred to "Nitrogen Cycling in Coastal Marine Environments" (Blackburn T.H. and Sorensen J. eds.) and "Nitrogen in the Marine Environment" (Carpenter E. and Capone D.G. eds.) for more comprehensive reviews.

Fig. 1.2 Simplified nitrogen cycle showing the main molecular transformations: 1. Nitrate assimilation, 2. Ammonia assimilation, 3. Ammonification, 4. Nitrification, 5. Denitrification, 6. Nitrogen fixation (after Brezonik, 1972).



Section 1.2: ORGANIC NITROGEN COMPOUNDS

1.2.1 Amino acids and proteins

Many studies on amino acids and proteins have been carried out as these compounds are major constituents of algae and may constitute the major fraction of total nitrogen in recent marine sediments (Henrichs and Farrington, 1987; Burdige and Martens, 1988; Haugen and Lichtentaler, 1991). Plankton usually contain 20-40% amino acids by weight, whilst amino acids account for 75-90% of their total nitrogen (Lee, 1988).

The distribution of amino acids in marine particulate matter and sediments reflects the balance between biological production and biological consumption. Phytoplankton take up inorganic nitrogen, for example nitrate and ammonium, and convert it to organic amino compounds. The terminal reaction during degradation of these organics generally yields ammonia. Most sedimentary amino acids are combined as peptides or proteins while only a minor portion (1-10%) exists in the free dissolved state (Henrichs and Farrington, 1980; Henrichs et al., 1984). Mayer and Rice (1992) calculated that protein concentrations in a coastal marine surface sediment accounted for up to 95% of the total sedimentary organic nitrogen. This percentage decreased quite rapidly over a short depth range, and it was suggested that this resulted from the mineralization of proteins to individual amino acids or other low molecular weight organic nitrogen volatiles. Burdige and Martens (1990) also observed significant decreases in the concentrations of total dissolved free amino acids in marine sediments with increasing depth, and suggested that amino acids liberated via the fermentation of proteins and other amino acid polymers are further

degraded during sedimentary diagenesis.

A number of pathways for the microbial degradation of amino acids have been suggested:

(1) Hydrolytic deamination.

The hydrolysis of an α-amino acid may result in the formation of α-hydroxy-carboxylic acids and ammonia; of an alcohol, CO_2 and ammonia; or of an aldehyde, lower acid and ammonia, as shown by the general equations:

$$RCH(NH_2)COOH + H_2O \rightarrow RCH(OH)COOH + NH_3 \quad \textbf{(1.1)}$$

$$RCH(NH_2)COOH + H_2O \rightarrow RCH_2 + CO_2 + NH_3 \quad \textbf{(1.2)}$$

$$RCH(NH_2)COOH + H_2O \rightarrow RCHO + HCOOH + NH_3 \quad \textbf{(1.3)}$$

(2) Decarboxylation with amine formation.

Amino acid hydrolysis may produce an amine and carbon doixide. The amine can then be further reduced to ammonia and an alcohol:

$$RCH(NH_2)COOH + H_2 \rightarrow RCH_2NH_2 + CO_2 \quad \textbf{(1.4)}$$

$$RCH_2NH_2 + H_2O \rightarrow RCH_2OH + NH_3 \quad \textbf{(1.5)}$$

(3) Reductive deamination

Degradation of the amino acid yields ammonia and a carboxylic acid:

$$RCH(NH_2)COOH + H_2 \rightarrow RCH_2COOH + NH_3 \quad \textbf{(1.6)}$$

(4) Ammonia formation without reduction.

Aerobic bacteria may produce ammonia from amino acids, without reduction.

$$RCH_2CH(NH_2)COOH + O_2 \rightarrow RCOOH + NH_3 + CO_2 \quad \textbf{(1.7)}$$

Examples of this reaction are the transformation of leucine into isovaleric acid {R = $(CH_3)_2CHCH_2$}, and of glutamic acid into succinic acid {R =

$COOCH_2CH_2$-}.

1.2.2. Quaternary amines

Quaternary amines (QAs) are ubiquitous in the intracellular solutions of marine plants and animals (Yancey et al., 1982; King, 1988a), where they are utilised for osmoregulation, preventing a build up of Na^+ ions, via diffusion from saline water, to toxic levels. The QAs, therefore, play an important role in regulating the concentrations of certain deleterious ions in marine organisms, where they can accumulate at very high concentrations without harming the organism or affecting their physiology (See Figure 5.11 for some examples of QAs).

High concentrations of certain QAs have been reported in anoxic marine sediments, where they are important substrates for both methanogenic and sulphate reducing bacteria (King, 1988b). QAs may also influence the fluxes of certain aliphatic amines (namely the methylamines) in marine sediments, as their microbially-mediated degradation (See Figure 5.12) can yield trimethylamine (TMA). Products of the subsequent degradation of TMA include the primary and secondary methylamines; monomethylamine (MMA) and dimethylamine (DMA).

1.2.3. Aliphatic amines

The production of aliphatic amines via the decarboxylation of amino acids has been reported in marine sediments (Stams and Hansen, 1984; Burdige, 1990; e.g. Equation 1.4). Hence, valine degrades to isobutylamine, 2-methylbutylamine is a product of isoleucine degradation, ethylamine is formed via the fermentation of

alanine and glycine degrades to produce monomethylamine and CO_2 (Scully, 1988).

The few investigations that have been carried out on the aliphatic amines have concentrated on the methylamines, and these are discussed in Section 1.2.4.

1.2.4. The Methylamines

The methylamines (MAs) are methylated analogue of ammonia, namely monomethylamine (MMA) dimethylamine (DMA) and trimethylamine (TMA), and these compounds have recently received considerable attention.

The MAs are ubiquitous in the water column and sediments of marine and lacustrine environments (Lee and Olsen, 1984; Glob and Sorensen, 1987; Sorensen and Glob, 1987; Abdul-Rashid, 1990), and have been detected in remote and urban aerosols (Van Neste et al., 1987; Abdul-Rashid, 1990). The MAs are also present in marine algae (Smith, 1971), benthic invertebrates (Sorensen and Glob, 1987) and marine teleosts (Daikoku, 1987). It is believed that dissolved MAs are taken up by marine flora and fauna along with other low-molecular weight organics such as carboxylic acids and QAs, for use in osmoregulation, as considerable amounts of the MAs have been detected in benthic invertebrates and algae in marine environments (Sorensen and Glob, 1987; Abdul-Rashid et al., 1991). A decrease in the water salinity can initiate diffusion of the MAs and QAs from the organisms, where they enter the nitrogen cycle and become available for bacterially-mediated mineralization (Oremland et al., 1982a; Large, 1983; King, 1984, Giani et al., 1984).

TMA is an important bacterial substrate in certain marine sediments (Oremland et al., 1982a; Giani et al., 1984), while MMA and DMA have also been preferentially utilised in cultures of marine bacteria (Budd and Spencer, 1968). It

preferentially utilised in cultures of marine bacteria (Budd and Spencer, 1968). It seems that the production and supply of MAs to the sediments, is necessary to sustain bacterial species that might not otherwise be able to flourish (King, 1984)

A decrease in the ratio of organic C/N has been observed with depth in some marine sediments (Arrhenius, 1952; Cowie and Hedges, 1992; Mayer and Rice, 1992). Formation of ammonia and subsequent incorporation into the sedimentary matrix may be partly responsible (Stevenson and Tilo, 1970; Stevenson and Cheng, 1972; Lee and Olsen, 1984). However, Muller (1977) found that ammonia alone could not account for the decrease, and suggested that adsorption of low molecular weight organic nitrogen species with low C/N ratios (e.g. the MAs) might also influence this phenomenon. The MAs are more basic than ammonia (see Table 3.1) and are protonated at seawater pH. They might, therefore, be expected to have a strong affinity for exchange sites on negatively-charged clay mineral surfaces. Adsorption of the MAs onto sediments may not be completely reversible (Wang and Lee, 1990), and may mean persistence of the MAs with depth in sediments and a potential role in controlling decreases in the sedimentary C/N ratio.

Section 1.3: THIS STUDY

1.3.1 Part A

The present study was undertaken to gain further insight into the role played by the MAs in the marine nitrogen cycle, and to compare the distributions of these compounds in a variety of marine and lacustrine sediments. Hence, sediment cores from Loch Etive and the Firth of Lorne (Scotland) and from a eutrophic freshwater

MAs in an environment where such factors as salinity stress (especially important in intertidal sediments) were not important for the resident organisms, and to see how this might influence abundance of the MAs.

The major part of the study, however, was concerned with the geochemical cycling of the MAs in a salt marsh in the Mersey Estuary (UK). Oglet Bay salt marsh contained three distinct zones of development, which were reflected by the relative abundances of salt marsh vegetation. The marsh was sampled through a tidal cycle in an attempt to monitor the effects of tidal inundation on the MA distribution. Although MA distributions in the sediments and porewaters of a salt marsh have been investigated previously (Wang and Lee, 1990), it has not, until now, been possible to determine all three MAs simultaneously.

Few studies on the speciation of the MAs between the porewater and sedimentary-associated pools have been carried out. For this reason, concentrations of porewater and sedimentary-associated MAs from the tidal cycle cores were normalised and expressed as mol/g wet sediment. In all other cases, concentrations were expressed in μM (porewaters) and μmol/g dry weight (sediments), as MA concentrations are most commonly expressed in these units in the literature. This enabled comparison with the results of previous studies.

Total organic carbon and nitrogen (TOC and TON) contents of some sediments were calculated, and other nitrogen species were measured (NO_3^-, NO_2^- and NH_4^+), in order to assess interactions between the geochemistries of the organic and inorganic species.

1.3.2 Part B

The River Mersey has a history of anthropogenic contamination, and has received considerable discharges of untreated sewage (see map showing sewage outfalls in Fig. 6.5) which contains high concentrations of volatile amines (Scully, 1988; Abdul-Rashid, 1990). It was during routine GC analysis of a Mersey water sample that an unknown peak was observed, which was subsequently identified as 1-aminopropanone (APR). Since APR is very soluble in water and is a sewage specific compound in the natural environment, a number of pilot studies were undertaken to evaluate its usefulness as a chemical marker for untreated sewage in water samples. The results of these studies are discussed in Chapter 6.

CHAPTER 2

MATERIALS AND METHODS

Section 2.1: REAGENTS AND STANDARD SOLUTIONS

Ammonium chloride, methylamine hydrochloride (both general purpose reagent grade), trimethylamine hydrochloride, hydrochloric acid (aristar), sodium nitrite (anhydrous), sodium nitrate (analar) and sodium hydroxide (aristar) were supplied by BDH Ltd. (Poole, UK). Cyclopropylamine, cyclobutylamine, ether (anhydrous), chloroacetone and potassium phthalimide were supplied by Aldrich Chemical Co. and dimethylamine hydrochloride by Sigma UK. All reagents (excepting chloroacetone which was distilled before use) were used without further purification. Water for blanks and standard solutions was obtained from a Milli-Q-Plus water purification system (Millipore, 18.2 ohms resistivity). Stock standard solutions of ammonia and the methylamines (1 M) were prepared by dissolution of the hydrochlorides in water. Concentrated HCl (1 ml) was added as a preservative and the solutions made up to 100 ml. The standards were refrigerated and checked regularly by GC. Working standards were prepared as required.

In order to minimise adsorption of amines onto the sediments (See Chapter 4, also Wang and Lee, 1990), all samples were made up to volume in seawater which had been collected from the Menai Straits (Anglesey, UK) and filtered. Prior to use, nitrogen gas (oxygen-free) was bubbled through the seawater (10 mins) to purge the solution of volatiles. Analysis of the treated seawater showed that volatile amines were efficiently removed.

Section 2.2: SAMPLE PREPARATION

2.2.1 Sample collection

Port Erin and New Brighton water samples were collected at the surface from a point on the Mersey estuary (New Brighton) and a grid network of stations in Port Erin Bay (Irish Sea, Chapter 6, Figures 6.5 and 6.6) using acid-cleaned, plastic bottles (ca 250 ml). All samples were immediately acidified with concentrated HCl and those from Port Erin frozen (-20°C) before transport to Liverpool by air.

Untreated sewage samples were obtained from treatment plants in Wigan (Greater Manchester) and St Helens (Merseyside), placed in autoclaved conical flasks (1 l) and incubated in a culture cabinet (15 °C). Samples (50 ml) were taken at regular intervals over the period of about 1 month. Duplicate samples from each site were treated with antibiotic (Penicillin, 400 mg/l) to investigate the influence of bacteria on the lifetime of APR.

Oglet Bay Sediment cores were taken from a series of different sites on Oglet Bay salt marsh using brass core tubes (50 mm i.d. x 0.3 m long). The core tubes were wrapped in aluminium foil and placed in a cool box containing dry ice (-78°C) before transport to the laboratory.

Priest Pot Sediment cores were taken using a spring-loaded corer containing perspex core tubes (60 mm i.d. x 0.15 m in length). In the laboratory, cores were

sectioned at 1.0 and 0.5 cm intervals and placed in electronically sealed plastic bags before transport to Liverpool in a cold box packed with ice.

Loch Etive and Firth of Lorne Sediment cores from Loch Etive and the Firth of Lorne were collected using a gravity corer (70 mm i.d. x 1.0 m long) as described by Ridgeway and Price (1987) and Pedersen et al. (1985). The perspex core tube was sealed at the base with a rubber bung before disengagement from the corer, after which a rubber bung was also fitted to the top of the tube. The undisturbed cores and overlying water were then transported back to the laboratory and left in cold storage overnight.

2.2.2 Sectioning of sediment cores

Sediment cores from Oglet Bay (Mersey Estuary, UK) were sectioned under nitrogen at 1 cm intervals using apparatus constructed in the laboratory workshop (Figure 2.1). This consisted of a wooden frame attached to the bench surface by a G-clamp. The core tube was clamped in the frame using wooden blocks and the thawed sediment core extruded using a nylon piston which was fitted with rubber 'O' rings for a good seal and operated by a threaded metal rod.

Loch Etive sediments were sectioned at the SMBA laboratories using a plastic pressure piston and handle. The cores were sectioned at 1 (0-20 cm), 2 (20-40 cm), 5 (40-60 cm) and 9 cm (60+ cm) intervals and were placed in nitrogen-filled bags before being transported to Liverpool in a cool-box.

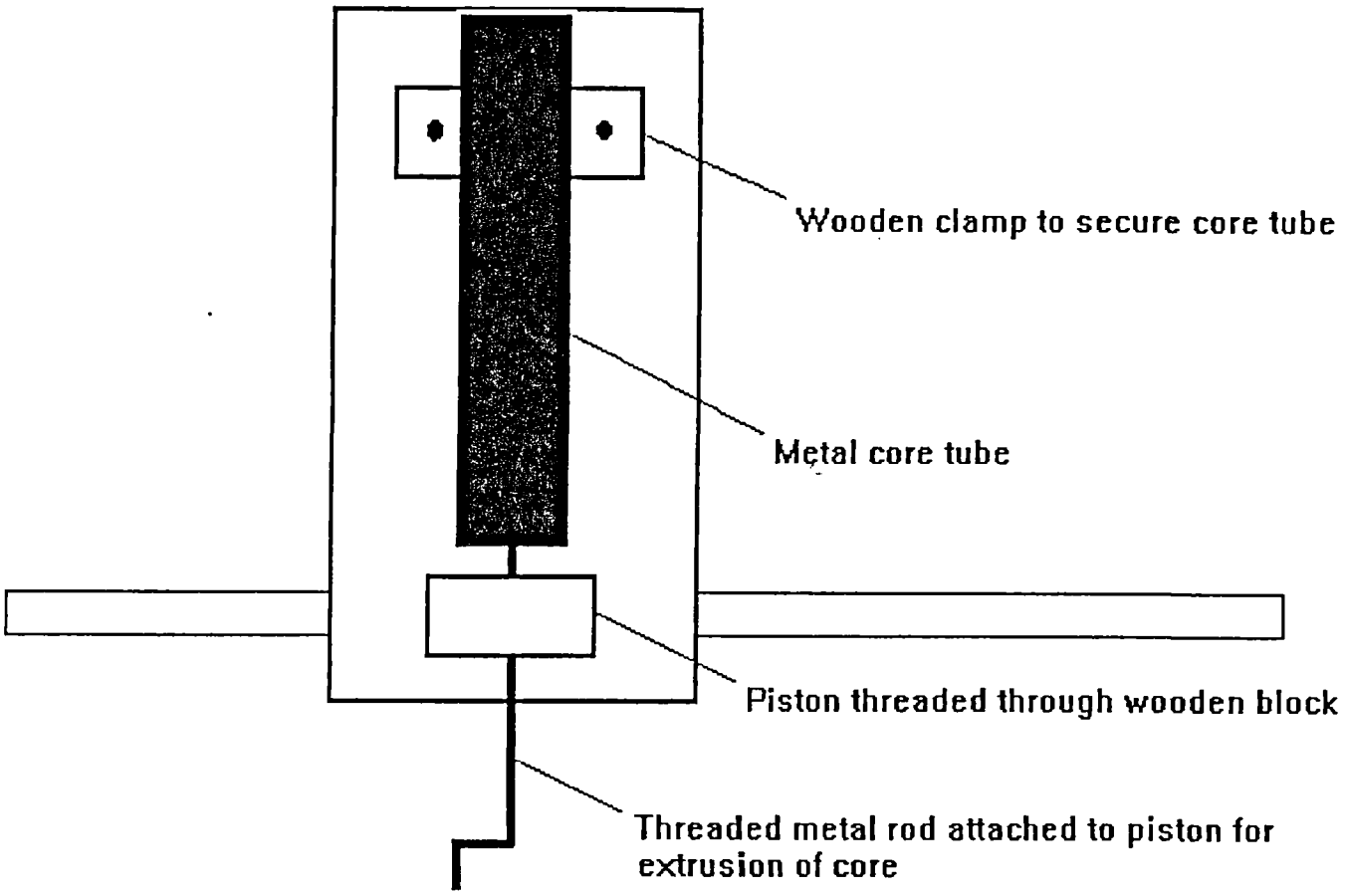


Figure 2.1 Core sectioning apparatus

2.2.3 Measurement of Porewater Content

The porewater content of each sediment section was determined. A portion of each sediment section was placed in a clean, dry glass vial and the weight recorded. The vials were then placed in an oven (80°C, 48 h) and the weight of the vial and dry sediment recorded. Porewater content was determined as the difference in weight between the wet and dry sediments.

2.2.4 Porewater Extraction

Porewaters were extracted from sediment samples using pressure filtration apparatus similar to that described by Reeburgh (1967), which was constructed in the departmental workshop (Figure 2.2). The sampler was operated manually using a G-clamp. A piston was used to squeeze the interstitial water through a 35 μm mesh, and a 0.5 μm (GF/F) glass fibre filter, into the collecting vial via a plastic tube.

Squeezing time varied according to porewater content and sample weight, but was normally not more than 10 minutes at full pressure. The process was carried out under nitrogen gas.

The percentage extraction of porewater was determined by measuring the porewater content of the sediments before and after extraction. Concentrations of porewater analytes were normalised, relative to these values.

2.2.5 Treatment of Sediment Samples for removal of Carbonate

Samples were treated with acid (HCl, 2M) to remove any carbonate before CHN analysis. Excess acid was added to samples in a clean plastic sterilin until

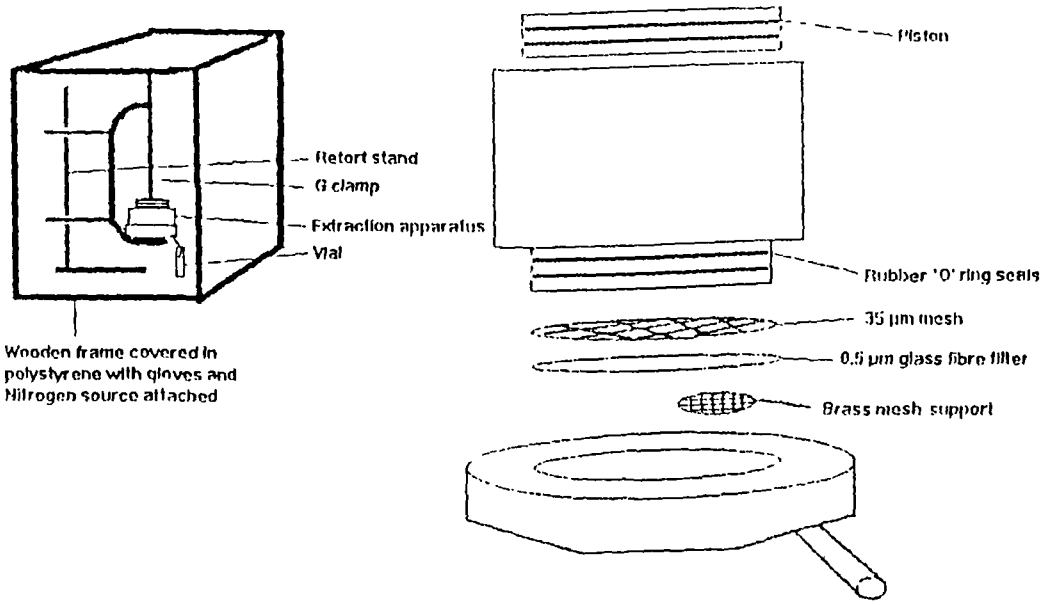


Figure 2.2 Porewater extraction apparatus

effervescence had ceased. Samples were then thoroughly rinsed with Milli-Q water and freeze-dried in preparation for CHN analysis.

2.2.6 Measurement of the Reproducibility of Sediment and Porewater Samples

The reproducibility of the method of porewater and sediment samples was measured for both Loch Etive and Oglet Bay samples. A sediment sample was homogenized, centrifuged and divided into four sections. Porewater was then extracted and the samples microdiffused (see below Section 2.3).

Section 2.3: MICRODIFFUSION

2.3.1 Glassware.

All glassware was acid-washed (1 M HCl, 24 hours), thoroughly rinsed with Milli-Q water, dried overnight in a fan-assisted oven at 200°C, and allowed to cool to room temperature before use. Clean, dry glassware was exposed for the shortest time possible to avoid atmospheric contamination.

2.3.2 Microdiffusion flask.

The microdiffusion flask was similar to that described by Riley and Sinhaseni (1957) for the determination of ammonia and total inorganic nitrogen in seawater. It was modified from a stoppered Quickfit Pyrex Erlenmeyer flask (150 ml, Figure 2.3).

A small collecting cup (20 mm in diameter) was supported by a 40 mm glass rod suspended from the base of the glass stopper (size B24/29). The flasks were

standardized by ensuring that cup and rod sizes were equal in all cases. The stopper was fitted with a PTFE sleeve to prevent loss of volatiles during microdiffusion, and secured using two stainless steel springs (Abdul-Rashid et al., 1991).

2.3.3 Internal standards.

An internal standard was used for the determination of MAs. A number of factors were taken into account when choosing these compounds. Chemical similarity to the MAs was most important, as it was necessary to use an internal standard with efficient recovery by microdiffusion. Many amines are ubiquitous in the natural environment and can also have an anthropogenic source (Scully, 1988), so these could not be used.

1-aminopropane and 2-aminopropane were unsuitable as they are present in many samples. Furthermore, the former undergoes a rearrangement to its isomer 2-aminopropane during microdiffusion or GC analysis (Abdul-Rashid, 1990). Chlorinated amines were deemed unsuitable as they cannot be detected using a nitrogen-phosphorus selective detector (NPSD). Satisfactory resolution from the MAs was required; ethylamine could not be used as it elutes with a retention time close to that of TMA.

Cyclopropylamine (CPA) was chosen as the internal standard for the microdiffusion process. It is a volatile amine of low molecular weight which has so far not been detected in any natural samples. Its retention time is reasonably close to those of the MAs, and its recovery and reproducibility were satisfactory (See Chapter 3)

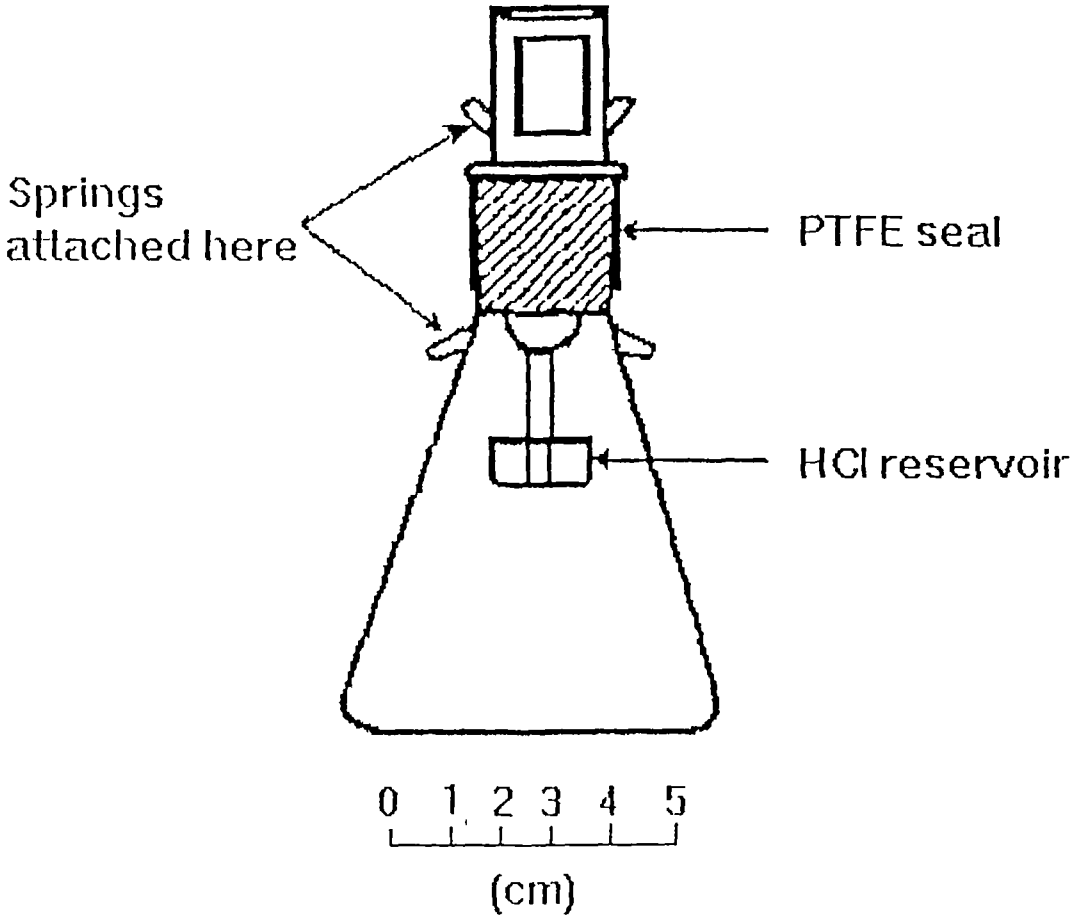


Figure 2.3 Cavett microdiffusion flask

2.3.4 Microdiffusion.

Ammonia and the MAs from natural samples were pre-concentrated by microdiffusion. Interstitial water samples (ca 1-2 ml) were treated with NaOH (6M, 2 ml). Cyclopropylamine (10^{-4} M, 0.25 ml) was added as an internal standard and the solution made up to volume (50 ml) with seawater. The solution was thoroughly mixed before being transferred to the Microdiffusion flask. Sediment samples (ca. 0.5 g) were weighed accurately, transferred to the Cavett Flask, and a well mixed solution of seawater (treated as above) was added. HCl (0.02 M, 200 μl) was added to the collecting cup, the flasks were sealed and the stoppers secured with stainless steel springs.

Reasonable recovery (see Section 3.4.3) of the analytes was achieved by incubation of the samples in a fan-oven at 60 °C for 24 hours. When this time had elapsed, the contents of the collecting cup were quantitatively transferred by a micropipette to a glass vial (0.8 ml Phase Sep. UK Ltd.). The pH of the solution was adjusted to 14 by addition of NaOH (0.5 M, 50 μl) and cyclobutylamine (CBA, 10^{-3} M, 20 μl) was added as a GC injection standard. The solution was made up to 300 μl with HCl (0.02 M) which was also used to rinse the collecting cup. Samples were stored in a freezer (-20 °C), if necessary, and were analysed as soon as possible.

2.3.5. Measurement of detection limits

Limits of detection for GC analytes were calculated according the baseline

signal (S) to noise ratio when the sample peak was at least twice the mean noise level (N) as shown in equation 2.1.

$$S/N \geq 2 \qquad (2.1)$$

2.3.5. Random GC analysis

In order to minimise any systematic errors, samples were analysed according to random number tables (Murdoch and Barnes, 1986).

2.3.6. Treatment of outlying data points

All data points were tested for credibility using the "Dixon's Q" formula (Miller and Miller, 1988) as shown below (Equation 2.2) and any outliers were discarded.

$$Q = [\text{supect value/nearest value}]/(\text{largest value - smallest value}) \qquad (2.2)$$

Section 2.4: ANALYTICAL INSTRUMENTATION

2.4.1 Gas chromatography

Analyses of ammonia and MAs were carried out using a Carlo Erba Fractovap Series 2350 gas chromatograph (GC) and a Carlo Erba Mega Series GC which were fitted with Nitrogen-Phosphorus Selective Flame Ionization Detectors (see Section 2.4.3). Samples were injected using an on-column injection system (250°C) onto a glass column (2.5 m x 2.0 mm i.d.) packed with Chromosorb 103

(60-80 mesh). Nitrogen (oxygen-free) was used as the carrier gas and the detector gases were hydrogen and air.

The GC operating conditions were similar to those used by Abdul-Rashid (1990). DMA and TMA were not fully resolved under these conditions, but a lower initial temperature resulted in deterioration of peak shape and poor reproducibility. The column was conditioned at 200 °C for 5 minutes between injections to purge the system of any remaining volatiles from the preceding run. Injections were carried out manually using a syringe (10 μl, Hamilton, USA) and 5 μl samples were injected. The syringe was rinsed with Milli-Q water at least 15 times between injections. Cyclobutylamine (CBA) which seems to be absent in natural systems was chosen for measuring injection reproducibility.

2.4.2 Nitrogen-Phosphorus Detector

The Nitrogen-Phosphorus Detector (NPD) is used for the selective determination of nitrogen and phosphorus- containing compounds with high sensitivity (Figure. 2.4). Thermoionic detection enables an enhanced response to compounds containing nitrogen and phosphorus (including inorganic species) to be achieved. In contrast, the response to other organic compounds is depressed. The detector gives no response with chlorinated compounds (see Section 2.2.3), or may give negative peaks if large amounts are present.

The NPD is a modified flame ionization detector (FID). A bead of alkali metal salt is mounted between the FID flame tip and the collector electrode. The flame temperature must be sufficiently high to volatilize the alkali metal salt and generate a stable population of alkali metal ions, which are necessary for the

thermionic process. The combustion products of nitrogen and phosphorus compounds undergo a complex series of reactions with the alkali metal ions. The resulting alkali metal compounds are collected on the electrode, causing an increase in the standing current which is proportional to the concentration of the compound. The sensitivity of the detector is governed by the alkali metal ion concentration, and therefore by the flame temperature. Table 2.2 shows the sensitivity of an NPD compared to that of other common GC detectors.

2.4.3 CHN Analysis

The carbon and nitrogen percentages of all sediments were measured using a Carlo Erba Elemental Analyzer Model 1106. Core sections were freeze-dried and finely-ground before analysis. Samples (approx. 1 mg) were weighed accurately into a tin capsule using a Cahn 21 Automatic Electrobalance (Scientific and Medical Products Ltd.). The sampler was then loaded with the capsules for injection into the combustion reactor at 1050°C. The combustion gases were carried in a constant flow of helium through to the catalytic section of the reactor in order to ensure complete oxidation to CO_2, H_2O, N_2 and N_xO_y. The gaseous mixture was then passed into a second reactor (oven temperature = 650°C) which was filled with copper in order to reduce the nitrogen oxides to N_2, and then through a chromatographic column packed with Poropak (50 to 80 mesh, Elemental Microanalysis Ltd.) at 100°C which separated N_2, CO_2 and H_2O. The eluting gases were analysed using a thermal conductivity detector, and peak areas were compared with those of a standard. Data were recorded on a Spectra Physics Minigrator. Operating conditions are shown in Table 2.2..

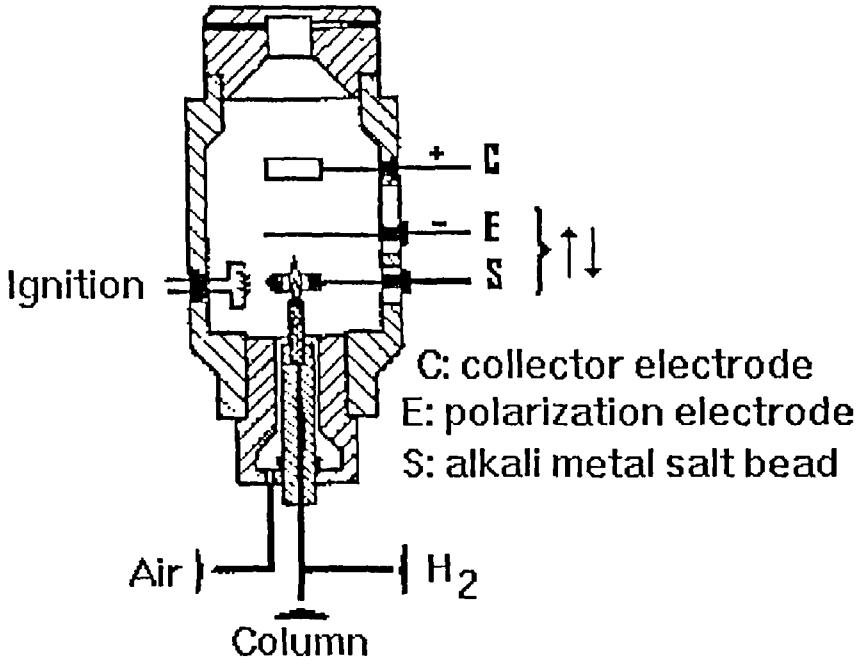


Figure 2.4 Nitrogen Phosphorus detector

Table 2.1 Sensitivity of some common GC detectors

Type of detector	MDL*+ (g ml^{-1})
FID	10^{-12}
NPD	10^{-14}
Flame Photometric	10^{-11}
Katherometer	10^{-9}
ECD	10^{-14}

Taken after Braithwaite and Smith (1985).

*** MDL = minimum detection limit**

+ Specific analytes were not reported

Table 2.2 Operational conditions of CHN Elemental Analyzer

CARRIER GAS:	He
Flow Rate:	20 ml min^{-1}
DETECTOR GAS:	O_2
Flow Rate:	40 ml min^{-1}
OVEN TEMPERATURES:	
Combustion Oven:	1030°C
Reduction Oven:	650°C
Chromatographic Oven:	100°C

2.4.4 Automatic analyser

An automatic analyser with segmented flow type was used for the determination of nitrate and nitrite. This apparatus was composed of four basic modules:

(1) sampler, (2) proportioning pump, (3) manifold and (4) colorimeter (and recorder). A diagram of the system is shown in Figure 2.5.

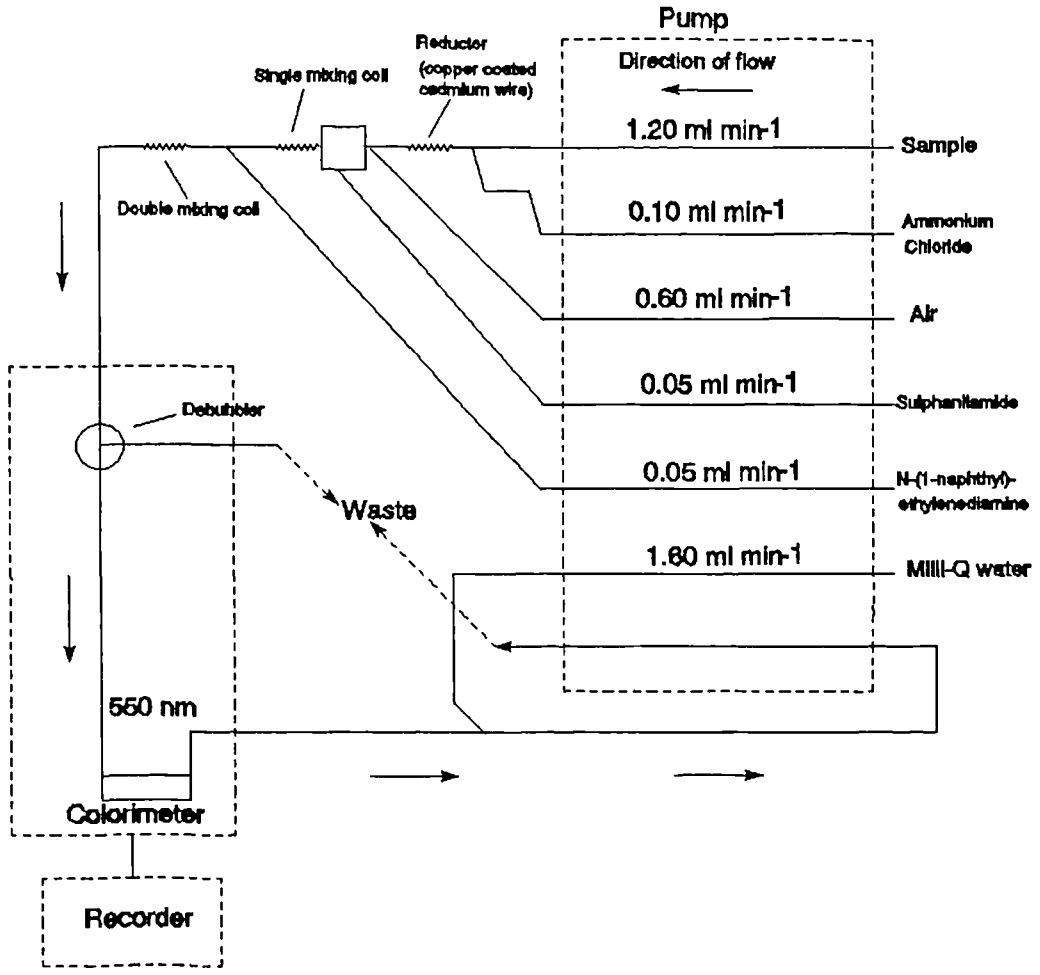
(1) **The sampler**: This consisted of a rotating platform holding up to forty sample cups and operated in a sequential manner via a stainless steel tube which drew the sample from a plastic cup (8 ml capacity). After sampling (60 seconds), the sampling tube was withdrawn and moved into a reservoir containing the wash solution (Milli-Q water). The wash was drawn (60 seconds), and the sampling tray was then rotated to allow the next sample to be taken. Sampling and wash times were controlled electronically (120 seconds maximum).

(2) **The proportioning pump**: The stream flow in the system was driven by a powerful peristaltic pump with rotating rollers. These moved at a constant speed over a bed along which plastic tubes were laid and held firmly. These tubes carried the sample (or wash) and reagents, the flow rate being determined by the internal diameter of the tubes (0.15 to 3.90 ml/min.).

(3) **The manifold**: This was made up of tubings, connectors and coils which carried the main stream and the various branches. The sample stream was segmented by air using a special connector (this occurred after the stream had passed through the

reduction column during nitrate determination). The reagent streams were then injected into the main segmented stream using **'h'** connectors. After each such addition the combined stream was passed through a coiled glass mixing tube of 2 mm i.d. (the coils were ca 2.5 cm in diameter and had between 10 and 20 turns). All reagent flows were combined with the main stream in this manner and in the order dictated by the analytical procedure. The output from the manifold was then fed to a simple double beam filter-**colorimeter** (4). This employed a flow-through cell of a capacity of less than 0.2 ml and a light path of 15 mm. The output was recorded using a chart recorder. A pumping tube was incorporated into the manifold design and arranged so that it drew the solution into the flow cell. This tube was chosen to give the highest flow rate through the cell while excluding air from the flow. The recorder output was a series of peaks representing the samples in the order in which they were sampled. The system was optimised to give high resolution of the analytical peaks. Samples were measured at 550 nm.

Figure 2.5. Diagram of auto analyser (including NO_3^- reduction column), used for the determination of NO_2^- and NO_3^- in porewater samples.



Section 2.5 DETERMINATION OF INORGANIC NITROGEN

2.5.1 Determination of nitrite

Pore water nitrite was measured according to the method Parsons *et al.* (1984). Hence, nitrite was reacted with *p*-aminobenzenesulphonamide (sulphanilamide) in acid solution. The resulting diazo compound was reacted with N-(1-naphthyl)-1,2-diaminoethane to form a highly coloured azo dye.

2.5.2 Preparation of reagents for nitrite analysis

Sulphanilamide (5 g) was dissolved in a mixture of concentrated hydrochloric acid (50 ml) and milli-Q water (300 ml). This solution was diluted with Milli-Q water (500 ml). The reagent was stable for many months.

N-(1-naphthyl)-1,2-diaminoethane dihydrochloride (0.5 g) was dissolved in Milli-Q water (500 ml). The solution was stored in a dark glass bottle and renewed monthly or whenever a strong brown colouration was seen to develop.

Sodium nitrite (0.425 g) was dried (110°C, 1 h), dissolved in Milli-Q water and diluted (500 ml). The solution was stored in a dark, glass bottle and chloroform (1 ml) was added as a preservative. This solution was stable for several months.

2.5.3 Experimental procedure for the determination of nitrite

Interstitial water samples (ca 1 ml) were diluted to 10 ml and placed in the sampling tray of the auto-analyser. Sampling time was 60 seconds (see Section 2.3.4). Sulphanilamide was then added (0.05 ml min^{-1}) and the sample passed through a glass mixing coil (10 turns). N-(1-naphthyl)-1,2-diaminoethane was then added (0.05 ml min^{-1}) and the sample passed through a second mixing coil (20 turns). The sample then passed into a colourimeter (see Section 2.3.4) and was measured in a cell (1 cm, 550 nm). Data were recorded on a chart recorder (See Figure 2.5).

2.5.4 Blank determination

The blank was measured by sampling Milli-Q water (10 ml) as described in Section 2.4.3. The resultant peak height was subtracted from all samples.

2.5.5 Nitrite calibration

Standardization was carried out in triplicate using aliquots of sodium nitrite solution (10 ml, 1 x 10^{-6} M) as described in Section 2.73. After deduction of the reagent blank **F** was calculated from equation 2.2:

$$F = 1 \times 10^{-4} / E_s \qquad (2.3)$$

where E_s is the mean extinction of standard measurements, corrected for the blank.

2.5.6 Determination of nitrate

Dissolved nitrate was determined using the method of Parsons *et al.* (1984) with some minor modifications. Nitrate was reduced to nitrite by passing the sample through a column containing cadmium wire coated with metallic copper. The resulting nitrite was determined as in Section 2.4.3. Any nitrite initially present in the sample was corrected for.

2.5.7 Preparation of reagents for nitrate analysis

Ammonium chloride. Stock ammonium chloride (125 g) was dissolved in Milli-Q water, made up to 500 ml and stored in a glass bottle.

Dilute ammonium chloride was prepared from concentrated ammonium chloride solution (25 ml), which was mixed with Milli-Q water, diluted to 1 l and stored in a glass bottle.

Reduction column. Cadmium wire (20 cm) was placed in a plastic tube which was then coiled to ensure good contact with the sample. A small plug of glass wool was placed at each end, and the tube was connected to the auto analyser manifold system. A 2% w/v solution of copper sulphate was then passed through until the blue colour was dissipated. The cadmium wire was immersed in solution at all times to prevent atmospheric oxidation of the copper. The column was washed thoroughly with dilute ammonium chloride (this was added constantly during nitrate analysis). The wire was reactivated periodically after continued use (as judged from a decrease in the F value obtained for the standard), by washing with 5% w/v HCl, followed

by Milli-Q water, until the pH of the decanted solution was > 5. The wire was then reactivated with copper sulphate as described above. Conversion efficiency of nitrate to nitrite did not fall below 84%.

2.5.8 Experimental procedure for nitrate analysis

Samples (5 ml) were transferred to the cups of the auto-analyser and placed on the sample tray. Sampling (60 seconds) was carried out and ammonium chloride added before the sample was pumped through the reduction column. After passing through the reduction column, the sample stream was segmented with air bubbles and sulphanilamide solution was added. The sample stream was then passed through a mixing coil (10 turns) after which N-(1-naphthyl)-ethylenediamine was added before mixing in another coil (20 turns). After debubbling, the sample was passed through the flow cell of the colourimeter and the resulting azo compound then determined as described in section 2.3.6.

2.5.9 Blank determination

A blank sample of milli-Q water was passed through the column, its absorbance measured, and this value subtracted from all samples.

Section 2.6: AMINOPROPANONE

2.6.1 Synthesis of Aminopropanone

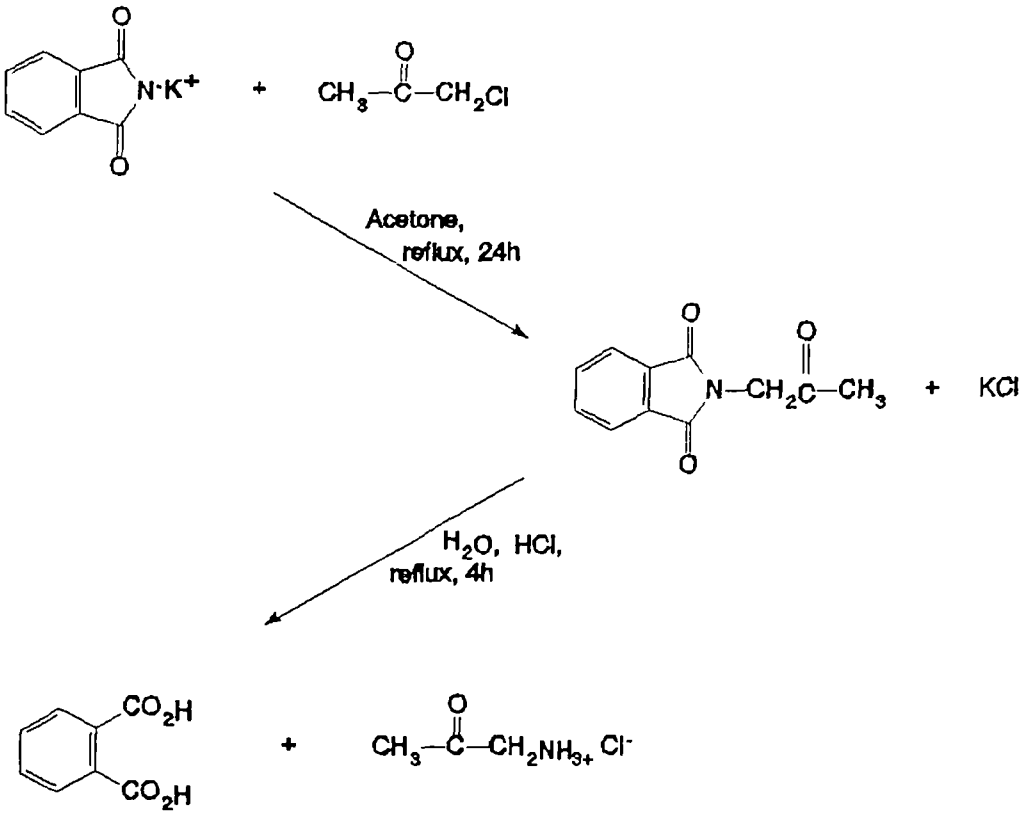
Aminopropanone was not commercially available, and was synthesised using a method adapted from Ellinger and Goldberg (1949) as shown in Figure 2.6.

Distilled chloroacetone was refluxed with potassium phthalimide in dry acetone (Analar, 24 hours). The solid product was then removed by filtration, dissolved in boiling ethanol, and filtered to remove any unreacted inorganic material. The filtrate was brought to the boiling point, and activated charcoal added. After removal of the charcoal, the filtrate was again heated to boiling point and water (100 °C) added. The solution was allowed to cool and phthalimidoacetone was separated as white crystals: **m.p. (118-120°C); ^{1}H nmr: δ 2.25 (3H, singlet, -CH_3), δ 4.5 (2H, singlet, -CH_2), δ 7.8 (4H doublet, -C_6H_4).**

Phthalimidoacetone was refluxed with hydrochloric acid (7 M; 2 h) in an oil bath. The solution was then allowed to cool in order that the resulting phthalic acid could crystallise, and was then refluxed for another 2 hours (if the solution was not cooled after the initial heating it was invariably found that the phthalic acid crystallised at the b.p. with considerable violence). The reaction was then cooled, phthalic acid removed and the filtrate evaporated to complete dryness using a rotary evaporator (40-50°C). The residue was heated in ethanol and alcoholic hydrogen chloride (3M). The insoluble ammonium chloride was rapidly filtered off and washed with boiling ethanol, the washings being combined with the filtrate which was rotary evaporated to dryness at 50°C. Aminopropanone hydrochloride was then recrystallised from ethanol and anhydrous ether (yellow white crystals). Melting pt. (77-80°C) and ^{1}H-nmr measurements were made to check on the compounds identity:

m.p. (77-80°C); ^{1}H-NMR: δ 2.25 (3H, singlet, -CH_3), δ 4.1 (2H, singlet, -CH_2), -NH_2 protons were unresolved. The nmr spectrum of APR is shown in Figure 2.7.

Figure 2.6 Reaction scheme showing the pathway by which 1-aminopropan-2-one was synthesised.



2.6.2 Microdiffusion of aminopropanone

Microdiffusion of aminopropanone was carried out as for the MAs (Section 2.3.4). However, during calibration with standard solutions of APR, it was found that the reproducibility the method was compromised by the formation of an artefact compound with a retention time which was almost double that of APR. Quantitative formation of this compound was controlled by lowering the injector temperature to 150 °C and injecting samples at neutral pH.

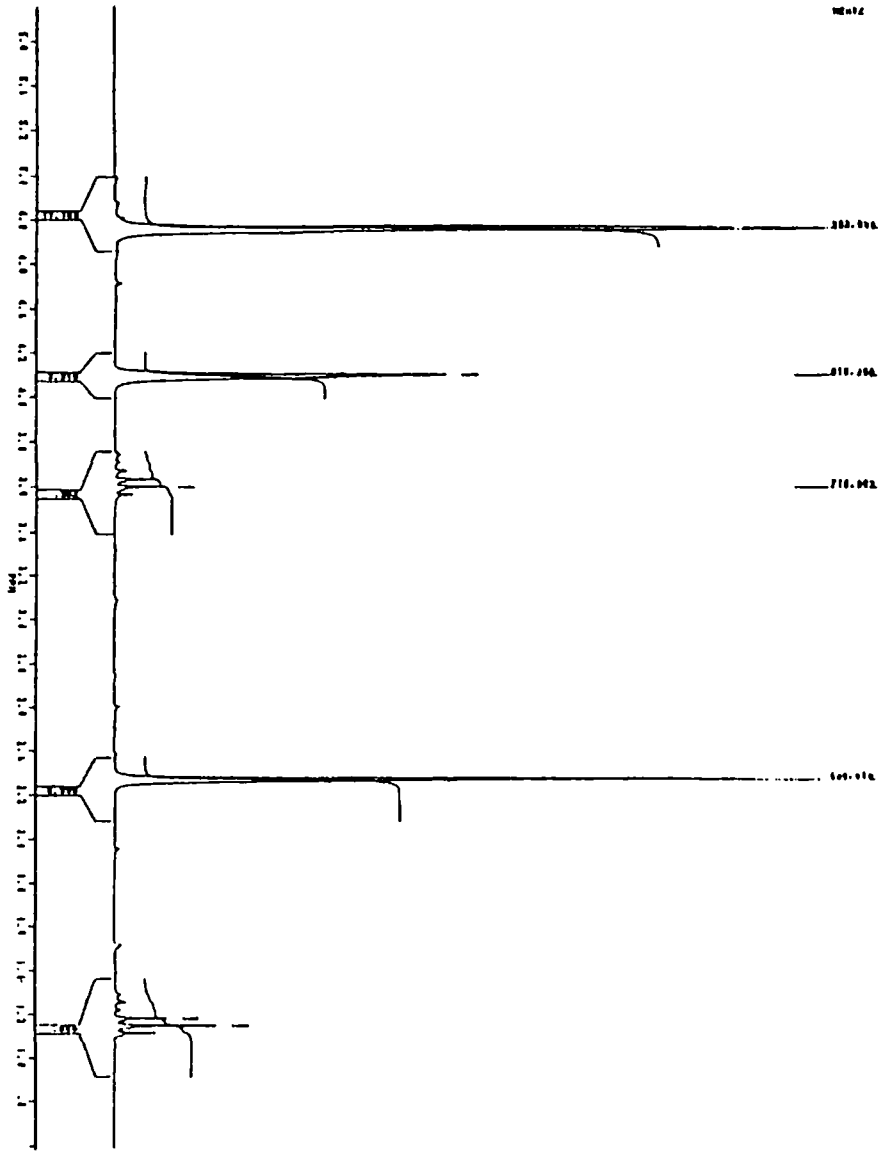


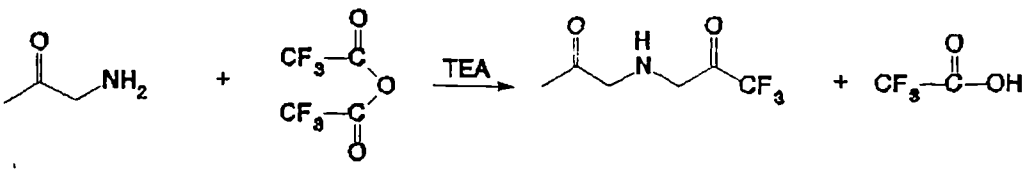
Figure 2.7. NMR of APR hydrochloride

2.6.3 GC-MS analysis of aminopropamone

A microdiffused sample of APR was adjusted to pH 12 with NaOH solution (50 μl, 0.5 M) and solvent extracted with hexane (500 μl). The extracted solvent was then acylated with trifluoroacetic anhydride (TFAA) according to the Pierce Handbook and General Catalogue (1989) as shown in Fig. 2.8.

The hexane extract (500 μl) was first mixed with triethylamine (0.05 M) in hexane (100 μl) and TFAA (100 μl) in glass reaction vials. The capped vials were heated in a fan-assisted oven (50 °C, 15 mins.). The vials were then cooled and Milli-Q water added to the sample (1.0 ml) with shaking for one minute. Aqueous ammonia (5 %, 1.0 ml) was then added with shaking (5 mins.). The solution was then centrifuged and the solvent phase injected onto the GC-MS system (1.0 μl).

Figure 2.8 reaction scheme for the derivatization of APR with TFAA.



2.6.4 Operating conditions

GC-MS analysis was performed using a Hewlett Packard 5970 Series Mass Selective Detector connected to a Hewlett Packard 5890 GC fitted with a HP-1 capillary column (12 m) and on-column injection.

The oven was programmed from an initial temperature of 30 °C (1.0 min) to 100 °C (10 mins.) at a rate of 3 °C min^{-1}.

The mass spectrum of APR is discussed in Chapter 6 (Figure 6.3).

CHAPTER 3

DEVELOPMENT OF THE ANALYTICAL METHOD

Section 3.1: INTRODUCTION

The methylamines (MAs) are methylated analogues of ammonia. A review of their occurrence in the environment can be found in Chapter 1. MAs are gases at room temperature and can be isolated as the solid hydrochloride salts. The lone pair of electrons on the nitrogen enables the free MAs to act as Lewis acids in aqueous solution (equation 3.1) which gives rise to their high solubility (see Table 3.1 for a list of physical properties).

$$CH_2—NH_2 \quad + \quad H_2O \longrightarrow \quad CH_2—NH_{3+} \quad + \quad OH^- \qquad (3.1)$$

The volatility and basicity of the MAs can cause problems in their analysis. However, these properties can also be exploited in analytical methods for their determination (Lee and Olsen, 1984; Abdul-Rashid, 1990; Wang and Lee, 1990; Abdul-Rashid *et al.*, 1991). Concentrations of the MAs in environmental samples are relatively low, and generally, it is necessary to concentrate them prior to analysis. For this reason, most methods which have been used for their determination involve a pre-concentration step (see below in Section 3.2). A variety of techniques have been employed in the determination of MAs, including high performance liquid chromatography (HPLC), gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). These are reviewed in Section 3.3.

Table 3.1 Physical properties of ammonia and the MAs

Name	Formula	Bp (°C)	Solubility	K_b	pK_b
Ammonia	NH_3	-33.35	89.9/100 g	1.79×10^{-5}	4.75
Monomethylamine	CH_3NH_2	-7.5	∞	45×10^{-5}	3.34
Dimethylamine	$(CH_3)_2NH$	7.5	∞	54×10^{-5}	3.27
Trimethylamine	$(CH_3)_3N$	3	∞	6.5×10^{-5}	4.19

Section 3.2: PRE-CONCENTRATION

3.2.1 Adsorbents

Sep Pak Cartridges

Sep-Pak C_{18} (octadecyl silyl-bonded silica) cartridges (SPC) have been employed widely for the pre-concentration of volatile amines from environmental samples. Kuwata et al. (1983) concentrated low molecular weight aliphatic amines using an SPC coated with 0.3 % phosphoric acid. A mixture of the amines (6 μg) was spiked onto the first of two cartridges in series and pure air (50-150 l, at 0.5-1.5 l/min) was passed through the system. Most of the amines were recovered from the first cartridge (87.0-93.4 %) indicating a good trapping efficiency. The adsorbed amines were recovered by elution with a mixture of ethanol and water (1:1 v/v, 1.5 ml) and analysed by GC.

Nishikawa and Kuwata (1984) used a similar method for the determination of C_1-C_4 amines. Ammonia and the amines in methanol were injected onto an SPC with a microsyringe, and pure nitrogen (5 l) was passed through the cartridge. The analytes were extracted with methanol and analyzed by HPLC as their derivatives with 7-Chloro-4-nitro-2,1,3-benzoxadiazole. Detection limits for MMA and DMA were 0.1 and 0.6 pmol respectively and recoveries were between 87 and 92 %. Similar methods have been developed for sampling MAs in the marine atmosphere (e.g. Van Neste et al., 1987). Sampled air was pumped through in-line glass fibre filters (efficiency > 99 % for particles > 0.01 μm), and then through SPCs in order to collect the gaseous amines (Kuwata et al., 1983; Nishikawa and Kuwata, 1984).

SPCs have also been used for the pre-concentration of volatile amines from aqueous samples. For example, da Costa et al. (1990) used SP cartridges for the pre-concentration of amines in rat urine samples, and achieved recoveries of 92-94 % (DMA and TMA only, MMA was not measured), although no details of the trapping solvent were reported.

Scully et al. (1988) estimated organic nitrogen in municipal waste water, by trapping amines and ammonia as copper complexes in pre-treated cartridges fitted to wash bottles. The latter contained effluent (150 ml), KOH (1 g) and KCl (50 g), and were purged with helium (2 h) until the cartridge was saturated (this was indicated by a colour change in the cartridge packing from green to blue). The amines were then eluted with KOH (1M) before saturation with KCl and extraction into benzene for GC analysis.

Activated Charcoal

Fuselli et al. (1982) compared activated charcoal traps with other solid adsorbents used for trapping amines (e.g. Carbopak B, Tenax GC). Standard solutions of MAs (in ether) were injected onto the traps, which were purged with known volumes of high purity N_2. Recoveries of the MAs were between 68 and 82 % for DMA and TMA respectively, which were much better than for the other adsorbents (Tenax GC 60/80 mesh and 60/80 Carbopack B).

3.2.2 Distillation

A refined distillation technique has been developed by Lee and Olsen (1984), who distilled amines from aqueous samples (20 ml) in an incubation flask. In their

determinations, a filter-paper wick, saturated with HCl was placed in a sealed flask, which was evacuated using a syringe through a septum stopper, the aqueous solutions having been treated with KOH to adjust the solution to pH 12. After gentle shaking (4 h, 60 °C), the wick was removed and washed with acid. The acid was evaporated to dryness and the residue was taken up in KOH for injection onto the GC.

A complex vacuum distillation procedure was also employed by Pons et al. (1985) for the determination of amines (including the MAs). They distilled a basic solution to dryness (35 °C, 15-20 Torr), the amines being trapped in flasks containing 0.1 M HCl. The acidic solutions were then pooled, evaporated and the residue of the amine salts dried in an oven (100 °C, 2 h). The salts were then dissolved in redistilled water and were introduced to a 35 ml flask which was evacuated using a vacuum pump. NaOH (1 ml, 6 M) was then added and the flask heated at 50°C (15 min.) before a headspace sample (5 ml) was taken and injected onto a GC.

Terashi et al. (1990) distilled aqueous samples (500 ml, pH 10) under vacuum. Recoveries using this method - which also included an extraction step - varied according to the type of sample (e.g. river water, sea water or sea sediment), but did not fall below 68 %.

3.2.3 Diffusion

Recently, a diffusion technique for the determination of volatile amines at nanomolar concentrations in seawater has been developed (Yang et al., 1993). Seawater samples (500-1000 ml) were treated with KOH solution to deprotonate the

amines. After separation of the resulting $Mg(OH)_2$ precipitate, the sample was circulated past a teflon membrane using a peristaltic pump. The free amines were concentrated through the membrane (24 h, 55° C) from a large volume of sample (pH > 12) into sulphuric acid (55 ml, 0.05 M) with added NaCl (2-4 %) to equalize the osmotic pressure gradient across the membrane. A static diffusion step was also necessary to further concentrate the solution and remove salts prior to analysis by GC. Recoveries for the MAs using this method were claimed to be 97.0-99.0 % at a concentration range of 2.5-2.9 μM. The detection limit for 500 ml of sample was 10 nM. For concentrations down to 5 nM, it was necessary to use 1 l of sample.

Another circulation diffusion method has been developed by Gibb et al. (unpublished results) for analysis of AMM and the MAs in aqueous solution. Filtered samples are introduced into a flow injection system, and treated with a mixed reagent of NaOH (deprotonation of the analytes) and EDTA (chelation of Mg^{2+} and Ca^{2+}) prior to diffusion across a gas permeable teflon membrane and into an acidic acceptor stream (HCl), where the MAs are protonated before subsequent analysis in an ion chromatograph.

Table 3.2 summarises the recoveries and detection limits (where available) of the methods discussed above.

Table 3.2 Pre-concentration of volatile amines

Pre-concentration step	Recovery (%)	Detection limit	Authors
Activated Charcoal			
	68.0-82.0	0.005 ppmv^{+}	Fuselli et al. (1982)
Cartridges			
SP Cartridges	87.0-93.4	0.02-0.05 ng	Kuwata et al. (1983)
	87-92	0.1-24 pmol	Nishikawa and Kuwata (1984)
	95-103*	1 pmol	Da Costa et al. (1990)
Chromosorb W (60/80) with $CuCl_2$ coating	8-53	0.9-5.2 ppb	Scully et al. (1988)
Distillation			
	62-65*	~15 ng	Lee and Olsen (1984)
	68.4-98.8	0.01-2 μg/l (water) 0.5-100 μg/kg (sediment)	Terashi et al. (1990)
Diffusion			
Teflon membrane	> 96.7	5-10 nM	Yang et al. (1993)

* DMA and TMA only

$^{+}$ Concentrations in air (30 l)

Section 3.3: ANALYTICAL DETERMINATION OF MAs

3.3.1 Colorimetry

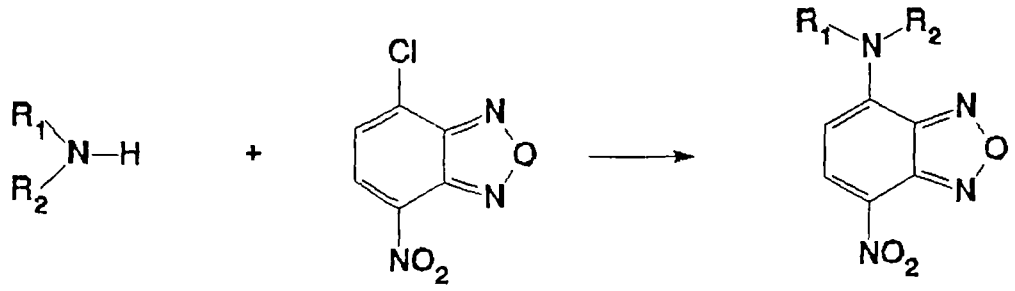
One of the earliest reported analytical techniques for the determination of the MAs and other low molecular weight amines was a colorimetric method described by Bystedt et al. (1959). Trimethylamine oxide (TMAO) was reduced to TMA using titanous chloride in neutral solution and the concentrations estimated colorimetrically as the picrate complex. Recoveries of TMAO were around 100 %.

3.3.2 High Performance Liquid Chromatography

The vast improvement in analytical instrumentation in recent years has influenced the search for a quick, efficient and simultaneous determination of the MAs. HPLC has been successfully employed for analysis of amino acids (e.g. by Lindroth and Mopper, 1979; Jones et al., 1981; Mopper and Lindroth, 1982; Burdige and Martens, 1990; Alberts et al., 1992). Amino acids (AAs) are generally derivatized with a fluorogenic reagent prior to analysis by HPLC. Fluorescent derivatives allow detection at the picomolar level (Lindroth and Mopper, 1979; Mopper and Lindroth, 1982).

Similar techniques have been applied to the determination of primary and secondary amines. For example, Nishikawa and Kuwata (1984) determined C_1-C_4 amines (including MMA and DMA) after derivativization with 7-Chloro-4-nitro-2,1,3-benzoxadiazole using reversed-phase HPLC (Figure 3.1).

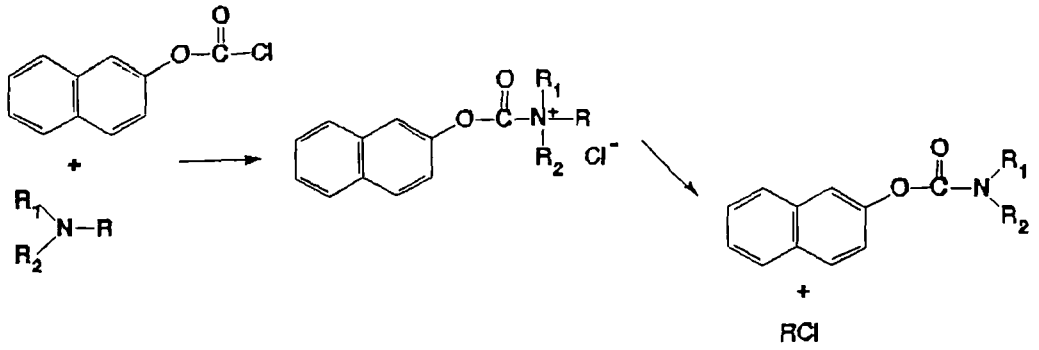
Figure 3.1 Reaction scheme for the derivatization of primary and secondary amines using 7-Chloro-4-nitro-2,1,3-benzoxadiazole (NBD-Cl) after Nishkawa and Kuwata (1984).



Chen and Farquharson (1979) derivatized ammonia and ethylenediamine with m-toluoyl chloride in NaOH (1 M) and followed this with extraction into dichloromethane. The samples were then washed with K_2CO_3 and water (10%) before analysis by HPLC. Phenyl isocyanate has also been employed for the determination of primary and secondary amines, by formation of the N,N-disubstituted ureas (Bjorkquist, 1981). The derivatives were chromatographed on a reversed-phase system with no pH suppression. UV monitoring (240-260 nm) allowed detection of the amines at the 1-10 ng level.

A similar method using phenyl isocyanate together with 1-naphthyl isocyanate as derivatization agents, to form disubstituted ureas has been used in the analysis of aliphatic amines in air samples (Andersson et al., 1984). Recoveries of the amines (namely n-propylamine, n-butylamine and allylamine) were between 87 and 100 % depending on the amounts added.

Figure 3.2 Scheme for the reaction of 2-naphthylchloroformate (NCF) with tertiary amines after Gubitz et al. (1981).



Neiderhauser and Fuller (1982) determined diethylamine (DEA, a secondary amine) in urine by derivatization with 3,5-dinitrobenzene and extraction into diethyl ether. The efficiency of this derivatization was reported to be greater than 90% (Neiderhauser et al., 1976). The residue from the benzoylation of urine was dissolved in ethanol and an aliquot (15 μl) was analysed by HPLC with UV detection (254 nm).

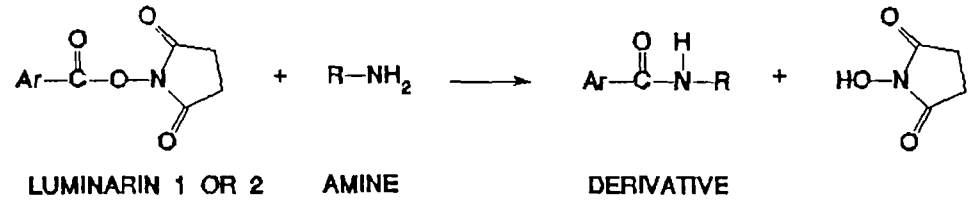
Luminarins are one of the unusual reagents which have been employed in the derivatization of primary and secondary amines (Tod et al., 1992; Fig. 3.3). These compounds had a quinolizinocoumarin structure and an N-hydroxysuccinimide ester reactive function. They react with primary and secondary amines under relatively mild conditions (50-80°C, 20-180 min.) without a catalyst and yield derivatives that are both fluorescent and chemiluminescent.

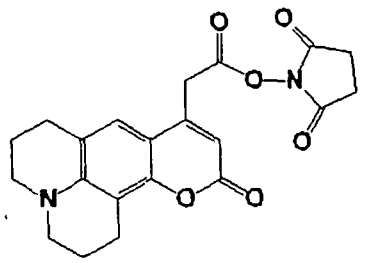
Derivatization reactions of this type are only useful for primary and secondary amines. However, Gubitz et al. (1981) adapted an elegant method for the

analysis of tertiary amines, which reacted with 2-naphthylchloroformate (NCF) in benzene to produce a quaternary amine derivative, which underwent dealkylation on heating (Figure 3.2) to yield a carbamate. The resulting tertiary amine was determined by HPLC using fluorescence detection (λ_{ex} 275 nm and λ_{em} 335 nm).

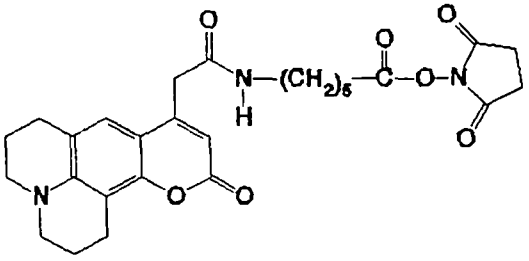
The MAs have also been determined by HPLC with electrochemical (EC) detection (or amperometric detection). Simple EC detector cells are available which contain only two electrodes (i.e. a working and a reference electrode). A pre-selected potential equal to, or greater than the half-wave potential of interest is applied constantly across the electrodes. As an electrochemically active species passes through the cell it is oxidised or reduced and the current generated in the flow cell is continually monitored. However, two electrode systems give a non-linear response as the voltage varies across the eluent with current flow changes. Thus electrochemical detectors now typically employ a three electrode cell. The additional electrode, which is known as the auxiliary or counter electrode, serves to carry any current generated in the flow cell, thus enabling the reference electrode to have a fixed potential (Braithwaite and Smith, 1985). Maruyama and Nagayoshi (1992) were able to measure volatile amines including DMA and TMA using this method. The three-electrode cell included a glassy carbon working electrode, platinum auxiliary electrode and Ag/AgCl reference electrode. Acetonitrile-phosphate buffer was employed as the mobile phase and ammonium acetate was added as a competing base to improve peak retention and shape. Peak potentials for DMA and TMA were 1280 and 1100 (mV *vs* Ag/AgCl) respectively. The method gave a linear response over the range 3-30 ng with a minimum detection limit of a few ng.

Figure 3.3: Reaction scheme showing the derivatization of amines by luminarins 1 and 2. After Tod et al. (1992)





LUMINARIN 1



LUMINARIN 2

Table 3.3: Derivatization agents used in the preparation of MAs for analysis by HPLC and GC-MS.

Reagent	Application	Source
Phenyl isocyanate	MMA, DMA	Bjorkvist (1981)
2-Naphthyl chloroformate	TMA	Gubitz et al. (1981)
3,5-Dinitrobenzene	MMA, DMA	Neiderhiser and Fuller (1982)
NBD-Cl	MMA, DMA	Nishikawa and Kuwata (1984)
1-Naphthyl isocyanate	MMA, DMA	Andersson et al. (1984)
Benzenesulphonyl chloride	MMA, DMA	Terashi et al. (1990)
p-Toluenesulphonyl chloride	DMA	da Costa et al. (1990)
2,2,2-Trichloroethyl chloroformate2	TMA	da Costa et al. (1990)d
Luminarin 1	MMA, DMA	Tod et al. (1992)
Luminarin 2	MMA, DMA	Tod et al. (1992)

3.3.4 Gas Chromatography

The most commonly employed method for the determination of volatile amines in environmental and biological samples is gas chromatography (GC; Di Corcia and Samperi, 1974; Dunn et al., 1976; Ohshima and Kawabata, 1978; Hippe et al., 1979; Dalene et al., 1981; Fuselli et al., 1982; Kuwata et al., 1983; Lowis et al., 1983; Lee and Olsen, 1984; Perez Martin et al., 1987; Glob and Sorensen, 1987; Scully et al., 1988; Lundh et al., 1991, Abdul-Rashid et al., 1991). It offers an easy and rapid technique for the determination of volatile amine compounds, and the use of a Nitrogen-Phosphorus detector (NPD) gives good sensitivity and specificity for nitrogenous compounds (Table 2.3). An important limitation of the use of GC is that amines are readily adsorbed onto glass columns which can lead to severe tailing of chromatographic peaks (Burchfield and Storrs, 1962), poor reproducibility (Dalene et al., 1981) and occasionally to total loss of the analyte (Casserlman and Barnard, 1974). The resolution of volatile amines by GC has been considerably improved by the use of low reactivity column packings. For example, satisfactory results have been obtained with cross-linked polystyrene material, typified by Chromosorb 103 (Casselman and Barnard, 1974; Abdul-Rashid, 1990; Abdul-Rashid et al., 1991), which was developed specifically for the GC separation of basic volatiles such as AAs (Dave, 1969). Not all workers have found this stationary phase to be adequate, however. Dunn et al. (1976) tested a number of column packings for resolution of the MAs and reported that Chromosorb 103 was difficult to pack and condition, and gave poor reproducibility. They found that columns packed with Chromosorb W containing 10 % amine 220 + 10 % KOH were most effective for the analysis of MAs in the concentration range of ng/μl.

Di Corcia et al. (1972) determined aliphatic amines in aqueous solution at the ppm level using two graphitized carbon blacks as column packings-namely graphitized sterling FT (Sterling FT-G) and Vulcan. These materials were supplied as Carbopak A and Carbopak B respectively. Three columns were packed as follows:

1) Sterling FT-G + KOH + 1.3 % PEG-20 M

2) Sterling FT-G + 0.2 % KOH + 0.5 % PEG-1500

3) Vulcan + 0.8 % KOH + 4% PEG-20 M.

KOH was added to neutralize any polar adsorbing sites on the column and was successful in improving column performance although tailing of chromatographic peaks was still evident. Tailing was fully suppressed by further addition of an appropriate amount of an organic compound with a high boiling point and medium polarity such as PEG-20 M (polyethylene glycol). Samples were analysed on a Carlo Erba GC equipped with a FI detector.

Fuselli et al. (1982) used GC with NP detection for analysis of the MAs (detection limit 50 pg). The column packing consisted of 60/80 Carbopak B/4% Carbowax 20 M/ 0.8% KOH. Kuwata et al. (1983) determined low molecular weight aliphatic amines using a glass column packed with GHP-1 (60-80 mesh) which is a spherically-shaped porous styrenedivinylbenzene copolymer. The phase was treated with KOH/methanol and, prior to use, KOH (10 μl) was injected at 170°C. NP detection was employed. Similar columns have been used by Lowis et al., 1983 for the determination of the MAs in the head space gas of biological samples, and by Lee and Olsen (1984) who also used a KOH impregnated column, the MAs being separated on a column packed with Carbopak B, which was coated

with Carbowax 20 M and KOH. The GC was equipped with an Antek 705 chemiluminescent detector (see also Van Neste et al., 1987). This chemilumescent technique was selective for highly volatile organic nitrogen compounds (VONs). The VONs were eluted from the GC column at low temperature, burned at 1100°C to NO, and reacted with ozone to form NO_2. As this excited molecule decayed, light was emitted and measured with a photomultiplier.

While determining volatile amines in seafoods, Perez Martin et al. (1987) assessed a number of column packings including Chromosorb 103 and encountered problems similar to those reported by Dunn et al. (1976). They eventually employed a column packed with 150 cm of 4 % Carbowax 20 M + 0.8 % KOH on Carbopak B, and 25 cm of untreated 80-100 mesh Chromosorb 103. The latter ensured that elution of the benzene solvent peak did not interfere with that of the internal standard. The column was conditioned overnight (220°C) and deionized water (10 μl) was injected several times to convert any K_2CO_3 to KOH (see Fuselli et al., 1982), and to flush out any traces of amines adsorbed around the injection port.

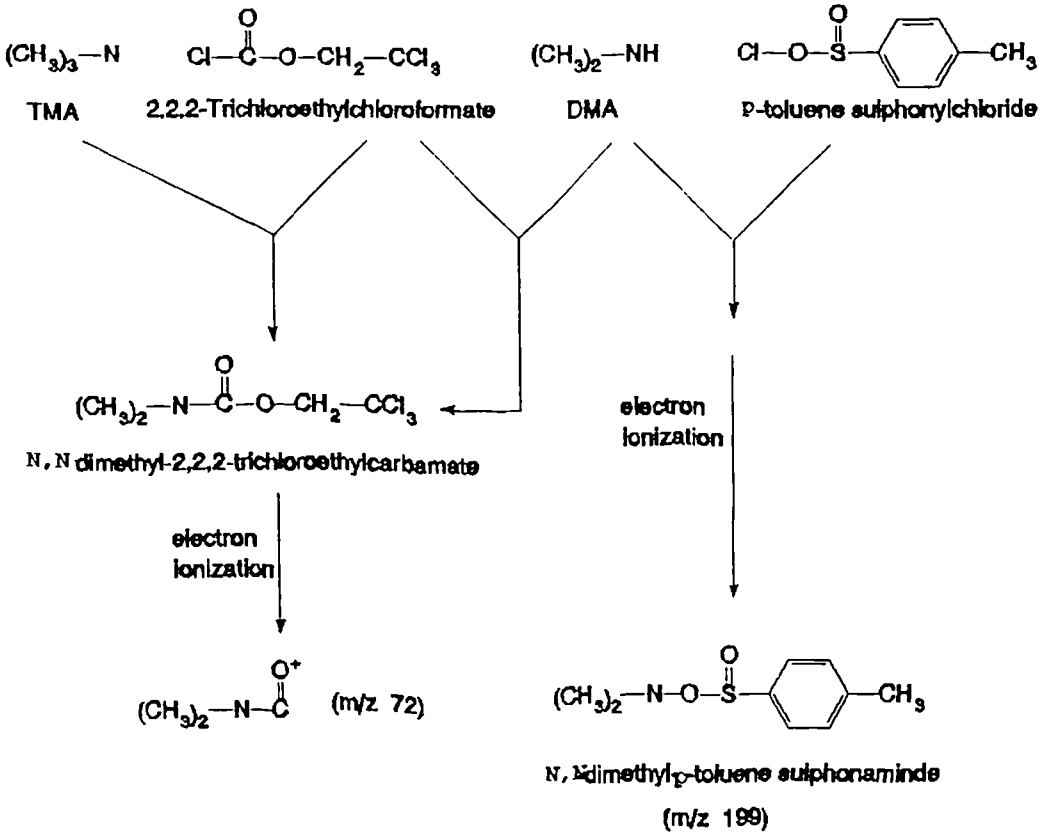
Although capillary columns have rarely been used in the analysis of volatile amines by GC, Scully et al. (1988) analysed volatile amines in wastewater using an SP-5 fused silica capillary column. However, a derivatization step was required, which involved the extraction of the amines and their derivatization with heptafluorobutyric anhydride (HFBA) prior to injection onto the GC.

3.3.5 Gas Chromatography-Mass Spectroscopy

GC coupled with mass spectroscopy (GC-MS) has been employed for the determination of amines in order to improve on the sensitivity of previous GC techniques. Pons et al. (1985) used packed column (4.8% PEG 20 M + 0.3 % KOH on 100-120 mesh Carbopak B) GC-MS for the determination of volatile amines in bacterial growth media. However, no recoveries or detection limits were reported. Terashi et al. (1990) determined primary and secondary aliphatic amines as the sulphonamide derivatives by capillary column GC-MS (SE- 54, 25 m x 53 mm I.D.) with selective ion monitoring. Detection limits were claimed to be 0.1-35 nM and 8-77 nmol/kg in water and sediment respectively.

The unsuitability of tertiary amines for derivatization was overcome by da Costa et al. (1990), who reacted TMA with 2,2,2- trichloroethylchloroformate to form the carbamate derivative of TMA (Figure 3.2, cf. Gubitz et al., 1981), while DMA was reacted with *p*-toluenesulphonyl chloride to form N,N-dimethyl-*p*-toluenesulphonamide. Capillary GC-MS with selective ion monitoring was employed. Recoveries of DMA, TMA and TMAO were between 93-103 %, and a detection limit of 1 pmol for each analyte was reported.

Figure 3.4: Reaction scheme showing the derivatization of DMA and TMA to form N,N-dimethyl-*p*-toluene sulphonamide and N,N-dimethyl-2,2,2-trichloroethylcarbamate. After da Costa et al. (1990).



Section 3.4: PRESENT STUDY

In summary, the volatility and basicity of the MAs can cause problems in their analysis, but their properties have also been used in the development of methods for amine analysis. A pre-concentration step is usually necessary prior to analysis. Some methods require a number of pre-concentration steps (Lee and Olsen,

1984; Scully et al., 1988) which can seriously reduce the recovery of the analytes.

Analyses of the MAs by HPLC and GC-MS both require a derivatization step which may not be suitable for tertiary amines (Nisikawa and Kuwata, 1984). Gubitz et al. (1981) used 2-Naphthylchloroformate (NCF) as a fluorogenic agent for the derivatization of drugs containing a tertiary amino group, but did not apply this method to volatile tertiary amines such as TMA. DaCosta et al. (1990) were able to form derivatives for DMA and TMA but found it necessary to use two separate reagents for their simultaneous determination on GC-MS.

GC is the most widely reported technique for the analysis of volatile amines. The volatility of the MAs has made it difficult to analyse these compounds on capillary columns without prior derivatization. For this reason, most workers have determined the free MAs on glass columns packed with a variety of materials. The use of special low reactivity column packings such as Chromosorb 103, has helped to eradicate problems such as peak tailing and poor reproducibility as reported in Section 3.3. (Burchfield and Storrs, 1962; Dalene et al., 1981).

In the present study, a cheap, simple method for analysis of ammonia and the MAs is reported. Only one pre-concentration step is required using a microdiffusion process which can be applied to any number of samples depending on oven space. The GC contains a glass column packed with untreated Chromosorb 103 (60-80 mesh) and an NP detector. A large number of samples can be analysed in a short space of time, and the column has been shown to be stable over a long period (over 20 000 injections), requiring only occasional repacking.

Section 3.5: PRESENT METHOD

3.5.1 Introduction

The method employed in this work was first developed in these laboratories (Abdul-Rashid, 1990; Abdul-Rashid et al., 1991). Although this is a straightforward procedure consisting of a simple pre-concentration step by microdiffusion (Riley and Sinhaseni, 1957) followed by analysis by packed column GC, it was found that the initial work by Abdul-Rashid (1990) required a considerable amount of refinement. The refined method is described below.

3.5.2 Microdiffusion

The major variable of this method is the microdiffusion step. Riley and Sinhaseni (1957) used a microdiffusion pre-concentration step for the determination of ammonia in seawater samples, and it was felt that this method might also be successfully employed for the determination of the MAs in aqueous solution (ammonia was also determined by microdiffusion in the present work).

Optimisation of the temperature and duration of the microdiffusion process were crucial in obtaining successful recoveries of the MAs. A balance had to be struck between reasonable recovery and the possibility of evaporation or loss of volatiles to the surrounding environment. It was also important to choose a timescale which made it possible to process large numbers of environmental samples. A series of time-related microdiffusion experiments (Abdul-Rashid et al., 1991), indicated that a 24 hour incubation offered the best compromise between the factors discussed above.

The ease and speed with which samples were pre-concentrated by microdiffusion compared favourably with the pre-concentration/derivatization methods reported by other workers. Scully et al. (1988) employed 8 preparation steps in the sample work-up procedure. The range of recoveries shown in Table 3.2 show that this diminished the percentage recovery of some compounds (Pyrrolidine 8 $\pm$ 2%) relative to others (isoamylamine 53 $\pm$ 1%). They reported that the greatest losses of amines occurred in the purge and trap systems, and from the XAD-2 and Dowex columns. Lee and Olsen (1984) also employed a number of manipulative steps prior to GC analysis. Terashi et al. (1990) determined DMA and TMA simultaneously but had to employ two derivatization agents prior to injection onto the GC-MS system.

3.5.3 Internal Standards

Abdul-Rashid (1990) reported that 1-aminopropane was not a suitable internal standard for microdiffusion in the determination of MAs, because of its low recovery ($\sim$10%) and tendency to rearrange to 2-aminopropane. For this reason Abdul-Rashid (1990) added 1-aminopropane after the microdiffusion step, which was obviously unsatisfactory. Hence, Cyclopropylamine (CPA) was chosen as a more suitable internal standard (see Section 2.2), and was added prior to microdiffusion. Cyclobutylamine (CBA) was added subsequently in place of 1-aminopropane, in order to measure the reproducibility of GC injections (Abdul-Rashid, 1990). A major advantage of using CPA and CBA is that, apparently, they are not naturally occurring. Hence, a wide variety of environmental samples can be analysed (both qualitatively and quantitatively) without fear of background levels of either CPA or

3.5.4 Chromatographic column

Samples were analysed using a Carlo Erba Fractovap Series 2350 GC equipped with a glass column (2.5 m x 2.0 mm i.d.) packed with Chromosorb 103 (a porous polymer). This packing was chosen as it has been specially designed for the resolution of volatile, basic, organic nitrogen compounds such as AAs and aliphatic amines (Phase Sep. Ltd).

The column was packed using suction and plugged with glass wool at both ends. Uniform packing was achieved by agitating the polymer particles in the column with an engraving vibrator. The column was then conditioned in the GC (48 h, 210 °C), with a current of nitrogen gas flowing through (15-20 ml/min). As the polymer contracts under prolonged heating, it was necessary to ensure that no gaps were evident in the packing before analyses were performed. Chromosorb 103 has an isothermal temperature limit of 250 °C (Dave, 1969) and a programmable limit of 300 °C (Jack Roberts, personal communication), so it was important that the oven temperature programme did not exceed these limits. It was also recommended that the detector temperature be at least 25 °C lower than the maximum temperature for the Chromosorb polymer (Carlo Erba instruction manual).

Since each GC cycle takes about 15 minutes, a large sample throughput is possible in a short timespan. The column was stable for long periods, and only occasional repacking was necessary (life expectancy about 20 000 injections, Abdul-Rashid et al., 1991).

3.5.5 Recovery, reproducibility and detection limits

Recoveries by microdiffusion are low for MMA and DMA (59 ± 5% and 60 ± 3% respectively), but are consistently reproducible for all three analytes. Lee and Olsen (1984) reported similar recoveries for DMA and TMA (65 ± 15% and 62 ± 15% respectively) but MMA could not be analysed or was below their detection limit (0.05 nmol g dry wgt.$^{-1}$). Recovery of TMA in this study was 100 ± 1%, while that of the internal standard CPA was 77 ± 2%. The tendency for the MAs to become adsorbed onto the sides of the microdiffusion flask and the collecting cup may be an factor governing the recovery of MMA and DMA, which are both more basic than TMA (Table 3.1). Adsorption onto the injector port and the glass column of the GC may also be of importance though this should also effect solutions of the MAs which are injected directly onto the column. Dalene et al. (1981) added ammonia (up to 5000 ppm) to suppress the adsorption of free amines (including the MAs) onto the glass column and column packing. Obviously, this was unsuitable for the present study as ammonia was one of the analytes, but is a useful suggestion for improvement of GC resolution in the analysis of amines.

Detection limits for the AMM and the MAs are shown in Table 3.5. and are an improvement on those reported by Abdul-Rashid et al. (1991). Detection limits were lowest for TMA which is consistent with the high recovery achieved for this MA. AMM had the highest detection limits (930 nM), but concentrations of AMM in environmental samples were generally much higher than for the other analytes.

Purged seawater blanks, which had been subjected to the microdiffusion procedure, were determined along with every batch of samples.

3.5.6 Discussion of present method

The present analytical scheme for the determination of MAs compares well with previously published methods. Good chromatographic separation of MAs was achieved with a minimal column preparation time. A number of volatile amines could be determined (see Fig 6.10) and the detection limits were suitably low for the analysis of environmental samples having very low MA concentrations (seawater, air etc.), but the lower recoveries of MMA and DMA relative to TMA remain a problem. Recently, a technique has been developed which employs an on-line diffusion process and requires minimal handling of samples and reagents (Yang et al., 1993). However, this method has not been used for the determination of amines in porewaters and sediments and requires large sample volumes to obtain detection limits comparable to those reported here. Yang et al. (1993) also employed two diffusion steps prior to analysis by GC, compared to the simple static diffusion step employed in the present method.

The conditions employed in the present method took account of all variable parameters. The ease of analysis enabled a large environmental study to be undertaken and distributions of the MAs were measured in samples from a wide variety of environments.

Table 3.4 Operating conditions for gas chromatograph

Carrier gas:	N_2 (oxygen-free)
Flow rate:	18.5 ml min^{-1}
Detector gases:	H_2, air
Flow rate:	34 ml min^{-1} (H_2), 230 ml min^{-1} (air)
Injector temperature:	250 °C
Detector temperature:	250 °C
OVEN TEMPERATURE: PROGRAMME:	
Temp. 1:	120 °C
Time 1:	1 min
Rate 1:	12 °C min^{-1}
Temp. 2:	200 °C
Time 2:	10 minutes
Temp. 3:	200 °C
Time 3:	5 minutes

Table 3.5 Percentage recoveries of ammonia and the MAs

Compound	Percentage recovery *	Coefficient of variation +	Detection limit (nM) ‡
AMM	60 %	± 9 %	930
MMA	59 %	± 3 %	48
DMA	60 %	± 5 %	10
TMA	100 %	± 1 %	5

* Recovery and reproducibility values were calculated for three samples at a concentration of 6×10^{-6} M.

\+ Each sample was analysed in triplicate.

‡ Detection limits were average values calculated for triplicate samples.

Table 3.6 Precision of the analytical method for Oglet Bay porewaters and sediments*.

Sediment	Porewaters
± 9 %	± 4 %

* The precision values were calculated as the mean of four samples for both the porewaters and sediments.

Figure 3.5: Gas chromatograms of AMM and the MAs in the sediments and porewaters of Oglet Bay.

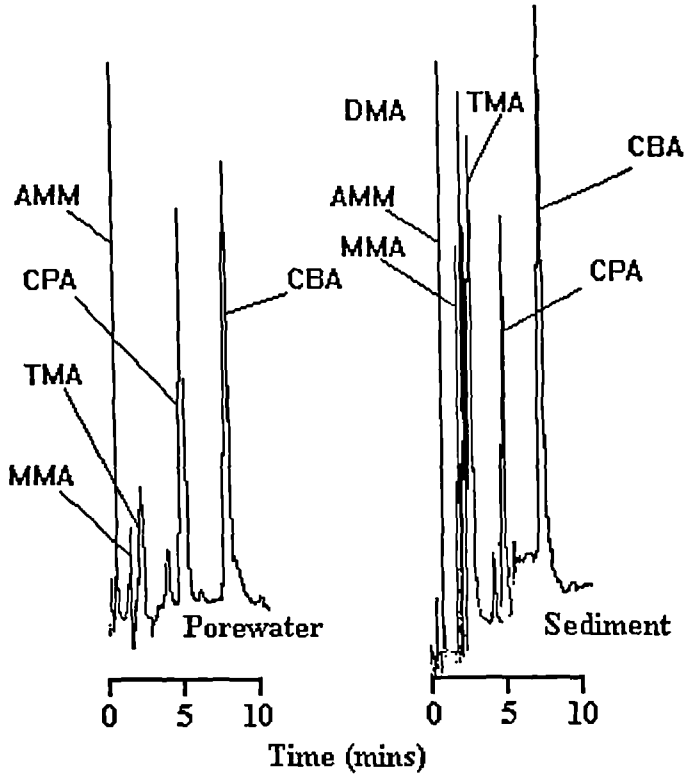
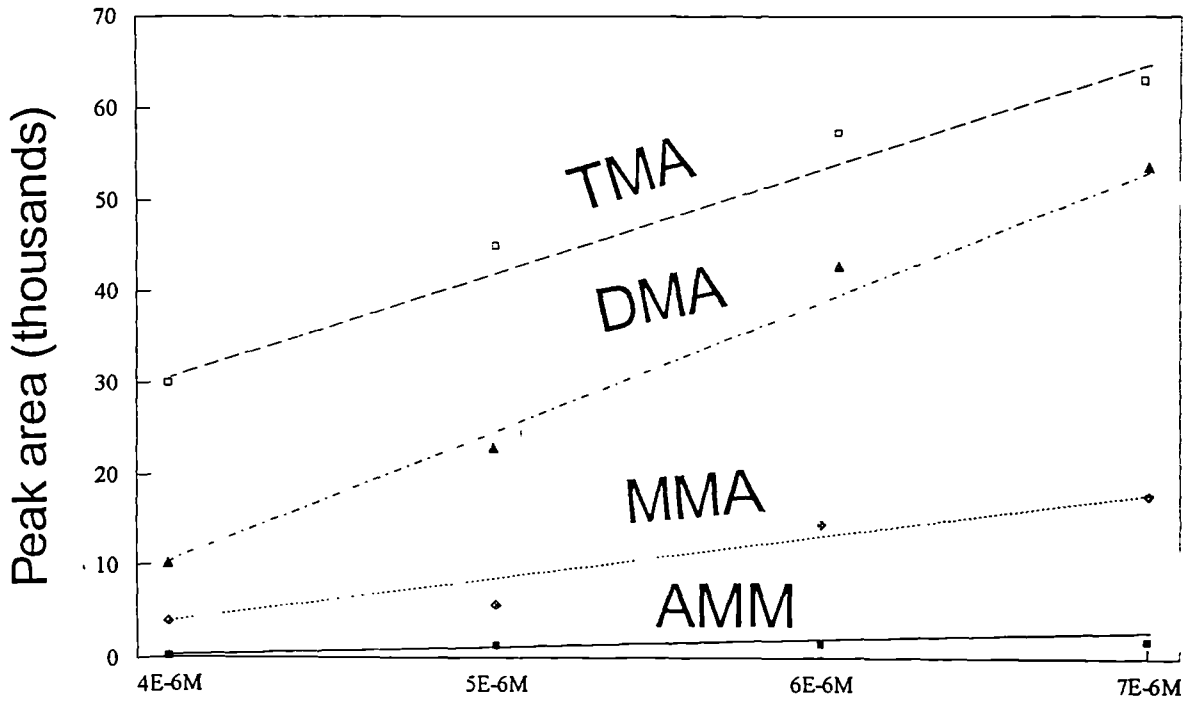


Figure 3.6: Calibration graph for AMM and the MAs.



AMM (r = 0.969869)
MMA (r = 0.996546)
DMA (r = 0.992562)
TMA (r = 0.996416)

CHAPTER 4

THE GEOCHEMISTRY OF THE METHYLAMINES IN A SERIES OF MARINE AND LACUSRTINE SEDIMENTS

Section 4.1: INTRODUCTION

The nitrogen cycle in natural waters and sediments has, thus far, generally been described in terms of well known inorganic nutrients such as AMM, NO_3^- and NO_2^-. Little attention has been devoted to the constituents of the organic nitrogen fraction, the roles of individual compounds in the cycling of organic nitrogen, and their contribution to the overall nitrogen cycle. This subject is discussed in more detail in Chapter 1.

Recently, the presence of MAs has been reported in a variety of organisms and environments including marine algae (*Dunaliella* and *Phaeodactylum* species, Abdul-Rashid, 1990), higher plants (King, 1988a), benthic invertebrates (Glob and Sorensen, 1987), seawater (Abdul-Rashid et al., 1991, Yang et al., 1993), freshwater lake sediments (Wang and Lee, 1990), coastal and open-ocean sediments (Lee and Olsen, 1984) and estuarine and salt marsh sediments (Glob and Sorensen, 1987; King, 1988b; Abdul Rashid, 1990; Wang and Lee, 1990).

The MAs constitute a considerable fraction of ON which, until recently, has attracted little attention. This is despite the fact that the MAs have been implicated in a number of important marine processes such as osmoregulation in marine organisms (Yancey et al., 1982; Sorensen and Glob, 1987; King, 1988a), and sedimentary methanogenesis (Lovley and Klug, 1983; King et al., 1988b).

Sediment composition and organic matter content can strongly influence the ability of the MAs to adsorb to sediments (Wang and Lee, 1990). The MAs are more basic than AMM and exist in the protonated form in the pH range associated with marine and brackish waters (i.e. 7.8-8.5). This could be an important factor

controlling adsorption of the MAs onto marine sediments. High [TMA_{pw}] have been reported in estuarine sediments with a high density of infauna (Glob and Sorensen, 1987), suggesting that biological processes can make an important contribution to the dissolved TMA pool in certain environments.

In the present study, the geochemistry of the MAs in a number of different sedimentary environments (both marine and lacustrine) was investigated. The biological and sedimentary characteristics of the sampling sites had previously been well documented (see Appendix A and below).

An attempt was made to evaluate the factors controlling the levels and speciation of the MAs in these locations. Although the behaviour of the MAs has been studied previously (Wang and Lee, 1990), this is the first report in which all three MAs have been analysed concurrently. Wang and Lee (1990) made an extensive study of MMA and DMA in a number of sediments of marine and lacustrine origin, and included in their study the seasonal sampling of a salt marsh sediment over a year. It was felt, however, that given the involvement of TMA in osmoregulation and methanogenesis (e.g. Sorensen and Glob, 1987; King et al., 1988b), its preclusion in the work of Wang and Lee (1990), might present an incomplete picture of the geochemical and biological importance of the MAs in sediments.

Furthermore, it was hoped that the present study, which involved the study of the MAs in sediment sections of 1 cm or less, would enable any variations in the [MAs] over small depth ranges to be observed. This was considered to be especially important in the surface sediments, where the density of benthic fauna and the rate of sedimentary diagenesis was expected to be greatest.

Concentrations of the MAs were expressed as μM and μmol/g dry weight (dw)

Concentrations of the MAs were expressed as μM and μmol/g dry weight (dw) for the porewaters and sediments respectively. These units are commonly employed by other workers and enabled comparisons to be made with other studies and sampling sites.

Section 4.2: SITE DESCRIPTIONS

4.2.1. Priest Pot

Priest Pot is a small (area 10^4 m^2, max. depth 3.9 m) eutrophic (summer chlorophyll-*a* levels up to 2300 μg/l, **Cranwell, 1986**) body of water in the English Lake District (54°22' N, 3°00' W), in which the bottom water periodically becomes anoxic (**Cohen, 1978**). A sediment core was taken when the lake was well mixed (i.e. the water column was no longer stratified) on 18/10/91.

4.2.2. Oglet Bay

A comprehensive site description for Oglet Bay salt marsh appears in Appendix A. Oglet Bay could be divided into three distinct zones reflecting marsh maturity. These were the upper marsh (UM), middle marsh (MM) and lower marsh (LM). The LM was the sampling site for the present study (Figure 4.1). This area of the marsh consists of intertidal mudflats, colonised exclusively on the surface by epipelic diatoms and euglenoid algae (Jemmett, 1991). This is in contrast to the UM and MM which were colonised to varying degrees by salt marsh higher plants although the MM was also colonised by the algal species mentioned above (see also Chapter 5).

A core was taken one day after a high spring tide (24/1/92). A study of the distribution and geochemistry of the MAs in the UM, MM and LM through a tidal cycle is presented in Chapter 5.

4.2.3. Loch Etive

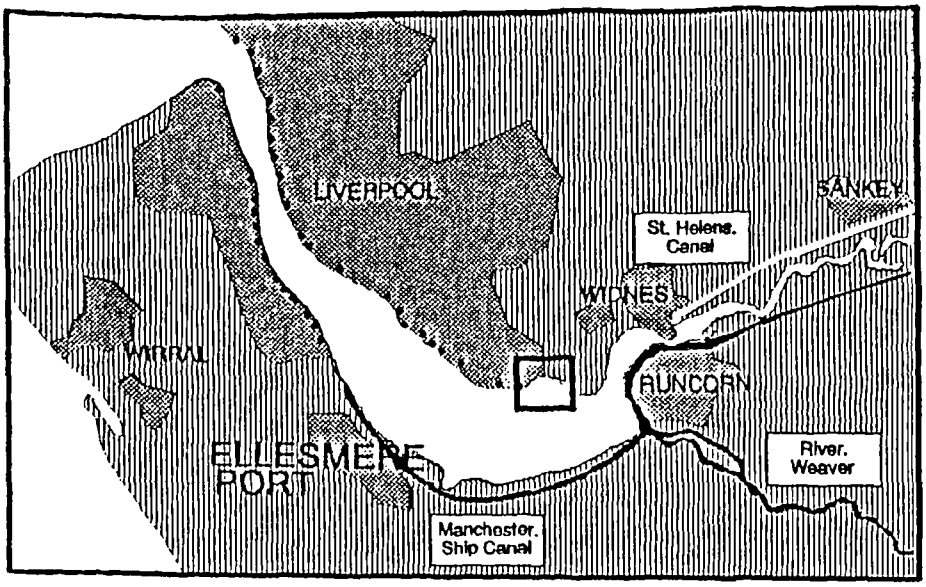
Two cores were taken from Loch Etive, a fjordic-type loch situated on the west coast of Scotland (Figure 4.2, and Appendix A). The loch is divided into an inner and outer basin, being separated by a shallow sill at Bonawe and connected to the Firth of Lorne by another shoal choked sill at Connel. Water circulation in the inner basin is poor, the water being replenished every 15 months on average. This leads directly to the presence of anoxic bottom waters. In contrast, the outer basin is estuarine in circulation showing a well-mixed, oxygenated water column. The sediment core from the inner basin was taken at a depth of 134 m (Lat. N 56° 27 35, Long. W 5° 11 13).

The sediment core, collected at a water depth of 49 m in the outer basin (Lat. N 56° 27 20, Long. W 5° 15 19), was oxic down to 5 cm, where the change in sediment colour from yellow to green/grey reducing sediments was reflected by the disappearance of benthic fauna. The inner basin had an oxic layer of about 1 cm and restricted faunal activity below this depth (Wood et al., 1973; Edwards and Edelsten, 1977; Ridgway, 1984; Ridgway and Price, 1985).

4.2.4. Firth of Lorne

A core was taken in the Firth of Lorne as an example of a coastal marine sediment (Figure 4.2). The Firth of Lorne is a body of water immediately adjacent

be observed in the zonation of the benthos in the Firth of Lorne. Faunal species such as *Pygospio elegans* and *Tellina tenuis*, which are known to be moderately tolerant to salinity fluctuations were abundant in regions of the sediment close to Loch Etive, whereas typically euryhaline species were more abundant with depth and distance from the Loch (Gage, 1974). The Firth of Lorne sediment core was taken at a depth of 34.3 m (Lat. N 56° 27 29, Long. W 5° 27 25).



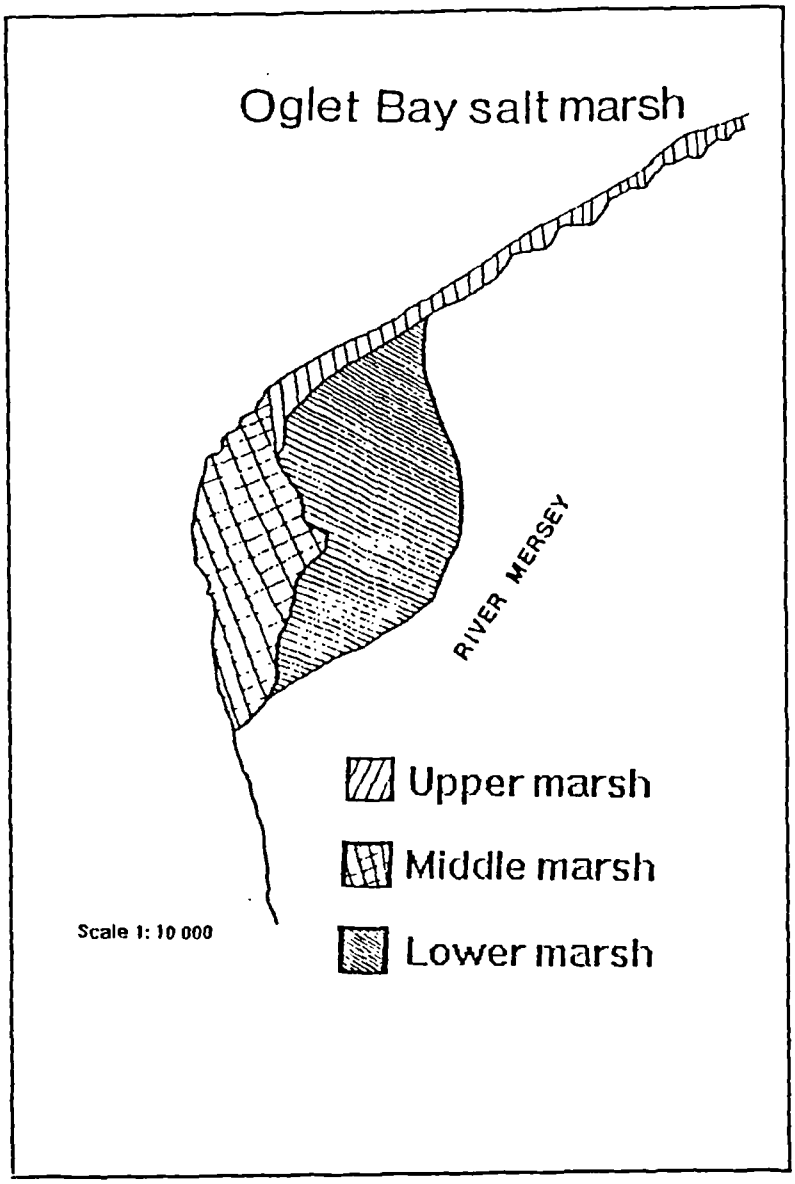


Figure 4.1. Diagram of Oglet Bay showing the zonation of the marsh and their relative areas.

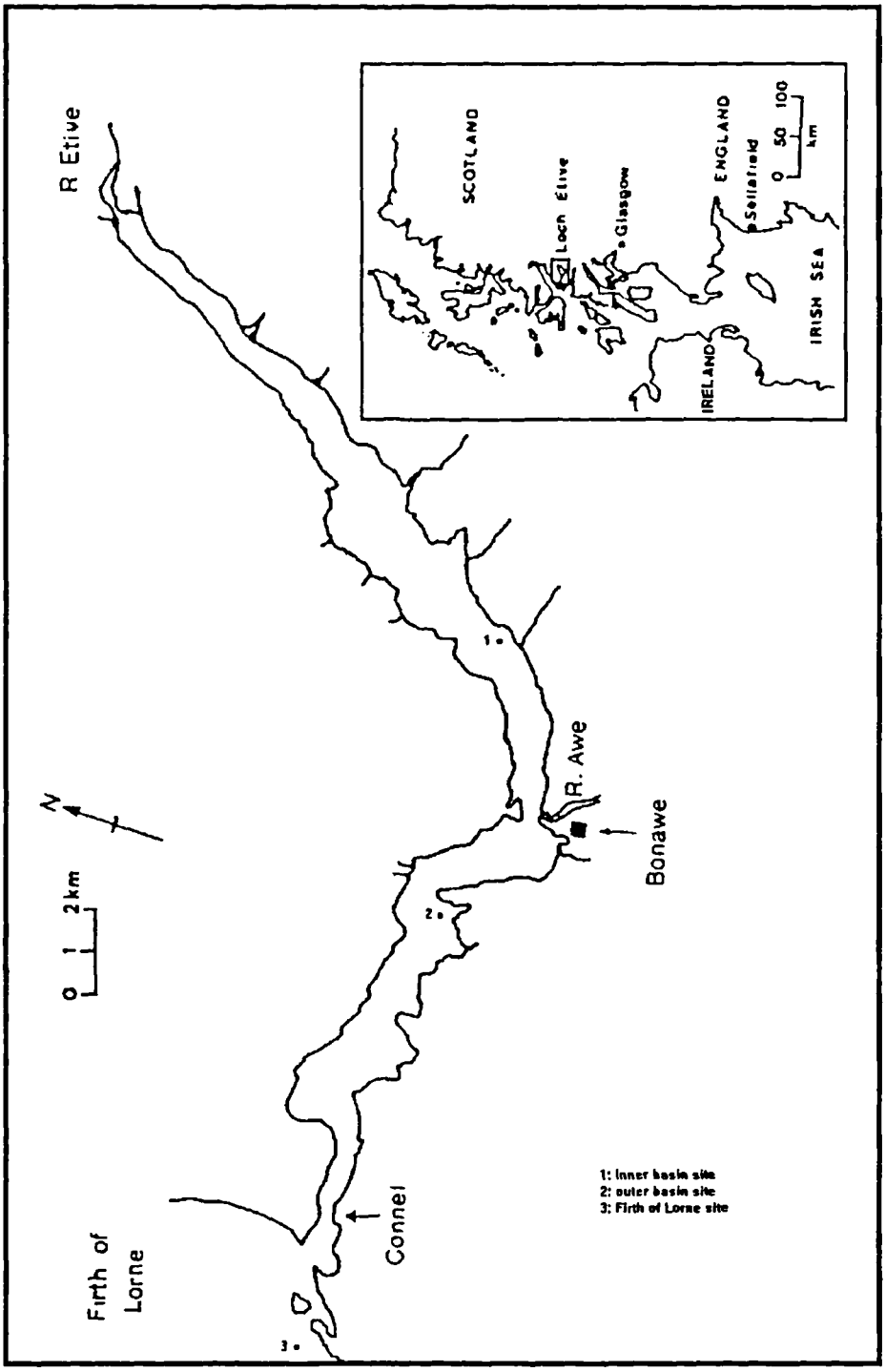


Figure 4.2. Map of Study area for Loch Etive and the Firth of Lorne Sampling sites are marked and the inset map shows the position of the study area relative to the West of Scotland.

Section 4.3: RESULTS

4.3.1. Oglet Bay

Distributions of the MAs in Oglet Bay are shown in Figure 4.3. Concentrations of porewater TMA were much higher than MMA and DMA, and showed clear surface enrichment (27 μM), although levels were rapidly depleted down to 10-11 cms (3 μM). This decrease in [TMA_{PW}] corresponded with a slight enrichment in [DMA_{PW}] in some core sections, however, there was no definite correlation between [DMA_{PW}] and [TMA_{PW}]. Maximum [DMA_{PW}] were between 5 and 6 μM, but the distribution down the core was more variable than for TMA_{PW}. Concentrations of MMA_{PW} were low, the compound being present above detection limits only at the 0-1 cm depth interval, and again at 14-17 cms. In this region of the core, [MMA_{PW}] increased (up to 6 μM), and coincided with a decrease in [DMA_{PW}].

The sedimentary MA pool was not dominated by one particular compound, but [MMA_S] were generally greater than [DMA_S] and [TMA_S] combined. Highest [MA_S] were seen in the top 1 cm of the sediment (5.5, 5.5 and 2.4 μmol/g dw for MMA, DMA and TMA, respectively) which then decreased down to the 8-9 cms depth interval (1.6, 0.5 and 1.0 μmol/g dw for MMA, DMA and TMA, respectively). All three MAs were persistent throughout the core. DMA_S was least abundant, though [DMA_S] and [TMA_S] converged at some points in the core. Less variation in [MA_S] was seen in the bottom half of the core although a subsurface maximum for the three compounds was apparent at the 12-13 cms depth interval.

4.3.2. Firth of Lorne

Concentrations of TMA_{PW} were similar to those observed for MMA at the sediment surface (Figures 4.4 and 4.6). Maxima for MMA and TMA in the porewaters were observed at 4-5 cms, but the highest concentration for MMA_{PW} (320 μM) was an order of magnitude greater than for TMA (12.5 μM). The decrease in $[TMA_{PW}]$ with depth corresponded to an increase in $[DMA_{PW}]$ at 9-10 cms. However, no clear relationship emerged as DMA_{PW} was not persistent throughout the core. Elevated $[MMA_{PW}]$ of up to 200 μM were observed towards the bottom of the core.

Interestingly, $[MMA_S]$ were lower in the Firth of Lorne sediment than in Loch Etive (approx. 10 μmol/g dw; see below) and the profile showed little variation below 5-6 cms (Figure 4.4). TMA_S was generally much less abundant (Figure 4.6), highest concentrations being observed at 2-3 cms (1 μmol/g dw). The maximum concentration of DMA_S was also observed at this depth interval (0.6 μmol/g dw) as shown in Figure 4.5.

4.3.3. Loch Etive

MMA_{PW} was persistent throughout the cores taken from the inner and outer basins of Loch Etive. However, concentrations of porewater MMA were generally lower than for the Firth of Lorne, and showed much less variation with depth. Highest $[MMA_{PW}]$ were 18 and 35 μM for the inner and outer basins, at 3-4 cms and 10-11 cms, respectively.

DMA_{PW} was much less abundant than the other MAs and was detected only in the outer basin, at the surface (2 μM) and at the 9-10 cms depth interval (0.3 μM). Of the two sites, TMA_{PW} was least abundant in the inner basin of Loch Etive.

[MMA_S] in the surface sediments of the inner and outer basins of Loch Etive were up to four times as high as the Firth of Lorne sediment with the highest levels being observed in the outer basin (up to 40 μM). Highest [DMA_S] and [TMA_S] were 2 and 2.5 μM respectively, and were observed for the outer basin core.

4.3.4 Priest Pot

This sediment was very fluid and was sectioned at 1 cm intervals above 3 cm. MMA was the most abundant MA in both the sediments and porewaters, reaching maximum concentrations of more than 60 μM at the 5.5-6.0 cms depth interval. Maximum concentrations of MMA_{PW} were higher than in most of the marine cores described above. [DMA_{PW}] and [TMA_{PW}] were never greater than 10 μM (Figure 4.7), and these MAs were not always present above detection limits in the porewaters. [DMA_{PW}] were generally similar to or higher than [TMA_{PW}] throughout, in contrast to the marine porewater observations.

The [MA_S] were much lower than in the cores from the other sites (maximum concentrations were 1.0, 0.4 and 0.1 μmol/g dw. for MMA, DMA and TMA respectively). Furthermore, as in the porewaters, DMA_S was in greater abundance than TMA_S. A subsurface maximum for the sedimentary MAs was observed at the 4.5-5.0 depth interval. Below this depth, [MA_S] decreased to levels seen at the sediment surface.

Figure 4.3. Distributions of the MAs in an estuarine salt marsh (Oglet Bay, LM).

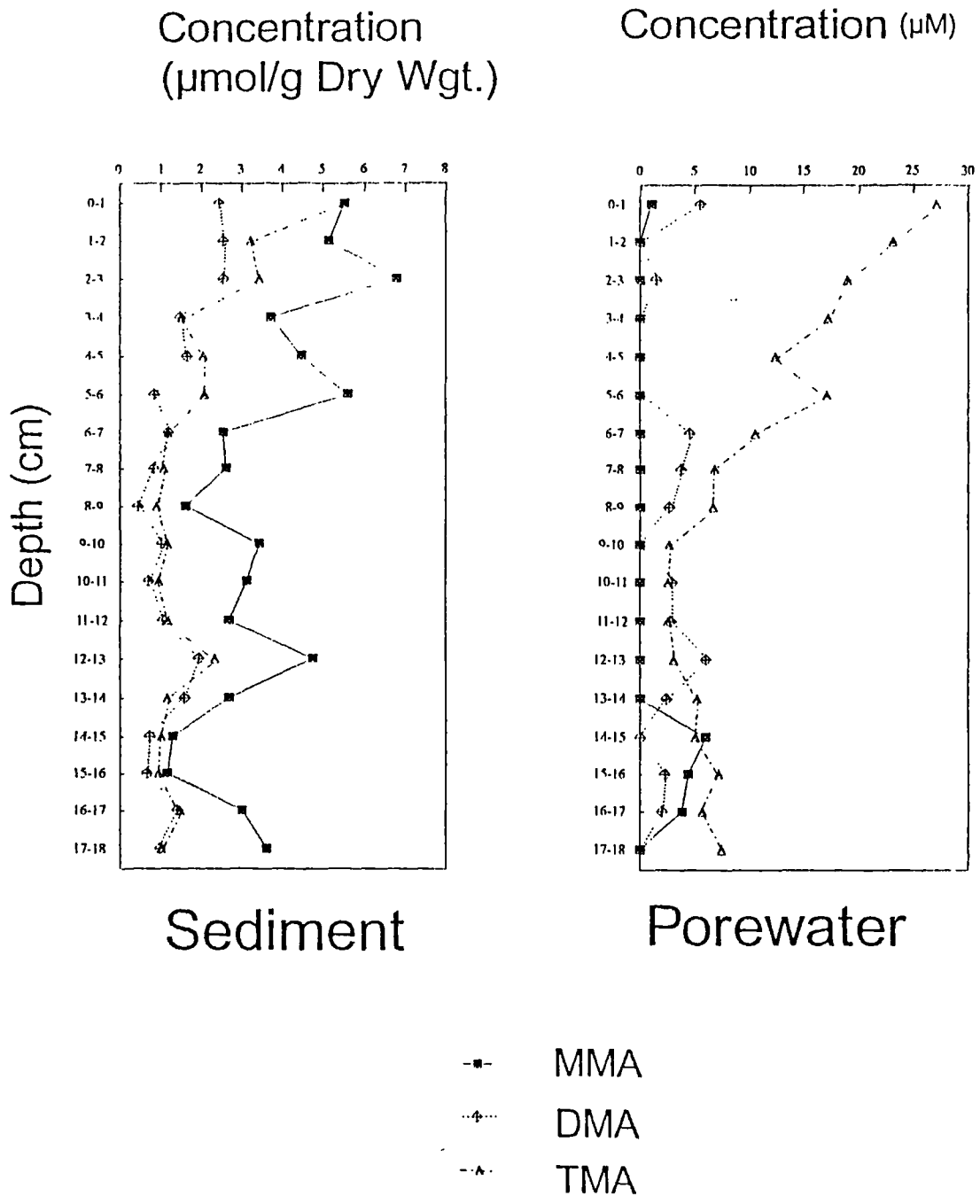


Figure 4.4. Distributions of MMA in a series of marine sediments.

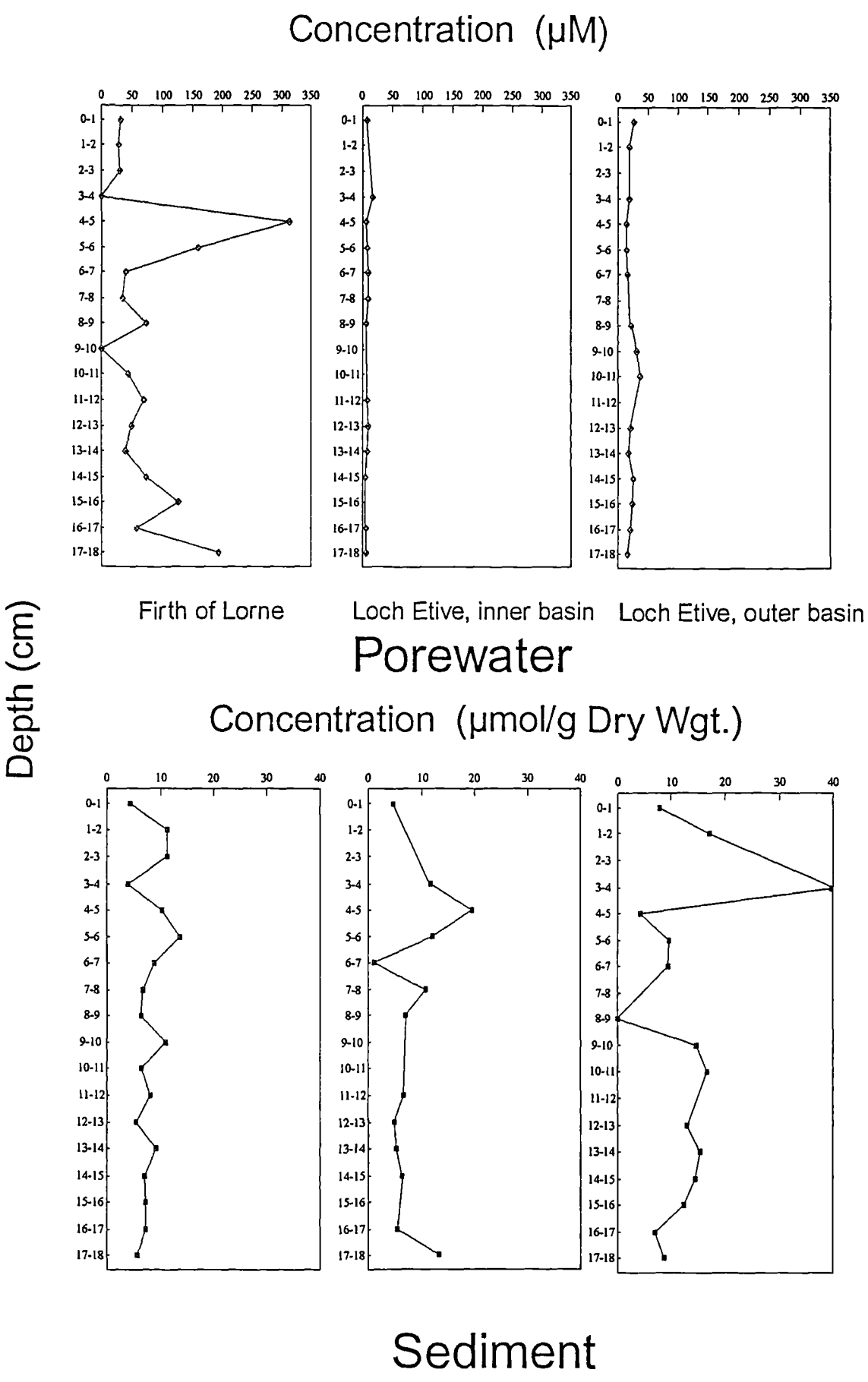


Figure 4.5. Distributions of DMA in a series of marine sediments.

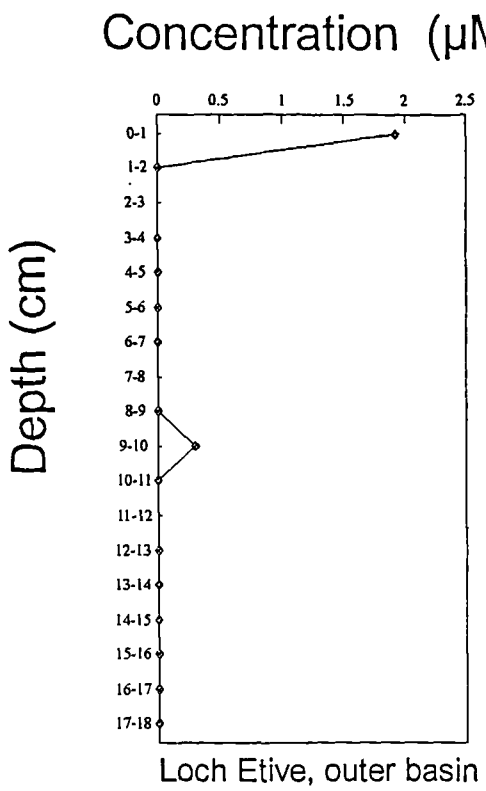


Figure 4.6. Distributions of TMA in a series of marine sediments.

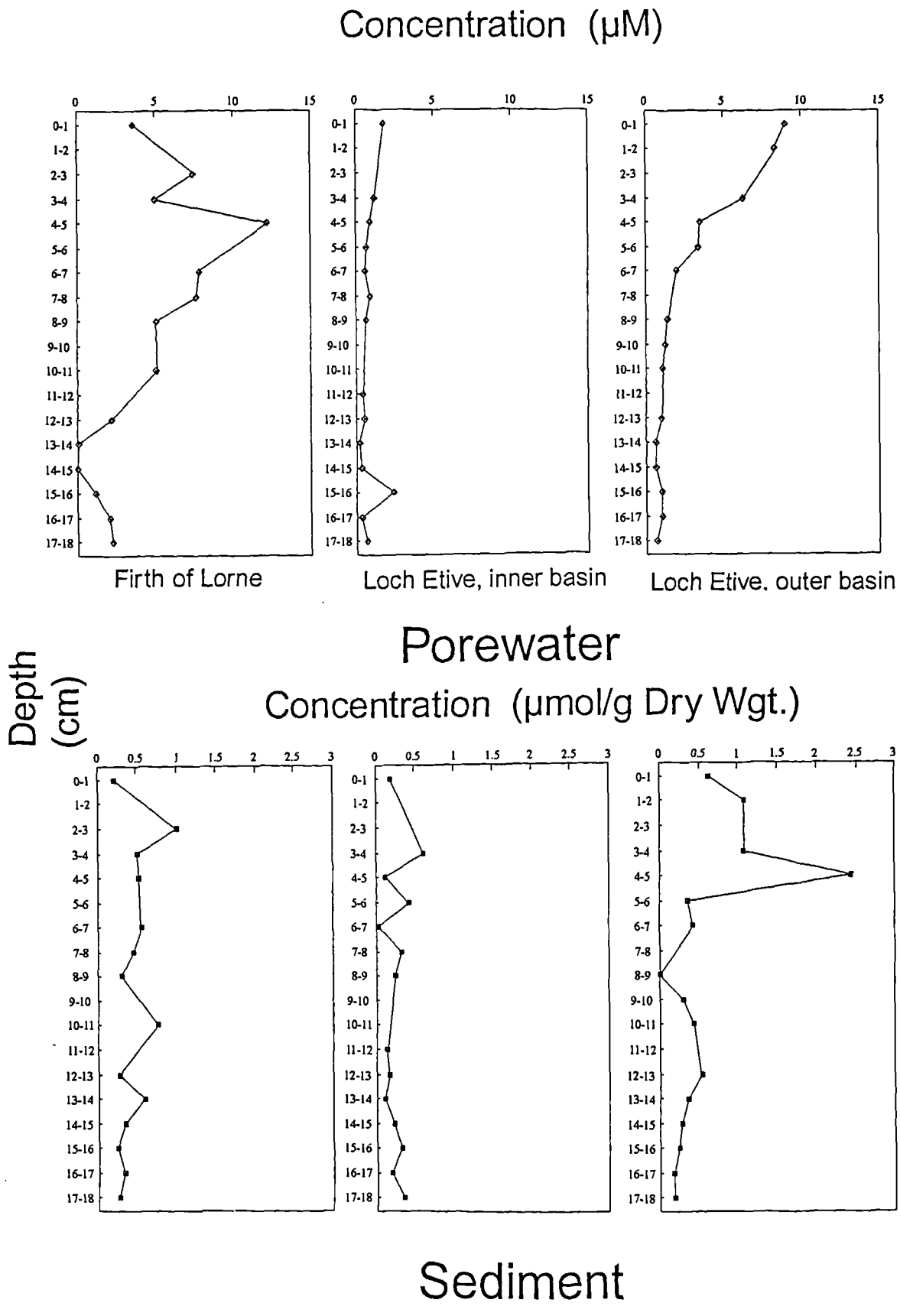


Figure 4.7. Distributions of the MAs in a lacustrine sediment core Priest Pot, UK).

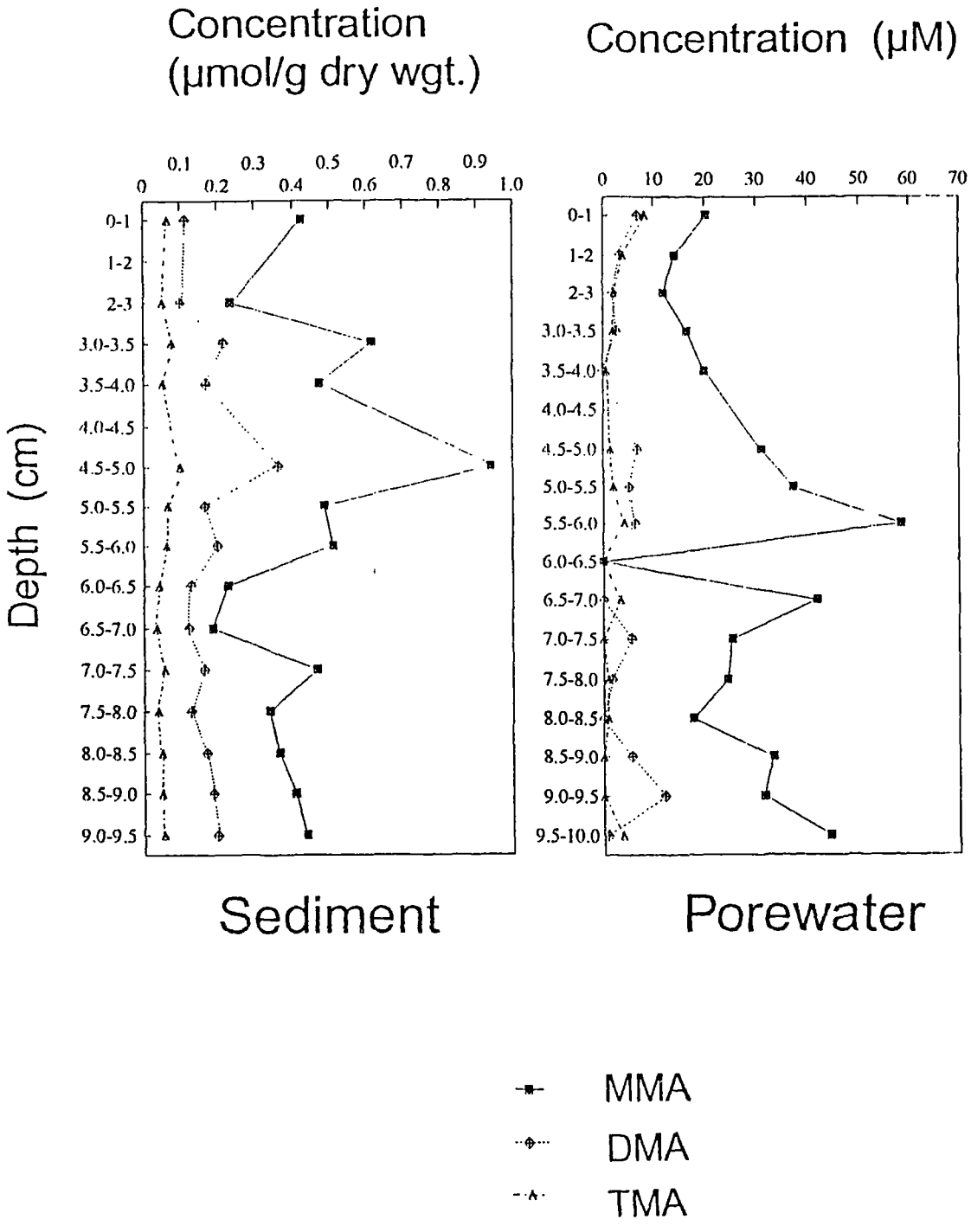


Figure 4.8. Distributions of porewater NO_3^- in sediments from Loch Etive and the Firth of Lorne.

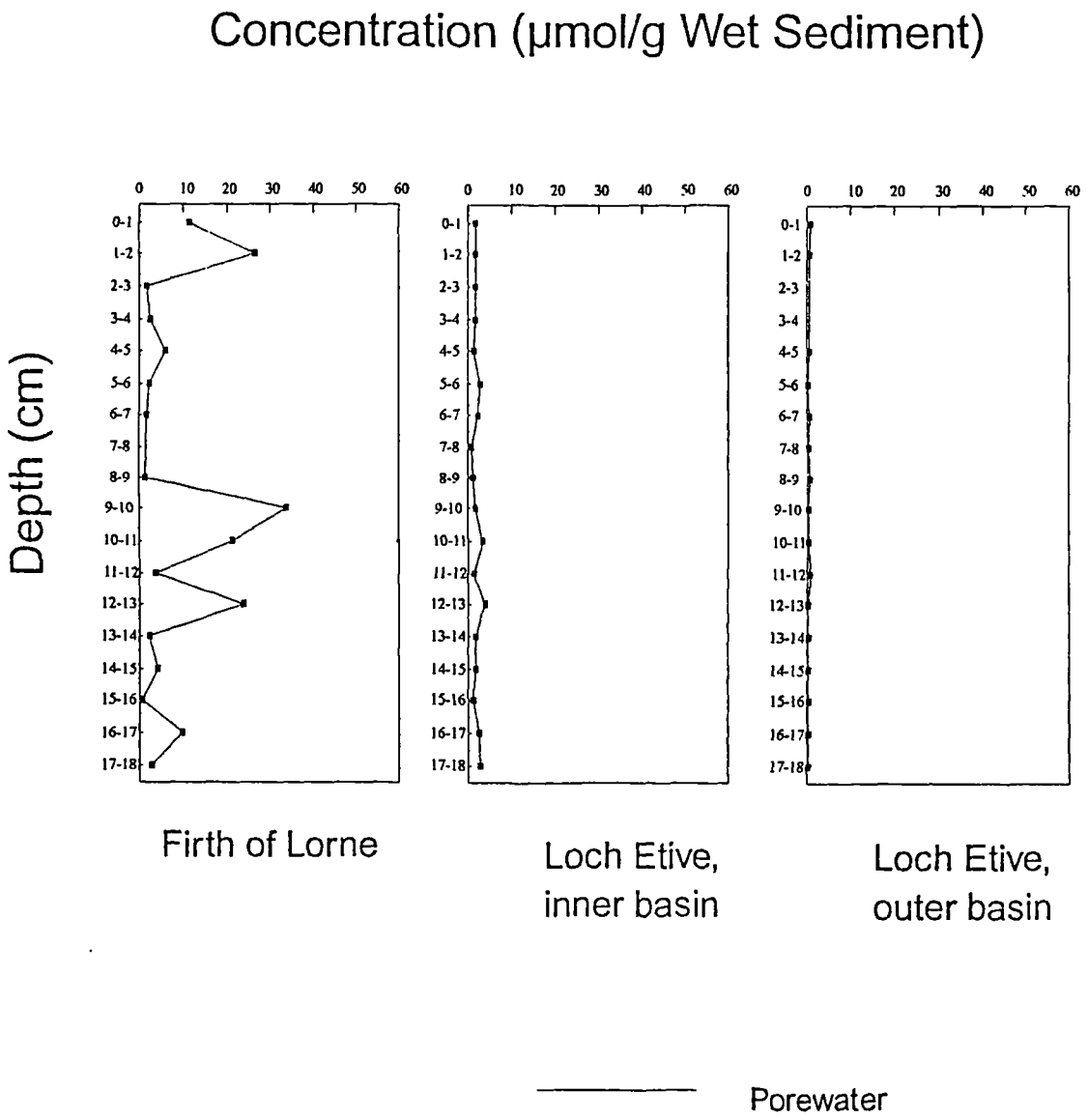


Figure 4.9. Distributions of AMM in cores from Loch Etive and the Firth of Lorne.

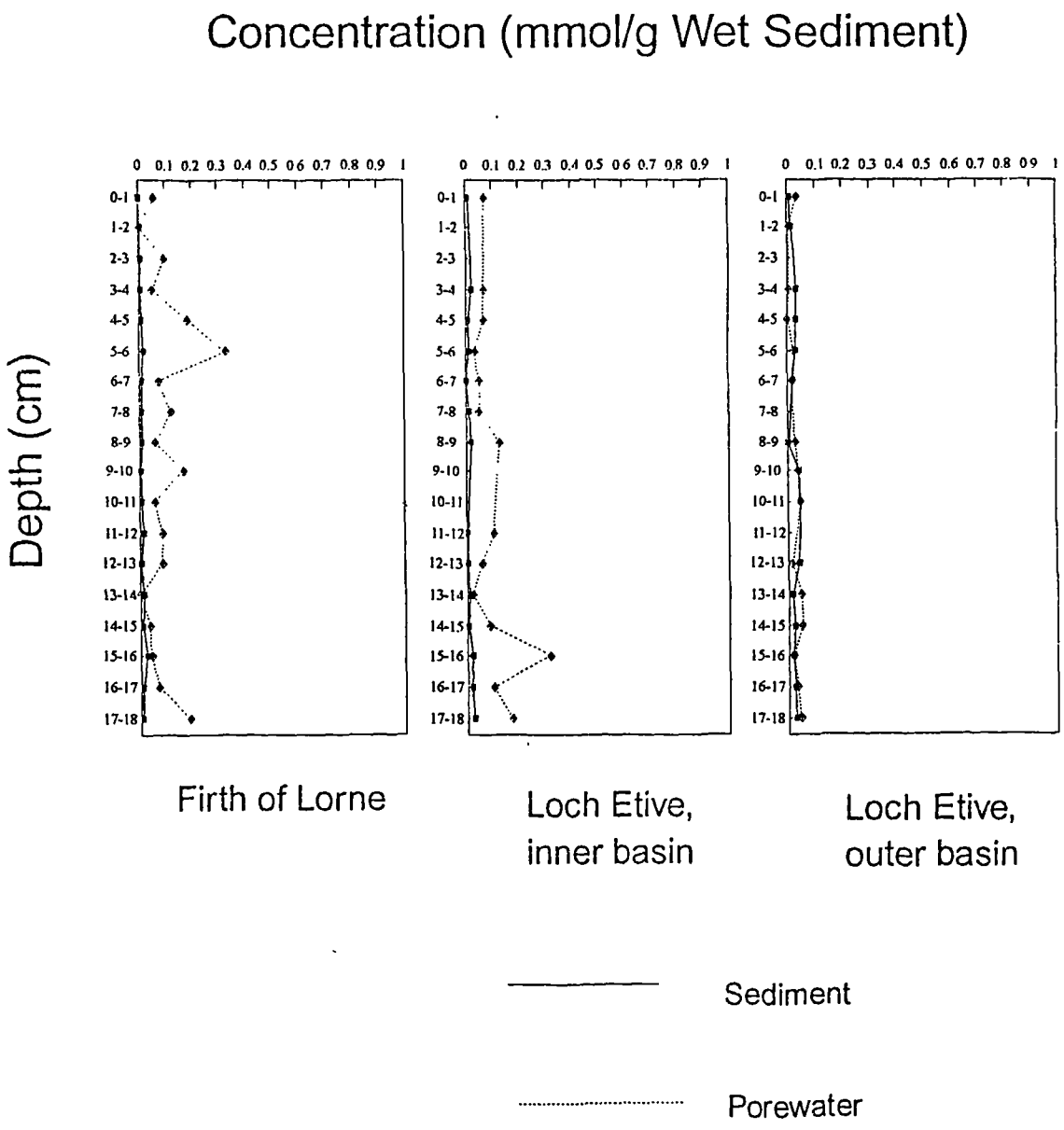


Figure 4.10. Distributions of porewater NO_2^- in sediments from Loch Etive and the Firth of Lorne.

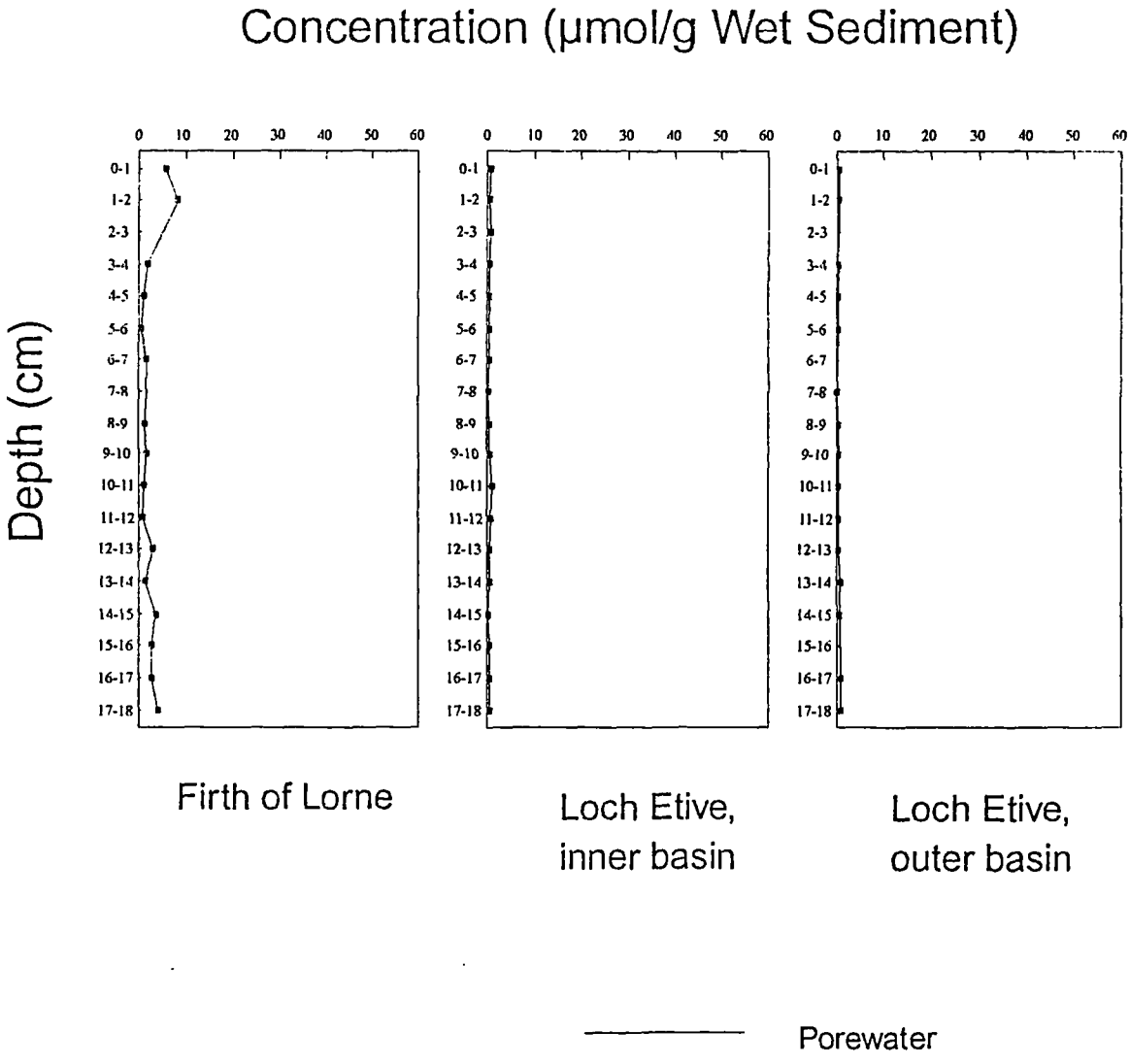


Table 4.1: Reported concentrations of the MAs

in a variety of sediments.

Sediment type	Porewater concentration (μM)			Authors
	MMA	DMA	TMA	
Coastal	bd	48	29	Lee and Olsen (1984)
Open ocean	bd	0.5	0.14	
Salt marsh	0.19*	1.06*	nd	Wang and Lee (1990)
Peru upwelling	0.1*	0.65*	nd	
Freshwater lake	0.22*	0.74*	nd	
Estuarine	0.12*	0.87*	nd	
Estuarine	nd	nd	15	Glob and Sorensen (1987)
	Sedimentary concentrations (nmol/g dry weight)			
Coastal	bd	70	22	Lee and Olsen (1984)
Open ocean	bd	24	2	
Salt marsh	2.9*	6.1*	nd	Wang and Lee (1990)
Peru upwelling	10.7*	25.3*	nd	
Freshwater lake	15.8*	25.5*	nd	
Estuarine	4.9*	6.1*	nd	

bd: below detection limits.

nd: not determined.

* average concentrations through the cores.

Section 4.3: DISCUSSION

Maximum concentrations of the MAs observed in these sediments were higher than any recorded to date (Table 4.1), and may be the consequence of a number of well documented biological and chemical processes. These are outlined below.

Osmoregulation

The high [TMA_{PW}] in the Oglet Bay surface sediment, and its depletion with depth, suggests that TMA is an important and significant constituent of these sediments, and is involved in a number of important processes. These intertidal sediments are inhabited by organisms which are tolerant to variations in the salinity of the porewaters, and specifically to the build up of a Na^+ gradient between the porewaters and the intracellular solutions of the organisms. In order to offset the Na^+ gradient, and avoid accumulation of toxic concentrations of Na^+, organic ions, such as quaternary amines (QAs), are accumulated to high concentrations by marine organisms, without physiological side effects (Yancey et al., 1982; King, 1988a).

TMA has been implicated as a product of the degradation of certain of these organic osmolytes, namely QAs (e.g. King et al., 1988b). When the sediments are exposed, and the salinity of the porewaters decreases, accumulated QAs diffuse from the organisms into the porewaters. King (1988a) suggested that the degradation of certain QAs may influence the marine flux of the MAs, and particularly TMA, and proposed a pathway by which this might occur (Figure 5.12).

Salinity stress would not be a problem for organisms inhabiting the Priest Pot sediment; and assuming an origin of TMA from QAs, this helps to explain the lower

[TMA_{PW}] which were observed in the Priest Pot core. Nevertheless, the presence of TMA_{PW} in Priest Pot sediments suggests that there are other sources of TMA, possibly from the decomposition of algal detritus (Abdul-Rashid, 1990), or from direct production by benthic organisms.

The sediments of Loch Etive and the Firth of Lorne are permanently submerged, but variations in the porewater geochemistries of these sediments might not be unexpected, given the significant freshwater input into Loch Etive and the subsequent mixing of the exported Loch Etive water mass in the Firth of Lorne (Gage, 1974). Furthermore, intertidal organisms are not the only species to accumulate or biosynthesise QAs. QAs are abundant in many types of marine organism (Yancey et al.,1982; Martin et al., 1982; King and Goldstein, 1983; Daikoku, 1985; King, 1988a). Excretion of QAs from the benthic organisms may occur even in a stable salinity environment, albeit at lower rates than from the infauna of Oglet Bay.

Sorensen and Glob (1987) measured [TMA_{PW}] in a number of estuarine sediments, and found that [TMA_{PW}] correlated positively with density of benthic fauna. Invertebrates isolated from the sediments also responded to immersion in low salinity water with the direct release of TMA from their body fluids. This implies that, although QAs probably play an important role in the marine flux of TMA, direct release or diffusion of TMA from the intracellular solutions of marine organisms may be just as important. This behaviour may also mimick biological changes associated with porewater salinity variation in an intertidal zone.

The concentration profiles of the Loch Etive sediments show that [TMA_{PW}] decreased with depth (Figure 4.6), the decrease coinciding with the maximum depth

of sedimentary biological activity (Ridgway and Price, 1985). Concentrations of TMA_{PW} were asymptotic below these depths (1 cm for the inner basin and 5 cm for the outer basin). The observed distributions suggest a role for the benthic fauna of Loch Etive in controlling the distributions of TMA in the porewaters. Isotope ratio data for the outer basin sediment is consistent with a marine origin (Harriman, unpublished results), however, the signal could not yield information concerning specific marine sources.

On the other hand, the elevated surface [TMA_{PW}] may also be directly associated with the deposition and degradation of algal detritus to the sediments (MMA was also detected at significant concentrations in the porewaters, and DMA_{PW} was present at the surface of the outer basin sediment). Dissolved organic carbon released by marine algae has been reported to consist mainly of low molecular weight labile compounds (e.g. Mague et al., 1980). Furthermore, culture experiments have shown that some brown and green marine algal species can produce and release MAs (Abdul-Rashid, 1990). Nevertheless, the low primary production of Loch Etive (approx. 70g C m^{-2} $year^{-1}$, cf. the Irish Sea. The Irish Sea, an environmental review, 1990), and the fact that the cores were taken in winter (24/2/92), when organic matter input rates would have been at a minimum, suggest that the relatively high [TMA_{PW}] are most likely to have another source.

It is interesting to note that Blackburn and Henriksen (1983) found that porewater fluxes of ammonia (associated with the degradation of fresh detritus) showed little variation in cores taken in July and November. They attributed this phenomenon to the buffering of the porewaters by the sedimentary ammonia pool during periods of low production. The high [TMA_{PW}] in the Loch Etive sediments

could arise via a similar buffering process.

At present, it is not possible to identify the sources of the porewater MAs (and TMA in particular) in Loch Etive. Algal or benthic sources are both entirely plausible, but it is expected that algal influence would diminish during the winter months. Isotope ratio values for the sedimentary organic matter are consistent with a marine source, but it would be useful to take isotope values from algal species associated with Loch Etive and also the resident infauna. These values, when conpared with the sedimentary organic carbon signal, may give more definite information on the source of the porewater MAs, such as bioturbation which is discussed below.

Bioturbation

TMA was more abundant with depth in the Firth of Lorne sediment than in the Loch Etive cores. Maxima for [MMA_{PW}] and [TMA_{PW}] were observed at 4-5 cms in this core but these MAs were persistent with depth. The porewater NO_3^- profile for the Firth of Lorne was consistent with an anoxic coastal sediment in the top half of the core (Figure 4.8), in that concentrations were highest in the upper 2 cms of the core and decreased to much lower levels below this depth. This was presumably a reflection of the involvement of NO_3^- in secondary reduction processes in the absence of oxygen (e.g. Chester, 1990). A corresponding increase was also observed for porewater AMM below the NO_3^- maximum (Figure 4.9) which presumably reflects the degradation of organic nitrogen in sediments (Blackburn and Henriksen, 1983). However, a subsurface maximum for [NO_3^-] was observed at 9-10 cms, which may indicate biological activity even at this depth in the sediments. The mucus-lined

burrows of many benthic animals result in oxic microenvironments at depth in many sediments. Aller and Aller (1986) suggested that burrow structures in marine sediments can have give rise to three dimensional localized enhancement of biological activity. The subsurface NO_3^- maximum and the persistence of MMA_{PW} and TMA_{PW} in this region of the sediment, may, therefore, reflect the colonisation by benthic fauna of deep sediments in the Firth of Lorne. Once they have been introduced into the sediments, the MAs can be utilised by sedimentary microbes, as the next section describes.

Microbial utilization

Budd and Spencer (1968) isolated a number of marine bacteria from a marine mud and found that *Micrococcus sp.* (an aerobic marine heterotroph) could grow on all three MAs, but utilised MMA and DMA in preference to TMA. It was suggested that this was due to the requirement of a specialised (possibly endogenic) demethylation step in the metabolism of TMA, which is not required for the lesser methylated amines. Hence, the decreasing $[TMA_{PW}]$ may have been the result of an unfavourable but necessary demethylation step resulting in the production of MMA and DMA, and may indicate that TMA was the sole source of these compounds in Oglet Bay. Production of MMA and DMA may have been offset by further rapid utilization by bacteria.

The oxic nature of the Loch Etive surface sediment implies that methanogens, which are exclusively anaerobic, were not active in removal of the MAs. A number of methylotrophic bacteria are capable of utilising some or all of the MAs as shown in Table 5.1 (Large, 1983), and may have been involved in their metabolism in these

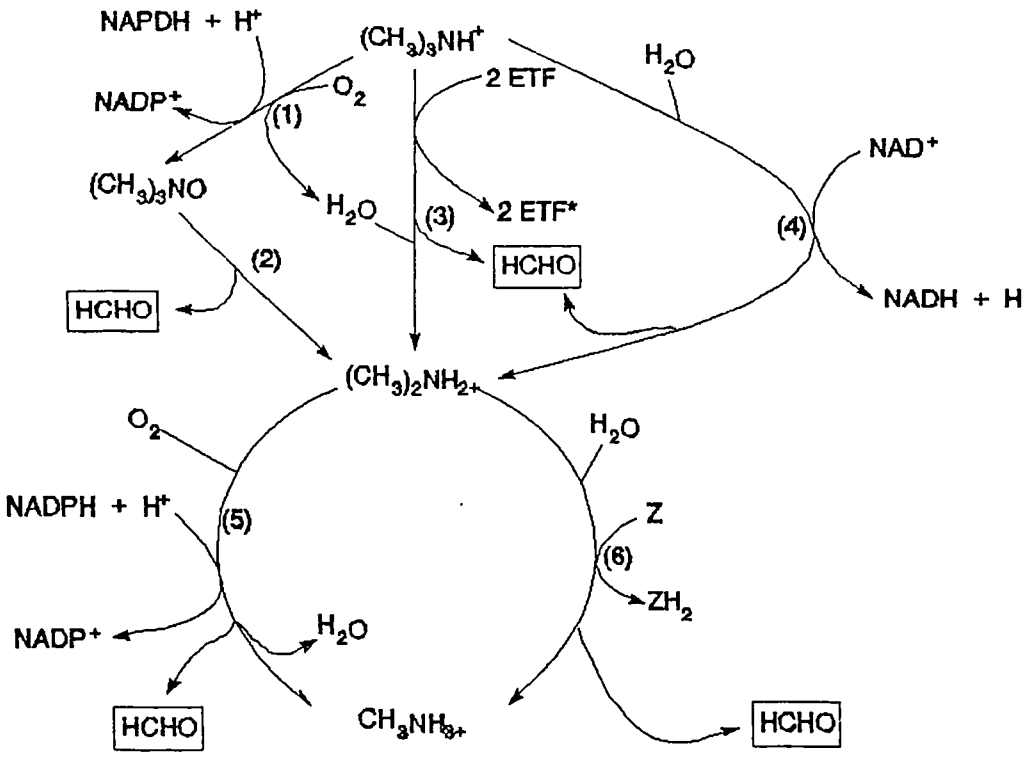
sediments. Furthermore, an aerobic pathway for the stepwise demethylation of the MAs has been reported (Figure 4.11). Selective utilisation of the MAs by the methylotrophs may depend on the appropriate enzymes (Figure 4.11), the lack of which may restrict them to the utilization of certain MAs, rather than the full suite of compounds, or preclude the uptake of MAs altogether.

The MAs (particularly TMA) have been reported as substrates for methanogenic bacteria in marine sediments where sulphate reducing bacteria utilise preferred substrates such as acetate (Patterson and Hespell, 1979; Lovely and Klug, 1982; King et al.,1983; King, 1984; Glob and Sorensen. 1987; King, 1988b). In this way, methanogens can exist in sediments with high concentrations of SO_4^{2-}, such as hypersaline sediments.

Dissolved SO_4^{2-} was not measured in the present study, but high concentrations of S^o have been reported for Oglet Bay LM (Paratono, personal communication), implying a high rate of sulphate reduction. Hence, the MAs may play a role as methanogenic substrates in these sediments. However, Budd and Spencer (1968) have suggested that the remineralization of organic nitrogen is a specialized process carried out by a flora of nitrogen-specific bacteria. If this is the case, then some methanogens may prefer the MAs, even when acetate is available.

Utilization of the MAs by methanogens in a freshwater water eutrophic lake has been reported (Lovely and Klug, 1983). Concentrations of major seawater ions such as SO_4^{2-} are low in freshwater environments, but acetogenic bacteria can also account for a major proportion of methane production in lacustrine sediments. Concentrations of the porewater MAs in Priest Pot decreased with depth in the surface sediment, which is consistent with their utilization as microbial substrates.

Figure 4.11 Enzymes involved in the conversion of TMA and DMA to MMA. (1) Trimethylamine mono-oxygenase; (2) trimethylamine N-oxide aldolase (EC 4.1.2.32); (3) trimethylamine dehydrogenase (EC 1.5.99.7); (4) trimethylamine dehydrogenase (NAD-linked); (5) dimethylamine mono-oxygenase; (6) dimethylamine dehydrogenase (EC1.5.99.-). **Abbreviations: ETF** electron-transferring flavoprotein (oxidised form), **ETF$^+$** electron transferring flavoprotein (semiquinone half-reduced form); **Z**, unidentified electron acceptor; **EC**, enzyme commission number; **ATP**, adenosine 5'-tri-phosphate; **ADP**, adenosine diphosphate; **NAD$^+$, NADH**, oxidised and reduced forms of nicotinamide adenine dinucleotide, respectively; **NADP$^+$, NADPH**, oxidised and reduced forms of nicotinamide adenine dinucleotide phosphate respectively; **NAD(P)$^+$, NAD(P)H**, signifies that *either* NAD$^+$ or NADP$^+$, NADH or NADPH may be involved in a particular reaction; P_i, inorganic phosphate. After Large (1983).



However, a subsurface maximum was then observed for MMA at 5.5-6.0 cms (60 μM), which was three times higher than at the surface. The higher [MMA_{pw}] relative to [TMA_{pw}] in Priest Pot suggests an alternative source of MMA. MMA has been reported as a direct product of the decarboxylation of glycine (GLY, see Figure 5.11), but this process has only been reported in marine sediments (Mead, 1971; Barker, 1981; Stams and Hansen, 1984; Burdige, 1990; Burdige and Martens, 1990). Meitzer (1977) stated that the decarboxylation of GLY to form MMA is catalyzed by coenzyme pyridoxamine 5'-phosphate, so, presumably, any microbe which can produce this enzyme is capable of converting GLY to MMA. Unfortunately, GLY was not measured in the porewaters of Priest Pot, but that does not preclude its occurrence in anoxic lacustrine sediments.

The adsorption of protonated MAs onto sedimentary exchange sites has been suggested as a major porewater removal process. Speciation of the MAs between the sediments and porewaters is discussed in greater detail in Chapter 5, but some ideas are outlined below.

Sedimentary exchange sites

Wang and Lee (1990) equilibrated sediments from a freshwater lake with seawater and distilled water. MAs were strongly adsorbed to the sediment in distilled water, but were desorbed on equilibration with seawater. This arose from the inability of the MAs to compete with the major seawater ions (which are absent in distilled water) for the available exchange sites on the sediment matrix. Wang and Lee (1990)

also investigated the effect of organic matter on the adsorption coefficients of the MAs by treating the sediment with hydrogen peroxide (30 %). This destroyed 45 % of the organic matter, and gave rise to a decrease of 54.6 and 31.6 % in the adsorption coefficients of the MAs (K=4.67 to K=1.38 and K=3.94 to 1.89 for MMA and TMA respectively in an organic-rich core from Peru; DMA was not measured). It was concluded that organic matter contents significantly affect the adsorption of the MAs onto sediments.

Priest Pot is an eutrophic body of water and the sediments have a high organic content (summer chlorophyll-*a* levels up to 2300 μg/l). Hence, the ratios of $[MA_S]/[MA_{PW}]$ were examined, for both the individual and total MAs, and compared with the marine sediments, to see if greater affinity of the MAs for adsorption onto freshwater sediments was reflected in the speciation of the MAs. No significant variation was seen between the cores, but there may have been a number of reasons for this. The size of the respective MA pools in the sediments may have influenced the speciation, along with the ongoing chemical and biological processes (such as buffering of the porewater MAs), which could have obscured any abiotic speciation of the MAs. However, recoveries of internal standard from Priest Pot sediment were low, reflecting the presence of available exchange sites. This phenomenon, along with the work of Wang and Lee (1990) influenced the sedimentary microdiffusion procedure described in Chapter 2.

Adsorption and chemical alteration of sedimentary MAs

The rapid decrease in $[TMA_{PW}]$ in the Loch Etive and Firth of Lorne sediments suggests the importance of removal processes, such as adsorption to the

sediments, or incorporation into the bacterial biomass. However, [TMA_S] also decreased with depth in Oglet Bay, so if adsorption to the sediments was the major removal process, then the MAs were too strongly adsorbed to be recovered by the present method (Wang and Lee, 1990). Lee and Olsen (1984) and Wang and Lee (1990) determined a "fixed amine fraction" in their sediments which was recovered via a strong leach (5 M HF/1 M HCl). This fraction apparently included MAs which had been incorporated into the sedimentary matrix. A similar gradual incorporation of MAs to the sediments might explain the decrease in the TMA_S pool. In this context, it is interesting to note that quaternary nitrogen was observed to increase in relative importance with increasing sediment depth and constituted up to 30 % of the total organic nitrogen with depth in sediments from the Peru Upwelling (Patience et al., 1992). It is possible that the protonated MAs could be chemically altered within the sediments in some way, or incorporated into the organic or inorganic matrix. This could explain the decreases in the abundances of the sedimentary MAs which were observed in Oglet Bay and Priest Pot.

Section 4.6: SUMMARY

(1) Initial investigation of the distributions of MAs showed that they were ubiquitous in both marine and lacustrine sediments, and that they were present at higher concentrations than previously reported.

(2) Concentrations of marine TMA_{PW} were highest in Oglet Bay, and probably reflected the importance, and greater turnover, of TMA as an osmolyte for benthic

organisms in the salt marsh.

(3) The high [MMA] in both porewaters and sediments from the freshwater lake, Priest Pot, suggest that MMA derives from sources other than TMA in this core.

(4) The observed decreases in abundance of TMA_{PW} with depth in the Loch Etive cores may reflect differences in the depth of benthic colonisation in these sediments. The highest concentrations of MAs were observed at depth in the outer basin sediment, which is oxic down to about 5 cm, and is more heavily colonised by infauna than the inner basin sediment which is oxic down to $\approx$ 1 cm. An algal source for TMA is possible, although fresh algal detritus would probably have had a minimal influence on the organic matter quality of the sediments in winter when these cores were taken.

(5) A decrease in the concentrations of the sedimentary MAs was also observed, and was most obvious in the Priest Pot and Oglet Bay cores. The MAs may become irreversibly adsorbed onto the organic and/or inorganic matrix in the sediments with increasing burial depth.

CHAPTER 5

THE GEOCHEMISTRY AND SIGNIFICANCE OF THE METHYLAMINES IN AN ESTUARINE SALT MARSH

Section 5.1: INTRODUCTION

The geochemistry of the MAs was investigated in an estuarine salt marsh (Oglet Bay, UK, Figure 4.1). Details of sampling, sectioning and extraction procedures are given in Chapter 2. Oglet Bay is a well-defined, dynamic salt marsh situated on the northern shore of the Inner Mersey estuary. A comprehensive site description can be found in Appendix A. Briefly, the marsh can be divided into three zones which were defined by their stage of maturity. These were described as the upper, middle and lower Marsh (UM, MM and LM). Cores were taken from a site in each zone through a tidal cycle, in order to investigate the spatial, temporal and geochemical variability of the MAs.

The UM was mature and heavily colonized by typical salt marsh plants such as *Spartina anglica*, *Puccenelia maritima* and *Aster tripolium*. Cores from this site contained extensive root systems and plant detritus (Figure 5.1). Sediments appeared oxic with grey/black anoxic microenvironments throughout the core, presumably reflecting the degradation of plant material in areas where the microbial oxygen demand exceeded the available supply in the porewaters.

The MM was also colonised by higher plants, albeit to a lesser extent and in a more haphazard fashion than the UM (Figure 5.2). Cores from this site were taken from an area of bare mud populated only by epipelic diatoms (*Navicula* and *Nitzschia* species) and euglenoid algae (Jemmett, 1991). This sediment appeared oxic in the upper 10 mm or so and quickly became anoxic/reducing below this depth, the colour of the sediment changing from brown to grey/black.

The LM was characterised by unconsolidated mud which was colonised only by algal species. The upper 2-4 mm of the sediment appeared oxic and this site was

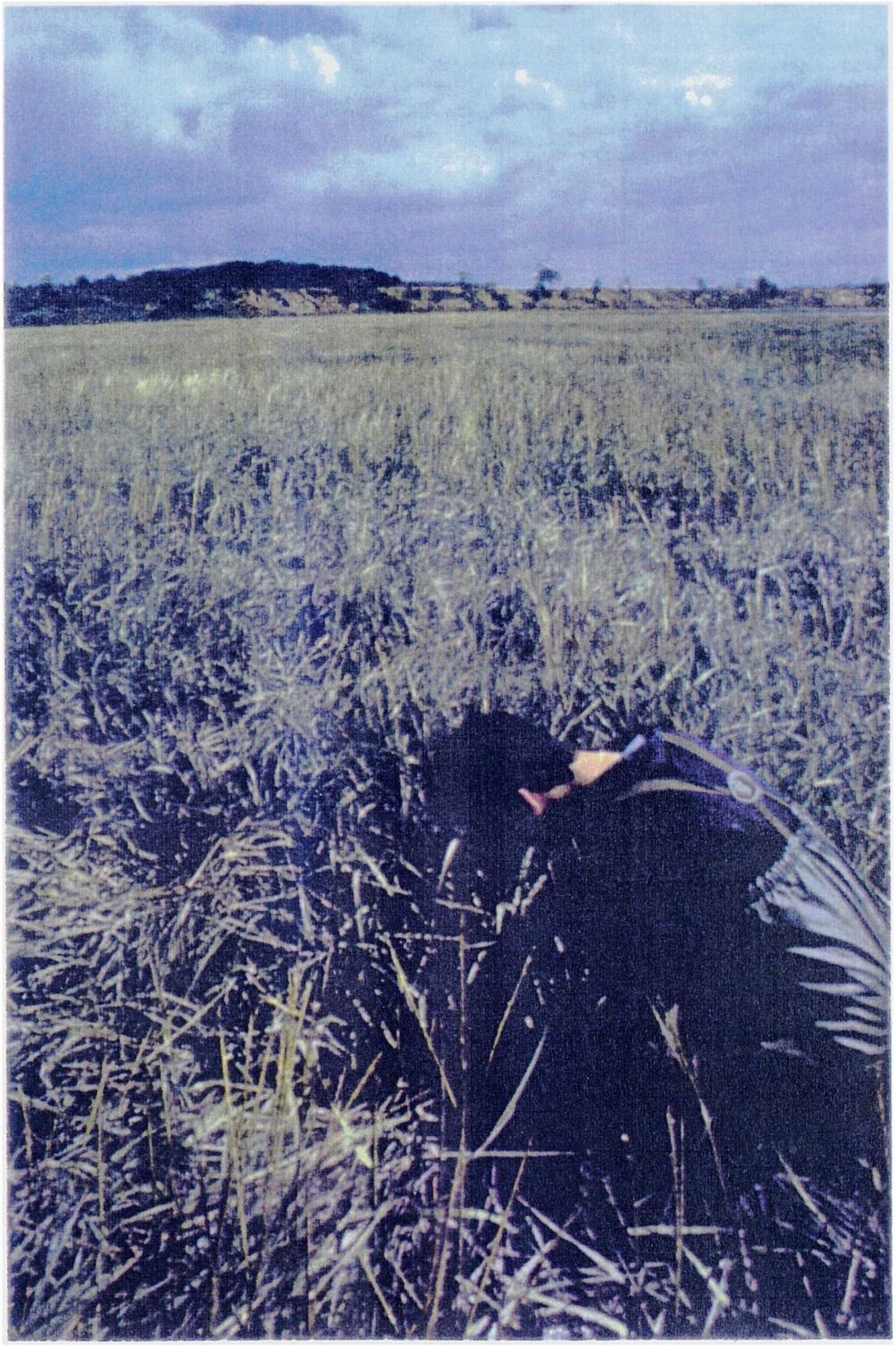
presumed to be free from the influence of higher plants (Figure 5.3).

The salt marsh was sampled through a tidal cycle in the period of late March and early April in 1992. Hence **Core Set 1** was taken one day after a high spring tide (20/3/92) when the entire marsh had been inundated. **Core Set 2** was taken 6 days later (26/3/92) around the time of the lowest neap tide, when the smallest area of the marsh was under tidal influence. **Core Set 3** was taken between this time and the next high spring tide (31/3/92) when all parts of the marsh were inundated except the UM. The last set of samples (**Core Set 4**) was taken just before the next high spring tide, when the entire marsh was submerged (3/4/92). The speciation of the dissolved and sedimentary MAs was investigated, hence all concentrations were normalised and expressed as mol/g wet sediment (ws).

A large number of cores were taken during this study. Therefore, three separate sections are presented to comprehensively discuss and compare the results for the UM, MM and LM.

Figure 5.1 overleaf shows a cross section of a UM sediment core, and views of the UM from both the shore and the river at low water.


COLLECT BAY SALT MARSH
UPPER MARSH CORE
4/8/92
TOP OF CORE



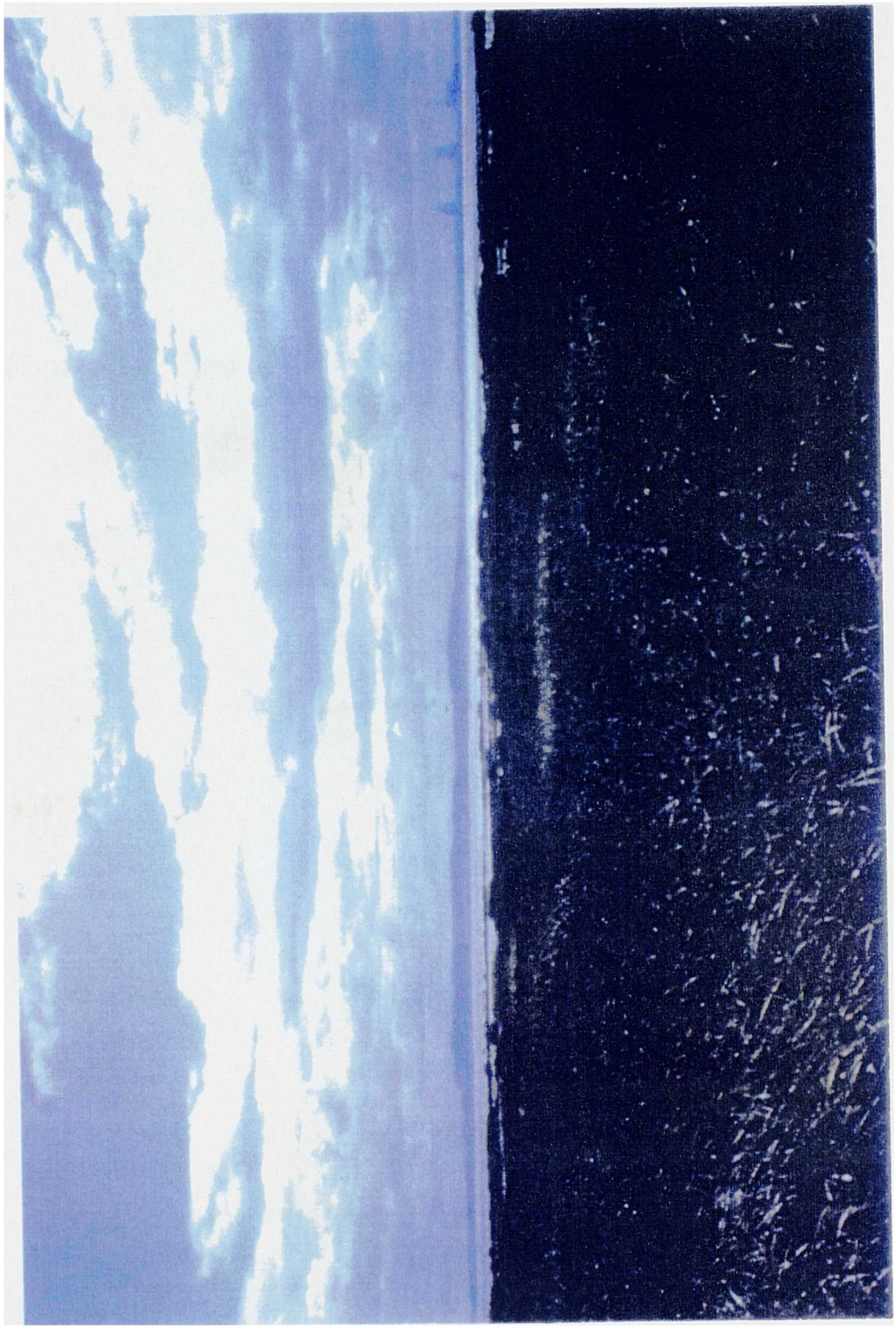
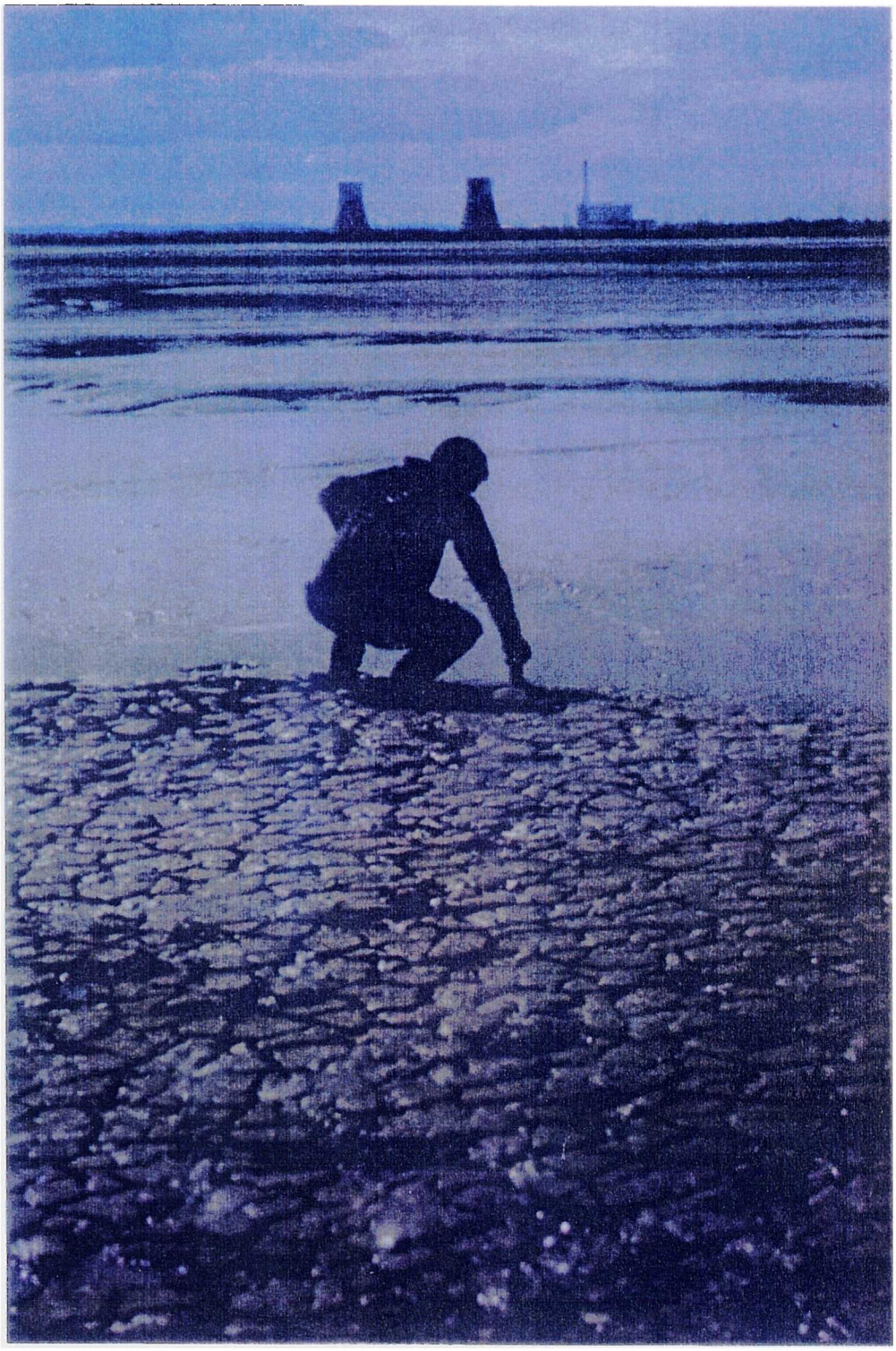


Figure 5.2 View of the MM looking shoreward at low water, which demonstrates the earlier stage of development of this zone of the marsh.

Figure 5.3 overleaf shows a cross section of a LM sediment core, and a view of the LM from the shore of the river at low water.


TOP
OF
CORE
OKLEY BAY SALT MARSH
LOWER MARSH CORE
4/8/92



Section 5.2: UPPER MARSH RESULTS AND DISCUSSION

5.2.1 Upper marsh results

Core Set 1 (20/3/92): MMA and DMA were completely absent from the porewaters and TMA was detected only in the upper 5 cms (Figure 5.4). Highest concentrations of porewater TMA ($[TMA_{PW}]$) were 3.2 μmol/g ws at the 0-1 cm depth interval, decreasing to zero by 4-5 cms.

$[MMA_S]$ and $[DMA_S]$ peaked in the upper 3 cms and gradually decreased to a depth of 10-12 cms. Subsurface maxima for both compounds were seen at 14-16 cms where $[MMA_S]$ were similar to those in the surface sediments. $[TMA_S]$ were highest at 0-1 cm, and decreased thereafter down to 5-6 cms. Below this depth, concentrations were non-zero and asymptotic in the 0.25-1.0 μmol/g ws range. MMA_S was the most abundant of the 3 analytes in the sediment (> 5 μmol/g ws) followed by TMA_S (up to 2.0 μmol/g ws) and DMA_S (up to 0.7 μmol/g ws).

Core Set 2 (26/3/92): All 3 amines were detected in the porewaters and sediment (Figure 5.6). $[MMA_{PW}]$ and $[DMA_{PW}]$ were up to 10 times higher than for Core Set 2. A sub-surface maximum was observed for MMA_{PW} at 9-10 cms, and the compound was persistent down to 16-17 cms. DMA_{PW} was only detected at 2-4 cms and again at 15-16 cms, although at much lower levels. $[TMA_{PW}]$ were comparable to $[MMA_{PW}]$ at the surface (8-16 μmol/g ws) but decreased rapidly down to 9 cms, although a subsurface maximum was observed at 9-10 cms (11 μmol/g ws). $[TMA_{PW}]$ were up to 20 times higher than $[TMA_S]$.

$[MMA_S]$ were very high in the surface sediment (up to 100 μmol/g ws), while

[DMA_S] and [TMA_S] were similar to Core Set 2. The profile of MMA_S showed no clear trend, but highest concentrations were observed at the surface (0-2 cms). [DMA_S] showed a maximum at 1-2 cms and a subsurface maximum at 7-8 cms.

Core Set 3 (31/3/92): Distributions of the MAs were distinctly different than in Core Set 1 (Figure 5.5). All 3 compounds were detected in the porewaters and sediments. The porewater profiles of MMA and DMA exhibited non-zero asymptotic concentrations below 4 cms having maximised in the 0-3 cms range. [MMA_{PW}] and [DMA_{PW}] were 2-10 times lower than in the sediment. [TMA_{PW}] (14μ mol/g ws) were up to 200 times higher than [TMA_S] in the surface of the core (0-1 cm).

Once again, MMA was the most abundant of the MAs in the sediment. [DMA_S] and [TMA_S] were comparable in this core (up to 2.5 μmol/g ws). All sediment-associated MAs exhibited subsurface maxima at 5-8 cms. Below this, concentrations decreased to surface sedimentary levels (0-2 cms).

Core Set 4 (3/4/92): MMA and DMA were absent from the porewater in this core and TMA was detected down to 7 cms (Figure 5.7). The TMA_{PW} depth profile was similar to Core Set 1. Concentrations declined from a maximum of 6.4 μmol/g ws at 0-1 cm, to zero at 6-7 cms.

The depth profiles of the sedimentary MAs were also similar to Core Set 1 but concentrations were 2-3 times higher. DMA and TMA showed a concentration maximum at 0-1 cm. The MMA_S profile varied down the core and maximised at 9-10 cms (19 μmol/g ws). [DMA_S] and [TMA_S] were less in the lower part of the core, and reached a fairly constant level below 8 cms. MMA was the most abundant

sedimentary amine and its concentration relative to [DMA_S] and [TMA_S] was similar to Core Set 1.

Inorganic geochemistry

Ammonia: Concentrations of sedimentary AMM were asymptotic with depth (< 0.1 mmol/g ws) throughout the tidal cycle in the UM (Figure 5.8). Concentrations of porewater AMM were always highest in the top half of the core and decreased with depth. A significant increase in [AMM_{PW}] was observed in Core Set 2 where the highest [AMM_{PW}], at 3-4 cms, was 3 times the maximum at 1-2 cms for Core Set 1 (0.6 compared to 0.2 mmol/g ws). In Core Set 3, the abundance of porewater AMM below the surface (0-1 cm) was decreased gradually. In the lower part of the core, however, an increase in [AMM_{PW}] was observed before a decrease to zero at 17-18 cms. Concentrations of porewater AMM decreased to sedimentary levels in Core Set 4 and were asymptotic in this range throughout the core (<< 0.1 mmol/g ws).

Nitrate: Significant concentrations of porewater NO_3^- were observed in Core Set 1 (Figure 5.9) with a maximum at 12-13 cms (0.65 mmol/g ws). The profile of NO_3^- with depth in the porewaters of Core set 2 was similar to that for AMM_{PW} in Core Set 4 (i.e. << 0.1 mmol/g ws). [NO_3^-] in the porewaters of Core Set 3 were again higher in the bottom part of the core but were not greater than 0.2 mmol/g ws. NO_3^- also increased in abundance with depth in Core set 4 down to a maximum at 9-10 cms and a second maximum at 12-13 cms (0.1 mmol/g ws at each depth) before decreasing down to a minimum at 15-16 cms (0.025 mmol/g ws). A further maximum may have occurred at 17-18 cms.

Nitrite: Concentrations of porewater NO_2^- were measured in Core Sets 1-3 only (Figure 5.10). NO_2^- was much less abundant than NO_3^- or AMM in all Core Sets (up to 10 μ mol/g ws). No significant variations in $[NO_2^-]$ were observed throughout.

Figure 5.4: Distributions of the MAs in Core Set 1 (UM, 20/3/92)

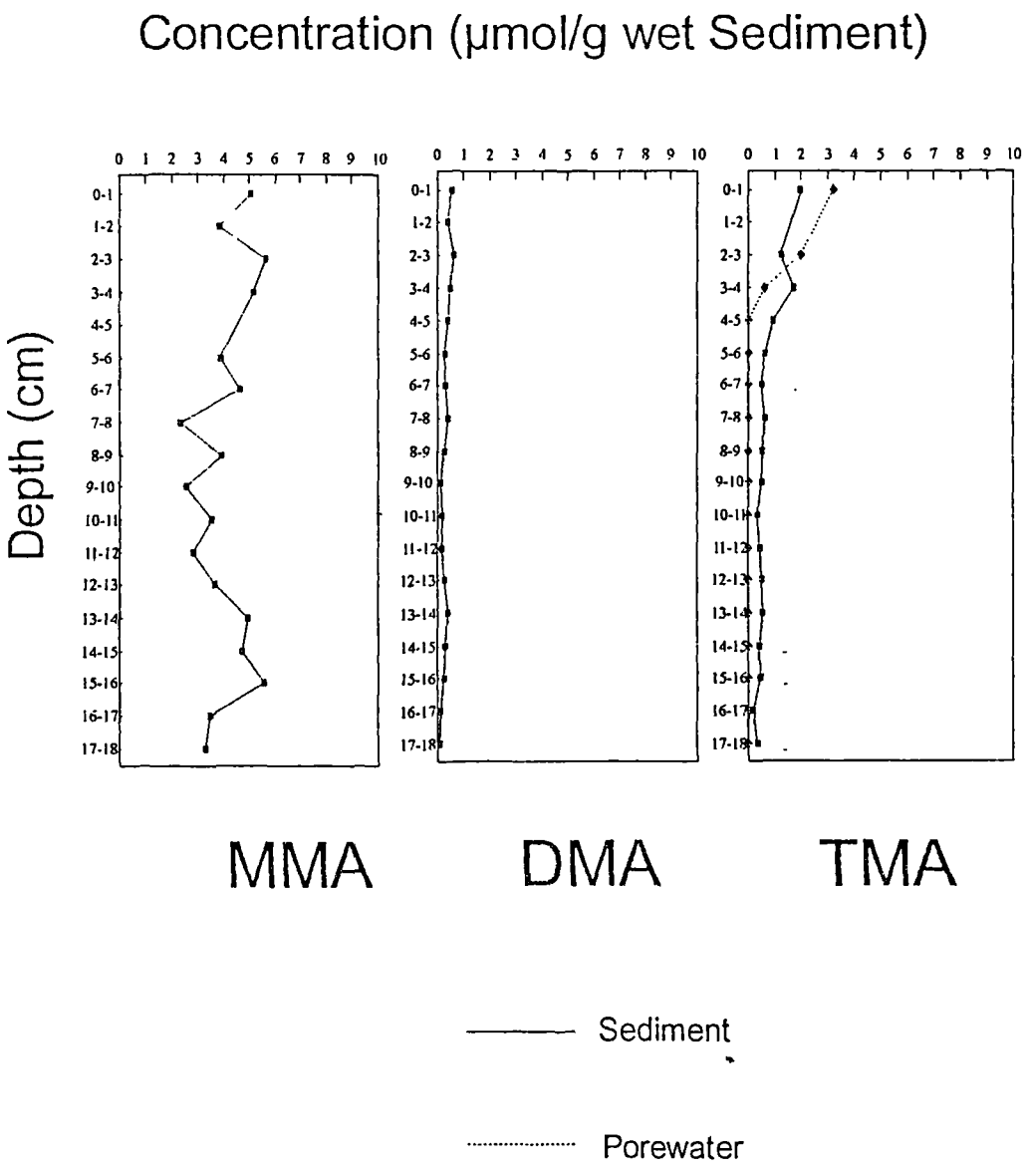


Figure 5.5: Distributions of the MAs in Core Set 2 (UM, 26/3/92)

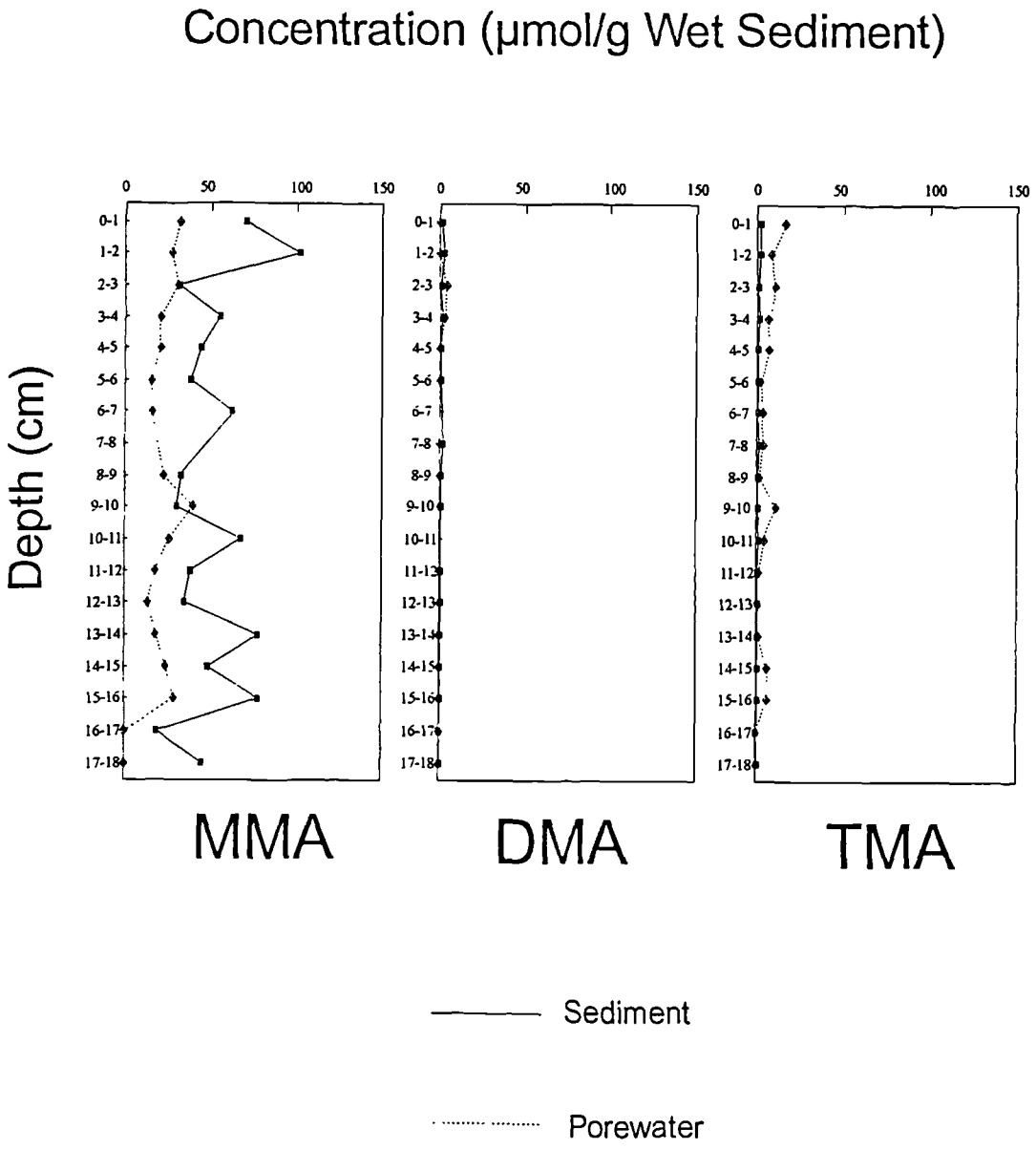


Figure 5.6: Distributions of the MAs in Core Set 3 (UM, 31/3/92)

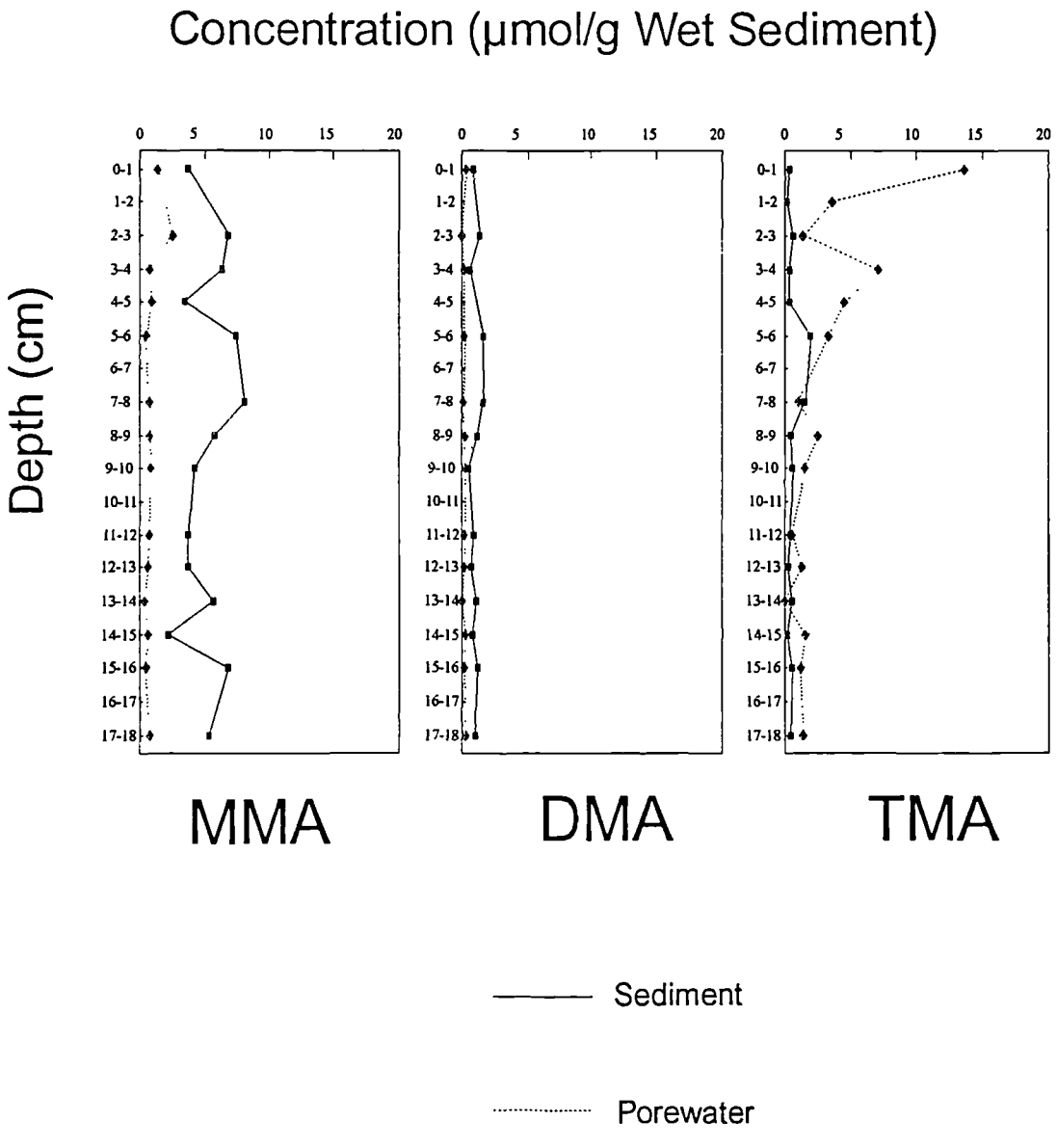


Figure 5.7: Distributions of the MAs in Core Set 4 (UM, 3/4/92)

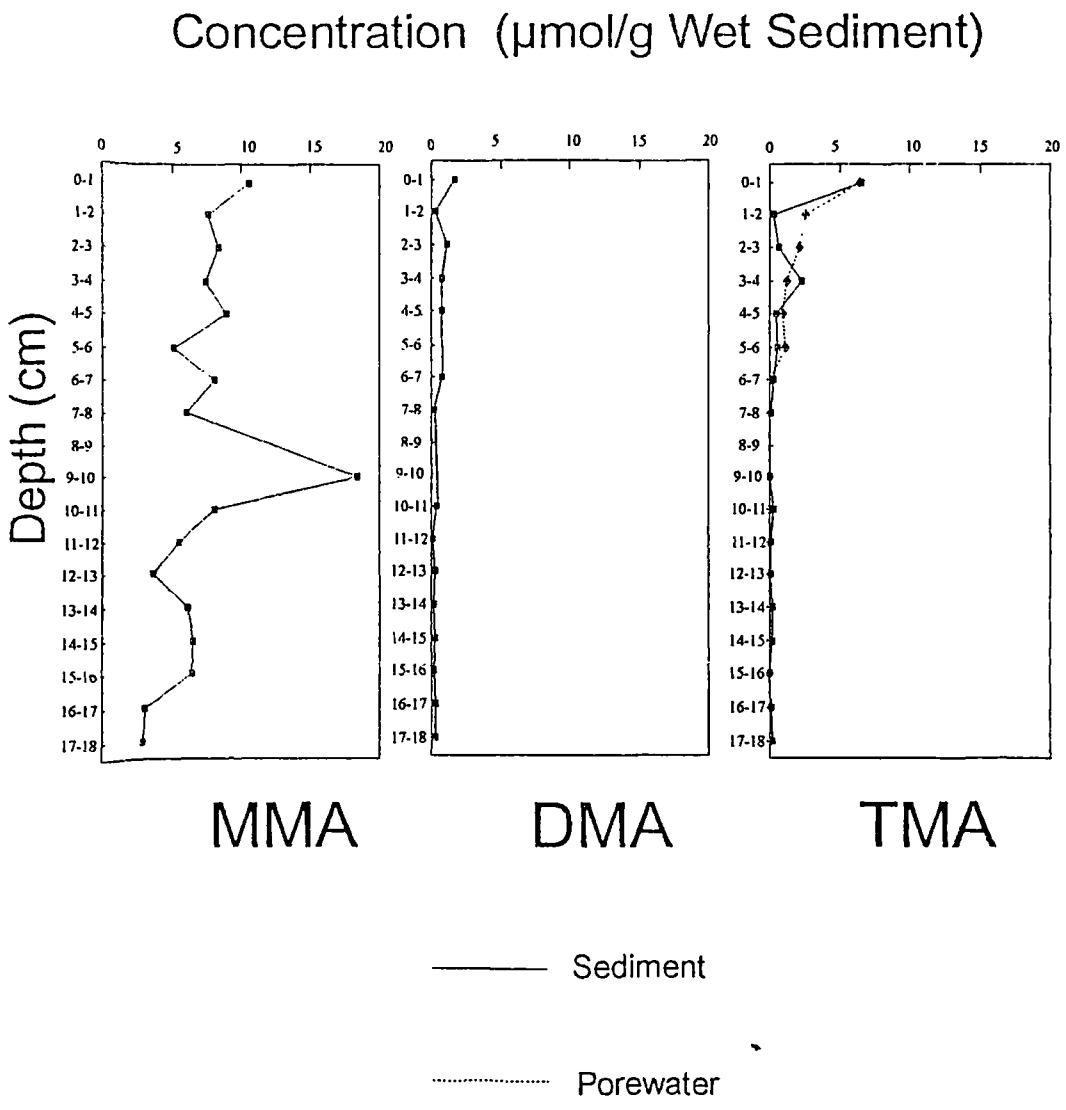


Figure 5.8 Distributions of AMM in the UM

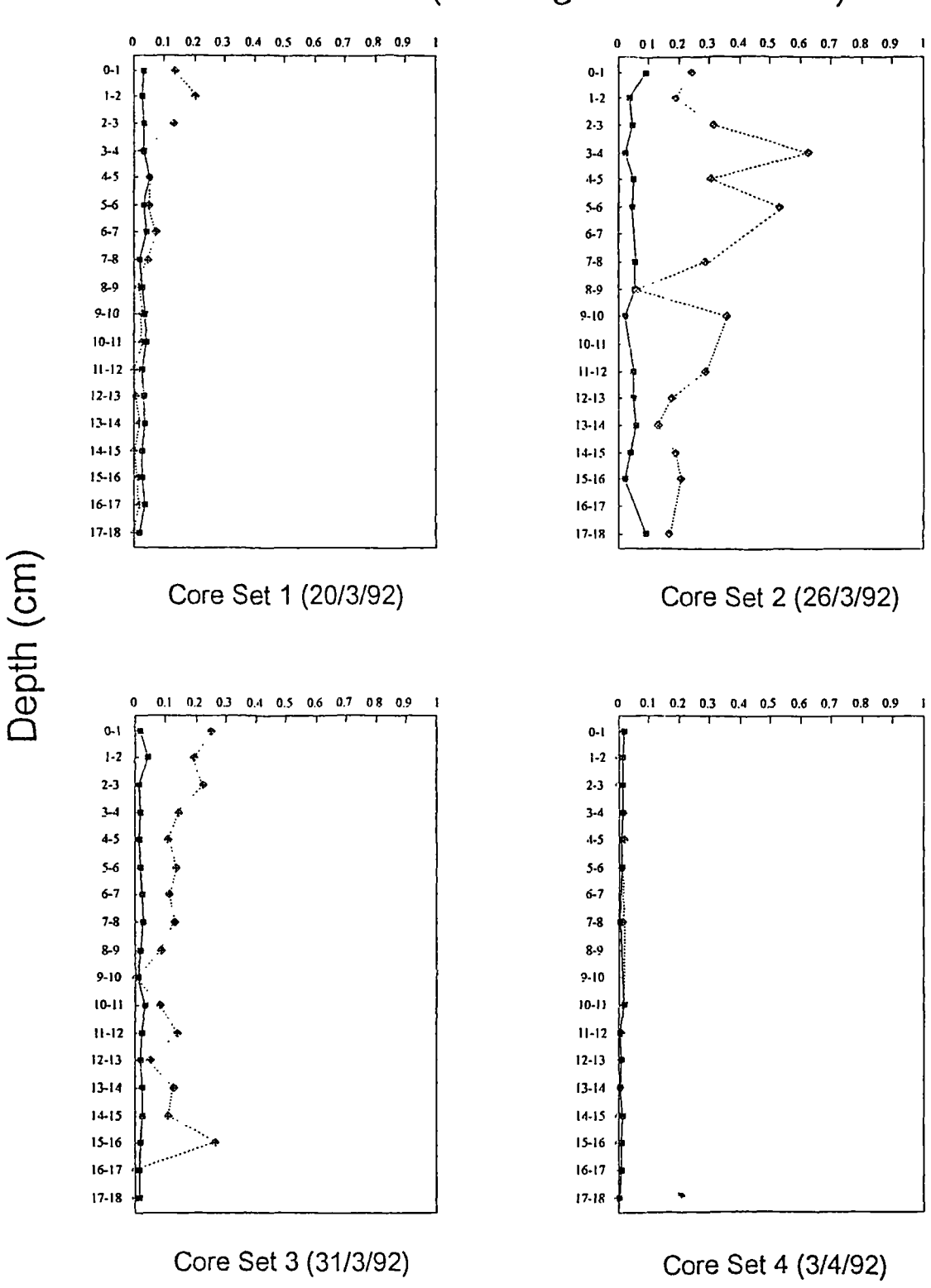


Figure 5.9: Distribution of porewater NO_3^- in the UM

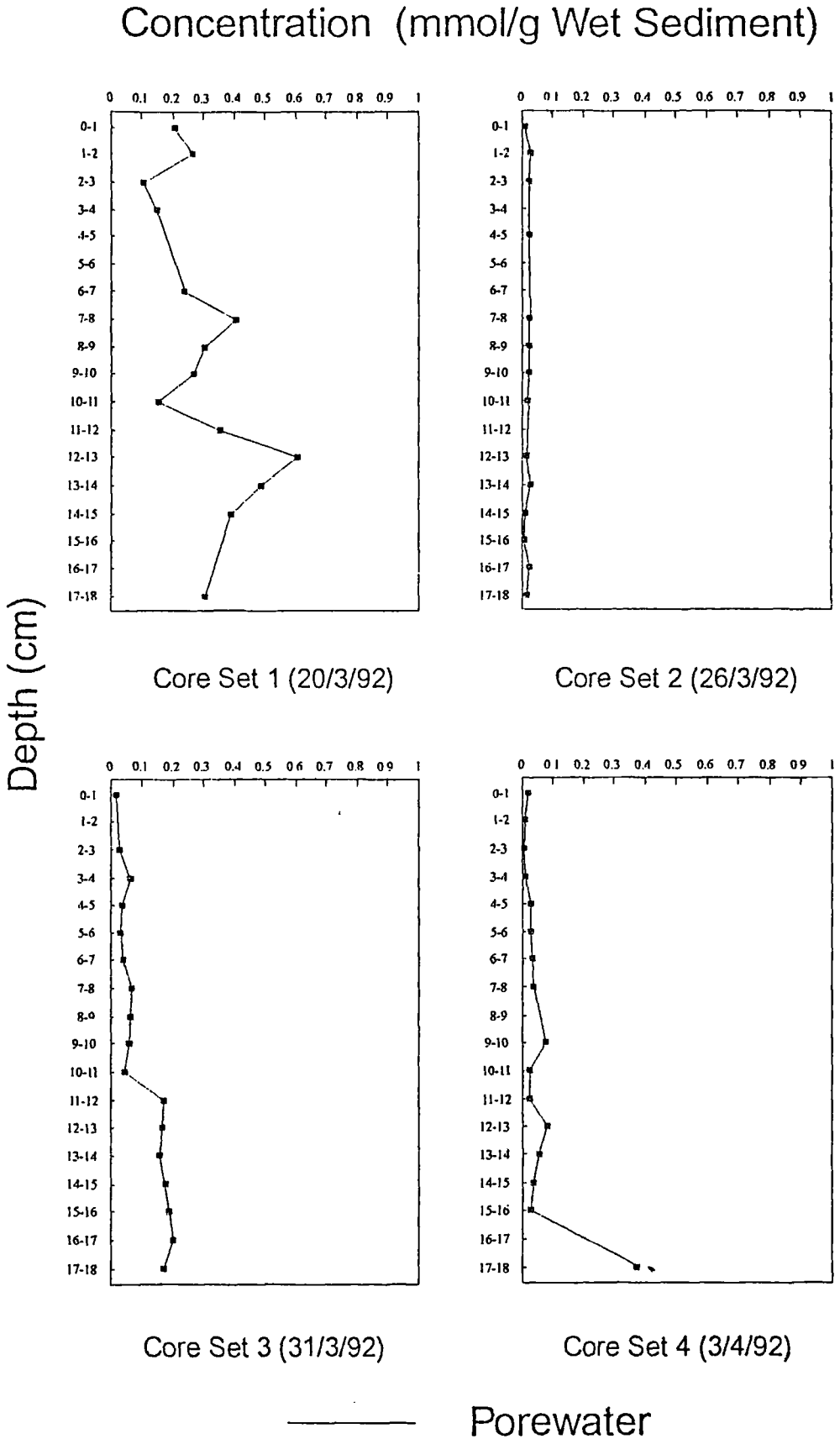
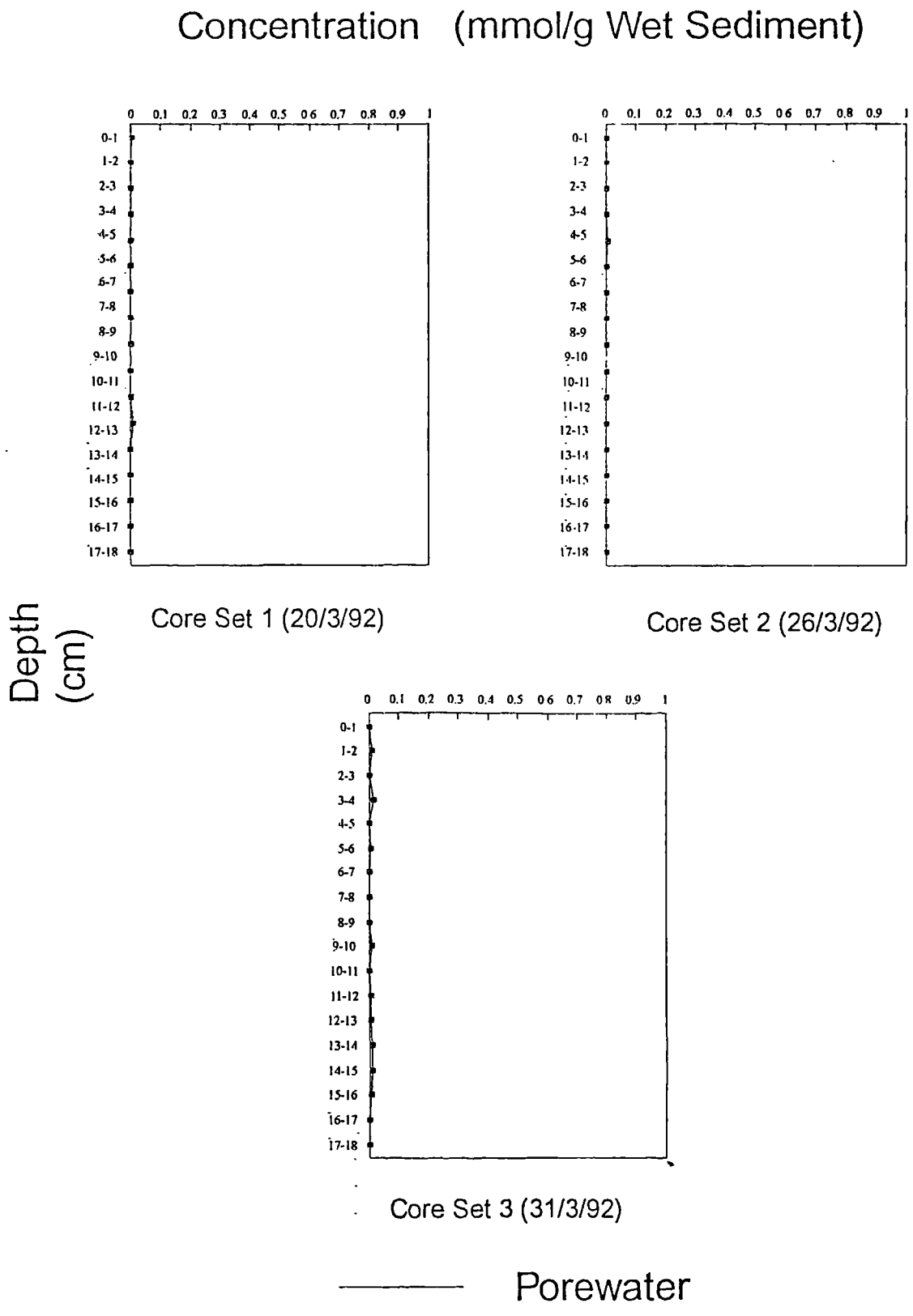


Figure 5.10: Distribution of porewater NO_2^- in the UM.



Sedimentary vs C/N ratio

In order to evaluate the contribution of the sedimentary MAs to decreases in the C/N ratio of the Oglet Bay sediments, the correlation of each MA vs the C/N ratio was evaluated. In the UM the results were as follows:

Core Set 1 A significant negative correlation between C/N ratio and MMA was observed at 95% confidence limits (r= -0.510) and for both DMA and TMA at 99.9% confidence limits (r= -0.747 and -0.885 respectively).

Core Sets 2 and 3 No significant correlation was seen for any of the compounds.

Core Set 4 DMA showed significant negative correlation with the C/N ratio at 99.9% confidence limits (r= -0.743).

5.2.2 Upper marsh discussion

Inorganic nitrogen geochemistry

Concentrations of AMM_{PW} were generally higher than AMM_S, but the pool sizes converged with depth. There was no obvious exchange between AMM_S and AMM_{PW} in the UM sediments, although such a process cannot be ruled out (Blackburn and Henriksen, 1983). Elevated levels of AMM_{PW} in surface sediments (generally < 8 cms) probably derive from decomposition of freshly deposited organic matter.

$[NO_3^-]$ in coastal and estuarine sediments are generally highest in the oxic surface sediments and decrease with depth below the redox boundary due to

involvement of NO_3^- in diagenesis as a secondary oxidant of organic matter.

At Oglet Bay, significant concentrations of NO_3^- were observed at all depths in the UM sediments (Figure 5.9). The upward diffusion of this porewater NO_3^- may have provided a source of nitrogen for the macrophyte rhizomes in the upper few cms of the sediment, leading to a depletion of the dissolved NO_3^- pool in this region. Highest concentrations of NO_3^- in the porewaters coincided with regions of the sediment where the porewater AMM pool was low. AMM is the preferred nitrogen source for phytoplankton and accounts for the majority of phytoplankton production (McCarthy et al.,1977; Nixon, 1981). NO_3^- and AMM are considered to be the principal forms of 'new' (allochthonous) and regenerated (autochthonous) nitrogen, respectively in the ocean (Dugdale and Georing, 1967). In estuaries and shallow marine systems, NO_3^- may be produced *in situ* via rapid oxidation of regenerated AMM (Kemp et al., 1982). Therefore, a portion of the AMM produced from the decomposition of organic matter may be reoxidised to NO_3^- before diffusion to the overlying water. It is possible, therefore, that the AMM produced in the porewaters of top half of the sediments was utilised by the macrophyes and resident infauna, while some of the 'excess' AMM produced at depth was oxidised to NO_3^- (Henriksen and Kemp, 1988).

In general, the profiles of the inorganic nitrogen compounds in the UM were different from those previously reported as "classical" cases in estuarine and near shore sediments, and provide evidence of the variability of this marine environment. Indeed, Valiela (1983) suggested that studies of NO_3^- in salt marsh sediments should take account of the unique and dynamic localised conditions associated with each salt marsh

Sedimentary organic matter content

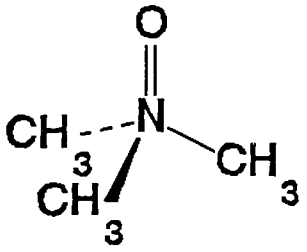
Total organic carbon (TOC) values of the sediments were up to 6% and were consistent with the high rates of sediment accretion at Oglet Bay (Appendix A). The rate of deposition of organic detritus in a salt marsh environment is higher than in most coastal or open ocean marine environments, due to the abundance of macrophytes, algae and the possible influence of untreated sewage (Valiela et al., 1975; Abdul-Rashid, 1990). The UM was the most mature area and was vegetated with native salt marsh plants. It can be reasonably assumed, therefore, that the organic content of these sediments consisted mainly of residual high molecular weight organic compounds associated with higher plant detritus (Figure 5.1).

Porewater MAs

Core Sets 1 and 4 (Figures 5.4 and 5.7) were taken a day before and after a high spring tide, respectively, when the entire marsh was inundated. The depth profiles of the MAs in the porewaters and sediments of these cores were very similar, TMA being the only compound detected in the surface porewaters in each core set.

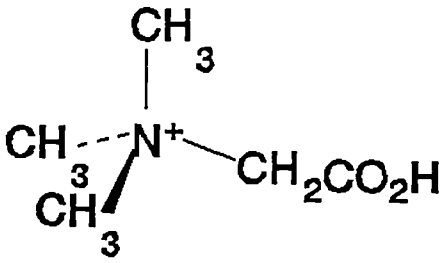
Changes in the salinity of the porewaters of intertidal sediments can result in the resident organisms accumulating toxic concentrations of ions such as Na^+ by osmosis, if they cannot regulate this process. It appears that many organisms accumulate certain organic ions at high concentrations within their living cells, in order to offset the Na^+ gradient. This uptake is not deleterious to the organisms and does not disrupt their physiology (King, 1988a). The depletion of TMA, and the absence of MMA and DMA, in the porewaters of the Core Sets 1 and 4 suggested that the MAs may be involved in this process. Concentrations of the dissolved MAs

Figure 5.11 Structures of some possible QA and AA precursors of the MAs.



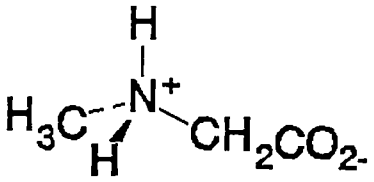
Trimethylamine oxide

(TMAO)



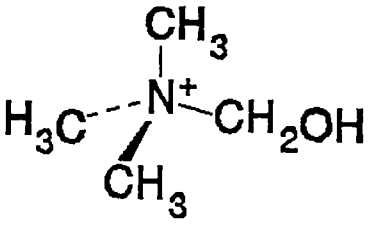
Glycine betaine

(GBT)



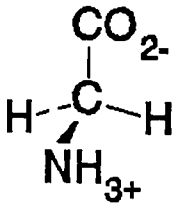
Sarcosine

(SAR)



Choline

(CHO)



Glycine

(GLY)

in the cores taken during the middle period of the tidal cycle (Sets 2 and 3) were considerably higher than Sets 1 and 4 (Figures 5.5 and 5.6). This increase in concentrations between spring tides presumably arose from the reverse of the uptake process, i.e. a diffusion of osmolytes from organisms in response to a decrease in the salinity of the porewaters. In Core Set 4, collected prior to the next spring tide, influx of fresh saline water presumably led once again to uptake of the porewater MAs to offset the ion gradient during tidal inundation of the UM. The diffusion of the MAs from benthic organisms has been suggested previously (Sorensen and Glob, 1987), along with other pathways which are discussed below.

Sources and fates of the porewater MAs

Quaternary amines (QAs) may be an important source of TMA to the Oglet Bay sediments. QAs such as glycine betaine (GBT), choline (CHO) and trimethylamine oxide (TMAO) are widely distributed in marine flora and fauna (Yancey et al., 1982; King, 1988a), and are related to strategies used for osmoregulation by a variety of organisms faced with salinity stress (see above). In certain extreme environments, QAs are a dominant nitrogen species, for example GBT levels in a hypersaline algal mat can account for up to 20 % of total mat nitrogen (King, 1988b) Some examples of QAs are shown in Figure 5.11.

Various QAs can give rise to the formation of TMA and an olefin via the Hoffman elimination, as seen in Equation 5.1 for β-alanine betaine (Miller and Springall, 1966; Allinger et al., 1971). Initially, it was feared that such a reaction might occur during microdiffusion of sediment samples (pH > 12), leading to the

detection of erroneously high [TMA] in the samples. However, the microdiffision of standard solutions of CHO and GBT (10 μ M, triplicate samples) did not yield TMA.

$$OH^{\cdot} + {}^{-}O_2C(CH_2)_2N(CH_3)_3 \longrightarrow H_2O + {}^{-}O_2CCH{=}CH_2 + N(CH_3)_3$$

(5.1)

It has been suggested that the fermentation of sedimentary QAs to yield MAs may significantly influence the flux of marine MAs (King, 1984). A number of pathways for the formation and metabolism of various QAs, including formation of DMA and TMA appear in Figure 5.12, although enzyme catalysts are not included. Hence, utilization of QA-derived MAs by methanogens is dependent on the prior fermentation of the QAs by sulphate reducing bacteria to produce TMA and acetate (King, 1984). Since sulphate reduction and methanogenesis are exclusively anaerobic processes (Goldhaber and Kaplan, 1975; Dubach and Bachofen 1985), any fermentation of the QAs in the oxic UM sediments is unlikely to have occurred. This suggests that the higher [MA_{PW}] of Core Sets 2 and 3 arise through direct diffusion of the MAs into the porewaters from benthic organisms and macrophytes.

MMA and DMA were not detected in the porewaters during the high spring tide, which raises a number of interesting questions about the source and behaviour of these compounds. They have not been reported as osmolytes, but this may have been due to limitations in the analytical techniques employed. King et al. (1983) and Lee and Olsen (1984) were unable to detect MMA in coastal sediments, although Wang and Lee (1990) reported the presence of MMA in porewaters and sediments

from a number of different sites. Significant concentrations of MMA have been detected in the sediments and porewaters from a variety of environments in the present study (Chapter 4), and also by Abdul-Rashid (1990) and Abdul-Rashid et al. (1991). Since all of the porewater MAs are likely to have been protonated (Table 3.1 for pK_a values), it is possible that they can all be used as osmolytes.

Porewater content of the sediments may influence the sedimentary speciation of the MAs. Porewaters accounted for up to 50 % of the sediments in the UM and MM, whereas the LM sediments were much more fluid (> 60 % porewater). Concentrations of dissolved MMA in the LM were consistently higher than those associated with the sediments. Furthermore, the dissolved MMA pool dominated the sedimentary pool in cores from Loch Etive and the Firth of Lorne which were composed of up to 90 % porewater. DMA has a lower pK_a value (i.e. more basic) than MMA (see Table 3.1) and is apparently more strongly adsorbed onto sediments than MMA or TMA (Wang and Lee, 1990). This may explain the higher concentrations of DMA in the sediments relative to the porewaters in nearly all cases in the present study.

$[TMA_{PW}]$ quickly declined with depth in Core Sets 2 and 3 (Figures 5.6 and 5.7) suggesting removal and/or bacterial uptake. Adsorption onto the sediment did not appear to be a major sink for TMA_{PW} in these cores, since there was no corresponding increase in the size of the TMA_S pool. The greater abundance of dissolved TMA_{PW} in the surface sediments was presumably related to the diffusion of TMA_{PW} into the porewaters from macrophytes and benthic organisms, and/or from the degradation of diffused QAs (King, 1988a, Figure 5.12).

A number of factors may have influenced the geochemical behaviour of the

MAs in the Oglet Bay UM sediments, including microbial production and utilization, adsorption, chemical alteration etc. These are outlined below:

Microbial production and utilization of the MAs

The MAs have been reported as suitable substrates for methylotrophic bacteria (Table 5.1) and other microbial inhabitants of marine sediments (Budd and Spencer, 1968; Large, 1983). Large (1983) proposed an aerobic pathway for methylotrophic degradation of the MAs, resulting in the formation of methanal (Figure 4.7). The rapid disappearance of TMA_{PW} relative to MMA_{PW} and DMA_{PW} with depth in Core Sets 2 and 3 may have arisen as a consequence of the activity of such bacteria, which could also have contributed significantly to the MMA_{PW} and DMA_{PW} pools.

Table 5.1 indicates that many bacterial strains do not have the capacity to utilise all three MAs. For example, the heterotrophic methylotrophs *Pseudomonas* AM1 and *Pseudomonas* MA only utilise MMA. This may be due to the fact that these organisms do not possess the appropriate enzymes for the tertiary and secondary demethylation steps (trimethylamine dehydrogenase and dimethylamine dehydrogenase, respectively). Furthermore, Budd and Spencer (1968) found that *Micrococcus sp.* (an aerobic marine heterotroph) could utilise all three MAs as a sole source of nitrogen, but had a preference for MMA and DMA. They suggested that removal of the first methyl group from TMA (a tertiary amine) was an unfavourable endogenic process not associated with demethylation of the primary and secondary MAs. While emphasising that results from culture experiments should be treated with caution when applied to nature, Budd and Spencer (1968) suggested that degradation of the MAs was a specialised process and that an extensive flora of marine bacteria

may be involved specifically in the regeneration of amine nitrogen. If true, this may mean that the high concentrations of the MAs observed in the porewaters of the UM are essential to the survival of certain strains of nitrogen bacteria, the activities of which may influence the abundance of the MAs in these sediments.

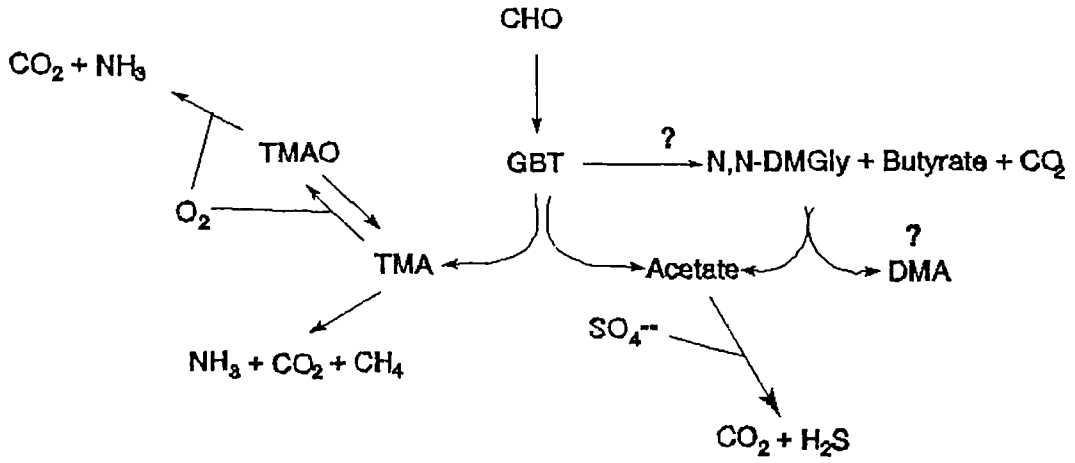
Sediment-porewater exchange

Blackburn and Henriksen (1983) reported that high [AMM_S] resulted from adsorption during periods of high productivity, and that this buffered the porewater AMM flux during winter and early spring when [AMM_{PW}] were much lower. In this way, the AMM_{PW} flux varied little throughout the year. It is conceivable that the MAs could behave analagously in the short term when processes such as osmoregulation could cause a dramatic decrease in surface concentrations of the porewater MAs. However, since MMA and DMA were not detected in the porewaters, it seems that any exchange between the sediments and porewaters occurring at this time, was rapidly offset by processes such as microbial utilization, as MMA and DMA have also been implicated as bacterial substrates (Table 5.1). The porewater AMM flux also varied considerably through the tidal cycle (Figure 5.8), so a longer seasonal study is required before conclusions can be drawn on the influence of the sedimentary MAs in buffering the porewater flux.

Effect of plant detritus on the sedimentary MA distribution

The high [MMA_S] and [DMA_S] relative to the porewaters may resulted from direct association with higher plant detritus, i.e. decomposition products of *Spartina* or could have occurred indirectly via adsorption of the MAs onto the organic

Figure 5.12 Pathways for the formation and metabolism of various QAs and MAs (including DMA and TMA). After King (1988a).



matrix. Mackin and Aller (1984) have reported that the presence of seagrass detritus etc., such as *Thalassia* may produce anomalously high NH_4^+ adsorption coefficients in the sediments. Valiela et al. (1985) observed increases in the absolute and relative concentrations of nitrogen during the decomposition of *Spartina alterniflora*. This may have been due to the complexation of nitrogenous compounds with plant phenolic compounds and lignin (Lee et al., 1980; Wilson et al., 1986).

The concentrations of the MAs were plotted against TOC to provide evidence of preferential adsorption to sediments with high TOC contents in the UM. MMA correlated significantly at 95% confidence limits in Core Set 2 (r= -0.511) and TMA at 90% confidence limits in Core Set 1 (r= -0.425), but no other correlation were observed. These measurements show little evidence of [MA_S] being influenced by TOC levels. However, significant changes in TOC were not observed in these cores (generally 40-45 mg/g sediment), so the influence of variation in the organic matter content of the UM sediments on sedimentary MA concentrations could not be gauged.

Irreversible adsorption of MAs

Lee and Olsen (1984) and Wang and Lee (1990) claimed to have measured a "fixed" methylamine fraction using a HF/HCl extraction (5M HF-1M HCl, 24 h). It may be that, in this study, the microdiffusion of sediment samples extracted only the loosely-held and exchangeable MAs. Therefore, the extent of a decrease in abundance of the MAs which was due to subsequent irreversible adsorption onto the sediment surface, or an incorporation into the sedimentary organic matrix cannot be quantified.

Wang and Lee (1990) carried out adsorption experiments with radiolabelled MAs and found that adsorption of the amines was irreversible over the time scale of their experiment (72 h). Their LiCl extraction of adsorbed ^{14}C-labelled amines removed only about 80% of the adsorbed MAs, which also suggests that adsorption may not be completely reversible.

The adsorption of various organic compounds by clay minerals retards their biodegradation (Wazolek and Alexander, 1979; Stotzky, 1980; Marshman and Marshall, 1981; Gordon and Millero, 1985). If the sedimentary MAs were unavailable to microbes, then processes such as those suggested below (along with irreversible adsorption) may also have been responsible for their removal.

Chemical alteration of sedimentary MAs

Patience et al. (1992) studied the functionality of ON in some recent organic-rich sediments from the Peru Upwelling and found at least four different ON-containing functional groups including amino and quaternary nitrogen. Amino nitrogen was found to account for (at most) 40-45 % of ON in the surface sediments, but this fraction declined rapidly with depth, whereas the proportion present as

(tentatively) quaternary nitrogen increased from 10-30 % with burial. Other workers have also reported a decrease in amino acid (AA) concentrations with depth and that AAs make up proportionately less of the total nitrogen deeper in the sediments (Emery et al., 1964; Whelan, 1977). This may indicate that amino nitrogen compounds (including the MAs) can be chemically altered within the organic matrix, thus reducing the fraction of sedimentary MAs detected.

The profiles of the sedimentary MAs in Core Sets 1 and 4 suggested that the MAs were being removed from or incorporated into the sediments in some way. Suggestions have been made as to the processes responsible, but a knowledge of the QA and AA pools in the cores would have been of considerable assistance in completing the picture, along with the percentage of adsorbed MAs recovered using the present method.

Influence of adsorbed MAs on the C/N ratio

MMA ranged from 9-13 % as a mean percentage of total sedimentary organic nitrogen (TON) through the cycle (Appendix B). Ranges for DMA and TMA were 1.0-2.7 % and 1.7-2.8 % respectively. Hence, the MAs represent a significant proportion of TON in the sediments, and the high percentage of MMA suggests that the MAs may strongly influence a lower C/N ratio in these sediments.

Freshly deposited and unaltered marine organic matter has C/N ratios of 6-8 (Redfield et al., 1963; Bordovsky, 1965; Walsh et al., 1981), whereas the C/N ratios of terrestrial plants may be as high as 36. Humic acids derived from partially degraded leaves may, however, in some cases approach the C/N ratios reported for marine source material (Nissenbaum and Kaplan, 1972; Ertel and Hedges, 1985).

The C/N ratio in the UM sediments ranged from 11-20, implying that the sources were neither purely marine or terrestrial, and that any terrestrial organic material must have been extensively reworked. Interestingly, there have been a number of reports of decreasing C/N ratios with depth in both deep sea and coastal sediments (Arrhenius, 1952; Mayer and Rice, 1992; Cowie and Hedges, 1992), this being the reverse of the expected trend for sinking particulates in the water column and many sediments (Holm-Hansen et al., 1966; Gordon, 1971; Lee and Cronin, 1982; Wakeham et al., 1984). It has been suggested that a decreasing C/N ratio in deep sea sediments with increasing depth is due to the presence of ammonium ions and low molecular weight organic nitrogen compounds, which are produced during the decomposition of organic matter and fixed in the interlayers of clay minerals (Stevenson and Tilo, 1970; Stevenson and Cheng, 1972). Certainly, Lee and Olsen (1984) claimed to have isolated a "fixed" MA fraction in the sediments.

MMA correlated significantly with the C/N ratio in Core Set 1 only. It seems strange that the elevated concentrations of MMA_S seen in Core Set 3 did not seem to influence the C/N ratio in the same way. Lee and Olsen (1984) deduced that since DMA and TMA made up about 0.5% of the total nitrogen in their shallow coastal sediments samples, it was unlikely that they would significantly alter the C/N ratio. However, they did not measure MMA which was by far the most abundant sedimentary MA in Oglet Bay.

DMA correlated significantly in Core Sets 1 and 4. DMA is the most basic of the MAs (Table 3.1) and may, therefore, adsorb to the cationic exchange sites on the sediments more strongly than MMA or TMA. The sedimentary DMA concentration was nearly always greater than in the porewater. [DMA_S], however, did

not vary significantly during the tidal cycle so it is possible that the organic signal giving rise to the [DMA_S] was effected by processes such as increased microbial utilization of DMA in the sediments at this time. However, Mayer and Rice (1992), reporting on the early diagenesis of protein in an intertidal sediment, suggested that the labile organic nitrogen fraction is altered with depth and the lower C/N ratios of remineralized materials arise through the replacement of algal detritus (rich in volatile nitrogen compounds) with more slowly decaying nitrogen products of microbial resynthesis.

5.2.3 Summary

(1) The profiles of the MAs in the sediments and porewaters of the UM showed significant variations throughout the period of sampling.

(2) The depleted surface [MA_{PW}] during the High Spring tides may have arisen through their uptake by higher plants and benthic organisms in response to saline water stress. Porewater depletion was not observed during the neap tide, when the UM was not inundated.

(3) MA exchange between the dissolved and sedimentary pools during periods of porewater depletion was not obvious, but any buffering may have been offset by the rapid microbial utilisation of MAs released from the sediments.

(4) The decreases in the [MA_S] throughout the cores were thought to be the result of irreversible adsorption onto the sediments and/or incorporation into the

sedimentary organic matrix. TOC varied little in the cores, so its effect on the [MA_S] pool was not obvious. Furthermore, as the extraction process may not measure all the sedimentary MAs, the influence of adsorption and incorporation into the sediments cannot, as yet, be quantified.

Section 5.3: MIDDLE MARSH RESULTS AND DISCUSSION

5.3.1 Middle Marsh results

Core set 1 (20/3/92): Concentrations of the MAs in the sediments and porewaters were similar to those found in the UM (Figure 5.13). TMA was the most abundant porewater MA, but all were present at significant concentrations. Highest $[MMA_{PW}]$ and $[DMA_{PW}]$ in the top 5 cms were about twice those of the deeper sections. $[TMA_{PW}]$ were 5 times higher than $[TMA_S]$ in the top 1 cm of the core, but quickly decreased to concentrations approaching those found in the sedimentary phase (2 μ mol/g ws). A subsurface maximum for TMA_{PW} was apparent at 5-6 cms (6.4 μ mol/g ws), which was greater than the maximum concentration at the surface. $[TMA_{PW}]$ declined down to a minimum at 8-9 cms where levels were again similar to $[TMA_S]$.

$[MMA_S]$ were highest in the top 9 cms of the core with a maximum at 6-7 cms (8 μ mol/g ws). $[DMA_S]$ were about 10 times lower than $[MMA_S]$ and varied little throughout the core. $[TMA_S]$ were in the range of 0.5-1.5 μ mol/g ws for most of the core, with levels decreasing below 13-14 cms.

Core Set 2 (26/3/92): TMA was present in high concentrations at the 0-1 cm depth interval, but was absent between 1-8 cms. However, at the 9-10 cms depth interval a subsurface maximum in $[TMA_{PW}]$ was observed (Figure 5.14). DMA_{PW} was only present between 9 and 11 cms, while MMA_{PW} was not detected above the 3-4 cms depth interval, and concentrations did not increase above 3 μ mol/g ws.

MMA_S was more abundant than DMA_S or TMA_S, whose profiles showed

MMA_S was more abundant than DMA_S or TMA_S, whose profiles showed minor variation with depth, and were in the same concentration range. A subsurface maxima of MMA_S was observed at 5-6 cms (7.5 μ mol/g ws). The sedimentary profiles of the MAs did not show any clear trends, except that concentrations seemed to be lower in the bottom few cms of the core.

Core Set 3 (31/3/92): Porewater concentrations of MMA and TMA were similar in this core while DMA was much less abundant (Figure 5.15). Concentrations of MMA_{PW} were also 2-3 times higher than the $[MMA_S]$ in the top 7 cms of the core. Maxima for $[MMA_{PW}]$ were observed at 2-3 and 16-17 cms, while the TMA_{PW} profile exhibited maxima at 7-8 and 10-11 cms. The TMA_{PW} profile was similar to Core Set 1 (see Fig. 5.13) showing a substantial decrease in $[TMA_{PW}]$ below 7-8 cms.

MMA_S was the most abundant MA in the sediments. Concentrations of MMA_S were highest below the surface, while DMA_S behaved asymptotically throughout the core. $[DMA_S]$ were much lower than $[MMA_S]$ but were again similar to $[TMA_S]$ in this Core Set. TMA_S was the only MA to show possible evidence of surface enrichment.

Core Set 4 (3/4/92): MMA_{PW} was not detected in this core, while DMA was detected only at 4-5 cms (Figure 5.16). Concentrations of TMA_{PW} were similar to Core Set 3. The profile exhibited a sharp decrease in TMA_{PW} concentration (cf. Core Sets 1 and 3).

MMA_S was the most abundant MA, and showed evidence of surface

surface sediments. DMA_S and TMA_S were 10 to 15 times less abundant than MMA_S, but depth profiles of these MAs exhibited similar trends. Hence, both profiles showed a maximum at 5-6 cms, but concentrations were higher than at 16-17 cms (2.5 μmol/g ws).

Inorganic geochemistry

Ammonia: Concentrations of AMM_{PW} were up to 0.5 mmol/g ws at the sediment surface, and decreased slightly with depth in Core Sets 1, 2 and 3 (Figure 5.17). However, $[AMM_{PW}]$ in the bottom few cms of the cores were similar to those observed at the surface. Highest concentrations of dissolved AMM were recorded in the middle of Core Set 4 at 8-9 cms (1.0 mmol/g ws), but maximum porewater concentrations above and below this depth were rarely above 0.5 mmol/g ws.

Maximum concentrations of AMM_S were approx. 0.25 mmol/g ws and were observed in Core sets 1 and 3. $[AMM_S]$ in the other two Core Sets were generally much lower.

Nitrate: Concentrations of porewater NO_3^- in Core Set 1 were very high (> 0.9 mmol/g ws in the top 1 cm) relative to those observed in subsequent Core Sets (Figure 5.18). High levels of NO_3^- also persisted with depth in Core Set 1. This was in stark contrast to the distributions of NO_3^- observed in Core Sets 2, 3 and 4 which were up to 20 times lower in the surface sediment. NO_3^- was more abundant in Core Set 2 than in 3 and 4, but did not approach the levels in Core Set 1.

Nitrite: As in the UM, no significant variation was seen in levels of NO_2^- in Core

Nitrite: As in the UM, no significant variation was seen in levels of NO_2^- in Core Sets 1 to 3 (Figure 5.19). Maximum concentrations were up to 9 μ mol/g ws and NO_2^- was not detected in many regions of the sediment.

Figure 5.13: Distributions of the MAs in Core Set 1 (MM, 20/3/92).

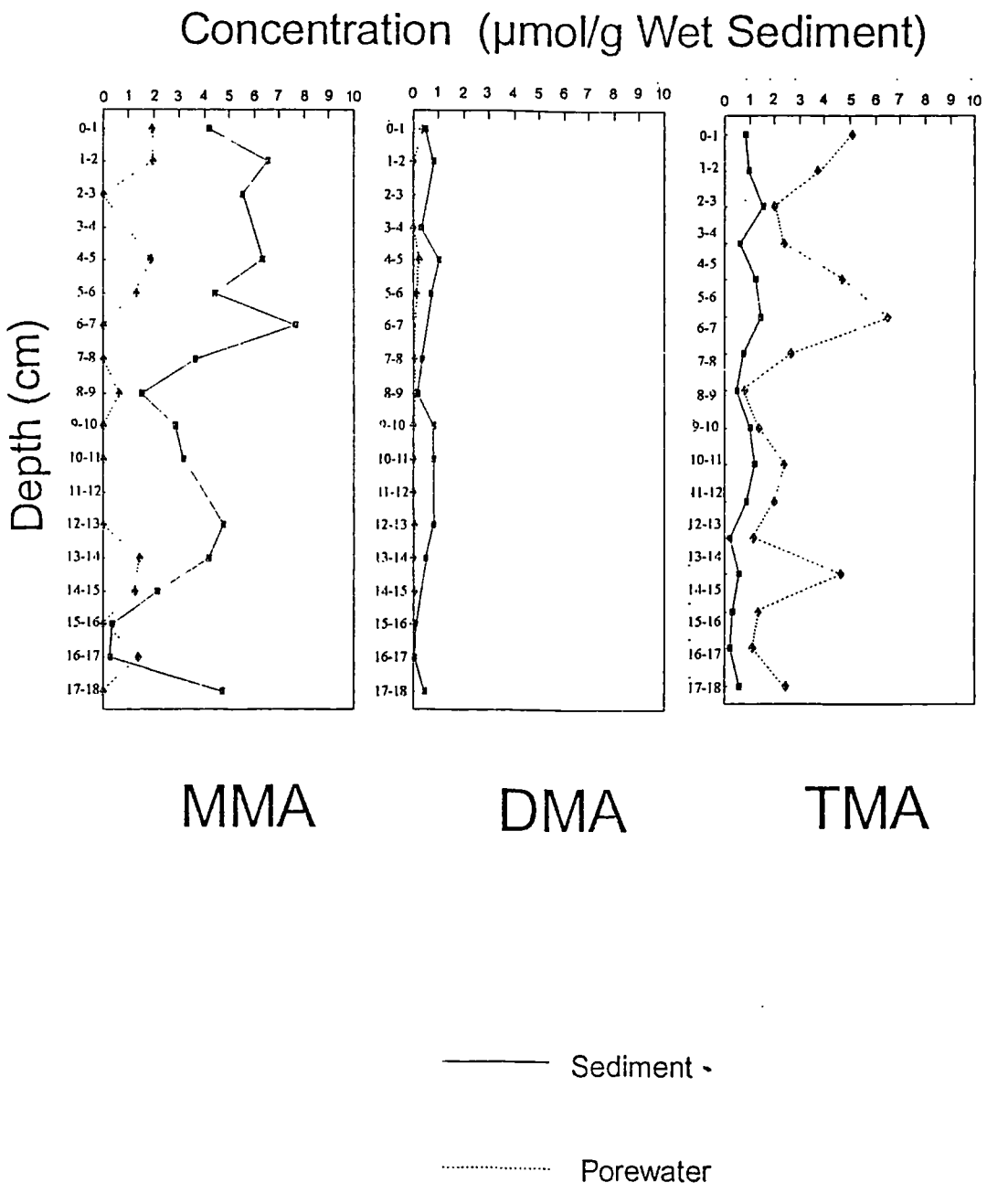


Figure 5.14: Distributions of the MAs in Core Set 2 (MM, 26/3/92).

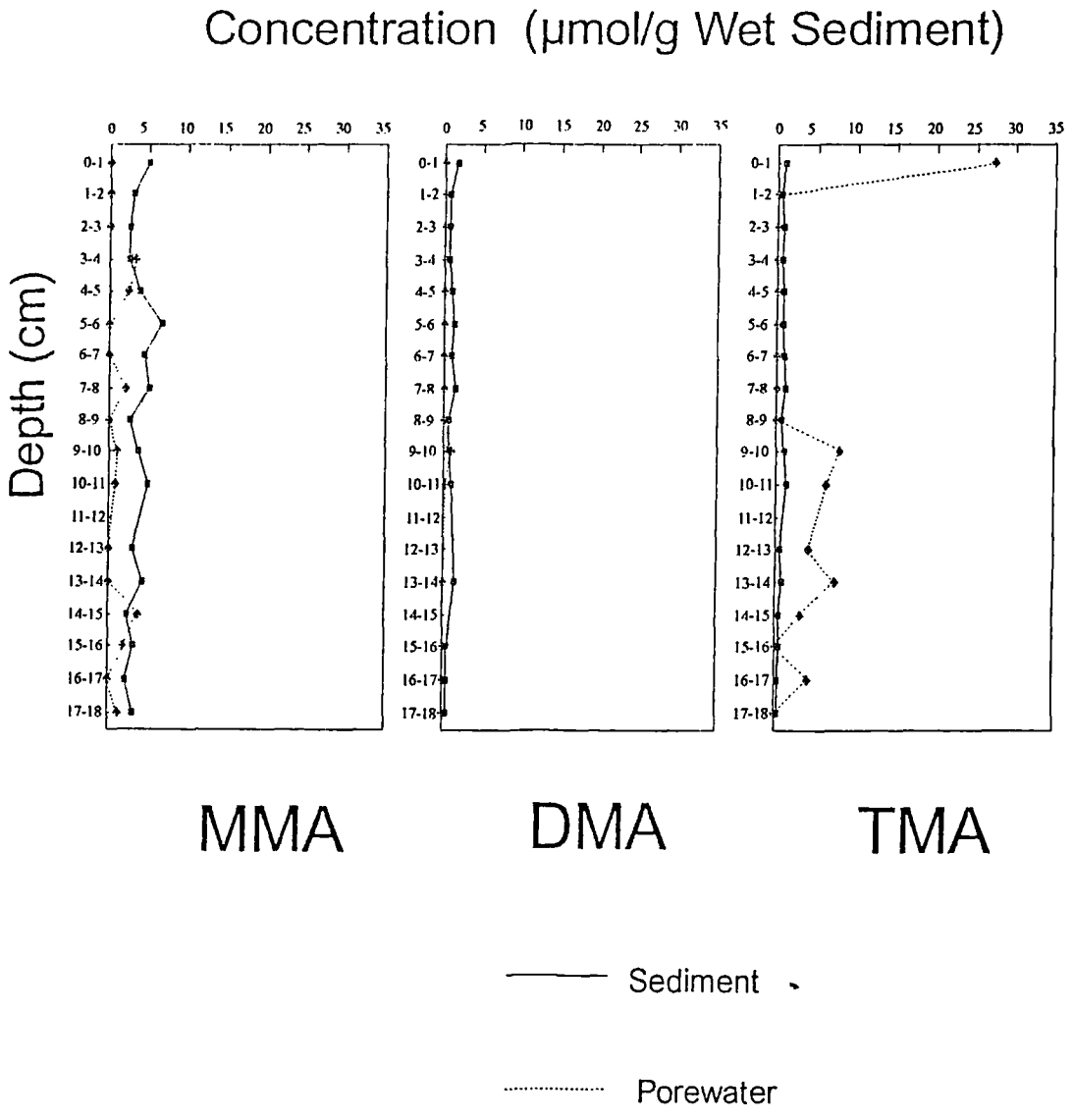


Figure 5.15: Distributions of the MAs in Core Set 3 (MM, 31/3/92).

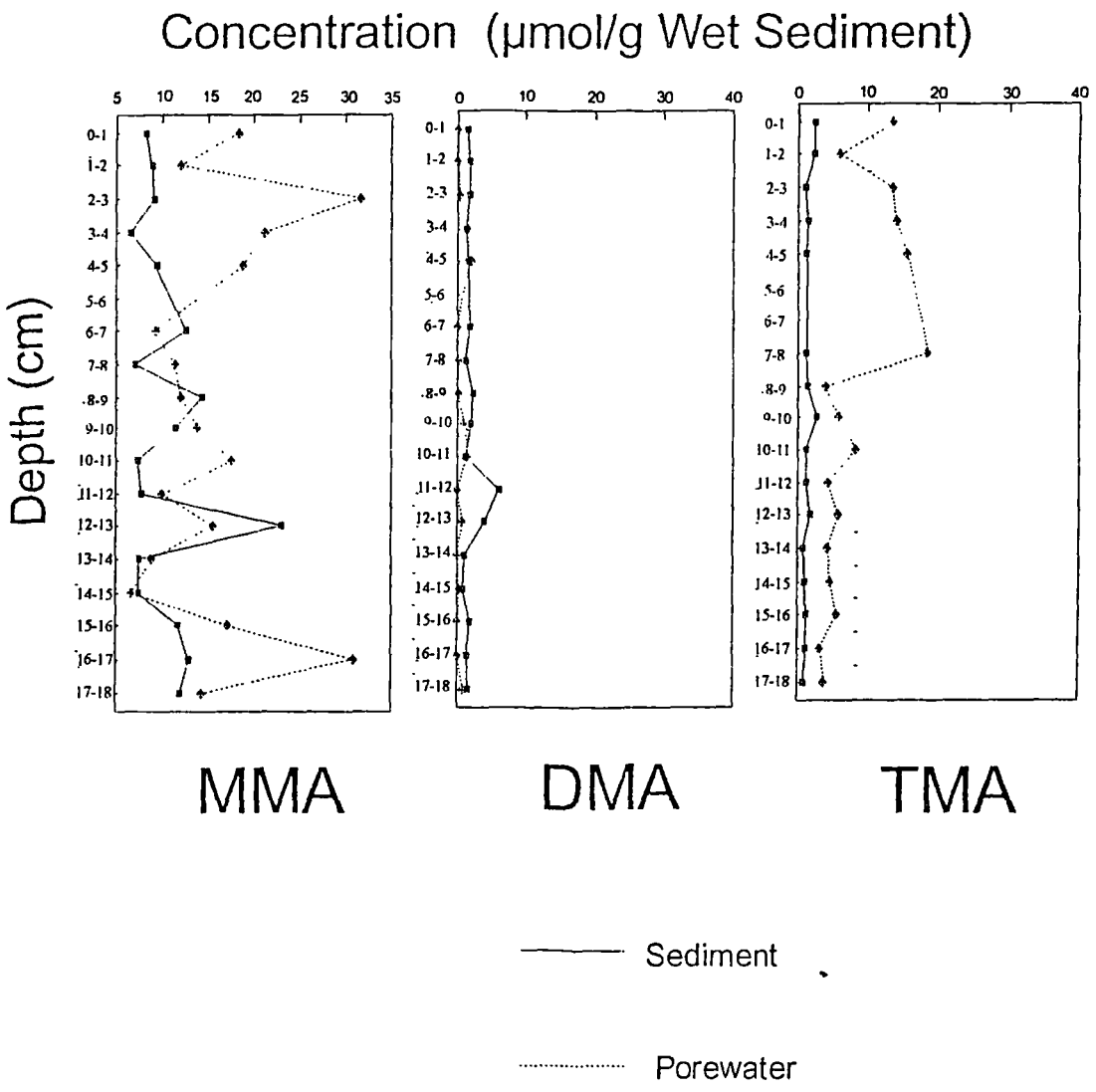


Figure 5.16: Distributions of the MAs in Core Set 4 (MM, 3/4/92)

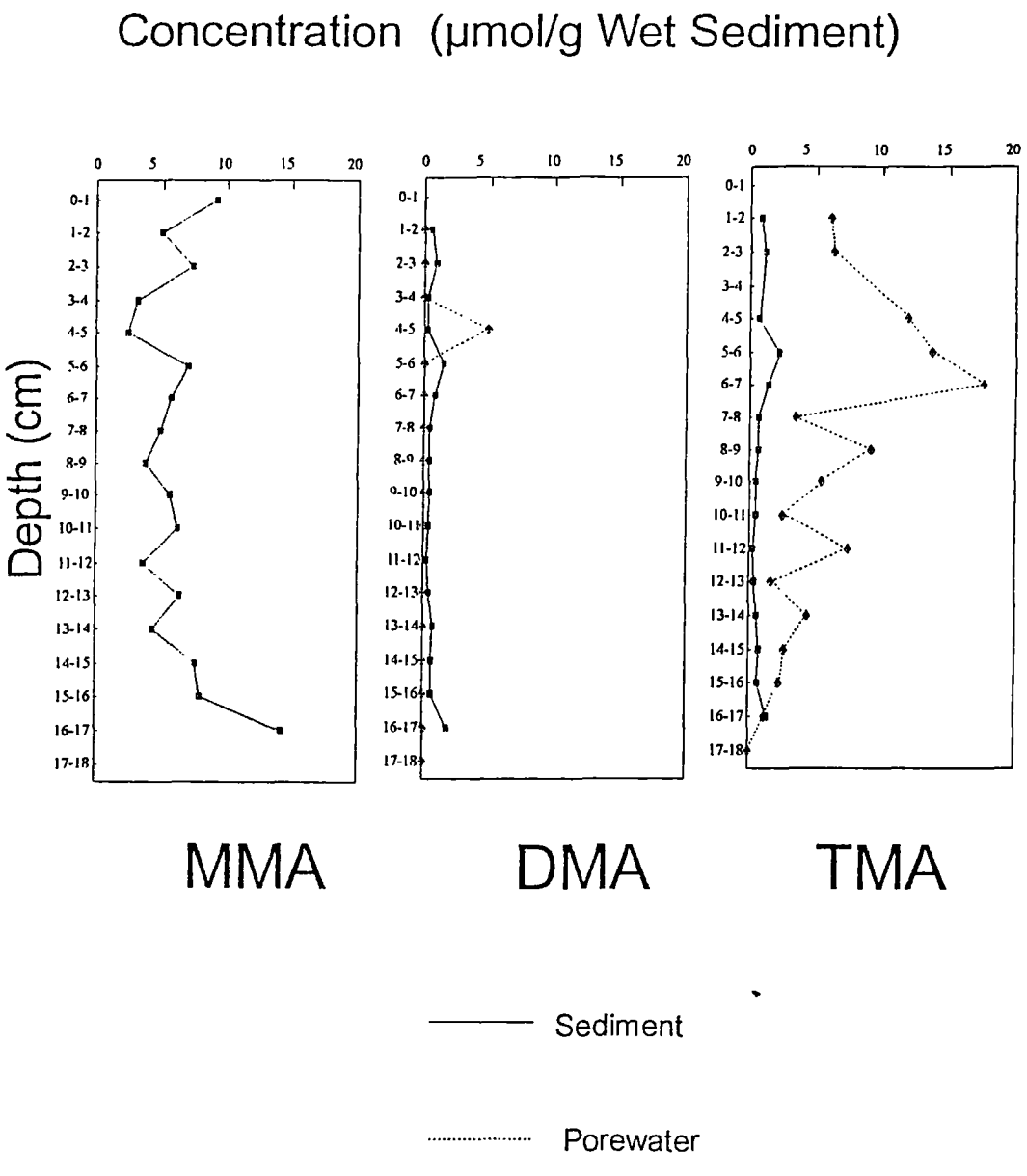


Figure 5.17 Distributions of AMM in the MM

Concentration (mmol/g Wet Sediment)

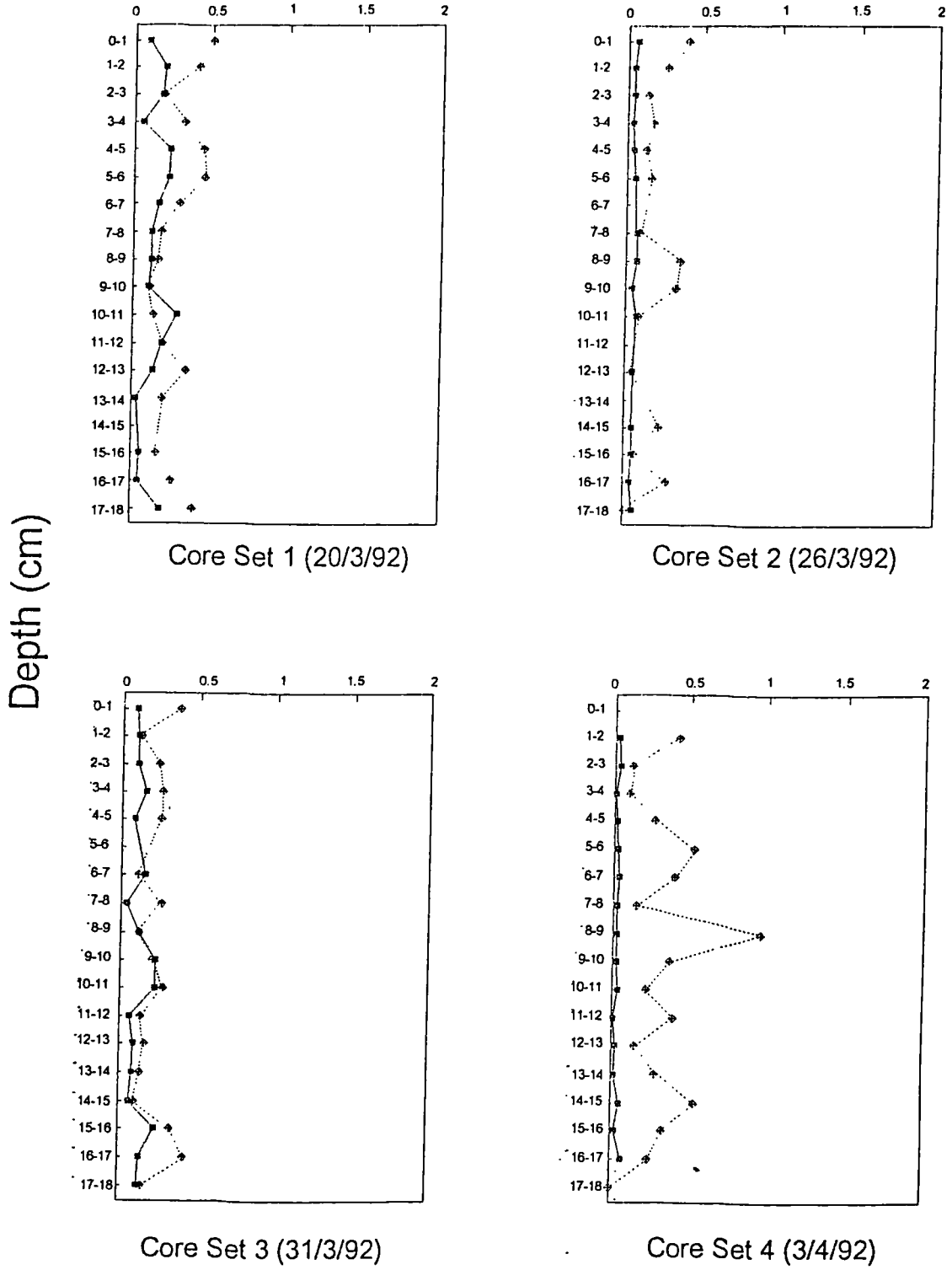


Figure 5.18: Distribution of porewater NO_3^- in the MM

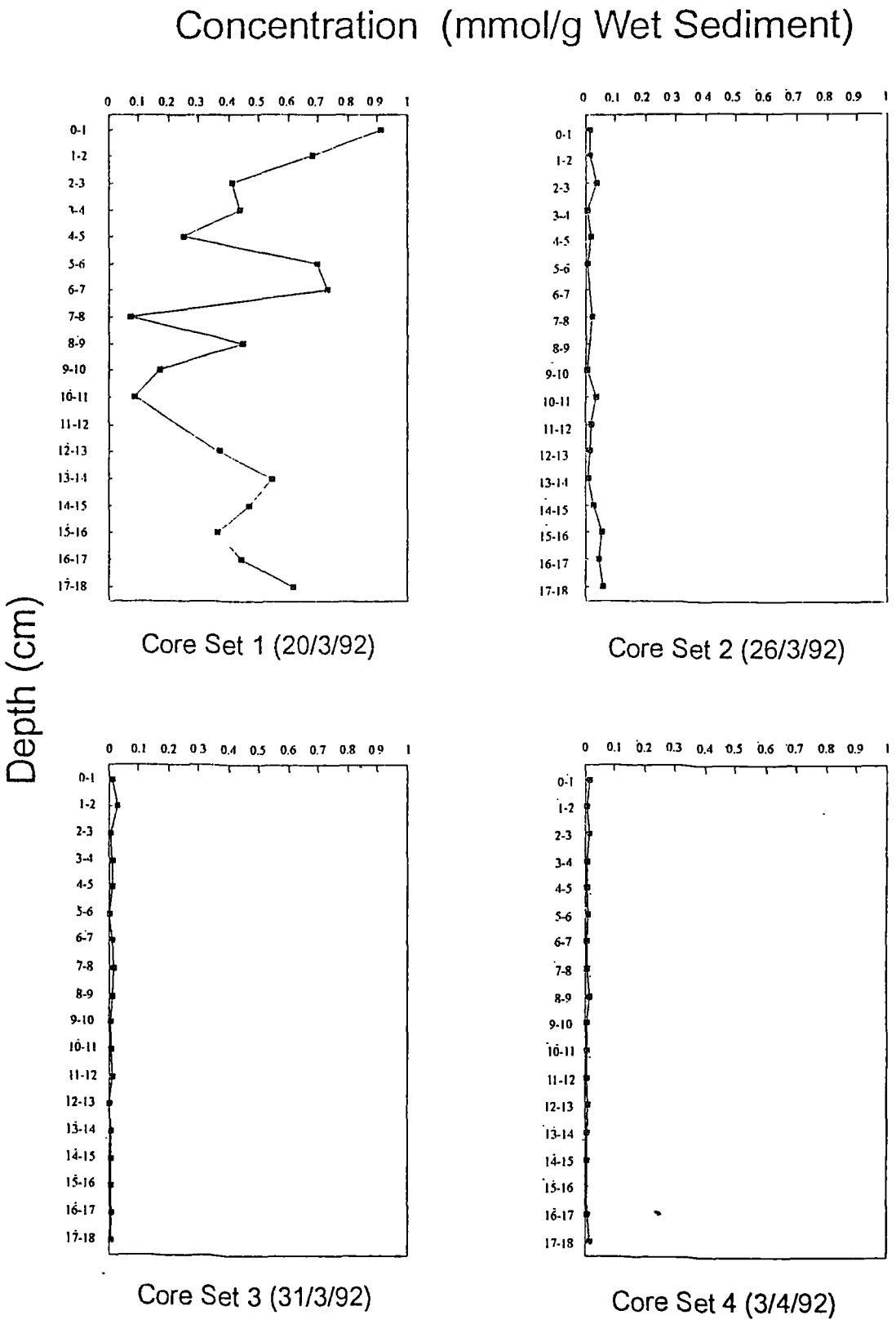
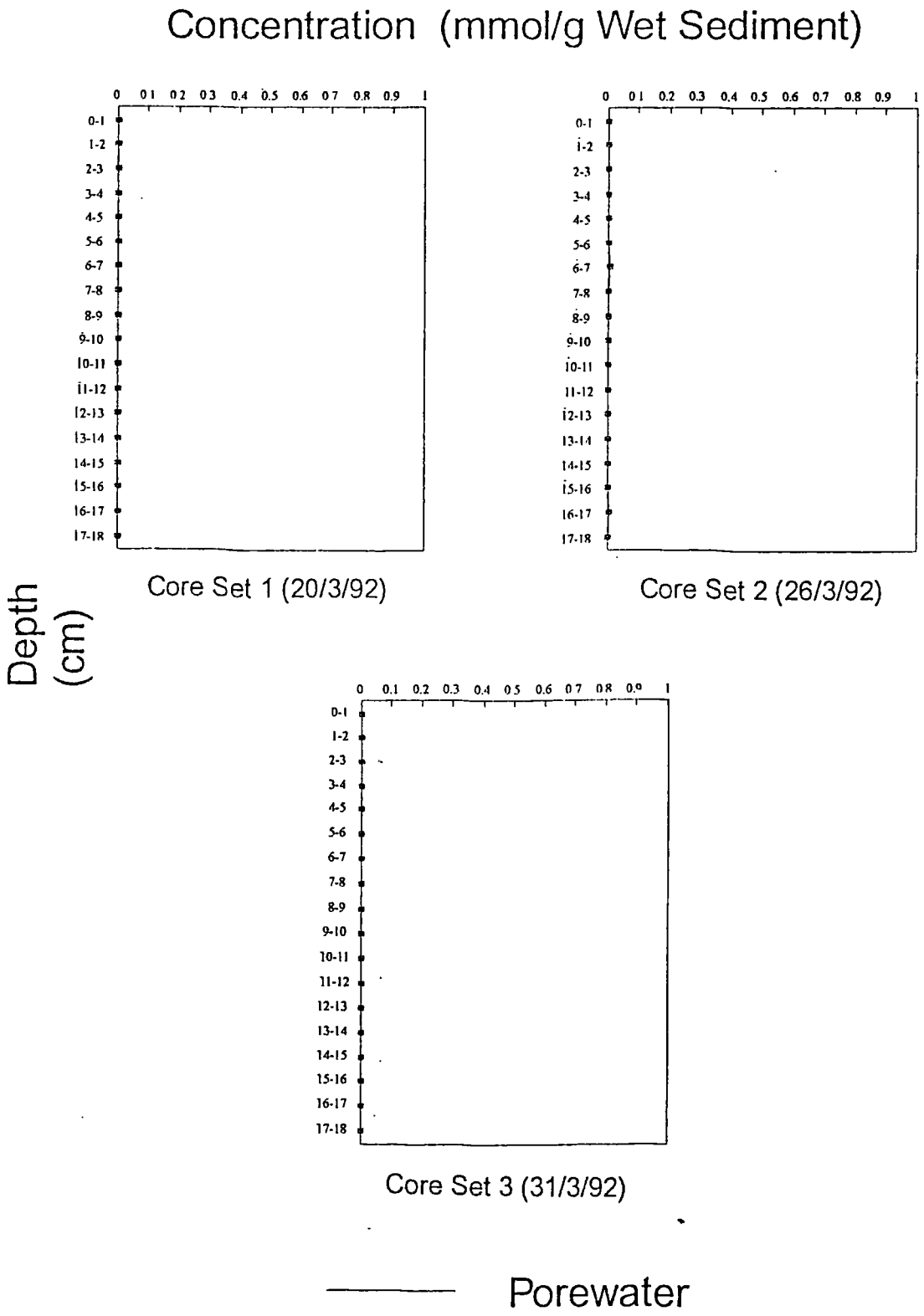


Figure 5.19: Distribution of porewater NO_2^- in the MM.



5.3.2 Middle Marsh discussion

Inorganic geochemistry

The very large decrease in the abundance of porewater NO_3^- between Core Sets 1 and 2 was interesting. The high [NO_3^-], even in the deeper part of Core Set 1, suggested an oxic environment (see UM). The lower [NO_3^-] in Core Sets 2-4, on the other hand, were consistent with an anoxic sediment, where NO_3^- is utilised as a secondary oxidant immediately below the oxic sediment surface (Figure 5.18). Further evidence of the highly anoxic nature of the MM sediment was shown by the absence of SO_4^{2-} in the porewaters of Core Set 3. Bacterial sulphate reduction only occurs under strictly anaerobic conditions.

Concentrations of porewater AMM did not vary markedly in Core Sets 1 to 3. The depletion in [NO_3^-] may have been the consequence of fresh organic deposition onto the MM during the high spring tide (e.g. sewage), and its subsequent oxidation via denitrification. Untreated sewage contains high concentrations of AMM, so the deposition of the anthropogenic organic material should have resulted in a noticeable increase in [AMM] in the MM surface sediment. The AMM profiles shown in Figure 5.17 suggest that this was not the case. Furthermore, increasing [AMM_{PW}], associated with increased degradation of organic matter were not observed.

Sedimentary organic matter quality

The MM samples were heavily colonised with epipelic diatoms and euglenoid algae (Jemmett, 1991). Mague et al. (1980) observed that most dissolved organic carbon released from phytoplankton consisted of low molecular weight compounds,

with dissolved free amino acids (DFAAs) comprising 5-10 % of the material. However, other studies (Jorgensen, 1982; Carlucci et al., 1984; Nagata and Kirchman, 1991) found that a higher percentage of this organic carbon (10-45 %) was released as DFAAs. Furthermore, algal degradation products and detritus are rich in labile organic nitrogen compounds (Mayer and Rice, 1992). It seems likely, therefore, that release of labile organic nitrogen compounds by the abundant benthic algae of the MM would strongly influence the sedimentary organic matter of these sediments (*cf.* higher plant influence in UM sediments).

The porewater MAs

Unlike the UM, MMA and DMA were continually present through the core, together with TMA. The porewater profiles in the UM suggested that osmoregulation was a major influence on the dissolved MA pool. It is more difficult to speculate on the porewater distributions in the MM, however. Tidal inundation of the MM was more regular, so the uptake and release of the MAs by the sedimentary organisms may have been more dynamic, resulting in porewater distributions which were more random than in the UM. On the other hand, the greater frequency of tidal incursion suggests that the dominant flora and fauna may be better adapted to salinity stress. This may inhibit the diffusion of the organic osmolytes from algae and other organisms into the porewaters, thereby implying that salinity stress had little influence on the porewater distributions of the MAs. Furthermore, the salinity of the MM porewaters probably fluctuated less than in the UM (Jemmett, pers. comm.), which may have led to a reduced flux of osmolyes from the resident organisms.

The profile of TMA_{PW} is of considerable interest. In Core Sets 1, 3 and 4

there was a gradual increase in [TMA_{pw}] with depth to the 5-8 cms horizon, followed by a sharp decrease below this depth. This trend was not seen for MMA_{pw} or DMA_{pw}. One reason for the different behaviour of TMA_{pw} may lie in the influence of the resident infauna. Glob and Sorensen (1987) and Sorensen and Glob (1987) found that [TMA_{pw}] was strongly correlated with the density of benthic infauna in a number of estuarine sediments. The most abundant species at these sites was the benthic invertebrate *Corophium volutator*, which is a common inhabitant of mudflats in the estuaries of northern Europe. *C. volutator* lives in U-shaped burrows that penetrate up to 6 cms into the mud (McClusky, 1968). It is also abundant at many sites in the Mersey Estuary including Oglet Bay. Indeed, highest populations of the organism at Oglet Bay were recorded in the MM, although *Tubificid* worms and *Nereis diversicolor* were the most abundant invertebrates populating this area of the marsh (Jemmett, personal communication).

Sorensen and Glob (1987) measured [TMA] in adult specimens of approx. 5 μmol g^{-1} fresh wgt., and tested *Corophium volutator* as a source of TMA by incubating 40 specimens (about 0.2 g fresh wgt.) in estuarine water of varying salinity (Table 5.2). It is clear from their data that the organism responded to lower salinities with a greater release of TMA than in water of higher salinity. This indicates that direct release of TMA from the body fluids to the porewaters occurs as a result of osmoregulation.

Given the significant release rates of TMA in waters of low salinity (Sorensen and Glob, 1987), it seems possible that [TMA_{pw}] in the MM sediments were directly influenced by the activities of benthic fauna during the sampling period. Significant faunal activity would not be expected at depth in anoxic sediments such as the MM,

but it is well known that the mucus-lined burrows of many benthic invertebrates can extend at least a few cms down into suboxic sediments (Aller and Aller, 1986; Glob and Sorensen, 1987).

Table 5.2: Release of TMA from the benthic invertebrate *Corophium volutator* in water of varying salinity (after Glob and Sorensen, 1987)

Salinity ($^{o}/_{oo}$)	Release rate (nmol TMA g^{-1} fresh wgt. h^{-1})
0.5	4000
12	20
34	200

The profile of [TMA_{PW}] in Core Set 2 (Figure 5.14) was somewhat different to those of 1, 3 and 4. [TMA_{PW}] were high at 0-1 cm, but were below detection limits between 1 and 8 cms. If the depletion of TMA_{PW} was a result of the osmoregulation requirements of the benthic organisms, then the algae colonising the sediment surface (significant populations, even in March) might have been expected to cause a depletion in concentrations of the MAs in the surface sediment also. The high [TMA_{PW}] seen at 0-1 cm are difficult to explain if this was the case as Core Set 2 was taken at the bottom of the neap tide when inundation of the sampling site probably did not occur. However, a decrease in the water level in the sediments at this time may have contributed to salinity differences with depth in the core and influenced this variation.

Since the MM sediment appeared anoxic below 1 cm, it is probable that methanogenesis would have occurred in this region of the marsh. The MAs have been reported as important substrates for methanogenic bacteria where bacterial sulphate reduction is the dominant microbial reaction (Oremland et al., 1982, King et al., 1983; Giani et al., 1984; King, 1988b). Presumably, uptake of MAs by methanogens should be observed in these porewater profiles, and this is discussed below.

Activity of methanogenic bacteria

Concentrations of dissolved SO_4^{2-} were measured in the porewaters of Core Set 3 and compared to the MA_{PW} profiles. SO_4^{2-} was not detected at all in the porewaters indicating that SO_4^{2-} was rapidly utilised below the sediment surface. Furthermore, high concentrations of S^o were reported in sediments from the LM site (Paratono, personal communication) indicating rapid utilization of SO_4^{2-} in LM

sediments, which also appeared anoxic.

TMA, which forms from the fermentation of CHO and GBT, is rapidly converted to methane by methanogens in the sulphate reduction zone, while acetate from CHO and GBT is simultaneously oxidised by sulphate reducing bacteria (King, 1984; Figure 5.12). A limited role for sulphate reducing bacteria (approx. 20 % during inhibition of methanogenesis) in the metabolism of TMA in marine sediments was also suggested by King (1984), although the preferred substrate was still acetate. This raises an interesting question on the source of the porewater MAs in these sediments. Certainly, the [MA_{pw}] decreased significantly in the top half of the core which is consistent with their utilization by methanogens. However, King (1984) implied that the utilization of QAs by methanogens is dependent on the prior fermentation of the QAs by sulphate reducing bacteria to produce TMA and acetate. If sulphate reducing bacteria were not active with depth in the MM, the methanogens must have utilised the MAs along with, or in preference to acetate. This may indicate a preference for amine nitrogen (Budd and Spencer, 1968).

The sedimentary MAs

Concentrations of sedimentary DMA were in the same range as in the UM, and were generally higher than in the porewaters. The distribution of DMA gave little indication once again as to its source other than from the degradation of TMA and QAs. Highest [DMA_S] (Core Set 3, 11-12 cms) may have been a consequence of a localised phenomenon such as the death of an organism, but corresponding increases in [MMA_S] and [TMA_S] were not observed.

Concentrations of MMA_S increased by a factor of 10 during the sampling

period, and highest levels were recorded at depth in Core Set 3 (23 μmol/g ws at 12-13 cms). Sedimentary concentrations of DMA and TMA were much lower and were fairly asymptotic throughout each Core Set, suggesting an alternative source of MMA. Unfortunately, GLY (a potential source of MMA) was not measured in the MM and it was not possible to speculate on the reasons for the increases in [MMA_s].

Influence of salinity on sedimentary MA distribution

Wang and Lee (1990) reported that salinity was a major factor influencing the adsorption capacity of sediments for the MAs. Hence, MAs were more strongly adsorbed to freshwater lake sediments than salt marsh sediments, presumably as a result of the inability of the MAs to compete with the major seawater ions for the available exchange sites on the sediment. The adsorption coefficients of the MAs associated with the salt marsh sediment varied little in either medium as exchange sites were presumably unavailable.

In this study, all zones of the marsh were inundated at various times, so it is likely that the sediments were equilibrated with waters of varying salinity, i.e. most exchange sites were occupied by major seawater ions. However, the frequency of tidal inundation and variations in the salinity of the porewaters (i.e. the concentrations of the major seawater ions) through the tidal cycle may have influenced the ability of the MAs to occupy available exchange sites in the various zones of the marsh. Furthermore, Burdige and Martens (1990) found that the porewaters of some marine coastal sediments appeared to be depleted in a wide range of AAs relative to the sediments (e.g. serine, threonine, arginine, valine, phenylalanine, isoleucine and leucine). In general these were either hydrophobic, uncharged polar, or basic AAs,

for which abiotic removal from solution (e.g. adsorption, incorporation into humic substances) was thought to play an important role in controlling their porewater concentrations (Rosenfield, 1979; Hedges and Hare, 1987; Henrichs and Farrington, 1987).

Certainly, [MMA_S] and [DMA_S] were much greater than in the porewaters of Core Sets 1 and 4, *cf.* TMA. This may reflect the greater basicity of DMA and MMA relative to TMA, leading to stronger adsorption of these MAs onto the sediments.

Balistrieri and Murray (1987) found that the major seawater ions significantly affected organic acid adsorption onto sediments, either by site competition or by the formation of a weakly or non-adsorbing complex between the major ion and the organic acid. The MAs do not have a carboxyl group, but the basic group may also be capable of taking part in complexation reactions. The average salinity of the MM porewaters was 11 $^{o}/_{oo}$ on 14/1/92 at low water (Jemmett, unpublished results), and if the pH of the porewaters can be assumed to have been between 7.8-8.5, then the MAs must have existed predominantly in the protonated form. As such, they may have been able to compete with the major seawater ions to form complexes with organic acids and adsorb onto the sediments. DMA is the most basic MA (see Table 3.1), hence, if complexation does occur, DMA should form stronger complexes than either MMA or TMA. This may be reflected in the high DMA_S pool size, relative to DMA_{PW} in the MM.

5.3.3 Summary

The geochemistry of the MAs in the MM seemed to be more variable than in the UM over the same period. A number of factors may effect this difference:

(1) The colonisation of the MM by benthic algae such as epipelic diatoms and euglenoid algae potentially supplies labile organic matter to the sediments, which may include MAs. Furthermore, these algae may also utilise the MAs as organic osmolytes.

(2) The osmotic uptake of the MAs in the MM may be influenced by the better adaption of the benthic organisms to salinity variations than in the UM, due to more frequent tidal inundation.

(3) The MM plays host to a significant invertebrate population including *Corophium volutator* which has been reported previously as a significant source of TMA. *C. volutator* and other invertebrates may, therefore, contribute significantly to the TMA_{PW} pool in the upper part of the MM cores. However, further work is necessary before definite conclusions can be drawn (e.g. correlation of the MAs with biomass and individual species within the same sample).

(4) $[SO_4^{2-}]$ were measured in the porewaters of Core Set 3 only, but were below detection limits, implying that most sulphate reduction was occurring close to the surface sediment. Sulphate reducing bacteria have been implicated as fermenters of QAs such as CHO and GBT producing TMA which is subsequently utilised by

methanogens. The absence of dissolved SO_4^{2-} in Core Set 3, and removal of the MAs with depth in the porewaters may indicate a preference for the MAs as substrates for methanogens, even when acetate is available.

(5) Variations in the salinity of the porewaters (and, therefore, of the concentrations of major seawater ions) may have influenced the ability of the MAs to compete with the major seawater cations for the available exchange sites on the sediments. However, the basicity of the MAs is probably extremely important in controlling their sedimentary distributions *cf.* concentrations of basic AAs in marine sediments relative to the porewaters. Also, the sedimentary DMA pool was nearly always greater than in the porewaters.

Section 5.4: LOWER MARSH RESULTS AND DISCUSSION

5.4.1 Lower Marsh results

Core Set 1 (20/3/92): MAs were detected throughout in the porewaters of this core (Figure 5.20). MMA_{PW} and TMA_{PW} were present at concentrations of up to 22 and 11 mol/g ws respectively. [MMA_{PW}] increased from 4 μmol/g ws in the surface of the core, to a maximum of 11 μmol/g ws at 8-9 cms. A further subsurface maximum was also observed at 14-15 cms. Concentrations of DMA_{PW} and TMA_{PW} decreased from 1 and 9 μmol/g ws respectively at the sediment surface, whilst DMA_{PW} was intermittently detected in the deeper parts of the core (maxima at 7-8 cms, 1.5 μmol/g ws). Concentrations of TMA_{PW} decreased gradually from 0-1 cm to a minimum at 6-7 cms (5.5 μmol/g ws), before gradually increasing to a subsurface maximum at 14-15 cms (11 μmol/g ws).

Sedimentary MMA increased from 4.5 μmol/g ws at the surface to 5.5 μmol/g ws at 6-7 cms. Below this depth, the profile showed non-zero asymptotic concentrations, except at 13-14 cms. The concentrations of DMA_S maximised at 6-7 cms (1 μmol/g ws). DMA_S was rarely as abundant as MMA_S, but concentrations were similar to those observed in the TMA_S pool. Concentrations of MMA_{PW} were higher than MMA_S in this core set, in contrast to the observations for the UM and MM.

Core Set 2 (26/3/92): Concentrations of dissolved MMA were again higher ($\geq$ 10 times) than in the sediments (Figure 5.21). The MMA_{PW} pool was much higher than in Core Set 1, and this was also the case for TMA_{PW} (8-38 μmol/g ws). Subsurface maxima in [MMA_{PW}] were measured at 11-12 and at 15-16 cms, where

[MMA_{PW}] were noticeably higher (> 100 μmol/g ws) than in any other core sample. DMA_{PW} was much less abundant (up to 0.1 μmol/g ws). The core was enriched in TMA_{PW} in the first few cms of the core and below the 7-8 cms depth interval where maximum concentrations were observed.

Concentrations of all sedimentary MAs were quite similar (0.5-8.5 μmol/g ws). MMA_S was most abundant, but its concentrations were low relative to those of the porewater. DMA_S and TMA_S showed minimal variation down the core.

Core Set 3 (31/3/92): Concentrations of MMA_{PW} and TMA_{PW} were similar to Core Set 2 (Figure 5.22), while [DMA_{PW}] were up to 8 times higher. TMA_{PW} concentrations decreased down to 6-7 cms but a subsurface maximum was observed at 2-3 cms (11 μmol/g ws), and a further subsurface maximum occurred below this depth (10-11 cms).

Concentrations of dissolved MMA were again higher than sedimentary MMA, as were [TMA_{PW}] vs [TMA_S].

Concentrations of MMA_S varied little throughout the core and reached a maximum concentration of 10 μmol/g ws at 12-13 cms. The profile of DMA_S also showed little significant variation throughout the core and was present at similar concentrations to DMA_{PW}. [TMA_S] were also asymptotic through the core.

Core Set 4 (3/4/92): DMA_{PW} was not detected in this core (Figure 5.23), whilst MMA_{PW} was only above detection limits at 3-5 cms. In contrast, the concentration profile of TMA_{PW} showed clear surface enrichment and concentrations gradually decreased down to 9 cms (0.5 μmol/g ws), then increasing in abundance to a

subsurface maximum at 16-17 cms (8 μmol/g ws). [MMA_{PW}] were up to 7 μmol/g ws but did not persist down the core.

Concentrations of MMA_S were much higher than in Core set 1 apart from the top few cms, and were most abundant at 11-12 and 14-15 cms (21 and 22 μmol/g ws). [DMA_S] were also similar to Core Set 1 and were up to 1 μmol/g ws. Concentrations of TMA_S varied little throughout the core and were again much less abundant than in the porewater.

Inorganic nitrogen

Ammonia: Concentrations of AMM_{PW} generally increased with depth in all Core Sets (Figure 5.24), but considerable fluctuations were observed between data points in Core Set 1. Highest [AMM_{PW}] were observed at 15-18 cms in Core Set 2 (up to 2.7 mmol/g ws), but concentrations approached these levels at 13-14 and 15-16 cms in Core Sets 1 and 3 respectively. Porewater AMM was least abundant in Core Set 4, with maximum concentrations of about 0.5 mmol/g ws.

Concentrations of sedimentary AMM were generally much lower than in the porewaters. The exception to this was the top 3 cms of Core Set 1, where concentrations approached 1.0 mmol/g ws. The profiles of AMM_S showed concentrations of up to 0.1 mmol/g ws with depth, with the exception of Core Set 1. This was similar to the concentrations and distributions of sedimentary AMM which were observed in the UM and MM.

Nitrate: In Core Set 1, concentrations of dissolved NO_3^- decreased rapidly from a maximum surface concentration of 0.6 mmol/g ws to 0.02 mmol/g ws at 1-2 cms

(Figure 5.25). [NO_3^-] below this depth did not increase above 0.1 mmol/g ws apart from the depth interval at 13-14 cms (0.2 mmol/g ws). Similar trends were observed in Core Sets 3 and 4, but subsurface maxima were apparent in Core Set 3 where concentrations were higher than at the surface (> 0.4 mmol/g ws). The NO_3^- profile for Core Set 2 were similar to that of Core Set 3, but fewer data points were available.

Nitrite: Concentrations of porewater NO_2^- were again much lower than for AMM or NO_3^- and no significant variations were observed in any of the Core Sets (Figure 5.26).

Figure 5.20: Distributions of the MAs in Core Set 1 (LM, 20/3/92).

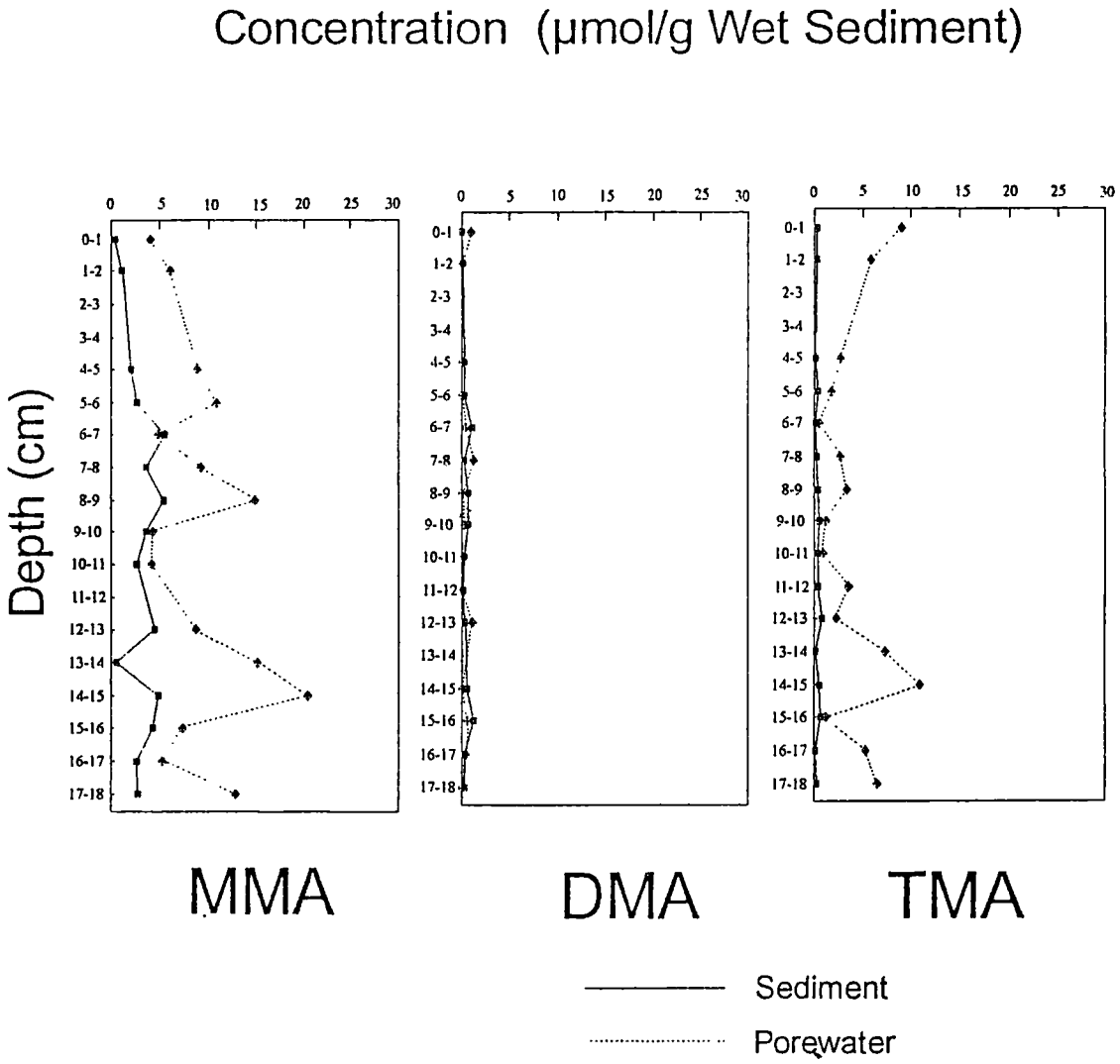


Figure 5.21: Distributions of the MAs in Core Set 2 (LM, 26/3/92)

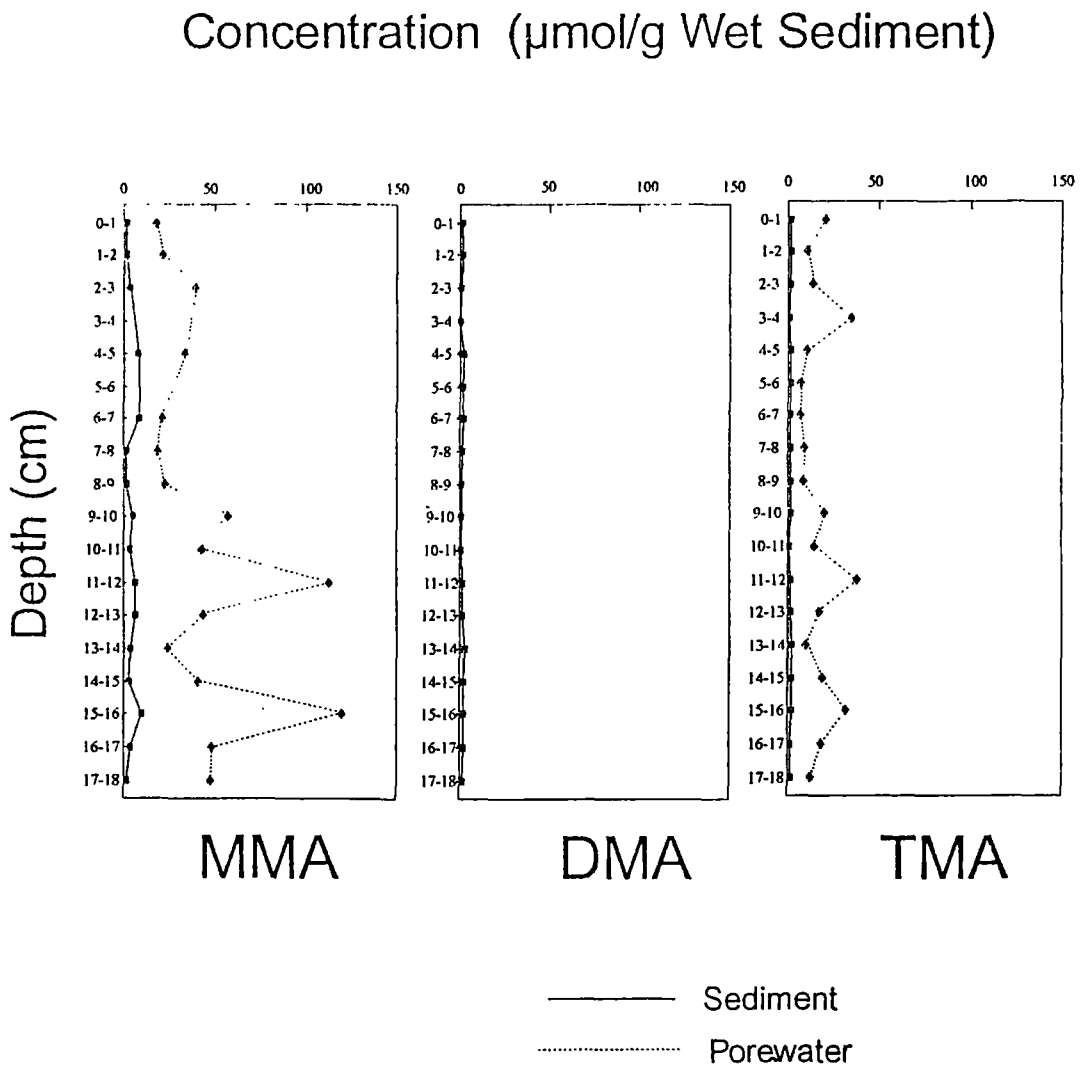


Figure 5.22: Distributions of the MAs in Core Set 3 (LM, 31/3/92)

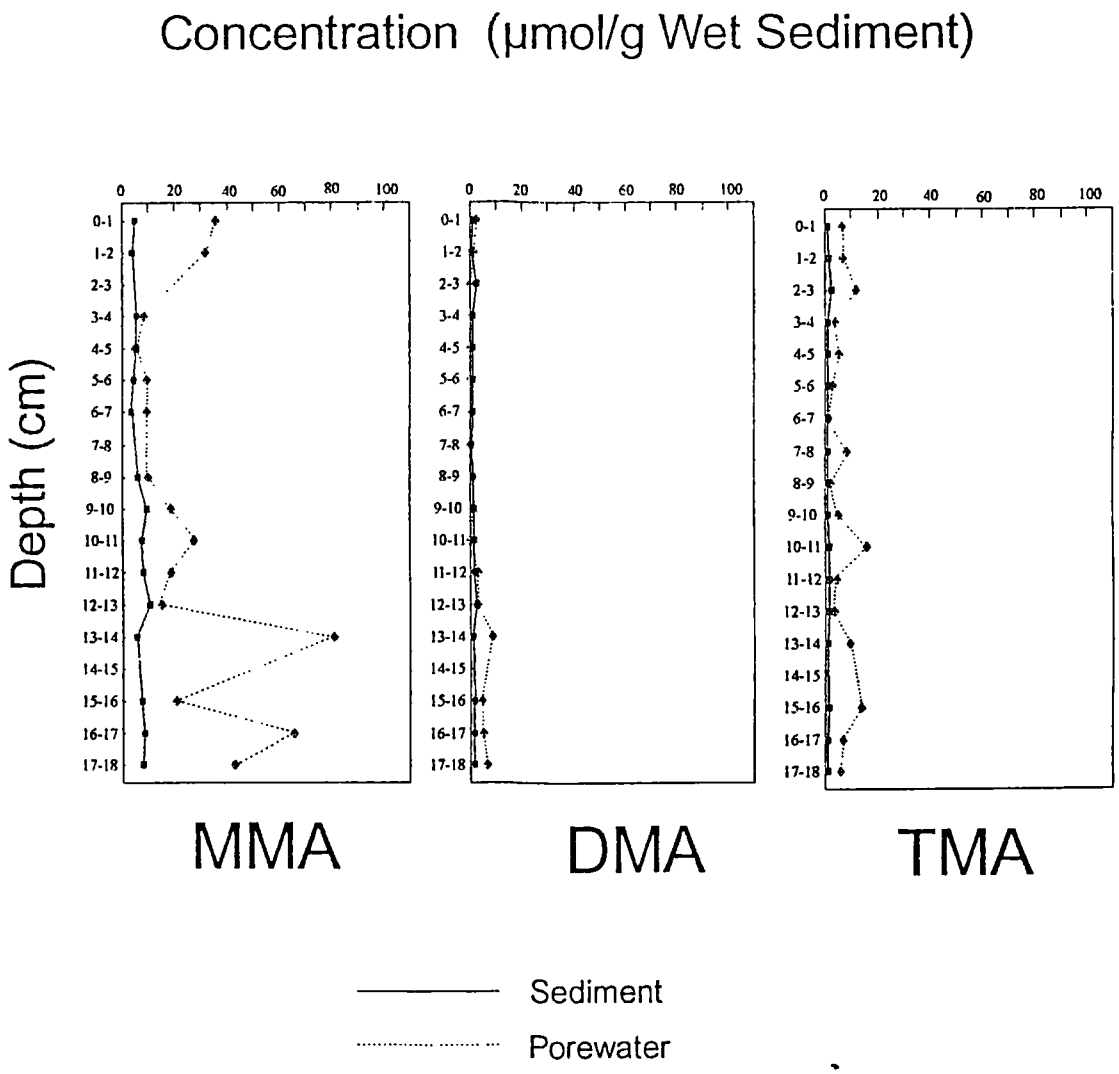


Figure 5.23: Distributions of the MAs in Core Set 4
(LM, 3/4/92)

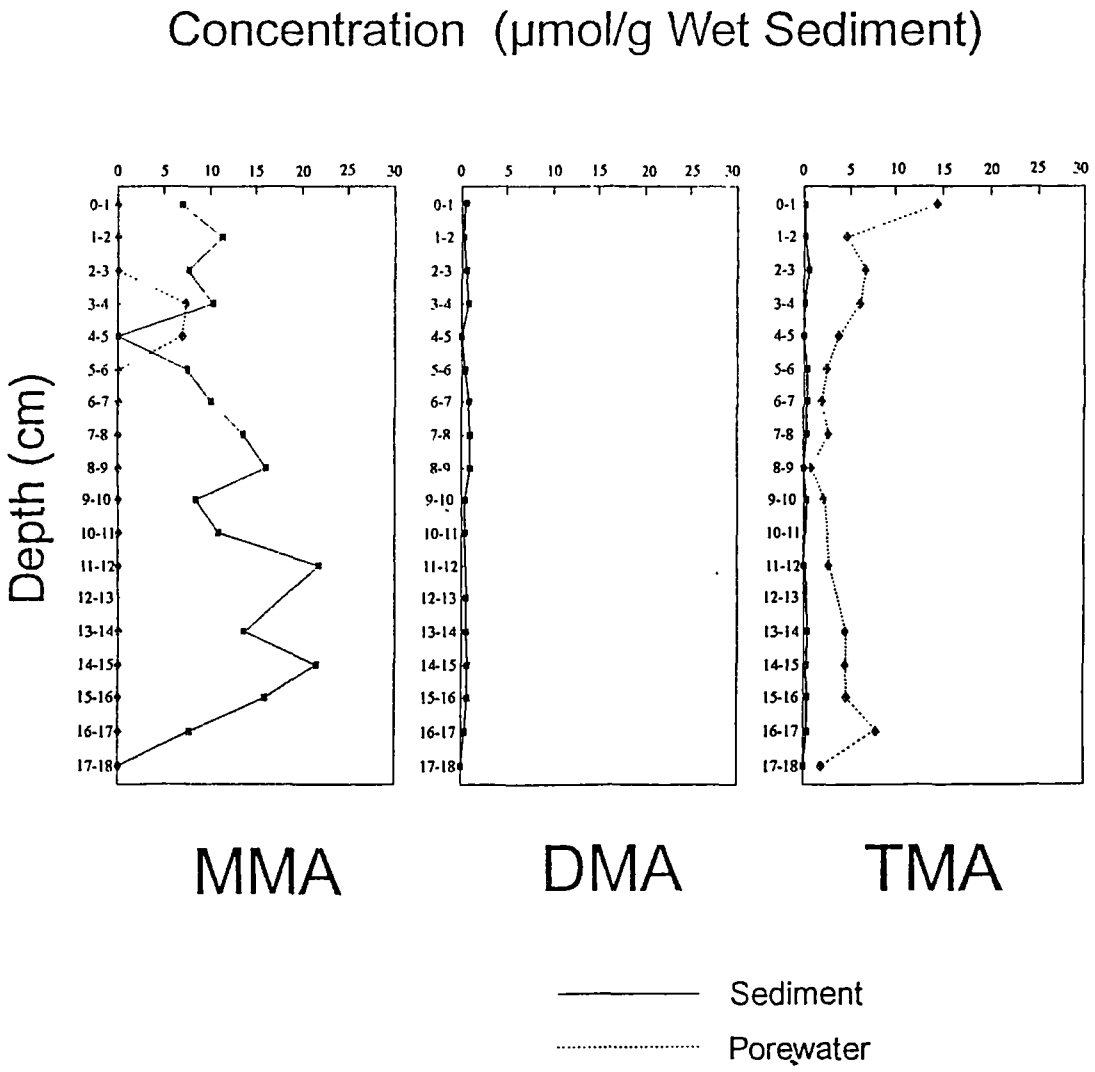


Figure 5.24 Distributions of AMM in the LM

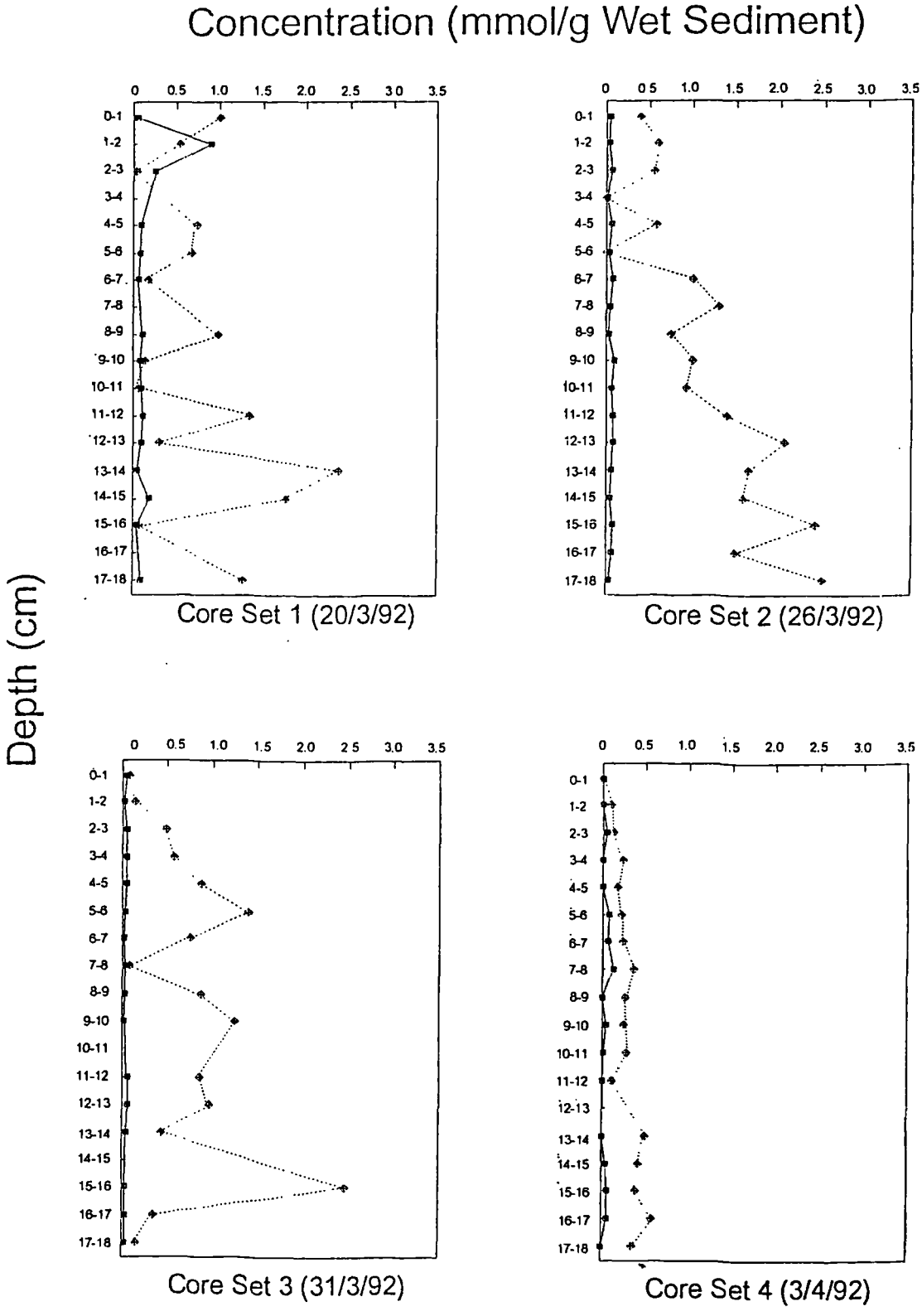


Figure 5.25: Distribution of porewater NO_3^- in the LM

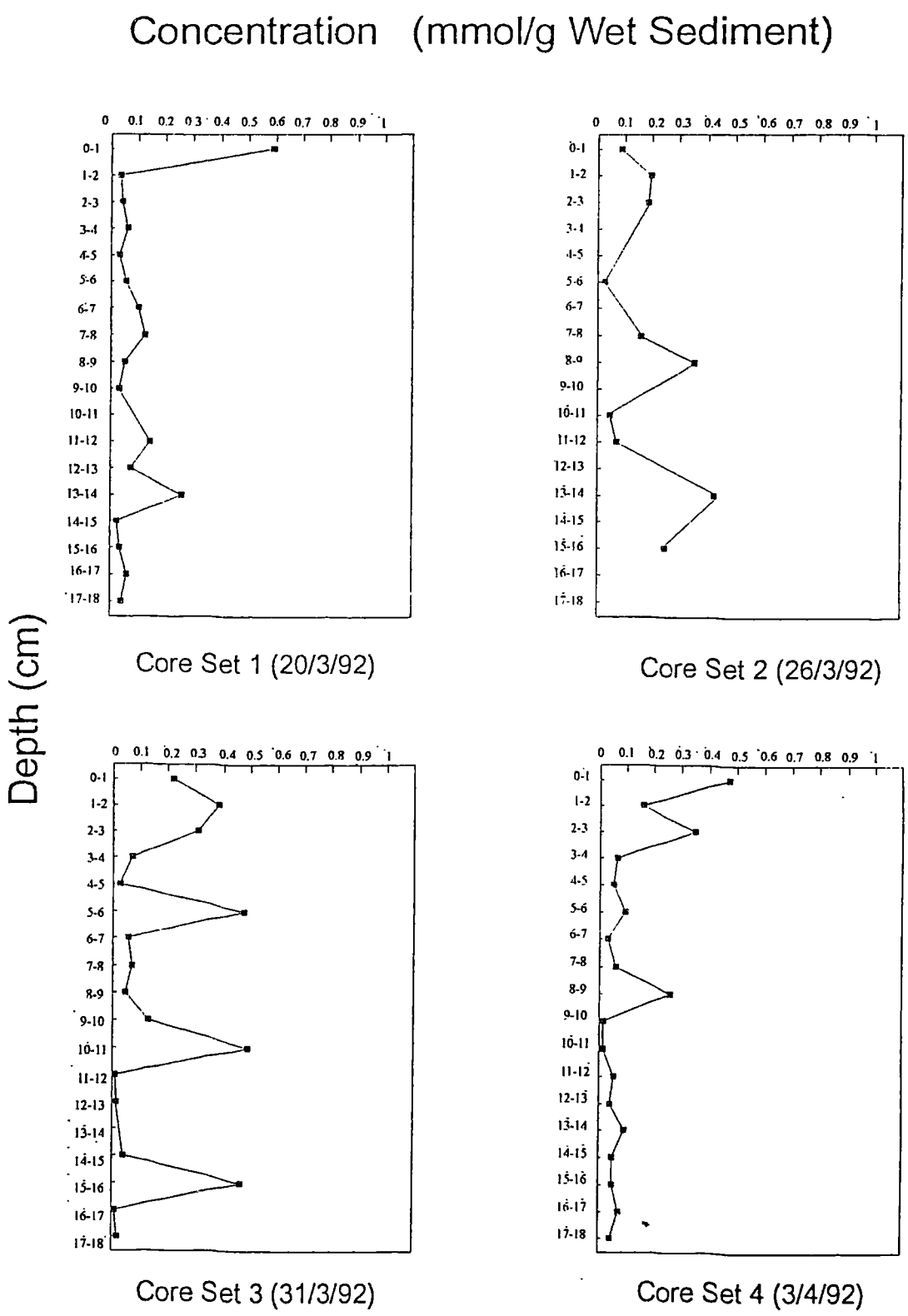
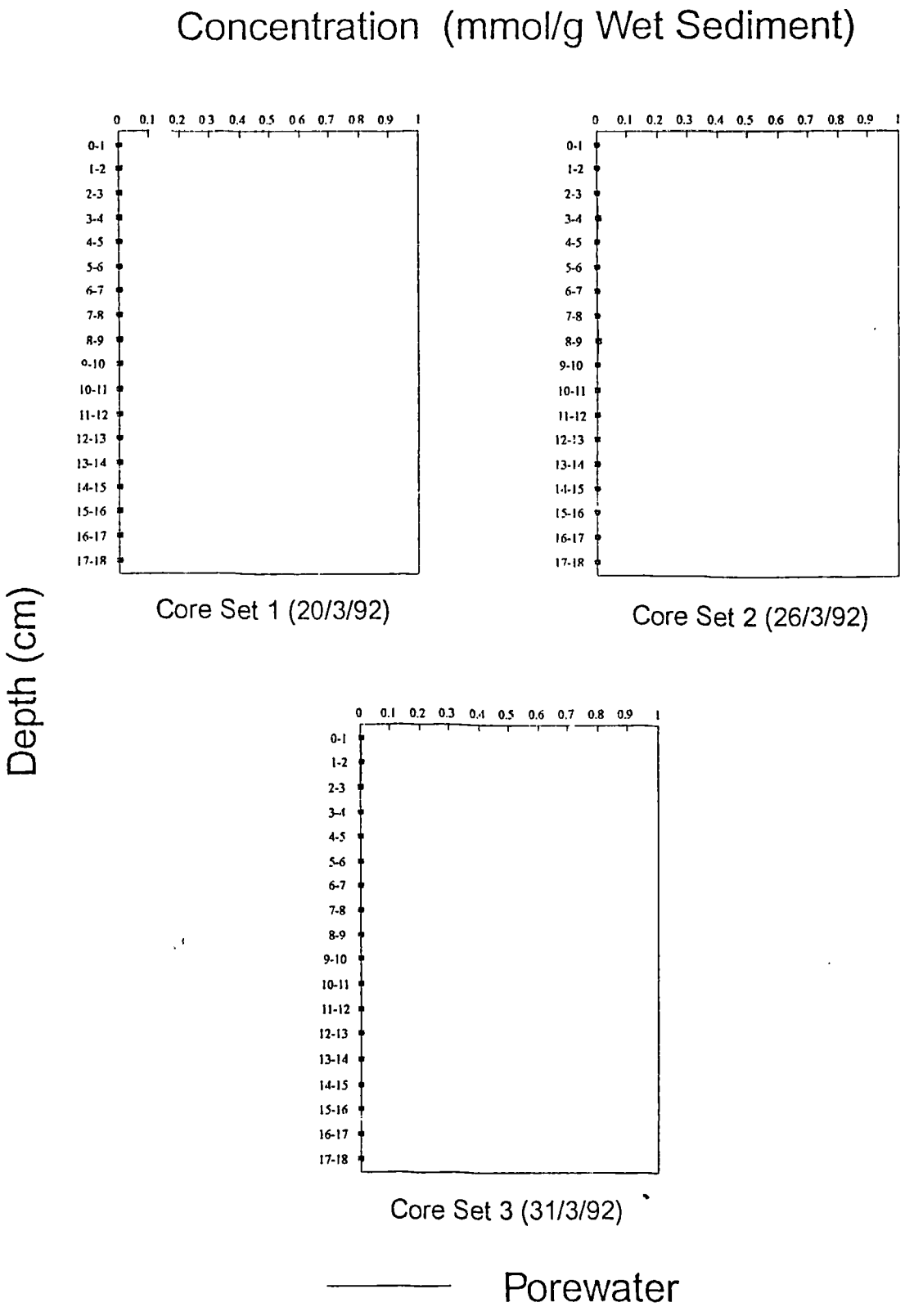


Figure 5.26: Distribution of porewater NO_2^- in the LM.



5.4.3 Lower Marsh discussion

Inorganic nitrogen geochemistry

The distributions of porewater AMM (Figure 5.24) contrasted with those observed for the UM and MM, where concentrations generally decreased with depth (UM), or behaved asymptotically (MM). Similar profiles have been observed previously in a salt marsh (Flax Pond, New York), where Wang and Lee (1990) observed low [AMM_{PW}] at the surface sediment, which increased significantly with depth. The general trend for porewater AMM concentrations in the LM cores was comparable with Flax Pond, which may indicate the similarity between sites, both sediments being anoxic below the upper few mm (Mackin and Swider, 1989).

A net export of AMM (and all other forms of nitrogen, apart from NO_3^-) has been reported for Flax Pond salt marsh (Valiela, 1983). This export trend may not be apparent from the data of Wang and Lee (1990), as cores were taken on a monthly basis. However, increased export of AMM from salt marshes has been reported with high tidal volumes (Jordan and Correll, 1991). This is consistent with the significant decrease in the surficial AMM_{PW} pool which was observed for Core Set 4. Hence, the depletion of porewater AMM in the surface sediment presumably arises as a consequence of AMM export either by diffusion to the overlying water and/or to the atmosphere.

The percentage export of AMM from salt marshes decreases with increasing maturity (Jordan and Correll,1991), probably due to the more consolidated nature of the sediment. This results in a more abundant flora and fauna in the surface sediments and gives rise to an additional source of AMM. Indeed, higher [AMM_{PW}] were observed in the UM surface sediments, when compared to the LM [AMM_{PW}] at

similar depth intervals.

The general decrease in the concentration of dissolved NO_3^- with depth in Core Sets 1 and 4 was consistent with the utilization of NO_3^- as a secondary oxidant during diagenesis (Figure 5.25). However, significant [NO_3^-] with depth were observed in Core Sets 2 and 3. The mechanisms involved in the remineralization of nitrogen, such as nitrification and denitrification, can be generally applied to salt marsh environments. However, local factors, especially hydrological ones, make for idiosyncratic conditions (Valiela, 1983). The maxima in the NO_3^- profiles below 9 cms may, therefore, have resulted from a localised phenomenon such as incursion of groundwaters rather than well-documented biological processes.

Sedimentary organic matter content

The LM sampling site was populated exclusively by epipelic diatoms and euglenoid algae (Jemmett, 1991). The TOC content of the LM ranged from 3-5 %, and were similar to the UM and MM values (Appendix B). However, the sedimentary organic matter was likely to be closer in character to that of the MM sediments, which were also populated by benthic algae. It can be reasonably assumed, therefore, that the LM sediments contained a higher percentage of labile, low-molecular weight organic compounds (AAs etc.) than the UM, which was dominated by higher plant detritus (see Section 5.2). Indeed, the high [GLY_S] (23 μmol/g ws) measured in Core Set 4, which were > 2 times the [MMA_S] at 0-4 cms, may provide evidence of the release of labile organic nitrogen compounds by the benthic algae colonising the surface sediment.

Porewater MAs

Highest porewater TMA concentrations in all Core Sets were measured in the surface sediment (0-1 cm) which also had the lowest TOC content (Figures 5.20-5.23). This may implicate TMA as a product of the degradation of organic matter, as the decrease in TMA_{pw} with depth was in contrast to the asymptotic behaviour of TOC below 0-1 cm (4-6 %, Appendix B) in the LM. Decreases in $[TMA_{pw}]$ with depth in the cores were in contrast to AMM_{pw}, and may reflect the microbial degradation of TMA_{pw}, as AMM is the most reduced form of nitrogen

Increased TMA_{pw} levels in the bottom half of Core Set 1 (13-15 cms) were very interesting. Concentrations of porewater MMA (which were slightly higher) also increased over this range. Bioturbation of the sediments would not be expected at this depth in an anoxic sediment, as dominant infaunal species such as *T. costatus*, *T. benedenii*, *N. diversicolor* are unlikely to inhabit the sediments at depths below 5-10 cm, and meiofaunal animals such as copepods and nematodes are restricted to the top 2-3 cm of LM sediment (Jemmett, pers. comm.). A significant increase was also observed for AMM and NO_3^- in the porewaters over this depth range (Figures 5.24 and 5.25). The presence of dissolved NO_3^- in the deeper sections of the LM cores may be a consequence of groundwater incursion (Valiela, 1983). Since it is possible that waste sewage effluents are also present in groundwaters (Williams et al., 1991), the high concentrations of MMA, TMA and AMM may reflect the influence of contaminated groundwater in these sediments (Scully et al, 1988).

Microbial utilization of MAs

The decrease in concentrations of TMA_{PW} with depth may indicate its utilization by methanogens (anaerobic microbes). High concentrations of elemental S° have been reported in LM sediments (Paratono, personal communication) and also in the anoxic sediments of Hale salt marsh which is in close proximity to Oglet Bay (Al-Lihaibi, 1991). This implies that the LM sediments are highly anoxic and can, therefore, provide an environment in which methanogenic bacteria can flourish. It has been suggested that sedimentary methanogens utilise the MAs only when they are unable to compete for acetate, as is the case when sulphate reducers are present (King et al., 1983; Winfrey and Ward, 1983; King, 1984). The TMA_{PW} profiles would, therefore, imply that sulphate reducers were active in the LM, and the depletion of dissolved SO_4^{2-} with depth in Core Set 3 of the MM suggests that high rates of sulphate reduction were occurring in Oglet Bay. If sulphate reduction did not occur, then the methanogens would have been expected to utilise acetate, leading to the possible preservation of TMA_{PW} with depth in the LM. Its continued depletion with depth suggests that the methanogens continued to use TMA as a substrate in the presence of acetate in the LM (*cf.* MM), and may provide further evidence to implicate some species of methanogens in the flora of marine bacteria which is dedicated specifically to the regeneration of amine nitrogen (Budd and Spencer, 1968).

Algal production of the MAs

Abdul-Rashid (1991) measured production of the MAs in a series of marine phytoplankton cultures (namely *Dunaliella minuta*, *Dunaliella tertiolecta*, *Chlorella*

salina, *Phyodactylum tricornutum*, *Phyodactylum lutheri*). [MMA] and [DMA] in the media were up to 0.6 and 0.4 μM, respectively, ~ 10 times higher than [TMA]. [MMA] and [DMA] were more than 100 times higher in cultures which had been inoculated against bacterial activity, while [TMA] remained at a similar level.

Caution is necessary in the interpretation of these results, because of the compositional differences between the culture medium and seawater and the fact that none of the studied species were benthic algae. However, the well-documented release of labile organic nitrogen by marine algae (Mague et al. 1980; Carlucci et al., 1984; Nagata and Kirchman, 1991) may explain the high surface [TMA_{PW}] and [MMA_{PW}] observed in the surface sediment.

Sedimentary MAs

MMA_S was the only amine to show significant variation in concentrations through the tidal cycle. The high levels in Core Set 4 (Figure 5.23) also coincided with high levels of sedimentary GLY in the top 4 cms. [GLY_S] were highest at 0-1 cm (23 μmol/g ws) and decreased to 15 μmol/g ws down to 4 cms (Horsfall, pers. comm.). The fermentation of AAs to compounds such as acetate, H_2 and MMA as end products, has been reported as an important process in organic matter cycling in marine sediments (Mead, 1971; Barker, 1981; Stams and Hansen, 1984; Burdige and Martens, 1990). Meitzer (1977) stated that the decarboxylation of glycine (GLY, see Figure 5.11) to form MMA is catalyzed by coenzyme pyridoxamine 5'-phosphate.

GLY has been widely reported as one of the most abundant AAs in marine sediments. Haugen and Lichtentaler (1991) studied AA diagenesis in sediments from the Oslofjord in Norway (oxic and anoxic) and found that the most abundant AAs

were GLY, aspartic acid and glutamic acid. Burdige and Martens (1990) measured dissolved free amino acids (DFAAs) in Cape Lookout Bight (an organic-rich coastal marine basin) and observed extremely high levels of GLY in surficial (0-2 cms) samples collected in March and April of 1985. In some samples they found GLY to account for between 42 and 68% of the total DFAAs (TDFAAs). They related this phenomenon to a temporary colonization of Cape Lookout Bight surface sediments by polychaete worms, since GLY is the most abundant AA in many benthic invertebrates (Awapara, 1962; Henrichs, 1980).

A comprehensive study of the invertebrates populating the LM has not been undertaken to date, however, the dominant infauna are polychaete worms (Jemmett, pers. comm.). Since [MMA_s] increased from 6-11 μmol/g ws with depth from 0-4 cms, it is tempting to speculate that the fermentation of sedimentary GLY contributed to the observed increase in [MMA_s] (Mead, 1971; Barker, 1981; Stams and Hansen, 1984; Burdige and Martens, 1990), and this is a worthwhile subject for future study.

Concentrations of MMA_s in the LM were highest in the lower half of Core Set 4; considerable variation in [MMA_s] (0-22 μmol/g ws) was also observed within this core. This contrasted with the asymptotic behaviour exhibited by MMA_s in all of the other LM cores. It is difficult to speculate on the origin of MMA at depth. A knowledge of [GLY] throughout this core would have helped to gauge the potential importance of GLY as a MMA precursor in these sediments. Another potential precursor of MMA is sarcosine (SAR, Figure 5.11) which is widely distributed in marine flora and fauna (Yancey et al., 1982). Degradation of this QA may yield MMA (Hippe et al., 1979). Again, the importance of SAR is unknown, but its geochemistry, in relation to the flux of MMA should be considered during future

investigations.

The increase in porewater AMM with depth in the LM suggested that organic matter was being degraded throughout the core, although sedimentary TOC levels did not vary significantly with depth. It would have been useful to measure dissolved organic carbon (DOC) in these samples, as increases in porewater AMM flux may have been a consequence of the oxidation of this fraction in Core Sets 1-4. The increase in concentrations of sedimentary MMA may also have resulted from the degradation of this organic matter, and the fact that MMA_{PW} was below detection limits suggests that the speciation of MMA may have favoured the sedimentary pool at this time. It may not be possible, however, to explain these profiles in a purely abiotic (i.e. adsorption) context, as bacteria also play an important role in controlling the MA pool in sediments (Oremland et al., 1982; King et al., 1983; Giani et al., 1984; King, 1988b).

5.4.3 Summary

(1) The inorganic nitrogen measurements suggested that the LM was highly anoxic below 1 cm. High concentrations of porewater NO_3^- with depth in Core Set 1 were unusual and suggested possible groundwater incursion. High porewater concentrations of AMM, MMA and TMA were also measured in this region of the sediment, and it was thought that this may arise through contamination of groundwaters by sewage effluent.

(2) The LM sediment surface was colonised exclusively by epipelic diatoms and euglenoid algae. High concentrations of GLY in the surface sediments of Core Set 4

may provide evidence of algal release of labile organic nitrogen to the sediments, but the MAs (also produced by algae) did not show consistent enrichment in the surface sediment of the LM.

(3) The decrease in [TMA_{PW}] with depth also suggested subsequent microbial utilization of TMA, which may, ultimately have contributed to the increasing levels of porewater AMM with depth in the sediments.

(4) As a result of some reported culture experiments, it has been reported that algae are capable of producing MAs and supplying them to the surrounding environment. There was little evidence of this in the LM surface sediments, but simultaneous microbial utilization of these MAs may have offset any production. Radiolabelling experiments would be a useful next step in assessing the benthic algal contribution to the MA pool in sediments.

(5) MMA was the only sedimentary-associated MA present at significant concentrations in the LM. The decrease with depth observed for sedimentary GLY (0-4 cms) corresponded to an increase in [MMA_S], and suggested fermentation of this AA to MMA. This phenomenon has been reported for marine sediments (Section 5.2).

CHAPTER 6

AMINOPROPANONE AS A MARKER FOR DISSOLVED SEWAGE IN WATER SAMPLES.

Section 6.1: INTRODUCTION

During the last decade, the problem of sewage pollution in estuaries and coastal waters has received considerable attention. Many methods of monitoring sewage or faecal contamination have been described: these have often employed natural and synthetic organic compounds as molecular markers. Nevertheless, to date, the counting of faecal coliforms remains the standard bacterial indicator of faecal pollution in wastewater and other waters, whilst total coliforms, which include faecal coliforms, are the standard indicator of pollution in drinking water. These parameters have been quantified in the past using a most probable number (MPN) technique, which consists of the quantification of the results from a dilution culture, using a Poisson model (Beliaeff and Mary, 1993). However, the validity of this method has been called into question in recent times. Berg et al. (1978) treated a number of fresh, filtered, primary effluent samples with sodium hypochlorite (NaOCl) which usually destroyed more than 99.999 % of the indigenous faecal coliforms, total coliforms, and faecal streptococci in primary sewage effluents. However, only 85 to 99 % of the indigenous viruses present were destroyed. Furthermore, viruses were recovered from five of eight chlorinated primary effluents in which faecal coliforms were below detection by standard MPN procedures.

Santiago-Mercado and Hazen (1987) took water samples from a number of marine, estuarine and freshwater sites around Puerto Rico in pristine waters, waters receiving treated and untreated sewage, and effluents from a tuna cannery and a rum distillery. They observed high densities of faecal coliforms at all sites when using membrane filter methods and concluded that the density estimates were, in many

cases, grossly overestimating the degree of recent faecal contamination. Furthermore, since the coliform *Escherichia coli* appeared to be a natural inhabitant of tropical waters, faecal contamination could have been indicated when none was present.

The unreliability of the MPN methods has led to the attempted use of natural molecular markers, such as faecal steroids, and particularly 5β(H)-cholestenan-3β-ol (coprostanol) and 5β(H)-cholestanone (coprostanone) as indicators of particulate sewage contamination in the marine environment (e.g. McCalley et al., 1981; Walker et al., 1982; Grimalt et al., 1990). Coprostanol is thought to be formed exclusively by stereo-specific bacterial reduction of cholesterol in human and higher animal gut systems, and is one of the principal sterols found in their faeces (Rosenfield et al., 1954; Rosenfield and Gallagher, 1964; Teshima and Kanazawa, 1978; Murtaugh and Bunch, 1967). Concentrations of more than 3 nmol/g have been reported in sediments adjacent to deepwater ocean outfalls for Sydney, a major Australian city with a population of 3.5 million (Nicholls et al., 1992). Brown and Wade (1984) measured coprostanol in the sediments surrounding sewage effluents in Chesapeake Bay and found good correlation of concentrations to proximity to the sewage outfall. They proposed that coprostanol analyses might be useful for deliniation of zones of contamination, if coupled with sediment dynamics. However, subsequent studies have cast doubt on an invariably anthropogenic source for coprostanol Pocklington et al. (1987) measured coprostanol in particulate organic matter from the Bedford Basin (Nova Scotia, Canada). They found that the pattern of quantitative variation in its concentration over an entire year was identical to that for natural phytosterols and for two independent chemical indicators of primary

production, suggesting sources of coprostanol within the marine biota. Grimalt et al. (1990) measured coprostanol in polluted and unpolluted waters and found it to be ubiquitous, again casting doubt on its source specificity. Venkatesan and Mirsadeghi (1992) found background levels of coprostanol (up to 40 ng g^{-1}) in the sediments of McMurdo Sound (Antarctica) which they attributed to seal faeces. It appears, therefore, that an unambiguous acceptance of coprostanol as a marker for sewage contamination is premature.

Nevertheless, Grimalt et al. (1990) suggested that coprostanol measurements should be complemented by determination of the 5α(H) isomer (present at high concentrations in sewage and urban wastewaters), as the ratios of these compounds gave a more accurate assessment of contamination in water and sediment samples. Coprostanone is also present in uncontaminated waters and sediments, but ratios of the 5α(H) and 5β(H) epimers of coprostanone were also observed to be useful in the assessment of sewage pollution (Grimalt et al. 1990).

Synthetic molecular markers have also been proposed for use as markers of sewage and industrial contamination, since these compounds also find their way into the sewage system. Linear alkylbenzenes (LABs) and associated compounds have been suggested as sewage markers by a number of workers (e.g. Eganhouse et al, 1984; Brunner et al., 1988; Raymundo, 1992). The usefulness of these compounds as dissolved tracers of the sewage plume is compromised by their affinity for particulates. Brunner et al. (1988), measuring linear alkylbenzenesulphonates (LAS) in sewage, found that at low concentrations of suspended solids, more than 90 % of the LAS were in the dissolved phase. When the concentration of solids increased to those found in raw sludge, however, more than 90 % of the LAS had been adsorbed

onto the particulate matter.

MAs have also been investigated as possible tracers of dissolved sewage, as their presence has been reported in wastewaters (Scully et al., 1988). However, although extremely high levels of these compounds have been found in dissolved sewage, their presence within marine biota (Glob and Sorensen, 1987) and their production by algae (Abdul-Rashid, 1990) and via the fermentation of AAs (Burdige and Martens, 1990) leads to problems with their use in the marine environment.

In this Chapter, a low molecular weight ketoamine (1-aminopropanone) has been identified in sewage-contaminated water samples in the dissolved phase. Analytical methodology is simple and almost identical to the procedure employed for MA determination (Chapter 2, Section 2.5. for details). Its potential as an indicator of sewage contamination has been assessed in a series of pilot studies described herein.

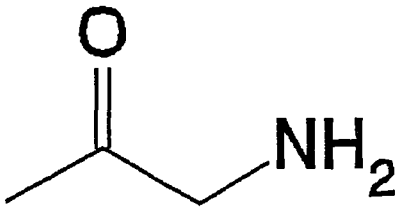
Section 6.2: PRESENT METHOD

6.2.1 Background

During analysis of Mersey water samples, Abdul Rashid (1990) detected an unknown compound with a retention time greater than that of the MAs. This compound was identified as 1-aminopropanone (APR, 1). APR is produced by the condensation of glycine and acetyl CoA, or by the oxidation of threonine (Marver et al., 1966; Gibbs et al.. 1974), and has long been identified as a component of urine (see Figure 6.1). It is present in primary treated sewage effluent, although GC analysis of a tertiary treated sample revealed only trace amounts (Abdul-Rashid,

analysis of a tertiary treated sample revealed only trace amounts (Abdul-Rashid, 1990).

Abdul-Rashid (1990) found that APR was also present in other samples from the Mersey and from Port Erin Bay (Isle of Man). Abdul-Rashid (1990) was unable to unambiguously identify APR, since his attempted synthesis of APR was unsuccessful. It was, therefore, important to attempt the chemical synthesis in order to confirm the compounds identity and to provide a standard to allow quantitative analysis (Chapter 2, Section 2.6).



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6.2.2 GC-MS analysis of APR

APR was identified by GC-MS analysis of its TFAA derivative, details of which are described in Chapter 2. The mass spectrum is shown in Figure 6.2. The odd molecular ion at m/z 169 indicates the presence of N, and the ion at m/z 126 is due to the loss of the CH_3C^+O ion. The ion at m/z 154 results from the elimination of a CH_3 group. The mass spectrum of the TFAA derivative was consistent with the structure of its APR fragmentation (Figure 6.2).

6.2.3 Synthesis of APR

APR is not commercially available, hence it was necessary to synthesise the compound. The synthetic scheme for of APR proposed by Ellinger and Goldberg (1949) was followed with some adaptions. The identity of the compound was checked by ^{1}H NMR (Figure 2.5), melting point and GC-MS. Details of these procedures and of the synthetic APR are reported in Chapter 2.

6.2.4 Calibration of the method

A major problem with the determination of APR was the formation of an artefact peak which was observed during GC analysis of standard solutions (Figure 6.3). The retention time of this unknown compound was about twice that of APR (approx. 27 mins.) and the size of the GC peak was observed to be proportional to the abundance of APR, suggesting formation of the compound *via* an inter-molecular reaction of APR. The artefact peak was also observed during direct injection of APR standards, which implied that the reaction was not occurring during incubation of microdiffusion samples, and was possibly catalysed by the basic sample pH (> 12) and injection temperature of the GC (250 °C).

Although formation of the artefact could not be prevented, a lowering the GC injection temperature to 150 °C and the injection of samples at neutral pH, enabled its abundance to be controlled, and made calibration of the method possible. APR detection limits were 100 nM.

Section 6.3: APR IN ENVIRONMENTAL SAMPLES

6.3.1 Background

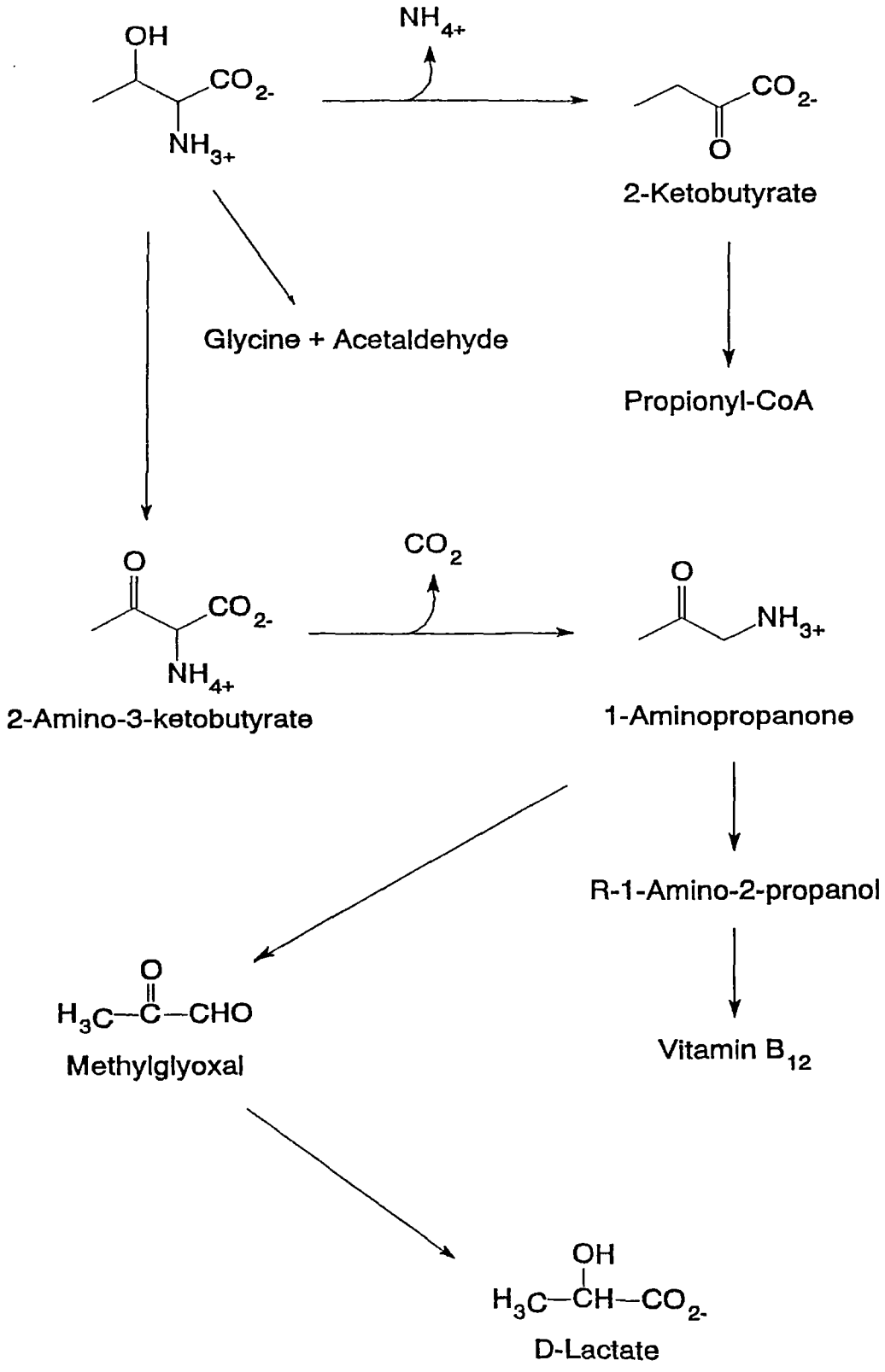
Subsequent to the successful synthesis and calibration of APR (Figure 6.4), the occurrence of APR in a series of environmental samples, including untreated sewage and seawater samples was monitored. Two sites were chosen for sampling of seawater: New Brighton on the Mersey estuary and Port Erin Bay on the Isle of Man (Figures 6.5 and 6.6 respectively). Untreated sewage samples were received from the NRA and were taken from treatment plants at St Helens and Wigan.

New Brighton samples were taken between 07:00 and 18:30 at 30 minute intervals through High and Low Water (14/2/91), to investigate the influence of tidal fluctuations on levels of APR in the seawater. Coprostanol was also measured to compare the behaviour of APR with that of another known sewage marker.

Port Erin Bay was chosen to monitor the dispersal and persistence of the sewage plume as there is only one outlet for the delivery of untreated sewage into the bay. Samples were taken at a series of sites in Port Erin Bay during one cruise (21/6/91) as shown in Figure 6.6.

Untreated sewage samples were incubated and APR levels monitored over 25 days in order to investigate the influence of indigenous microbes on the abundance of APR. Duplicate samples from each location were inoculated with antibiotic (cf. Abdul-Rashid, 1990).

Figure 6.1 Production of APR as a result of the metabolism of threonine (After Metzler, 1977).



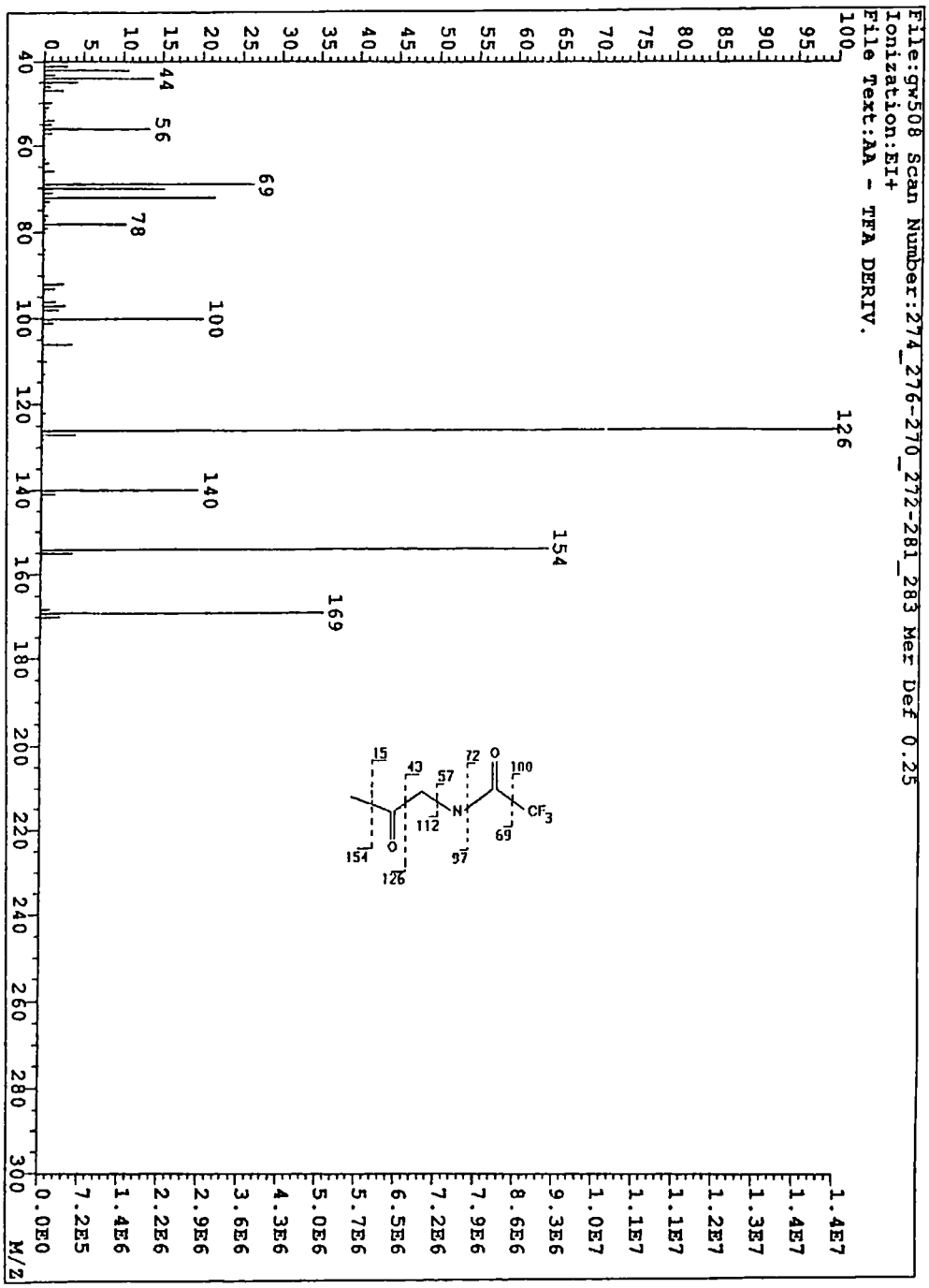


Figure 6.2. Mass spectrum of the TFAA derivative of APR.

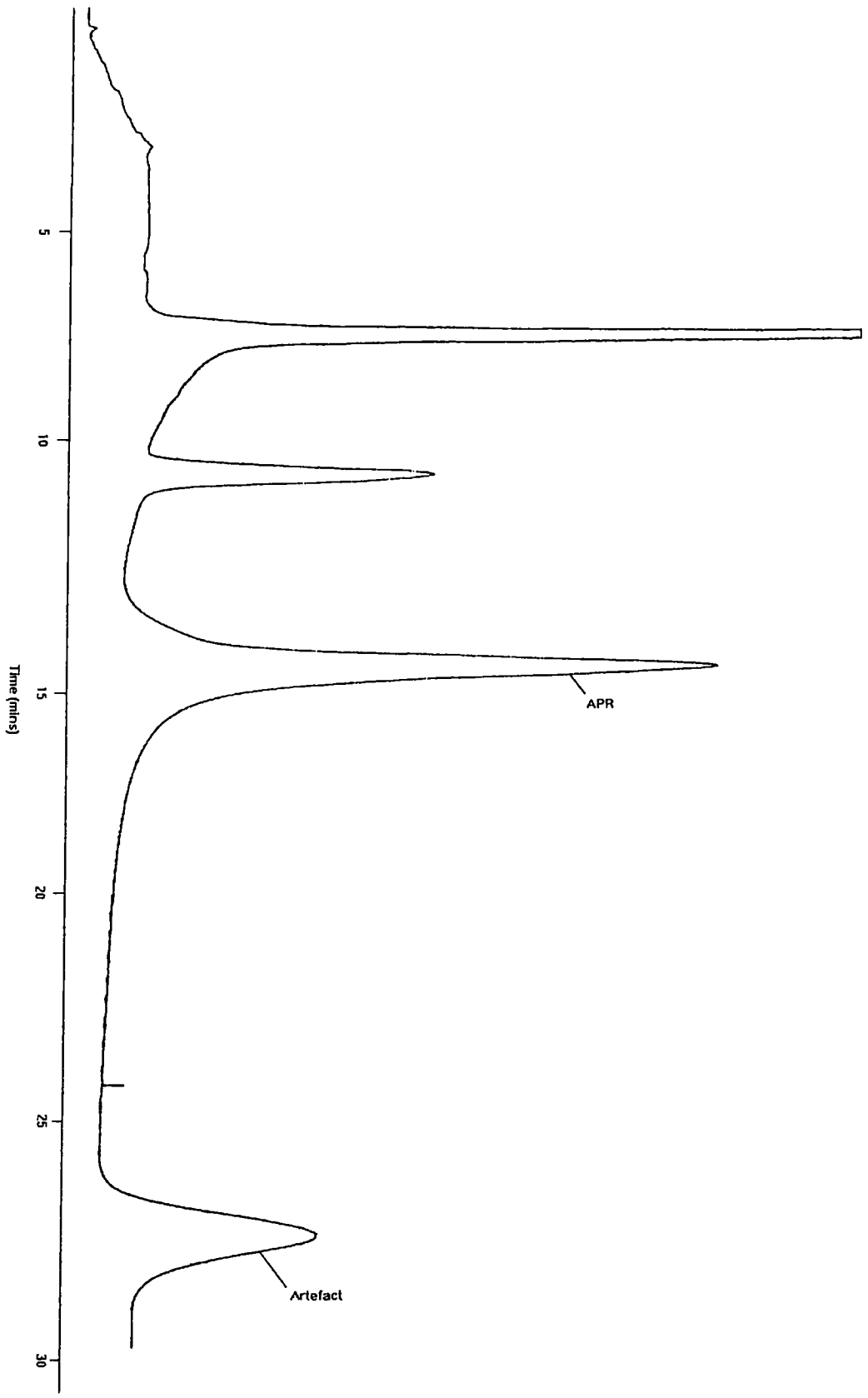


Figure 6.3 GC trace of APR showing artefact formation.

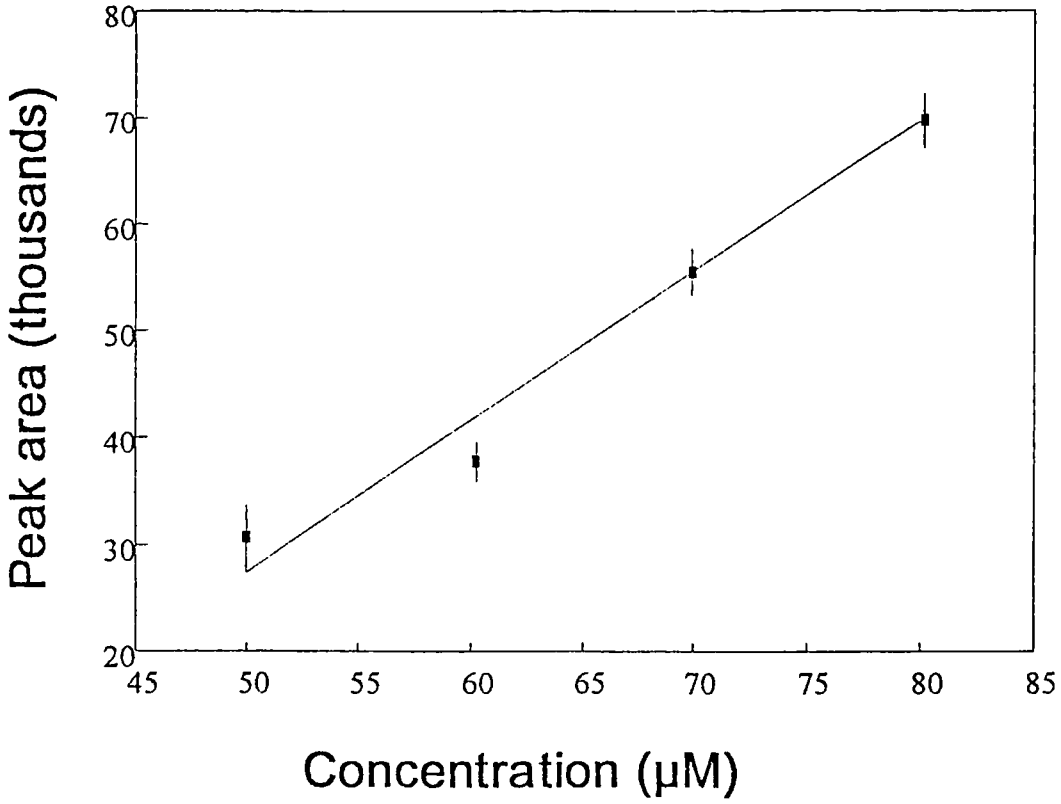
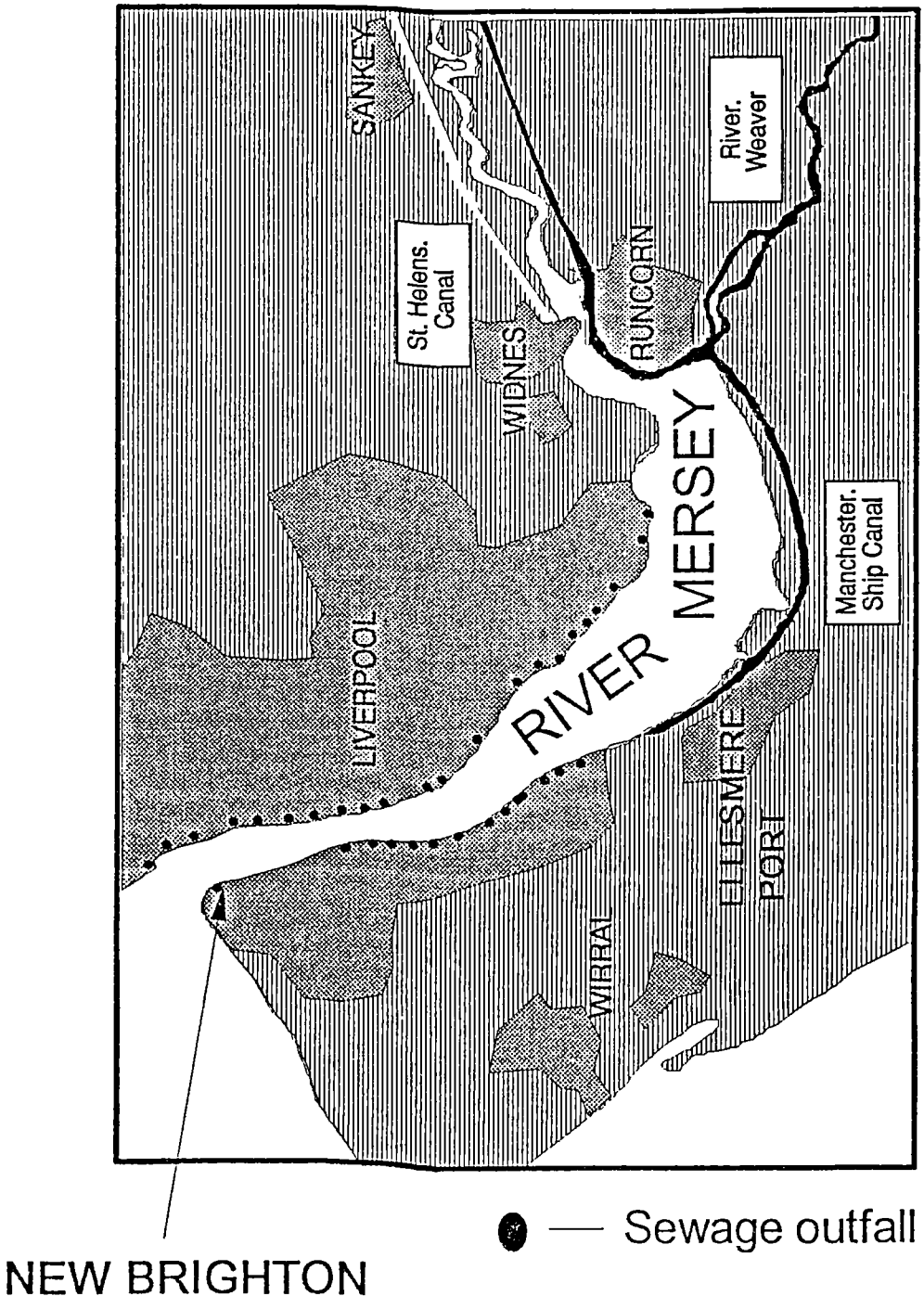


Figure 6.4. Calibration of APR

Figure 6.5 Map of the Mersey Estuary, showing the location of sewage outfalls, and the sampling point at New Brighton.



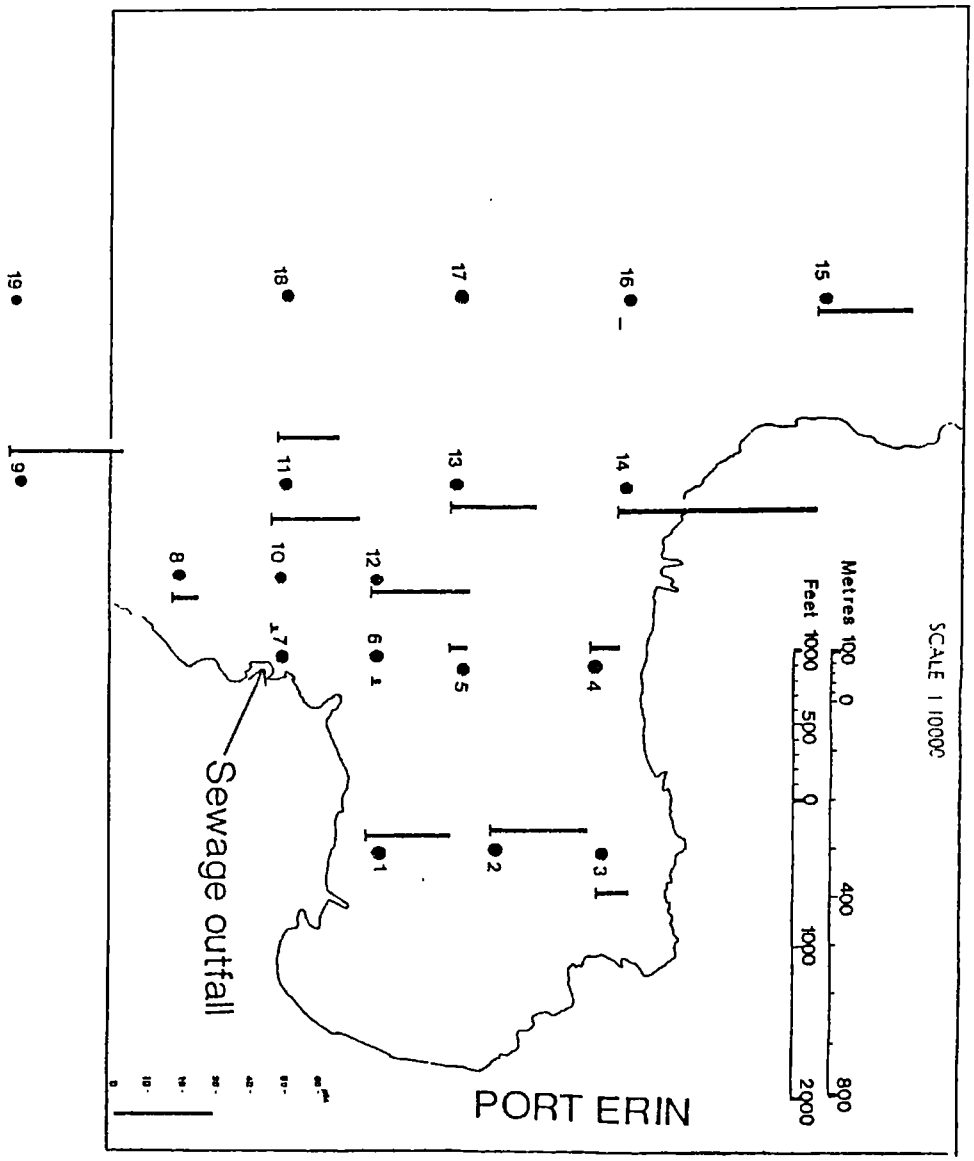


Figure 6.6 Distributions of APR at a series of stations from Port Erin Bay, Isle of Man.

Section 6.4: RESULTS AND DISCUSSION

6.4.1 Results

New Brighton

APR was present in all samples, generally in concentrations of $\leq$ 10 μM (Figure 6.7). [APR] was above 10 μM only once (24 μM) about one hour before low water. Coprostanol was present at lower concentrations but its behaviour was similar to that of APR, apart from a period of about 3 hours (samples 15 to 21).

Port Erin Bay

APR was present in 16 of the 19 samples, although concentrations varied quite markedly (Figure 6.8). The highest concentrations of APR were observed at station 14 (57 μM) which was distant from the outfall (approx. 650 m). [APR] at station 7, closest to the outfall, were much lower (1.6 μM), but significant concentrations were detected at station 10 (25 μM) which was also close to the outflow. Significant [APR] were detected at stations which formed a transect across the mouth of the bay (9-14). Significant levels were also seen in the inner bay (Stations 1-4, $\leq$ 29 μM) and 15 (Irish Sea, 27 μM).

Sewage samples

Initial concentrations of APR in the St Helens samples were higher than in the Wigan samples (Figures 6.9 and 6.10 respectively), and were similar to that of station 14 in the Port Erin water samples. Significant differences in the abundance and persistence of APR were not observed in the antibiotic and untreated samples.

APR decreased in concentration for the duration of the sampling period but was persistent in all samples, and [APR] had not dropped below ~ 10 μM when the final samples were taken (25 days).

6.4.2 Discussion

A typical GC trace of APR in a contaminated water sample is shown in Figure 6.10. The results presented here suggest that APR has potential as a marker of raw sewage. It was detected at significant concentrations in both contaminated seawater and untreated sewage samples. APR concentrations in samples taken through a tidal cycle at New Brighton correlated well with coprostanol determined in water samples from the same site, with the exception of a 180 minute period during the ebb tide, when coprostanol concentrations were considerably enhanced. The observed increase in coprostanol concentrations may have been associated with sediment resuspension during the ebb tide, as coprostanol is predominantly associated with the particulate and sedimentary phases (Brown and Wade, 1984; Grimalt et al., 1990; Venkatesan and Mirsadeghi, 1992). The resuspension of large amounts of sedimentary material is expected given the high tidal range of the Mersey (~ 10 m).

Grimalt et al. (1990) detected coprostanol at concentrations up to 0.04 nM in pristine water samples, while samples from New Brighton yielded concentrations which were nearly 3 orders of magnitude higher ($\leq$ 20 nM), and were closer to those observed by Grimalt et al. (1990) for coastal areas receiving urban inputs ($\leq$ 1.5 nM). Readmann et al. (1986) detected coprostanol at concentrations of up to 23 nmol/g at Eastham Ferry in the inner Mersey estuary implying considerable sewage

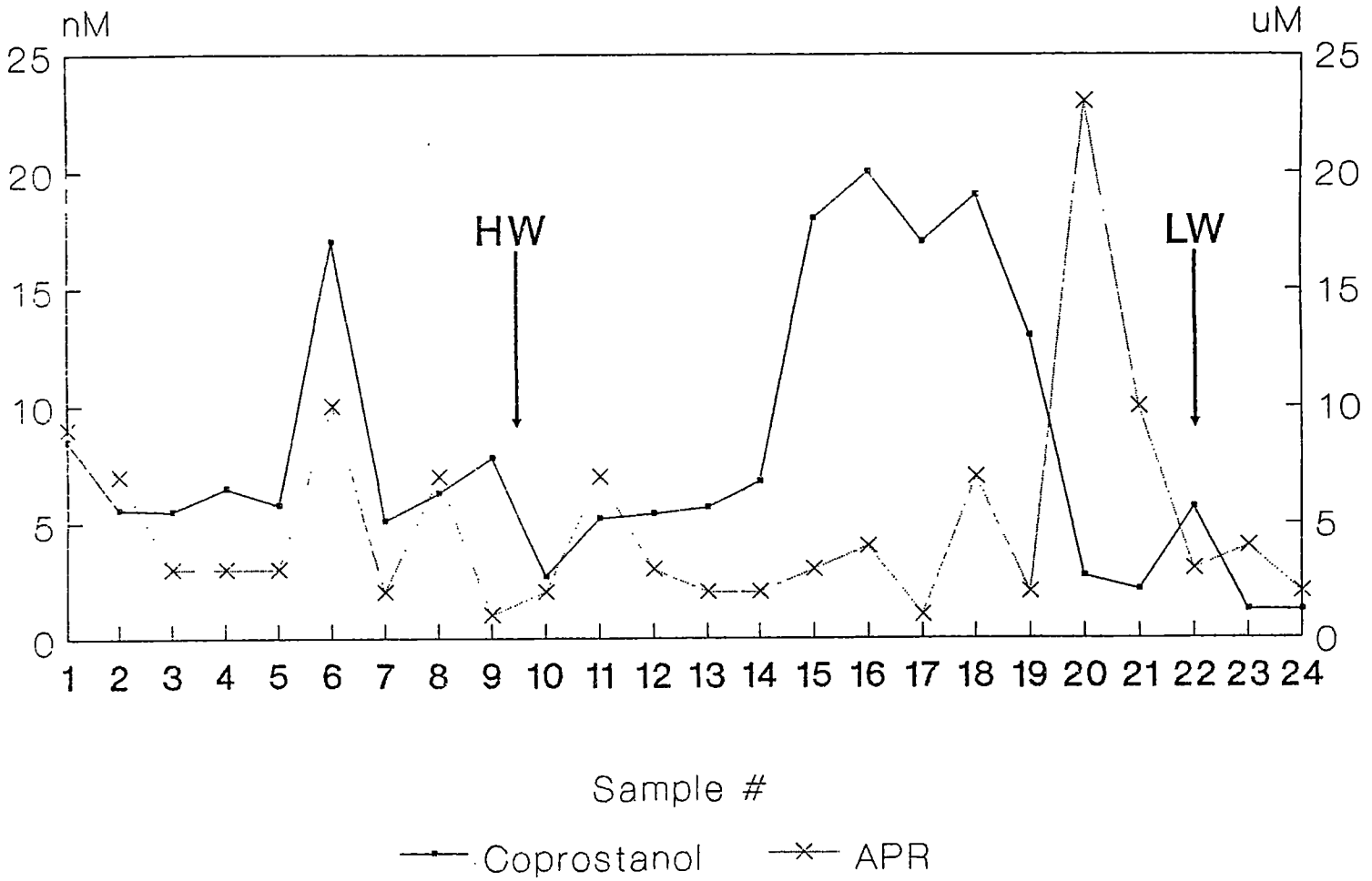
contamination of sediments (see Nicholls et al., 1992).

The fact that higher concentrations of APR were present in the Port Erin samples was interesting for a number of reasons. Firstly, given the number of sewage outfalls on the Mersey (see Figure 6.5), a higher sewage discharge might have been expected and higher concentrations observed at sites on the estuary as compared to more open sites such as Port Erin Bay. However, in interpreting the results from the present study, it is necessary to take account of the mixing and prevailing currents in the Mersey estuary which may result in the influence of the discharged sewage being confined to regions in the vicinity of the respective outfalls and the inner estuary. An example of this may be seen in the case of the Mersey oil spill of August 1989. A discharge of crude oil occurred from the Shell refinary at Stanlow in Ellesmere Port which polluted a considerable area of the inner estuary but did not penetrate extensively into the waters and coastline of the outer estuary (Davies and Wolff, 1990; Jemmett,1991).

Port Erin has only one waste outfall, but the characteristic path of the sewage plume subsequent to discharge is across the mouth of the bay to the opposite headland (Hawkins, personal communication), and is consistent with the high concentrations of APR at stations 9-14 (Fig. 6.6). Obvious signs of extensive sewage discharge have been reported on the coastline in the region of station 14 (Allen, personal communication) which suggests that the sewage plume may not have mixed significantly by the time it has reached stations 14 and 15.

The sewage plume has also been known to enter the shore regions of the inner bay depending on the prevailing winds (Allen, personal communication). This may explain the elevated [APR] seen at stations in the inner bay (Stations 1-4). It

Figure 6.7. APR and coprostanol levels in water samples from New Brighton.



Samples taken 1/2 hourly - (14/2/91)

also suggests a major pollution problem in Port Erin inner bay, if the undiluted discharge from the outfall (which was presumably placed in order to facilitate rapid dilution and dispersal of the sewage) is penetrating into the inner bay. It also implies a health risk to anyone in contact with the water from this area.

The observed lifetime of APR in the sewage samples from St Helens and Wigan (see also Figure 6.11) suggests that it may be persistent in the environment. The fact that no significant enhancement in preservation of APR was observed in samples with added antibiotic may have indicated that: (1) The microbial degradation of APR was negligible, and that the volatile nature of the compound may have been responsible for the decreasing concentrations in time. (2) Insufficient antibiotic was added, and the activity of the indigenous microbes was not reduced. Hence, rates of metabolism of APR were comparable to those in the untreated samples.

It would have been useful to have compared the behaviour of other dissolved compounds associated with untreated sewage (such as urea) to that of APR. However, urea has been reported as an excretion product of marine animals (Walsh et al., 1990) and is utilised by marine phytoplankton (Cochlan and Harrison, 1991). Coprostanol was measured but its strong affinity for the particulate phase limited its usefulness in the assessment of APR as a sewage indicator

Figure 6.8. Distributions of APR with time in a set of sewage samples, one of which contained antibiotic (Wigan treatment plant).

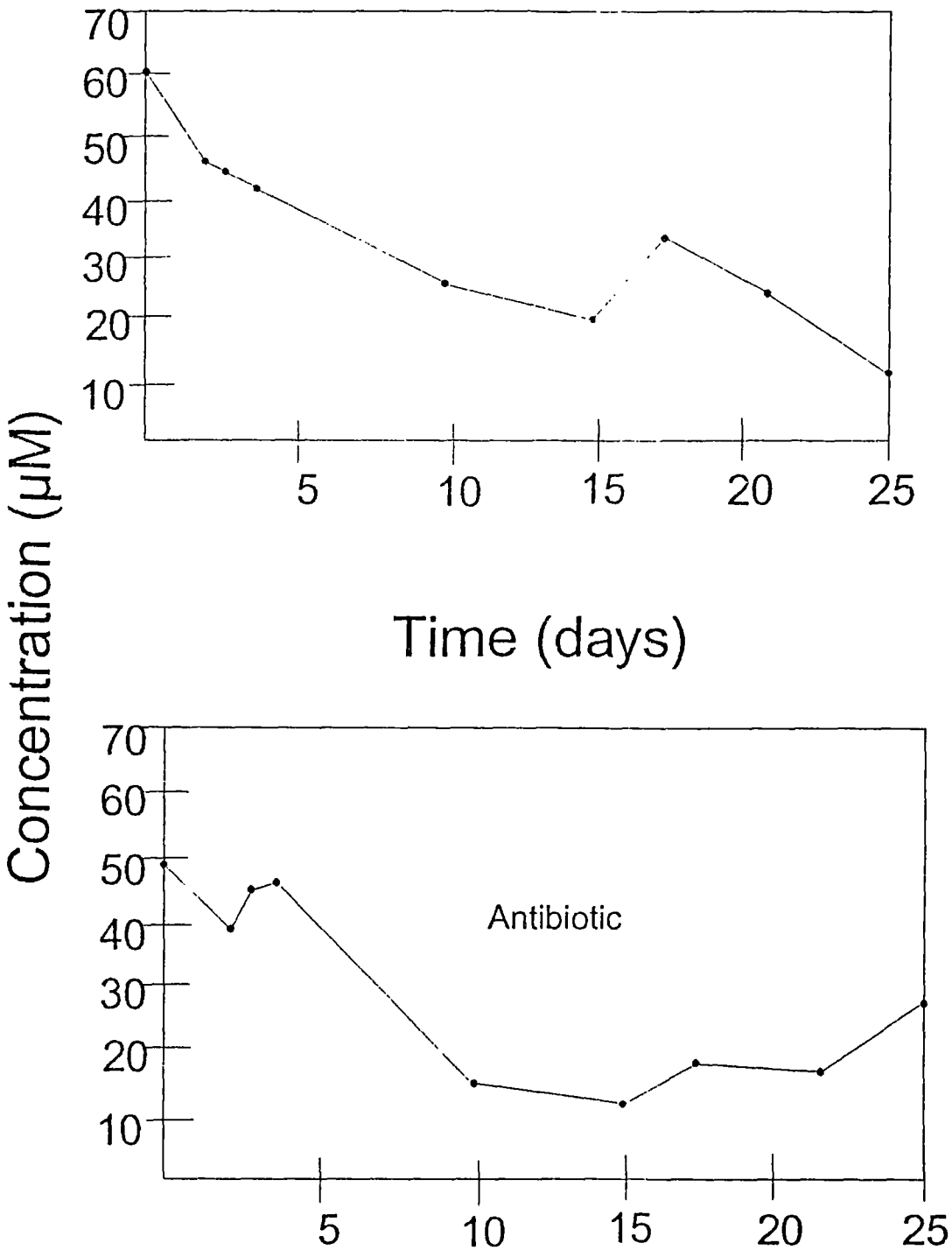
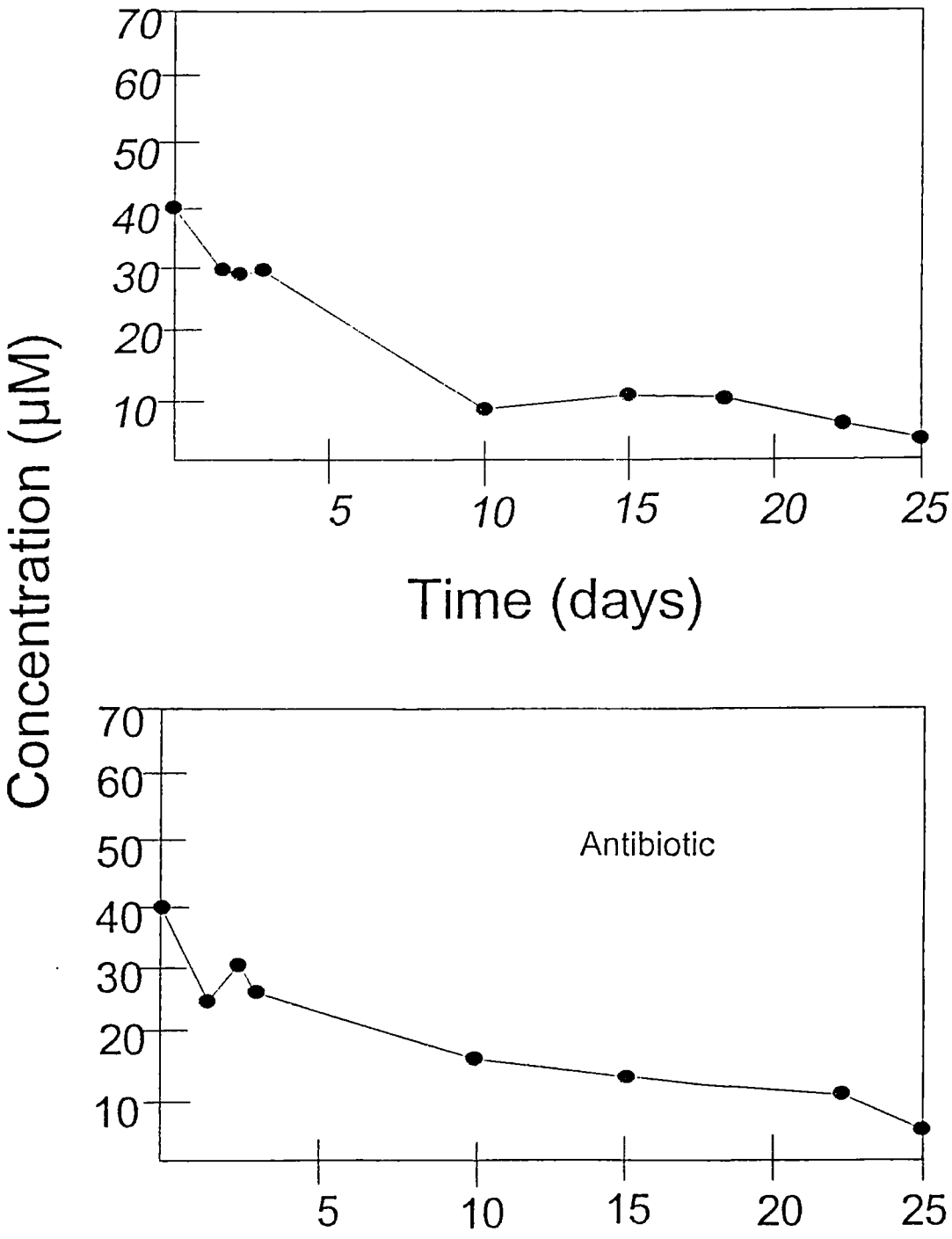


Figure 6.9. Distributions of APR with time in a set of sewage samples, one of which contained antibiotic (St Helens treatment plant).



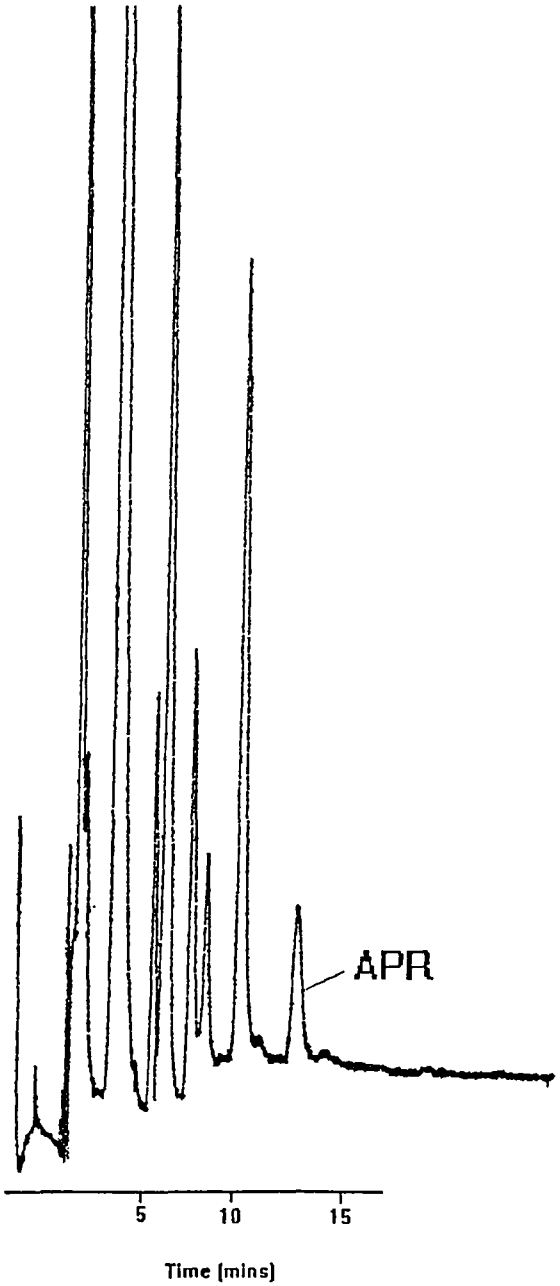


Figure 6.10. Gas chromatogram of a contaminated groundwater sample from Barcelona, Spain.

Section 6.5: SUMMARY

(1) APR (a component of urine) was isolated in sewage and environmental samples and showed potential as a marker for sewage contamination in water samples. The analytical protocol was simple and straightforward (50 ml sample), and compared favourably with the determination of other known markers. Coprostanol, for example, required a much larger volume of sample (1 l in the present study) because of its adsorbance onto particulates, and required derivatization prior to analysis by GC.

(2) APR appeared to be persistent in the environment (> 25 days) and was found in seawater at levels comparable those associated with untreated sewage.

(3) Concentrations of APR in sewage samples treated with antibiotic differed negligibly from untreated samples suggesting that the antibiotic was not sufficiently strong or that the indigenous microbes had a minor influence on the degradation of APR.

CHAPTER 7

CONCLUSIONS AND FUTURE WORK

Section 7.1: CONCLUSIONS

The MAs were detected at significant concentrations in all of the sediments studied, and their levels were higher than any previously reported either for marine or lacustrine sediments.

MA distributions in salt marsh sediments from Oglet Bay through a tidal cycle showed considerable variation in the different zones of the marsh. The depletion of the porewater MAs in the UM during tidal inundation indicated uptake by salt marsh macrophytes for the purpose of osmoregulation. This pattern was not observed for the MM and LM which were only sparsely colonised by marine flora.

The higher concentrations of the MAs observed in the MM and LM, which were more frequently inundated, may have indicated better adaption of the organisms, indigenous to those zones, to salinity stress, therefore leading to minimal uptake of the porewater MAs for osmoregulation. The MM was host to a variety of benthic invertebrates, including *Corophium volutator*, which releases large amounts of TMA (Sorensen and Glob, 1987). Such a source may provide an explanation for the high MM concentrations of dissolved TMA.

GLY concentrations were high in the LM at 0-1 cm (Core Set 4) and decreased with depth down to 4 cms. This corresponded with an increase in levels of sedimentary MMA. The fermentation of GLY to MMA has been reported previously in marine sediments (Burdige and Martens, 1990), and it seems reasonable to assume that this process could occur in the LM.

Although Oglet Bay was the most dynamic environment studied, similar, or higher, concentrations of the MAs were observed in all sediments analysed in the

present study. High porewater concentrations of the MAs observed in the lacustrine Priest Pot sediment may have resulted from high rates of organic matter degradation. The subsurface maxima of MA concentrations in both the porewaters and sediments suggested production of the MAs via the remineralization of organic nitrogen detritus. Similar concentration maxima were observed for MMA and TMA in the porewaters of a sediment core from the Firth of Lorne. This contrasted with distributions of the porewater MAs in the adjacent Loch Etive sediments, where concentrations were at their highest in the surface sediments. It was suggested that the elevated surface concentrations may have indicated algal production of the MAs, and/or their association with sinking algal detritus.

The low generally asymptotic concentrations of sedimentary TMA indicated that adsorption from the porewaters is not a significant geochemical process for TMA. However, it has been suggested that exchange of sedimentary AMM compensates for depletion in the porewaters of marine sediments (Blackburn and Henriksen, 1983). High concentrations of porewater TMA and MMA were observed in the Loch Etive and Firth of Lorne cores at a time when organic productivity would have been expected to be minimal, so a similar buffering process may have been occurring for these ammonia analogues.

A reported decrease in sedimentary amino nitrogen with depth in sediments from the Peru Margin corresponded with an increase in compounds described (tentatively) as quaternary nitrogen (Patience et al., 1992). This may indicate a chemical transformation of volatile organic nitrogen species such as AAs and amines associated with incorporation into the sedimentary organic matrix. Certainly, significant increases in the sedimentary MA pools with depth were not observed in

the present study. Wang and Lee (1990) claimed to have measured a "fixed MA fraction" in sediments. This may be a fraction of the MAs which are irreversibly adsorbed, or recovered from the organic matrix. An equilibrium between strongly adsorbed and exchangeable MAs, such as that suggested for AMM in the porewaters (Blackburn and Henriksen, 1983), may also occur, and would help to explain the asymptotic concentrations of the sedimentary MAs which were frequently observed.

In the present study, MMA, DMA and TMA have been concurrently determined and quantified in sediments for the first time. They appear to constitute a significant fraction of organic nitrogen, and play a role in marine processes such as osmoregulation, and may be important constituents of the sedimentary nitrogen cycle. They certainly need to be taken into account when considering nitrogen budgets in the geosphere.

Section 7.2: FUTURE WORK

7.2.1 The MAs

There are a number of ways in which the geochemical study of the MAs, initiated in the present study, could be carried forward. The work of Wang and Lee (1990) suggested that organic matter significantly influences the adsorption of the MAs to sediments, but the effect of variation in TOC's on levels of sedimentary MAs was not investigated. In the present study, longer cores where fluctuations in TOC content were more obvious, may have shed light on these effects. However, the wide range of chemical and biological processes which can occur in sediments are likely to obscure any obvious trends.

Radiolabelling experiments and stable isotope measurements may yield more

information on sources amd fates of the MAs. Radiochemical studies would enable monitoring of labelled MAs introduced into the sediments, while stable isotope measurements of the MAs by GC-MS could also identify bacterial depletion of the MA pool. Analysis by capillary GC-MS would require prior derivatization of the MAs, leading to problems in the determination of TMA (see Chapter 3), but could still yield valuable information of the geochemistries of MMA and DMA.

Microbial utilization rates of the MAs have been measured previously (King, 1988; Wang and Lee, 1990), but all experiments were carried out in the laboratory. *In situ* incubations, although more difficult to set up, may replicate the natural environment more effectively. Since incorporation into the sedimentary biomass may be a significant sink for the sedimentary MAs (the present method cannot distinguish between biological uptake and abiotic adsorption), stable isotope measurements on the sedimentary MAs should provide useful source information. Furthermore, introduction of radiolabelled MAs to the sediments should indicate the proportion of the sedimentary MA pool which is actually extracted using the microdiffusion procedure.

Since *Tubificid* worms and *Nereis diversicolor* were the most abundant invertebrates in the sediments of the MM, it would have been of great interest to have studied their behaviour in this region of the marsh, including MA content of the organisms and the depth of their burrows in the MM sediments. Unfortunately, the MM at Oglet has matured very rapidly and is now very similar to the UM in terms of colonisation by salt marsh vegetation. However, this work could provide useful results if carried out in any sedimentary environment.

7.2.2 APR

APR has been identified as a potentially useful marker for dissolved sewage, and is present at significant concentrations in environmental water samples The analytical technique employed is simple and allows rapid analysis of water samples. The presence of an artefact peak presents problems in the quantification of this compound, however. A derivatization agent which reacts with the keto group would be necessary, as compounds which complex on the nitrogen, such as TFAA, would also react with amines and amino acids in natural water samples.

Comparison of APR with another dissolved sewage marker would be a useful exercise, whilst speciation experiments are also necessary to enable measurement of the true concentrations of APR which are present in the dissolved phase in the environment.

APPENDIX A

DESCRIPTION OF SAMPLING SITES

Oglet Bay site description

Oglet Bay is situated on the Northern shore of the Inner Mersey estuary, and to the east of the Hale Head lighthouse (Figure 4.1). Up until the early 1980's, Oglet Bay was described as a narrow band of saltmarsh, maybe 100 metres wide (Fairhurst and Buxton, 1982). Since then, the marsh has accreted rapidly, and is characterised by high rates of fine sediment accretion with an annual accretion rate of perhaps 6-7 cm of mud (Jemmett, 1991). The increase in the rate of accretion can be primarily attributed to two processes:

(1) The movement of the erosive low water channel to a position more central to the estuary;

(2) The colonisation of areas of bare mud by cord grass, *Spartina anglica* (Charman, 1990).

The marsh has a narrow band of vegetation characteristic of the strand line at the upper limit of regular tidal inundation, with species such as *Plantago coronupus, Honkenya peploides* and the grass *Festuca rubra* in abundance. Freshwater seepage from the bordering clay cliffs is marked by isolated stands of *Phragmites australis* and *Juncus geradii* (Haslam, 1972). The upper marsh, which is at the level of 9.4m CD, tends to be species less diverse, but with a vegetation characteristic of a marsh in mid-succession. The upper marsh is colonised by typical halophytes such as *Aster tripolium, Puccinellia maritima* and *Spartina anglica.*

A large tidal creek, whose bed level is at approximately 6m CD, and which separates the upper and middle marsh, is an important channel for the tidal flood and drainage of waters from the marsh surface. The middle marsh is characterised by isolated patches of `common saltmarsh grass' *Puccinellia maritima,* the biennial `scurvy grass' *Cochlearia officinalis* in early spring and summer, the perennial `sea aster' *Aster tripolium* and small swards of *Spartina anglica.* In between the

vegetation cover, there is clear evidence of extensive microalgal growth, which composes of epipelic diatoms (*Navicula* and *Nitzschia* species) and euglenoid algae (Jemmett *pers comms*).

At lower positions on the marsh, from approximately 8.5 to 8.0 m CD, the marsh is much more monospecific with large and almost continuous stands of *Spartina*. A more diverse vegetation with *Aster tripolium* and the saltmarsh pioneer *Salicornia europea* (Grant et al., 1986), are associated with the shallow creek dendrites which ramify through this area of marsh.

The distribution of *Spartina* on the marsh tends to be less continuous as the frequency of tidal influence increases towards the lower limit of macrophyte vegetation. In these areas, the presence of dense microalgal blooms is a common feature of the sediment surface. The lowermost limit of macrophyte vegetation at approximately 7.7 m CD, is demarked by a steeply sloping channel to intertidal mudflat.

Loch Etive site description

Loch Etive is a sea loch or fjord situated 5 km north of Oban on the west coast of Scotland (Figure 4.2). The loch is 28 km long and is divided into two distinct basins by a shallow sill at Bonawe: the inner, deeper basin having a maximum depth of 170 m compared with a maximum depth of 60 m in the outer basin. The loch is connected to the coastal waters of the Firth of Lorne via an extensive shoal-choked sill at Connel. The large catchment area (> 1300 km^2), small surface area (approx. 26 km^2) and high rainfall (> 2000 mm^1) lead to a considerable fresh-water influence within the loch. The outer basin is estuarine in circulation showing a well-mixed, oxygenated water column. In contrast, circulation in the inner basin is more restricted, with a dominant fresh/brackish flow moving seawards at the surface, below which is an intermediate water mass characterised by a density range of 10-22 σ_t. Major exchange of water between the two basins is restricted by the Bonawe sill such that a dense, often oxygen-poor, body of water occurs at depth in the inner basin. Periodic

flushing renews these bottom waters.

The population within the catchment area of Loch Etive is very low. The only sizeable community in the region is Oban (population approx. 10 000) which lies outside the catchment, and here industrial activity is minimal. The nearest sizeable industrial area is Glasgow and the Clyde Valley which lie over 100 km to the south east.

The contrast in environmental setting of the outer and inner basins is also reflected in the character of the sediments. The silts and clays of the outer basin generally show a well developed red-brown surface extending down to 5 cm below the sediment/water interface. Below, the reduced sediments tend to green-grey. There is an abundant macrofaunal presence in the upper sediemnts, dominated by two species of worm, *Capitella capitata* and *Nepthys hombergi*, and occasional bivalves, especially, *Macoma balthica.* At depth, below the oxygenated zone, macrofaunal activity is restricted or absent. In contrast, the often oxygen-poor conditions of the inner basin promote a sediment with only 1 cm oxidising surface below which dark-grey to black sediments are usual. Faunal activity is more restricted than in the outer basin, with only the tube worm *Spirochaetopterus typicus* being prevalent. Hence, in these latter sediments biomixing, in contrast to irrigation, is negligible (Wood et al., 1973; Edwards and Edelsten, 1977; Ridgway, 1984; Ridgway and Price, 1985).

APPENDIX B

DATA TABLES*

* For each table (% TON) should read (% TN) and applies to the individual MAs.

Oglet Bay, UM (20/3/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	33.73	1.75	11.82	0.58	19.67	2.58	10.70
1-2	39.17	3.69	12.63	0.39	7.00	0.90	2.77
2-3	40.76	3.78	12.14	0.55	10.13	1.34	3.19
3-4	44.52	3.20	11.20	0.55	10.88	1.23	5.16
4-5	47.81	3.65	12.38	0.38	-*	0.95	2.44
5-6	43.10	2.96	14.75	0.40	8.81	0.78	1.99
6-7	57.96	2.92	15.48	0.23	10.71	0.86	1.56
7-8	53.12	3.12	14.84	0.36	4.98	1.08	1.94
8-9	51.23	2.37	14.77	0.24	11.16	0.98	2.06
9-10	58.82	3.04	14.83	0.29	5.65	0.36	1.50
10-11	60.29	2.30	14.78	0.24	10.29	0.65	1.39
11-12	57.70	3.19	14.61	0.31	5.95	0.52	1.36
12-13	53.34	3.22	14.16	0.40	7.64	0.68	1.43
13-14	56.10	3.00	14.22	0.40	11.16	1.11	1.65
14-15	54.14	3.23	14.69	0.44	9.86	0.77	1.18
15-16	54.47	2.86	13.97	0.30	13.15	0.84	1.50
16-17	47.92	3.19	14.11	0.25	7.36	0.32	0.60
17-18	48.54	3.40	14.96	0.04	6.52	0.25	1.06

* Any values which were rejected as outliers are not included in these tables.

Oglet Bay, UM (26/3/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	48.08	3.41	14.12	0.36	7.43	2.22	1.05
1-2	48.24	3.24	14.88	0.02	0	0	0.65
2-3	52.23	4.13	12.77	0.63	11.09	2.71	1.48
3-4	58.56	4.62	12.72	0.51	9.23	1.20	0.69
4-5	46.07	3.37	13.68	0.34	6.97	1.93	1.12
5-6	48.97	3.76	13.03	0.81	13.24	3.52	4.75
6-7	44.95	3.00	14.97	-	-	-	-
7-8	45.73	2.84	16.04	0.82	19.14	4.79	4.83
8-9	47.00	3.32	14.17	0.53	11.77	2.94	1.31
9-10	47.46	2.94	16.40	0.38	9.66	1.64	1.77
10-11	48.21	2.85	16.90	-	-	-	-
11-12	44.50	2.58	17.28	0.37	9.86	2.92	1.58
12-13	39.87	2.75	14.51	0.34	9.26	2.24	1.03
13-14	54.52	4.08	13.37	0.53	9.37	2.25	1.39
14-15	46.39	3.01	15.43	0.24	4.91	2.30	0.66
15-16	43.62	2.67	16.39	0.60	17.11	3.58	1.93
16-17	46.91	3.04	-	-	-	-	-
17-18	54.22	3.52	15.38	0.49	10.19	2.33	1.28

Oglet Bay, UM (31/3/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs* (mg/g)	MMA*	DMA	TMA
0-1	49.86	3.80	13.13			3.68	5.05
1-2	46.32	3.41	13.62			6.58	6.26
2-3	53.79	3.91	13.75			1.68	2.27
3-4	51.88	3.92	13.24			2.84	2.96
4-5	47.37	3.51	13.50			2.62	2.48
5-6	44.58	2.97	15.00			3.18	1.90
6-7	41.16	2.06	19.99			-	4.67
7-8	44.62	3.06	14.56			5.47	4.22
8-9	45.98	3.14	14.63			2.25	1.53
9-10	45.95	2.98	15.39			1.72	1.69
10-11	44.91	3.01	14.97			-	3.80
11-12	41.40	2.51	16.50			2.50	2.76
12-13	43.55	3.08	14.14			2.37	1.84
13-14	47.57	3.42	13.88			2.35	2.17
14-15	49.63	3.44	14.44			2.18	2.50
15-16	50.26	3.56	14.11			2.37	1.84
16-17	46.05	3.28	14.05			2.35	2.17
17-18	49.72	3.54	14.07			2.18	2.50

* MMA > 100 %

Oglet Bay, UM (3/4/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	46.75	3.52	13.13	1.47	20.25	3.88	17.73
1-2	49.91	3.61	13.62	0.56	14.26	0.60	0.66
2-3	51.17	3.81	13.75	0.71	14.78	2.42	1.53
3-4	54.18	4.10	13.24	0.78	12.22	1.47	5.38
4-5	49.68	3.65	13.50	0.71	16.37	1.77	1.24
5-6	58.25	5.42	15.00	0.69	6.39	5.34	1.03
6-7	47.89	3.33	19.99	0.62	16.28	1.77	0.86
7-8	44.38	2.89	14.56	0.44	14.20	0.64	0.40
8-9	-	3.08	14.64	-	-	-	-
9-10	44.56	3.00	15.40	0.23	-	7.77	0
10-11	43.56	2.98	14.97	0.61	18.22	1.11	1.01
11-12	39.48	2.54	16.50	0.38	14.52	0.37	0.22
12-13	41.53	2.77	14.14	0.28	8.85	0.85	0.32
13-14	-	3.01	13.88	0.45	13.69	0.49	0.70
14-15	39.14	3.01	14.43	0.48	14.56	0.90	0.66
15-16	51.99	2.80	14.11	0.45	15.44	0.56	0
16-17	42.54	-	14.05	-	-	-	-
17-18	44.29	2.80	14.07	0.24	7.10	0.94	0.55

Oglet Bay, MM (20/3/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	42.37	2.40	17.66	0.41	11.82	1.77	3.41
1-2	47.69	2.98	16.00	0.60	14.86	2.19	3.13
2-3	46.24	2.76	16.78	0.64	13.57	4.30	5.40
3-4	49.74	3.18	15.68	0.12	1.16	0.84	1.88
4-5	49.62	3.28	15.11	0.63	12.80	2.60	3.59
5-6	49.02	3.48	14.09	0.50	8.58	1.65	4.05
6-7	45.54	3.29	13.46	0.93	15.72	3.65	8.97
7-8	40.68	2.67	15.27	0.35	9.24	1.06	2.78
8-9	41.16	2.19	18.30	0.16	4.65	0.71	2.17
9-10	45.36	2.77	16.37	0.36	6.93	2.34	3.55
10-11	43.44	2.70	16.11	0.40	7.94	2.46	4.28
11-12	44.22	2.83	15.63	0.43	10.58	1.71	3.05
12-13	39.21	0.060		-	-	1.61	0.90
13-14	43.25	2.57	23.96	0.34	10.97	-	-
14-15	41.95	3.48	12.04	0.22	4.14	0.55	1.64
15-16	42.65	3.39	6.29	0.06	0.72	0.28	0.81
16-17	43.59	3.22	13.52	0.04	0.59	0.16	0.66
17-18	45.83	3.68	12.46	0.41	8.68	0.98	1.51

Oglet Bay, MM (26/3/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	43.98	2.97	14.87	0.54	11.31	4.47	2.43
1-2	48.34	3.36	14.41	0.30	6.05	1.58	1.26
2-3	35.74	2.10	17.10	0.29	8.01	2.51	3.28
3-4	47.80	3.53	13.56	0.28	4.98	1.41	1.59
4-5	51.25	3.69	13.90	0.39	6.86	2.07	1.73
5-6	48.69	3.63	13.41	0.63	12.40	3.08	1.88
6-7	47.15	3.27	14.43	0.45	9.14	2.33	2.34
7-8	44.42	2.76	16.07	0.55	12.54	4.07	3.45
8-9	44.74	3.03	14.75	0.29	6.12	1.66	1.67
9-10	48.14	3.63	13.29	0.38	6.86	1.51	2.08
10-11	45.65	3.24	14.07	0.52	10.32	2.64	3.13
11-12	46.01	3.21		-	-	-	-
12-13	45.81	3.55	12.93	0.25	5.71	-	1.27
13-14	45.72	3.05	15.00	0.47	9.57	3.83	2.07
14-15	45.70	3.08	14.88	0.21	5.33	-	1.40
15-16	46.75	3.40	13.75	0.30	6.28	1.32	1.15
16-17	43.64	1.86	13.28	0.22	8.39	2.26	1.46
17-18	44.40	2.83	16.28	0.28	7.67	1.31	1.06

Oglet Bay, MM (31/3/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	43.34	2.41	18.05	0.89	22.86	4.76	9.41
1-2	44.74	3.23	14.24	0.96	18.60	4.45	6.81
2-3	47.60	3.49	13.62	0.88	17.70	4.37	2.97
3-4	-	-	-	-	-	-	-
4-5	51.36	4.09	12.56	0.89	15.63	3.20	3.00
5-6	48.80	3.69	13.24	-	-	-	-
6-7	45.71	3.10	14.74	0.99	27.42	4.67	-
7-8	45.40	3.25	14.04	0.69	14.59	3.21	3.64
8-9	46.86	3.23	14.52	1.29	30.09	5.81	4.18
9-10	44.34	3.30	13.45	1.20	23.47	5.17	7.89
10-11	45.65	3.05	14.98	0.72	16.24	3.37	3.83
11-12	45.50	3.39	13.43	1.15	15.38	15.01	3.63
12-13	39.65	2.60	15.27	2.05	59.92	12.50	6.46
13-14	44.01	3.08	14.36	0.69	16.43	3.22	2.68
14-15	45.27	3.19	14.19	0.69	15.77	2.66	3.20
15-16	43.37	3.18	13.65	1.07	25.06	4.74	3.79
16-17	44.65	3.15	14.19	1.12	27.94	4.05	3.59
17-18	46.02	3.19	14.41	1.04	25.50	4.12	2.91

Oglet Bay, MM (3/4/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	44.51	2.93	18.05	0.61	21.00	-	-
1-2	43.44	1.95	14.24	0.46	17.00	2.47	4.13
2-3	45.98	1.84	13.62	0.68	26.98	4.07	5.82
3-4	47.65	3.47		0.23	6.02	0.69	-
4-5	50.03	2.70	12.56	0.25	5.95	1.00	2.36
5-6	-	-	13.24	-	-	-	-
6-7	39.75	0.90	14.74	0.58	42.88	7.82	14.16
7-8	44.45	1.26	14.04	0.43	25.95	3.43	4.73
8-9	48.62	1.99	14.52	0.36	12.69	2.16	3.20
9-10	46.19	3.12	13.45	0.46	12.19	1.17	1.50
10-11	44.39	3.02	14.98	0.50	14.05	1.14	1.48
11-12	42.95	2.75	13.44	0.28	8.74	0.79	0.83
12-13	-	-	15.27	-	-	-	-
13-14	43.07	2.96	14.36	0.40	9.76	1.96	1.63
14-15	45.29	3.39	14.19	0.64	15.12	1.66	1.98
15-16	41.68	2.64	13.65	0.65	20.28	2.07	2.20
16-17	43.94	2.73	14.19	1.23	35.02	5.48	4.70
17-18	-	-	14.41	-	-	-	-

Oglet Bay, LM (20/3/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	30.88	4.58	17.68	0.06	0.79	0.11	0.49
1-2	48.40	4.06	13.14	0.14	2.28	0.36	0.70
2-3	48.11	4.43	12.76	-	-	-	-
3-4	-	4.39	-	-	-	-	-
4-5	48.21	4.00	13.19	0.22	4.36	0.75	0.44
5-6	46.86	3.18	15.80	0.29	6.84	0.96	1.24
6-7	47.11	2.93	16.13	0.56	15.39	3.32	0.59
7-8	48.25	3.12	15.46	0.35	9.51	0.82	0.80
8-9	42.25	3.15	17.81	0.54	13.93	1.75	1.41
9-10	43.07	3.28	14.36	0.40	8.90	1.79	1.60
10-11	40.91	2.19	17.80	0.28	9.74	1.18	2.02
11-12	46.72	3.33	14.65	0.22	5.19	0.52	1.02
12-13	47.86	3.38	14.84	0.47	10.71	0.90	2.33
13-14	45.54	3.28	15.18	0.06	1.29	-	0.44
14-15	47.29	2.94	14.63	0.49	13.26	1.52	1.95
15-16	44.07	3.36	15.39	0.51	10.30	2.98	1.92
16-17	46.35	3.24	14.54	0.25	6.65	0.81	0.34
17-18	47.90	2.69	14.06	0.28	8.37	0.92	1.12

Oglet Bay, LM (26/3/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	31.63	1.98	15.97	0.36	4.46	6.29	7.53
1-2	48.24	3.68	13.12	0.35	2.91	2.94	3.74
2-3	49.10	3.92	12.52	0.44	6.47	1.88	2.87
3-4	42.38	2.89	14.68	10.09	-	1.40	1.58
4-5	42.88	2.98	14.38	0.38	17.54	6.85	5.08
5-6	40.90	2.87	14.28	0.37	4.02	4.36	4.54
6-7	42.78	3.22	13.30	0.90	17.91	4.92	5.02
7-8	46.35	3.12	14.88	0.34	3.90	3.45	3.47
8-9	40.06	2.27	17.66	0.28	4.21	3.78	4.54
9-10	39.94	2.22	18.01	0.52	15.53	3.15	4.68
10-11	39.37	2.19	18.56	0.41	11.91	2.65	4.30
11-12	45.50	3.15	14.46	0.68	13.98	3.37	4.41
12-13	49.81	3.82	13.03	0.67	11.09	3.18	3.74
13-14	49.19	3.50	14.07	0.68	7.15	6.09	6.27
14-15	42.99	2.97	14.49	0.58	5.94	6.43	7.02
15-16	39.17	2.77	14.14	0.99	22.77	6.71	6.42
16-17	41.79	2.92	14.31	0.52	7.44	5.56	4.69
17-18	47.34	3.12	15.18	0.37	3.62	4.38	3.81

Oglet Bay, LM (31/3/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	29.56	1.54	16.66	0.71	32.76	5.78	7.68
1-2	46.59	3.80	12.60	0.60	10.74	1.62	3.52
2-3	42.76	3.30	14.32	1.49	31.99	6.26	6.80
3-4	43.88	3.38	13.22	0.69	15.19	2.49	2.80
4-5	38.60	2.77	14.69	0.71	18.66	3.36	3.58
5-6	41.74	3.04	17.50	0.58	14.04	2.41	2.70
6-7	49.95	3.54	14.72	0.52	9.41	2.58	2.56
7-8	47.50	3.64	14.89	0.71	-	1.74	2.13
8-9	43.99	3.02	14.87	1.12	18.92	3.37	3.00
9-10	37.67	2.48	16.38	0.9	37.61	5.79	4.59
10-11	43.52	3.16	15.81	1.08	23.13	4.81	4.84
11-12	46.38	3.83	14.26	1.26	20.22	3.36	3.22
12-13	47.34	3.67	12.26	0.86	27.60	5.45	3.75
13-14	47.23	3.84	11.79	0.92	13.62	2.50	2.61
14-15	43.52	2.88	13.44	-	-	-	-
15-16	46.38	3.39	12.81	1.07	21.51	3.58	4.63
16-17	47.34	3.75	12.43	1.04	21.13	3.58	3.20
17-18	44.51	3.50	15.86	0.22	22.39	3.93	2.47

Oglet Bay, LM (3/4/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	32.88	1.97	16.66	0.51	23.63	1.72	0.47
1-2	44.73	3.56	12.60	0.81	21.48	0.83	0.45
2-3	44.77	3.14	14.32	0.62	16.36	1.56	1.83
3-4	46.11	3.49	13.22	0.79	20.06	2.16	0.45
4-5	42.18	2.87	14.69	-	-	-	-
5-6	35.43	2.02	17.50	0.57	24.60	1.64	1.88
6-7	42.63	2.90	14.72	0.78	23.50	2.33	1.17
7-8	45.22	3.05	14.89	1.03	30.01	2.53	1.38
8-9	53.76	3.62	14.87	1.17	29.89	2.38	-
9-10	23.11	2.62	16.38	0.64	21.73	1.56	0.98
10-11	42.60	2.71	15.81	0.77	26.90	1.42	-
11-12	44.18	3.10	14.26	1.64	47.45	5.54	-
12-13	49.06	4.00	12.26	0.05	-	1.20	-
13-14	48.42	4.11	11.79	1.01	22.36	1.29	0.90
14-15	52.60	3.91	13.44	1.53	37.12	1.37	0.64
15-16	48.98	3.82	12.81	1.16	27.98	1.45	0.94
16-17	50.00	4.02	12.43	0.59	12.87	0.96	0.84
17-18	48.58	3.06	15.86	-	-	-	-

Firth of Lorne (24/2/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	35.15	3.00	14.17	0.08	2.55	0.10	0.18
1-2	35.49	3.00	14.27	0.30	7.98	0.85	1.51
2-3	36.14	2.96	13.81	0.18	5.45	0.38	0.69
3-4	36.22	2.74	14.49	0.09	2.46	0.18	0.45
4-5	35.52	3.02	15.08	0.36	11.35	0.47	0.83
5-6	36.14	2.85	14.17	-	14.71	0.84	-
6-7	36.02	2.94	15.45	0.46	8.38	0.60	0.76
7-8	35.35	3.10	15.75	0.26	7.64	0.33	0.76
8-9	35.68	2.74	15.82	0.20	7.84	0.36	0.54
9-10	38.08	2.88	16.81	-	15.60	1.00	-
10-11	34.98	2.76	15.18	0.22	7.34	0.34	1.23
11-12	35.60	2.98	15.72	0.41	9.86	0.36	0.43
12-13	35.76	2.86	15.36	0.23	6.23	0.33	-
13-14	34.69	3.02	14.09	0.30	10.64	0.95	0.96
14-15	35.06	3.00	14.52	0.19	9.32	0.88	0.66
15-16	35.04	2.73	14.14	-	8.74	0.37	0.41
16-17	35.09	2.75	13.65	0.35	7.22	0.45	0.47
17-18	34.42	2.98	14.45	0.49	6.04	0.30	0.43

Loch Etive, Inner Basin (24/2/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	37.63	4.35	12.97	0.03	0.63	0.06	0.04
1-2	37.34	4.42	14.77	-	-	-	-
2-3	49.63	5.22	13.99	-	-	-	-
3-4	34.14	5.76	13.04	0.13	1.97	0.08	0.15
4-5	43.72	5.33	12.79	0.16	2.81	0.12	0.03
5-6	36.21	5.44	12.54	0.13	2.03	0.22	0.10
6-7	50.13	5.35	12.78	0.01	0.15	0.01	0.01
7-8	45.86	5.90	11.74	0.09	1.31	0.12	0.06
8-9	40.32	5.75	11.98	0.06	0.95	0.08	0.05
9-10	35.49	5.60	11.82	-	-	-	-
10-11	49.68	5.79	12.19	-	-	-	-
11-12	25.47	5.06	11.99	0.08	1.43	0.08	0.04
12-13	41.08	5.30	12.09	0.04	0.64	0.04	0.03
13-14	47.26	5.59	12.00	0.04	0.63	0.05	0.02
14-15	51.57	5.72	12.69	0.04	0.70	0.06	0.04
15-16	19.22	3.77	15.67	0.15	3.45	0.29	0.17
16-17	32.63	4.87	14.02	0.06	1.09	0.10	0.05
17-18	28.61	4.68	14.23	0.17	3.02	0.43	0.11

Loch Etive, Outer Basin (24/2/92)

(% of TON)

Depth (cm)	TOC (mg/g)	TON (mg/g)	C/N	MAs (mg/g)	MMA	DMA	TMA
0-1	-	-	-	-	-	-	-
1-2	57.06	0.44	13.43	0.12	23.15	2.10	2.06
2-3	-	0.60	-	-	-	-	-
3-4	42.03	-	11.96	-	-	-	-
4-5	34.95	0.61	11.94	0.07	6.31	0.44	5.05
5-6	28.95	0.90	12.04	0.12	12.62	0.54	0.67
6-7	20.92	0.85	13.22	0.14	14.92	1.17	0.96
7-8	29.33	0.62	12.22	-	-	-	-
8-9	25.40	0.82	12.41	-	-	-	-
9-10	22.04	1.30	12.37	0.26	18.56	1.10	0.52
10-11	15.79	0.97	12.73	0.28	31.59	2.91	1.15
11-12	17.09	1.26	11.88	-	-	-	-
12-13	18.06	1.14	11.96	0.25	19.19	1.95	1.12
13-14	29.87	0.66	12.15	0.17	23.36	1.55	0.78
14-15	21.33	0.69	12.74	0.19	25.62	1.08	0.69
15-16	34.02	0.57	12.03	0.12	18.93	1.02	0.52
16-17	21.01	0.88	12.30	0.10	10.79	0.57	0.38
17-18	27.60	0.69	12.02	0.10	13.54	0.69	0.42

APPENDIX C

EQUATIONS FOR THE NORMALISATION OF ENVIRONMENTAL CONCENTRATIONS OF AMMONIA AND THE METHYLAMINES.

The equations relating to the normalisation of the ammonia and MA concentrations in porewater and sediment samples are as follows:

$$C_p \text{ (mol/l)} = C \times R \times D \qquad (1)$$

$$C_p \text{ (mol/g wet sediment)} = C \times D \times R \times P_W \qquad (2)$$

$$C_S \text{ (mol/g dry sediment)} = C \times (W_S/1) \times R \qquad (3)$$

$$C_S \text{ (mol/g wet sediment)} = C \times (W_S/1) \times R \times P_S \qquad (4)$$

Where: C_p = porewater concentration

C = concentration before normalisation

R = recovery of internal std.

D = dilution factor

P_W = percentage porewater in wet sample

C_S = sedimentary concentration

W_S = wgt. of sediment sample

P_S = percentage sediment in wet sample

References

Abdul-Rashid M.K. (1990) Studies of aliphatic amines and other volatile organic compounds in the marine environment. **PhD Thesis.**

Abdul-Rashid M.K., Riley J.P., Fitzsimons M.F. and Wolff G.A. (1991) Determination of volatile amines in sediment and water samples. **Analytica Chimica Acta** 252, 223-226.

Aller J.Y. and Aller R.C. (1986) Evidence for localised enhancement of biological activity associated with tube and burrow structures in deep sea sediments at the HEBBLE site, western North Atlantic. **Deep Sea Research** 33A, 755-790.

Al-Lihaibi S.S. (1991) Organic sulphur in recent marine sediments. **Ph.D. Thesis,** University of Liverpool, UK.

Alberts J.J., Filip Z., Price M.T., Hedges J.I. and Jacobsen T.R. (1992) Cu-O oxidation products, acid hydrolyzable monosaccharides and amino acids of humic substances occurring in a salt marsh estuary. **Organic Geochemistry** 18(2), 171-180.

Allinger N.L., Cava N.P., DeJongh D.C., Johnson C.R., Lebel N.A. and Stevens C.L. (1971) **"Organic Chemistry"** Worth, New York.

Andersson K., Hallgren C., Levin J-L and Nilsson C-A. (1984) Determination of the aliphatic amines by reversed phase high-performance liquid chromatography using 1- naphthyl isocyanate derivatives. **Journal of Chromatography** 312, 482-488.

Arrhenius G. Sediment cores from the East Pacific. (1952) In **"Reports of the Swedish Deep-Sea Expedition 1947-1948"** (H. Petterson ed.) Goteberg Volume V, pp 1-227.

Awapara J. (1962) Free amino acids in invertebrates: A comparative study of their distribution and metabolism. In **"Amino Acid Pools"** (T. Holden ed.) pp 158-175.

Barker H.A. (1981) Amino acid degradation by anaerobic bacteria. **Annual Review of Biochemistry** 50, 23-40.

Bather J.M. and Riley J.P. (1954) **Journal du Conseil** 20, 145-152.

Beliaeff B. and Mary J.Y. (1993) The "most propable number" estimate and its confidence limits **Water Research** 27(5), 799-805.

Berg G., Dahling D.R, Brown G.A and Berman D. (1978) Validity of faecal streptococci as indicators of viruses in chlorinated primary sewage effluents. **Applied and Environmental Microbiology** 36, 880-884.

Bjorkqvist B. (1981) Separation and determination of aliphatic and aromatic amines by high-performance liquid chromatography with ultraviolet detection. **Journal of Chromatography** 204, 109-114.

Blackburn T.H. and Henriksen K. (1983) Nitrogen cycling in different types of sediment from Danish waters. **Limnology and Oceanography** 28(3), 477-493.

Bordovskiy O.K. (1965) Transformation of organic matter in bottom sediments and its early diagenesis. **Marine Geology** 3, 83-114.

Braithwaite A. and Smith F.J. (1985) **"Chromatographic Methods".**

Brezonik. K. (1972) *in* **Nutrients in Natural Waters** (K. Thurman ed.)

Brown R.C. and Wade T.L. (1984) Sedimentary coprostanol and hydrocarbon distribution adjacent to a sewage outfall. **Water Research** 18, 621-632.

Brunner P.H., Capri S., Marcomini A. and Giger W. (1988) Occurrence and behaviour of linear alkylbenzenesulphonates, nonylphenol, nonylphenol mono- and nonylphenol diethoxylates in sewage and sewage sludge treatment. **Water Research** 22(12), 1465-1472.

Budd J.A. and Spencer C.P. (1968) The utilisation of alkylated amines by marine bacteria **Marine Biology** 2, 92-101.

Burchfield H.P. and Storrs E.E. (1962) **"Biochemical Applications of Gas Chromatography"** Academic Press, New York.

Burnett W.C. and Schaeffer O.A. (1980) Effect of ocean dumping on $^{13}C/^{12}C$ ratios on marine sediments from the New York Bight. **Estuarine and Coastal Marine Science** 11, 605-611.

Burdige D.J. and Martens C.S. (1988) Biogeochemical cycling in an organic-rich coastal marine basin: 10. The role of amino acids in sedimentary carbon and nitrogen cycling. **Geochimica et Cosmochimica Acta** 52, 1571-1584.

Burdige D.J. and Martens C.S. (1990) Biogeochemical cycling in an organic-rich coastal marine basin: II: The Sedimentary Cycling of DFAA's. **Geochimica et Cosmochimica Acta** 54, 3033-3052.

Buscail R. (1986) Biogeochemical processes of incorporation, transformation and migration of organic matter at the sediment-water interface: Simulation by ^{14}C labelled compounds. **Advances in Organic Geochemistry** 10, 1091-1097.

Bystedt J., Swenne L. and Aas H.W. (1959) Determination of TMAO in fish muscle. **Journal of Science Food and Agriculture** 10, 301-304.

Carlucci A.F., Craven D.B. and Henrichs S.M. (1984) Diel production and microtrophic utilization of dissolved free amino acids in waters off Southern California. **Applied and Environmental Microbiology** 48, 165-170.

Casselman A.A. and Bannard R.A.B. (1974) Gas chromatography of some polyamines on three porous polymer columns. **Journal of Chromatography** 88, 33- 40.

Charman K. (1990) The current state of *Spartina anglica* in Britain. In "***Spartina anglica*-a research review**" (A.j. Gray and P.E.M. Benham, eds.). Institute of Terrestrial Ecology, NERC, 69-71.

Chen E.C.M. and Farquharson R.A. (1979) Analysis of trace quantities of ammonia and amines in aqueous solutions by reversed-phase high-performance liquid chromatography using *m*-toluoyl derivatives. **Journal of Chromatography** 178, 358- 363.

Chester R. (1990) **"Marine Geochemistry"** Chapman and Hall, Hampshire, England.

Cochlan W.P. and Harrison P.J. (1991) Uptake of nitrate, ammonium, and urea by nitrogen-starved cultures of *Micromonas pusilla* (Prasinophycae): transient responses. **Journal of Phycology** 27, 673-679.

Cohen Y. (1978) Consumption of dissolved nitrous oxide in an anoxic basin, Saanich Inlet, British Columbia **Nature** 272, 235-237.

Cowie G.L. and Hedges J.L. (1992) Sources and reactivities of amino acids in a coastal marine environment. **Limnology and Oceanography** 37(4), 703-724.

Cranwell, P.A. (1986) Esters of acylic and polycyclic isoprenoid alcohols: Biochemical markers in lacustrine sediments. **Geochimica et Cosmochimica Acta**

da Costa K.A., Vrbanac J.J. and Zeisel S.H. (1990) The measurement of dimethylamine, trimethylamine and trimethyamine N-oxide using capillary gas chromatography-mass spectrometry. **Analytical Biochemistry** 187(2), 234-239.

Daikoku T. (1985) Studies on the salinity adaptations in fish. VI. Distribution of trimethylamine oxide on the various tissues and organs in some teleosts. **Bulletin Baika Women's College (Japan)** 20, 125-137.

Dalene M., Mathiasson L. and Jonsson J.A. Trace analysis of free amines by gas-liquid chromatography. **Journal of Chromatography** (1981) 207, 37-46.

Dave S.B. (1969) A comparison of the chromatographic properties of porous polymers. **Journal of Chromatographic Science** 7, 389-399.

Davies N.J. and Wolff G.A. (1989) The Mersey oil spill, August, 1989: A case of the sediments contaminating the oil? **Marine Pollution Bulletin** 21(10), 481-484.

Di Corcia A. and Samperi R. Gas chromatographic determination at the parts per million level of aliphatic amines in aqueous solution. **Analytical Chemistry** (1974) 46(8), 977-981.

Dugdale R.C. and Goering J.J. (1967) Uptake of new and regenerated forms of nitrogen in primary production. **Limnology and Oceanography** 12, 196-206.

Dunn S.R., Simenhoff M.L. and Wesson L.R. (1976) Gas chromatographic determination of free mono-, di- and trimethylamines in biological fluids. **Analytical Chemistry** 48(1), 41-44.

Edwards A. and Edelsten D.J. (1977) Deep water renewal of Loch Etive: a three basin Scottish fjord. **Estuarine and coastal marine science** 5, 575-595.

Eganhouse R.P., Blumfield D.L. and Kaplan I.R. (1983) Linear alkylbenzenes as molecular tracers of domestic wastes in the marine environment. **Environmental Science and Technology** 17, 523-530.

Ellinger and Goldberg (1949) A new route to imazoles. **Journal of the Chemical Society** 1, 263-266.

Emery K.O., Stitt C. and Saltman P. (1964) Amino acids in basin sediments. **Journal of Sedimentary Petrology** 34, 433-437.

Ertel J.R. and Hedges J.I. (1985) Sources of sedimentary humic substances: vascular plant debris. **Geochimica et Cosmochimica Acta** 49, 2097-2017.

Fairhurst C.P. and Buxton N.E. (1982) Salt Marsh Vegetation in the Mersey Estuary. **Unpublished Report to NCC.**

Fitzsimons M.F. (1989) Distributions of the methylamines in a series of algal cultures. **BSc (Hons) Dissertation.**

Fuselli S., Benedetti G. and Mastrangeli R. (1982) Determination of methylamines in air using activated charcoal traps and gas chromatographic analysis with an alkali flame detector (AFD). **Atmospheric Environment** 16(12), 2943-2946.

Gage J. (1974) Shallow-water zonation of sea-loch benthos and its relation to hydrographic and other physical features. **Journal of the Marine Biological Association of the UK** 54, 223-249.

Gallagher J.L. (1974) Sampling macro-organic matter profiles in salt marsh plant root zones. **Soil Science Society of America Proceedings** 38, 154-155.

Gibbs B.F., Itiaba K.,and Crawhall J.C. (1974) A gas chromatographic method for the determination of aminoketones in urine and serum. **Biochemical medicine 11**, 165-170.

Giani D., Giani L., Cohen Y. and Krumbien W.E. (1984) Methanogenesis in the hypersaline solar lake (Sinai). **FEMS Microbiology Letters 25**, 219-224.

Glob E. and Sorensen J. (1987) Determination of dissolved and exchangeable trimethylamine pools in sediments. **Journal of Microbiological Methods 6**, 347-355.

Goldhaber M.B. and Kaplan I.R. (1975) Controls and consequences of sulphate reduction rates in recent marine sediments. **Soil Science 119(1)**, 42-55.

Gordon D.C. (1971) Distribution of particulate organic carbon and nitrogen at an oceanic station in the Central Pacific. **Deep Sea Research 18**, 1127-1134.

Gordon A.S. and Millero F.J. (1985) Adsorption mediated decrease in the biodegradation rate of organic compounds. **Microbial Ecology 11**, 289-298.

Grimalt J.O., Fernandez P., Bayona J.P.and Albaiges J.(1990) Assessment of faecal sterols and ketones as indicators of urban sewage inputs to coastal waters. **Environmental Science and Technology 24**, 357-363.

Gubitz G., Wintersteiger R. and Hartinger A. (1981) Fluorescence derivatization of tertiary amines with 2-naphthyl chloroformate **Journal of Chromatography 218**, 51- 56.

Haslem S.J. (1972) Biological flora of the British Isles. *Phragmites communis* Trin. **Journal of Ecology, 60**, 585-610.

Haugen J.E. and Lichtentaler R. (1991) Amino acid diagenesis, organic carbon and nitrogen mineralization in surface sediments fron the inner Oslofjord, Norway. **Geochimica et Cosmochimica Acta 55**, 1649-1661.

Hanson R.B. (1977) Nitrogen fixation (acetylene reduction) in a salt marsh amended with sewage sludge and organic carbon and nitrogen compounds. **Applied and Environmental Microbiology 33(4)**, 846-852.

Henrichs S.M. (1980) Biogeochemistry of dissolved free amino acids in marine sediments. **Ph.D. thesis** MIT/WHOI.

Henrichs S.M. and Farrington J.W. (1980) Amino acids in interstitial waters of marine sediments: A comparison of results from varied sedimentary environments. **Advances in Organic Geochemistry** pp 435-443.

Henrichs S.M., Farrington J.W. and Lee C. (1984) Peru upwelling region sediments near 15oS. 2. Dissolved free and total hydrolyzable amino acids. **Limnology and Oceanography 29**, 20-34.

Henrichs S.M. and Farrington J.W. (1987) Early diagenesis of amino acids and organic matter in two coastal marine sediments. **Geochimica et Cosmochimica Acta 51**, 1-15.

Henriksen K. and Kemp W.M. (1988) Nitrification in estuarine and coastal marine sediments. In **"Nitrogen Cycling in Coastal Marine Environments"** (T.H. Blackburn and J. Sorensen eds.), SCOPE, John Wiley and Sons Ltd, pp 207-249.

Hippe H., Caspari D., Fiebig K. and Gottschalk G. (1979) Utilization of trimethylamine and other N-methyl compounds for growth and methane formation by *Methanosarcina barkeri*. **Proceedings of the National Academy of Science of the USA 76**, 494-498.

Holm-Hansen O., Strickland J.D.H. and Williams P.M. (1966) A detailed analysis of biologically important substances in a profile off southern California. **Limnology and Oceanography 11**, 548-569.

Howes B.L., Dacey J.W.H. and King G.M. (1984) Carbon flow through oxygen and sulphate reduction pathways in salt marsh sediments. **Limnology and Oceanography 29(5)**, 1037-1051.

Howes B.L. (1985) Effects of sampling technique on measurements of porewater constisuents in salt marsh sediments. **Limnology and Oceanography 30(1)**, 221-227.

Jemmett A.W.L. (1991) An investigation into the heavy metals, sediment and vegetation of a Mersey Estuary salt marsh. **Ph.D. Thesis,** University of Liverpool, U.K.

Jones B.N., Paabo S. and Stein S. (1981) Amino acid analysis and enzymatic sequence determination of peptides by an improved o-phthaldialdehyde precolumn labelling procedure. **Journal of Liquid Chromatography 22**, 565-586.

Jordan T.E. and Correll D.L. (1991) Continuous automated sampling of tidal exchanges of nutrients by brackish marshes. **Estuarine, coastal and shelf science 32**, 527-545.

Jorgensen N.O.G. (1982) Heterotrophic assimilation and occurrence of dissolved free amino acids in a shallow estuary. **Marine Ecology-Progress Series 8**, 145-159.

Kemp W.M., Wetzel R.L., Boynton W.R., D'Elia C.F. and Stevenson J.C. (1982) Nitrogen cycling and estuarine interfaces. In **"Estuarine Comparisons"** (Kennedy V.S. ed.), Academic Press, New York. pp 209-230.

Kim K.H. and Burnett W.C. (1988) Accumulation and mixing of Peru Margin sediments. **Marine Geology** 80, 181-194.

King G.M., Klug M.J. and Lovley D.R. (1983) Metabolism of acetate, methanol and methylated amines in intertidal sediments of Lowes Cove, Maine. **Applied and Environmental Microbiology** 45(6), 1848-1853.

King G.M. (1984) Metabolism of trimethylamine, choline and glycine betaine by sulphate reducing bacteria in marine sediments. **Applied and Environmental Microbiology,** 48(4), 719-725.

King G.M. Distribution and metabolism of quaternary amines. (1988a) In **"Nitrogen Cycling in Coastal Marine Environments"**
(T.H. Blackburn and J. Sorensen eds.), SCOPE, John Wiley and Sons Ltd, pp 143-173.

King G.M. (1988b) Methanogenesis from methylated amines in a hypersaline algal mat. **Applied and Environmental Microbiology** 54(1), 130-136.

King P.C. and Goldstein L. (1983) Organic osmolytes and cell volume regulation in fish. **Molecular Physiology** 4, 53-66.

Kremling K. (1983) In **"Methods of seawater analysis"** (K. Grasshoff, M. Ehrhardt,and K. Kremling eds.) Verlag Chemie GmbH, D-6940 Weinheim, Germany. 259-260.

Kuwata K., Akiyama E., Yamazaki Y., Yamasaki H. and Kuge Y. (1983) Trace Determination of Low Molecular Weight Aliphatic Amines in Air by Gas Chromatography. **Analytical Chemistry** 55, 2199-2201.

Large P.J. (1983) **"Methylotrophy and methanogenesis".**

Lee C., Howarth R.W. and Howes B.L. (1980) Sterols in decomposing *Spartina alterniflora* and the use of ergosterol in estimating the contribution of fungi to detrital nitrogen. **Limnology and Oceanography** 25, 290-303.

Lee C. and Cronin C. (1982) The vertical flux of particulate organic nitrogen in the sea: decomposition of amino acids in the Peru upwelling area and the equatorial Atlantic. **Journal of Marine Research** 40, 227-251.

Lee C. and Olsen B.L. (1984) Dissolved, exchangeable and bound amines in marine sediments: Initial results. **Organic Geochemistry** 6, 259-263.

Lee C. (1988) Amino acid and amine biogeochemistry in marine particulate material and sediments. In **"Nitrogen Cycling in Coastal Marine Environments"** (Blackburn and Sorensen eds.) SCOPE, John Wiley and Sons Ltd. pp 125-141.

Lindroth P. and Mopper K. (1979) HPLC determination of sub-picomole amounts of amino acids by precolumn fluorescense derivitization with o-phthaldialdehyde. **Analytical Chemistry** 51, 1667-1674.

Lovley D.R. and Klug M.J. (1983) Methanogenesis from methanol and methylamines and acetogenesis from hydrogen and carbon dioxide in the sediments of a eutrophic lake. **Applied and Environmental Microbiology** 45, 1310-1315.

Lowis S. Eastwood M.A. and Brydon W.G. (1983) The measurement of methylamines in biological material using a gas chromatographic head space gas technique.
Journal of Chromatography 278, 139-143.

Lundh T. and Akesson B. (1991) Methods for the determination of dimethylamine and dimethylamine-N-oxide in air plasma and urine samples. **International Journal of Environmental and Analytical Chemistry** 44, 81-86.

McCalley D.V., Cooke M. and Nickless G. (1981) Effect of sewage treatment on faecal sterols. **Water Research** 15, 1019-1025.

McCarthy J.J., Taylor W.R. and Taft J.L. (1977) Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. **Limnology and Oceanography** 22, 996-1011.

McLusky D.S. (1968) Some effects of salinity on the distribution and abundance of *Corophium volutator* in the Ythan estuary. **Journal of the Marine Biological Association of the UK** 43, 443-454.

Mackin J.E. and Aller R.C. (1984) Ammonium adsorption in marine sediments. **Limnology and Oceanography** 29, 250-257.

Mackin J.E. and Swider K.T. (1989) Organic matter decomposition pathways and oxygen consumption in coastal marine sediments. **Journal of Marine Research** 47, 681-716.

Mague T.H., Friberg E., Hughes D.J. and Morris I. (1980) Extracellular release of carbon by marine phytoplankton; a physiological approach. **Limnology and Oceanography** 25(2), 262-279.

Marshmann N.A. and Marshall K.C. (1981) Bacterial growth on proteins in the presence of clay minerals. **Soil Biology and Biochemistry** 13, 127-134.

Maruyama M. and Nagayoshi T. (1992) Reversed-phase liquid chromatographic behaviour of alkylamines with amperometric detection and its application to trace analysis **Journal of Chromatography** 594, 159-163.

Marver H.S., Tshudy D.P., Perlroth M.G. Collins A. and Hunter G (1966) The determination of aminoketones in biological fluids. **Analytical Biochemistry** 14, 53-60.

Mayer L.M. and Rice D.L. (1992) Early diagenesis of protein: A seasonal study. **Limnology and Oceanography** 37(2), 280-295.

Mead G.C. (1971) The amino acid-fermenting Clostridia. **Journal of General Microbiology** 67, 47-56.

Metzler D.E. **"Biochemistry: The chemical reactions of living cells."** Academic: New York (1977) pp 444-460.

Miller I.T. and Springall H.D. (1966) **"The organic chemistry of nitrogen"** Clarendon Press, Oxford.

Miller J.C. and Miller J.N. (1993) **"Statistics for Analytical Chemistry"** Ellis Horwood.

Mopper K. and Lindroth P. (1982) Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. **Limnology and Oceanography** 27, 336-347.

Muller P.J. (1977) C/N ratios in Pacific deep-sea sediments: Effect of inorganic ammonium and organic nitrogen compounds sorbed by clays. **Geochimica et Cosmochimica Acta** 41, 765-776.

Murdoch J. and Barnes J.A. (1986) **"Statistical Tables for Science, Engineering, Management and Business Studies"**, MacMillen Press Ltd, London.

Murtagh J.J. and Bunch R.L.(1967) Sterols as a measure of faecal pollution. **Journal of the Water Pollution Control Federation** 39, 404-409.

Nagata T. and Kirchman D.L. Release of dissolved, free and combined amino acids by bacterivorous marine flagellates. **Limnology and Oceanography** (1991) 36(3), 433-443.

Neiderhiser D.H. and Fuller R.K. (1982) High-performance liquid chromatographic method for the determination of diethylamine, a metabolite of disulfiram in urine. **Journal of Chromatography** 229, 470-474.

Nicholls P.D., Leeming R. and Cresswell G. (1992) Survey of Sydney's coastal environment. **Australian Wastewater Association Water Journal** 19, 32-33.

Nishikawa Y. and Kuwata K. (1984) Liquid chromatographic determination of low molecular weight aliphatic amines in air via derivatization with 7-Chloro-4-Nitro-2,1,3-Benzoxadiazole. **Analytical Chemistry** 56, 1790-1793.

Nissenbaum A. and Kaplan I.R. (1972) Chemical and isotopic evidence for in situ origin of marine humic substances. **Limnology and Oceanography 17**, 570-582.

Nixon S.W. (1981) Remineralization and nutrient cycling in coastal marine ecosystems. In **"Estuaries and Nutrients"** (Nielson B.J. and Cronin L.E. eds.), Humana Press, Clipton, NJ. pp 111-138.

Ohshima H. and Kawabata T. (1978) Mechanism of the *N*-Nitrosodimethylamine formation from trimethylamine and trimethylamine oxide. **Bulletin of the Japanese Society of Scientific Fisheries 44(1)**, 77-81.

Oremland R.S., Marsh L. Des Marais D.J. (1982a) Methanogenesis in Big Soda Lake, Nevada: An Alkaline , Moderately Hypersaline Desert Lake. **Applied and Environmental Microbiology 43(2)**, 462-468.

Oremland R.S., Marsh L.M. and Polcin S. (1982b) Methane production and simultaneous sulphate reduction in Anoxic, Salt Marsh Sediments. **Nature 296**, 143- 145.

Patience R.L., Baxby M., Bartle K.D., Perry D.L., Rees A.G.W. and Rowland S.J. (1992) The functionality of organic nitrogen in some recent sediments from the Peru upwelling region. **Organic Geochemistry 18(2)**, 161-169.

Parsons T.R., Maita Y. and Lalli C.M. (1984) **"A Manual of Chemical and Biological Methods for Seawater Analysis".** Pergamon Press.

Patterson J.A. and Hespell R.B. (1979) TMA and MMA as growth substrates for rumen bacteria and *Methanosarcina barkeri*. **Current Microbiology 3**, 79-83.

Pedersen T.F., Sholkowitz E.R. and Malcolm S.J. (1985) A lightweight gravity corer for undisturbed sampling of soft sediments. **Canadian Journal of Earth Sciences 22**, 133-135.

Perez Martin R.I., Franco J.M., Molist P. and Gallardo J.M. (1987) Gas chromatographic method for the determination of volatile amines in seafoods. **International Journal of Food Science and Technology 22**, 509-514.

Pocklington R., Leonard J.D. and Crewe N.F. (1987) Coprostanol as an indicator of faecal contamination in seawater and marine sediment. **Oceanologica Acta 10(1)**, 683-689.

Pons J-L., Rimbault A., Darbord J.C. and Leluan G. (1985) Gas chromatographic-mass spectrometric analysis of volatile amines produced by several strains of *Clostridium*. **Journal of Chromatography 337**, 213-221.

Raymundo C.C. (1992) Linear alkylbenzenes in marine and estuarine sediments. **Ph.D. Thesis**, University of Liverpool, UK.

Readmann J.W., Preston M.R. and Mantoura R.F.C. (1986) An integrated technique to quantify sewage, oil and PAH pollution in estuarine and coastal environments. **Marine Pollution Bulletin** 17(7), 298-308.

Redfield A.C., Ketchum B.H. and Richards B.H. The influence of organisms on the composition of seawater. In **"The Sea"** (M.N. Hill ed.) New York pp 26-27.

Reeburgh W.S. (1967) An improved interstitial water sampler. **Limnology and Oceanography** 12, 163-165

Ridgway I.W. The behaviour of organic matter and minor elements in Scottish sea lochs. **PhD thesis**, University of Edinburgh (unpublished).

Ridgway I.M. and Price N.B. (1987) Geochemical associations and post-depositional mobility of heavy metals in coastal sediments: Loch Etive, Scotland. **Marine Chemistry** 21, 229-248.

Riley J.P. and Sinhaseni P. (1957) The determination of ammonia and total inorganic nitrogen in Seawater. **Journal of the Marine Biological Association UK** 36, 161- 168.

Rosenfield R.S., Fukushima D.K., Hellman L and Gallagher J.F.(1954) The transformation of cholesterol to coprostanol. **Journal of Biological Chemistry** 211, 301-311.

Rosenfield R.S. and Gallagher J.F.(1964) Further studies of the biotransformation of cholesterol to coprostanol. **Steroids** 4, 515-520.

Santiago-Mercado J. and Hazen T.C. (1987) Comparison of four membrane filter methods for faecal coliform enumeration in tropical waters. **Applied and Environmental Microbiology** 53(12), 2922-2928.

Scully F.E. Jr, Howell G.D., Penn H.H., Mazina K. and Johnson J.D. (1988) Small molecular weight organic amino nitrogen compounds in treated municipal waste water. **Environmental Science and Technology** 22, 1186-1190.

Sorensen J. and Glob E. (1987) Influence of benthic fauna on trimethylamine concentrations in coastal marine sediments. **Marine Ecology-Progress Series** 39, 15- 21.

Stams A.J.M. and Hansen T.A. (1984) Fermentation of glutamate and other compounds by *Acidaminobacter hydrogenoformans* gen. nov. sp. nov., an obligate anaerobe isolated from black mud. Studies with pure cultures and mixed cultures with sulphate-reducing and methanogenic bacteria. **Archives of Microbiology** 137, 329- 337.

Stevenson F.J. and Tilo S.N. (1970) Nitrogenous constituents in deep sea sediments. In **"Advances in organic geochemistry"** (Hobson G.D. and Speers G.C.) Pergamon Press, Oxford pp 227-253.

Stevenson F.J. and Cheng C.N. (1972) Organic geochemistry of the Argentine Basin sediments: carbon-nitrogen relationships and quaternary correlations. **Geochimica et Cosmochimica Acta** 36,653-671.

Stotzky G. (1980) Surface interactions between clay minerals and microbes, viruses and soluble organics, and the probable importance of these interactions to the ecology of microbes in soil. In **"Microbial Adhesion to Surfaces"** (R.C.W. Berkeley et al. eds.) Ellis-Horwood, West Sussex pp 231-247.

Terashi A., Hanada Y., Kido A. and Shinohara R. (1990) Determination of primary and secondary aliphatic amines in the environment as sulphonamide derivatives by GC-MS. **Journal of Chromatography** 503, 369-375.

Teshima S. and Kanazawa (1978) Occurrence of coprostanol, 24-ethylcoprostanol and 5-stanols in marine sediments. **Journal of the Oceanography Society of Japan** 34, 85-92.

Tod M., Legendre J.Y., Chalom J., Kouwatli H., Poulou M., Farinotti R. and Mahuzier G. (1992) Primary and secondary amine derivatizativation with luminarins 1 and 2: separation by liquid chromatography with peroxyoxylate chemiluminescence detection. **Journal of Chromatography** 594, 386-391.

Valiela I., Teal J.M. and Sass W.J. (1975) Production and dynamics of salt marsh vegetation and the effects of experimental treatment with sewage sludge: Biomass, production and species composition. **Journal of Applied Ecology** 12, 973-981.

Valiela I. (1983) Nitrogen in salt marsh ecosystems. In **"Nitrogen in the Marine Environment"** (E.J. Carpenter and D.G. Capone eds.), Academic Press pp 649-678.

Valiela I., Teal J.M., Allen S.D., Van Etten R., Goehringer D. and Volkmann S. (1985) Decomposition in salt marsh ecosystems: the phases and major factors affecting disappearance of above-ground organic matter. **Journal of Experimental Marine Biology and Ecology** 89, 29-54.

Valls M., Bayona J.M. and Albeiges J. (1989) Use of trialkylamines as an indicator of urban sewage in sludges, coastal waters and sediments. **Nature** 337, 722-724.

Van Neste A., Duce R.E. and Lee C. (1987) Methylamines in the marine atmosphere. **Geophysical Research Letters** 14(7), 711-714.

Wakeham S.G., Lee C. Farrington J.W. and Gagosian R.B. (1984) Biogeochemistry of particulate organic matter in the oceans: Results fron sediment trap experiments. **Deep Sea Research** 31, 509-528.

Walsh J., Premuzic E.T. and Whittledge T.E. (1981) Fate of nutrient enrichment on continental shelves as indicated by C/N content of bottom sediments. In **"Ecohydrodynamics"** (J.C.J. Nihoul ed.) Elsevier pp 13-49.

Walsh P.J., Danulat E. and Mommsen T.P. (1990) Variation in urea excretion in the gulf toadfish *Opsanus beta.* **Marine Biology** 106, 323-328.

Wang X. and Lee C. (1990) The distribution and adsorption behaviour of aliphatic amines in marine and lacustrine sediments. **Geochimica et Cosmochimica Acta** 54, 2759-2774.

Wazolek P.C. and Alexander M. (1979) Effect of desorption rate on the biodegradation of n-alkylamines bound to clay. **Journal of Agricultural and Food Chemistry** 27, 410-414.

Whelan J.K. (1977) Amino acids in a surface sediment core of the Atlantic abyssal plain. **Geochimica et Cosmochimica Acta** 41, 803-810.

Williams G.M., Smith B. and Ross C.A.M. (1991) The migration and degradation of waste organic waste organic compounds in groundwater. In **"Advances in Organic Geochemistry Part II: Advances and applications in energy and the natural environment.** (C.B. Eckardt, J.R. Maxwell, S.R. Larter and D.A.C. Manning eds.) Pergamon Press pp 531-543.

Wilson J.O., Buchsbaum R., Valiela I. and Swain T. (1986) Decomposition in salt marsh ecosystems: phenolic dynamics during decay of litter of *Spartina alteriflora.* **Marine Ecology-Progress Series** 9, 177-187.

Winfrey M.R. and Ward D.M. (1983) Substrates for sulphate reduction and methane production in intertidal sediments. **Applied and Environmental Microbiology** 45, 193-199.

Wood B.J.B., Tett P.B. and Edwards A. (1973) An introduction ot the phytoplankton, primary productivity and relevant hydrography of Loch Etive. **Journal of Ecology** 61, 569-585.

Yancey P.H., Clark M.E., Hand S.C., Bowlus R.D. and Somero G.N. (1982) Living with water stress: Evolution of osmolyte systems. **Science** 217, 1214-1222.

Yang X-H., Lee C. and Scranton M.I. (1993) Determination of nanomolar concentrations of individual dissolved low molecular weight amines and organic acids in seawater. **Analytical Chemistry** 65, 572-576.